



Will pre-adaptation buffer the impacts of climate change on novel species interactions?

Emily Herstoff and Mark C. Urban

E. Herstoff (emily.herstoff@stonybrook.edu) and M. C. Urban, Dept of Ecology and Evolutionary Biology, Univ. of Connecticut, 75 North Eagleville Rd., Unit 3043, Storrs, CT 06269, USA. Present address of EH: SUNY Stony Brook, Dept of Ecology and Evolution, 650 Life Sciences Building, Stony Brook, NY 11794-5245, USA.

Species are expected to alter their ranges as climates change. Climate-induced range expansions of predators could threaten evolutionarily naïve prey populations, leading to high mortality at the invasion front. If prey can apply existing defenses against local predators to novel predation threats induced by climate change, mortality threats will be less than expected. Here, we examine if spotted salamander larvae *Ambystoma maculatum* from populations that coexist with native red-spotted newts *Notophthalmus viridescens* survive better when exposed to a novel predator, the marbled salamander *Ambystoma opacum*. We show that regional mean winter temperatures warmed 2.0°C over 116 yr in the region, and that *A. opacum* survival increases in ponds with higher winter temperatures. Hence as winters continue to warm, this apex predator will likely colonize ponds north of their current range limit. Next, we performed common garden experiments to determine if local adaptations to native *N. viridescens* and exposure to *A. opacum* or *N. viridescens* kairomones (predator chemical cues) altered *A. maculatum* survival in predation trials. We did not find evidence for local adaptation to *N. viridescens*. However, *A. maculatum* from high-*N. viridescens* ponds that were reared with *A. opacum* kairomones suffered significantly higher mortality from the native predator *N. viridescens*. This outcome suggests an unanticipated interaction between local adaptation and plastic responses to novel kairomones from a potentially range-expanding predator. Current projections of biodiversity losses from climate change generally ignore the potential for eco-evolutionary interactions between native and range-expanding species and thus could be inaccurate.

General circulation models predict that global land temperatures will increase 1.8–4.0°C by the year 2100 (IPCC 2007). Species with high dispersal capacity might track their optimal temperatures as climate change shifts thermal habitats across the landscape. Indeed, evidence suggests that many species have already begun to shift their ranges in accordance with the predictions of climate change (Parmesan and Yohe 2003, Angert et al. 2011, Chen et al. 2011). These range expansions will lead to novel species interactions as expanding species encounter naïve populations for the first time. The evolutionary history of populations might play a substantial role in mediating these interactions. For instance, populations that have adapted to a native predator might fare better when exposed to an ecologically similar predator that has expanded its range with climate change. Surprisingly few studies have examined how evolutionary history and novel species interactions jointly determine effects of global climate change (for an exception see Sanford and Swezey 2008). Here, we evaluate for the first time how local adaptation to a native predator affects prey survival with a functionally similar predator that is potentially expanding its range northward with warmer winters.

In particular, we study how warmer winters might lead to the expansion of the apex temporary pond predator, the marbled salamander *Ambystoma opacum*, into northern New England and its subsequent effects on naïve prey populations of spotted salamanders *Ambystoma maculatum*. During the past 116 yr, winters in southern New Hampshire have warmed by 2.1°C (Fig. 1). As a result, ice cover now disappears from southern New England lakes an average of 16 d earlier than it did 150 yr ago (Hodgkins et al. 2002). General circulation models predict a further rise in mean winter temperatures of 4°C by 2100 for this region (Christensen et al. 2007). Shifts in aquatic communities, due in part to reduced ice cover, have already been observed in pond communities (Quinlan et al. 2005, Smol et al. 2005). Whereas previous studies have focused on the direct effects of climate change such as changes in phenology or physiological stresses on organisms, few studies have examined indirect effects of climate change such as shifts in predators and competitors (Corn 2005). Amphibian survival and reproduction are sensitive to temperature and water availability, which could make them more sensitive to rapid climate change (Carey and Alexander 2003). However, warmer ponds might also allow new species to colonize habitats that were previously

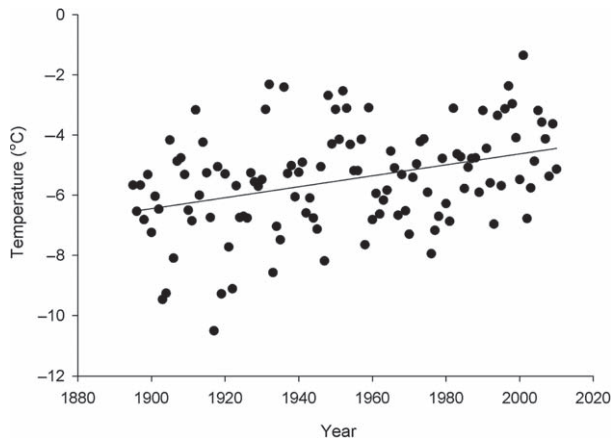


Figure 1. Average monthly temperature from 1895–2010 for meteorological winter (December, January and February) in southern New Hampshire (climate division 2). Line indicates ordinary least-squares fit to data (linear regression, slope = $0.018^{\circ}\text{C yr}^{-1}$, $p < 0.001$, $R^2 = 0.12$). Data obtained from National Oceanic and Atmospheric Administration, National Climatic Data Center.

unavailable to them, which in turn could substantially alter aquatic food webs (Quinlan et al. 2005). Thus, warming winters in New England could shift community composition in temporary ponds by allowing apex predators such as *A. opacum* to inhabit ponds from which they traditionally have been excluded.

Ambystoma opacum is an apex predator that occurs in temporary ponds throughout eastern North America (Urban 2007a). *Ambystoma opacum* does not currently inhabit the northern region of *A. maculatum*'s range in North America (Fig. 2). Unlike most temporary pond-breeding amphibians, *A. opacum* breeds in the fall and its larvae overwinter under ice cover. Warmer winters with less ice cover could create newly suitable habitats at the northern edge of *A. opacum*'s

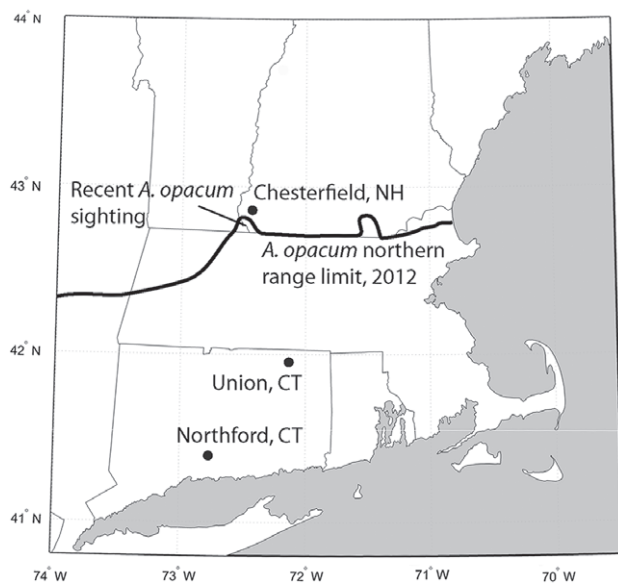


Figure 2. Current range of *A. opacum* in New England based on data from Petranka (1998) and RAARP (2010). Locations of field study sites of winter survival are also indicated.

range in central New England. Northward movement by *A. opacum* could facilitate high mortality in maladapted *A. maculatum* prey populations that have not faced selection from this predator in contemporary history, assuming that drift and selection on linked traits would erode traits under relaxed selection. However, local adaptation to a similar native predator, the red-spotted newt *Notophthalmus viridescens*, could lessen the impact of this novel predator if *A. maculatum* respond to *N. viridescens* in a manner that also reduces their risk from the novel *A. opacum*. Other research shows that *A. maculatum* populations have adapted to different predator communities in ponds separated by as little as 100 m, including evolving differences in foraging rates (Urban 2007b) and morphology (Urban 2010). These local adaptations have occurred despite substantial gene flow in this species (Zamudio and Wiczorek 2007), which indicates that strong selection can overcome moderate gene flow in this system (Nosil et al. 2005). Moreover, microgeographic adaptations have been shown for *A. maculatum* in response to other selection regimes such as salt from road run-off (Brady 2012), suggesting that local adaptation might be common in this species. Thus the opportunity exists for local adaptation in response to *N. viridescens* predation across the same spatial scales as local adaptations have been observed previously.

By coupling existing defenses for native predators to invading predators, certain native prey populations might be pre-adapted to survive novel predation threats (Edgell and Neufeld 2008). Previous research suggests that *A. maculatum* from our New Hampshire study site recognize kairomones from both *A. opacum* and *N. viridescens* (Urban 2008b). Kairomones are the chemical cues produced by predators as they consume and digest prey. These northern populations also survived better than more southern populations when exposed to *A. opacum* (Urban 2008b). However, it remains unknown if kairomone recognition and higher survival reflects something particular to the populations assayed in this prior experiment or a more generalized response because most of the *A. maculatum* populations chosen for this prior experiment came from ponds inhabited by high densities of *N. viridescens*.

In this manuscript, we first develop a link between *A. opacum*'s overwinter survival and changing winter temperatures in New England. We hypothesized that *A. opacum* overwinter survival would be greater in ponds with higher winter temperatures. Then we perform common garden experiments to evaluate the survival of *A. maculatum* larvae originating just beyond the current northern range limit of *A. opacum* based on *A. maculatum*'s local evolutionary history with or without predaceous *N. viridescens* and exposure to kairomones from either native *N. viridescens* or novel *A. opacum*. We tested to see if *A. maculatum* have become locally adapted to *N. viridescens*, and hypothesized that pre-existing local adaptations in high-*N. viridescens* ponds would enable *A. maculatum* to better survive in predation experiments both when exposed to native *N. viridescens* and the novel predator *A. opacum*. Specifically, we predicted that *A. maculatum* from high-*N. viridescens* ponds would evolve adaptive plasticity that would promote their survival with *A. opacum*, which feeds in a similar manner and is also gape-limited. We then evaluated kairomone-mediated

growth differences as a possible explanation of differences in survival among treatments and populations.

Material and methods

Natural history and study site

Ambystoma maculatum is a relatively large (15–25 cm total length) terrestrial salamander found throughout eastern North America. Each spring, *A. maculatum* adults migrate into temporary ponds to mate and to lay eggs. Small (~15 mg) aquatic larvae hatch after eight to ten weeks and metamorphose by late summer. Predaceous *A. opacum* larvae frequently co-occur with *A. maculatum* larvae in southern New England. Based on its densities (up to 3 m⁻²) and consumption rates, *A. opacum* is one of *A. maculatum*'s most important predators in southern New England (Urban 2007a).

Unlike most temporary pond amphibians that breed in the spring, *A. opacum* breeds in the autumn, and hence larvae must overwinter under ice cover. The density and the proportion of ponds inhabited by *A. opacum* steadily decreases from south to north in New England up to their northern range boundary at the border between Massachusetts and New Hampshire and Vermont, suggesting a possible climatic origin to their range boundary (Urban 2008b). *Ambystoma opacum* only rarely occur north of the Massachusetts state border (Fig. 2), and we have never recorded it at our long-term study site in southwestern New Hampshire. However, *A. opacum* has recently been reported in Hinsdale, New Hampshire (RAARP 2010), the town just south of our Chesterfield, New Hampshire study site. Climate change might fuel the northward range expansion of *A. opacum* if climate change enhances its overwinter survival.

At our New Hampshire study site, the most important gape-limited predator in temporary ponds is *N. viridescens* (Urban 2007a). *Notophthalmus viridescens* is widely distributed throughout eastern North America, and has a complex, variable life cycle, with adults returning to aquatic habitats following a terrestrial eft stage (Petranka 1998). The aquatic adult *N. viridescens* are generalist predators that readily prey upon *A. maculatum* larvae (Bishop 1941, Petranka 1998). Similarities can be drawn between the native *N. viridescens* and range-expanding *A. opacum*. Both predators are suction-feeding salamanders that use a combination of active hunting and sit-and-wait techniques (Hassinger et al. 1970, Martin 1974), and both species are important gape-limited predators in temporary ponds in New England (Urban 2007a). The gape of *A. opacum* is larger than *N. viridescens*, which allows it to feed for a longer period of time on growing *A. maculatum* larvae (Urban 2007a).

Ambystoma opacum survival and winter ice cover

In this experiment, we hypothesized that warmer winter pond temperatures would promote *A. opacum* survival. We monitored winter water temperatures in three sets of nine ponds distributed among three study sites in New England. The three sites are distributed along a latitudinal gradient in *A. opacum* occurrence and density from southern

to central New England (Urban 2007a). These sites were located in Northford, Connecticut (high-density *A. opacum* site), Union, Connecticut (moderate-density site), and Chesterfield, New Hampshire (no *A. opacum* ever sampled) (Fig. 2). Tidbit temperature loggers (Onset Computer Corporation, Bourne, MA, USA) were anchored to the bottom of each pond at the point of maximum depth. We recovered temperature loggers in the spring after the ice melted.

We conducted area-standardized dipnet surveys in the fall and spring at each pond at the two Connecticut study sites with *A. opacum* populations. We then calculated *A. opacum* winter survival as spring densities divided by fall densities. In one pond in Union, Connecticut, we estimated a greater density of larvae in the spring than in the fall, and here we set percent survival to its maximum of 100. We only analyzed survival for the ten ponds inhabited by *A. opacum* in the fall. We used logistic regression to analyze the relationship between percent *A. opacum* winter survival and minimum winter pond temperature. Substantial overdispersion necessitated a quasibinomial model (Crawley 2007).

Common garden experimental setup

Here we tested if local adaptation to *N. viridescens* and exposure to kairomones from different predator species increased *A. maculatum* survival in predation trials. We collected *A. maculatum* egg masses from three high- and three low-*N. viridescens* density ponds in Pisgah State Park, Chesterfield, New Hampshire. *Notophthalmus viridescens* density was determined based on earlier surveys and verified by surveys in the year of collection (Table 1). In high-newt-density ponds, newts occurred at average densities of 0.362 newts m⁻², whereas no newts were found during sampling of any of the low-newt-density ponds (Table 1). Eggs were collected within a few days of being laid and transported back to the laboratory on ice. Because not all eggs were laid at the same time, egg collection occurred over a two-week span. Collected eggs were held at 6°C in an incubator (Percival Scientific, Perry, IA, USA) on a 12-h light/dark cycle so that all eggs collected would be initiated in the outdoor enclosure at similar developmental stages and on the same date. Prior research suggests that these incubator temperatures

Table 1. Mean newt densities per meter squared in New Hampshire study ponds. Ponds were sampled over four years (2002–2004, 2011) with dip nets (17 × 25 cm, with 1.4 mm mesh). An area 0.8 m long was swept once every 20 m² (2002–2004) or every 10 m² (2011) of pond area, and total dip net sweeps were counted. Newt density was calculated by dividing the total number of newts collected by the total area sampled. We never detected newts in the low-newt ponds; high-newt ponds had an average density of 0.362 newts m⁻² (standard error = ± 0.231).

Pond name	Newt density	Mean newt density per meter squared	Standard error
P2	Low	0	0
P3	Low	0	0
P19	Low	0	0
P12	High	0.225	0.190
P25	High	0.645	0.418
P29	High	0.215	0.0845

minimize developmental rate without long-term fitness consequences (Urban 2008b). After egg collection was complete, egg masses were cut into equal-sized sections and placed in individual 19-l containers filled to 15 cm (\pm 1 cm), a natural depth for egg mass development in ponds, with treated, aged well water. In both years, egg stage did not significantly differ between high- and low-newt ponds (ANOVA, $p > 0.4$). For predation trials, we placed three sets of four eggs from each of the ponds sharing the same *N. viridescens* predation risk into each container. For the growth experiment, we placed six eggs from each family in each container. These rearing containers were maintained in an outdoor fenced enclosure covered with 50% shade cloth to simulate natural tree canopy cover.

We used a randomized block experimental design. This design ensured similar rearing conditions for the larvae, except for the kairomones received by the developing *A. maculatum* three times weekly. We ended the experiment six weeks after hatching, after which predation by these strongly gape-limited predators becomes minimal.

Kairomone treatments included *A. opacum* only, *N. viridescens* only, equal parts of both *A. opacum* and *N. viridescens* (growth experiment only), or a control (water with no predators that contained live 10 *A. maculatum* larvae, to replicate the larvae used for food in predator containers). In the growth experiment, we tested if kairomones generated differences in growth that might mediate survival with predators. The mixed kairomone treatment in the growth experiment allowed us to evaluate if growth differences were additive or not. Kairomone treatments were created by allowing each predator to consume *A. maculatum* larvae ad libitum, filtering equal amounts of water from each predator's container through 150 μ m mesh to remove any food resources, and then adding one liter of chemical cues to each experimental unit. Previous research shows that kairomones from each of the predator species elicits different behavioral responses (Urban 2008b). Whereas *N. viridescens* kairomones induced faster growth in *A. maculatum*, the larvae decreased their growth rate when exposed to *A. opacum* kairomones (Urban 2008b). Additionally, *A. maculatum* from ponds with greater *A. opacum* risk foraged more actively, likely in order to reduce the amount of time the *A. maculatum* larvae exist at a size vulnerable to *A. opacum* predation (Urban 2007b). For kairomone treatments, three predators of the same species and pond origin were raised together in 68-l containers with leaf litter for shelter. *Notophthalmus viridescens* were collected from the Chesterfield, New Hampshire study site, and *A. opacum* were collected from three ponds at the Northford, Connecticut site.

Kairomones and *A. maculatum* survival in predation trials

In 2010, we performed experiments to determine if local adaptations of *A. maculatum* larvae to native *N. viridescens* predation risk would confer a survival advantage against potentially range-expanding *A. opacum*. We predicted that local adaptation to *N. viridescens* would allow *A. maculatum* from high-density *N. viridescens* ponds to survive better with *N. viridescens* as well as the novel predator *A. opacum*.

Mixed-population groups of *A. maculatum* from the same pond type (high- or low-*N. viridescens* density) were reared with kairomones from *A. opacum* only, *N. viridescens* only, or a control, and fed a dense 24 ml allotment of zooplankton once weekly.

Predation trials were conducted in 1135 l cattle tanks (Newell Rubbermaid, Atlanta, GA, USA) filled to 32 cm with treated, aged well water and contained a standardized amount of leaf litter to shelter the animals. Predators were either two *A. opacum* or three *N. viridescens* randomly selected from the kairomone containers. These predator densities are within the range of densities in natural ponds (Petranka 1998). In each trial, 24 *A. maculatum* were randomly selected, individually photographed, and allowed to interact with predators until 12 or fewer larvae remained, or a maximum of 7 d had passed. Surviving *A. maculatum* were re-identified using photographs to compare spot patterns (a method verified previously Urban 2010). In total, six predation trials were completed with each predator species for a total of 12 trials. Due to space limitations, all trials could not all be run concurrently and thus were conducted over 15 d (with *A. opacum*) and 17 d (with *N. viridescens*). We used a temporal block term in statistical models to account for possible temporal autocorrelation in responses.

We evaluated differences in survival versus pond type (high- versus low-*N. viridescens*) and kairomone treatment in a mixed-effects generalized linear model with binomial errors and temporal block entered as a random effect. Significant overdispersion was not detected in analyses with either predator species (Likelihood ratio test; $p > 0.1$). The best method to determine the significance of fixed effects in mixed-effects models remains controversial (Bolker et al. 2009). Randomization tests applied to categories of treatments have not yet been developed for mixed effects models with binomial errors in the R function lmer. We therefore used likelihood ratio tests to evaluate the significance of fixed-effects between models calculated with maximum likelihood (Pinheiro and Bates 2000). We then used probability values calculated from z-values to assess significant differences among individual treatment types.

Kairomones and *A. maculatum* growth rate

After finding that *A. maculatum* from high-*N. viridescens* ponds survived poorly when they developed with novel *A. opacum* kairomones, we designed an experiment to test one potential explanation. Previous work suggested that *A. maculatum* from our study site recognized both *A. opacum* and *N. viridescens* kairomones, and *A. maculatum* decreased its growth rate when exposed to *A. opacum*, but not *N. viridescens* kairomones (Urban 2008b). We predicted that the survival patterns observed in the previous experiment might occur if *A. maculatum* larvae from high-*N. viridescens* ponds grew slower when exposed to *A. opacum* kairomones than other population-treatment combinations. These smaller larvae would then encounter higher risk from the gape-limited *N. viridescens* adults (Results). We examined this hypothesis by evaluating if snout-vent length differed among the larvae in the predation trials depending on kairomone treatment and the presence of *N. viridescens* in *A. maculatum*'s natal

ponds. Using the program ImageJ (Rasband 1997–2011), we averaged three profile measurements of the salamander, from the tip of its snout to the midpoint of the body above the vent. We used a mixed-effects model with treatment and predation regime entered as fixed factors and the specific predation trial entered as a random effect. We used log-likelihood tests to evaluate overall significance of treatments and estimated the significance of individual treatment types based on confidence intervals generated from the posterior distribution of 10 000 parameter estimates obtained by a Markov Chain Monte Carlo approach in the 'pvals.fnc' available in the languageR package in R.

We further tested if survival patterns with newts could be explained by growth differences induced by kairomones and predation regime in a second experiment. In this experiment, we carefully controlled per capita resources so that random differences in container survival did not contribute to variation in growth among experimental units. Sibling groups of *A. maculatum* were reared with kairomones from *A. opacum* only, *N. viridescens* only, equal mixes of both predator kairomones, or a control. The mixed kairomone treatment allowed us to explore if growth responses were additive or not. After hatching, all but three randomly selected *A. maculatum* larvae were removed from each container, and three siblings from each bucket were wet-weighed to obtain an initial mass estimate. The initial masses of *A. maculatum* hatchlings did not differ among pond types, kairomone treatments, or their interaction ($LR_1 = 0.07$, $p = 0.794$; $LR_3 = 5.42$, $p = 0.144$; $LR_3 = 4.66$, $p = 0.198$; respectively).

The remaining *A. maculatum* larvae were fed weekly one 24-ml allotment of a dense natural zooplankton mixture per salamander. Salamander survival was checked weekly, and food allotments were adjusted per capita. After six weeks, the remaining larvae were collected, wet-weighed and preserved. In total, 15 sets of sibling *A. maculatum* from each pond type (high- or low-*N. viridescens* density) were reared with each of the kairomone treatment types for a total of 120 experimental units.

We evaluated mean *A. maculatum* size-specific growth from each container after verifying that growth was exponential for the period of the experiment. Size-specific growth was calculated as the difference between ln-transformed initial mean masses of hatchlings and final masses of survivors in each container divided by time. We used a mixed-effects model with pond identity as a random effect because individuals from a given population are related and thus likely to be more similar in growth. We used log-likelihood tests to evaluate overall significance of treatments and estimated p-values in the same way as for the snout-vent length data.

Results

Ambystoma opacum survival and winter ice cover

At the Connecticut sites where *A. opacum* coexist with *A. maculatum*, the winter survival of *A. opacum* larvae increased significantly with higher minimum winter water temperatures (Fig. 3; logistic regression; $t_8 = 2.56$; $p = 0.034$). Moreover, *A. opacum* larvae survived only if the temperature of the bottom pond water was greater than the average

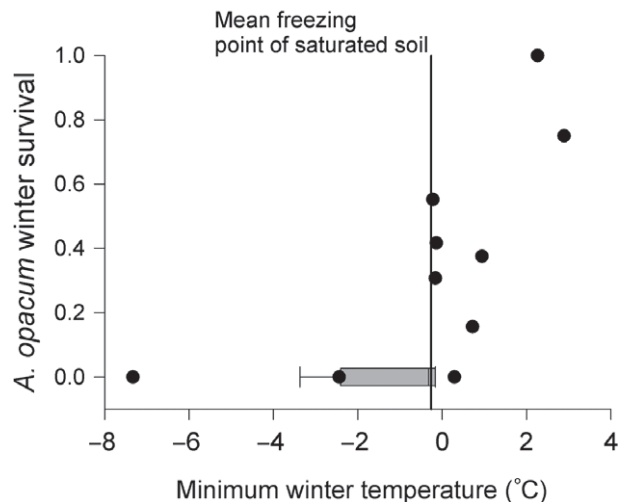


Figure 3. Proportional survival of overwintering *A. opacum* larvae in relation to minimum winter temperature ($^{\circ}\text{C}$) recorded at maximum pond depth for ten ponds at the Northford and Union, Connecticut sites (circles). Vertical line signifies mean freezing point of wetted soils (-0.16°C). The box plot shows the median (central vertical line), 25th and 75th percentiles (box boundaries) and 5th and 95th percentiles (whiskers) of minimum winter pond temperatures in nine ponds in Chesterfield, New Hampshire in the same year.

freezing point of mud (-0.16°C , Kozłowski 2004). The ponds sampled at the New Hampshire site, about 20 km north of the northernmost range limit of *A. opacum*, had a median minimum winter pond temperature of -0.32°C . All water temperatures in New Hampshire were at or below the freezing point of mud (see box plot in Fig. 3), although one-third of ponds had minimum temperatures exactly at the -0.16°C freezing boundary.

Kairomones and *A. maculatum* survival in predation trials

In trials where *A. opacum* was the predator, the survival of *A. maculatum* was not significantly influenced by kairomone treatment, pond type, or their interaction (Fig. 4A, $p > 0.3$). In trials with *N. viridescens* as the predator, a marginally significant interaction was detected between kairomone treatment and pond type (Fig. 4B, Likelihood ratio $_{\text{degrees of freedom}} [\text{LR}_2] = 5.91$; $p = 0.052$). This effect became significant when we assumed that time was a fixed, rather than a random, effect in the model ($p = 0.047$). In particular, *A. maculatum* larvae from high-*N. viridescens* ponds reared with *A. opacum* kairomones survived significantly worse than larvae from the other treatments and pond types ($z_{25} = 2.27$; $p = 0.024$).

Kairomones and *A. maculatum* growth rate

We expected that *A. maculatum* from high-*N. viridescens* ponds raised with *A. opacum* kairomones would grow the slowest, making them smaller and at greater risk of predation. These size differences would therefore explain

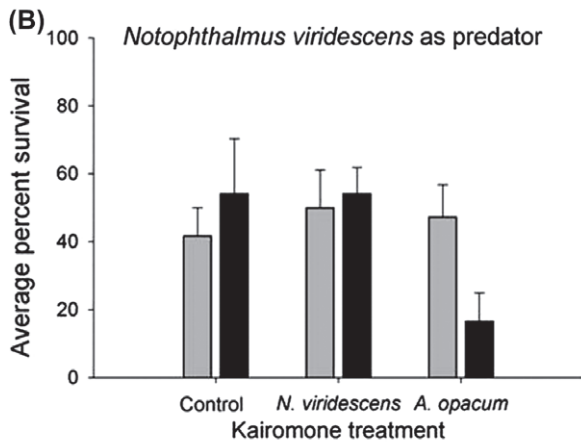
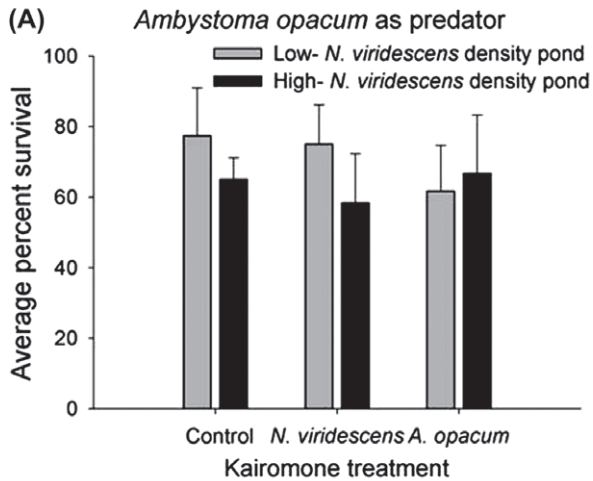


Figure 4. Average percent survival of *A. maculatum* in predator trials facing either (A) the novel predator, *A. opacum* or (B) the native predator, *N. viridescens*. *Ambystoma maculatum* originated from low-*N. viridescens* (grey) or high-*N. viridescens* (black) density ponds, and were reared with different kairomone types before predator trials. Error bars represent average percent survival, \pm one standard error.

the low survival in this treatment in the predation trials. We first evaluated if longer *A. maculatum* larvae in predation trials survived better as predicted. Next we evaluated if size, as measured by SVL, differed among population and kairomone treatments in a manner consistent with observed differences in survival when *N. viridescens* was the predator. In predation trials, longer larvae survived significantly better as expected based on the gape-limitation of *N. viridescens* ($LR_1 = 27.45$; $p < 0.001$). The interaction between kairomone treatment and population type did not significantly explain snout-vent length variation (Fig. 5; $LR_2 = 3.06$; $p = 0.217$) and thus does not support a size basis for survival differences among population and treatment combinations. Pond type did not significantly explain size variation ($LR_1 = 0.38$; $p = 0.537$). Only kairomone treatment significantly explained snout-vent length variation ($LR_2 = 11.43$; $p = 0.003$) because larvae exposed to *A. opacum* were shorter than those exposed to control conditions or *N. viridescens* kairomones. Although the trend was similar to that with *N. viridescens* as the predator,

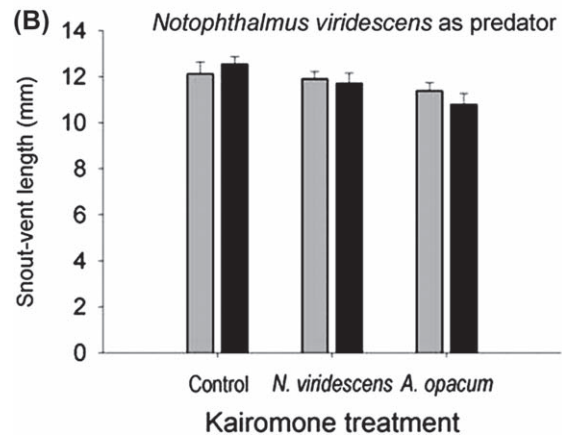
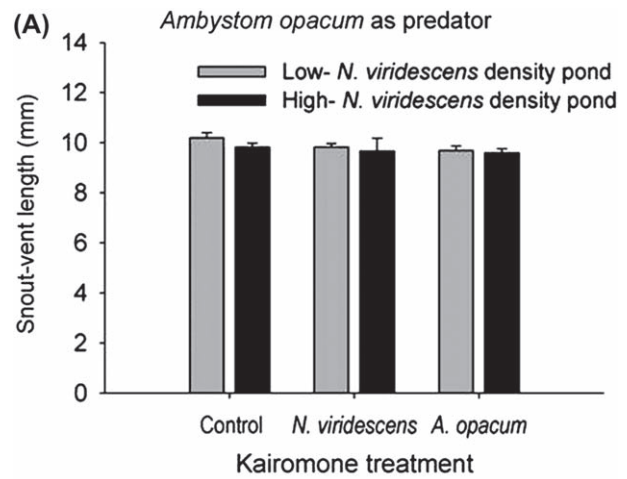


Figure 5. Snout-vent lengths of *A. maculatum* used in predation trials facing either (A) the novel predator, *A. opacum* or (B) the native predator, *N. viridescens*. Bars show the prey's average size, \pm one standard error, for *A. maculatum* from low-*N. viridescens* density (grey) and high-*N. viridescens* density ponds (black) reared with various kairomone types.

kairomone treatment did not significantly affect growth in larvae exposed to *A. opacum* predation ($LR_2 = 1.87$; $p = 0.393$).

In the more carefully controlled growth experiment, the kairomone treatment by pond type interaction again was not significant ($LR_1 = 0.91$; $p = 0.823$), providing further evidence that detected survival differences did not originate from induced size differences as expected. Pond type had no significant effect on growth (Fig. 6; $LR_3 = 0.22$; $p = 0.638$). Kairomone treatment significantly affected growth ($LR_3 = 37.91$; $p < 0.001$). In particular, *A. maculatum* larvae grew significantly slower relative to controls when raised with *A. opacum* kairomones (Markov Chain Monte Carlo test; 95th highest posterior density intervals = -0.406 , -0.260) and these values overlapped with the mixed *N. viridescens*-*A. opacum* kairomone treatment, but not the *N. viridescens*-only treatment, suggesting that cues were not additive. Kairomones from *N. viridescens* did not significantly reduce growth compared to the control.

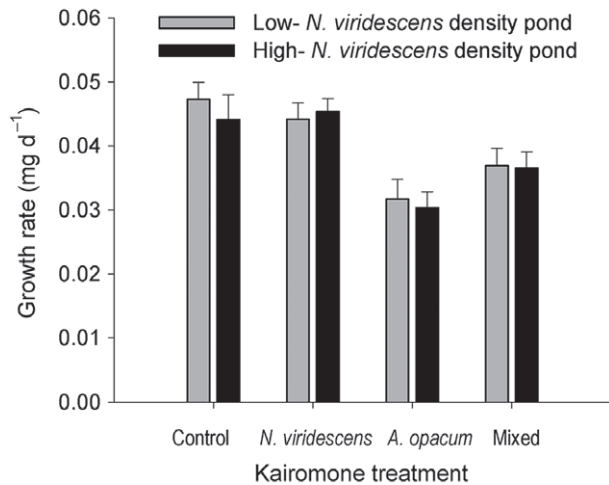


Figure 6. Daily growth rate of *A. maculatum* from low-*N. viridescens* (grey) and high-*N. viridescens* (black) density ponds in response to kairomone treatment. Error bars represent \pm one standard error.

Discussion

Our study suggests that warming winter temperatures could allow *A. opacum* to expand its range northwards to our study site in Pisgah, New Hampshire. Winter temperature determines range limits in many species (Crozier 2004, Battisti et al. 2005, Ling et al. 2009), and thus multiple species are likely to expand their ranges in response to the warmer winters expected with global climate change. Indeed, current evidence indicates that many species are dispersing into colder regions at higher latitudes and elevations (Parmesan and Yohe 2003, Battisti et al. 2005, Angert et al. 2011, Chen et al. 2011). In our research, we found that winter temperatures in southern New England have risen about 2°C in the last 116 yr and that high winter pond temperatures significantly increase *A. opacum*'s overwinter survival in New England temporary ponds. *Ambystoma opacum* were not found in any ponds in which the water froze to the bottom (Fig. 3). All of the sampled ponds at our New Hampshire study site – situated above the current range of *A. opacum* – had minimum water temperatures at or below the freezing point of mud, suggesting that *A. opacum* would not have survived in sampled ponds during this winter. However, winter temperatures are projected to rise an additional 4°C over the next 90 yr (Parmesan and Yohe 2003, Christensen et al. 2007). Several factors indicate that colonization by *A. opacum* could occur soon at our New Hampshire study site. First, one-third of sampled New Hampshire ponds had minimum temperatures at the exact threshold for *A. opacum* overwinter survival and thus any warming will make those ponds suitable. Second, *A. opacum* were recently reported in Hinsdale, the nearest town south of our study site (RAARP 2010). Third, *A. opacum* are capable of relatively long-distance dispersal: *A. opacum* generally disperse 200–400 m, and some individuals travel more than 1.2 km (Gamble et al. 2007). Fourth, we commonly observed *A. opacum* breeding in ponds that are usually unsuitable for larval survival in our southern sites,

suggesting frequent colonization of sink habitats (Fig. 3). Hence, we expect *A. opacum* to expand its northern range limit into southern parts of Vermont and New Hampshire as temperatures warm in the region.

However, we did not find evidence that New Hampshire *A. maculatum* are pre-adapted via their current interactions with *N. viridescens* to deal with range-expanding *A. opacum*. We predicted that *A. maculatum* from high-*N. viridescens* ponds would possess adaptive plasticity that would also promote their survival with *A. opacum*, which feeds in a similar manner and is also gape-limited. In contrast to our prediction, *A. maculatum* survival with *A. opacum* did not differ significantly based on its evolutionary history with *N. viridescens* (Fig. 4A). Thus, we found no support for the hypothesis that adaptation to a native predator species increased prey survival with a similar novel predator species. This result might have occurred either because local *A. maculatum* populations have not become locally adapted to *N. viridescens* predation or because local adaptations to *N. viridescens* do not confer a survival advantage with *A. opacum*. Given that populations from high-*N. viridescens* ponds did not survive better than those from low-*N. viridescens* ponds when exposed to *N. viridescens*, the former reason explains the patterns most parsimoniously.

Kairomone treatment also did not significantly affect *A. maculatum* survival, suggesting that any traits that developed as the result of long-term kairomone induction did not affect interactions with the predator. Based on the growth experiment, we know that *A. maculatum* from New Hampshire differentiate between kairomones from *N. viridescens* and *A. opacum* and grow less only when they sense *A. opacum*. Furthermore, we found that *A. maculatum* reared in the mixed *A. opacum* and *N. viridescens* kairomone treatment grew similarly to those reared with *A. opacum*-only rather than the *N. viridescens*-only kairomones. One explanation for this pattern is that the fitness benefits of slower growth in response to *A. opacum* predation outweigh the costs of this trait in the presence of *N. viridescens*. We confirmed that both predators preferentially preyed on smaller individuals. Despite this existing size plasticity in response to *A. opacum* kairomones, kairomone treatment did not affect survival except for the high-*N. viridescens* ponds in the *N. viridescens* predation trials. One explanation is that size selection is completely soft, such that predators still can prey upon larger individuals once smaller individuals have been eaten and thus generate no difference in absolute predation rates among treatments. With *N. viridescens* kairomones, New Hampshire *A. maculatum* do not alter activity and do not differ in growth relative to control conditions (Urban 2008b), which provides one reason why *N. viridescens* cues had no significant effect on survival in this experiment.

We predicted that *A. maculatum* populations that face high densities of *N. viridescens* in their natal ponds would survive better when encountering *N. viridescens*, owing to local adaptation. In contrast to this prediction, high-*N. viridescens* populations fared no better in predation trials with *N. viridescens* than did low-*N. viridescens* populations. We expected local adaptation to *N. viridescens* based on evidence for local adaptation in *A. maculatum* to other

predators across similar spatial scales, including *A. opacum* (Urban 2007b) and the *Dytiscus* diving beetle (Urban 2010). Local adaptive differentiation might not occur among New Hampshire populations if selection from *N. viridescens* is weak, variable in time, or if gene flow is greater (Storfer et al. 1999) among these populations than in other sites. However, long-term data does not indicate high year-to-year variation in *N. viridescens* densities (Table 1), and ponds are separated by similar distances compared to the same ones across which we observe local adaptation. *Notophthalmus viridescens* selection is likely to be weaker than selection from *A. opacum* because the two predators differ in how long they can successfully attack *A. maculatum* larvae. *Ambystoma maculatum* can grow into a size refuge from *N. viridescens* within 1–2 weeks, whereas it takes twice as long to reach a size refuge from *A. opacum*. Although *A. maculatum* survived better in our time-constrained experiments with *A. opacum* relative to *N. viridescens*, it is possible that in studies run over more realistic time periods, predation from *A. opacum* would reduce *A. maculatum* numbers to a greater extent than predation from *N. viridescens*.

We detected an unexpected significant interaction between kairomone treatment and *N. viridescens* predation regime. Only about 20% of individuals survived if they came from populations with high-*N. viridescens* densities and had received novel *A. opacum* kairomones, whereas 50% of individuals survived from all other treatment combinations. This outcome suggests that an evolutionary difference between high- and low-*N. viridescens* populations interacts with plastic responses to *A. opacum* kairomones to decrease survival.

Smaller size cannot explain this result. All populations – not just the high-*N. viridescens* ones – were smaller when exposed to *A. opacum* kairomones. We currently do not know what this evolutionary difference between ponds might entail. *Notophthalmus viridescens* usually inhabit more permanent ponds, and pond permanence correlates with a suite of associated factors including less canopy cover, more aquatic vegetation, and a greater diversity of predator species (Urban 2004). Any of these correlated factors might select for traits that might alter predator responses. Future work will be needed to ascertain which traits differ between high-*N. viridescens* and low-*N. viridescens* ponds and which environmental factors select for this trait divergence. Overall, results suggest the possibility of unexpected interactions between adaptation to local selection regimes and induced reactions to novel range-expanding species.

We found that *A. maculatum* responded to *A. opacum* kairomones by reducing growth even though these northern populations do not currently encounter *A. opacum*. Southern populations of *A. maculatum* that coexist with *A. opacum* reduce growth in a similar way when encountering *A. opacum* cues (Urban 2008a), suggesting that the trait might be an adaptive response that has not disappeared in *A. opacum*'s absence. This response might be retained from historical interactions with *A. opacum* during the last glacial maximum or it might occur owing to gene flow from southern populations that currently face predation from *A. opacum*. Phylogenetic reconstructions suggest that *A. maculatum* expanded their range northwards from southern refugia following glacial retreat (Zamudio and Savage

2003). *Ambystoma opacum* is likely to have shared similar glacial refugia, which would suggest that the predator shares its evolutionary history with *A. maculatum*. Yet, local adaptations to predators can occur rapidly and over short spatial scales (Reznick et al. 1990). Hence, we might expect that relaxed selection will lead to the evolution of lost adaptive responses. This explanation assumes either high maintenance costs or strong pleiotropy between the unused trait and other traits under current selection. Other defenses have been shown to remain even though they serve no detectable purpose now (Peckarsky and Penton 1988), suggesting that these hidden plastic defenses might often be expressed long after selection has disappeared. In addition, the location of New Hampshire populations close to the northern range of *A. opacum* supports the idea that gene flow maintains adaptive plasticity even if weak selection operates against it. To determine if gene flow between populations affords protection to these southern New Hampshire *A. maculatum*, a future study should obtain *A. maculatum* far north of the current range limit of *A. opacum* and evaluate responses to *A. opacum*.

Conclusions

Most predictions about the impacts of climate change on biodiversity assume that species do not interact and do not evolve (Thomas et al. 2004, Malcolm et al. 2006). However, an increasing number of studies predict that species interactions and local adaptation will mediate the effects of climate change on species persistence (Gienapp et al. 2008, Gilman et al. 2010, Hoffmann and Sgro 2011, Norberg et al. 2012, Urban et al. 2012). In particular, climate change effects on top predators are expected to have strong effects on biodiversity (Zarnetske et al. 2012). Here we explore if evolutionary differentiation determines the coexistence of a range-expanding top predator and its naïve prey populations. The prey populations differed in survival when exposed to novel *A. opacum* kairomones, but only when they originated from ponds with high densities of native newts. This outcome indicates that evolved differences among populations linked to newt predation or some other correlated selection regime determine predation risk in communities of both native and novel predators. Hence, to predict biodiversity patterns when climate change creates novel species interactions might often require an understanding of evolutionary history, not just ecological context.

Acknowledgements – Research was supported by NSF award DEB-1119877 and a Univ. of Connecticut large faculty grant. Special thanks to the South Central Connecticut Regional Water Authority and Yale Univ. Forest for access to field sites. We thank J. MacLellan, A. Shepack, E. Didan and J. Rack for assistance in the field and with experiments.

References

- Angert, A. L. et al. 2011. Do species' traits predict recent shifts at expanding range edges? – *Ecol. Lett.* 14: 677–689.
- Battisti, A. et al. 2005. Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. – *Ecol. Appl.* 15: 2084–2096.

- Bishop, S. C. 1941. The salamanders of New York. – N. Y. State Mus. Bull. 324: 1–365.
- Bolker, B. M. et al. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. – Trends Ecol. Evol. 24: 127–135.
- Brady, S. P. 2012. Road to evolution? Local adaptation to road adjacency in an amphibian (*Ambystoma maculatum*). – Sci. Rep. 2: 235.
- Carey, C. and Alexander, M. A. 2003. Climate change and amphibian declines: is there a link? – Divers. Distrib. 9: 111–121.
- Chen, I.-C. et al. 2011. Rapid range shifts of species associated with high levels of climate warming. – Science 333: 1024–1026.
- Christensen, J. H. et al. 2007. Regional climate projections. – In: Solomon, S. et al. (eds), Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge Univ. Press, pp. 847–940.
- Corn, P. S. 2005. Climate change and amphibians. – Anim. Biodivers. Conserv. 28: 59–67.
- Crawley, M. J. 2007. The R book. – Wiley.
- Crozier, L. G. 2004. Field transplants reveal summer constraints on a butterfly range expansion. – Oecologia 141: 148–157.
- Edgell, T. C. and Neufeld, C. J. 2008. Experimental evidence for latent developmental plasticity: intertidal whelks respond to a native but not an introduced predator. – Biol. Lett. 4: 385–387.
- Gamble, L. R. et al. 2007. Fidelity and dispersal in the pond-breeding amphibian, *Ambystoma opacum*: implications for spatio-temporal population dynamics and conservation. – Biodivers. Conserv. 139: 247–257.
- Gienapp, P. et al. 2008. Climate change and evolution: disentangling environmental and genetic responses. – Mol. Ecol. 17: 167–178.
- Gilman, S. E. et al. 2010. A framework for community interactions under climate change. – Trends Ecol. Evol. 25: 325–331.
- Hassinger, D. D. et al. 1970. The early life history and ecology of *Ambystoma tigrinum* and *Ambystoma opacum* in New Jersey. – Am. Midl. Nat. 84: 474–495.
- Hodgkins, G. A. et al. 2002. Historical changes in lake ice-out dates as indicators of climate change in New England, 1850–2000. – Int. J. Climatol. 22: 1819–1827.
- Hoffmann, A. A. and Sgro, C. M. 2011. Climate change and evolutionary adaptation. – Nature 470: 479–485.
- IPCC 2007. Climate change 2007: synthesis report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. – IPCC.
- Kozłowski, T. 2004. Soil freezing point as obtained on melting. – Cold Regions Sci. Technol. 38: 93–101.
- Ling, S. D. et al. 2009. Climate-driven range extension of a sea urchin: inferring future trends by analysis of recent population dynamics. – Global Change Biol. 15: 719–731.
- Malcolm, J. R. et al. 2006. Global warming and extinctions of endemic species from biodiversity hotspots. – Conserv. Biol. 20: 538–548.
- Martin, J. B. 1974. Analysis of feeding behavior in the newt *Notophthalmus viridescens*. – Can. J. Zool. 52: 277–282.
- Norberg, J. et al. 2012. Eco-evolutionary responses of biodiversity to climate change. – Nat. Clim. Change doi: 10.1038/NCLIMATE1588
- Nosil, P. et al. 2005. Reproductive isolation caused by natural selection against immigrants from divergent habitats. – Evolution 59: 705–719.
- Parmesan, C. and Yohe, G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. – Nature 42: 37–42.
- Peckarsky, B. L. and Penton, M. A. 1988. Why do Ephemerella nymphs scorpion posture: a “ghost of predation past”? – Oikos 53: 185–193.
- Petranka, J. W. 1998. Salamanders of the United States and Canada. – Smithsonian Books.
- Pinheiro, J. C. and Bates, D. M. 2000. Mixed-effects models in S and S-Plus. – Springer.
- Quinlan, R. et al. 2005. Food web changes in arctic ecosystems related to climate warming. – Global Change Biol. 11: 1381–1386.
- RAARP 2010. New Hampshire reptile and amphibian reporting program town distribution maps. – In: Department, N. F. G. (ed.), Nongame & endangered wildlife program. New Hampshire Reptile and Amphibian Reporting Program.
- Rasband, W. S. 1997–2011. ImageJ. – U. S. National Inst. of Health, < <http://imagej.nih.gov/ij/>>.
- Reznick, D. N. et al. 1990. Experimentally induced life-history evolution in a natural population. – Nature 346: 357–359.
- Sanford, E. and Swezey, D. S. 2008. Response of predatory snails to a novel prey following the geographic range expansion of an intertidal barnacle. – J. Exp. Mar. Biol. Ecol. 354: 220–230.
- Smol, J. P. et al. 2005. Climate-driven regime shifts in the biological communities of arctic lakes. – Proc. Natl Acad. Sci. USA 102: 4397–4402.
- Storfer, A. et al. 1999. Adaptive coloration and gene flow as a constraint to local adaptation in the streamside salamander, *Ambystoma barbouri*. – Evolution 53: 889–898.
- Thomas, C. D. et al. 2004. Extinction risk from climate change. – Nature 427: 145–148.
- Urban, M. C. 2004. Disturbance heterogeneity determines freshwater metacommunity structure. – Ecology 85: 2971–2978.
- Urban, M. C. 2007a. Predator size and phenology shape prey survival in temporary ponds. – Oecologia 154: 571–580.
- Urban, M. C. 2007b. Risky prey behavior evolves in risky habitats. – Proc. Natl Acad. Sci. USA 104: 14377–14382.
- Urban, M. C. 2008a. The evolution of prey body size reaction norms in diverse communities. – J. Anim. Ecol. 77: 346–355.
- Urban, M. C. 2008b. Salamander evolution across a latitudinal cline in gape-limited predation risk. – Oikos 117: 1037–1049.
- Urban, M. C. 2010. Microgeographic adaptations of spotted salamander morphological defenses in response to a predaceous salamander and beetle. – Oikos 119: 646–658.
- Urban, M. C. et al. 2012. A crucial step toward realism: responses to climate change from an evolving metacommunity perspective. – Evol. Appl. 5: 154–167.
- Zamudio, K. R. and Savage, W. K. 2003. Historical isolation, range expansion, and secondary contact of two highly divergent mitochondrial lineages in spotted salamanders (*Ambystoma maculatum*). – Evolution 57: 1631–1652.
- Zamudio, K. R. and Wicczorek, A. M. 2007. Fine-scale spatial genetic structure and dispersal among spotted salamander (*Ambystoma maculatum*) breeding populations. – Mol. Ecol. 16: 257–274.
- Zarnetske, P. L. et al. 2012. Biotic multipliers of climate change. – Science 336: 1516–1518.