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Productivity and significance of headwater streams: population structure and biomass of the black-bellied salamander (*Desmognathus quadramaculatus*)

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SUMMARY

1. Headwater streams are a significant feature of the southern Appalachian landscape, comprising more than 70% of the total stream length in the region. Salamanders are the dominant vertebrate within headwater-riparian forest ecosystems, but their ecological role is not clearly understood.
2. We studied a population of black-bellied salamanders (*Desmognathus quadramaculatus*) at a headwater stream in the southern Appalachian Mountains using radio-telemetry and mark-recapture methods. The length and area of headwater streams in the region were estimated using GIS.
3. Home ranges of radio-tracked salamanders were relatively small (mean = 1.06 m^2). Adult salamanders in our telemetry study inhabited edge microhabitats significantly more often than either stream or riparian microhabitats, and the same trend was observed in the mark-recapture study.
4. We estimated the population density at this site to be 11 294 salamanders ha^{-1}, amounting to 99.30 kg ha^{-1} of biomass, an estimate that is six times greater than reported in previous studies. The majority of this biomass was found within the stream, but 22% was found in the surrounding riparian habitat more than 1 m from the stream. Using headwater stream length and area estimates, we extrapolated biomass estimates for black-bellied salamanders inhabiting stream and riparian microhabitats across the study region.
5. We report one of the largest estimates of secondary consumer biomass for a headwater ecosystem, attesting to the overall productivity of headwater streams. Headwaters are known to be important for ecological and ecosystem processes and our biomass estimates suggest that salamanders are a critical component to these systems.

Keywords: biomass, *Desmognathus quadramaculatus*, headwater stream, population estimation, salamander

Introduction

Headwater streams are estimated to account for at least three-quarters of stream and river channel length within the United States (Meyer & Wallace, 2001). Headwater streams represent the smallest tributaries in the landscape, and include first and second order streams (Meyer *et al*., 2003). Though often overlooked and underappreciated elements of the landscape, headwater streams harbour a tremendous biodiversity (Naiman & Decamps, 1997). Headwaters serve important ecosystem services including flood control, sediment retention, water purification, cycling of nutrients and transfer of energy to downstream

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ecosystems, and support of biological diversity (Vannote *et al.*, 1980; Ward, 1989; Meyer & Wallace, 2001; Gomi, Sidle & Richardson, 2002). Headwater streams in temperate climates are largely detritus-based ecosystems with little to no primary productivity (Wallace *et al.*, 1999; Gomi *et al.*, 2002). As such, headwater systems depend on allochthonous inputs from the surrounding riparian habitat, which are then utilized by numerous invertebrate detrivores (Vannote *et al.*, 1980). The relationship between invertebrate production, detritus breakdown and nutrient cycling in Appalachian headwater streams has been extensively studied (e.g. Wallace *et al.*, 1999), but the biomass of higher trophic levels supported by primary and secondary production has not been addressed in detail.

Among the most prominent consumers of invertebrates in Appalachian headwater streams are salamanders (Davic & Welsh, 2004). The southern Appalachian Mountain region harbours an extraordinary diversity of salamanders (e.g. *Aneides*, *Desmognathus*, *Eurycea*, *Gyrinophilus*, *Plethodon*, and *Pseudotriton*), which are most abundant in headwater streams and the associated riparian habitats (Petranka, 1998). These are all lungless salamanders of the family Plethodontidae that use dermal respiration to breathe across the surface of the skin. Numerous Appalachian plethodontid salamander genera (except *Aneides* and *Plethodon*) are stream-associated and have biphasic life cycles that include an aquatic larval stage followed by a semi-terrestrial adult stage (Petranka, 1998). Appalachian salamanders are the top vertebrate predators of both aquatic and terrestrial invertebrate detrivores within these fishless headwater habitats (Southerland *et al.*, 2004). These trophic associations ultimately affect the breakdown of leaf litter and the release and transfer of nutrients (Davic, 1983; Wyman, 1998; Davic & Welsh, 2004). The low metabolic rate of salamanders results in an efficient assimilation of ingested food into high-quality protein, which makes up more than 50% of the wet weight biomass of salamanders (Burton & Likens, 1975a).

The ecology of Appalachian salamander assemblages has been the focus of research for many years (e.g. Organ, 1961; Hairston, 1987), but studies of plethodontid salamander movement, population structure and abundance are difficult to conduct because of their semi-fossorial behaviour, nocturnal activity and high variation in capture numbers between sampling periods. Their ecological significance has consequently been difficult to assess (e.g. Bailey, Simons & Pollock, 2004; Dodd & Dorazio, 2004). Few published studies have detailed salamander biomass and there is considerable ambiguity within these studies. For example, Petranka & Murray (2001) estimated the biomass of a riparian associated salamander community (including terrestrial *Plethodon* spp.) to be 16.53 kg ha^{-1}, seven times greater than biomass estimates reported by Burton & Likens (1975b) (1.77 kg ha^{-1}). Traditionally, area-constrained searches using mark-recapture (MRC) methods have been used in these studies as well as in studies of movement and home range (e.g. Camp & Lee, 1996), but MRC methods result in episodic observations that may not reveal fine-scale movements. However, new insights may be provided by recent technological advances in radio-telemetry (i.e. miniaturization of radio-transmitters), which allow continuous observations. Using multiple research approaches to focus on different spatial and temporal scales of movement and population structure may enable a clearer understanding of the ecological role of a species.

Black-bellied (*Desmognathus quadramaculatus* Holbrook) salamanders are the largest of the desmognathine salamanders reaching >21 cm in total length and weighing >20 g (Petranka, 1998; WEP unpubl. data). They are relatively long-lived (at least 13 years; Bruce, 1988; Castanet, FrancillonVieillot & Bruce, 1996) with aquatic larvae that metamorphose at 2–4 years into semi-terrestrial salamanders. Adult black-bellied salamanders are closely associated with headwater and low order streams where they reproduce and forage on terrestrial and emergent aerial insects (Davic, 1983; Petranka, 1998). The large mass, long life, and previously reported population structure of black-bellied salamanders (Camp & Lee, 1996) make them particularly appealing subjects to emphasize the production of headwaters streams that has previously been underestimated.

Here, we used radio-telemetry and classic MRC methods to assess habitat use, population structure, abundance, and biomass of a population of black-bellied salamanders. We also conducted spatial analyses of the study region using GIS to estimate the total length and area of headwater streams and the potential biomass that black-bellied salamanders contribute across the landscape. The combination of these research methods allows a thorough representation of movement, microhabitat use, population structure, and biomass, all of which are essential to extrapolating salamander population size and biomass across the region. Our objectives in studying black-bellied salamanders were: (1) to estimate the large biomass supported by a headwater stream; (2) to measure movements, home range, and habitat use; (3) to estimate population size and structure; (4) to measure biomass distribution across size classes and microhabitats; and (5) to estimate the length and area of headwater streams inhabitable by black-bellied salamanders in order to extrapolate salamander biomass across the study region.

Methods

Study area

The studied population of black-bellied salamanders was located at a headwater stream in the Blue Valley Experimental Forest of the Highlands Ranger District (hereon HRD), Nantahala National Forest, Macon Co., NC, U.S.A. (UTM 17S 0295741E, 3876678N; elevation 780 m). The origin of this spring-fed stream was 15 m upstream from the study area, where it emerged from the mountainside. The stream was located in a second-growth, mixed deciduous forest dominated by *Rhododendron* spp. in the riparian zone. Canopy coverage, measured with a spherical crown densiometer, averaged 97.8% over the stream. The stream gradient was low (0.267 cm^{-m}), and the surrounding riparian habitat extended away from the stream at a 30° angle. The average wetted width of the stream was 5.8 m (±1.50 SD), with <3.0 cm of flowing water over the sand and bedrock stream bed. The stream was separated from the riparian habitat by a bank that was <0.5 m high. Other stream salamander species found at the study area include seal salamanders (*Desmognathus monticola* Dunn), Ocoee salamanders (*Desmognathus ocoee* Nicholls), Blue Ridge two-lined salamanders (*Eurycea wilderae* Dunn), and Blue Ridge spring salamanders (*Gyrinophilus porphyriticus* Blatchley). Spring salamanders were rarely encountered as larvae in the stream; Ocoee salamanders and two-lined salamanders were found three times as often and seal salamanders twice as often as black-bellied salamanders in the riparian habitat. The relative abundance of the riparian salamander assemblage was estimated using data collected during night surveys of the riparian habitat (JAC unpubl. data). Environmental and habitat characteristics at this site were typical of at least 20 sites in the region that are part of a larger study (Crawford & Semlitsch, 2007; WEP, JAC, & RDS unpubl. data).

Radio-telemetry

To estimate the home range size of individual salamanders, we collected twelve adult black-bellied salamanders with an average mass of 17.0 g (±2.52 SD) from the study site. Animals were transported to the laboratory at Highlands Biological Station where they were surgically implanted with radio-transmitters. We used model BD-2H transmitters (14 × 6.5 × 3.5 mm; 0.85 g; expected battery life of 25–28 days) with internal helical antennae from Holohil Systems Ltd. (Carp, ON, Canada). Animals were anesthetized in a 1000 mg L^{-1} solution of ethyl-3 aminobenzoate methanesulfonate (MS-222) (Peterman & Semlitsch, 2006). Transmitters were intracoelomically implanted (Johnson, 2006) through a 6-mm incision made anterior to the hind limb; incisions were closed with four sutures (Ethicon, Inc., Somerville, New Jersey, U.S.A., coated Vicryl synthetic absorbable suture, J443). Animals recovered from anesthetization within 27 min (±4 SD, $n = 12$) and were returned to their respective capture points 36–48 h later. Implanted transmitters were <6.5% of the total mass of each monitored salamander.

Over an 18-day period (20 June to 8 July 2005), salamanders were located at least twice every 24 h: one to three times between 2200 and 0200 h EST, and once more between 0900 and 1300 hours EST. Wire flags were used to mark each locality, which was designated as either stream (had flowing water), edge (<0.5 m from water-riparian interface), or riparian (>0.5 m from water-riparian interface). We measured the cardinal direction and length (to the nearest cm) of all movements using a measuring tape and compass. At the conclusion of the study, salamanders were collected from the field and transmitters were surgically removed following the above procedures.

To calculate minimum home range areas, the movements of each radio-tracked salamander were drawn to scale on graph paper. For each individual, the outermost points were connected to form a polygon. We calculated the area of the polygon as an estimate of home range (i.e. minimum convex polygon home range; Camp & Lee, 1996). A *G*-test for goodness- of-fit (Sokal & Rohlf, 1995) was calculated to determine if salamanders used the three microhabitats equally.

Mark-recapture

To estimate population size and structure, we conducted a MRC study. The area for MRC was delineated around the home ranges of the salamanders that were monitored through telemetry. A 30 m × 8.5 m area encompassing both the stream and the surrounding riparian habitat was marked off and divided into three 10 m × 8.5 m sections. Due to the variable width of the stream (3.9–8.0 m), the amount of edge and riparian habitat sampled on each side of the stream ranged from 0.25–2.3 m (mean = 1.50 m). The entire study area was intensively surveyed every five to seven nights (2200–0300 EST) between 17 June and 5 August 2005 for a total of 11 sampling periods. All surveys were conducted at night because the focal species is nocturnal, and surface activity occurs at night. No cover objects were turned during sampling; only surface active animals were collected. This allowed for non-destructive sampling of the study area. Three experienced researchers participated in each survey; researchers were randomly assigned to search a 10-m section each night.

All black-bellied salamanders encountered during a sampling period were captured by hand (*sensu* Camp & Lovell, 1989). Total length, snout-vent length (SVL), and mass were measured using a ruler and Pesola® (Pesola AG, Baar, Switzerland) spring scale (accurate to 0.1 g) respectively. Details on each salamander's location were recorded and microhabitat was classified as either stream, riparian, or edge. Each salamander was then given a unique identification mark through toe clipping. Salamanders were released at the point of capture following data collection and marking.

Mark-recapture data were sorted by SVL (mm) into arbitrary10-mm size classes (<50, 50–59, 60–69, 70–79, 80–89, ≥90). All salamanders in our study were metamorphosed juveniles and adults; black-bellied salamanders generally metamorphose between 32 and 43 mm SVL and are sexually mature at >70 mm SVL (Bruce, 1985, 1988, WEP unpubl. data). MRC data were analysed using the CAPTURE extension of Program MARK (MARK, 2004 Version 4.2). Population sizes (n) were estimated in CAPTURE using closed population models. Each size class was analysed separately. The best fit model was selected from twelve candidate models in CAPTURE by assessing goodness-of-fit and chi-square likelihood comparisons with other probable models (White *et al*., 1982).

G-tests for goodness-of-fit were used to determine microhabitat use by each size class and for the entire population. Only original capture data were used in these analyses to avoid pseudoreplication (Hurlbert, 1984) to prevent possible bias associated with some individuals being caught repeatedly. All means are presented as ±SD and statistical tests were significant at $\alpha = 0.05$.

Headwater stream estimation

The length and area of headwater streams in the southern Appalachian region was estimated using ArcGIS (ESRI; Redlands, CA, U.S.A.; v 9.0). Data layers were obtained from the United States Geological Survey seamless data distribution website (USGS, 2007) and the United States Forest Service. Stream networks were delineated from 10-m resolution digital elevation data (e.g. Tarboton, Bras & Rodriguez-Irturbe, 1991). Stream estimation was refined by using the coordinates of 25 known headwater streams from the region. Streams were then assigned an order based upon the Strahler method (Strahler, 1964). Only first and second order streams (i.e. headwaters) between 490 m and 1680 m in elevation that were flowing through forested areas were selected for estimation of black-bellied salamander habitat area and biomass calculations; these are the broad scale characteristics that correspond with abundant black-bellied salamander populations (Hairston, 1949; Organ, 1961). Forest habitat delineations were made using 2001 National Land Cover Data (NLCD). Headwater stream area was estimated using the average width from 16 known first and second order streams (WEP & JAC unpubl. data).

Results

Radio-telemetry

Eleven of the twelve radio-telemetered salamanders were tracked for the duration of the study. Each was located an average of 39.6 times (±9.6, $n = 472$ total locations). One radio-telemetered animal could not be detected after 5 days and was omitted from data analyses. The maximum movement observed during this study was 5.05 m, with a mean movement of 1.03 m between consecutive locations (±0.82, $n = 183$). Home range area varied from 0.10 to 3.01 m^2 (mean = 1.06 m^2, ±0.96). There was no observed affect of salamander mass or microhabitat association on the calculated home range area ($P = 0.390$ and 0.493 respectively). The number of salamander locations in each microhabitat differed significantly from random ($G = 75.60$, d.f. = 2, $P < 0.0001$). Salamanders were more frequently located in edge microhabitat than in riparian or stream microhabitats ($P < 0.0001$). The number of locations did not differ significantly between stream and riparian microhabitats ($P = 0.76$).

Mark-recapture

We marked a total of 204 black-bellied salamanders (*D. quadramaculatus*) during the study, and recaptured animals 260 times (mean = 1.30 recaptures, range 0–8; Table 1). Salamanders showed differential microhabitat use that corresponded with size class. Salamanders <59 mm SVL were more frequently encountered in the stream (64.3%) than either the riparian (26.8%) or edge (8.9%). Salamanders >59 mm SVL were found in edge microhabitat 48.6% of the time, while the proportion of captures in the stream and riparian were 23.6% and 27.8% respectively. When microhabitat use was examined by size class, only salamanders <50 mm and 80–89 mm SVL used a microhabitat significantly more than expected (stream and edge, respectively; Table 1). Overall, the entire population used riparian habitat less than expected, but a clear shift in microhabitat use from stream to edge was seen as SVL length increased.

Based on MRC data, the best fit population estimation model for the three smallest size classes (<70 mm SVL) was the constant capture probability model (M_o), whereas the best fit model for all size classes >70 mm SVL incorporated temporal variation and capture heterogeneity (M_{th}; Williams, Nichols & Conroy, 2002). These models estimated the total population size of black-bellied salamanders in our 255 m^2 study area to be 288 salamanders (95% CI = 241–422; Table 2), yielding a density estimate of 11,294 ha^{-1}. The mean mass of each size class was calculated from capture data (Table 2) and was used to generate a wet-weight biomass estimate of 2.532 kg (95% CI = 2.191–3.533 kg) for this population, equivalent to 99.30 kg ha^{-1} (95% CI = 85.94–138.56 kg ha^{-1}).

To assess the distribution of biomass among microhabitats, the total biomass was multiplied by the proportion of novel captures for each size class in each microhabitat (Table 3). Both the stream and edge microhabitats had proportionally more biomass than their respective capture proportions (Table 3). More than 78% of black-bellied salamander biomass was found within 0.5 m of the stream (47% along the edge and 31.3% within the stream) and 21.7% was found beyond the stream edge, in the adjacent riparian habitat.

Headwater stream estimation

The Nantahala National Forest boundaries encompass 642 672.08 ha of western North Carolina, of which

Table 1 Capture and (recapture) data for all size classes (snout-vent length, mm) across three microhabitats and the respective proportion of captures for each size class therein (calculated as size class microhabitat capture/total size class capture)

	Edge		Riparian		Stream				
Size (mm)	Number captures	Per cent captures	Number captures	Per cent captures	Number captures	Per cent captures	Total Number captures	G-value	*P*-value
<50	4 (2)	14.3	1 (2)	3.6	23 (9)	82.1	28 (13)	30.242	<0.0001
50–59	11 (4)	39.3	4 (0)	14.3	13 (8)	46.4	28 (12)	5.542	0.0655
60–69	14 (13)	46.7	8 (4)	26.7	8 (9)	26.7	30 (26)	2.281	0.3197
70–79	22 (32)	46.8	11 (10)	23.4	14 (17)	29.8	47 (59)	4.010	0.1346
80–89	22 (51)	52.3	10 (14)	23.8	10 (13)	23.8	42 (78)	6.428	0.0401
90+	14 (45)	48.3	6 (18)	20.7	9 (9)	31.0	29 (72)	3.361	0.1862
Total	87 (147)	42.7	40 (48)	19.6	77 (65)	37.7	204 (260)	19.565	<0.0001

Significance was calculated using a *G*-test for goodness-of-fit assuming equal distribution across all three microhabitats.

Table 2 Population and associated biomass estimates calculated from mark-recapture data collected on a 255 m^2 study area. Size is snout-vent length (mm). Mean mass was calculated from all individuals measured during the study within each size class

Size (mm)	Mean mass (g)	Population size (SE)	95% CI (population) Lower	Upper	Biomass (g)	95% CI (biomass) Lower (g)	Upper (g)
<50	1.92	44 (7.97)	35	68	84.48	67.20	130.56
50–59	3.89	59 (14.40)	42	102	229.51	163.38	396.78
60–69	6.12	38 (3.95)	34	50	232.56	208.08	306.00
70–79	9.46	62 (8.13)	53	87	586.48	501.38	823.02
80–89	15.04	47 (4.06)	43	61	723.80	646.72	917.44
90+	17.77	38 (4.83)	34	54	675.26	604.18	959.58
Total		288 (43.34)	241	422	2532.09	2191.48	3533.38
Total m^{-2}		1.13 m^{-2}	0.95 m^{-2}	1.65 m^{-2}	9.93 g m^{-2}	8.59 g m^{-2}	13.86 g m^{-2}
Total ha^{-1}		11 294 ha^{-1}	9451 ha^{-1}	16 549 ha^{-1}	99.30 kg ha^{-1}	85.94 kg ha^{-1}	138.56 kg ha^{-1}

All mass and biomass estimates are in grams (wet-weight) unless otherwise noted.
CI, confidence interval.

Table 3 Biomass distribution of black-bellied salamanders based on the proportion of captures of each size class in each microhabitat (see Table 1). Percent capture is the proportion of the population captured in each microhabitat

Microhabitat	Per cent Capture	Biomass (per cent)	Biomass (kg ha^{-1})	95% Confidence interval Lower (kg ha^{-1})	Upper (kg ha^{-1})
Stream	37.7	31.3	31.12	26.56	44.44
Edge	42.7	47.0	46.66	40.62	64.60
Riparian	19.6	21.7	21.52	18.76	29.52
Total	100	100	99.30	85.94	138.56

138 275.31 ha are within the HRD. Based on ArcGIS analyses, a total of 4559.93 km of streams flow through the HRD, 76.7% of which are low order streams (3489.16 km). To estimate the area of these streams, we first calculated the average width of sixteen first and second order streams (stream order designated through the GIS stream order analysis); streams were measured in the field using a metre tape, The width of first and second order streams did not differ significantly ($F = 0.3835$, d.f. = 1, 14 $P = 0.546$) and had a mean width of 3.2 m (±1.24, $n = 16$). An estimated 1119.41 ha of low order streams traverse the HRD.

Because a substantial portion of the black-bellied salamander biomass was found in the riparian habitat, we adjusted the estimate of headwater stream area in the HRD to include 1.5-m wide riparian-edge zones along both sides of these streams (1 m riparian, 0.5 m edge). The size of this riparian-edge zone was based on the average width of riparian and edge microhabitat searched during our MRC surveys. Including this riparian-edge habitat to both sides of streams increases the black-bellied salamander habitat to 1819.04 ha (Table 4). Using the biomass distributions reported in Table 3, microhabitat-weighted biomass estimates were made for the entire HRD. Results from this extrapolation exercise predict that the total HRD black-bellied salamander biomass is 71 441.18 kg (95% CI = 61 669.20 – 100 460.69 kg; Table 4). Based on data for black-bellied salamanders from sites in the same region, Crawford & Semlitsch (2007) reported that riparian corridors 3.2 m in width would incorporate 50% of individuals in populations of this species, and that corridors 4.7 m in width would incorporate 95% of the individuals. Using these riparian-edge widths in the HRD biomass calculations yields estimates of 76 995.34 kg (50% population) and 81 896.11 kg (95% population).

Discussion

We estimated black-bellied salamander population density to be 11 294 ha^{-1} (95% CI = 9451–16 549 ha^{-1}) which, although large, is not extraordinary compared to other reports of salamander density (Table 5). Though numerically less abundant and dense than smaller species of *Desmognathus* and *Eurycea*, the large mass of black-bellied salamanders

Table 4 Extrapolation of black-bellied salamander habitat and biomass within the Highlands Ranger District, North Carolina. The 1.5-m riparian is the average width of riparian habitat surveyed in this study, 3.2-m and 4.7-m are distances reported by Crawford & Semlitsch (2007) that encompass 50% and 95% (respectively) of the black-bellied salamander populations in riparian habitat along southern Appalachian headwater streams*

Riparian width (m)*	Microhabitat	Area (ha)	Biomass (kg)	95% Confidence interval	
				Lower (kg)	Upper (kg)
None	Stream-edge†	1119	68 174	58 808	96 062
1.5	Riparian	700	3267	2861	4399
	Total‡	1819	71 441	61 669	100 461
3.2	Riparian	1889	8821	7725	11 878
	Total‡	3008	76 995	66 533	107 940
4.7	Riparian	2938	13 722	12 017	18 476
	Total‡	4057	81 896	70 825	114 538

*Riparian widths were multiplied by 2 in area and biomass estimates to account for habitat on both sides of the stream; 0.5 m of edge microhabitat was subtracted from each riparian width prior to area and biomass calculations.
†Stream-edge estimates include edge microhabitat on both stream banks; calculated from the average Nantahala headwater stream width (3.2 m) and edge width (0.5 m) surveyed.
‡Includes stream-edge estimations.

Table 5 Summary of published density and biomass estimates from salamander studies within the Appalachian Mountain region

Species	Density (ha^{-1})	Biomass ($kg\ ha^{-1}$)	Reference	Forest type
DQUA	11 294	99.30	This study	MD; 2nd growth
DQUA, EWIL, GPOR*	120 667	78.83	Davic, 1983†	OHD; 2nd growth
DQUA¶	22 667	36.00	Davic, 1983†	OHD; 2nd growth
DQUA**	13 333	27.33	Davic, 1983†	OHD; 2nd growth
DCAR, DWRI, EWIL, PGLU, PJOR, PYON	18 486	16.53	Petranka & Murray, 2001	MD; old growth
DQUA, DOCH, DMON, PGLU, PJOR	5961–9935	6–10	Hairston, 1987	MH; 2nd growth
DQUA††	4000	6.00	Davic, 1983†	OHD; 2nd growth
DQUA, DOCH, DMON, PJOR, PGLU	N/A	2.29	Hairston, 1987	
PCIN, DFUS, EBIS‡, GPOR, NVIR§	2950	1.77	Burton & Likens, 1975b	MD; 2nd growth
DQUA	14 100	N/A	Camp & Lee, 1996	N/A

DCAR, *Desmognathus carolinensis*; DFUS, *D. fuscus*; DMON, *D. monticola*; DOCH, *D. ochrophaeus* (now *D. ocoee*); DWRI, *D. wrighti*; DQUA, *D. quadramaculatus*; EBIS, *E. bislineata*; EWIL, *Eurycea wilderae*; GPOR, *Gyrinophilus porphyriticus*; PCIN, *Plethodon cinereus*; PGLU, *P. glutinosus*; PJOR, *P. jordani*; PYON, *P. yonahlossee*; MD, mixed deciduous; MH, mixed hardwood; OHD, oak-hickory-deciduous.
*These species comprise the salamander guild found within the stream, including larval forms; published in Davic & Welsh (2004).
†Density and biomass estimates of in-stream salamanders are an average of estimates made in June, August, and October; published in Davic & Welsh (2004).
§Both larval and adult salamanders included in estimates.
‡Terrestrial eft stage only.
¶, **, ††Larval, Juvenile, and Adult life stages respectively.

leads to a conservative biomass estimate of 99.30 kg ha^{-1} (95% CI = 85.94–138.56 kg ha^{-1}), which is six times greater than any previously reported salamander biomass for the Appalachian region (Table 5). This biomass estimate does not include larval forms, which Davic (1983); see Table 5) estimated can attain 56 kg ha^{-1} (mean = 36 kg ha^{-1}). Using the mean larval biomass reported by Davic (1983) would result in a total population biomass estimate of 135.3 kg ha^{-1} in our study.

The biomass reported above is from a single stream-dependent species, which is part of a multi-species assemblage. Three other stream-breeding plethodontid salamander species are also abundant at this site (*D. monticola*, *D. ocoee*, and *E. wilderae*) and a fourth (*G. porphyriticus*) was occasionally observed. Both of the *Desmognathus* and *Eurycea* species were more abundant in the riparian habitat than black-bellied salamanders (Crawford & Semlitsch, 2007; JAC unpubl. data). Petranka & Murray (2001) included

D. carolinensis (Dunn; sister species to *D. ocoee*) and *E. wilderae* in their streamside biomass estimates (Table 5). These two species had a combined biomass of 8.50 kg ha^{-1} which, when added to our biomass estimate (with larval biomass added from Davic, 1983), yields an approximate biomass for the entire stream salamander community of 143.8 kg ha^{-1}. This community biomass estimate is three to six times greater than that of fish communities studied in a headwater stream from Virginia (Neves & Pardue, 1983). The biomass of fish communities was lowest in upper stream reaches where our study was conducted and where salamanders are most abundant.

The assemblage and relative abundances of salamanders varies from stream to stream (Organ, 1961), but our study site is not exceptional for the southern Appalachian region in the abundance of black-bellied salamanders or other species observed (Davic, 1983; Camp & Lee, 1996; Petranka & Smith, 2005; JAC & WEP pers. obs.). Removal sampling conducted at five headwater streams in the region resulted in density estimates for black-bellied salamanders ranging from 0.71 to 1.90 m^{-2} (mean 1.05 m^{-2}), making the current study stream (1.13 salamanders m^{-2}) average for the region (WEP unpubl. data). Radio-telemetry of adult black-bellied salamanders showed that they have relatively small home ranges (mean = 1.06). However, this home range size was still nearly 25 times greater than home ranges reported by Camp & Lee (1996); mean = 0.1207 m^2) who studied a population at the southern range extent using MRC methods. Population density differences between sites (1.41 m^{-2} reported by Camp & Lee (1996) versus 1.13 m^{-2} in our study) may affect home range size, though numerous other variables such as meteorological, geographical, and altitudinal variation must also be considered. Adult salamanders in our telemetry study inhabited edge microhabitats more frequently than either stream or riparian microhabitats, and this same trend was observed in the MRC study. Similar to Camp & Lee (1996), we found that smaller individuals (<60 mm SVL) primarily inhabited stream microhabitats. This pattern can be explained by the physiological constraints of smaller salamanders. All plethodontid salamanders need to maintain moist skin to facilitate dermal respiration, but smaller salamanders desiccate at a faster rate, limiting their ability to disperse far from the stream (Spotila, 1972). The abundance of salamanders in edge habitat is likely facilitated by the prevalence of burrows in the bank created by woodland jumping mice (*Napaeozapus insignis*; Brannon, 2005). The use of burrows and the semi-fossorial nature of black-bellied salamanders (and other plethodontid species) make observing these species difficult. Radio-telemetry allowed salamanders to be tracked within these burrows; they were often located underground >1 m from the entrance.

Using MRC to study this population, we were able to make detailed abundance estimates for each size class using closed population models. We are confident that the assumptions of closed population models [i.e. no immigration, emigration, birth, or death; equal catchability of each animal during each period; and no loss of marks (Bailey *et al*., 2004)] were satisfactorily met in our study because we sampled over a discrete time period and home range data for this population and others (Camp & Lee, 1996) suggest that movement into or out of the population are limited and unlikely. We have no way of determining if deaths occurred during the study, and newly metamorphosed juveniles were not frequently encountered. Black-bellied salamanders metamorphose between 32 and 43 mm SVL (Bruce, 1985; WEP unpubl. data); only 17 salamanders in this size range were captured during our study.

Although black-bellied salamanders are generally considered to be one of the most aquatic species of the desmognathine assemblage (Petranka, 1998), they frequently forage in riparian habitat. Petranka & Smith (2005) found that 4.5% of the black-bellied salamanders observed were in the riparian habitat (maximum distance from stream = 35 m) and Crawford & Semlitsch (2007) report nocturnal captures of black-bellied salamanders in the riparian habitat (95% of captures within 4.7 m). All salamanders captured in our study were within 2.3 m of the aquatic interface. Salamanders captured in the riparian habitat surrounding the stream accounted for 37.7% of original captures and 21.7% of the population biomass (21.52 kg ha^{-1}). The riparian component of the black-bellied salamander biomass alone exceeds previous estimates of Petranka & Murray (2001). The amount of biomass found in salamanders is significant and has been found to be greater than that of birds and small mammals in New Hampshire (Burton & Likens, 1975b), leading to the conclusion that the biomass of stream salamanders within the southern

Appalachians likely exceeds that of both birds and small mammals (Hairston, 1987; Petranka & Murray, 2001, this study).

The prevalence of headwater streams throughout the southern Appalachian Mountain region further emphasizes the significance of salamanders to overall ecosystem function. Spatial analysis of the HRD corroborates previous estimates that headwater streams can account for more than 70% of the total stream channel length (Leopold, Wolman & Miller, 1964; Meyer & Wallace, 2001); they comprised 76% of the total stream length within the HRD. These streams are prevalent across the mountainous southern Appalachian landscape, creating a relatively even distribution of salamander habitat. The total cumulative biomass of black-bellied salamanders reported here seems to be a rather extreme, abstract number, but this is only because biologists have not yet begun to quantify the significance of headwater streams within the context of landscape and ecosystem level goods and services (Meyer & Wallace, 2001). It should be noted that the study site for this research is not identifiable on a 1 : 24 000 scale map of the region, nor were any of the twenty-five streams used to ground-truth the stream network maps, emphasizing our under-appreciation of these habitats. An adequate understanding of the extent of headwater streams is essential for comprehension of broad-scale ecosystem and landscape level processes.

We acknowledge that this study was limited to a single stream site, perhaps limiting generalizations on a regional scale, but we consider this population of black-bellied salamanders and the studied stream to be typical of many other sites studied (WEP unpubl. data). The ecological significance of salamanders in terms of biomass was first highlighted by Burton & Likens (1975a,b) and has been revisited by Hairston (1987) and Petranka & Murray (2001), each time with a revised increase of headwater salamander biomass (Table 5). Our estimate of the biomass of black-bellied salamanders far exceeds previous biomass estimates for salamanders and is exceptional for a secondary vertebrate consumer. The biomass of salamanders detailed within this study is an astounding figure, but it is also a testament to our lack of understanding of the productivity of headwater systems. Unfortunately, we are no closer to elucidating the role that this biomass plays in ecosystem processes. Future research should focus on closing the gap in our understanding of the role that salamander biomass has within greater ecosystems processes such as nutrient cycling and sequestration and lotic connectivity.

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