

# Microgeographic evolution of metabolic physiology in a salamander metapopulation

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**Abstract.** The Metabolic Theory of Ecology explains ecological variation spanning taxonomic organization, space, and time based on universal physiological relationships. The theory depends on two core parameters: the normalization constant, a mass-independent measure of metabolic rate expected to be invariant among similar species, and the scaling coefficient, a measure of metabolic change with body mass commonly assumed to follow the universal 3/4 scaling law. However, emerging evidence for adaptive microevolution of metabolic rates led us to hypothesize that metabolic rate might exhibit evolved variation among populations on microgeographic scales. To evaluate our hypothesis, we explored evidence for evolved variation in the scaling coefficient and normalization constant within a spotted salamander (*Ambystoma maculatum*) metapopulation in Connecticut, USA. We measured standard metabolic rate in common-garden raised spotted salamanders from 22 different populations and tested for the effects of six ecological variables suspected in advance to select for divergent physiology. We found that metabolic rate rose with body mass with a log-log slope of 0.97 that was statistically different from the expected 3/4 scaling law. Although we found no evidence for interpopulation variation in the scaling coefficient, we found evidence for interpopulation variation in the normalization constants among populations. Metabolic variation was best explained by differences in population density among ponds. Our results provide mixed support for Metabolic Theory of Ecology assumptions about parameter invariance and illustrate how fundamental physiological processes such as metabolic rate can evolve across microgeographic spatial scales.

**Key words:** *Ambystoma maculatum*; amplify; eco-evolutionary; interference competition; metabolic theory of ecology; microevolution; pace-of-life; positive feedback; standard metabolic rate.

## INTRODUCTION

The Metabolic Theory of Ecology is one of a few general theories proposed to explain ecological variation spanning taxonomic organization, space, and time (Brown et al. 2004, West 2005). Derived from fundamental scaling relationships from biological first principles, metabolic theory seeks to explain ecological processes and patterns by scaling up behavior, somatic growth, population growth, and material flux using a common allometric relationship between body mass and metabolic rate (Kleiber 1932, Ernest et al. 2003, Savage et al. 2004). Metabolic Theory of Ecology has remarkable explanatory power, explaining ecological patterns ranging from population dynamics and community structure (McCoy and Gillooly 2008, Hatton et al. 2015) to material and energy flux through ecosystems (Allen et al. 2005, Allen and Gillooly 2009, Allgeier et al. 2015).

At the core of Metabolic Theory of Ecology are two parameters: the normalization constant ( $a$ ), which is a mass-independent measure of metabolic rate characteristic of a broad taxonomic group, and the scaling coefficient ( $b$ ), which is the rate at which metabolic rate changes with body size ( $M$ )

$$\text{Whole organism metabolic rate} = aM^b.$$

The scaling coefficient can be derived from theory to take a value of 0.75 (West 1997), and empirical estimates are typically close to this value (Kleiber 1932, Savage et al. 2004). Given this consistency, the Metabolic Theory of Ecology generally assumes that the scaling coefficient does not vary among or within taxa (Brown et al. 2004). In contrast, the normalization constant is based on direct measurements of metabolic rate. Although residual variation around linear fits of metabolic rate to body mass has been noted (Ernest et al. 2003, Brown et al. 2004), its use often entails the implicit assumption that metabolic physiology is highly conserved, varying only across large macroevolutionary distinctions (e.g., multicellularity and endothermy), and can be ignored for

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comparisons among similar species or for populations within species. That these key parameters are typically assumed to vary little within or among related species is a useful feature of Metabolic Theory of Ecology (Gillooly 2001, Brown et al. 2004) and mirrors research traditions in physiological ecology that have long emphasized macroevolutionary and continental scales and have relied upon broad taxonomic comparative analyses (Garland and Adolph 1991, Garland and Carter 1994, Gaston et al. 2009, Chown and Gaston 2016).

However, alternative theories such as the Metabolic Level Boundaries Hypothesis (Glazier 2005, 2010, 2015, Glazier et al. 2011) and empirical data that do not match Metabolic Theory of Ecology predictions (e.g., Dell et al. 2011) have challenged the general assumption of parameter invariance. Additionally, a growing body of research has begun to reveal evolved intraspecific variation in metabolic physiology (Burton et al. 2011, Pettersen et al. 2018). For example, evolved interpopulation variation in the scaling coefficient has been shown in a spring-dwelling isopod (Glazier et al. 2011, 2020), and recent work with Poeciliid fishes indicates rapid evolution of interpopulation variation in the normalization constant (Auer et al. 2018, Polverino et al. 2018). These examples and others underscore the potential relevance of microevolutionary dynamics for metabolic physiology in general, and the Metabolic Theory of Ecology, specifically.

In addition to a burgeoning microevolutionary perspective, theories that integrate metabolic physiology and behavioral ecology suggest new predictions for correlated responses. First, the Pace-of-Life hypothesis posits that metabolic physiology, life-history, and behavior exhibit positive trait covariances and that taxa can be characterized by their evolution along a fast-slow continuum (Ricklefs and Wikelski 2002, Wikelski et al. 2003, Biro and Stamps 2008, Careau et al. 2008, Reale et al. 2010, Montiglio et al. 2018, Wright et al. 2019). Thus, correlations between metabolic rate and foraging rate, boldness, aggression, dominance, somatic growth, development, and maturation are generally positive and taxonomically widespread (Biro and Stamps 2008, 2010, Konarzewski and Książek 2013, Mathot and Frankenhuis 2018, Mathot et al. 2019). Second, adaptation of metabolic physiology to ecological variation, such as temperature or predation, usually depends on energy availability (Burton et al. 2011, Careau et al. 2014). An important role for resource limitation in metabolic adaptation has long been recognized (McNab 1986, Mueller and Diamond 2001, Cruz-Neto and Bozinovic 2004, Steyermark 2005). However, the context-dependent hypothesis detailed in Burton et al. (2011) considers how the fitness costs of energy consumption shape the primary axis of metabolic adaptation. The rationale is that low-energy environments will usually favor the evolution of low metabolic rates, whereas a wider range of metabolic strategies can persist in high-energy environments (Bozinovic et al. 2009, Burton et al. 2011, Careau

et al. 2011). These integrative theories are advancing the field by merging the mechanistic approach of metabolic physiology with microevolutionary models of trait evolution and integration (Pettersen et al. 2018).

In this study, we explored evidence for evolution of metabolic physiology in a spotted salamander (*Ambystoma maculatum*) metapopulation in northeastern Connecticut, USA. Previous work in spotted salamanders shows that despite gene flow and genetic drift, spatial heterogeneity in predation risk, population density, and water chemistry can generate clear signatures of adaptation in foraging behavior, morphology, and physiology (Urban 2007a, 2010, 2013, Brady 2012, Urban and Richardson 2015; Giery et al. 2021b). Notably, this trait variation emerges across distances less than the average dispersal distance of adult spotted salamanders, demonstrating the microgeographic scale at which evolutionary differentiation can occur in this system (Richardson and Urban 2013). These observations led us to hypothesize that metabolic physiology (more specifically, the scaling coefficient and normalization constant) might also vary adaptively on a microgeographic scale. To test our hypothesis, we measured standard metabolic rate in common-garden-raised spotted salamanders from 22 populations. We then assessed if metabolic physiology was correlated with six ecological gradients known to impose antagonistic selection across this metapopulation (Skelly 2004, Urban and Richardson 2015) and that have induced the evolution of metabolic physiology in other systems (predation [Glazier et al. 2011, 2020, Auer et al. 2018], temperature [Álvarez et al. 2006, Moffett et al. 2018], hydroperiod/temporal constraint [Conover and Present 1990, Wikelski et al. 2003], population density [Reid et al. 2011, 2012], productivity [McNab 1986, Mueller and Diamond 2001, Steyermark 2005, Bozinovic et al. 2009]). We also evaluated support for the context-dependent hypothesis to see if these selective agents interact with energy availability.

## METHODS

### *Study system*

We studied a series of temporary ponds supporting breeding aggregations of the spotted salamander (*Ambystoma maculatum*). The spotted salamander is a large terrestrial salamander found in eastern United States and Canada. Each spring, adults move from upland terrestrial habitat into temporary ponds to mate and lay eggs (several dozen to several hundred per female). Small (~15 mg) aquatic larvae hatch from eggs after 4–7 weeks. Spotted salamander larvae must survive numerous predator species in the ponds, including larval marbled salamanders (*Ambystoma opacum*), before metamorphosing into terrestrial juveniles by late summer when most temporary ponds dry. Marbled salamanders breed in the autumn, their larvae grow throughout the winter and consequently reach a size large enough to

prey on larval spring-breeding amphibians (Urban 2007c). Studies suggest that larval marbled salamanders are one of the most important predators of spotted salamander larvae in the region. Marbled salamanders are gape-limited, and thus escape for spotted salamander larvae often occurs through rapid growth into a size refuge (Urban 2007b, 2008). Experiments verified that marbled salamander larvae induce selection for larger-bodied spotted salamander larvae (Urban 2010) with further study revealing genetic differences in foraging among local ponds correlated with marbled salamander predation risk at a more southern site (Urban 2013). At the site studied here, both marbled salamander density and population density, as measured by egg density, were positively associated with the evolution of higher foraging rates (Urban and Richardson 2015).

#### *Ecological variation*

We collected environmental factors across the developmental period (May–June) of larval spotted salamander in 2017 and 2018 (Appendix S1: Table S1). We measured mean water temperature using hourly readings from Hobo temperature loggers (UA-001-08) (Onset Computer Corporation, Bourne, Massachusetts, USA) and measured hydroperiod as the mean number of weeks that a pond retained water from the beginning of the year based on periodic field visits across two years. We measured periphyton productivity by suspending weighted  $23 \times 3$  cm clear plastic strips (four strips per pond) attached to a  $9 \times 4$  cm floating platform at the point of maximum depth in a pond (tin fishing weights attached to the bottom of the strip ensured vertical orientation in the water column). From May until the end of June, we carefully removed one strip per pond every two weeks, submerged it in 14 mL of 95% ethanol in a light-proof vial, and kept it on ice until we returned to the lab and measured chlorophyll *a*. We measured chlorophyll *a* in periphyton following plastic strip immersion in 95% ethanol for 12–24 h following the methods of Webb et al. (1992). Extracts were read on a Turner Aquaflor probe (Turner Designs Inc., San Jose, California, USA) as per EPA method 445 (Arar and Collins 1997). Concentrations of dissolved organic carbon, nitrogen, and phosphorus were measured monthly from April to July from 1 L of water collected just below the pond surface. Samples were kept dark and on ice during collection. Water samples were then filtered through Whatman GF/F filters and analyzed within 24 h following Giery and Layman (2017). Total N and total P were analyzed at the University of Connecticut Center for Environmental Sciences and Engineering with a Lachat QuikChem 8500 Flow Injection Ion Auto Analyzer (Lachat Instruments, Milwaukee, Wisconsin, USA) and Shimadzu TOC-L Total Organic Carbon (TOC) Analyzer (Shimadzu Scientific Instruments, Columbia, Maryland, USA). For all abiotic variables, we used the mean across all samples in our analyses. As a measure of

population density, we included spotted salamander larval density. We calculated the densities of marbled salamanders and spotted salamanders as the number of larvae collected per pond surface area ( $m^2$ ) based on area-standardized (by standardizing the length of the sweep for a net of known width and recording number of sweeps per pond) dip net surveys in the spring and early summer, respectively. We used these dip net surveys to also calculate the total density of known invertebrate predators of spotted salamander larvae (Urban 2007c) including *Aeshna* and *Libellula* dragonflies, *Chauliodes* fishflies, *Dytiscus* diving beetle larvae, and the egg predator, the *Ptilostomis* caddisfly, as well as *Hirudinea* leeches (Appendix S1: Table S1).

#### *Common garden rearing*

In April 2017, we collected fertilized egg masses from 22 study ponds within 24–48 h of laying and raised them in a common garden to minimize environmental influences on larval phenotypes. Due to protracted laying dates among ponds, egg masses were held in incubators at 4°C with natural diurnal/nocturnal light ratios until all egg masses were at the same developmental stage. Once developmental stages were synchronized, they were moved to an outdoor rearing facility in Storrs, Connecticut where egg masses were housed individually in 19-L containers filled with 18 cm of aged well water under 50% shade cloth. Egg mass location within the common garden was randomly assigned to avoid location effects on population variation. Eggs hatched in mid-May and we divided larvae from each egg mass among three blocks of 19-L containers filled with 18 cm of aged well water under 50% shade cloth. Container location was randomized within blocks to limit the potential for environmental covariation among replicates (see Appendix S1 for additional details). Larvae were fed wild-caught and cultured zooplankton prey daily and received chemical cues from captive larval marbled salamanders fed a diet of zooplankton, wood frog (*Rana sylvatica*) tadpoles, and spotted salamander larvae.

#### *Measuring standard metabolic rate*

In July, six-week-old larvae were moved from the common garden to incubators (Percival Scientific model I-41; Percival, Perry, Iowa, USA) set to 12.3°C with a 12-h light/dark cycle. The 12.3°C temperature replicated mean spring temperatures of ponds in the study region. Prior to metabolic rate measurement, the larvae were kept singly at 12.3°C for 72 h without food. This period served to acclimate individuals and allowed for complete digestion of previously ingested food.

We measured standard metabolic rates following Lighton (2018). Briefly, metabolic chambers were constructed from 20 mL glass scintillation vials fitted internally with an optical sensor (SP-Ost3-NAU; PreSens, Regensburg, Germany). Chambers were connected to a

multichannel oxygen meter (PreSens OXY-10) through external optical probes. Aged and conditioned tap water was saturated with oxygen at the time chambers were sealed. We allowed all individuals to acclimate to the chambers for 45 minutes before recording. After acclimation, we measured oxygen consumption for 75 minutes. Oxygen concentrations were always above 50% atmospheric concentration within respirometry chambers. After trials were completed, larvae were euthanized with Tricaine methanesulfonate (MS-222) and weighed.

Chambers were large enough for larvae to move freely. However, visual inspection of larvae during incubation showed that they remained motionless after several minutes of acclimation. We therefore consider our measures as reflecting standard metabolic rate following definitions in Careau et al. (2014). We controlled for the potential for background microbial oxygen consumption rate by including blank chambers with only water in each run. Oxygen consumption ( $\text{mg O}_2/\text{h}$ ) was estimated with linear fits to data following inspection of data. From 431 measures of healthy postprandial individuals, 58 traces were characterized by poor fits ( $r^2 < 0.85$ ), and we removed these individuals from the analysis (Appendix S1: Table S3).

#### *Statistical analysis*

Body mass and standard metabolic rate were  $\log_{10}$ -transformed for analysis. We removed four negative metabolic rates because negative metabolic rates are not realistic and these negative values had high leverage. However, analyses including these points resulted in no qualitative changes (Appendix S1: Table S4). For all analyses, we used the `rstanarm` function (Goodrich et al. 2020) in R (version 4.0.2) to apply a Bayesian Markov chain Monte Carlo (MCMC) algorithm with weakly informative priors for coefficients (mean = 0, variance = 100) and a default prior for variances (exponential with rate = 1), following standard recommendations (Gelman et al. 2013). We ran three chains for 5,000 samples with a burn-in of 3,000. We visually assessed chains for autocorrelation and evaluated within-chain convergence using Geweke-Brooks plots and assessed inter-chain convergence using the Gelman-Rubin diagnostic and increased burn-in time if models did not converge. The MCMC method samples from the posterior distribution to generate 95% credible intervals for independent factors, which were used to make statistical inferences.

To test for interpopulation variation in standard metabolic rate, we first tested for among-population heterogeneity of allometric slopes ( $b$ ) with a linear mixed model:  $\log(\text{metabolic rate}) \sim \log(\text{body mass}) + \text{population} + \log(\text{body mass}) \times \text{population}$ , with population coded as a random effect (Appendix S1: Fig. S2). We found no evidence of a differential slope between  $\log_{10}(\text{body mass})$  and  $\log_{10}(\text{metabolic rate})$  among populations by comparing models with and without this interaction term ( $\Delta\text{LOOIC} = -14.3$ ; more negative values

indicate greater support for the simpler model). Thus, for the remainder of our study we examined variation in standard metabolic rate using the residuals from the common slope of the  $\log_{10}$ - $\log_{10}$  relationship among all populations.

We used the R package `loo` (Vehtari et al. 2019) to calculate the Leave-One-Out Information Criterion (LOOIC) to evaluate model performance and choose the best model. This model selection method has been found to excel over alternative Bayesian methods such as Deviance Information Criterion (Vehtari et al. 2017).

We included each factor predicted to be related to standard metabolic rate as fixed factors in the regression models. We also added random effects that accounted for population, family, day of trial, and chamber. Here, “family” denotes the identity of the egg mass to control for phenotypic covariance among siblings. “Day of trial” provides a temporal block in case results varied through time, and “chamber” identifies the chamber used during each respirometry trial. The best random-effects model, as measured by lowest LOOIC, included all random effects. We first evaluated support for each full model (i.e., models 1–7 in Table 1) against the random-effects-only model using LOOIC. Next, we combined mechanisms supported individually to find a best comprehensive model by applying an iterative backward selection procedure and LOOIC to simplify the model to the set that maximized model performance (as measured by lowest LOOIC).

We had multiple variables that measured different aspects of potential and observed productivity, including dissolved nutrients (total organic carbon, total nitrogen, and total phosphorus) and periphyton. Because many of these were correlated (Appendix S1: Fig. S3), we first created a latent variable informed by each of these variables using the `bsem` function in `blavaan`, a Bayesian version of the `lavaan` program for structural equation and latent variable modeling. We then simplified the latent variable by removing factors with low standardized contributions and comparing the models using LOOIC. This process resulted in a model reduced to just total nitrogen with more complex models not providing additional insights. We confirmed this result by evaluating these factors in a standard regression backward elimination approach and again found that the nitrogen-only model had the highest performance. Henceforth, we use total dissolved nitrogen as our estimate of pond productivity.

## RESULTS

### *Scaling coefficients and normalization constants*

We measured standard metabolic rate in 369 spotted salamander larvae from 22 populations in Yale-Myers forest with a median of 18 replicates per population (minimum = 6, maximum = 26). Metabolic rate rose strongly with body mass showing a log-log slope of 0.97

TABLE 1. Summary of focal environmental variables, coefficient estimates, and model performance.

Variable	Measure	Standardized estimates	$\Delta$ LOOIC <sup>†</sup>
Individual tests			
1. Predator density	marbled salamander density	-0.011 (-0.035, 0.014)	+1.0
2. Predator density	invertebrate predator density	+0.015 (-0.009, 0.037)	+0.3
3. Temperature	water temperature	+0.019 (-0.003, 0.042)	-0.1
4. Hydroperiod	<i>n</i> weeks inundated	+0.003 (-0.021, 0.028)	+0.7
5. Population density	larval density	<b>+0.022 (0.001, 0.042)</b>	<b>-1.8‡</b>
6. Productivity	<i>N</i>	+0.020 (-0.003, 0.043)	-0.3
Context-dependent tests			
7a. Predator density	marbled salamander density $\times$ <i>N</i>	+0.108 (-0.093, 0.309)	+2.0
7b. Predator density	invertebrate predator density $\times$ <i>N</i>	+0.002 (-0.030, 0.033)	+2.1
7c. Temperature	water temperature $\times$ <i>N</i>	+0.005 (-0.023, 0.035)	+1.9
7d. Hydroperiod	hydroperiod $\times$ <i>N</i>	-0.010 (-0.034, 0.014)	+0.1
7e. Population density	larval density $\times$ <i>N</i>	+0.006 (-0.025, 0.040)	-1.0

Notes: Models 7a–e test the context-dependent hypothesis with interactions between focal variables and productivity (*N*). Bold-face type indicates coefficient estimates that do not overlap zero and LOOIC values that represent better models than the random-effects-only model. Values in parentheses are 95% credible intervals.

<sup>†</sup> LOOIC of best model minus intercept-only model LOOIC. More negative numbers indicate better models.

<sup>‡</sup> Best model among all combinations of supported factors.

(95% credible intervals: 0.91, 1.03), which does not include the expected value given the 3/4 scaling law (Fig. 1A). Although we found no evidence for interpopulation variation in the scaling coefficient (slope), model comparisons revealed different population intercepts, suggesting a moderate level of variation among populations in normalization constants (Fig. 1B, Appendix S1: Table S5,  $\Delta$ LOOIC = -3.1).

### Environmental gradients

As hypothesized, the normalization constant varied along at least one environmental gradient that was expected to impose differing selection on populations. We found no evidence that predation or hydroperiod played a role in the evolution of metabolism (Table 1). Temperature, population density, and productivity effects were included in models that were supported by model comparison, however, only the coefficient for population density had a credible interval that did not overlap with zero (Table 1). Temperature was positively associated with metabolic rate variation among populations, but the gain in model performance was slight ( $\Delta$ LOOIC = LOOIC(model with focal factor) - LOOIC(base model without fixed factors) = -0.1, where negative values suggest model support) and the slope overlapped with zero (Fig. 2A;  $\beta_{\text{temp}} = 0.019$ , 95% credible interval -0.003, 0.042). Higher population densities were associated with higher metabolic rate,  $\Delta$ LOOIC = -1.8) and the slope did not overlap with zero ( $\beta_{\text{comp}} = 0.022$ , 95% credible interval 0.001, 0.042; Table 1, Fig. 2B). Productivity, as measured by total nitrogen, also was moderately related to metabolic rate ( $\Delta$ LOOIC = -0.3), but the slope slightly overlapped with zero ( $\beta_{\text{prod}} = +0.020$ , 95% credible interval: -0.003, 0.043). These three variables were not highly correlated,

except for a moderate correlation between nitrogen and temperature (Appendix S1: Fig. S3;  $\rho = +0.45$ ).

Our data failed to support the context-dependent hypothesis, whereby other selective agents are expected to interact with productivity such that low-productivity constrains evolutionary responses to other agents. Although we found some model support for the population density and productivity interaction ( $\Delta$ LOOIC = -1.0), the slope overlapped with zero ( $\beta_{\text{int}} = +0.006$ , 95% credible interval -0.025, 0.040), and the model did not perform as well as other single-factor models (Table 1). Moreover, when we evaluated a model that combined all supported mechanisms (temperature, population density, and productivity) and iteratively removed factors that did not contribute to lower LOOIC, the best overall model was the single-factor population density model, with the next best model being the productivity and population density model ( $\Delta$ LOOIC = -1.8 vs. -1.1, relative to a model without fixed factors; Appendix S1: Table S6).

### DISCUSSION

Our overarching hypothesis of microgeographic evolutionary differentiation of metabolic physiology in spotted salamanders was supported by differences among nearby populations that correlate with a suspected agent of natural selection. This finding contrasts with assumptions about the evolutionary conservation of metabolic physiology. Importantly though, microgeographic variation was only observed for the normalization constant and not the metabolic scaling coefficient. Our data indicate that microgeographic variation in this system is most likely driven by population density, with temperature and productivity having moderate effects on the normalization constant (Table 1). The other putatively

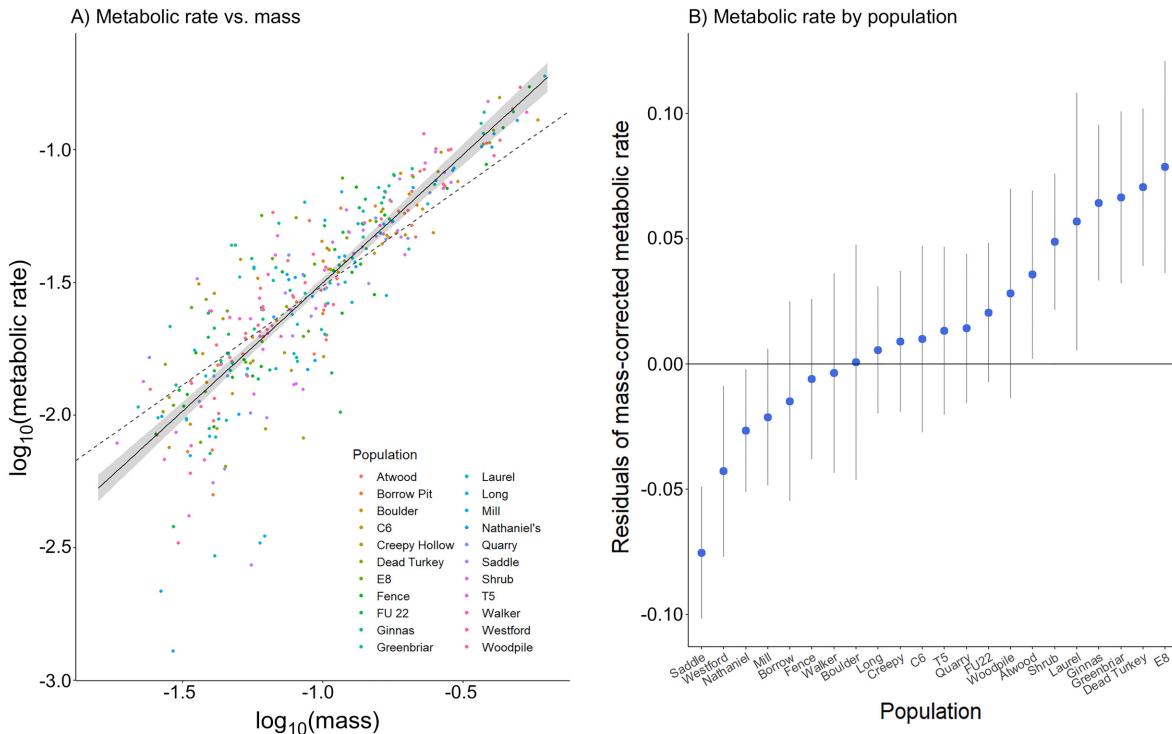


FIG. 1. (A) Log-log plot of metabolic rate on mass illustrating the overall allometry of metabolic rate ( $\text{mg O}_2/\text{h}$ ) and body mass ( $\text{g}$ ), the scaling coefficient, in spotted salamander larvae. Solid line indicates the scaling coefficient as estimated from our data ( $b = 0.97$ ), the dashed line indicates the hypothetical value,  $b = 0.75$ , and centered at the mean mass from our data. (B) While slopes were similar, populations differed in their mean mass-corrected metabolic rate normalization constant. Populations means  $\pm$  SE are arranged in order of increasing metabolic rate.

selective agents, predator density and hydroperiod, were not supported by our analyses, and we found no support for the context-dependent hypothesis driven by interactions with productivity.

#### Species invariant scaling coefficient

The assumption of an invariant scaling coefficient has been a source of controversy in the Metabolic Theory of Ecology for decades (reviewed in Glazier 2010). Here, results suggest that the scaling coefficient remains relatively invariant within the focal metapopulation despite strong ecological gradients that simultaneously drive microgeographic variation in behavior, morphology, and physiology. Interestingly, besides major differences between our study and that of Messerman and Leal (2020), our scaling coefficient estimates for spotted salamanders were similar in magnitude and statistically indistinguishable ( $b = 0.87$ , 95% credible interval = 0.39–1.32, Appendix S1), a result consistent with the Metabolic Theory of Ecology’s assumption of an invariant scaling coefficient. In contrast, we found evidence for inter-population variation in normalization constants across at least one environmental gradient suspected to be a source of selection on metabolic physiology.

#### Population density and standard metabolic rate

Density dependence in *Ambystoma* salamanders (and other larval amphibians) is pervasive and well documented (Wilbur and Collins 1973, Wilbur 1976). Density-dependent growth and survival is typical for *Ambystoma* larvae, usually negative, and driven by both exploitative and interference competition (Petranka 1989, Smith 1990, Van Buskirk and Smith 1991, Walls 1998). Given how conspecifics interact in this system, we suspect competition plays a prominent role. Below, we discuss how intraspecific competition might select for higher standard metabolic rates.

One possible explanation for the observed pattern is that the intensity of exploitative competition is positively correlated with population density and favors increased standard metabolic rate. This could happen if individuals with high metabolic rate forage more successfully for sparse resources or better defend them, an explanation fitting the “performance” or “intake” model of metabolic physiology commonly observed in ectotherms (Careau et al. 2008, 2014, Mathot et al. 2019). For example, juvenile Atlantic salmon (*Salmo salar*) with low standard metabolic rates fail to acquire feeding territories at high population density and when food is limited (Reid et al. 2011, 2012). While plausible, this explanation seems

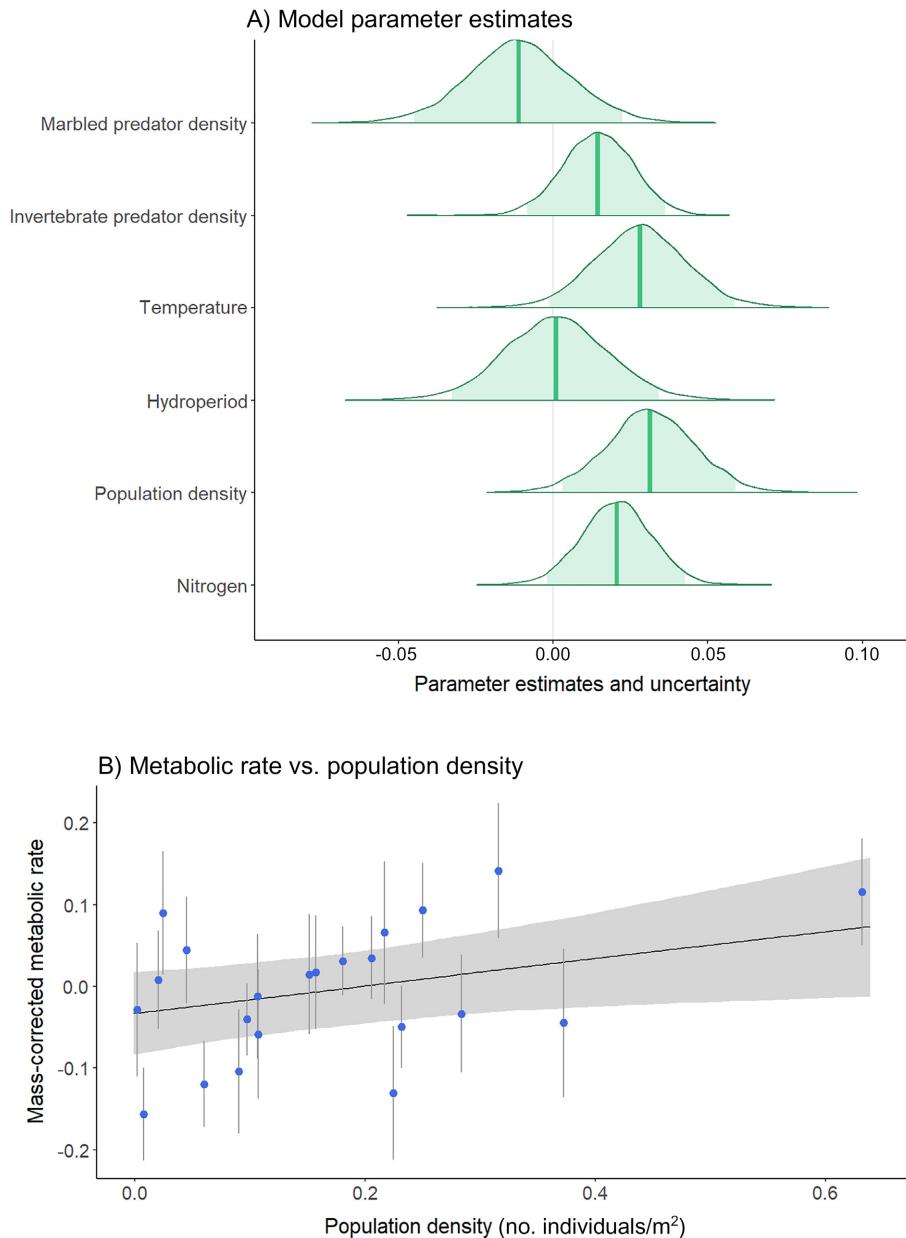


FIG. 2. (A) Parameter estimates for factors explaining differentiation among populations in standard metabolic rate, ordered by coefficient value. Bar represents median estimate from Markov chain Monte Carlo (MCMC) samples, while the shaded areas indicate the 95% credible intervals. When the green shading does not overlap with zero, the coefficient is considered significant. (B) Estimated relationship between mass-corrected metabolic rate and population density. Shaded area indicates the 95% credible interval.

unlikely for two reasons. First, previous work in this system doesn't show a strong relationship between food (zooplankton) and population density that might indicate ongoing exploitative competition (Urban and Richardson 2015). Second, absent and weak correlations between population and food density are not unusual for *Ambystoma* salamanders (Petranka 1989, Van Buskirk and Smith 1991). Both lines of evidence complicate arguments about the importance of exploitative

competition in this case. Ultimately, weak linkages between population density and food availability in this system make population density a poor proxy for exploitative competition intensity.

Alternatively, we speculate that high-frequency interference competition favors the evolution of high metabolic rates. This hypothesis is based on two suppositions. The first is that rapid growth is favored in populations with high rates of direct intraspecific interactions. In

*Ambystoma* salamanders, direct intraspecific interactions frequently take the form of cannibalism, grazing of tails and appendages, and aggression: costs that are highest for small individuals in dense populations (Smith 1990). Thus, a size refuge favoring rapid growth is likely to arise via interference competition in dense populations, similar to that seen in response to gape-limited predation by marbled salamanders (Urban 2007b). If so, we might expect selection for rapid growth to mitigate the costs of interference competition, just as it does for predation risk. We also suppose that somatic growth exhibits positive covariance with standard metabolic rate in this system. Indeed, evolved covariance among metabolism, foraging, and aggression is common in vertebrates and overwhelmingly positive (Stamps 2007, Biro and Stamps 2008, 2010, Careau et al. 2008, Reale et al. 2010, Careau and Garland 2012, Metcalfe et al. 2016). If we assume other processes involved with growth, such as allocation to tissues, and foraging rates are similar or increase across the population density gradient, then salamander adaptation to high density environments should favor high metabolic rates. Although we do not have direct evidence for growth rate differences for these populations, dense populations in this metapopulation exhibited higher foraging rates in previous work (Urban and Richardson 2015). Considered together, these data suggest that high population density favors the evolution of rapid growth supported by correspondingly high rates of foraging and metabolism.

#### *Microgeographic variation of metabolic physiology*

The fine spatial scales at which populations differentiate has led ecologists to reconsider the relevance of microevolution for population and community patterns (Richardson and Urban 2013, Urban et al. 2020). These new insights demonstrate how environmental variation at fine spatial scales can translate into evolved intraspecific variation, despite high potential for gene flow, if selection is relatively strong, or due to barriers to gene flow such as selection against migrants (Garant et al. 2007, Richardson and Urban 2013, Richardson et al. 2014). The development and application of this microevolutionary framing to metabolic physiology is ongoing (Burton et al. 2011, Pettersen et al. 2018) and yielding increasingly compelling evidence that metabolic physiology fits squarely within a modern eco-evolutionary framework that emphasizes the potential for contemporary and microgeographic evolution (Glazier et al. 2011, Auer et al. 2018).

These advances demand a close reevaluation of the assumptions underpinning purely ecological (or macroevolutionary) models such as Metabolic Theory of Ecology. Emerging evidence showing that fundamental physiological processes such as metabolic rate evolves across microgeographic scales might account for unexplained variation in mass-specific metabolic rates and indicate new research directions. Indeed, increasingly widespread appreciation for the fine scale of physiological

adaptation highlights the importance of another assumption of Metabolic Theory of Ecology that linkages between population density and metabolic rate are unidirectional. Specifically, the Metabolic Theory of Ecology predicts that metabolic rate controls population density because high metabolic rate reduces the hypothetical carrying capacity (Brown et al. 2004:1780). While reasonable, this hypothesis does not take into account the effects of population density on the evolution of metabolic rate. For example, our data indicate the potential for a positive eco-evolutionary feedback between population density and foraging and metabolic rates. Dense populations appear to evolve higher metabolic rates, which should reduce carrying capacity and increase density dependence, possibly limiting extremely dense populations while strengthening selection on rapid growth through metabolic processes. This finding is relevant for Metabolic Theory of Ecology and other ecological models because it suggests that adaptation to intraspecific competition can amplify the ecological effect of consumers in dense populations, supporting an increasingly identified pattern of evolution amplifying spatial ecological patterns related to resource uptake (Urban et al. 2020). Ultimately, these findings highlight the clear relevance of adaptation-mediated feedbacks, and eco-evolutionary dynamics in general, for the physiological underpinnings of any universal ecological theory.

#### *Caveats and additional considerations*

We designed a common garden experiment in which we raised individuals from eggs to larvae to reduce environmental contributions and reveal genetically differentiated responses. Common garden experiments are the most common approach for testing for genetic divergence in complex traits (Kawecki and Ebert 2004, Urban et al. 2020). However, observed trait variation could also occur due to maternal effects or transgenerational plasticity. In this study and previous research, we excluded four of the most common sources of maternal effects, including maternal habitat choice, care, egg provisioning, and environmental cues of predation for both mother and offspring (Urban 2013; Appendix S1). Yet, we cannot exclude multigenerational maternal effects because we did not raise salamanders for several generations in the laboratory (~6 yr for this species), and thus conclusions about genetic determination should be treated with caution. Theoretical and empirical observations do suggest that the observed differences in metabolic rates likely enhance local fitness and thus reflect local adaptation (Urban 2007b, 2013). However, reciprocal transplant experiments would be needed to test this adaptive hypothesis directly.

#### *Conclusion*

Past and current efforts to develop an integrative research tradition within physiological ecology have

generally overlooked the relevance of microevolutionary variation (Garland and Adolph 1991, Chown and Gaston 2016). Although recent cases have demonstrated evolved intraspecific variation in metabolic physiology, they generally compare populations separated by long distances or across barriers impermeable to gene flow (Bozinovic et al. 2009, Glazier et al. 2011, Auer et al. 2018, Moffett et al. 2018, Polverino et al. 2018). Our findings contribute to mounting evidence for fine-scaled, evolutionary differentiation in metabolic rate, broadening a realization that metabolic physiology evolves readily on small scales (Kawecki and Ebert 2004, Garant et al. 2007, Richardson et al. 2014, Urban et al. 2020). However, our finding that the scaling coefficient does not vary among populations indicates that the components of metabolic physiology may differ in their evolvability. Thus, broad assumptions about the evolutionary conservation and taxonomic invariance of metabolic physiology could be supported in some cases. Ultimately, our results provide key evidence for evolved differentiation in metabolic physiology within a metapopulation and make clear that a microevolutionary perspective on physiological ecology is warranted.

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/ecy.3488/supinfo>

## OPEN RESEARCH

Data (Giery et al. 2021a) are available on the Dryad digital repository: <https://doi.org/10.5061/dryad.djh9w0w0v>.