Notes and Discussion Piece

The Diet of the Cumberland Plateau Salamander (Plethodon kentucki) in an Old Growth Forest of Southeastern Kentucky

ABSTRACT.—Examining the diet of salamanders is important for understanding their effects on invertebrate communities and the interactions among sympatric salamander species. We examined the diet of the Cumberland Plateau Salamander, *Plethodon kentucki* (Mittleman), in an old growth forest in southeastern Kentucky. A total of 763 prey items were recovered from 73 salamanders with an average of 10.75 prey items per stomach. The four most important prey groups were Formicidae (ants), Araneae (spiders), Coleoptera (beetles), and Collembola (springtails). Overall, we found a total of 58 different prey types in the stomach contents from 20 invertebrate orders. This study represents one of the few successful uses of nonlethal gastric lavage methods on a large plethodontid salamander and the first description of *P. kentucki* diet identified to family and genus. Future work should examine diet throughout the year, compare prey species composition to sympatric salamander species, and look at local prey abundances and diversity to explore salamander foraging behavior.

INTRODUCTION

Terrestrial lungless salamanders are known to reach very high densities in certain forested ecosystems of the United States (Bailey *et al.*, 2004; Dodd and Dorazio, 2004) and can be an important component in the top down regulation of invertebrate populations (Davic and Welsh, 2004; Semlitsch *et al.*, 2014). Wyman (1998) estimated a population of *Plethodon cinereus* in New York could consume 1.5 million prey items/ha/year. It is generally thought plethodontid salamanders are opportunist euryphagous predators and prey acquisition is typically only related to prey availability and microhabitat selection (Jaeger, 1981; but *see* Paluh *et al.*, 2015).

Numerous studies have examined the diets of large (*i.e.,* adult SVL > 4.0 cm) salamanders in the genus *Plethodon* (Oliver, 1967; Rubin, 1969; Whitaker and Rubin, 1971; Powders and Tietjen, 1974; Jensen and Whiles, 2000; Lewis *et al.*, 2014). However, the diets of 37% (20 of 54 extant species) of North American *Plethodon* salamanders are still entirely unknown (Duellman, 1954; Holman, 1955; Brandon, 1965; Oliver, 1967; Rubin, 1969; Whitaker and Rubin, 1971; Powders and Tietjen, 1974; Fraser, 1976a; Bailey, 1992; Mitchell *et al.*, 1996; Petranka, 1998). Furthermore, very few studies have identified prey items beyond the taxonomic level of order. Though prey information at the level of order can be useful, prey identified to more specific taxa can be used to identify changes in specific prey availability, to compare the relative importance of prey items between similar species and regions, and to gain knowledge of taxon-specific feeding behaviors.

The Cumberland Plateau Salamander [*Plethodon kentucki* (Mittleman)] is a large terrestrial lungless salamander confined to the Cumberland Plateau of the central Appalachian Mountains (Mittleman, 1951; Highton and MacGregor, 1983). Bailey (1992) examined the diet of *P. kentucki* in West Virginia and identified prey to the level of order. In this study we examined the diet of adult *P. kentucki* from an old growth forest in southeastern Kentucky in order to evaluate the overall importance of prey groups to this regionally endemic species.

METHODS

We analyzed stomach contents of adult *P. kentucki* (4.0 cm or greater; Mittleman, 1951) and excluded juvenile salamanders due to possible ontogenetic differences in prey composition. We collected salamanders over a 75 m sampling transect on a ridgetop (locally known as Whitaker Branch, 37°5′16.73″N, 82°59′16.99″W) within the forest of Eastern Kentucky University's Lilley Cornett Woods Appalachian Ecological Research Station (LCW), Letcher County, Kentucky. The study area is an upland old growth mixed mesophytic forest at 340 m elevation (Braun, 1950; Martin, 1975). The forest primarily consists of live Eastern Hemlock (*Tsuga canadensis*) stands, with mixed oak (*Quercus sp.*), and American beech (*Fagus grandifolia*). *See* Martin and Shepherd (1973) and Martin (1975) for a complete list of vegetation at the study site.

We located salamanders by overturning rocks, logs, other cover objects, and sifting through dense leaf litter. Sampling was conducted during the early mornings and early evenings over eight samplings from 26 Apr. to 27 May 2016. Salamanders were placed in plastic bags with a moistened paper towel and transported to the research laboratory at LCW. All salamanders were anesthetized in a solution of 1g Maximum Strength Orajel®/1 liter of aged tap water (Cecala *et al.*, 2007) and were removed from the anesthetic solution upon failure to right themselves after being flipped over. First, using a caliper, we measured snout-vent length (SVL: from the tip of the snout to the posterior angle of the vent) to the nearest millimeter. We then inserted an approximately 6.0 cm long piece of 1.3 mm OD PTFE tubing (Zeus Inc., catalog number AWG24) into the salamander's esophagus until there was resistance. Aged tap water was then pumped into the tubing using Nipro 3 mL syringes with 22 gauge needles. Pumping was repeated until all food items had been removed (Fraser, 1976a). We placed salamanders in a recovery container of aged tap water and returned them to their approximate location of capture within 2.5 h. Immediately after removal, we identified stomach contents to the lowest possible taxonomic level and placed prey items into labeled vials containing 70% ethanol. Vials are stored in the Branson Museum collection at Eastern Kentucky University, Richmond, Kentucky.

Prey items were identified to order and in some cases family, genus, and species. In order to evaluate the overall importance of the stomach contents, prey groups are recorded with percent occurrence (the percentage of salamanders that ate a prey group) and percent abundance or percent total (the total number of prey items in a specific group divided by all prey items; adapted from Felix and Pauley, 2006). Empty stomachs were not included in percent analyses.

<center>RESULTS</center>

We stomach flushed 73 adult *P. kentucki* (mean ± SE SVL = 5.9 ± 0.319 cm; range = 4.1–7.9 cm). Fifteen individuals had prominent mental glands (males), two individuals were gravid females, and 56 individuals were of unknown sex. Seventy-one of 73 individuals contained at least one prey item in their stomachs. We recovered 763 prey items. Salamander stomachs contained an average of 10.75 prey items of 58 prey types from 20 invertebrate orders (Table 1; Fig. 1). Forty-two prey types were identified to the level of family or genus (Table 2). The four most important prey taxa accounted for nearly 75% of all prey items: Formicidae (ants), Araneae (spiders), Coleoptera (beetles), and Collembola (springtails) (Table 1; Fig. 1).

The diversity of ants found in the stomachs of *P. kentucki* was greater than all other prey types that could be identified to family or genus. Individuals were identified to four subfamilies and 12 genera (Table 2). Salamanders fed on each ant species in similar quantities throughout the sampling period, suggesting there were no changes in prey availability. However, 70% of the salamanders that fed on ants consumed individuals belonging to the genus *Pheidole*. Spiders appeared to be important prey; unfortunately, many could not be identified due to advanced digestion or method-based damage. Of those spiders that could be identified, members of the families Theridiidae (cobweb weavers) and Thomisidae (crab spiders) were the most common. The diversity of coleopterans was second to that of ants—nine families were identified. Nearly 70% of the beetles were adults, but adults and larvae were each found in 50% of the salamanders that ate coleopterans. Adults from the families Scarabaeidae (scarab beetles) and Curculionidae (true weevils) were the most frequently consumed coleopterans. Collembolans were identified to three families from three orders: individuals from Poduromorpha (Hypogastruridae) were the least common, and members from Symphypleona (Sminthuridae: globular springtails) and Entomobryomorpha (Isotomidae: smooth springtails) were more common and had similar abundances and frequencies. Micro-gastropods (Stylommatophora) were found in nearly 25% of the salamander stomachs, suggesting land snails were an important component of salamander diets. The diversity of land snails was fairly large with eight species from six genera. Snails from the genera *Glyphyalinia* and *Ventridens* were the most frequently consumed. Among salamander stomachs containing dipterans, adult flies occurred in more than 80% and larva occurred in 30%. Overall, larval prey from three orders (Coleoptera, Diptera, and Lepidoptera) made up approximately 6% of all food items.

TABLE 1.—Prey types and frequencies in the stomachs of adult *Plethodon kentucki* (n = 73) from southeastern Kentucky from Apr.–May 2016

Prey category	Occurrence (%)	Total items (%)
Hymenoptera		
Formicidae	73.24	44.04
Araneae	61.97	10.35
Coleoptera	57.75	9.96
Collembola	42.25	10.22
Diptera	28.17	3.41
Acari	26.76	4.19
Gastropoda	23.94	2.75
Lepidoptera (Larvae)	18.31	1.83
Chilopoda		
Geophilomorpha	16.90	1.97
Oligochaeta	15.49	2.10
Opiliones	12.68	1.18
Diplopoda	11.27	1.31
Pseudoscorpiones	8.45	0.92
Blattodea		
Isoptera	4.23	0.66
Orthoptera		
Gryllidae	2.82	0.26
Blattodea		
Blattidae	1.41	0.13
Isopoda	1.41	0.13
Ephemeroptera	1.41	0.13
Empty stomach	2.82	–
Unidentifiable prey	32.39	4.46

DISCUSSION

Ants have been reported as the most important prey group in many eastern North American *Plethodon* salamanders: *P. albagula* (Oliver, 1967; Milanovich *et al.*, 2008), *P. amplus* (Rubin, 1969), *P. cinereus* (Cochran, 1911; Bellocq *et al.*, 2000), *P. cylindraceus* (Fraser, 1976b), *P. electromorphosis* (Duellman, 1954), *P. glutinosus* (Powders and Tietjen, 1974; Bailey, 1992; Jensen and Whiles, 2000; Hutton, pers. obs.), *P. grobmani* (Brandon, 1965), *P. jordani* (Powders and Tietjen, 1974), *P. kentucki* (Bailey, 1992), *P. metcalfi* (Whitaker and Rubin, 1971), *P. petraeus* (Jensen and Whiles, 2000), *P. richmondi* (Hutton, pers. obs.), *P. shermani* (Whitaker and Rubin, 1971; Lewis *et al.*, 2014), and *P. wehrlei* (Hall, 1976; Pauley, 1978). Here, we report that ants were the most important prey item of *P. kentucki* in southeastern Kentucky, in terms of both the percent of salamanders that consumed them and the total number of prey items. However, only a few *Plethodon* salamander diet studies have identified ants or other prey to family or genus or provided specific life stage information. Lewis *et al.* (2014) found ten distinct ant taxa in the stomachs of *P. shermani* from North Carolina, with *Aphaenogaster fulva* and *A. rudis* most common. Paluh *et al.* (2015) found *A. picea* in to be the most abundant ant in the stomachs of *P. cinereus* from Ohio. In *P. kentucki* stomachs, we found *Aphaenogaster sp.* to only comprise a fraction of total ant diversity (Table 2), with the majority of individuals belonging to the genus *Pheidole*. Sympatric *Plethodon richmondi* and *P. glutinosus* that were also sampled in our study area, both consumed a large diversity of ant genera, the majority of which were also *Pheidole* (Hutton, pers. obs.). Therefore, a regional or microhabitat difference may be responsible for the observed differences in the relative ant species composition and abundances within *Plethodon* stomach contents.
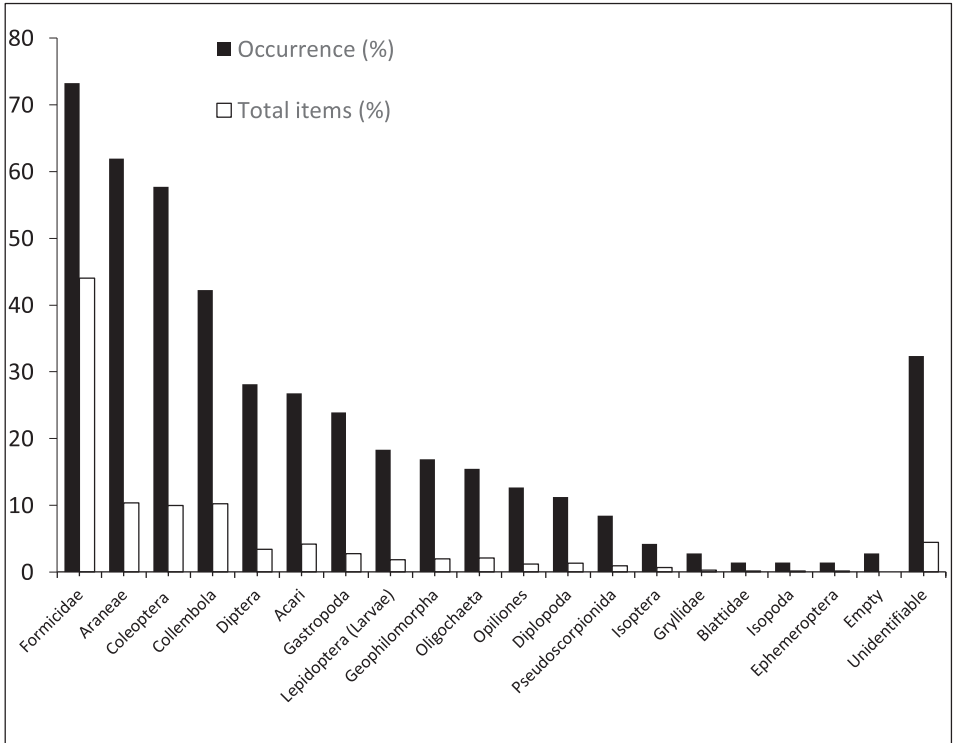
FIG. 1.—Prey categories found in adult *Plethodon kentucki* (n = 73), expressed as percent occurrence and percent of total diet, from southeastern Kentucky from Apr.–May 2016

Similarly to the *P. kentucki* in our study, coleopterans from the families Scarabidae and Carabidae (ground beetles) were among the most frequent in the diet of *P. albagula* from Arkansas (Milanovich *et al.*, 2008). In *P. shermani* stomach contents, Carabidae and Curculionidae were the most frequently observed families and were followed by individuals from the family Elateridae (click beetles; Lewis *et al.*, 2014). In *P. amplus* from North Carolina, Rubin (1969) found individuals from Curculionidae and Carabidae to comprise the majority of the coleopteran diet. However, unlike in our *P. kentucki*, the previous studies noted adult coleopterans were more frequent and numerous than larvae, suggesting larvae may be more important to the *P. kentucki* in our study than to the other species.

Lewis *et al.* (2014) identified spiders in the stomach contents of *P. shermani* to five families, individuals from the family Linyphiidae (sheet weavers) were most common. Salticid (jumping spiders) spiders were also present in their diet, similarly to the *P. kentucki* in our study (Lewis *et al.*, 2014). Oliver (1967) found larval dipterans from two families (Blephariceratidae and Stratiomyidae) in the diet of *P. albagula*. Whitaker and Rubin (1971) also found larval dipterans more frequently than adult individuals in both *P. metcalfi* and *P. shermani*. Additionally, Jensen and Whiles (2000) also reported larval dipterans more frequently than adults in both *P. petraeus* and *P. glutinosus*. In our study however, larval flies were less important than adults, which could be attributed to seasonal differences but further investigation is needed.

The most important prey items found in *P. kentucki* from West Virginia (listed in order of importance) were ants, coleopterans, micro-gastropods, spiders, pseudoscorpions, collembolans, mites, and dipterans (Bailey, 1992). Bailey (1992) found prey from 18 orders but did not include life stages of prey items and sampling periods were not included in the methods. Therefore, we are unable make any comparisons

TABLE 2.—Prey types found in the stomachs of adult *Plethodon kentucki* identified beyond the level of order and their percent occurrence and makeup with each group, from southeastern Kentucky from April–May 2016

Prey category	Occurrence (%)	Total items (%)	Prey category	Occurrence (%)	Total items (%)
Araneae			Formicidae		
Unidentified Spider	81.82	70.89	*Pheidole sp.*	71.15	43.45
Theridiidae	25.00	16.46	Unidentified Formicidae	42.31	12.80
Thomisidae	9.09	7.59	*Amblyopone sp.*	15.38	2.68
Xysticus sp.	2.27	3.80	*Lasius sp.*	13.46	24.40
Salticidae	2.27	1.27	*Camponotus sp.*	9.62	1.49
Coleoptera			*Formica sp.*	7.69	5.95
Unidentified Larvae	46.34	30.26	*Aphaenogaster sp.*	7.69	1.79
Unidentified Adult	24.39	14.47	*Temnothorax sp.*	5.77	5.06
Scarabaeidae	17.07	18.42	*Myrmecina americana*	5.77	0.89
Curculionidae	17.07	9.21	*Stenamma sp.*	1.92	0.60
Carabidae	7.32	6.58	*Hypoponera sp.*	1.92	0.30
Staphylinidae	7.32	3.95	*Ponera pennsylvanica*	1.92	0.30
Tenebrionidae	7.32	9.21	*Pyramica sp.*	1.92	0.30
Silphidae	4.88	2.63	Gastropoda		
Elateridae	2.44	1.32	Unidentified Snail	52.94	47.62
Elateridae Larvae	2.44	1.32	*Glyphyalinia indentata*	17.65	14.29
Dytiscidae	2.44	1.32	*Ventridens suppressus*	11.76	9.52
Nitiulidae	2.44	1.32	*Discus sp.*	5.88	4.76
Collembola			*Glyphyalinia sp.*	5.88	4.76
Sminthuridae	46.67	29.49	*Polygyridae sp.*	5.88	4.76
Isotomidae	40.00	33.33	*Punctum minutissimum*	5.88	4.76
Unidentified Collembola	40.00	25.64	*Strobilops labyrinthicus*	5.88	4.76
Hypogastruridae	13.33	11.54	*Ventridens sp.*	5.88	4.76
Diptera			Isoptera		
Unidentified Adult	80.00	65.38	Rhinotermitidae	-	-
Unidentified Larvae	30.00	30.77			
Tabanidae	5.00	3.85			

beyond the order level or explore the influences of seasonality on prey composition. In West Virginia, micro-gastropods, pseudoscorpions, and diplopodans were eaten more frequently than in southeastern Kentucky (Bailey, 1992). Conversely, coleopterans, spiders, and collembolans were found in more individuals from Kentucky than West Virginia. Despite these differences, individuals from southeastern Kentucky and West Virginia appear to have generally similar diets.

Plethodon kentucki is a euryphagous salamander that consumes a wide diversity of ants, spiders, beetles, and micro-gastropods. To our knowledge, this is the fourth study to nonlethally examine the diet of a large terrestrial plethodontid; Fraser (1976a; b) and Lewis *et al.* (2014) were the first investigations. Future studies should nonlethally examine salamander diet across their known ranges and sample through the various seasons in order to better understand the mechanisms behind prey acquisition, composition, and possible selection. Invertebrates should also be collected in order to compare total community diversity and abundance with salamander stomach contents. Lastly, species level dietary information should be gathered to compare sympatric salamander prey selection and composition.

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