A TEST OF FOOD PARTITIONING BETWEEN THE AQUATIC LARVAE OF TWO PARAPATRIC SPECIES OF TWO-LINED SALAMANDER (EURYCEA BISLINEATA SPECIES COMPLEX) IN A ZONE OF SYMPATRIC CONTACT

Carlos D. Camp
Piedmont University, ccamp@piedmont.edu

S. Michael Owensby
Piedmont University, sowensby0325@lions.piedmont.edu

Emily Benfield
Piedmont University, emilybenfield3@gmail.com

Jessica A. Wooten
Piedmont University, jwooten@piedmont.edu

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Carlos D. Camp, S. Michael Owensby, Emily Benfield, Jessica A. Wooten

Department of Biology, Piedmont University, Demorest, Georgia 30535

ABSTRACT

Phylogenetically related species with similar ecologies often partition resources when in sympatry. Food is an important factor in the co-occurrence of sympatric salamanders, and food partitioning occurs in a variety of sympatric, similar species. Several members of the Two-lined Salamander (*Eurycea bislineata*) species complex are largely parapatric but co-exist within a narrow zone of sympatric contact. Because larvae of these salamanders frequently occur in very high densities, we tested the hypothesis that larvae of the Blue Ridge Salamander (*E. wilderae*) and the Southern Two-lined Salamander (*E. cirrigera*) partition food in sympatry in northeastern Georgia. We predicted that the diets of these two species would differ in sympatry and that the respective diet of each species would differ between allopatric and sympatric populations. Both species fed largely on the aquatic larvae of Trichoptera and Diptera, and their diets reflected the available insect fauna of the respective streams. There was no significant difference between the species in sympatry or between allopatric and sympatric populations of either species. Although we found no evidence of food partitioning, we cannot rule out interspecific competition that may manifest itself in some resource other than food.

Key Words: resource partitioning, interspecific competition, salamander, diet, *Eurycea cirrigera*, *Eurycea wilderae*

INTRODUCTION

Although often applied to differences in resource use between sympatric species regardless of cause, resource partitioning was originally defined as an adaptive response to competition between ecologically similar forms (Walter 1991). One group of animals with species that exhibit resource partitioning is salamanders. While salamanders are largely dietary generalists, the food niche is a key factor relating to coexistence in multispecies assemblages (Vignoli et al. 2017). Examples of food partitioning between salamanders can be found among both terrestrial (e.g., Stuczka et al. 2016) and aquatic (e.g., Cudmore 2014) species.

The Two-lined Salamander (*Eurycea bislineata*) species complex (Jacobs 1987) includes a number of unnamed, evolutionary lineages that share parapatric borders (Pierson et al. 2021). One lineage is currently included within the Blue Ridge Two-lined Salamander (*E. wilderae*) and occurs in the Blue Ridge Physiographic ecoregion. Another lineage is currently recognized as the Southern Two-lined Salamander (*E. cirrigera*) and occurs in the Piedmont ecoregion. Hereafter referred to as *E. wilderae* and *E. cirrigera* for simplicity, the two forms contact each other along the foothills of the Blue Ridge in

the southern Appalachian Mountains (Kozak and Montanucci 2001) where they share a narrow zone of sympatry (Camp et al. 2000).

Phylogenetically close species are more likely to exhibit interspecific competition because of similar ecological requirements (Crombie 1947). Not only are these two species close phylogenetically, having at one time been considered conspecific (Jacobs 1987), but they are extraordinarily similar ecologically. The aquatic larvae of both species are particularly abundant in small streams, with densities of each species occasionally exceeding 100 m^{-2} (Peterson and Truslow 2008; Nowakowski and Maerz 2009). Larval forms of the two species are morphologically cryptic with a broad overlap in body sizes (Bruce 1985; Gustafson 1993). They have similar diets, feeding primarily on the larvae of aquatic insects (e.g., Johnson and Wallace 2005; Barrett et al. 2012).

Ecological similarity and high densities suggest the strong possibility of competition between the larvae of these two taxa. Our purpose was to test the hypothesis that the aquatic larvae of *E. wilderae* and *E. cirrigera* exhibit food partitioning where they occur in sympatry. Dietary shifts from allopatry to sympatry is a potential signal of food partitioning (Arlattaz et al. 1997). Therefore, we predicted that, if food partitioning occurs between the two study species, then diets of the larvae of the two species would (1) differ significantly from each other in sympatry and (2) show distinct dietary shifts from respective allopatric populations.

MATERIALS AND METHODS

In the summer of 2015, we collected 71 *Eurycea* larvae from a section of Nancytown Creek (NC) in Habersham County in the upper Piedmont ecoregion at an elevation of 347 m (34.512 N; 83.473 W) where the two species are known to occur sympatrically (Camp et al. 2000). This section flowed by montane uplands and flat lowlands simultaneously and received water from tributaries emptying into Nancytown Creek from opposite sides. On the east side, a small, montane tributary drained a steep hillside. To the west lay a flat bottomland pocked with beaver ponds that overflowed into the creek. We chose a 50-m section that consisted of pools and riffles with abundant flat rocks on a substrate dominated by sand and gravel.

For comparative purposes, we also chose streams supporting allopatric populations that were well within the respective ranges of the two species (Jacobs, 1987). We collected 28 *E. cirrigera* from a stream in Fulton County, Georgia (FC; 33.571 N; 84.713 W), located in the middle of the Piedmont. FC is a small, rocky channel fed by overflow from Bear Creek, which empties into the Chattahoochee River. We collected 31 *E. wilderae* from a second-order tributary (Flat Creek) of Cooper Creek of the Toccoa River system (Hiwassee River drainage) located in the Blue Ridge province in Union County, Georgia (CC; 34.755 N; 84.004 W). This creek flowed over rocky substrates with abundant pools and riffles.

We collected salamander larvae by hand aided by rectangular aquarium nets. We over-anesthetized all specimens in a solution (1 g l^{-2}) of ethyl-3 aminobenzoate methanesulfonate (MS-222). We then preserved them in 10% formalin and stored them in 70% ethanol. Following the study, we deposited all specimens in the Georgia Museum of Natural History located at the University of Georgia, Athens (accession numbers = GMNH 51188–51445).

Although morphological characters can distinguish metamorphosed individuals of *E. wilderae* and *E. cirrigera* (Jacobs, 1987; Camp *et al.*, 2000), no such traits are known for larvae. Therefore, prior to preservation, we removed ~0.5 cm of the tail tips of all specimens collected in sympatry and stored them in absolute ethanol for genetic determination of species. We genotyped species using a portion of the mtDNA gene cytochrome b (*cyt b*, 1012 bp), setting allopatric specimens (*E. cirrigera* from FC; *E. wilderae* from CC;) as comparative outgroups. We extracted DNA and used primers for *cyt b* described by Lamb and Beamer (2012) to sequence the appropriate gene segment following methods described by Wooten et al. (2010).

We sampled the communities of potential prey. At each of the three sites, we sampled five quadrats, each measuring 10 x 12.5 cm using aquarium nets. We chose the quadrats throughout stream reaches where we collected larval salamanders. Gathering all of the substrate down to bedrock, we preserved all items in 70% ethanol. We then searched each sample under a dissecting microscope and identified all invertebrate organisms to the same categories as the stomach contents. We combined the data from all five quadrats from each site for an overall view of the invertebrate fauna.

To test for food partitioning, we dissected individual salamanders under a dissecting microscope and teased apart the stomach and lower intestine. We identified food items to taxonomic group: order for arthropods and class for non-arthropod food items (e.g., bivalves). Considering acanthocephalans, tapeworms, and nematodes to be probable parasites, we did not include them in our analyses.

We analyzed potential differences in diet using contingency tables, setting population in columns and the number in each prey taxon in rows. We made separate comparisons between sympatric (NC) and allopatric (FC) *E. cirrigera*, between sympatric (NC) and allopatric (CC) *E. wilderae*, and between the two species in sympatry (NC). Because we used the data for the sympatric populations in each of two separate analyses, we adjusted the alpha level to 0.033 for significance testing (Narum, 2006).

RESULTS

The most abundant invertebrates found in the stream samples were insect larvae of the orders Trichoptera and Diptera (Table I). Larval trichopterans were the most abundant food item in the guts of sympatric (NC) *E. wilderae* and *E. cirrigera* (Table I). Larval dipterans were the most abundant in the guts of allopatric (FC) *E. cirrigera*. There were very few food items in allopatric (CC) *E. wilderae*.

Table I. Numbers of invertebrate organisms found in occupied streams and stomachs of sympatric (NC) and allopatric (FC and CC) *Eurycea cirrigera* and *E. wilderae*. Columns headed with species suffixes are numbers of prey items identified from gut tracts of salamander larvae.

Taxon	NC_{stream}	$NC_{wilderae}$	$NC_{cirrigera}$	CC_{stream}	$CC_{wilderae}$	FC_{stream}	$FC_{cirrigera}$
Trichoptera	514	15	17	112	0	51	6
Plecoptera	46	1	4	29	0	3	5
Ephemeroptera	83	2	0	15	1	14	1
Coleoptera	82	2	0	0	0	0	0
Megalopatera	2	6	2	2	0	1	2
Diptera	223	4	9	69	0	35	16
Hemiptera	0	0	1	0	0	0	1
Odonata	0	0	0	0	0	1	0
Collembola	0	0	0	0	0	0	1
Amphipoda	0	3	3	1	1	2	1
Decapoda	0	0	0	1	0	0	0
Hydracarina	3	0	0	0	0	0	0
Bivalvia	0	0	0	0	0	0	1
Gastropoda	0	0	0	5	0	3	0
Acanthocephala	0	4	0	0	0	0	4
Platyhelminthes (Cestoda)	0	1	0	0	0	0	1
Nematoda	1	0	0	0	2	9	39

Of the 71 *Eurycea* larvae collected from NC, 32 were genotyped to *E. wilderae*, 20 of which contained identifiable food items. Twenty-three of the 39 *E. cirrigera* contained identifiable food. Unidentifiable remains comprised very little of the food volume.

Contingency analysis indicated no significant difference in the diets of sympatric (NC) and allopatric (FC) *E. cirrigera* ($\chi^2 = 7.887$; $P = 0.4445$). Neither was there a significant difference between sympatric (NC) and allopatric (CC) *E. wilderae* ($\chi^2 = 8.706$; $P = 0.1908$), although this may have been due to the small number of CC food items. Moreover, we found no significant difference in the diets of sympatric (NC) *E. cirrigera* and *E. wilderae* ($\chi^2 = 10.738$; $P = 0.1505$). For the most part, foods found in the guts of the two sympatric salamander species reflected those available in the stream (NC; Figure 1).

Fig. 1 Proportion of total invertebrate taxa collected from stream samples and from gut tracts of sympatric *Eurycea cirrigera* and *E. wilderae* at Nancytown Creek (NC) in Habersham County, Georgia. Total *N* invertebrates collected in stream = 954; total *N* invertebrates found in *E. cirrigera* stomachs = 36; total *N* for *E. wilderae* = 33. Counts do not include presumed parasites (Acanthocephala, Cestoda, Nematoda). Invertebrate groups are in descending order of abundance in the stream fauna.
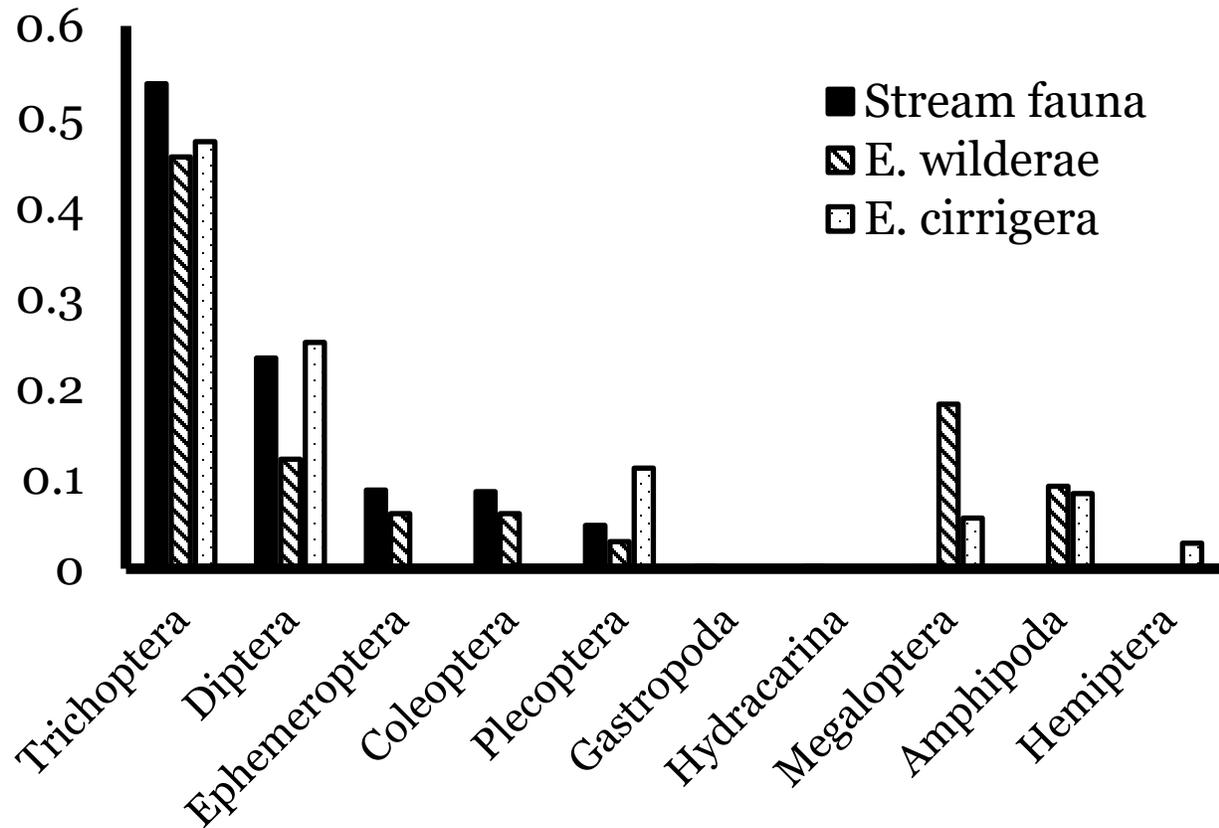
DISCUSSION

We hypothesized that larvae of these two species would exhibit food partitioning, predicting differences in their respective diets in sympatry and between allopatric and sympatric populations of the same species. However, our results failed to support either of these predictions. Similar ecologies and potentially high densities of both species suggest the likelihood of interspecific competition. While we cannot rule out the possibility of partitioning within invertebrate taxa, we found no indication of food partitioning in the measures that we used.

If the lack of food partitioning reflects an absence of competition, then food may not be a limiting factor, a prerequisite for interspecific competition (Sale 1974). Similar-sized terrestrial salamanders (genus *Plethodon*) compete with each other so strongly that they not only partition food but display character displacement in feeding morphology (Adams, 2000, 2004, 2010; Adams & Rohlf, 2000; Adams, West & Collyer, 2007). Terrestrial salamanders are limited in their foraging opportunities by levels of

soil moisture and humidity, which may further limit the abundance of arthropod prey (Feder, 1983; Grover, 1998). Therefore, terrestrial salamanders may face a much more food-limited environment than aquatic larvae of *Eurycea*.

Another possibility is that segregation by habitat mitigates the effect of competition in our study system. Larvae of these two species segregate, at least in part, by microhabitat, with *E. wilderae* being more dominant in riffles and *E. cirrigera* predominating in pools (Pierson et al. 2021). While we did not design our study to identify species by microhabitat, we did collect larvae in nearly equal numbers in the same stretch of stream where the two species were in very close proximity to one another.

Interspecific competition, or its adaptive outcome, has often been presumed without robust supportive evidence (Wiens 1977; Suart and Losos 2013). Therefore, while we cannot eliminate the possibility of interspecific competition, other metrics (e.g., experiments in mesocosms; Tran et al. 2015) will be necessary to confirm its presence. What we can say is that we found no evidence for competition in the form of food partitioning between the larval forms of *E. cirrigera* and *E. wilderae*.

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