

# Variability in functional response curves among larval salamanders: comparisons across species and size classes

T.L. Anderson, C. Linares, K.N. Dodson, and R.D. Semlitsch

**Abstract:** Predator species and body size represent critical factors that have differential effects on prey populations, as well as overall community structure. However, investigations of how morphologically-similar predator species, simultaneous to variation in predator body size, influence lower trophic levels are infrequently performed. We tested whether predator species and body size influenced the functional response curve of three larval ambystomatid salamanders (Ringed Salamander, *Ambystoma annulatum* Cope, 1886; Spotted Salamander, *Ambystoma maculatum* (Shaw, 1802); Marbled Salamander, *Ambystoma opacum* (Gravenhorst, 1807)) while eating congeneric prey. We combined larval salamanders of varying body sizes with up to six prey densities within experimental microcosms. We tested for the shape of the functional response curve and obtained parameter estimates for attack rate and handling time for each predator size – species combination. We found variability among both species and size classes, with a combination of type I and type II functional response curves. Large size classes of predators had higher attack rates than smaller size classes, but equivalently-sized larvae of different species exhibited differences in attack rates and handling time. Our study shows that predation risk varies depending on the size structure and diversity of predators present in a food web, and that grouping predators by either species or size class may reduce the ability to predict changes in community structure resulting from such interactions.

**Key words:** *Ambystoma*, functional redundancy, functional response, intraguild predation, Ringed Salamander, Spotted Salamander, Marbled Salamander.

**Résumé :** L'espèce et la taille du corps des prédateurs sont d'importants facteurs clés qui ont des effets différentiels sur les populations de proies ainsi que sur la structure globale de la communauté. Les études sur les incidences concurrentes d'espèces de prédateurs de morphologie semblables et de variations de la taille du corps des prédateurs sur les niveaux trophiques inférieurs sont rares. Nous avons vérifié si l'espèce et la taille du corps des prédateurs influencent la courbe de réponse fonctionnelle de trois salamandres ambystomatidés larvaires (salamandre annelée, *Ambystoma annulatum* Cope, 1886; salamandre maculée, *Ambystoma maculatum* (Shaw, 1802); salamandre marbrée, *Ambystoma opacum* (Gravenhorst, 1807)) se nourrissant de proies congénères. Nous avons combiné des larves de salamandre de tailles différentes à jusqu'à six densités de proies au sein de microcosmes expérimentaux. Nous avons caractérisé la forme de la courbe de réponse fonctionnelle et obtenu des estimations de la fréquence des attaques et du temps de manipulation pour chaque combinaison de taille et d'espèce de prédateur. Nous avons constaté une variabilité tant sur le plan de l'espèce que de la classe de tailles, avec une combinaison de courbes de réponse fonctionnelle de type I et de type II. Les classes de grandes tailles de prédateur présentaient des fréquences d'attaques plus élevées que les classes de tailles plus petites, mais des larves de taille semblable d'espèces différentes étaient caractérisées par des fréquences d'attaques et des temps de manipulation différents. L'étude démontre que le risque de prédation varie selon la structure de taille et la diversité des prédateurs présents dans un réseau trophique, et que le regroupement des prédateurs par espèce ou par classe de tailles pourrait limiter la capacité de prédire les changements de la structure des communautés découlant de telles interactions. [Traduit par la Rédaction]

**Mots-clés :** *Ambystoma*, redondance fonctionnelle, réponse fonctionnelle, prédation intraguild, salamandre annelée, salamandre maculée, salamandre marbrée.

## Introduction

Predator species are often combined into functional categories and treated as interchangeable entities within food webs, which ultimately contribute to structuring ecological communities (reviewed in Chalcraft and Resetarits 2003a). It has been increasingly documented that not all predator species are equivalent in consuming or otherwise influencing prey populations, indicating that organisms which occupy similar trophic levels are not necessarily redundant in their ecological role (Chalcraft and Resetarits 2003a; Loreau 2004; Resetarits and Chalcraft 2007). Furthermore, investigations of closely-related (i.e., the same genus) and (or)

morphologically-similar organisms have not been thoroughly tested to determine whether they have similar effects on prey, i.e., their functional equivalence (Resetarits and Chalcraft 2007). The few studies that have tested for such effects have found that even species which are very similar in morphology do not necessarily influence prey populations in an analogous manner (Resetarits and Chalcraft 2007; Rudolf et al. 2014).

Similar to grouping species into functional categories, classification of all individuals of a single species into one group further minimizes the impacts that intraspecific variation has on their ecological role (Bolnick et al. 2011; Rudolf and Lafferty 2011). For example, size or age structure within a population often influ-

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ences species interactions and ecological communities (Persson et al. 2003; Woodward et al. 2005; Wissinger et al. 2010; Miller and Rudolf 2011; Rudolf and Rasmussen 2013a). Simultaneous comparisons of these two different components of functional redundancy (i.e., similarity within and among predator species and size classes) has received little attention, yet likely plays a critical role in determining both population and community structures (Rudolf et al. 2014).

One important element of predator–prey interactions is the per capita consumption rate of prey by predators, or their functional response (Holling 1959). Variability in the shape of a predator's functional response, as determined by changes in attack rates and handling times, are important to understand because such differences can lead to regulation and (or) (de)stabilizations in both predator and prey population growth rates and dynamics (Berryman 1992; Bolnick et al. 2011). Generally, functional response curves follow one of three patterns (Holling 1959): type I, or a linear relationship between the number of prey killed and the initial prey abundance; type II, where the number of prey killed increases but then saturates at high prey densities; type III, where the number of prey killed is reduced at low prey densities, increases at intermediate prey densities, and then saturates at high prey densities (i.e., a sigmoidal function). The type and overall shape of a predator's functional response can vary based on many factors, including predator and prey sizes (Aljetlawi et al. 2004; McCoy et al. 2011; Kalinkat et al. 2013), temperature (Rall et al. 2012), the presence of other species (e.g., alternative prey or additional predators; Schmidt et al. 2014; Stier and White 2014; Paterson et al. 2015), or the physical complexity of the environment (Hossie and Murray 2010; Toscano and Griffen 2013).

Despite the numerous investigations of variability in functional response curves in response to various biotic or abiotic, few studies have tested how multiple trait axes simultaneously influence such responses. Species and size classes may exhibit dissimilar functional response curves for many reasons, including predator-specific avoidance by prey, increased experience and reduced gape limitations (i.e., handling time) for larger predators, allometric differences in head size among species that provide foraging advantages, or because of greater evolutionary history among species (Abrams 2000). While the exact mechanism that induces differences in functional responses may be difficult to discern, identification of whether this relationship between predator and prey differ in type and shape is a critical first step.

We tested the hypothesis that different size classes of three congeneric predator species vary in their functional response parameters (attack rate and handling time). Our study system consisted of three predatory larval salamanders: Ringed Salamander (*Ambystoma annulatum* Cope, 1886), Marbled Salamander (*Ambystoma opacum* (Gravenhorst, 1807)), and Spotted Salamander (*Ambystoma maculatum* (Shaw, 1802)). Larval salamanders represent a model system for testing size and species differences because many species co-occur in ponds and prey upon one another, including the study species (Urban 2007; Anderson and Semlitsch 2014). Larvae are also gape-limited predators, making the size ratio between predators and prey a key determinant of their foraging abilities (Urban 2007; Anderson et al. 2013). We tested whether functional response curves varied for two size classes each of *A. annulatum* and *A. opacum* when consuming hatchlings of *A. maculatum*. We also examined the functional response curve of larval *A. maculatum* when consuming hatchlings of *A. annulatum*. We expected that all predators would show saturating functional response curves (type II), and that smaller size classes of each species would have lower maximum per capita consumption rates and greater han-

dling times than larger size classes. Because of morphological similarities among species and a general lack of information on larval salamander functional response curves, we did not have any a priori expectations for significant differences among predator species in attack rates or handling times.

## Materials and methods

### Study system

Our three focal pond-breeding salamanders co-occur in ponds in Missouri, USA (Hocking et al. 2008; Peterman et al. 2014). These species have variable life histories, including breeding strategy (aquatic eggs vs. terrestrial eggs) and phenology (fall-breeding vs. spring-breeding), but also represent distinct subclades with the family Ambystomatidae (Williams et al. 2013). *Ambystoma opacum* breed in late August through November in Missouri. After mating, females form terrestrial nests in partially dried pond basins and they brood the eggs until the pond is flooded. The larval stage lasts for approximately 7–9 months, with metamorphosis occurring from April throughout July (Petranka 1998). *Ambystoma annulatum* are an Ozark endemic that breed in ponds and lay aquatic eggs from mid-September through November (Semlitsch et al. 2014). The larval stage lasts for approximately 7–9 months, with metamorphosis occurring as early as April and lasting through early July (Semlitsch et al. 2014). *Ambystoma maculatum* breed from late February to early April (Sexton et al. 1990; R.D. Semlitsch and T.L. Anderson, accepted manuscript<sup>1</sup>). Females also lay aquatic eggs masses and the larval stage lasts for approximately 3–5 months, with metamorphosis occurring from June to October (R.D. Semlitsch and T.L. Anderson, accepted manuscript<sup>1</sup>).

Larval *A. annulatum* and *A. opacum* are potential predators on early stages of the spring-breeding *A. maculatum* because of size differences that develop from the phenology differences (Urban 2007; Anderson and Semlitsch 2014). However, there are a suite of factors that can influence the relative size differences between fall-breeding and spring-breeding species (i.e., the predation risk), including the timing of breeding migrations or larval density (Anderson and Semlitsch 2014; Anderson et al. 2015). Predation by fall-breeding species is also more prevalent at higher larval densities, but depends on the amount of temporal overlap among fall-breeding and spring-breeding species, which ranges from approximately 30–60 days (Anderson and Semlitsch 2014; Anderson et al. 2015). Larval *A. maculatum* can also persist in permanent ponds through the fall, especially under high densities which delays their metamorphosis (Phillips 1992; R.D. Semlitsch and T.L. Anderson, accepted manuscript<sup>1</sup>; T.L. Anderson, unpublished data). Therefore, their larvae can be present when *A. opacum* and *A. annulatum* breed, providing the opportunity for reciprocal predation to occur.

### Experiment 1: fall-breeding predators

We conducted experiment 1 in an environmentally-controlled animal care room at the University of Missouri in Columbia, Missouri, USA. We set up 60 aquaria (37.85 L; 51 cm × 27 cm × 33 cm) on four racks, with 5 aquaria on each of three rows. We filled each aquarium with approximately 30 L of charcoal-filtered UV-sterilized water. Paper was wrapped around the outer sides of all aquaria to limit disturbance and visual cues, except one end that had single sheet which could be lifted up for observations. In each aquarium, we added 12 pieces of cover constructed from gray window screen (20 cm × 5 cm with a 1.6 mm pore size) in an irregular bowtie shape (Drake et al. 2014). Water temperature was approximately 22 °C throughout the experiment and the light:dark cycle was set to

<sup>1</sup>R.D. Semlitsch and T.L. Anderson. Structure and dynamics of Spotted Salamander (*Ambystoma maculatum*) populations in Missouri. Herpetologica. Accepted manuscript.

mimic the natural light conditions of mid-April (14 h light : 10 h dark).

We collected approximately 15 egg masses of *A. maculatum* during the first week of April 2014 from ponds at Fort Leonard Wood (FLW) in Pulaski County, Missouri, USA. We collected larval *A. opacum* and *A. annulatum* predators from ponds at FLW using dip nets during the third week of April 2014, but in different ponds than where the eggs were collected; however, all three species were sympatric at all collection sites. We collected 15 individuals for each of two size classes (large and small) of each predatory salamander (for size ranges see the Results). For *A. opacum*, the size classes were collected from separate ponds because we observed very little size variance within a pond. For *A. annulatum*, the size classes were split across two ponds.

We transported predators and prey back to the laboratory; each predator was placed in an individual plastic container (17 cm × 12 cm × 9 cm) in a mix of pond water and aquarium water and held overnight prior to the start of the experiment (approximately 24 h). We combined egg masses in three large plastic containers filled with pond water and added a small amount of water each day until they all hatched (approximately 21 April) to acclimate them to the experimental conditions. We took a dorsal photograph of each predator with a ruler placed under each container to measure predator head width (HW) and snout-vent length (SVL) (Rasband 2012).

We randomly assigned prey densities ( $n = 4, 8, 12, 16, \text{ or } 20$  prey), predator species, and size class treatments to each aquarium and added them on 22 and 24 April, respectively. We had three replicates of each density – species – size class combination. We also set up a single control tank with 12 hatchlings, all of which survived to the end of the experiment. Though this is a reduced number of control replicates, other studies have similarly shown nearly 100% survival of hatchling ambystomatids in the absence of predators at up to 20 hatchlings (Anderson et al. 2013; Drake et al. 2014). Our second experiment using larval *A. maculatum* as predators also had controls at each density with 100% survival (see below). We terminated the experiment after 46 h and counted the remaining number of hatchlings.

Based on initial analysis of the experiment (see Results), we discovered prey densities were not high enough to attain clear asymptotes for the functional response. Therefore, we conducted one additional density treatment ( $n = 50$  hatchlings, 2 replicates) in April 2015. The reduced number of replicates at high prey densities is a common design in functional response studies. The experimental setup and duration were identical to the treatments in 2014, including similarly-sized predators, but was conducted in the greenhouse where experiment 2 occurred (see below). Environmental conditions (e.g., water temperature, light:dark cycles) were otherwise similar to the setup in 2014, so we do not expect location and year differences had a substantial bias on the outcome of the experiment though we recognize the confounding effect in the experimental design.

### Experiment 2: spring-breeding predator

We tested the ability of the spring-breeding larval *A. maculatum* to consume hatchlings of *A. annulatum* during the fall of 2014. We did not conduct predation trials on *A. opacum*, as we could not capture enough hatchlings of this species without unduly injuring them. We performed these trials indoors in a greenhouse on the University of Missouri campus due to a switch in available laboratory space. This venue permitted a natural light regime and maintained a relatively constant temperature (approximately 20–25 °C), both of which were similar to experiment 1. We conducted this experiment in plastic containers (32 cm × 19 cm × 11 cm) that were filled with 5 L of aged well water. We set up 30 total containers on five racks of a shelving unit, with white paper strips again placed around containers to limit visible disturbances and eight pieces of shade cloth added as cover. The entire array was covered

with a cotton sheet to limit direct sunlight and minimize disturbance. We collected eggs of *A. annulatum* from FLW on 28 September that hatched 3 days later in the greenhouse. We collected larval *A. maculatum* predators from FLW on 2 October that were also transported back to the greenhouse and housed in individual containers until the experiment began on 3 October. Both predators and prey were introduced in a similar manner to the previous trials. In this set of trials, we used six prey densities ( $n = 4, 8, 12, 16, 20, \text{ and } 30$  hatchlings of *A. annulatum*), but only one predator size class of larval *A. maculatum* because we did not observe substantial size variance at our collection sites. We also had one control container of each prey density but with no predator. For both experiments, the prey densities used reflect natural densities of hatchling salamanders (Petranka 1998). We terminated the experiment after 48 h and counted the number of survivors per container.

### Analysis

We first compared whether predator SVL varied among the three species, as well as size classes within species for *A. annulatum* and *A. opacum*, using a one-way analysis of variance (ANOVA). We created a five-level dummy variable for the species–size combinations because we did not have a complete factorial design (e.g., only one size class of *A. maculatum*) that would permit an interaction among these factors. We also performed an analysis of covariance (ANCOVA) on the relationship of HW and SVL (centered prior to analysis) by species.

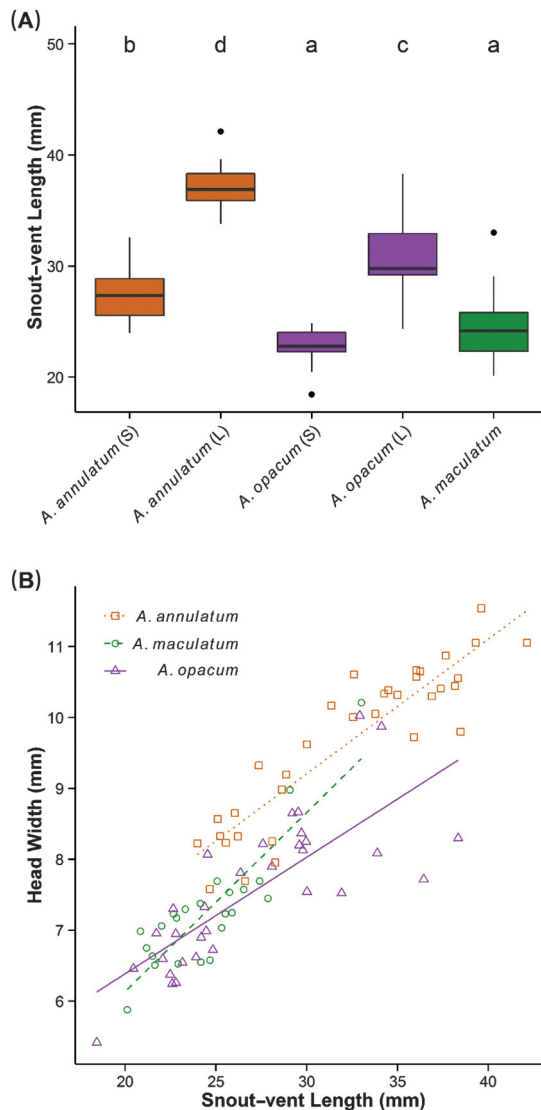
Our primary goal initially was to describe the shape of larval salamander functional response curves. Our analysis followed Juliano (2001), where we initially tested for the effects of prey abundance on a two-column matrix of survival data (number killed, number alive) using generalized linear models with a binomial error distribution. We conducted this analysis separately for each predator species and size class combination. We started with a cubic model of prey density, which is analogous to a type III response, but no cubic terms were significant and therefore removed. Some treatments did not show significant quadratic terms (type II response) and differences in Akaike's information criterion (AIC) scores between models supported type I over type II responses. We therefore fit type I functional responses to all species – size class combinations. However, we also wanted to account for prey depletion in the analysis (Juliano 2001). We therefore also used the Rogers random predator equation with the type II response for all species – size class combinations, except for large *A. annulatum* where the model did not converge, as this model accounts for prey depletion (Juliano 2001). The Rogers random predator equation is

$$N_e \sim N_0[1 - \exp[a(N_e h - PT)]]$$

where  $N_e$  is the number of prey eaten,  $a$  is attack rate,  $h$  is handling time,  $P$  is the number of predators,  $T$  is the temporal duration (h), and  $N_0$  is initial prey density. Attack rate and handling time are not directly quantified in this analysis, but rather are estimated from model parameters. Because we used smaller containers in experiment 2, predator densities were higher compared with those in experiment 1. To make parameter estimates comparable across the two experiments, we multiplied parameter estimates from experiment 2 by 0.44, as this value would be the equivalent predator density in experiment 2 if conducted in the same-sized arena as experiment 1. We calculated all maximum-likelihood estimates of  $a$  and  $h$  using the `mle2` function in the “`bbmle`” package within R (Bolker 2012; R Core Team 2015).

Finally, we assessed whether SVL as a continuous variable would predict predation rates. To do so, we fit a single Rogers random predator equation functional response to all the data where *A. annulatum* and *A. opacum* were predators. We used this subset of data because fitting all the data within one model did not allow for us to account for the different-sized containers with

**Fig. 1.** (A) Snout-vent length (mm) of Ringed Salamanders (*Ambystoma annulatum*), Marbled Salamanders (*Ambystoma opacum*), and Spotted Salamanders (*Ambystoma maculatum*). S and L refer to small and large size classes of each species, respectively. (B) Relationship between head width (mm) and snout-vent length (mm) among species. Colour online.



*A. maculatum* as a predator. We extracted the residuals from this model and regressed them on SVL. We compared cubic, quadratic, and linear versions of this regression model using AIC (Burnham and Anderson 2002).

## Results

Predator size significantly varied between species and size classes (SVL:  $F_{[4,84]} = 72.82$ ,  $p < 0.001$ ). Tukey post hoc tests revealed that size classes within *A. opacum* and *A. annulatum* (i.e., large vs. small) were significantly different from each other in SVL (Fig. 1A). Additionally, *A. maculatum* were similarly-sized in SVL to small *A. opacum*, but smaller than either size class of *A. annulatum* and large *A. opacum* (Fig. 1A). All species showed significant positive relationships of HW and SVL (Fig. 1B). The slope of HW and SVL for *A. maculatum* appeared to be steeper ( $\beta = 0.25$  (*A. maculatum*) vs. 0.19 (*A. annulatum*) and 0.16 (*A. opacum*)), but the relationship of SVL and HW was not significantly different among species (ANCOVA interaction term:  $F_{[2,83]} = 2.12$ ,  $p = 0.13$ ; Fig. 1B). Assuming homogeneous slopes, the

intercept of *A. annulatum* was significantly higher than that of *A. maculatum* and *A. opacum*, which were not different from each other ( $F_{[2,85]} = 27.74$ ,  $p < 0.001$ ).

Comparison of type I responses across treatments showed that smaller size classes had lower attack rates (i.e., reduced slopes) than large size classes and that *A. annulatum* had only slightly higher attack rates than *A. opacum* (Table 1; Figs. 2A–2E). After accounting for the different size containers, *A. maculatum* had the lowest attack rates (Table 1).

For type II responses, both attack rate and handling time were significantly different from zero for small *A. annulatum*, small *A. opacum*, and *A. maculatum* (Table 1; Figs. 2A, 2C, 2E). The small *A. annulatum* and small *A. opacum* showed similar attack rates, but the handling time of the former was twice as high as the latter (Table 1). The handling time of *A. maculatum* was similar to small *A. opacum*, but had a lower attack rate compared with small *A. opacum* and small *A. annulatum* (Table 1). The large size class of *A. opacum* had a higher attack rate and lower handling time than all other predators, but only attack rate was significantly different from zero (Table 1).

The residuals of the functional response curve were significantly predicted by a cubic relationship with SVL (cubic vs. quadratic:  $\Delta\text{AIC} = 3.6$ ; cubic vs. linear:  $\Delta\text{AIC} = 24.1$ ). There was also a significant interaction of SVL and predator species (ANCOVA interaction term:  $F_{[3,60]} = 3.86$ ,  $p = 0.01$ ). Predation rate was relatively higher for smaller *A. opacum*, which then reversed at approximately 29 mm for SVL (Fig. 3). Above 29 mm SVL, relative predation rate was greater for *A. annulatum*, peaking around 35 mm SVL. Predation rate approximately saturated for *A. opacum* as SVL increased, which approached being equivalent to *A. annulatum* again at approximately 39 mm SVL (Fig. 3); at this point, the foraging rate of *A. annulatum* began to decline.

## Discussion

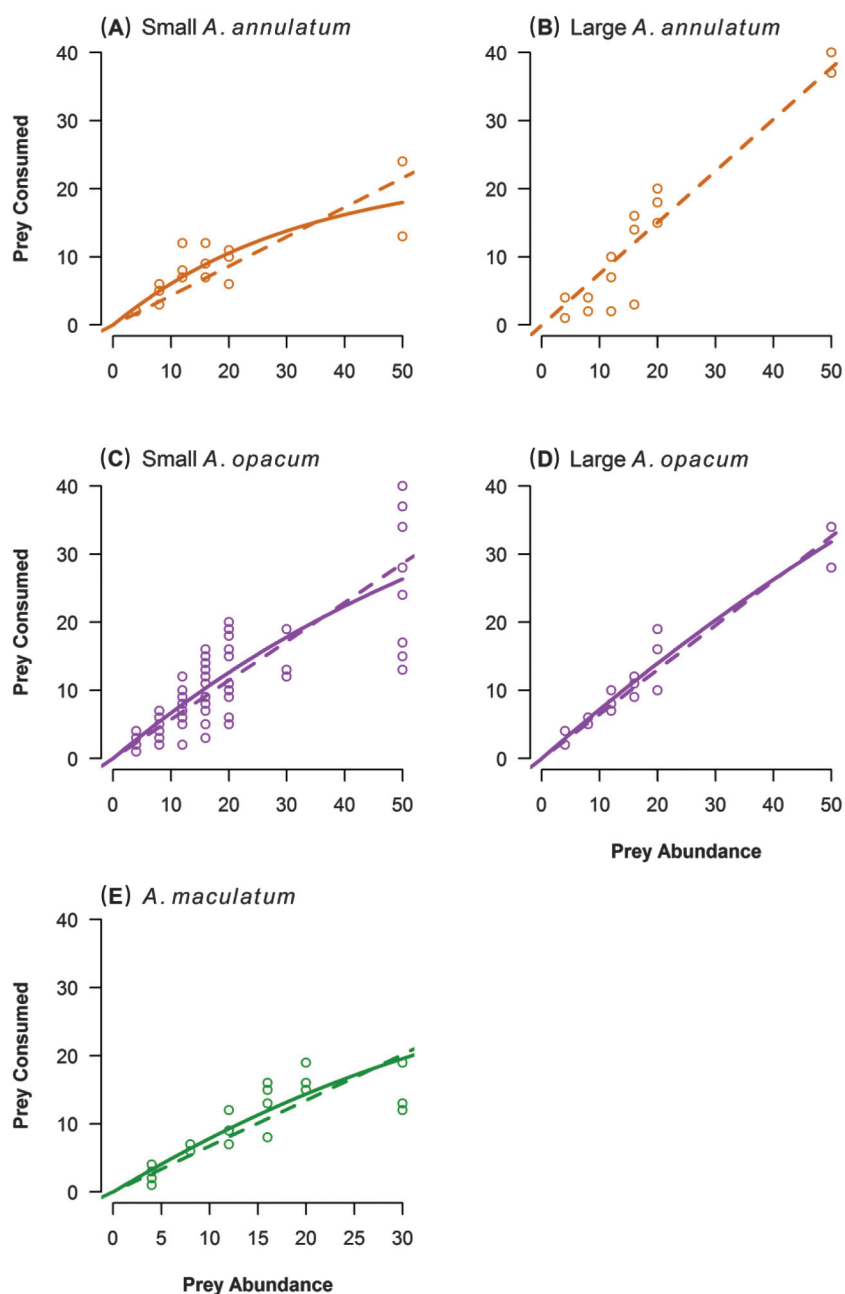
Predator species and body size can both play critical roles in determining community structure (Persson et al. 2003; Resetarits and Chalcraft 2007; Rudolf and Rasmussen 2013a). Yet, joint investigations of these two attributes (species identity and size) are rarely performed in predator–prey studies and the only studies to our knowledge to have tested these features in a synergistic manner found complex relationships among them (Rudolf and Rasmussen 2013b; Rudolf et al. 2014). We found similar complex patterns, such that neither predator species nor size alone consistently and predictably affected the functional response curve shape and associated parameters (i.e., attack rate and handling time). We saw stronger effects of size, regardless of species, as the attack rates were generally lower for smaller salamanders and that the functional response shifted from type II (saturation) to type I (no saturation) for small to large predators. However, because our a priori classifications of size were not equal (i.e., small *A. annulatum* and large *A. opacum* were more similarly-sized), our results show that equivalently-sized predators of different species had dissimilar functional responses. Furthermore, we were able to identify distinct switch points in relative predation rates based on body size among species. While community-level consequences may not be predictable from short-term experiments such as our study, both species identity and body size appear to be critical aspects of predator–prey interactions such that independent investigations of each factor may not fully capture the complexity of these interactions.

Population size structure is a critical factor that influences many ecological communities (Miller and Rudolf 2011). For example, Rudolf et al. (2014) found greater differences between size classes of the same predator species relative to cross-species comparisons on community structure. Because larval salamanders are gape-limited, predation is frequently influenced by the relative size difference between individuals that stems from either

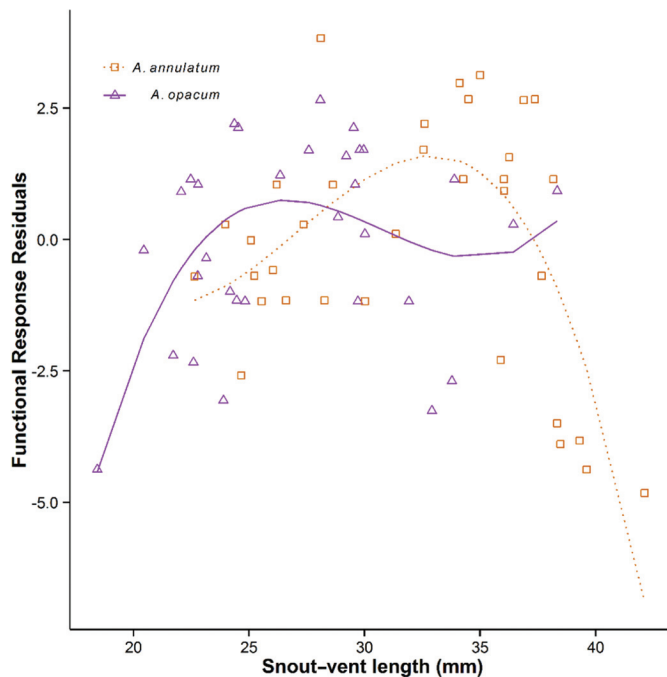
**Table 1.** Parameter estimates ( $\pm$ SE) for attack rate ( $a$ ) and handling time ( $h$ ) for each predator species (Spotted Salamander, *Ambystoma maculatum*; Ringed Salamander, *Ambystoma annulatum*; Marbled Salamander, *Ambystoma opacum*) and size class.

Species	Size	$a$ (type I)				$a$ (type II)				$h$ (type II)			
		Estimate	SE	Lower	Upper	Estimate	SE	Lower	Upper	Estimate	SE	Lower	Upper
<i>Ambystoma maculatum</i>	NA	0.673 (0.296)	0.045	0.348	0.516	0.0422 (0.019)	0.0068	0.0288	0.0555	1.36 (0.60)	0.348	0.678	2.044
<i>Ambystoma annulatum</i>	Small	0.432	0.034	0.348	0.516	0.0240	0.0045	0.0153	0.0330	1.59	0.485	0.643	2.542
<i>Ambystoma opacum</i>	Small	0.573	0.023	0.527	0.618	0.0244	0.0016	0.0212	0.0276	0.65	0.155	0.346	0.954
<i>Ambystoma opacum</i>	Large	0.652	0.027	0.594	0.710	0.0270	0.0039	0.0197	0.0350	0.37	0.278	-0.169	0.922
<i>Ambystoma annulatum</i>	Large	0.755	0.044	0.661	0.848	NA	NA	NA	NA	NA	NA	NA	NA

**Note:** Only one size class of *A. maculatum* was used. The type II response would not converge for large *A. annulatum*. Estimates in parentheses for *A. maculatum* represent the adjusted attack rate and handling time after accounting for the different-sized containers. Lower and upper are 95% confidence intervals. NA, not available.

**Fig. 2.** Functional response curves for (A, B) Ringed Salamanders (*Ambystoma annulatum*), (C, D) Marbled Salamanders (*Ambystoma opacum*), and (E) Spotted Salamanders (*Ambystoma maculatum*). For *A. opacum* and *A. annulatum*, the prey species was hatchling *A. maculatum*; for *A. maculatum*, the prey species was *A. annulatum*. Lines indicate predicted responses and symbols are raw data points. Solid lines indicate a type II functional response and broken lines indicate a type I functional response. Colour online.

**Fig. 3.** Residuals of functional response curves for Ringed Salamander (*Ambystoma annulatum*; squares and dotted line) and Marbled Salamander (*Ambystoma opacum*; triangles and solid line) as a cubic function of individual body size (snout-vent length; mm). Symbols represent individual data points and lines are predicted relationships. Colour online.



phenology differences or overlapping generations (Urban 2007; Wissinger et al. 2010; Anderson et al. 2013; Anderson and Semlitsch 2014). While we observed that both fall-breeding species (*A. annulatum* and *A. opacum*) could consume hatchlings of the spring-breeding *A. maculatum*, similarly-sized larvae of these predators were not functionally equivalent: small *A. annulatum* and large *A. opacum* showed different responses (type II vs. type I), despite the latter having a smaller head size for a given SVL. Furthermore, we identified switch points in predation rates, where intermediate-sized *A. annulatum* had higher rates than *A. opacum*, but the latter had a greater predation rate at small body sizes and, on average, a more consistent and higher relative predation rate compared with *A. annulatum*. This indicates that communities composed of different species of fall-breeding species may result in variable threats of intraguild predation to spring-breeding species, especially if substantial intra- or inter-specific structure exists among predators.

One possible explanation for higher attack rates in *A. opacum* at a given size compared with *A. annulatum* is that the former species often inhabits more ephemeral ponds, which could result in higher baseline foraging rates that are needed to complete metamorphosis in ponds prior to summer. This adaptive behavior would not be apparent for *A. annulatum*, as they inhabit more permanent water bodies and are subjected to this selection pressure less often. Intraspecific aggression in *A. opacum* has also been shown to peak during the middle of the larval period, which would coincide with the size classes used in our study (Mott and Sparling 2010), also possibly explaining their relatively higher attack rates.

Our finding that spring-breeding amphibians can consume fall-breeding species is to our knowledge a novel finding, though not necessarily unexpected. Most larval salamanders have diverse diets and will generally consume most organisms within their gape limitations (Taylor et al. 1988). Predation risk to fall-breeding species by congeners would only occur when metamorphosis is delayed

in *A. maculatum*, which may stem from factors such as density-dependent competition (Phillips 1992; Anderson and Semlitsch 2014; Anderson and Whiteman 2015). Intraguild predation risk would not occur in ephemeral ponds that would eliminate *A. maculatum*, or in years where metamorphosis of *A. maculatum* is completed prior to breeding of fall species, highlighting the need to understand life-history variation in the context of species interactions. Understanding such transitions in the role of predator and prey are increasingly important as community structure and ontogenetic pathways shift with climate change (Yang and Rudolf 2010).

Despite our high observed rates of predation, all three salamander species in this study often co-occur in ponds in Missouri (Hocking et al. 2008; Peterman et al. 2014). This syntopic distribution pattern is common to many ambystomatids across the southeastern United States, as is experimental evidence of strong interspecific interactions that would suggest exclusionary processes (Walls and Williams 2001; Brodman 2004). Mechanisms must therefore exist that permit larval co-existence. Differential survival due to consumptive effects of predation (Semlitsch 1987; Walls 1995; Anderson and Semlitsch 2016), nonconsumptive effects of top predators (Relyea and Yurewicz 2002; Kishida et al. 2011), or abiotic constraints (Peterman et al. 2014) may all contribute to variable densities of fall-breeding larval salamanders, which would have subsequent effects on prey species, including spring-breeding congeners. Behavioral shifts in response to predators, a type of nonconsumptive effect, may have influenced survival of our prey species in this study, as well as be a mechanism of co-existence in natural populations. Walls (1995) showed that larval *A. maculatum* did not behaviorally respond to cues of *A. opacum* with increased use of structural refuge, potentially explaining why *A. opacum* was a more voracious predator on *A. maculatum*. Alternatively, Shaffery and Relyea (2015) found that *A. maculatum* decreased activity with larval dragonfly predators early in ontogeny, suggesting their behavioral response may be species-specific. Mathis et al. (2003) and Crane et al. (2012) found that larval *A. annulatum* respond to predator cues with decreased activity, but the former study found that this response decreased as ontogeny progressed, indicating that larval behavior shifts as they increase in size. At the same time, freshly hatched larvae as used in our study have the lowest escape response relative to later ontogenetic stages (Landberg and Azizi 2010), making it possible they exhibited little to no antipredator behavior.

Because most food webs consist of many predatory species and size classes, further work is needed to understand how their functional responses change under more natural conditions, including variable predator densities (Schmidt et al. 2014; Stier and White 2014) or habitat heterogeneity (Hossie and Murray 2010). Furthermore, functional response curves often are starting points for developing predator-prey population models that can help predict population growth rates and when and where coexistence is likely. Incorporating predator species identity and size class into such population models may help explain stability in long-term community patterns, as has been shown in other systems (e.g., Persson et al. 2003). However, developing models that show changes in population growth rate are still difficult for amphibians because population regulation occurs in two habitat types (aquatic and terrestrial), with the latter usually being the more influential factor on population dynamics (Vonesh and De la Cruz 2002; Harper et al. 2008). Therefore, translating aquatic survival with different predator species and size classes is only part of the equation in determining how such predation influences long-term population dynamics.

While our study provides a starting point for furthering our understanding species- and size-specific predation rates, more questions remain on factors that differentiate whether predators exhibit functional redundancy. We only assessed one aspect of ecological redundancy (foraging rates); it is likely that our species are both more and less redundant in other aspects of their ecol-

ogy. Our study emphasizes the need for more comparisons into the potential axes of ecological redundancy (e.g., across species or individuals within a species), as a growing body of literature suggests functional redundancy is unlikely (Chalcraft and Resetarits 2003b; Resetarits and Chalcraft 2007; Rudolf and Rasmussen 2013b; Rudolf et al. 2014). Whether experimental findings would show similar patterns under more natural conditions is an important next step, especially as functional response parameters may vary in heterogeneous natural communities (Duijns et al. 2015). Identifying disconnects between natural and experimental studies can help predict the importance of species identity and size structure in predator–prey interactions, as well as their influences on community structure.

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