

# Widespread rapid reductions in body size of adult salamanders in response to climate change

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## Abstract

Reduction in body size is a major response to climate change, yet evidence in globally imperiled amphibians is lacking. Shifts in average population body size could indicate either plasticity in the growth response to changing climates through changes in allocation and energetics, or through selection for decreased size where energy is limiting. We compared historic and contemporary size measurements in 15 *Plethodon* species from 102 populations (9450 individuals) and found that six species exhibited significant reductions in body size over 55 years. Biophysical models, accounting for actual changes in moisture and air temperature over that period, showed a 7.1–7.9% increase in metabolic expenditure at three latitudes but showed no change in annual duration of activity. Reduced size was greatest at southern latitudes in regions experiencing the greatest drying and warming. Our results are consistent with a plastic response of body size to climate change through reductions in body size as mediated through increased metabolism. These rapid reductions in body size over the past few decades have significance for the susceptibility of amphibians to environmental change, and relevance for whether adaptation can keep pace with climate change in the future.

**Keywords:** Appalachians, body size, climate, evolution, metabolism, salamanders

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## Introduction

Appalachian habitats are expected to experience shifts in seasonal temperature and moisture conditions under projected climate change scenarios, and species that occupy these habitats are expected to respond to these new conditions through changes in ecology, physiology, or genetics (Ohlberger, 2013). Scientists have reported climate-induced alterations to phenotypic traits in many species, such as changes in body size (Barnosky *et al.*, 2003; Millien *et al.*, 2006; Gardner *et al.*, 2011; Secord *et al.*, 2012), shifts in distributions through range expansions or contractions (Galbreath *et al.*, 2009), or changes in key demographic events such as breeding, flowering, or migration (Millien *et al.*, 2006; Blaustein *et al.*, 2010; Gardner *et al.*, 2011; Sheridan & Bickford, 2011).

Environmental changes can affect biophysical processes that ultimately affect survival and fitness (Gardner *et al.*, 2011). If warmer environmental temperatures limit activity, foraging can be restricted resulting in reduced body size (Adolph & Porter, 1993; Sears, 2005a). Further, warmer body temperatures can result in higher energetic expenditure leading to decreased

growth even if energy intake remained constant (Sears, 2005b). For example, Cheung *et al.* (2013) used metabolic modeling to show how projected changes in global temperatures were expected to reduce the body size of marine fishes through alteration of temperature and oxygen-dependent metabolic processes.

Amphibians, because of their ectothermic physiology and constant need for moisture are particularly susceptible to such environmental changes (Wells, 2007). Evidence is growing that amphibians are showing multiple responses to climate, including shifts in breeding phenology (Todd *et al.*, 2011), shifts in elevational distributions (Raxworthy *et al.*, 2008), phenotypic shifts in color pattern (Gibbs & Karraker, 2006), and climate-induced reductions in body size associated with reduced survivorship (Reading, 2007). Body size is a critical trait for amphibians because it plays a prominent role in many aspects of their ecology and evolution. Reductions in body size can directly affect physiology through changes in thermal and energetic requirements, and thus affect activity patterns and survival with resulting consequences on population demography (Blaustein *et al.*, 2010). Changes in body size are often associated with differences in foraging behavior, diet, and activity patterns, thereby influencing growth rates, reproductive output, and predator–prey relationships (Beaupre, 1995; Reading, 2007).

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Inter- and intraspecific interactions such as territoriality, predation, and competition are also body size-dependent, and changes in body size relationships may generate indirect effects on community composition mediated by these interactions (Sheridan & Bickford, 2011).

Salamanders in the genus *Plethodon* are particularly sensitive to warming and drying trends as they are terrestrial, lungless organisms completely dependent upon cutaneous respiration for gas exchange and water uptake (Wells, 2007). Plethodontid salamanders respond to environmental change through varied mechanisms. For instance, many species have shifted their distributions over evolutionary time to remain within particular climate zones (Kozak & Wiens, 2010), and plethodontid species richness is positively correlated with cool moist conditions (Marshall & Camp, 2006). In addition, thermal limitations on physiology restrict both dispersal and distribution in some plethodontid species (Bernardo *et al.*, 2007). Recent climate models predict widespread range contractions for all Appalachian salamander species starting by 2020 (Milanovitch *et al.*, 2010), with greatest effects predicted for high-elevation populations in the southern Appalachians.

Because changes in body size are predicted as a major response to global climate change (Daufresne *et al.*, 2009), we tested whether shifts in mean body size have occurred in populations of Appalachian woodland salamanders. We then determined whether body size shifts were associated with geography, and with real-time changes in climate at those specific locations using data downloaded from Climate Wizard. Finally, we modeled annual activity and metabolic expenditure of a large (10 g) and a small (1 g) plethodontid salamander at three latitudes over the 55-year study period using actual climate data downloaded from the National Center for Environmental Prediction (NCEP) database. Modeling would test whether observed changes in body size were consistent with expectations

of changes in metabolism associated with actual climate change over that same time period.

## Materials and methods

### Field surveys

During June–October 2011 and March–June 2012, we resurveyed 78 historic (Highton, 2005) collecting sites comprised of 212 populations of 15 *Plethodon* taxa once in each season (Spring, Summer, Fall). This transect covered 767 km (Fig. 1) and ranged from 401 to 1687 m elevation (Table S1). At each site, we sampled three 50 × 3 m diurnal cover object plots; at some sites we also sampled two 50 m nocturnal Visual Encounter Survey (VES) transects. We identified each capture to species, sex, and age class, and measured snout-vent-length (SVL), mass, and body temperature. We also included measurements of 157 *Plethodon* individuals sampled in an identical way from 18 sites in Great Smoky Mountain National Park in 2009, seven of which were not resampled in 2011–2012 (Caruso & Lips, 2013), for a total of 85 unique sites.

We compared mean adult SVL for each population using measurements from wild caught animals we made in 2009 and in 2011–2012 and measurements made on museum specimens (Adams & Church, 2008, 2011) collected from those same populations between 1950 and 1996. We captured 1193 *Plethodon* salamanders from 102 populations and 15 species at 64 of the 85 sites sampled in 2009 and in 2011–2012. Twenty-one sites were lacking salamanders, which prevented us from including those sites in this analysis. We added another 8257 measurements from preserved adult specimens collected from those same sites (Adams & Church, 2008, 2011) for a final database of 9450 individual measurements.

It is not possible to completely replicate the sampling methods of Highton, but given the goals of our work we attempted to match effort as much as possible based on conversations with Highton and his students. We designed a survey approach to sample all individuals and all possible microhabitats at a site, and we corrected for effort. Most importantly, there is little variation in collecting practices in our analyses because the bulk of the data we analyzed is in fact Highton's data, collected in his standardized way. Our comparisons were made between measurements of animals sampled

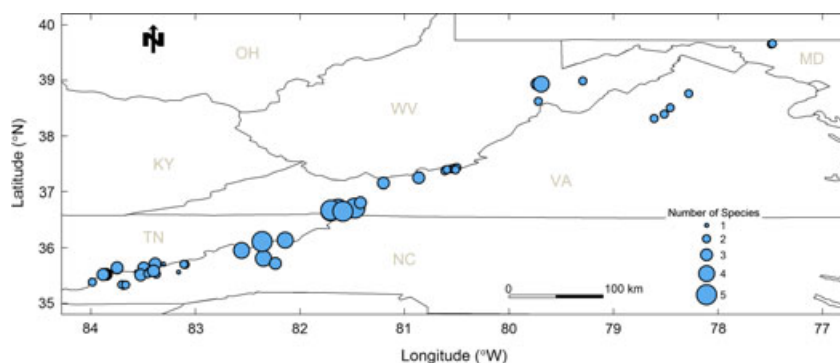


Fig. 1 Location of all study sites. Size of the dot represents the original number of species in each community.

predecline (Highton data collected from 1950 to 1979) and those sampled postdecline [a mixture of Highton specimens (1980–1996) and our field data (2009–2012)]. Highton collected as many individuals of as many species as possible, and essentially collected every individual encountered at a site. He was interested in hybrid zones as well as in phenotypic variation and collected tissues of both adults and juveniles. We also sampled all individuals encountered, as our original purpose was to search for disease. For this analysis however, we only used specimens of putative adult size for comparison based on literature values established for other purposes (e.g., Adams & Church, 2008, 2011) and excluded any obvious juveniles. As a result, we can eliminate inherent bias in the measurements of SVL.

We can also eliminate preservation as a contributor to reduction in body size. Museum specimens can shrink from preservation, but this would reduce any observed pattern, as this would affect the historic samples and not measurements of recently sampled live animals. Since the fresh material was the most recent, and also the smallest, preservation biases cannot explain the reduced body size over time.

### Statistical analyses

All analyses were performed in R version 2.15.2 (R Development Core Team, 2012). We fit linear models and mixed effects models using Markov Chain Monte Carlo (MCMC) in WinBUGS 1.4 (Spiegelhalter *et al.*, 2003) using the R2WinBUGS package (Sturtz *et al.*, 2005). For all models, we used noninformative, uniform priors, estimated posterior distributions of model parameters with three chains, 50 000 iterations, and discarded 20 000 burn-in iterations. We reduced the autocorrelation of our sample by retaining every 90th iteration, with the result that posterior distributions were estimated from 1002 simulations. We checked all models for convergence by examining MCMC chains and the  $\hat{R}$  statistic (Gelman & Rubin, 1992). All models presented in the results showed good mixture in MCMC chain plots and all model  $\hat{R}$  statistics were  $\leq 1.04$ . We determined parameter coefficient significance if the 95% credible interval (CRI) did not overlap zero.

For all species, we modeled the relationship between the SVL and year of survey as a linear mixed effects model (lme); species were modeled as a random intercept, while the year of survey was modeled as a fixed slope ( $\beta_1$ ). We used this lme model to determine the relationship between SVL and year of survey both with and without our field measurements included. This allowed us to assess the role of sampling biases in either historic or current sampling (e.g., if only the largest animals were captured in historic sampling). We modeled each species using either a linear model (lm; number of populations  $< 5$ ) or a linear mixed effects model (number of populations  $\geq 5$ ). For both of these models, the year of survey was modeled as a fixed effect ( $\beta_1$ ) while we modeled the intercept as a random effect for each population (lme) or as a fixed effect for all populations (lm).

We calculated body size change through time both as the percent body size reduction, and as the standardized difference in mean body size per generation, relative to within-

population standard deviation as indicated by Haldane Ratio (e.g., Gingerich, 2009). This allowed us to compare body size evolution in terms of changes in standard deviations per generation across species. With this approach, we detected differences in SVL for each population (Table 1) between pre- (1957–1979) and postdecline (1980–2012) time periods as identified by Highton (2005). Standardized mean differences were used as a measure of body size change through time using Bayesian linear models with average body size of each species, site latitude and elevation, and change in precipitation and temperature as covariates. We selected the best model by Deviance Information Criterion (DIC; Spiegelhalter *et al.*, 2002). We used climate data from Girvetz *et al.* (2009) to determine the spatial variation in both precipitation and temperature from 1951 to 2006 of the 64 field sites represented by individuals used in body size measurements. We determined the average body size of each species based on our full dataset of 9450 measurements.

### Biophysical Models

To examine whether changes in body size might have resulted from changing climates, we used a biophysical modeling approach to estimate whether annual duration of activity and annual metabolic expenditure had changed over the sampling period. To estimate activity, humid operative temperatures ( $T_{eh}$ ) were calculated for each minute of the day as:

$$T_{eh} = T_a + \frac{\gamma^*}{\Delta/P_a + \gamma^*} \left( \frac{R_{abs} - \epsilon_s \sigma T_a^4}{c_p g_{Hr}} - \frac{D}{\gamma^* P_a} \right) \quad (1)$$

where,

$$\gamma^* = \frac{\gamma(1/g_{vs} + 1/g_{vc} + 1/g_{va})}{1/g_{Hc} + 1/g_{Hr}} \quad (2)$$

to estimate equilibrium temperatures resulting from heat flux and water loss (Campbell & Norman, 1998). Symbol definitions for all equations can be found in Table 2. Next, using  $T_{eh}$ , body temperatures for each time step were calculated as

$$T_{b2} = T_{eh} + e^{(-t/\tau)}(T_{b1} - T_{eh}) \quad (3)$$

In the above equations,  $R_{abs}$ ,  $T_a$ ,  $g_{Hr}$  were calculated using equations in the Appendix of Sears *et al.* (2011). An individual was considered to be active if its body temperature fell between 6.5 and 22 °C (Spotila, 1972; Feder, 1983) and water loss did not exceed 10% of its body mass (Feder & Londos, 1984). Using these body temperatures, we determined whether an individual was active over the course of a given day on a minute-by-minute basis from 1957 to 2011.

Water loss was calculated as

$$W = A \frac{D}{r} \quad (4)$$

We estimated activity using relative humidities of 90%, 95%, and 100%. We approximated the surface areas of salamanders as that of a similar sized cylinder (filled with water) of the same mass. We simulated the activities of a small sized salamander (1 g, similar to *P. cinereus*) and a larger

**Table 1** Change in body size of each species of *Plethodon* by decade. Results of statistical models (slope, 95% CRI), time period, number of sites, years sampled, sample size, and average SVL (in mm). Single asterisks indicate the six species with significant reductions in body size and the double asterisk indicates the one species with significant increase in body size based on 95% CRI

Species	Slope (95% CRI)	Decade	Sites	Years	Sample Size	Average SVL
<i>aureolus</i> <sup>a</sup>	0.034 (−0.059, 0.013)	1970s	1	1979	1	50.00
		1980s	1	1988	18	49.11
		2010s	1	2011	1	52.35
* <i>cheoah</i> <sup>b</sup>	−0.012 (−0.021, −0.004)	1960s	4	1962, 1964, 1966, 1969	61	51.42
		1970s	3	1971, 1972, 1979	217	50.88
		1980s	2	1983–1985	60	50.53
		2010s	4	2011, 2012	53	49.51
* <i>cinereus</i> <sup>b</sup>	−0.017 (−0.020, −0.013)	1950s	11	1957–1959	1,841	42.62
		1960s	25	1960–1969	1,413	42.01
		1970s	14	1970–1974, 1976–1979	720	40.74
		1980s	8	1980–1982, 1984–1988	73	39.16
		1990s	1	1992, 1993	5	36.96
		2010s	34	2011, 2012	592	39.34
* <i>cylindraceus</i> <sup>a</sup>	−0.054 (−0.085, −0.025)	1960s	1	1965, 1966	3	68.64
		1970s	3	1970–1973	102	63.99
		1980s	2	1981, 1987, 1988	16	63.52
		1990s	1	1994	7	59.97
		2010s	3	2011, 2012	16	55.23
<i>glutinosus</i> <sup>b</sup>	0.006 (−0.011, 0.023)	1950s	6	1957–1959	195	61.21
		1960s	13	1960, 1961, 1963, 1968, 1969	191	59.32
		1970s	3	1971, 1974, 1978	27	55.87
		1980s	2	1984, 1987	8	57.13
		2000s	1	2009	4	57.88
		2010s	14	2011, 2012	60	60.70
* <i>jordani</i> <sup>b</sup>	−0.014 (−0.020, −0.008)	1960s	14	1960–1965, 1967–1969	378	53.86
		1970s	7	1971–1975, 1978, 1979	639	52.95
		1980s	3	1981, 1984, 1986–1988	87	53.59
		1990s	1	1994	2	54.97
		2000s	13	2009	117	50.32
		2010s	8	2011, 2012	92	52.33
<i>metcalfi</i> <sup>a</sup>	−0.005 (−0.028, 0.019)	1960s	1	1962, 1964	14	54.59
		1970s	1	1974	16	52.78
		2000s	1	2009	5	53.36
		2010s	1	2011	6	53.59
<i>montanus</i> <sup>b</sup>	−0.005 (−0.011, 0.0004)	1960s	5	1960, 1961, 1963, 1964, 1969	197	52.13
		1970s	10	1970–1973, 1976–1979	760	52.11
		1980s	7	1981–1983, 1985, 1987–1989	236	52.39
		1990s	3	1994, 1996	13	51.80
		2010s	12	2011, 2012	138	51.31
<i>nettingi</i> <sup>a</sup>	0.009 (−0.009, 0.026)	1950s	1	1950, 1957–1959	84	41.26
		1960s	1	1960, 1961, 1964, 1965, 1967, 1969	241	41.09
		1970s	1	1970, 1972–1974, 1977	35	41.66
		2010s	1	2012	3	40.13
<i>richmondi</i> <sup>b</sup>	−0.006 (−0.021, 0.006)	1960s	2	1960, 1961, 1963, 1964, 1969	81	43.35
		1970s	5	1970–1973, 1976–1979	108	44.37
		1980s	1	1981, 1987	2	44.61
		2010s	6	2011, 2012	41	43.31
<i>serratus</i> <sup>b</sup>	0.001 (−0.018, 0.020)	1960s	5	1961, 1963, 1965, 1968, 1969	37	39.54
		1970s	4	1971, 1972, 1974	22	40.01
		1980s	2	1982, 1988	2	37.62
		2000s	6	2009	8	38.98
		2010s	3	2011, 2012	6	39.50

Table 1 (continued)

Species	Slope (95% CRI)	Decade	Sites	Years	Sample Size	Average SVL
<i>teyahalee</i> <sup>a</sup>	−0.019 (−0.055, 0.018)	1960s	3	1961–1965, 1967	20	66.01
		1970s	1	1971, 1972	8	64.04
		1980s	1	1987	3	57.98
		2000s	1	2009	6	67.38
		2010s	3	2011, 2012	8	58.72
<i>*ventralis</i> <sup>a</sup>	−0.021 (−0.042, −0.0001)	1960s	3	1962, 1963, 1965, 1969	60	38.79
		1970s	1	1971, 1974, 1975, 1978	39	37.97
		1990s	1	1996	1	36.88
		2000s	3	2009	18	36.32
<i>**welleri</i> <sup>a</sup>	0.026 (0.008, 0.044)	1960s	1	1960, 1969	27	36.24
		1970s	1	1972, 1976, 1977	117	36.06
		1980s	1	1980, 1981, 1984	35	37.57
		2010s	1	2011, 2012	12	38.71
<i>*yonahlossee</i> <sup>a</sup>	−0.097 (−0.185, −0.004)	1970s	2	1970–1973, 1979	29	60.40
		1980s	1	1981	2	56.66
		2010s	2	2011	5	49.11

<sup>a</sup>results of linear models; <sup>b</sup>results of linear mixed effects.

Table 2 List of symbols and definitions used in biophysical models

Symbol	Definition
$T_{eh}$	Humid operative temperature
$T_a$	Air temperature
$\gamma^*$	Apparent psychrometer constant
$\gamma$	Thermodynamic psychrometer constant
$\Delta$	Slope of saturation vapor pressure vs. temperature function
$P_a$	Atmospheric pressure
$R_{ibs}$	Absorbed longwave and shortwave radiation
$\epsilon_s$	Surface emissivity
$\sigma$	Stefan–Boltzmann constant
$D$	Vapor deficit of air
$g_{vs}$	Surface conductance for vapor
$g_{vc}$	Coat conductance for vapor
$g_{va}$	Boundary layer conductance of vapor
$g_{Hc}$	Coat conductance for heat
$g_{Hr}$	Sum of boundary layer and radiative conductances
$T_{b1}$	Initial body temperature
$T_{b2}$	Final body temperature
$W$	Total water loss
$A$	Surface area of organism
$r$	Whole body resistance to water loss

bodied salamander (10 g, similar to *P. glutinosus*). We then summed the number of minutes of activity across each year from 1957 to 2011. In addition, given an individual's size and body temperature, we estimated energetic expenditure for each hour of activity. We estimated hourly standard metabolic rate using:

$$\ln(\text{standard metabolic rate}) = 0.102(\text{temperature}) + 0.681[\ln(\text{mass})] - 4.489$$

(Homyack *et al.*, 2010). Activity and energetic expenditure was calculated for each of three locations that spanned the latitudinal range of our study sites. The three sites, from south to north, were Mt. Rogers National Recreation Area, VA (LIM; 36.3695°N, 81.6344°W; 1172 m elev.), Mountain Lake, VA (ATW 37.4161°N, 80.5097°W; 1247 m elev.), and Catoclin National Park, MD (FDR; 39.6539°N, 77.4875°W; 401 m elev.). Climatic data for these sites were downloaded from the NCEP climatic database using the RNCEP tool in the R Statistical Package (Kemp *et al.*, 2012).

## Results

Using a large dataset of 9450 adult body size measurements from 15 *Plethodon* species from 102 populations (Fig. 1), we found an 8% reduction in the average adult body size across all species over the 55-yr study period ( $\beta_1 = -0.019$ ; 95% CRI =  $-0.021, -0.017$ ). This trend remained significant even after we removed the contemporary field measurements ( $\beta_1 = -0.028$ ; 95% CRI =  $-0.033, -0.023$ ). Within species, we found significant changes in body size over time for seven species, and found that six exhibited a significant reduction in body size (Fig. 2; Table 1). We detected reductions of 2% (*P. cheoah*), 3% (*P. jordani*), 6% (*P. ventralis*), 7% (*P. cinereus*, *P. cylindraceus*), and 18% (*P. yonahlossee*; Fig. 2; Table 1). Body size in *P. welleri* increased by 2% over that time period.

We investigated four correlates of reduction in body size: latitude, elevation, climate change, and average species size. Neither average species size (DIC =  $-276.6$ ;



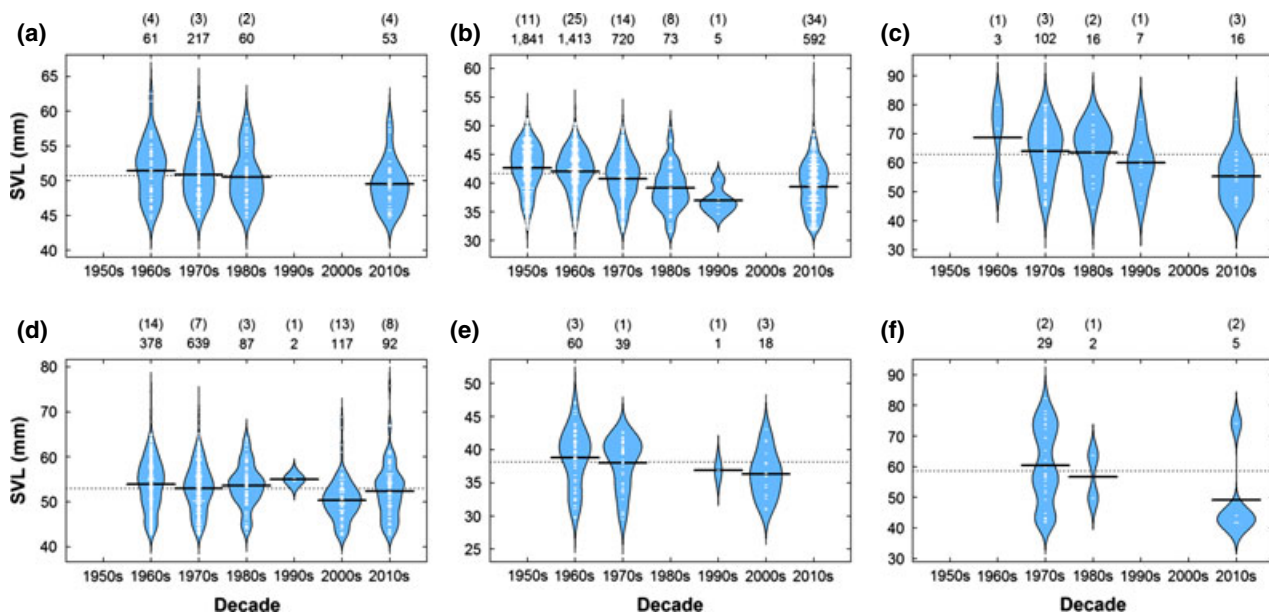


Fig. 2 Shifting size distributions over time for *Plethodon cheoah* (a), *P. cineris* (b), *P. cylindraceus* (c), *P. jordani* (d), *P. ventralis* (e), and *P. yonahlossee* (f). The number of sites sampled in each decade is represented by the number in parentheses above the sample size of animals measured for that time period.

95% CRI = -0.002, 0.001; Figure S1), elevation (DIC = -279.1; 95% CRI = -0.00002, 0.0000004), change in temperature (DIC = -277.2; 95% CRI = -0.70, 0.34), nor change in precipitation (DIC = -277.8; 95% CRI = -0.04, 0.13) explained the magnitude of change in body size. We discovered strong environmental associations with changes in body size, where populations at lower latitudes ( $\beta_1 = 0.014$ ; 95% CRI = 0.00, 0.03; Figure S2) that experienced both an increase in temperature and decrease in precipitation ( $\beta_1 = 3.693$ ; 95% CRI = 0.73,

6.84; Fig. 3) showed the greatest reductions in body size; latitude (DIC = -283.3) explained this slightly better than did change in temperature and precipitation (DIC = -282.3).

Biophysical modeling showed that annual energy expenditure increased across sites across the sampling period, with different responses for each size class with respect to site ( $P < 2.2e-16$ ) and year ( $P = 2.86e-5$ ; Fig. 4). At LIM, metabolic expenditure increased by 7.14% and 7.17% for 1 g and 10 g salamanders, respec-

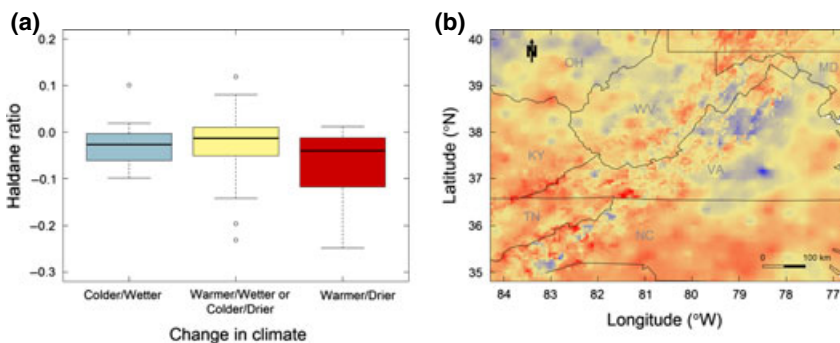


Fig. 3 (a) Grand mean changes in standardized difference in mean body size per generation, relative to within-population standard deviation for populations in areas that have become colder and wetter (blue), warmer and drier (red), and either colder and drier or warmer and wetter (khaki). To compare among species and populations with different generation times, we converted body size change into Haldane Ratios. The greatest body size reductions, as indicated by Haldane Ratios, were found in populations that experienced both an increase in temperature and a decrease in precipitation (95% CRI = 0.732, 6.837). (b) Spatial distribution of actual climate trends during the study period; areas with the darkest reds experienced the greatest amount of both warming and drying, blue colors are areas that have become both colder and wetter (data from Girvetz *et al.*, 2009).

tively. At ATW, metabolic expenditure increased by 7.86% and 7.88% for 1 g and 10 g salamanders, respectively. At FDR, metabolic expenditure increased by 7.60% and 7.62% for 1 g and 10 g salamanders, respectively. We detected no increase in the annual duration of activity for any of the three sites across the sampling years ( $P < 0.060$ ) although sites differed in the duration of activity ( $P < 2e-16$ ; Fig. 4). Because relative differences across sites were similar across different levels of relative humidity, we only report results for 100% relative humidity. The durations of activity (mean  $\pm$  1 SD) were  $1953 \pm 105$  h at LIM,  $1791 \pm 100$  h at ATW, and  $1618 \pm 97$  h at FDR. We found no differences in activity between small (1 g) and large-bodied (10 g) salamanders ( $P = 0.078$ ).

## Discussion

Organisms will need to adapt, acclimate, migrate, or risk extinction in response to changing climates (Chevin *et al.*, 2010; Sears & Angilletta, 2011). Our results are consistent with plethodontid salamanders responding to climate change through reductions in body size as mediated through increased metabolism. We found a strong association between body size reduction and latitude. Further, body size change was most accentuated in regions that have become warmer and drier over the

past 55 years, which in eastern North America tends to occur at lower latitudes. Observed body size reductions were spatially and temporally correlated with patterns of documented climate change, and are consistent with climate change projections for this group of salamanders (Milanovitch *et al.*, 2010).

Our data show significant body size reductions in adults over time that is consistent with several potential mechanisms operating through organismal life-history traits (e.g., age at maturity, size at maturity, reduced growth rates). Reduced body size has been proposed as the third universal response to global warming (Daufresne *et al.*, 2009). They hypothesized that a shift in mean body size within a community could be explained by a decrease in mean body size within a population or an increase in the proportion of small species. Our analysis showed no evidence for a shift in the proportion of small species: first, we found no difference in response among the three size classes of salamanders, and secondly, one species from each size class showed significant reduction in body size. Our findings support the population body size shift hypothesis, which could be explained by a decrease in individual body size, a decrease in the minimal adult body size, or an increase in the proportion of juveniles (Daufresne *et al.*, 2009). Our measurements were taken from animals within the range of putative adult body size and from which we

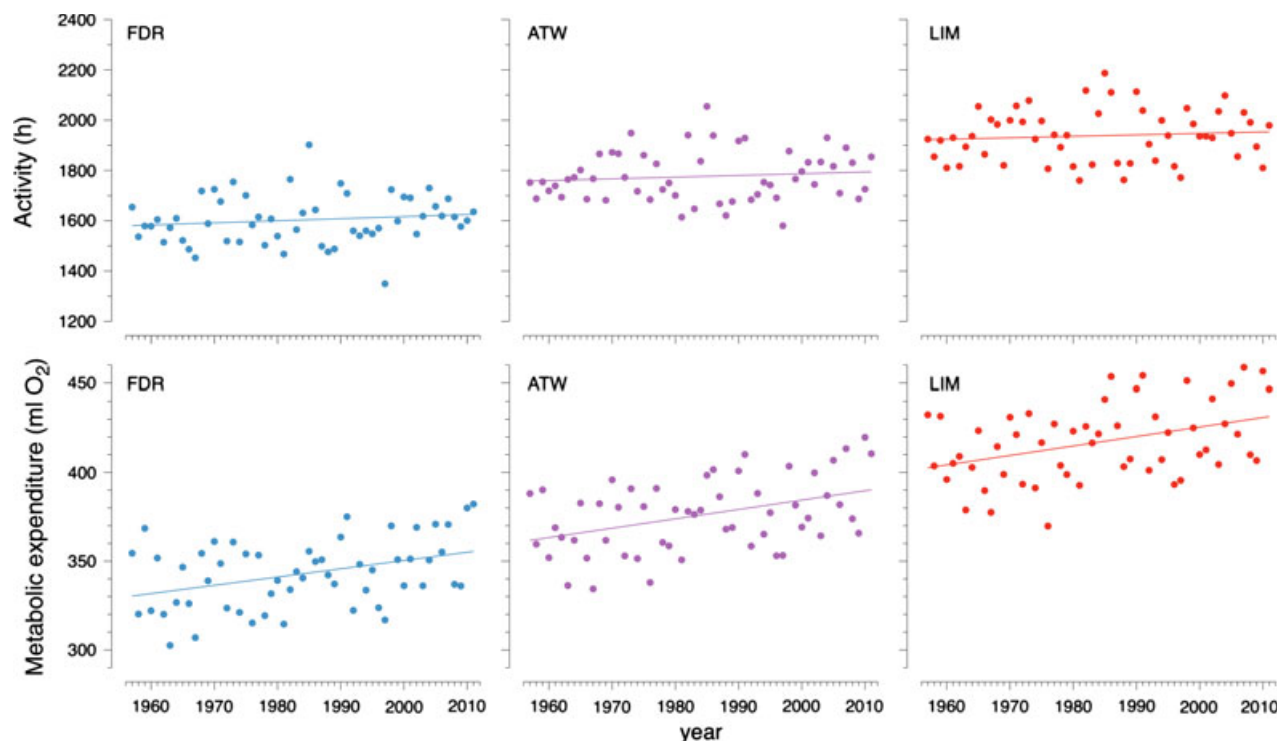


Fig. 4 Results of modeling annual activity (upper row) and annual energy expenditure (bottom row) for a 10 g *Plethodon* at Catoclin Mountain N.P., MD (FDR), Mountain Lake area, VA (ATW), and Mt. Rogers National Recreation Area, VA (LIM).

excluded any obvious juveniles, thus the third possible factor is unlikely. Examination of body size distributions (Fig. 2) shows a reduction in the number of individuals in the largest adult size classes but no change in the number of the smallest adult size classes. Additional analyses and studies are needed to determine the underlying mechanism behind these changes, but both genetic responses and plasticity may represent possible adaptive strategies of these species to spatially or temporally heterogeneous environments.

One of the main questions regarding the impacts of climate change on organisms is the degree to which changes are genetically based, and whether adaptation can keep pace with climatic changes. Shifts in average population body size could result from either plasticity in the growth response of salamanders to changing climates through changes in allocation and energetics, or from selection for decreased size where energy is limiting. We were unable to discriminate between phenotypic plasticity and genetic adaptation to climate change; however, the patterns observed here represent rapid body size changes over a period of relatively few generations. Indeed, while many recent studies have shown phenotypic changes associated with climate change, few have definitively shown that these were genetically based and were thus unable to rule out the alternative of environmentally induced responses (Gienapp *et al.*, 2008; but see Emerson *et al.*, 2010 for an example of evolutionary adaptation to climate change).

With respect to a potential plastic response to climate, we were able to provide a potential mechanism for reduced body size through energetics: increases in metabolic expenditures might lead to reduced growth if food or foraging time is limited. In general, organisms reared at higher temperatures achieve smaller body sizes at equivalent developmental stages than siblings reared at lower temperatures (Atkinson, 1994; Angilletta *et al.*, 2004). That said, evidence suggests that Plethodontid salamanders do not follow this pattern (Bernardo & Reagan-Wallin, 2002; Adams & Church, 2008). However, we acknowledge that other related mechanisms could generate a similar pattern. For instance, if individuals become energetically limited, they could allocate less energy to activity, maintenance, reproduction, or storage to offset increased expenditure (Ohlberger, 2013). If less energy was allocated to activity, potential reductions in foraging success might also lead to energetic constraints on growth. To distinguish these potential mechanisms would require both species and population specific data regarding thermal preferences and thermal sensitivities of physiologic function (Ohlberger, 2013). Unfortunately, as in most systems, such data are scarce or inadequate.

Regardless of whether the effect is genetic or environmental, the degree of body size reduction we documented in *Plethodon* was both large and rapid. For the six species displaying significant trends, body size reduced by an average of nearly 8% across the time period examined. When standardized for within-population variation, this corresponds to approximately a 1% body size reduction per generation in these species. This magnitude of change is on par with some of the largest phenotypic changes observed in contemporary populations (Hendry *et al.*, 2008; Uyeda *et al.*, 2011). Thus, these changes represent some of the fastest responses to environmental perturbations ever recorded and lend support to the observation that phenotypic responses, particularly those related to anthropogenic disturbance, are both more rapid and more extreme than those observed in natural contexts or over longer time periods (Hendry *et al.*, 2008). The rapidity and the widespread extent of these changes across so many species in a biodiversity hotspot may signal rapid adaptation to novel environmental conditions.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** List of all resurveyed field sites by state, latitude, longitude, elevation and species analyzed.

**Figure S1.** Change in Haldane Ratio, the standardized difference in mean body size per generation, relative to within-population standard deviation among populations, species, and body size categories (CRI =  $-0.002$ ,  $0.001$ ). Each triangle represents the average change in Haldane Ratio for each population for each species of *Plethodon* salamander. Species are listed from smallest to largest in average body size. The horizontal dotted line indicates no change.

**Figure S2.** Latitudinal variation in reduction in body size for the full dataset of 16 species of *Plethodon* salamanders ( $\beta_1 = 0.014$ ; 95% CRI =  $0.004$ ,  $0.025$ ). Color of each dot indicates the amount of change in Haldane Ratio (the standardized difference in mean body size per generation, relative to within-population standard deviation) with ‘hotter’ colors indicating more change and ‘cooler’ colors indicating less change.