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# Will climate change reduce the effects of a pesticide on amphibians?: partitioning effects on exposure and susceptibility to contaminants

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## Abstract

Several studies suggest that global climate change could increase the toxicity of contaminants, but none of these studies explicitly integrate the effects of climate change on both susceptibility and duration of exposure to pollution. For many amphibian and aquatic insect species, exposure to contaminants is probably greatest during their fully aquatic embryonic and larval stages because these stages cannot readily escape water bodies where many contaminants accumulate and concentrate. Hence, by accelerating embryonic and larval development, global warming might reduce the duration of contaminant exposure for these taxa. To test this hypothesis, we isolated the effects of a temperature gradient (13–25 °C) on susceptibility (toxicity at a controlled exposure duration) and exposure of the streamside salamander, *Ambystoma barbouri*, to the herbicide atrazine (0, 4, 40, and 400 $\mu g\,L^{-1}$) by quantifying growth, survival, hatching, and metamorphosis under an atrazine exposure duration that was either constant or that depended on time to metamorphosis (and thus temperature). Increasing atrazine concentrations reduced growth, delayed hatching and metamorphosis, and decreased embryonic and larval survival. Increasing temperatures enhanced growth, accelerated development, and reduced survival for embryos but not larvae. With the exception of growth, increasing temperatures generally did not enhance the toxicity of atrazine, but they did generally ameliorate the adverse effects of atrazine by accelerating development and reducing the duration of atrazine exposure. The actual effects of climate change on contaminants remains difficult to predict because temperature changes can affect chemical use, uptake, excretion, biotransformation, fate, transport, and bioavailability. However, this work highlights the importance of explicitly considering how climate change will affect both exposure and toxicity to contaminants to accurately assess risk.

*Keywords:* *Ambystoma barbouri*, amphibian decline, atrazine, climate change, contaminant, exposure, global warming, pollution, susceptibility, toxicity

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## Introduction

Understanding the impacts of global climate change for life on earth represents one of the most formidable challenges of our time (Vitousek *et al*., 1997; Parmesan & Yohe, 2003; Root *et al*., 2003; Rohr *et al*., 2008a; Rohr & Raffel, 2010). Climate change is expected to differentially affect homeostasis and other physiological mechanisms of species (Gordon, 2003; Raffel *et al*., 2006; Martin *et al*., 2010), and thus there is good reason to believe that climate change will affect biotic responses to many natural and anthropogenic factors (Vitousek *et al*., 1997; Blaustein & Kiesecker, 2002; Rohr & Madison, 2003). One potentially important and ubiquitous stressor whose effects could be modified by climate change is pollution (Hojer *et al*., 2001; Schiedek *et al*., 2007; Noyes *et al*., 2009). In the European Union and United States, there are approximately 100 000 registered chemicals being released into the environment (EU 2001; Kiely *et al*., 2004). In the United States, coal combustion emits >120 million tons of toxic industrial waste (The National Research Council, 2006) and there are over a billion tons of pesticide products used annually (Kiely *et al*., 2004). Clearly, wildlife is commonly encountering pollution, which can threaten its existence (Rohr *et al*., 2006a). Indeed, pollution is considered the second greatest threat to aquatic and amphibious species behind habitat loss in the United States (Wilcove & Master, 2005), and wildlife is likely to simultaneously encounter pollution and climate change more regularly in the future (Vitousek *et al*., 1997; Noyes *et al*., 2009). Nevertheless, both pollution and climate change are considered two of the most understudied stressors in conservation science (Lawler *et al*., 2006).

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Several studies have examined the interactive effects of climate and chemical contaminants on biota. Some of these studies suggest that climate change will increase the toxicity of contaminants (Monserrat & Bianchini, 1995; Hojer *et al*., 2001; Capkin *et al*., 2006; Cherkasov *et al*., 2006; Khan *et al*., 2006; Osterauer & Kohler, 2008; Delorenzo *et al*., 2009) or that contaminants will reduce tolerances to extreme temperatures (Heath *et al*., 1994; Gaunt & Barker, 2000; Lannig *et al*., 2006; Patra *et al*., 2007), perhaps because an organism has finite resources to allocate to competing selection pressures (Rohr *et al*., 2003b) and thus multiple stressors tend to decrease energy available for detoxification or temperature regulation (Noyes *et al*., 2009). In contrast, there are several studies revealing that excretion or tolerance of chemicals is positively associated with temperature (Horne & Dunson, 1994; Morgan *et al*., 2001; Maruya *et al*., 2005; Talent, 2005; Paterson *et al*., 2007; Harwood *et al*., 2009; Weston *et al*., 2009). In all of these studies, the duration of chemical and temperature exposures were controlled across treatments, but it is well known that both temperature and contaminants can affect the growth and development of species (Akkerhuis *et al*., 1999; Rohr *et al*., 2003a, 2004).

For many amphibian and aquatic insect species, exposure to contaminants is probably greatest during their fully aquatic embryonic and larval life-history stages (as opposed to their more terrestrial adult stages) because these stages cannot readily escape water bodies where many contaminants accumulate and concentrate (Rohr & Palmer, 2005). Hence, temperature increases associated with global climate change might accelerate embryonic and larval development, reducing the length of the aquatic stages of certain amphibian and insect species and thus the duration of their contaminant exposure. If climate change reduces exposure to contaminants more than it enhances contaminant toxicity, then its net effect could be positive for some species. Consequently, we must quantify changes to both duration of exposure and susceptibility to adequately assess risk (Rohr *et al*., 2006b; Rohr *et al*., 2008c), which has not been attempted in previous climate change-contaminant studies. In this paper, we refer to susceptibility or toxicity as the effect of a chemical at a controlled exposure duration. In contrast, effects of a chemical that can be isolated to alterations in the duration of the chemical–organismal interaction are referred to as effects driven by changes in exposure to the chemical, rather than changes in susceptibility.

Amphibians in particular might be sensitive to climate change–contaminant interactions because their permeable skin offers little resistance to evaporative water loss or contaminant uptake (Rohr & Madison, 2003; Quaranta *et al*., 2009) and, given that they are ectotherms, amphibians can be sensitive to temperature changes. Indeed, several studies suggest that exposure to contaminants and climate change scenarios might be worse for amphibians than either stressor alone (Materna *et al*., 1995; Boone & Bridges, 1999; Broomhall 2002; Broomhall 2004; Rohr & Palmer, 2005). Moreover, amphibians are considered to be the most threatened vertebrate taxon on the globe (Stuart *et al*., 2004).

Here, we test for an interaction between a temperature gradient and exposure to the herbicide atrazine on growth, survival, and hatching and metamorphic timing of the streamside salamander, *Ambystoma barbouri*, isolating effects of temperature on both exposure and susceptibility to atrazine. Atrazine is the second most commonly used pesticide in the United States (Kiely *et al*., 2004), is a widespread contaminant in freshwaters where amphibians develop, and is persistent and mobile relative to most other current-use pesticides (Rohr & McCoy, 2010). For amphibians, authors have reported that atrazine affects behavior and physiology (Larson *et al*., 1998; Rohr *et al*., 2003a, 2004; Rohr & Palmer, 2005), elevates mortality (Storrs & Kiesecker, 2004; Rohr *et al*., 2006b; Rohr *et al*., 2008c), increases infections and suppresses immunity (Brodkin *et al*., 2007; Rohr *et al*., 2008b; Rohr *et al*., 2008c), disrupts the endocrine system (Hayes *et al*., 2002; Hayes *et al*., 2003), and induces community-wide, indirect effects (Boone & James, 2003; Rohr & Crumrine, 2005). A recent meta-analysis revealed that all of these effects appear to be general, with the exception of elevated mortality (Rohr & McCoy, 2010). Further, climate change has also been shown to enhance the risk posed by atrazine exposure for several freshwater organisms (Gaunt & Barker, 2000; Rohr & Palmer, 2005; Osterauer & Kohler, 2008).

The focal amphibian, *A. barbouri*, is found in central Kentucky, southern Indiana, and southern Ohio (Petranka, 1998), a region of frequent atrazine use (Solomon *et al*., 1996). It is a species of conservation concern because of its small geographic range, isolated populations, and local declines (Petranka, 1998). We hypothesized that increasing temperatures would increase the toxicity of atrazine (i.e. increase susceptibility) for *A. barbouri*, given that several previous studies have shown that extreme temperatures increase the toxicity of contaminants and because energy used to manage extreme temperatures might reduce energy that could be used for detoxification. In contrast, we also hypothesized that increasing temperatures would accelerate *A. barbouri* development, reducing its aquatic exposure to, and thus the effects of, atrazine (i.e. reduce atrazine exposure). However, no hypotheses were made regarding the net effect of these expected temperature-induced changes to atrazine susceptibility and exposure.

## Materials and methods

### *Animal collection and maintenance*

Fourteen *A. barbouri* egg clutches were collected from Fossil Creek (Jessamine County, KY, USA) in February 2004 soon after they were laid. The eggs were separated (Harrison stage 18–28), placed into an aquarium, and mixed thoroughly to randomize genetic variation. We conducted two experiments on *A. barbouri*, one that began with, and thus emphasized, effects on embryos and another that began with, and emphasized, effects on larvae. For both experiments, 12 arbitrarily chosen *A. barbouri* were placed into each of 64 glass bowls (3.7 L), 16 located in each of four Percival environmental chambers maintaining a 12-h light : dark photoperiod. Each of the 64 bowls contained a submerged, translucent, gray semicircular glass refuge plate (9 cm radius, 1 cm from the bottom) and 2 L of charcoal-filtered, dechlorinated municipal water (pH $\sim$8) that was constantly aerated through a glass pipet. All larvae were fed live blackworms, *Lumbriculus variegatus*, *ad libitum* throughout each experiment.

### *Dosing and water changes*

A stock solution of technical grade atrazine (purity: 99%, Chemservice, West Chester, PA, USA) was prepared by dissolving 8.984 mg atrazine $mL^{-1}$ of acetone. This stock solution was kept in an amber glass bottles to minimize photodegradation and stored at $-20\,^{\circ}C$. Glass bowls were treated with either 165 mL of acetone, or 1.6, 16.5, or 165 mL of stock solution, producing nominal concentrations of 0, 4, 40, and 400 $\mu g\,L^{-1}$ of atrazine, respectively. The highest concentration approximates the expected environmental concentration of atrazine when first registered and the two lowest concentrations are commonly found in freshwater environments (Rohr & McCoy, 2010). Flame ionization detection gas chromatography was used to verify actual concentrations of stock solutions (A. Elskus, unpublished results). We did not include a water control in our design because similar solvent concentrations were shown to have no significant effects on *A. barbouri* survival, life history, or behavior (Rohr *et al*., 2003a). A previous study revealed that atrazine degradation under similar experimental conditions was negligible over a 1-week period (Rohr *et al*., 2004). Hence, for the duration of the experiments (until all embryos hatched/died or until all larvae metamorphosed/died for the embryo and larval experiments, respectively), full water changes were conducted for each bowl each week and appropriate atrazine concentrations were reapplied based on treatment assignment.

### *Embryonic experiment*

For the embryonic experiment, two environmental chambers were set at 13 °C and two were set at 19 °C. We chose a 6 °C spread because the Intergovernmental Panel on Climate Change predicts that, by 2100, global temperatures could increase as much as 5.8 °C due to global warming (Houghton *et al*., 2001). Each chamber had four replicates of each of the four atrazine concentrations. Embryos that hatched were left in their bowls. We did not partition the effects of temperature on duration of exposure vs. its effects on susceptibility to atrazine in this experiment because the embryo stage is short (typically 1–3 weeks) relative to the larval stage (usually $>8$ weeks), and thus we expected little temperature-driven variation in embryonic exposure to atrazine. Furthermore, after hatching, unlike after metamorphosis, the larvae remain in the aquatic environment where atrazine can accumulate and concentrate. The embryonic experiment lasted until all embryos hatched or were dead (49 days), at which point, all live larvae were euthanized with MS-222 and preserved in neutral buffered formalin. All bowls were checked daily for hatching and mortality and larval mass was determined from preserved specimens.

### *Larval experiment*

For the larval experiment, we wanted to cover a greater range of temperatures than in the embryonic experiment because larvae typically experience greater variability in temperature as a result of the larval period being longer than the embryonic period. Furthermore, larvae tend to experience warmer temperatures than embryos because temperatures tend to increase after eggs are laid (Petranka, 1998). Hence, each of the four environmental chambers was set at a different temperature, 16, 19, 22, and 25 °C. The intent of this 9 °C gradient is not to simulate climate change but to generate regression parameters that could be implemented in climate change models if one chose to do so.

Like in the embryonic experiment, each chamber had four replicates of each of the four atrazine concentrations. However, in an effort to partition the effects of temperature on exposure and susceptibility to atrazine, two replicates of each atrazine concentration in each chamber were transferred to water without atrazine (but with acetone) once the first larva metamorphosed, 19 days into the experiment (from the 25 °C chamber). These eight replicates in each chamber received the acetone control treatment for the remainder of the experiment. Thus, half the replicates in each chamber had 19 days of atrazine exposure and the other half were exposed to atrazine until they metamorphosed. This provided a standardized atrazine-exposure-duration for each temperature and an atrazine-exposure duration that varied with temperature, the scenario that occurs in nature. Unexposed larvae from the initial pool of field-collected clutches were used in this experiment, not the larvae from the embryonic experiment. These larvae were reared identically before use in this experiment (as described above in 'Animal collection and maintenance'), homogenized, and then distributed haphazardly among the replicates. Nineteen days should not be interpreted as the length of the larval period at 25 °C because the larvae had developed before their use in the experiment.

We quantified mortality and metamorphosis daily, and this experiment lasted until all larvae metamorphosed or died (78 days from the start of the experiment). Upon metamorphosis or death, salamanders were euthanized with MS-222 and preserved in neutral buffered formalin, and the mass of

metamorphs was determined from preserved specimens. In both the embryonic and larval experiments, temperatures and treatments were assigned randomly, and temperatures were verified with a digital thermometer at water changes.

### Statistical analyses

Atrazine concentration has a log relationship with most *A. barbouri* response variables tested in previous studies (Rohr *et al*., 2003a, 2004; Rohr & Palmer, 2005; Rohr *et al*., 2006b), and thus atrazine concentration was log-transformed before all analyses here, which improved statistical fit (based on $R^2$). For the embryonic experiment, hatching day and larval mass were log transformed and analyzed with the general linear model, with atrazine nested within temperature treatments. This ensured proper error degrees of freedom for the effect of temperature (only two error degrees of freedom). We tested for the main effects of temperature and atrazine (a continuous predictor) and their interaction. For embryonic and larval survival, the effects of temperature, atrazine, and their interaction were analyzed using the generalized linear model with a binomial error distribution, a logit link function, and a type III likelihood ratio test. Importantly, any statistical interaction between temperature and atrazine would indicate that the toxicity of atrazine would likely be altered with changes in temperature associated with climate change.

In the embryonic experiment, the timing of hatching influenced how long individuals were larvae, which consequently influenced the amount of time available for larval growth and death. Hence, in our statistical models testing for effects of atrazine and temperature on log larval mass at the end of the experiment and larval survival (number of surviving larvae/number of surviving embryos), we incorporated log hatching day as a covariate to ensure that effects on these responses were independent of timing of hatching. In addition, arcsine-square-root-transformed survival was used as a covariate for the larval mass analyses because survival can affect competition for resources and thus growth rates. For larval survival and mass, there were no significant interactions between the covariates and log atrazine or temperature, indicating that we met the assumptions of homogeneity of slopes. Hence, interactions with the covariates were dropped from these models.

The larval experiment could not be analyzed the same way as the embryonic experiment because we did not have replication of each temperature and thus we needed to take a regression-based approach to test for any factors containing the effect of temperature. For each temperature-by-exposure-duration combination, we calculated a slope parameter for the relationship between log atrazine concentration and arcsine-square-root-transformed survival and between log atrazine concentration and log-transformed day of, and mass at, metamorphosis with survival as a covariate (arcsine-square-root transformed). We controlled for survival in the day of, and mass at, metamorphosis analyses because density can influence timing of, and size at, metamorphosis, even when amphibians are fed *ad libitum* (Rohr *et al*., 2004; there was, again, no evidence of an interaction between covariates and predictors in these models). Thus, for each response variable, we generated two atrazine slope parameters for each of the four temperatures, one for the 19-day atrazine exposure and one for the case where atrazine exposure persisted until metamorphosis. We emphasize the slope parameter because it represents the effect size for the atrazine treatment. To test whether the strength of the atrazine effect (i.e. the slope) depended on both temperature and the duration of atrazine exposure, we conducted a split-plot analysis in the general linear model where the atrazine slope parameters for the two exposure duration treatments were nested within the continuous temperature treatment.

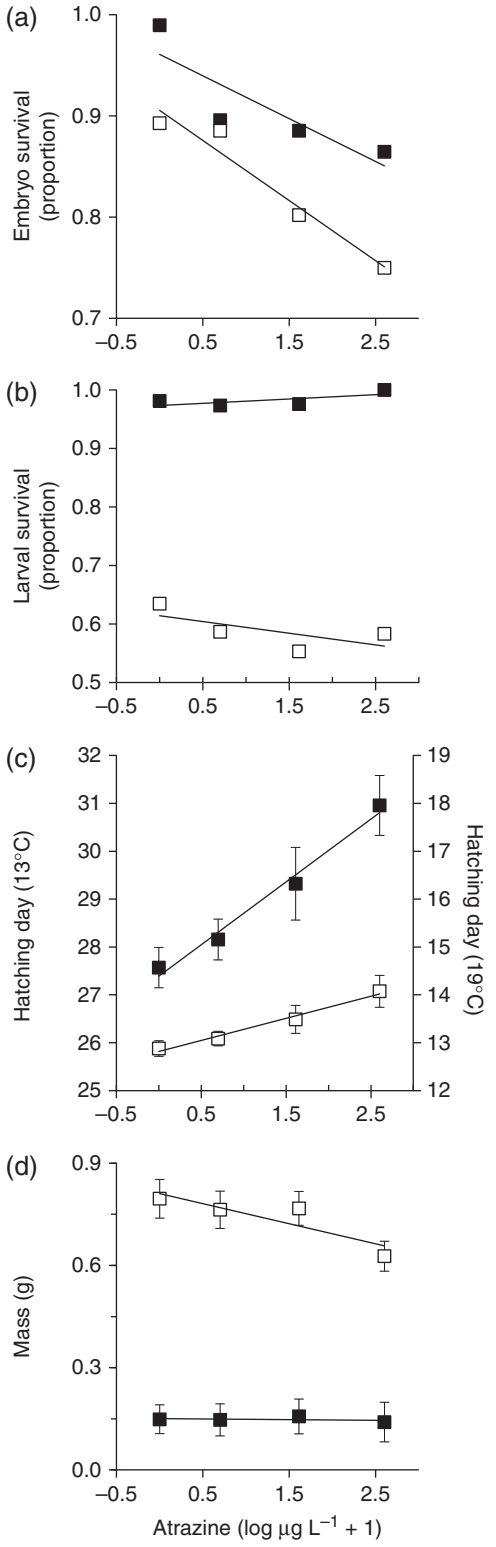
Unlike statistical factors that included the effect of temperature, factors that only included the effects of atrazine or exposure duration could use all 64 replicates. Thus, the effects of these factors and their interactions on larval survival (controlling for the effect of temperature) were tested using the generalized linear model with a binomial error distribution, a logit link function, and a type III likelihood ratio test. The factorial effects of atrazine and exposure duration on log day of, and mass at, metamorphosis (controlling for the effect of temperature) were tested using the general linear model and with arcsine-square-root survival as a covariate. Dunnet's multiple comparison tests were used to compare responses in the solvent control treatment to responses at each of the atrazine concentrations. We used the effect size estimator partial eta-squared ($\eta_p^2$) to compare the relative strengths of significant susceptibility- and exposure-mediated interactions between temperature and atrazine. All statistical analyses were conducted with STATISTICA 9.1 (Statsoft Inc., Tulsa, OK, USA).

## Results

### Embryonic experiment

Despite both embryos and larvae being more likely to die at 19 than 13 °C ($\chi^2 = 3.827$, $P = 0.050$; $\chi^2 = 8.867$, $P = 0.003$, respectively; Fig. 1a and b), A. *barbouri* hatched sooner ($F_{1,2} = 1242.35$, $P < 0.001$; Fig. 1c) and were heavier ($F_{1,2} = 22.34$, $P = 0.041$; Fig. 1d) at the warmer temperature, even after controlling for survival and hatching differences among replicates in the mass analyses. Atrazine significantly delayed hatching ($F_{1,58} = 32.96$, $P < 0.001$; Fig. 1c), reduced embryonic ($\chi^2 = 16.554$, $P < 0.001$, Fig. 1a), but not larval, survival ($\chi^2 = 0.272$, $P = 0.602$, Fig. 1b), and reduced larval mass ($F_{1,56} = 4.045$, $P = 0.049$; both survival and hatching day were covariates; Fig. 1d). All concentrations of atrazine induced significant embryonic mortality ($P < 0.011$), but only 400 $\mu g\,L^{-1}$ significantly delayed hatching ($P < 0.001$) and reduced larval mass ($P = 0.011$) relative to the solvent control. We did not detect a significant interaction between temperature and atrazine treatments for timing of hatching ($F_{1,58} = 0.62$, $P = 0.434$; Fig. 1c), embryonic survival ($\chi^2 = 0.135$, $P = 0.713$, Fig. 1a), or larval survival ($\chi^2 = 0.003$, $P = 0.955$; Fig. 1b).

However, atrazine decreased larval mass more at 19 than at 13 °C (temperature × atrazine: $F_{1,56} = 7.627$, $P = 0.008$; both survival and hatching day were covariates; Fig. 1d).




*Larval experiment*

There was a trend for atrazine concentration to be a significant negative predictor of larval survival ($\chi^2 = 3.082$, $P = 0.079$, Fig. 2). Given that Rohr *et al.*, (2004) has already shown that the tested atrazine concentrations, under similar experimental conditions, significantly reduced the survival of larval *A. barbouri*, a one-tailed test might be justified, which would place this *P*-value below the alpha significance threshold. This effect of atrazine on survival, however, did not significantly depend on the temperature or exposure duration treatments given our statistical power ($P > 0.15$; Fig. 3a). Atrazine delayed metamorphosis ($F_{1,54} = 9.013$, $P = 0.004$; Fig. 4a) and reduced mass at metamorphosis ($F_{1,54} = 7.886$, $P = 0.007$; Fig. 4b), and temperature was positively associated with developmental rate ($F_{1,2} = 198.725$, $P = 0.005$; Fig. 4a), but did not have a significant effect on size at metamorphosis ($F_{1,2} = 13.405$, $P = 0.067$; Fig. 4b).

The effects of atrazine on development and size at metamorphosis, however, significantly depended on both the temperature and exposure duration treatments, resulting in a significant interaction between temperature and duration for the slope of the atrazine effect on timing of, and size at, metamorphosis ($F_{1,2} = 19.137$, $P = 0.0485$; $F_{1,2} = 39.539$, $P = 0.024$, respectively; Fig. 3b and c). Given this significant interaction, we admonish that the main effects should thus be interpreted with caution. When the atrazine exposure duration was 19 days regardless of temperature, the effect of atrazine on timing of, and size at, metamorphosis was moderate and independent of temperature (Fig. 3b and c). Hence, given this atrazine exposure duration, temperature did not significantly affect susceptibility to, or toxicity of, atrazine. However, when atrazine exposure duration was allowed to depend on temperature (up to 78 days of exposure), exposure duration increased with decreasing temperatures (Fig. 4a). Consequently, increasing temperatures decreased the effects of atrazine on both timing of, and size at, metamorphosis presumably by reducing exposure, rather than susceptibility, to atrazine (Fig. 3b and c).

**Fig. 1** Effects of temperature (13 °C, closed symbols and 19 °C, open symbols) and atrazine concentrations (0, 4, 40, and 400 µg L$^{-1}$) on *Ambystoma barbouri* (a) embryonic survival, (b) larval survival, (c) timing of hatching, and (d) larval mass after 49 days of treatment exposure (least squares means with log hatching day as a covariate). Shown are best-fit lines and weighted means in (a and c) and least squares means (controlling for hatching day) in (b and d) ($n = 8$). Standard errors are shown only for data that are normally distributed (i.e. not binomially distributed data). See 'Results' for statistics.

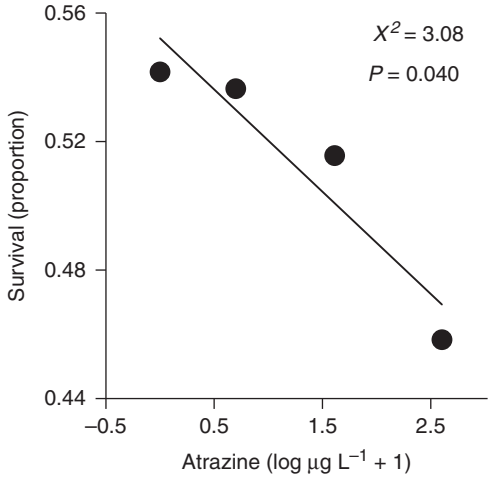



**Fig. 2** Effects of atrazine concentrations (0, 4, 40, and 400 µg L$^{-1}$) on the survival of larval *Ambystoma barbouri* averaging across temperature and atrazine exposure duration treatments. Shown are means ($n = 16$), a best-fit line, and a one-tailed $P$-value.

The size of the temperature-by-atrazine effect on larval mass mediated by changes in exposure (i.e. in the larval experiment) was 12 times larger ($\eta_P^2 = 0.803$) than the effect mediated by changes in susceptibility (i.e. in the embryonic experiment; $\eta_P^2 = 0.066$). Hence, although atrazine had a greater adverse effect on growth at warmer than cooler temperatures when the duration of atrazine exposure was controlled (embryonic experiment), this increase in toxicity was small in comparison to the positive effects of increased temperature by accelerating *A. barbouri* development and thus reducing their exposure to atrazine (larval experiment).

## Discussion

In this study we provide evidence that by increasing mean temperatures, climate change might reduce the duration of the obligate aquatic embryonic and larval stages of *A. barbouri*, and perhaps other amphibians, reducing their duration of exposure to chemical contaminants that often wash into and accumulate in aquatic ecosystems. Temperature increases generally did not increase *A. barbouri* susceptibility to atrazine (the exception was growth in the embryonic experiment), but temperature was negatively associated with atrazine exposure duration, thus reducing the strength of atrazine effects on various *A. barbouri* endpoints. That is, by reducing atrazine exposure, elevated temperatures reduced the effects of atrazine on time of, and size at, metamorphosis (Fig. 3b and c).

The effects of environmentally realistic concentrations of atrazine observed in this study, specifically the reductions in embryonic and larval survival, decreases in larval mass, and delays in hatching, match the findings of Rohr *et al*. (2004) for the same species and atrazine concentrations. The exception was that Rohr *et al*. (2004) detected accelerated metamorphosis in response to atrazine rather than the delayed metamorphosis observed here. However, a recent meta-analysis on the effects of atrazine on amphibians revealed that atrazine can both accelerate and delay metamorphosis and that the effect of atrazine on metamorphosis is likely nonlinear and context dependent (Rohr & McCoy, 2010). Hence, the effects of atrazine on this highly plastic trait are considerably variable. By delaying development at relatively low temperatures, atrazine might increase the duration of exposure to aquatic contaminants in general.

The strength of atrazine effects increased with increasing atrazine concentration and exposure duration, suggesting that the effects of atrazine on amphibian populations will likely depend on both of these factors. In addition, the effects of atrazine in nature will likely depend on the strength of negative density dependence for the focal species (Vonesh & De la Cruz, 2002). Negative density dependence can ameliorate the adverse effects of contaminants later in life by reducing competition for the survivors of the contaminant exposure (Rohr *et al*., 2006b). Although the duration of atrazine exposure appears to modulate atrazine effects, some effects of early-life exposure to atrazine can be delayed and might even be permanent, such as effects on the endocrine system, gonadal development, behavior, and survival (Stoker *et al*., 1999; Cooper *et al*., 2000; Hayes *et al*., 2002; Hayes *et al*., 2003; Rayner *et al*., 2005; Rohr & Palmer, 2005; Rohr *et al*., 2006b). It is unclear whether these important and potentially permanent effects of atrazine depend more so on the duration or the timing of atrazine exposure. If they depend more on the timing of exposure, then the effect of climate change might be more difficult to predict than if they depend more on the duration of exposure.

Several studies have examined the effects of climate change scenarios on the toxicity of chemicals to aquatic organisms. Increasing temperatures were shown to increase the toxicity of atrazine to catfish (Gaunt & Barker, 2000) and to amplify the adverse effects of amphibian exposure to the insecticides carbaryl, endosulfan, and esfenvalerate (Materna *et al*., 1995; Boone & Bridges, 1999; Broomhall, 2002; Broomhall, 2004). Similarly, atrazine exposure increased salamander desiccation risk up to 8 months after atrazine exposure (Rohr & Palmer, 2005). These results are consistent with atrazine reducing *A. barbouri* larval mass more at 19 than at 13 °C in our embryonic experiment, where the atrazine exposure duration was 49 days (as opposed to 19 days in the

larval experiment). Atrazine did not reduce larval mass more at high than low temperatures in the larval experiment at a constant exposure duration probably because the duration treatment was much shorter than in the embryonic experiment (only 19 vs. 49 days for the embryonic experiment). Further, the standardized effect sizes revealed that the temperature-enhanced toxicity of atrazine on larval growth was considerably smaller than the reduction in exposure to atrazine associated with elevated temperatures. Moreover, for the most part, we did not find consistent evidence that the effects of contaminant exposure increased with increasing temperature. Rather, for most endpoints, our findings suggest that climate change might reduce *A. barbouri* exposure to contaminants by reducing the duration of aquatic exposure to chemicals via a shortening of the larval period.

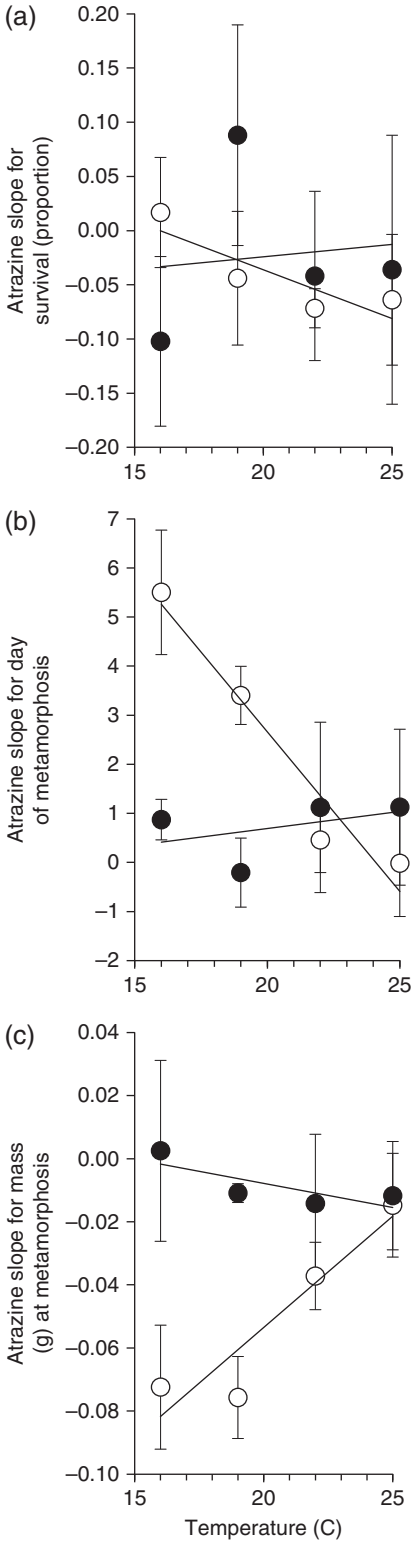



**Fig. 3** Effects of temperature (16, 19, 22, and 25 °C) and atrazine exposure durations (19 days, closed symbols or until metamorphosis, open symbols) on the relationship between atrazine concentrations (0, 4, 40, and 400 μg $L^{-1}$) and *Ambystoma barbouri* (a) larval survival, (b) timing of metamorphosis, and (c) mass at metamorphosis. Shown are slope parameters for the relationship between log atrazine concentrations and each response, the standard errors of these parameters, and best-fit lines. See 'Results' for statistics.

The impact of climate change on risk from chemical contamination is clearly complicated, but undoubtedly will require assimilating the effects of climate change on both exposure and susceptibility to contaminants. Projected increases in temperature will likely accelerate development for embryonic and larval stages of many aquatic insects and amphibians, reducing their aquatic exposure to contaminants; however, this increase might also enhance chemical toxicity, emphasizing the importance of quantifying the net effect of both processes. Temperature changes can differentially affect chemical uptake, excretion, biotransformation, fate, transport, and bioavailability and can affect the neuroendocrine system of species, all in directions that are difficult to predict (Noyes *et al*., 2009) and that were not explicitly accounted for in the present study. Furthermore, climate change will alter climatic factors other than temperature, such as precipitation, which too might affect exposure and susceptibility to contaminants. The use and demand of pesticides and industrial chemicals might also be considerably altered by climate change (Noyes *et al*., 2009), influencing exposure to contaminants independent of how climate change might affect chemical exposure through altered developmental rates. For example, a recent long-term experiment and meta-analysis revealed that elevations in $CO_2$ associated with global climate change typically reduce arthropod herbivory (Stiling & Cornelissen, 2007; Stiling *et al*., 2009), suggesting that climate change might reduce insecticide use. However, most of these studies did not simultaneously increase temperature and $CO_2$, which is

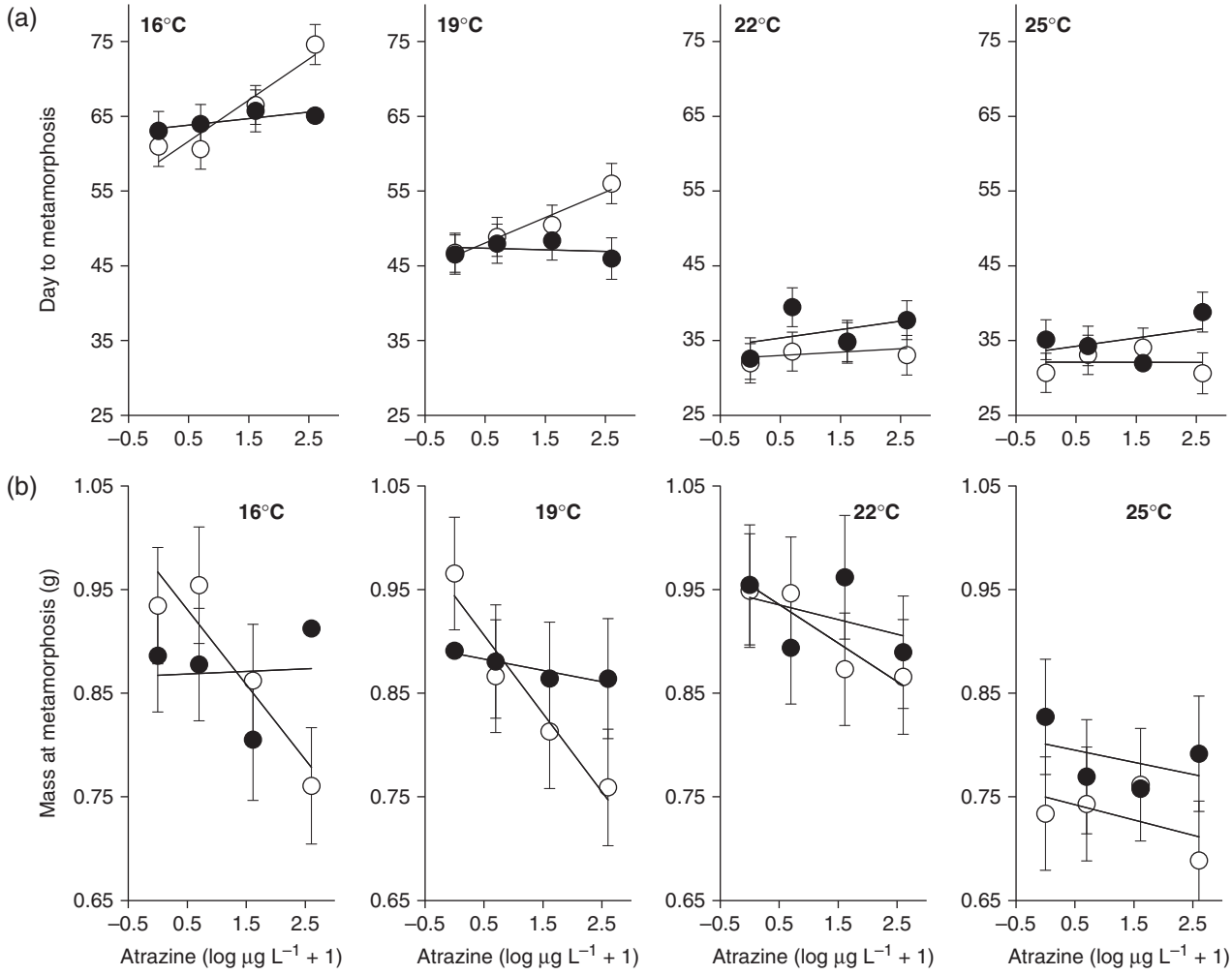



**Fig. 4** Effects of temperature (16, 19, 22, and 25 °C), atrazine concentrations (0, 4, 40, and 400 µg $L^{-1}$), and atrazine exposure durations (19 days, closed symbols or until metamorphosis, open symbols) on *Ambystoma barbouri* (a) timing of metamorphosis and (b) mass at metamorphosis. Shown are means ($n = 2$), standard errors, and best-fit lines. Some data points do not have error bars because one of the two replicates had no animals metamorphose. See 'Results' for statistics.

expected with climate change, and it is also unclear how climate change will affect the use of other pesticides or industrial chemicals.

Although our study suggests that climate change could ameliorate the adverse effects of atrazine exposure, this is undoubtedly an oversimplification of how pollution and climate change will interact in the future. Even if climate change reduces the adverse effects of contaminants for certain species, this should not be misconstrued as climate change being beneficial for these species or biota in general. Climate change could have a net adverse effect on a species despite it reducing a species' exposure to contaminants. To comprehensively understand the combined effects of climate change and pollution, scientists will undoubtedly have to more thoroughly integrate the effects of changed climates on both susceptibility and exposure to contaminants.

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