Research

Salamander evolution across a latitudinal cline in gape-limited predation risk

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General predictions of community dynamics require that insights derived from local habitats can be scaled up to explain phenomena across geographic scales. Across these larger spatial extents, adaptation can play an increasing role in determining the outcome of species interactions. If local adaptation is common, then our ability to generalize measures of species interaction strength across communities will be limited without an additional understanding of the genetic variation underlying interaction traits. In the context of predator–prey interactions, prey individuals commonly are expected to reduce risky foraging behaviors and subsequent growth under predation threat. However, rapid growth into a large body size can defend against gape-limited predators, creating a tradeoff between increased predation risk due to elevated foraging activity and decreased predation risk due to large size. Here I combine field observations, natural selection experiments, and common garden assays to understand potential adaptations of spotted salamander *Ambystoma maculatum* larvae to gape-limited and gape-unconstrained predators. Field observations and natural selection trials suggested antagonistic selection on prey body size among ponds dominated by gape-limited predator salamanders *A. opacum* and gape-unconstrained beetle larvae *Dytiscus*. In common garden experiments, prey from sites with high gape-limited predation risk grew larger than those from other sites, suggesting the evolution of rapid growth into a prey size refuge. Larvae from all sites grew to a large size when exposed to the gape-limited *N. viridescens* predator's kairomones. Hence, induced rapid growth into a size refuge may be an adaptive response to gape-limited predation risk. Results point to an important role for cross-community generalizations based on functional classifications of predators by their gape constraints and inter-site genetic variation in prey growth rates and behaviors.

Community ecologists increasingly are called upon to apply locally derived insights to regional and geographic scales (Hanski and Gaggiotti 2004, Holyoak et al. 2005). Evolutionary dynamics are likely to affect species interactions at larger spatial scales as gene flow becomes more restricted and as selection regimes more divergent (Garant et al. 2007). Therefore, scaling up predictions about species interactions from local to geographic scales often will require information about spatial variation in both ecological and evolutionary factors (Travis 1996, Thompson 2005, Urban and Skelly 2006). In particular, predicting the outcome of predator–prey interactions across a landscape often might require information about phenotypic variation in key defensive traits across natural predation gradients (Parejko and Dodson 1991, Storfer and Sih 1998, Trussell 2000). In this paper, I examine evidence for the evolution of growth and behavioral plasticity in spotted salamander larvae *Ambystoma maculatum* from three sites distributed across a natural latitudinal cline in predator composition and gape-limitation.

Prey populations generally encounter diverse predator assemblages. These predator species will differ in numerous traits that determine their interactions with prey. Therefore,

functional descriptions of predator traits are needed to generate predictions that apply across diverse taxa, systems and spatial scales (Chalcraft and Resetarits 2003). The relative difference in body size between predator and prey offers one such generalization because many predators cannot capture prey above a certain gape size threshold (Wilson 1975, Paine 1976, Hambright 1991, Persson et al. 1996). In general, a useful functional distinction can be made between those predator species that are gape-limited and those that are gape-unconstrained with respect to a particular prey species (Wilbur 1988, Urban 2007b). One potential consequence of a predator's gape-limitation is natural selection for prey to grow rapidly into a body size refuge (Paine 1976, Case 1978, DeAngelis et al. 1985, Day et al. 2002). Along these lines, a recent demographic model of optimal prey foraging and growth predicts a fitness tradeoff between slow and rapid growth (Urban 2007a). Slow prey growth can limit the predation risk associated with risky foraging behavior. However, slow prey growth also extends the period of time during which prey are susceptible to gape-limited predators. In contrast, rapid growth into a large body size can provide an eventual morphological refuge from gape-limited predators despite

the initial high mortality costs of risky foraging behavior. The optimal growth rate given these contrasting fitness consequences of slow and rapid growth is mediated by the prevalence of gape-limited versus gape-unconstrained predators. High gape-limited predation risk can select for rapid prey growth under a broad range of conditions (Urban 2007a). On the other hand, high gape-unconstrained predation risk can favor a slow growth strategy underlain by risk-averse behaviors as predicted by classic prey growth/predation risk models (McPeek and Peckarsky 1998). Therefore, we might expect the evolution of rapid growth in prey populations faced with stronger gape-limited predation risk and slow growth in prey populations faced with stronger gape-unconstrained predation risk.

Interactions between spotted salamander *Ambystoma maculatum* larvae and their predators in temporary ponds in New England offer an ideal system in which to test this theory. *Ambystoma maculatum* face diverse predator species that differ in their distribution over local and geographic landscapes (Urban 2004, 2007b). Pronounced changes in predator composition occur in New England ponds as the predatory marbled salamander *Ambystoma opacum* steadily declines in distribution and abundance from south to north until it reaches its northernmost range limit in central New England (Fig. 1). Prior research shows that *A. maculatum* adaptively reduces foraging in response to dragonfly predation (Yurewicz 2004). This aspect of their biology, together with geographic variation in predation risks from different species, might select for the evolution of induced life history traits (Tollrian and Harvell 1999). However, we do not know if *A. maculatum* respond with different induced behaviors and growth patterns in a manner consistent with regional adaptations to predator gape-limitation.

Here, I evaluate two underlying assumptions of a model of adaptive prey growth in natural communities in response to predator gape-limitation (Urban 2007a) by assessing if gape-limited predation risk varies across natural landscapes and if gape-limited and gape-unconstrained predators generate opposing selection on prey body size. To describe the geographic variation in gape-limited predation risk in New England, I evaluated the density and composition of gape-limited and gape-unconstrained predator species co-occurring with focal *A. maculatum* salamander larvae distributed across three sites located along a 160 km latitudinal gradient in southern New England, USA. To determine if predator gape-limitation shapes prey survival in the field, I estimated the predator-specific size refuge threshold, the size below which prey become vulnerable to gape-limited predator species. I tested if field prey survival was low when prey individuals were small enough to fit easily within their predators' gapes. Next, I measured selection gradients on *A. maculatum* size during exposure to gape-limited *A. opacum* larvae and gape-unconstrained diving beetle larvae *Dytiscus* spp. I predicted that the gape-limited predator would impose strong selection for larger prey body size, whereas the gape-unconstrained predator would impose no selection on prey body size because they could capture prey of any size. I also evaluated how developmental plasticity influenced survival because the prey larvae under selection were raised under different predator chemical cues (hereafter kairomones). I predicted that prey larvae exposed to a predator's kairomones would develop a set of traits that defended against the predator during selection trials (Benard 2006, Laurila et al. 2006).

Next, I assessed evidence for the model's major prediction that either fixed or induced rapid growth rates would evolve in geographic regions where prey populations coexist
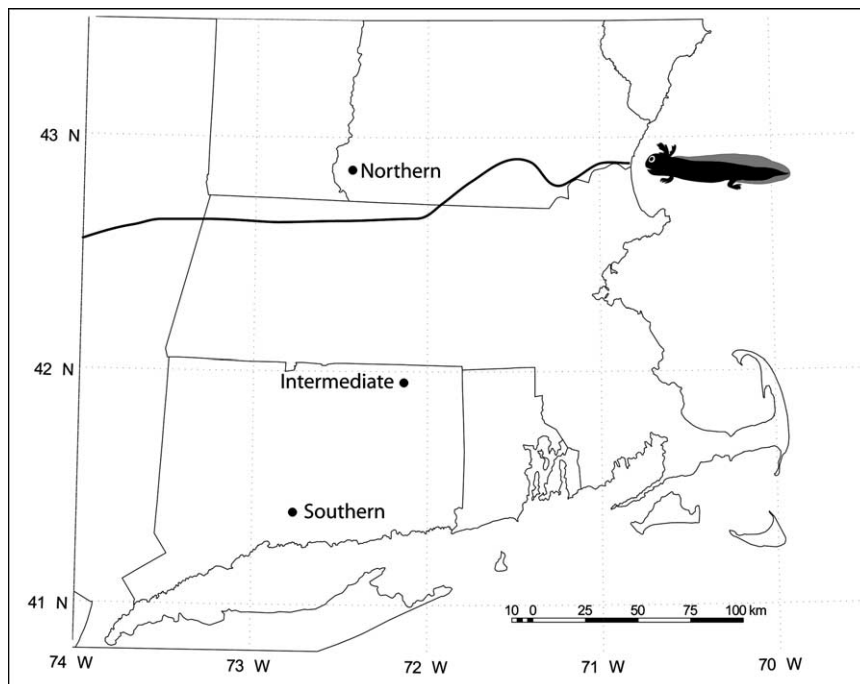

Figure 1. Map of study locations, including the southern (Northford, CT), intermediate (Union, CT), and northern (Chesterfield, NH) sites. The bold line indicates the northern range limit of gape-limited marbled salamanders (*Ambystoma opacum*) (after Petranka 1998). The distribution of *A. opacum* is associated with a cline in total gape-limited predation risk across the study region.

with high densities of gape-limited predators. I assayed phenotypic variation in *A. maculatum* larvae originating from different sites that were raised under common garden conditions. I predicted that particular predator species would induce specific defenses in *A. maculatum* larvae as has been demonstrated across a range of aquatic taxa (Tollrian and Harvell 1999, Van Buskirk 2001, Relyea 2003, Laurila et al. 2006). I expected that larvae from southern sites with high gape-limited predation risk would grow rapidly and forage actively outside of a structurally complex, physical refuge habitat. I predicted that larvae from all sites would grow larger and more frequently leave structural refuges when exposed to kairomones from the widely distributed and gape-limited red spotted newt *Notophthalmus viridescens*. However, I expected that only larvae from the two southern sites would grow rapidly in response to gape-limited *A. opacum* predator kairomones because the northern site lacked this predator. Kairomones from gape-unconstrained *Dytiscus* larvae were expected to elicit lower final masses and reduced activity in larvae from all sites because *A. maculatum* cannot grow out of risk from this predator. Lastly, I evaluated alternative abiotic hypotheses for site-level mass differences, including variation in pond hydroperiod and water temperature.

Methods

Natural history and site description

The focal prey species, Ambystoma maculatum, is a relatively large (15–25 cm total length) terrestrial salamander found throughout much of eastern North America. Each spring, A. maculatum adults emerge from underground burrows and migrate into temporary ponds to reproduce (Petranka 1998). Females produce about 200 eggs, which hatch into aquatic larvae after 4–8 weeks. Early hatchlings suffer high mortality from a broad range of vertebrate and invertebrate species (Urban 2004). Surviving individuals initiate metamorphosis when ponds begin to dry or with the onset of cool autumn temperatures. I surveyed 40 breeding populations of *A. maculatum* and their predators across three sites along a latitudinal gradient in southern New England, USA from 2002–2004 (Fig. 1). Hereafter I refer to them as the southern (Northford, CT), intermediate (Union, CT) and northern (Chesterfield, NH) sites. Adjacent sites were separated by 80 km (160 km total). At each site, I identified 12–14 breeding ponds for A. maculatum within a 2 km² area. These ponds generally dried by early August. However, four ponds held water year-round once during the study's time span.

Predator composition, gape-limitation and size

To assess variation in predator composition across sites, I sampled each pond at each site every two weeks from 2002–2004 beginning when *A. maculatum* hatched and continuing until a pond dried or no larvae were found over two subsequent surveys. In 2004, I sampled the southernmost site weekly to obtain finer resolution data during *A. maculatum*'s most vulnerable post-hatching stages. Prey and predator populations were sampled by sweeping all representative microhabitats in each pond with a 17×25 cm dip net with 1.4 mm mesh with effort set proportional to pond area (about one sweep per 20 m²). The number of dip net sweeps could be converted into a total pond area sampled because net area and sweep length were kept constant. I calculated population densities as the number of individuals collected of each species divided by the pond area sampled by dip net sweeps. I preserved *A. maculatum* and their potential predators in 70% ethanol.

Predators were identified to genus (invertebrates) or species (vertebrates) under a $7–30 \times$ stereoscope using standard literature (Merritt and Cummins 1996, Petranka 1998). Potential predator species of *A. maculatum* larvae were defined by their ability to consume *A. maculatum* or similar amphibian larvae (Stenhouse 1985, Schneider 1997, Petranka 1998, Brunkhurst 2004, Urban 2004). These predators were categorized as gape-limited or gape-unconstrained based on a literature review (Stenhouse 1985, Wilbur and Fauth 1990, Wissinger 1992, Wilbur 1997) (Supplementary material Appendix 1). I only counted gape-limited predators if their gape or labium surpassed the mean minimum head width of newly hatched *A. maculatum* (2 mm). Gape-limited predators included salamanders, fish and libellulid dragonfly larvae. Salamanders and fish often are gape-limited because they hunt by suctioning prey through their mouths (van Wassenbergh et al. 2006). Similarly, small libellulid dragonflies cannot readily handle prey larger than their labium widths (Wissinger 1992). In contrast, the gape-unconstrained predators in the study were able to grasp prey larger than their capture apparatus (e.g. stiff prementum in *Aeshnidae* dragonfly larvae, Wilbur 1997) or deliver immobilizing and necrotoxic venom (*Dytiscus*, Formanowicz 1982).

I measured gape-limited predator size as the maximum cross-sectional dimension of each predator's gape or labium (for dragonfly larvae) with a digital caliper (± 0.1 mm). A maximum of 10 individuals of each predator species was measured for each pond on each sampling date. From these measurements, I calculated the mean gape-limited predator size as the average of each species weighted by its relative field density. Some predators were not preserved due to low population numbers. *Notophthalmus viridescens* adults only were preserved in 2004 and in a supplementary collection in 2005. *N. viridescens* from 2002–2003 were assigned the mean size from the appropriate pond, when available, or the site mean calculated from 2004–2005 collections across all dates. Four metamorphic *A. opacum* predators also were noted in the field but not preserved. In these cases, *A. opacum* size was estimated from the mean size for all ponds at that site and on that date.

Analyses of variance (ANOVAs) were performed to evaluate variation in gape-limited, gape-unconstrained, and total predator densities with respect to the fixed factor, site of origin. I also performed an ANOVA of gape-limited predator sizes with respect to site. Similar analyses were performed for mean focal predator densities. All analyses were performed in S-Plus ver. 7.0. Data was ln-transformed to meet the normality assumption of parametric statistics (Neter et al. 1996). For all predator response variables, nonsignificant modified Levene test statistics suggested that the ANOVA assumption of constant variance was not violated (Neter et al. 1996).

Prey size refuge thresholds and survival

The prey size refuge threshold was calculated as a dimensionless ratio of the maximum prey size captured by a predator divided by its maximum gape size (Wissinger 1992, Urban 2007b). I dissected the stomachs of 50 *A. opacum* larvae and 48 *N. viridescens* adults from field collections to estimate the mean size refuge thresholds for these two important gape-limited predators. Prey width was measured as its maximum cross-sectional width. For Libellulids, for which stomach contents could not be identified, I used the mean size threshold of 0.8 reported for the libellulid dragonfly species (Wissinger 1992) that were recorded in this study.

I tested if *A. maculatum* survival in the field was affected by the ratio of mean larval head width to mean gape-limited predator size. Maximum head width, the maximum cross-sectional body dimension in larvae, likely governs *A. maculatum*'s survival during encounters with the study's gape-limited predators. I measured *A. maculatum* head widths with an ocular micrometer for all individuals up to a maximum of 20 per sample. In larger samples (>20), I measured 20 haphazardly selected individuals. Prey survival was calculated as the proportional change in larval density for each pond divided by the maximum proportional change in larval density across all ponds on that date to account for weekly changes in pond area that occur with precipitation. For this analysis, I used the finer resolution data collected from the southern site during the first four vulnerable weeks of larval development in 2004 in order to capture the dynamics during this high-mortality period. I explicitly tested whether early field survival (arcsin-transformed to correct for non-normality) in 14 ponds was greater when *A. maculatum* larvae were larger than the average predator's gape limit at that time. Unfortunately, the lack of fine-resolution and early-season data precluded a meaningful application of this test to the two other sites. I analyzed two mixed-effects ANOVA models, one with a fixed factor that indicated whether larval size was above or below the size refuge threshold estimated for salamander predators (0.37) and one parameterized for dragonfly predators (size refuge threshold $=0.80$). I included population as a random effect to account for repeated measures on the same populations over time (von Ende 2001).

Selection gradients on fixed and induced traits

I measured the natural selection gradients imposed on *A. maculatum* phenotypes by gape-limited *A. opacum* larvae and gape-unconstrained *Dytiscus* larvae. I predicted that *A. opacum* would induce selection for larger *A. maculatum* body size while gape-unconstrained *Dytiscus* would induce no directional selection on *A. maculatum* body size. I also evaluated selection associated with developmental plasticity in response to different kairomones. I predicted that a larva exposed to a specific predator's kairomone during development would express an induced phenotype that would protect it from future attacks from that predator species.

Selection experiments were performed in 2002 (exposure to *Dytiscus*) and 2003 (*A. opacum*) using larvae raised under the same conditions used in the phenotypic assay experi-

ment (see next section). *A. maculatum* larvae used in the selection trials were raised under *A. opacum*, *Dytiscus* and no-kairomone conditions. Selection gradients could be calculated based on head width (the expected body dimension under selection by an engulfing predator) and based on kairomone-induced phenotypic variation (by evaluating if kairomone treatment affected larval survival). Four egg masses from each of four ponds from each site were collected in the same way as the phenotypic assay experiment. Egg masses were sampled from the same ponds as in the phenotypic assay experiment, except for one pond that was replaced with another to avoid disrupting a field experiment and for the addition of one new pond per site. I separated nine eggs from each egg mass, and then combined one egg from each of the four egg masses collected per pond to form nine mixed clutch replicates. Three of these replicates were exposed randomly either to one of two kairomone treatments or to a control treatment.

Larvae were exposed to free-ranging *Dytiscus* predators six weeks after hatching and to free-ranging *A. opacum* predators three weeks after hatching. The shorter development time for prey in the *A. opacum* experiment was done to match the early vulnerability of small *A. maculatum* to *A. opacum* in the field (Urban 2006). After about three weeks, *A. opacum* no longer can capture most *A. maculatum* because of their larger size. In contrast, *Dytiscus* prey on *A. maculatum* throughout their development and reach their highest densities late in the season (Urban 2006). Therefore, I compared selection gradients measured during periods that coincided with the maximum predation threat observed in the field. Following kairomone induction, one *A. maculatum* larva was selected randomly from each of the 12 treatment and population combinations (three treatments × four populations) within each site. These 12 larvae were exposed together either to two *A. opacum* larvae or one *Dytiscus* larva in six replicated selection arenas (three sites × two predators × six replicates for a total of 36 trials). The density of predators per arena matched the mean maximum densities of the two predators in all study ponds in which they occurred in 2002 (*A. opacum* and *Dytiscus* mean densities per arena: 2.00 and 0.83 individuals, respectively).

Prior to selection trials, each *A. maculatum* larva was photographed dorsally and in profile so that head width could be measured and spotting patterns recorded. Surviving individuals were identified after selection by their idiosyncratic spotting patterns. Spotting patterns were used to re-identify all *A. maculatum* individuals after two weeks in a pilot study (unpubl.). All trials for each predator species were conducted simultaneously with different field-collected predators. Individual predators were selected to be within one standard deviation of the mean field gape sizes observed at the developmental stage of *A. maculatum* larvae used in the experiment. Predators were starved 24 h before trials. Selection trials were conducted in 60-l wading pools (A $=0.80$ m^2) to which I added a standardized mixture of substrate leaf litter and zooplankton. *A. maculatum* larvae were allowed to acclimate to mesocosm conditions for 24 h before predator introduction. Following predator introduction, mesocosms were censused twice daily ($\sim$07:00 and 19:00). Any predators that died or reached metamorphosis were replaced with similarly sized predators. A trial ended

when half the larvae remained or 10 days had elapsed. This study design was needed to ensure that surviving larvae remained at the end to calculate a selection gradient given the differential consumption rates of the two predator species.

I evaluated survival as a measure of one component of larval fitness under predation threat. Prey survival was analyzed with respect to site, population of origin nested within site, kairomone treatment, maximum head width, and their interactions in a logistic generalized linear model. I pooled data across replicates because the interaction between covariates and mesocosm was not significant (Sokal and Rohlf 1995). I also estimated gradients for head width alone and with its quadratic term to estimate the potential for stabilizing or disruptive selection gradients (Lande and Arnold 1983). I converted the non-linear gradients estimated for head width from logistic regressions into linear standardized gradients by multiplying coefficients by a conversion factor and the standard deviation of the pre-selection trait (Janzen and Stern 1998).

Lastly, I evaluated the consequences of phenotypic variation for prey survival in selection experiments. The number of survivors over time was evaluated in a mixed-effects linear model with replicate arena (the random factor) nested in time to account for repeated observations.

Phenotypic assays

I raised A. maculatum larvae that originated from different sites under common garden conditions and under exposure to one of three predator kairomone treatments or a control treatment. The experiment was conducted to determine whether A. maculatum larvae from sites with different predator regimes expressed differences in fixed growth and activity overall or expressed differences in growth and activity depending on kairomone treatment. Three egg masses were collected from three ponds within each site. Four sets of three eggs (one for each treatment level) were haphazardly sampled from each egg mass (three sites × three ponds × three egg masses × four treatments = 108 replicates). Ponds were selected based on their high natural predator densities during initial surveys. Egg masses were collected from divergent locations within each pond to ensure the sampling of diverse families. All eggs were collected within three days of their laying to minimize environmental conditioning. Eggs were transported in ice-cooled, aerated containers to Greeley Laboratory (New Haven, CT) where they were maintained in an incubator at 8.0°C so that all eggs could be initiated in the outdoor enclosure at similar stages on the same date. These short exposures to cool temperatures at the egg stage had no effect on future larval mass or survival (unpubl.). Once all eggs were collected, I haphazardly separated four sections of three eggs from each egg mass with a sterilized scalpel and staged them (Harrison 1969). The mean developmental stage (33.7 ± 1.7 SD) of these egg sections was not significantly different across sites (p = 0.19). By collecting eggs in the field soon after laying and by keeping them cool in the meantime, I could begin the experiment with randomly sampled animals that did not differ significantly in developmental stage.

The experiment was initiated when each set of three eggs representing a clutch was placed in a 19-l container of aged, de-chlorinated water randomly located in an outdoor and shaded (50% shade cloth to mimic natural canopy cover) experimental compound in New Haven, CT and randomly assigned a treatment regime. To each container, I added 3.0 ± 0.1 SD g of mixed deciduous leaves collected from the dry margin of a temporary pond (chiefly Acer rubrum and Quercus rubra) to replicate natural habitat. Leaves were placed to one randomly selected side of a plastic hardware cloth divider to establish two equal sections of open and structurally complex (hereafter refuge) habitats for behavioral assessments. To feed A. maculatum larvae, I added to each container twice a week 20-ml of homogenized zooplankton that had been collected from natural ponds and from a Daphnia magna culture.

I randomly assigned one of the treatments (A. opacum, N. viridescens or Dytiscus kairomones or control) to the four egg sections from each clutch. These three predator species were chosen based on their high relative abundances at study sites and different gape constraints. Treatments consisted of twice-weekly exposure to 1 l of predator-conditioned water or control water. Predator kairomones were collected by sampling water from 68 l containers in which field-caught predators were raised separately and fed A. maculatum larvae ad libitum. Dytiscus larvae cannot be identified to species (Larson et al. 2000), so I mixed Dytiscus larvae from different source ponds to homogenize any potential species-specific differences. Control water was handled in the same way as predator water except that it lacked predators. All water additions were filtered with 70-micron Nitex mesh to prevent the possible differential introduction of food resources across treatments.

After placing egg clusters into outdoor containers, I recorded hatching survival and initial activity (percent that moved away from empty ova). Both of these traits did not differ significantly among sites (p > 0.19). I did not record the masses of hatchlings because weighing the small and easily injured hatchlings would have caused significant mortality. Based on my experience in the system, initial hatchling size does not appear to differ among sites. In previous research performed on the same populations, I found that egg sizes did not vary significantly among sites, that the initial masses of A. maculatum hatchlings did not differ significantly among sites, and that initial masses were not correlated with final A. maculatum masses in common garden experiments (Urban 2006, 2008). The experiment was ended when the first larva was observed at the stage preceding metamorphosis. At this stage, I did not witness the mass loss that is sometimes observed as salamanders undergo metamorphosis. Instead, final stage was positively and significantly related to final mass ($F_{1,100} = 9.7$, p = 0.002). Also, the non-linear term in a polynomial regression of final mass versus stage was not significant ($F_{2,99} = 9.7$, p = 0.239), indicating that mass did not decline at the last stage. This analysis suggests that mass differences were not affected by metamorphic changes in late-stage larvae.

For each replicate container, I evaluated mean final larval mass and mean activity outside leaf-litter refuges (both day and night). Final mass was recorded as the mean wet mass per container using a digital scale (± 0.1 mg). Refuge use was evaluated as the mean proportion of surviving larvae

recorded outside refuge habitat during the day (11:00–14:00) and night (23:00–02:00) averaged over two survey dates: 15 July and 8 August 2002. These dates corresponded roughly to the two developmental periods used in the selection trials (see next section). A videotaped pilot study demonstrated that larval activity returned to pre-disturbance levels after a 5 min interval following observer approach. A subset of nine containers was approached in random order, five minutes were allowed to pass, and then successive observations were made on each container every minute for a total of 10 observations per unit. Nighttime surveys were aided by a headlamp fitted with a red filter, which had no observed effect on larval feeding.

I analyzed a multivariate analysis of variance (MANOVA) of foraging behavior and final mass with respect to the fixed factors, site, pond nested in site, treatment, and their interactions. I evaluated this initial MANOVA because I expected that foraging behaviors and final masses would be correlated. I also treated refuge use during day and night as independent traits, assuming that different genes influence these complex behaviors. I analyzed mean trait values per container and nested pond within site to account for local variation. Site was made up of three levels (northern, intermediate and southern sites). The treatment factor included four levels: control and exposure to *Dytiscus*, *A. opacum*, and *N. viridescens* kairomones. I also performed separate 'protected' ANOVA's on each univariate response variable when multivariate results were significant (Scheiner 2001).

I also evaluated a series of analysis of covariance (ANCOVA) models with larval masses as the response variable and the same factor structure as the initial ANOVA, except that continuous site variables that signified alternative selection regimes were substituted for the site classification factor. In this way, I evaluated if larval mass variation was related to differences in mean regional gape-limited predator densities, pond temperatures, and pond hydroperiods. I calculated mean pond temperatures from six ponds at each site (18 ponds total) as the average of the median water temperatures recorded hourly with temperature loggers placed 10 cm below the water's surface at the point of maximum pond depth for the duration of larval development. Pond permanence was measured as the number of days from egg laying to pond drying or the end of the developmental period (set to 1 September) averaged across three years for all 40 ponds sampled for predator composition. As in the initial model, pond was nested within these factors to account for local scale factors. I expected that *A. maculatum* masses would be greater in regions with higher gape-limited predation risk. One might also expect that larvae from cooler sites would grow more rapidly than larvae from warmer sites when raised at the same temperature (e.g. countergradient selection Conover and Schultz 1995, Skelly 2004). Finally, shorter development times due to early pond drying also might select for rapid growth (Storfer and Sih 1998).

All statistical tests were performed in S-Plus ver. 7.0. I used type III sums of squares for calculations whenever groups were unbalanced (Neter et al. 1996). A priori orthogonal contrasts were applied based on predictions: site, southern vs northern; southern vs intermediate; predator treatment, control vs each predator treatment. With the

exception of daytime refuge use, non-significant modified Levene test statistics demonstrated that the ANOVA assumption of constant variance was not violated (Neter et al. 1996). For daytime refuge use, I examined results from the Kruskal-Wallis non-parametric ANOVA (Zar 1999) and found that statistical outcomes remained qualitatively unchanged.

Results

Predator assemblages

The most commonly collected predators were the caudates *N. viridescens* adults and *A. opacum* larvae, the anuran *Rana clamitans*, odonate larvae including *Sympetrum*, *Aeshna*, *Pachydiplax* and *Leucorrhinia*, the coleopteran *Dytiscus*, the hemipterans *Lethocerus* and *Notonecta*, and the megalopteran *Chauliodes* (Supplementary material Appendix 1). Together these 11 taxa made up 95% of the predators sampled. The largest gape-limited predators were the salamanders, *A. opacum* and *N. viridescens*, with median gape sizes of 8.07 and 6.21 mm, respectively (Supplementary material Appendix 1).

Mean gape-limited predator densities decreased from south to north (Fig. 2A; $F_{2,36} = 4.4$, $p = 0.019$) while mean
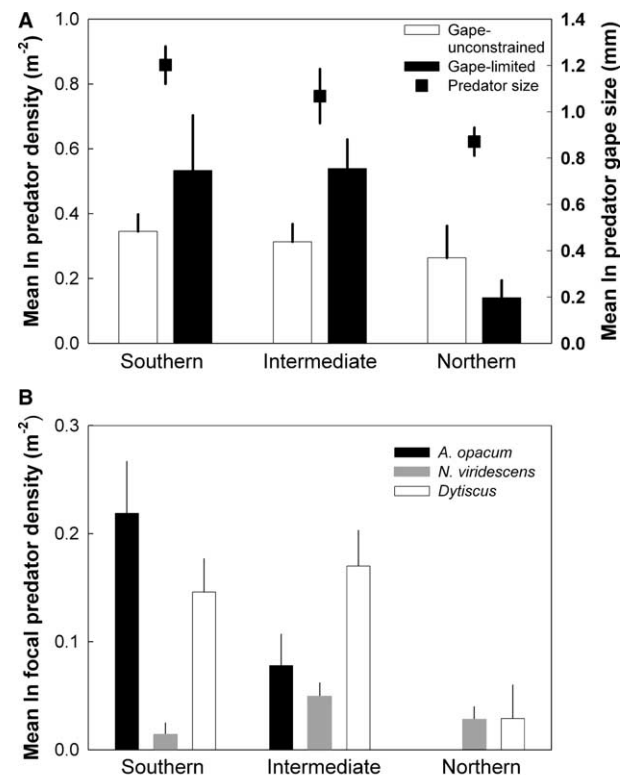

Figure 2. (A) Mean ln-transformed densities (black bars) and sizes (black boxes) of gape-limited predators of *A. maculatum* larvae decline from south to north among sites. Gape-unlimited predator densities (white bars) remain constant along the same cline. (B) Mean maximum (early spring) ln-transformed densities of focal predators along the same latitudinal gradient. For all metrics, SE are indicated.

gape-unconstrained predator densities remained constant ($F_{2,36} = 0.1$, p = 0.940). Total predator density followed a similar pattern with lowest predator densities at the northernmost site ($F_{2,36} = 3.9$, p = 0.029). Mean predator size also declined along the same latitudinal gradient ($F_{2,36} = 5.8$, p = 0.006). Significant site differences were attributed to larger gape-limited predator densities and sizes at the southern as compared to the other sites (p < 0.05, Fisher LSD). The mean ln density of these three focal predators varied markedly among study sites (overall MANOVA; $F_{6,70} = 5.5$, p < 0.001; Fig. 2B). *N. viridescens* was broadly distributed across the site, whereas *A. opacum* declined in density from south to north and was entirely absent from the northern site ($F_{2,36} = 7.4$, p = 0.002). *Dytiscus* densities also differed among sites ($F_{2,36} = 7.8$, p = 0.002). These patterns were consistent with those found for the individual populations from each site used in the phenotypic assays.

Field observations of gape-limitation

The mean size refuge threshold for *A. opacum* larvae and *N. viridescens* adults calculated from field diets was 0.37 ± 0.13 and 0.37 ± 0.17 SD, respectively (Supplementary material Appendix 2). Based on estimated and literature-derived size refuge thresholds and relative prey–predator size ratios in the field, the two salamanders, *A. opacum* and *N. viridescens*, usually were the only gape-limited predators large enough to capture *A. maculatum* larvae at any developmental stage. Even though libellulid dragonfly larvae such as *Sympetrum* spp. were highly abundant in the field and had a greater estimated size threshold (0.8), they were seldom large enough to capture *A. maculatum* larvae.

Ambystoma maculatum larvae had lower relative survival in the field at the southern site when their head widths were less than estimated salamander size refuge thresholds (Fig. 3; $F_{1,13} = 5.0$, p = 0.044) but not less than the larger thresholds calculated for libellulid dragonfly larvae ($F_{1,13} = 2.6$, p = 0.130). This result accords with the fact that few libellulids ever grow large enough to pose a threat to *A. maculatum* larvae and suggests that salamanders pose the strongest gape-limited predation risk in the system. This effect was not due solely to prey-size-dependent survival. Prey size, without factoring in gape-limited predator size, was not related statistically to prey survival ($F_{1,13} = 2.0$, p = 0.181).
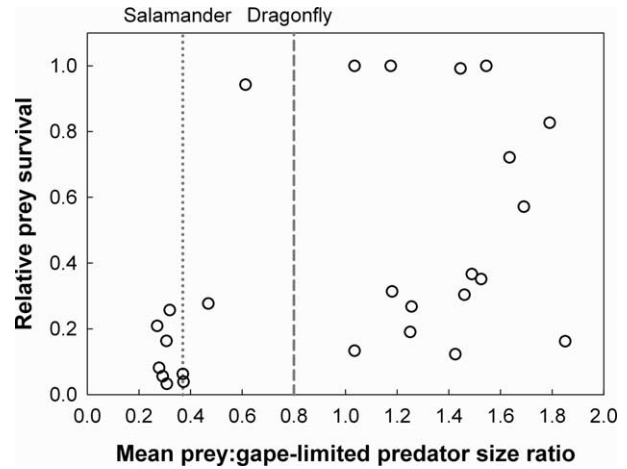

Figure 3. Relationship between relative prey survival and the ratio of mean prey head width to mean predator gape width in ponds at the southern site during the first four weeks of development in 2004. Prey survival was calculated relative to the maximum change in larval density among all ponds for that week to account for changes in pond areas with precipitation. Droplines indicate the estimated average size thresholds at which the salamanders, *A. opacum* and *N. viridescens*, and dragonfly larvae encounter difficulties in handling larger prey items. Each point represents a pond on a specific sampling date during *A. maculatum*'s early development.

Selection gradients on fixed and induced traits

Predator kairomone treatment, site, and population of origin were not related significantly to individual prey survival in the *Dytiscus* prey selection experiment (Table 1). However, the interaction between site, predator treatment, and head width was significant in the *A. opacum* experiment ($F_{4,198} = 4.7$, p = 0.001). This interaction term was significant because larvae from the intermediate site were more susceptible to *A. opacum* when at a small size and raised under *Dytiscus* kairomones than larvae from the southern site raised under similar conditions (Supplementary material Appendix 3; planned contrast, t = 2.2, p = 0.029). In line with its observed gape-limitation, *A. opacum* was more likely to prey upon small *A. maculatum* larvae (Fig. 4A; $B_{avggrad} \pm SE = +0.048 \pm 0.021$, p = 0.010) while gape-unconstrained *Dytiscus* was more likely to prey upon large *A. maculatum* larvae (Fig. 3B; $B_{avggrad} \pm SE = -0.131 \pm 0.039$, p < 0.001). Non-linear selection gradients were not

Table 1. Results from a logistic model of the survival of *A. maculatum* larvae under predation threat by free-ranging *A. opacum* and *Dytiscus* larvae in relation to site, pond nested in site, maximum head width, and their interactions. Significant p-values in bold.

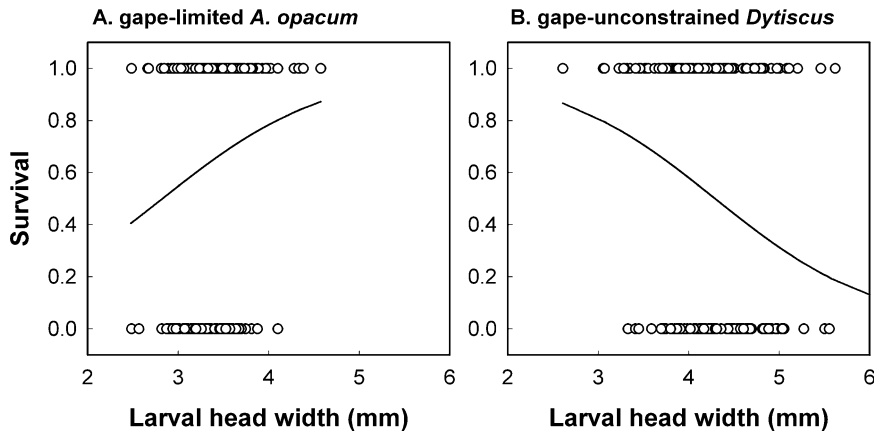| Trait	*A. opacum*		*Dytiscus* sp.	
	F_{DF}	p	F_{DF}	p
Site	$1.6_{2,213}$	0.196	$0.4_{2,213}$	0.661
Pond (nested in site)	$0.8_{9,201}$	0.623	$0.5_{9,201}$	0.900
Treatment	$0.2_{2,211}$	0.836	$2.2_{2,211}$	0.110
Head width	$6.0_{1,210}$	**0.015**	$12.1_{1,210}$	**<0.001**
Site × treatment	$0.7_{4,197}$	0.569	$0.5_{4,197}$	0.749
Site × head width	$0.5_{2,195}$	0.627	$1.2_{2,195}$	0.308
Treatment × head width	$0.7_{2,193}$	0.478	$1.0_{2,193}$	0.382
Site × treatment × head width	$4.7_{4,189}$	**0.001**	$0.5_{4,189}$	0.730

Figure 4. Selection on prey head width by (A) *A. opacum* and (B) *Dytiscus* larvae. Circles indicate either the survival (1) or death (0) of a larva with the indicated head width. The predicted fit for the log odds-ratio obtained from logistic regression has been back-transformed to survival probabilities. At each point, this fit estimates the average expected survival probability of a larva in the prey selection trials with a given head width.

supported statistically for *A. opacum* (p = 0.156). However, *Dytiscus* selection on prey size appears to be marginally disruptive (p = 0.070), strongly favoring small larvae and weakly favoring the largest larvae (based on a cubic spline visualization, Schluter 1988). Lastly, *A. opacum* and *Dytiscus* densities were negatively correlated across ponds within sites ($r_s = -0.52$; p < 0.02), indicating that prey populations can experience antagonistic selection regimes across natural landscapes.

Next, I analyzed differences in prey survival among sites during selection trials. Survival did not differ among sites under predation by the broadly distributed *Dytiscus* larvae (Fig. 5). However, northern populations survived significantly better under *A. opacum* predation than intermediate and southern sites over time (site × time: $F_{2,322} = 7.3$, p < 0.001).

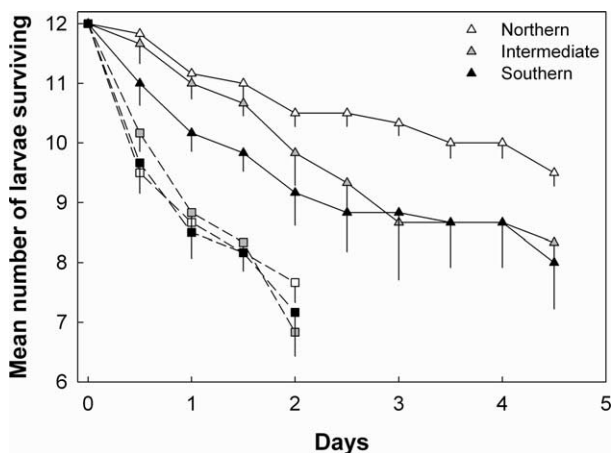Figure 5. The number of larvae surviving over time in the *A. opacum* (top, △) and *Dytiscus* (bottom, □) selection experiments. Values are depicted for the time period in which all replicates were under selection in each experiment. Separate lines indicate the northern (white symbols), intermediate (gray) and southern (black) sites. Each point depicts the mean number (±SE) of surviving larvae in each of six mesocosms.

Phenotypic assays

MANOVA results showed that *A. maculatum* larvae differed in their mass and refuge use across sites (Table 2; $F_{6,130} = 4.1$, p < 0.001) and plastic reactions to different predator kairomones ($F_{9,198} = 2.5$, p = 0.009). Variation in larval mass was explained significantly by the main effects site, kairomone treatment, and population of origin (p < 0.02). In line with predictions, larvae from the intermediate and southern sites exposed to the most intense gape-limited predation risk were larger than those originating from the northern site (Fig. 6). Individuals from all sites grew to a larger size when exposed to kairomones from *N. viridescens* – a finding that matches the predicted increase in growth rate under threat by a broadly distributed, gape-limited predator (Fig. 7A; planned contrast: t = 2.9, p = 0.015). In contrast to predictions, larvae were not larger when exposed to kairomones from the gape-limited *A. opacum* larvae (t = −0.0, p = 0.98). Nor did larvae significantly reduce their growth when confronted with kairomones from the gape-unconstrained *Dytiscus* larva (t = −1.1, p = 0.27).

I next evaluated a series of alternative hypotheses about potential mechanisms underlying this regional variation in larval masses, including regional gape-limited predator densities, pond permanence, and pond water temperatures. The significant decline in gape-limited predator densities with increasing latitude has already been discussed (Fig. 2A). Pond permanence differed significantly among sites (Supplementary material Appendix 4; $F_{2,37} = 4.0$, p = 0.026) because the intermediate site had, on average, more permanent ponds (mean developmental period = 123 days) while the southern site had more temporary ponds (99 days). Mean pond water temperatures did not differ significantly among sites (Supplementary material Appendix 4; $F_{2,14} = 0.4$, p = 0.671). A likely explanation for little variation in mean water temperatures across sites is because *A. maculatum* adults lay eggs successively later in the season at more northern sites and thus their larvae experience close to the same developmental temperatures as larvae at the southern site. Neither pond permanence nor

Table 2. Results from multivariate and univariate analyses of the variance explained in larval masses and the proportion of larvae outside of refuge based on site of origin, pond nested within site, kairomone treatment, the interaction between site and treatment, and pond nested in this interaction. Results are from the means of mass and refuge use for 108 replicate containers of *A. maculatum* larvae, originating from three sites, raised under common garden conditions, and exposed to one of three kairomone treatments or a control. Multivariate results are listed first, followed by ANOVA results. Each column includes the F-statistic, subscripted degrees of freedom and parenthesized p-values. F-statistics for MANOVA are given for the Pillai trace. Significant p-values in bold.

Response	Source of variation				
	Site	Pond in site	Treatment	Site × treatment	Pond in (site × treatment)
All traits (MANOVA)	$4.1_{6,130}$ (**<0.001**)	$1.4_{18,198}$ (0.137)	$2.5_{9,198}$ (**0.009**)	$0.6_{18,198}$ (0.864)	$0.7_{54,198}$ (0.903)
Final mass (ln)	$4.3_{2,66}$ (**0.018**)	$3.5_{6,66}$ (**0.005**)	$3.5_{3,66}$ (**0.019**)	$0.8_{6,66}$ (0.605)	$1.1_{18,66}$ (0.401)
Prop. outside refuge day (asin)	$5.7_{2,72}$ (**0.005**)	$0.9_{6,72}$ (0.511)	$2.7_{3,72}$ (0.053)	$0.3_{6,72}$ (0.954)	$0.3_{18,72}$ (0.937)
Prop. outside refuge night (asin)	$4.2_{2,72}$ (**0.018**)	$0.8_{6,72}$ (0.548)	$2.8_{3,72}$ (**0.047**)	$1.9_{6,72}$ (0.089)	$0.9_{18,90}$ (0.529)

pond water temperature was associated significantly with variation in *A. maculatum* masses (p > 0.3). As expected, gape-limited predator densities were significantly correlated with the observed gradient in larval masses ($F_{1,66} = 7.7$, p = 0.007).

Larval mass was explained by an interaction term between activity outside of refuges during both the day and night ($F_{3,98} = 4.0$, p = 0.002). Therefore, structural refuge use and variation in opportunities for foraging were correlated with mass differences among sites and treatments. Overall, behavioral results highlighted differences in activity outside leaf-litter refuges depending on site, time of day, and predator treatment (Fig. 7B). Larvae spent more time outside refuges at night than during the day. Activity outside of refuges during day and night depended on both site (Table 2; day, p = 0.005, night, p = 0.018) and predator treatment (day, p = 0.053, night, p = 0.047). Site differences in daytime behavior occurred because larvae from the southern site spent more time in refuge habitat than those from the northern site during the day (t = −1.6, p = 0.104) and more time in refuge habitat than those from the intermediate site during the night (t = −2.5, p = 0.014). Both *A. opacum* and *Dytiscus* kairomones induced increased leaf-litter refuge use during the day. This effect of higher refuge use during the day was greatest for *Dytiscus* when analyzed across all sites (planned contrast; t = −2.2, p = 0.030).

Discussion

The relationship between a predator's body size and the prey size that it most easily captures often mediates the strength of predator–prey interactions (Wilson 1975). Paine (1976) argued that natural selection should favor rapid early growth in prey populations that can avoid size-limited predators by entering a body size refuge. A recent model (Urban 2007a) suggests a tradeoff between slow and rapid prey growth depending on the prevalence of gape-limited and gape-unconstrained predators. Here, I show that a geographic cline in gape-limited predation risk was associated with increases in the final masses of *A. maculatum* larvae raised under common garden conditions. I also found novel evidence for the induction of larger prey body size in response to the widely distributed gape-limited *N. viridescens* predator. The strong patterns revealed here and backed by spatiotemporal analyses of predator threats, experimental evaluations of natural selection, and common garden phenotypic assays suggest that simple functional determinations of size-selectivity among predators may explain a sizeable proportion of the overall variation in prey growth and activity in this system.

Gradient in gape-limited predation risk

Gape-limited predator size and density decreased from south to north in the study area while threats from gape-unconstrained predators remained fairly constant (Fig. 2A). *Ambystoma maculatum* larval survival was low in natural ponds when their heads fit easily into salamander mouths (Fig. 3). Other research shows that early *A. maculatum* field survival was explained by the presence or absence of gape-limited *A. opacum* predators and not by other potential predators or environmental factors (Urban 2007b). Hence, the latitudinal gradient in gape-limited predation risk in the study area, due predominantly to the distribution of *A. opacum*, likely creates a gradient in predation risk for *A. maculatum*. Specifically, southern larvae experience a strong pulse of predation risk at small sizes relative to northern populations lacking the *A. opacum* predator (Urban 2006). Similar gradients in gape-limited predation risk exist in diverse ecosystems (lakes, Brooks and Dodson 1965, streams, Reznick et al. 1990, oceans, Sogard 1997, old fields, Schmitz 1998) and thus gape-limited predation gradients may be common.
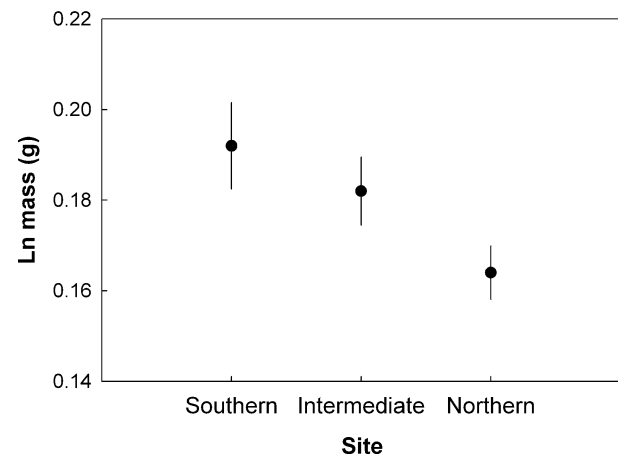

Figure 6. Ln-transformed final mass ($\pm$ SE) by site of origin. Southern populations grew larger than northern populations in accord with predictions about latitudinal variation in gape-limited predation risk and contrary to that expected by countergradient selection for temperature-dependent growth rates.
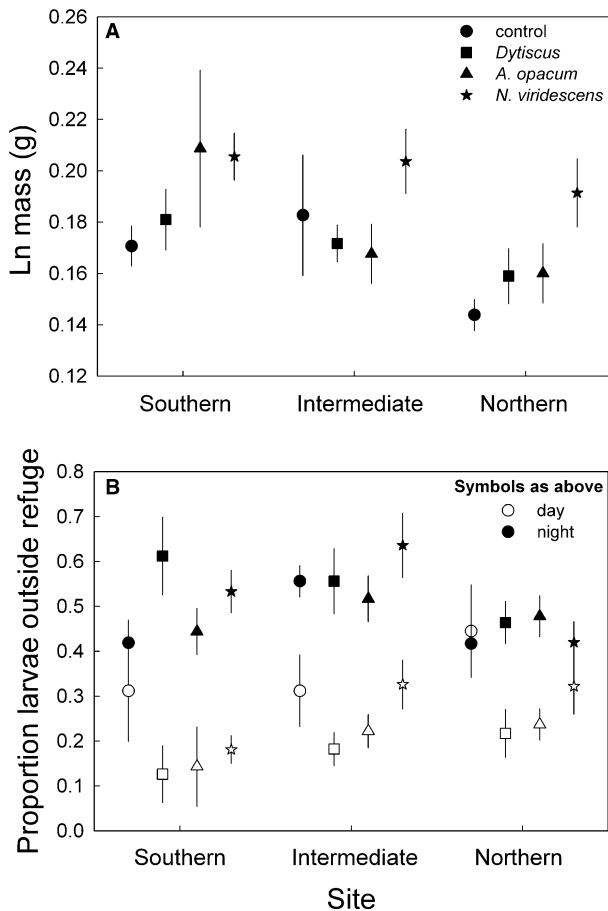
Figure 7. Common garden assays of induced responses to predator kairomones from species depicted in order of increasing gape-limitation on *A. maculatum* larvae: *Dytiscus*, *A. opacum* and *N. viridescens* (see legend for symbols) for the northern, intermediate and southern sites. Traits analyzed include (A) larval mass and (B) the proportion of larvae outside of refuge habitat differentiated by time of day (○ = day; ● = night). Larvae from all sites were significantly larger when exposed to *N. viridescens* kairomones and reduced activity outside of leaf-litter refuges when exposed to *Dytiscus* and *A. opacum* kairomones (p < 0.05). Bars represent SE.

Antagonistic selection

Differences in gape-limitation between *A. opacum* and *Dytiscus* likely generate antagonistic selection on *A. maculatum* growth rate both across geographically distributed sites and across ponds within sites. Gape-limited *A. opacum* larvae were more likely to consume small prey as was expected given their gape-limitation, whereas gape-unconstrained *Dytiscus* larvae were more likely to consume large prey (but not the largest ones as suggested by a marginally significant disruptive selection gradient). *Dytiscus* might eat larger larvae if the predator optimally forages for prey with higher energy content per unit handling time (Schoener 1971) or if larger prey tend to be more active and therefore more obvious to *Dytiscus* (Lima and Dill 1990). *Dytiscus* might have difficulty capturing the largest prey sizes even if it is not constrained by its gape if other prey traits correlated with prey body size, such as swimming ability, affect prey escape (Fitzpatrick et al. 2003). On a cautionary note, because multiple correlated traits might be involved in predator escape and because some of the potentially

important traits have not been measured in this research, it cannot be said with certainty which prey traits are under direct selection and which ones are under correlated selection.

Divergent selection could result in different genetic responses among distant sites or among local populations dominated by either predator. Antagonistic selection among ponds would be expected to be strongest at the southern-most site where *A. opacum* is densest. Strong local heterogeneous selection in the south could lead to the evolution of stronger phenotypic plasticity than at other sites (Pigliucci 2001). However, I found no evidence for a significant site by treatment interaction (Table 2) that would indicate this pattern. One explanation might be that each population has adapted locally such that variable population-level plasticity obscures patterns at the regional level. Future research should be directed toward estimating variation in plasticity across many local populations to provide a statistically robust test of this idea.

Development under different kairomone treatments affected *A. maculatum* survival exposed to free-ranging *A. opacum*, but not *Dytiscus*. Prey survival under *A. opacum* predation was determined by a significant three-way interaction between kairomone treatment, site, and larval head width. This interaction occurred because selection was stronger for large larvae raised under *Dytiscus* kairomones versus control conditions at the intermediate site as opposed to the southern site (Supplementary material Appendix 3). Hence, unstudied traits that are induced by *Dytiscus* kairomones and that vary across sites probably contributed to *A. maculatum* survival. In contrast, kairomone treatment did not affect prey survival in the *Dytiscus* selection experiment. One reason for this weak treatment effect might be that much of the body size variation selected upon by *Dytiscus* was generated within replicate containers rather than across treatment types. Intraspecific size variation in larval salamanders in mesocosms and natural ponds is common (Wilbur and Collins 1973, Brunkow and Collins 1996) and *A. maculatum* larvae compete aggressively for limited resources (Walls 1998). Hence, strong intraspecific competition for limited resources for growth in response to gape-limited predation threats might expand a prey population's size distribution which, in turn, could increase predation risks for smaller competitors.

Evolution of fixed rapid growth

Individuals from the southern and intermediate sites were expected to grow rapidly through either a fixed or an induced adaptive response. Consistent with this former prediction, southern populations grew to larger sizes than northern populations. Interestingly, northern populations survived better than southern populations in selection experiments with *A. opacum*, but not with *Dytiscus* (Fig. 5). However, this pattern of high survival in northern populations would have been reversed if selection experiments had been conducted after most larvae from the southern population had reached a size refuge from *A. opacum* while northern populations were still small enough to be eaten. Overall, results suggest that southern populations adopted riskier foraging behavior to grow quickly under threat by a gape-limited predator despite the initial

risks involved. Other studies show that zooplankton also evolve a larger body size in response to size-selective predators (Leibold and Tessier 1991, Spitze 1991). Hence, adaptive prey body size clines caused by size-selective predation gradients may be more common than generally appreciated.

Correlative associations between phenotypes and hypothesized variation in gape-limited predator selection must be interpreted with caution. However, I was able to refute two alternative explanations for observed patterns. The larval mass cline across sites (Fig. 6) was not explained by either mean pond hydroperiod or mean pond water temperatures. However, consistent with the main hypothesis, among-site variation in larval masses was explained significantly by regional variation in gape-limited predator densities.

Evidence for induced rapid growth

Phenotypic plasticity can evolve when selection regimes vary over space or time, fitness tradeoffs exist for the trait under study across this environmental variation, and an organism can discern reliable cues related to its selective environment (Tollrian and Harvell 1999). This study provides new evidence that rapid growth into a size refuge, independent of longer developmental periods (Crowl and Covich 1990, Black 1993, Chase 1999) or changes in body width without changes in body size (Brönmark and Miner 1992, Kishida and Nishimura 2004), can evolve as an induced response to gape-limited predators. Kairomones from gape-limited newts induced plastic increases in larval masses at all sites, reflecting the newt's strong gape-limitation and even distribution across the study area.

Ambystoma maculatum larvae did not grow larger in response to kairomones from gape-limited *A. opacum* larvae. In subsequent experiments performed under similar conditions, *A. maculatum* larvae always maintained or decreased final mass when exposed to *A. opacum* kairomones (Urban 2006). Why was there an induced increase in growth in response to gape-limited newts but not in response to gape-limited *A. opacum*? One reason might be that fixed growth rates have evolved among sites instead of plastic ones in response to *A. opacum*. In this system, the southern sites grew more rapidly overall than their northern counterparts, suggesting the possible evolution of increased fixed growth rates and a possible associated decrease in selection for induced rapid growth. In addition, the relative costs of rapid growth in response to *A. opacum* might be higher than those for *N. viridescens*. *Ambystoma opacum*'s mean gape size is 30% larger than *N. viridescens*' gape size (Supplementary material Appendix 1). Therefore, prey can grow more quickly into a size refuge from the smaller-gaped *N. viridescens* than they can for *A. opacum*. Lastly, a significant population effect for larval mass suggests that growth rates might be locally differentiated (Table 1). Other research on the same populations has demonstrated genetically divergent foraging and growth patterns among local populations (Urban 2007c, 2008). Thus, microgeographic variation in growth rates might be as important as geographic differences in this system.

Dytiscus kairomones also did not induce lower larval masses relative to control conditions as expected. Yet, *Dytiscus* kairomones strongly reduced the daytime activity of *A. maculatum* outside of refuge habitat and thus ostensibly limited their foraging opportunities. However, this lost time foraging in the day was made up for, at least in part, by increased foraging at night (Fig. 7B). This nocturnal behavior likely reflects enhanced feeding opportunities at the pond surface caused by aggregations of zooplankton (Petranka and Petranka 1980) rather than avoidance of *Dytiscus* larvae, which remain active at night (Hampton and Duggan 2003). In general, complex behavioral adjustments to predator presence through time will frustrate more simplistic evaluations of mean behavioral patterns.

With respect to the hypothesized relationship between predator gape-limitation and adaptive prey growth, I assume that body size and microhabitat preference are the key traits that are directly under selection. However, other size-correlated prey traits also may affect the outcome of predator–prey interactions besides body size and therefore should be considered in future research. For instance, swimming speed in aquatic organisms often increases with body size and thus may offer an additional benefit to large body size (Van Buskirk and Schmidt 2000, Fitzpatrick et al. 2003). Prey might evolve rapid growth even in response to gape-unconstrained predators, so long as the costs of higher mortality due to risky foraging behavior do not negate the multiple fitness benefits of rapid growth. Size-correlated increases in swimming velocity might explain why *Dytiscus* kairomones did not induce significantly slower growth.

Conclusions

Community ecology too often ignores the implications of evolution in its understanding of species interactions (Travis 1996, Thompson 2005). The effects of evolutionary divergence are expected to become increasingly important over larger spatial scales as the swamping effect of local gene flow declines and selection regimes change. This study's combination of field observations, measurements of selection intensities, and common garden-derived estimates of phenotypic divergence provides a rich perspective on how evolution by natural selection and community context interact to generate observed predator–prey interactions across geographic scales. Most, if not all, prey populations encounter antagonistic selection from diverse predators across natural landscapes (Parejko and Dodson 1991, Trussell and Etter 2001, Brodie et al. 2002, Relyea 2002). Therefore, quantifying the ecological and evolutionary size-based mechanisms underlying similarities among divergent predator–prey interactions will provide a means to generalize insights about local community dynamics to multiple communities distributed over broader geographic scales.

References

Benard, M. F. 2006. Survival tradeoffs between two predator-induced phenotypes in Pacific treefrogs (*Pseudacris regilla*). – Ecology 87: 340–346.

Black, A. R. 1993. Predator-induced phenotypic plasticity in *Daphnia pulex*: life history and morphological responses to *Notonecta* and *Chaoborus*. – Limnol. Oceanogr. 38: 986–996.

Brodie, E. D., Jr. et al. 2002. The evolutionary response of predators to dangerous prey: hotspots and coldspots in the geographic mosaic of coevolution between garter snakes and newts. – Evolution 56: 2067–2082.

Brönmark, C. and Miner, J. G. 1992. Predator-induced phenotypical change in body morphology in crucian carp. – Science 258: 1348–1350.

Brooks, J. L. and Dodson, S. I. 1965. Predation, body size, and composition of plankton. – Science 150: 28–35.

Brunkhurst, E. P. 2004. Predatory interactions between insects and spotted salamander larvae, *Ambystoma maculatum*, in vernal pools. – Univ. of Rhode Island, p. 109.

Brunkow, P. E. and Collins, J. P. 1996. Effects of individual variation in size on growth and development of larval salamanders. – Ecology 77: 1483–1492.

Case, T. J. 1978. On the evolution and adaptive significance of postnatal growth rates in the terrestrial vertebrates. – Q. Rev. Biol. 53: 243–282.

Chalcraft, D. R. and Resetarits, W. J. J. 2003. Mapping functional similarity of predators on the basis of trait similarities. – Am. Nat. 162: 390–402.

Chase, J. M. 1999. To grow or to reproduce? the role of life-history plasticity in food web dynamics. – Am. Nat. 154: 571–586.

Conover, D. O. and Schultz, E. T. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. – Trends Ecol. Evol. 10: 248–252.

Crowl, T. A. and Covich, A. P. 1990. Predator-induced life-history shifts in a freshwater snail. – Science 247: 949–951.

Day, T. et al. 2002. The role of size-specific predation in the evolution and diversification of prey life histories. – Evolution 56: 877–887.

DeAngelis, D. L. et al. 1985. The influence of Naticid predation on evolutionary strategies of bivalve prey: conclusions from a model. – Am. Nat. 126: 817–842.

Fitzpatrick, B. M. et al. 2003. Morphology and escape performance of tiger salamander larvae (*Ambystoma tigrinum mavortium*). – J. Theor. Biol. 297A: 147–159.

Formanowicz, D. R. 1982. Foraging tactics of larvae of *Dytiscus verticalis* (Coleoptera: Dytiscidae): the assessment of prey density. – J. Anim. Ecol. 51: 757–767.

Garant, D. et al. 2007. The multifarious effects of dispersal and gene flow on contemporary adaptation. – Funct. Ecol. 21: 434–443.

Hambright, K. D. 1991. Experimental analysis of prey selection by largemouth bass: role of predator mouth width and prey body depth. – Trans. Am. Fish. Soc. 120: 500–508.

Hampton, S. E. and Duggan, I. C. 2003. Diel habitat shifts of macrofauna in a fishless pond. – Mar. Freshwater Res. 54: 797–805.

Hanski, I. and Gaggiotti, O. E. 2004. Ecology, genetics and evolution of metapopulations. – Elsevier.

Harrison, R. G. 1969. Harrison stages and description of the normal development of the spotted salamander, *Amblystoma*

punctatum (Linn.). – In: Harrison, R. G. (ed.), Organization and development of the embryo. Yale Univ. Press, pp. 44–66.

Holyoak, M. et al. 2005. Metacommunities: spatial dynamics and ecological communities. – Univ. of Chicago Press.

Janzen, F. J. and Stern, H. S. 1998. Logistic regression for empirical studies of multivariate selection. – Evolution 52: 1564–1571.

Kishida, O. and Nishimura, K. 2004. Bulgy tadpoles: inducible defense morph. – Oecologia 140: 414–421.

Lande, R. and Arnold, S. J. 1983. The measurement of selection on correlated characters. – Evolution 37: 1210–1226.

Larson, D. J. et al. 2000. Predaceous diving beetles (Coleoptera: Dytiscidae) of the Nearctic region with emphasis on the fauna of Canada and Alaska. – NRC Research Press.

Laurila, A. et al. 2006. Population divergence in growth rate and antipredator defences in *Rana arvalis*. – Oecologia 147: 585–595.

Leibold, M. and Tessier, A. J. 1991. Contrasting patterns of body size for *Daphnia* species that segregate by habitat. – Oecologia 86: 342–348.

Lima, S. L. and Dill, L. M. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. – Can. J. Zool. 68: 619–640.

McPeek, M. A. and Peckarsky, B. L. 1998. Life histories and the strengths of species interactions: combining mortality, growth, and fecundity effects. – Ecology 79: 867–879.

Merritt, R. W. and Cummins, K. W. 1996. An introduction to the aquatic insects of North America. – Kendall/Hunt Publishing.

Neter, J. et al. 1996. Applied linear statistical models. – McGraw-Hill.

Paine, R. T. 1976. Size-limited predation: an observational and experimental approach with the *Mytilus–Pisaster* interaction. – Ecology 57: 858–873.

Parejko, K. and Dodson, S. I. 1991. The evolutionary ecology of an antipredator reaction norm: *Daphnia pulex* and *Chaoborus americanus*. – Evolution 45: 1665–1674.

Persson, L. et al. 1996. Size-specific interactions in lake systems: predator gape limitation and prey growth rate and mortality. – Ecology 77: 900–911.

Petranka, J. W. 1998. Salamanders of the US and Canada. – Smithsonian Inst.

Petranka, J. W. and Petranka, J. G. 1980. Selected aspects of the larval ecology of the marbled salamander *Ambystoma opacum* in the southern portion of its range. – Am. Midl. Nat. 104: 352–363.

Pigliucci, M. 2001. Phenotypic plasticity: beyond nature and nurture. – Johns Hopkins Univ. Press.

Relyea, R. A. 2002. Local population differences in phenotypic plasticity: predator-induced changes in wood frog tadpoles. – Ecol. Monogr. 72: 77–93.

Relyea, R. A. 2003. How prey respond to combined predators: a review and an empirical test. – Ecology 84: 1827–1839.

Reznick, D. N. et al. 1990. Experimentally induced life-history evolution in a natural population. – Nature 346: 357–359.

Scheiner, S. M. 2001. MANOVA: multiple response variables and multispecies interactions. – In: Scheiner, S. M. and Gurevitch, J. (eds), Design and analysis of ecological experiments. Oxford Univ. Press, pp. 99–115.

Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. – Evolution 42: 849–861.

Schmitz, O. J. 1998. Direct and indirect effects of predation and predation risk in old-field interaction webs. – Am. Nat. 151: 327–342.

Schneider, D. W. 1997. Predation and food web structure along a habitat duration gradient. – Oecologia 110: 567–575.

Schoener, T. W. 1971. Theory of feeding strategies. – Annu. Rev. Ecol. Syst. 2: 369–404.

Skelly, D. K. 2004. Microgeographic countergradient variation in the wood frog, *Rana sylvatica*. – Evolution 58: 160–165.

Sogard, S. M. 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. – Bull. Mar. Sci. 60: 1129–1157.

Sokal, R. R. and Rohlf, F. J. 1995. Biometry: the principles and practice of statistics in biological research. – Freeman.

Spitze, K. 1991. *Chaoborus* predation and life-history evolution in *Daphnia pulex*: temporal patterns of population diversity, fitness' and mean life history. – Evolution 45: 82–92.

Stenhouse, S. L. 1985. Interdemic variation in predation on salamander larvae. – Ecology 66: 1706–1717.

Storfer, A. and Sih, A. 1998. Gene flow and ineffective antipredator behavior in a stream-breeding salamander. – Evolution 52: 558–565.

Thompson, J. N. 2005. The geographic mosaic of coevolution. – Univ. of Chicago Press.

Tollrian, R. and Harvell, C. D. 1999. The ecology and evolution of inducible defenses. – Princeton Univ. Press.

Travis, J. 1996. The significance of geographical variation in species interactions. – Am. Nat. 148: S1–S8.

Trussell, G. C. 2000. Phenotypic clines, plasticity, and morphological tradeoffs in an intertidal snail. – Evolution 54: 151–166.

Trussell, G. C. and Etter, R. J. 2001. Integrating genetic and environmental forces that shape the evolution of geographic variation in a marine snail. – Genetica 112/113: 321–337.

Urban, M. C. 2004. Disturbance heterogeneity determines freshwater metacommunity structure. – Ecology 85: 2971–2978.

Urban, M. C. 2006. Evolution and ecology of species interactions across multiple spatial scales. Forestry and environmental studies. – Yale Univ, p. 289.

Urban, M. C. 2007a. The growth-predation risk tradeoff under a growing gape-limited predation threat. – Ecology 88: 2587–2597.

Urban, M. C. 2007b. Predator size and phenology shape prey survival in temporary ponds. – Oecologia 154: 571–580.

Urban, M. C. 2007c. Risky prey behavior evolves in risky habitats. – Proc. Natl Acad. Sci. USA 104: 14377–14382.

Urban, M. C. 2008. The evolution of prey body size reaction norms in diverse communities. – J. Anim. Ecol. 77: 346–355.

Urban, M. C. and Skelly, D. K. 2006. Evolving metacommunities: toward an evolutionary perspective on metacommunities. – Ecology 87: 1616–1626.

Van Buskirk, J. 2001. Specific induced responses to different predator species in anuran larvae. – J. Evol. Biol. 14: 482–489.

Van Buskirk, J. and Schmidt, B. R. 2000. Predator-induced phenotypic plasticity in larval newts: tradeoffs, selection and variation in nature. – Ecology 81: 3009–3028.

van Wassenbergh, S. et al. 2006. Scaling of suction feeding performance in the catfish *Clarias gariepinus*. – Physiol. Biochem. Zool. 79: 43–56.

von Ende, C. N. 2001. Repeated measures analysis: growth and other time-dependent measures. – In: Scheiner, S. M. and Gurevitch, J. (eds), Design and analysis of ecological experiments. Oxford Univ. Press, pp. 134–157.

Walls, S. C. 1998. Density dependence in a larval salamander: the effects of interference and food limitation. – Copeia 1998: 926–935.

Wilbur, H. M. 1988. Interactions between growing predators and growing prey. – In: Ebenman, B. and Persson, L. (eds), Size-structured populations. Springer, pp. 157–172.

Wilbur, H. M. 1997. Experimental ecology of food webs: complex systems in temporary ponds. – Ecology 78: 2279–2302.

Wilbur, H. M. and Collins, J. P. 1973. Ecological aspects of amphibian metamorphosis. – Science 182: 1305–1314.

Wilbur, H. M. and Fauth, J. E. 1990. Experimental aquatic food webs: interactions between two predators and two prey. – Am. Nat. 135: 176–204.

Wilson, D. S. 1975. The adequacy of body size as a niche difference. – Am. Nat. 109: 769–784.

Wissinger, S. A. 1992. Niche overlap and the potential for competition and intraguild predation between size-structured populations. – Ecology 73: 1431–1444.

Yurewicz, K. L. 2004. A growth/mortality tradeoff in larval salamanders and the coexistence of intraguild predators and prey. – Oecologia 138: 102–111.

Zar, J. H. 1999. Biostatistical analysis. – Prentice Hall.

Supplementary material (available online as Appendix O16334 at <www.oikos.ekol.lu.se/appendix>). Appendix 1–4.