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Distribution and habitat of *Desmognathus welteri*, Black Mountain Salamander, in West Virginia

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ABSTRACT

We conducted field and literature surveys to clarify the range and habitat of the Black Mountain Salamander, *Desmognathus welteri*, in West Virginia. This semi-aquatic species is known from 17 locations in Logan, Wyoming, Mercer, Summers, and McDowell counties. It appears the range of *D. welteri* is restricted to the Cumberland Plateau region in the southernmost section of the state within the Guyandotte, Bluestone, and Big Sandy River drainages. Populations are limited to high gradient streams at relatively high elevations that are associated with mature hardwood forests. It is likely additional populations will be discovered, but we suggest the species is of special concern due to specific habitat requirements and a high degree of habitat degradation within the area of the state where they occur.

INTRODUCTION

The first Black Mountain Salamander, *Desmognathus welteri* Barbour, collected in West Virginia was taken in 1938 by Neil Richmond at Blair Mountain, Logan County. At the time, *D. welteri* was synonymous with *D. fuscus*, Northern Dusky Salamander, and was identified as such. Ten years later another specimen was collected at Pineville in Wyoming County by W. R. DeGarmo, and also catalogued as *D. fuscus*. The next record of the species in the state was by Hutton (1980). *Desmognathus welteri* was named a likely addition to the herpetofauna of West Virginia by Green and Pauley (1987). The first published record of *D. welteri* in West Virginia was by McCleary and Orr (1987), who reported the species from Mercer County. Later electrophoretic and morphological data proved the species was a part of the West Virginia herpetofauna (McCleary 1989). Additional surveys by Seeman (1996) extended the range north into Summers County and west to Wyoming County. Researchers at Marshall University found new populations while surveying in Wyoming and McDowell counties (Pauley, unpublished data). Current range maps for *D. welteri* do not include West Virginia (Conant and Collins 1998, Petranka 1998). It was the purpose of this study to investigate the range of *D. welteri* in West Virginia, and describe the habitat of the species.

METHODS

First- through third-order streams with permanent water flow and minimal habitat disturbance were identified on topographical maps within the perceived range of *D. welteri*. Streams were surveyed one person-hour between April and November of 2000. Environmental parameters measured included water temperature and pH. Notes on the canopy cover and stream size were taken at each stream, and elevation and gradient of streams were determined from USGS topographical quadrangles. Several vouchers of *D. welteri* were collected from most new populations and deposited in the West Virginia Biological Survey (WVBS) at Marshall University. Inspections of *D. fuscus* specimens in the WVBS were conducted to determine if other specimens of *D. welteri* were collected but misidentified.

RESULTS

Sixteen streams in Mingo, Logan, Wyoming, Mercer, and McDowell counties were surveyed for new populations (Felix 2001). *D. welteri* was detected in five of these, including Marsh Fork in Mercer County, an unnamed tributary of Pinnacle Creek, Lick Creek, and Turkeywallow Branch in Wyoming County, and Larken Branch in McDowell County (Table 1, Figure 1).

Streams where *D. welteri* was observed were always associated with mature deciduous forests and ranged in elevation from 400-730 meters. Gradient of these streams ranged from 29.1 to 82.3 m/km and averaged 50.7 m/km. Cover was present in all streams in the form of large, flat rocks. Water temperatures ranged from 13 to 20 °C (avg = 17 °C) and pH ranged from 7.5 to 8.3.

Streams where *D. welteri* was not observed varied in the extent and degree of disturbance. Some streams contained heavy loads of sediments possibly

associated with mining activities in their headwaters. Others lacked canopy coverage over large portions due to adjacent roads. Yet others were apparently undisturbed in terms of physical structure.

DISCUSSION

To date, the distribution of *D. welteri* in West Virginia includes the southern counties of Logan, Wyoming, Mercer, Summers, and McDowell, which include the Big Sandy, Guyandotte, and Bluestone River drainages. Including sites from the present study and previous studies, there are 17 known sites where *D. welteri* occurs in West Virginia (Table 1, Figure 1). Two of these, Blair Mountain and Pineville, are too vague to be assigned to a specific stream.

Various investigators have discussed the distribution of this species in the state. Reasons for the inclusion of *D. welteri* in north-central West Virginia by several authors (Barbour 1971, McCleary and Orr 1987) remains unclear. It is possible that an isolated population exists here, but more likely this discrepancy is a result of misidentification. Juterbock (1975, 1984) discussed the problematic nature of identifying this species. A population described in Pocahontas County (McCleary 1989) was possibly the result of introduction of individuals by fisherman (pers. comm., L. Orr, Kent, OH). It is believed the release of unused bait salamanders has introduced salamander species outside their normal range (Martof 1953, Redmond 1980). Density of salamanders was low at the time of discovery (McCleary 1989), and subsequent surveys have turned up no additional specimens (Seeman 1996). For these reasons we have not included this site in our list of known sites. It has been suggested this species may occur in the New, Gauley, and Greenbrier River drainages (McCleary 1989, Seeman 1996). We believe this is unlikely since multiple studies in the area, including a systematic survey of the New River Gorge National River (Pauley 1993), have not detected the presence of the species.

It appears this species has invaded the state from three river drainages. The species apparently immigrated into southeastern West Virginia along the Bluestone River, which enters the state from an area of Virginia where *D. welteri* occurs (Mitchell and Reay 1999). Invasion of the southwestern portion of the state likely occurred via the Big Sandy River, which flows into an area of Kentucky that contains this species (pers. comm., J. MacGregor, Nicholasville, KY). The presence of the species in the Guyandotte River drainage is interesting because this river originates in southern West Virginia and flows into the Ohio River in an area that is distant from known populations. It is possible that *D. welteri* entered the headwaters of the Guyandotte from the Tug Fork of the Big Sandy River and spread downstream from there.

In West Virginia, *D. welteri* appears to be limited to small- to medium-sized streams with moderate to high gradients at relatively high elevations (426-762 meters) flowing through mature deciduous forests. Shade provided by these forests likely results in lower water temperatures, which may be important to the maintenance of oxygen levels needed for cutaneous respiration (Whitford and Hutchinson 1965). The apparent absence of this species from some streams may be a result of many factors. A large percent of streams surveyed in southern West Virginia were impacted by a number of land use practices, such as coal mining activities and tree removal along streams adjacent to roads. Mining activities are known to contribute to the absence of certain species from watersheds through alteration of physical and chemical attributes (Kucken et al. 1994, Mathews and Morgan 1982). In Tennessee, this species was never observed in streams where strip mining had altered streambank vegetation or streams with high silt or metal concentrations (Redmond 1980).

Additional populations of this species will no doubt be discovered in the future. We predict the range of the species in West Virginia will eventually extend throughout the Cumberland Plateau region including Mingo, Logan, Wyoming, Raleigh, Summers, Mercer, and McDowell counties. Topography in this area is characterized by steep ravines and streams flowing through high-gradient hollows. Topography becomes flatter to the north, and streams are likely not suitable for the species. It has been suggested that this species has been competitively excluded from the New River drainage by Black-bellied Salamanders, *D. quadramaculatus* (Seeman 1996). If this is true, the New River would represent a barrier to the range extension of *D. welteri.* Surveys for this species are especially crucial because the entirety of their range coincides with an area extensively mined for coal. The Blair Mountain population provides an ominous example of this situation. The presence of the species in West Virginia was unknown until this site had likely been destroyed by a mountaintop removal operation.

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Table 1. Localities for D. *welteri* in West Virginia. * Localities with vouchers in WVBS.

Location	County	Watershed	Coordinates	Source
1. Blair Mountain*	Logan	Guyandotte	NA	T.K. Pauley unpubl. data
2. Pineville*	Wyoming	Guyandotte	NA	T.K. Pauley unpubl. data
3. Still Run	Wyoming	Guyandotte	37° 37' 30" 81° 25' 00"	Seeman 1996
4. Marsh Fork	Wyoming	Guyandotte	37° 37' 24" 81° 27' 30"	T.K. Pauley unpubl. data
5. Trib. Of Gooney Otter Creek	Wyoming	Guyandotte	37° 30' 32" 81° 20' 46"	T. K. Pauley unpubl. data
6. Unnamed trib. Pinnacle Creek*	Wyoming	Guyandotte	37° 28' 56" 81° 23' 16"	Present study
7. Lick Creek*	Wyoming	Guyandotte	37° 29' 10" 81° 30 25"	Present study
8. Turkeywallow Branch*	Wyoming	Guyandotte	37° 31' 24" 81° 35' 54"	Present study
9. Bear Creek*	Mercer	Bluestone	37° 31' 30" 81° 08' 01"	McCleary 1989 Seeman 1996
10. Farley Branch	Mercer	Bluestone	37° 51' 46" 81° 51' 23"	McCleary 1989 Seeman 1996
11. Marsh Fork*	Mercer	Bluestone	37° 30' 04" 81° 08' 55"	Present study
12. Tony Hollow	Summers	Bluestone	37° 31' 00"	Seeman 1996 81° 55' 00"
13. Indian Branch	Mercer	Bluestone	37° 29' 30" 81° 01' 00"	Seeman 1996
14. Laurel Branch	Mercer	Bluestone	37° 29' 00" 81° 01' 00"	Seeman 1996
15. Double Cabin Branch	Mercer	Bluestone	37° 28' 00" 81° 01' 00"	Seeman 1996
16. Middle Fk. Slaunch Fork*	McDowell	Big Sandy	37° 23' 22" 81° 53' 33"	T.K. Pauley unpubl. data
17. Larken Branch*	McDowell	Big Sandy	37° 22' 18" 81° 55' 24"	Present study

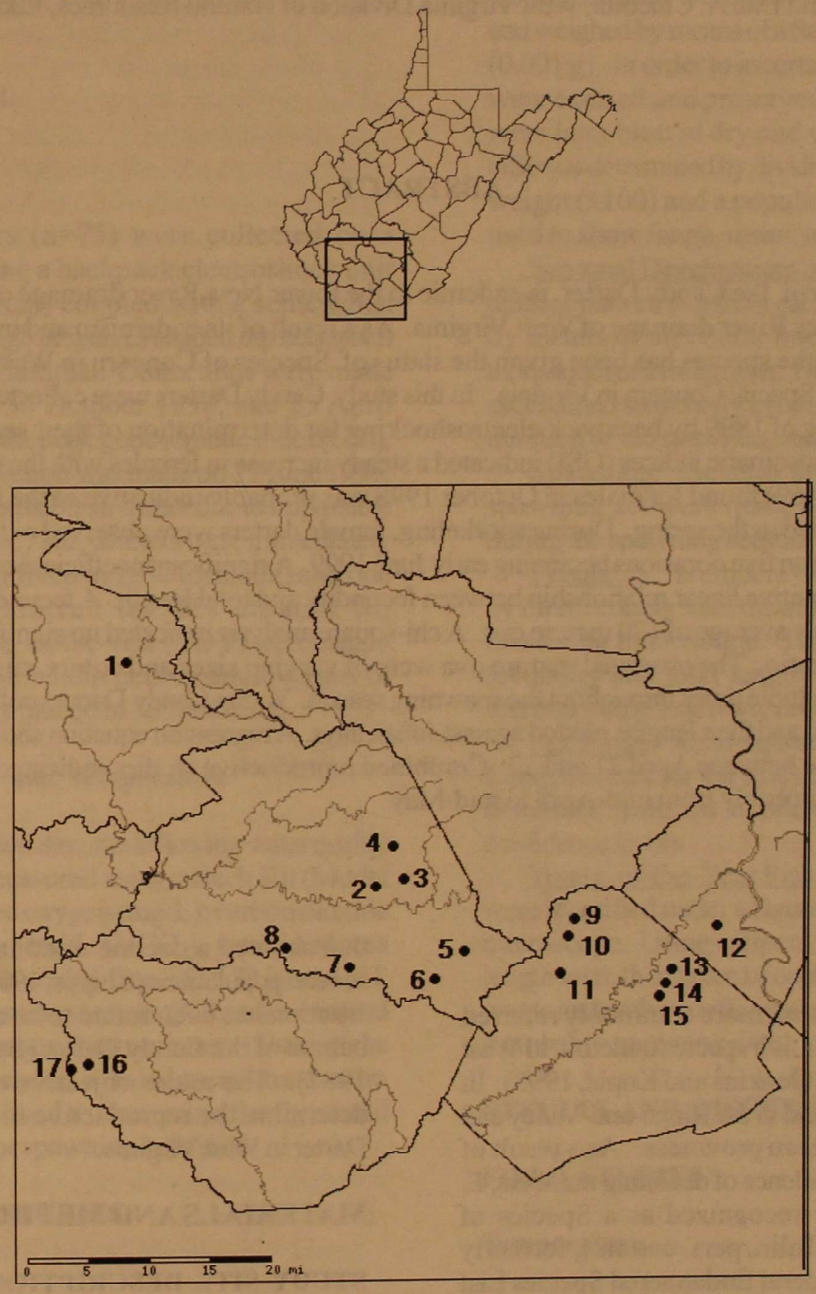

Figure 1. Location of known populations of *D. welteri* in southern West Virginia.

Numbers correspond to locations in Table 1.

Reproductive Biology 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 the Gauley River drainage of West Virginia. As a result of its endemism and evidence of declining population numbers, the species has been given the status of Species of Concern in West Virginia and at the federal level and State Special Concern in Virginia. In this study, Candy Darters were collected in the summer and fall of 1998 and spring of 1999 by backpack electroshocking for determination of their seasonal reproductive biology. Seasonal gonosomatic indices (GSI) indicated a steady increase in females with the peak (7.137) in April 1999. A peak GSI (0.469) found for males in October 1998 was probably indicative of the low number of adult males (mature) collected in the spring. During snorkeling, female darters were observed exhibiting behaviors of possible nest-guarding on five occasions beginning early June 1999. A significant coefficient of correlation ($r=0.70$; $p<0.05$) indicated a positive linear relationship between fecundity and total length. A fecundity analysis showed females to contain a low average of 170 mature ova. A chi-square analysis indicated no significant deviation from the expected 1:1 sex ratio. The observed mature ova were of varying sizes and colors, suggesting that Candy Darters may spawn multiple times throughout the spawning season. Young Candy Darters collected in the summer of 1999 were measured and their lengths plotted against Julian days. A regression equation showed the approximate date of spawning to be between April 21 and 22. Combined reproductive studies indicated that Candy Darters spawn in late spring, probably from mid-April to mid-May.

INTRODUCTION

Etheostoma osburni, more commonly referred to as the Candy Darter, is a species endemic to West Virginia and Virginia (Jenkins and Kopia, 1995). In West Virginia, it is found in the Ridge-and-Valley and the Appalachian Plateau provinces. 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). 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 West Virginia this species has been given state status of Species of Concern and in Virginia has been given status of State Special Concern (Williams et al., 1989) as a result of studies that showed a decline or complete extirpation of several populations (Chipps, 1992). In many streams, siltation has been found to be a major threat to the habitat of the Candy Darter (Berkman and Rabeni, 1987). The major objective of this study was to determine the reproductive biology of the Candy Darter in West Virginia.

MATERIALS AND METHODS

STUDY SITE DESCRIPTION

Candy Darters were collected at 38°12'54"N, 80°30'18"W on the South Fork of the Cherry River in Nicholas County near Richwood, West Virginia. Substrate consists primarily of shale and sandstone types of rock – small to large boulders mixed with cobble, pebbles, and sand. Swift chutes between the larger rubble and boulders are common throughout the South Fork, as are shallow to deep pools and

shallow runs (Chipps et al., 1994). Usually, adult Candy Darters inhabit swift riffles and runs (mainly males) and the swifter portions of pools (mainly females).

FIELD STUDIES

Collections

Candy Darters (n=75) were collected on a seasonal basis using a backpack electroshock unit (115 volt AC current) coupled 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. To establish the peak gonosomatic index(GSI), one additional collection was made on 17 June 1999. No collection was possible in winter due to impassible conditions to the study site. Darters were immediately placed on ice, then fixed in 10% formalin, and one week later transferred to 70% ethanol for preservation. Young-of-the-year (YOY) were caught by means of D-shaped dip nets in shallow runs or pools and promptly placed in 70% ethanol.

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$CaCO_3$) ; and pH. Additionally, water temperature (°C) was measured with an instantaneous thermometer. During the reproductive season, a minimum-maximum thermometer was used to determine the threshold temperature (°C) for spawning activities.

LABORATORY STUDIES

Reproduction

To study the reproduction of the Candy Darter, attempts were made to determine the duration of the reproductive season by: (1) calculating seasonal GSI, (2) observing changes in seasonal development of ova, and (3) observing the spawning act. Additionally, YOY were measured against Julian days to determine an approximate spawning date. Observations were also made on fecundity of females and changes in sex ratio.

Gonosomatic Index (GSI). Prior to any excisions, Candy Darters were blotted dry on a paper towel and weighed by means of a Sartorius analytical balance (0.001 g). In order to ascertain seasonal GSI, gonads were excised and preserved in 70% ethanol. They were later blotted dry and weighed (0.001g). The GSI was determined by dividing gonad weight by body weight (x100) and a population range diagram was used to show range, mean, and standard deviation.

Seasonal Development of Ova. Diameters of one tenth of mature ova from each female were measured by means of an ocular micrometer to the nearest thousandth of a millimeter. Diameters and ranges were ascertained seasonally in order to determine time and duration of the spawning season.

Spawning. Several attempts to observe the spawning act were made by snorkeling upstream during the spawning season.

Fecundity. Numbers of eggs were determined by direct counts with the aid of a Bausch and Lomb dissecting microscope. The relationship between fecundity and total length was determined and a regression line tested with a coefficient of correlation.

Sex Ratio. A chi-square test was applied to all Candy Darters for the study period to determine any departure from the expected 1:1 sex ratio (0.05 confidence level).

Young-of-the-Year Fishes. Young-of-the-year were identified under a Bausch and Lomb dissecting microscope. Using calipers, measurements of total length were then taken of each fish. A regression line was tested with a coefficient of correlation to estimate a probable spawning date.

RESULTS AND DISCUSSION

FIELD STUDIES

Temperature

Temperature data from the South Fork of the Cherry River suggested that this portion of the river is relatively cold. During mid afternoon in July, the water temperature was measured to be 19° C, whereas in October and April the temperature was 11 °C. Minimum-maximum temperatures were recorded at two-week intervals during the period that was expected to be the spawning season (Table 1). From

08 April 1999 to 29 July 1999, temperatures ranged from 10 °C to 28 °C.

Water Chemistry

Water quality parameters indicated that the South Fork of the Cherry River is of good water quality (Schoolcraft, 1999). Dissolved oxygen remained high throughout the year at 9 mg/L in July and April and 8 mg/L in October. The carbon dioxide level was 5 mg/L during the fall and spring but was 10 mg/L in the summer. Free acidity remained 0.0 mg/L throughout the year. Total alkalinity was a constant 17.1 mg/L $CaCO_3$, while the total hardness remained a steady 34.2 mg/L $CaCO_3$. Hydrogen ion concentration (pH) measurements from July and April were neutral at 7.0 and 7.1, respectively. The pH in October was 8.0.

LABORATORY STUDIES

Gonosomatic Index (GSI)

An additional collection of Candy Darters was made on 17 June 1999 to aid in determining the duration of the spawning season. Gonad and body weights were measured from 38 males and 37 females taken from July and October of 1998 and April and June of 1999. The ratio of these two measurements, or the GSI, was plotted graphically to show seasonal changes (Fig. 1). Average GSI for females showed a steady increase from July 1998 and peaked in April 1999 (7.137). Thomas (1970) reported that gonad sizes in darters were reduced in summer and early fall, began increasing in October, and reached maximum size in the spring. The lowest overall GSI average for males (0.077) and females (0.753) occurred in June 1999. This would be consistent with evidence that suggests spawning season is April to May. However, males from this study showed a peak GSI average in October 1998 (0.469). This inconsistency may be due to the fact that of the males collected on 23 April 1999, three were juveniles, and thus had immature gonads. A small sample size of males may also be the cause of the low GSI during the expected spawning season.

Seasonal Development of Ova

In an attempt to determine the time and duration of the spawning period, diameter measurements were made of one tenth of mature ova from female Candy Darters. In agreement with Thomas (1970), females collected on 30 October of 1998 contained enlarged ova; however, none were mature. Nonetheless, measurements were made on these ova, which ranged in diameter from 0.79 to 0.96 mm and had a mean diameter of 0.85 mm. Eleven females with mature eggs were collected. Three of the 11 females were collected on 14 July 1998 and were not included in the diameter measurements. These females had very few mature ova, and it is likely that extreme environmental conditions caused the late maturation of these eggs. Remaining females were all taken in April 1999. The average diameter of a mature ovum was 1.46 mm with a range of 0.82 to 1.84 mm (Table 2).

Because the spawning period was thought to be later than suggested in the literature (due to the ripe females collected in July, 1998), biweekly collection of females for seasonal development of ova began on 02 June 1999 and continued until 29 July 1999. Attempts were made to collect females on two separate dates in May of 1999. However, collection attempts with a kick-seine or dip net were not possible due to high water. No females with mature ova were found after the collection date in April 1999. Therefore, results on seasonal development of ova are inconclusive with respect to the time and duration of the spawning period for Candy Darters.

Spawning

Seven trips were designated as snorkeling dates in an attempt to observe spawning activity or post-spawning behaviors (e.g. nest-guarding) in Candy Darters. No spawning was observed on any date. Although males were elusive, females were seen throughout much of the snorkeling period and references were made as to their positions in the river. No mature females were shocked from riffles on 23 April 1999. On 07 May 1999 and again on 22 May 1999, snorkeling showed that many females were in the "glides". Beginning 02 June 1999, females were observed in the heads of riffles every two weeks until the last snorkeling date on 29 July 1999. Females generally did not move more than a half meter from the rock crevice in which they were first spotted. On all occasions females returned to this point, only to dart away and enter one or more different crevices. It is believed that female Candy Darters join males in riffle areas to spawn (Jenkins and Burkhead, 1994).

Although the spawning act has never been observed in the natural habitat, collection of prenuptial adults suggested that they spawn in mid- to late spring (Jenkins and Burkhead, 1994), most likely in April and May (Stauffer et al., 1995).

Females may have been carrying out one of three possible behaviors: (1) darting in and out of several crevices in search of male spawning partners; (2) guarding a chosen pocket within the rocks, prior to the arrival of a male (or males) to fertilize ova; (3) guarding fertilized eggs following spawning activities. Given the high water velocity of the river, it is possible that eggs are deposited and fertilized deep within a crevice to avoid being washed downstream. Because males were never collected far from a riffle, with exception of juveniles, it is possible that they establish a territory and defend it throughout the year. In terms of energy, it would be very costly for a male to move around unnecessarily within swift riffles. Therefore, possibilities one and three would be the more likely explanations of the behaviors observed by females. However, because seasonal GSI data and seasonal development of ova data showed no mature eggs after collection on 23 April 1999, nest-guarding (possibility three) is most likely the cause of the behavior of the females.

Fecundity

Fecundity refers to the total number of mature ova within the ovaries of a female. Females (N=8) ranged in total length from 60.45 to 71.4 mm and averaged 65.59 mm in length. They contained an average of 170.4 mature eggs with a range of 104 to 224 (Table 2). A linear regression of fecundity versus total length was computed, which showed the following relationship: $y = -414.9 + 8.9227x$, where y is the total number of eggs and x is total length in millimeters (Fig. 2). A significant coefficient of correlation ($r = 0.70$; $p<0.05$) indicated a positive linear relationship between fecundity and body length.

The average number of mature ova seen in Candy Darter females represents low fecundity and is considered an advanced characteristic, which is seen in many other darter species (Holbrook, 1975). In the ovaries of female Candy Darters, ova were found to vary in size and color. Any single egg that had turned yellow in color (as opposed to milky white) was considered mature. The most mature ova were somewhat clear and yellow-orange in color. A single ovary in which there are varying stages of ova development suggests that a female may spawn more than one time during a season (Shelton, 1963). Winn (1958) reported all *Etheostoma* species displayed one of the following spawning categories: one to several, several, or many spawns. It is probable, then, given the varying sizes of ova found within a single female, that Candy Darters also engage in multiple spawnings.

Sex Ratio

In July of 1998, 15 Candy Darters were collected. Nine of these were males and six were females. The October collection revealed 12 males and nine females, while seven males and 10 females were collected in April 1999. A chi-square analysis indicated no significant deviation from the expected 1:1 sex ratio.

Young-of-year (YOY) Fishes

Young-of-Year fishes were collected on three separate occasions for estimation of an approximate spawning date. On 10 July 1999, 19 July 1999, and 03 September 1999, a total of nine YOY were captured ranging in size from 19.6 to 39.2 mm. Total lengths were plotted against Julian days and the following regression equation resulted: $y = -29.499 + 0.2665x$, where y is total length in millimeters and x is the Julian day (Fig. 3). A high correlation coefficient ($r=0.931$; $p<0.05$) indicated that the regression equation would be reliable for determining a date at which larval fish would be size 0.0 mm (i.e. the date of egg fertilization). Using the regression equation showed the approximate spawning date to be between Julian days 110 and 111, correlating to April 21 and April 22, respectively. These dates are consistent with GSI and ova diameter data, which showed females had the highest GSI and the largest ova in April.

Appearances of young Candy Darters varied according to size. These fish had the overall body shape of adults but dorsal saddles across the backs of the fish varied. They did not take on the characteristics of the adults in that saddles were not solidly pigmented. Rather, the saddles consisted of clusters of small dots of pigments. The largest of these fish (collected on 03 September 1999, or Julian day 246) had very distinguishable saddles. However, they were not quite solid. Also, the young fish had six or seven dorsal saddles, opposed to five in adults.

Vertical bars were not apparent on these fish. Their sides were lined with a row of 10 - 12 large spots. On fish taken 03 September 1999, the rows of spots were larger and more elongated. Fin ray and spine counts correlated with those of adults, but often the last one or two dorsal spines were extremely small and difficult to distinguish.

CONCLUSIONS

Due to the paucity of information on the life history of *Etheostoma osburni*, this study focused primarily on reproductive aspects of the species. Candy Darters from the South Fork of the Cherry River in Nicholas County, West Virginia, were collected in the summer and fall seasons of 1998 and spring of 1999. Results of gonosomatic indices (GSI) showed that females had the highest average gonad weight to body weight ratio of 7.137 in the spring season (April). Males, however, had a peak GSI of 0.469 in the previous fall season. A small sample size in the spring, in which three of the seven collected males were juveniles, was the probable cause of the unexpectedly low spring GSI. Average diameter of a mature ovum was found to be 1.46 mm. In the stream, females in the presumed peak of their spawning activities were collected on April 23, 1999. By means of snorkeling, an attempt was made to observe Candy Darters spawning in their natural habitat. Beginning in early June, females were found to have moved to the heads of riffles from their usual "glides". Although no spawning was observed, females were observed darting in and out of various rock crevices, always returning to the same place.

After all possibilities were analyzed, these females were assumed to be exhibiting behaviors of nest-guarding. An analysis of fecundity showed female Candy Darters have a low average number of mature ova (170.4). Finding mature ova in different developmental stages suggests this species engages in multiple spawnings. A positive linear relationship ($r = 0.70$) between fecundity and body length was found by means of a linear regression despite a small sample size. All Candy Darters collected were counted and subjected to a chi-square analysis, which showed no significant deviation from the expected sex ratio of 1:1. Twenty-eight males and 25 females were taken during the three collecting seasons. Nine larval Candy Darters were collected and measured in the summer of 1999. A linear regression analysis was performed on total lengths plotted against Julian days. The regression equation that resulted shows that these fish were size 0.0 mm (the approximate date of spawning by adults) between April 21 and 22, 1999.

ACKNOWLEDGMENTS

We are grateful to the Non-Game Heritage Program, West Virginia Division of Natural Resources, for funding the project. We thank the following persons for laboratory and field assistance: Kirk Barnett, Tom Bassista, Steve Foster, Eric Higginbotham, Ben Lowman, Tara Rose, and Jason Morgan. We express appreciation to Drs. Mary Etta Hight, Mike Little, and Tom Pauley for guidance and assistance in the project. Special appreciation goes to Mary Jo Smith for typing the manuscript.

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LIST OF TABLES

Table 1. Temperature data from the South Fork of the Cherry River from 07 April 1999 to 19 July 1999.

Date	Current Temp (°C)	Minimum Temp. (°C)	Maximum Temp. (°C)
4/7/99	15	11	18
4/23/99	13	11	19
5/7/99	15	11	18
5/21/99	13	11	19
6/2/99	15	10	18
6/17/99	17	NA	NA
6/26/99	21	15	23
7/10/99	22	20	28
7/29/99	22	19	28

Table 2. Data for seasonal development of ova and fecundity for *Etheostoma osburni*.

Fish #	# of Eggs	Body Length (mm)	Average Egg Diameter (mm)
403	107	60.45	0.819
404	104	62.70	1.323
408	211	65.65	1.844
410	145	64.50	1.440
411	180	70.90	1.789
412	224	71.40	1.507
413	170	63.60	1.485
417	222	65.55	1.443

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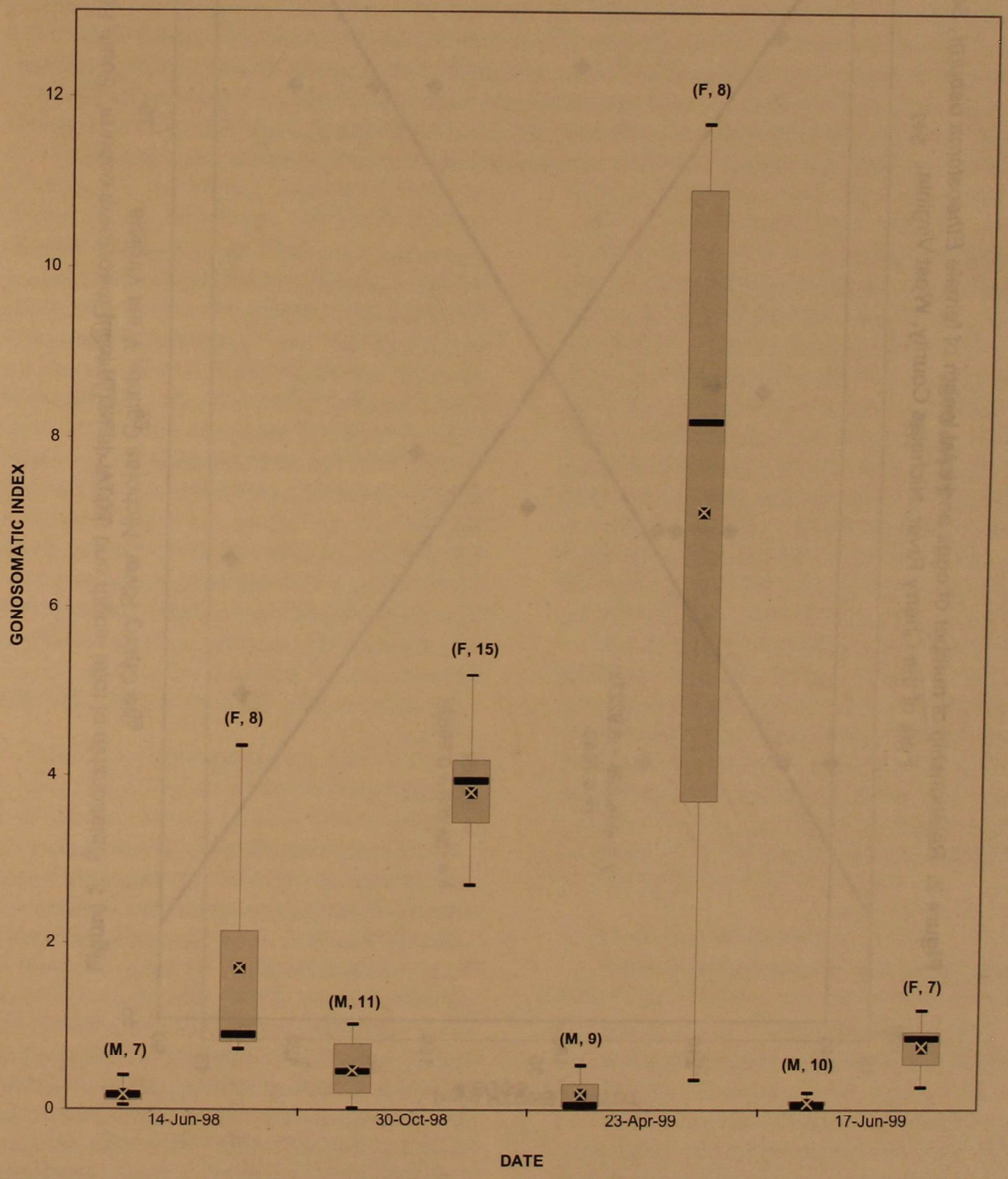
Figure 1. Seasonal variation of gonosomatic indices (GSIs) for *Etheostoma osburni,* South Fork of the Cherry River, Nicholas County, West Virginia. A vertical line represents the range, a box is standard deviatioin, a horizontal line (within box) is the median, and a darkened square with a white 'x' represents the mean. Sex (M=male, F=female) and sample size are in parentheses.



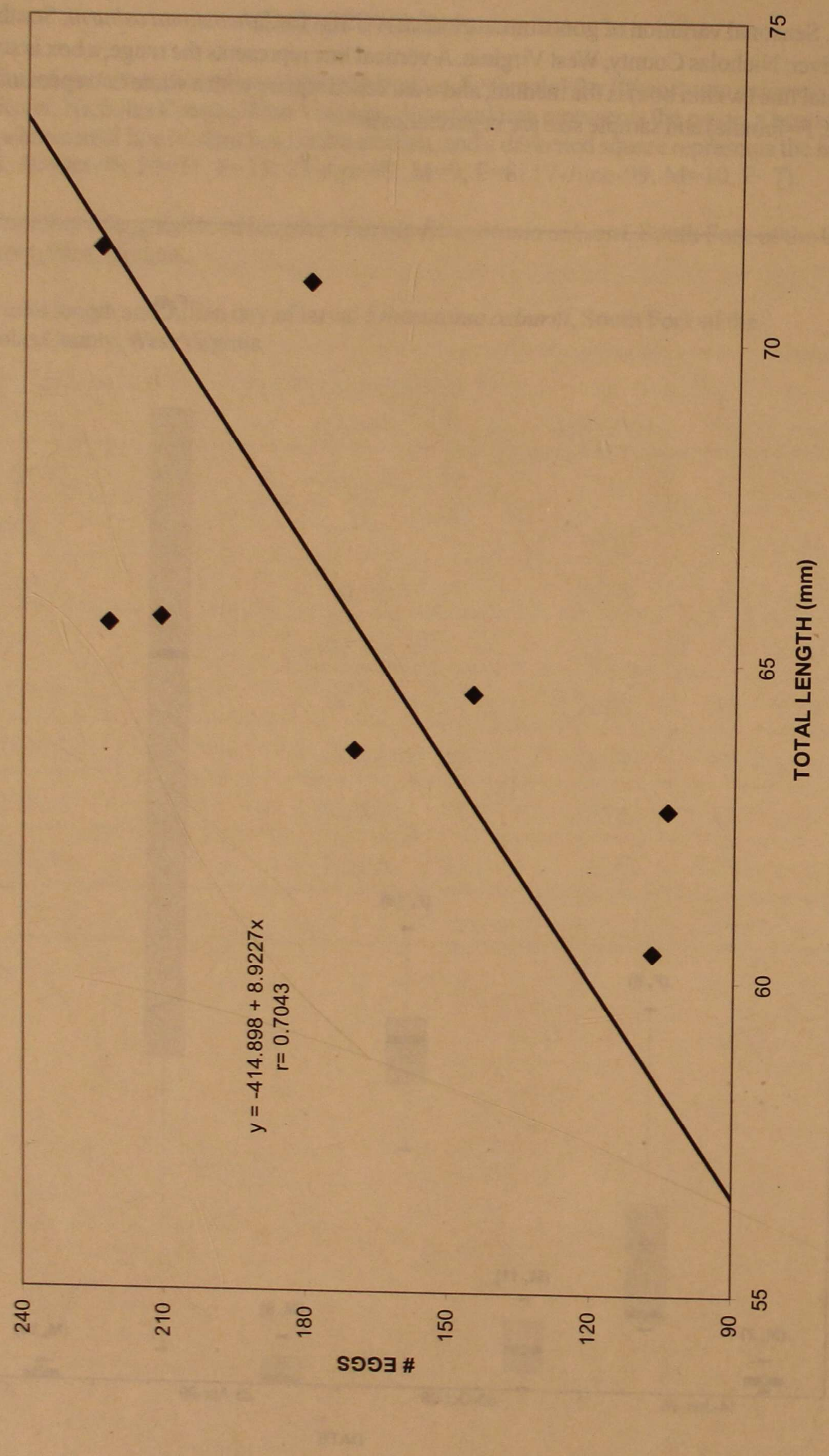


Figure 2. Relationship of number of eggs and total length of female *Etheostoma osburni*, South Fork of the Cherry River, Nicholas County, West Virginia.

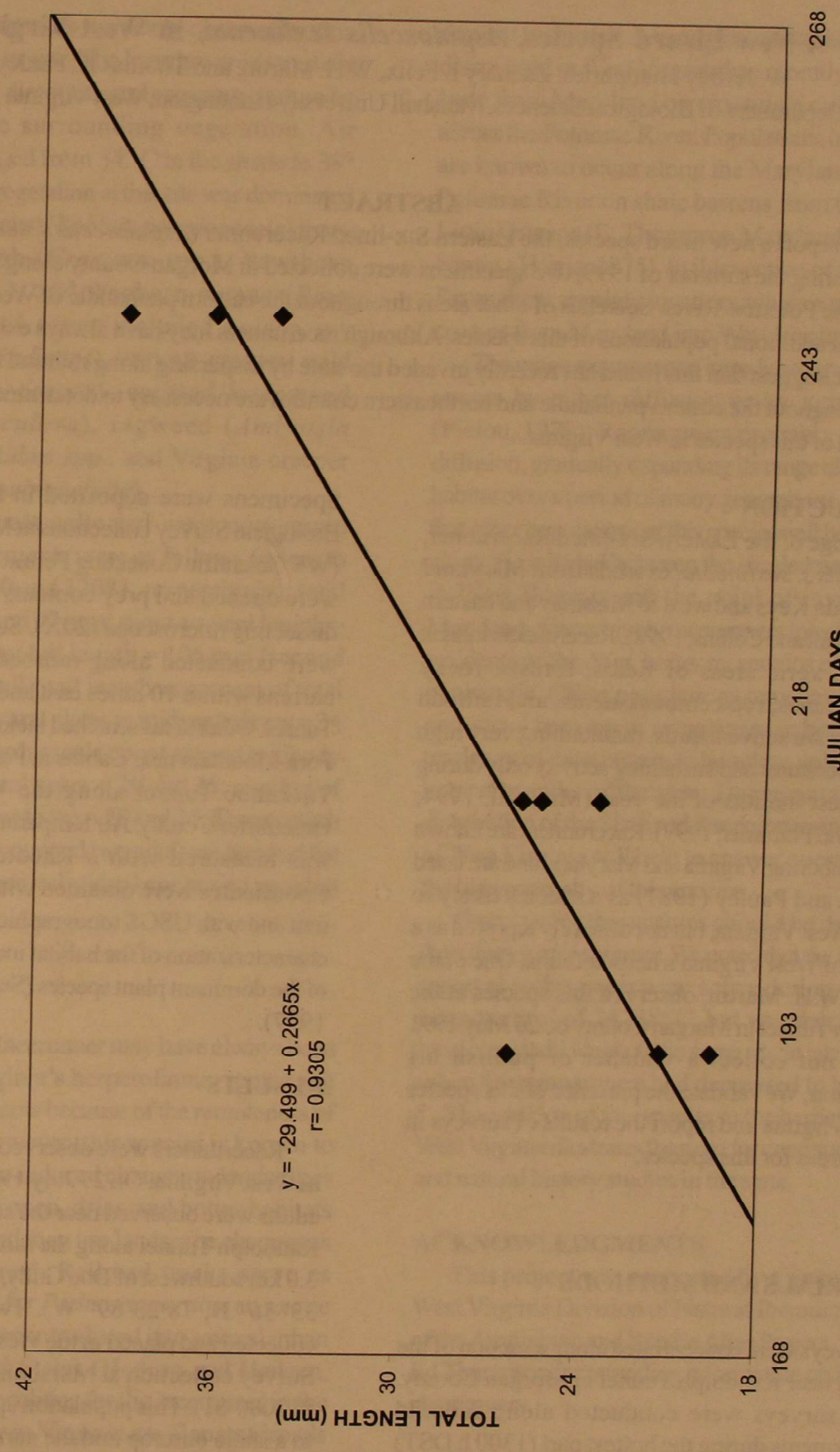


Figure 3. Relationship of total length and Julian day of larval *Etheostoma osburni*, South Fork of the Cherry River, Nicholas County, West Virginia.

A New Lizard Species, *Aspidoscelis sexlineata*, in West Virginia

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ABSTRACT

We report a new lizard species, the Eastern Six-lined Racerunner (*Aspidoscelis s. sexlineata*), in West Virginia. During the summer of 1999, two specimens were collected in Morgan County along the CSX railroad bordering the Potomac River. Searches of other areas throughout the eastern panhandle of West Virginia failed to document additional populations of this species. Although racerunners may have always existed in West Virginia, we suggest that this lizard has recently invaded the state by dispersing along railroad tracks. Further studies throughout the eastern panhandle and northeastern counties are necessary to determine the actual distribution of this species in West Virginia.

INTRODUCTION

The range of the Eastern Six-lined Racerunner, *Aspidoscelis s. sexlineata*, extends from Maryland to the Florida Keys and west to Missouri and eastern Texas (Conant and Collins, 1998). Racerunners inhabit hot, open, xeric areas of fields, forests, rocky outcrops, dunes, road embankments, and railroad cuts. These are active lizards, maintaining very high body temperatures and sustaining activity only during the warmest months of the year (Mitchell, 1994; Ransom and Plummer, 1999). Racerunners are known from neighboring Virginia and Maryland and are listed by Green and Pauley (1987) as a species likely to occur in West Virginia, but not officially reported as a member of West Virginia's herpetofauna. One of the authors, W.H. Martin, observed this species at the Randolph Tunnel in Morgan County on 26 May 1991 but did not collect a voucher or publish his observation. We validate the presence of this species in West Virginia and report the results of surveys in several areas for this species.

MATERIALS AND METHODS

Surveys were concentrated along a section of the railroad near Randolph Tunnel in Morgan County. Visual surveys were conducted along railroad embankments during the hottest part (1300 h DST) of the day. Lizards were collected by stunning them with rubber bands and catching them by hand. Specimens were deposited in the West Virginia Biological Survey collections at Marshall University (WV Scientific Collecting Permit 47-199). Stomachs were opened and prey contents examined under a dissecting microscope (20X). Subsequent searches were conducted along railroad tracks and shale barrens within 10 miles east and west of Randolph Tunnel. Other areas searched included a ridge of North Fork Mountain near Cabins in Pendleton County and Tuckahoe Tunnel along the Virginia border in Greenbrier County. Air temperature (°C) at each site was measured with a Rheotemp thermometer. Coordinates were obtained with a hand-held GPS unit and with USGS topographical maps. Additional characterization of the habitat included identification of the dominant plant species (Strausbaugh and Core, 1977).

RESULTS

Racerunners were observed at only one location in West Virginia. On 27 July 1999 at 1450 h, twelve adults were observed near the southwest entrance of Randolph Tunnel along the railroad, approximately 3.0 km southwest of Doe Gully, Morgan County (39° 35' 56" N, 78°23'89" W). Two individuals were collected and placed in the West Virginia Biological Survey collection at Marshall University (WVBS #12080-81). This population appears to be restricted to a shale outcrop and the surrounding shrubs less than 10 m from the railroad track. The outcrop has been blasted out on all sides and stands approximately

8.5 m high. Racerunners were only observed within an area 20 x 60 m in size. The lizards were extremely active, basking in direct sun and escaping capture by darting into the surrounding vegetation. Air temperatures ranged from 34° C in the shade to 38° C in the sun. The vegetation at this site was dominated by young black locust (*Robinia psuedoacacia*) trees, and included ash (*Fraxinus* spp.), hawthorn (*Cretaegus* spp.), and staghorn sumac (*Rhus typhina*). Ground cover included poison ivy (*Toxicodendron radicans*), various grasses, wild carrot (*Daucus carota*), spotted knapweed (*Centaurea maculosa*), ragweed (*Ambrosia artemisiifolia*), *Rubus* spp., and Virginia creeper (*Parthenocissus quinquefolia*).

Both individuals collected were non-gravid females. Measurements were as follows (refers to specimens #12080 and 12081, respectively): total length = 171 mm and 97 mm; snout-to-vent length = 65 mm and 63 mm; tail length = 106 mm (second specimen lost its tail); tail length as percent of total length = 62.0%; ventral plates in midventral row = 34 and 30; dorsal granular scale count around midbody = 80 and 78; femoral pores = 29 and 25; number of lamellae on right fourth toe = 28 and 25. The stomach of one individual contained two adult spiders and the stomach of the other individual contained an adult wasp.

DISCUSSION

The Six-lined Racerunner may have always been a part of West Virginia's herpetofauna, remaining undiscovered until now because of the remoteness of the population. However, this species is known to benefit from human-induced changes to landscapes which create more open, drier, and hotter habitats (Mitchell, 1994), and may use landscape changes as a means of dispersal. Railroad tracks serve as dispersal corridors for *Podarcis muralis,* an exotic lizard which has been introduced into several urban areas in the United States (Hedeen and Hedeen, 1999). All known localities for the racerunner in the Shenandoah Valley in Virginia are along railroads (Mitchell, 1994), which suggests that the species has used the tracks as dispersal corridors from more eastern localities. It is possible that the population discovered in West Virginia has recently invaded the state from Maryland by crossing a railroad trussle across the Potomac River. Populations of this species are known to occur along the Maryland side of the Potomac River on shale barrens from Old Town to Little Orleans (E. Thompson Maryland DNR, pers. comm.; Harris, 1975). In this section of the Potomac River, there are eight locations where a railroad track crosses from Maryland into West Virginia.

The range expansion of introduced species usually occurs by either diffusion or by jump-dispersal (Pielou, 1979). Racerunners probably disperse by diffusion, gradually expanding its range along suitable habitat over a period of many generations. We suggest that other populations of this species will be discovered along the railroad between the single known locality in West Virginia and the point of invasion from Maryland. Alternatively racerunners may be "natural" residents of the drier, hotter mountains of the eastern panhandle. Other populations may have remained unnoticed because of remoteness or because of the tendency of racerunners to be active only during the hottest months of the year. Documentation of the distribution of the Six-lined Racerunners in other parts of West Virginia will help to answer questions about the biogeography of this species.

Surveys for this species should be done on hot days during mid-summer. We noted that the individuals reported in this paper were active during midday in temperatures of 34 -38° C, but we were unable to locate any individuals at the same site in late afternoon when the temperature had decreased to below 30° C. The addition of this species to the herpetofauna of West Virginia illustrates the need for continued surveys and natural history studies in the state.

ACKNOWLEDGMENTS

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