

Diet of the southern ravine salamander *Plethodon richmondi*

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ABSTRACT - The diets of many small North American *Plethodon* species are poorly studied despite their important roles in forest ecosystems. Using a non-lethal gastric lavage method, we examined the spring-season diet of 31 southern ravine salamanders (*Plethodon richmondi*) from a second-growth forest in south-eastern Kentucky (USA). We recovered and identified a total of 452 prey items from 14 different prey groups. The three most important prey groups were Formicidae (ants), Acari (mites and ticks), and Collembola (springtails). Together, these groups accounted for more than 80 % of all prey items. Examining the diets of terrestrial salamanders may help us better understand their roles in the regulation of invertebrate communities and the transfer of accessible nutrients back to the soil.

INTRODUCTION

Terrestrial lungless salamanders (Plethodontidae) are an integral component of many forested ecosystems. In the eastern United States, terrestrial salamanders are often abundant (Bailey et al., 2004; Dodd & Dorazio, 2004), with recent density estimates of up to 5.26 individuals/m² (Hernández-Pacheco et al., 2019). Thus, terrestrial salamanders can play an important role in the top-down regulation of invertebrate populations (Hairston, 1987; Davic & Welsh, 2004; Walton et al., 2006; Semlitsch et al., 2014). In turn, terrestrial salamander populations can influence the rate of detritus and leaf litter decomposition (Wyman, 1998; Hickerson et al., 2017), which regulates the amount of CO₂ released from forest floors into the atmosphere each year (Wyman, 1998).

Salamanders typically employ a euryphagous feeding strategy (but see Paluh et al., 2015), where the diversity, amount, and frequency of prey acquired is directly dependent on the spatial and temporal availability of prey in the salamander's microhabitat (Jaeger, 1981). Numerous studies have examined the diets of large (i.e., adult snout-vent-length, SVL > 60 mm), terrestrial, eastern North American plethodontid salamanders (i.e., Oliver, 1967; Rubin, 1969; Whitaker & Rubin, 1971; Powders & Tietjen, 1974; Jensen & Whiles, 2000; Lewis et al., 2014; Hutton et al., 2017). However, the diets of most small (i.e., adult SVL < 60 mm) North American *Plethodon* species are unknown or poorly studied. Furthermore, the majority of previous *Plethodon* dietary studies only identified prey items to the taxonomic level of order and only provided percent occurrence or raw numerical count data. While this dietary information can be useful, identification of prey to the lowest taxonomic level, along with estimates of individual prey volumes, can provide researchers with the data resolution necessary to examine specific differences in ontogenetic, seasonal, and inter-species diet composition.

Plethodon richmondi (southern ravine salamander) is a small (less than 138 mm total length, TL; Petranka, 1998) terrestrial lungless salamander with a broad distribution in eastern Kentucky, northern North Carolina, north-eastern Tennessee, western Virginia, and southern West Virginia. The species is commonly found under flat rocks, damp rotting logs, and in leaf litter on rocky wooded slopes in tracts of mature forest. In Kentucky, these animals are generally active on the forest floor during periods of damp, mild weather from fall through spring and they are particularly abundant on the forest floor from March through May.

In this study, we examined the spring (March - May) diet of *P. richmondi*, from a second growth forest in south-eastern Kentucky. We used a non-lethal gastric lavage technique to identify stomach contents to the lowest possible taxonomic resolution, with these data we have been able to report 1) the first description of *P. richmondi* diet, and 2) evaluate the importance of prey groups/types to the overall diet.

MATERIALS & METHODS

Our study area was located in the University of Kentucky's Robinson Forest (RF), a 5983 ha experimental forest located in the interior rugged section of the Cumberland Plateau in Breathitt and Knott Counties, Kentucky USA. Robinson Forest is a 90-year-old, second growth forest with vegetation consisting of typical, mixed mesophytic forests of the region; dominant tree species included white oak (*Quercus alba*), tulip tree (*Liriodendron tulipifera*), Eastern hemlock (*Tsuga canadensis*), and chestnut oak (*Quercus prinus*) (for more details, see Phillippi & Boebinger, 1986).

In the spring (March-May) of 2016 and 2017, *P. richmondi* were located by sifting through dense leaf litter, as well as by turning over logs, rocks, and other cover objects near first-order streams. Upon capture, the salamanders were placed in individual containers and brought back to the dry lab at RF for processing. Salamanders were anesthetised in a solution

of 1 g Maximum Strength Orajel®/1 liter of aged tap water (Cecala et al., 2007). They were removed from the solution when they could no longer right themselves after being flipped over. Then to obtain their stomach contents the salamanders were subjected to a non-lethal gastric lavage technique (Fraser, 1976a; Hantak et al., 2016; Hutton et al., 2017). Salamanders were placed on their dorsum on a folded paper towel, and an approximately 6.0 cm long piece of water-lubricated tubing (1.3 mm OD PTFE tubing were used, Zeus Inc., catalog number AWG24) was slowly inserted into the esophagus until there was resistance. Distilled water was then pumped (Nipro® 3 mL syringes with 22-gauge needles) into the tubing. As in previous studies, salamander stomachs were pumped at least two additional times after the last prey item was extracted to verify removal of all contents (Cecala et al., 2007; Bondi et al., 2015). After lavage, each salamander was measured for snout-vent length (SVL: from the tip of the snout to the posterior angle of the vent) and total length (TL: from tip of the snout to the tail's terminus) to the nearest 0.01 mm with a digital caliper, and mass to the nearest 0.1 g with a digital scale. If possible, sex was determined based on the presence of mental glands in males, or eggs visible through the abdominal walls in females. Salamanders were then placed in a recovery container of aged tap water until they could right themselves and responded to tapping, which took approximately 15 minutes. Salamanders were returned to their exact location of capture within 1.5 h.

Stomach contents were retained on the paper towels and immediately identified to family, genus, and species, if possible, using a dissecting microscope along with appropriate keys and guides (Peckarsky, 1990; Merritt & Cummins, 1996; Fisher & Cover, 2007; Bradley, 2012; Evans, 2014). Additionally, invertebrate life stage (larval or adult) was reported, if applicable. The individual prey items were then grouped based on order/class and life stage.

To calculate prey volume, we measured the length and width of each prey item to the nearest 0.01 mm using a digital caliper and estimated volume as a prolate spheroid using the equation (Dunham, 1983):

$$\text{Prey Volume } (v_x) = \left(\frac{4\pi}{3}\right) \left(\frac{\text{length}}{2}\right) \left(\frac{\text{width}}{2}\right)^2$$

The relationship between individual *P. richmondi* size (i.e., mass, SVL, and TL) and total consumed prey volume were analyzed using individual linear regressions in the statistical program R (Version 3.4.3). The Shapiro-Wilk test was used to assess the normality of the data residuals. Salamander size (SVL and TL) were log-transformed to meet the normality assumption of linear regression.

Relative occurrence (RO) of each prey group was calculated using the equation:

$$\text{RO} = (P*100)/T$$

where P is the total number of occurrences of a given prey type, and T is the total number of prey items recovered (Loveridge & Macdonald, 2003).

Individual prey volumes and RO were used to calculate importance values (I_x) for each prey group (Holomuzki, 1980; Davic, 1983). Specifically, I_x values estimate the relative weight of importance of a particular prey type or group to the overall diet in the animals sampled. To estimate group I_x values, the volumetric and relative occurrence (RO) data of each prey item and type are calculated. Importance values (I_x), ranging from 0 to 1, were calculated for each prey group/type using the equation (Powell et al., 1990; Anderson & Mathis, 1999):

$$I_x = \frac{\left[\left(\frac{n_x}{N}\right) + \left(\frac{v_x}{V}\right) + \left(\frac{f_x}{F}\right)\right]}{3}$$

Where n_x , v_x , and f_x represent the number of a prey type, the volume of the prey type, and frequency or the number of stomachs containing that prey type, respectively, and N, V, and F represent their sums across all prey types (Hantak et al., 2016). These importance values were used to compare the overall importance of a particular prey group/type to the overall diet of *P. richmondi*. After processing, prey samples were placed into individually labeled vials containing 70 % ethanol. Samples are stored in the Branson Museum collection at Eastern Kentucky University, Richmond, Kentucky.

RESULTS

We stomach flushed a total of 31 *P. richmondi*, SVL ranged from 20.00–57.20 mm (mean \pm SD = 40.86 \pm 7.93), TL ranged from 38.54–101.07 mm (mean \pm SD = 76.46 \pm 17.33), and mass ranged from 0.1–1.2 g (mean \pm SD = 0.75 \pm 0.31). Overall, 14 individuals were identified as adult females, 3 were reproductive males, and 14 were of unknown sex. All individuals had at least one prey item in their stomachs. We recovered a total of 452 prey items, and on average, individuals contained 14.6 \pm 10.1 prey items in their stomachs. We found no relationship between total consumed prey volume and salamander mass ($R^2 = -0.028$; $P = 0.683$), SVL ($R^2 = -0.034$; $P = 0.891$), or TL ($R^2 = 0.013$; $P = 0.249$).

Overall, we found 49 distinct prey types from 14 invertebrate prey groups. These results are summarised in Figure 1 and shown in detail in Supplementary Materials (Table S1). The three most important prey (I_x), which made up 62% of the overall importance (1.519 of 2.419 total I_x) and 82% of the total prey occurrence, were Formicidae (ants: $I_x = 0.659$, RO = 29.20%), Acari (mites/ticks: $I_x = 0.514$, RO = 28.76%), and Collembola (springtails: $I_x = 0.346$, RO = 24.56%; Fig. 1, Table S1).

Among the ants, prey from the genus *Pheidole* contributed to approximately 50 % of both the overall Formicidae I_x and RO. *Pheidole* also comprised 12 % of the overall prey importance and 15.3 % of all prey items consumed. Mites from the superfamily Galumnoidea, were the most important and frequent among the Acari (34 % and 42 %, respectively), comprising 7.1 % of the overall prey importance and 12 % of all prey items. Lastly, springtails from the family Isotomidae, were the most important and frequent among the Collembola

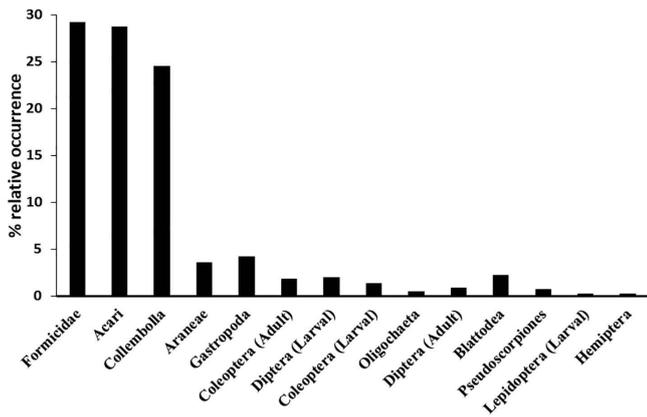


Figure 1. Prey groups found as stomach contents in *Plethodon richmondi* (n=31), expressed as percent relative occurrence, from south-eastern Kentucky (USA) from March-April 2016 and 2017

(62 % and 55 %, respectively) and comprised 8.9 % of the total prey importance and 13.5 % of all prey items.

Arachnids (Acari, Araneae, and Pseudoscorpiones) accounted for approximately 30 % of all prey importance and occurrence. Araneae were found to be the fourth most important prey group ($I_x = 0.178$) in terms of volumetric importance (i.e., I_x), although they only comprised 3.5 % of all prey items. Unfortunately, many of the spiders could not be identified further than order due to advanced digestion, but of those that could be identified, most were either cobweb (Theridiidae) or ground (Gnaphosidae) spiders. The fourth most common prey were gastropods (snails) and comprised 4.2 % of all prey and were the fifth most important group ($I_x = 0.170$). Overall, larval prey comprised Coleoptera (beetles), Diptera (flies), and Lepidoptera (moths), accounted for 8.4 % of the total importance and just 3.5 % of all prey items.

DISCUSSION

This is the first study to describe the spring diet of *P. richmondi* and calculate individual prey group importance values. We reported the importance values for 14 prey groups to adult *P. richmondi* from a population in Kentucky to the taxonomic level of family, genus, and species, and our data demonstrate that *P. richmondi* spring diet is primarily composed of adult-staged, terrestrial invertebrates, with Formicidae, Acari, and Collembola being the three most volumetrically important and frequently consumed prey.

We found ants to be both the most important and numerous prey ($I_x = 0.659$, RO = 29.20 %) consumed by *P. richmondi*. Previous studies have similarly reported ants as one of the most important prey groups in many eastern North American *Plethodon* (Cochran, 1911; Duellman, 1954; Oliver, 1967; Rubin, 1969; Whitaker & Rubin, 1971; Powders & Tietjen, 1974; Hall, 1976; Fraser, 1976b; Camp & Bozeman, 1981; Bailey, 1992; Bellocq et al., 2000; Jensen & Whiles, 2000; Milanovich et al., 2008; Lewis et al., 2014; Paluh et al., 2015; Hantak et al., 2016; Hutton et al., 2017). King et al. (2013) estimated that ants can account for more than 95 % of the macroinvertebrate population in eastern temperate hardwood forests. Thus, the general importance of ants to

Plethodon species is likely a result of their abundance and availability in salamander microhabitats relative to other potential invertebrate prey.

We identified ants in the spring diet of *P. richmondi* from nine species/genera, with members of *Pheidole* and *Lasius* comprising 53 % and 23.5 % of all ants, respectively. Similar to our results, Hutton et al. (2017) reported 12 species/genera of ants in the south-eastern Kentucky spring diet of *P. kentucki*, 43 % were *Pheidole* and 24 % were *Lasius*. Unsurprisingly, studies conducted outside of Kentucky or in other seasons have produced somewhat different results. Paluh et al. (2015) reported ants in the north-eastern Ohio fall (September and October) diet of *P. cinereus* from ten species/genera with *Aphaenogaster picea*, followed by two species from the genus *Lasius* comprising the majority of ants consumed. Lewis et al. (2014) reported ants in the south-western North Carolina summer diet of *P. shermani* from ten species/genera with *Aphaenogaster* comprising up to 46 % of the ant prey and 27 % of all prey items consumed. In our study, *Aphaenogaster* comprised only 0.8 % of the ants consumed by *P. richmondi*. Differences in regions, sampling seasons, species distributions, species assemblages, and microhabitats are likely responsible for the observed differences in the species of ants in the diets of these eastern *Plethodon* salamanders.

In this study, Acari (mites) were found to be the second-most important and frequently consumed prey group ($I_x = 0.514$, RO = 28.76 %). Despite their importance in *P. richmondi*, Duellman (1954) reported only three mite prey items in the diet of the closely related *P. electromorphus*. Acari have been found to feature prominently in the diets of *P. amplus*, *P. glutinosus*, and *P. wehrlei* (Rubin, 1969; Hall, 1976) and have been reported as the second-most consumed prey in *P. jordani*, *P. serratus*, and *P. websteri* (Powders & Tietjen, 1974; Camp & Bozeman, 1981). Numerous studies throughout the eastern range of *P. cinereus*, have also reported Acari to comprise a large portion of their diet (Cochran, 1911; Maglia, 1996; Bellocq et al., 2000); specifically, Hantak et al. (2016) found Acari to comprise 26 % of the diet of this species. Similarly, Hutton et al. (2017) reported Acari in 27% of *P. kentucki* diets. It is uncertain if seasons, species assemblages, and or microhabitat are responsible for the variation of Acari in the composition of the diet.

We found Collembola (springtails) to be the third-most important and frequently consumed prey group ($I_x = 0.346$, RO = 24.56 %) in the diet of *P. richmondi*. In the diet of *P. electromorphus*, Duellman (1954) reported Collembola to constitute just 2 % of the diet. However, Collembola (springtails) have been reported to constitute a significant portion of diet in *Plethodon* (Cochran, 1911; Fraser, 1976a; Powders & Tietjen, 1974; Camp & Bozeman, 1981; Jensen & Whiles, 2000; Bellocq et al., 2000). Specifically, Hantak et al. (2016) found Collembola to comprise 17 % of the dietary importance in *P. cinereus* and Hutton et al. (2017) reported Collembola in 42 % of *P. kentucki* stomachs and comprised 10 % of all prey. In addition to differences in Acari and Collembola in the diet of *P. electromorphus* and *P. richmondi*, Duellman (1954) reported that Isopoda (isopods) were the second-most abundant prey, however, we failed to detect isopods in any *P. richmondi*. Overall, the differences in the

diet compositions between these two closely related species are likely due to the influence of sampling season and site conditions on invertebrate prey diversity, abundance, and availability.

Plethodon richmondi is a euryphagous salamander that during spring time consumes a diverse assemblage of primarily mid-sized, terrestrial, adult invertebrates, principally consisting of Formicidae, Acari, Collembola, Araneae, and Gastropods. Additional studies should prioritise evaluating the diet of *P. richmondi* during other times of the year. Further, the non-lethal gastric lavage technique should be used to examine the diets of other small *Plethodon* salamanders to identify prey to the lowest taxonomic level and so better understand the role of these salamanders in the regulation of invertebrate communities. These studies should include the estimation of prey volumes to allow for the calculation of importance values, which can be used to make a more discerning examination of spatial and temporal variation in salamander diets among species, age groups, and between sexes.

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