Effects of Environmental Variables on *Plethodon cinereus* Abundance in the Bruce Peninsula

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Abstract

Ecosystems and biodiversity are at risk because of current and rapid climate change and increases in land-use. Monitoring indicator species, such as woodland amphibians, may be an effective tool for maintaining species and ecosystem health. Using long-term monitoring data of eastern red-backed salamanders (*Plethodon cinereus*) in the Bruce Peninsula, Ontario, Canada, the present study aimed to assess population trends through time and potential environmental predictors for population trends. Salamander abundance showed a statistically significant increase between 2009-2017. However, environmental variables: soil temperature, air temperature and precipitation within the last 24 hours, were unable to explain this trend. Further monitoring studies, with more environmental variables measured, are needed to help tease apart these relationships and provide insight into greater ecosystem health.

Introduction

Ecosystems are experiencing a vast amount of change, including climate and land-use change, which threaten species' survival and ultimately results in decreased biodiversity (Travis 2003, Hanski 2011). The degree to which species are affected by such environmental changes is taxon-specific, but amphibians are among the worst affected (Hopkins 2007). Conditions often change along a gradient and so different species are affected along a gradient as well (Emmett et al. 2004, Chen et al. 2011, Noss 1990). Those species that are most sensitive to environmental change or perturbations from the normal range of conditions are termed indicator species, and are the first to be affected (Noss 1990). This attribute allows them to indicate when the rest of their ecosystem may be at risk as well (Noss 1990).

Species can be sensitive to changes for a variety of reasons. For example, some species are limited to a very specific range, some species are entirely dependent on other species that may be sensitive themselves, and others have robust physiological constraints that dictate their habitable environment (Thuiller et al. 2005, Hopkins 2007, Chen et al. 2011). There are also many ways this sensitivity is demonstrated in situ. In some cases, species range shifts are observed directly. In others, differences in abundances may result in response to environmental change (Lemoine et al. 2007, Chen et al. 2011). Woodland salamanders, such as the eastern red-backed salamander (*Plethodon cinereus*), are one example of an indicator species whose sensitivity comes as a result of physiological adaptations (Feder 1983, Hopkins 2007). These salamanders are entirely terrestrial and lack lungs (Feder 1983). Their missing lungs contribute to their unique respiratory mechanism where they breath via their moist skin (Feder 1983). This specific way of breathing dictates the types of environments and range of environmental conditions they can live in and makes them dependent on their microclimate (Feder 1983, Demaynadier & Hunter 1998, Moore 2005). Eastern red-backed salamanders are usually in cool and moist environments in mature forests where there are plenty of fallen logs (Heatwole 1962, Zorn et al. 2004). On hot and dry days it has been noted that these salamanders will go underground, indicating once again how important their microclimate is to their survival and well-being (Ontario Nature 2019). Respiration via their skin and reliance on the microclimate make them indicator species since they are very sensitive to changing conditions, especially conditions relating to soil, water and air quality and changes in temperature (Feder 1983, Hopkins 2007). Understanding which environmental conditions affect these species, and in what way these changes are affecting populations, is vital in maintaining populations as well as monitoring greater ecosystem health.

The eastern red-backed salamander is regularly monitored in northeastern North American forests due to its relatively large and stable populations, as well as its role as an indicator species (Demaynadier & Hunter 1998, Zorn et al. 2004, Hopkins 2007). The Bruce Peninsula in Ontario, Canada is one area where the eastern red-backed salamander has been part of a long-term monitoring program (Bruce Peninsula Biosphere Association 2019). The Bruce Peninsula contains the last continuous forest surrounding the populated south of the province and is almost entirely protected area (Parks Canada 2019). This region is also comprised of deciduous and mixed forests which the eastern red-backed salamander makes its habitat (Bruce Peninsula Biosphere Association 2019). Over the last fourteen years, this species has been surveyed for abundances in the Bruce Peninsula and the environmental conditions of the area have been monitored as well (Cavan et al. 2017). This study aims to analyse the monitoring program data in order to shed light on eastern red-backed salamander abundance trends through time, and potential environmental predictors for such trends. This assessment will provide insight into the species' population trends as well as potential ecosystem-level environmental risks.

Materials and Methods

Data Collection and Description

The dataset used was collected by the Government of Canada spanning the years 2004-2017 (McAfee 2004). The study sites were chosen and set up in the north end of the Bruce Peninsula in Ontario, Canada in 2003 and sampling began a year later in 2004. The chosen plots included Cameron Lake Dunes, Pendall Point, Emmett Lake, South Cameron Lake, Harmony

Acres, Robins Property, Johnstone Property and Behmann Property. Salamander abundance was sampled using artificial cover boards (ACO), which were set up 10m in from the perimeter of a plot and 5m apart from each other. For 8 weeks every spring, plots were visited on a weekly basis in the mornings to assess the ACOs for salamander count. Samples were taken in the morning as conditions were wetter and cooler, and thus more favourable to salamanders. Data about the salamander was recorded such as species, sex, age, as well as observer, ACO number and plot number. At this time, environmental variables that may contribute to salamander counts were also recorded, including: soil pH, soil temperature (°C), soil moisture, Beaufort wind class, Beaufort sky class, air temperature (°C), and precipitation in the last 24 hours (mm).

Data Cleaning and Analysis

The R packages used in this analysis were “tidyverse” (Wickman et al, 2019), “MuMIn” (Barton, 2017) and “PerformanceAnalytics” (Peterson et al, 2019). The dataset was first filtered to include only the years 2009 and later. This was done as prior to 2009, there was inconsistency in data collection, and site visitation: only 5 sites continued to be sampled after 2009 and some variables such as soil moisture stopped being sampled after 2004. Unavailable data (NAs) were removed from analysis. Only the variables precipitation in the last 24 hours, soil temperature, and air temperature were selected because the other environmental variables were not measured consistently and included a lot of missing data.

The data was grouped by year and by site to calculate the means of the chosen environmental variables per site per year. The same process was done to calculate the standardized abundance: the counts per site per year were divided by the number of ACOs sampled to give a per site per year average. This standardized abundance accounted for any years or sites in which more ACOs were sampled than others so as not to skew abundance of any given year. The sum of these averages for that year was calculated and the results were graphed to produce the abundance over time graph.

Furthermore, the three environmental variables were then assessed for multicollinearity. This was necessary so that if any variables were collinear they would not be used within the same model, and only the variables that accounted for most of the variation would be used. Correlation was evaluated using the chart.Correlation() function and normality was also checked using this function. The data was shown to not be perfectly normal but it was decided not to transform the data as any transformations tried (log and square root transforms) did not affect normality. This is due to the fact that variables like precipitation had a lot of zeros and so any transformation had little to no impact. Additionally, homoscedasticity and normality of residuals was assessed. No multicollinearity was confirmed using the variance of inflation factors (VIF) test.

The assumptions of the linear regression model – normality, no multicollinearity, no correlation, homoscedasticity and linear relationship – were met to an acceptable degree and so a linear regression model was done. Using the three environmental variables as independent variables, fifteen models were created and run. These fifteen models included all interactions and variable combinations. An AICc was used due to the small sample size of the data, $N/K<40$, to determine the model that best fit the data. The models with the lowest AICc was selected and graphed. To account for differences in site we additionally ran linear mixed models. AICc measures were also calculated for the linear mixed models.

Results

Checking assumptions

The chart.Correlation() function gave information on the correlations, normality, and homoscedasticity. As shown in Figure 3, there was no correlation between the three variables. As for normality, the air temperature and soil temperature data were fairly normal but the precipitation values were not. To double check for multicollinearity, variance inflation factors were calculated. All VIF values were less than three, thus there was no suggestion of collinear values (Table 1).

Linear regression models

The abundance of Eastern Red-Backed Salamanders significantly increased over time (Figure 2). The linear regression model revealed that there was a positive slope and a significant p-value (Table 4, Figure 2). As well, time (i.e. years) was able to explain around 51% of the variation in salamander abundance.

After comparing the regression models using AICc, we were left with 4 top models all with similar AICc values (Table 2). These models included all predictor variables in their own model with no other interaction or variable involved as well as an intercept only model (Table 2). Despite having the best AICc values, none of the models were able to explain salamander abundance. All had large p-values and adjusted R^2 values of zero (Table 3, Figure 4). As well, the same analysis was done using a linear mixed effects model with site as the only random variable and with all the same models used in the simple linear regression analysis. This did not change the results - the intercept only model was still the best model. Thus, we concluded that the site did not need to be included as a random variable.

It should also be noted that because of the standardization of the salamander counts, we needed to calculate the means of each independent variable based on the site and year. These mean values match with each standardized salamander abundance for each site and year. Since we were using the means, we checked for outliers for each of the calculated means. There were six outliers identified: one in 2014 for the air temperature, three in 2011 and one in 2017 for precipitation, and one in 2009 for soil temperature. After running the analysis with the outliers removed, the final result did not change. The best model was still the intercept only model and there were no models with a significant p-value. For this reason, we left our final results with outliers in the data.

Discussion

The single clearest pattern that emerged from our analysis was a relative increase in salamander abundance over time. The magnitude of increase is small, and may even be a reflection of abundance that is stable over time, which agrees with a previous study of this species in southern Ontario (Seburn and Mallon 2017). If the trend is to be interpreted as an increase in population size, it can be explained in a few ways. Population increase could be caused by non-environmental changes such as a decrease in predation or competition, northward migration, or other demographic effects. None of which rely on invoking environmental variation to explain abundance. However, this does not mean environmental variation has no effect on eastern red-backed salamander abundance.

The scope of our work does not allow us to draw any concrete conclusions regarding the effect of environmental variables on salamander abundance in the Bruce Peninsula. Previous studies do clearly indicate a correlational relationship between certain environmental factors such as pH and soil moisture, and abundance (Riedel et al. 2008, Bondi et al. 2016). The absence of a detectable signal could simply be due to a mismeasurement of the available data. For example, in one study, soil moisture was measured 12cm into the ground at the time of survey (Riedel et al. 2006). Here, precipitation was used as a proxy of soil moisture and was measured as amount of rainfall the previous night. It is unclear if this could explain the lack of signal in Figure 4C. Rainfall could also be measured as or converted to a binary variable indicating rainfall or no rainfall. It is conceivable that it is not the amount of rainfall that affects abundance, but rather simply whether or not it rained. Making this variable binary could have made effects more detectable.

Soil pH, soil moisture, wind and sky class were left out of the entire analysis as potential explanatory variables due to little consistency in their sampling over time. The latter two variables describe the speed of winds and clearness of the sky on the day of survey respectively. As mentioned, variables relating to soil have been shown to affect salamander abundance. Therefore, had they been included in this study, it is more likely to have caused a detectable effect. Additionally, five years from 2004 to 2008 went unused here due to a different sampling method that had been used during that time and inconsistencies in measurement of variables.

Given the limitations of the current study, there are some potential extensions and considerations that can be made to allow a more thorough probing of the data. Most importantly, it would be useful to have the ability to include explanatory variables that are more certain to affect abundance such as soil moisture and pH. The current study also only measured abundance during the spring and summer months, and so it could be useful to make the surveys multi-seasonal. All survey sites used were contained within the Bruce Peninsula, which is not a very large area. Increasing the latitudinal gradient could also prove very useful in drawing more tangible conclusions.

Conclusion

Although citizen science observations have indicated eastern red-backed salamanders being absent in locations they were known to inhabit previously, in this small region of the Bruce Peninsula, populations appear to be stable or growing. It could be that the known reduction in global amphibian population may be restricted to certain localities, and does not affect locations in southern Ontario. Most research focuses on global frog declines as well, and so declines may only affect certain taxa and not others. However, it is difficult to be able to draw broad-scale conclusions about amphibian or even salamander abundance given the small geographic scale, and to some extent the temporal scale of the available data. With climate change currently causing a shift in species' biogeographic responses, it is important to keep doing studies similar to this, especially on known indicator species.

Tables and Figures

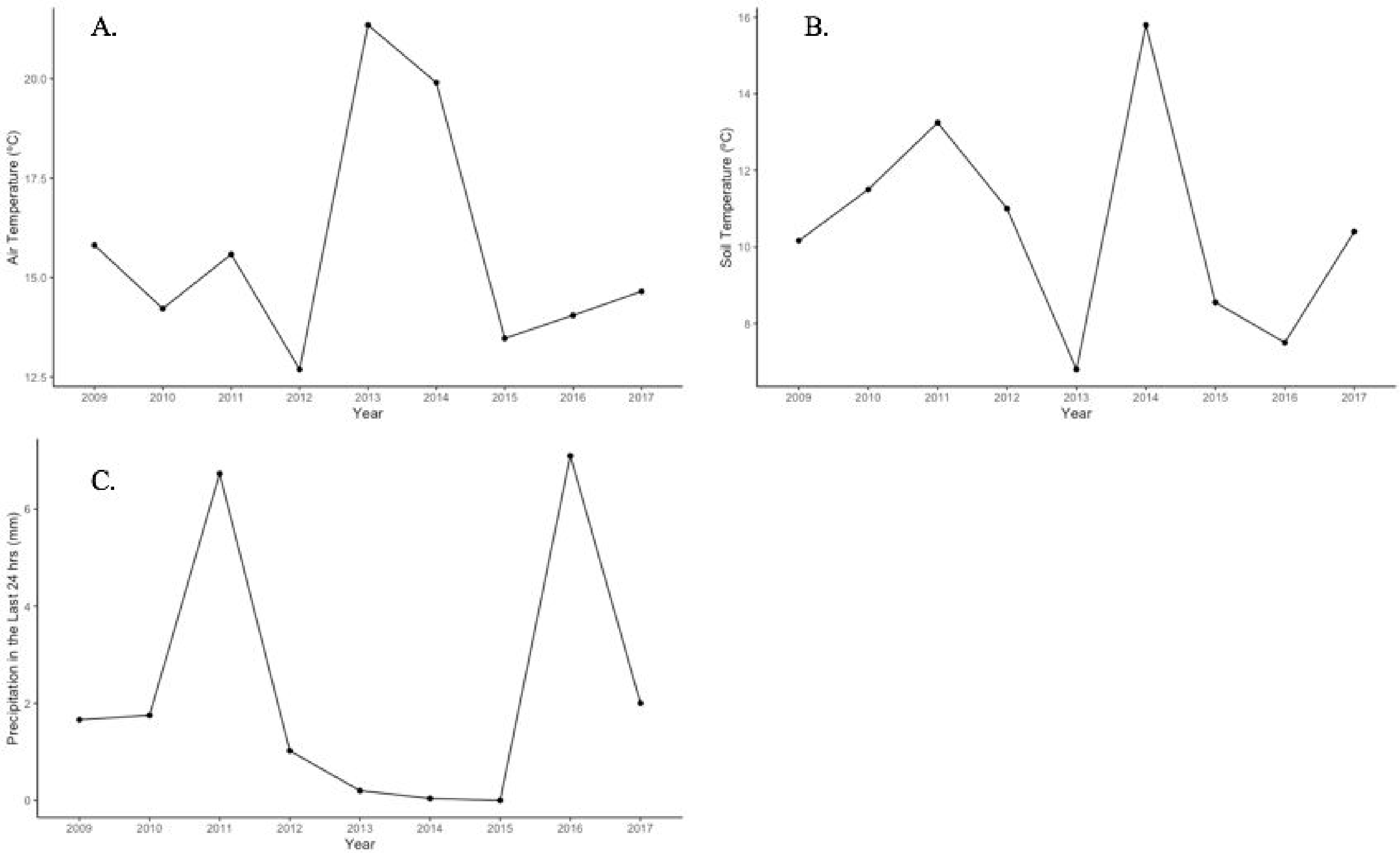

Figure 1. Plots of all predictor variables taken as a mean for each year from 2009 to 2017: A. Air temperature (°C) B. Soil temperature (°C) C. Precipitation in the last 24 hours (mm).

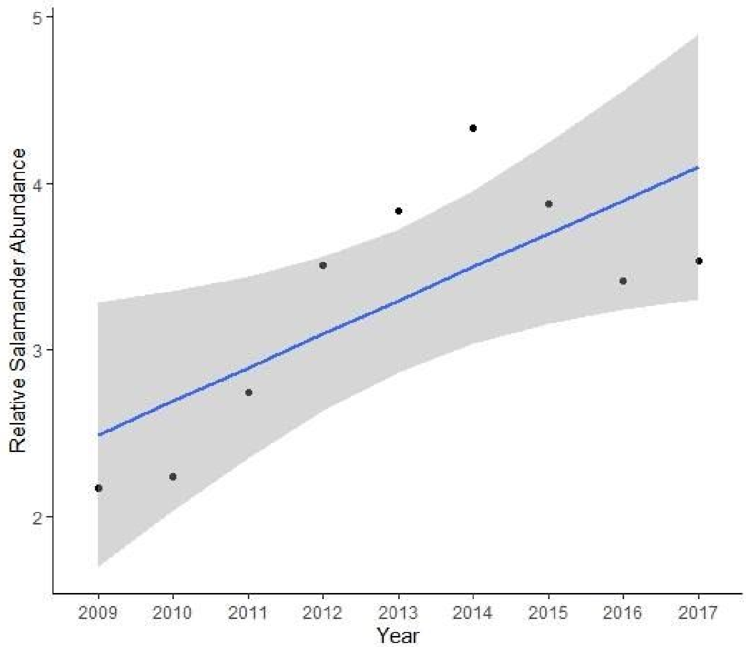

Figure 2. The standardized salamander abundance from 2009 to 2017 with the regression line plotted. The statistics are given in Table 4.

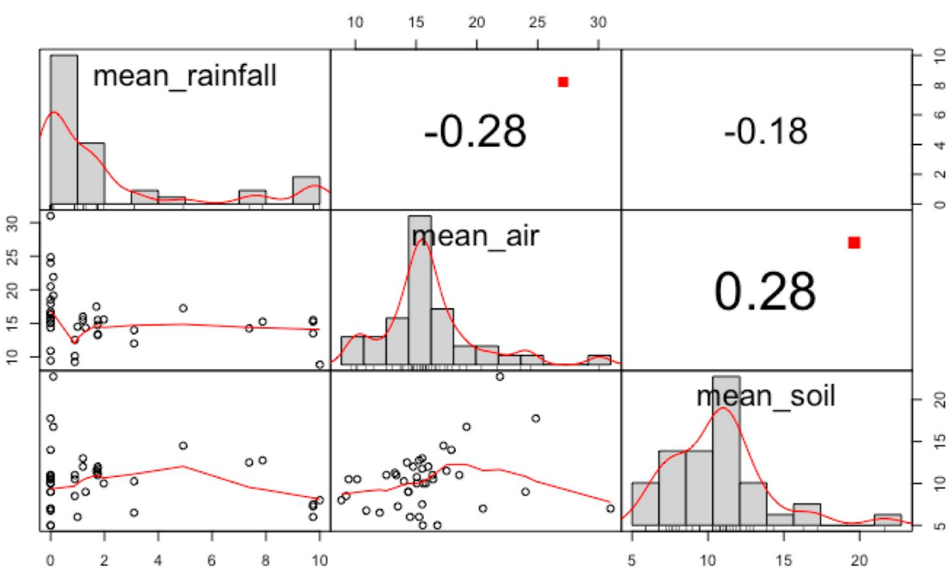

Figure 3. Checking assumptions. A correlation chart with the correlation coefficients between the variables. Histograms for each variable and their residuals are shown.

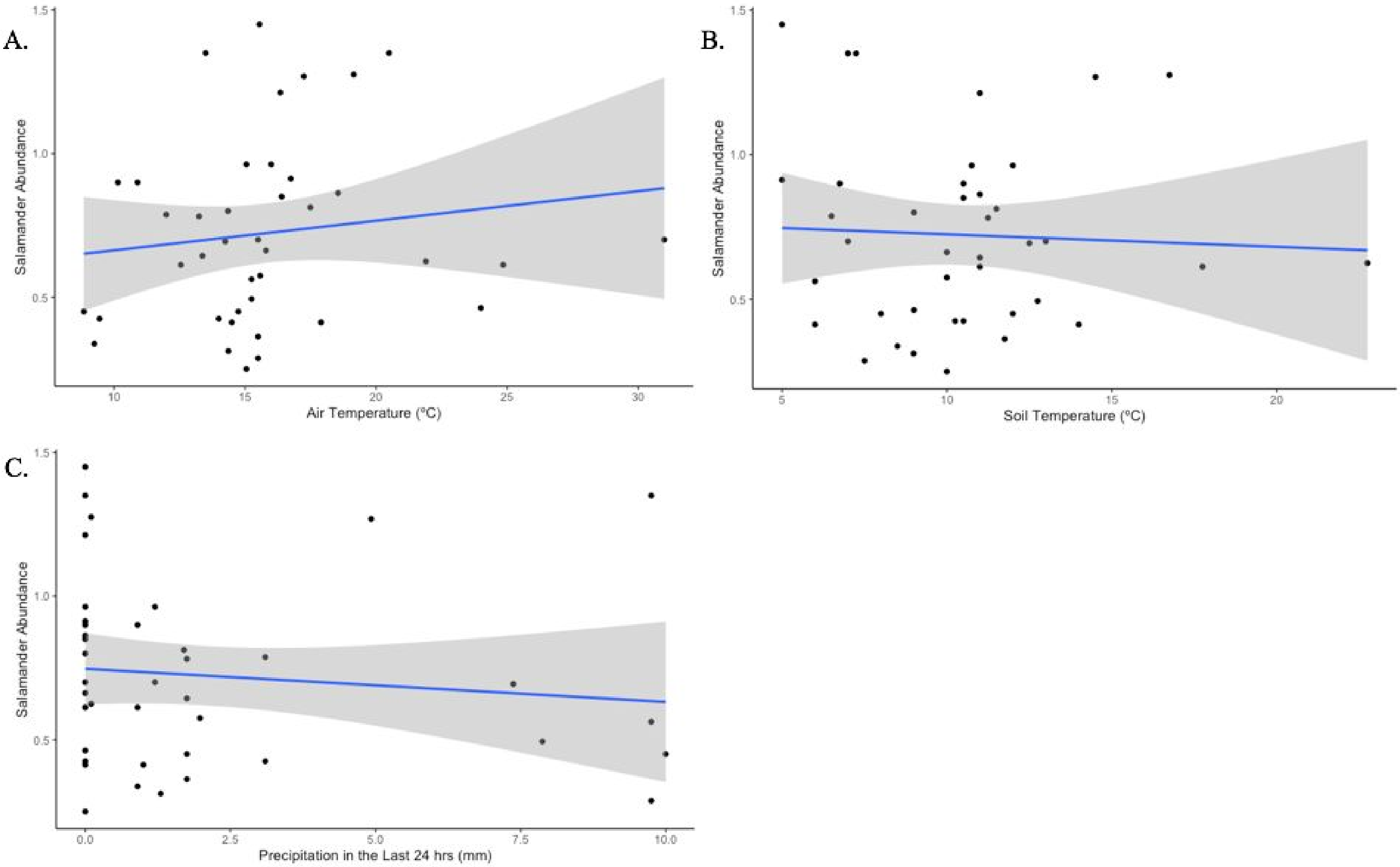

Figure 4. Linear regression models of each predictor variable on salamander abundance: A. Air temperature (°C) B. Soil temperature (°C) C. Precipitation in the last 24 hours (mm). All statistics are given in Table 3.

Table 1. Variance inflation factors for the three predictor variables.

Predictor	**GVIF**
Mean precipitation	1.098545
Mean air temperature	1.156916
Mean soil temperature	1.098287

Table 2. List of linear models and their AICc values. Bolded and shaded rows are the four lowest AICc models. "Average" represents the standardized salamander abundance.

Model #	Model	AICc
1	lm(average ~ mean_rainfall * mean_air * mean_soil, data = salamander_new)	39.23311
2	lm(average ~ mean_rainfall * mean_air, data = salamander_new)	32.32256
3	lm(average ~ mean_rainfall * mean_soil, data = salamander_new)	32.40086
4	lm(average ~ mean_soil * mean_air, data = salamander_new)	32.26190
5	lm(average ~ mean_rainfall + mean_air + mean_soil, data = salamander_new)	31.97120
6	lm(average ~ mean_rainfall + mean_air, data = salamander_new)	29.75010
7	lm(average ~ mean_rainfall + mean_soil, data = salamander_new)	30.04784
8	lm(average ~ mean_air + mean_soil, data = salamander_new)	29.67373
9	**lm(average ~ mean_rainfall, data = salamander_new)**	**27.76272**
10	**lm(average ~ mean_air, data = salamander_new)**	**27.52819**
11	**lm(average ~ mean_soil, data = salamander_new)**	**28.19979**
12	lm(average ~ mean_rainfall * mean_air + mean_soil, data = salamander_new)	34.68040
13	lm(average ~ mean_rainfall * mean_soil + mean_air, data = salamander_new)	34.40085
14	lm(average ~ mean_soil * mean_air + mean_rainfall, data = salamander_new)	34.70349
15	**lm(average ~ 1, data = salamander_new)**	**25.94770**

Table 3. Statistics table for the top four models according to their AICc values (listed in Table 2).

Model	Predictor	Response	Sample size	df	F-statistic	R^2	Adj. R^2	P-value	Intercept	Predictor slope	Predictor Std. error
9	Air temp.	Salamander abundance	40	38	0.7307	0.01887	-0.006953	0.398	0.55990	0.01031	0.01206
10	Precipitation	Salamander abundance	40	38	0.5043	0.0131	-0.01287	0.482	0.74684	-0.01153	0.01624
11	Soil temp.	Salamander abundance	40	38	0.08583	0.002254	-0.024	0.7711	0.767590	- 0.004316	0.014730
15*	No variable	Salamander abundance	40	39	0.3211			<2e-16	0.72266		0.05077

* Model 15 is the intercept only model. For this reason, there is no R^2 and the p-value and standard error are the intercept associated values. Additionally, the F-statistic is the residual standard error.

Table 4. Statistics table for the linear regression model of salamander abundance over time, from 2009 to 2017.

Predictor	Response	Sample size	df	F-statistic	R^2	Adj. R^2	P-value	Intercept	Predictor slope	Predictor Std. error
Time (Years)	Salamander abundance	9	7	9.416	0.5736	0.5127	0.01811	-484.5213	0.24229	0.07896

Citations

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