

Diet of Larval Red Salamanders (*Pseudotriton ruber*) Examined Using a Nonlethal Technique

KRISTEN K. CECALA, STEVEN J. PRICE, AND MICHAEL E. DORCAS¹

Department of Biology, Davidson College, Davidson, North Carolina 28035-7118, USA

ABSTRACT.—Stream salamanders may play important roles as predators within streams, but we know little about actual predation by stream salamanders on other organisms. Because larval stream salamanders are more abundant within streams than adults, feed and forage throughout the year, and may spend multiple years in streams before transformation, larvae may play a more important role than adults in trophic interactions within streams. We conducted a study using larval Red Salamanders (*Pseudotriton ruber*) to determine (1) the prey composition of larval salamanders, (2) whether feeding rates are affected by stream water temperature, (3) whether larval size affects the diversity of prey items, and (4) whether nonlethal stomach flushing is an effective technique for examining the diet of larval salamanders. We found that larvae consumed a wide diversity of prey items including individuals of the families Chironomidae (36.52% of prey items) and Sphaeriidae (15.17%) as well as terrestrial prey (7.87%) and other salamanders (2.25%). We also found that feeding rates were negatively correlated with stream water temperature, and larger larvae consumed a wider diversity of prey items than smaller individuals. Our results also suggest that nonlethal stomach flushing did not affect survivorship. These findings suggest that larval Red Salamanders are generalist predators that can play important trophic roles in stream ecosystems.

In the absence of fish, salamanders are often the top predators in first-order streams and may structure stream communities via predation (Davic and Welsch, 2004). Examining diet composition and factors that affect the diet of stream salamanders is the first step in developing a more complete understanding of their role as predators within streams. Diet studies on stream salamanders have generally been conducted on adults (Hamilton, 1932; Burton and Likens, 1976; Lowe et al., 2005; but see Caldwell and Houtcooper, 1973; Rudolf, 1978; Petranka, 1984), despite a greater abundance of larval salamanders in most stream habitats where they occur (Davic, 1983). Furthermore, many stream-salamander species have larval periods lasting from 2–3 yr. During these long larval periods, larvae often feed throughout the year (Bruce and Castanet, 2006), whereas adults may exhibit seasonal foraging activity around streams (Petranka, 1998). Consequently, because of their abundance, long larval periods, and more consistent foraging patterns, the impact of larval stream salamanders on trophic interactions may be more important than that of adult salamanders (Wyman, 1998; Davic and Welsch, 2004).

Red Salamanders (*Pseudotriton ruber*) occur throughout the eastern United States in springs and streams (Petranka, 1998), and their larvae spend up to three years in aquatic habitats prior to metamorphosis (Bruce, 1972). Larval *P. ruber* range from 11–52 mm in snout-vent length (SVL; Petranka 1998), which exceeds the size range of most stream-salamander larvae (e.g., *Desmognathus fuscus* 8–20 mm, *Eurycea cirrigera*, 7–32 mm, Petranka 1998) found within their range. Adult *P. ruber* have been documented as consumers of a wide variety of animals (Petranka, 1998), but little is known about the diet of larval *P. ruber*. In this study, we describe the diet of larval *P.*

ruber, determine whether feeding rates vary temporally and with temperature, and examine the relationship between *P. ruber* size and the number of prey items consumed. Finally, we examine the practicality of using a nonlethal stomach-flushing technique on stream-salamander larvae by comparing recapture rates between stomach-flushed and non-stomach-flushed individuals.

MATERIALS AND METHODS

We analyzed stomach contents of 253 *P. ruber* larvae and 23 recently transformed *P. ruber* collected from three streams in the western Piedmont of North Carolina, concentrating on a stream at Cowan's Ford Wildlife Refuge as the primary study site. Salamanders were collected primarily by dip-netting from February through April 2006 and by funnel traps (Willson and Dorcas, 2003) at Cowan's Ford Wildlife Refuge from May until August 2006. As part of a larger mark-recapture study, funnel trapping consisted of three consecutive trapping days every other week. Water temperature was collected at the Cowan's Ford Wildlife Refuge stream at 30-min intervals using a temperature-sensitive data-logger (Tidbit Stowaways, Onset Computer, Inc. Part TBI32-20b50; Pocasset, Massachusetts).

The SVL of all larvae examined was between 18 mm and 56 mm (measured to the posterior edge of the cloaca to the nearest millimeter). We also examined recently transformed individuals (individuals with visible gill scars) that measured between 47 mm and 56 mm SVL. After capture, we individually marked each animal with visible implant elastomer (Nauwe-laerts et al., 2000; $N = 465$) to ensure that the salamanders were stomach flushed only once throughout the study. We arbitrarily assigned animals for stomach flushing. Following all procedures, individuals were returned to their point of capture within approximately 90 min (maximal processing time).

¹ Corresponding Author. E-mail: midorcas@davidson.edu

Because this study was part of a larger mark-recapture study, we wished to examine stomach contents without mortality associated with dissection. Thus, we used a nonlethal procedure modified from the technique outlined by Griffiths (1986) to remove stomach contents. Within four hours of capture, we anesthetized individuals with a solution of 1 g Orajel®/1 liter of tap water (Cecala et al., 2007). Larvae were removed from anesthesia, rinsed with distilled water, and placed on a moist paper towel. We used a sterile 1-ml syringe (Becton Dickinson, Franklin Lakes, New Jersey) with a 20-gauge needle and attached a sterile 30 mm long, 1.19 mm diameter section of Silastic tubing (Dow Corning, Catalog number 508-003) to the end of the needle to insert into each salamander's oral cavity. We inserted 5–10 mm of tubing into the oral cavity of each salamander and then pumped 1 ml of distilled water into the gastric cavity. After removing the tube, we palpated food items out of the salamander. Although we could not conclusively verify that we removed all contents of the gastric cavity, the translucent nature of the ventral surface of larval *P. ruber* allowed us to determine whether the stomach contained additional items. If necessary, we repeated the procedure until visual examination revealed no remaining prey items. Stomach contents were preserved in 95% ethanol, and we identified all items to the lowest taxonomic group possible. In some instances, we were able to identify items to family, but others were identified only to phylum.

We used linear regression to examine the relationship between 72-h stream water temperature means and the arcsine-transformed proportion (Sokal and Rohlf, 1981) of individuals with stomach contents at Cowan's Ford Wildlife Refuge. We analyzed our results with 72-h stream water temperature means because these temperatures are reflective of temperatures salamanders would have experienced during foraging prior to capture. We also used linear regression to examine the relationship between SVL and number of prey items consumed. To examine the effects of stomach flushing on survival, we compared recapture rates for individuals stomach-flushed ($N = 253$) and those not stomach-flushed ($N = 212$) with a contingency table assuming equal recapture rates. All statistical tests were conducted using Minitab (vers. 12.1), and an alpha of 0.05 was used to determine significance for all tests.

RESULTS

We obtained 178 food items from 68 larval *P. ruber* (Table 1). Overall, 26.87% (68 of 253 larvae) of stomach-flushed larval *P. ruber* contained stomach contents. Food items were identified into 29 different taxonomic prey groups. None of the recently transformed *P. ruber* contained stomach contents ($N = 23$). The most frequently consumed prey were midges (family Chironomidae) and fingernail clams (family Sphaeriidae), which comprised 36.52% and 15.17%, respectively, of all organisms ingested (Table 1). Although fully aquatic, approximately 8% of their diet was composed of terrestrial prey such as spring-tails (order Collembola; 2.25%) or terrestrial beetles (order Coleoptera; 3.39%; Table 1). On rare occasions,

P. ruber larvae consumed other salamanders (2.25%). We were able to identify only one sample of salamander prey item, a larval *Eurycea* species.

In May, 21.8% of salamanders contained stomach contents, but when water temperature was highest in July, only 8.1% of salamanders contained prey items (Fig. 1). We found that stream water temperature negatively influenced the proportion of individuals with prey items in their stomach (Fig. 1; linear regression, $Y = -0.116X + 2.574$, $N = 6$, $R^2 = 0.77$, $F = 13.65$, $P = 0.021$). We found no relationship between the SVL of individuals and the number of prey items they consumed (linear regression, $N = 68$, $R^2 < 0.01$, $F = 0.03$, $P = 0.86$).

Our recapture rate of stomach-flushed individuals (32.81%) did not differ from the recapture rate of non-stomach-flushed individuals (33.07%; $\chi^2_{df=2} < 0.01$, $P = 0.99$). All salamanders appeared to recover fully from anesthesia after stomach flushing, and we observed no mortality associated with the procedure within 90 min after the procedure (the time within which all animals were released at their point of capture).

DISCUSSION

Pseudotriton ruber larvae consumed a wide variety of prey items, suggesting that they are generalist predators similar to many other amphibians (Burton and Likens, 1976; Brophy, 1980; Parker and Goldstein, 2004). Their frequent consumption of detritivores such as midges (family Chironomidae) supports claims by Davic and Welsh (2004) that larval stream salamanders may play an indirect role in detritus decomposition and nutrient cycling within aquatic systems (Davic, 1983; Wyman, 1998). Our results also highlight the role of stream amphibians as vectors of energy transfer between terrestrial and aquatic habitats (Lowe et al., 2005) as a result of their consumption of terrestrial organisms and other salamanders (Table 1). Although adults of many stream-salamander species, such as Spring Salamanders (*Gyrinophilus porphyriticus*) and Black-Bellied Salamanders (*Desmognathus quadramaculatus*) are known to consume other salamanders (Bruce, 1979; Gustafson, 1994; Petranksa, 1998); prior to this study, we knew little about intraguild predation rates of larval stream salamanders. The discovery of predation on other salamanders by larval *P. ruber* indicates that these salamanders may affect local guild dynamics (Camp, 1997), but based on our results, their effect is likely minimal (2.25% of diet, Table 1).

We found that higher temperatures may reduce foraging activity in larval *P. ruber*. Our results support predictions by Bruce and Castanet (2006) that sporadic, rapid bone growth in *P. ruber* larvae was caused by variable activity patterns associated with large temperature ranges experienced by these salamanders. Conversely, prey items may become less active or abundant in streams during the summer and, thus, less available for consumption (Matthews et al., 1991). Or, as with most ectotherms, higher temperatures may increase digestion rates causing a subsequent decrease in the discovery of stomach contents.

TABLE 1. Diet composition of larval *Pseudotriton ruber* from streams in the western Piedmont of North Carolina. Prey items were identified to the lowest two taxonomic levels available.

Taxonomy of prey items	% of prey items	% of individuals with item	Habitat
Diptera, Chironomidae	36.52	54.41	Aquatic
Pelecypoda, Sphaeriidae	15.17	26.47	Aquatic
Insecta, Ephemoptera	6.18	8.82	Aquatic
Insecta, Coleoptera	3.39	7.35	Terrestrial
Nematoda	3.39	2.94	Aquatic
Insecta, Plecoptera	2.81	1.47	Aquatic
Insecta, Lumbriculida	2.25	5.88	Aquatic
Decapoda, Cambaridae	2.25	4.41	Aquatic
Entognatha, Collembola	2.25	4.41	Terrestrial
Diptera, Tipulidae	2.25	5.88	Aquatic
Insecta, Tricoptera	2.25	8.82	Aquatic
Caudata, Plethodontidae	2.25	5.88	Aquatic
Diptera, Simuliidae	1.69	2.94	Aquatic
Clitellata, Hirundinea	1.12	2.94	Aquatic
Malacostraca, Isopoda	1.12	2.94	Aquatic
Maxillopoda, Copepoda	1.12	2.94	Aquatic
Myriapoda, Chilopoda	1.12	2.94	Terrestrial
Neuroptera, Corydalidae	1.12	4.41	Aquatic
Basommatophora, Physidae	1.12	2.94	Aquatic
Annelida	0.56	1.47	Aquatic
Clitellata, Tubificidae	0.56	1.47	Aquatic
Malacostraca, Amphipoda	0.56	1.47	Aquatic
Insecta, Odonata	0.56	1.47	Aquatic
Insecta, Lepidoptera	0.56	1.47	Aquatic
Insecta, Coleoptera	0.56	1.47	Aquatic
Mollusca, Pelecypoda	0.56	1.47	Aquatic
Mollusca, Gastropoda	0.56	1.47	Aquatic
Turbellaria, Seriata	0.56	1.47	Aquatic
Unknown	2.25	4.41	-

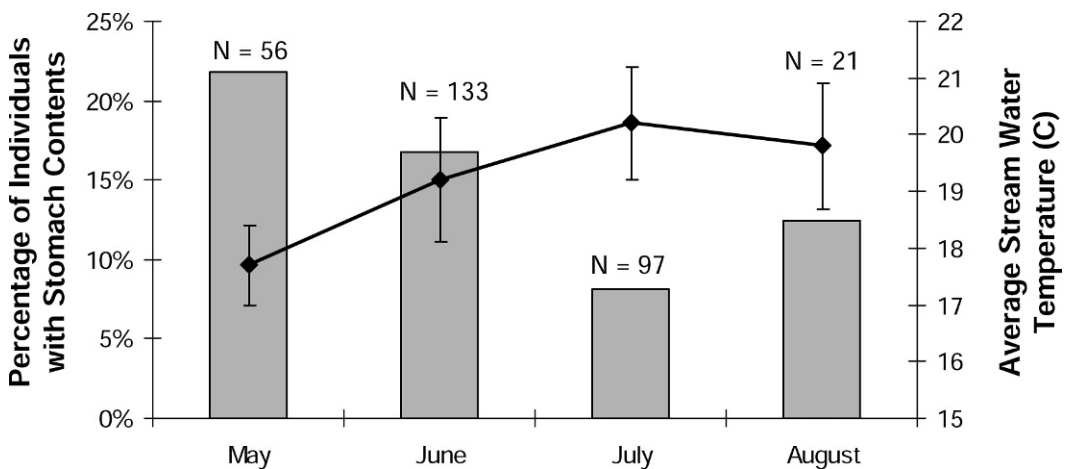


FIG. 1. Monthly proportion of larval *Pseudotriton ruber* containing prey items is negatively associated with average water temperature in a North Carolina Piedmont stream. Mean water temperature during sampling days for each month are shown with error bars representing the average daily minimum and maximum temperature. The number of stomach-flushed individuals is indicated for each month.

Similar to other studies examining the foraging strategies of larval salamanders (Sites, 1978; Sih and Petranks, 1988), we found no correlation between salamander size and number of prey items. Optimal-foraging strategy predicts consumption of smaller numbers of large prey as predators grow (Stephens and Krebs, 1986), but as with many other organisms (Pyke, 1984), large larval *P. ruber* continue to consume small as well as large prey items. Furthermore, conclusions regarding size of prey are difficult to draw because of a lack of information about prey availability and abundance within the streams we studied.

Our data support nonlethal stomach flushing as an effective alternative to dissection as a method of examining diet in larval amphibians. Although many diet studies have relied on dissection (e.g., Burton and Likens, 1976), some previous studies have used stomach-flushing techniques on other animals such as American Alligators (*Alligator mississippiensis*; Fitzgerald, 1989) and Smooth Newts (*Triturus vulgaris*; Griffiths, 1986). Because our recapture rates of stomach-flushed individuals were similar to non-stomach-flushed individuals, we conclude that this technique had minimal negative effects other than the energetic loss of the meal and that nonlethal stomach flushing appears to be an effective technique for examining the stomach contents of larval salamanders.

Acknowledgments.—We thank G. E. Vaughan, J. J. Hall, and S. F. McCorkle of the Duke Power Company Environmental Laboratory for identifying preserved stomach contents; C. Hickman and three anonymous reviewers for providing comments that improved the manuscript; Davidson College Herpetology Laboratory members, particularly L. A. Harden, N. Diluzio, and S. Rhodes, for assistance with animal collection; and D. Seriff and K. Coffey of the Mecklenburg County Natural Resources Division for allowing us access to study sites. All procedures were approved by the Davidson College Animal Care and Use Committee, (3-05-17; 2006). Manuscript preparation was aided by the Environmental Remediation Sciences Division of the Office of Biological and Environmental Research, U.S. Department of Energy through Financial Assistance Award number DE-FC09-96SR18546 to the University of Georgia Research Foundation. Funding was provided by: Davidson College Department of Biology; a Grant-in-Aid-of-Research from the National Academy of Sciences, administered by Sigma Xi, The Scientific Research Society; a Yarbrough Research Grant administered by the Collegiate Academy, North Carolina Academy of Science; Duke Power; and National Science Foundation grants (DEB-0347326) to MED.

LITERATURE CITED

- BROPHY, T. E. 1980. Food habits of sympatric larval *Ambystoma tigrinum* and *Notophthalmus viridescens*. *Journal of Herpetology* 14:1–6.
- BRUCE, R. C. 1972. The larval life of the Red Salamander, *Pseudotriton ruber*. *Journal of Herpetology* 6:43–51.
- BRUCE, R. C. 1979. Evolution of paedogenesis in salamanders of the genus *Gyrinophilus*. *Evolution* 33:998–1000.
- BRUCE, R. C., AND J. CASTANET. 2006. Application of skeletochronology in aging larvae of salamanders *Gyrinophilus porphyriticus* and *Pseudotriton ruber*. *Journal of Herpetology* 40:85–90.
- BURTON, T. M., AND G. E. LIKENS. 1976. Energy flow and nutrient cycling in salamander populations in the Hubbard Brook experimental forest, New Hampshire. *Ecology* 56:1068–1080.
- CALDWELL, R. S., AND W. C. HOUTCOOPER. 1973. Food habits of larval *Eurycea bislineata*. *Journal of Herpetology* 7:386–388.
- CAMP, C. D. 1997. The status of the Black-Bellied Salamander (*Desmognathus quadramaculatus*) as a predator of heterospecific salamanders in Appalachian streams. *Journal of Herpetology* 31:613–616.
- CECALA, K. K., S. J. PRICE, AND M. E. DORCAS. 2007. A comparison of the effectiveness of recommended doses of MS-222 (tricaine methanesulfonate) and Orjel® (benzocaine) for amphibian anesthesia. *Herpetological Review* 38:63–66.
- DAVIC, R. D. 1983. An Investigation of Salamander Guild Predation in a North Carolina Stream: An Experimental Approach. Unpubl. Ph.D. diss., Kent State University, Kent, OH.
- DAVIC, R. D., AND H. H. WELSH. 2004. On the ecological roles of salamanders. *Annual Review of Ecology, Evolution, and Systematics* 35:405–434.
- FITZGERALD, L. A. 1989. An evaluation of stomach flushing techniques for crocodilians. *Journal of Herpetology* 23:170–172.
- GRIFFITHS, R. A. 1986. Feeding niche overlap and food selection in smooth and palmate newts, *Triturus vulgaris* and *T. helveticus*, at a pond in mid-Wales. *Journal of Animal Ecology* 55:201–214.
- GUSTAFSON, M. P. 1994. Size-specific interactions among larvae of the plethodontid salamanders *Gyrinophilus porphyriticus* and *Eurycea cirrigera*. *Journal of Herpetology* 28:470–476.
- HAMILTON, W. J., JR. 1932. The food and feeding habits of some eastern salamanders. *Copeia* 1932:83–86.
- LOWE, W. H., K. H. NISLOW, AND G. E. LIKENS. 2005. Forest structure and stream salamander diets: Implications of terrestrial-aquatic connectivity. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie* 29:279–286.
- MATTHEWS, G. B., R. A. MATTHEWS, AND B. HACHMÖLLER. 1991. Mathematical analysis of temporal and spatial trends in the benthic macroinvertebrate communities of a small stream. *Canadian Journal of Fisheries and Aquatic Sciences* 48:2184–2190.
- NAUWELAERTS, S., J. COECK, AND P. AERTS. 2000. Visible implant elastomer as a method for marking adult anurans. *Herpetological Review* 31:154–155.
- PARKER, M. L., AND M. I. GOLDSTEIN. 2004. Diet of the Rio Grande Leopard Frog (*Rana berlandieri*) in Texas. *Journal of Herpetology* 38:127–130.
- PETRANKA, J. W. 1984. Ontogeny of the diet and feeding behavior of *Eurycea bislineata* larvae. *Journal of Herpetology* 18:48–55.

- PETRANKA, J. W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, DC.
- PYKE, G. H. 1984. Optimal foraging theory: a critical review. *Annual Review of Ecology and Systematics* 15:523–575.
- RUDOLF, D. C. 1978. Aspects of the larval ecology of five plethodontid salamanders of the western Ozarks. *American Midland Naturalist* 100:141–159.
- SIH, A., AND J. W. PETRANKA. 1988. Optimal diets: simultaneous search and handling of multiple prey loads by salamander larvae. *Behavioral Ecology and Sociobiology* 23:335–339.
- SITES, J. W., JR. 1978. The foraging strategy of the Dusky Salamander, *Desmognathus fuscus* (Amphibia: Urodela: Plethodontidae): an empirical approach to predation theory. *Journal of Herpetology* 12:373–383.
- SOKAL, R. R., AND F. J. ROHLF. 1981. *Biometry*. W.H. Freeman and Company, San Francisco, CA.
- STEPHENS, D. W., AND J. R. KREBS. 1986. *Foraging Theory*. Princeton University Press, Princeton, NJ.
- WILLSON, J. D., AND M. E. DORCAS. 2003. Quantitative sampling of stream salamanders: a comparison of dipnetting and funnel trapping techniques. *Herpetological Review* 34:128–130.
- WYMAN, A. H. 1998. Experimental assessment of salamanders as predators of detrital food webs: effects of invertebrates, decomposition and the carbon cycle. *Biodiversity and Conservation* 7:641–650.

Accepted: 7 May 2007.