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Microgeographic divergence of functional responses among salamanders under antagonistic selection from apex predators

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A predator's functional response determines predator–prey interactions by describing the relationship between the number of prey available and the number eaten. Its shape and parameters fundamentally govern the dynamic equilibrium of predator–prey interactions and their joint abundances. Yet, estimates of these key parameters generally assume stasis in space and time and ignore the potential for local adaptation to alter feeding responses and the stability of trophic dynamics. Here, we evaluate if functional responses diverge among populations of spotted salamander (*Ambystoma maculatum*) larvae that face antagonistic selection on feeding strategies based on their own risk of predation. Common garden experiments revealed that spotted salamander from ponds with varying predation risks differed in their functional responses, suggesting an evolutionary response. Applying mechanistic equations, we discovered that the combined changes in attack rates, handling times and shape of the functional response enhanced feeding rate in environments with high densities of gape-limited predators. We suggest how these parameter changes could alter community equilibria and other emergent properties of food webs. Community ecologists might often need to consider how local evolution at fine scales alters key relationships in ways that alter local diversity patterns, food web dynamics, resource gradients and community responses to disturbance.

1. Introduction

Community ecologists seek to understand and predict the abundances of interacting species in time and space [1]. In many environments, trophic interactions dominate [2,3], and thus predator–prey interactions can strongly determine the stability and dynamics of natural communities [3–6] as well as their responses to natural and unnatural disturbances [7,8]. These interactions have been codified in hundreds of predator–prey models of varying complexity, which form the basis of our understanding of community coexistence, trophic ecology, resource harvests and biological control [9–13].

The vast majority of studies that measure or predict predator–prey interactions assume that predator–prey interactions remain constant in time and space. Yet, empirical syntheses suggest that key ecological traits often vary substantially within species [14] because they sometimes evolve rapidly [15] and across fine spatial scales [16]. Traits involved in species interactions might often come under particularly strong selection, given their close links to fitness (e.g. survival with predators) [17–19]. The growing realization that evolution occurs rapidly and over fine spatial scales suggests a critical need to explore evolutionary explanations for community dynamics and patterns [20–26].

A predator's functional response to prey density plays a disproportionate role in determining the dynamic stability of predator–prey interactions and

Figure 1. Predictions for parameter evolution in a functional response curve. Compared to a baseline (blue, bottom), higher prey consumption can evolve independently through (i) lower handling times (orange, third highest), (ii) higher attack rates (purple, highest) or (iii) a sigmoidal relationship, i.e. higher exponent (green, second highest). Inset displays the same results at lower prey abundance. We standardized changes to correspond to 20% more prey eaten at prey abundance = 300. Baseline parameters from equation (2.1): $a = 1.0$, $h = 0.0067$, $q = 1$; sigmoidal: same as baseline but $q = 1.13$; lower handling time: same as baseline but $h = 0.005$; higher attack: same as baseline but $a = 2.04$. Prey abundance range corresponds to the range of cladoceran densities (per l) observed in the wild. (Online version in colour.)

regulation of prey populations [27]. The functional response describes the relationship between number of prey available and the number eaten by a predator in a given period [10]. Three parameters describe the shape and slope of the functional response: (i) the maximum attack rate of the predator on prey; (ii) the time it takes to catch, handle and digest prey; and (iii) an exponent determining if the relationship is saturating or sigmoidal [9]. Switching between prey types, high costs associated with foraging on rare prey or other negative frequency-dependent feeding decisions can promote a sigmoidal functional response characterized by less feeding at low prey densities [10]. By identifying which of these parameters change, researchers can obtain a more mechanistic interpretation of the relative importance of varying attack rates, handling times or frequency-dependent foraging decisions across environments or populations [28–30].

We hypothesize that local adaptive evolution can alter predator–prey interactions by modifying the parameters of the predator’s functional response. We investigated larval spotted salamanders (*Ambystoma maculatum*) that inhabit neighbouring ponds and encounter varying levels of risk from the marbled salamander (*Ambystoma opacum*) [31]. We predict that spotted salamanders experiencing high risk from marbled salamanders will evolve higher foraging rate than those facing other predator species. Prey encountering predators are normally expected to reduce foraging rate because it increases attack rate by visual predators [32,33]. However, this prediction assumes that predation risk does not vary with prey size. If prey can forage intensely and grow rapidly into a size refuge from gape-limited predators for which gape size or capture apparatus constrains the ability to handle large prey, then an optimal strategy is to forage intensely early during development to enter a size refuge rapidly and thereafter face limited risk [34]. Analytical theory predicts that rapid foraging is optimal in response to gape-limited predators if prey can grow fast enough to reach a size refuge [34]. Alternatively, prey populations might evolve more cautious foraging behaviours if prey cannot grow fast enough to reach

a size refuge, predation risk accelerates with foraging or rapid growth accrues other costs [34–36].

First, we evaluated if functional responses differ among salamanders exposed to different risks from gape-limited predators. We predicted that predation risk should select for higher consumption of prey, specifically at lower prey abundances. Previous work demonstrates that marbled salamanders impose strong natural selection on spotted salamanders to grow into a size refuge from this gape-limited predator. By contrast, gape-unconstrained predators like dragonfly larvae or *Dytiscus* diving beetles select for reduced foraging in alternative habitats [34,37]. Common garden experiments indicate that individuals that co-occur with marbled salamanders forage more frequently than individuals experiencing less predation from marbled salamanders and proportionally more predation risk from gape-unconstrained predators [38]. These differences in foraging rate among spotted salamander populations, in turn, probably also depend on prey density. We expected proportionately more foraging effort at low densities because marbled salamanders also deplete zooplankton resources that would otherwise be shared with spotted salamanders [39]. Thus, predators potentially decrease prey availability while simultaneously selecting for intense foraging in spotted salamanders to reach a size refuge [40]. Selection for greater prey consumption can occur via each mechanism that contributes to the functional response, including (i) shorter handling times, (ii) higher attack rates, or (iii) an altered shape (figure 1). However, we predicted that higher attack rates would be most likely in high-predation ponds because increasing this trait elevates consumption at low prey abundances. More efficient handling times would elevate food intake at higher prey abundances that might not be available in nature and might require modifying more genetically conserved functions such as metabolism [41]. Alternatively, a sigmoidal relationship might characterize prey from high-risk ponds if attack rates at low prey densities accrue high costs. For instance, spotted salamanders might expend more energy searching for food or attract greater mortality

risks from predators [38] than the benefits gained in a low-resource environment. We also evaluated the degree to which differences in functional responses between high- and low-predation ponds depend on genetically determined traits, predator cue-induced plasticity or their combination via a genotype-by-environment interaction. Based on previous research, we predicted that both genetic and plastic mechanisms would interactively shape functional responses.

2. Material and methods

(a) Natural history

The spotted salamander inhabits the eastern US and Canada. Each spring, adults migrate from upland terrestrial habitat into temporary ponds to mate and lay eggs. Small (approx. 15 mg) aquatic larvae hatch from eggs after four to seven weeks. Spotted salamander larvae must survive numerous predator species in the ponds, including marbled salamanders (*Ambystoma opacum*), *Aeshna* dragonfly larvae and *Dytiscus* diving beetle larvae, before metamorphosing into terrestrial juveniles by late summer before most ponds dry.

The congeneric marbled salamander breeds in the autumn; their larvae grow under ice and, consequently, reach a size large enough to prey on larval spring-breeding amphibians [42]. The larval marbled salamander is one of the most important predators of spotted salamander larvae in the region [31,43]. Marbled salamanders are strongly gape-limited, and spotted salamander larvae often escape through rapid growth into a size refuge [40]. Experiments verify that marbled salamander larvae induce selection for larger bodied spotted salamander larvae [43]. By contrast, gape-unconstrained predators like dragonflies and diving beetles either prey equally on different sized larvae or prefer larger spotted salamanders, as occurs for *Dytiscus* diving beetles [31,37,43].

Earlier studies revealed genetic differences in foraging among local ponds correlated with marbled salamander predation risk for a fixed number of prey [38,40]. Despite the proximity of ponds, these genetic differences in foraging behaviours were not explained by neutral gene flow [44] or non-genetic maternal effects [40]. Also, a comparison of multiple competing hypotheses for selection regime indicated that marbled salamander predation risk best explained divergent foraging rates among ponds [39]. Analyses of neutral genetic variation revealed population structure that was associated with predator selection regime [44]. These neutral genetic differences combined with the high philopatry of this species [42], which returns year after year to their natal pond, suggests that each pond hosts a population, although gene flow can be moderately high, especially between ponds that share the same selection regime [44]. Here, we evaluate the effect of local evolution in response to predation risk on specific feeding mechanisms, functional responses, predator–prey interactions and their inferred stability.

(b) Study sites

We evaluated the functional responses of spotted salamander from four ponds at a long-term research site on Totoket Mountain in southern CT, USA. Across this region, ponds differ in marbled salamander prevalence and abundance [31,38,43]. Marbled salamanders occur every year and at high densities in some ponds and rarely and at low densities in other ponds. These local differences remain consistent across years [37] and reflect the differential overwintering survival of marbled salamander larvae across ponds [37]. However, ponds with limited marbled salamander predation risk are not predator-free, but host gape-unconstrained predators like dragonflies and diving beetles [31].

For the experiment, we chose four ponds where spotted salamanders face either low or high marbled salamander predation risk. Spotted salamanders from the B-13 and B-1 ponds face low-marbled salamander predation risk: marbled salamanders occurred in B-13 and B-1 in 45% and 55% of 11 sampled years and occurred at low densities in those years (mean density = 0.08 and 0.14 larvae m^{-2} for B-13 and B-1, respectively). In the other two ponds, spotted salamanders consistently face high marbled salamander predation risk (B-7 and B-9). Across 11 years, the B-7 and B-9 ponds hosted marbled salamanders every year and at densities 3–5 times higher than other study ponds (mean density = 0.28 and 0.45 larvae m^{-2} for B-7 and B-9, respectively). Focal ponds occur in close proximity (minimum distance = 120 m between B-7 and B-13; maximum distance between B-9 and B-1 = 930 m), and share similar physical characteristics aside from predation threat levels [39]. See electronic supplementary material, table S1 for additional characteristics, including density of gape-unconstrained predators (mainly diving beetles and large dragonfly larvae), intraspecific density, hydroperiod and temperature.

(c) Common garden experiment

We collected 12 egg masses from each of the four ponds after fertilization within less than 1 day of egg laying and raised them in a common garden to limit environmental trait induction. Each egg mass was placed in 18 cm of aged well water in a 19 l container under 50% shade cloth until eggs hatched. Once eggs hatched, the larvae were fed wild zooplankton from study ponds ad libitum, including *Daphnia ambigua*, the species used in feeding trials. At 7 days after hatching, two sets of 10 larvae from each container were collected and brought into the laboratory. Each set of larvae was exposed to either water conditioned by live marbled salamanders fed spotted salamander larvae or control water handled the same way but without predators. These cues have been shown to induce plastic reactions in spotted salamander larvae in response to marbled salamanders [38,45].

(d) Feeding trials

We measured functional responses when larvae were two to three weeks old, which corresponds to a period of high-predation risk for spotted salamander larvae from marbled salamanders and when maximal foraging differences were observed previously [38]. During each feeding trial, a larva was placed in an individual 900 ml rectangular glass container in a temperature-controlled incubator (Percival Scientific model I-41; Perry, IA, USA). Each larva was starved for 24 h prior to functional response experiments. We set lighting to natural daylength and temperature to 13.2°C, the mean measured in study ponds during this period.

We evaluated functional responses of spotted salamanders feeding on *D. ambigua*, a zooplankton that occurs naturally in study ponds that is readily consumed by spotted salamander larvae [40]. We conducted four 24 h foraging trials. In each temporal block, larvae from two ponds from each of the two predation regimes (i.e. high versus low-marbled salamander predation risk) was fed one of eight abundances of prey, and two replicates were split between treatments with or without marbled salamander chemical cues, generating a total of 64 experimental units ($2 \times 2 \times 2 \times 8$). Each population and cue treatment group were fed 10, 20, 40, 60, 80, 100, 200 and 300 *D. ambigua*. These initial numbers were determined based on a pilot experiment suggesting that saturation was approached at 300 *D. ambigua* per litre. These prey densities reflect the range of cladoceran densities ($0\text{--}240\ l^{-1}$) found in natural ponds at the field site. We obtained chemical cues from marbled salamander larvae in 38 l containers fed spotted salamander larvae ad libitum using methods previously demonstrated to elicit plastic anti-predator responses [43]. We also added the algae *Nannochloropsis* to each container as food for *D. ambigua*.

(final concentration approx. 1×10^6 cells ml^{-1}). After 24 h, uneaten zooplankton were collected with 80 μm mesh and preserved for enumeration under dissecting microscopes. The four replicate trials were completed in 4 days to lessen differences due to growth and development of spotted salamanders. Maintaining prey densities during the experiment was desirable but impractical for this system because counting the number of remaining prey in experimental vessels requires destructive filtering and enumeration under a microscope. At the end of trials, we wet-weighted salamanders to the nearest 0.0001 g.

(e) Statistical analyses

We evaluated feeding for each group of salamanders in relation to available food and predator chemical cues. We parametrized functional response curves using the R interface for Stan Bayesian inference in the *RSTAN* package in R (v. 3.5.2). A Bayesian mixed-effects framework was chosen to fit a nonlinear function with a non-normal error distribution, random effects and a hierarchical design. We used the standard functional response model (Holling's disc equation [9,29,46]) where the number of prey eaten (N_e) depends on number of predators (P) and the period of feeding (T) as well as the number of prey given (N_g), handling time (h), attack rate (a) and an exponent (q) that varies between 1 and 2 for a type II purely saturating response and a type III logistic response, respectively

$$N_e = \frac{PTaN_g^q}{1 + h a N_g^q} \quad (2.1)$$

We measured functional responses on one predator for 1 day, and thus PT simplifies to 1.

We assumed a Poisson distribution using a log-link. We also parametrized a negative binomial model and compared posterior predictions against the results from the simpler Poisson model. Both models replicated the mean and variance of feeding rate in an accurate and similar way, but the Poisson model matched the observed variance among observations slightly better than the more complex negative binomial model (electronic supplementary material, table S2). Hereafter, we present results from the Poisson model.

We included predation environment, population, and chemical cue as factors potentially affecting attack rate (a), handling time (h) and the exponent (q) differentiating between type II and type III responses. We also included larval weight as a covariate based on commonly observed mass-scaling relationships whereby attack rate is expected to increase with consumer mass and handling time is expected to decline to meet mass-specific increases in metabolism [47]. We added random effects that accounted for day of trial and family. We assumed weakly informative priors [48] for all model coefficients ($\mu = 0$, $\sigma = 100$) except for the q -exponent, which we assumed was uniformly distributed between 1 and 2. We assumed a half-normal distribution ($\mu = 0$, $\sigma = 10$) for the standard deviation of error terms to avoid negative variances. We ran three chains with randomly determined initial values each for 10 000 iterations after a burn-in period of 100 000 iterations and retained every fifth draw, which resulted in well-mixed chains for all parameters using Gelman–Rubin statistics and low autocorrelation. To test inferences, we calculated 95% credible intervals from the posterior distributions of slopes (larval weight) or differences among groups (predation environment, pond, chemical cue) and evaluated if they overlapped zero.

3. Results

Chemical cue did not affect attack rates or handling times (electronic supplementary material, figure S1; attack rate median

difference $\pm$ credible intervals, CIs: 0.002 [−0.015, 0.019]; handling time median difference: −0.0001 [−0.0025, 0.0023]). Larger larvae had lower attack rates and shorter handling times (electronic supplementary material, figure S3; attack rate median slope = −0.0095 [−0.0021, −0.0072]; handling time median slope = −0.0018 day [−0.0021, −0.0015]).

The median difference in the maximum attack rate between high- and low-predation environments was significantly different from zero (0.082 [0.045, 0.117]; figure 2a). In particular, salamanders from the two high-risk ponds (B-7 and B-9) had higher attack rates than the low-risk population, B-1, but not B-13 (figure 2b). Handling times also varied among ponds such that salamanders from high-predation ponds had lower handling times than those from low-predation ponds (median difference: −0.004 day [−0.007, −0.001]; figure 2c). This difference was mostly because B-7 salamanders had a shorter handling time than others. High-predation ponds also hosted salamanders with lower q -exponents than low-predation ponds (median difference: −0.142 [−0.091, −0.038]; figure 2d), and three exponents overlapped with one, indicating a type II functional response. Much of this difference was due to B-1, which diverged from the other populations with $q = 1.19$ [1.10, 1.28], indicating a more sigmoidal relationship for this population.

Overall, the three parameters combined to produce greater feeding rates for salamanders from the two high-predation ponds (figure 3). These changes generally resulted in higher proportional feeding rates at lower prey abundances (approx. 10%, figure 4). In particular, B-9 larvae ate 30% more prey at low prey abundances than B-1 (electronic supplementary material, figure S2). Differences between high- and low-predation ponds were not different from zero at intermediate prey abundances and were different, but less pronounced at higher prey abundances (figure 4). B-7 had a proportionately larger feeding response than other ponds at high prey availability (electronic supplementary material, figure S2).

4. Discussion

Despite the importance of functional responses for understanding predator–prey interactions, coexistence, community stability and applied questions of biological control, few studies have evaluated if functional responses can evolve. Those exceptional studies that have evaluated the evolution of functional responses focused on how prey evolution altered the functional response of a non-evolving predator [28,49]. For instance, Livdahl [28] demonstrated that mosquitoes that evolved higher defences altered their predator's functional response. Tully *et al.* [50] found no evidence that predaceous mites diverged in their functional responses on three genetic clones of springtails. Surprisingly, no study to our knowledge has evaluated if predator populations evolve different functional responses.

We demonstrate that spotted salamanders from nearby ponds differed in functional responses in common garden experiments, mostly due to variation in maximum attack rates. Spotted salamander larvae face varying threats from the gape-limited marbled salamander and other gape-unlimited predators such as *Dytiscus* diving beetles and *Aeshnid* dragonfly larvae. These two functionally divergent types of predators have been shown theoretically and empirically to induce antagonistic selection on body size in prey

Figure 2. Spotted salamander functional responses differ between high- and low-predation environments. (a) We estimate the median (square) and 80% (thick red line) and 95% (thin grey line) credible intervals for the difference in parameters between predation environments using samples from the posterior distribution. For handling time, the median symbol is larger than the credible intervals, which do not overlap zero. (b–d) We document the median and 95% credible intervals of the (b) attack rates, (c) handling times and (d) q -exponents split by population and whether they experience low (blue) or high (red) predation risk, again estimated from samples from the posterior distribution of these model parameters. (Online version in colour.)

Figure 3. Divergence of spotted salamander functional responses between salamanders from high- and low-predation ponds. We used estimated parameters to predict the overall functional responses of each pond. All salamanders showed a saturating increase in prey consumption, but differed in the rate of increase, saturation level and shape. Inset displays patterns at lower prey abundances. See figure 4 for estimated credible intervals. (Online version in colour.)

[34,38–40,43]. More specifically, gape-limited predators like marbled salamander larvae select for intense feeding to grow into a size refuge, whereas gape-unlimited predators like beetle larvae select for more cautious prey that feed and grow more conservatively to avoid attracting attention from visual predators [38,39,43]. Across the landscape, predator communities differ in ways that exert divergent selection on feeding behaviours. Previous studies found that spotted salamanders from high- and low-marbled salamander populations diverged

genetically in foraging rate and overall feeding on fixed quantities of prey [38,39]. These foraging differences are correlated with higher growth in laboratory experiments and higher growth and survival rates in the field [38,43]. These changes occur within the dispersal distance of spotted salamanders, and genetic studies suggest that these so-called microgeographic evolutionary changes are made possible by strong selection against migrants from populations that experience selection from divergent predator communities [16,44].

Figure 4. Percentage difference in spotted salamander functional responses between high- and low-predation ponds calculated from posterior predictions. Dark line indicates the median per cent difference and light ribbon indicates 95% credible intervals. The x -axis is displayed on a log₁₀ scale to allow visualization of differences at both low and high values of prey availability. (Online version in colour.)

However, these previous experiments held prey density constant, precluding an understanding of the density-dependence of different foraging strategies and the specific feeding mechanisms involved. By examining feeding rates at varying prey densities, we assessed if foraging responses have adapted to maximize prey intake, especially at the low densities expected in ponds with marbled salamanders, which are not only predators on spotted salamanders, but also competitors for shared zooplankton resources. We also wanted to understand the degrees to which maximum attack rate, handling time or more complicated density-dependent (i.e. saturating type II versus sigmoidal type III) responses might evolve between selective environments. The evolution of functional response shape is particularly interesting because it can alter the stability of predator–prey interactions, potentially leading to extirpation of prey (see ‘Dynamic stability’ below).

We found that salamanders from high-predation risk ponds employed a mixed strategy of higher attack rates, shorter handling times and less sigmoidal functional responses relative to those from low-predation risk ponds. The largest effects occurred for attack rate and type of functional response, with smaller and more variable changes to handling time. The overall effect was to raise the feeding rate of salamanders from high-predation risk ponds as expected by theory [34] and predictions, especially at the low prey abundances found in high-predation ponds (figure 4). Experimental results validated *a priori* predictions based on our understanding of the natural history of predator–prey interactions and resources in the system. However, additional differences among ponds, most notably intraspecific densities and hydroperiod, could also play a role in the observed divergence. Identifying the source of natural selection with certainty is not possible without direct manipulations of natural selection in the field [51], but at present, we cannot reject a contribution from selection related to marbled salamander predation.

(a) Origin effects

Despite finding overall differences in functional response parameters between predation regimes as predicted, we also found substantial variation between ponds within each predation regime. For example, salamanders from ponds B-13 and B-7 had similar parameters and functional responses despite

belonging to different predation regimes. One explanation is that these two ponds lie within 120 m of each other, well within the dispersal distances of spotted salamanders [44]. Thus, gene flow might be weakening evolution in response to each respective predation regime. Other selective agents, besides marbled salamanders, might also affect the evolution of feeding responses. In previous research, we evaluated if seven alternative hypothesized factors explained evolved foraging rate variation (at a fixed prey density) among ponds and found that only marbled salamander prevalence contributed significantly to foraging variation [39]. Here, too, we evaluated alternative selective factors in electronic supplementary material, table S2. Although we lack the power to draw statistical inferences with only four ponds, we found little evidence that gape-unconstrained predators such as *Dytiscus* diving beetles or dragonfly larvae or water temperature correlated strongly with functional response variation. However, high intraspecific density was strongly correlated with lower maximum attack rates, longer handling times and more sigmoidal shapes, which could suggest a role for competition in affecting evolutionary divergence among populations. At two other sites with less intense gape-limited predation risk, intraspecific competition was correlated with foraging rate [39] and thus could interact with predation risk to determine responses at this site. Because marbled salamanders reduce spotted salamander densities via predation, we cannot separate these factors under correlated field conditions. Hydroperiod (days with water during development) also covaried with the height of the functional response, suggesting that longer developmental periods might affect functional response evolution.

(b) Effects of predator cues and weight

We predicted that chemical cues from marbled salamanders would induce different functional feeding responses. By contrast, we found no effect of cues on promoting the plasticity of functional feeding responses, which is likely to occur if predation risks are relatively constant in ponds or if cues are unreliable [52]. In this system, some traits are plastically induced by predator cues and some are not. For instance, marbled salamander cues induced plastic responses in activity and growth patterns [38,43,45], but did not affect spotted salamander tail-fin depth despite being under strong selection by marbled salamanders [37]. Another potential reason entails time-dependent plasticity. Previously, we found that marbled salamander cues reduced spotted salamander growth by 20% early in development [45], but not later in life [43], suggesting a narrow, early window of plasticity. Future experiments should assess how functional responses not only change in response to population of origin and chemical cues, but also developmental stage.

Larger larvae had lower attack rates and shorter handling times (electronic supplementary material, figure S3), which supports a less rapid rise to the functional response curve, but a higher overall saturation point. Consistent with predictions, handling time decreased for larger bodied individuals. Across a wide range of taxa, handling time generally decreases with body mass, probably because higher mass-specific metabolic demands require more efficient digestion rates [47]. In contrast with metabolic theory, we found that attack rates decreased with body size. One potential explanation is that larger larvae face lower risks from marbled salamanders as they grow into a size refuge [34,43], but higher risks from

other predators such as diving beetles that prefer larger prey [37]. Thus, larger larvae might be shifting towards more conservative foraging strategies as they grow into a size refuge from marbled salamanders.

(c) Dynamic stability

The type II (decelerating intake) functional response destabilizes predator–prey dynamics and prevents prey control because predators consume a smaller proportion of prey when prey numbers are increasing [46,53,54]. Type III (sigmoidal) functional responses, however, can control prey numbers in the low-density zone of accelerating prey consumption. We found evidence for a type III functional response for one low-predation pond (B-1), suggesting that the evolution of more dynamic stability is possible. However, stabilization only occurs for the narrow range at low prey densities before the functional response saturates, producing a bifurcation point for stability and coexistence.

Theory suggests that evolution can either stabilize or destabilize predator–prey dynamics. Similar to the empirical case, most models evaluate how prey defensive adaptations alter stability [55]. When prey adapt defences in proportion to predator abundance, evolution might stabilize the interaction by allowing rare predators to rebound by eating less defended prey [49,56]. However, assuming a saturating, type II functional response can destabilize the interaction if predators become satiated on vulnerable prey and runaway cycling spirals to extinction [49]. Experiments support these theories. For instance, one experiment demonstrated that the evolutionary cycling of defended and undefended algae generated aperiodic and unstable cycles with grazing rotifers [20,57,58]. Theory focusing on prey preference suggests that evolution can promote coexistence of multiple prey types by inducing adaptive switching behaviour [59]. Lastly, predator–prey coevolution generally stabilizes interactions and promotes coexistence, but the outcome depends on relative evolutionary and trade-off surfaces [60]. Overall, predator evolution probably enhances stability and coexistence when it dampens prey oscillations through enhanced attack rates or prey switching, whereas evolution of prey defences might more readily destabilize dynamics under realistic assumptions of saturating predator functional responses.

(d) Caveats and additional considerations

We designed a common garden experiment in which we raised individuals from eggs to larvae to reduce environmental contributions and reveal genetically diverged responses. Common garden experiments are the most common approach for testing for genetic divergence in complex traits [26,61]. However, observed trait variation could also occur due to maternal effects or transgenerational plasticity. In previous research, we excluded four of the most common sources of maternal effects, including maternal habitat choice, care, egg provisioning and environmental cues of predation for both mother and offspring [40]. We cannot exclude multi-generational maternal effects because we did not raise salamanders for several generations in the laboratory (approx. 6 years for this species), and thus conclusions about genetic determination should be treated with caution, given that adaptive maternal effects could also explain the observed changes in the functional response. Theoretical and empirical observations suggest that these differences in functional responses probably

enhance local fitness and thus reflect local adaptation [34,40]. However, reciprocal transplant experiments would be needed to test this adaptive hypothesis directly.

Despite fitting mechanistic equations to observations rather than simply describing statistical patterns, the practice remains an exercise in constrained curve-fitting. Various combinations of parameters could generate similar observed patterns (e.g. figure 1). Although we fit equations to reveal mechanistic insights, we remain cautious about interpretations until independent traits can be isolated and tested. In the mosquito example, behavioural assessments eventually suggested that predation induced the evolution of less risky behaviours, which reduced attack rates from visual predators [62]. In our system, previous behavioural assessments at low densities of prey suggest that spotted salamander larvae from high-risk ponds evolve more active foraging [39,40], providing additional and independent evidence supporting attack rates as more likely to evolve compared to handling times. The degree to which type II versus type III functional responses diverge among ponds will require additional experiments focused on behavioural assays conducted at low prey densities. Given the complexity of our design, we could not maintain constant prey densities or fit a dynamic model that accounts for prey depletion, and therefore, absolute parameter estimates should be viewed with caution. However, the relative differences among predation regimes that we explored should be robust to these methodological concerns.

Although mechanistic equations can suggest possible effects on stability and coexistence, ultimately experiments are required to test these predictions. For instance, future experiments could evaluate if zooplankton coexist with larval salamanders from high- and low-predation risk ponds by tracking dynamics across many prey generations in large experimental mesocosms or field enclosures. Also, manipulations of prey densities could assess if a basin of attraction exists, such that prey numbers recover from rarity [63]. However, the long-lived nature of the predators in this study (up to 30 years) makes the system untenable for evaluating joint predator–prey population dynamics. Shorter-generation systems of interacting species will remain useful for testing predictions about predator–prey eco-evolutionary dynamics [20,57,64]. Yet insights from non-model, long-lived species will continue to be useful in suggesting the generality of outcomes in real, wild systems.

5. Conclusion

Results suggest that salamanders from ponds with varying predation risk diverged in their functional responses over fine spatial scales. These changes probably further reduce prey densities, and at least in one case, might promote coexistence. The experimental set-up along with extensive previous research indicates that these differences are genetically determined, at least in part. Overall, functional responses provide the key linkage between predator and prey abundances, and thus finding that they can evolve suggests important consequences for our understanding of trophic dynamics, species coexistence, community diversity and even applied questions related to biological control. As a first step, biologists should not initially assume that functional responses remain fixed in theory or empirical systems, but rather accept that functional responses might evolve in response to natural selection. Appreciating

the dynamic interplay between evolution and ecology, although undoubtedly more complex, should facilitate a more predictive and complete understanding of trophic ecology.

Ethics. The salamanders in this study were collected under an approved Connecticut State Wildlife Scientific Collection permit. Experiments were conducted in accordance with the approved IACUC protocol A12-008.

Data accessibility. Data are available at the Dryad Digital Repository: <https://doi.org/10.5061/dryad.j0zpc86cd> [65].

Authors' contributions. All authors conceived of the study, collected data and contributed to writing the manuscript. M.C.U. analysed and led manuscript writing.

Competing interests. We declare we have no competing interests.

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