Terrestrial Salamanders Maintain Habitat Suitability under Climate Change despite Trade-Offs between Water Loss and Gas Exchange*

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ABSTRACT

Physiological acclimation has the potential to improve survival during climate change by reducing sensitivity to warming. However, acclimation can produce trade-offs due to links between related physiological traits. Water loss and gas exchange are intrinsically linked by the need for respiratory surfaces to remain moist. As climates warm and dry, organisms may attempt to lower desiccation risk by limiting water loss but at a cost of inhibiting their ability to breathe. Here we used laboratory experiments to evaluate the trade-off between water loss and gas exchange in a fully terrestrial, lungless salamander (*Plethodon metcalfi*). We measured acclimation of resistance to water loss and metabolic rates in response to long-term exposure to temperature and humidity treatments. We then integrated the trade-off into a simulation-based species distribution model to determine the consequences of ignoring physiological trade-offs on energy balance and aerobic scope under climate change. In the laboratory, we found a close association between acclimation of resistance to water loss and metabolic rates indicative of a trade-off. After incorporating the trade-off into our simulations, we found that energy balance and aerobic scope were reduced by 49.7% and 34.3%, respectively, under contemporary climates across their geographic range. Under future warming scenarios, incorporating the trade-off lowered the number of sites predicted to experience local extirpation by 52.2% relative to simulations without the trade-off; however, the number of sites capable of supporting the energetic requirements for reproduction declined from 44.6% to 32.6% across the species' geographic range. These experiments and simulations suggest that salamanders can maintain positive energy balance across their geographic range under climate change despite the costs associated with trade-offs between water loss and gas exchange.

Keywords: acclimation, climate change, trade-offs, water loss, metabolism, gas exchange, mechanistic species distribution models.

Introduction

Climate change threatens survival at a physiological level by pushing organisms closer to lethal thermal tolerances (Pinsky et al. 2019) and challenging their capacity to balance energy budgets (Huey and Kingsolver 2019). To reduce the threat of warming, animals can reversibly adjust the sensitivity of physiological traits depending on environmental conditions (Somero 2010). These reversible responses are often referred to as acclimation or acclimatization, depending on whether the response occurs from a single variable in a laboratory or from multiple environmental variables in a field setting, respectively (Roberts et al. 1997). Organisms with a greater capacity to acclimate are predicted to experience less decline under climate change (Stillman 2003; Somero 2010; Gunderson and Stillman 2015), but studies often focus on the benefits of acclimation in single trait without considering the potential consequences for related or linked traits (Seebacher et al. 2015). Because of the integrated nature of physiological traits (Mykles et al. 2010), incorporating the links between physiological traits during acclimation may produce more realistic predictions for habitat suitability under climate change.

Dependencies between physiological traits produce trade-offs that influence the capacity of an organism to respond to environmental change. We define a trade-off as any increase in a physiological trait that necessitates a reduction in a related trait (Garland 2014). Gas exchange and water loss are fundamentally linked physiological traits due to shared chemical and physical pathways. Specifically, diffusion of gases across a respiratory surface—such as lungs, leaves, or skin—requires a moist and permeable surface for gas exchange (Maina 1998). Any reductions in moisture to the respiratory surface would thereby constrain the ability of plants or animals to respire. This trade-off influences variation in water loss and metabolic rates across plants, insects, and many vertebrates (Woods and Smith 2010). The pattern also holds for individuals during acclimation. Animals that increase their resistance to water loss (i.e., a reduction in water loss rates) often experience a simultaneous reduction in metabolic rate (Addo-Bediako et al. 2001; Riddell et al. 2018*a*). As warming and drying continue under climate change, plants and animals may struggle to meet their respiratory requirements while attempting to avoid desiccation by rapid water loss. Predictive models that are capable of incorporating complex physiological relationships, such

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as mechanistic species distribution models (SDMs), will help to explore the effect of these trade-offs on organismal performance.

Here we evaluated the trade-off between resistance to water loss (r_i) and volume of oxygen consumption ($\dot{V}O_2$) in an acclimation experiment on a widely distributed terrestrial salamander (*Plethodon metcalfi*). These terrestrial salamanders are an excellent system to evaluate this physiological trade-off because, being lungless, water loss and gas exchange occur across the same surface—the skin. We combined a long-term acclimation experiment that simulated nightly bouts of activity under various temperature and humidity treatments with flow-through respirometry to measure acclimation of r_i and $\dot{V}O_2$. We then integrated the trade-off between water loss and gas exchange into a simulation-based SDM that estimated energy balance and aerobic scope across the southern Appalachian Mountains under the "business as usual" climate warming scenario. We ran simulations under various scenarios to demonstrate the effect of ignoring acclimatization, considering acclimatization in only a single trait, and the trade-off between the two related physiological traits. We predicted that the trade-off would substantially reduce energy balance and aerobic scope across the geographic range under climate warming scenarios and undermine the capacity of salamanders to avoid local extirpation under climate change.

Methods

Laboratory Experiments

We evaluated the physiological trade-off between water loss and gas exchange using a long-term acclimation experiment in the laboratory on *Plethodon metcalfi*, one of the most widely distributed and abundant salamanders of the *Plethodon jordani* species complex (Hairston 1980). Salamanders were collected from nature ($n = 156$) along an elevational gradient to account for potential geographic variation in physiology (Riddell and Sears 2015). We collected salamanders at night in the spring of 2015 from randomly generated coordinates on Coward Mountain in the Balsam Mountain Range (35°21′3.8″N, −83°4′25.5″W). After collection, salamanders were immediately transported to the laboratory at Clemson University. We received the appropriate permissions to collect salamanders (North Carolina Wildlife Commission: 16-SC00746; US Fish and Wildlife Service: MA90761B-0) and conduct experiments in the laboratory (Clemson University Institute for Animal Care and Use Committee: 2014-024).

Salamanders were maintained in a monthlong acclimation period before the experiment. During this time, salamanders were held under saturated conditions under a cool, cycling temperature regime in an incubator (I-36VL; Percival, Perry, IA) in individual containers (17 cm × 17 cm × 12 cm) with wet paper towels. We generated the temperature profile to simulate the thermal cycle that salamanders experience in the spring (Riddell et al. 2018*a*). The acclimation period was designed to minimize physiological differences that may be caused by acclimatization from their site of collection and expose individuals to field conditions. After the monthlong acclimation period, we measured the physiological traits of each individual using a flow-through system (see description below).

After the initial physiological measurements, individuals were randomly divided into four treatments ($n = 36$ for each treatment). The treatments consisted of either a warm or a cool temperature cycle and either a wet or a dry humidity cycle in a full factorial design. The cool temperature cycle was programmed to fluctuate between 8.5° and 15°C, and the warm temperature cycle fluctuated between 15° and 21.5°C. The warm temperature cycle represents a scenario that salamanders would experience under the business as usual climate warming scenario (Riddell et al. 2019). The treatments also regulated the vapor pressure deficit (VPD) by fluctuating the relative humidity to maintain either a wet (0.25 kPA) or a dry (0.5 kPa) scenario, which also replicated relevant vapor pressures for salamanders (Riddell et al. 2019).

Each night during the experiment, salamanders were moved to an open-air activity chamber (17 cm × 17 cm × 12 cm) to simulate nocturnal activity and expose individuals to their treatment in the incubators. The activity chambers consisted of dried soil and a mesh lid to allow salamanders to experience the humidity in the incubator. We monitored the temperature and humidity in the enclosures using iButtons (Hygrochrons; Maxim Integrated, San Jose, CA) to validate the treatment conditions. After a 3-h exposure, salamanders were returned to their containers with wet paper towels to rehydrate. Salamanders were weighed to the nearest 0.001 g before and after each exposure. After five nights of exposure, we measured the physiological rates using the flow-through system (see below). We continued to expose and measure individuals for 4 wk to assess change in physiological rates. We assessed the health of each salamander daily through visual inspection and by measuring the change in mass relative to a baseline mass determined at the beginning of the experiment. We did not expose salamanders to their treatment if they had not rehydrated to within 10% of their baseline mass from their previous exposure, which occurred in only 1.1% of total exposures (22 out of 1,921 exposures). We also randomized the shelf, location on the shelf, and incubators throughout the experiment to minimize any effects related to the incubator.

Flow-Through System

We measured r_i and $\dot{V}O_2$ using a flow-through respirometry system (Sable Systems, Las Vegas) capable of precisely controlling the temperature and VPD. Physiological rates were measured at constant temperature (18°C) and VPD (0.5 kPa). We used a subsample pump (SS4; Sable Systems) to pump air into a bubbler bottle (provided by Sable Systems) to saturate the air. We then used a dew point generator (DG4; Sable Systems) to remove water vapor to the desired VPD (0.5 kPa). The air was then divided into separate chambers, and flow rates were controlled using a flow manifold (180 mL/min). The airstream then passed into individual acrylic chambers (16 cm × 3.5 cm; volume ca. 153 mL) containing a single salamander. The salamanders were suspended on a hardwire mesh platform to minimize their ability to behaviorally reduce water loss rates and standardize posture that mimicked the same posture during activity. We then sampled each constantly flowing chamber using a multiplexer (MUX8; Sable Systems), followed by a water-vapor analyzer (RH300; Sable Systems) and a dual differential oxygen analyzer (Oxzilla; Sable Systems). The airstream was scrubbed of water vapor and carbon dioxide before any measurements of oxygen consumption and flow rate.

We ensured that salamanders remained inactive during the experiment by assessing irregularities in the vapor pressure readings. During activity, water loss rates can become highly variable and exceed 18-fold inactive values. We removed any readings indicative of activity or when salamanders urinated during the experiment. The series of equations used to transform voltage changes into meaningful physiological values can be found in previously published literature (Riddell et al. 2017). Physiological traits were measured three times for each individual over a period of 2.5 h, and we analyzed the average value across the three measurements for each individual.

Physiological Simulations

We simulated the consequences of physiological trade-offs using a mechanistic SDM. The model generates predictions for annual activity and energy balance across landscapes from broadscale climatic data by simulating activity for each night of the year (Riddell et al. 2018*b*). We leveraged the model to predict energy balance as a surrogate of habitat suitability for the *P. jordani* species complex based on the assumption that all seven species within the complex have similar physiological requirements (Kozak and Wiens 2006; Gifford and Kozak 2012). We used climatic data from microclim, a data repository that estimates the relevant microhabitat for any species of interest (Kearney et al. 2014). We then used Python (ver. 2.7) to model organismal performance in local microhabitat conditions associated with salamander habitat. We used first principles to estimate humid operative temperature (T_{eh}) for salamanders, which incorporated the effect of evaporative cooling on body temperatures (Riddell et al. 2018*b*), though the effect of evaporative cooling was very low, as conditions during activity were generally saturated.

In the simulations, salamanders were active at night under thermal conditions that were suitable for activity, which ranged from 5° to 25°C (Spotila 1972; Riddell et al. 2018*b*). We assumed that salamanders remained active on the forest floor until they lost 10% of their body mass to water loss—an amount of time that was determined by the combination of local humidity and physiological resistance to water loss—or if environmental conditions exceeded thermal preferences (Feder and Londos 1984; Caruso et al. 2014). If inactive or during the day, salamanders remained underground, and we estimated body temperature on the basis of the soil depth that salamanders typically experience (Riddell et al. 2018*b*). Salamanders assimilated and burned energy while active given established empirical thermal performance curves under the assumption that they are generally not limited by prey availability (Gifford and Kozak 2012; Clay and Gifford 2017; Riddell et al. 2018*b*). These nightly simulations were run across the southern Appalachian Mountains for an entire year to identify regions that met the annual energetic requirements for survival and reproduction (Riddell et al. 2018*b*). Salamanders also maximized energy balance by avoiding surface activity if energetic costs exceeded energy intake during activity. Avoiding negative energy balance by seeking underground microhabitats simulates the sensitivity of activity to harsh environmental conditions, a key aspect in the natural history of terrestrial salamanders (Heatwole 1962).

We determined whether a site became locally extirpated by calculating the depletion of annual lipid reserves, estimated by the mass-specified amount of triglycerides for a given plethodontid salamander (Riddell et al. 2018*b*). We assumed an average-sized salamander (3 g) to focus on the effect of the trade-off, though body mass can play an important role in determining energy balance across landscapes (Riddell et al. 2018*b*). The body size also corresponds to the minimum age of maturation for females in this species complex (Hairston 1983). We determined whether salamanders acquired sufficient energy for reproduction at a particular site by evaluating whether annual energy balance exceeded 1.2 kJ, half of the energy required to produce an average clutch size (10 eggs) every other year (Fitzpatrick 1973; Hairston 1983; Gifford and Kozak 2012). We defined reproductive potential as the proportion of sites within the geographic range of the species that exceeded 1.2 kJ/yr.

The simulations were executed using classes in Python (ver. 2.7), which assigns attributes to individuals at the beginning of the simulation (e.g., skin resistance, mass, etc.) and subsequently tracks attributes of interest (e.g., energy balance, mass lost from water loss) through time. The code for the simulation can be found at http://www.github.com/ecophysiology. Here we leveraged these simulations to explore the potential trade-off between water loss and gas exchange under the business as usual warming scenario (Representative Concentration Pathway 8.5), corresponding to a future with the highest predicted greenhouse gas emissions.

Incorporation of Trade-Off

Previous simulations indicated that the trade-off between water loss and gas exchange determined the phenotypic variation of physiological rates in *Plethodon* salamanders (Riddell et al. 2018*a*). In this analysis, we explicitly incorporated the costs of acclimation by adjusting metabolic rates, energy assimilation rates, and maximal $\dot{V}\text{O}_2$ with changes in r_i. As a result, we incorporated potential costs associated with higher resistance to water loss. We estimated the maximal metabolic rates (MMRs) based on empirical evidence that maximal rates are roughly fourfold higher than resting metabolic rates (RMRs; Withers 1980). Using this empirical data, we used DataGraph (Visual Data Tools) to generate the following hypothetical function for MMR:

$$\begin{aligned}\text{MMR} = 1.914 + (-0.217 \times T_{eh}) + (0.237 \times T_{eh}^2)\\ + (-0.007 \times T_{eh}^3),\end{aligned}$$

where T_{eh} represents the humid operative temperature. The function was selected based on the best fit with empirical estimates of MMR ($r^2 = 0.99$). We generated the function for an average-sized salamander (3 g), and consequently the function does not incorporate the sensitivity of MMR to mass. Though beyond the scope of this study, mass likely has a positive relationship with aerobic scope and energy balance, suggesting that juveniles may be more sensitive to warming than adults (Riddell et al. 2018*b*). For each hour of the day, we estimated the MMR (fig. 1) and subtracted the RMR to estimate the aerobic scope at any time point during the day.

We then ran various simulations to illustrate the consequences of including acclimation with or without the trade-off. In simulations with acclimation, salamanders acclimated r_i based on the elevation using empirical evidence from geographic variation in r_i (Riddell and Sears 2015). Individuals that lived at or below 1,000 m exhibited an r_i of 7 s/cm, the highest resistance to water loss, which also occurs at the edge of their lower elevational limit. For individuals at or above 3,000 m, individuals exhibited the lowest r_i (4 s/cm), which also coincides with the most-saturated air (Riddell and Sears 2015). Between 1,000 and 3,000 m, individuals adjusted their resistance proportional to the difference between the maximum and minimum r_i, modeled after the observed geographic variation in water loss (Riddell and Sears 2015).

In simulations with the trade-off, we reduced energy assimilation, RMR, and MMR depending on the degree of acclimation (fig. 1). Individuals that exhibited the highest r_i (7 s/cm) exhibited the greatest reduction in the energetic traits—a 36% reduction based on acclimation responses in laboratory experiments (Riddell et al. 2019). Individuals with the lowest r_i exhibited the maximal, nonacclimated phenotypes, and individuals with r_i between 4 and 7 s/cm acclimated proportionally based on their elevation. Therefore, simulations incorporated the benefits of acclimation of r_i by increasing activity time and also the costs associated with lower energy assimilation and aerobic scope. Though estimated, these traits were generated from empirical evidence on the geographic variation in physiological traits as well as the extent to which individuals acclimated in controlled laboratory experiments. We then generated spatially explicit hypotheses that predicted energy balance across the southern Appalachian Mountains and calculated the proportion of sites that met the criteria for positive energy balance, reproductive potential, and local extirpation within the species' geographic range.

Statistical Analyses

We conducted statistical analyses in R (ver. 3.4.2; R Core Team 2017) to analyze the physiological responses from the experiment. We conducted three separate analyses. First, we evaluated the trade-off using linear regressions between the change in r_i and $\dot{V}O_2$ for each individual. In separate analyses, we also evaluated the relationship between the change in a physiological trait (r_i and $\dot{V}O_2$) and the initial value of the trait. These separate analyses were intended to test for any effect of mass or treatment that affected a physiological trait independent of the other trait. For each analysis, we included the effect of experimental treatment and their interaction as factors. Mass was also included as a covariate as well as its interaction with treatment because of previous studies (Riddell et al. 2019). We then used a type II ANCOVA to determine the degree of association between the two traits or the initial values of either trait. We reported adjusted R^2 values to describe the proportion

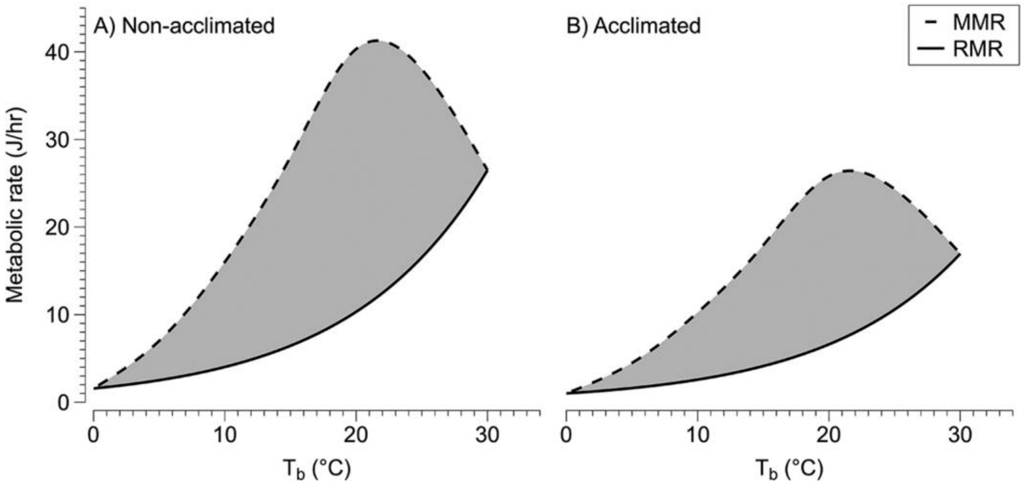

Figure 1. Hypothetical acclimation in the thermal sensitivity of aerobic scope. *A*, The empirically determined thermal sensitivity of the resting metabolic rate (RMR; solid line) in a nonacclimated adult (3 g) and the hypothetical maximal metabolic rate (MMR; dashed line) represented a roughly fourfold increase in metabolic expenditure. The aerobic scope is illustrated by the gray fill between RMR and MMR. *B*, The empirically determined degree of acclimation in RMR (solid line) and the hypothetical reduction in MMR (dashed line) due to the trade-off between acclimation of r_i and $\dot{V}O_2$. Aerobic scope in an acclimated individual was predicted to be much lower than in a nonacclimated individual because of the underlying physiological trade-off. A color version of this figure is available online.

of explained variation. For the simulations, we generated a script that calculated the proportion of sites within the species range complex of *P. jordani* with positive energy balance, that experienced local extirpation, and that maintained sufficient energy for reproduction (http://www.github.com/ecophysiology). The analysis for the simulations evaluated our spatially explicit hypotheses on the energetic requirements for reproduction using the geographic range defined by the International Union for Conservation of Nature (http://www.iucnredlist.org).

Results

Laboratory Experiments

Physiological responses to the acclimation experiment supported the trade-off between water loss and gas exchange. The degree to which individuals increased r_i coincided with the degree to which $\dot{V}\text{O}_2$ decreased within an individual (fig. 2*A*; $F_{1,118} = 34.51$, $P < 0.001$, $R^2 = 0.22$). On average, individuals increased r_i by 21.8%, and $\dot{V}\text{O}_2$ declined by 36.4% over the course of the experiment. However, the experiment revealed a high degree of variation in the magnitude of acclimation. Individuals that did not change r_i also did not change $\dot{V}\text{O}_2$ (fig. 2*A*). In a few cases, individuals that reduced r_i (i.e., water loss rates increased) exhibited an increase in $\dot{V}\text{O}_2$. At the other end of the spectrum, individuals increased r_i by as much as 52.6% (initial $r_i = 4$ s/cm; final $r_i = 6.1$ s/cm), which coincided with a 71.4% reduction in $\dot{V}\text{O}_2$ (initial $\dot{V}\text{O}_2 = 350$ μL/min; final $\dot{V}\text{O}_2 = 100$ μL/min). Neither the treatments nor the mass explained significant variation in the relationship between the change in r_i and $\dot{V}\text{O}_2$ ($P > 0.05$). The highly correlated responses between r_i and $\dot{V}\text{O}_2$ suggest that increasing the physiological barrier to water loss also inhibits gas exchange across the integument.

Our experiments revealed a high degree of individual variation in physiological plasticity. For r_i, individuals that began the experiment with a low r_i (i.e., a high water loss rate) exhibited the greatest increase in r_i (fig. 2*B*; $F_{1,111} = 41.95$, $P < 0.001$, $R^2 = 0.43$). Mass was also positively associated with the increase in r_i in the warm treatment, as indicated by the interaction between mass and temperature treatment ($F_{1,111} = 2.27$, $P = 0.036$). For $\dot{V}\text{O}_2$, individuals that began the experiment with the highest $\dot{V}\text{O}_2$ exhibited the greatest reduction in $\dot{V}\text{O}_2$ over the course of the experiment (fig. 2*C*; $F_{1,111} = 580.73$, $P < 0.001$, $R^2 = 0.84$). Neither mass nor experimental treatment was associated with the change in $\dot{V}\text{O}_2$. These patterns indicate that our population exhibited a spectrum of plasticity that ranged from very high to low plasticity.

Physiological Simulations

Salamanders maintained much of their suitable habitat under contemporary climate scenarios despite the costs associated with the trade-off. By incorporating the acclimation of r_i, average energy balance within the species range increased by 166.6%, from a negative average in simulations without acclimation (mean ± SD = −1.6 ± 2.4 kJ) to a positive average after including acclimation of r_i (1.1 ± 1.8 kJ; fig. 3*A*, 3*B*). Incorporating acclimation also increased the annual sum of aerobic scope by 4% across the species range (fig. 3*D*, 3*E*), and sites capable of sustaining reproduction increased from 18.2% to 67.2% with acclimation. Incorporating the trade-off with acclimation reduced the average energy balance by 49.7% (0.5 ± 1.3 kJ) across the species range (fig. 3*C*); however, the number of sites in positive energy balance only decreased from 80.4% to 77.4% with the trade-off. Annual aerobic scope decreased by 34.3% after including the trade-off relative to simulations that incorporated only acclimation of r_i.

Though energy balance and aerobic scope declined with the trade-off, sites within the species range did not experience any local extirpation under contemporary climatic conditions (fig. 3*C*), whereas in simulations that incorporated only acclimation, 2.1% of the sites experienced local extirpation (fig. 3*B*). The trade-off also reduced the number of sites with reproductive

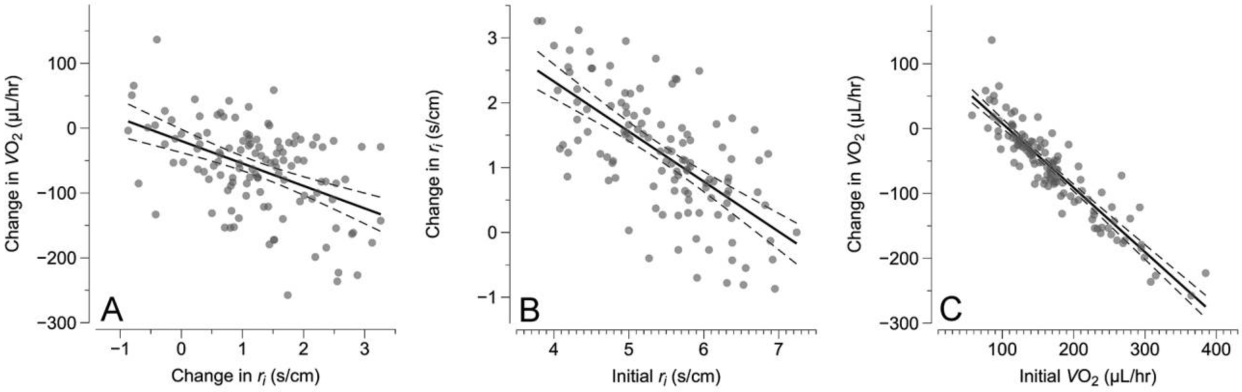

Figure 2. Acclimation in $\dot{V}\text{O}_2$ influenced by the degree of change in r_i. *A*, Degree of acclimation in volume of oxygen consumption ($\dot{V}\text{O}_2$) was associated with the change in resistance to water loss (r_i) over the course of a 1-mo acclimation experiment. *B*, Individuals that began the experiment with a low r_i exhibited the greatest increase in r_i. *C*, Individuals that began the experiment with high $\dot{V}\text{O}_2$ exhibited the greatest reduction in $\dot{V}\text{O}_2$. The figure illustrates the physiological effect of the trade-offs and individual variation in physiological plasticity.

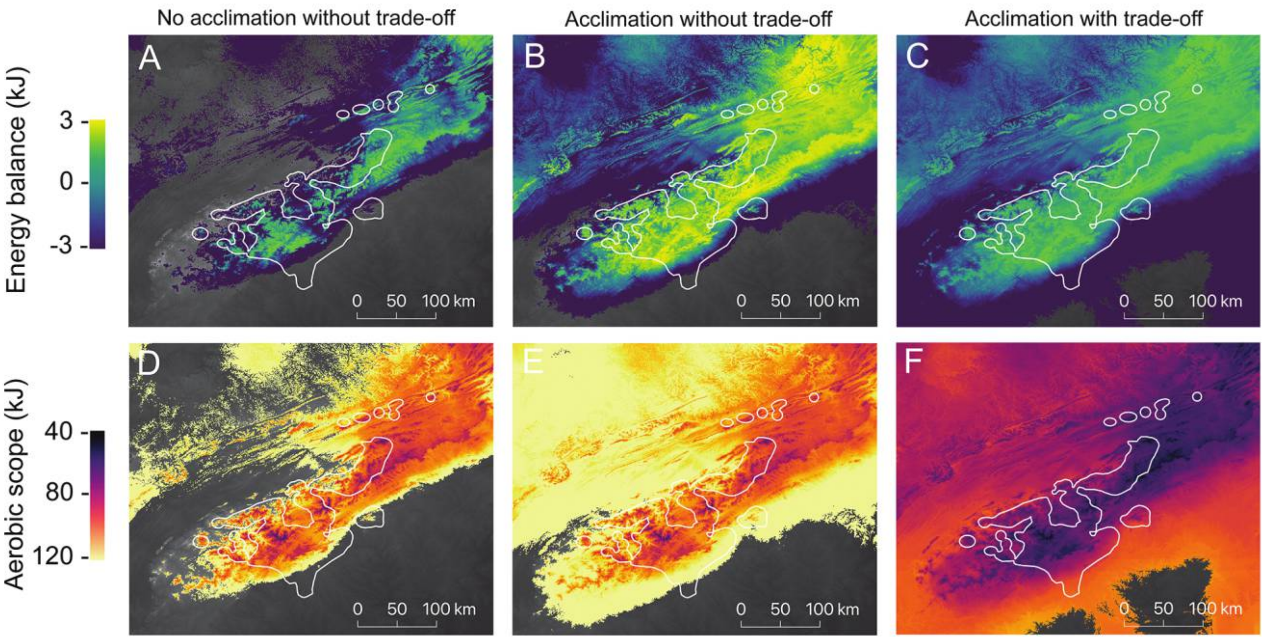

Figure 3. Habitat suitability is maintained despite costs associated with physiological trade-off. Simulations included scenarios without acclimation in r_i and without the physiological trade-off (*A*, *D*), acclimation in r_i without the physiological trade-off (*B*, *E*), and acclimation in r_i with the physiological trade-off (*C*, *F*). Shown are the annual energy balance (*A*–*C*) and the annual sum of aerobic scope (maximal metabolic rate − resting metabolic rate; *D*–*F*) throughout the southern Appalachian Mountains, with the geographic range of the *Plethodon jordani* species complex shown in white. Energy balance and aerobic scope increased by including acclimation of r_i without the trade-off. By including the trade-off, aerobic scope was greatly reduced throughout the geographic range, but the majority of locations maintained positive energy balance throughout the species' existing geographic range.

potential from 67.2% with only acclimation to 44.9% with the trade-off and acclimation. The costs associated with trade-offs during acclimation were high for aerobic scope and reproductive potential, but salamanders maintained most of their suitable habitat and experienced fewer local extirpations under contemporary conditions.

Under the business as usual climate warming scenario, habitat suitability was maintained after including the trade-off. No matter the simulation, climate warming increased the number of sites that experienced local extirpation relative to contemporary climates (fig. 4*A*–4*C*). However, including acclimation and the trade-off resulted in the lowest number of sites experiencing local extirpation. Including the effect of acclimation increased the number of sites with positive energy balance from 28.8% to 53.1% (fig. 4*A*, 4*B*). By incorporating the physiological trade-off, the proportion of sites with positive energy balance declined to 44.5% (fig. 4*C*). In simulations with acclimation, 44.7% of the sites were capable of reproduction, and by incorporating the trade-off, reproductive potential declined to 32.6%.

Across all simulations, aerobic scope increased by 42.9% over the course of the next century of warming. Under climate warming, incorporating the trade-off reduced aerobic scope by 33.5% relative to simulations with only acclimation of r_i (fig. 4*E*, 4*F*). Including the trade-off also reduced the number of sites that experienced local extirpation from 20.2% to 9.6%, reducing the number of sites experiencing climate-driven extirpation by 52.2%. Including the physiological trade-off reduced energy balance and the number of sites with reproductive potential, but fewer sites experienced local extirpation.

Discussion

Acclimation has the potential to dramatically reduce the energetic costs associated with climate warming for a wide variety of ectotherms (Gunderson and Stillman 2015; Seebacher et al. 2015), but few studies have explicitly explored trade-offs related to reversible acclimation in an ecological context. Our experiments provided physiological evidence for a trade-off between avoiding desiccation and respiration. We found that the degree to which salamanders increased resistance to water loss coincided with the simultaneous reduction in metabolic rate. The pattern suggests that increasing the barrier to water loss across the skin also interferes with gas exchange. Using our simulation-based approach, we found that salamanders maintained positive energy balance throughout most of their range under the worst case climate warming scenario, even with the costs associated with lower rates of energy assimilation. The simulations revealed a dramatic reduction in aerobic scope by incorporating the trade-off (figs. 3, 4), which may reflect the low-energy lifestyle characterized by lungless salamanders (Feder 1983). However, incorporating the trade-off also reduced the number of sites with reproductive potential, providing a clear fitness-related cost of physiological trade-offs during acclimation.

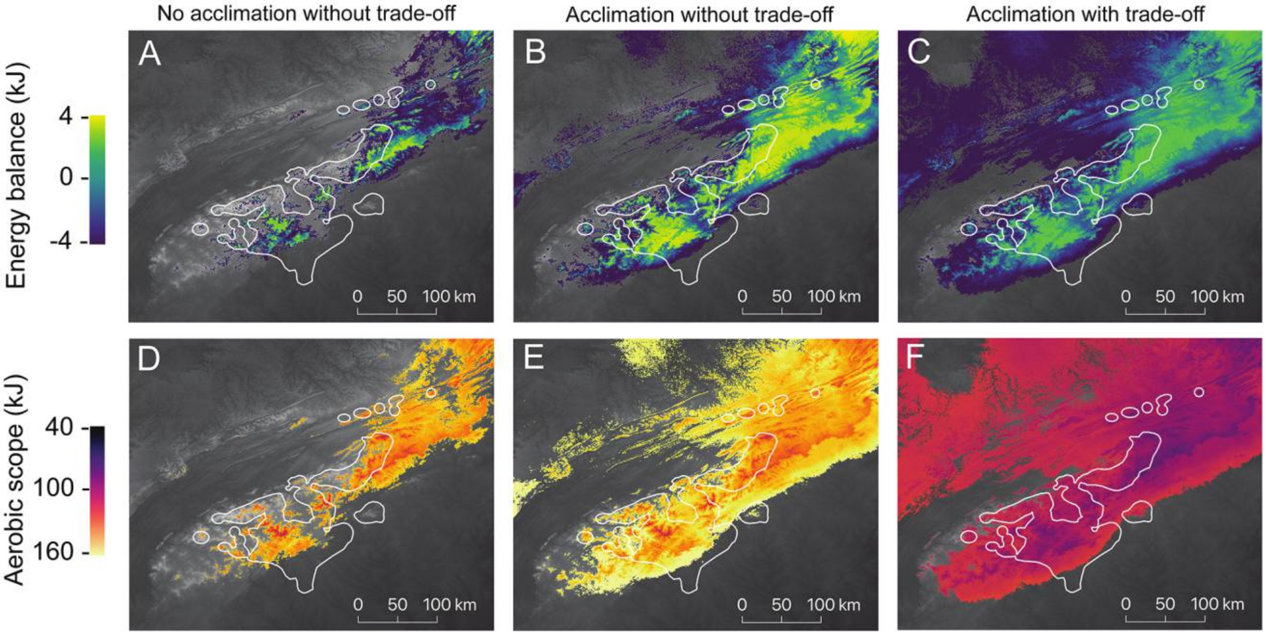

Figure 4. Salamanders maintain suitable habitat under climate change with simulated trade-off. Simulations under the business as usual climate warming scenario (Representative Concentration Pathway 8.5) included those without acclimation in r_i and without the physiological trade-off (*A*, *D*), acclimation in r_i without the physiological trade-off (*B*, *E*), and acclimation in r_i with the physiological trade-off (*C*, *F*). Shown are the annual energy balance (*A*–*C*) and the annual sum of aerobic scope (maximal metabolic rate − resting metabolic rate; *D*–*F*) throughout the southern Appalachian Mountains, with the geographic range of the *Plethodon jordani* species complex shown in white. Similar to simulations under contemporary climate scenarios, aerobic scope was much lower when including acclimation and the trade-off compared with scenarios without acclimation or the trade-off. Despite the lower aerobic scope, salamanders still maintained positive energy balance throughout much of their geographic range under the worst case climate warming scenario.

We predicted that acclimation to climate change will reduce reproductive potential in terrestrial salamanders by reducing energy balance. In plethodontines, females generally reproduce biennially (Tilley and Bernardo 1993), but in the most southerly latitudes, some populations reproduce annually and at a younger age (Highton 1962). This geographic variation in life-history traits may be due to shorter hibernation periods that widen foraging opportunities and time for ova maturation (Highton 1962). Interestingly, using the same logic, climate warming could increase reproductive output in populations at higher latitudes by extending the activity season. For our species, climate change might erode reproductive potential by increasing energetic costs and lowering rates of energy assimilation. Therefore, warming may translate into lower reproductive output through smaller clutch sizes or fewer opportunities to reproduce. However, reproductive potential declined from 44.9% to 32.4% under the worst case climate warming scenario, indicating that the majority of regions with reproductive potential under contemporary climates are likely to persist. The reduction in reproductive potential is an important cost to physiological trade-offs, but they do not fully undermine the potential resistance of salamanders to warming. Predicting further costs to fitness will depend on the mechanisms that link the trade-off and their influence to related complex phenotypes.

The links between water loss and metabolic rates are tightly coupled in salamanders because these fluxes occur simultaneously across the skin (Feder 1983). The diffusion of oxygen requires a moist surface for gas exchange to occur (Feder and Burggren 1985; Maina 2002). Any reduction in moisture to the skin would thereby reduce the capacity for oxygen to dissolve into the skin and underlying capillary beds. Salamanders limit water loss through perfusion and vascular regression, which would also reduce the amount of blood delivered to the skin for gas exchange (Brown 1972; Burggren and Moallf 1984; Riddell et al. 2019). In previous studies that examined gene expression in this experiment, salamanders without physiological flexibility constitutively express higher levels of ALOXE3 (Riddell et al. 2019), a gene underlying the production of a lipid barrier to reduce water loss rates across the skin (Champagne et al. 2015). Salamanders have the capacity to reduce desiccation risk by increasing the dermal barrier to water loss, but these responses likely constrain respiration over longer periods of time. These underlying mechanisms may be fundamental to limiting amphibians to cooler and more moist habitats (Peterman and Semlitsch 2014; Gade and Peterman 2019). Further studies into the pathways that link water loss and gas exchange are critical for predicting complex physiological responses to environmental change.

Trait-specific responses to the experimental treatments indicate that these traits may also become uncoupled. Our experiments indicated that temperature and mass influenced acclimation in r_i but not in $\dot{V}O_2$. These differences indicate that water loss may be regulated independently of gas exchange because of physiological changes along the oxygen transport pathway (Storz et al. 2010). The first adjustable process in the exchange of oxygen is ventilation. Without lungs, salamanders cannot adjust pulmonary ventilation, but acclimation in rates of buccal pumping may be critical for modulating gas exchange (Whitford and Hutchison 1965), especially given the expression of novel surfactant proteins that likely facilitate buccopharyngeal respiration (Lewis et al. 2018). Alternatively, salamanders might adjust the concentration of hemoglobin, hematocrit, or oxygen-binding affinity (Gatz et al. 1974). Changes along the oxygen transport pathway would allow salamanders to avoid desiccation under warm temperatures by lowering water loss rates, while simultaneously promoting their ability to respire. Impeding respiration likely has consequences for other complex phenotypes, such as foraging ability and competitiveness.

A more complete understanding of trade-offs requires knowledge of the behavioral consequences of physiological acclimation. Metabolic rates reflect the energetic demands required for maintenance, growth, and reproduction, but they also support energetically intensive behaviors, such as foraging and competition (Huey and Pianka 1981; Gillooly et al. 2001). Metabolic rates in anurans, for instance, vary depending on the energetic costs associated with demands for capturing certain prey (Taigen and Pough 1983), with RMRs being positively associated with the estimated foraging intensity for particular prey. Across insects, sit-and-wait predators exhibit some of the lowest RMRs among insects of similar body mass (Greenstone and Bennett 1980). Organisms that maintain higher RMRs after acclimation also tend to outmatch competitors during agonistic behaviors (Seebacher and Wilson 2006; Wilson et al. 2007). The influence of metabolic acclimation on competition and foraging behavior in salamanders is less clear but requires more attention (Gifford 2016), especially given the role of interspecific competition in determining species range boundaries (Gifford and Kozak 2012).

A species' geographic range can reflect the physiological mechanisms that constrain organisms to specific environmental conditions—a fundamental insight underlying the development of the macrophysiology discipline (Chown et al. 2004). Associations between physiological tolerance and the extent of a species' geographic range have been found for a wide variety of terrestrial and aquatic taxa (Sunday et al. 2010). In salamanders, species that inhabit a wider range of temperatures also exhibit greater breadth in their ability to assimilate energy (Clay and Gifford 2018), and similarly, species with greater plasticity in metabolic rates also tend to have larger geographic ranges (Markle and Kozak 2018). The trade-off between desiccation risk and respiration may therefore influence the outcome of competitive interactions near the warm edges of a geographic range. The temperature-dependent outcome of competition requires further investigation, especially given inconsistencies between studies of competition in salamanders (Hairston 1951, 1980; Nishikawa 1990; Clay and Gifford 2016; Hoffacker et al. 2018). The integration of thermally mediated competition and physiological plasticity will ultimately improve predictions of shifts in the geographic range due to abiotic and biotic factors.

Predicting the loss of biodiversity under climate change will require a greater understanding of the trade-offs between related traits. The last decade has witnessed a rapid rise in the complexity of predictions of habitat suitability under climate change, from both a mechanistic and a statistical perspective (Sears et al. 2016; Carlo et al. 2017; Briscoe et al. 2019; Enriquez-Urzelai et al. 2019). More recent predictive models for salamanders have focused on the impact of physiological and behavioral strategies for limiting the impact of climate change (Riddell et al. 2018*b*; Lyons and Kozak 2019; McEntire and Maerz 2019). Despite these advances, most predictions of extinction risk have yet to incorporate any kind of mechanism (Urban et al. 2016). To advance predictions structured in mechanism, models require the flexibility to incorporate such complex trade-offs into a framework that integrates across the biological hierarchy of organization to make predictions about organismal performance, survival, and reproduction (Kearney and Porter 2019). Such an integration will improve our ability to predict the impending loss of biodiversity while also enriching quantitative theory in ecology (Houlahan et al. 2017).

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