

# ECOGRAPHY

## Research

### Salamander morph frequencies do not evolve as predicted in response to 40 years of climate change

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The global climate is changing rapidly, yet biotic responses remain uncertain. Most studies focus on changes in species ranges or plastic responses like phenology, but adaptive evolution could be equally important. Studying evolutionary responses is challenging given limited historical data and a poor understanding of genetically variable traits under selection. We take advantage of a historical dataset to test for an adaptive response to climate change in a widespread, polymorphic amphibian, the eastern red-backed salamander *Plethodon cinereus*. We resurveyed color morph frequencies across New England to test for an adaptive shift in response to climate change. We modeled historical and present-day morph proportions as a function of climate and tested the accuracy of predictions both within and across different time periods. Our models showed moderate accuracy when predicting morph frequencies within time periods, but poor accuracy across time periods. Despite substantial changes in climate and significant relationships between morph frequency and climate variables within periods, we found no evidence for the predicted shift in morph frequencies across New England. The relationship between climate and color morph frequencies is likely more complex than originally suggested, potentially involving the interplay of additional factors such as microclimate variation, land use changes, and frequency-dependent selection. Model extrapolation and changes in the correlation structure of climate variables also likely contributed to poor predictive ability. Evolution could provide a means to moderate the effects of climate change on many species. However, we often do not understand the direct links between climate variation, traits, and fitness. Therefore, forecasting climate-mediated evolution remains an ongoing and important challenge for understanding climate change threats to species.

Keywords: adaptation, *Plethodon cinereus*, polymorphism

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#### Introduction

Human-induced climate change is affecting species distributions, species abundances, and ecosystem functions worldwide (Walther et al. 2002, Parmesan 2006, IPCC 2014). For decades, biologists have been trying to predict the substantial, and



often idiosyncratic, responses of species to climate change (Barry et al. 1995, Parmesan and Yohe 2003, Lenoir et al. 2010, Chen et al. 2011, Crimmins et al. 2011). In response to changing conditions, species can track suitable climatic conditions by dispersing (Chen et al. 2011), shifting phenology (Parmesan and Yohe 2003, Visser and Both 2005), persisting in microclimate refugia (Ashcroft et al. 2012, Scheffers et al. 2014), or adapting to changing conditions in place (Hoffmann and Sgro 2011). Given the complexity of ecological systems, the frequent lack of proximal environmental data at species-relevant scales, and the scarcity of detailed historical data, the relative importance of these different responses remains unclear for most species (Lavergne et al. 2010, Shaw and Etkerson 2012, Urban et al. 2016). These factors make it difficult to evaluate which species are most at risk of extinction caused by climate change.

The extent to which we currently understand the relationship between climate change and species responses is largely centered on changes in species distributions or phenology. However, for some species these responses are unfeasible given the magnitude and rate of climate change in combination with habitat loss and fragmentation. Consequently, adapting to changing conditions in place may be a potentially critical response, especially in dispersal-limited species (Norberg et al. 2012, Urban et al. 2012). Rapid adaptive responses require sufficient standing genetic variation among individuals in order to match the rate of environmental change (Skelly et al. 2007, Barrett and Schluter 2008). Convincing examples of adaptive responses to climate change can be found across a diversity of taxa (Bearhop et al. 2005, Franks et al. 2007, Buckley et al. 2012, Chirgwin et al. 2015), yet in some species, constraints such as lack of genetic variation have prevented adaptation to climate change (Hoffmann et al. 2003). Due to the multidimensional selective pressures created by climate change, we seldom know which traits are under selection or whether those traits are heritable and are underlain by sufficient genetic variation (Hoffmann and Sgro 2011). Additionally, we often lack the historical data needed to validate our predictions, which makes adaptive evolution the least understood of the responses to climate change (Merilä and Hendry 2014, Urban et al. 2014).

Given the above challenges, polymorphic species offer several advantages for studying potentially adaptive evolutionary responses to climate change. First, genetic variation in the form of alternative morphs is already present, and the long-term persistence of alternative morphs suggests that they confer differential fitness advantages in time or space (Forsman et al. 2008, Forsman and Wennersten 2016). Second, spatial variation in morph frequencies across climatic gradients often suggests a climate-morph link (Galeotti et al. 2003, Lepetz et al. 2009, Antoniazza et al. 2010). Third, the ability to score morphs easily in the field provides greater opportunities to document variation across space and time. As a result, the availability of a large-scale, detailed historical dataset for a dispersal-limited species with a hypothesized climate-related

polymorphism provides an exceptional opportunity to assess adaptive evolution in response to climate change.

Our study explores the relationship between climate and evolutionary responses in a widespread, polymorphic amphibian, the eastern red-backed salamander *Plethodon cinereus*. *Plethodon cinereus* has two common color morphs (Fig. 1 inset) that are characterized by the presence ('striped') or absence ('unstriped') of a dorsal stripe. The relative proportions of these morphs vary across the species range in eastern North America (Gibbs and Karraker 2006, Moore and Ouellet 2015). The color polymorphism is genetically determined, although the exact genetic architecture remains unknown and may vary across localities (Highton 1959, 1975). As a lungless, terrestrial salamander, *P. cinereus* relies entirely on cutaneous respiration, and the need to maintain moist skin constrains its activity, including foraging, growth, and reproduction (Feder 1983, Peterman and Semlitsch 2013, 2014). Given this sensitivity to environmental conditions, climate has long been hypothesized to be a primary driver maintaining the color polymorphism in *P. cinereus*.

Multiple lines of evidence suggest that this polymorphism is linked to climate. Geographic patterns of morph frequencies suggest unstriped morphs are cold-intolerant (Lotter and Scott 1977, Gibbs and Karraker 2006). Likewise, temporal variation in morph frequencies at some sites indicates that unstriped morphs are less likely to be active on the surface and will retreat to underground refugia in cold temperatures (Lotter and Scott 1977, Moreno 1989, Anthony et al. 2008). Finally, population-specific physiological and mechanistic data show metabolic differences between the color morphs corresponding to differential heat tolerance (Moreno 1989, Petruzzi et al. 2006). The lower metabolic rates of unstriped morphs may allow them to occupy drier habitats (Fisher-Reid et al. 2013). As a result, warmer and drier conditions are generally expected to select for higher unstriped morph frequencies. However, the relationship between morph frequencies and climate has also been questioned based on inconsistent patterns between populations and insignificant differences in the response of color morphs to temperature or moisture (Petruzzi et al. 2006, Fisher-Reid et al. 2013, Moore and Ouellet 2015).

Here, we test for an evolutionary response to climate change in *P. cinereus* by resurveying sites originally sampled by Lotter and Scott (1977) in the early 1970s. Lotter and Scott (1977) surveyed morph frequencies at 50 locations across New England and concluded that the regional clines in color morph frequencies indicated adaptation to local temperatures. Our study addresses four research questions: 1) is there a relationship between climate and unstriped morph frequency in the New England region? If so, 2) has regional climate shown sufficient change since the 1970s that we would expect to see changes in morph frequencies? If yes, then 3) what evolutionary change is predicted by the climate-morph relationship and the magnitude of observed climate change? Finally, 4) do we observe this predicted evolutionary response? Given the previously proposed climate-morph link

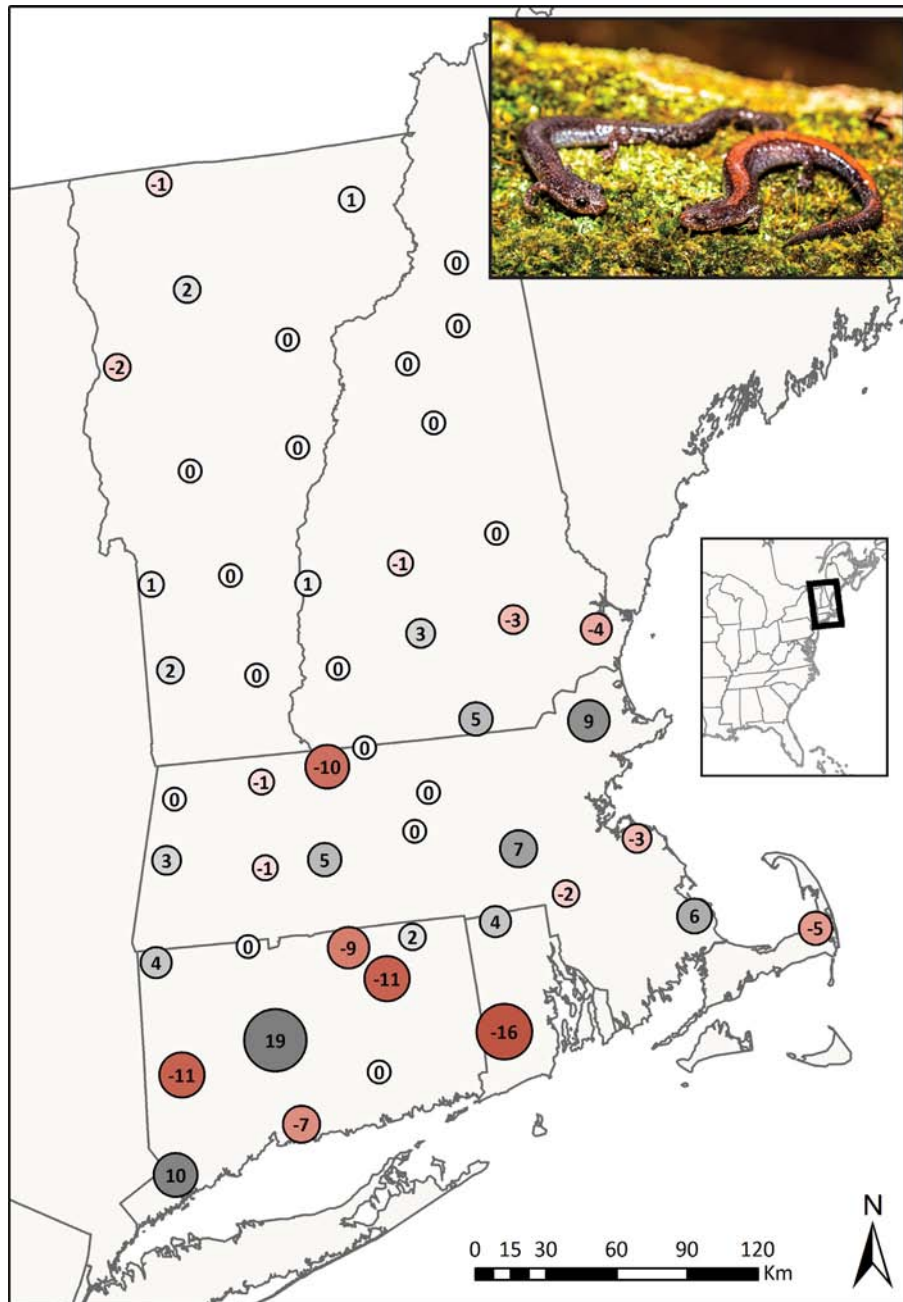


Figure 1. Map of survey locations showing change in proportion of unstriped morphs between 1970 and 2015. Circles are scaled to the magnitude of change in unstriped morph proportions (percentage, specified by the number within each circle), with increasing grey tints indicating increasing frequency of unstriped morphs and increasing red tints indicating increasing frequency of striped morphs. Inset: photo of *Plethodon cinereus* morphs, unstriped (left) and striped (right). Photo copyright: Binbin Li (used with permission).

and an average generation time of 2–3 yr (Sayler 1966), we hypothesized that changes in selection resulting from climate change have caused changes in morph frequencies across New England since the 1970s. We expected that models based on correlations between climate and morph frequencies would accurately predict morph frequencies both within and between time periods (i.e. 1970s and 2015). We specifically predicted an increase in the proportion of unstriped individuals at sites that have become warmer and drier during the last 40 yr.

## Methods

### Salamander surveys

In 2015, we resampled the 50 localities that Lotter and Scott (1977) surveyed in 1971–1973. Like Lotter and Scott (1977), we searched for salamanders under cover objects including fallen logs, bark fragments, and rocks, in wet leaf litter, and inside decaying logs, and at each site recorded the morph (striped, unstriped, erythristic (fully red), or

amelanistic) of a minimum of 100 *P. cinereus* individuals (Supplementary material Appendix 1 Table A1). The sites selected for the resurvey are detailed in Supplementary material Appendix 1 Fig. A1 and Table A2. Because the original sampling localities were specified only at the level of towns and counties, we selected resampling locations using a combination of town center coordinates (Lotter and Scott 1977) and vegetation and topographic descriptions provided in Lotter's (1975) dissertation appendix. We matched specific localities (e.g. state parks) in seven of the eight cases in which they were provided (the eighth is now a residential block). The total area searched during each survey varied among sites but was consistent with areas covered by the original surveys (1–3 km in diameter; Lotter and Scott 1977).

We divided the resurvey effort into five time periods, spanning 14 May to 11 October 2015, and conducted each resurvey in the month that most closely matched the original survey time (see Supplementary material Appendix 1 Table A2 for original and resurvey dates). Intra-annual temporal surveys by Lotter (1975) and AEE (unpubl.) indicated no significant differences in morph proportions across repeated survey efforts during the months when sampling occurred, suggesting that morph frequencies observed following the same procedures are comparable across years. Additionally, we sampled two sites across multiple months and found no significant differences in morph proportions (Supplementary material Appendix 1 Table A3).

### Predictor variables

We developed a set of nine climatic predictor variables relevant to salamander biology and ecology (Supplementary material Appendix 1 Table A4). Five of these variables (water balance and temperature variables) were further refined for the seasonal activity period of terrestrial salamanders across the survey area (April–November, determined by literature searches; Cochran 1911, Lotter and Scott 1977, Nagel 1977, Jaeger 1979, Gergits and Jaeger 1990, Dawley and Crowder 1995, Leclair et al. 2008). Temperature variables were derived from TopoWx daily data (Oyler et al. 2015). Monthly water balance data recently recalculated using TopoWx temperature data were provided by Dobrowski et al. (2013). The spatial resolution of both data sets is 30 arc-seconds. We used the average of each variable for the 10 yr prior to the historic and current salamander surveys as the predictor. We used a decadal average to more accurately capture average climates experienced by surveyed populations throughout the lifetime of adults (up to 8–9 yr, Leclair et al. 2006). Lotter (1975) conducted his surveys from 1971–1973, so we used a decadal average of 1962–1971; for current surveys we used 2005–2014. For water balance metrics, we used data from 2005–2010 because more recent data were not available. For sites that had more than one sampling location (subsites, Supplementary material Appendix 1 Table A2), we used the mean of the variable values at each location. We did not weight subsites based on sample size since that information was not available for Lotter's (1975) data. We found only

one case where there is a significant difference in the morph frequencies across subsites (Supplementary material Appendix 1 Table A3) and exploratory analysis of the 2015 data showed no evidence that weighting subsites based on sample numbers improved model accuracy or precision ( $R^2=0.70$ ,  $RMSE=37.93$ ).

To account for spatial autocorrelation in sampling, we selected a subset of spatial eigenfunctions that significantly accounted for spatial structure in morph data for each time period. First, we calculated spatial eigenfunctions from the current sampling site coordinates using distance-based Moran's eigenvector maps (dbMEMs, Legendre and Legendre 2012). When sites were represented by more than one sampling location (Supplementary material Appendix 1 Table A2), we used the geometric median of the subsite coordinates. Second, we built a full multiple linear regression model for each time period where the response was either the proportion of unstriped morphs at each site during each time period or the change in unstriped morph proportions across time periods and the predictors were the full set of dbMEMs. Finally, we used forward selection (permutation p-value of 0.05 for adding a dbMEM variable to the model) to retain significant dbMEM predictors, using the full model-adjusted  $R^2$  as the stopping criterion (Blanchet et al. 2008). We checked correlations and variance inflation factors (VIFs) between significant dbMEM predictors and climate variables to ensure correlations and VIFs were less than  $|0.7|$  and 10, respectively. All predictor variables were calculated using R ver. 3.2.3 (R Development Core Team) and the packages ncd4 ver. 1.16 (Pierce 2015), raster ver. 2.6-7 (Hijmans 2015), PCNM ver. 2.1-4 (Legendre et al. 2012), pracma ver. 1.6.4 (Borchers 2015), and usdm ver. 1.1-18 (Naimi et al. 2014).

### Models of color morph frequency and climate

To test for a relationship between climate variables and unstriped morph frequency for each time period ('1970' and '2015' hereafter) we used a Markov chain Monte Carlo (MCMC) approach to fit Bayesian generalized linear mixed models in the MCMCglmm package, ver. 2.22 (Hadfield 2010). We first created a set of candidate models for each time period, consisting of all combinations of predictor variables that had correlations and VIFs less than  $|0.7|$  and 10, respectively (see Supplementary material Appendix 1 Table A5 for candidate model lists). To allow for comparison between the two survey periods, we also ran any additional factor combinations that were run in the other time period, regardless of variable correlation and VIF. For each candidate model, we modeled the proportion of unstriped individuals where our response variable was a two-vector count of unstriped individuals and individuals of all other color morphs (striped, erythristic and amelanistic morphs pooled) at each site (see Supplementary material Appendix 1 Table A1 for morph counts). We used multinomial2 (binomial) as the model family, and included three significant dbMEMs as random slopes (see Results). We fitted all random effects and residuals

using an uninformative inverse Wishart prior with  $V=1$  and  $nu=0.002$ , where  $V$  is an estimate of variance and  $nu$  is a parameter for the degree of belief in  $V$  (Hadfield 2010). We ran all models for 2 500 000 iterations, with a burn-in of 500 000 iterations, and a thinning interval of 250 to reduce autocorrelation. These parameters provided 8000 samples from the posterior distribution for each model parameter. For a subset of these models and for all final models, we ensured suitable convergence and mixing using Heidelberger and Welch (1983), Geweke (1991), and Gelman and Rubin (1992) diagnostics, as well as visual inspection of trace plots and posterior distributions plots to check that distributions were symmetrical.

To select our best climatic model for each time period, we used leave-one-out cross-validation to build each model in the candidate set with data from 49 out of 50 sites and then predicted morph counts for the withheld site. We repeated this process 50 times, sequentially leaving out and predicting each site. We then calculated log-likelihood of the observed unstriped morph count based on morph predictions for each site, and summed the log-likelihoods across all sites to rank the candidate models based on overall log-likelihood (see Supplementary material Appendix 1 Table A5 for full list).

To evaluate the fit and transferability of our best 1970 and 2015 models, we compared the observed and predicted proportion of unstriped individuals under four scenarios: 1) the 1970 model predicting unstriped morph proportions observed in the 1970s, 2) the 2015 model predicting 2015 unstriped morph proportions, 3) the 1970 model predicting 2015 unstriped morph proportions based on 2015 climate predictors (forecast model), and 4) the 2015 model predicting 1970s unstriped morph proportions based on 1970 climate predictors (hindcast model). Sites were over- or underpredicted if observed values fell outside the 95% highest density posterior intervals (HDPI) of the model prediction. We rounded HDPI values to the nearest whole number since the MCMC predict function cannot generate HDPI values that equal exactly zero (e.g. if the lower HDPI is 0.005, it is rounded down to 0). We estimated model precision using Pearson's  $r$  correlation and relative model accuracy using root mean squared error (RMSE) based on point estimates of unstriped morph proportions. We also estimate the pMCMC statistic, which tests whether the parameter is significantly different from zero and is calculated as two times the smallest MCMC estimate of the probability that a parameter value is either 1)  $< 0$  or 2)  $> 0$ .

### Models of changes in morph frequency and climate

Because our predictive models performed poorly across time periods, a posteriori, we asked whether change in morph proportions was correlated with changes in any of our climate variables. To calculate change in climate variables and color morph proportions, we subtracted the 1970s data from the 2015 data. Because our 'change' predictors showed reduced correlation structure, we created a new candidate model set using a more conservative correlation cut-off of 0.4 (see

Supplementary material Appendix 1 Table A6 for candidate model list). We fitted Bayesian generalized linear mixed models to these data, where the response variable was the change in unstriped morph proportion, assuming a Gaussian distribution. We used one random effect, the variance of the difference between the proportion of unstriped morphs in the 1970s and 2015 to account for measurement binomial error variance (J. Hadfield pers. comm.). We found no significant spatial eigenfunctions for the change models (dbMEMs, see results). We fitted these models and checked model convergence as described above. Because we were not using the change model for prediction, we ranked models using DIC in the package MuMIn ver. 1.15.6 (Barton 2012) instead of using cross-validation. Additionally, we tested for significant site-specific changes in color morph proportions between the two surveys using Fisher's exact tests, followed by Holm-Bonferroni sequential correction for multiple tests.

## Results

### Relationship between color morph frequency and climate

We found significant relationships between climate variables and unstriped morph proportion in the study area during each of the survey periods (Table 1). For each time period, our best morph-climate model showed moderate fit and accuracy (Table 2, Fig. 2a, c). However, the most important predictors differed across time periods (Supplementary material Appendix 1 Table A5). The best (highest cross-validation score) 1970 model included average freezing degree days (FDD), average actual evapotranspiration from April–November (AET), and average number of days above the critical thermal maximum (CTmax). In this model, increases in FDD and AET both had a significant positive effect on the proportion of unstriped morphs (pMCMC=0.0001 and pMCMC=0.011 respectively, Table 1), whereas increases in CTmax had a marginally significant positive effect on unstriped morph proportion (pMCMC=0.084, Table 1). By contrast, the best 2015 model included only average maximum temperature from April–November (Tmax0411), which had a significant positive effect on unstriped morph proportion (pMCMC=0.0001, Table 1). For both 1970 and 2015, three spatial eigenfunctions (MEM1, MEM2, and MEM6) accounted for a significant spatial signal in the salamander morph data. None of these dbMEMs were correlated with our climate predictors above  $|0.7|$  and all VIFs were less than four. All candidate models had VIFs less than ten, and only two were greater than five.

### Regional climate change

The four important climate variables included in our best models (AET, FDD, CTmax and Tmax0411) changed significantly between the 1970s and 2015 (all paired t-tests,  $p < 0.0001$ , Fig. 3). Mean FDD decreased by 229 degree days,

Table 1. MCMCglmm model parameters for the best (highest log-likelihood value) morph frequency-climate models for each survey period (1970 and 2015). Climate variables included in best models are FDD (freezing degree days), AET (actual evapotranspiration from April to November), CTmax (number of days above critical thermal maximum, base 32°C), and Tmax0411 (mean monthly maximum temperature from April to November). HDPI represents the 95% highest density posterior interval. The pMCMC statistic tests whether the parameter is significantly different from zero and is calculated as two times the smallest MCMC estimate of either 1) the probability that a parameter value is < 0 or 2) the probability that a parameter value is > 0.

Model	Variable	Posterior mean (95% HDPI)	pMCMC
1970	FDD	0.0047 (0.0031, 0.0063)	0.0001
	AET	0.1483 (0.0388, 0.2576)	0.0110
	CTmax	0.2467 (-0.0333, 0.5437)	0.0842
2015	Tmax0411	1.3150 (0.9119, 1.7443)	0.0001

mean AET increased by 8.9 mm, mean CTmax increased by 1.5 d, and mean Tmax0411 increased by 0.8°C. These changes correspond to overall warmer temperatures with an increase in days above 32°C, a reduction in days below freezing, and overall drier conditions. We also found a significant difference in the overall correlation structure between the 2015 and 1970s correlation matrices (Mantel test,  $z=29.04$ ,  $p=0.001$ ), with variables becoming increasingly correlated over time (Table 3).

### Evolutionary predictions based on morph frequency-climate models

Both the forecast and hindcast models predicted changes in morph frequency. However in both cases, the models predicted larger changes in unstriped morph proportions than were actually observed (Fig. 2b, d). For example, the best 1970s model predicted an overall increase in unstriped morph frequency of 27.4% across the 50 sites but we observed a non-significant increase of only 0.4%, (Pearson's chi-squared=0.371,  $p=0.543$ ). In the forecast model, predictive accuracy was poor and 82% of sites were incorrectly predicted with a strong bias for overprediction of unstriped morph proportions (Table 2). By comparison, the hindcast model tended to underpredict unstriped morph proportions (Table 2). Full observed and predicted data with credible intervals are provided in Supplementary material Appendix 1 Table A7 and Table A8.

Table 2. Model performance for each morph-frequency-climate model under four prediction scenarios; 1970 model predicting 1970 morph proportion, 2015 model predicting 2015 morph proportion, 1970 model predicting 2015 morph proportion (forecast), and 2015 model predicting 1970 morph proportion (hindcast).

Model	Precision (Pearson's $r$ )	Accuracy (RMSE)	Overprediction	Underprediction
			no. sites (%)	no. sites (%)
1970→1970	0.74	7.67	13 (26%)	9 (18%)
2015→2015	0.74	7.70	15 (30%)	9 (18%)
1970→2015 (forecast)	0.72	35.09	39 (78%)	2 (4%)
2015→1970 (hindcast)	0.69	9.50	10 (20%)	19 (38%)

### Evolutionary change in morph frequency based on change in climate

We observed no change in the overall (pooled) proportion of unstriped morphs between the two survey periods ( $z=0.70$ ,  $p=0.48$ , Supplementary material Appendix 1 Table A1). Morph frequency changed significantly at only one site, after correcting for multiple tests (site 9;  $p=0.0004$ , see Supplementary material Appendix 1 Fig. A1 for site locations), where the proportion of unstriped individuals decreased by 16%. The proportions of a third, rarer erythristic color morph also appeared stable between the two surveys (Supplementary material Appendix 1 Table A1) with only one site experiencing a significant change at the 5% level after applying the Holm-Bonferroni correction (site 50,  $p=0.001$ ; see Supplementary material Appendix 1 Fig. A1 for site locations). Unsurprisingly given the lack of change in morph proportions, none of the climate predictors that we examined explained a significant amount of the variance in the change in unstriped morph proportion over time. The best change model contained only an intercept, with all nine univariate models within 5 DIC points of the null model (Supplementary material Appendix 1 Table A6). The change model had no significant spatial eigenfunctions.

### Discussion

Despite a significant relationship between climate and *Plethodon cinereus* morph frequencies within each time period and an overall warming of the study region, we found no evidence for the predicted evolutionary change in morph frequencies in response to 40 yr of climate change. Morph frequencies appeared stationary across virtually all sites, even though the significant changes in climate we detected have occurred over a time span encompassing approximately 13 generations of salamanders, which has been enough time for evolution to occur in other species (Kinnison and Hendry 2001). This outcome could be explained if *P. cinereus* color morph proportions are not directly related to climate, selection from climate change is weak, or the morph-climate interaction is more complex than a simple linear response over time. Other factors that may complicate the color morph response include 1) interacting selective processes, 2) the decoupling of macro- and microscale climate and behavioral buffering, and 3) variability in the strength and direction of selection over time.

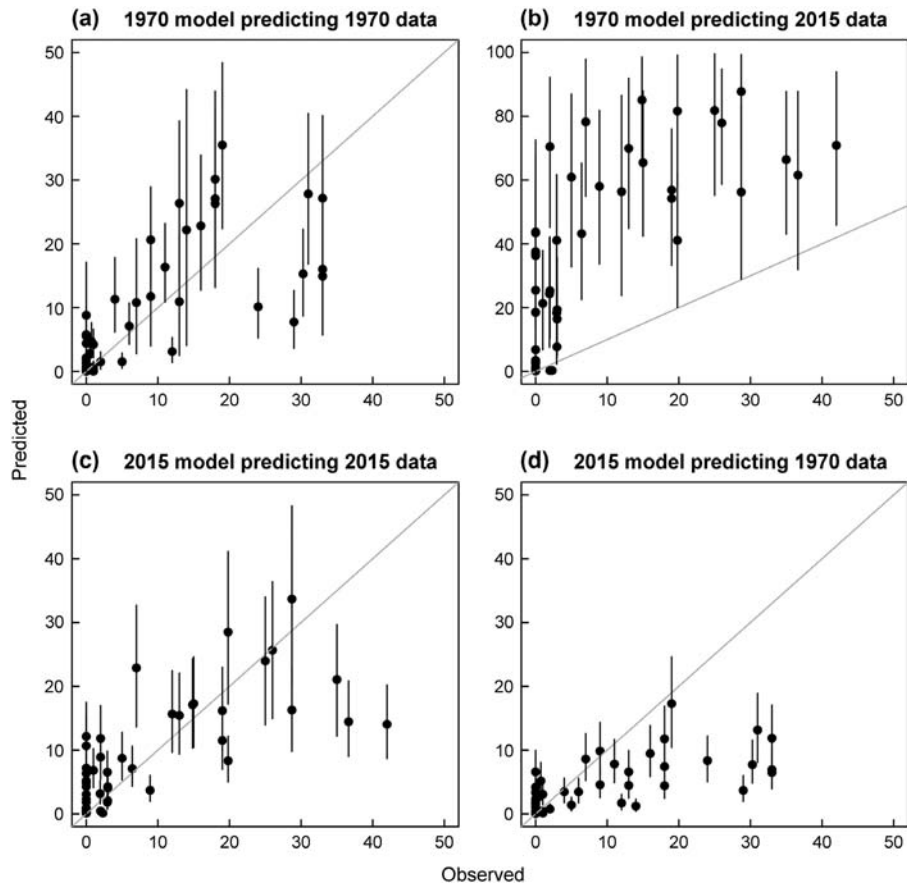


Figure 2. Proportion of *Plethodon cinereus* unstriped morphs observed during field surveys against the number predicted by models under four scenarios: (a) 1970 model predicting 1970 morph proportion, (b) 1970 model predicting 2015 morph proportion (forecast), (c) 2015 model predicting 2015 morph proportion, and (d) 2015 model predicting 1970 morph proportion (hindcast). Error bars show 95% highest density posterior intervals. Diagonal line is the 1:1 line that signifies perfect model prediction. Note different y-axis scale in (b).

The presence of a morph–climate relationship but lack of a predictable temporal response points to the basic challenge of determining the ultimate cause of selection over space and time (Merilä and Hendry 2014). In other polymorphic species, morphs often differentiate along multiple ecological axes other than climate (Jones et al. 1977, Hoffman and Blouin 2000, Roulin 2004). Previous work on the *P. cinereus* polymorphism has largely identified ways in which unstriped individuals are at a competitive disadvantage compared to striped individuals (Reiter et al. 2014): they have a poorer quality diet (Anthony et al. 2008, Stuczka et al. 2016), higher levels of stress hormones (Davis and Milanovich 2010), and higher levels of predation (Lotter and Scott 1977, Moreno 1989, Venesky and Anthony 2007). Despite these apparent disadvantages, the unstriped morph has persisted over evolutionary time (Fisher-Reid and Wiens 2015), indicating that additional factors, such as the proposed ability to exploit warmer and drier microclimates (Lotter and Scott 1977, Fisher-Reid et al. 2013) possibly due to a lower metabolic rate (Moreno 1989, Petrucci et al. 2006), likely convey fitness benefits to unstriped individuals. Frequency-dependent selection mediated by visual predators, such as blue jays *Cyanocitta cristata*, has also been implicated as a mechanism

maintaining this color polymorphism (Fitzpatrick et al. 2009). However, the distribution of known visual predators of *P. cinereus* (e.g. blue jays) does not coincide with morph variation in New England, and color morphs also experience selective pressure from other predators, which may perceive and discriminate between the color morphs differently (Venesky and Anthony 2007, Kraemer and Adams 2014). More research is needed to evaluate the degree to which differences in predation contribute to regional variation in morph frequencies.

The lack of temporal response in morph frequencies over the past 40 yr may also be related to the decoupling of coarse climate predictors (such as ours) from microclimates that can vary profoundly over centimeters for small, dispersal-limited species like terrestrial salamanders (Heatwole 1962, Feder 1983, Peterman and Semlitsch 2013, 2014, Storlie et al. 2014). For example, aspect and elevation can have a substantial impact on microclimate conditions, with low elevation, south-facing slopes typically experiencing warmer temperatures and drier habitats (Fu and Rich 2002), which may favor unstriped individuals. Macroscale climate also interacts with site-specific changes in land use, such as logging and urbanization, which can alter regional and

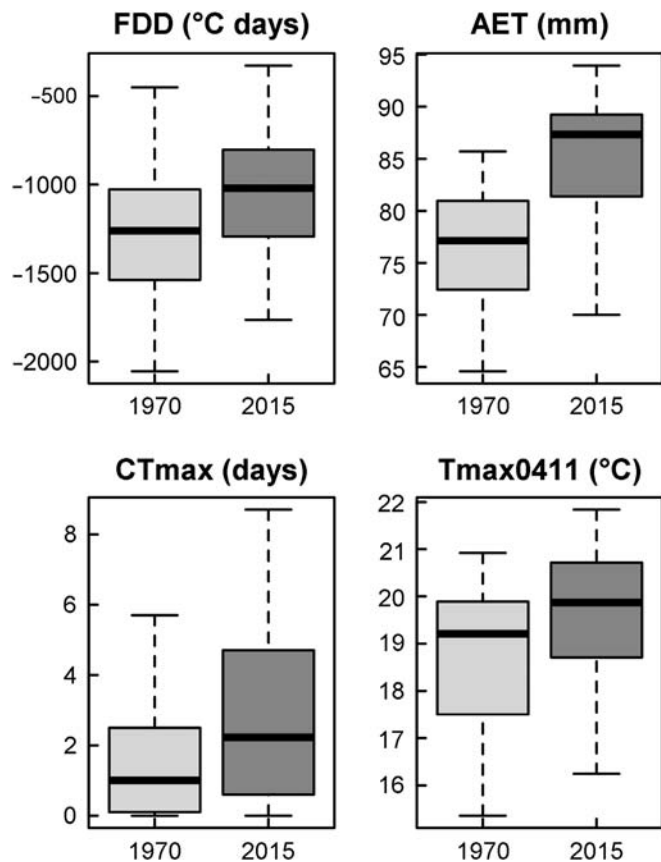


Figure 3. Box plots of climate variables: FDD (freezing degree days), AET (actual evapotranspiration from April to November), CTmax (number of days above critical thermal maximum, base 32°C), Tmax0411 (mean monthly maximum temperature from April to November) from 50 survey locations for 1970 and 2015 survey periods. Horizontal black line represents the median, while the bottom and top of the box represent the 25th and 75th percentiles; whiskers extend to the minimum and maximum values.

microscale forest composition and climate (Jemison 1934). In recent decades, anthropogenic disturbances, particularly suburbanization, have increased across the northeastern United States (Jeon et al. 2014), inducing changes in meso-scale climate, e.g. urban heat-island effects. Thus, site-level variation in forest type, land-use change, and land-use history may play important roles in mediating regional effects of

Table 3. Correlation structure within time periods (1970 and 2015) for climate variables included in the best models; AET (actual evapotranspiration from April to November), CTmax (number of days above critical thermal maximum, base 32°C), FDD (freezing degree days), Tmax0411 (mean monthly maximum temperature from April to November).

		1970			
		AET	CTmax	FDD	Tmax0411
2015	AET	–	0.42	0.55	0.79
	CTmax	0.65	–	0.42	0.69
	FDD	0.71	0.58	–	0.84
	Tmax0411	0.87	0.81	0.86	–

climate on morph frequencies (Gibbs and Karraker 2006). For example, Cosentino et al. (2017) demonstrated a relationship between *P. cinereus* striped morph frequencies and forest cover in warmer regions of the species' range, but this relationship weakens in colder regions. Because up to 75% of plethodontid salamander populations shelter underground at any one time (Connette and Semlitsch 2015), a combination of behavioral plasticity in response to local weather conditions (Muñoz et al. 2016) and microclimate heterogeneity mediated by mesoscale changes in land use could be sufficient to buffer populations from changes in macroscale climate observed over the past four decades. Examining if and how site-level variation in multiple environmental stressors such as land use and climate affect our ability to model and predict the evolutionary responses of *P. cinereus* populations is an important follow up to this research.

Lastly, surveys spaced over four decades might miss potentially important temporal variability both in climate and morph frequency changes. The temporal and spatial variability in climate and an increasing frequency of extreme events (Buckley et al. 2012, Vasseur et al. 2014) might explain some site-specific responses better than simple hypotheses equating morph frequencies to mean climate variables (Gibbs and Karraker 2006, Moore and Ouellet 2015, Nadeau et al. 2017). This result has been demonstrated in repeated surveys of polymorphic land snail species, which show correlations between morph frequency changes and climate variation across multiple time periods (Cameron and Pokryszko 2008, Johnson 2011). These studies suggest that long periods between resurvey efforts may conceal temporal variation in selective pressures, which act in the opposite direction to the overall predicted trend, thus mediating expected climate-driven responses. The 40-yr temporal resolution of our surveys and the coarse spatial and temporal resolution of the climate data mean that we are unable to account for changes in the strength and direction of selection on morph frequencies over time (Siepielski et al. 2009).

Even over the relatively short time span of our study, we found substantial changes in the correlations among climate factors. These changing correlations likely contributed to the poor predictions and asymmetries in the temporal transferability of climate models in our study and others (Dobrowski et al. 2011). Changing correlations among predictors make it difficult to understand the actual mechanistic driver of distribution patterns and thereby extrapolate to the future (Braunisch et al. 2013). Moreover, shifting correlated predictors could buffer or exacerbate climate effects such as we might see for integrated and more biologically informed measures like actual evapotranspiration. These factors should be considered in future studies given that many future climates are predicted to have no modern analog (Williams et al. 2007), and current modeling approaches often cannot accurately predict distributions when extrapolated into unknown climatic regimes (Araújo et al. 2005, Fitzpatrick and Hargrove 2009, Veloz et al. 2012).

In our study we encountered a number of limitations including 1) variation in weather conditions before and



during survey periods and 2) an inability to resurvey identical locations for the majority of sites. Weather conditions prior to surveys can affect salamander surface activity and detection probability and thus may affect population-level inferences (Connette and Semlitsch 2015, Muñoz et al. 2016). However, two large-scale studies found no significant intrapopulation differences in surface activity between *P. cinereus* color morphs under different weather conditions, suggesting both color morphs respond similarly to changes in temperature and moisture-related conditions across most of the activity period (Anthony et al. 2008, Muñoz et al. 2016). The lack of differences between morphs in surface activity is also supported by repeated temporal surveys at site 7 (Fenton, Mansfield, CT) (Lotter and Scott 1977; AEE unpubl.). Additionally, we controlled for the effects of earlier retreat of unstriped individuals in the fall (Lotter and Scott 1977, Anthony et al. 2008) by matching survey and resurvey months as closely as possible. Thus, we do not expect that weather and seasonal differences in survey effort should have a substantial impact on our results. Finally, while we lacked specific information regarding the location of the original survey sites, in addition to our careful matching of site descriptions from Lotter (1975), we did not select sites based on elevation or aspect. Therefore, we did not systematically bias sites towards specific microclimates. Moreover, a significant difference in morph proportions between subsites within a site was detected at only one of the sites where subsites were sampled in 2015, and both morphs were still detected (Supplementary material Appendix 1 Table A3). These data indicate that small-scale spatial and temporal variation is unlikely to substantially alter our regional-scale findings.

Climate has changed substantially in the New England USA region over the last 40 yr, yet we did not observe the expected evolution of morph frequencies. This result calls into question the simple climate–morph link thought to exist for this species. In our study, we were fortunate in having a detailed historical dataset to test for a predicted adaptive response in a species with known genetic and ecological diversity. We demonstrate that, even for well-studied species like *P. cinereus*, we have yet to fully understand the link between traits and climate change (Merilä and Hendry 2014, Urban 2015). As climate continues to change rapidly in the coming decades, additional field and experimental research efforts are needed to understand these links more fully and predict the degree to which evolution can mitigate future impacts.

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Supplementary material (Appendix ECOG-03588 at <[www.ecography.org/appendix/ecog-03588](http://www.ecography.org/appendix/ecog-03588)>). Appendix 1.

Ecography

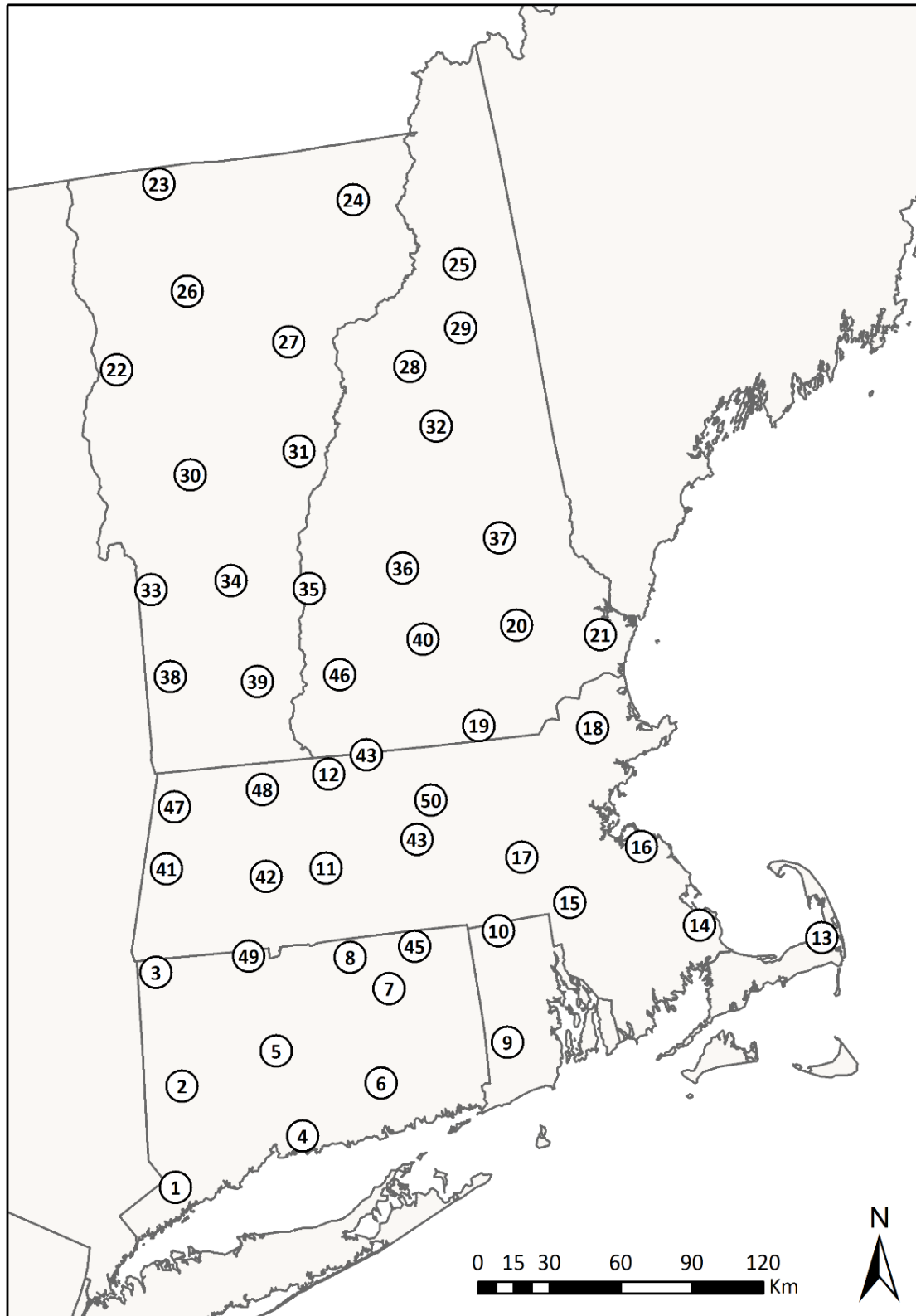
**ECOG-03588**

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**Supplementary material**

**Appendix 1**

**Fig. A1:** Map of survey locations showing site numbers.



**Table A1:** *Plethodon cinereus* color morph count data from the 1970s (Lotter and Scott 1977) and 2015 surveys.

Site #	1970s Surveys				2015 Surveys			
	Striped	Unstriped	Erythristic	Amelanistic	Striped	Unstriped	Erythristic	Amelanistic
1	81	19	0	0	72	29	0	0
2	69	31	0	0	81	20	0	0
3	67	33	0	0	64	37	0	0
4	67	33	0	0	74	26	0	0
5	84	16	0	0	65	35	0	0
6	87	13	0	0	87	13	0	0
7	76	33	0	0	81	19	0	0
8	71	29	0	0	81	20	0	0
9	82	18	0	0	98	2	0	0
10	89	11	0	0	85	15	0	0
11	76	24	0	0	72	29	0	0
12	88	12	0	0	101	2	0	0
13	86	14	0	0	92	9	0	0
14	87	13	0	0	81	19	0	0
15	88	9	2	1	91	7	2	0
16	80	18	2	0	86	15	0	0
17	82	18	0	0	75	25	0	0
18	67	33	0	0	58	42	0	0
19	93	7	0	0	87	12	0	1
20	94	6	0	0	99	3	0	0
21	91	9	0	0	94	5	1	0
22	95	5	0	0	97	3	0	0
23	99	1	0	0	100	0	0	0
24	99	1	0	0	98	2	0	0
25	100	0	0	0	100	0	0	0
26	100	0	0	0	127	3	0	0
27	97	0	3	0	100	0	0	0
28	100	0	0	0	100	0	0	0
29	98	0	2	0	101	0	0	0
30	100	0	0	0	100	0	0	0
31	100	0	0	0	101	0	0	0
32	100	0	0	0	101	0	0	0
33	98	2	0	0	99	3	0	0
34	100	0	0	0	100	0	0	0
35	100	0	0	0	99	1	0	0
36	99	1	0	0	106	0	0	0
37	100	0	0	0	100	0	0	0
38	99	0	1	0	98	2	0	0
39	100	0	0	0	102	0	0	0
40	100	0	0	0	98	3	0	0
41	100	0	0	0	95	3	2	0
42	99	1	0	0	106	0	0	0
43	100	0	0	0	100	0	0	0

Site #	1970s Surveys				2015 Surveys			
	Striped	Unstriped	Erythristic	Amelanistic	Striped	Unstriped	Erythristic	Amelanistic
44	100	0	0	0	100	0	0	0
45	96	4	0	0	102	7	0	0
46	87	0	13	0	94	0	10	0
47	112	0	10	0	101	0	0	0
48	111	1	25	0	98	0	7	0
49	92	0	8	0	89	0	11	0
50*	362	2	52	0	102	0	0	0
Totals	4848	417	118	1	4638	411	33	1
%	90.0%	7.7%	2.2%	0.02%	91.2%	8.1%	0.6%	0.02%

\* In the 1970s survey, site 50 counts were derived from museum specimens rather than a field survey as in 2015.

**Table A2:** *Plethodon cinereus* survey locations (2015) and survey dates for 1970s and 2015.

Site #	2015 Site Name	Township	State	Latitude	Longitude	2015 Survey Date	1970 Survey Date
1	Leonard Bradley Park	Wilton	CT	41.1827	-73.4311	9 Oct 2015	Oct 1971
2	Mine Hill Preserve	Roxbury	CT	41.5596	-73.3375	9 Oct 2015	Oct 1971
3	Edith Scoville Memorial Park	Salisbury	CT	42.0006	-73.3980	8 Oct 2015	Oct 1971
4	North Farms Park	North Branford	CT	41.3152	-72.7656	14 May 2015	Apr 1973
5	Sunset Rock	Plainville	CT	41.6470	-72.8424	10 Oct 2015	Oct 1971
6	Devil's Hopyard State Park	East Haddam	CT	41.4743	-72.3400	14-15 May 2015	Apr 1973
7	Univ. Conn. Forest; Fenton River	Mansfield	CT	41.8238	-72.2356	12-13 June 2015; 30 June 2015	Jun 1973
8	Shenipset State Forest	Somers	CT	41.9616	-72.4085	10 Oct 2015	Oct 1971
9	Browning Mill Pond	Exeter	RI	41.5608	-71.6832	15 May 2015	May 1973
10	Black Hut State Management Area	Burrillville	RI	41.9820	-71.6454	16 May 2015	May 1973
11	Mt Holyoke State Park	Amherst	MA	42.3075	-72.4709	16 Sep 2015	Sep 1971
12	Northfield State Forest	Northfield	MA	42.6586	-72.3930	18 May 2015	May 1973
	Northfield Town Forest	Northfield	MA	42.6596	-72.4190		
13	Nickerson State Park	Brewster	MA	41.7690	-70.0311	9-10 June 2015	Jun 1972
14	Myles Standish - Middle	South Carver	MA	41.8897	-70.6321	9-10 June 2015; 6 Aug 2015; 20 Sept 2015	Jun 1972
	Myles Standish - Cranberry Rd	South Carver	MA	41.8395	-70.6907		
	Rocky Gutter WMA	South Carver	MA	41.8527	-70.8428		
15	Norton - Great Woods	Norton	MA	42.0102	-71.2192	6 Aug 2015; 19 Sept 2015	Jul 1973
	Norton - Gilbert Hills	Norton	MA	42.0491	-71.2667		
16	Whitney & Thayer Woods	Cohasset	MA	42.2340	-70.8240	16-17 May 2015	May 1973
	Wompatuck State Park	Hingham	MA	42.2169	-70.8619		
	Scituate Town Forest	Scituate	MA	42.2077	-70.7814		



Site #	2015 Site Name	Township	State	Latitude	Longitude	2015 Survey Date	1970 Survey Date
17	Ashland State Park	Ashland	MA	42.2455	-71.4690	11 Oct 2015	Oct 1971
18	Boxford Wildlife Sanctuary	Boxford	MA	42.6402	-70.9911	5 Aug 2015	Jul 1971
	Wildcat Conservation Area	Boxford	MA	42.6895	-71.0166		
19	Silver Lake State Park	Hollis	NH	42.7600	-71.5996	4-5 Aug 2015	Aug 1971
	Big Dickerman Town Forest	Hollis	NH	42.7829	-71.6061		
20	Bear Brook State Park	Allenstown	NH	43.1149	-71.3255	2 Aug 2015	Aug 1971
21	Stratham Hill Park	Stratham	NH	43.0408	-70.8919	3 Aug 2015	Aug 1973
	Stratham Hill - Elementary	Stratham	NH	43.0286	-70.8815		
22	Mt Philo	Charlotte	VT	44.2779	-73.2138	28-29 Jul 2015	Jul 1971
23	Lake Carmi State Park	Franklin	VT	44.9528	-72.8682	29-30 Jul 2015	Jul 1971
24	Brighton State Park	Brighton	VT	44.7941	-71.8504	30 Jul 2015	Jul 1971
25	White Mt. National Forest	Milan	NH	44.5075	-71.3364	31 Jul 2015	Sep 1971
	Mill Brook Trail - York Pond Rd	Milan	NH	44.4979	-71.3429		
26	Smugglers' Notch	Cambridge	VT	44.5382	-72.7909	11 Jun 2015	Jun 1973
27	Groton State Forest, Nature Trail	Groton	VT	44.2857	-72.2646	28 Jul 2015	Jul 1971
	Groton State Forest, Owls Head	Peacham	VT	44.2980	-72.2952		
28	Franconia Notch	Lincoln	NH	44.1421	-71.6812	11 Jun 2015	Jun 1973
29	Jefferson Notch 1 (Jeff. Notch Rd)	Randolph	NH	44.2579	-71.3824	31 Jul 2015; 1 Aug 2015	Aug 1971
	Jefferson Notch 2 (Mt. Clinton Rd)	Jefferson	NH	44.2300	-71.4065		
30	Green Mt. National Forest	Rutland	VT	43.8483	-72.9003	27 Jul 2015	Aug 1971
31	Podunk Wildlife Mngt Area	Strafford	VT	43.8886	-72.3313	11 Jun 2015	Jun 1973
32	White Mt. National Forest	Gorham	NH	43.9060	-71.5890	1 Aug 2015	Jul 1971
33	Pond Mountain B&B, backyard	Wells	VT	43.4369	-73.1788	25-26 Jul 2015	Aug 1971
34	Okemo State Park	Ludlow	VT	43.4314	-72.7611	27 Jul 2015	Aug 1971

Site #	2015 Site Name	Township	State	Latitude	Longitude	2015 Survey Date	1970 Survey Date
35	Moody Park	Claremont	NH	43.3577	-72.3662	17 Sep 2015	Sep 1971
36	Winslow State Park	Wilmot	NH	43.3897	-71.8660	17 Sep 2015	Sep 1971
37	Thompson Town Forest	Thompson	NH	43.4549	-71.3439	1 Aug 2015	Aug 1971
38	Arlington State Forest	Arlington	VT	43.0209	-73.1851	25 Jul 2015	Aug 1971
	Fisher-Scott Pines Park	Arlington	VT	43.1027	-73.1367		
39	Townshend State Park	Townshend	VT	43.0416	-72.6925	26 Jul 2015	Aug 1971
40	Vincent State Forest	Deering	NH	43.1157	-71.8082	2 Aug 2015	Aug 1971
41	Kennedy Park	Lenox	MA	42.3830	-73.2787	24 Jul 2015	Jul 1971
42	Tob Hill Rd Town parcel	Westhampton	MA	42.3064	-72.7802	16-17 Sept 2015	Sep 1971
43	Lawrence Brook WMA	Royalston	MA	42.7118	-72.1865	18 Sep 2015	Sep 1971
44	Rutland State Park	Rutland	MA	42.3690	-71.9880	19 Sep 2015	Sep 1971
45	Black Pond YMCA	Woodstock	CT	41.9719	-72.0737	13 Jun 2015	Jun 1972
46	Bear Den Geological Park	Gilsum	NH	43.0253	-72.2680	18 May 2015	May 1971
47	Mt. Greylock - Bellows Pipe	Adams	MA	42.6734	-73.1391	19 May 2015	Jul 1971
	Mt. Greylock - Slopes	Adams	MA	42.6121	-73.2001		
	Mt. Greylock - Visitor Center	Adams	MA	42.5536	-73.2122		
48	Catamount State Forest	Colrain	MA	42.6348	-72.7406	19 May 2015	Sep 1971
49	Tunxis State Forest	Hartland	CT	42.0162	-72.9201	12 Jun 2015	Jun 1971
50	Wachusset Mountain	Princeton	MA	42.5079	-71.8926	18 Sep 2015	NA (Museum collection)

**Table A3:** Polymorphic 2015 sites that had more than one survey location. Striped+Other column includes counts of striped, erythristic, and amelanistic morphs. Includes a test of subsite morph differences (Chi-squared with simulated p-value). Two sites include a Chi-squared test at the same subsite, but in different sampling months. Four other sites with more than one survey location were monomorphic for striped morphs so not included in the analysis (Sites 25, 27, 29 and 47).

Site #	Site Name	Unstriped	Striped+Other	Chi-squared	Simulated p-value
12	Northfield State Forest	0	53	2.162	0.247
	Northfield Town Forest	2	48		
14	Myles Standish - Cranberry Rd	19	71	2.606	0.352
	Myles Standish - Middle	0	6		
	Rocky Gutter WMA	0	4		
15	Norton - Great Woods	2	84	20.618	0.001
	Norton - Gilbert Hills	5	9		
16	Whitney & Thayer Woods	8	46	0.866	0.657
	Wompatuck State Park	2	19		
	Scituate Town Forest	5	21		
19	Silver Lake State Park	10	54	2.212	0.206
	Big Dickerman Town Forest	2	34		
38	Arlington 1	1	42	0.041	1.000
	Arlington 2	1	56		
SITES SAMPLED ACROSS TWO MONTHS:					
14	Myles Standish - Cranberry Rd, June	1	17	3.269	0.112
	Myles Standish - Cranberry Rd, September	18	54		
15	Norton - Great Woods, August	1	9	2.934	0.217
	Norton - Great Woods, September	1	75		

**Table A4:** Predictor variables averaged over 1962–1971 for Lotter and Scotts 1970s survey and 2005–2014 for our 2015 resurvey.

Predictor	Description	Months Included
Actual evapotranspiration (AET)	The supply component of the climatic water balance; accounts for the concurrent availability of water and energy.	April- November*
Water deficit (DEF)	The unmet demand component of the climatic water balance; accounts for the concurrent availability of water and energy.	April- November*
Mean monthly mean temperature (Tmean0411)	Average monthly mean temperature; calculated from daily mean temperatures.	April- November
Mean monthly maximum temperature (Tmax0411)	Average monthly maximum temperature; calculated from daily maximum temperatures.	April- November
Mean monthly minimum temperature (Tmin0411)	Average monthly minimum temperature; calculated from daily minimum temperatures.	April- November
Growing degree days, 0°C base (GDD)	Average annual sum of degrees above 0°C; calculated from daily mean temperatures.	January- December
Freezing degree days (FDD)	Average annual sum of degrees below 0°C; calculated from daily minimum temperatures.	January- December
Frost free days (FrFD)	Number of days between the last 0°C day in the spring and the first 0°C day in the fall; calculated from daily minimum temperatures.	January- December
Number of days above critical thermal max (CTmax)	Number of days where maximum temperature is above <i>Plethodon cinereus</i> critical thermal max ( $> 32^{\circ}\text{C}$ )†.	January- December

\* Current water balance data are a six-year average of 2005–2010.

† Spotila JR (1972) Role of temperature and water in the ecology of lungless salamanders. *Ecological Monographs*, **42**, 95-125.

**Table A5:** Candidate model sets ordered by log-likelihood values (lnL, best models at top) for 1970 and 2015. Grey shading indicates candidate models that included variables correlated above |0.7|. These were included to allow all models to be compared between time periods. See Supplementary materials Appendix 1, Table A4 for the full name of each abbreviated climate variable.

<b>Model</b>	<b>1970 lnL</b>	<b>Model</b>	<b>2015 lnL</b>
AET + CTmax + FDD	-240.25	Tmax0411	-236.69
AET + FDD	-252.07	CTmax + Tmean0411	-237.40
AET + Tmean0411	-253.29	Tmean0411	-238.62
AET + GDD	-257.45	CTmax + Tmax0411	-246.36
AET + CTmax + Tmean0411	-261.71	CTmax + GDD	-246.44
AET + CTmax + GDD	-263.15	AET + CTmax + Tmean0411	-253.58
Tmean0411	-266.92	AET + Tmean0411	-254.32
Tmax0411	-268.47	GDD	-254.70
CTmax + Tmean0411	-272.49	CTmax + FDD	-261.83
CTmax + Tmax0411	-272.69	AET + CTmax + GDD	-262.05
AET + DEF	-278.05	AET + CTmax + FDD	-265.99
GDD	-279.49	AET + CTmax + DEF	-266.53
CTmax + GDD	-282.57	AET + GDD	-267.59
AET + CTmax + Tmin0411	-284.25	CTmax + Tmin0411	-271.77
AET + CTmax + DEF	-285.97	AET + DEF	-276.18
AET + CTmax + FrFD	-292.77	CTmax + DEF	-276.64
CTmax + FDD	-303.61	AET + CTmax + Tmin0411	-279.09
AET + Tmin0411	-305.72	AET + CTmax + FrFD	-285.36
AET + FrFD	-320.16	AET + FDD	-288.03
FDD	-325.99	CTmax + FrFD	-301.88
CTmax + Tmin0411	-363.72	AET + Tmin0411	-308.48
AET	-389.87	FDD	-308.68
AET + CTmax	-391.75	AET + FrFD	-316.54
DEF	-395.62	DEF	-320.47
CTmax + FrFD	-404.23	AET + CTmax	-332.65
Tmin0411	-407.08	Tmin0411	-333.50
CTmax + DEF	-413.25	AET	-363.00
FrFD	-462.68	CTmax	-366.83
CTmax	-485.72	FrFD	-415.48

**Table A6:** Candidate change models ordered by change in DIC values. See Supplementary materials Appendix 1, Table A4 for the full name of each abbreviated climate variable.

<b>Model</b>	<b>DIC</b>	<b>delta DIC</b>	<b>weight</b>
null model	-288.2	0.0	0.3
CTmax	-285.4	2.8	0.1
Tmean0411	-285.2	3.0	0.1
GDD	-285.2	3.0	0.1
AET	-285.1	3.2	0.1
Tmax0411	-284.9	3.3	0.1
Tmin0411	-284.9	3.3	0.1
DEF	-284.7	3.5	0.1
FDD	-284.5	3.7	0.0
FrFD	-283.7	4.5	0.0
DEF + CTmax	-282.3	6.0	0.0
CTmax + Tmax0411	-282.0	6.2	0.0
AET + Tmean0411	-281.9	6.3	0.0
AET + Tmax0411	-281.8	6.4	0.0
DEF + GDD	-281.7	6.5	0.0
AET + Tmin0411	-281.7	6.6	0.0
DEF + Tmean0411	-281.6	6.6	0.0
Tmax0411 + Tmin0411	-281.6	6.6	0.0
FDD + Tmean0411	-281.6	6.7	0.0
CTmax + FDD	-281.4	6.8	0.0
DEF + Tmin0411	-281.4	6.9	0.0
DEF + FDD	-281.3	6.9	0.0
FDD + Tmax0411	-281.2	7.0	0.0
FDD + Tmin0411	-281.1	7.1	0.0
FrFD + GDD	-280.7	7.6	0.0
CTmax + rFFD	-280.6	7.6	0.0
FrFD + Tmean0411	-280.5	7.7	0.0
AET + FrFD	-280.4	7.9	0.0
FrFD + Tmin0411	-280.3	8.0	0.0
FDD + FrFD	-279.9	8.3	0.0
DEF + CTmax + FDD	-278.6	9.6	0.0
AET + Tmax0411 + Tmin0411	-278.3	9.9	0.0
DEF + FDD + Tmin0411	-278.1	10.1	0.0
DEF + FDD + Tmean0411	-278.0	10.3	0.0
CTmax + FDD + Tmax0411	-277.9	10.3	0.0
AET + FrFD + Tmean0411	-277.1	11.1	0.0
AET + FrFD + Tmin0411	-276.9	11.3	0.0
FDD + FrFD + Tmean0411	-276.9	11.4	0.0
CTmax + FDD + FrFD	-276.5	11.7	0.0
FDD + FrFD + Tmin0411	-276.3	11.9	0.0
global model	-109.2	179.1	0.0

**Table A7:** Observed and predicted unstriped morph frequencies (unstriped morph percent with 95% credible intervals) for the 1970s survey.

Site	Observed: 1970	Predicted: 1970 model predicting 1970 data	Predicted: 2015 model predicting 1970 data
1	19	36 (22--48)	17 (10-25)
2	31	28 (17-40)	13 (8-19)
3	33	15 (6-25)	6 (4-10)
4	33	27 (16-40)	12 (7-17)
5	16	23 (13-34)	9 (6-14)
6	13	26 (14-39)	7 (4-10)
7	30	15 (9-22)	8 (5-12)
8	29	8 (4-13)	4 (2-6)
9	18	26 (13-41)	4 (2-7)
10	11	16 (11-23)	8 (5-12)
11	24	10 (5-16)	8 (5-12)
12	12	3 (1-5)	2 (1-3)
13	14	22 (4-44)	1 (0-2)
14	13	11 (3-22)	4 (2-7)
15	9	21 (13-29)	10 (6-14)
16	18	30 (17-44)	7 (5-11)
17	18	27 (14-41)	12 (7-17)
18	33	16 (6-29)	7 (4-10)
19	7	11 (3-21)	9 (5-13)
20	6	7 (4-11)	3 (2-6)
21	9	12 (4-22)	5 (3-7)
22	5	2 (0-3)	1 (0-3)
23	1	0 (0-0)	0 (0-1)
24	1	0 (0-0)	0 (0-0)
25	0	0 (0-0)	0 (0-0)
26	0	0 (0-0)	0 (0-0)
27	0	0 (0-0)	0 (0-0)
28	0	0 (0-0)	0 (0-0)
29	0	0 (0-0)	0 (0-0)
30	0	0 (0-1)	0 (0-1)
31	0	0 (0-0)	0 (0-0)
32	0	0 (0-1)	0 (0-1)
33	2	2 (0-3)	1 (0-2)
34	0	0 (0-1)	0 (0-1)
35	0	2 (1-3)	3 (2-5)
36	1	1 (0-2)	0 (0-0)
37	0	2 (1-4)	2 (1-3)
38	0	4 (2-8)	4 (2-7)
39	0	9 (2-17)	7 (4-10)
40	0	1 (0-3)	1 (0-2)

<b>Site</b>	<b>Observed: 1970</b>	<b>Predicted: 1970 model predicting 1970 data</b>	<b>Predicted: 2015 model predicting 1970 data</b>
41	0	2 (1-4)	2 (1-3)
42	1	4 (2-7)	3 (1-5)
43	0	2 (1-3)	2 (1-4)
44	0	6 (3-8)	3 (2-5)
45	4	11 (6-18)	3 (2-6)
46	0	1 (0-2)	1 (0-2)
47	0	0 (0-1)	0 (0-1)
48	1	5 (2-8)	5 (3-8)
49	0	6 (2-10)	2 (1-3)
50	0	3 (1-5)	3 (1-5)



**Table A8:** Observed and predicted unstriped morph frequencies (unstriped morph percent with 95% credible intervals) for the 2015 survey.

Site	Observed: 2015	Predicted: 2015 model predicting 2015 data	Predicted: 1970 model predicting 2015 data
1	29	34 (20–48)	88 (70–99)
2	20	29 (17–41)	82 (58–99)
3	37	14 (9–21)	62 (32–88)
4	26	26 (15–36)	78 (59–95)
5	35	21 (12–30)	66 (43–88)
6	13	15 (9–22)	70 (45–92)
7	19	16 (10–23)	54 (33–76)
8	20	8 (5–12)	41 (20–62)
9	2	12 (7–17)	70 (45–92)
10	15	17 (10–25)	65 (42–88)
11	29	16 (10–23)	56 (29–83)
12	2	3 (2–5)	24 (8–42)
13	9	4 (2–6)	58 (34–82)
14	19	12 (7–17)	57 (37–75)
15	7	23 (14–33)	78 (55–98)
16	15	17 (10–24)	85 (66–99)
17	25	24 (14–34)	82 (55–100)
18	42	14 (9–20)	71 (46–94)
19	12	16 (10–22)	56 (24–87)
20	3	7 (4–10)	41 (19–62)
21	5	9 (5–13)	61 (33–87)
22	3	4 (2–7)	19 (5–36)
23	0	2 (1–3)	3 (1–6)
24	2	0 (0–1)	0 (0–1)
25	0	0 (0–1)	0 (0–0)
26	2	0 (0–0)	0 (0–1)
27	0	0 (0–1)	1 (0–1)
28	0	0 (0–0)	1 (0–1)
29	0	0 (0–0)	0 (0–0)
30	0	1 (0–2)	3 (1–5)
31	0	0 (0–1)	1 (0–2)
32	0	1 (0–2)	3 (1–6)
33	3	2 (1–3)	18 (2–39)
34	0	1 (0–2)	3 (1–7)
35	1	7 (4–10)	21 (7–38)
36	0	0 (0–1)	3 (1–6)
37	0	3 (1–5)	18 (8–30)
38	2	9 (5–13)	25 (10–42)
39	0	12 (7–17)	43 (16–73)
40	3	2 (1–4)	8 (4–11)

<b>Site</b>	<b>Observed: 2015</b>	<b>Predicted: 2015 model predicting 2015 data</b>	<b>Predicted: 1970 model predicting 2015 data</b>
41	3	4 (2-6)	16 (7-27)
42	0	7 (4-11)	38 (16-59)
43	0	4 (2-7)	19 (8-29)
44	0	6 (4-10)	36 (19-56)
45	6	7 (4-11)	43 (22-65)
46	0	2 (1-4)	7 (3-11)
47	0	1 (0-2)	3 (1-4)
48	0	11 (7-15)	44 (19-68)
49	0	5 (3-7)	37 (14-60)
50	0	5 (3-8)	25 (14-37)