_Contributed Paper_

# Climate Change, Multiple Stressors, and the Decline of Ectotherms

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**Abstract:** _Climate change is believed to be causing declines of ectothermic vertebrates, but there is little evidence that climatic conditions associated with declines have exceeded critical (i.e., acutely lethal) maxima or minima, and most relevant studies are correlative, anecdotal, or short-term (hours). We conducted an 11-week factorial experiment to examine the effects of temperature (22 °C or 27 °C), moisture (wet or dry), and atrazine (an herbicide; 0, 4, 40, 400 μg/L exposure as embryos and larvae) on the survival, growth, behavior, and foraging rates of postmetamorphic streamside salamanders_ (Ambystoma barbouri), _a species of conservation concern. The tested climatic conditions were between the critical maxima and minima of streamside salamanders; thus, this experiment quantified the long-term effects of climate change within the noncritical range of this species. Despite a suite of behavioral adaptations to warm and dry conditions (e.g., burrowing, refuge use, huddling with conspecifics, and a reduction in activity), streamside salamanders exhibited significant loss of mass and significant mortality in all but the cool and moist conditions, which were closest to the climatic conditions in which they are most active in nature. A temperature of 27 °C represented a greater mortality risk than dry conditions; death occurred rapidly at this temperature and more gradually under cool and dry conditions. Foraging decreased under dry conditions, which suggests there were opportunity costs to water conservation. Exposure to the herbicide atrazine additively decreased water-conserving behaviors, foraging efficiency, mass, and time to death. Hence, the hypothesis that moderate climate change can cause population declines is even more plausible under scenarios with multiple stressors. These results suggest that climate change within the noncritical range of species and pollution may reduce individual performance by altering metabolic demands, hydration, and foraging effort and may facilitate population declines of amphibians and perhaps other ectothermic vertebrates._

**Keywords:** amphibian decline, atrazine, climate change, critical thermal maxima, global warming, pollution, toxicity

Cambio Climático, Estresantes Múltiples y la Declinación de Ectotermos

**Resumen:** _Se cree que el cambio climático es el causante de la declinación de vertebrados ectotérmicos, pero existe poca evidencia de que las condiciones climáticas asociadas con declinaciones han excedido máximas o mínimas críticas y la mayoría de los estudios relevantes son correlativos, anecdóticos o a corto plazo (horas). Realizamos un experimento factorial de 11 semanas para examinar los efectos de la temperatura (22 °C ó 27 °C), la humedad (mojado o seco) y la atrazina (un herbicida; exposición de embriones y larvas a 0, 4, 40, 400 μg/L) sobre la supervivencia, el crecimiento, el comportamiento y las tasas de forrajeo de salamandras_ (Ambystoma barbouri) _ribereñas postmetamórficas, una especie de interés para la conservación. Las condiciones climáticas evaluadas estuvieron entre la máxima y la mínima críticas de las salamandras por lo que este experimento cuantificó los efectos a largo plazo del cambio climático dentro del rango no-crítico de dichos cambios. A pesar de una gama de adaptaciones de comportamiento para condiciones cálidas y secas (p.ej.: enterramiento, uso de refugios, agrupamientos con coespecíficos y reducción de actividades) las salamandras exhibieron una pérdida significativa de masa y una mortalidad significativa en todas_

*las condiciones, a excepción de las condiciones húmedas y frescas que fueron las condiciones climáticas más cercanas a aquellas en las que se encuentran más activas en la naturaleza. La temperatura de 27 °C representó un riesgo mayor de mortalidad que las condiciones secas; las muertes ocurrían rápidamente a esta temperatura y más gradualmente bajo condiciones frescas y secas. El forrajeo disminuyó bajo las condiciones secas, lo que sugiere que hubo costos de oportunidad para conservar agua. La exposición al herbicida atrazina disminuyó aditivamente los comportamientos para preservar agua, la eficiencia de forrajeo, la masa y el tiempo transcurrido hasta la muerte. De ahí la hipótesis de que el cambio climático moderado puede causar declinaciones poblacionales es todavía más plausible bajo escenarios con estresantes múltiples. Estos resultados sugieren que el cambio climático dentro del rango no-crítico de especies y la contaminación pueden reducir el desempeño individual al alterar las demandas metabólicas, la hidratación y el esfuerzo de forrajeo y pueden facilitar declinaciones poblacionales de anfibios y, tal vez, otros vertebrados ectotérmicos.*

**Palabras Clave:** Atrazina, calentamiento global, cambio climático, contaminación, declinación de anfibios, temperatura máxima crítica, toxicidad

## Introduction

Evidence is mounting that global climate change contributes to shifts in the phenology, distribution, and abundances of organisms and alters species interactions and physiology (e.g., Parmesan 2006; Martin et al. 2010; Todd et al. 2011). Although projections suggest that climate change could cause the extinction of up to 37% of species worldwide (Thomas et al. 2004), links between climate change and actual species declines remain controversial (Buckley & Roughgarden 2004; Thuiller et al. 2004). This is especially the case for ectothermic vertebrates, such as amphibians and reptiles (e.g., Carey & Alexander 2003; Araujo et al. 2006; Chamaille-Jammes et al. 2006). These taxonomic groups, which are in global decline (Gibbons et al. 2000; Stuart et al. 2004), are thought to be particularly sensitive to changes in temperature and moisture because they are generally poikilothermic and have limited dispersal abilities (Gibbons et al. 2000). Amphibians also face challenges with staying hydrated because their permeable skin provides little resistance to evaporative water loss (Heatwole et al. 1969; Spotila 1972).

Part of the reason for the controversy regarding the role of climate change in declines of amphibians and reptiles is that most relevant studies are correlative or anecdotal (e.g., Rohr & Raffel 2010; Li et al. 2013). Manipulative studies are needed to better assess the plausibility of climate change as a factor in declines of ectothermic vertebrates (Gibbons et al. 2000; Carey & Alexander 2003; Raffel et al. 2013). Although manipulative studies on the behavioral, thermal, and hydric physiology of amphibians and reptiles were common until the 1970s (Hutchison 1961; Brattstrom 1963; Spotila 1972), they are less so now (Carey & Alexander 2003; Li et al. 2013), perhaps because tests of the critical limits of species are less ethically acceptable now. Results of these classic studies showed that actual temperatures and moisture levels associated with recent amphibian and reptile declines rarely exceeded critical maxima or minima (Carey & Alexander 2003; Sinervo et al. 2010; Li et al. 2013). The critical thermal or hydric maximum or minimum is the mean temperature or moisture level at which individuals respond with disorganized locomotion which prevents escape from conditions that will rapidly lead to death (Hutchison 1961).

Most studies of critical maxima or minima were conducted under unnatural but highly controlled laboratory conditions in which researchers emphasized short-term mortality responses (on the order of hours) to gradients of moisture or temperature alone. Hence, a complete understanding of how species respond to combined but independent changes in both moisture and temperature under ecologically relevant conditions is lacking. Such an understanding is important given that climate change is not occurring uniformly across the globe; some areas are getting warmer, some cooler, some drier, and some wetter (Loaiciga et al. 1996; Wetherald & Manabe 1999). Also lacking is a thorough understanding of how changes in climate within the noncritical range (i.e., between the critical maxima and minima) affect fitness over longer periods than assessed in classic critical maximum studies and what other factors might interact with climate change to affect amphibians. Although some authors propose that climatic shifts within the noncritical range are causing amphibian and reptile declines (e.g., Whitfield et al. 2007; Sinervo et al. 2010), empirical evidence supporting the plausibility of this explanation remains limited (but see Kiesecker et al. 2001; Bustamante et al. 2010).

One common factor that may alter risk of mortality associated with climate change is exposure to contaminants. For instance, the herbicide atrazine is the second most commonly used pesticide in the United States (Kiely et al. 2004) and is mobile and persistent relative to other currently used pesticides (Rohr & McCoy 2010). Aquatic exposure of larval streamside salamanders (*Ambystoma barbouri*) to environmentally relevant concentrations of atrazine is associated with reduced water-conserving behaviors after metamorphosis, elevated desiccation risk under laboratory conditions (filter paper-lined dishes [Rohr & Palmer 2005]), and increased mortality when reared in soil-filled terraria (Rohr et al. 2006). What

remains unclear, however, is whether atrazine exposure affects amphibian responses to climate change in an additive, synergistic, or antagonistic manner.

We exposed larval streamside salamanders to atrazine and then tracked their behaviors, foraging rates, mass change, and survival in soil-filled terraria for 11 weeks after metamorphosis under crossed (i.e., all possible combinations) moisture (wet or dry) and temperature (22 °C or 27 °C) conditions. We based selection of these moisture and temperature regimes on conditions expected with climate change (Loaiciga et al. 1996; Wetherald & Manabe 1999). These conditions are not near thermal or hydric critical maxima or minima for ambystomatid salamanders (Hutchison 1961; Spotila 1972). Hence, we emphasized the long-term effects of climatic change within noncritical ranges of moisture and temperature on streamside salamander behavior and fitness proxies (e.g., survival, mass loss). The streamside salamander is a species of conservation concern because it has a restricted range with very little gene flow among populations, some of which are in decline (Petranka 1998). Furthermore, data suggest that species with limited ranges may be narrowly adapted and thus more susceptible to contaminants and climate change (Fioramonti et al. 1997).

Owing to the importance of water availability for maintaining vital functions in amphibians (Spotila 1972) and for dictating global amphibian richness patterns (Buckley & Jetz 2007), we expected moisture to be a more important predictor of streamside salamander mortality than temperature. Specifically, we expected warm and dry conditions to provide the greatest mortality risk of the 4 moisture and temperature treatments. Given that atrazine exposure increases risk of desiccation (Rohr & Palmer 2005) and that evaporative water loss is greater at higher temperatures, we expected that atrazine would increase dehydration (measured as loss of mass) and mortality under warm conditions. Finally, we expected that the suite of behavioral responses induced by atrazine, dryness, and cooler temperatures would reduce streamside salamander foraging rate and thus offer additional insight into gradual effects of the treatments on mortality risk.

## Methods

We exposed streamside salamanders to atrazine from the embryo stage (April 2002) to metamorphosis (August 2002) as described in Rohr et al. (2004). Briefly, we placed 31 eggs (from 38 clutches whose genetic variation was homogenized) in each of 48 aquaria containing 9.5 L of water (pH 8). Streamside salamander embryos and larvae develop in streams from February to June (Petranka 1998), when temperatures are cool. Thus, we reared the embryos and larvae at 15 °C. After metamorphosis, how-

ever, streamside salamanders can be exposed to the high summer temperatures of Kentucky (U.S.A.) (see below).

We randomly assigned 1 of 4 treatments to each aquarium: solvent (dimethylsulfoxide) or 4, 40, or 400 $\mu$g/L of technical grade atrazine (80% pure) (ICN Laboratories, Aurora, OH, U.S.A.) dissolved in solvent. We did not include a water control because the solvent did not have effects on the behavior, growth, or survival of this species in a previous study (Rohr et al. 2003a). The highest concentration of atrazine was approximately the estimated environmental concentration when atrazine was originally registered for use in the United States (Rohr & McCoy 2010). We changed aquarium water completely each week and reapplied the appropriate treatment. Chronic exposure to the 2 lowest atrazine concentrations occurs in nature (Rohr & McCoy 2010). We sampled water weekly and determined atrazine concentration means were 4.95 $\mu$g/L (SE 0.30), 38.70 $\mu$g/L (2.14), and 386.04 $\mu$g/L (23.24) in the 3 respective treatments (Rohr et al. 2004). Given the similarity between the nominal and actual concentrations and potential error in our atrazine assay, we report nominal concentrations hereafter. Atrazine exposure occurred only before metamorphosis.

In August 2002, we constructed 160 terraria to evaluate the long-term effects of atrazine exposure, temperature, and moisture on postmetamorphic streamside salamander behavior, growth, and survival. Each terrarium had a cylindrical transparent plastic container (18.5 cm diameter, 7.5 cm high) with 3, 3.2-mm holes drilled in the top to allow moderate air circulation; 450 g of a 3:1 ratio of organic top soil to sphagnum moss (soil depth of ∼5 cm); and 3, 5 × 5 × 0.5 cm plastic refuge plates placed equidistant from one another. We placed 3 metamorphs from the same atrazine or solvent container in each terrarium. Individuals were uniquely toe clipped so they could be identified throughout the experiment. We recorded the mass and length of all metamorphs just before they were placed in the terraria. Metamorphs are typically 2–3 cm in snout-vent length (Rohr et al. 2004). The terraria were evenly distributed among 4 incubators, 2 at 22 °C and 2 at 27 °C (12:12 h day:night photoperiod). Most streamside salamanders metamorphose between April and June and the average temperature during this period is 22 °C in Kentucky (Meoweather 2013). Hence, 22 °C is a reasonable present-day reference temperature to which many metamorphs would be exposed. In addition, the 5 °C difference between the 2 temperatures is a possible future climate scenario for Kentucky due to global warming under worst-case carbon dioxide emissions (Houghton et al. 2001). Average daytime air temperature during the summer in Kentucky, however, is 30.5 °C and thus both tested temperatures are within the present, natural temperature variability over the geographic range of streamside salamanders.

Within each incubator, we randomly assigned half the terraria to wet treatment and half to dry treatment. To implement the moisture treatment, each week we weighed the terraria with soil in it and added water to bring the soil mass up to 400 g (42.0% water by mass) and 750 g (nearly saturated, ~0 kPa, 78.7% water by mass) for the dry and wet terraria, respectively. This approach minimized the dependence of moisture on the temperature treatments (Supporting Information). The terraria at both temperatures, on average, lost approximately 1% of their mass each week. Hence, we implemented a 2 × 2 × 4 experimental design with 2 temperature, 2 moisture, and 4 atrazine treatments nested within each incubator. There were 10 replicates (5 in each incubator) of each of the 16 treatment combinations.

Each terrarium received one Herptivite-dusted (vitamins and minerals), 1-week-old cricket per live salamander each week. Thirteen times during the experiment (once weekly at terrarium weighing and on 2 other occasions), we recorded salamander behaviors in each terrarium (9:00–15:00). For each individually identified salamander, we quantified whether they were in refuge (under a refuge plate or buried), moving, huddled with a conspecific, or climbing up the walls of the terrarium. We also recorded the number of refuge plates occupied. After each behavioral observation, we identified individual salamanders by their toe clips and placed them on the tops of separate refuge plates in an effort to reduce temporal autocorrelation among the behavioral observations. This required the salamanders to return to refuge and find conspecifics with which to huddle, if they so chose. In the last week of the 11-week experiment, we recorded mass and length of each surviving salamander. Streamside salamanders spend a substantial portion of their terrestrial lives under refugia and in soil (Petranka 1998). Thus, determining survival occasionally required pulling animals out of the soil. To minimize salamander disturbance, we only assessed survival weekly when we weighed terraria.

During the last week of the experiment, we conducted foraging trials to evaluate how moisture and previous atrazine exposure affected salamander foraging rates. These trials were only conducted on salamanders in the 2, 22 °C incubators because most of the salamanders exposed to 27 °C had died by this point in the experiment. We recorded whether or not individuals were buried (i.e., at time 0; 10:00) and then added 4 crickets per surviving salamander to each terrarium. Every hour for the next 4 h, we recorded the number of crickets remaining and the number of salamanders that were buried. We focused on burying behavior in these foraging trials because buried salamanders cannot forage on crickets (they bury themselves rather than form burrows). We added 4 crickets to each terrarium in the hopes that there would be ad libitum food over the 4-h trials.

## Statistical Analyses

We conducted statistical analyses in R statistical software (R Development Core Team 2013). We analyzed normally distributed data (e.g., percent mass loss, weeks alive) and binomially distributed data (behavior, survival) with the lme and lmer functions, respectively (nlme package). Salamanders that lived until the end of the experiment were assigned a time of death of 11 weeks, which is a conservative estimate of their true time of death given that some fraction would have certainly lived longer. We did not conduct a censored survival analyses because we are unaware of any survival-analysis software package that properly accounts for the nested nature of our experimental design (i.e., salamanders nested in terraria and terraria nesting in environmental chambers). Atrazine concentration had a log relation with most streamside salamander response variables tested in previous studies (e.g., Rohr et al. 2004, 2006, 2011). Thus, we log transformed atrazine concentrations before all analyses (which improved statistical fit on the basis of $R^2$) and treated this variable as a continuous predictor. We used one-tailed tests for atrazine effects on survival, mass change, inactivity, and huddling because atrazine is negatively associated with these response variables in streamside salamanders (e.g., Rohr et al. 2004, 2006; Rohr & Palmer 2005).

We used individual salamander mass at the start of the experiment as a covariate in the behavior and mass change analyses, and survival as a covariate in the huddling analyses because huddling is dependent on the presence of conspecifics. Individual salamanders were nested within terraria for repeated-measures behavioral observations, terrarium was nested within environmental chamber for all responses, and salamander, terrarium, and environmental chamber were all treated as random effects to ensure proper error structure. These mixed-effects models allowed us to use the total number of animals in the experiment (480) to test for effects of initial mass; the number of terraria (160) to test for effects of atrazine, moisture, and interactions; and the number of environmental chambers (4) to test for the main effect of temperature. In these models, we tested for all main effects and 2- and 3-way interactions among temperature, moisture, and atrazine with log-likelihood ratio tests. We also used log-likelihood ratio tests to compare the responses of salamanders that were not exposed to atrazine with those exposed to 4, 40, and 400 $\mu$g/L of atrazine (no alpha adjustments made).

In the foraging trials, mean number of crickets eaten per salamander and mean proportion of salamanders that were buried (angularly transformed) were treated as repeated-measures Gaussian variables; time was the repeated-measures independent variable, and moisture and log atrazine were crossed categorical and continuous predictors, respectively. We blocked by environmental chamber (conducted only at 22 °C because of too much
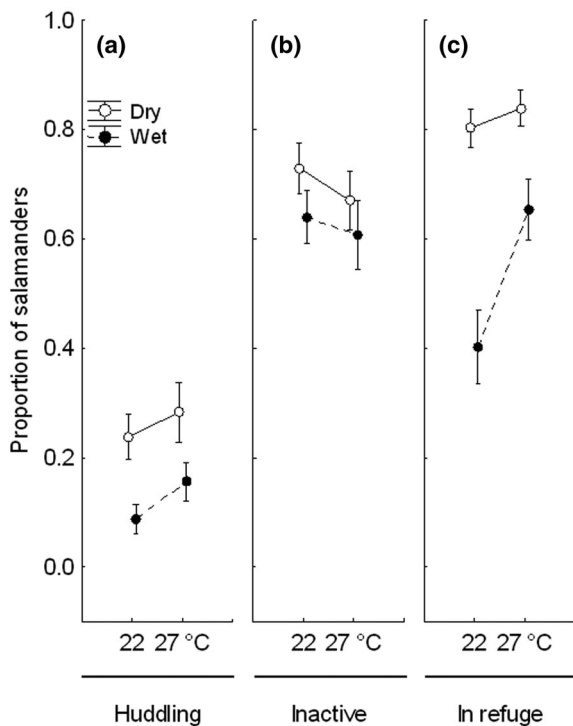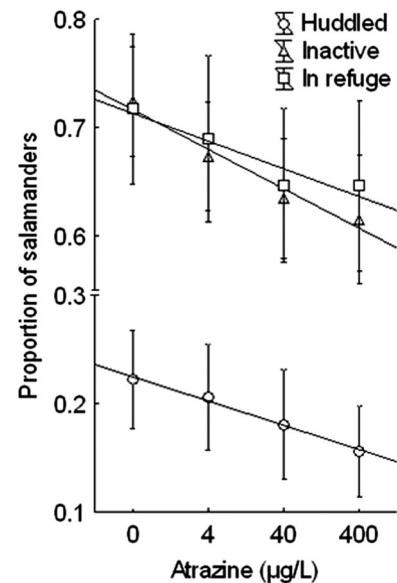
*Figure 1. Effects of moisture and temperature on the proportion of streamside salamanders (a) huddled with conspecifics, (b) inactive, and (c) in a refuge (under refuge plate or buried). Shown are least-squares means (95% CI) of observations made throughout the experiment averaged across herbicide (atrazine) concentrations (n = 40).*

mortality at 27 °C), treated environmental chamber as a random variable, controlled for the number of salamanders per terrarium in the foraging analyses, and determined the significance of effects with *F*-ratio tests.

## Results

### Behavior

Moisture and temperature treatments interacted significantly to affect the proportion of salamanders huddled with conspecifics or in refugia (huddling, $\chi^2_1 = 3.96$, $p = 0.047$; refuge use, $\chi^2_1 = 9.14$, $p = 0.003$; all other interactions, $p > 0.294$). Each behavior occurred less often in cool (22 °C) and wet conditions than in warm and dry conditions (Figs. 1a & b). Salamanders were less active under dry than wet conditions ($\chi^2_1 = 23.27$, $p < 0.001$; all interactions $p > 0.257$) and at cool than warm temperatures, although not significantly so ($\chi^2_1 = 1.33$, $p = 0.248$) (Fig. 1c). Although climbing on the walls of the terraria was rare, this behavior occurred significantly more often in wet than dry conditions (dry,
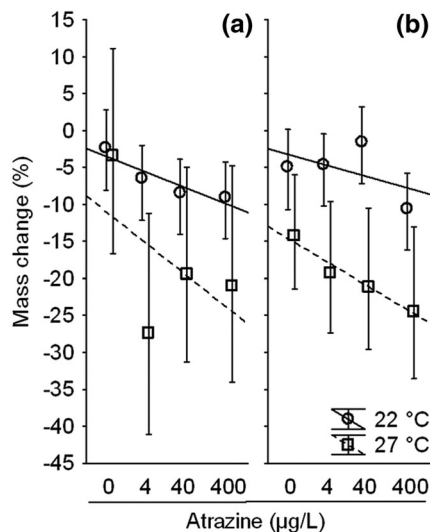


*Figure 2. Effects of atrazine (an herbicide) concentration (log scale) on the proportion of streamside salamanders huddling with conspecifics, inactive, and in a refuge (under refuge plate or buried). Shown are best-fit lines and least-squares means (95% CI) of observations made over 9 weeks averaged across moisture and temperature treatments (n = 40). The 3 behaviors are not mutually exclusive.*

mean [SE] = 0.00 [0.00]; wet, 0.68 [0.19]; $\chi^2_1 = 5.08$, $p = 0.024$; all other effects $\chi^2_1 > 0.16$, $p > 0.687$).

Aquatic atrazine exposure was negatively associated with huddling ($\chi^2_1 = 7.85$, $p = 0.003$) and refuge use ($\chi^2_1 = 5.41$, $p = 0.020$) (Fig. 2) and positively associated with salamander activity ($\chi^2_1 = 5.21$, $p = 0.011$). For each behavior, solvent controls were significantly different from the 400 $\mu$g/L ($p = 0.001$, $p = 0.011$, $p = 0.008$, respectively) and 40 $\mu$g/L concentration ($p = 0.013$, $p = 0.037$, $p = 0.003$, respectively). For the 4 $\mu$g/L concentration, only activity was significantly different from the controls (activity, $p = 0.004$; huddling, $p = 0.141$; refuge use, $p = 0.0885$).

### Salamander Mass Change

There were no significant differences in salamander mass among atrazine concentrations when they were initially placed in the terraria (atrazine $\chi^2_1 = 3.66$, $p = 0.300$; all interactions $p > 0.273$). By the end of the experiment, there was a significant negative relation between the percentage of salamander mass change and atrazine concentration ($\chi^2_1 = 6.21$, $p = 0.006$; 400 vs. 0 $\mu$g/L $p = 0.002$; 40 vs. 0 $\mu$g/L $p = 0.054$; 4 vs 0 $\mu$g/L $p = 0.016$) (Fig. 3). At 27 °C, each $\log_{10}$ increase in atrazine concentration was associated with an

*Figure 3. Effects of atrazine (an herbicide) concentration (log scale), temperature, and (a) wet and (b) dry moisture conditions on mean (95% CI) percent change in mass of streamside salamanders from the beginning to the end of the experiment (controlling for mass at the start of the experiment). Shown are best-fit lines and means (95% CI; n = 10).*

approximately 4% reduction in salamander mass (Fig. 3). Salamanders also lost more mass at 27 °C than 22 °C ($\chi^2_1 = 8.96$, $p = 0.003$) (Fig. 3), but there was no significant effect of moisture ($\chi^2_1 = 0.21$, $p = 0.643$) or any treatment interactions on percent mass change ($\chi^2_1 < 0.70$, $p > 0.403$) (Figs. 3a & b).

## Survival

Moisture and temperature treatments interacted significantly to affect the proportion of salamanders surviving ($\chi^2_1 = 5.49$, $p = 0.019$) and how long they survived ($\chi^2_1 = 24.41$, $p < 0.001$) (Fig. 4a). This interaction was driven by higher and sooner mortality in terraria at 27 °C than 22 °C under wet conditions and by lower and slower mortality at 27 °C than 22 °C under dry conditions (Fig. 4a). Temperature was a larger mortality factor than moisture. By the end of the experiment, there was >80% mortality of salamanders exposed to 27 °C across both moisture treatments and approximately 60% mortality of salamanders exposed to the dry terraria across both temperature treatments (Fig. 4a). There was a negative association between atrazine concentration and how long salamanders survived ($\chi^2_1 = 3.20$, $p = 0.037$) (Fig. 4b), but this association was not significant ($\chi^2_1 = 2.37$, $p = 0.062$) for proportion surviving. The atrazine-by-temperature interaction was not significant (Fig. 4b). There was a positive relation between salamander mass at the start of the experiment and the probability of survival

(coefficient 0.69 [SE 0.44], $\chi^2_1 = 2.59$, $p = 0.053$) and time of death during the experiment (coefficient 1.42 [0.65], $\chi^2_1 = 4.88$, $p = 0.014$).

## Burying Behavior in the Foraging Trials

Burying behavior was not significantly different among the 3 atrazine concentrations (Fisher's least significant difference test [LSD]: $p > 0.666$), but all 3 were significantly different from the control treatment (atrazine $F_{3,58} = 3.33$, $p = 0.026$, Fisher's LSD $p < 0.044$; 0 μg/L atrazine, mean [SE] = 0.262 [0.045]; 4 μg/L, mean = 0.081 [0.042]; 40 μg/L, 0.130 [0.051]; 400 μg/L, 0.104 [0.042]). These results indicate the relation between atrazine concentration and burying was not linear and thus regression analyses would not be ideally suited to these data. Therefore, we pooled the 3 atrazine concentrations and treated atrazine as categorical (presence or absence) for this response variable only.

Burying behavior exhibited a significant time-by-moisture-by-atrazine interaction ($F_{4,248} = 3.09$, $p = 0.016$) (Fig. 5). Just before crickets were added (hour 0), there was a moisture-by-atrazine interaction. Atrazine exposure (relative to controls) reduced burying behavior in the dry, but not the wet, terraria ($F_{1,62} = 5.90$, $p = 0.018$) (Fig. 5). This was because almost no salamanders were buried in the wet terraria regardless of treatment (Fig. 5). Within the first hour of adding crickets to the terraria, 83% of the buried salamanders in the dry terraria that had not been exposed to atrazine had emerged to forage, which eliminated the moisture-by-atrazine interaction ($F_{1,62} = 0.005$, $p = 0.946$) (Fig. 5). The proportion of salamanders buried did not change over time in the wet terraria, but in the dry terraria many more salamanders were buried 4 h after cricket additions than were buried before or 1 h after cricket additions (Fig. 5). However, by the end of the experiment in the dry terraria, a smaller fraction of the atrazine-exposed salamanders had buried themselves than those never exposed to atrazine (Fig. 5). Consequently, the strongest atrazine-by-moisture interaction on burying behavior occurred 4 h after addition of crickets ($F_{1,62} = 8.06$, $p = 0.006$) (Fig. 5).

## Foraging Rate and Duration

Fewer salamanders were buried in the wet than dry terraria (Fig. 5), and salamanders in the wet terraria ate more crickets over 4 h than those in the dry terraria ($F_{1,61} = 12.10$, $p < 0.001$) (Fig. 6). Most salamanders were not buried in the wet terraria or in the dry terraria 1 h after cricket additions (Fig. 5). For this subset of the data, previous atrazine exposure decreased foraging efficiency (i.e., crickets eaten per hour aboveground) of salamanders that were not buried (Fig. 6). However, in the dry terraria, atrazine-exposed salamanders foraged
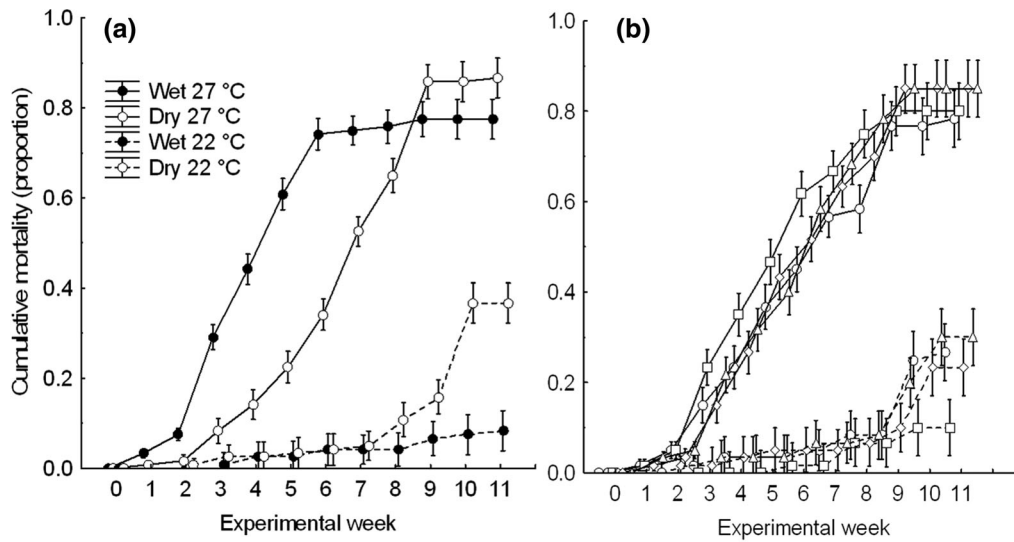
*Figure 4. Mean (SE) cumulative streamside salamander mortality as a function of (a) moisture and temperature treatments (n = 40) and (b) atrazine (an herbicide) concentrations (n = 20) (27 °C solid lines and 22 °C dashed lines; data are averaged across moisture treatments).*
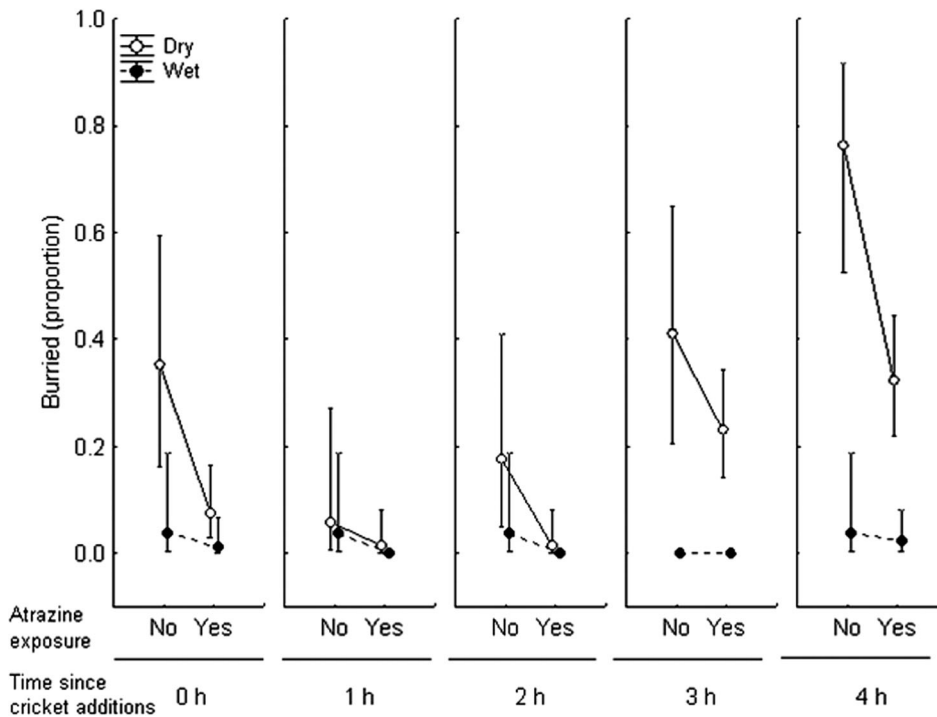


*Figure 5. Effects of atrazine (an herbicide) and moisture on the proportion (mean [95% CI]) of streamside salamanders that had buried themselves at a particular time interval in the foraging trials (n = 30 salamanders for atrazine exposed wet or dry, n = 10 for atrazine absent wet or dry). Observations made at 0 h occurred before crickets were added to the terraria; observations made at hours 1–4 occurred after crickets were added.*

longer because they were less likely to bury themselves 2–4 h after cricket additions relative to the controls (Fig. 5). Consequently, atrazine exposure had a positive effect on the number of crickets eaten per salamander in the dry terraria 4 h after cricket additions, whereas the effect of atrazine on this response was negative for the salamanders in dry terraria 1 h after cricket additions and was negative for salamanders in wet terraria at all time points (Fig. 6). Hence, the cumulative number of crickets eaten depended significantly on time, moisture level, and previous atrazine exposure (time × moisture × atrazine $F_{1,61} = 4.97$, $p = 0.029$) (Fig. 6).

## Discussion

### Effects of Temperature and Moisture Treatments

Streamside salamanders exhibited considerable behavioral plasticity in response to the temperature and
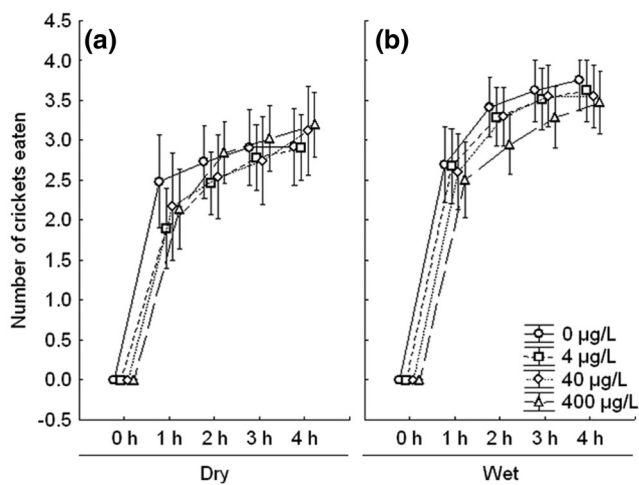
*Figure 6. Mean (95% CI) cumulative number of crickets eaten per streamside salamander when salamanders were exposed to atrazine (an herbicide) (concentration log scale) in (a) dry and (b) wet conditions (n = 10 terraria).*

moisture treatments. Both huddling with conspecifics and refuge use are common and adaptive responses to dryness that reduce exposed surface area and water loss of amphibians (e.g., Heatwole 1960; Gehlbach et al. 1969; Rohr & Madison 2003). Both of these behaviors were synergistically affected by moisture and temperature (Figs. 1a & b); the greatest level of huddling and refuge use occurred under warm and dry conditions and the least occurred under cool and wet conditions. This might be expected given that evaporative water loss occurs more rapidly at higher temperatures and thus as temperatures increase rates of rehydration could be offset by dehydration. Although it is possible that huddling is simply a by-product of all the salamanders within a terrarium being attracted to the moistest soil patch (i.e., involuntary rather than voluntary), this seems unlikely to be the only driver of huddling because at least some salamander species are attracted to the odor of conspecifics under xeric conditions (Rohr & Madison 2003), a response that would facilitate huddling.

In contrast to huddling and refuge use, locomotor activity increased with moisture and temperature. Reduced activity should allow amphibians to increase body contact with moist substrates and decrease exposed surface area, which should facilitate rehydration and decrease further dehydration, respectively. Indeed, activity can increase the rate of water loss up to 200% above the rate for resting amphibians, largely because of increases in exposed surface area (Heatwole et al. 1969).

Activity presumably increased with temperature because the metabolic rate of ectotherms increases with temperature. However, activity did not increase exponentially with temperature as does metabolic rate

(Gillooly et al. 2001). Hence, activity actually decreased with increasing temperature relative to metabolic rate, a response that should minimize water loss at higher temperatures. Although climbing on the sides of the terraria was rarely observed, it occurred only in the moist terraria, consistent with the observation of Jaeger (1978) that salamanders climb plants to access prey only on wet nights.

Despite this suite of presumably adaptive behaviors to minimize the fitness consequences of adverse environmental conditions, salamanders still lost considerable mass and had high mortality. The effect of the treatments on salamander mass change was likely a function of at least 3 factors: metabolic rate, dehydration, and foraging activity differences among the treatments. For instance, salamanders lost significantly more mass at 27 °C than 22 °C, a response that was likely a product of both increased metabolic demands and evaporative water loss at the higher temperature.

At the start of our foraging trials, nearly 40% of salamanders not exposed to atrazine were buried in the dry terraria. Of these buried salamanders, 83% emerged from the soil within 1 h of cricket additions (Fig. 5); this is likely an adaptive response to detect surface prey from underground. Ambystomatid salamanders bury themselves deeper as conditions get drier (Heatwole 1962). Hence, it is unclear whether streamside salamanders would have buried themselves deeper if we had provided more soil and whether surface prey detection would have attenuated with increasing soil depth.

Despite the streamside salamander's likely adaptation to detect surface prey from underground, salamanders in the dry terraria (that were not exposed to atrazine) consumed fewer crickets than those in the wet terraria because they foraged for a shorter time. Nearly 80% of salamanders in the dry terraria abandoned foraging and buried themselves in the soil by the end of the 4-h foraging trial, whereas almost none of the salamanders in the wet terraria buried themselves. Similarly, plethodontid salamanders voluntarily abandon foraging sooner in dry than moist conditions (Feder & Londos 1984). Importantly, half as many of the salamanders in the dry terraria were buried at the beginning of the foraging trial as were buried at the end when they were presumably more satiated. This suggests that streamside salamanders face conflicting selection pressures in dry environments (Rohr et al. 2003b); there was a trade-off between foraging when hungry and the expression of water-conserving behaviors that require reduced activity. It remains uncertain, however, the degree to which prey availability and hunger might exacerbate desiccation risk under future climates.

Moist conditions were more detrimental to salamander survival at the higher temperature, whereas dry conditions were more detrimental at the lower temperature. Furthermore, temperature was a better predictor of

mortality than moisture, with >80% of the salamanders dying at the warmest temperature. This was contrary to our original hypothesis that moisture would be a bigger mortality factor for streamside salamanders than temperature because moisture is considered the primary factor dictating the distribution of amphibians (Buckley & Jetz 2007). In addition to climate, salamander size at the start of the experiment was a significant positive predictor of survival. This result is consistent with the results of previous studies that show smaller metamorphs have lower terrestrial survival (Berven 1990; Scott 1994) and suggests that smaller individuals within a species might be at greater risk of mortality from climate change.

Mortality occurred most rapidly under warm and wet conditions, consistent with evidence linking amphibian and reptile declines in Costa Rica to increases in temperature and moisture (Whitfield et al. 2007). The cause of the rapid and high mortality of streamside salamanders under warm and moist conditions is unknown. One plausible explanation is that moist conditions allow salamanders to move more and warm conditions increase their metabolism, both of which would elevate the rate of energy loss relative to the other 3 moisture-by-temperature treatment combinations. This, in turn, might lead to earlier and greater mortality when food is restricted (as it was here). If other species of amphibians also perform poorly under warm and wet conditions, it may require a rethinking of amphibian responses to climate change. The combination of warm and dry conditions was the second-most deadly treatment in our study. Several authors suggest that this combination of environmental conditions has been at least a contributing factor to a number of amphibian and reptile species declines (e.g., Pounds et al. 1999; Kiesecker et al. 2001).

### Effects of Atrazine

In short-term tests (hours) conducted in filter-paper-lined dishes, streamside salamanders exposed to atrazine were more active and lost more water weight relative to controls when tested 8 months after exposure to atrazine (Rohr & Palmer 2005). We obtained similar results but with a longer term experiment conducted under more natural conditions. Early-life exposure of streamside salamanders to atrazine was negatively associated with inactivity, refuge use, burying, huddling with conspecifics, foraging efficiency, and mass gain later in life. Our results provide additional evidence that atrazine exposure can have persistent effects on amphibians. The negative association between mass gain and atrazine was likely a function of atrazine reducing water-conserving behaviors, increasing energy expenditures (because of hyperactivity), and diminishing prey consumption (because of reduced foraging efficiency under moist conditions).

Although atrazine was associated with less burying and reduced foraging efficiency under dry conditions,

atrazine also increased the duration of sojourns from cover that lengthened time spent foraging. Consequently, under dry conditions, previous atrazine exposure did not significantly affect the total number of crickets eaten because the longer time spent foraging offset the reduced foraging efficiency. Under wet conditions, however, most of the salamanders did not bury themselves and thus their foraging durations were similar across atrazine treatments. Thus, atrazine negatively affected cricket consumption under wet conditions by reducing foraging efficiency. These results highlight the complex and perhaps unexpected ways factors can interact with climate.

The effects of atrazine on behavior, foraging, and loss of mass manifested as adverse effects on performance and significantly shortened salamander time to death and substantially ($p = 0.062$) reduced the proportion of salamanders surviving until the end of the experiment. These results are consistent with a previous study (Rohr et al. 2006) in which early-life exposure of streamside salamanders to atrazine elevated mortality later in life when maintained under similar conditions as in this study (approximately 24 °C and nearly saturated soil). Although atrazine was associated with reduced mass and survival, the magnitude of this effect was small relative to the effects of the temperature and moisture treatments on these responses. In addition, there was little evidence that the effect of atrazine on survival strongly depended on the tested temperature or moisture conditions.

Global warming is expected to accelerate the larval development of amphibians and aquatic insects and thus may reduce their exposure to chemical contaminants that concentrate in water bodies (Rohr et al. 2011). Consequently, the net effects of climate change and any contaminant will be challenging to forecast and require the quantification of how climate change affects both exposure and sensitivity to chemicals (Rohr et al. 2011).

### Implications for Conservation

We found a suite of behaviors streamside salamanders use to compensate for shifts in moisture and temperature that occur naturally and that may become more common as climate changes. Despite these behaviors, streamside salamanders experienced considerable mass loss and mortality in all but cool and moist conditions (closest to their preferred natural climate), which indicates these adaptations cannot fully compensate for prolonged adverse environmental conditions.

Consistent with a recent validated physiological model accounting for lizard declines in Mexico (Sinervo et al. 2010), our findings support the notion that moderate shifts in temperature or moisture, within the noncritical range of ectotherms, can cause both sharp and gradual mortality in ectothermic vertebrates, perhaps by

altering metabolic demands, hydration, or foraging effort. Moreover, this hypothesis became even more plausible when the persistent effects of exposure to a common abiotic factor, the herbicide atrazine, were considered. Atrazine exposure additively increased mortality risk of postmetamorphic salamanders above that caused by elevated temperature and dryness. This result suggests that common stressors may increase the fitness consequences of climate change. Furthermore, for many species of amphibians, the loss of postmetamorphic individuals has the largest effect on populations because density-mediated compensation in survival at this stage of life is less common than during the embryo and larval stages (Vonesh & De la Cruz 2002; Rohr et al. 2006). Even though few, if any, amphibian or reptile population declines are known to be associated with temperature or moisture levels that exceed their critical maxima or minima, this study provides rare empirical evidence to support the hypothesis that population declines are plausible even if critical maxima or minima are not exceeded. Hence, our findings indicate climate change within the noncritical range of species and pollution can reduce performance (mass and survival), potentially facilitating species declines. Although our results bolster the plausibility of climatic shifts within the noncritical range causing substantial amphibian mortality, it does not demonstrate that climate change was the cause of any declines and does not incorporate evolutionary responses to climate change. Further studies are needed to demonstrate more conclusively a causal link between climate change and the present losses of herptofaunal diversity.

## Acknowledgments

## Supporting Information

The mean (±95% confidence interval) mass of "wet" and "dry" terraria after one week of exposure to 22 or 27° (Appendix S1). Supporting Results: We weighed the terraria at the end of each week to quantify water loss and to know how much water to add to the terraria in an effort to keep moisture content within the two moisture treatments constant. (Appendix S2). The authors are solely responsible for the content of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

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