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PIT TAG RETENTION IN SMALL (205-370 MM) AMERICAN EELS, *ANGUILLA ROSTRATA*

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ABSTRACT

Passive integrated transponders (PIT tags) are commonly used in ecological studies of aquatic and terrestrial animals. Researchers have used PIT tags in American eels (*Anguilla rostrata*) to study growth rates, home range size, and migration. The placement of PIT tags within the body of American eels differs among studies, including insertion in the dorsal musculature behind the head, the dorsal musculature near the dorsal fin origin, and the abdominal cavity. Retention rates of PIT tags for American eels are reported in the literature, but researchers have not previously compared and reported tag retention rates among different tagging locations. The objective of this study was to compare retention rates of PIT tags placed in the dorsal musculature behind the head, the dorsal musculature near the dorsal fin origin, and the abdominal cavity of American eels. Eighteen American eels were collected during fall 2006 from the Millville dam eel ladder on the lower Shenandoah River, West Virginia. Each eel was PIT tagged in three locations and tag retention was monitored for nine weeks. Tag retention in the dorsal musculature near the dorsal fin origin (100%) and the abdominal cavity (100%) were higher than the retention rate of tags placed behind the head (88%). Our results, consistent with previous literature, support an overall high retention rate of PIT tags in American eels. Previous studies have primarily focused on large eels and our results document high tag retention rates in small eels (205-370 mm total length).

INTRODUCTION

Passive integrated transponders (PIT tags) are small microchips with coiled antennas enclosed in biocompatible glass. These tags are typically injected into the body cavity or muscle tissue of an animal. Researchers have used PIT tags on many species, such as bats (*Eptesicus fuscus;* Bubb et al. 2002), crayfish (*Pacifastacus leniusculus*; Neubaum et al. 2005), ferrets (*Mustela putorius furo* and *Mustela nigripes*; Fagerstone and Johns 1987), frogs (*Rana muscosa*; Matthews 2003), king crabs (*Paralithodes camtschaticus*; Donaldson et al. 1992), penguins (*Pygoscelis adeliae*; Ballard et al. 2001), quail (*Colinus virginianus*; Carver et al. 1999), rattlesnakes (*Sistrurus miliarius*; Jemison et al. 1995), seals (*Mirounga leonine*; Galimberti et al. 2000), sea turtles (*Chelonia mydas* and *Caretta caretta*; Broderick and Godley 1999), and sea urchins (*Strongylocentrotus franciscanus*; Shelton et al. 2006). These "passive" tags remain dormant in the body cavity or muscle tissue until activated by a handheld or automated reader. The reader sends a low frequency signal to the microchip, which activates the tag, and retrieves an individual alphanumeric sequence code. These sequence codes are unique to each tag, thus allowing researchers to identify individual animals (Prentice et al. 1990a).

In fisheries, PIT tags were first used for mark-recapture studies of coho and Chinook salmon (*Oncorhynchus kisutch* and

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Oncorhynchus tshawytsch; Prentice and Park 1984). Since this initial study, PIT tags have gained extensive use in fisheries research, including studies of arctic grayling (*Thymallus arcticus*; Buzby and Deegan 1999), black rockfish (*Sebastes melanops*; Parker and Rankin 2003), creek chub (*Semotilus atromaculatus*; Smithson and Johnston 1999), damselfish (*Pomacentrus amboinensis*; McCormick and Smith 2004), lampreys (*Petromyzon marinus*; Quintella et al. 2005), red drum (*Sciaenops ocellatus*; Jenkins and Smith 1990), sculpin (*Cottus bairdii*; Ruetz et al. 2006), striped bass (*Morone saxatilis*; Jenkins and Smith 1990), sturgeon (*Acipenser oxyrinchus desotoi*; Clugston 1996), and sunfish (*Lepomis cyanellus* and *Lepomis megalotis*; Smithson and Johnston 1999). Researchers have also used PIT tags to study movements of fishes associated with fishways and dams, as PIT tag readers can be set up directly where fish pass, allowing for scanning of individual fish (Prentice et al. 1990b; Burke and Jepson 2006).

PIT tags have many advantages to other methods of mark-recapture in fisheries, including fin clipping, branding, use of dyes and pigments, and coded wire tags (Harvey and Campbell 1989; Jenkins and Smith 1990; Prentice et al. 1990b). These other tags suffer from faded identification numbers, unrecognizable marks, tag loss, or the need to sacrifice the study animal to obtain information (Prentice and Park 1984; McFarlane et al. 1990; Gibbons and Andrews 2004). Handling time of tagged animals is reduced with PIT tags relative to other forms of tagging (Prentice et al. 1990b). Further, PIT tags are internal and often permanent (except for tag loss), have little influence on animal behaviors, and allow for identification of individuals (Prentice and Park 1984; Jenkins and Smith 1990; Prentice et al. 1990a). Also, PIT tags often have high tag detection rates (95-100%), as well as reader accuracy near 100% (Prentice et al. 1990b; McCutcheon et al. 1994).

Despite the overall high success rate of PIT tags, tag retention rates differ among species and the area of tag placement. Tag loss ranges from 0% in largemouth bass (*Micropterus salmoides*; Harvey and Campbell 1989) to 99.4% in paddlefish (*Polyodon spathula*; Onders et al. 2001). Most studies on bony fish, however, report tag loss rates of less than 5% (Prentice and Park 1984; Moore 1992; Ombredane et al. 1998; Das Mahaptra et al. 2001; Dare 2003). In American eel (*Anguilla rostrata*) studies, PIT tag retention rates range from 89% to 100% (Table 1; Morrison and Secor 2003; Verdon and Desrochers 2003; Verdon et al. 2003; Thomas 2006). Tag loss can result from incorrect tagging techniques, rejection of the tag by the animal's body, or migration of the tag within the body (Gibbons and Andrews 2004). Tag loss is a serious problem for mark-recapture studies (McFarlane et al. 1990). For example, recaptured animals (with lost tags) counted as newly-tagged animals bias the ratio of recaptured animals to tagged animals (Arnason and Mills 1981; Stobo and Horne 1994; Schwartz and Stobo 1999), although biases associated with tag loss are correctable with estimates of retention rates.

PIT tag retention is influenced, in part, by tag placement (Elbin and Burger 1994; Gibbons and Andrews 2004). Gibbons and Andrews (2004) state that "the success of tagging technique is determined almost solely by placement of the tag", and recommend that researchers perform pilot tag retention studies before using PIT tags. Studies of PIT tag retention in American eel have not compared different tagging locations. Our study objective was to quantify retention rates of three PIT tag placements in American eels. This research was a pilot study to determine PIT tag placement for a study of upstream migration in American eels, but results will also apply to other PIT tag studies of American eels.

MATERIALS AND METHODS

COLLECTION AND LABORATORY SETTING

Eighteen American eels were collected in October 2006 from the Millville Dam eel ladder on the lower Shenandoah River, West Virginia. Eels were collected in a 4.76 mm mesh net attached to the upstream end of the eel ladder. Once in the laboratory, eels were held in a 380 L recirculation-system holding tank with constant water flow, and fed minnows and crayfish. Water temperatures within the tank ranged from 13.4˚C – 18.8˚C over the duration of the study. The eels were acclimated to this tank setting for two months prior to the start of the study.

PIT TAGGING METHODS

Before insertion of PIT tags, eels were anesthetized with a clove oil solution (10 L H_20, 1.2 mL clove oil, and 12 mL of ethanol) following methods of Anderson et al. 1997 [1]. Prior to tagging, eels remained in the clove oil solution for approximately five minutes. Each eel was tagged in three separate locations with PIT tags (Destron-Fearing TX1411L, size 12.50 mm x 2.07 mm) using a modified syringe with a 12-gauge needle. Tagging locations included the dorsal musculature near the dorsal fin origin, the abdominal cavity, and the dorsal musculature behind the head (Figure 1). Eel lengths and corresponding individual PIT tag numbers were recorded. After being tagged, the eels were placed in an aerated recovery tank for approximately 20 minutes before being returned to the holding tank.

PIT tag retention was checked weekly for a period of five weeks and then bi-weekly for four additional weeks, for a total of nine weeks. To check tag retention, eels were removed from the tank and anesthetized (as described above). A handheld PIT tag reader (Destron-Fearing, Mini Portable Reader 2, Part # 800-0249-01) was used to check each eel for all three PIT tags. After tag checks, eels were placed in a recovery tank for 20 minutes before return to the holding tank. Shed tags were located in the substrate of the holding tank following the study.

RESULTS

Eels ranged in length from 205 to 370 mm, with a mean length of 311.6 mm (Table 2). Tag retention was relatively high in all three tagging locations. Retention rates were highest (100%) in the dorsal musculature near the dorsal fin origin and in the abdominal cavity. Lower retention rates (88%) for the dorsal musculature near the head resulted from two lost tags within the first week of the study (Table 2). Both shed tags were located in the tank, confirming tag loss and not tag or scanner malfunctions.

Figure 1. PIT tag location for American eels included 1) dorsal musculature behind the head, 2) dorsal musculature near the dorsal fin origin, and 3) the abdominal cavity.

Table 1. Synthesis of PIT tag retention rates from American eel studies.

Study	Location of Study	Duration	Eel Length (mm)	Tag Location	Tag Retention
Thomas (2006)	Laboratory	6 months	≥ 500	Dorsal musculature	100%
Morrison and Secor (2003)	Hudson River, NY	2 months	Mean = 457	Visceral cavity	89%
Verdon and Desrochers (2003)	St. Lawrence River, NY	1998-1999	Mean = 471.7 (1998) Mean = 468.7 (1999)	Behind the head	98%
Verdon et al. (2003)	Richelieu River, Quebec	1997-1999	Mean = 379.7	Dorsal Musculature	93.9%

Table 2. PIT tag retention in small American eels (205 – 370 mm total length) during a nine week laboratory study (P = tag present, H = head tag shed, D = dorsal tag shed, and A = abdominal tag shed).

	Week								
Length (mm)	1	2	3	4	5	6	7	8	9
205	P	P	P	P	P	P	P	P	P
270	H	-	-	-	-	-	-	-	-
275	P	P	P	P	P	P	P	P	P
280	P	P	P	P	P	P	P	P	P
295	P	P	P	P	P	P	P	P	P
300	P	P	P	P	P	P	P	P	P
300	P	P	P	P	P	P	P	P	P
305	P	P	P	P	P	P	P	P	P
310	P	P	P	P	P	P	P	P	P
310	P	P	P	P	P	P	P	P	P
330	P	P	P	P	P	P	P	P	P
340	H	-	-	-	-	-	-	-	-
340	P	P	P	P	P	P	P	P	P
340	P	P	P	P	P	P	P	P	P
345	P	P	P	P	P	P	P	P	P
345	P	P	P	P	P	P	P	P	P
350	P	P	P	P	P	P	P	P	P
370	P	P	P	P	P	P	P	P	P

DISCUSSION

PIT tag retention in this study was 100% for American eels tagged in the dorsal musculature and in the body cavity, and 88% for eels tagged behind the head. These results are similar to those published in the literature (Table 1). In a 6-month laboratory study, Thomas (2006) also found 100% retention for American eels tagged in the dorsal musculature. Conversely, Verdon et al. (2003) reported a lower retention rate for eels tagged in the dorsal musculature, with a success rate of 93.9%. Morrison and Secor (2003) also found lower retention rates (89%) for eels tagged in the abdominal cavity relative to our study.

Estimates of PIT tag retention rates from laboratory studies may overestimate retention rates from natural environments. Lower retention rates reported by Verdon et al. (2003) and Morrison and Secor (2003) were from eels in riverine environments, and eels within rivers may have a higher likelihood of tag loss than those in captivity (Thomas 2006). The range of water temperatures (13.4˚C – 18.8˚C) during this study may have reduced eel activity. Temperature triggers activity in eels, and temperatures $\geq$ 20˚C are associated with peak increases in eel activity (Knights and White 1998; Verdon and Desrochers 2003; Verdon et al. 2003). Eels that are migrating upstream, like those reported by Verdon et al. (2003), may be more likely to lose tags than more sedentary eels in laboratory holding tanks.

Thomas et al. (2006) suggested that retention rates may be influenced by eel size. Thomas et al. (2006) tagged eels $\geq$ 500 mm – larger than mean eel lengths from other PIT tag studies (Table 1). Verdon and Desrochers (2003) found higher retention rates (98%) for eels tagged behind the head, as compared to this study (88%). The larger mean length (464.8 mm) of the eels tagged by Verdon and Desrochers (2003) relative to our study (311.6 mm) may explain differences in retention rates. The low mean length of the eels in our study (311.6 mm), however, indicates that high tag retention is possible in small eels.

Loss of PIT tags may result from incorrect tagging technique or rejection of the tag by the animal's body. Also, migration of a tag within an animal's body, and tag or scanner malfunctions mimic tag loss, because undetected tags are assumed lost. Most tag losses result from improper implantation of the tag (Gibbons and Andrews 2004). Dare (2003) attributed inexperience of the tagging crew to high tag loss in spring Chinook salmon (*Oncorhynchus tshawytscha*), because tag loss decreased with an increase in experience of the tagging crew (Dare 2003). Tag migration may increase with the size of the tagged animal (Gibbons and Andrews 2004). Camper and Dixon (1988) reported tag migration in 44% of the kingsnakes (*Lampropeltis getulus holbrooki*) and 71% of collared lizards (*Crotaphytus c. collaris*) with abdominally-placed PIT tags. Prentice and Park (1984) reported migration of abdominally-paced PIT tags in 5% of juvenile fall Chinook salmon (*Oncorhynchus tshawytscha*). Tag migration, however, is unlikely to influence tag detection in American eels, because tag readers can easily scan the entire body of the eel.

Tag loss in our study probably resulted from either improper implantation or tag rejection. The two tags shed during the first week of this study likely exited through the tagging wound. Feldheim et al. (2002) found that lemon sharks (*Negaprion brevirostris*) shed PIT tags through unclosed wounds a few days after tagging. Prentice and Park (1984) also reported shed tags (through the tagging wound) in coho and Chinook salmon (*Oncorhynchus kisutch* and *Oncorhynchus tshawytsch*) within a few days after tag placement.

Our results support two body locations for PIT tag placement in American eels – the abdominal cavity and the dorsal musculature near the dorsal fin origin. Tags inserted into the abdominal cavity, however, may increase risk of mortality in American eels owing to the proximity of the tagging syringe needle to internal organs, but we did not experience tagging mortality. Our study was relatively short-term (9-weeks) and longer-term studies are needed to further understand differential rates of retention among tagging locations. Additionally, laboratory-based studies restrict eel movements and provide relatively benign environments, hence, our laboratory results may not allow inference to tag-retention rates of American eels within riverine habitats.

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SEXUAL SIZE DIMORPHISM IN DUSKY SALAMANDERS, *DESMOGNATHUS* (AMPHIBIA: CAUDATA: PLETHODONTIADE) IN WEST VIRGINIA

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ABSTRACT

Sexual size dimorphism (SSD) occurs when one sex attains a larger mean adult body size than the other. Sexual size dimorphism has been documented extensively in various vertebrate species, including amphibians and reptiles. We investigated SSD in desmognathine salamanders in West Virginia. We found that the five species of *Desmognathus* in West Virginia exhibit a male-biased SSD, which is typical of this genus. In all desmognathine species, except *D. quadramaculatus*, males exhibited a significantly larger snout-vent length (SVL) than females; however, when total length (TL) was considered, we found that in all species, males were significantly larger. Furthermore, we found that for the head measurements, males were significantly larger than females in all species. Research has demonstrated that male desmognathine salamanders continue to grow after reaching sexual maturity; whereas, after maturation females allocate most of their energy to reproduction instead of growth. More research to investigate variation in adult body size across the landscape is necessary to outline true body size differences among these populations. It is also clear that research is needed to document intra- and inter-specific variation in SSD and aid in explaining the determinants of SSD in *Desmognathus*.

INTRODUCTION

Sexual size dimorphism (SSD) is a phenomenon in which males and females have a difference in mean adult body size. Sexual size dimorphism has been documented extensively in both amphibians (Bakkegard and Guyer 2004; Bruce 2000; Malmgren and Thollesson 1999; Bruce 1993; Shine 1979) and reptiles (Krause and Burghardt 2007; Gregory 2004; Rivas and Burghardt 2001; Butler et al. 2000; King et al. 1999; Shine 1994; Shine 1978), as well in other vertebrates (Ehrlich et al. 1988; González-Solís 2004). The presence of either male- or female-biased sexual size dimorphism reflects the adaptation of males and females to different ecological or reproductive roles in nature (Bruce 2000).

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There have been at least two mechanisms proposed for the development of sexual size dimorphism: sexual selection and ecological causation through natural selection (González-Solís 2004; Bruce 2000; Shine 1989; Darwin 1874); however, the exact causative mechanism and determinants of SSD have yet to be uncovered.

Desmognathine salamanders exhibit male-biased SSD; whereas, in many *Plethodon* species there is either a female-biased SSD or males and females are of equal body size (Bruce 2000; Bruce 1993; Shine 1979). In most *Desmognathus* salamanders, males tend to reproduce earlier and at smaller sizes than females, yet males will achieve a larger adult body size than females (Hom 1988; Tilley 1980). Differences in morphology between males and females have been attributed to the male continuing to grow after sexual maturity is reached; whereas, the female allocates energy to the production of eggs and does not grow after the attainment of sexual maturity.

The salamander genus *Desmognathus* is comprised of at least 20 species that range in body size from the smallest, *Desmognathus aeneus* (Seepage Salamander) and *Desmognathus wrighti* (Pygmy Salamander) to the largest dusky salamanders, *Desmognathus quadramaculatus* (Black-Bellied Salamander) and *Desmognathus welteri* (Black Mountain Dusky Salamander). Desmognathine salamanders exhibit a unique stepwise community structure and habitat gradient where the largest salamanders are the most aquatic and exhibit a biphasic life history to the smallest salamanders are the most terrestrial and exhibit direct development. These salamanders are very abundant along swift-flowing streams and seepages throughout the Appalachian Mountains of eastern North America.

Sexual size dimorphism in desmognathine salamanders has been previously documented for specific populations throughout the range (Crespi 1996; Bruce 1993; Harrison 1967; Organ 1961); however, in West Virginia, these data are lacking. The objective of this study is to document the occurrence of SSD in five species of desmognathine salamanders found in West Virginia. One of the limitations of this research is that there is intraspecific variation in body size in desmognathine salamander populations across the landscape. Because of this variation, it is difficult to combine data sets from different populations; however, we used homogeneity of variance to determine if the differences among populations were great enough to mask any observable differences in body size among populations. All data met the homogeneity of variance test. Because of this we believe that our data are sufficient to use to investigate the level of SSD for each of the five desmognathine species found in West Virginia.

MATERIALS AND METHODS

ANIMALS

Specimens selected for this study are maintained in the West Virginia Biological Survey (WVBS) located in the Vertebrate Museum at Marshall University, Huntington, West Virginia. We measured five desmognathine species including *Desmognathus welteri* (Black Mountain Dusky Salamander), *D. quadramaculatus* (Black-Bellied Salamander), *D. fuscus* (Northern Dusky Salamander), *D. monticola* (Seal Salamander), and *D. ochrophaeus* (Allegheny Mountain Dusky Salamander) from various localities throughout West Virginia. Only sexually mature specimens were used and in order to confirm or reject maturity, dissection of each specimen was performed. Specimens were considered adult by the presence of convoluted ova ducts in females or the presence of multiple lobed testes in males.

MEASUREMENTS

Select standard measurements were used following Tilley (1981) and are defined in Table 1. All measurements were made with Fowler® digital calipers to the nearest 0.01mm (unless otherwise noted) with a computer connection that allowed the measurement value to be directly entered into Microsoft Excel. Because specimens were preserved, mass was not determined, and as a note, specimens preserved in formalin typically shrink 5-6%. Snout-vent length (SVL) was measured from the tip of the snout to the distal (posterior) margin of the cloaca. Total length (TL) was measured from the tip of the snout to the tip of the tail. Tail length (TAL) was calculated by subtracting SVL from TL. Salamanders that possessed a broken, regenerated, or regenerating tail were not included in the analysis. Head height (HDHM) and head

Table 1. Morphological measurements of preserved specimens of *Desmognathus* salamanders. All measurements are in mm, unless noted.

Measurement	Abbreviation	Location
Snout-Vent Length	SVL	Tip of snout to posterior (distal) margin of the cloaca
Tail Length	TAL	Calculated as TL minus SVL
Total Length	TL	Tip of snout to tip of tail
Head Height at Jaw Musculature	HDHM	Height of the head at the musculature of the jaw
Head Width at Angle of Jaw	HDWM	Head width at the musculature of the jaw
Head Height in between Nostrils	DVTN	Height of the head at the thinnest portion located in between the nostrils
Tail Circumference	TC	Circumference of the tail

width (HDWM) were measured at the jaw musculature. The tip of the snout between the nostrils was considered the thinnest part of the head (DVTN). Tail circumference (TC) was measured by wrapping a piece of number eight kite string around the tail and making a mark with a permanent marker. The string was then placed linearly on a ruler and the measurement was recorded.

STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS v.10.0 (Chicago, Illinois). Except where noted, a one-way analysis of variance (ANOVA) was used to test for the effect of sex (i.e., male or female) on morphological body size measurements in each of the five species. In all analyses, sex was treated as a fixed effect. All variables were transformed by computing natural logarithms. This transformation served to improve homogeneity of variances and normality of error terms in all analyses. Normality for each of the transformed independent variables (i.e., male and female for each measure of body size for each species) was tested using the Shapiro-Wilk (W) test statistic. Homogeneity of variance was tested using Levene statistic for each of the transformed independent variables. Body size difference between males and females was considered significant at $p \leq 0.05$.

An index of sexual dimorphism was calculated for each morphological variable for each species. The degree of difference between males and females for each morphological measurement was calculated as: [(larger measurement – smaller measurement)/smaller measurement) * 100]. All morphological measurement values reported in the text are reported as: (mean ± standard error).

RESULTS

All independent variables were transformed using the natural logarithm in order to satisfy the assumption of normality. In all cases, the transformed variables satisfied Levene's homogeneity of variance test.

Desmognathus ochrophaeus, D. quadramaculatus, D. welteri, D. fuscus, and *D. monticola* varied markedly in size; the mean male SVL for *D. ochrophaeus* was nearly half the size of the mean male SVL for *D. welteri* (Table 2). *Desmognathus quadramaculatus*, the largest desmognathine salamander in many parts of its geographic range, possessed a smaller mean male SVL than its ecological equivalent, *D. welteri* (Felix 2001).

In all species, except *D. quadramaculatus* ($p=0.06$), males were significantly larger in SVL than the females; however, the degree of difference between the sexes among species varied (Table 2). The TAL was not significantly different between males and females, except for *D. quadramaculatus* and males (51.07 mm ± 0.93) possess a significantly longer tail than females (47.57 mm ± 1.21). The mean TL of all species was significantly different with males attaining a larger TL than females (Table 2).

Table 2. Mean ± standard error (SE) for body size measurements for five species of desmognathine salamanders including *Desmognathus ochrophaeus*, *D. fuscus*, *D. monticola*, *D. quadramaculatus*, and *D. welteri*. N= sample size; SVL=snout-vent length; TAL= tail length; TL=total length; HDWM=head width at jaw musculature; HDHM=head height at jaw musculature; DVTN=height of head between nostrils; TC=tail circumference. The percentage is the amount of difference exhibited between females and males of a species, which was calculated as: [(larger measurement - smaller measurement)/smaller measurement]*100.[1]

Species	Sex	N	SVL	TAL	TL	HDWM	HDHM	DVTN	TC
ochrophaeus									
	Female	43	36.22±0.48	34.80±0.92	71.02±1.28	4.48±0.06	2.39±0.05	1.63±0.03	11.66±0.31
	Male	68	38.40±0.39	37.09±0.76	75.50±1.03	5.01±0.06	2.66±0.05	1.83±0.03	12.43±0.22
			6.02%***	6.58%	**6.31%*****	**11.83%*****	**12.55%*****	**12.27%*****	**6.60%***
fuscus									
	Female	31	45.99±1.20	44.29±1.29	90.28±2.19	6.08±0.19	3.64±0.16	2.26±0.11	16.80±0.55
	Male	72	51.26±0.67	45.57±1.02	96.84±1.49	7.26±0.11	4.01±0.08	2.60±0.05	18.41±0.37
			11.46%***	2.89%	**7.27%***	**19.41%*****	**10.16%****	**15.04%*****	**9.58%***
monticola									
	Female	58	55.95±0.78	52.97±1.19	108.92±1.79	7.78±0.12	4.59±0.09	2.64±0.08	20.71±0.35
	Male	61	59.97±0.95	54.91±1.20	114.88±1.84	8.52±0.14	4.94±0.11	2.90±0.07	22.59±0.38
			7.18%***	3.66%	**5.47%***	**9.51%*****	**7.63%***	**9.85%*****	**9.08%*****
quadramaculatus									
	Female	34	63.51±1.62	47.57±1.21	111.08±2.57	9.25±0.22	5.54±0.20	3.16±0.12	25.93±0.78
	Male	59	67.51±1.26	51.07±0.93	118.58±2.08	10.09±0.19	6.03±0.16	3.58±0.10	27.35±0.55
			6.30%	**7.36%***	**6.75%***	**9.08%****	**8.84%***	**13.29%***	5.48%
welteri									
	Female	10	66.68±1.65	56.44±1.58	123.12±2.60	9.47±0.10	5.75±0.22	3.35±0.09	25.92±0.87
	Male	14	74.65±2.42	58.57±2.41	133.21±3.79	11.04±0.33	6.64±0.28	4.03±0.18	28.02±0.90
			11.95%*	3.77%	**8.20%***	**16.58%*****	**15.30%***	**20.30%****	8.10%

[1]Bold indicates a significant effect of sex. Level of significance indicated by an asterisk: *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$

For the morphological measurements of the head, males of each species possessed a significantly larger head than the females (Table 2). *Desmognathus monticola* exhibited the smallest percent difference between males and females for head measurements: for HDWM, HDHM, and DVTN, 9.51%, 7.63%, 9.85%, respectively. Whereas, *D. welteri* exhibited the largest percent difference between males and females for head measurements: for HDWM, HDHM, and DVTN, 16.58%, 15.30%, and 20.30%, respectively (Table 2). Tail circumference was significantly different between males and female for each species, except *D. quadramaculatus* (p=0.11) and *D. welteri* (p=0.12).

DISCUSSION

Amphibians experience indeterminate growth; however, growth rates decline considerably after attaining sexual maturity (Jørgensen 1992). Furthermore, among amphibian species, most exhibit a female-biased SSD; however this is more distinct in anurans, rather than in caudates. This is an interesting phenomenon because most desmognathine salamander species exhibit male-biased SSD, whereas most other species in Plethodontidae exhibit either female-biased SSD or no SSD.

Research has demonstrated that male desmognathine salamanders continue to grow after reaching sexual maturity; whereas after maturation females allocate most of their energy to reproduction instead of growth. Body size differences, as well as other phenotypic differences, between males and females are mainly influenced by developmental processes and growth that occurs before maturation (Shine 1990). We found that the level of difference among morphological measurements among species varied interspecifically. This may be a direct reflection of sexual or natural selection that is occurring or has occurred historically in that particular species. Primary influences on interspecific and intraspecific variation in body size are duration of the larval period and juvenile period (Beachy 1995). Using skeletochronology, Castanet et al. (1996) demonstrated that in desmognathine salamanders: (1) variation in body size among species is directly correlated with age at maturation; (2) growth slows following the attainment of sexual maturity; and (3) differences in body size between males and females is a reflection of interspecific differences in age at maturation.

For the desmognathine salamander species included in this study, significant intraspecific SSD was found. In all species, except *D. quadramaculatus*, males were significantly larger than females in SVL. These data support other data found in the literature which suggest that male desmognathine salamanders achieve a larger adult body size than females (Bruce 2000; Bruce 1993; Bruce 1988; Shine 1979). Sexual selection involving male-male competition and aggression probably plays a role in male-biased SSD in desmognathine salamanders (Bruce 2000).

For populations throughout the geographic range of *D. quadramaculatus*, a male-biased SSD has been described (Bruce 1993; Bruce 1988). It is possible that the level of SSD in West Virginia populations is not as distinct as what has been documented for other populations within the geographic range of *D. quadramaculatus*. It is noteworthy that in West Virginia, populations of *D. quadramaculatus* exhibit considerably smaller adult body sizes (maximum male SVL 88.0 mm; maximum female SVL 81.8 mm) than conspecific populations in other parts of the geographic range (Wooten and Pauley, unpublished data).

For other desmognathine species, SSD can either be female-biased or that males and females achieve similar adult body sizes.

For instance *D. wrighti*, males are either larger (Organ 1961), or females are slightly larger (Crespi 1996) and for *D. aeneus*, males and females are either the same size or males are slightly larger (Harrison 1967) or females are larger (Bruce 2000). More research to investigate how populations of desmognathine salamanders vary in adult body size and what level of SSD exhibited are necessary to outline true body size differences among populations. It is also clear that more research is needed to document intra- and inter-specific variation and aid in explaining the determinants of SSD in *Desmognathus*.

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REVISED NOTES ON THE RANGE OF THE CHEAT MOUNTAIN SALAMANDER, *PLETHODON NETTINGI*, (AMPHIBIA: CAUDATA)

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ABSTRACT

Plethodon nettingi, the Cheat Mountain Salamander, is a federally threatened species that is endemic to only five counties in the Allegheny Mountains of eastern West Virginia. Over the past 30 years, I have surveyed more than 1300 sites, of which approximately 80 contained *P. nettingi*. The total range of *P. nettingi* extends from the Blackwater River Canyon in Tucker County south to Thorny Flat in Pocahontas County. Since 1976, of the 80 populations that are known to support *P. nettingi,* one population and possibly two others have been extirpated and two have been reduced in size. Approximately 60 of these 80 populations are on federal- or state-owned land which provides them protection, but nearly all of these populations are impacted by roads, trails, ski slopes, or clearcuts. These perturbations plus competitive interactions with *P. cinereus* (Eastern Red-backed Salamander) and *Desmognathus ochrophaeus* (Allegheny Mountain Dusky Salamander) place several *P. nettingi* populations in peril.

INTRODUCTION

Plethodon nettingi, the Cheat Mountain salamander, is a small woodland salamander endemic to the high elevations of the Allegheny Mountains of West Virginia. Green (1938) described it from specimens collected at Barton Knob, Randolph County. Highton and Grobman (1956) considered it a subspecies of *P. richmondi* but later Highton and Larson (1979) assigned it full species status. The United States Fish and Wildlife Service (USFWS) listed it as a threatened species on September 28, 1989 (Pauley 1991a).

Initially, the range of *P. nettingi* was thought to be limited to red spruce forest above 1067m of the Cheat Mountains in Randolph and Pocahontas counties (Green 1938; Brooks 1945, 1948). Subsequently, it was found in Pendleton and Tucker counties (Highton 1971, 1986; Pauley 1980, 1981, 1986, 1987). More recent work by Pauley (1991b) extended the total range to the western edge of Grant County. *Plethodon nettingi* is presently known to occur in five counties: Randolph, Pendleton, Pocahontas, Tucker, and the most western edge of Grant County along the Allegheny Front. The total range extends from Blackwater River Canyon (Tucker County) in the north, south to Thorny Flat (Pocahontas County) (approximately 92 km) and from Cheat Mountain in the west, east to the Allegheny Front (approximately 31 km) (Pauley 2005).

Calise (1978) and Clovis (1979) did not find *P. nettingi* associated with any particular type of vegetation. However, Pauley (2007) described the typical habitat as stands of conifers such as Red Spruce (*Picea rubens*) and occasionally Eastern Hemlock (*Tsuga canadensis*) or stands of mixed deciduous forests at elevations above 610m in the northern part of the known range (i.e., Spruce Knob in Pendleton County and areas north), to above 1067m in the southern part of the known range (i.e., south of Spruce Knob). Pauley (2007) also found that the forest floor is usually covered with the liverwort *Bazzania trilobata* and that the habitat typically contains rock

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outcrops, emergent rocks, boulder fields, or narrow ravines lined with Great Rhododendron (*Rhododendron maximum*).

It is the purpose of this paper to describe the total range of *P. nettingi* and assess factors that could contribute to its limited range and disjunct distribution.

MATERIALS AND METHODS

Data presented here are from surveys conducted for *P. nettingi* from 1976 through 2006. Site selection was based on elevation, i.e., above 610 m in the northern part of the potential *P. nettingi* range and above 1067m in the southern part of the potential range. All sites were searched typically for one-person hour and during the day rather than night because of the remote areas where many surveys were conducted. Searches for salamanders involved turning rocks, logs, and leaf litter. Salamander data collected included species, size classes, and gender. Habitat characteristics recorded at sites consisted of dominant plant species in the canopy, understory, shrub and herbaceous levels and presence or absence of emergent rocks or boulder fields.

RESULTS

During these 30 years, over 1300 sites were surveyed and *P. nettingi* was located in 135. After determining the areal extent of these 135 sites it was found that several were part of the same population so it now appears that there are approximately 80 disjunct populations. The total range from north to south extends approximately 92 km from Blackwater River Canyon (Tucker County) south to Thorny Flat on Cheat Mountain (Pocahontas County). The west to east range varies in distance from less than 3.2 km at the southern tip of the range to approximately 31 km near the northern end. In the north, the range extends south from the northern rim of Blackwater River Canyon to Cheat Mountain where it stops at Thorny Flat (Pocahontas County) and from Blackwater River Canyon southeast to the Allegheny Front at Dolly Sods (Tucker and Grant counties) where it continues south along the Allegheny Front and Spruce Mountain to Spruce Knob (Pendleton County). South of Barton Knob on Cheat Mountain (Randolph County), which is the type locality, the range becomes narrow and is confined to Cheat Mountain (Pocahontas County) to the west and Back Allegheny Mountain (Pocahontas County) to the east (Figure 1).

Within its range, *P. nettingi* was not found on Cheat Mountain between Red Run and Suter Run (#8) or on the entirety of Middle Mountain (#11) (Figure 1). Mountains south of the range with elevations that could support *P. nettingi* but do not include Allegheny Mountain (#16), Gauley Mountain (#17) and Yew Mountains (#18). Mountains with apposite elevations immediately north of its range but where it does not occur include the most northern sections of Backbone Mountain (#1), Canaan Mountain (including Brown Mountain) (#2 and #5), Cabin Mountain (#4), and Allegheny Front (#14) (Figure 1).

DISCUSSION

The disjunct distribution of *P. nettingi* throughout its range has been attributed, in part, to clearcuts and forest fires between 1870 and the mid 1900s (Pauley, 2008, in press). These events may be responsible for the hiatus in the distribution of *P. nettingi* on Cheat Mountain between Red Run and Suter Run. This area was logged and heavily mined for coal during the early 1900s (Clarkson 1990) providing a reasonable possibility for forest fires that could have eliminated *P. nettingi*. However, *P. nettingi* is found south of Red Run and north of Suter Run as well as east on Shavers Mountain where mining activities and clearcuts also occurred. In addition, there

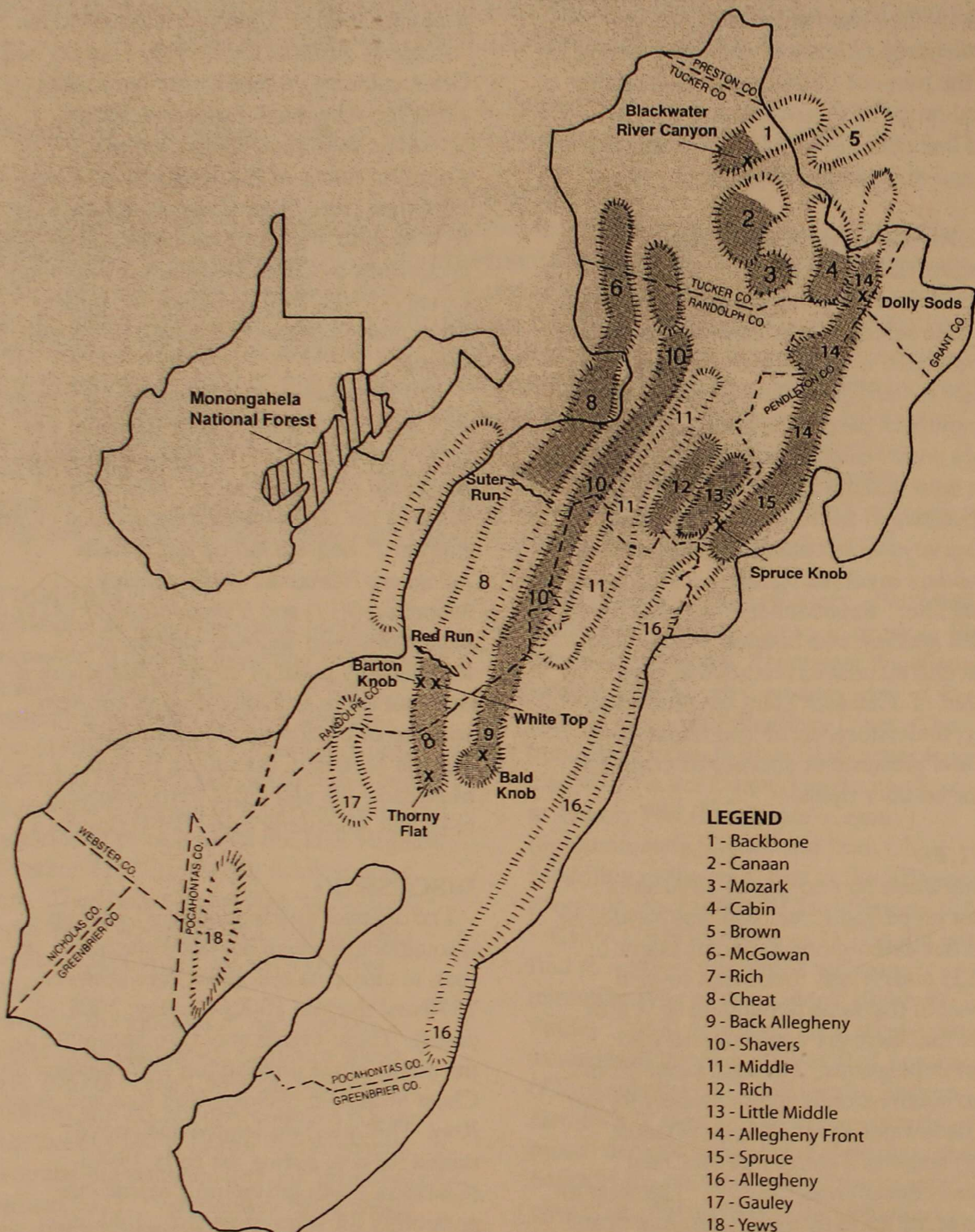

Figure 1: Total range of the Cheat Mountain Salamander, *Plethodon nettingi*. Mountains with *P. nettingi* are shaded and mountains without *P. nettingi* are not shaded. Mountains are not to scale and locations are approximate.

are several areas in this gap with large emergent rocks, boulder fields, and narrow ravines lined with Great Rhododendron, habitats that probably served as refugia for *P. nettingi* from clearcuts and forest fires in other parts of its range. Additional research will be necessary to ascertain why *P. nettingi* is absent in this area of Cheat Mountain.

Middle Mountain, including Burner Mountain, has elevations that exceed 1067m and reach over 1219 m in some areas. While elevations at the 44 sites surveyed on Middle Mountain are appropriate for *P. nettingi* populations, vegetation is not typical of that found in *P. nettingi* habitat. The forest canopy is mostly a mix of deciduous species with scattered Red Spruce. The lack of *Bazzania trilobata* on the forest floor in most areas may be indicative of drier soils. Why Middle Mountain lacks *P. nettingi* habitat has yet to be determined. It is interesting that *P. nettingi* has been located at one site each on both Little Middle Mountain (#13) and Rich Mountain (#12). Both mountains are within 8.0 km of Middle Mountain and between Middle Mountain and Spruce Mountain where several populations have been found (Figure 1). The site on Little Middle Mountain is over 1402 m with Red Spruce and Yellow Birch and the site on Rich Mountain is over 1219 m with Red Oak (*Quercus rubra)* and Yellow Birch. *Plethodon nettingi* was found in emergent rocks on Rich Mountain.

The most southern point of the range of *P. nettingi* is near the top of Thorny Flat, which is the southern terminus of Cheat Mountain. In 1938, *P. nettingi* extended downslope to West Virginia Route 66 approximately 0.5 km south of where the population is located today (Maurice Brooks, personal communication), but after numerous searches from 1978 through 2003, they have not been found below 1439 m. Gauley Mountain and the Yew Mountains, which are south of Cheat Mountain, have typical *P. nettingi* habitat in appropriate elevations, but after finding over 2100 forest salamanders in 87 sites in this area, I do not believe *P. nettingi* occurs south of Thorny Flat. There is a large valley that separates Cheat and Back Allegheny mountains from Gauley Mountain and the Yew Mountains that may be a natural barrier that has prevented any southern expansion of *P. nettingi*.

At the northern end of its range, typical *P. nettingi* habitat does not extend beyond the northern rim of Blackwater River Canyon. While there are numerous red spruce stands, most are xeric in nature. One hundred and fourteen sites have been examined on the northern sections of Canaan, Backbone, Cabin, and Allegheny Front mountains. Several of these sites, especially on Cabin Mountain and Allegheny Front, have few to no salamanders due to the extremely dry conditions. Some of this area was burned to the bedrock during the early 1900s and has never regained enough soil to support lungless salamanders such as *P. nettingi* (Allard and Leonard 1952; T.K. Pauley, personal observation).

Since I started working with *P. nettingi* in 1976, I have found that one historical population no longer exists (White Top), two others are probably extirpated (Bald Knob and Mozark Mountain), and two others have been reduced in size (Barton Knob and Thorny Flat) (Figure 1). Strip mine activities destroyed the population at White Top and reduced the size of the population at Barton Knob. Clearcuts and forest fires most likely terminated the populations at Bald Knob and Mozark Mountain and reduced the size of the population at Thorny Flat.

While there are most likely populations within the total range yet to be found, I believe this paper describes the complete range of *P. nettingi* and a high percentage of all existing populations. Data on the range and distribution provides a basis from which conservation biologists can work to provide the maximum protection for this federally protected species. It is clear that *P. nettingi* has a tenuous existence. Of the approximately 80 populations, 60 are on state- or federally-owned lands and are potentially afforded more protection than those on private lands. Unfortunately, nearly all populations on public lands are impacted by some type of habitat disturbance such as ski slopes, roads, utility rights-of-way, and clearcuts and in many locations populations are bisected into subpopulations by roads, hiking trails, utility rights-of-way, or ski slopes. Given these disturbances and competitive stress from *P. cinereus* (Eastern Red-backed Salamander) and *Desmognathus ochrophaeus* (Allegheny Mountain Dusky Salamander) (Pauley 2005), many of these disjunct populations could be imperiled. It is important for the natural survival of this species that conservation measures recommended in the Recovery Plan (Pauley 1991a) be followed so that future generations can enjoy this West Virginia endemic species.

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SURVEY OF ABANDONED COAL MINES FOR AMPHIBIANS AND REPTILES IN NEW RIVER GORGE NATIONAL RIVER, WEST VIRGINIA

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ABSTRACT

Thousands of abandoned coal mines exist throughout most of West Virginia. In some cases, these abandoned mines serve as surrogate caves for many species of animals including amphibians and reptiles. From 1989 through 1992, we conducted extensive inventories in upland habitats for amphibians and reptiles throughout the New River Gorge National River, West Virginia. During this time we searched 40 abandoned coal mines and 25 had at least one species of amphibian or reptile. In these 25 mines, 23 had amphibians (12 species of salamanders, one species of toad, and three species of frogs) and five had reptiles (two species of lizards and two species of snakes).

INTRODUCTION

The importance of caves as refugia for amphibians and reptiles has been reported in the literature for several years (Reese 1934;Mohr 1944; Dearolf 1956; Humphries 1956; Cooper 1962a; Cooper 1962b; Franz 1967; Cooper and Cooper 1968; Resetarits 1986; Saugey et al. 1985; Saugey et al. 1988; Garton et al. 1993; Buhlmann 2001; Dodd et al. 2001; Himes et al. 2004; Taylor and Mays 2006).

Published studies of the use of abandoned mines by amphibians and reptiles are somewhat limited. Five species of salamanders have been observed in abandoned mines in Ouachita Mountains of Arkansas including *Plethodon caddoensis* (Caddo Mountains Salamanders) (Saugey et al. 1985), *Desmognathus brimleyorum* (Ouachita Dusky Salamander) (Heath et al. 1986), *Eurycea multiplicata* (Many-ribbed Salamander) (Saugey et al. 1988), *Plethodon albagula* (Western Slimy Salamander) (Saugey et al. 1988, Trauth et al. 2004), and *Plethodon serratus* (Ouachita Red-backed Salamander, now the Southern Red-backed Salamander) (Saugey et al. 1988). *Plethodon idahoensis* (Coeur d'Alene Salamanders) have been found in two abandoned mines in Montana (Montana Fish, Wildlife & Parks and the Natural Heritage Program) and *Rana palustris* (Pickerel Frogs) have been observed in mines in the Ouachita Mountains of Arkansas (Health et al. 1986; McAllister et al. 1995).

Coal mining commenced in the New River Gorge in the late 1800's and continued into the mid 1900's. This nearly century-long coal mining activity left approximately 200 open portals, 125 structures, 150 refuse piles, and 50 ponds within 115 separate mine and well areas in what we now know as the New River Gorge National River (Armstrong and Yuill 1989).

The New River Gorge was incorporated into the National Park system in 1978 as New River Gorge National River (NERI) and today the park includes over 70,000 acres along 53 miles of the New River. Land encompassed within the NERI is characterized by high ridges and steep hillsides with precipitous cliffs.

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MATERIALS AND METHODS

From 1989 through 1992, we conducted inventories throughout the NERI for amphibians and reptiles that might appear in the varied habitats of the upland areas. During this three-year study, we examined over 1,000 upland sites throughout NERI including 40 abandoned coal mines. Inventories included both day and night searches and were restricted to the twilight zones in mines because of the danger of roof falls and gases (methane, carbon monoxide, and carbon dioxide). Specimens observed were identified to species and life stages (adults, subadults, and larvae) were noted. Studies were conducted from March through October and consisted of searching the floor, walls and ceilings of mines with flashlights and turning rocks and other cover objects within the mines.

RESULTS

Of the 40 mines surveyed, 25 had at least one species of amphibian or reptile. In these 25 mines, 23 had amphibians (12 species of salamanders, one species of toad, and three species of frogs) and five had reptiles (two species of lizards and two species of snakes). The most common species of salamanders were *Eurycea lucifuga* (Cave Salamander), *E. longicauda* (Long-tailed Salamander), *Plethodon glutinosus* (Northern Slimy Salamander), *Gyrinophilus p. porphyriticus* (Northern Spring Salamander), *Desmognathus fuscus* (Northern Dusky Salamander), *D. monticola* (Seal Salamander), and *P. wehrlei* (Wehrle's Salamander). *Rana clamitans melanota* (Northern Green Frog) was the most commonly observed species of frog. We only found six reptiles (two species of lizards and two species of snakes) in the mines. A complete list of all species, frequency of occurrences, and dates observed is presented in Table 1.

DISCUSSION

Abandoned coal mines serve as surrogate caves and underground refugia for subterranean and epigean species. Prior to the construction of mines, species presently observed in these underground retreats most likely congregated in natural caves and underground cavities. Abandoned mines have allowed researchers the opportunity to observe these species in heretofore hidden refugia. Today abandoned mines represent an important man-man habitat for many cave-dwelling species throughout West Virginia.

Cave-dwelling species are characterized as troglobites, troglophiles, or trogloxenes. Troglobitic (TB) species are those found only in caves; troglophilic (TP) species are those found in caves and epigean habitats; and trogloxenic (TX) species are those in which part of their life cycle is spent in caves (Culver 1986). Species found in caves in small numbers that are not dependent on caves for any part of their life cycle are considered to be accidentals (AC). Of the 16 species of amphibians observed in mines in the NERI, two are troglophilic, four are probably trogloxenic, and the remaining accidentals. All species of reptiles observed are accidentals.

Eurycea lucifuga (TP) – Cave Salamanders are found in numerous caves in the southeastern counties of West Virginia (Reese 1934; Hutchinson 1956; Cooper 1960; Cooper 1962a; Cooper 1962b; Green and Brant 1966; Storage 1977; Green and Pauley 1987; Garton et al. 1993; Carey 1973; Rosevear 1995; Longenecker 2000; Osbourn 2005). They were the most common species found in the mines in NERI occurring in nine of 40 examined. All nine mines had water pools and in most cases small streams were present, which are ideal nesting habitats for this species. We observed all size classes indicating that reproduction efforts are successful in these

Table 1. Date, life stage and cave dwelling status of species of amphibians and reptiles observed in abandoned coal mines in New River Gorge National River.

Species	Dates Observed	Larvae	Subadult	Adult	Total	Cave Dwelling Status[1]
Eurycea lucifuga	8/1991 5/1992 6/1992	--	1	24	25	TP
Eurycea longicauda	8/1991 5/1992 6/1992	--	--	15	15	TX
Plethodon glutinosus	8/1991 6/1992	--	--	15	15	TX
Desmognathus monticola	1/1990 8/1991	--	--	10	10	AC
Gyrinophilus porphyriticus	5/1991 8/1991	1	--	9	10	TP
Desmognathus fuscus	8/1991	--	--	9	9	AC
Plethodon wehrlei	5/1992 6/1992	--	3	6	9	TX
Notophthalmus v. viridescens	5/1991 8/1991 6/1992	--	5	--	5	AC
Rana clamitans melanota	5/1991 8/1991 6/1992	--	1	4	5	AC
Desmognathus ochrophaeus	1/1990 8/1991	--	--	3	3	AC
Sceloporus undulatus	6/1989 7/1989	--	--	3	3	AC
Diadophis punctatus edwardsii	5/1992	--	--	2	2	AC
Eurycea cirrigera	8/1991	--	--	2	2	AC
Bufo americanus	6/1992	--	--	1	1	AC
Coluber constrictor	8/1991	--	--	1	1	AC
Desmognathus quadramaculatus	5/1992	--	--	1	1	AC
Eumeces fasciatus	5/1991	--	--	1	1	AC
Plethodon kentucki	6/1992	--	--	1	1	UK
Pseudacris crucifer	8/1991	--	--	1	1	AC
Rana palustris	8/1991	--	--	1	1	AC

(1) TB – Troglobitic , TP – Troglophilic, TX – Trogloxenic, AC – Accidental, UK – Status Unknown

man-made habitats. Occurrence of Cave Salamanders in the mines of NERI expanded their range in West Virginia approximately 40 miles west from known populations in limestone caves of Greenbrier County.

Gyrinophilus porphyriticus (TP) – Spring salamanders are commonly found in epigean habitats such as first and second-order streams and seeps (Green and Pauley 1987; Petranka 1998). They are known to occur in caves throughout their total range (Dearolf 1956; Cooper and Cooper 1968, Barbour 1971; Culver 1973; Mount 1975; Martof et al. 1980; Cliburn and Middleton 1983; Redmond and Scott 1996; Buhlmann 2001; Dodd et al. 2001) including West Virginia (Reese 1934, Green and Brant 1966; Brandon 1967; Cooper 1960; Cooper 1962b; Cooper and Cooper 1968; Carey 1973; Rutherford and Handley 1976; Storage 1977; Newsom 1991; Garton et al. 1993; Rosevear 1995; Osbourn 2005). We found 10 spring salamanders in two mines with streams.

Eurycea longicauda (TX) – Long-tailed Salamanders occur statewide in West Virginia (Green and Pauley 1987) and were the second most common species observed in the mines. Occurrences of this species in caves have been reported in West Virginia (Hutchinson 1956; Cooper 1960; Cooper 1962a; Cooper 1962b; Green and Brant 1966; Carey 1973; Storage 1977; Rosevear 1995; Longenecker 2000; Osbourn 2005) as well as in other parts of their total range (Bishop 1941; Culver 1973; Mohr 1944; Smith 1948; Hutchinson 1958; Myers 1958; Cooper 1960; Franz 1964; Franz 1967; Martof et al. 1980; Holsinger 1982; Cliburn and Middleton 1983; Buhlmann 2001; Dodd et al. 2001; Taylor and Mays 2006). Mohr located the eggs of *E. longicauda* (1943) and large numbers of adults (1944) in an abandoned mine in southeastern Pennsylvania.

Plethodon glutinosus (TX) – Several studies have reported slimy salamanders in caves and in some instances in large numbers (Noble and Marshall 1929, Smith 1948, Humphries 1956, Peck 1974, Brode and Gunter 1958, Cliburn and Middleton 1983, Saugey et al. 1985, Wells and Gordon 1958, Saugey et al. 1988, Buhlmann 2001, Dodd, et al. 2001, Himes et al. 2004, Taylor and Mays 2006, Trauth et al. 2006). They have been reported in caves in West Virginia by Reese (1934), Cooper (1962a), Green and Brant (1966), Carey (1973), Rutherford and Handley (1976), Storage (1977), Garton et al. (1993), Longenecker (2000), and Osbourn (2005). Western Slimy Salamanders (*P. albagula*) have been reported in abandoned mines in Arkansas (Heath et al. 1986, McAllister et al. 1995). We found them in four mines and in one mine we found 11 in crevices of the walls and ceiling.

Plethodon kentucki (Status Unknown) – Cumberland Plateau Salamanders are similar in appearance to *P. glutinosus* and occupy habitat similar to *P. glutinosus* (Green and Pauley 1987). Like *P. glutinosus*, *P. kentucki* most likely retreat to subterranean habitats during the summer months (Bailey and Pauley 1993). Pauley (1993) found *P. kentucki* in rock crevices in numerous locations throughout the NERI suggesting that they could move to underground refugia such as caves and mines during warm, dry periods. Because we found just one individual in a mine, their cavernicole classification is not known.

Plethodon wehrlei (TX) – Wehrle's Salamanders most likely deposit their eggs in subterranean nests; thus caves and mines could provide excellent nesting sites. However, to our knowledge nests have not been located in either of these subterranean refugia. *P. wehrlei* have been observed in caves throughout much of their range (Reese 1934; Netting 1933; Netting et al. 1946; Pope and Fowler 1949; Fowler 1951;

Newman 1954; Hutchinson 1958; Cooper 1962a; Green and Brant 1966; Carey 1973; Storage 1977; Martof et al. 1980; Gross 1982; Holsinger 1982; Green and Pauley 1987; Osbourn 2005). Based on their apparent commonality in caves and their underground nesting retreats, we believe they are trogloxenic. We found nine specimens in five mines.

Rana palustris (AC) – A survey of the literature reveals that Pickerel Frogs are the most common anurans found in caves in the eastern United States (Brode 1958; Dodd et al. 2001; Schaaf and Smith 1970; Knight 1972; Buhlmann 2001). Heath et al. (1986) and McAllister et al. (1995) reported them in abandoned mines in Arkansas. In the NERI we located just one individual in a mine.

Northern Green Frogs (*R. clamitans melanota*), an accidental species, were the most common anuran observed in our study. The results of our study were contrary to other studies (Buhlmann 2001, Resetarits 1986) that reported few green frogs compared to pickerel frogs. Based on our findings and data reported in Garton et al. (1993), Rosevear (1995), Longenecker (2000), and Osbourn (2005), pickerel frogs do not utilize caves or mines as part of their natural history in West Virginia as they have been reported to do in other parts of their range and should be considered accidental here instead of trogloxenic.

All other anurans found in mines were considered accidentals (Table 1).

Six other species of salamanders were found in mines that are considered to be accidentals. Two species, *Desmognathus fuscus* (10 individuals in three mines) and *D. monticola* (10 individuals in four mines), were relatively abundant in small streams that flowed through the mines. Since both of these species are common in first-order streams throughout NERI (Pauley 1993), it is not surprising they were in streams associated with abandoned mines. Red Efts (*Notophthalmus v. viridescens*) were the next most common accidentals observed. Five individuals were found in five separate mines, which indicates that these were haphazard occurrences. The other accidental species, Mountain Dusky Salamanders (*D. ochrophaeus*), Southern Two-lined Salamanders (*E. cirrigera*), and Black-bellied Salamanders (*D. quadramaculatus*), were uncommon in mines with three, two, and one occurrences respectively.

All four species of reptiles, *Sceloporus undulatus* (Eastern Fence Lizard), *Eumeces fasciatus* (Common Five-lined Skink), *Coluber constrictor* (Northern Black Racer), and *Diadophis punctatus edwardsii* (Northern Ring-necked Snake) were accidentals.

Caves and their man-made counterparts, deep coal mines, are unique ecosystems that serve as refugia during winter as well as dry, hot periods in the summer for several species of amphibians and reptiles in West Virginia. Abandoned coal mines can also be an important habitat for some phases in the life histories of these species.

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FOOD HABITS OF THE CANDY DARTER, *ETHEOSTOMA OSBURNI* (HUBBS AND TRAUTMAN), IN THE CHERRY RIVER, WEST VIRGINIA

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ABSTRACT

Etheostoma osburni, the candy darter, is endemic to the lower New River drainage of West Virginia and Virginia, and Gauley River drainage of West Virginia. As a result of this endemism and evidence of declining population numbers, the species has been given the status of Special Concern in both states. In this investigation, candy darters were collected in the summer and fall of 1998 and spring of 1999 by backpack electroshocking for determination of seasonal food habits. Analysis of gut contents showed feeding activity to be high in the summer season and low in fall and spring seasons. The family Heptageniidae (mayflies) occupied the largest total percent volume within stomachs in summer and fall seasons. In the spring season, the family Baetidae (mayflies) was highest in volume. The families Baetidae and Chironomidae (midges) were found in all stomachs collected in the summer season. The family Heptageniidae occurred most frequently in fall season, while the family Baetidae was most common in stomachs from the spring season. Ivlev Electivity Index showed dipteran families Chironomidae and Tipulidae (craneflies) to have positive selection values in the summer season, indicative of active searching by candy darters for these food items. In the fall season, all families, with the exception of the family Chironomidae showed positive selection values. All benthic families taken from spring stomachs showed negative selection values, with the exception of family Baetidae. Overall studies on the food habits suggested that the candy darters are most likely opportunistic feeders.

INTRODUCTION

Etheostoma osburni, more commonly referred to as the candy darter, is a species endemic to West Virginia and Virginia (Jenkins and Kopia 1995). As a result of this endemism and evidence of declining numbers, *E. osburni* is federally recognized as a Species of Concern (SOC) (B. Tolin, pers. comm.), formerly Category 2 of the Federal Endangered Species List (Williams et al. 1989;Chipps 1992). The status of SOC means that the species may require protection under the Endangered Species Act. However, there are insufficient data pertaining to threats and population numbers to prepare a proposal for federal listing (B. Tolin, pers. comm.). In many West Virginia streams, siltation has been found a major threat to the habitat of the candy darter (Berkman and Rabeni 1987). The primary objective of this investigation was to determine the seasonal food habits of the candy darter in West Virginia.

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MATERIALS AND METHODS

FIELD STUDIES

COLLECTIONS

Candy darters were collected at

38°12'54"N, 80°30'18"W on the South Fork of the Cherry River in Richwood, West Virginia. Substrate consists primarily of shale and siltstone types of rock – small to large boulders mixed with cobble, pebbles, and sand. Usually, adult candy darters inhabited swift riffles and runs.

Fishes. Candy darters were collected on a seasonal basis using a backpack electroshock unit (AC current) with a seine, when water levels were high, or with D-shaped dip nets when water levels were minimal. Collections were made on 14 July 1998, 30 October 1998 and 23 April 1999. No collection was possible in winter due to impassible conditions leading to the study site. Darters were immediately placed on ice (until death) to prevent regurgitation, then fixed in 10% ethanol for preservation.

Benthos. Triplicate benthic samples were taken from the riffle areas with a Surber sampler. They were preserved in 70% ethanol and returned to the laboratory for identification to the lowest possible taxonomic level (Merritt and Cummins 1996; Peckarsky et al. 1990; Wiggins 1977).

WATER CHEMISTRY AND TEMPERATURE

On each collection date, the following water quality parameters were measured using a Hach Kit (Model AL-36B): dissolved oxygen (mg/L), carbon dioxide (mg/L), alkalinity (mg/L $CaCO_3$), total hardness (mg/L $CaCO_3$), free acidity (mg/L), and pH. Additionally, water temperature (°C) was measured with an instantaneous thermometer.

LABORATORY STUDIES

FOOD HABITS

Digestive tracts of the candy darters were excised from the esophagus to the beginning of the small intestine with microdissecting scissors, and then placed in 70% ethanol. Food items of each stomach were identified to the lowest possible taxonomic level with a Bausch and Lomb dissecting microscope using Merritt and Cummins (1996), Peckarsky et al. (1990), and Wiggins (1977) as taxonomic guides. The volume that each food item occupied was estimated according to a point method devised by Hynes (1950). In this method, each stomach is allocated a specific number of points based in fullness (distended =30, full =20, three-quarters full =15, half-full =10, one-quarter full =5, trace =2, empty =0). Point values awarded to each type of food item were used to calculate the following numbers: (1) percentage total volume = total number of points per given food items/total points awarded per season, and (2) percentage frequency of occurrence = number of stomachs in which an item appeared/total number of stomachs per season.

IVLEV ELECTIVITY INDEX (FORAGE RATIOS)

A comparison of feeding habits of the candy darter with the availability of potential food resources in the natural habitat was calculated by the Ivlev Index of Electivity (Ivlev 1961):

$$E = r_i - p_i / r_i + p_i$$

where E = measure of Electivity, r_i = the relative abundance of prey item in the gut, and p_i = the relative abundance of the same prey in the environment (Surber samples). The percentage or proportion of the relative abundance of prey items in the gut and in the environment was calculated. Food items with one percent or more were given an Index of Electivity. The index has a possible range of -1 to +1; positive values indicate active selection of prey items by the predator, negative values indicate avoidance or inaccessibility of prey items to the predator, and zero values indicate random selection of prey items from the environment.

RESULTS AND DISCUSSION

FIELD STUDIES

TEMPERATURE

Temperature data of the South Fork of the Cherry River suggested that this portion of the river is a cold water habitat. During midafternoon in July the water temperature was measured at 19°C, whereas in October and April the temperature was 11°C (Table 1).

WATER CHEMISTRY

Water quality data collected during this study are recorded in Table 1 (Schoolcraft 1999). These data are typical of most other Appalachian mountain streams (i.e. cold, sterile, and low buffering capacity).

FOOD HABITS

SUMMER (14 JULY 1998)
HYNES POINT METHOD

Percent total volume calculations showed that candy darter stomachs contained mostly mayflies (Figure 1). The mayfly families Heptageniidae and Baetidae represented 26 and 22%, total volume, respectively. The caddisfly family Rhyacophilidae had the third highest value (21%). All other families in this season had percentages less than 15.

When calculating percent frequency of occurrence values, the mayfly family Baetidae and the dipteran family Chironomidae were found to have occurred in 100% of all stomachs for the season. Perhaps these are preferred food items of candy darters, or they may occur in such high numbers in the stream that little effort must be put forth to find them. The families Heptageniidae (mayflies), Hydropsychidae (caddisflies), and Simuliidae (black flies) were all found in over 70% of the stomachs. The other represented families occurred in less than 35% of all stomachs.

Adult candy darters have been found to consume immature benthic organisms (Jenkins and Kopia 1995) including mayfly and caddisfly larvae, as well as chironomid midges and water mites (Jenkins and Burkhead 1994).

IVLEV ELECTIVITY INDEX (FORAGE RATIOS)

A value is a ratio of items in the gut to items in the environment - in this case, the Surber sample. A negative value indicates that a predator (i.e. candy darter) is not actively searching for an item of prey. In this study, all families, with the exception of Chironomidae, Tipulidae and Hydropsychidae, exhibited values that indicate negative selection (Figure 2). Chironomid midges had the highest value (0.7), while Hydropsychidae had a value less than 0.1. This value, approaching 0, may indicate that members of this family are chosen at random as a source of food by the candy darter. The caddisfly family Glossosomatidae had the lowest value (<-0.9), suggesting that these caddisflies may be inaccessible to the fish. Indeed, these caddisflies build cases of rocks and/or vegetable matter, out of which they are rarely found (Wiggins 1977). The dipteran family Tipulidae, which occurred in stomachs often or in high volumes, may have a high electivity index due to the organism's requirement of atmospheric oxygen. To maintain proper oxygen levels, tipulids must stay just below the surface of the water, making , making them very accessible to the fish (Merritt and Cummins, 1996).

FALL (30 OCTOBER 1998)
HYNES POINT METHOD
The family Heptageniidae was, as in July, the dominant family by percent total volume with 37% (Fig. 3). The family Hydropsychidae, however, had the second highest value of 34%. All other represented families contained within stomachs of this season, obtained values less than 5%.
The family Heptageniidae also had the highest percent frequency of occurrence (65%), while the family Hydropsychidae was found in 47% of the stomachs for the fall season. The family Tipulidae occurred in 23% of stomachs and all other families were found in abundance of less than 20%. Because feeding activity is reduced in the fall, the families Heptageniidae and Hydropsychidae may be more suited to colder temperatures than other representative families. These families may occur in greater relative abundance and become a more easily obtainable food source for candy darters. However, results were based on relatively few individuals and thus, may be misleading.

IVLEV ELECTIVITY INICES (FORAGE RATIOS)
Values of Ivlev's Electivity Index for October stomachs showed different values than in July. Chironomidae was the only family found to have a negative value (Fig. 4). All other families had forage ratios of 0.4 or greater. These results reiterate the assumption that benthic organisms are found in less abundance due to colder temperatures (and perhaps other physicochemical parameters), causing the candy darters to become active searchers for food.

SPRING (23 APRIL 1999)
HYNES POINT METHOD
In April, the percent total volumes indicated that mayflies accounted for the most space occupied in candy darter stomachs (Figure 5). The families Baetidae and Heptageniidae were found in total volumes of 58% and 33%, respectively. Three other families, Chironomidae, Ephemerellidae (mayflies), and Perlidae (stoneflies), occupied total volumes of less than one percent each.

Percent frequency of occurrence showed that the famlies Baetidae and Heptageniidae were in 88% and 75% of all stomachs, respectively. Nineteen percent of stomachs contained chironomid midges while less than 10% had families Ephemerellidae or Perlidae.

IVLEV ELECTIVITY INDEX (FORAGE RATIOS)
Forage ratios determined by the Ivlev Electivity Index indicated that the only family potentially being actively pursued for was Baetidae (mayflies) (Figure 6). The family Heptageniidae was just on the negative side of the Ivlev's scale, indicating that these benthic organisms may have been chosen at random. All other represented families had values less than -0.4, indicating that they may be inaccessible to candy darters.

CONCLUSIONS

Due to the paucity of dietary information on the candy darter, this investigation focused on the food habits of the species. Candy darters were collected in the summer and fall seasons of 1998 and spring season of 1999 from the South Fork of the Cherry River in Nicholas County, West Virginia. Food habit study showed that candy darters consumed mostly mayflies. Percentages of total values of benthic organisms determined by the Hynes point method showed that stomachs of candy darters collected in the summer season contained mostly the mayfly families Heptageniidae (26%) and Baetidae (22%). In the fall season, family Heptageniidae occupied the greatest volume (37%) in stomachs. In the spring season, families Baetidae (58%) and Heptageniidae (33%) were the dominant benthic organisms by volume. Percent frequency of occurrence calculations, also determined by the Hynes point method, showed the families Baetidae and Chironomidae to occur in 100% of stomachs from the summer collection. Sixty-five percent of stomachs from fall contained family Heptageniidae, while 47% had the caddisfly family Hydropsychidae. High percentages of stomachs contained families Baetidae (88%) and Heptageniidae (75%) during the spring.

Forage ratios were determined by Ivlev's Electivity Index. The families Hydropsychiidae, Chironomidae, and Tipulidae exhibited positive selection values in the summer, meaning that these organisms were easily obtainable or actively pursued for by candy darters. In the fall season, all families except Chironomidae had positive values, while in the spring, only family Baetidae was found to have a positive selection value.

ACKNOWLEDGMENTS

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Table 1. Water quality data from the South Fork of the Cherry River for the three seasons of *Etheostoma osburni* collections.

Parameter	14 July 1998	30 October 1998	23 April 1999
Dissolved O_2 (mg/L)	9	8	9
CO_2 (mg/L)	10	5	5
Free Acidity (mg/L)	0	0	0
Alkalinity (mg/L $CaCO_3$)	17.1	17.1	17.1
Hardness (mg/L $CaCO_3$)	34.2	34.2	34.2
pH	7.0	8.0	7.1
Temperature (°C)	19	11	11

Figure 1. Percent total volume of benthic organisms found within stomachs of *Etheostoma osburni* collected on 14 July 1998 (Summer) from the South Fork of the Cherry River, Nicholas County, West Virginia.

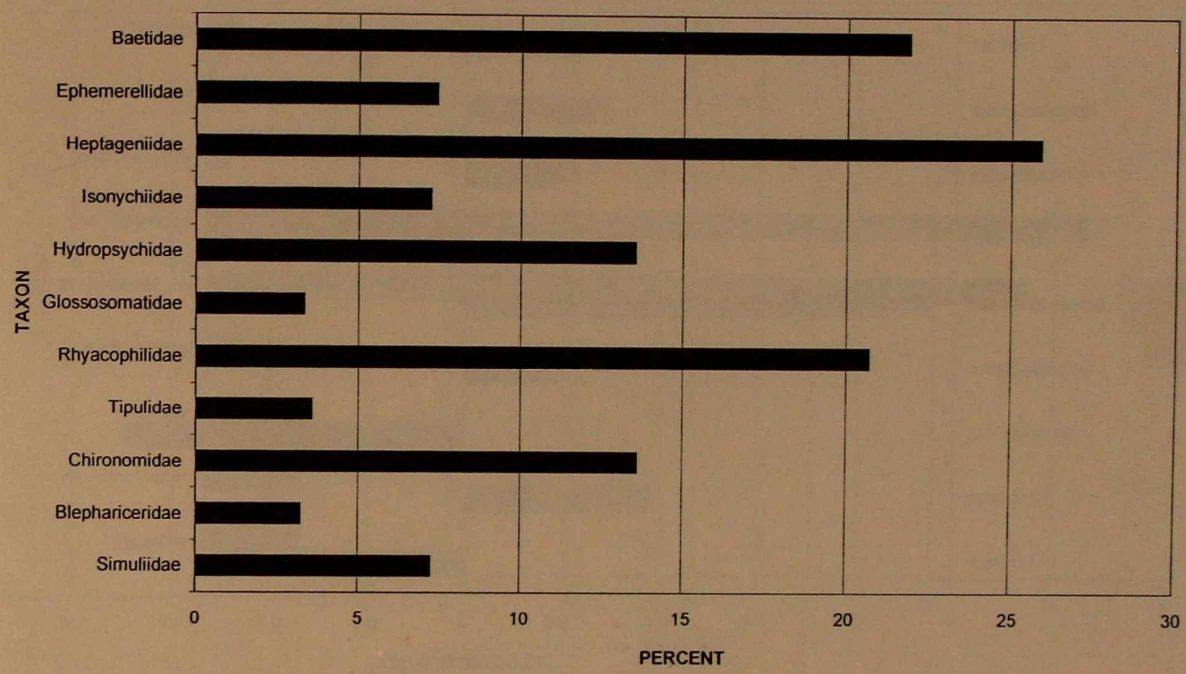


Figure 2. Forage ratios determined by the Ivlev Electivity Index for stomach content analyses of *Etheostoma osburni* collected on 14 July 1998 (Summer) from the South Fork of the Cherry River, Nicholas County, West Virginia.

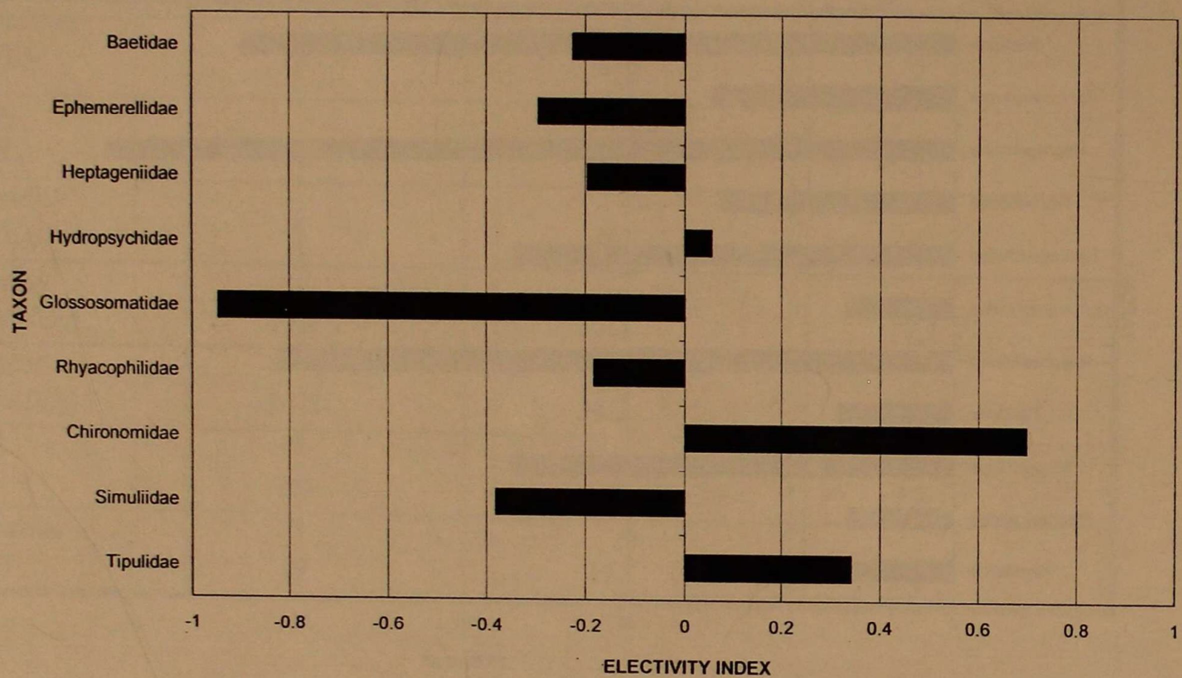


Figure 3. Percent total volume of benthic organisms found within stomachs of *Etheostoma osburni* collected on 30 October 1998 (Fall) from the South Fork of the Cherry River, Nicholas County, West Virginia.

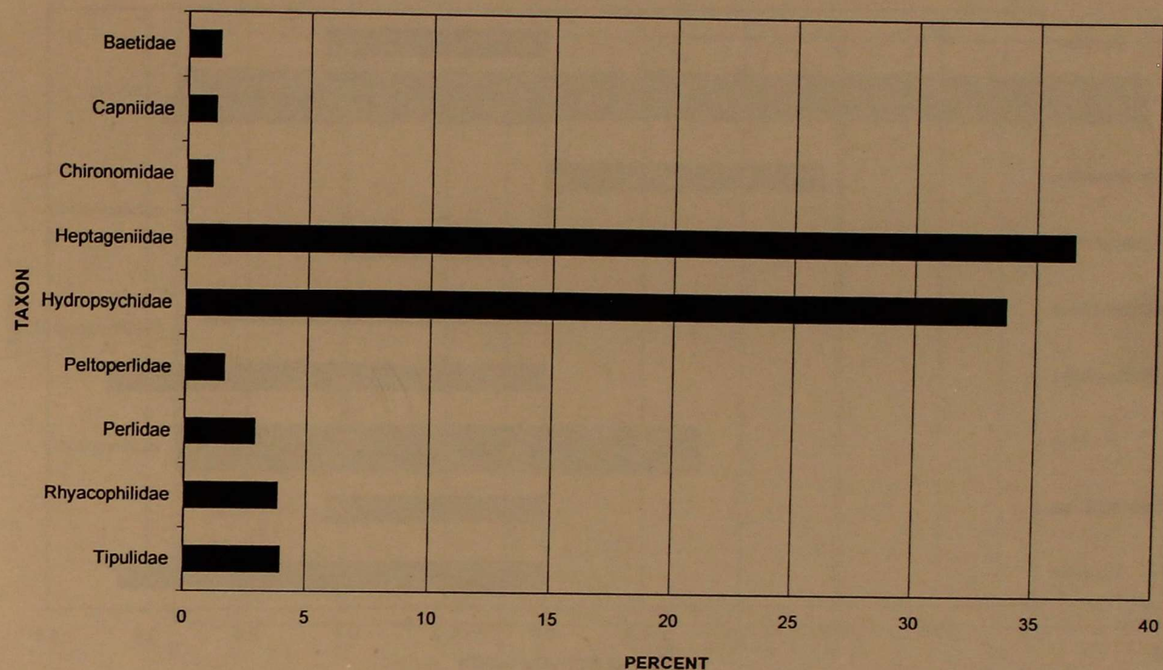


Figure 4. Forage ratios determined by Ivlev Electivity Index for stomach content analyses of *Etheostoma osburni* collected on 30 October 1998 (Fall) from the South Fork of the Cherry River, Nicholas County, West Virginia.

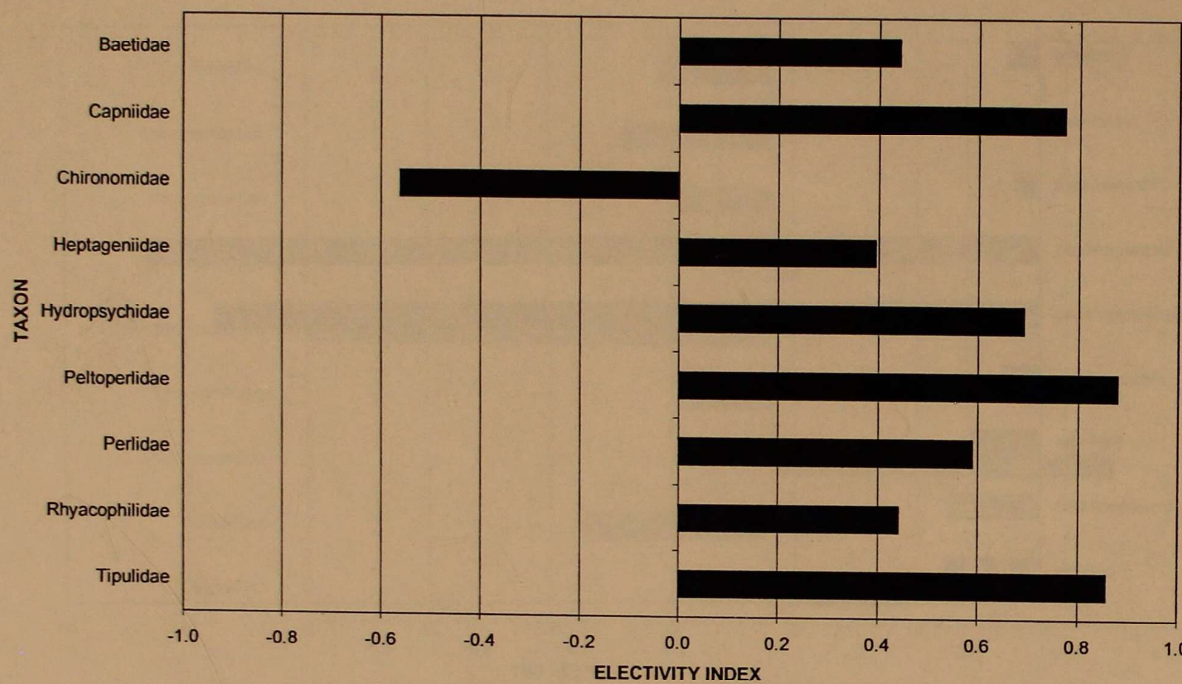


Figure 5. Percent total volume of benthic organisms found within stomachs of *Etheostoma osburni* collected on 23 April 1999 (Spring) from the South Fork of the Cherry River, Nicholas County, West Virginia.

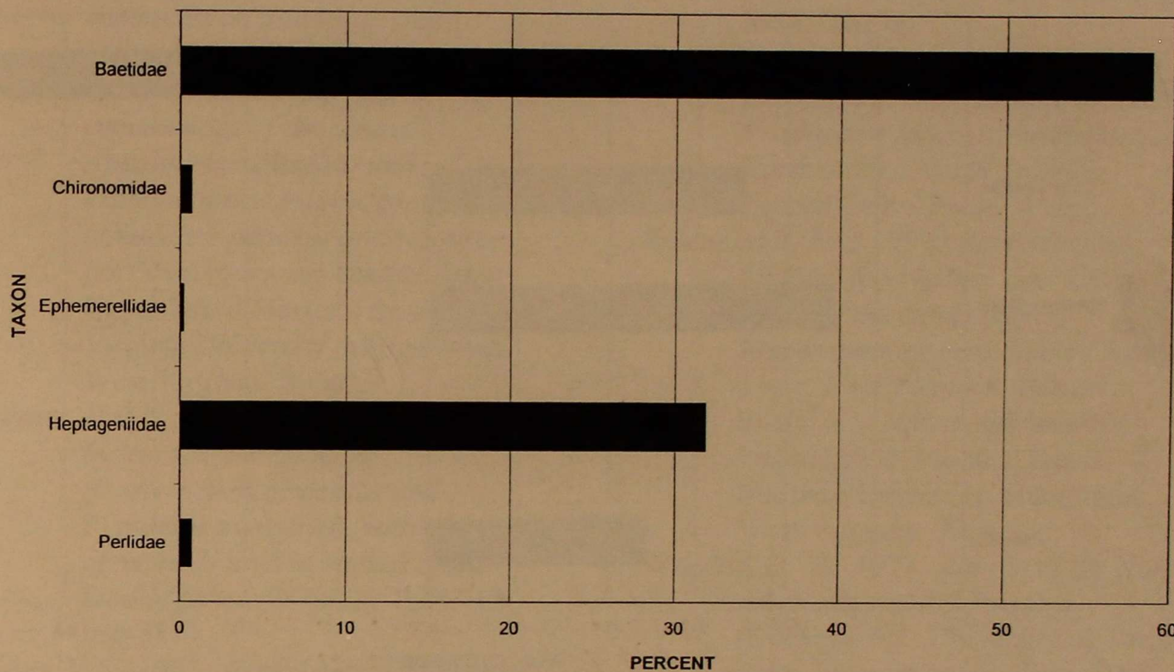
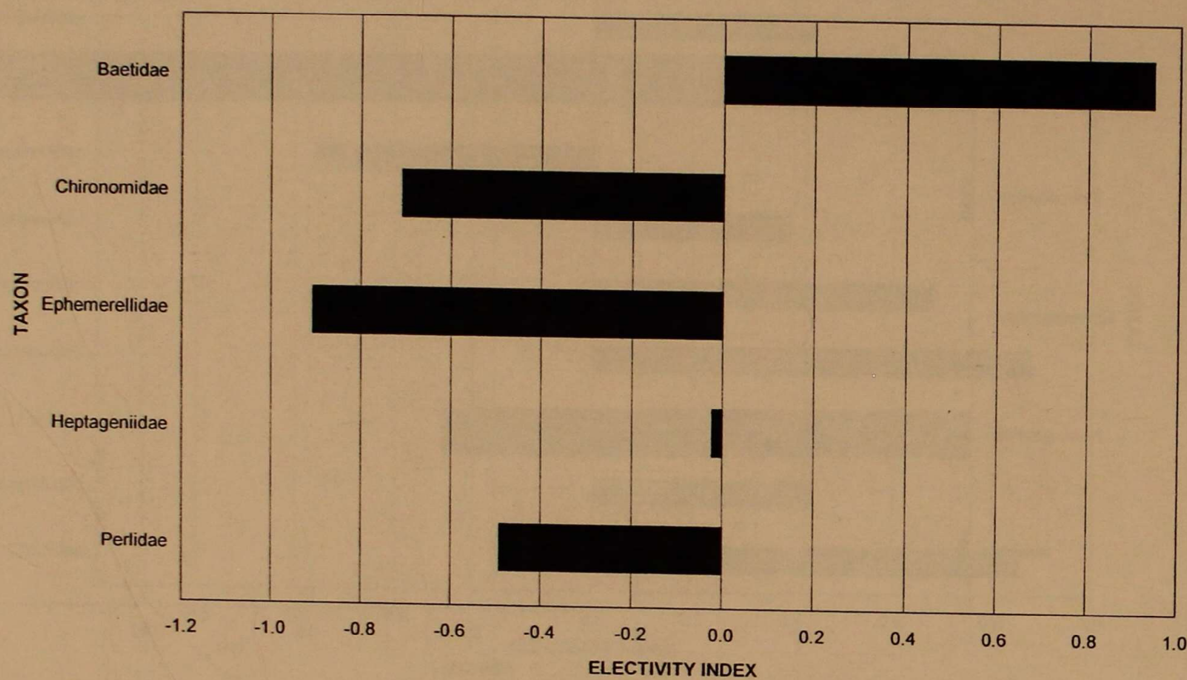


Figure 6. Forage ratios detremined by the Ivlev Electivity Index for stomach content analyses of *Etheostoma osburni* collected on 23 April 1999 (Spring) from the South Fork of the Cherry River, Nicholas County, West Virginia.



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AMPHIBIANS OF THE UPLAND HABITATS WITHIN THE NEW RIVER GORGE NATIONAL RIVER, WEST VIRGINIA

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ABSTRACT

Amphibian surveys were conducted in the New River Gorge National River (NERI) between March 1989 and December 1992. Various techniques, including visual encounter surveys, auditory sampling, pitfall traps, and dip nets were used to determine the presence of amphibian species in the NERI. The objectives of this study were to determine amphibian species composition and richness, including documentation of species of special concern, found in upland habitats of the NERI. Thirty amphibian species, including 21 salamander species and nine anuran species, were recorded at 723 sites out of 1134 sites examined in the NERI. The NERI contains 61.8% of salamander and 64.3% of anuran species found in all of West Virginia. Six amphibian species of special concern were found in the NERI including *Ambystoma jeffersonianum* (Jefferson Salamander), *Aneides aeneus* (Green Salamander), *Desmognathus quadramaculatus* (Black-bellied Salamander), *Eurycea lucifuga* (Cave Salamander), *Pseudotriton montanus diastictus* (Midland Mud Salamander), and *Pseudotriton ruber ruber* (Northern Red Salamander). It is crucial that these species are protected through established conservation measures so that they continue to exist in the NERI. The National Park Service has maintained road puddles and roadside ditches, erected gates and/or placed large boulders at the entrance of mine portals, and has reduced the amount of recreational use in and around sensitive habitat areas, including rock faces, emergent rocks, and wetlands. These conservation measures will help conserve amphibian and reptile species diversity in the NERI.

INTRODUCTION

With significantly more species at risk for extinction than any other vertebrate group, reptiles and amphibians are among the most threatened groups worldwide (Gardner et al., 2007; IUCN, 2006). Habitat change is the primary cause of population declines for amphibian species and many researchers agree that this is true for the loss of biodiversity throughout the world (Gardner et al., 2007; Sala et al., 2000). In order to establish management plans for amphibian species, the need to conduct herpetofaunal searches to determine species richness and to assess the stability of populations is essential; however, without prior knowledge of the reptile and amphibian species richness of a particular geographic area, the establishment and implementation of conservation management plans would be ineffective.

The New River Gorge National River (NERI) is a free-flowing, rugged whitewater, northward-flowing river located in Fayette, Raleigh, and Summers counties in southern West Virginia (Figure 1). Specifically, the NERI extends from the Bluestone Dam in Hinton, West Virginia (Summers County) northward to the New

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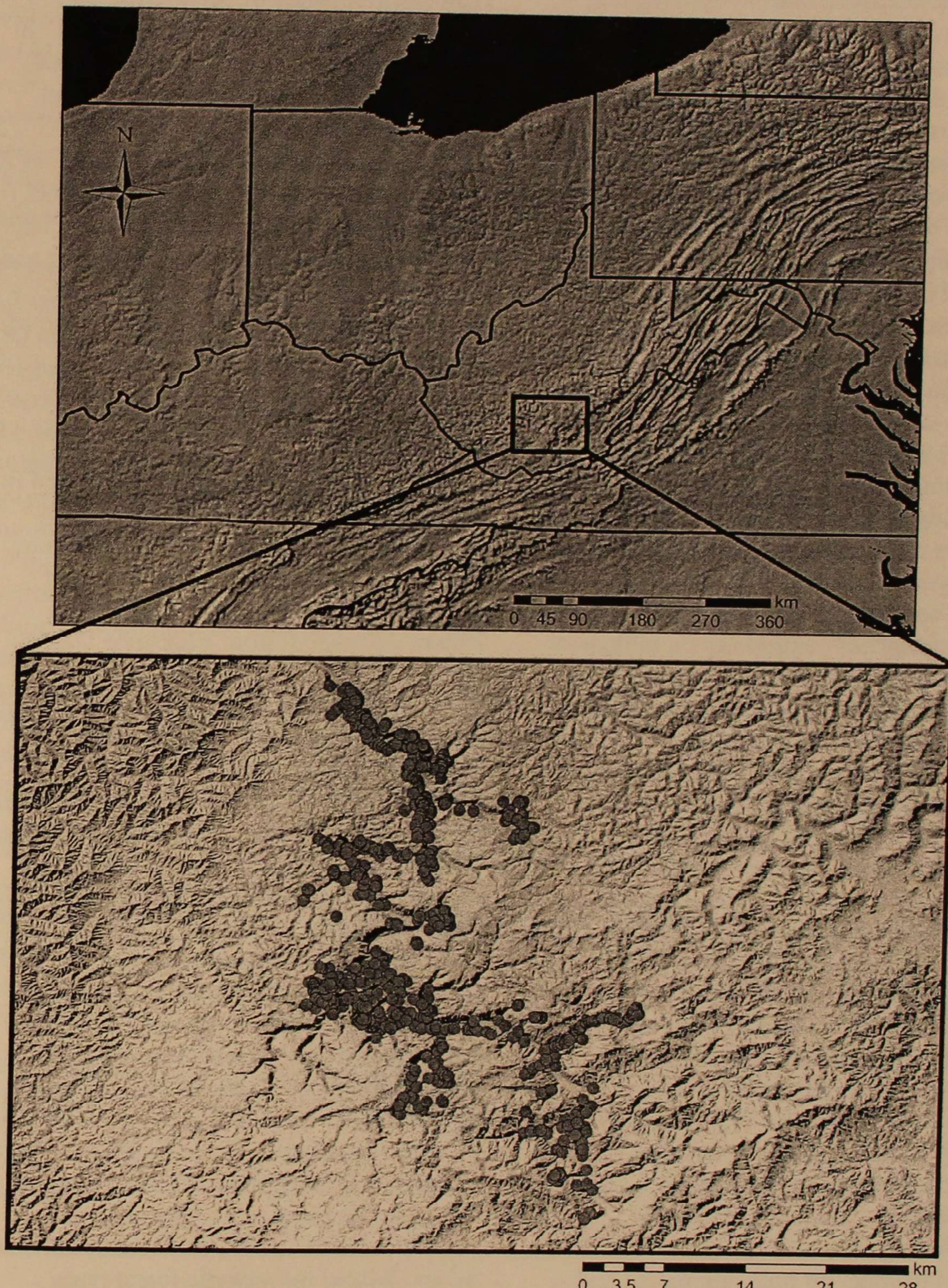

Figure 1. Location of the New River Gorge National River in West Virginia. The lower map is the distribution of the survey sites in the New River Gorge National River in which amphibians were discovered.

River Bridge in Fayetteville, West Virginia (Fayette County). Included within the NERI are more than 85 km of waterway that dissects the Allegheny Plateau. Because of the northward-flowing nature and bisection of this physiographic province, the NERI acts as a corridor for southern species to move into available habitats in West Virginia (Core, 1966).

The NERI is an especially interesting region due to its long and varied geological history. The New River is one of the oldest waterways in North America and over millions of years the Appalachian Mountains have been altered by uplifting and subsequent erosion. Upland regions of NERI provide a rich diversity of habitats for both reptiles and amphibians, many of which are species of special concern (i.e., uncommon species or species that are declining throughout major portions of their range). In addition to serving as a refuge for some species, NERI provides a geographical barrier that may limit the east-west distribution of other species. The ranges of several Appalachian salamanders extend into NERI where they are replaced by closely related sibling species on the western side of the New River (i.e., *Plethodon glutinosus* and *Plethodon kentucki*) (Montani and Pauley, 1992; Bailey and Pauley, 1991; Jewell and Pauley, 1991; Highton, 1972). These specialized cases of distribution where species ranges meet but have little or no overlap are especially interesting because they provide a unique opportunity to study species interactions, including competition and predation, as well as current and historical biogeography.

The upland regions of NERI provide a rich diversity of habitats for both amphibian and reptile species. Until this study, little information was available on amphibian species found in the NERI. Furthermore, Grafton and Grafton (1988) reported that little information was available on species richness, frequency of occurrence, and distribution of vertebrates in the NERI. In light of this, the purpose of this study was to: (1) determine amphibian species that occur in the upland habitats of the NERI; (2) survey as many sites as possible in the NERI throughout every season from 1989-1992 for amphibian species; (3) identify amphibian species of special concern in the NERI; and (4) assess amphibian fauna and provide information relevant to the conservation of natural resources for the NERI.

MATERIALS AND METHODS

STUDY AREA

Herpetofaunal surveys were conducted in the NERI from 1989 through 1992; only amphibian species data are presented here. The NERI includes more than 85 km of waterways and encompasses more than 292km^2 extending from Bluestone Dam in Hinton, West Virginia northward to New River Bridge located just north of the United States Highway Route 19 at Fayetteville, West Virginia (National Park Service, 2006). The NERI falls nearly 228.6m in the 80.5 km from Bluestone Dam to New River Bridge and consists of a 304.8m deep, 1.6 km wide gorge (National Park Service, 2006) (Figure 1).

We considered upland habitats to be the area located from the riparian zone to the rim of the canyon; surveys were conducted in the floodplains and riparian zones, but these data are not presented here. Major upland habitat types found in the NERI include vernal pools, roadside ditches, first-, second-, and third-order streams, wetlands, mixed deciduous forests, quarries, rock outcrops, roadrut pools, and abandoned coal mines. Mixed mesophytic forest types include oak-hickory, mixed oak, oak-maple, oak-yellow pine, hemlock-hardwoods, northern hardwoods, cove hardwoods, and bottomland and floodplain hardwoods (National Park Service 2006). Diverse vegetation types occur within the NERI and some common tree species include: *Acer saccharum* (Sugar Maple), *Acer rubrum* (Red Maple), *Fagus grandifolia* (American Beech), *Tilia americana* (American Basswood), *Pinus strobus* (White Pine), *Quercus rubra* (Red Oak), *Quercus alba* (White Oak), and *Liriodendron tulipifera* (Yellow-Poplar).

SURVEY METHODS

All voucher specimens collected from the NERI are maintained in the West Virginia Biological Survey (WVBS) at Marshall University, Huntington, West Virginia. Due to the sensitive nature of the amphibian populations in the NERI, exact localities of surveys are not published. All scientific and common names were taken from Crother (2000).

Terrestrial surveys were conducted by walking through habitats turning cover objects with a four-pronged potato rake, and capturing specimens by hand. Because amphibians are nocturnal, surveys were conducted at night using flashlights. Typically, nocturnal surveys were conducted beginning approximately 30 minutes after dark and continued until midnight during rainfall or within 24 hours of a rainfall. Either one-gallon or five-gallon pitfall traps were buried in selected habitats; in some cases, drift fences were used in association with pitfall traps.

Aquatic surveys were conducted in both lentic and lotic habitats; lentic habitats included vernal pools, permanent pools, road puddles, and roadside ditches and lotic habitats included first-, second-, and third-order streams, springs, and seeps. Surveys for adult and juvenile salamanders were conducted in streams and seeps by flipping cover objects and capturing individuals by hand. We surveyed for salamander larvae in small gravel beds and sandy pools with tea strainers and small aquarium dip nets. Within streams, funnel traps were used to survey for all life stages of salamanders. Anuran larvae were sampled by sweeping a dip net through ponds and road puddles; after identification, larvae were returned to the place of collection.

Table 1. Total number of sites surveyed for upland amphibian species in the New River Gorge (1989-1992).

Year	Number of Sites with Amphibians	Total Number of Sites Surveyed
1989	100	147
1990	77	172
1991	246	351
1992	300	464
Total	**723**	**1134**

RESULTS

A total of 1134 sites was surveyed within the NERI between March 1989 and December 1992 (Table 1). Within those sites, amphibian species were found at 723 sites (63.8%). There are 34 salamander and 14 anuran species known to occur in West Virginia (WVDNR, 2003). Of these, 21 salamander and nine anuran species were found in the NERI; our surveys indicated that at least 62.8% of salamander and 64.3% of anuran species found in West Virginia occur in the NERI (Table 2). We documented six species of special concern in the NERI including *Ambystoma jeffersonianum* (Jefferson Salamander), *Aneides aeneus* (Green Salamander), *Desmognathus quadramaculatus* (Black-bellied Salamander), *Eurycea lucifuga* (Cave Salamander), *Pseudotriton montanus diastictus* (Midland Mud Salamander), and *Pseudotriton ruber ruber* (Northern Red Salamander) (Table 3). Nine new county records for salamanders were documented in the NERI including *Ambystoma jeffersonianum* (Jefferson Salamander; Fayette County), *Desmognathus ochrophaeus* (Allegheny Mountain Dusky Salamander; Fayette County), *Plethodon kentucki* (Cumberland Plateau Salamander; Fayette and Raleigh counties), *Plethodon wehrlei* (Wehrle's Salamander; Fayette County), *Hemidactylium scutatum* (Four-toed Salamander; Fayette County), *Pseudotriton montanus diastictus* (Midland Mud Salamander; Fayette County), *Aneides aeneus* (Green Salamander; Summers County), *Eurycea lucifuga* (Cave Salamander; Fayette County).

Based on known distributions (Green and Pauley 1987), we determined the potential number of amphibian species that may be present in the NERI and compared that with the number of species that was actually found. We documented 20 of the 22 salamander species (90.9%) and nine of the nine anuran species (100%) thought to occur in the NERI (Table 4).

DISCUSSION

To date, this research provides the most comprehensive list of amphibian species in the NERI (Table 5). We now know that more than 60% of West Virginia's amphibian species occur in habitats within the NERI. The amphibian surveys that took place in the NERI increased the number of documented amphibian species in Fayette, Raleigh, and Summers counties in West Virginia. Furthermore, six species of special concern were found within the NERI and conservation of their habitats, as well as population monitoring, should continue.

Habitats, such as those found in the NERI and in most areas throughout West Virginia, have been previously disturbed by timbering, coal mining, recreation, and other forms of habitat degradation; however, while not supporting these destructive activities, it is noteworthy that some of these areas provide unique habitats for various vertebrate species. Even though many of these disturbed habitats will eventually disappear due to secondary succession, puddles in old railroads and haul roads and ditches along the sides of roads provide breeding habitats for several amphibian species; vernal pools in old strip mine sites are commonly used by amphibians during both breeding and nonbreeding seasons; old field areas in strip mine sites and other disturbed areas provide habitat for several herpetofaunal species and mammals; and abandoned deep mines have provided habitats for several salamander species including *Eurycea lucifuga*, and some small mammals (i.e., *Neotoma floridana*).

Managed areas, where human influence may directly affect species composition, should be a priority in the conservation of amphibian and reptile species diversity

Table 2. Amphibian species found during surveys conducted between 1989 and 1992 in the New River Gorge National River. Bold font indicates a species of special concern.

Salamander Species	Common Name
***Ambystoma jeffersonianum* (Green, 1827)**	**Jefferson Salamander**
Ambystoma maculatum (Shaw, 1802)	Spotted Salamander
***Aneides aeneus* (Cope and Packard, 1881)**	**Green Salamander**
Desmognathus fuscus (Green, 1818)	Northern Dusky Salamander
Desmognathus monticola Dunn, 1916	Seal Salamander
Desmognathus ochrophaeus Cope, 1859	Allegheny Mountain Dusky Salamander
***Desmognathus quadramaculatus* (Holbrook, 1840)**	**Black-bellied Salamander**
Eurycea cirrigera (Green, 1830)	Southern Two-lined Salamander
Eurycea l. longicauda (Green, 1818)	Long-tailed Salamander
***Eurycea lucifuga* Rafinesque, 1822**	**Cave Salamander**
Gyrinophilus p. porphyriticus (Green, 1827)	Northern Spring Salamander
Hemidactylium scutatum (Schlegel, 1838)	Four-toed Salamander
Notophthalmus v. viridescens (Rafinesque, 1820)	Red-spotted Newt
Plethodon cinereus (Green, 1818)	Eastern Red-backed Salamander
Plethodon glutinosus (Green, 1818)	Northern Slimy Salamander
Plethodon kentucki Mittleman, 1951	Cumberland Plateau Salamander
Plethodon richmondi Netting and Mittleman, 1938	Southern Ravine Salamander
Plethodon wehrlei Fowler and Dunn, 1917	Wehrle's Salamander
***Pseudotriton montanus diastictus* Bishop, 1941**	**Midland Mud Salamander**
***Pseudotriton r. ruber* (Latreille, 1801)**	**Northern Red Salamander**
Anuran Species	
Bufo a. americanus Holbrook, 1836	Eastern American Toad
Bufo fowleri Hinckley, 1882	Fowler's Toad
Hyla versicolor/chrysoscelis Cope, 1880	Gray/Cope's Gray Treefrog
Pseudacris brachyphona (Cope, 1889)	Mountain Chorus Frog
Pseudacris c. crucifer (Wied-Neuwied, 1838)	Northern Spring Peeper
Rana catesbeiana Shaw, 1802	American Bullfrog
Rana clamitans melanota Rafinesque, 1820	Northern Green Frog
Rana palustris LeConte, 1825	Pickerel Frog
Rana sylvatica LeConte, 1825	Wood Frog

Table 3. Species of special conern discovered during amphibian surveys in the New River Gorge National River. Descriptions of the ranks are listed below.

Species	Common Name	State Rank*	Global Rank**
Ambystoma jeffersonianum (Green, 1827)	Jefferson Salamander	S3	G4
Aneides aeneus (Cope and Packard, 1881)	Green Salamander	S3	G3G4
Desmognathus quadramaculatus (Holbrook, 1840)	Black-bellied Salamander	S3	G5
Eurycea lucifuga Rafinesque, 1822	Cave Salamander	S3	G5
Pseudotriton montanus diastictus Bishop, 1941	Midland Mud Salamander	S1	G5T5
Pseudotriton r. ruber (Latreille, 1801)	Northern Red Salamander	S3	G5

*S1 - Five or fewer documented occurrences, or very few remaining individuals within the state. Extremely rare and critically imperiled or because of some factor(s) making it especially vulnerable to extirpation; S3 - twenty-one to 100 documented occurrences. May be somewhat vulnerable to extirpation (http://www.wvdnr.gov).

**G3 - Twenty-one to 100 documented occurrences; either very rare and local throughout its range or found locally in a restricted range; vulnerable to extinction. G4 - Common and apparently secure globally, though it may be rare in parts of its range, especially at the periphery. G5 - Very common and demonstrably secure, though it may be rare in parts of its range, especially at the periphery. G5T5 - The species is very common and demonstrably secure, though it may be rare in parts of its range, especially at the periphery and T5 indicates that the subspecies is critically imperiled (http://www.wvdnr.gov).

Table 4. Amphibian species that potentially could be found within the NERI. Species discovered during the 1989-1992 surveys are indicated with an "X". This species list was made using current, known ranges for each amphibian species known to occur in West Virginia.

Scientific Name	Common Name	Found in the NERI
Salamander Species		
Ambystoma jeffersonianum	Jefferson Salamander	X
Ambystoma maculatum	Spotted Salamander	X
Ambystoma opacum	Marbled Salamander	
Notophthalmus v. viridescens	Eastern Red-spotted Newt	X
Desmognathus fuscus	Northern Dusky Salamander	X
Desmognathus monticola	Seal Salamander	X
Desmognathus ochrophaeus	Allegheny Mountain Dusky Salamander	X
Desmognathus quadramaculatus	Black-bellied Salamander	X
Plethodon cinereus	Red-backed Salamander	X
Plethodon richmondi	Ravine Salamander	X
Plethodon glutinosus	Northern Slimy Salamander	X
Plethodon hoffmani	Valley and Ridge Salamander	
Plethodon kentucki	Cumberland Plateau Salamander	X
Plethodon wehrlei	Wehrle's Salamander	X
Hemidactylium scutatum	Four-toed Salamander	X
Gyrinophilus p. porphyriticus	Northern Spring Salamander	X
Pseudotriton montanus diastictus	Midland Mud Salamander	X
Pseudotriton r. ruber	Northern Red Salamander	X
Aneides aeneus	Green Salamander	X
Eurycea cirrigera	Southern Two-lined Salamander	X
Eurycea l. longicauda	Long-tailed Salamander	X
Eurycea lucifuga	Cave Salamander	X
Anuran Species		
Bufo a. americanus	American Toad	X
Bufo fowleri	Fowler's Toad	X
Pseudacris brachyphona	Mountain Chorus Frog	X
Pseudacris c. crucifer	Northern Spring Peeper	X
Hyla versicolor/chrysoscelis	Gray/Cope's Gray Treefrog	X
Rana catesbeiana	American Bullfrog	X
Rana clamitans melanota	Green Frog	X
Rana sylvatica	Wood Frog	X
Rana palustris	Pickerel Frog	X

Table 5. A complied list of the observed amphibian species by habitat type in the New River Gorge National River. This list may not be a complete list as we acknowledge that some species may be found in one or more habitat types other than what is listed here.

Habitat	Species Observed
Vernal Pool	*Pseudacris brachyphona*
	Pseudacris c. crucifer
	Rana catesbeiana (subadult)
	Rana clamitans melanota
	Rana palustris
	Rana sylvatica
	Eurycea cirrigera (permanent pond only)
	Ambystoma jeffersonianum
	Ambystoma maculatum
Roadside Ditch	*Bufo a. americanus*
	Bufo fowleri
	Pseudacris brachyphona
	Rana sylvatica
	Rana clamitans melanota
	Rana catesbeiana
	Hemidactylium scutatum
	Ambystoma jeffersonianum
	Ambystoma maculatum
	Notophthalmus v. viridescens
Stream	*Rana sylvatica*
	Rana clamitans melanota
	Desmognathus fuscus
	Desmognathus monticola
	Desmognathus ochrophaeus
	Desmognathus quadramaculatus
	Eurycea cirrigera
	Eurycea l. longicauda
	Gyrinophilus p. porphyriticus
	Pseudotriton r. ruber
Wetlands	*Pseudotriton montanus diastictus*
	Ambystoma jeffersonianum
	Ambystoma maculatum
	Eurycea cirrigera
	Bufo a. americanus
	Bufo fowleri
	Rana clamitans melanota
	Rana sylvatica
	Pseudacris c. crucifer
	Rana catesbeiana
	Notophthalmus v. viridescens

Table 5 *continued.*

Mixed Deciduous Forest	*Hemidactylium scutatum*
	Plethodon cinereus
	Plethodon glutinosus
	Plethodon kentucki
	Plethodon richmondi
	Plethodon wehrlei
Quarry	*Bufo fowleri*
Rock Outcrop	*Aneides aeneus*
	Plethodon kentucki
	Plethodon glutinosus
Roadrut Pool	*Bufo a. americanus*
	Pseudacris brachyphona
	Bufo fowleri
	Notophthalmus v. viridescens
Abandoned Mines	*Eurycea lucifuga*
	Gyrinophilus p. porphyriticus
	Desmognathus monticola
	Eurycea cirrigera
	Eurycea l. longicauda
	Plethodon glutinosus
	Desmognathus fuscus
	Desmognathus ochrophaeus
	Plethodon kentucki
	Bufo a. americanus
	Pseudacris c. crucifer
	Desmognathus quadramaculatus
	Rana clamitans melanota
	Notophthalmus v. viridescens
	Plethodon wehrlei
	Rana palustris

(Meyers and Pike, 2006). In light of the importance of these unique habitats, the National Park Service (NPS) has taken steps to ensure the conservation of amphibian species throughout the NERI. Roadrut pools and roadside ditches are now preserved in order to allow amphibian species to use these habitats during breeding and nonbreeding seasons. Abandoned deep mine portals have been gated and/or large boulders put in the entrance to allow utilization of the habitat by small vertebrates while preventing public access. Conservation of amphibian and reptile species diversity is essential, given the current conservation status of these species worldwide.

Future research in the NERI, as well as throughout West Virginia, should include ecological studies of herpetofaunal species, especially species of special concern. Detailed studies of all aspects of biology and ecology of critical habitats including, but not limited to, emergent rocks, rock outcrops, streams, road puddles, and other wetlands should be conducted. These baseline inventories when combined with subsequent monitoring can be used to detect and evaluate spatial and temporal trends in the richness and abundance of both amphibian and reptile species (Tuberville et al., 2005).

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THE LAND SNAILS OF NEW RIVER GORGE, WEST VIRGINIA

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ABSTRACT

The Land Snails of New River Gorge Scenic River (NRG) in southern West Virginia were surveyed during the spring and summer of 2003. The gorge is approximately 80 km long and lies roughly between the towns of Hinton and Fayetteville WV. Twenty-five locations throughout the Gorge were selected to represent a variety of elevations and habitat types. Snails were collected using random search methods. The snails were then returned to the lab, cleaned and identified to species. Specimens representing 48 species were found. *Mesomphix rugeli* is reported herein as a first record for the state. All other species were previously known to be residents of the state. A rather large population of the small (<10 mm greatest length) tropical invasive species *Opeas pyrgula* was found on a hillside in the small village of Thurmond WV.

INTRODUCTION

Publications on the land snails of West Virginia are fairly rare and those that are available are quite old. G. K. MacMillan (1949) published "The land snails of West Virginia". Stanley Brooks (1935), Brooks and Kutchka (1937), Brooks and Kutchka (1938), Briscoe (1963), Hubricht (1972) and Taylor and Counts (1976) have all made contributions to the understanding of West Virginia land snail populations. Les Hubricht's (1985), "The Distribution of the Native Land Mollusks of the Eastern United States", is always a valuable tool for anyone doing field studies on land snails of the eastern United States.

Twenty years ago I had collected *Punctum smithi* in NRG. To this date, that is the only known locality for the state. I approached the state WVDNR Natural Heritage Program about attempting to determine the current status of *P. smithi* in the park and during the conversations that followed it was decided that I should look at the entire snail population of the NRG.

METHOD

During the summer of 2003, twenty-five sites were visited and snails were collected. The sites were located throughout the entire length of the New River Gorge National Scenic River (Figure 1) in southern West Virginia. Sites were established in a variety of habitat types and elevations. Habitats ranged from moist moss-covered deep-wooded areas to exposed dry talus-covered road cuts. Elevations ranged from 288 meters near the river to 702 meters on the higher cliffs of the Gorge walls. After the sites were identified, two to four hours of search time were spent at each site. Longer periods were spent if the hunting proved productive. Random searching was the preferred method. Rocks were rolled, leaves scattered and low branches and leaves observed for the presence of snails. Live snails were taken only if no dead shells could be found. GPS readings were taken at all sites with a hand-held Magellan Sport Trak. These data are presented in Table 1.

The specimens were returned to the lab for cleaning, identification to species and

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cataloging. All specimens were accessioned to the Marshall University Malacological Collections. Burch (1962) and Pilsbry (1940), (1946), (1948) were utilized in identification of specimens. All scientific names used in this paper follow Turgeon et al (1998).

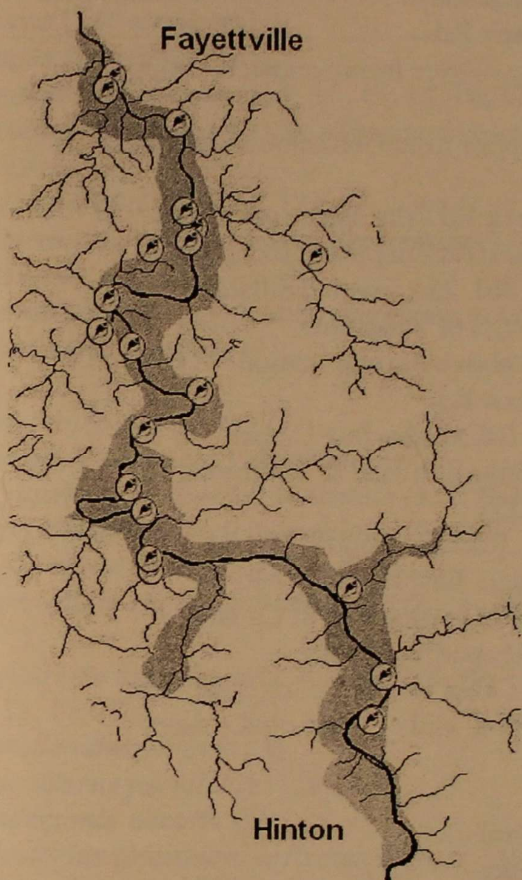

Figure 1. New River Gorge National River, West Virginia with collecting sites indicated.

Table 1. Locations of collecting sites within New River Gorge National River

Col. Site	UTMN	UTME	Elev. M	Date Col.	County	Location
1	4201419	492817	326	08/09/03	Fayette	Thurmond
2	4186474	495453	607	07/26/03	Raleigh	Glade Creek Rd
3	4198803	494328	326	07/26/03	Raleigh	Glade Creek Rd
4	4180627	509046	396	07/26/03	Fayette	Across New River from Sandstone Rock outcrop
5	4189627	585675	425	07/26/03	Fayette	WV 41, uphill from Quinnimont- dry shale rock face
6	4190128	582998	454	07/26/03	Fayette	Glade Creek Rd - dry shale rock face
7	4189710	495027	308	07/26/03	Fayette	Glade Creek Rd - dry shale rock face
8	4191001	494152	424	05/24/03	Fayette	along Co. Rd. 25 - wooded hillside
9	4178220	508300	564	06/14/03	Summers	Co. Rd. 20, S of Sandstone
10	4213359	492999	299	05/24/03	Fayette	Rockwall above Fayette Station
11	4187140	495356	607	07/06/03	Raleigh	Glade Creek Rd
12	4185431	506979	599	06/14/03	Summers	Claypool Rd. at Meadow Creek
13	4199642	492451	354	07/26/03	Fayette	RR crossing Glen Jean to Thurmond Rd. (Co. Rd. 25)
14	4194254	494971	436	05/24/03	Fayette	along Co. Rd. 25 - wooded hillside
15	3524798	490682	346	08/09/03	Fayette	Army Camp, East side of the River
16	4205366	497900	311	08/09/03	Fayette	Just South of boat access at Cunard
17	4191007	494151	430	05/24/03	Fayette	Co. Rd. 25, north of Prince
18	4203801	504916	702	06/14/03	Fayette	Babcock Cabin Rd., Babcock State Park
19	4211056	496970	288	06/14/03	Fayette	Co. Rd. 85-2, end of Road on Keeney's Creek
20	4204176	495362	524	08/09/03	Fayette	Cunard
21	4206235	497318	439	08/09/03	Fayette	near Cunard
22	4204584	497613	305	08/09/03	Fayette	near Cunard
23	4196349	498230	379	05/24/03	Fayette	C0. Rd. 25
24	4213557	493164	465	05/24/03	Fayette	Lansing Rd, Under New River Bridge (U.S. 19)
25	4212819	492772	329	05/24/03	Fayette	Near Fayette Station

Table 2. The species are listed by locations where they were found with additional statewide distribution information derived from published literature and my personal collections (Sites where they were found are indicated within parenthesis). Names that were in prior use are included but not bolded.

Species (site numbers)	Location
Allogona profunda (6)	Scattered statewide
Anguispira alternata (1, 5, 11, 13, 14, 16, 17, 22)	Every county in the state
Appalachina sayana (=M*esodon sayanus),* (3, 6, 14, 21) –	Cabell, Mason and all eastern and southern counties
Discus patulus (8, 9, 11, 13)	Every county in the state
Euchemotrema *(Stenotrema)* ***fraternum*** (2, 4) – Statewide	Statewide
Euconulus fulvus (1) Eastern highlands counties	Eastern highlands counties
Gastrocopta armifera (1)	Absent from most of central counties in the state
Gastrocopta contracta (1)	All counties except Northern Panhandle
Nesovitrea *(Retinella)* ***electrina*** (2)	Eastern highlands counties
Glyphyalinia *(Retinella)* ***indentata*** (1, 2, 9, 15, 17)	All counties except Northern Panhandle
Glyphyalinia *(Retinella)* ***lewisiana*** (2, 4)	Scattered statewide in 5 counties
Glyphyalinia *(Retinella)* ***rhoadsi*** (5)	All counties except Northern Panhandle
Glyphyalinia *(Retinella)* ***wheatleyi*** (8, 11, 18, 21)	Statewide except Northern Panhandle and counties along Ohio River
Haplotrema concavum (2, 4, 5, 6, 7, 8, 10, 11, 12, 13, 15, 17, 18, 21, 23, 25)	Statewide in every county
Hawaiia minuscula (1, 3)	South central and Eastern counties
Helicodiscus parallelus (1, 9)	Statewide
Hendersonia occulta (2)	Southern and Eastern counties
Inflectarius *(Mesodon)* ***inflectus*** (4, 5, 6, 11)	Known only from Fayette
Inflectarius *(Mesodon)* ***rugeli*** (2)	Monroe, Mingo, Fayette and Kanawha
Mesodon mitchellianus (1, 2, 5, 11, 15)	Monroe, Kanawha, Fayette, Wayne, Ohio, Boone, Summers, Wood, Clay, Pleasants and Putnam
Mesodon thyroidus (1, 4 5, 6, 8, 9, 10, 11, 15, 16)	Statewide in every county
Mesodon zaletus (4, 6, 9, 12, 16) –	Eastern and southern counties
Mesomphix cupreus (2, 3, 4, 5, 6, 7, 8, 9, 12, 20, 22, 23, 25)	Statewide
Mesomphix inornatus (2, 5, 6, 7, 8, 9, 10, 11, 12, 17, 18, 20, 21, 23, 25)	Statewide in every county
Mesomphix rugeli (2)	Not previously reported from West Virginia
M***esomphix capnodes*** (4)	Kanawha and Monroe counties
Neohelix (Triodopsis) ***albolabris*** (4, 5, 6, 17)	Statewide
Neohelix (Triodopsis) ***dentifera*** (3, 6, 14, 18)	Eastern highlands
Novisuccinea *(Succinea)* ***ovalis*** (6, 10)	
Paravitrea capsella (1, 2, 11)	Kanawha, McDowell, Mercer, Monroe and Fayette counties

Table 2. *Continued*

Paravitrea reesi (1, 15)	5 or 6 southern counties
Patera appressa *(=Mesodon appressus),* (1, 2, 4, 5, 6, 11, 17, 20, 25)	Kanawha, Fayette, Wayne, Mingo, Summers, Logan and Clay
Patera (Mesodon) panselenus (3, 20)	Southern half of the state
Pupoides albilabris (1)	Wood, Mingo and several eastern counties
Stenotrema edvardsi (4, 10, 11, 12)	6 or 8 southern tip counties
Stenotrema hirsutum (1, 16)	Eastern and southern counties
Stenotrema stenotrema (1, 4, 5, 6, 9, 10, 13, 19, 24, 25)	All southern counties
Striatura ferrea (1)	Eastern Half of the state
Striatura meridionalis (1)	Eastern highlands
Triodopsis fraudulenta (6, 7, 8, 9)	Eastern highlands
Triodopsis tridentata (2, 3, 4, 5, 6, 10, 11, 12, 13, 18, 25)	Statewide
Vallonia pulchella (1)	Raleigh, Preston, Greenbrier, Ohio, Mason, Cabell and Randolph
Ventridens demissus (4, 7, 15, 19)	Statewide
Ventridens intertextus (1)	Statewide except north central and northern panhandle
Ventridens ligera (1, 2, 3, 4, 6, 7, 15, 16, 22)	Statewide
Xolotrema denotatum *(=Triodopsis denotata),* (2, 5, 18, 19)	Statewide
Zonitoides arboreus (1)	Every county except northern panhandle

RESULTS

Forty-seven native species of shelled land snails, plus the invasive land snail *Opeas pyrgula.* (Figure 2) were identified. Even though much time and effort were spent in looking for *Punctum smithi* at the location where they had been previously found, none was found during this survey. *Mesomphix rugeli* (W. G. Binney, 1879) is reported here for the first time as a resident of the state.

DISCUSSION

The single population of *Opeas pyrgula* was found in the nearly abandoned small village of Thurmond in Fayette Co. West Virginia. While, at the turn of the twentieth century, Thurmond was a thriving mining community with a large steam locomotive repair facility, it is now inhabited by fewer than a dozen families. Hundreds of specimens of this snail were found in the middle of town, on a steep hillside that had been chemically treated to remove a dense covering of kudzu. It seems apparent that the snail came in on a potted plant at some time in the past and became adjusted to the environment and thrived. It was not found anywhere else in the Park.

One of the collecting sites shown on the map appears to be out of the park area. It is in fact in Babcock State Park. This site sits on the canyon rim and represents the highest point in the survey. The park is within the authorized boundary of the New River Gorge National River and it seems appropriate to include the specimens collected there.

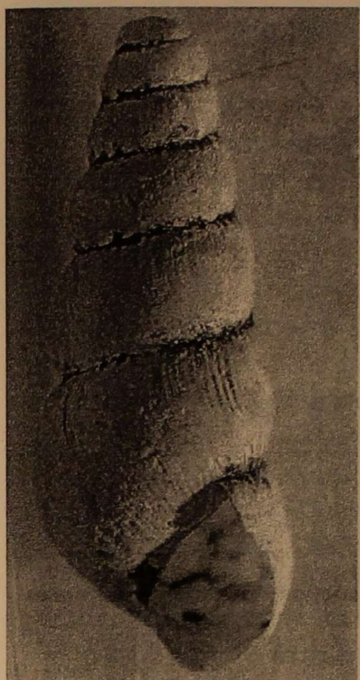

Figure 2. *Opeas pyrgula*

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CHRYSOMYA RUFIFACIES (DIPTERA: CALLIPHORIDAE) FROM WEST VIRGINIA.

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ABSTRACT

Chrysomya rufifacies (Macquart), the hairy maggot blow fly, was collected for the first time in West Virginia. A single adult male was trapped in the north central portion of the state (Preston Co.) in August 2006, and third instars were collected the following month from two sunlit pig (*Sus scrofa* L.) carcasses at a southwestern site in Cabell Co. along the Ohio River. Third instars of *C. rufifacies* colonized sunlit carcasses in an open field at the latter location, but were not found on shaded (forested) carcasses. This species has now been reported from 15 states in the continental U.S., with West Virginia representing the most northeastern extent of its range. As the range of *C. rufifacies* expands one would expect this species to become more important in medicolegal cases, however, the utility of this carrion fly as a geographic indicator will decrease in those cases where postmortem movement of decedents is suspected.

INTRODUCTION

The hairy maggot blowfly, *Chrysomya rufifacies* (Macquart), a species indigenous to the Australian and Asian regions of the Old World tropics, was introduced into Latin America in the late 1970's (Jiron 1979) and subsequently spread to the continental U.S. (Texas) in 1980 (Richard and Ahrens 1983). This blow fly species is now well established in Florida (Mertins 1991, Steck and Butler 1991), Louisiana (Martin et al. 1996), Texas (Richard and Ahrens 1983), and California (Greenberg 1988). Additional collections of this species have been reported from Arizona (Baumgartner 1986), Colorado (De Jong and Chadwick 1997), Arkansas (Meek et al. 1998), Nebraska (Figarola and Skoda 1998), Alabama (Wells 1999), Tennessee (Shahid et al. 2000), and North Carolina (Tomberlin et al. 2006). It has also been collected in Michigan (Merritt, unpublished data) and Kentucky (Haskell, unpublished data) (fide' Shadid et al. 2000), and reported from Indiana (by N. Haskell, 2006, personal communication). Thus West Virginia represents the 15[th] state record for this blowfly species. The reader is directed to Baumgartner (1993) for a review of this invasive species, and to Baumgartner (1993) and Shahid et al. (2000) for extensive bibliographies. *Chrysomya rufifacies* could gain increased importance in forensic investigations if it becomes more widely established in the United States, but its usefulness as a geographical marker would be expected to diminish.

This communication is to document collections of *C. rufifacies* in two geographically disjunct regions of West Virginia: an adult collection in the north central portion of the state (Hazelton, Preston Co.) and larval collections from pig carcasses in the southwest (Green Bottom Wildlife Management Area, Cabell Co.) along the Ohio River. This is the first record of this species in the state even though extensive carrion insect collection efforts have been carried out in recent years (James Amrine, 2006, personal communication).

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MATERIALS AND METHODS

Adult collections. One adult male *Chrysomya rufifacies* was collected in a hand held sweep net on 3 Aug 2006 near Hazelton, WV (Preston Co., elevation ≈ 620 m) by VAD. Daily collections over decaying meat bait at this site had been ongoing from 1 July through 22 August 2006. This specimen has been deposited in the West Virginia University entomology collection. Larval collections. Four ≈ 16 kg pigs (*Sus scrofa* L.) were killed on 19 May 2006 by shooting with a .22 caliber bullet to the head, bagged in individual 42 gallon heavy duty plastic contractor clean-up bags, and frozen. Pigs were removed from the freezer, allowed to thaw, then placed at the Green Bottom Wildlife Management Area (Cabell Co., 39° 31' 13" N and 82° 17' 53" W, elevation ≈ 170 m) field site.

Two experimental plots (one shaded, one sunlit) were established at the field site with two pigs (one "naked", one "clothed") placed on the ground in each plot at 1:00 PM (hour zero) on 17 Sep 2006. Carcasses were covered with 2.5 cm hexnetting staked firmly to the ground to prevent disturbance by scavengers.

Dominant forest canopy species at the shaded plot were *Acer negundo* L. (box elder), *Juglans nigra* L. (black walnut), *Sida hermaphrodita* (L.) (Virginia mallow), and *Verbesina alternifolia* (L.) (wing stem), with an understory of *Elymus* sp. (wild rye), *Pilea pumila* (L.) (clear weed) and *Viola papilionacea* Pursh (common blue violet).

The sunlit plot was an open, mowed field dominated by *Allium vinale* L. (wild onion), *Rumex crispus* L. (curly dock), seedlings of an unidentified species of *Rumex* sp., *Asclepias syriaca* L. (purple milkweed), and an unidentified member of the mustard family (Brassacaceae). Bordering the sunlit plot some 15 m from the carcasses were dense stands of *Solidago canadensis* L. (golden rod) and *Aster pilosa* Willd. (aster).

Ambient temperatures were recorded with a digital thermometer 3 or 4 times a day and maggot mass temperatures were recorded with the first observance of larval aggregates (at 31 h). Twelve larval collections were made from 31 h through 168 h. *Chrysomya rufifacies* third instars have been deposited (as whole specimens in bottles of 70% ethanol) in both the WVU entomology collection in Morgantown, WV, and the state insect collection (WV Department of Agriculture, Charleston, WV). Reared adults (2 females; 1 male) were deposited in the WVU entomological collection with an additional 2 females deposited in the WV state insect collection.

RESULTS

Nearly 200 adult carrion flies were collected daily (1 hour each day) from 1 July through 22 August 2006 at the Preston County site, and yet only a single specimen of *C. rufifacies* was identified as a result of that collection effort.

Chrysomya rufifacies third instars were first observed on both "naked" and "clothed" sunlit carcasses at 144 h, and again at 168 h, after carcasses had been placed at the Cabell County site. These instars made up only a small proportion of the total maggot masses which were clearly dominated by *Phormia regina,* with *Cochliomyia macellaria* present to a lesser extent. No third instars of *C. rufifacies* were observed on either shaded carcass.

Nine adult *Chrysomya rufifacies* (8 females; 1 male) emerged from the 14 third instars placed on potting soil after a period of 11 days at room temperatures (admittedly poorly controlled) that fluctuated from 18 °C to 23 °C.

DISCUSSION

This communication constitutes the first report of *Chrysomya rufifacies* from West Virginia. Previous field studies have demonstrated a predisposition for *C. rufifacies* to visit larger carcasses in open pasture areas rather than forested settings (vide' Baumgartner, 1993). Our Green Bottom observations of *C. rufifacies* instars colonizing open sunlit pig carcasses, but not shaded ones, corroborates those habitat generalizations. Baumgartner (1993) also noted that *C. rufifacies* exploited and emerged from human corpses that were in an advanced stage of decomposition in Texas and Florida (citing personal communications with N. Haskell and J. Butler, respectively). This too, comports with our findings, as our collections of third instars were from sunlit carcasses in advanced stages of decomposition.

Our single attempt of placing both small (n = 5, 7 to 9 mm in length) and large (n = 9, 12 to 14 mm) *C. rufifacies* third instars on soil in order to obtain adults for voucher specimens showed that larvae immediately burrowed into the soil. The 9 adults (8 females; 1 male) emerging after 11 days at room temperatures suggested that the five smaller third instars did not complete pupation successfully.

There is also some evidence that *C. rufifacies* could possibly displace other native species (especially *C. macellaria*) and dominate the blowfly fauna (vide' Wells and Greenberg 1992, Baumgartner 1993). While such a competitive displacement seems potentially realistic in more southern climates it remains to be seen how *C. rufifacies* fares, competitively, in the more northern reaches of its range. For this reason, future monitoring of *C. rufifacies* to determine its interaction with *C. macellaria*, and perhaps other blowfly species in West Virginia, appears desirable.

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