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A TEST OF PREDATOR AVOIDANCE BY LARVAE OF THE BLUE RIDGE TWO-LINED SALAMANDER (*EURYCEA WILDERAE*) IN APPALACHIAN STREAMS

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ABSTRACT

Larval Blue Ridge Two-lined Salamanders (*Eurycea wilderae*) are significant components of Appalachian streams, reaching densities up to 100/m^2. Not surprisingly, these salamanders fall prey to many types of predator. In order to test the hypothesis that larval *E. wilderae* actively avoid predators, we paired them against a variety of predators of this species. Predators included Banded Sculpins (*Cottus carolinae*), Chattahoochee Crayfish (*Cambarus howardi*), and Spring Salamanders (*Gyrinophilus porphyriticus*), both adult and larval. We placed larvae in a 1-m metal trough filled with water with a predator placed in a cage at one end. A control treatment consisted of an empty cage. For each trial, we placed a larval *E. wilderae*. After the larva stopped in one place for 10 min, we measured the distance between the larva and the cage. We ran 18–20 replicates for each of the five treatments. A Kruskal-Wallis test showed no significant difference between any of the treatments in mean distance. Disagreement between our results and those of other workers suggests the possibility of interspecific or interpopulation variation in anti-predator behavior. In addition, because of their high densities and wide variety of predators, larval *E. wilderae* may not suffer sufficient predatory pressure from a particular species of predator to evolve appropriate behavioral responses.

Key Words: predator, prey, behavioral avoidance, salamander, larvae, Appalachian streams, *Eurycea wilderae*

INTRODUCTION

Predation is a critical process influencing the distribution and abundance of many organisms, especially in aquatic ecosystems. In fact, aquatic taxa can sort themselves among habitats according to the presence of their predators (Barr and Babbitt, 2002). Moreover, predator-induced, behavioral responses can dramatically affect population and community dynamics (e.g., Reznick et al., 2001; Binckley and Resetartis, 2003; Vonesh et al., 2009).

How a prey species responds to potential predators depends on a number of factors. For one thing, anti-predator responses vary depending on the type of predator. In stream systems, prey organisms respond differently to invertebrate versus vertebrate predators (Wooster and Sih, 1995). Responses also vary with individual prey species such as variance in vulnerability of prey species determining how small fish respond to larger fish predators (Schlosser, 1987). In some cases, aquatic species including both fish and amphibians (Kraus 1993; Lutterschmidt et al., 1994; Chivers and Smith, 1998) respond to alarm signalling ("Schreckstoff").

Anti-predator responses can be further complicated by the prey species' stage of development, particularly when different stages are associated with different ecological niches. Predator-avoidance behaviors, for instance, are most evident in the youngest stages of fishes (Fuiman and Magurran, 1994). How prey species respond to familiar predator species versus unfamiliar ones is another influential factor (Sanson et al., 2001), and some species such as Brook Trout (*Salvelinus fontinalis*) can learn to recognize predators (Mirza and Chivers, 2000).

While lungless salamanders of the family Plethodontidae have long been recognized for their ecological importance to terrestrial systems (e.g., Burton and Likens, 1975), a better understanding of the role of plethodontid larvae in aquatic systems has recently been emerging. Aquatic larvae are important components of headwater streams of the Appalachian Mountains, having a biomass that significantly influences both energy flow and nutrient cycling (Milanovich et al., 2015). Densities of some plethodontid species such as the Blue Ridge Two-lined salamander (*Eurycea wilderae*) can reach as high as 100/m^2 (Peterson and Truslow, 2008).

Several stream predators feed on larvae of the plethodontid genus *Eurycea*. Potentially important predators include Banded Sculpins (*Cottus carolinae*; Tumlison and Cline 2002), various crayfish species (Gamradt and Katz, 1996; Cruz and Robelo, 2005), and Spring Salamanders (*Gyrinophilus porphyriticus*; Burton, 1976; Bruce, 1979).

Plethodontid salamanders have well developed visual systems, and visual communication through displays and movements convey information about aggression and other social cues (Jaeger and Forester, 1993). Plethodontids also use chemoreception to identify predators to avoid them (Madison et al., 1999). Therefore, it is likely that plethodontid larvae recognize and avoid predators. Because of their numerical importance to their respective ecosystems, how plethodontid larvae respond to potential predators may be important in determining the availability of resources to the surrounding members of the ecosystem.

While larval ambystomatid salamanders respond to the presence of predatory fish (e.g., Holomuzki, 1986; Semlitsch, 1987), studies of interactions between *Eurycea* larvae and their predators have yielded mixed results. Larvae of the Northern Two-lined Salamander (*E. bislineata*) shift activity schedules in the presence of predatory Brook Trout (Barr and Babbitt, 2007). Moreover, they have a negative association with Brook Trout across spatial scales (Barr and Babbitt, 2002). Larval *G. porphyriticus* can reduce the survival of larval *E. wilderae* (Beachy, 1994) and inhibit the nighttime activity of larval *E. cirrigera* (Gustafson, 1994). In addition, adult *E. cirrigera* avoid chemical cues from *G. porphyriticus* (Marvin and Cupp 2018), while larval *Eurycea* have been shown to avoid chemical cues of predatory fish (Petranka et al., 1987; DeSantis et al., 2013). On the other hand, larval *E. cirrigera* from an Indiana population did not respond to chemical stimuli from crayfish within their native ranges (Vollmer and Gall, 2014), and larvae of *E. wilderae* from Georgia showed no reaction to the presence of fish predators (Lombard and Camp, 2020).

Because the *E. wilderae* tested by Lombard and Camp (2020) were from a fishless stream, the absence of a response by these larvae may have been due to the fact that the predators tested were not from their resident stream. Our purpose was to test the hypothesis that *E. wilderae* larvae respond to the presence of predators with which they reside, i.e., crayfish and *G. porphyriticus*.

MATERIALS & METHODS

In order to test larval response to potential predators, we collected larvae (n = 60) of *E. wilderae* from the same stream used in the study by Lombard and Camp (2020). This stream is a small, headwater stream on the campus of Piedmont University in Demorest, Habersham County, Georgia. We collected predatory Chattahoochee Crayfish (*Cambarus howardi*) and larval *G. porphyriticus* from the same stream. In addition, we collected adult *G. porphyriticus* from a tributary of the West Fork of Wolf Creek located in Sosebee Cove in Union County, Georgia.

We housed all specimens in aerated tanks at 21 C for several days before testing began. The lights were kept off, although natural lighting from two windows gave a photoperiod of ~12.5 hr.

We had four treatments, which were as follows: 1) a predator-free control; 2) a larval *G. porphyriticus*; 3) an adult *G. porphyriticus*; and 4) an adult *C. howardi*. We replicated each treatment 20 times except for the larval *G. porphyriticus*, which treatment had 18 replicates.

In the avoidance trials, we followed the methods of Lombard and Camp (2020), using the same metal troughs used in that study. Each trough was 1 m in length and lined with Press-n-Seal® to prevent leaks. At the end of each trough, we placed a screen cage (9.5 x 8.5 x 10 cm) with an aerator to house a predator. The control treatment consisted of an empty cage with an aerator.

For each trial, we filled a trough with water, placing a predator in the aerated cage. To prevent a predator's escape, we covered each cage with a screen lid. We placed a larval *E. wilderae* in the middle of the trough and checked its position every 10 min. Once the salamander remained in the same place for two consecutive observations (10 min), we measured the distance of the salamander from the cage. For each subsequent trial, we replaced the water and used a different larva. We ran all trials with only natural light from the windows. We never used a larval *E. wilderae* more than once. Once the trials were completed, we released all subjects at their original streams with the exception of the larval *G. porphyriticus*, which died prematurely.

For statistical analysis, we added the results of the 20 trials run earlier by Lombard and Camp (2020) with the predatory *C. carolinae* as an additional treatment. Because of the non-normal nature of some of the data, we tested for differences among treatments in mean distance using a Kruskal-Wallis. We ran these analyses using SPSS ver. 24.

RESULTS

The distance (mean ± 1 SE) from the predator's cage for each of the five treatments was 35.48 ± 6.51 cm for adult *G. porphyriticus*, 47.70 ± 6.90 cm for larval *G. porphyriticus*, 43.53 ± 7.47 cm for *C. howardi*, 37.23 ± 7.30 cm for *C. carolinae*, and 39.48 ± 7.22 cm for the control (Figure 1). There was no significant difference among the five treatments ($H = 3.060$, DF = 4, $p = 0.545$).

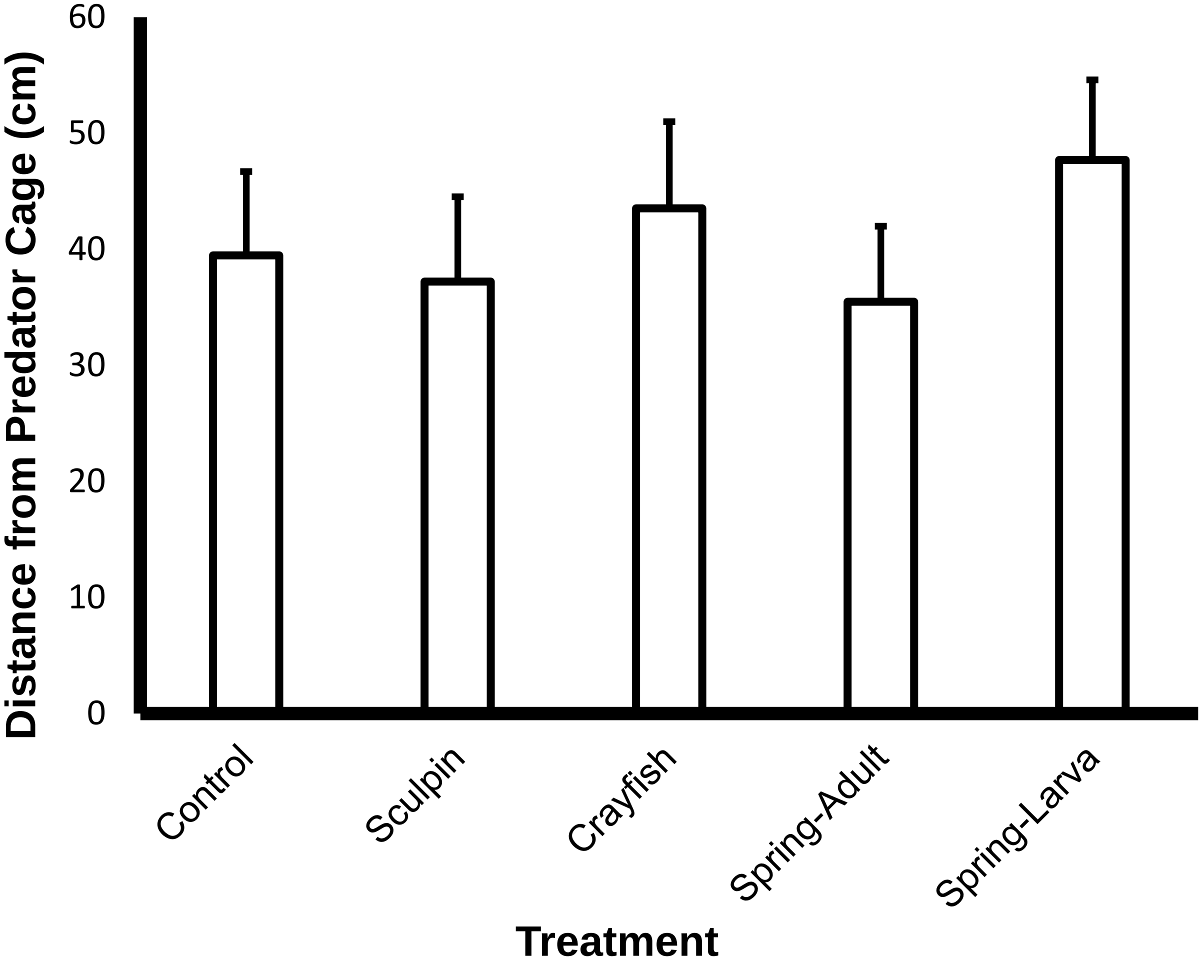

Figure 1. Mean distance from larval *Euryca wilderae* and control cage or cage containing one of the following predators: Banded Sculpin (*Cottus carolinae*), Chattahoochee Crayfish (*Cambarus howardi*), adult or larval Spring Salamander (*Gyrinophilus phorphyriticus*).

DISCUSSION

In our study, *E. wilderae* larvae did not avoid any of the tested predators. Our results did not support our hypothesis that larval salamanders would avoid predators (i.e., crayfish, *G. porphyriticus*) that came from the same stream. Similarly, Vollmer and Gall (2014) found that larvae of the closely related *E. cirrigera* did not recognize predatory crayfish. In contrast, Petranka et al. (1987) found that larval *E. cirrigera* avoided water conditioned with predatory fish. The mixed results of these studies suggest that predator-avoidance behavior might be species or even population specific.

Differences in anti-predator behavior among populations of the same species could result from variation in the species of predator that are present or differences in predator density. Variation in behavior could also result from discrepancies in the ability of *Eurycea* larvae to hide from predators, either because of differences in refuge availability or in cryptic coloration of the larvae themselves. Larval *Eurycea*, which are brown and spotted, differ markedly in appearance from the respective adults, which are brown, yellow, or orange with longitudinal stripes (Petranka, 1998). Cryptic coloration is generally more important to the predator-avoidance toolkit of larval fishes than it is to adults (Fuiman and Magurran, 1994). The larvae of both *E. wilderae* and *E. cirrigera* exhibit inter-population variation in color (Benfield et al., 2015), a possible adaptive response to variance in predation pressure.

Another possible reason for the lack of avoidance of predators by larval *E. wilderae* may be a synergism between the high density of this species and the diversity of predators with which they must contend. Larval *E. wilderae* are extremely abundant in Appalachian streams, reaching densities approaching or even exceeding 100/m^2 (Peterson and Truslow 2008). Moreover, the diversity of predators including fish, salamanders, and crustaceans translates into predator pressure coming from many different directions. Given the sheer numbers of *Eurycea* larvae and the wide variety of visual and chemical cues emitted by different predators, the larval population of *E. wilderae* simply may not suffer sufficient pressure from a single predator species to select for behavioral avoidance of that particular predator.

In contrast to our results with larval *E. wilderae*, Marvin and Cupp (2018) demonstrated that adult *E. cirrigera* avoid adult *G. porphyriticus*, suggesting the possibility of a difference between life-history stages in anti-predator behavior. Selection for the enhancement of adult survivorship sometimes works in opposition to selection for greater larval survival, forcing a tradeoff between larval and adult ontogenetic stages. Such tradeoffs between larval and adult ontogenetic stages in species with biphasic life cycles are not uncommon (e.g., Sentinella et al., 2013; Lindstedt et al., 2016).

Our study adds another piece to the emerging picture of predator-prey interactions involving larval plethodontids. Even so, it is obvious that an abundance of unanswered questions remain. In particular, the possibility of inter-population differences in anti-predator responses would seem an interesting and fruitful avenue for future research in this area.

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