Questioning the use of an amphibian colour morph as an indicator of climate change

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Abstract

The effects of recent climate changes on earth ecosystems are likely among the most important ecological concerns in human history. Good bioindicators are essential to properly assess the magnitude of these changes. In the last decades, studies have suggested that the morph proportion of the eastern red-backed salamander *(Plethodon cinereus),* one of the most widely distributed and abundant vertebrate species in forests of eastern North America, could be used as a proxy for monitoring climate changes. Based on new discoveries in the northern areas of the species' range and on one of the largest compilation ever made for a vertebrate in North America (236 109 observations compiled from 1880 to 2013 in 1148 localities), we demonstrate however that climatic and geographic variables do not influence the colour morph proportions in *P. cinereus* populations. Consequently, we show that the use of colour morph proportions of this species do not perform as an indicator of climate change. Our findings indicate that bioindicator paradigms can be significantly challenged by new ecological research and more representative databases.

Keywords: amphibian, bioindicator, climate change, colour morph, *Plethodon cinereus*, salamander

Introduction

In the last decades, organisms have been used to assess the possible effects of climate change on ecosystems (Walther *et al.*, 2002; Parmesan & Yohe, 2003; Root *et al.*, 2003). Amphibians are strongly influenced by environmental temperatures (Beebee, 1995; Gibbs & Breisch, 2001) and consequently, have the potential to be used as indicators of climate change. Among the amphibians of North America, the eastern red-backed salamander *(Plethodon cinereus)* is one of the most studied amphibian species, because of its abundance, broad geographical distribution, and ecological importance in northeastern forests (Burton & Likens, 1975; Petranka, 1998; Wyman, 1998). These characteristics, coupled with the species' sensitivity to habitat disturbances (Hocking *et al.*, 2013), mean that *P. cinereus* has the qualities of a good bioindicator (Holt & Miller, 2010). Moreover, because this salamander is an ectotherm and needs environmental heat sources to maintain its metabolic rate, it can be affected by temperature variables (Bobka *et al.*, 1981; Homyack *et al.*, 2011; Caruso *et al.*, 2014). Two colour phenotypes of this species, the red-backed (striped) and lead-backed (unstriped) morphs (Moore & Ouellet,

2014), are commonly found in most populations, but in very different proportions.

Geographic variations in the proportions of *P. cinereus* colour morphs have been the subject of numerous studies over the last century (Anthony *et al.*, 2008). Many authors have suggested that the proportions of the two morphs can vary with the climate, either in space or time (Lotter & Scott, 1977; Gibbs & Karraker, 2006). For example in New England (50 localities, ~5000 observations), a higher frequency of the lead-backed morph was reported in warmer regions (5% to 33%, mean = 17%) as compared to colder regions (0% to 4%, mean = 0.4%) (Lotter & Scott, 1977). Another compilation of colour morph proportions for *P. cinereus* (558 localities, 50 960 observations), although mostly limited to the species' range in the United States, showed that the probability of encountering the red-backed morph increased with latitude, longitude, and elevation, and decreased over time (Gibbs & Karraker, 2006). Moreover, other studies have indicated that the lead-backed morph withdraws earlier than the red-backed morph from the soil surface in autumn (Lotter & Scott, 1977; Moreno, 1989; Anthony *et al.*, 2008). As a result, the ratio of colour morphs of *P. cinereus* has been proposed as a good indicator of climate warming (Gibbs & Karraker, 2006). However, the possible role of climatic variables in the distribution of colour morphs of this species has never been unanimously agreed upon (Petruzzi *et al.*, 2006). For example in recent years,

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we observed a high prevalence of the lead-backed morph in some locations in Québec, Canada, at the northern limit of the species' range.

To test the validity of using this species as an indicator of climate change, we performed additional inventories in the northern part of the species' range and attempted to compile all available data on colour morph frequencies. Here, we present an analysis on 236 109 observations of *P. cinereus* (Fig. 1) compiled from 1880 to 2013 (sample size per area ≥ 10; 1148 localities; mean and median number of *P. cinereus* per area: 209 and 39, respectively), which greatly exceeds the two most important compilations for this species (Gibbs & Karraker, 2006; Adams & Church, 2008).

Materials and methods

Data compilation

We conducted an exhaustive literature review to compile all available data on the prevalence of *P. cinereus* colour morphs

in North America. In addition to our own data sets, 401 people or institutions were contacted to complement the published literature (see Supporting Information) and to access unpublished field records, when available. Most data were collected at the site scale, but some were collected at the township or the county scale. For each area, location (latitude, longitude), sampling period, and the number of red- and lead-backed morphs were noted. Other colour phenotypes (e.g., erythristic; Moore & Ouellet, 2014) were rare (*N* < 400) and were pooled with the red-backed morph. When unavailable from the literature or authors of the inventory, the latitude and longitude were determined using Google Earth (version 7.1.1.1580, 2013). Elevation was calculated using a digital elevation model (1 km resolution). Latitude ranged from 35.8 to 48.8° North, and longitude from −60.3 to −93.6° West.

Climatic and geographic variables

Climatic variables used in this study are mean annual temperature, number of frost-free days, and total precipitation. Mean climate values of the sampling period were used if
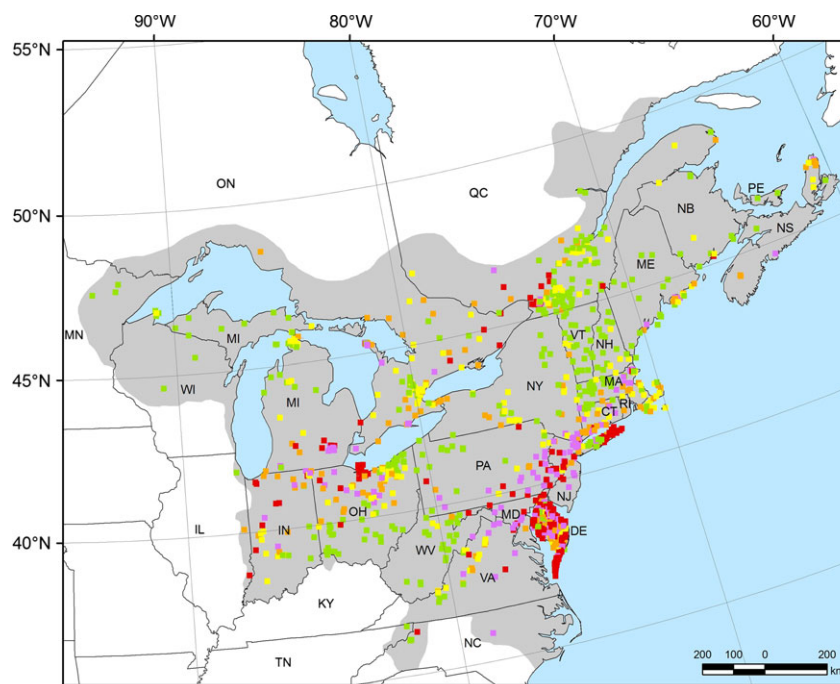

Fig. 1 Site locations and frequencies of the lead-backed morph of the eastern red-backed salamander (*Plethodon cinereus*). Squares show the lead-backed morph frequencies for each locality: 0–5% (green), 6–15% (yellow), 16–30% (orange), 31–50% (purple), and >50% (red). The shaded area represents the species' range, modified from Petranka (1998). Number of observations and mean lead-backed morph frequency across the species' range: Canada – New Brunswick (NB: 325; 8%), Nova Scotia (NS: 8496; 17%), Ontario (ON: 71 217; 16.1%), Prince Edward Island (PE: 86; 2%), Québec (QC: 10 882; 13%). United States – Connecticut (CT: 2739; 24%), Delaware (DE: 5537; 36%), Illinois (IL: 33; 73%), Indiana (IN: 17 882; 22%), Maine (ME: 9292; 19%), Maryland (MD: 17 039; 24%), Massachusetts (MA: 9093; 13%), Michigan (MI: 10 004; 31%), Minnesota (MN: 101; 0%), New Hampshire (NH: 15 281; 4%), New Jersey (NJ: 2183; 45%), New York (NY: 16 335; 16%), North Carolina (NC: 394; 9%), Ohio (OH: 11 047; 23%), Pennsylvania (PA: 16 374; 29%), Rhodes Island (RI: 200; 15%), Tennessee (TN: 17; 0%); Vermont (VT: 6775; 2%), Virginia (VA: 18 885; 13%), West Virginia (WV: 4145; 1%), Wisconsin (WI: 136; 3%).

salamanders were monitored for more than 1 year. Climate data for years prior to 1913 (10 localities) were not available. Latitude, longitude, and elevation were used as geographic variables. Operative year of the sampling period was calculated as the average of the initial and final year of sampling. Other climatic variables were tested, such as annual mean of daily minimum and maximum temperatures, annual mean of daily mean relative humidity, annual growing degree-day summation over 0, 3, and 5 °C, growing season duration, and total snowfall. Although similar, responses to these variables were weaker than those for other associated climatic variables. Consequently, these variables were considered poorer predictors than the ones used.

We estimated the average yearly climate conditions of each site using the BioSIM software (version 10.2.5.39, 2013) developed by the Canadian Forest Service (Régnière & St-Amant, 2007). These estimates are based on climatic variables interpolated from eight nearby weather stations in Canada and the United States (1910–2013), adjusted for differences in elevation, latitude, and longitude.

Statistical analyses

To evaluate the possible relationship between the morph proportions of *P. cinereus* and year of sampling, climatic as well as geographic variables (Figs 2 and 3), we applied a probit transformation to the frequency of the lead-backed
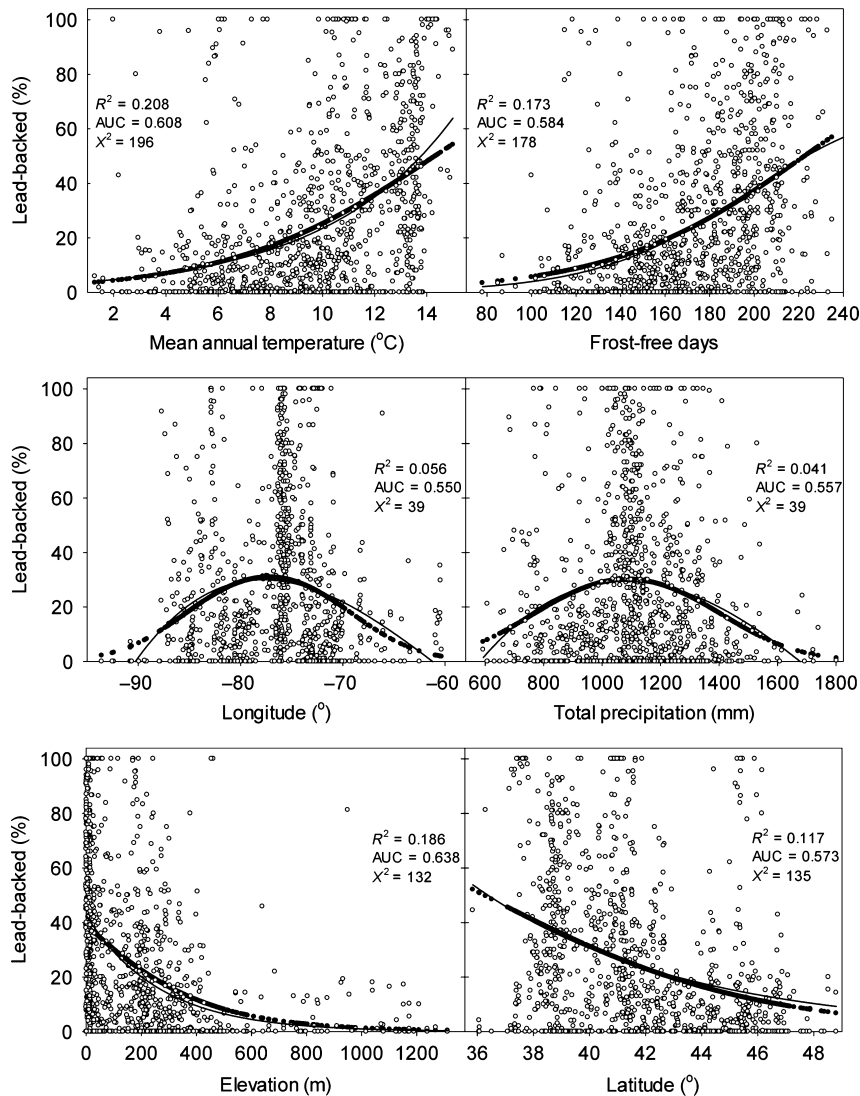

Fig. 2 Relationships between the frequency of the lead-backed morph of *P. cinereus* and climatic or geographic variables, for 236 109 observations from 1148 populations sampled between 1913 and 2013. Shown are the observed data (white circles), predicted data (black circles), and observed trend (black line). Frost-free days = longest uninterrupted period without frost in the year, AUC = area under the receiver operating characteristic (ROC) curve. The values of the root mean square error (RMSE) and RMSE (%) ranged from 0.257–0.283, and from 100–110, respectively.
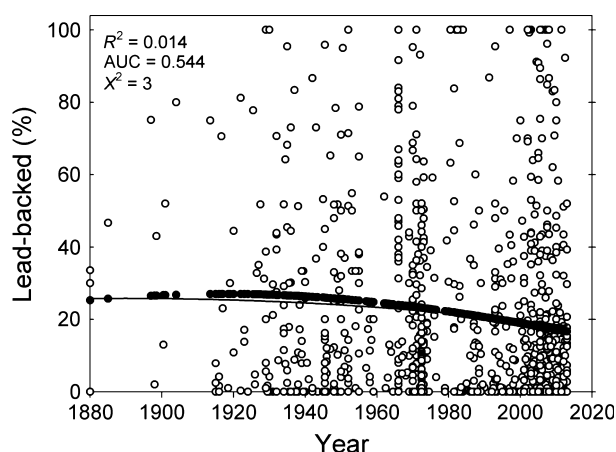
Fig. 3 Relationship between the frequency of the lead-backed morph of *P. cinereus* and the year (or average years) of sampling for 228 846 observations from 970 populations sampled between 1880 and 2013. Shown are the observed data (white circles), predicted data (black circles), and observed trend (black line). AUC = area under the receiver operating characteristic (ROC) curve. The values of the root mean square error (RMSE) and RMSE (%) were 0.286 and 110, respectively.

morph. The statistical analyses were performed using the LOGISTIC procedure with the Williams' method to model overdispersion (SAS Institute Inc. 2002-2010, version 9.3). This technique is used when proportional data are not normally distributed (Hosmer & Lemeshow, 2000). The quality of each predictor was assessed with three statistic tests. The coefficient of determination of the regression between observed and predicted proportions (R^2) was used to evaluate the quality of the fit, a value of 1 indicating a perfect fit. The root mean square error (RMSE; the square root of the mean square difference between observed and predicted proportions) was calculated to evaluate the precision of the predictions. The area under the curve (AUC) of the receiver operating characteristic (ROC; Hosmer & Lemeshow, 2000) was also calculated, which evaluates the ability of the predictor to discriminate between presence and absence of lead-backed morph. A value of 1.0 represents a perfect fit (discrimination), while an area of 0.50 represents a worthless model (no discrimination). A value below 0.70 is considered as a poor discriminative model.

One set of data (Highton, 1977) had a high concentration in time (effective year = 1965), space (Delmarva Peninsula: Delaware, Maryland, and Virginia), and in lead-backed morph frequencies (Fig. 1). We tested the possible overrepresentation effect of these data in our dataset with a simulation procedure (1000 iterations) which sampled 25% of the observations in this region (without replacement). Although Ontario had the greatest number of *P. cinereus* observations for a province or a state ($N = 71\ 217$, 90 localities), these data did not show the same concentration in time (sampling year = 1976 to 2013), space or lead-backed morph frequencies (Fig. 1) as Highton's (1977) data. Therefore, no particular tests were done for the Ontario data.

Results and discussion

Relationship between colour morph and climatic and geographic variables

Our most significant result is the absence of an accurate and strong relationship between the frequency of the lead-backed morph and the climatic and geographic variables tested (Fig. 2). Although a probit analysis showed a statistically significant and possible relationship between the frequency of the lead-backed morph and all the variables tested ($\alpha = 0.05$, $P < 0.001$), the very large sample size in this study is partly responsible of the low P value, leading to an incorrect interpretation of the results (Murtaugh, 2014). In this context, the accuracy of the relationships was assessed with three different statistic tests. These statistics showed that the climatic and geographic variables used were poor or invalid predictors of the lead-backed morph frequency (Fig. 2). Moreover, the use of data from Highton (1977) in the dataset (178 localities; 7263 observations; see also Materials and methods) could have led to an overrepresentation of these data linked to the concentration of climatic and geographic variables of the Delmarva Peninsula, situated at the southern limit of the species' range. Removing these data lowered all R^2 and AUC, and increased RMSE (%) statistic values, rendering poorer predictions of the lead-backed morph frequency.

This main result is also supported by the relatively high lead-backed morph frequencies observed both at the northern and southern limit of the species' range (Fig. 1). In the northern part of the *P. cinereus*' range (southern Québec), we found ten sites with very high (50% to 100%) frequencies of the lead-backed morph (15 to 618 observations; 124 to 172 frost-free days), including five monomorphic lead-backed populations (28 to 138 observations; 124 to 164 frost-free days). Moreover, we found three sites at the extreme northern limit of the species' range, in Québec and Ontario, with relatively high (15% to 21%) lead-backed morph frequencies (48 to 581 observations; 107 to 127 frost-free days). This contradicts other studies that associated a high prevalence of the lead-backed morph to warmer climates (Lotter & Scott, 1977; Moreno, 1989; Gibbs & Karraker, 2006; Anthony *et al.*, 2008). Compared to these studies, our data set covers all the species' range, including strong representation in the northern part of the range (Canada: 91 006 observations).

Evidence from other studies has also raised doubts about the use of this salamander as an indicator species for climate on a large scale. First, morph frequencies can vary widely over small distances (Fig. 1; Anthony *et al.*, 2008; Fisher-Reid *et al.*, 2013), which suggests that

temperature is not the only controlling factor. Second, some studies did not report an early hibernation of the lead-backed morph (Petruzzi *et al.*, 2006), contrary to other reports (Lotter & Scott, 1977; Anthony *et al.*, 2008). Third, certain studies found no effect of temperature, or an inverse relationship at the local scale (Petruzzi *et al.*, 2006; Anthony *et al.*, 2008). Combined with our results, these reports show that temperature alone does not govern the spatial distribution of the lead-backed morph in *P. cinereus*. Moreover, with one exception (Gibbs & Karraker, 2006), other studies evaluating the effect of climatic variables on the colour morph proportions of *P. cinereus* were conducted at a relatively small scale (Lotter & Scott, 1977; Moreno, 1989; Anthony *et al.*, 2008), as compared to our study.

Plethodon cinereus are highly dependent on the moisture in its environment, not only to keep its body hydrated but also to keep its skin moist and functional as a respiratory organ. Substrate moisture, through its effect on absorption and loss of water, is one of the most important factors in the ecology of this species (Heatwole & Lim, 1961). Past studies have also suggested that soil moisture may influence the proportions of *P. cinereus* colour morphs, since the lead-backed morph is reputedly less tolerant to desiccation (Test, 1955). In this study, the variables that could have been related to soil moisture (total precipitation, annual mean of daily mean relative humidity, total snowfall) were not correlated with morph frequencies (Fig. 2), in agreement with other reports (e.g., Mitchell & Woolcott, 1985).

Relationship between colour morph and sampling year

Gibbs & Karraker (2006) have reported that the frequency of the lead-backed morph increased by 6% (from 20% to 26%) from the early 1900's to the mid 2000's. Our data do not agree with this observation. Even if the previously mentioned 178 sites of Highton (1977) are used in the analysis, which could have caused an overrepresentation of these data linked to the year of observations, the accuracy of the relationship was very low (1148 localities, $P < 0.001$, $R^2 = 0.027$, RMSE = 0.286, RMSE (%) = 110, AUC = 0.565, $\chi^2 = 32$). Without Highton's data, the relation becomes non-significant (970 localities, $P = 0.250$, Fig. 3). Given the warming trend in annual temperatures reported within the range of *P. cinereus* over the last century (Gibbs & Karraker, 2006; Houle *et al.*, 2007), this result reinforces the conclusion that the frequency of the lead-backed morph is not related to temperature. The most reasonable hypothesis to explain the existence of visual polymorphism across a geographical and phylogenetic range as wide as that of *P. cinereus* is

that natural selection acts directly on appearance (Fitzpatrick *et al.*, 2009). Natural selection could be the driving force to explain the morph frequency in given areas.

Our results demonstrate that the frequency of the red- and lead-backed phenotypes of *P. cinereus* is not affected by climate. As a result, the colour morph proportions of this species would not be useful as an indicator of climate change. Our study shows that the usefulness of a bioindicator is dependent on the data used to build the relationships, and that additional data can significantly challenge bioindicator paradigms. Bioindicators should thus be used with caution if the dataset is limited.

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Supporting Information

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

Data S1. Additional Acknowledgements and References used for the database.