1-1-1991

Ecology of the green salamander : Aneides aeneus (Cope and Packard), in West Virginia

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ECOLOGY OF THE GREEN SALAMANDER,

ANEIDES AENEUS (COPE AND PACKARD), IN WEST VIRGINIA

A Thesis

Presented to

the Faculty of the Graduate School

of Marshall University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Ronald A. Canterbury

THIS THESIS WAS ACCEPTED ON 8 12 91
Month Day Year

as meeting the research requirement for the master's degree.

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Leonard Deutsch
Dean of Graduate School

ACKNOWLEDGEMENTS

I would like to extend my deepest appreciation to Dr. Thomas K. Pauley, my major professor, for his guidance and encouragement during my graduate work at Marshall University. His expertise in the field of salamander ecology proved invaluable to the completion of this thesis. I would also like to thank Drs. Dan K. Evans and Michael E. Seidel for serving on my graduate committee. Their ideas and suggestions were very helpful throughout my research on Aneides aeneus.

I wish to thank Drs. Margaret A. Bird and Michael L. Little of Marshall University for supplying fruit fly media, stock bottles, and Drosophila cultures used to feed salamanders that were kept in the laboratory. Dr. Little also provided assistance on egg volume analysis.

A deep appreciation is also extended to Dr. Jim Joy of Marshall University for his assistance in photographing sperm of Aneides aeneus. I thank Dr. Jim Arnold and Charles C. Shiflet for their help in the photography. Appreciation is also extended to Dr. Wayne Elmore for his help with the dehydration studies and Dr. Thomas E. Weaks for his assistance in identification of the mosses found in Aneides aeneus habitat.

Special thanks is extended to Jeffrey E. Bailey and Ronnie D. Jewell for their assistance in some of the field work. I am grateful to superintendents Robert Doolittle of Holly River State Park and Mike Smith of Beartown State Park for their help and use of the parks. I thank Dr. C. J. McCoy of Carnegie Museum of

Natural History and Greg Schneider of Museum of Zoology, University of Michigan for loan of specimens used in the spermatogenic wave study.

In addition to the above-mentioned, appreciation is extended to Drs. Frank Gilliam, Donald C. Tarter, Ralph Taylor, and Chris Waldron of Marshall University for loaning equipment used in this study, and for providing suggestions about this manuscript.

I must thank my wife, Beverly, for her understanding and help during the long hours of writing this manuscript. Much love and thanks go to my mother, Dollie, and family for their assistance in the field work and for their financial support.

Financial aid was also provided in part by a grant presented by the Graduate School of Marshall University to offset some of the travel expenses and to purchase equipment. Without this funding the study could not have been completed.

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INTRODUCTION AND LITERATURE REVIEW

Much has been written about the ecology of the green salamander, Aneides aeneus (Cope and Packard). The life history and ecology of southern populations of this species have been studied intensively by Gordon (1952), Woods (1968), and Snyder (1971). Gordon collected data in Highlands, North Carolina on distribution, habitat selection, population dynamics, and the ability of A. aeneus to withstand water loss as compared to Plethodon jordani melaventris. Woods conducted a similar study in Mississippi, and Snyder examined the adaptive brooding behavior of A. aeneus. In addition to these studies, further research has been conducted by Dr. Paul V. Cupp, Jr. of Eastern Kentucky University and by Dr. J. William Cliburn and students at the University of Southern Mississippi. Cupp (1971, 1979, and 1980) observed territorial behavior, Fall courtship, and mating in A. aeneus in Kentucky. Cliburn's reseach focused on the comparative climbing abilities and vertical stratification of A. aeneus and P. glutinosus (Cliburn and Porter 1986 and 1987). Cliburn also examined A. aeneus responses to a magnetic field (Cliburn and Pihkala 1986, Pihkala and Cliburn 1986). Baltar (1983), a former student of Cliburn's, found that vertical stratification of some cliff dwelling plethodontid salamanders is related to the competitive survival of the Mississippi population of A. aeneus.

Other studies that must be mentioned include Juterbock's research on the endangered Ohio populations (Juterbock in

Pfingsten and Downs 1989), karotype analyses (chromosomes, DNA sequences, and evolution) of the genus Aneides by Macgregor and Jones (1977), and Lee and Norden (1973) examined the gut contents of 25 specimens collected from Cooper's Rock State Forest, Monongalia County, West Virginia. In addition, Wake (1963) clarified the osteology and taxonomic relationships of the genus Aneides. Dunn (1926) considered A. aeneus to be the most primitive member of the genus, but Wake (1966) showed that the more recently described A. hardii, a southwestern plethodontid confined to high elevations in south central New Mexico, is the more primitive. Bruce (1968) examined the role of the Blue Ridge Embayment in the zoogeography of this species. Wylie (1966 and 1971) has published several papers on A. aeneus in West Virginia and Norden (1974) examined brooding behavior of this species in the state.

Because of the specific habitat requirements, sedentary nature, and colonial existence of this species which points to a limited gene flow, it was suggested by Gordon (1952) and Woods (1968) that further studies on geographical variation were needed. In addition, little was known about the population biology/ecology of A. aeneus in the northern portion of its range.

The purpose of this thesis was to examine the ecology of A. aeneus in the northern portion of its range (West Virginia) with an emphasis on the population biology of this species. The following questions were addressed.

1. Does any geographical variation in life history and reproductive biology (i.e., time of mating and egg laying) occur between populations in the north (West Virginia) and south (southwestern North Carolina and northeastern Mississippi)? What degree of synchrony exists within or between populations (Chapter II)?

2. Does any altitudinal variation in body size occur between populations in West Virginia (Chapter III)?

3. Does any variation in behavior (i.e., competitve relationship with other rock-dwelling plethodontid salamanders) exist between populations (Chapter IV)?

The location, topography, and shape of West Virginia places it in five physiographic provinces ranging from the Allegheny Plateau east through the higher elevations of the Allegheny Mountains and rugged terrain of the Ridge and Valley Province, to the Great Appalachian Valley and Blue Ridge Mountain Provinces in the Eastern Panhandle. The elevation of these provinces ranges from 73 m at Harpers Ferry, Jefferson County to 1482 m at Spruce Knob, Pendleton County (Green and Pauley 1987). These provinces also differ in weather patterns. For example, the annual mean temperatures range from 12 °C in the Allegheny Plateau to 9.7 °C in the Allegheny Mountains (U.S. Department of Commerce 1973). Annual average precipitation varies from 110.2 cm in the Allegheny Plateau to 136.6 cm in the mountains and 93.0 cm in the Ridge and Valley section (U.S. Department of Commerce 1973).

Since _A_. _aeneus_ occurs in disjunct colonies throughout its range and probably has a limited gene flow, adaptations to such differences in topography, elevation, and weather may lead to geographical variation between populations. On the other hand, a long period of isolation and close association with its climbing or arboreal mode of life coupled with its specific habitat requirements may have resulted in little variation between populations.

DESCRIPTION OF THE SPECIES

Aneides aeneus is a completely terrestrial (the larval stage occurs in the egg) member of the family of lungless salamanders, the Plethodontidae. The generic common name for *Aneides* is climbing salamanders, which alludes to the arboreal and rock-climbing tendencies of several species of the genus (Conant 1975). Members of *Aneides*, unlike all other plethodontids, have the posterior portion of the maxilla sharply edged without teeth, vomerine and parasphenoid teeth separated, tips of the phalanges Y-shaped, and the tongue attached in front (Green and Pauley 1987). Wake (1966) considered the genus *Aneides* to be closely related to the genus *Plethodon* and to be the most specialized and advanced genus in the tribe Plethodontini. Gordon (1952) considered *Aneides* to be the most terrestrial of the eastern United States plethodontids.

Aneides aeneus is the only member of its genus found east of the Mississippi River and is distinguished from its western congeners by having nasals in which the width equals or exceeds the length (Wake 1966). It is a small to medium-sized salamander that may attain a total length of 12.7 cm. The dorsal color is dark brown with irregularly shaped, lichenlike, light green to yellow patches over the body. The specific epithet aeneus (Latin aeneus, of bronze or copper) refers to the bronze, lichenlike patches (Gordon 1967). The ventral surface is lighter than the dorsum and unmarked. Aneides aeneus (Figure 1), like other arboreal and rock-climbing members of the genus Aneides, has a flattened head and body, relatively long limbs with squared-off, expanded toe tips, and a long, somewhat prehensile tail. The head is large and noticeably wider posterior to the protruberant eyes. This widening is caused by enlargement of the jaw musculature, and is exaggerated in the adults, especially males. There are 14 or 15 costal grooves (Gordon 1967, Woods 1968, Conant 1975, Green and Pauley 1987).

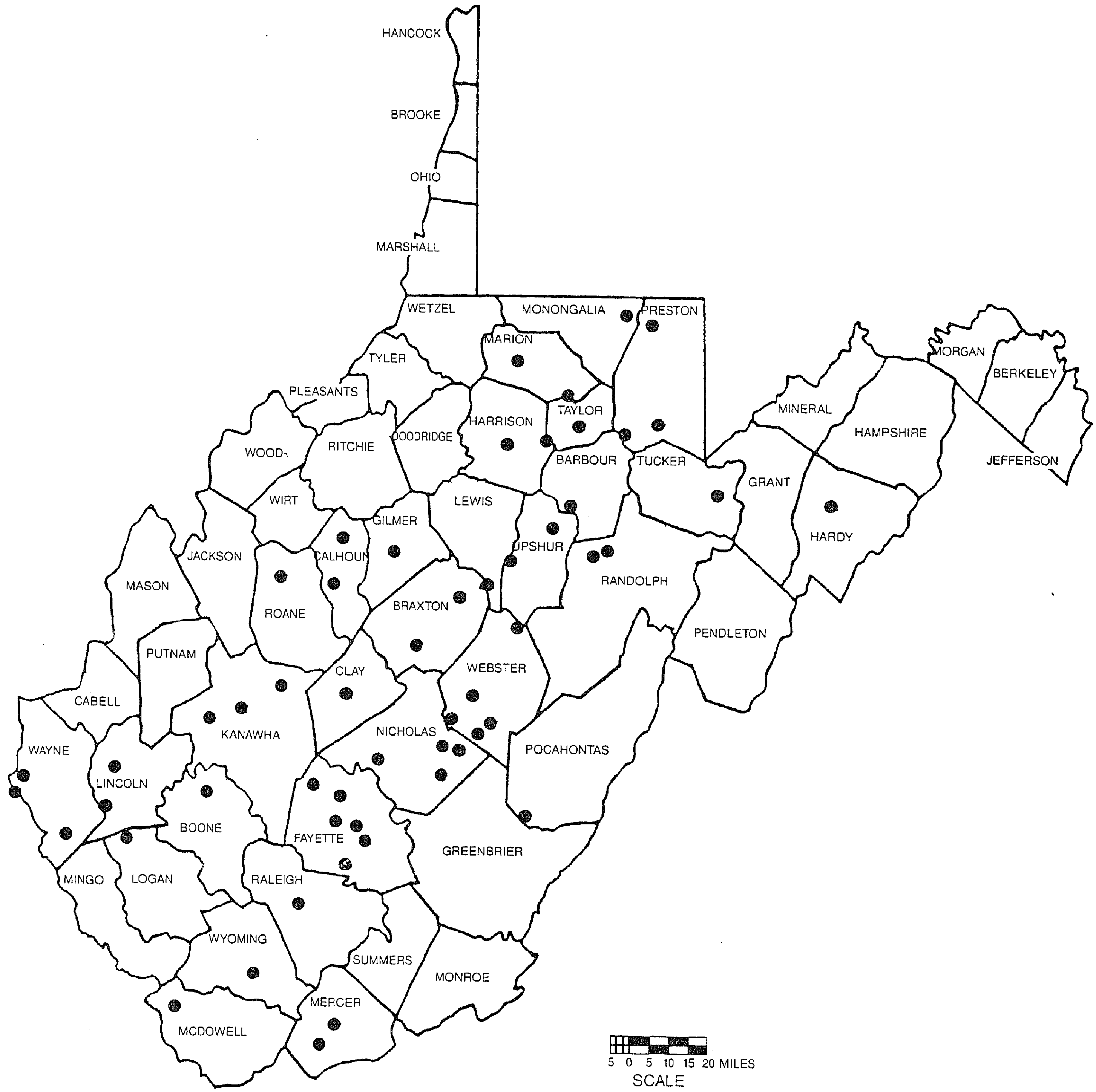
Aneides aeneus is distributed from southwestern Pennsylvania, western Maryland, and southern Ohio to west central Alabama and northeastern Mississippi. It is found in disjunct populations throughout the Allegheny Plateau and isolated populations occur in the southern Blue Ridge Mountains from southwestern North Carolina to northeastern Georgia (Gordon 1967, Woods 1968, Conant 1975, Green and Pauley 1987). It has never been found above 1333 m in elevation (south slope of Cold Mountain, Transylvania County, North Carolina); the lowest elevation known is 139 m (Gordon 1967). Aneides aeneus appears

Figure 1. Dorsal view of the green salamander, *Aneides aeneus*. This plethodontid salamander has lichenlike blotches over the body and is anatomically adapted for climbing and living in rock crevices. Note the elongated phalanges with terminal expansions, the long, slender tail, and the flattened head and body.

to be absent from apparently suitable habitat throughout the Great Smoky Mountains National Park (Gordon 1967). Gordon has suggested that the prolonged brooding period of the female, which consumes most of the active portion of the annual cycle, prevents _A. aeneus_ from inhabiting elevations exceeding 1333 m. In West Virginia, _A. aeneus_ occurs predominately in the central counties of the Allegheny Plateau from Monongalia and Preston counties in a southwesterly direction to the Big Sandy River (Green and Pauley 1987). Figure 2 shows its distribution in West Virginia and was based on records from the West Virginia Biological Survey and personal records of Dr. Thomas K. Pauley and the author. The highest elevation it has been recorded in West Virginia is 936 m at Canyon Rim Trail in the Monongahelia National Forest, Tucker County and the lowest is 333 m at Cabwaylingo State Forest. It appears to be absent from suitable habitat in Bear Heaven Camp Ground (1045 m), Monogahelia National Forest, Randolph County (Canterbury and Pauley, unpublished data 1990). This supports data collected by Gordon (1967) on altitudinal distribution of this species. Bear Heaven is apparently too far north and too high in elevation to allow enough time to complete reproduction.

Aneides aeneus inhabits narrow crevices on rock outcrops in mesophytic forests. These rock outcrops must be damp but not wet, situated where the atmosphere is humid, and well protected from sun and direct rain (Gordon 1952 and 1967, Woods 1968, Conant 1975). The nature of the rock formation is immaterial,

Figure 2. Geographical distribution of Aneides aeneus in West Virginia. Solid cicles indicate records of populations of which voucher specimens are available in the West Virginia Biological Survey, Marshall University.


HANCOCK
BROOKE
OHIO
MARSHALL
WETZEL
MONONGALIA
PRESTON
MARION
TYLER
PLEASANTS
MORGAN
BERKELEY
MINERAL
TAYLOR
HARRISON
HAMPSHIRE
JEFFERSON
WOOD
RITCHIE
DODDRIDGE
BARBOUR
TUCKER
GRANT
WIRT
LEWIS
GILMER
UPSHUR
HARDY
JACKSON
CALHOUN
RANDOLPH
MASON
ROANE
BRAXTON
PENDLETON
PUTNAM
CLAY
WEBSTER
CABELL
KANAWHA
NICHOLAS
WAYNE
POCAHONTAS
LINCOLN
BOONE
FAYETTE
GREENBRIER
MINGO
LOGAN
RALEIGH
WYOMING
SUMMERS
MONROE
MERCER
MCDOWELL
5 0 5 10 15 20 MILES
SCALE

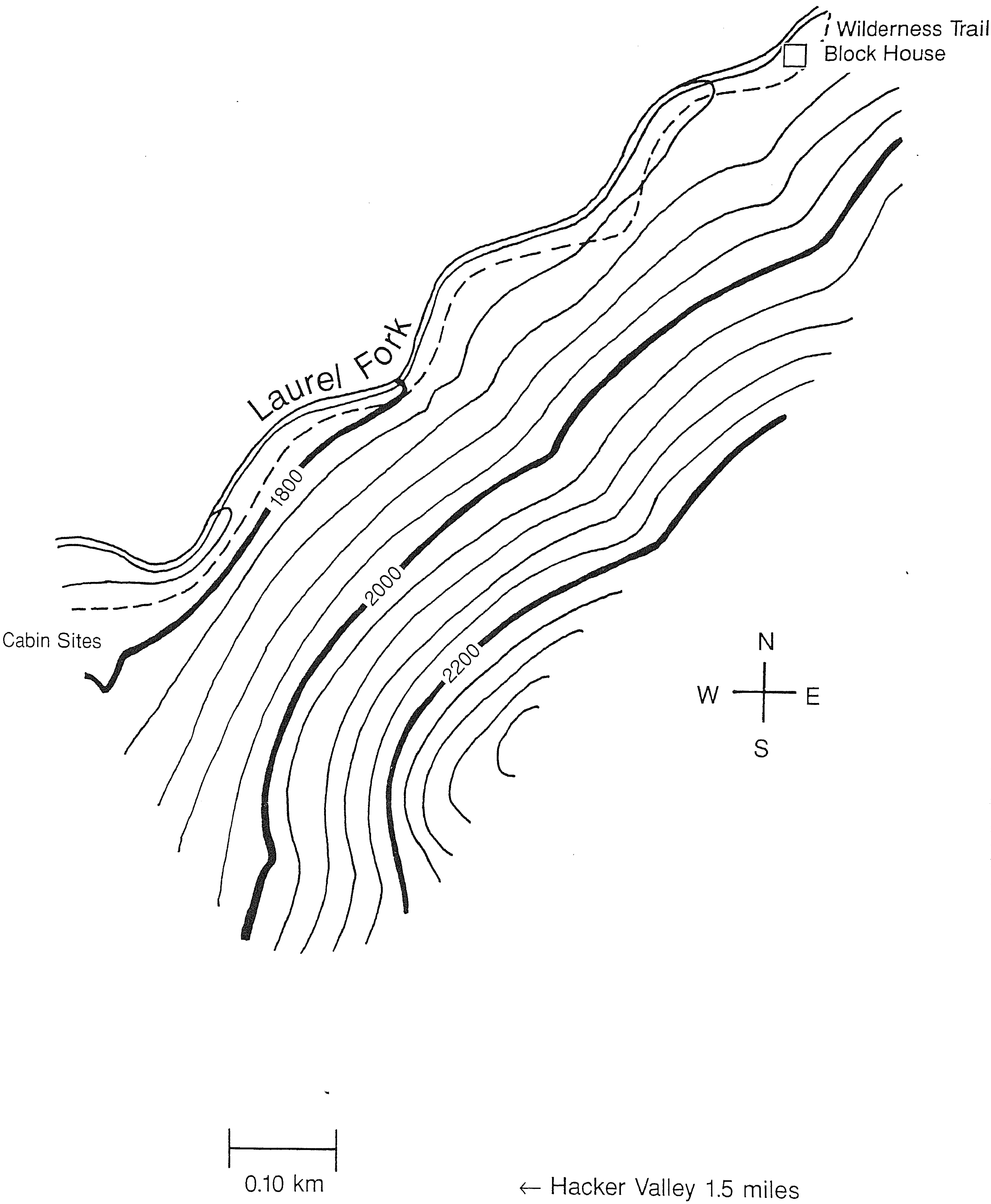
but its geological condition as to crevice depth and position appears to be a limiting factor (Gordon and Smith 1949). Netting and Richmond (1932) collected A. aeneus near Cooper's Rock State Forest, Monongalia County, West Virginia in sandstone crevices. Brooks (1948) found that the only habitat utilized by this species in Summersville, Nicholas County, West Virginia was sandstone cliffs. Walker and Goodpaster (1941) found it in limestone solution pockets in Ohio, and Gordon and Smith (1949) revealed its presence in granite crevices in North Carolina. Gordon (1952) and Woods (1968) concluded that A. aeneus is a cliff-dwelling species found in damp rock outcrops and resorts to arboreal and other residences only where suitable habitat is lacking. Gordon (1952) states that in eastern Kentucky, southwestern Virginia, and adjacent portions of Tennessse A. aeneus occurs in arboreal or arboreal and rock crevice habitat. In all other portions of its range it is found chiefly in rock crevices. He indicated that the region of arboreal habitat coincides with the undifferentiated mixed mesophytic forest of Braun (1941), while the rock habitat generally occurs in regions of segregated forests of the mixed mesophytic type. Pope (1928) found Aneides aeneus under bark strips of fallen logs and Dr. N. B. Green also collected it from bark strips (West Virginia Biological Survey). Dr. Thomas K. Pauley (personal communication, 1989) stated that he has also found it under bark of rotten logs. Pitfall traps set in the New River Gorge National River (Kates Br., Raleigh County) during the 1989 summer produced a single specimen in an area without rock outcrops, suggesting arboreal habits where rock crevice habitat is missing.

DESCRIPTION OF THE STUDY AREA

This study was conducted at Holly River State Park (Nature's Rock Garden and Wilderness Trails) located in Hacker Valley, Webster County, West Virginia. Holly River State Park lies 35.2 km directly north of Webster Springs, 54.4 km south of Buckhannon, and 5.9 km east of Pickens, Randolph County, a small community just west of the Appalachian Divide. Holly River State Park is found within the Allegheny Mountains Physiographic Province and covers 3,359 ha where the elevation ranges from 485 to 909 m. The total study area at Nature's Rock Garden (Figure 3) covers 1.5 ha and consisted of several ravines and rugged hillsides along Laurel Fork, a tributary to Holly River. The area averages 545 m in elevation and is predominately mature, dense mesophytic forest. Nature's Rock Garden Trail begins at the cabin area and covers a circular loop of about 0.8 km. Many of the rocks where A. aeneus were found lie along this trail and near the park cabins. The Wilderness Trail portion of the study area starts at cabin 1 and ends where it joins Laurel Fork. The substratum as well as the rock outcrops are sandstone, thus the soil is very rocky.

This area has the greatest annual average precipitation (170.2 cm) in the state. The mean annual temperature for Holly

Figure 3. An outline of the study area showing 40-foot contour lines. Enlargement from U. S. Geological Survey topographic map, Goshen Quadrangle. Adapted from techniques of Pauley (1977).


Wilderness Trail
Block House
Laurel Fork
1800
2000
2200
Cabin Sites
N
W
E
S
0.10 km
← Hacker Valley 1.5 miles

River is 10.2 °C (U. S. Department of Commerce 1990) and these conditions (temperature and moisture) allow for great salamander diversity including a dense population of *A. aeneus*.

CHAPTER I

HISTORY OF ANEIDES AENEUS IN WEST VIRGINIA

Aneides aeneus was first collected in West Virginia on 15 August 1900 by W. P. Moore from Baileysville in Wyoming County (Wildlife / Heritage Data Base 1983). Surveys in the 1930's - 1960's by Mr. Neil D. Richmond and Dr. N. Bayard Green produced specimens representing statewide populations of A. aeneus. These specimens are part of the West Virginia Biological Survey Collections at Marshall University. These historical populations form the bulk of information upon which the present distribution of A. aeneus is documented in Amphibians And Reptiles In West Virginia (Green and Pauley 1987). The purpose of this section of my research was to survey historical sites in an attempt to determine if A. aeneus appeared to be declining in West Virginia. These surveys were needed to update the status of this species since it is listed as a species of special concern by the West Virginia Division of Natural Resources.

Several historical populations of Aneides aeneus appear to be declining or have been extirpated. This is particularly true of populations in the western part of the state where human activity has decimated populations. The population at Cabwaylingo State Forest in Wayne County seemed to be very small and appeared to be declining. There are numerous specimens from this locality in the West Virginia Biological Survey, but the highest number of individuals recorded at this locality during the present study was seven on 11 October 1989. Only one breeding female was found at this locality on 9 July 1990. Aneides aeneus were found only at two locations in Cabwaylingo State Forest. These are referred to as sites A which has three rock outcrops and site B containing nine rock outcrops. No salamanders were found on other nearby rock outcrops (site C). Site A and B are 0.8 mi. apart, and site C is on the opposite side of the road from site A. This low density is obvious when compared to Holly River State Park (block house path site). Both sites had similar habitats (about the same number of rocks and crevices in damp, mesophytic forests), but each of the eight outcrops at the block house path site contained as many as 20-30 salamanders (Appendix II). At locality B, a single specimen was observed on an exposed rock along the road near the edge of the forest on 11 October 1989. However, this rock was found crushed on 15 January 1990 and suggested that habitat destruction by humans may contribute to the decline of this species. The picnic areas found at Cabwaylingo State Forest site A as well as the dirt road separating sites A and C may support the hypothesis that A. aeneus is declining partly because of habitat destruction.

Other populations in the western part of the state may be declining in numbers. For example, three visits to the Henry Ferguson Branch site produced no more than four individuals per visit (Table 1). The Louisa, Echo, and Myra populations (Table 1 and 2) were extirpated and have been replaced by farms and

Table 1. Historical Populations surveyed for A. aeneus. Part I.

Locality	County	Date	Number observed	Age class A= adult I= immature
Cabwaylingo State Forest	Wayne	11 Oct 1989 29 Oct 1989 15 Jan 1990 17 Feb 1990 9 July 1990 7 April 1990	7 0 1 1 1 0	A & I I I A
1 mi. N Echo along Rt. 52	Wayne	22 Feb 1990	0	
1 mi. E Lousia Wayne	Wayne	22 Feb 1990	0	
Big Br. 3.5 mi. NW Fort Gay	Wayne	22 Feb 1990	0	
Henry Ferguson Br. 4 mi. NE Fort Gay	Wayne	22 Feb 1990 8 March 1990 27 April 1990	1 2 4	I I A

Table 2. Historical populations surveyed for A. aeneus. Part II.

Locality	County	Date	Number Observed	Age Class A= Adult I=Immature
0.5 mi. N Myra along Mud R.	Lincoln	8 March 1990	0	
Beartown State Park	Greenbrier	29 May 1990 19 June 1990	0 0	
Scarbro 4-H Camp	Fayette	2 July 1990	0	
Grandview State Park	Raleigh	22 May 1990	0	
Babcock State Park	Fayette	22 May 1990	0 6	
Rockcamp; 3 mi. S Gassaway	Braxton	3 June 1990	7	A & I
Coon Creek, 4 mi. S Gassaway	Braxton	3 June 1990	2	A
Coopers Rock State Forest	Monongalia	30 May 1991 31 May 1991 24 June 1991 25 June 1991	21 1 5 2	A & I A ♀ w/eggs A & I A

houses. Populations at other locations such as Beartown State Park on Droop Mountain appeared to be declining for unknown reasons. Several A. aeneus were collected from this site in 1935 and 1940, but Dr. Thomas K. Pauley found only a single specimen on 29 May 1981. Dr. Pauley and I surveyed this site on 29 May 1991 and on 19 June 1990 and no A. aeneus were found. Only Plethodon wehrlei were found in the rock crevices which leads me to believe that interspecific competiton for crevices between these two species may play a significant role in the competitive survival of A. aeneus. Competition, rather than other natural events such as changes in habitat and microclimatic conditions, was suspected to be aiding in the decline since the habitat at this site still appeared suitable (damp rock outcrops with narrow crevices, moderate temperatures and high relative humidity situated in ravine of mesophytic forest) for A. aeneus. It still remains uncertain as to whether this population exists as a very small one or whether it has been extirpated.

Aneides aeneus has been reported to be declining in North Carolina and possibly throughout the southern Appalachians (Juterbock in Pfingsten and Downs 1989), and is listed by the States of Ohio and Maryland as an endangered species because of its specific habitat requirements and occurrence in disjunct populations. Since about half the known range of this species occurs in West Virginia, extirpation of local populations may have severe consequences. The effects of man's activities, including lumbering and clearing of forested areas, on limiting the distribution of this species has long been noted in the literature. Its spotty distribution in Ohio due to such activities has been noted by Dr. N. B. Green (in Gordon 1952). Baltar (1983) studied factors effecting survival of *A. aeneus* in Mississippi and concluded that survival was becoming increasingly difficult as technological advancements were made in the region. However, field surveys in the New River Gorge National River have revealed 15 previously unknown populations. These populations appear to be reproducing since first year immatures were collected at several sites. In addition, large numbers of this species have been observed by Dr. Thomas K. Pauley while conducting field surveys on the West Virginia University Forest near the historical site at Cooper's Rock State Forest (Table 2).

CHAPTER II

NATURAL HISTORY OF THE GREEN SALAMANDER, *ANEIDES AENEUS* (COPE AND PACKARD), IN WEST VIRGINIA

INTRODUCTION: The life history and ecology of *Aneides aeneus* was first described by Gordon (1949 and 1952). Gordon (1952) documented its habitat and community relationships, life history and annual cycle, and population dynamics in North Carolina. He identified the vegetative, invertebrate, and vertebrate communities associated with *A. aeneus*. Although habitat selection in this species has been investigated by Gordon (1952), Woods (1968), and Baltar (1983), no studies have been made on why *A. aeneus* inhabits some rock outcrops but not others. Dr. Thomas K. Pauley said that he has considered this question for years while conducting ecological studies on salamanders throughout West Virginia, but has never had the opportunity to investigate it (personal communication 1989). Therefore, one purpose of this study was to determine why *A. aeneus* inhabited some rock outcrops but not others at Holly River State Park. Some of the factors that limit the distribution of plethodontid salamanders include: vegetation, temperature, moisture, and direction of slope or aspect (Pauley 1977). This chapter examines the relationship between these limiting factors and the distribution of *A. aeneus* in its restricted habitat. The following objectives were considered:

1. Microenvironmental factors such as crevice temperature,

crevice relative humidity, and aspect of the rock outcrops were examined for *Aneides aeneus*. For comparison, data were also collected on *P. glutinosus*.

Gordon (1952), Woods (1968), and Snyder (1971) reported a "buffering effect" of the crevice as indicated by the difference in temperatures between crevices and ambient environment. Gordon (1952) found a difference of 5.6 °F in maximum temperature between the crevice and the ambient environment. Gordon and Smith (1949) reported the average temperature for five days (June 16 to 21) in 1948 in seven nesting crevices to vary only slightly from 18 °C. In addition, Gordon (1952) also found a persistence of high ambient relative humidity, but he did not record the relative humidities inside the rock crevices. Snyder (1971) found a reduced amplitude (2 °C vs 6 °C) of daily fluctuations between the crevice temperatures and ambient air temperatures one meter from the rock face as well as a time lag of about 2-4 hours in the crevice temperatures as compared with the ambient temperature. The relative humidity of the crevice air did not fluctuate with the relative humidity of the outside as temperature did, but both remained relatively high. Snyder (1971) reported that the nesting crevices used by *A. aeneus* provide a microenvironment of high relative humidities and constant temperatures. He also indicated that the presence or absence of a female in a crevice had little effect on these parameters since a vacant nest crevice had about the same values. Thus, it seems logical that differences in such environmental factors as temperature and moisture may determine the presence of A. aeneus on some rock outcrops but not others. Temperature and moisture are also influenced by the direction of slope and rock exposure (aspect). Snyder (1971) found the direction of slope exposure to be of little significance in determining the presence or absence of A. aeneus. However, the relationship between exposure, temperature, and moisture and what, if any, role they play either singly or in combination in determining the presence of A. aeneus was unclear.

2. There may be a difference in vegetation types associated with rocks outcrops inhabited by A. aeneus vs rocks without this species (plants as indicators of occurrence of A. aeneus).

3. Another purpose of this thesis was to clarify the life history and reproductive biology of this species in West Virginia since it was unclear whether populations in the northern edge of its range follow the same patterns as those in northeastern Mississippi and southwestern North Carolina. To determine what degree of synchrony exists in the annual cycle between A. aeneus in West Virginia and more southern populations, the following questions were examined during this study: 1. Does A. aeneus in West Virginia follow the same seasonal patterns of spring emergence, breeding, fall aggregation, and hibernation periods as populations in the southern Blue Ridge Mountains and Mississippi? Since populations in North Carolina differed somewhat in their annual cycle from the Mississippi population it was assumed that northern populations found in West Virginia would show some

adaptation to northern limits of their range and thus a different annual cycle. 2. Does monthly surface abundance fluctuate and what is the relationship between surface abundance and microclimatic conditions? 3. Does the spermatogenic wave and ovarian egg cycle of West Virginia Aneides support a spring and fall courtship and mating period as suggested by Snyder (1971) and Cupp (1980)?

Gordon (1952) summarized the life history and annual cycle of A. aeneus in the southern Appalachians. He found the annual cycle of this species includes four periods: The breeding period, dispersal and aggregation period, hibernation, and post-hibernation aggregation and dispersal period. The breeding period which involves mating, egg laying, and hatching lasts from late May to late September. Gordon (1952) found that mating probably occurred in spring (late May and early June), but data collected by Snyder (1971) and Cupp (1980) have indicated that A. aeneus is apparently both a spring and fall breeder. Egg laying in the southern Appalachians occurs from late May into early July, but is concentrated in the first two weeks of June. In that region, A. aeneus nests have been found only in rock crevice situations where the female deposits from 10 to 30, whitish-yellow, eggs. Eggs are normally deposited on the ceiling of the nest crevice and guarded by the mother for about 12 weeks (84-91 days, average 86) until hatching in late August or early September. Snyder (1971) found one female to brood for 117 days. Gordon found the dispersal and aggregation period to extend from late September to November. During this time, hatchlings and adult females leave the breeding crevices and move with other members of the population to the vicinity of deep crevices in the rock outcrops. Hibernation occurs in deep, anastomosing rock crevices from November through late April. The animals emerge from their winter retreats in late April and early May and enter the post-hibernation aggregation and dispersal period. During this time, they disperse to outlying breeding crevices as the weather warms.

Woods (1968) found a shorter incubation period of 82 days in the Mississippi population as well as differences in the annual cycle. Woods divided the annual cycle into two periods: (1) The breeding period lasting from late March to November and (2) The dispersal and aggregation period lasting from November to March.

In addition to these studies mentioned above, this thesis also examined the feeding habits of this species and whether populations throughout the state feed on the same invertebrate taxa. Lee and Norden (1973) found *A. aeneus* to be primarily insectivorous, but it is still unclear whether any seasonal patterns exist or if populations feed on the same invertebrate species.

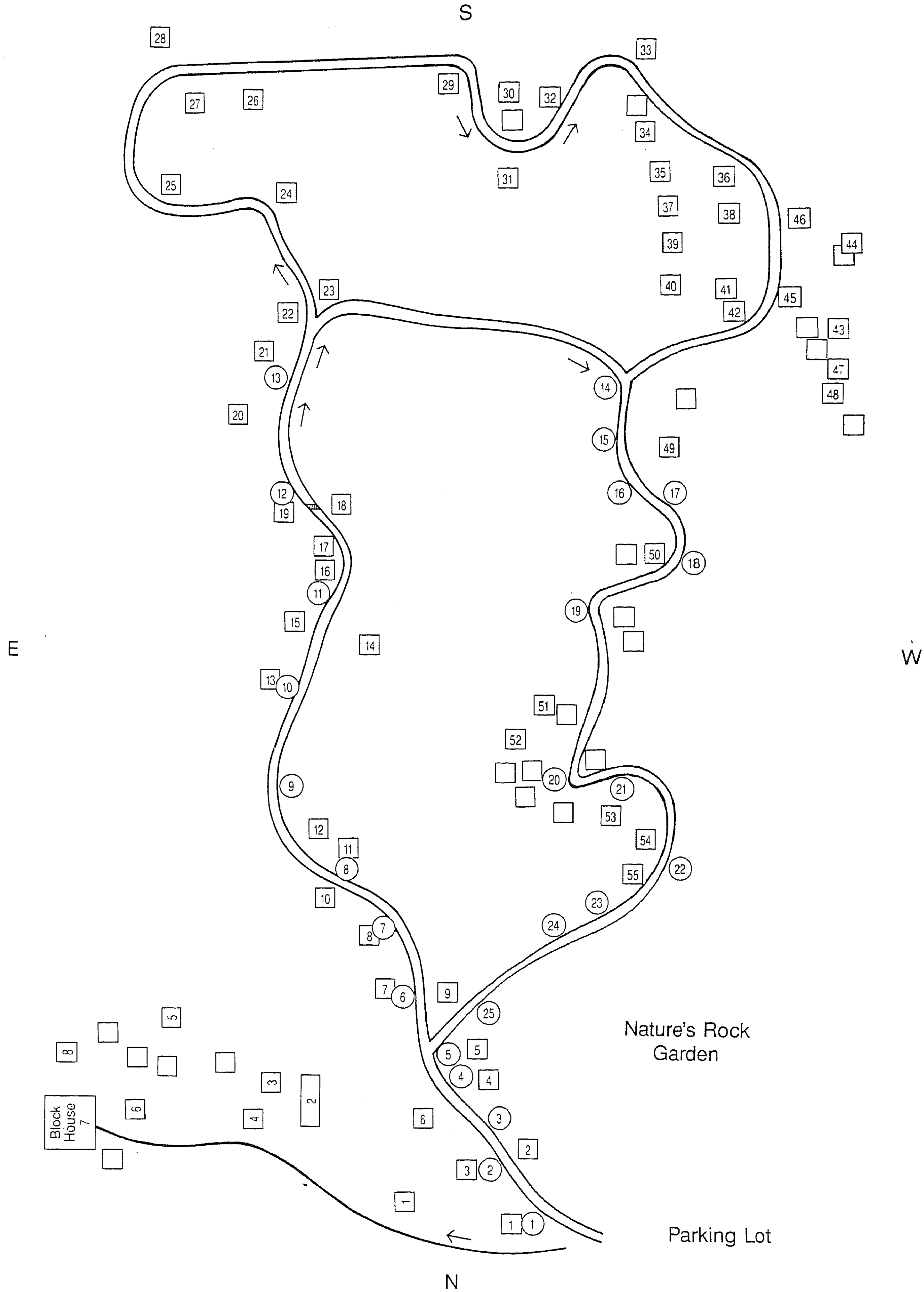
METHODS AND MATERIALS:

Habitat Selection

Most large rocks in the study area were surveyed for *A. aeneus* from the second week in May 1990 until the end of the study in early June 1991 to determine which rocks had salamanders. Sixty-three rocks were marked consecutively with a number using red fingernail polish. Rocks 1-55 were in Nature's Rock Garden area and rocks 1B-8B were in the Wilderness Trail area (Figure 4). These rocks are listed in Appendix II and denoted whether or not they contained salamanders. Environmental data (temperature and relative humidity) were obtained by use of a Hanna stick hygrometer (model HI 8565) and pocket thermometer. Both ambient and crevice temperatures and relative humidities were recorded at Holly River State Park and Beartown State Park (Appendix I). Crevice temperature and relative humidity were recorded by placing the instruments inside the crevice within a few centimeters of the salamander. Ambient readings were obtained by placing the instruments on the rock face or one m from the rock and 2 m above ground. Aspect was obtained with a compass.

Two of the four strata of the floral community proposed by Dumas (1956) were surveyed for this study. These included trees and mosses and liverworts (bryophytes). The floral study was made from June through mid-September 1990. Trees were identified using Strausbaugh and Core (1977) and counted by the true census method in four vertical transects 30 m in length and 3 m wide. Two of these transects were placed in areas having rocks with A. aeneus (one each at rocks 2B-6B or area 1 and rocks 26-28 or area 2). The other two were placed in areas having rocks without A. aeneus (one each at rocks 4-7 or area 3 and 47-52 or area 4).

Figure 4. Marked rocks in Holly River State Park study area. Rock 7B was a man-made cement building with cracks that was inhabited by _A_. _aeneus_.



S
E
W
N
Block House 7
Nature's Rock Garden
Parking Lot

Bryophytes were collected from selected rocks inhabited by and not inhabited by A. aeneus, and transported to the laboratory for identification using the methods of Conrad (1956). For comparison, trees and bryophytes were identified at sites B (rocks with A. aeneus) and C (rocks without A. aeneus) in Cabwaylingo State Forest. One transect 20 m in length was placed at each site on 9 July 1990 to survey trees. Bryophytes were surveyed on 29 October 1989 using the same methods mentioned above.

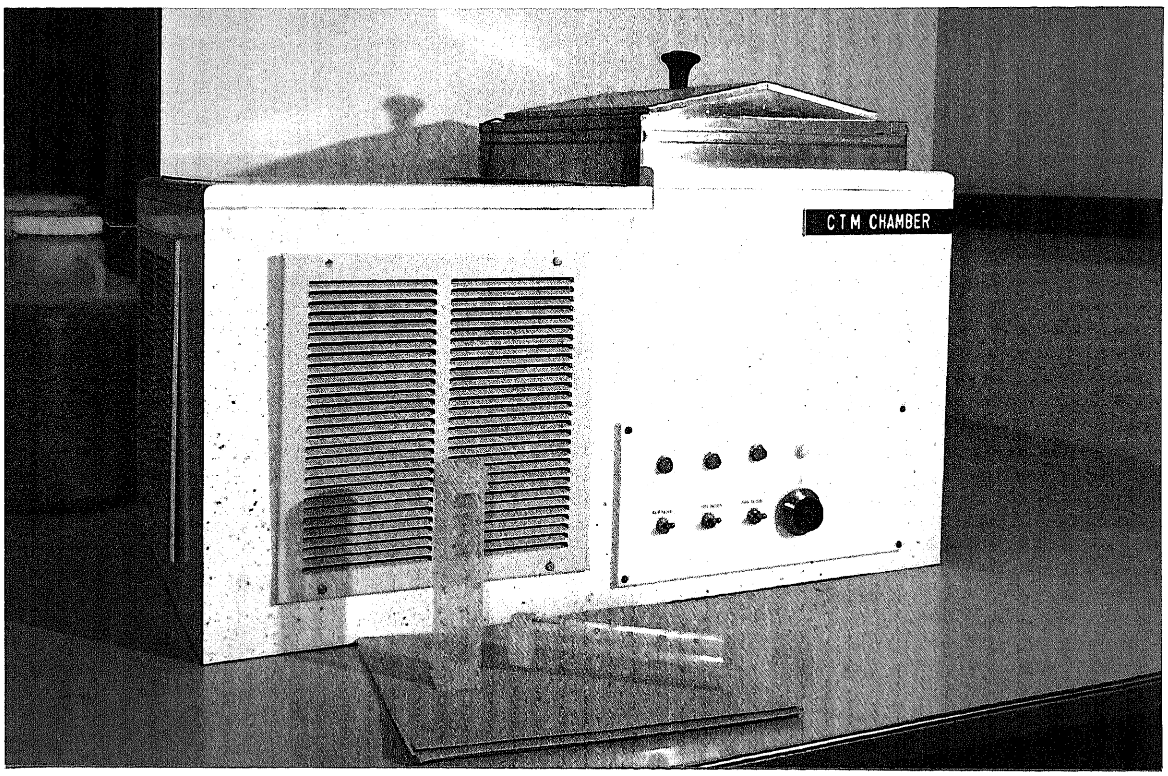
T-tests and Chi-square contingency tests were used to test for any significant difference between rocks with and without A. aeneus.

Microclimatic Factors

Temperature and relative humidity inside and outside the rock crevices were recorded as indicated above. These environmantal factors were compared with Critical Thermal Maxima (CTM) and dehydration values obtained in the laboratory. Twenty-seven salamanders were collected for CTM studies from Holly River State Park (Pickens Road). Nine of these salamanders were collected on 13 October 1990, 16 on 23 October 1990, and two on 28 November 1990. They were placed in a refrigerator at 9 °C until use. The upper thermal limit (CTM) which A. aeneus could tolerate was determined by using the methods of Pauley (1978a). Salamanders were tested individually by placing them in a perforated plastic bottle with a thermometer inserted through the lid and submerged in the water bath or CTM chamber (Figure 5). The water bath was cooled from room temperature (23 °C) to refrigerator temperature (9 °C) where they were kept before the start of the experiment to avoid biasing the study. The temperature of the water bath was increased 1 °C per three minutes to prevent thermal shock. The CTM was determined at the point where the salamander turned upside down and could not right itself (Hutchinson 1961). The snout-vent length (SVL) of each specimen was measured to the nearest 0.1 mm from the tip of the snout to the anterior angle of the cloaca with a vernier caliper. Critical Thermal Minima (ctm) could not be ascertained because endpoints could not be determined (salamanders never turned over). Although, it must be pointed out that several other investigators obtained ctm of other species using similar methods.

Dehydration rates of A. aeneus were determined by following the methods of Pauley (1978b). Eight A. aeneus collected from Holly River State Park on 3 June 1991 by the author and three collected from Cooper's Rock State Forest by Dr. Thomas K. Pauley on 30 May 1991 were used for this study. They were dehydrated by placing each animal in a plastic bottle of known weight inside a cylinder with 200 mg of anhydrous calcium chloride as a dehydrant. Air (23 +/- 1.0 °C) was pumped into the cylinder via an air pump. An opening with a rubber tube inserted in a flask of distilled water was placed at the opposite end of the cylinder. The presence of air bubbles in the water of this flask

Figure 5. Critical Thermal Maxima chamber used to test for CTM of _A_. _aeneus_.


CTM CHAMBER

indicated that air was passing through the cylinder (Figure 6). All salamanders were weighed every 30 minutes until death at room temperature. T-tests were used to test for any significant difference between the microhabitat of *A. aeneus* and *P. glutinosus* in Holly River State Park.

Reproductive Strategy

The purpose of this study was to detemine the time of mating and egg deposition in the Holly River State Park population, with emphasis on how their reproductive strategy compares with populations in North Carolina and Mississippi. Field surveys were made three times a week in May 1990 and almost daily in June 1990 to detemine the time of mating and egg laying. Field surveys were also made every day from the last week in August through mid-September 1990 to determine the time of hatching. In addition, weekly visits were made from mid-September to early November to determine whether fall courtship and mating occurred in this population. Salamanders were marked (toe clipped) from 28 May 1990 to 3 June 1991 using the methods of Twitty (in Ferner 1979) and sexed externally with hand held lens. External sexing techniques followed the methods of Gordon (1952) and Cupp (1980). Gordon found sexing in the field could be accomplished by separating the cloacal lips and examining for fine papillae located anteriorly. Males possess papillae, but females have a smooth vent (Bishop 1943). Cupp sexed *A. aeneus* by examining for the presence of the mental hedonic gland found in males in

Figure 6. Dehydration apparatus used to test moisture loss.


Calcium
CERTIFIED
C77-500

reproductive condition. The marking system used facilitated recognition of each salamander. Laboratory studies of spermatogenic wave and ovarian egg analyses were conducted and compared with results obtained from field studies. Correlation between field and laboratory data revealed the time of mating and egg laying.

The reproductive cycle (time of mating and egg laying) was ascertained by examining 115 specimens representing populations throughout the state. These specimens were from the West Virginia Bilogical Survey at Marshall University, Huntington, West Virginia; Carnegie Museum of Natural History, Pittsburgh, Pennsylvania, and Museum Of Zoology, University of Michigan, Ann Harbor, Michigan. All months except November, December, January, and February were represented in this study.

Time of mating was determined by examining the pathway of sperm through the reproductive system (spermatogenic wave). Spermatozoa are produced in the posterior region of the testes, migrate to the anterior region before moving through the anterior and posterior regions of the vasa deferentia, and into the cloaca before deposition (Saylor 1966). Methods for this study were modified somewhat from those of Pauley and England (1969). Tissues from testes and vas defenentia were placed on a cover slip containing glycerol-egg albumen and smashed. Tissues were heat-fixed on a hot plate for five minutes and stained with Wright's stain for three minutes. Samples were rinsed with distilled water before dehydration with a series of 35, 50, 70, 85, and 100% isopropyl alcohol solution for 10 minutes each. Tissues were then rehydrated by first placing them in a solution containing one-half methyl salicylate and one-half 100% isopropyl alcohol and then in pure methyl salicylate for 10 minutes each. They were mounted on a glass slide with permount and examined for spermatozoa (Figure 7).

The period of egg deposition was detemined by examining the size and volume of ovarian eggs (Figure 8). This average egg volume method followed the procedures of Fitzpatrick (1972) and Little (personal communication 1990). Eggs were removed from the ovary and all fat was removed from around the eggs. The number of eggs per cluster was recorded and placed in a 5 ml volumetric flask with distilled water. The volume of the egg cluster was determined by water displacement which was removed with a 1 cc syringe and recorded. The average egg volume was determined by simply dividing the total volume of the egg cluster by the number of eggs in the cluster.

Sex was determined by examination of the gonads (sexually mature males have lobed testes and sexually mature females have highly coiled oviducts) and individuals that were at least 45.0 mm in snout-vent length were considered sexually mature.

Seasonality And Annual Cycle

Field surveys for *A. aeneus* were conducted from 11 October 1989 - 3 July 1991. Three to 10 field trips were made each month at all hours during both day and night. The mark-recapture

Figure 7. Testis with sperm of A. aeneus (400X).

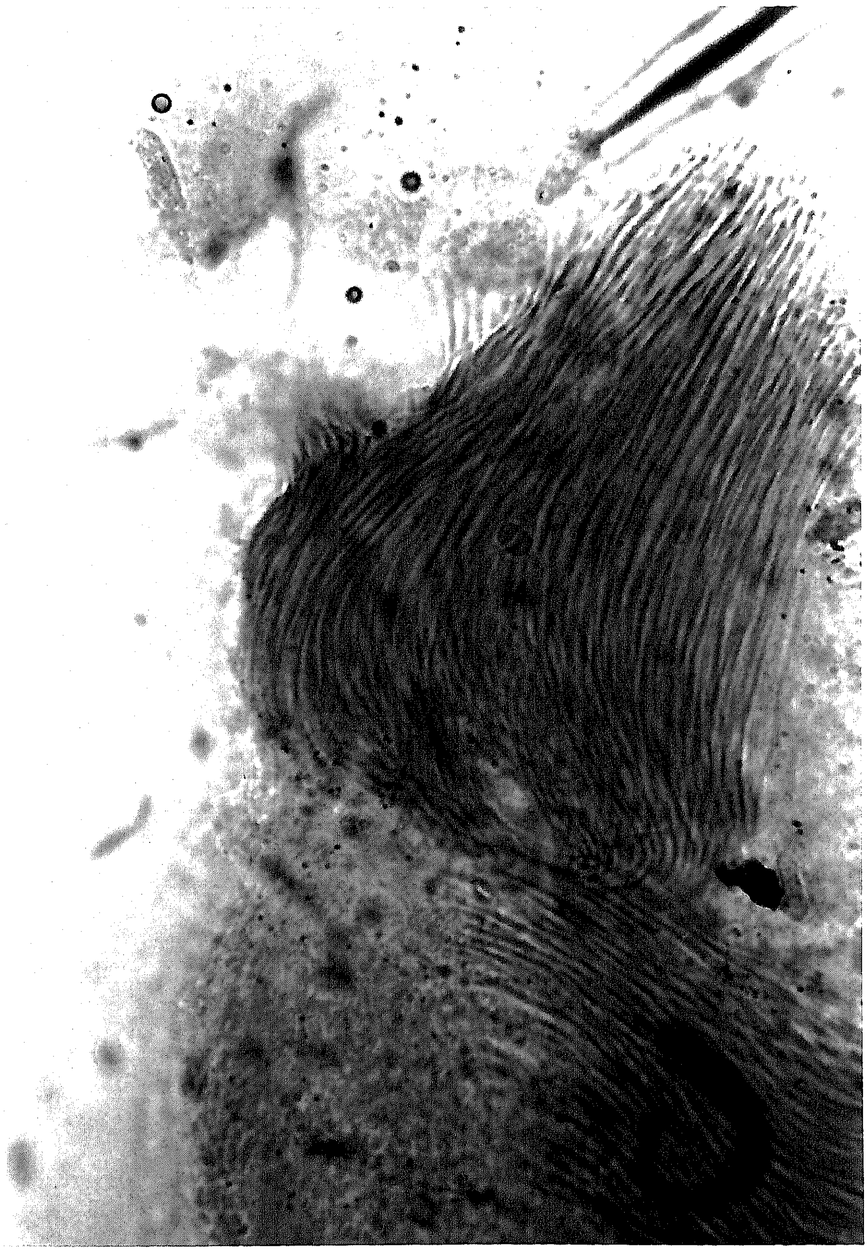


Figure 8. Ovarian eggs of A. aeneus (5X).

technique mentioned above provided information on movement and seasonal activity (spring emergence, fall aggregation, etc.) by recording the position (rock number and aspect) of each salamander recaptured. Surface abundance was determined by recording all salamanders observed at the beginning, middle, and end of each month and computing the mean value per month. The mean number of salamanders observed per month was plotted against the mean monthly temperature and precipitation (U. S. Department of Commerce 1991). To determine if different age classes or sexes were active at different times of the year, the SVL and sex of specimens were recorded.

Feeding Habits

The following hypotheses were tested in this study to determine and compare the prey items consumed by populations of *A. aeneus* in the state:

1. Different age classes feed on different invertebrate species.
2. Seasonal variation in prey items consumed would exist.
3. Different populations feed on the same invertebrate taxa.

Ninety-six specimens from the West Virginia Biological Survey representing populations throughout the state and collections from every month (except November and December) were examined for prey items. Prey items were removed from the stomach and small intestine and identified using Borrer and White

(1970) and Borrer and Delong (1970). Salamanders were measured for SVL and classified into age classes according to their size (Woods 1968). Specimens were also grouped by monthly capture dates so that any seasonal patterns in prey items could be examined. Prey items consumed by each age (size) class were compared taxonomically and by size. Prey items identified from the 96 specimens were used to compute the percentage of each invertebrate group represented. T-test was used to test for any significant differences between mean number of invertebrate groups consumed per month.

RESULTS:

Habitat Selection

Appendix I shows the environmental readings recorded from the rock outcrops. There was no significant difference in the environmental factors (temperature or relative humidity) between rocks inhabited vs rocks not inhabited by this species (t-tests, $p>0.05$). Crevice temperatures were about the same on rocks with and without *A. aeneus*. Crevice relative humidities were similar on both groups of rock outcrops. Ambient temperature or relative humidity were about the same on or around both groups of rocks. Temperature and relative humidity values recorded on the rock face and one m from the rock were usually the same or about the same and were recorded as one value for ambient readings. Rocks without and without *A. aeneus* had the same exposures, and *A. aeneus* was found on rock outcrops with all aspects (north, south, east, and west).

There appeared to be no difference between the floral composition (trees or bryophytes) on or around rock outcrops with and without A. aeneus. The frequency of occurrence of trees found on or around rocks with and without A. aeneus in Holly River State Park or Cabwaylingo State Forest were very similar and are shown in Tables 3 and 4. Ten bryophytes were identified from the rock outcrops with and without A. aeneus in Holly River State Park and four species were found on the rock outcrops in Cabwaylingo State Forest (Table 5). There appeared to be no difference between species of bryophytes found on both groups of rocks in Holly River State Park or Cabwaylingo State Forest. However, there appeared to be a relationship between salamander occurrence and bryophyte density. Therefore, the hypothesis that there is a relationship between salamander occurrence and bryophyte cover (salamander occurrence is dependent or contingent upon bryophyte density) was tested. Chi-square contingency test indicated that the four possible classes of rocks (rocks with salamanders and high bryophyte cover, rocks with salamanders and low bryophyte cover, rocks with no salamanders and high bryophyte cover, rocks with no salamanders and low bryophyte cover) were significantly different ($p<0.001$). Table 6 shows results of this test. Rocks with 75% or more bryophyte cover were considered as high. Therefore, a relationship between the presence or absence of salamanders and the amount of bryophyte cover appeared to exist. Salamanders were present on rocks with low bryophyte

Table 3. Frequency of occurrence of trees in Holly River State Park. Areas 1 and 2 had rocks with A. aeneus and areas 3 and 4 had rocks without this species.

Species	Area 1	Area 2	Area 3	Area 4
Magnolia acuminata Cucumber Tree	6	4	4	3
Tsuga canadensis Hemlock	4	7	7	3
Acer rubrum Red Maple	6	8	8	9
Acer saccharum Sugar Maple	5	5	3	6
Quercus alba White Oak	3	2	3	4
Quercus rubra Red Oak	3	4	4	2
Fagus grandifolia American Beech	5	4	3	7
Liriodendron tulipifera Yellow Poplar	5	4	2	2
Total	37	38	34	36

Table 4. Frequency of occurrence of trees in Cabwaylingo State Forest study area at sites B (rocks with _A_. _aeneus_) and C (rocks without _A_. _aeneus_).		
Species	Site B	Site C
Acer rubrum Red Maple	6	4
Acer saccharum Sugar Maple	1	2
Tsuga canadensis Hemlock	6	7
Betula lenta Black Birch	3	4
Fagus grandifolia American Beech	5	4
Ulmus americana American Elm	2	1
Aesculus glabra Ohio Buckeye	5	5
Quercus alba White Oak	3	2
Quercus rubra Red Oak	3	1
Total	34	30

Table 5. Bryophytes observed on rock outcrops with and without A. aeneus in Holly River State Park and Cabwaylingo State Forest.

Holly River State Park	Cabwaylingo State Forest
Bazzania trilobeta	Polystrichum commune
Dicranum fulvum	Dicranum fulvum
Dicranum majus	Dicranum scoparium
Dicranum scoparium	Thuidium delicatulum
Rhodobryum roseum	
Brachythecium salebrosum	
Thuidium delicatulum	
Hypnum imponens	
Leucobryum glacum	

Table 6. Chi-square contingency test for significant difference between rocks with and without A. aeneus at Holly River State Park.

Class	Number Of Rocks Observed
No Salamander / High Bryophyte Cover	23
No Salamander / Low Bryophyte Cover	11
Salamander Present / High Bryophyte Cover	3
Salamander Present / Low Bryophyte Cover	26
X^2= 17.214	

cover and absent onrocks with high bryophyte density.

Microclimatic Factors

Crevice temperatures were 0.5-2 °C warmer and cooler than ambient temperatures during cold and hot weather, respectively (Appendix I). Relative humidities (inside and outside the crevices) were usually high (greater than 60 per cent) except for several days of observations (Appendix I).

There was no significant difference (t-test, $p>0.01$) in the crevice temperatures or crevice relative humidity between crevices occupied by A. aeneus and P. glutinosus (Figure 9).

Critical Termal Maxima values were higher for adult A. aeneus than immatures and averaged 33.9 °C for adults and 31.6 °C for immatures (Table 7). The CTM usually increased with increasing snout-vent length (SVL).

Figure 10 shows the dehydration results where the percentage of weight loss was greater for immatures (8.0%/60 minutes) than in adults (6.0%/60 minutes). The average SVL of the adults was 51.7 mm while immatures averaged 33.9. The mean percentage of body weight loss at death was also higher for immatures than for adults (Table 8).

Reproductive Strategy

Field and laboratory studies indicated that mating occurred in the spring (late May and early June) and possibly the fall (September or October). Single pairs of salamanders were

Figure 9. Mean temperature and relative humidity recorded inside crevices occupied by A. aeneus or P. glutinosus on 20 May 1990 in Holly River State Park.

Figure 9. Mean temperature and relative humidity recorded inside crevices occupied by *A. aeneus* or *P. glutinosus* on 20 May 1990 in Holly River State Park.

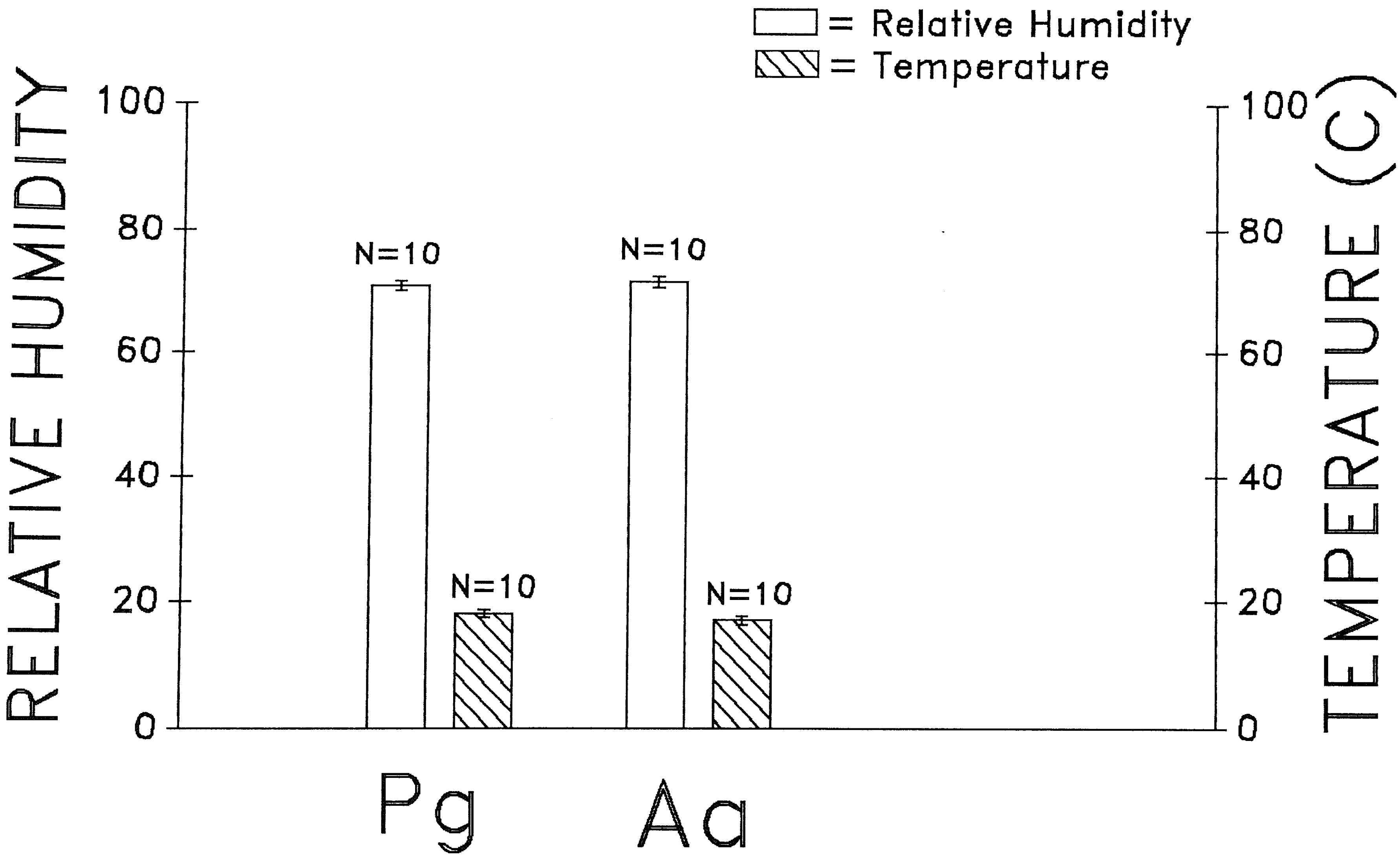

= Relative Humidity
= Temperature
RELATIVE HUMIDITY
TEMPERATURE (C)
100
80
60
40
20
0
N=10
N=10
N=10
N=10
Pg
Aa

Table 7. Critical Thermal Maxima (CTM) of *A. aeneus*.

Adults (N=10)		Immatures (N=17)	
SVL	CTM (°C)	SVL	CTM (°C)
44.8	33.0	32.4	31.2
42.6	33.0	30.0	30.8
58.0	35.0	31.6	31.0
56.8	34.8	32.5	31.4
52.8	33.9	31.4	31.0
52.6	33.7	31.7	31.0
53.1	34.0	34.8	31.8
53.6	34.2	35.6	32.0
52.8	34.0	35.8	32.1
50.0	33.5	33.8	31.5
Mean = 51.7	Mean = 33.9	32.4	31.3
		33.9	31.5
		36.0	32.0
		36.2	32.5
		37.0	32.8
		35.6	32.0
		34.9	31.8
		Mean = 33.9	Mean = 31.6

Figure 10. Dehydration rates shown as cumulative percentage of body weight loss over time until death.

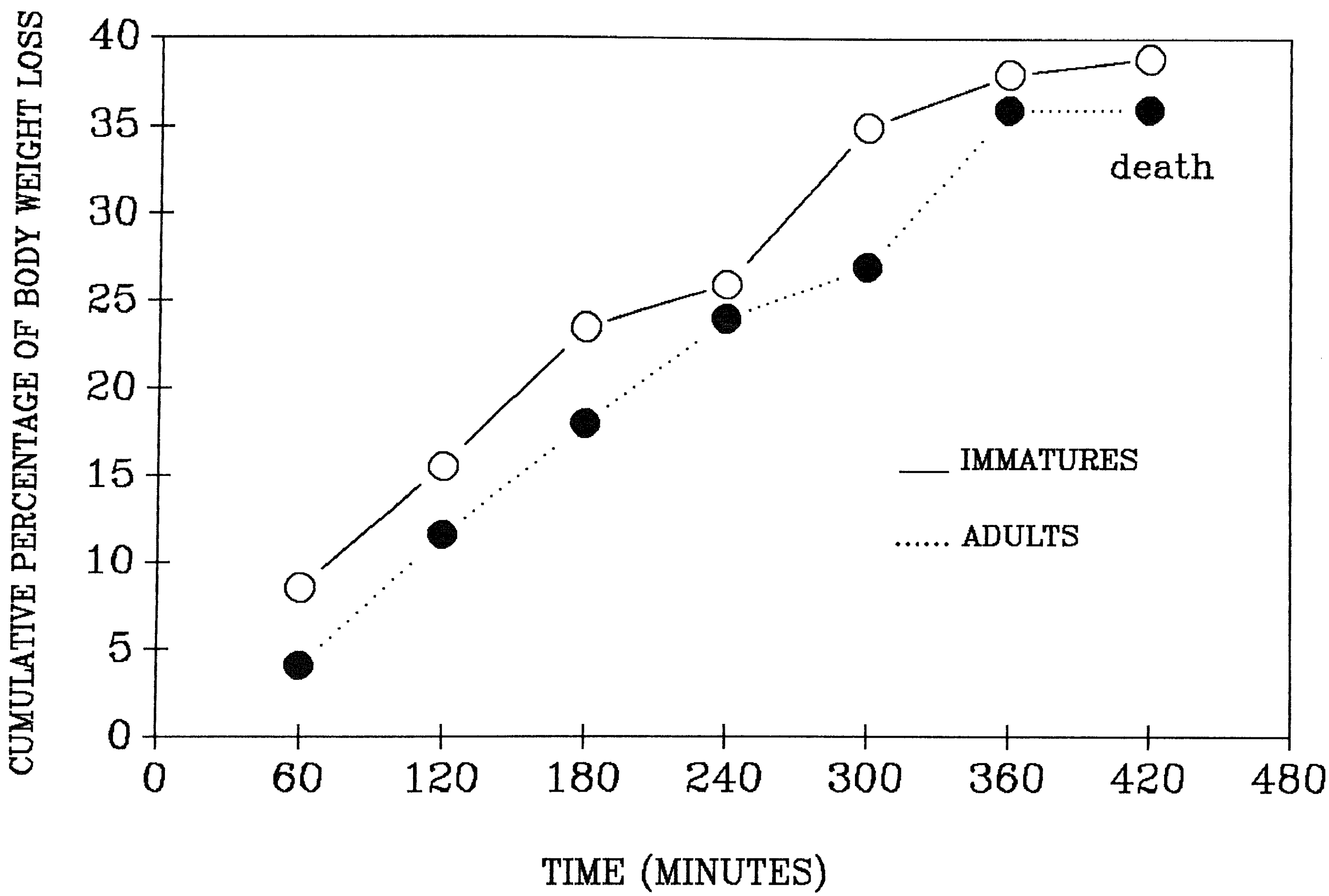

CUMULATIVE PERCENTAGE OF BODY WEIGHT LOSS
0
5
10
15
20
25
30
35
40
0
60
120
180
240
300
360
420
480
TIME (MINUTES)
death
IMMATURES
ADULTS

Table 8. Dehydration results of *A. aeneus* expressed as the percentage of body weight loss until death.

Age Class	N	Mean	Range
Adult	7	36.4	26.5-45.0
Immature	4	38.9	20.8-43.3

observed in crevices together prior to egg laying. Six unmarked pairs (male and female) of salamanders were found in separate crevices in the spring from 19 May - 3 June 1990. Single marked pairs were found together in October 1990 (Table 9). Table 10 indicates the number of marked pairs found togther on 3 June 1991. During both spring and fall, these pairs were curled around each other and the males possessed well developed hedonic glands and all females were gravid. The movement of sperm through the reproductive tract (spermatogenic wave) in _A_. _aeneus_ is demonstated in Table 11. In late May, 71.4% of the males contained spermatozoa in the posterior vasa deferentia and 28.9% were devoid of spermatozoa. None of these males contained spermatozoa in the testes during this time. By early June, most of the males had presumably deposited their spermatozoa and only 38.9% contained spermatozoa in the posterior vasa deferentia. In addition, 44.4 % of the June males were devoid of spermatozoa. Therefore, it was assumed that mating occurred at this time (late May and early June). The 38.9% of the males that had spermatozoa in the posterior vasa deferentia probably did not mate. Sperm production for the fall mating season began in June (16.7% of the males contained spermatozoa in the testes) or July (84.6% contained spermatozoa in the testes). By August, the spermatozoa had migrated to anterior and posterior vasa deferentia. All specimens examined from late October were devoid of spermatozoa indicating that mating probably occurred in early October.

Egg deposition in 1990 occurred during the second week in June

Table 9. Pairs of *A. aeneus* observed in crevices together during the fall (October) mating period.

Marked Pair ♂	♀	Rock #	Date
123	122	2B	10-9-1990
64	160	6B	10-9-1990
140	44	2B	10-9-1990

Table 10. Marked pairs observed in crevices together on 3 June 1991 in Holly River State Park.

Marked Pair ♂	♀	Rock #
205	129	2B
7	8	2B
112	107	1B
20	9	9
57	155	7B

Table 11. Spermatogenic wave of A. aeneus in West Virginia.

		Percentage of males with sperm			
Month	N	Testis	AVD	PVD	Devoid of sperm
March	3	33.3	67.7	67.7	--
April	4	25	50	50	--
May	7	0	42.9	71.4	28.9
June	18	16.7	33.3	38.9	44.4
July	13	84.6	--	--	15.4
August	11	90.9	45.5	27.3	--
September	1 *			--	--
October	6	--	--	--	100

* This specimen had sperm present in the testes and anterior vasa deferentia

AVD = Anterior vas deferens
PVD = Posterior vas deferens

(9-14). Thirteen brooding females were observed during this period (Table 12). Ten of these females were actually observed in the egg-laying process. Eggs were placed in clusters in secluded, damp crevices that contained little or no moss. Females were found to attach the eggs to the roofs of horizontal crevices and to the sides of vertical crevices. Two complete clutches were observed on 3 June 1991 (rock 6B and 25). Many non-brooding females were observed in the population and were toe clipped to facilitate recognition. Recapture of some of these marked females in the fall 1990 showed them to be gravid and indicated that a biennial egg-laying cycle was occurring (Table 13). Since these females did not have a clutch in the 1990 summer breeding period it was assumed that they would produce a clutch in 1991. Marked female number 129 was recaptured on 3 June 1991 in the same breeding crevice that female 43 used the previous year. This female was with male 205 and was seen in the egg laying position later that same day. Laboratory results on ovarian egg analyses correlate with these field results (Table 14). Brooding females collected in June (voucher specimens number 50 and 422 from West Virginia Biological Survey) contained developing eggs which indicated that eggs started developing during this time. Table 14 also shows that developing eggs in June and July averaged less than 1 μl in volume. Ovarian egg analyses indicated that egg development takes two years to complete. Eggs averaged 14.6 μl in volume at the end of their first year of development and beginning of their second year.

Table 12. Incubation period of brooding females in Holly River State Park during the 1990 breeding season.

Marked ♀	Rock #	Date of Egg Laying	Hatching Date	Length of Incubation (Days)
43	2B	9 June	31 August	82
48	7B	9 June	31 August	82
12	25	9 June	3 September	85
24	2B	10 June	8 September	89
67	25	10 June	8 September	89
42	1B	10 June	9 September	90
75	11	10 June	9 September	90
125	2B	10 June	9 September	90
39	7B	13 June	9 September	87
33	25	14 June	9 September	86
3	9	---	---	--
74	7B	---	---	--
118	28	---	3 September	--

Table 13. Marked gravid females observed in the fall 1990, but were non-brooding females during the 1990 summer breeding season.

Marked ♀	Recapture Date
8	10/23
9	12/22
82	10/9
83	10/9
84	10/9
85	10/9
92	10/9
107	10/9
120B	10/9
129	10/9
155	10/13
177	10/13

Table 14. Average monthly ovarian egg volume (AEV) of <u>A</u>. <u>aeneus</u> in West Virginia.

Month	N	First year AEV (µl)	Month	N	Second year AEV (µl)	Month	N	Third year AEV (ul)
June *	6	D	June	10	14.6	June ♦	3	22.1
July	5	D	July	5	16.0	July ☼	3	40.0
August	6	1.7	August	7	17.5			
October	3	2.8	October	--	--			
March	4	3.0	March	--	--			
April	1	4.6	April	--	--			
May	7	9.0	May	2	21.2			

* Eggs start developing in June. Developing eggs (D) were less than 1 µl in volume. Eggs averaged 14.6 µl in volume at the end of the first year of development.

♦ Mature eggs at end of second year of development

☼ Eggs collected from nest crevice

Maturity was reached when the eggs averaged 22.1 μl in volume at the end of the second year of devlopment. Eggs were deposited at this time and reached an average egg volume of 40.0 μl by late July. Females remained with the eggs during the brooding period which averaged 87 days (extremes 82 and 90 days). This was determined by continuous observations over a three month period. The number of eggs per cluster in 1990 averaged 18 and ranged from 12-27 egg. Females examined in the laboratory during ovarian egg analyses contained from 12-30 eggs with an average of 22 (Table 15). Hatching occurred between 31 August and 9 September 1990. Some of hatching was noted to have occurred between 0130 and 0230 hr.

Further evidence for a biennial egg laying cycle was obtained by monitoring the 13 marked females after the hatchlings had departed from their nest crevices. During this time, these females moved around on the rock outcrops and mixed with other members of the population. Most of these females were recaptured on several occasions and always appeared devoid of eggs (Table 16).

Seasonality And Annual Cycle

Aneides aeneus were found to emerge from their deep wintering crevices in late April and early May. The animals were found to remain at the entrance of the deep, anastomosing rock crevices until mid-May when the weather was warm enough for dispersal. Field observations on marked individuals indicated

Table 15. Egg cluster counts for marked brooding females in Holly River State Park (June 1990) and specimens from the West Virginia Biological Survey.

Field		Labor	
Marked ♀	# Eggs	Voucher Specimen # *	# Eggs
3	20	221	24
12	12	3561	15
24	20	2555	16
33	19	129	23
39	21	58	28
42	22	3798	12
43	18	2062	18
48	16	3560	30
67	16	48	16
74	--	2655	26
75	12	3799	15
118	15	3562	19
125	17	1074	26
-- ♦	17	1912D	20
-- ♦	27	776	28
		617	30
		98	22

* Includes 17 Of 43 females examined in the laboratory.

♦ Unmarked females observed on 3 June 1991

Table 16. Recapture dates of 13 females that produced a clutch in the 1990 breeding season.

Marked ♀	Recapture Dates
3	12/22/1990
12	3/23/1991
24	---
33	---
39	---
42	---
43	6/3/1991
48	3/23/1991
67	6/3/1991
74	---
75	---
118	6/3/1991
125	---

that they moved either to adjacent crevices on the same rock outcrop or to nearby adjacent rock outcrops. For example, male number 20 was toe-clipped on rock 8 on 30 July 1990 and was found in a crevice with gravid female number 9 on rock 9 on 3 July 1991. Dispersal appeared to be completed by late May as indicated by not finding any movement of marked salamanders. During the May and June mating season, single adult males and single pairs of salamanders were found in separate crevices (Table 10). After mating, females either stayed in the same crevices where mating occurred or moved to nearby adjacent crevices to deposit their eggs. Thirteen breeding crevices of females with eggs in 1990 averaged 9.9 cm wide, 15.3 cm deep, 11.3 cm high, and 1.8 m above ground (Table 17). These breeding crevices used for mating and/or egg laying contained no moss and were free of surface runoff. The breeding period lasted from late May to late October and incorporated spring and fall mating, egg laying, and hatching (see section on reproductive strategy mentioned above).

The length of time females remained in the nest crevice with their young depended upon the activity of the young. Post-hatching association of the females and their broods extended for about a month. The initial dispersal of a few hatchlings occurred on 23 September 1990 and most had departed from their hatching sites by 23 October 1990. Hatchlings were observed to move upward on the rock faces towards the moss covered ledges and crevices. No hatchlings were observed on 11 November 1990. The

Table 17. Measurements of breeding crevices of brooding females in Holly River State Park during the 1990 breeding period.

Marked ♀	Width (cm)	Depth (cm)	Height (cm)	Type Of Crevice - Horizontal (H) Vertical (V)	Height Above Ground (m)
03	5.1	5.3	10.7	V	2.7
12	3.8	1.9	12.5	V	1.5
24 *	23.9	10.2	13.5	V	2.0
33	10.2	20.6	8.0	H	1.0
39	30.5	15.5	17.2	H	1.1
42	12.0	10.2	10.0	H	1.9
43	30.5	20.0	10.7	H	1.5
48	12.7	25.1	7.6	H	2.0
67	5.1	7.6	8.9	V	2.1
74	32.3	13.2	14.0	H	3.6
75	15.2	22.2	10.7	H	1.1
118	3.8	20.3	15.0	V	1.0
125 *	23.9	10.2	13.5	V	2.0

* Found in the same crevice together.

brooding females left their nest crevices when all the hatchlings had departed and did not return the following year to the nest crevices, but were found in nearby crevices on the same rock outcrop. The nest crevices were occupied by different brooding females that existed as non-brooders the previous year. For example, gravid female number 129 was found in the nest crevice of number 43 on rock 2B on 3 June 1991. Marked female number 212 was found in the nest crevice occupied by number 42 the previous year on rock 1B and an unmarked brooding female was observed in the nest crevice of female number 67 on rock 25.

Immatures were found to move more than adults during the breeding season. All movement was between crevices on single rock outcrops and no marked individuals were observed to move between rock outcrops during this time. Immatures were seldomly recaptured in the same crevices and were unpredictable in crevice positions on the rock outcrops. Some were never observed more than once in the same crevice, but a few individuals such as number 2 was recaptured repeatedly in the same crevice. Brooding females remained in their nest crevices and were only occasionally found outside their crevices on the rock outcrops. Adult males were found to move more than non-brooding females. Outside the mating periods, males apparently were not territorial since two males were found togther on several occasions at several different localities. In addition, as many as 12 individuals (males, females, and immatures) were found in the same crevice on rock 6B on several occasions.

During the fall dispersal and aggregation period (late September to November) considerable movement by all age classes and sexes took place. Table 18 indicates movement that occurred between crevices on a single rock outcrop, and Table 19 shows marked individuals found to move between adjacent rock outcrops. Table 18 also indicates that some movement occurred in August and early September. October was the best time to survey the population size since large numbers of both marked and unmarked salamanders were found on the rock outcrops during the fall dispersal and aggregation period (refer to Appendix III and compare the number toe-clipped in October with the other months). By mid-November all salamanders had entered deep, anastomosing crevices on the rock outcrops, since no individuals were observed on 14 November 1989 and on 11 November 1990. Most individuals apparently hibernated on the same rock outcrops they were observed on during breeding period by simply retreating to deeper crevices. For example, marked individuals number 3, 9, 23, 49, and 77 were observed on 22 December 1990 on the same rock outcrops where they were active during summer and fall. Marked individual number 34, 64, 77, 78, and 88 were also observed on the same rocks on 27 March 1991. Marked specimens that moved to other nearby rock outcrops indicated that some migration to more suitable winter crevices occurred (Table 19). Aneides aeneus did not remain in an inactive state of hibernation, but were active

Table 18. Distance travelled between adjacent crevices on single rock outcrops by marked *A. aeneus*.

Marked Salamander	Time Span Movement Took Place (1990)	Number Times Recaptured In Time Frame Movement Took Place	Distance Travelled (cm)
7	6/3 - 6/22	2	8.1
8	8/5 - 8/25	2	6.5
34	9/23 - 10/13	2	11.5
44	9/23 - 10/13	2	6.1
49	8/31 - 10/9	1	3.7
60	9/23 - 10/13	1	3.4
64	9/23 - 10/13	2	5.8
70	9/23 - 10/13	1	10.6
94	10/9 - 10/13	1	4.9
97	10/9 - 10/13	1	15.8
120B	10/9 - 10/23	2	5.1
124	10/9 - 10/23	1	8.9
144	10/9 - 10/23	2	10.2
147	10/9 - 10/23	2	7.6
160	10/9 - 10/23	2	8.9

Table 19. Distance travelled (m) between rock outcrops by marked A. aeneus.

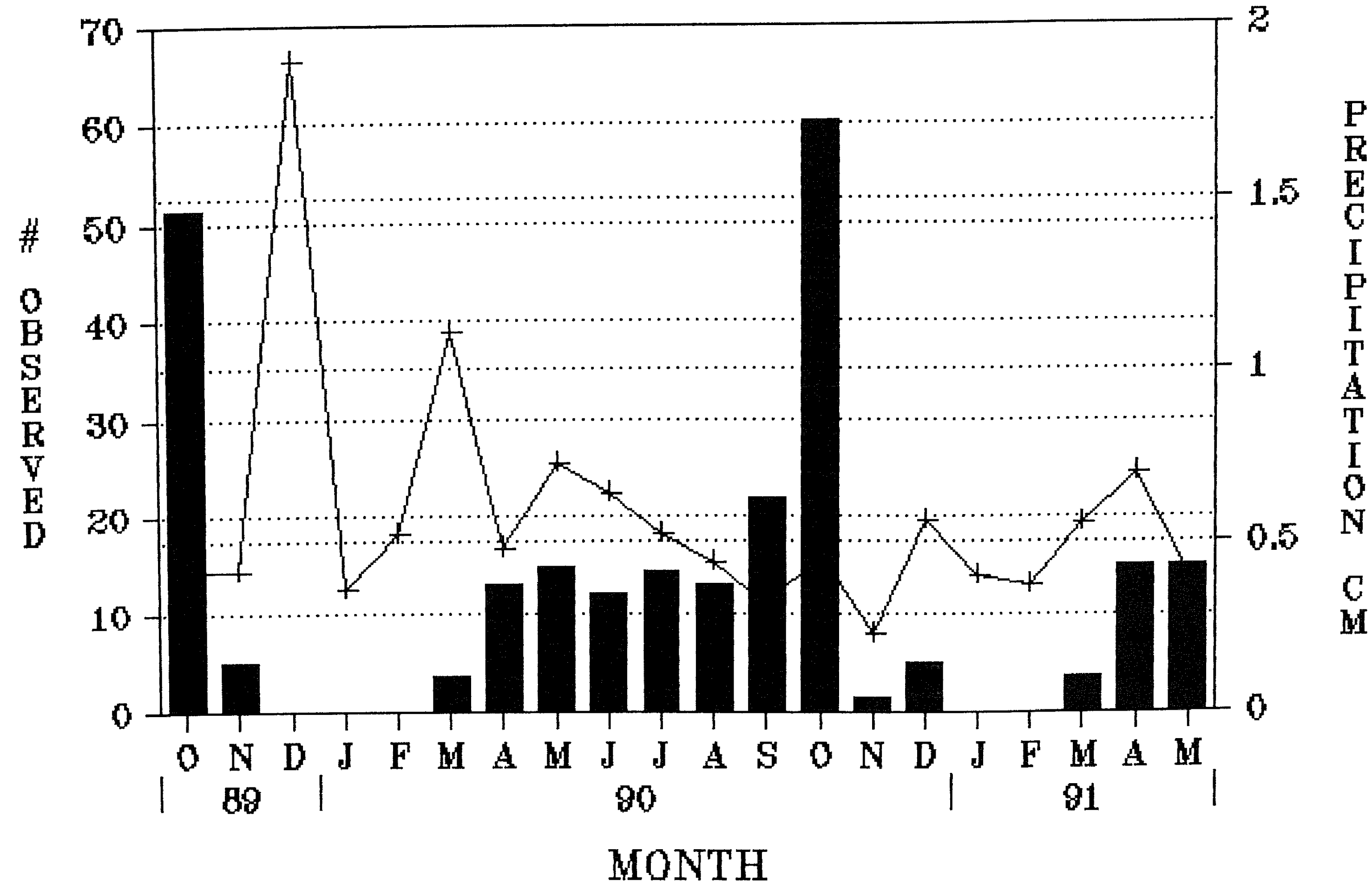
Marked Specimen	Age Class A= Adult I= Immature	Rock Outcrops Where Movement Took Place – From	To	Distance Travelled (m)	Time Period In Which Movement Took Place
9 *	A ♀	1	9	25.6	12/22/1990 - 6/3/1991
20 *	A ♂	8	9	6.4	7/30/1990 - 6/3/1991
23	I	2B	3B	7.6	8/25/ - 9/3/1990
29	A ♂	25	20	46.3	9/8/ - 10/13/1990
29 *	A ♂	20	25	46.3	10/13/1990 - 6/3/1991
40	I	25	22	33.8	8/25/ - 10/23/1990
87	I	8	9	6.4	10/9/ - 10/23/1990
128	I	4B	6B	27.7	10/9/ - 10/23/1990
140	A ♂	4B	2B	16.8	10/9/ - 10/13/1990
153	I	6B	7B	49.4	10/13/ - 10/23/1990

* These individuals were believed to have moved during the spring dispersal period for mating purposes.

throughout the year during favorable weather conditions. Marked individuals number 130 and 203 were observed on 16 November 1990, four unmarked individuals were collected on 28 November 1990, and nine were seen on 22 December 1990, and seven individuals were observed on 27 March 1991 in Holly River State Park. Populations in the western part of the state were active during the 1989 winter, and individuals were found at the mouths of their crevices in January, February, and March (Table 1 and 2). There appeared to be very little differences in the seasonal activity of the age classes; although, immatures were not observed as much as adults during the spring mating season. For example, only adult males and pairs of salamanders were observed on 3 July 1991, and no immatures were seen. However, this was believed to be due to the mating activity of the adults rather than any behavioral difference in the immatures since they were active at all other times along with the adults. Aneides aeneus was most abundant during the Fall dispersal and aggregation period in September and October. October had low amouts of precipition but provided the most optimal combination of temperature and precipitation for this species. They were least abundant and active during the winter months when most precipitation was in the form of snow and during the hot summer months when precipitation was low (Figures 11 and 12).

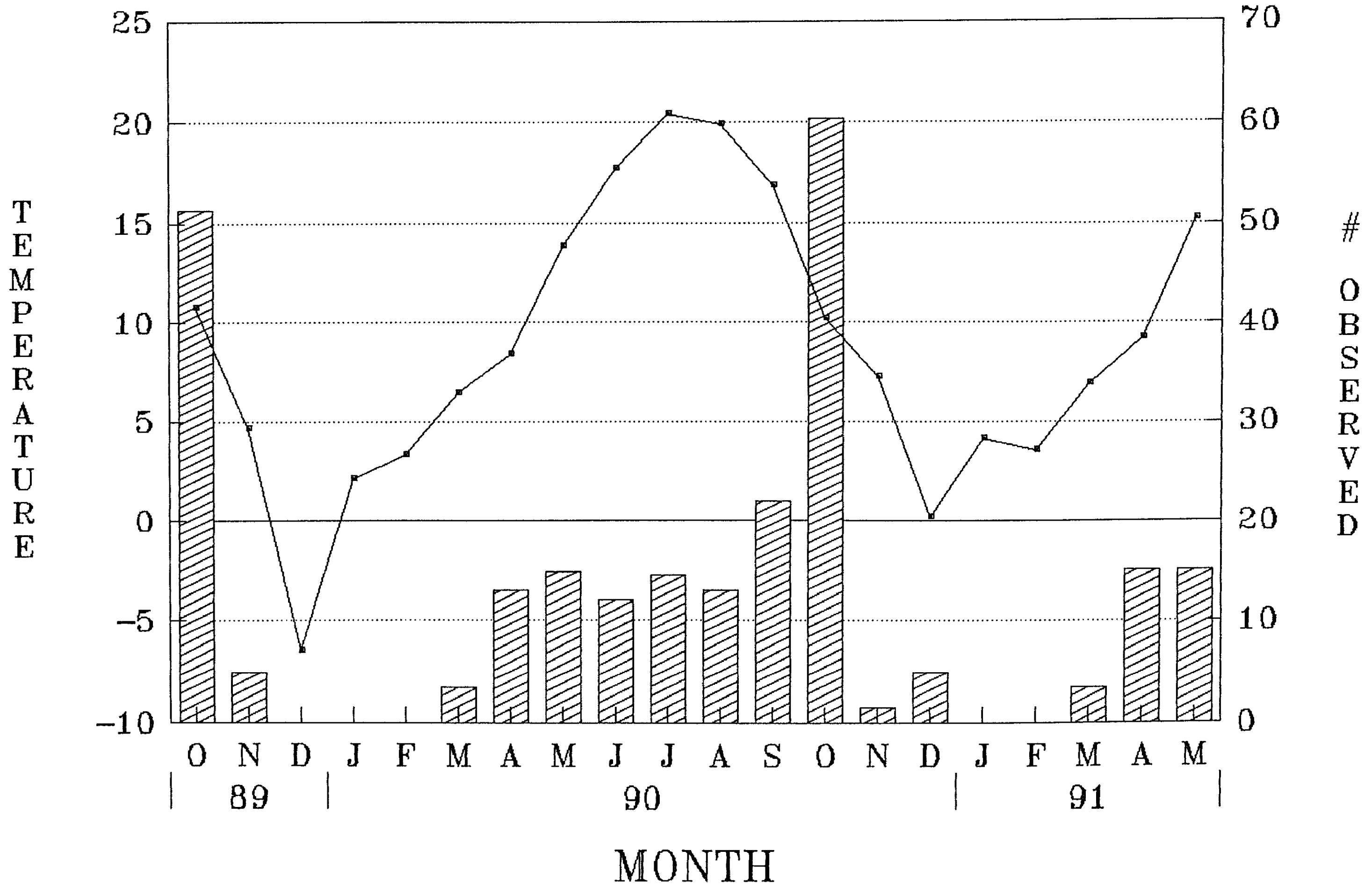
Aneides aeneus appeared to be mostly nocturnal, but was also found active and out on the rock outcrops during cloudy and rainy days. Salamanders became more diurnal than nocturnal during the

Figure 11. Average monthly surface abundance of *A. aeneus* plotted against average monthly precipitation. Vertical bars represent salamander surface abundance.



70
60
50
40
30
20
10
0
OBSERVED
2
1.5
1
0.5
0
PRECIPITATION CM
O N D J F M A M J J A S O N D J F M A M
89
90
91
MONTH

Figure 12. Average monthly surface abundance of A. aeneus plotted against mean monthly temperature. Vertical bars represent salamander surface abundance.



TEMPERATURE
25
20
15
10
5
0
-5
-10
OBSERVED
70
60
50
40
30
20
10
0
O N D J F M A M J J A S O N D J F M A M
89
90
91
MONTH

winter months when temperatures were warmer in the day than at night. For example, two individuals were found out during the day on 27 November 1990 at 1400 hr. (18 °C), but no individuals were active that same night (-6 °C).

Aneides aeneus were also found to be very agile and would jump from the rock outcrops and/or break their tails to avoid capture. No aggressive behavior by immatures or adults was observed in the field.

Feeding Habits

Of the 96 specimens examined, 39 (40.6%) were devoid of food and 24 of these contained pieces of digested food in the large intestine. Insects constituted 92.1% of all food items recovered and were found in all specimens containing food items. Spiders and mites made up the remaining 7.9% of the prey items consumed. Hymenopterans and coleopterans were the major insects consumed and comprised 93.9% of the diet. Ants made up 82.5% of all hymenopterans consumed while orthopterans, dipterans, hemipterans, and lepidopterans constituted the remaining 16.1% of insects consumed. Table 20 summerizes the percentages that each taxonomic group comprised. All age classes were examined and showed considerable variations in body size, but not in the size of food items consumed, except for adults who took larger coleopterans than other age classes. Adults (n=38) averaged 49.7 mm in SVL and 8.2 mm in head width (HW), immatures (n=15) averaged 39.1 mm in SVL and 6.9 mm in HW, and hatchlings

Table 20. Gut analyses of *A. aeneus* specimens from the West Virginia Biological Survey.

Invertebrate Group	Percent Recovery
Coleoptera	23.7
Diptera Culicidae	 1.8
Hemiptera	1.3
Hymenoptera Formicidae	60.2 49.6
Lepidoptera	0.53
Orthoptera	4.5
Mites	5.0
Spiders	2.9

(n=6) averaged 15.4 mm in SVL and 3.8 mm in HW. All age classes were found to feed on the same invertebrate species, but hatchlings, probably due to their size, consumed more mites than any of the other invertebrates. There was no significant difference (t-test, $p>0.05$) between the invertebrate groups consumed per month.

DISCUSSION:

Habitat Selection

Rocks with and without A. aeneus in Holly River State Park had no significant difference in temperature or relative humidity values inside and outside the rock crevices. This suggested that these two factors did not determine the presence or absence of this species on the rock outcrops. However, these two environmental factors probably become crucial outside the coves, ravines, and gorges that harbor suitable habitat of this species throughout its range. Both of these microclimatic factors probably present unsuitable conditions in the mountain ridges where rock outcrops tend to be drier than in the coves.

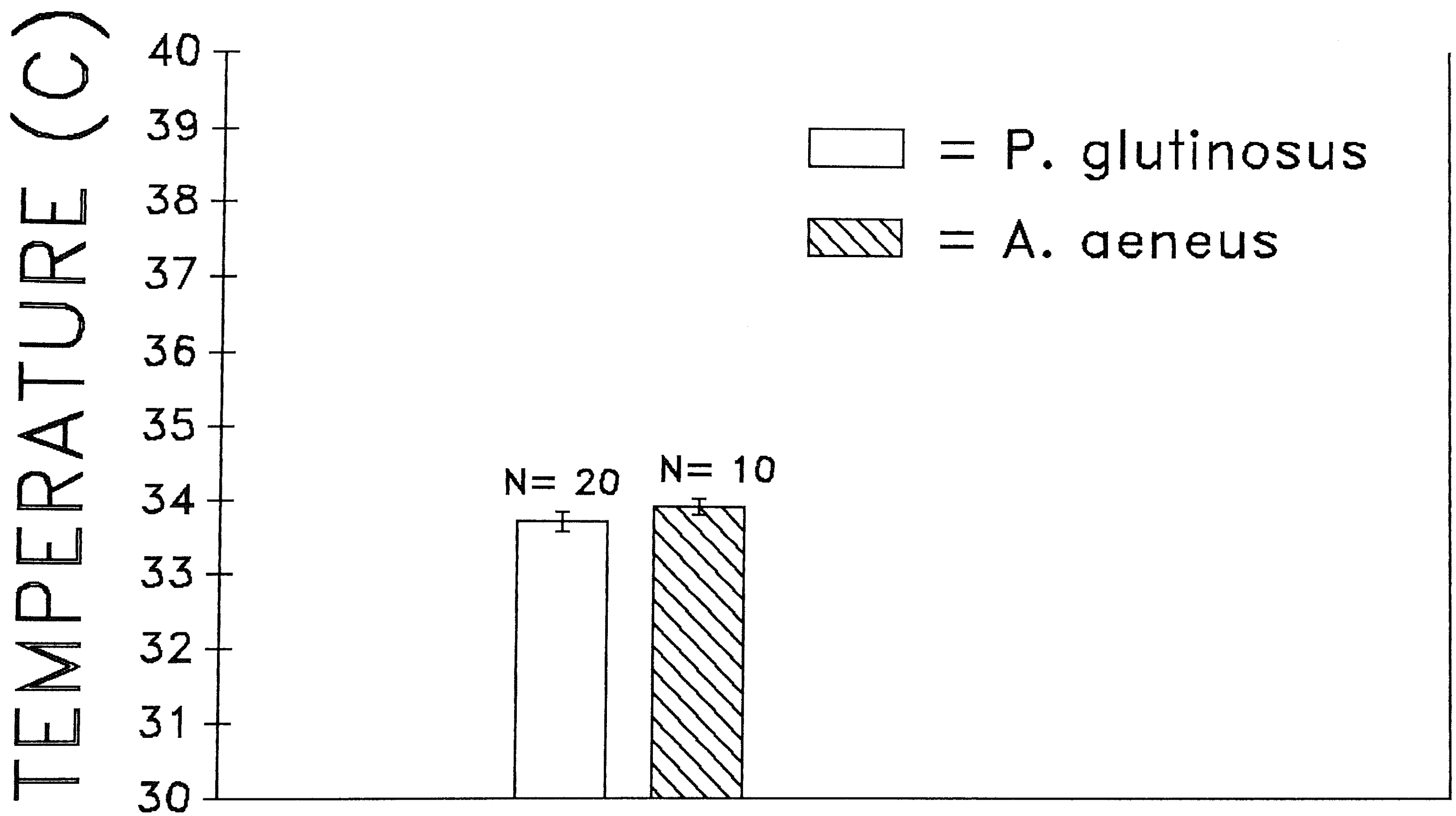
Since no difference in tree cover or bryophyte species was found between rocks with and without A. aeneus, it was assumed that plants did not determine the presence or absence of A. aeneus on rock outcrops in Holly River State Park and Cabwaylingo State Forest. On the other hand, bryophyte abundance, unlike bryophyte diversity, did determine the presence or absence of this species. The only difference between rocks with and without this species was the availability of crevices and/or the amount of bryophyte cover. Aneides aeneus was missing from rocks completely covered in bryophytes which covered and filled in the crevices. Any rock that lacked crevices, whether covered in bryophytes or not, were without salamanders. Therefore, it was assumed that crevice availability and amount of bryophyte cover determined the presence or absence of this species.

Microclimatic Factors

Gordon (1952) indicated that high temperatures are frequently more critical to an organism than low ones. Deep rock crevices not only provide relief from high temperatures but they also provide buffering from low ones as well. Results from this study support the crevice "buffering effect" and high relative humidity characteristics of A. aenues habitat found by Gordon (1952), Woods (1968), and Snyder (1971).

Plethodon glutinosus has a CTM of 33.7 °C (Jeffrey Bailey, personal communication 1991) which is about the same as A. aeneus. There was no significant difference in CTM between these two species (Figure 13). Field results and CTM values indicated that these two species can survive in the same niche, but probably rarely do so because of competition for space and food. Aneides aeneus, because of its flattened head and body and ability to climb to higher rock outcrops, probably has an adaptive advantage over P. glutinosus. This has been

Figure 13. Comparison of critical thermal maxima of A. aeneus and P. glutinosus showing no significant difference between the two.



TEMPERATURE (C)
40
39
38
37
36
35
34
33
32
31
30
N= 20
N= 10
= P. glutinosus
= A. aeneus

demonstrated by Baltar (1983) who found that vertical stratification among three species of cliff dwelling salamanders (*A. aeneus*, *P. glutinosus*, *P. dorsalis*) limits interspecific competition for food and space. She disclosed that *A. aeneus* occupies the highest positions on the cliff, *P. dorsalis* occupies the lowest positions, and *P. glutinosus* inhabited intermediate levels. *Aneides aeneus* instinctively climbs when confronted with a suitable vertical surface, thus the tendency for this species to climb is innate (Baltar 1983). It is an ancestrally arboreal species that is well equipped for climbing both anatomically and physiologically (Gordon 1952, Baltar 1983). Anatomical adaptations include its toe pads, prehensile tail, and flattened body. Physiologically it is capable of withstanding much water loss. The ability of *A. aeneus* in other parts of its range to withstand greater amounts of water loss than other competitve salamanders has been demonstrated by Gordon (1952). He compared the rates of desiccation of *A. aeneus* to *P. j. melaventris* and found that *A. aeneus* is more resistant to desiccation. Gordon concluded that *A. aeneus* is able to escape space competition with *P. j. melaventris* by climbing to the higher and drier positions on the rock outcrop that were inaccessable to *P. j. melaventris*. When the results of the present study (desiccation rates of *A. aeneus*) were compared to those of *P. cinereus* (Pauley 1978b) it was concluded that *A. aeneus* has a better ability to withstand water loss than *P. cinereus*. Since *P. cinereus* is more resistant to dry air than *P. glutinosus* (Bishop 1947) it was concluded that

A. aeneus is probably able to withstand greater desiccation than P. glutinosus. Baltar (1983) indicated that the ability of A. aeneus to withstand greater desiccation than P. glutinosus was advantageous in lessening the competition between these two species since A. aeneus has the ability to climb to higher and drier positions on the rock outcrop. Littleford et al. (1947) also correlated the ability to survive desiccation with ecological habitat selection in plethodontid salamanders. Gordon (1952) and MacMahon (1964) demonstrated that A. aeneus is relatively resistant to desiccation in dry situations (very few plethodontids occupy more xeric situations than A. aeneus in terms of normal microhabitat and ovipositional sites).

Plethodon wehrlei occurrs on the rock outcrops in Beartown State Park in sympatry with A. aeneus. This may be one of the few places in the the state where it occurs in the same habitat with A. aeneus. Dehydration rates of A. aeneus demonstrate that it is able to withstand greater amounts of water loss than P. wehrlei (Pauley 1978b). Combining the results of Bishop (1947) and Pauley (1978b) disclosed that Plethodon wehrlei may be more of a competitor with A. aeneus than P. glutinosus since it has a higher tolerance for water loss. Plethodon wehrlei is also less stout and more flattened than P. glutinosus which allows it to reach more of the high and narrow crevices occupied by A. aeneus. This hypothesis is tested in Chapter IV.

Reproductive Strategy

Gordon (1952) found that mating occurred in late May and early June. He observed single pairs of salamanders occupying a crevice together prior to egg laying, and freshly killed males collected in June had spermatophores. Snyder (1971) observed courtship behavior in late May and picked up a gravid female containing a spermatophore stalk in her cloaca in September. This female was accompanied by an adult male. Diurnal fall courtship and mating has also been observed by Cupp (1971) in Kentucky on 17 October 1970. Cupp observed the distinct circling pattern described by Noble and Brady (1930) for several species of plethodontid salamanders in which the males often exhibited some circling behavior as a result of their turning and nosing the cloacae of females. He also observed a circling tail walk prior to and after spermatophore deposition.

The present study also indicated and provided further evidence for a spring and fall mating periods. Salthe and Mecham (1974) found that an extended season of courtship interrupted by a period of winter inactivity to be typical of many temperature zone plethodontids.

Field data and ovarian egg analyses disclosed that egg deposition in West Virginia populations of A. aeneus is highly correlated to populations in southwestern North Carolina. However, egg deposition did not cover as an extensive time period in Holly River State Park as in southwestern North Carolina. Egg deposition was concentrated in the first two weeks of June in both areas, but it did not extend into July as in southwestern North Carolina where the summers are probably a little longer. The number of eggs produced by females during this study was similar to those in the south. Lee and Norden (1973) found five females collected from Cooper's Rock State Forest to contain 20-32 eggs which correlates with females from Holly River State Park as well as populations throughout the state. The length of the incubation period and time of hatching were also similar to those in southwestern North Carolina and northeastern Mississippi (Table 21). Although similarities in the reproductive strategy of *A. aeneus* were found between populations in West Virginia and those in the south, some geographical variations or adaptations (such as a biennial egg laying cycle) in the northern edge of its range appeared to exist. Not only may biennial egg laying in *A. aeneus* be an adaptation to northern conditions, but it may also be a means of controlling population numbers and reducing intraspecific competition for mates, food, and space.

Seasonality And Annual Cycle

Aneides aeneus was found to show similar behavior in terms of post-hibernation aggregation and dispersal period to populations in North Carolina and Mississippi. Movement during this period was also observed by Gordon (1952) and Woods (1968). Cupp (personsal communication 1991) reported that he observed aggression in males in mating crevices, and that they compete intraspecifically for territories. This would support evidence

Table 21. Comparison of breeding periods among populations of *A. aeneus* from southwestern North Carolina (Gordon 1952 and Snyder 1971), northeastern Mississippi (Woods 1968), and West Virginia.

	Sw NC	Ne Miss	WVa
Length Of Breeding Period	Late May - late Sept.	March - Nov.	Late May - late Oct.
Egg Laying Period	Late May - early June		Early June - mid June
Number Of Eggs	A = 17 R = 10 - 30		A = 22 R = 10 - 30
Length Of Incubation Period (days)	A = 86 R = 84-91	82	A = 87 R = 82-90
Hatching Dates	Late Aug - early Sept.		Late Aug - early Sept.

A = Average, R = Range.

collected in this study and by Gordon (1952) which indicates that males establish territories and wait for the arrival of females during the spring mating season. Gordon also observed single pairs of salamanders occupying a crevice together prior to egg laying. Females in Holly River State Park, were found either to remain in the same crevices where mating occurred or to move to adjacent crevices for egg depostion. Since this was also noted by Gordon (1952) it would appear that some factor such as crevice suitability (i.e., its depth, shelter, etc.) determined whether the female remained in the mating crevice to deposit her eggs or moved to a more suitable crevice.

Not much was known about the behavior of immatures during the breeding period, but this study demonstrated that immatures moved more than adults who were usually sedentary during this time. However, this study and results of Gordon (1952) indicated that some movement by adults did take place during the breeding period. Males and non-brooding females moved more than brooding females who were busy guarding their clutch. Movement during this time was probably due to the need for damper and more secluded crevices during the hot summer weather. Movement may also been due to intraspecific competition for space and food. Although A. aeneus was found to be primarily sedentary during the breeding period, considerable movement (especially between rock outcrops) took place during the fall dispersal and aggregation period. This movement may be necessary for territorial establishment and finding of mates during the fall mating season.

In addition, some movement may have been necessary in areas where crevices were not suitable for hibernation. Gordon (1952) and Woods (1968) concluded that A. aeneus was either sedentary or migratory depending upon whether their crevices were suitable for both breeding and hibernation or breeding only. Individuals of A. aeneus were usually sedentary in areas where crevices were suitable for both breeding and hibernation, but migrated from rock outcrops having crevices suitable for breeding only. Woods (1968) found immatures to move as much as 30.6 m. He found that Immatures and adults but not hatchlings move to a hibernaculum during the fall, and no A. aeneus were observed at the hibernaculum during the breeding season. This differs somewhat from the Holly River population where most rocks were used for breeding and hibernation and no rocks were used just for hibernation.

Post-hatching association of the females and hatchlings as well as the positions of the hatchlings during the fall aggregation and dispersal period were also examined by Gordon (1952) and Woods (1968) who found a post-hatching association of about one month. They disclosed that the length of time the young remained with their mother depends upon the activity of the young rather than the females. Gordon was not able to determine whether females returned to the same nest crevices during subsequent breeding periods, but results of this study indicated that they did not return to their nest crevices. However, the position of breeding females after the breeding period and their absence in the nest crevices during the next breeding period may not hold true for all populations, since biennial egg laying has never been observed in other populations of this species. In addition, other results obtained by this study would support the literature that A. aeneus is not territorial outside the mating seasons. Gordon (1952) found as many as 11 adults and immatures in a single crevice, and he never observed A. aeneus to demonstrate any aggressive encounters towards other members of its species. This close association of individuals within a crevice has also been observed by Juterbock (in Pfingsten and Downs 1989). The role that territoriality plays in the ecology of this species such as whether females and immatures establish territories and whether males defend territories only during periods of courtship is tested further in Chapter IV.

Aneides aeneus was probably most abundant during the fall dispersal and aggregation period for mating and hibernation purposes. They were less visible or abundant during the hot and drier summer months which indicated their need for the cooler, damper rock crevices. The relationship between surface abundance, movement, and environmental factors seems unclear, but it appeared that A. aeneus moved more during the cooler and wetter months of spring and fall rather than during the warmer, and usually drier summer months which probably forced them to move deeper into the rock outcrops.

Aneides aeneus is typically known to be nocturnal, but was occasionally found to be diurnal, especially during the winter months. Brandon and Huheey (1975) stated that its diurnal habits may explain the similarity between its color pattern and the appearance of the lichen encrusting rocks around which it lives. Others have also found it to be out during cloudy days and periods of light rain (Gordon 1952, Juterbock in Pfingsten and Downs 1989, and Cupp 1971). Winter activity has also been reported by Woods (1968) who found smaller individuals to appear first during warmer periods and disappear first during cooler periods. A correlation between winter activity and its diurnal habits was found in the present study, since individuals were out during the day in November, December, and March. For example, eight salamanders were observed out during the day on 22 December 1990, but no salamanders were active at night.

The present study showed tail breakage or autotomy to be a common occurrence in this species, but Wake (1966) stated that autotomy was rare in *A. aeneus*, a climbing species that uses its tail in locomotion.

Feeding Habits

This study indicated that there was no variation in the types of prey items consumed between populations. Lee and Norden (1973) found that 25 specimens from Cooper's Rock State Forest fed mostly on insects which comprised 75.7% of the diet. They found that beetles, mosquitoes, and ants comprised 68.5% of all items consumed and that the types of food consumed were similar for all size groups. Their study as well as results collected in the present study indicated that A. aeneus feeds mainly on beetles and ants throughout the year and that no seasonal patterns in the types of prey consumed appeared to exist.

SUMMARY: This study showed that some other factor other than crevice temperature, crevice relative humidity, vegegation types, and aspect, such as crevice availability and amount of bryophyte cover, determine the presence or absence of A. aeneus on rocks at Holly River State Park and Cabwaylingo State Forest.

Aneides aeneus were found to have spring and fall mating periods and a biennial egg laying cycle. Sperm and egg production was found to begin in June and early July. Eggs were deposited in the first two weeks of June and were incubated for an average of 87 days. Hatching occurred in late August and early September. Other periods of the annual cycle such as Fall dispersal and aggregation were similar to those in North Carolina and Mississippi. As in other areas of its range, A. aeneus was found to move between rock outcrops, but most rocks were used for both breeding and hibernation.

This study also showed that A. aeneus is active during warmer periods of winter, especially during the day. Aneides aeneus was found to be insectivorous and to feed mainly on coleopterans and formicides throughout the year. No differences in the types of prey items consumed appeared to exist between the age classes or between months.

CHAPTER III

POPULATION DYNAMICS AND MORPHOLOGICAL VARIATION

INTRODUCTION: Population dynamics in A. aeneus has been studied by Gordon (1952) in southwestern North Carolina and by Juterbock (in Pfingsten and Downs 1989) in Ohio. Gordon found that changes in the visible population were due to the annual cycle. He concluded that during the breeding period little flucuation in the total visible population occurred, i.e,, the population remained relatively stable until hatching in early September. The population was again reduced during the dispersal and hibernation period but increased in numbers during the following post-hibernation aggregation. The population became stable with little fluctuation in numbers or distribution during the next breeding period.

Any departure from the expected 1:1 sex ratio is believed to be due to the nest guarding phase of females which renders some of them inaccessable in their secluded breeding crevices (Gordon 1952, Juterbock in Pfingsten and Downs 1989). Juterbock found no differences between the age/sex categories in the proportion of individuals recaptured in single rather than multiple years.

Much variation occurs in color pattern of A. aeneus, but neither color pattern or any other characteristic has been analyzed geographically. Gordon (1967) has suggested a possible correlation of pigment intensity and altitude. Mock (1977) published an abstract on altitudinal variation in size in

A. aeneus.

The purpose of this study was to estimate the population size at Holly River State Park, examine the relationships between population size, age classes, and sex ratio, and to test for any morphological differences between populations of A. aeneus in West Virginia. This study examined the hypothesis that A. aeneus, because its long and close association with the rock crevice habitat and limited gene flow, would show very little morphological variation between populations.

METHODS AND MATERIALS:

The mark-recapture method of Schnabel (method T) as described in Smith (1980) was used to estimate the size of the population. As indicated earlier, each specimen captured in the field was sexed and measured for SVL to determine age class and sex ratio. Spermatogenic wave and ovarian egg analyses performed in the laboratory indicated that sexual maturity was reached at about 42.0 - 45.0 mm SVL. All individuals with SVL equal to or greater than 45.0 mm were classified as sexually mature adults while those less than 42.0 mm were classified as immatures. Indivduals that fell into the 42.0 -44.9 mm range were classified according to cloacal appearance (males possess genital papillae but females do not). Hatchlings were measured on or a few days after hatching. The number of salamanders in each age class was plotted against the average SVL for the age class. Chi-square test was used to test for any significant difference in the sex ratio.

Seventeen external morphological characters were meaured on 169 museum specimens of A. aeneus to examine for any external morphological differences between populations and between sexes. Specimens were from the West Virginia Biological Survey and Carnegie Museum of Natural History. Specimens were categorized into three geographic groups: 1) northern group (n=71) which consisted of populations in the Allegheny Plateau north of the New and Kanawha Rivers; 2) southern and western group (n=62) which consisted of populations in the Allegheny Plateau south of the New and Kanawha Rivers; 3) high elevation group (n=36) which consisted of populations in the Allegheny Mountains Physiographic Province. Measurements were made with a vernier caliper to the nearest tenth of a millimeter. Specimens with a SVL of at least 45.0 mm were considered adults and were sexed externally by examination of the cloaca. Sexing was verified by using the same specimens for spermatogenic wave and ovarian egg analyses and found to be 98.7% accurate. Most of the external characters measured were from Gross (1982) and consisted of the follow: head height (HH), head length (HL), width at base of jaw (JW), width at base of the head or at the widest point (WW), width at base of eye (BE), inside diameter of eye (II), outside diameter of eye (OO), eye height (HE), eye length (LE), length of third toe of hind legs (TL), femur length (FL), amount of webbing of hind legs (WH), amount of webbing of front legs (WF), body length between the legs (BL), tail length (TA), and snout-vent length from anterior angle of cloaca (SVL). The number of costal grooves (CG) were also recorded.

Morphological data were initially analyzed using principal component analysis to test for any differences between the groups. Six principal components were extracted in this study. Subsequently, external morphometric measurements were adjusted for size differences by dividing each character by the SVL. These resulting numbers were analyzed by analysis of variance (MANOVA) to test for any significant differences. Student-Newman-Keuls (SNK) procedures were used to show groupings of the mean, and all tests were performed at the 0.05 significance level.

RESULTS:

One hundred seventy-one salamanders were toe clipped (Figure 14) in this study and the Schnabel method of estimating the population produced 459 individuals. This would represent a density of about 366 individuals per ha. Of the 171 individuals that were marked, 53 were immatures, 62 were males, and 56 were females. Appendix IV shows the SVL of all marked specimens. Immatures (Figure 15) averaged 35.7 mm (range = 26.9 - 43.6) in SVL, males averaged 52.3 mm (range = 42.2 - 66.4) in SVL, and females averaged 57.8 mm (range = 42.1 - 67.0). Although females were slightly larger, there was no significant difference between the size of males and females (t-test, $p>0.05$). Several of the adults were larger than the 12.7 cm record (Conant 1975) for this

Figure 14. Marked (toe clipped) adult female _A_. _aeneus_.

Figure 15. Dorsal view of an immature A. aeneus.

species (Table 22).

Hatchlings averaged 22.3 mm in total length, TL, (range = 18.7 - 25.0), 14.4 mm in SVL (range = 12.5 - 15.2), and 3.5 mm in head width, HW, (range = 2.7 - 4.1). Tables 23 and 24 show the measurements of 49 hatchlings. Hatchlings did not grow much their first fall and winter. Four individuals measured on 13 October 1990 were 25.0, 25.0, 24.7, and 21.5 mm in TL, and two individuals measured on 23 October 1990 were 29.3 and 25.2 mm in TL. Twenty-one first year immatures measured in May 1990 averaged 29.4 mm (range = 28.4 - 30.3) in TL, 17.9 mm (range 17.4 - 18.9) in SVL, and 3.9 mm (range 3.0 - 4.0) in HW (Table 25).

Three age classes were observed in the population (Figure 16), and Table 26 shows that the sex ratio did not differ significantly from the expected 1:1 ratio ($p>0.05$).

No significant difference was observed between the groups or between the sexes. Principal component analysis showed no clustering and indicated that there was very little morphological variation between the groups or between the sexes. Figure 17 shows an example of the scattered individuals (absence of clustering) when principal component 1 is plotted against principal component 2. In addition, no sexual dimorphism was found both within and between the groups.

DISCUSSION:

The mark-recapture study indicated that the Holly River State Park population is probably a dense population, since

Table 22. Total length (TL) measurements of A. aeneus adults that were larger than 14.0 cm record.

Specimen	Sex	TL(cm)
89	♀	14.6
69	♀	14.3
59	♂	14.3
64	♀	14.1
20	♂	14.1
60	♀	14.4

Table 23. Measurements of hatchlings from Holly River State Park during the September 1990 hatching period. All measurements were recorded in mm. Part I.

Specimen	TL	SVL	HW	Specimen	TL	SVL	HW
1	23.4	14.2	3.8	17	20.7	13.3	3.0
2	23.1	14.0	3.6	18	20.2	13.1	3.1
3	19.2	12.6	2.9	19	21.5	13.5	3.3
4	18.9	12.6	2.7	20	18.8	12.7	2.7
5	25.0	15.2	4.0	21	18.9	13.0	2.9
6	22.0	13.9	3.5	22	19.6	13.3	3.0
7	23.9	14.8	3.9	23	24.6	14.7	4.0
8	23.6	14.1	3.9	24	23.8	14.2	3.8
9	21.1	13.5	3.3	25	22.6	13.7	3.5
10	24.8	14.9	4.1	26	22.9	14.0	3.7
11	18.7	12.5	2.9	27	21.0	13.4	3.2
12	20.0	13.3	3.1	28	24.2	14.7	3.9
13	19.1	13.0	2.9	29	22.1	13.8	3.3
14	23.3	14.6	3.5	30	18.8	12.5	2.8
15	24.6	15.0	4.0	31	19.1	12.9	3.0
16	18.7	12.8	2.7	32	24.4	14.9	3.9

Table 24. Measurements of hatchlings from Holly River State Park during the September 1990 hatching period. All measurements were recorded in mm. Part II.

Specimen	TL	SVL	HW	Specimen	TL	SVL	HW
33	25.0	15.0	4.1	42	24.7	15.2	4.0
34	24.8	14.8	4.1	43	23.5	14.7	3.8
35	24.0	14.7	3.9	44	22.8	14.5	3.6
36	23.6	13.6	3.8	45	19.0	12.6	2.9
37	18.9	12.5	2.9	46	20.7	12.9	3.4
38	24.5	14.8	4.0	47	24.6	15.0	4.0
39	23.9	14.6	3.9	48	23.8	14.7	3.8
40	22.9	13.9	3.7	49	19.9	12.9	3.0
41	25.0	14.9	4.0				

Table 25. Measurements of 21 first year immatures from Holly River State Park in mid-May 1990.

Specimen	TL	SVL	HW
1	28.4	17.4	3.0
2	29.6	18.6	4.0
3	28.9	17.8	3.4
4	28.5	17.4	4.0
5	29.9	18.8	4.0
6	30.3	18.7	4.0
7	29.0	18.5	4.0
8	28.5	17.9	4.0
9	29.0	18.0	4.0
10	30.0	18.6	4.0
11	28.9	17.8	3.8
12	29.9	18.7	4.0
13	29.4	18.1	4.0
14	29.7	18.3	3.8
15	29.8	18.5	4.0
16	28.4	17.5	3.8
17	30.0	18.7	4.0
18	29.3	18.6	4.0
19	26.9	18.1	3.9
20	30.1	18.9	4.0
21	28.6	17.7	3.9

Figure 16. Age classes plotted against average snout-vent lengths.

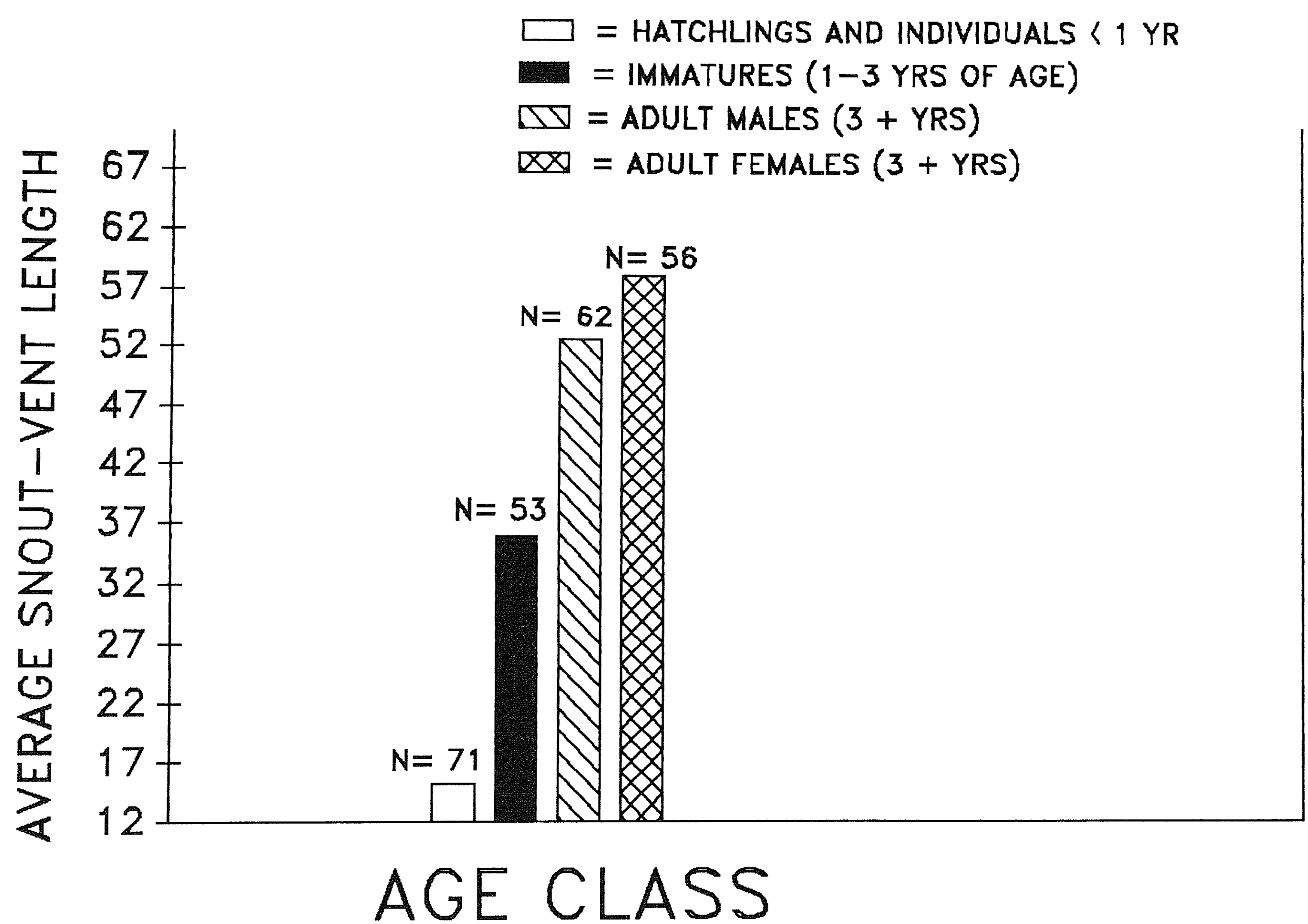
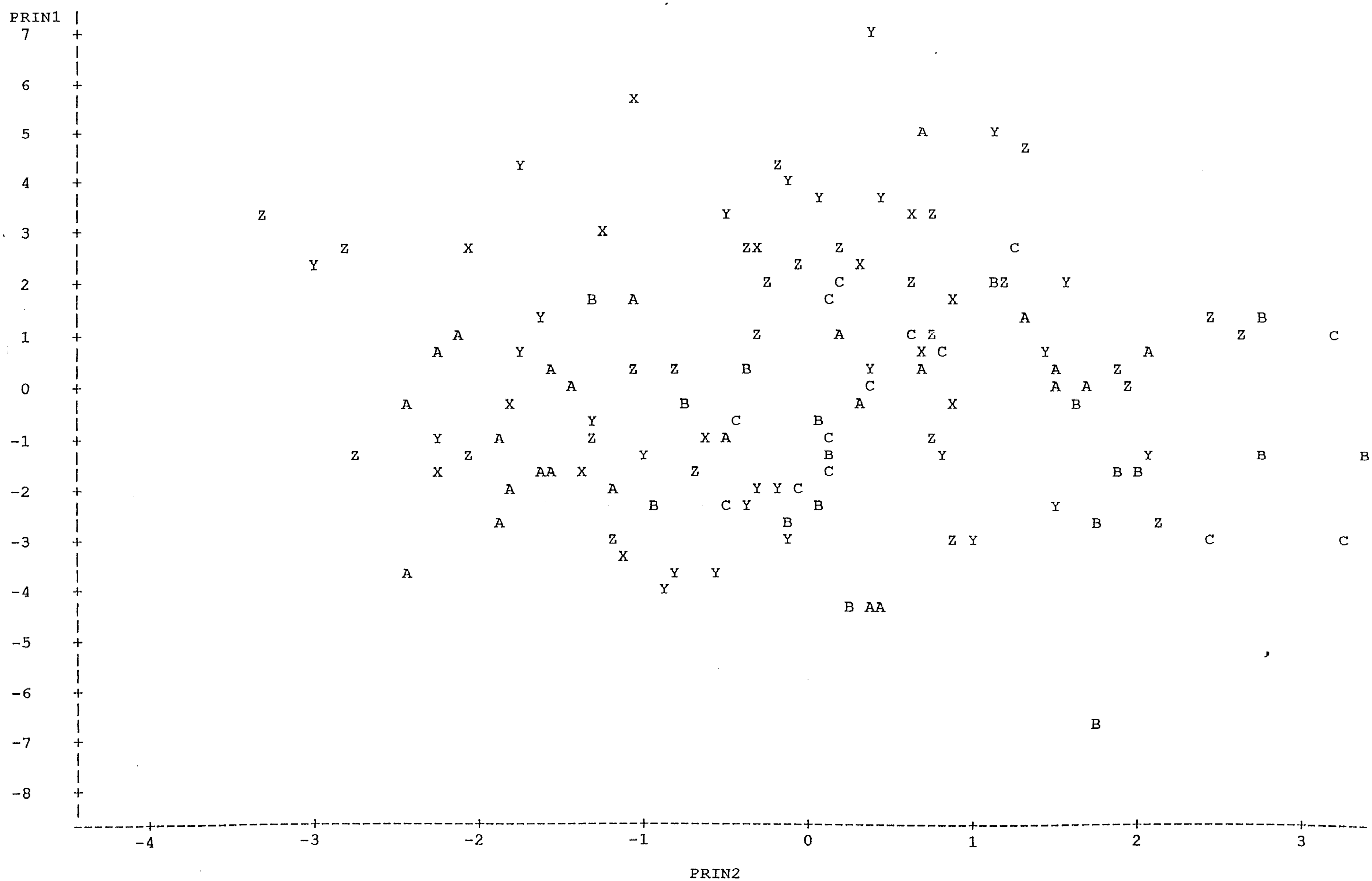

= HATCHLINGS AND INDIVIDUALS < 1 YR
= IMMATURES (1-3 YRS OF AGE)
= ADULT MALES (3 + YRS)
= ADULT FEMALES (3 + YRS)
AVERAGE SNOUT-VENT LENGTH
67
62
57
52
47
42
37
32
27
22
17
12
N= 71
N= 53
N= 62
N= 56
AGE CLASS

Table 26. Yates' Correction Chi-square test to determine sex ratio.

Class	Observed	Expected
Male	62	59
Female	56	59
$X^2 = 0.22$		

Figure 17. Principal Component Analysis showing principal component 1 plotted against principal component 2. Abbreviations: A= Northern Females, B= Southern Females, C= Mountain Females, X= Mountain Males, Y= Northern Males, Z= Southern Males.

PLOT OF PRIN1*PRIN2 SYMBOL IS VALUE OF TAXON



NOTE: 5 OBS HIDDEN

return visits usually produced several unmarked specimens. Gordon (1952) reported a maximum of 41 individuals in his 0.37 ha study area. The Holly River State Park population appeared to be large as indicated by its high density and large size of some of the individuals. Gordon (1952) also found females to be slightly larger than males. Woods (1968) found specimens less than 60 mm in TL could be classified as immatures, individuals between 60-80 mm TL were consided subadults or juveniles, and adults were greater than 80 mm. He indicated that sexual maturity was reached in the spring of their third year. This classification scheme differs somewhat from hatchling, immature, and adult system observed in this study; although, sexual maturity must be reached in the third year of growth since three age classes having different snout-vent lengths were observed. Woods (1968) reported that newly hatched specimens in Mississippi averaged 15.5 mm in TL, 10.2 mm in body length (BL), and 2.8 mm in HW. Hatchlings from this area as well as in other northern populations are larger than in the south. Juterbock (in Pfingsten and Downs 1989) found hatchlings from Ohio to range from 23.5-25.0 mm in TLs and 15-15.5 mm in SVLs.

Gordon (1952) found that four different populations in the Southern Blue Ridge Mountains did not differ significantly from the expected 1:1 ratio, unless the results from all four populations were combined.

This study also disclosed that very little morphological variation exists among populations found in West Virginia.

However, it is expected that populations in the Southern Blue Ridge Mountains may show morphological variations from West Virginia populations, since Highton (1991 personal communication) found protein (electrophoretic) differences between northern and southern populations. Cupp (1980) indicated that sexual dimorphism in A. aeneus is not very great. Females lack the mental hedonic gland, and jaw musculature is large but not as well-developed as in males. This would support the results of this study which disclosed that there is very little, if any, sexual dimorphism in this species.

SUMMARY:

The results of this study indicated that A. aeneus exist as a dense population (366 individuals per ha) at Holly River State Park. Individuals in the population were classified into one of three age classes (hatchlings, immatures, and adults) depending upon their SVL. Sexual maturity appeared to be reached in the spring or summer of their third year of growth. Hatchlings are larger than in southern populations and do not grow much during their first winter. The population does not differ significantly from the expected 1:1 sex ratio and has about egual numbers of individuals in each age class. Very little morphological variation was found within and between populations, and there also appears to be very few differences between the sexes. Some characters studied such as head height, when considered singly, showed some variation between populations. Since all populations probably show some morphological variation, this has little significance and cannot merit separation into subspecies.

CHAPTER IV

INTRA- AND INTERSPECIFIC COMPETITION IN THE GREEN SALAMANDER, ANEIDES AENEUS

INTRODUCTION: Competition for limited resources is generally believed to be conveyed through exploitation, interfernce, or a combination of both (Wrobel et al. 1980). Competition in terrestrial salamanders has been an intriguing area of study in the field of salamander ecology. Several investigators have performed laboratory tests to determine if competition was occurring and what for resources. Many studies have been conducted to determine how competition influences the spatial distribution of salamanders. Jaeger (1972) found that P. cinereus and P. shenandoah competed for food, and that P. cinereus was able to exclude its congener from habitats preferred by both species. Hairston (1981) concluded that competition was important in determining the altitudinal distribution of two Plethodon species.

Territoriality in A. aeneus was studied by Cupp (1979 and 1980) who found males to be aggressive against conspecific males. However, not much is known about the behavior of males towards females and immatures or whether males are territorial only during the breeding season. It is also unclear whether females and immatures establish territories. Gordon (1952) observed males and brooding females to snap at inserted objectes. In the present study, adults were observed to snap at pieces of wire used in capturing the animals and indicated some type of aggressive or defensive behavior. In addition, movement on the same rock outcrops and between rocks (Chapter II) was believed to have been caused by intraspecific competition.

Baltar (1983) found that vertical stratification was important in limiting competition for food, space, and moisture between A. aeneus and other cliff dwelling plethodontid salamanders in Mississippi. In West Virginia, a state with unusual topography, shape, and a wide range of elevations, it is not known whether localized populations of A. aeneus competes interspecifically with the different sympatric species or whether vertical stratification limits such competition. Field observations on vertical statification suggested that at least some interspecific competition was occurring. Plethodon kentucki was found to occur higher on the rock outcrops with A. aeneus at Cabwaylingo State Forest than did P. glutinosus and Desmognathus ochrophaeus at Holly River State Park. This suggested that competition was probably more intense between A. aeneus and P. kentucki. In addition, only P. wehrlei was found in the rock outcrops at Beartown State Park which according to museum records probably had a large population of A. aeneus at one time. The extent of the competitive relationship and vertical statification between A. aeneus and P. wehrlei has not been studied.

The purpose of this study was to determine if A. aeneus found in West Virginia competes intraspecifically and/or interspecifically with other rock-dwelling plethodontids such as P. kentucki, P. glutinosus, D. ochrophaeus, and P. wehrlei. The objective of the interspecific tests was to determine which species was A. aeneus closest competitor and whether A. aeneus acted equally aggressive toward all other rock-dwelling plethodontids or whether these different species ellicit different responses.

The three species of Plethodon that occur on the rock outcrops with A. aeneus are primarily subterraneous and are not anatomically well equipped for climbing. Plethodon kentucki, Cumberland Plateau salamander, resembles the slimy salamander, P. glutinosus, but is smaller and flatter. It ranges from the New River in West Virginia southwest to western Virginia and eastern Kentucky in the Cumberland Plateau section of the Appalachian Plateau Physiographic Province (Green and Pauley 1987). Figure 18 shows a dorsal view of P. kentucki. Its larger sibling species, P. glutinosus may grow up to 20.3 cm long and like P. kentucki is characterized by its heavy, somewhat rounded trunk and tail, short stubby limbs, and short phalanges which lack terminal expansions. Its range extends from central New York south to central Florida and west to eastern Oklahoma and Louisiana (Green and Pauley 1987). P. glutinosus (Figure 19) has a statewide distribution in West Virginia (Green and Pauley 1987). Plethodon wehrlei, Wehrle's salamander, (Figure 20) is medium-sized and the toes of the hind feet are partially webbed. It may be better adapted for climbing than its congeneres

Figure 18. Dorsal view of P. kentucki.

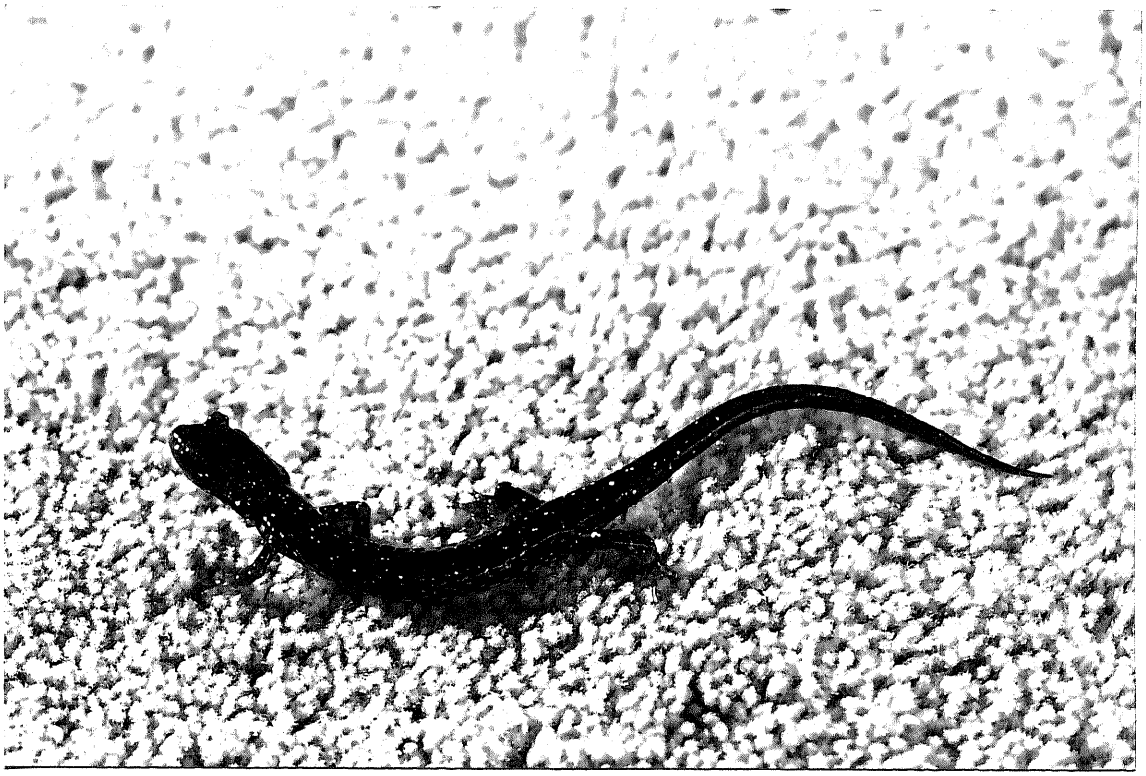
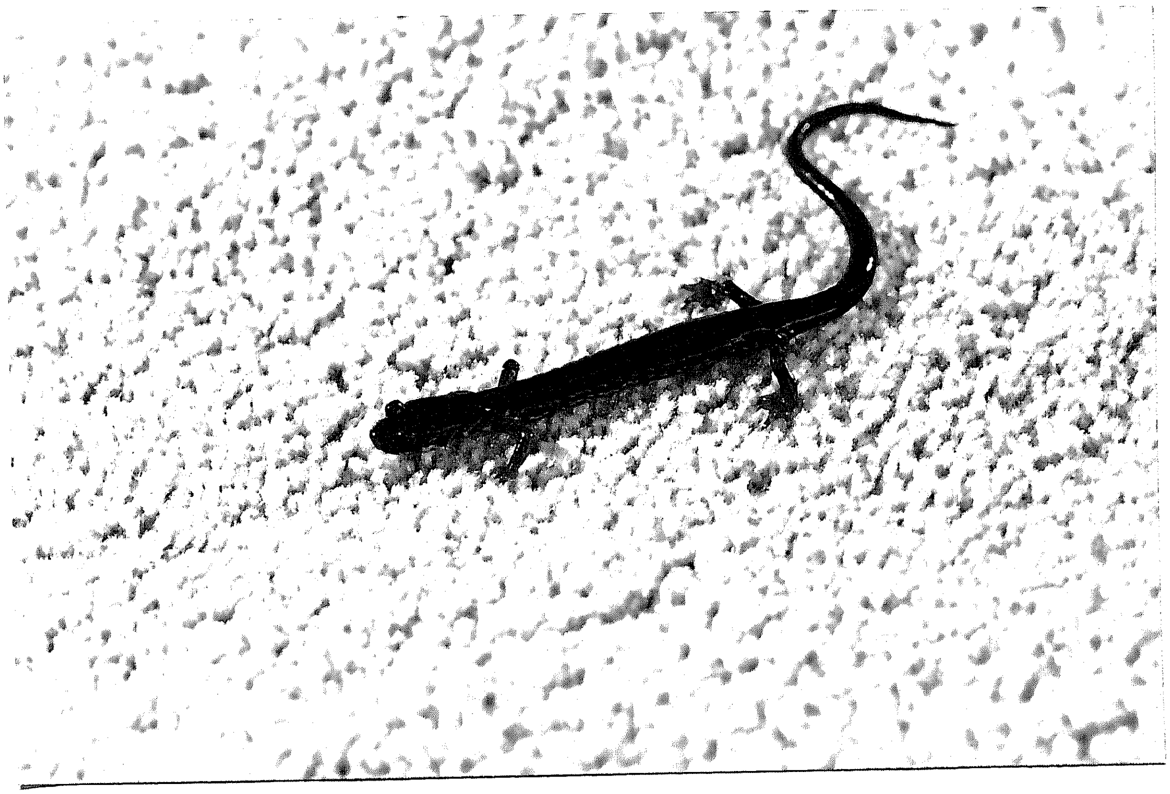


Figure 19. Dorsal view of _P_. _glutinosus_.

Figure 20. Dorsal view of P. wehrlei.



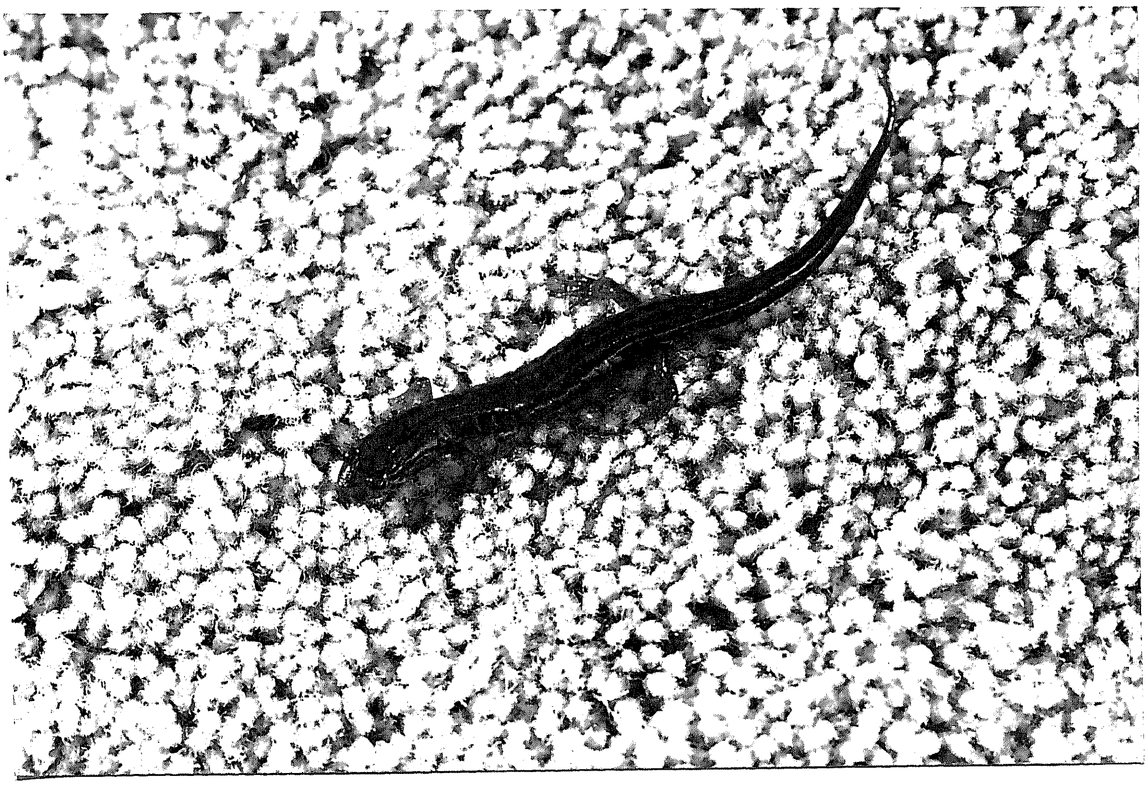
mentioned above. It ranges throughout the Appalachian Plateau from southwestern New York to northwestern North Carolina (Green and Pauley 1987). In West Virginia P. wehrlei ranges from the western edge of the Ridge and Valley Province throughout the Allegheniеs and the Appalachian Plateau Province with the exception of the Cumberland portion (Green and Pauley 1987).

Desmognathus ochrophaeus, mountain dusky salamander (Figure 21), is a small salamander that may attain a total length of 10.2 cm. It is more terrestrial than other members of its genus that occurs in the state and often stays far from water, especially during the summer. It was found on the rock outcrops at Holly River State Park from June - October 1990. It ranges from New York to northeastern Alabama, but in West Virginia it occurs mainly in the mountainous and eastern counties (Green and Pauley 1987).

METHODS AND MATERIALS:

Aneides aeneus were collected at various times throughout the study from Holly River State Park and Cabwaylingo State Forest to test competitive interactions. Plethodon glutinosus and D. ochrophaeus were collected from Holly River State Park, and P. kentucki were collected from Beech Fork State Park, Wayne County. Plethodon wehrlei were collected from North Bend State Park, Ritchie County. The sex of each specimen was detemined by external examination of the cloaca or by the presence of a mental hedonic gland, except for D. ochrophaeus which were sexed using

Figure 21. Dorsal view of D. ochrophaeus.



the methods of Orr (in Pfingsten and Downs 1989). Each specimen was measured for SVL and placed into one gallon jars. The jars were placed on their sides with approximately one inch potting soil. Each jar was labelled to facilitate recognition of the resident salamander and was placed in a controlled temperature chamber at 20 °C with a photoperiod of 12L:12D. The soil was sprayed with water twice a week and the salamanders were fed Drosophilia melanogaster twice a week. Salamanders were allowed one month to mark their territories with pheromones before any tests were performed.

Tests were performed by placing an intruding salamander into a chamber occupied by a resident salamander. The resident salamander was also handled to avoid biasing the procedures. One hour observations were made on each interaction under a red lamp. Tests were performed all times of the year so any behavioral differences during specific times of the year such as the breeding season could be monitored. Specific behavorial states targeted for study were taken from Jaeger et al. (1982), and included the following: Resident salamander - any individual on its own marked territory; Intruder - any salamander that entered the territory of another animal; and Defender - any salamander whose territory was invaded. During each of these states the following events were recorded: Initiator - the first salamander that moved from its original position; Aggressor - any salamander that threatened or attacked the other (Thurow 1975); Appeaser - any salamander that moved in order to avoid an attack (Thurow

1975); Escaper - any salamander that attempted to flee the area either by burrowing in the substrate, climbing the side of the jar, or running away after an attack (Tristram 1977); Complacency - no reaction by an opponent to a threat or attack while having the body in a resting or normal position, or a submissive posture defined by Jaeger et al. (1982) as being flat on the ground with all legs extended outward was also recorded. In addition, the number of times butting, snapping, biting, and snout-pressing occurred as defined by Cupp (1980) was also recorded. Butting was an aggressive act involving the resident charging the intruder and ramming its head or snout directly into the head or body of the intruder. Snapping involved the resident rapidly opening and closing its mouth without contacting the intruder, but within close proximity to the intruder. Biting was a rapid event in which the jaws of one salamander contacted some body region of the other salamander. Snout-pressing involved the resident pressing its snout with elongate premaxillary teeth along the back of the intruder.

RESULTS:

Intraspecific competition was observed when male A. aeneus were introduced into test chambers occupied by inhabited male residents. Of 35 such introductions, 29 resulted in aggressive encounters where resident males were the aggressors in 25 instances and winners in 26. Males used in this trial averaged 51.7 mm in SVL (Table 27). Salamander interactions performed in

Table 27. Snout-vent lengths (mm) of *A. aeneus* males used in intraspecific competition tests.

Specimen	SVL (mm)
A1	55.8
A2	55.3
A3 *	57.3
A4	54.1
A5	51.7
A6	48.4
A7 *	49.1
A8	52.2
A9	49.7
A10	50.9
A11	47.6
A12 *	48.9

* Died in competition chamber during study

this test are presented in Tables 28-30. Figure 22 summarizes the results of intraspecific competition between A. aeneus males. The initiator (IN) or first animal to move (i.e., raise head in response to pheromone or move to explore the new surroundings) was usually the intruding salamander (65.5%). Resident males were the initiators in 35.5% of the trials. Aggressive interactions (AG) were made by resident males in 86.2% of the trials and by intruders in 13.8%. Aggression was in the form of biting, snout-pressing, snapping, and butting. Twenty bites were observed during the 29 aggressive encounters. Thirteen or 75.0% of these bites were by resident males who bit the intruding salamander. Eight of the thirteen bites by the resident males were to the head and/or snout of the intruding salamander. The other five bites were to the body of the intruder. When the resident male bit the head and/or snout of the intruder, the intruder escaped by moving quickly away and/or by climbing the wall of test chamber. Bites to the body sometimes resulted in reciprocal aggression by the intruding salamander. Snout-pressing (SP) occurred in 12 instances and was initiated by the resident male in 8 of the 12 interactions or 66.7% of its occurrence. Snapping (SN) occurred in five instances and was initiated by resident males in three instances (60.0%). Butting (BU) occurred in three instances and was performed by resident males in one instance (33.3%). Introduced males initiated the aggression in four of the 29 aggressive encounters or 13.8% of the time. This initial aggressive behavior was in the form of

Table 28. Trials performed in intraspecific competition between A. aeneus males. Part I.

Trial: Intruder	Trial: Resident	IN	AG	RA	WI	E	E: climb	E: run	NI
A2	A1	A1	A1	-	A1	A2	Y	Y	-
A10	A4	A10	A4	-	A4	A10	Y	-	-
A4	A6	A4	A6	-	A6	A4	Y	-	-
A9	A2	-	-	-	-	-			Y
A1	A5	A1	A5	A1	A5	A1	Y	Y	-
A10	A1	A10	A1	-	A1	A10	Y	Y	-
A4	A8	A8	A8	-	A8	A4	-	Y	-
A7	A10	A10	-	-	-	-			Y
A9	A7	A9	A7	-	A7	A9	-	Y	-
A6	A2	A6	A2	A6	A6	A2	-	Y	-
A11	A9	A11	A11	A9	A9	A11	Y	Y	-

Abbreviations: A= Aneides aeneus IN= Initiator, AG= Aggressor, RA= Reciprocal Aggressor, WI= Winner, E= Escaper, NI= No Interaction, Y= Yes.

Table 29. Trials performed in intraspecific competition between A. aeneus males. Part II.

Trial: Intruder	Trial: Resident	IN	AG	RA	WI	E	E: climb	E: run	NI
A7	A4	A4	A7	–	A7	A4	Y	–	–
A10	A8	A8	A8	–	A8	A10	–	Y	–
A6	A11	A6	A11	–	A11	A6	–	Y	–
A2	A5	A2	A5	A2	A5	A2	–	Y	–
A1	A9	A1	A9	A1	A9	A1	–	Y	–
A8	A6	A6	A6	A8	A6	A8	–	Y	–
A5	A10	A10	A5	–	A5	A10	Y	–	–
A8	A11	A8	A11	–	A11	A8	–	Y	–
A2	A10	A10	–	–	–	–			Y
A9	A1	A9	A1	A9	A1	A9	Y	Y	–
A11	A2	A11	A2	A11	A2	A11	Y	–	–
A6	A5	–	–	–	–	–			Y
A4	A11	–	–	–	–	–			Y

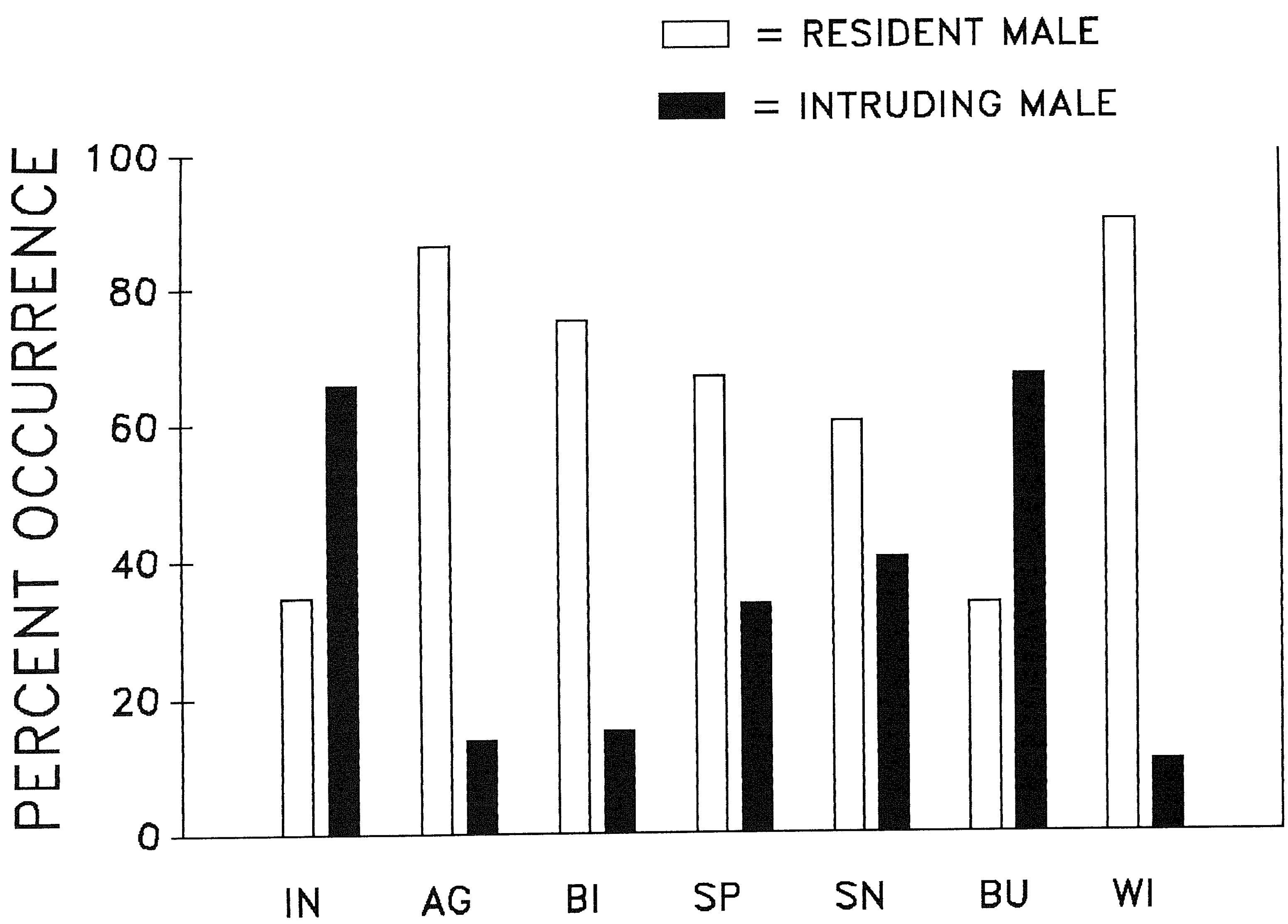
Abbreviations: A= Aneides aeneus, IN= Initiator, AG= Aggressor, RA= Reciprocal Aggressor, WI= Winner, E= Escaper, NI= No Interaction, Y= Yes.

Table 30. Trials performed in intraspecific competition between A. aeneus males. Part III.

Trial		IN	AG	RA	WI	E			NI
Intruder	Resident						climb	run	
A1	A10	-	-	-	-	-			Y
A8	A9	A8	A8	A9	A9	A8	Y	-	-
A1	A4	A1	A4	-	A4	A1	Y	-	-
A2	A6	A2	A6	-	A6	A2	Y	Y	-
A11	A1	A11	A1	A11	A1	A11	-	Y	-
A5	A2	A2	A2	-	A2	A5	Y	-	-
A5	A9	A9	A9	A5	A9	A5	Y	-	-
A10	A6	A10	A6	-	A6	A10	Y	Y	-
A9	A4	A9	A4	-	A4	A9	Y	Y	-
A11	A5	A5	A5	-	A5	A11	Y	-	-
A1	A6	A6	A6	-	A6	A1	Y	Y	-

Abbreviations: A= Aneides aeneus, IN= Initiator, AG= Aggressor, RA= Reciprocal Aggressor, WI= Winner, E= Escaper, NI= No Interaction, Y= yes.

Figure 22. Summary of intraspecific competition results observed in _A_. _aeneus_ males. Abbreviations: IN= Initiator, AG: Aggressor, BI= Biter, SP= Snout-pressing, B= Butting, WI= Winner.


= RESIDENT MALE
= INTRUDING MALE
PERCENT OCCURRENCE
100
80
60
40
20
0
IN
AG
BI
SP
SN
BU
WI

approach and butting, or snapping. Introduced males showed butting in 67.7% of its occurrence and snapping in 40% (Figure 22). Intruders showed reciprocal aggression (i.e., returned the aggression) in nine instances (Tables 28-30). Reciprocal aggression was by biting (five instances or 15.0%) and snout-pressing (four instances or 33.3%). Four of these returned bites were to the body of the resident male and resulted in prolonged bite holds by both salamanders that lasted from one to five minutes. Intruding males were winners in two cases involving bite holds. The other returned bite by the intruder was to head of the resident male and resulted in the only victory by an intruder where the resident male initiated the aggression. Thus, intruding males were winners (WI) in three of 29 aggressive encounters or 10.3% of the time (Figure 22). Subordinate males were found to escape by climbing the walls of the test chambers in 65.5% of the aggressive encounters and to run in 34.5%. In the 6 trials where no interactions occurred, climbing on the part of one or both salamanders was believed to have prevented such aggressive behaviors. No appeasement and complacency were observed in this study.

These same males were tested under neutral conditions (i.e., by placing them into unoccupied chambers and putting the mouths of their chambers together). No aggressive behavior by either salamander was observed during 10 such trials.

Aggressive encounters were not observed in female _A_. _aeneus_ when non-resident females were introduced into the test chambers inhabited by resident females. Thirty-nine trials and field observations (two brooding females were found in the same nest crevice on two different occasions - Pickens Road and rock 2B) indicated that female _A_. _aeneus_ were not territorial and did not compete intraspecifically for mating and/or brooding crevices. Females used in intraspecific trials averaged 51.9 mm in SVL (Table 31). The interactions between these females are presented in Table 32. Adults were also tested against immatures and were found not to act aggressively towards the immatures, except for male A5 who snapped at two different immatures. Conversely, immatures were found not to establish territories and attempt to exclude adults. Adults used in these tests averaged 54.1 mm in SVL and immatures averaged 32.9 mm (Table 33). Table 34 shows trials performed during intraspecific tests between adults and immatures.

Aggressive behavior and interspecific competition were observed in male _A_. _aeneus_ when tested with _P_. _glutinosus_ and _P_. _kentucki_. Of 20 trials where _P_. _glutinosus_ or _P_. _kentucki_ were introduced into the test chambers of _A_. _aeneus_, 10 resulted in aggressive encounters. The same salamanders used in intraspecific competition between adult _A_. _aeneus_ were used in tests against _P_. _glutinosus_ and _P_. _kentucki_.
Plethodon glutinosus used in this interspecific test averaged 64.3 mm in SVL and _P_. _kentucki_ averaged 57.9 mm (Table 35). Tables 36 and 37 show the results obtained when _A_. _aeneus_ were introduced into the chambers of _P_. _glutinosus_ or _P_. _kentucki_.

Table 31. Snout-vent lengths (mm) of *A. aeneus* females used in intraspecific competition tests.

Specimen	SVL (mm)
A1	48.7
A2	52.8
A3	47.7
A4	55.1
A5	54.1
A6	55.8
A7 *	46.6
A8 *	48.2
A9	54.6
A10 *	54.9
A11 *	50.1
A12	48.9
A13	50.2
A14	53.2
A15 *	58.1

* Died during competition study

Table 32. Trials performed in intraspecific competition between female *A. aeneus*.

Trial Intruder	Trial Resident	Trial Intruder	Trial Resident	Trial Intruder	Trial Resident
A1	A3	A8	A15	A12	A4
A2	A5	A13	A6	A1	A11
A10	A14	A9	A7	A4	A2
A3	A13	A7	A12	A2	A4
A14	A8	A1	A9	A5	A11
A14	A7	A9	A11	A13	A5
A14	A1	A12	A1	A4	A9
A11	A7	A2	A5	A14	A13
A1	A7	A5	A12	A5	A3
A11	A4	A2	A1	A7	A14
A12	A9	A4	A11	A2	A12
A7	A1	A3	A5	A12	A14
A11	A13	A1	A4	A9	A2

Table 33. Snout-vent lengths (mm) of salamanders used in intraspecific competition tests between adult and immature (I) A. aeneus.

Specimen	Sex Or Age Class	SVL
A1	♂	50.6
A2	♂	54.7
A3	♀	49.9
A4	♀	56.3
A5	♂	58.7
A6	♂	52.6
A7	♂	51.9
A8	♂	55.4
A9	♀	47.3
A10	♂	56.6
A11 *	♀	56.4
A12 *	♀	58.8
A13	I	32.6
A14	I	34.0
A15 *	I	31.7
A16	I	30.1
A17	I	32.2
A18	I	36.6

* Died during competition study

Table 34. Trials performed in intraspecific competition between adult and immature *A. aeneus*.

Trial Intruder	Trial Resident	Trial Intruder	Trial Resident
A13	A1	A11	A15
A17	A12	A1	A13
A14	A2	A2	A14
A16	A2	A12	A16
A17	A5 *	A5	A17
A16	A11	A2	A13
A13	A5 *	A12	A15
A14	A1	A5	A14
A15	A11	A1	A16
A15	A12	A2	A15

* Aggressively attacked immature intruder.

Table 35. Snout-vent lengths of _P_. _glutinosus_ and _P_. _kentucki_ used in interspecific tests against _A_. _aeneus_.

Specimen	Sex	SVL	Specimen	Sex	SVL
PK1	♀	51.0	PK11	♂	60.7
PK2	♀	48.9	PK12	♂	60.9
PK3	♀	50.9	PG1	♀	62.8
PK4	♂	60.3	PG2	♂	61.4
PK5	♂	61.8	PG3	♂	66.0
PK6	♂	59.5	PG4	♀	64.7
PK7	♂	61.0	PG5	♂	65.3
PK8	♂	60.5	PG6	♂	65.6
PK9	♂	60.4	PG7	♂	64.2
PK10	♂	59.2	PG8	♀	64.8

Table 36. Results of interspecific tests between A. aeneus and P. glutinosus or P. kentucki. Part I.

Trial		IN	AG	WI	E			NI
Intruder	Resident					climb	run	
PK5 ♂	A5 ♂	PK5	A5	A5	PK5	-	Y	-
PK12 ♂	A10 ♂	PK12	A10	A10	PK12	-	Y	-
PK4 ♂	A1 ♂	PK4	A1	PK4	A1	Y	-	-
PK3 ♀	A2 ♀	--	--	--	--			Y
PK2 ♀	A5 ♀	--	--	--	--			Y
PG4 ♀	A9 ♀	--	--	--	--			Y
PK1 ♀	A12 ♀	--	--	--	--			Y
PG1 ♀	A1 ♀	--	--	--	--			Y
PG2 ♂	A6 ♂	--	--	--	--			Y
PK7 ♂	A4 ♂	PK7	A4	PK7	--			-

Abbreviations: A= Aneides aeneus, PK= Plethodon kentucki, PG= Plethodon glutonosus, IN= Initiator, AG= Agressor, WI= Winner, NI= No Interaction, Y= Yes.

Table 37. Results of interspecific tests between A. aeneus and P. glutinosus or P. kentucki. Part II.

Trial Intruder	Trial Resident	IN	AG	WI	E climb	E run	NI
PG6 ♂	A5 ♂	--	--	--	--		Y
PK11 ♂	A9 ♂	--	--	--	--		Y
PK10 ♂	A4 ♂	--	--	--	--		Y
PK8 ♂	A6 ♂	A6	A6	PK8	A6 Y	-	-
PK9 ♂	A2 ♂	--	--	--	--		Y
PG2 ♂	A8 ♂	PG2	A8	PG2	A8 Y	-	-
PG3 ♂	A1 ♂	PG3	A1	PG3	A1 Y	Y	-
PG5 ♂	A9 ♂	PG5	A9	PG5	A9 Y	Y	-
PG6 ♂	A10 ♂	PG6	A10	PG6	A10 Y	-	-

Abbreviations: A= *Aneides aeneus*, PK= *Plethodon kentucki*, PG= *Plethodon glutinosus*, IN= Initiator, AG= Aggressor, WI= Winner, E= Escaper, NI= No Interaction, Y= Yes.

These results are summarized in Figure 23. This figure shows that intruding Plethodon were the initiators (IN) in 90.0% of the aggressive encounters, but A. aeneus were the aggressors (AG) in all 10 or 100% of the aggressive encounters. Aggression was initaited in the form of snapping in two instances and snout-pressing in the other eight. Aneides aeneus was considered the winner in both instances where it snapped at the intruding Plethodon, since the intruder moved quickly away and attempted to escape from the chamber. However, Plethodon returned the aggression in eight instances by snapping and biting. Aneides aeneus escaped by climbing in 75.0% of instances where P. glutinosus and P. kentucki returned the aggression. Thus, P. glutinosus and P. kentucki were considered winners (WI) in 80.0% of the aggressive encounters. In addition, A. aeneus climbed in 50.0% of the trials where aggressive encounters did not occur.

Aneides aeneus were introduced into the test chambers of P. glutinosus and P. kentucki. Of 26 such introductions, 13 or 50.0% resulted in aggressive encounters (Tables 38 and 39). Aneides aeneus were the initiators (IN) in 61.5% of the aggressive encounters and Plethodon were the aggressors (AG) and winners (WI) in all cases (Figure 24). Aggression was iniatiated in the form of approach and chasing in six instances, biting in four instances (two each to the head and body of A. aeneus), snapping in one instance, and butting in two instances. Male Plethodon were more aggressive than females (Tables 38 and 39).

Figure 23. Summary of results obtained when P. glutinosus or P. kentucki were introduced into test chambers of A. aeneus. Abbreviatios: IN= Initiator, AG= Aggressor, WI= Winner.

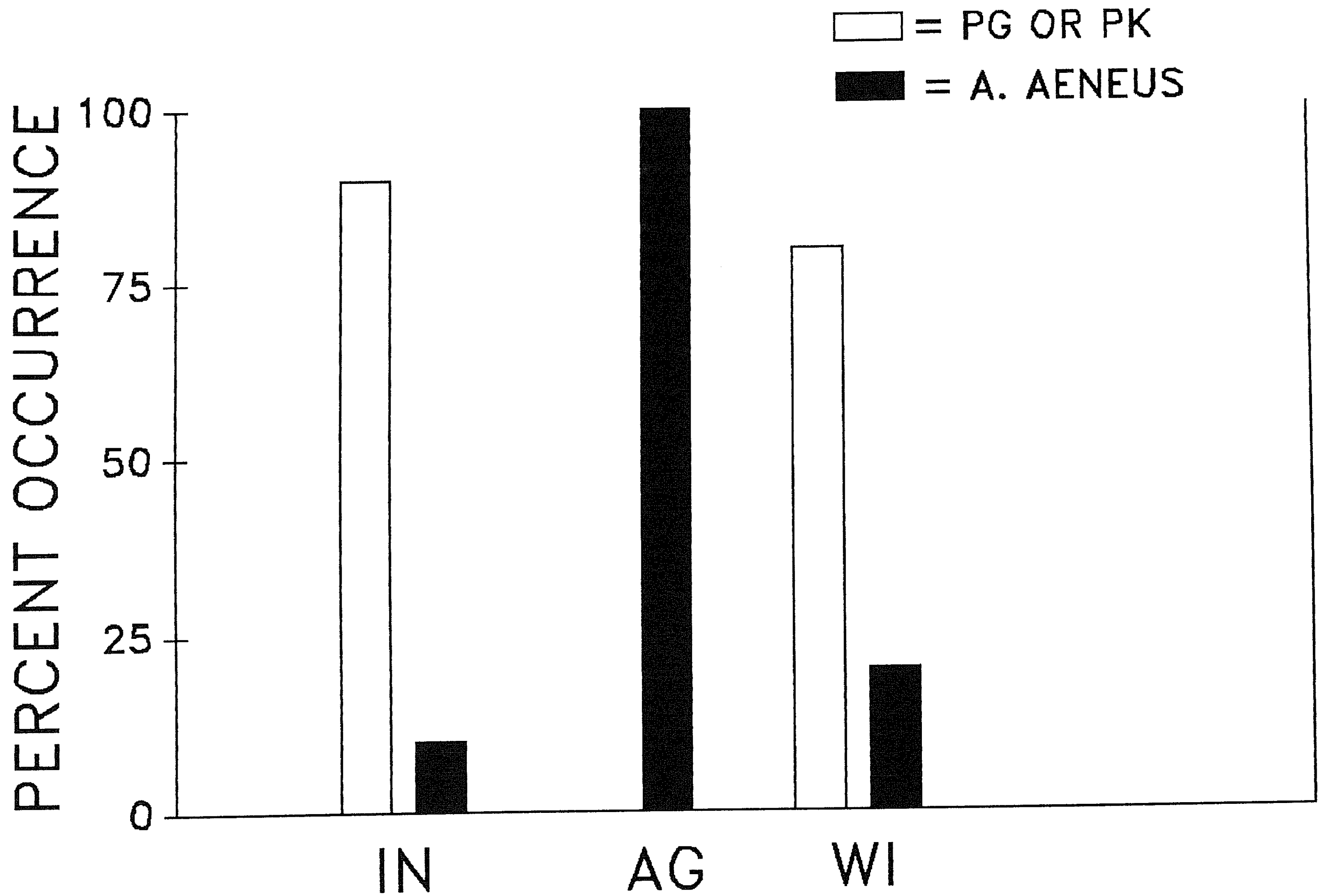

= PG OR PK
= A. AENEUS
PERCENT OCCURRENCE
100
75
50
25
0
IN
AG
WI

Table 38. Results of interspecifc tests between A. aeneus and P. glutinosus or P. kentucki. Part III.

Trial Intruder	Trial Resident	IN	AG	WI	E climb	E run	NI
A6 ♂	PG6 ♂	PG6	PG6	PG6	A6 Y	Y	-
A9 ♂	PG5 ♂	A9	PG5	PG5	A9 Y	-	-
A10 ♂	PK12 ♂	A10	PK12	PK12	A10 Y	-	-
A1 ♂	PG2 ♂	PG2	PG2	PG2	A1 Y	-	-
A2 ♂	PG3 ♂	A2	PG3	PG3	A2 Y	Y	-
A4 ♂	PG2 ♂	--	--	--	--		Y
A5 ♂	PK6 ♂	PK6	PK6	PK6	A5 Y	-	-
A2 ♂	PK5 ♂	--	--	--	--		Y
A1 ♂	PK4 ♂	--	--	--	--		Y
A4 ♂	PK8 ♂	A4	PK8	PK8	A4 Y	-	-
A4 ♀	PG4 ♂	--	--	--	--		Y
A2 ♀	PG8 ♀	--	--	--	--		Y
A3 ♀	PG3 ♀	A3	PG3	PG3	A3 Y	-	-

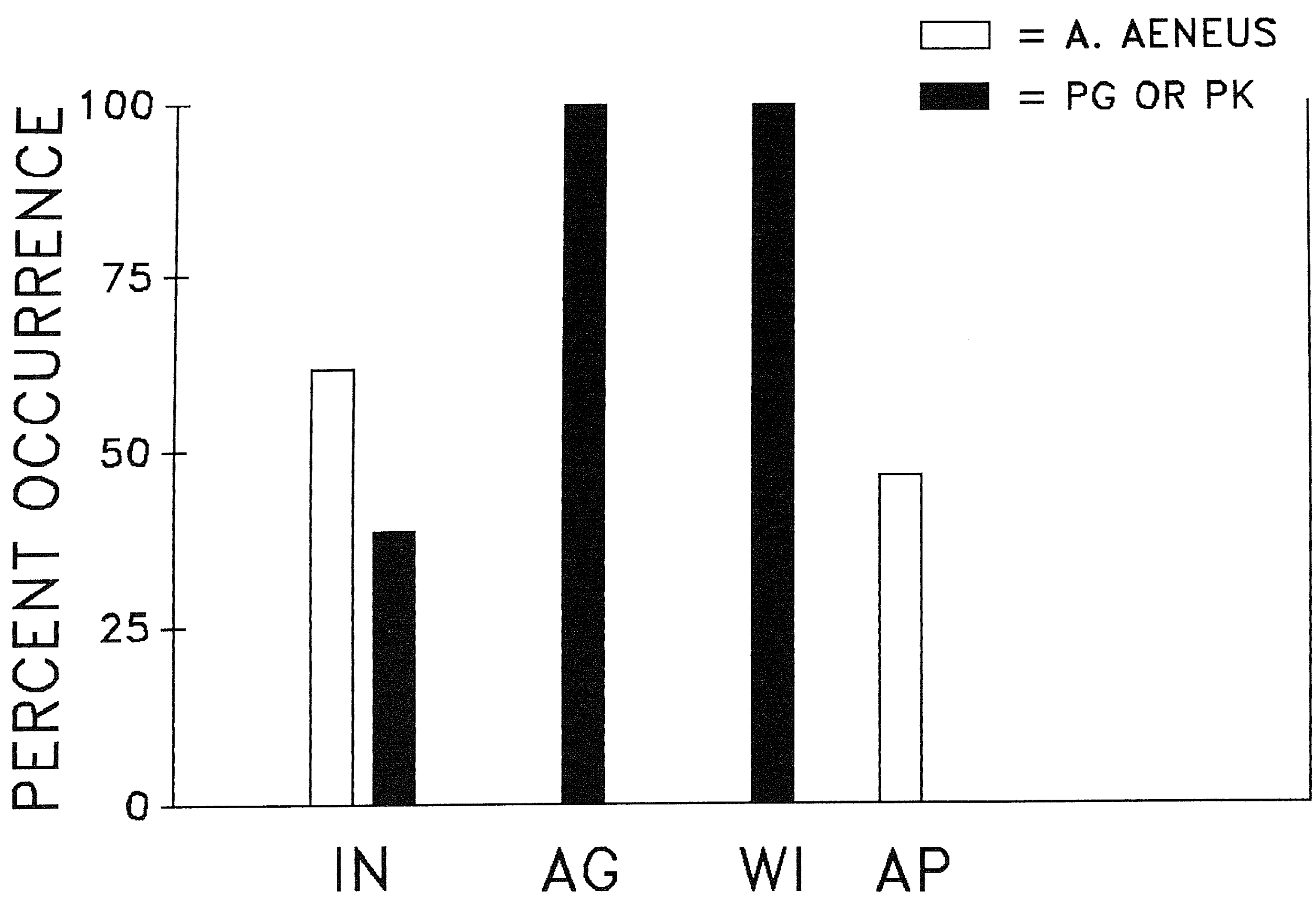
Abbreviations: A= Aneides aeneus, PK= Plethodon kentucki, PG= Plethodon glutinosus, IN= Initiator, AG= Aggressor, WI= Winner, E= Escaper, NI= No Interaction, Y= Yes.

Table 39. Results of interspecific tests between A. aeneus and P. glutinosus or P. kentucki. Part IV.

Trial Intruder	Trial Resident	IN	AG	WI	E climb	E run	NI
A1 ♀	PG1 ♀	PG1	PG1	PG1	A1 Y	-	-
A4 ♀	PG4 ♀	--	--	--	--		Y
A5 ♀	PK3 ♀	--	--	--	--		Y
A4 ♂	PG7 ♂	--	--	--	--		Y
A1 ♀	PK1 ♀	--	--	--	--		Y
A2 ♀	PK2 ♀	A2	PK2	PK2	A2 Y	-	-
A1 ♂	PG7 ♂	--	--	--	--		Y
A2 ♀	PG3 ♀	--	--	--	--		Y
A3 ♀	PG8 ♀	--	--	--	--		Y
A4 ♂	PG4 ♂	A4	PG4	PG4	A4 Y	Y	-
A5 ♂	PG1 ♂	PG1	PG1	PG1	A5 Y	Y	-
A4 ♂	PG8 ♀	A4	PG8	PG8	A4 -	Y	-
A1 ♂	PG3 ♂	--	--	--	--		Y

Abbreviations: A= Aneides aeneus, PK= Plethodon kentucki, PG= Plethodon glutinosus, IN= Initiator, AG= Aggressor, WI= Winner, E= Escaper, NI= No Interaction, Y= Yes.

Figure 24. Summary of results obtained when A. aeneus were introduced into test chambers of P. glutinosus or P. kentucki. Abbreviations: IN= Initiator, AG= Aggressor, WI= Winner, AP= Appeaser.



= A. AENEUS
= PG OR PK
PERCENT OCCURRENCE
100
75
50
25
0
IN
AG
WI
AP

Aneides aeneus escaped by climbing in 92.3% of the aggressive encounters, and climbed in 58.3% of the trials were aggression did not occur. Appeasement (AP) was shown by *A. aeneus* in six of the 13 instances (46.2%) where aggression occurred.

Two aggressive encounters were observed when *A. aeneus* and *P. glutinosus* or *P. kentucki* were placed in neutral chambers. Eight trials under neutral conditions resulted in two aggression attacks by *P. glutinosus*. *Aneides aeneus* escaped by climbing and were observed to climb in all six of the trials where aggression did not occur. Both attacks by *P. glutinosus* were snaps at the body of *A. aeneus*.

Aggressive behavior and interspecific competition were observed when *P. wehrlei* were introduced into the tests chambers of *A. aeneus*. *Aneides aeneus* used in this study averaged 54.0 mm in SVL and *P. wehrlei* averaged 61.8 mm in SVL (Table 40). Of 26 such trials, 11 resulted in aggressive encounters (Tables 41 and 42). Figure 25 summarizes the results obtained when *P. wehrlei* were introduced into the chambers occuppied by *A. aeneus*. *Plethodon wehrlei* were the initiators (IN) in 72.7% of the aggressive encounters, but *A. aeneus* were the aggressors (AG) in all cases. Aggression was initiated in the form of snapping in nine instances (at head, body, and tail), defensive approach in one instance, and biting in one instance (to tail). *Aneides aeneus* were the winners (WI) in 8 instances, but reciprocal aggression by *P. wehrlei* occurred in three instances where it bit the body of *A. aeneus*. In these three cases,

Table 40. Snout-vent lengths (mm) of A. aeneus and P. wehrlei used in interspecific competition tests.

Specimen	Sex	SVL	Specimen	Sex	SVL
A1	♂	55.6	PW1	♂	62.0
A2	♂	54.7	PW2	♀	61.7
A3	♀	49.8	PW3	♀	62.7
A4	♀	50.3	PW4	♂	60.0
A5	♂	58.7	PW5	♂	63.2
A6	♂	52.6	PW6	♀	63.1
A7	♂	59.9	PW7	♂	61.8
A8	♂	55.4	PW8	♂	62.4
A9	♀	60.5	PW9	♂	59.7
A10	♂	56.7	PW10	♀	61.7
A11	♀	47.7			
A12	♀	45.8			

Table 41. Results of interspecific tests between A. aeneus and P. wehrlei. Part I.

Trial: Intruder	Trial: Resident	IN	AG	WI	E: climb	E: run/burrow	NI
PW1 ♂	A10 ♂	--	--	--	--		Y
PW9 ♂	A6 ♂	--	--	--	--		Y
PW4 ♂	A5 ♂	--	--	--	--		Y
PW6 ♀	A4 ♀	PW6	A4	A4	PW6 -	Y	-
PW3 ♀	A5 ♂	A5	A5	A5	PW3 -	Y	-
PW10 ♀	A7 ♂	PW10	A7	A7	PW10 -	Y	-
PW2 ♀	A3 ♀	--	--	--	--		Y
PW5 ♂	A6 ♂	--	--	--	--		Y
PW9 ♂	A7 ♂	PW9	A7	A7	PW9 Y	-	-
PW8 ♂	A8 ♂	A8	A8	A8	PW8 -	Y	-
PW4 ♂	A1 ♂	--	--	--	--		Y
PW10 ♀	A8 ♂	--	--	--	--		Y
PW8 ♂	A9 ♀	PW8	A9	PW8	A9 Y	-	-

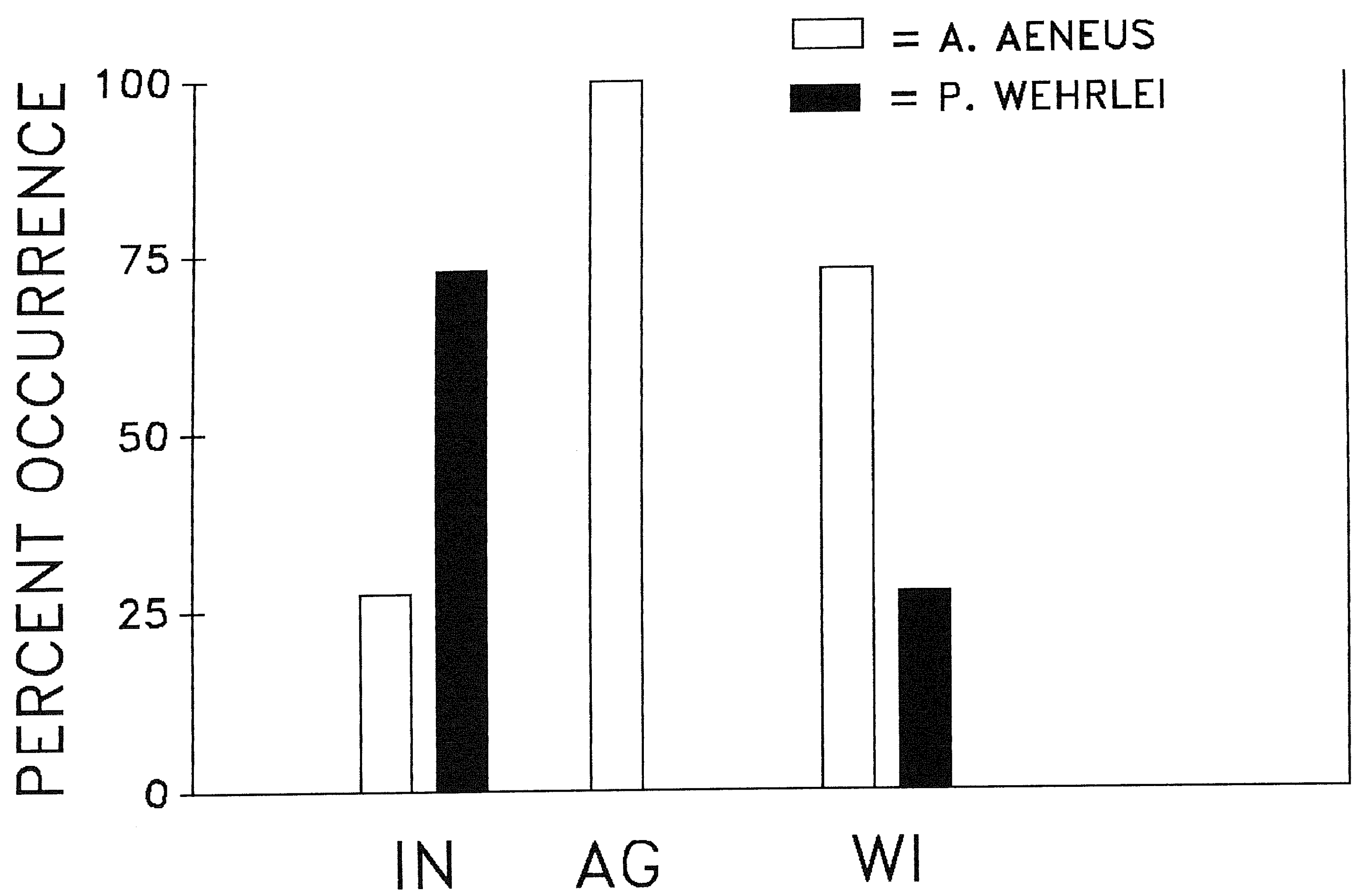
Abbreviations: A= Aneides aeneus, PW= Plethodon wehrlei, IN= Initiator, AG= Aggressor, WI= Winner, E= Escaper, NI= No Interaction, Y= Yes.

Table 42. Results of interspecific tests between <u>A</u>. <u>aeneus</u> and <u>P</u>. <u>wehrlei</u>. Part II.

Trial: Intruder	Trial: Resident	IN	AG	WI	E	E: climb	E: run/burrow	NI
PW7 ♂	A5 ♂	--	--	--	--			Y
PW1 ♂	A2 ♂	--	--	--	--			Y
PW4 ♂	A10 ♂	PW4	A10	PW4	A10	Y	-	-
PW5 ♂	A4 ♀	--	--	--	--			Y
PW2 ♀	A3 ♀	--	--	--	--			Y
PW6 ♀	A8 ♂	--	--	--	--			Y
PW7 ♂	A2 ♂	PW7	A2	A2	PW7	-	Y	-
PW6 ♀	A4 ♀	--	--	--	--			Y
PW7 ♂	A3 ♀	--	--	--	--			Y
PW1 ♂	A1 ♂	A1	A1	A1	PW1	-	Y	Y
PW9 ♂	A10 ♂	PW9	A10	PW9	A10	Y	-	-
PW3 ♀	A9 ♀	--	--	--	--			Y

Abbreviations: A= <u>Aneides</u> <u>aeneus</u>, PW= <u>Plethodon</u> <u>wehrlei</u>, IN= Initiator, AG= Agressor, WI= Winner, E= Escaper, NI= No Interaction, Y= Yes.

Figure 25. Summary of results obtained when _P_. _wehrlei_ were introduced into test chambers of _A_. _aeneus_. Abbreviations: IN= Initiator, AG= Aggressor, WI= Winner.


= A. AENEUS
= P. WEHRLEI
PERCENT OCCURRENCE
100
75
50
25
0
IN
AG
WI

P. wehrlei were considered winners since A. aeneus retreated by climbing the walls of the test chambers. Plethodon wehrlei escaped by burrowing in 12.5% of the aggressive encounters, climbing in 12.5%, and running in 62.5%. Appeasement occurred once by P. wehrlei and A. aeneus climbed in eight out of 15 instances (53.5%) were aggression did not occur.

When the resident-intruder roles were reversed, P. wehrlei were the aggressors and winners in all instances. Of 23 trials performed, 11 resulted in aggressive encounters (Tables 43 and 44). Figure 26 summarizes results obtained when A. aeneus were introduced into the chambers of P. wehrlei. Aneides aeneus were the initiators in 63.6% of these encounters. Aggression was initiated by snapping in nine instances (at head, body, and tail) biting in two (once each to the head and tail of A. aeneus). Aneides aeneus escaped by climbing in 63.4% of the aggressive encounters and climbed in 75.0% of the trials where aggression did not occur.

These same salamanders were tested under neutral conditions which resulted in two aggressive encounters (both were snaps to the body of the other salamander) out of 10 trials. Aneides aeneus was the aggressor and winner in one of the encounters while P. wehrlei was the aggressor and winner in the other. Aneides aeneus climbed in all of the neutral interactions.

Desmognathus ochrophaeus were introduced into the test chambers of A. aeneus and resulted in five aggressive encounters

Table 43. Results of interspecific tests between A. aeneus and P. wehrlei. Part III.

Trial		IN	AG	WI	E		NI
Intruder	Resident				climb	run	
A1 ♂	PW1 ♂	--	--	--	--		Y
A2 ♂	PW2 ♀	--	--	--	--		Y
A5 ♂	PW4 ♂	A5	PW4	PW4	A5 Y	 -	-
A7 ♂	PW5 ♂	PW5	PW5	PW5	A7 Y	 -	-
A10 ♂	PW9 ♂	A10	PW9	PW9	A10 Y	 Y	-
A3 ♀	PW3 ♀	--	--	--	--		Y
A8 ♂	PW7 ♂	--	--	--	--		Y
A4 ♀	PW6 ♀	--	--	--	--		Y
A6 ♂	PW8 ♂	PW8	PW8	PW8	A6 Y	 -	-
A9 ♀	PW10 ♀	A9	PW10	PW10	A9 Y	 Y	-
A3 ♀	PW7 ♂	A3	PW7	PW7	A3 -	 Y	-
A5 ♂	PW8 ♂	A5	PW8	PW8	A5 Y	 Y	-

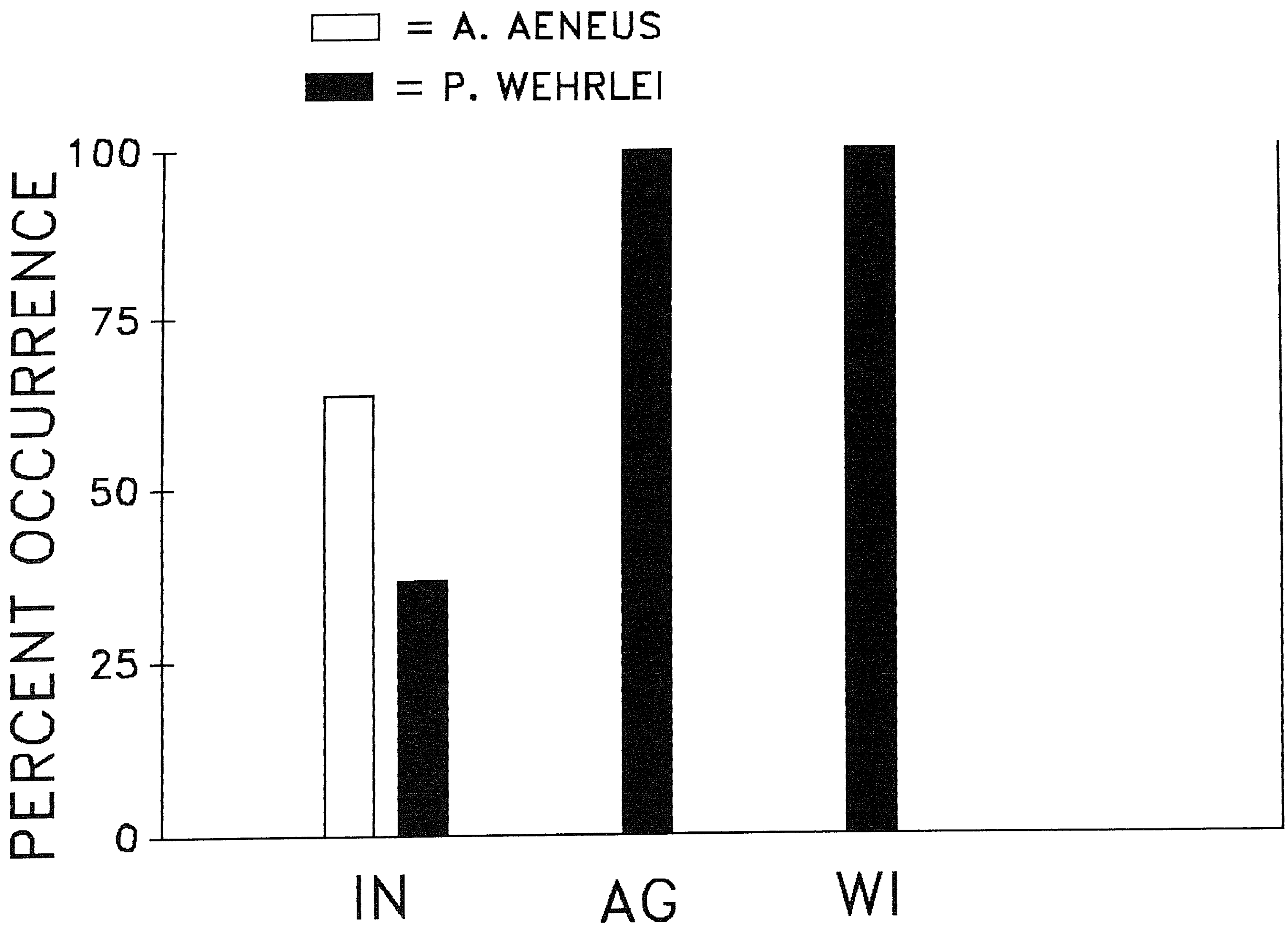
Abbreviations: A= Aneides aeneus, PW= Plethodon wehrlei, IN= Initiator, AG= Aggressor, WI= Winner, E= Escaper, NI= No interaction, Y= Yes.

Table 44. Results of interspecific tests between A. aeneus and P. wehrlei. Part IV.

Trial		IN	AG	WI	E		NI
Intruder	Resident				climb	run	
A9 ♀	PW1 ♂	--	--	--	--		-
A1 ♂	PW4 ♂	--	--	--	--		Y
A6 ♂	PW6 ♀	A6	PW6	PW6	A6 -	Y	-
A10 ♂	PW10 ♀	PW10	PW10	PW10	A10 Y	Y	-
A8 ♂	PW2 ♀	--	--	--	--		Y
A2 ♂	PW5 ♂	A2	PW5	PW5	A2 Y	Y	-
A7 ♂	PW9 ♂	A7	PW9	PW9	A7 Y	-	-
A4 ♀	PW3 ♀	--	--	--	--		Y
A5 ♂	PW5 ♂	--	--	--	--		Y
A1 ♂	PW2 ♀	--	--	--	--		Y
A10 ♂	PW7 ♂	--	--	--	--		Y

Abbreviations: A= Aneides aeneus, PW= Plethodon wehrlei, IN= Initiator, AG= Aggressor, WI= Winner, E= Escaper, NI= No Interaction, Y= Yes.

Figure 26. Summary of results obtained when A. aeneus were introduced into test chambers of P. wehrlei. Abbreviations: IN= Initiator, AG= Aggressor, WI= Winner.


= A. AENEUS
= P. WEHRLEI
PERCENT OCCURRENCE
100
75
50
25
0
IN
AG
WI

out of 20 trials. The same adults used in the interspecific tests against P. wehrlei were used in this study. Table 45 shows the snout-vent lengths of immature A. aeneus and adult D. ochrophaeus. The results of the interspecific tests between these two species are shown in Tables 46 and 47. Figure 27 summarizes these results from this study and indicates that D. ochrophaeus were the initiators (IN) in 60.0% of the aggressive encounters, but A. aeneus were the aggressors and winners in all cases. Aggression was in the form of snaps at the body of D. ochrophaeus who attempted to escape by running (60%), burrowing (20%), and climbing (60%). Aneides aeneus climbed in 53.3% of the trials where aggression did not occur.

DISCUSSION:

The above results indicated that male A. aeneus are territorial and exclude conspecific males from established territories. This would support data collected in the field which suggested that males arrive first at the breeding crevices and establish territories. Gordon (1952) also found that males apparently arrive first at the breeding crevices and establish territories. Territoriality would enhance the chances that mating occurs since females may arrive and enter crevices containing a resident male without interference from competiting males.

Residents became aggressive and attacked intruders in the test chambers to the extent that 89.7% of the males retreated.

Table 45. Snout-vent lengths (mm) of *A. aeneus* immatures (I) and *D. ochrophaeus* used in interspecific competition tests.

Specimen	Sex	SVL
A1	I	32.6
A2	I	34.0
A3 *	I	31.7
A4	I	30.1
A5	I	32.2
A6	I	36.6
DO1	♀	38.4
DO2	♂	33.6
DO3	♀	37.9
DO4	♂	34.1
DO5	♂	32.8
DO6	♀	38.0
DO7	♀	38.4
DO8 *	♂	33.9

* Died during competition study.

Table 46. Results of interspecific tests between A. aeneus and D. ochrophaeus. Part I.

Trials: Intruder	Trials: Resident	IN	AG	WI	E: climb	E: run/burrow	NI
DO2 ♂	A6 I	--	--	--	--	--	Y
DO7 ♀	A2 ♂	A2	A2	A2	DO7 -	DO7 Y	-
DO6 ♀	A5 ♂	DO6	A5	A5	DO6 -	DO6 Y	-
DO1 ♀	A2 I	--	--	--	--	--	Y
DO5 ♂	A6 ♂	--	--	--	--	--	Y
DO6 ♀	A7 ♂	DO6	A7	A7	DO6 Y	DO6 Y	-
DO3 ♀	A1 I	--	--	--	--	--	Y
DO4 ♂	A4 I	--	--	--	--	--	Y
DO5 ♂	A5 I	--	--	--	--	--	Y
DO1 ♀	A6 I	--	--	--	--	--	Y

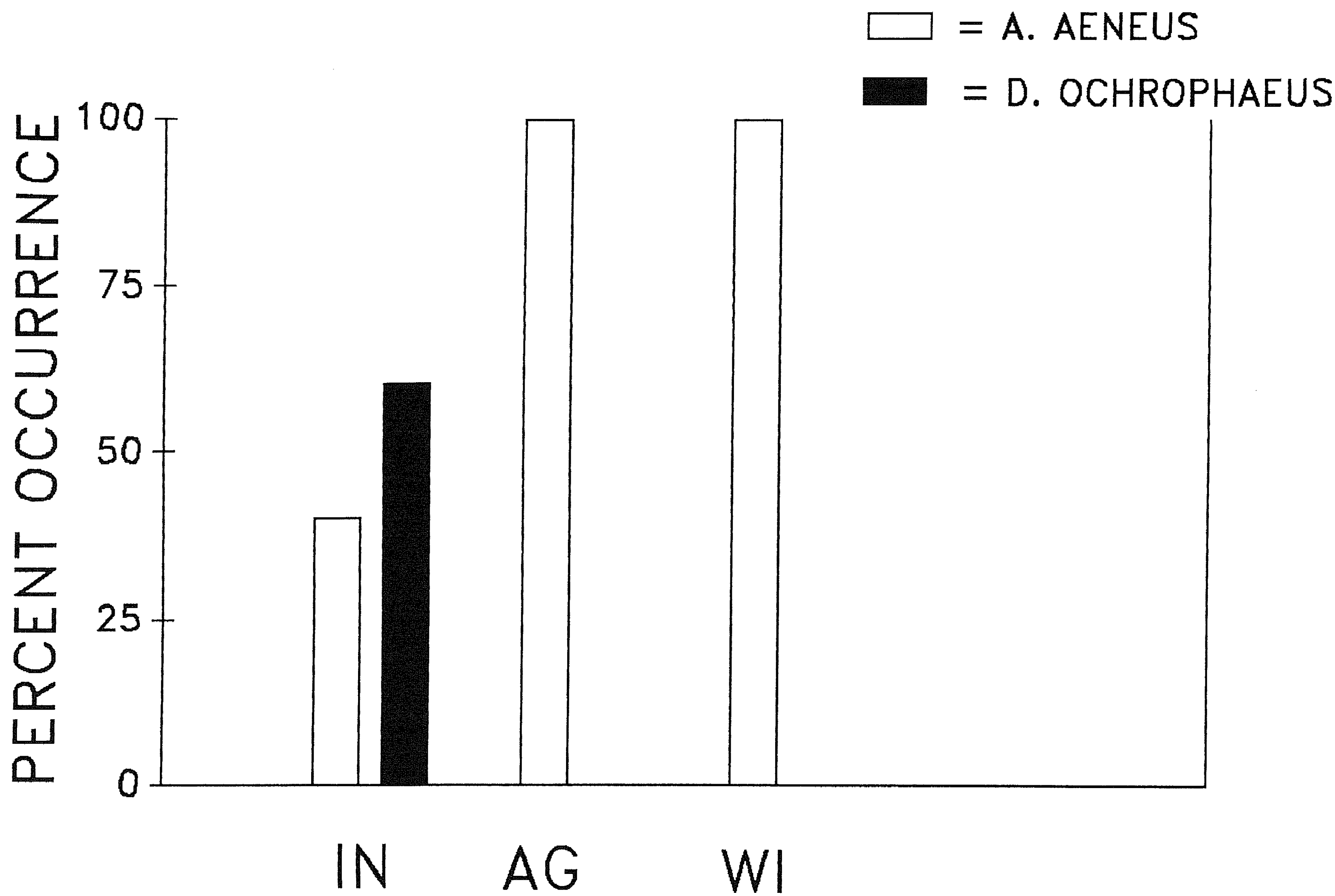
Abbreviations: A= Aneides aeneus, DO= Desmognathus ochrophaeus, IN= Initiator, WI= Winner, E= Escaper, NI= No Interaction, Y= Yes.

Table 47. Results of interspecific tests between A. aeneus and D. ochrophaeus. Part II.

Trial Intruder	Resident	IN	AG	WI	E climb	E run/burrow	NI
DO2 ♂	A10 ♂	--	--	--	--		Y
DO4 ♂	A3 ♀	--	--	--	--		Y
DO7 ♀	A9 ♀	--	--	--	--		Y
DO2 ♂	A4 ♀	--	--	--	--		Y
DO5 ♂	A1 I	--	--	--	--		Y
DO6 ♀	A6 I	--	--	--	--		Y
DO4 ♂	A2 I	--	--	--	--		Y
DO3 ♀	A6 ♂	A6	A6	A6	DO3 -	Y	-
DO5 ♂	A10 ♂	DO5	A10	A10	DO5 -	Y	-
DO7 ♀	A5 I	--	--	--	--		Y

Abbreviations: A= Aneides aeneus, DO= Desmognathus ochrophaeus, IN= Initiator, AG= Aggressor, WI= Winner, E= Escaper, NI= No Interaction, Y= Yes.

Figure 27. Summary of results obtained when _D_. _ochrophaeus_ were introduced into test chambers of _A_. _aeneus_. Abbreviations: IN= Initiator, AG= Aggressor, WI= Winner.


= A. AENEUS
= D. OCHROPHAEUS
PERCENT OCCURRENCE
100
75
50
25
0
IN
AG
WI

Cupp (1980) found that 95.6% of the intruding males retreated during laboratory observations of _A. aeneus_ from Kentucky. Cupp (1980) has shown that when a male was introduced into a chamber with a resident male, the intruder either began exploring or remained motionless for a few minutes and then commenced to move. The resident was usually stationary on one side of the chamber and would orient his head in the direction of the intruder and follow his movements until the intruder came near or contacted the resident. The resident usually initiated aggression in the form of butting, snapping, biting, or snout-pressing. The intruding salamander would either flee or return the aggression. If the intruder returned the aggression then lateral head-swinging and bite-holds would usually occur until one male would flee. These same behavioral acts were also observed in this study.

Cupp (1980) indicated that the well-developed secondary sex characteristics such as enlarged jaw musculature (Gordon 1952) and elongated maxillary and premaxillary teeth (Dunn 1926), which are important in courtship, may also function in territorial defense. The large muscles of the head may be important in butting, lateral head- swinging, biting, and maintaining bite-holds, while the teeth may be used for snout-pressing as well as lateral head-swinging and biting. The yellowish-orange mental hedonic gland (Cupp 1971) plays an important role during coutship and appears well developed in most aggressive males.

Cupp (1980) found that the concept of territoriality in

A. aeneus was supported by the observations in which resident-intruder roles were reversed, and indicated that a resident's familiarity with his territory gave him an advantage over the intruder.

In addition, Cupp concluded that since the resident won in 95.6% of the encounters, size was apparently not a significant factor in territorial defense among male *A. aeneus*. This same reseasoning was also indicated by the fact that residents won 89.7% of the encounters in the present study.

The drive or motivation to retain a territory for mating purposes appears advantageous for this rather sedentary species. Field observations collected in this study complement Gordon (1952) in that adults were often found in the same crevices during the breeding season. Displacement studies have disclosed that males and females will return to crevices from which they have been removed (Gordon 1961, Cliburn and Pihkala 1986). Together, these behavioral acts may enhance the chances of a resident male successfully courting and inseminating a female.

Females were found not to show territorial behavior in the laboratory. Gordon (1952) also reported that two or more brooding females may occupy a single nest crevice. Since females apparently were not territorial and had no reason to move to adjacent rock outcrops to find available territories, this might explain why they appeared not to move between adjacent rock outcrops (Table 19) and why two or more females could be found in a single nest crevice. However, Snyder (1971) observed aggression between gravid females in a nest crevice. It may be that gravid and brooding females are less aggressive than males. In addition, there may be behavioral differences among females within a population which could be related to crevice availability, population size, and/or some other factor. There may also be some behavioral differences within males of a population, since one adult male was found to exclude immatures from his territory. Immatures were found not to establish territories which would explain why they were found to move more than adults (see Chapter II).

Laboratory interspecific competition tests suggested that intruding salamanders were usually the initiator while residents were usually the aggressors. Initiation was usually in the form of head raising or exploratory movement. Plethodon glutinosus and P. kentucki were believed to be more aggressive than A. aeneus since they showed defensive action (approach with body up in the aggressive position) while in their own chambers and in neutral conditions. During these situations, P. glutinosus and P. kentucki were always winners. The results of this study also suggested that A. aeneus was able to escape interspecific competition by climbing. Plethodon kentucki may be more of a competitor with A. aeneus than P. glutinosus because of its flatter head and body which allow it occupy higher and narrower rock crevices. Competition is probably not very keen between A. aeneus and P. glutinosus because of the chunky body of P. glutinosus and its inability to climb and inhabit higher rock crevices. Woods (1968) observed competition between these two species and found that *P. glutinosus* is *A. aeneus* closest competitor and proposed that vertical statification was limiting competition between the two species. Although Baltar (1983) studied the same population in Mississippi and did not observe any aggression between *A. aeneus* and *P. glutinosus*, she was able to show through field and laboratory tests that vertical statification was probably a means of limiting competition between these two species. In West Virginia, *P. wehrlei* may be the closest competitor of *A. aeneus* because of its flatter body and better climbing ability than *P. glutinosus* and *P. kentucki*. Competition with *D. ochrophaeus* was believed to be minimal because of the inability of *D. ochrophaeus* to climb. It may be more of a space competitor with immature *A. aeneus* than adults because of the similarities in size. Size appears to play a role in interspecific competition since larger intruding *Plethodon* were usually winners against smaller *A. aeneus*, but smaller sized *D. ochrophaeus* and similar- sized intruding *P. wehrlei* were defeated. *Aneides aeneus* acted aggressively against larger *D. ochrophaeus* females (for example DO7, gravid female, and females DO6, and DO3 were attacked by male *A. aeneus*). In addition, sex may may also play a role since 38 of 54 (70.4%) aggressive encounters were by male vs male, 12 (22.2%) were by female vs male in which the male was the aggressor in seven instances, and only four involved female vs female. Further studies are needed on competition to determine why males are more aggressive than females and to determine what limited resources are involved in the competitive interactions.

SUMMARY: Aggressive behavior and territoriality were observed in male A. aeneus but not in females and immatures. Males probably establish territories to increase their chances of successfully courting and inseminating females. Females are probably not territorial and do not compete intraspecifically for brooding crevices. Immatures do not establish territories and compete with each other as well as with adults for space and other limited resources. However, interspecific competiton may play a significant role in the spatial and community relationships of rock-dwelling plethodontid salamanders. Such competition is believed to have been reduced through vertical stratification. Aneides aeneus is a relict member of its family and may have become adapted to its specialized rock crevice habitat which reduced competition with other plethodontid salamanders. It is believed that P. glutinosus, P. kentucki, and P. wehrlei have only recently and secondarily become adapted to rock habitats, while the more specialized A. aeneus has had a long and close association with this habitat and is able to reduce interspecific competition because of its climbing ability. Plethodon wehrlei and A. aeneus have been found in sympatry in a few areas throughout the state. Since P. wehrlei is probably better adapted at climbing than P. glutinosus and P. kentucki, it is believed that P. wehrlei is the closest competitor of A. aeneus.

Interspecific competition for food, space, and moisture may prove to be one factor influencing the numbers of A. aeneus in the southern Appalachians were it is thought to be in decline. Wynn et al. (1988) descrided a new species of rock-dwelling plethodon, P. petraeus, from Georgia. This species has a flattened body and expanded toe pads for climbing and is able to occupy the higher and narrower crevices normally occupied by A. aeneus. Therefore, it may be in competition with A. aeneus for vital resources in their restricted habitat.

THESIS CONCLUSION

Results of this study showed that Aneides aeneus is declining in areas where its habitat has been altered or destroyed, but is abundant in dense populations throughout the state in areas having suitable habitat. Habitat selection studies indicated that its occurrence is dependent of bryophyte density with a inverse relationship between the two (high salamander abundance on rocks with low bryophyte density).

Aneides aeneus was found to have a spring (late May and early June) and fall (September and October) mating periods. Field observations and spermatogenic wave and ovarian egg analysis disclosed a biennial egg laying cycle with egg laying occurring in the first half of June. The average incubation period was 87 days and hatching occurred in late August and early September.

The annual cycle was divided into four period as populations in the Southern Blue Ridge Mountains: (1) The breeding period, lasting from late May to October, includes mating, egg laying, and hatching; (2) The dispersal and aggregation period occurs from late September to November; (3) Hibernation occurs from mid-November through late April; (4) Post-hibernation aggregation and dispersal period occurs from late April and early May.

Aneides aeneus was found to be active throughout the year and was more diurnal than nocturnal during the winter months. It reached a peak surface abundance during the Fall dispersal and aggregation period in October. Movement on the same rocks or between adjacent rocks took place throughout the year, but was concentrated more during fall dispersal and aggregation. Immatures were found to move more than adults and males moved more than females. Brooding females were completely sedentary.

Aneides aeneus was found to be insectivorous and to feed mainly on beetles and ants. Different populations and all age classes were found to feed on the same invertebrate species. No differences in the types of prey items consumed per month were noted.

The Holly River State Park population was found to be dense (366 individuals per ha) and to have an egual sex ratio. Measurements of SVLs of hatchlings, immatures, and adults indicated that sexual maturity is reached in the spring of their third year. Principal component analysis disclosed that there was very little morphological differences between populations and that sexes were vertually indistinguishable.

Laboratory competition studies supported field observations that male A. aeneus are territorial while females and immatures did not appear to compete intraspecifically for crevices and space. Plethodon wehrlei and P. kentucki were found to be its closes competetors because of their flatter body and better climding abilities than P. glutinosus and D. ochrophaeus.

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ABSTRACT

The ecology of the green salamander, Aneides aeneus, in West Virginia was studied to determine habitat selection, reproductive strategy, seasonal activity, feeding habits, population size, morphological variation, and competitve behavior.

The study showed that there was a highly significant correlation between salamander abundance and bryophyte density. Aneides aeneus was found to have a spring (late May and early June) and fall (September or October) mating periods. Field and laboratory results (spermatogenic wave and ovarian egg analysis) disclosed a biennial egg laying cycle. Egg laying occurred during the first half of June and the incubation period averaged 87 days.

Aneides aeneus was found to be active during favorable weather conditions in winter, but was reached a peak surface abundance in the Fall dispersal and aggregation period (October). It was also found to feed mainly on beetles and ants and to be active during the day as well as night.

The Holly River State Park population was found to contain 366 individuals per ha and to have three age classes (hatchlings, immatures, and adults). Very little morphological variation was found between populations throughout the state and principal component analysis disclosed little sexual dimorphism.

Territoriality and aggressive behavior were observed in male A. aeneus, but not in females and immatures. Aneides aeneus were

also found to compete interspecifically with other rock-dwelling plethodontid salamanders (*Plethodon glutinosus*, *P. kentucki*, *P. wehrlei*, and *Desmognathus ochrophaeus*). *Plethodon wehrlei* and *P. kentucki* were found to be its closest competetors because of their flatter body and better climbing abilities.

APPENDIX I

ENVIRONMENTAL READINGS RECORDED AT HOLLY RIVER STATE PARK

Temperature (°C)

Rock Number	Outside Crevice (Ambient)	Inside Crevice	Date
9	17	15	11-14-1989
7B	16	15	11-14-1989
4	4.5	5	01-09-1990
9	4.5	5	01-09-1990
7B	3.5	4	01-09-1990
7B	3	4	01-09-1990
7B	3	4	01-09-1990
7B	13.5	14	05-13-1990
12	13.5	14	05-13-1990
1B	14	15	05-14-1990
20	15	14	05-18-1990
25	15	14	05-18-1990
7B	13	13	05-18-1990
8B	13	13	05-18-1990
10	16	15	05-18-1990
7B	16	15	05-18-1990
7B	16	16	05-18-1990
7B	16	16	05-18-1990
49	16.5	16	05-20-1990
50	16.5	16	05-20-1990
9	14	15	05-27-1990
20	16.5	16	05-27-1990

8B	16	15	05-27-1990
7B	16	16	05-27-1990
9	15	14	05-28-1990
9	15	15	05-28-1990
7B	16	15	05-28-1990
*	16	15	05-29-1990
*	18	17	06-19-1990
7B	20	19	06-19-1990
7B	20	19	06-22-1990
25	20.5	20	06-22-1990
9	22.5	22	07-30-1990
1	23	21	08-02-1990
2	23	21	08-02-1990
2	22.5	21	08-02-1990
2	23	21	08-02-1990
2	22.5	21	08-02-1990
3	23	22	08-02-1990
4	23	22	08-02-1990
6	23	22	08-02-1990
6	22.5	22	08-02-1990
7	23	22	08-02-1990
20	23	22	08-02-1990
8	22.5	22	08-02-1990
2B	21	20	08-05-1990
2B	20	19	08-18-1990
2B	20.5	19.5	08-18-1990

19	21	21	08-25-1990
5	20	19	09-03-1990
9	20.5	20	09-03-1990
53	22	21	09-08-1990
25	21	20	09-08-1990

Relative Humidity

Rock Number	Outside Crevice (Ambient)	Inside Crevice	Date
9	48.5	49.3	11-14-1989
7B	48.2	49.4	11-14-1989
4	36.8	36.5	01-09-1990
9	53.6	53.3	01-09-1990
7B	62.4	62.2	01-09-1990
7B	69.8	69.7	01-09-1990
7B	68.0	67.7	05-13-1990
12	67.8	67.6	05-13-1990
7B		73.7	05-14-1990
12	75.0	73.5	05-14-1990
8B		74.4	05-14-1990
7B	66.8	66.3	05-18-1990
10	74.5	74.4	05-18-1990
7B	74.1	73.9	05-18-1990
7B	73.1	72.9	05-18-1990
7B	73.0	72.7	05-18-1990
49	73.1	72.9	05-20-1990
50	73.0	72.7	05-20-1990
9	79.9	81.8	05-27-1990

20	83.1	82.4	05-27-1990
8B	84.4	83.4	05-27-1990
7B	84.5	83.7	05-27-1990
8B	83.1	80.5	05-27-1990
9	81.4	76.7	05-28-1990
9	85.7	85.5	05-28-1990
7B	86.4	88.7	05-28-1990
7B		86.1	05-28-1990
7B	84.0		06-22-1990
1B	89.4	89.0	06-22-1990
7B	94.9	93.9	06-22-1990
7	92.9	92.5	06-22-1990
7	96.4	96.2	06-22-1990
8	82.5	82.5	07-30-1990
19	83.3	83.0	07-30-1990
20	77.5	77.5	07-30-1990
9	78.3	77.8	07-30-1990
11	79.4		07-30-1990
16	78.5		07-30-1990
25	79.4		07-30-1990
3	80.7		07-30-1990
4	83.8	82.7	07-30-1990
6	82.7		07-30-1990
6	83.4		07-30-1990
2B	94.0	94.5	08-05-1990
2B	87.4		08-05-1990

2B	82.3	83.0	08-18-1990
3B		84.9	08-18-1990
4B	87.2		08-18-1990
5B	88.2		08-18-1990
6B	89.7	89.5	08-18-1990
7-B	90.0	89.6	08-18-1990
1	78.6		08-25-1990
2	84.3	84.0	08-25-1990
28	84.3		08-25-1990
27	93.9	93.4	08-25-1990
7	59.2	59.9	08-31-1990
2B	64.6	64.3	08-25-1990
53	54.8	54.3	09-03-1990
49	67.2	67.0	09-04-1990
9	70.4	70.4	09-08-1990
53	69.0	68.4	09-08-1990
25	72.2	72.8	09-08-1990

* = Readings from Beartown State Park

APPENDIX II

POSITION OF MARKED SALAMANDERS ON NUMBERED ROCKS

Marked Rock	Marked Salamander	Date Marked
1	9	07-30-1990
2	--	
3	10	07-30-1990
	72	09-08-1990
	178	10-13-1990
	179	10-13-1990
4	--	
5	--	
6	--	
7	--	
8	19	07-30-1990
	20	07-30-1990
	77	10-09-1990
	92	10-09-1990
	172	10-13-1990
9	2	05-28-1990
	3	05-28-1990
	49	08-31-1990
	50	08-31-1990
	79	10-09-1990
	80	10-09-1990
	82	10-09-1990
	83	10-09-1990

	84	10-09-1990
	85	10-09-1990
	87	10-09-1990
	88	10-09-1990
	89	10-09-1990
	90	10-09-1990
	170	10-13-1990
	173	10-13-1990
	174	10-13-1990
	175	10-13-1990
	177	10-13-1990
10	--	
11	75	09-08-1990
12	--	
13	--	
14	4	05-28-1990
	100	10-09-1990
	169	10-13-1990
	209	06-03-1991
15	--	
16	73	09-08-1990
17	--	
18	--	
19	--	
20	5	05-28-1990
	34	08-18-1990

	69	09-08-1990
	70	09-08-1990
	78	09-08-1990
	93	10-09-1990
	94	10-09-1990
	95	10-09-1990
	97	10-09-1990
	98	10-09-1990
	168	10-13-1990
21	103	10-09-1990
	104	10-09-1990
22	28	08-05-1990
	38	08-25-1990
	99	10-09-1990
	102	10-09-1990
23	53	09-03-1990
24	--	
25	12	07-30-1990
	13	07-30-1990
	29	08-05-1990
	30	08-18-1990
	32	08-18-1990
	33	08-18-1990
	40	08-25-1990
	55	09-03-1990
	59	09-03-1990

	60	09-03-1990
	62	09-03-1990
	67	09-08-1990
	68	09-08-1990
	164	10-13-1990
	165	10-13-1990
	167	10-13-1990
26	115B	10-09-1990
	119	10-09-1990
	120A	10-09-1990
	130	10-09-1990
	203	11-16-1990
27	54	09-03-1990
28	27	08-05-1990
	105	10-08-1990
	107	10-09-1990
	108	10-09-1990
	109	10-09-1990
	110	10-09-1990
	112	10-09-1990
	113	10-09-1990
	114	10-09-1990
	115A	10-09-1990
	117	10-09-1990
	118	10-09-1990
	157	10-13-1990

	158	10-13-1990
	159	10-13-1990
	182	10-13-1990
	183	10-13-1990
	184	10-13-1990
	185	10-13-1990
	187	10-13-1990
	188	10-13-1990
	189	10-13-1990
	190	10-13-1990
29	--	
30	--	
31		
32	--	
33	35	08-25-1990
	135	10-13-1990
34	65	09-08-1990
	134	10-13-1990
35	--	
36	--	
37	--	
38	--	
39	--	
40	52	09-08-91
41	--	
42	--	

43	--	
44	133	10-13-1990
45	--	
46	--	
47	--	
48	--	
49	--	
50	--	
51	--	
52	--	
53	--	
54	--	
55	132	10-13-1990
1B	42	08-25-1990
	137	10-13-1990
	212	06-03-1991
2B	7	05-28-1990
	8	05-28-1990
	14	07-30-1990
	22	08-05-1990
	23	08-05-1990
	24	08-05-1990
	37	08-18-1990
	43	08-31-1990
	44	08-31-1990
	45	08-31-1990

	122	10-09-1990
	124	10-09-1990
	125	10-09-1990
	127	10-09-1990
	138	10-13-1990
	139	10-13-1990
	150	10-13-1990
	205	06-03-1991
3B	15	07-30-1990
	17	07-30-1990
	25	08-05-1990
	152	10-13-1990
	192	10-23-1990
	193	10-23-1990
4B	47	08-31-1990
	63	09-03-1990
	128	10-09-1990
	129	10-09-1990
	140	10-09-1990
	142	10-09-1990
	194	10-23-1990
	204	06-03-1991
5B	149	10-13-1990
	195	10-23-1990
	197	10-23-1990
6B	64	09-03-1990

	120B	10-09-1990
	143	10-09-1990
	144	10-09-1990
	145	10-09-1990
	147	10-09-1990
	148	10-09-1990
	160	10-09-1990
	162	10-09-1990
	163	10-09-1990
	153	10-13-1990
	154	10-13-1990
	180	10-13-1990
	198	10-23-1990
	199	10-23-1990
	207	06-03-1991
7B	39	08-31-1990
	48	08-31-1990
	57	09-08-1990
	58	09-08-1990
	74	09-08-1990
	155	10-13-1990
	200	10-23-1990
	202	10-23-1990
	208	06-03-1991
8B	1 unmarked specimen	

APPENDIX III

MARK AND RECAPTURE DATES OF *ANEIDES AENEUS*

MARKED SALAMANDER	CAPTURE (MARKING) DATE	RECAPTURE DATE
2	5-28-1990	6-10, 6-14, 9-8, & 10-9-1990
3	5-28-1990	6-3, 6-8, 6-17, 7-1, 10-9, 10-13, & 12-22-1990
4	5-28-1990	6-14, 10-9, & 10-13-1990
5	5-28-1990	6-3-1990
7	5-28-1990	6-3, 6-11, 6-22, 8-5, 8-18, & 8-25-1990; 6-3-1991
8	5-28-1990	6-3, 8-5, 8-18, 8-25, 10-13, & 10-23-1990; 6-3-1991
9	7-30-1990	12-22-1990 & 6-3-1991
10	7-30-1990	9-3, 9-8, & 10-13-1990
12	7-30-1990	6-9, 6-10, 6-11, 6-12, 6-13, 6-14, 6-17 6-22, 7-1, 7-30, 8-2, 8-5, 9-3, & 9-8-1990; 3-23-1991
13	7-30-1990	8-2 & 9-23-1990
14	7-30-1990	--
15	7-30-1990	--
17	7-30-1990	--
18	7-30-1990	--
19	7-30-1990	--
20	7-30-1990	6-3-1991
22	8-5-1990	--
23	8-5-1990	9-3, 10-13, & 12-22-1990
24	8-5-1990	6-10, 6-11, 6-12, 6-13, 6-14, 6-17, 6-22 7-1, 7-30, 8-18,

		8-25, 9-3, 9-8, 9-23, & 10-23-1990
25	8-5-1990	--
27	8-5-1990	--
28	8-5-1990	8-25-1990
29	8-5-1990	9-8 & 10-13-1990;
30	8-5-1990	9-8-1990
32	8-5-1990	9-8-1990
33	8-5-1990	6-14, 6-17, 6-22, 7-1, 9-8, 9-9, 10-13, & 10-23-1990
34	8-5-1990	9-23, 10-9,& 10-13-1990; 3-27-1991
35	8-25-1990	--
37	8-25-1990	10-9-1990
38	8-25-1990	--
39	8-31-1990	6-13, 6-14, 6-17, 6-22, 7-1, 7-30, 8-25, 9-3, 9-8, 9-9, & 9-23-1990
40	8-25-1990	10-23-1990
42	8-25-1990	6-10, 6-11, 6-12, 6-13, 6-14, 6-17, 6-22, 7-1, 7-30, 9-3, 9-8, 9-9, 9-23, 10-13, & 10-23-1990
43	8-31-1990	6-9, 6-10, 6-11, 6-12, 6-13, 6-14, 6-17, 6-22, 7-1, 7-30, 8-5, 8-18, 8-25, 9-3, 9-8, 9-23, 10-9, & 10-23-1990; 6-3-1991
44	8-31-1990	9-23, 10-9, & 10-13-1990
45	8-31-1990	--
47	8-31-1990	--
48	8-31-1990	6-9, 6-10, 6-11, 6-12, 6-13, 6-14, 6-17, 6-22, 7-1, 7-30,

		8-5, 8-25, 8-31, 9-3,9-8, 9-23, 10-13, & 10-23-1990; 3-23-1991
49	8-31-1990	10-9 & 12-22-1990
50	8-31-1990	--
52	9-3-1990	--
53	9-3-1990	--
54	9-3-1990	10-9 & 10-13-1990
55	9-3-1990	--
57	9-8-1990	6-3-1991
58	9-8-1990	--
59	9-3-1990	--
60	9-3-1990	9-23 & 10-13-1990
62	9-3-1990	10-9-1990
63	9-3-1990	--
64	9-3-1990	9-23, 10-9, & 10-13-1990; 3-27-1991
65	9-8-1990	--
67	9-8-1990	6-10, 6-11, 6-12, 6-13, 6-14, 6-17, 6-22, 7-1, 7-30, 8-5, 8-18, 9-3, 9-8, & 9-9-1990; 6-3-1991
68	9-8-1990	--
69	9-8-1990	--
70	9-8-1990	9-23 & 10-13-1990; 3-27-1991
72	9-3-1990	9-8, 9-23, & 10-13-1990
73	9-8-1990	--
74	9-8-1990	6-13 & 9-23-1990
75	9-8-1990	6-10, 6-11, 6-12, 6-13, 6-14, 6-22, 7-1, 8-31, 9-9, 10-9, &

		10-13-1990
77	10-9-1990	10-13, 11-16, & 12-22-1990; 3-27-1991
78	9-8-1990	9-23-1990 & 3-27-1991
79	10-9-1990	--
80	10-9-1990	--
82	10-9-1990	--
83	10-9-1990	--
84	10-9-1990	--
85	10-9-1990	--
87	10-9-1990	10-23-1990
88	10-9-1990	3-27-1991
89	10-9-1990	--
90	10-9-1990	--
92	10-9-1990	10-13-1990
93	10-9-1990	10-13-1990
94	10-9-1990	10-13-1990
95	10-9-1990	--
97	10-9-1990	10-13-1990
98	10-9-1990	10-13-1990
99	10-9-1990	--
100	10-9-1990	--
102	10-9-1990	--
103	10-9-1990	--
104	10-9-1990	--
105	10-9-1990	--
107	10-9-1990	6-3-1991

108	10-9-1990	--
109	10-9-1990	--
110	10-9-1990	10-13-1990
112	10-9-1990	6-3-1991
113	10-9-1990	--
114	10-9-1990	--
115A	10-9-1990	--
115B	10-9-1990	--
117	10-9-1990	--
118	10-9-1990	7- 30, 8-25, 9-3, 9-8, & 10-13-1990; 6-3-1991
119	10-9-1990	10-13-1990
120A	10-9-1990	10-13-1990
120B	10-9-1990	10-13 & 10-23-1990
122	10-9-1990	--
123	10-9-1990	--
124	10-9-1990	10-23-1990; 6-3-1991
125	10-9-1990	6-10, 6-11, 6-12, 6-13, 6-14, 6-17, 6-22, 7-1, 7-30, 8-18, 8-25, 8-31, 9-3, 9-8, 9-9, 9-23, 10-9, & 10-23-1990
127	10-9-1990	--
128	10-9-1990	10-23-1990
129	10-9-1990	6-3-1991
130	10-9-1990	11-16-1990
132	10-13-1990	--
133	10-13-1990	--
134	10-13-1990	--

135	10-13-1990	--
137	10-13-1990	10-23-1990
138	10-13-1990	--
139	10-13-1990	--
140	10-9-1990	10-13-1990
142	10-9-1990	10-23-1990
143	10-9-1990	10-13 & 10-23-1990
144	10-9-1990	10-13 & 10-23-1990
145	10-9-1990	10-13 & 10-23-1990
147	10-9-1990	10-13 & 10-23-1990
148	10-9-1990	10-13 & 10-23-1990; 3-27-1991
149	10-13-1990	--
150	10-13-1990	10-23-1990
152	10-13-1990	--
153	10-13-1990	10-23-1990
154	10-13-1990	--
155	10-13-1990	6-3-1991
157	10-23-1990	--
158	10-13-1990	--
159	10-13-1990	--
160	10-9-1990	10-13 & 10-23-1990
162	10-9-1990	--
163	10-9-1990	10-23-1990
164	10-13-1990	--
165	10-13-1990	--
167	10-13-1990	--

168	10-13-1990	--
169	10-13-1990	--
170	10-13-1990	--
172	10-13-1990	--
173	10-13-1990	--
174	10-13-1990	--
175	10-13-1990	--
177	10-13-1990	--
178	10-13-1990	--
179	10-13-1990	--
180	10-13-1990	--
182	10-13-1990	--
183	10-13-1990	--
184	10-13-1990	--
185	10-13-1990	--
187	10-13-1990	--
188	10-13-1990	--
189	10-13-1990	--
190	10-13-1990	--
192	10-23-1990	--
193	10-23-1990	--
194	10-23-1990	--
195	10-23-1990	--
197	10-23-1990	--
198	10-23-1990	--
199	10-23-1990	--

200	10-23-1990	--
202	10-23-1990	--
203	11-16-1990	--
204	6-3-1991	--
205	6-3-1991	--
207	6-3-1991	--
208	6-3-1991	--
209	6-3-1991	--
210	6-3-1991	--
212	6-3-1991	--

APPENDIX IV

SNOUT-VENT LENGTHS OF MARKED SALAMANDERS

MARKED SALAMANDER	SEX/AGE CLASS	SVL (mm)
2	immature	32.0
3	♀	54.5
4	immature	28.7
7	♂	52.4
8	♀	50.6
9	♀	44.4
10	immature	26.9
12	♀	52.7
13	♂	58.1
14	♂	56.4
15	♂	49.6
17	♂	51.7
18	♂	55.3
19	♂	55.6
20	♂	65.3
22	♂	56.3
23	immature	40.9
24	♀	56.6
25	♀	43.6
27	♀	44.2
28	immature	29.8
29	♂	45.3
30	♂	51.2

32	♂	47.8
33	♀	55.8
34	immature	43.1
35	♀	46.8
37	♂	48.0
38	♂	45.3
39	♀	58.8
40	immature	27.4
42	♀	53.9
43	♀	57.0
44	♀	48.3
45	immature	34.0
47	♂	44.9
48	♀	58.0
49	♂	50.6
50	immature	33.0
52	♀	56.5
53	♂	53.2
54	♀	47.5
55	immature	43.5
57	♂	58.0
58	immature	42.6
59	♂	63.0
60	♀	65.8
62	immature	31.6
63	immature	29.3

64	♂	66.4
65	♂	50.6
67	♀	47.6
68	immature	30.5
69	♀	65.6
70	♂	55.4
72	immature	29.0
73	immature	29.9
74	♀	58.7
75	♀	57.7
77	♂	56.3
78	♀	48.6
79	♂	52.6
80	♂	53.3
82	♀	55.0
83	♀	51.6
84	♀	58.4
85	♀	60.1
87	immature	34.2
88	♂	51.8
89	♀	67.0
90	♂	60.0
92	♀	57.0
93	♀	51.4
94	immature	32.0
95	immature	35.0

97	immature	33.0
98	♀	52.0
99	immature	31.9
100	immature	33.6
102	♂	51.6
103	♂	43.1
104	♂	43.0
105	immature	42.0
107	♀	65.0
108	immature	33.1
109	♂	51.1
110	immature	32.7
112	♂	43.7
113	♂	42.2
114	immature	41.6
115A	immature	27.6
115B	immature	34.5
117	♀	45.1
118	♀	55.3
119	♂	45.3
120A	♀	49.5
120B	♀	59.6
122	♀	62.5
123	♂	60.4
124	♀	43.1
125	♂	49.2

127	immature	27.7
128	immature	29.7
129	♀	51.0
130	♂	49.6
132	immature	34.5
133	immature	31.7
134	♂	51.7
135	♂	46.0
137	immature	33.5
138	♀	57.4
139	immature	30.9
140	♂	45.4
142	immature	43.5
143	♂	52.9
144	♂	49.4
145	♂	45.6
147	♀	59.8
148	♀	48.2
149	♂	57.4
150	immature	35.2
152	immature	35.5
153	immature	33.0
154	♂	50.4
155	♀	51.4
157	immature	32.6
158	immature	34.0

159	immature	34.7
160	♀	60.8
162	♂	55.7
163	immature	41.4
164	♂	55.6
165	♂	54.7
167	♂	58.7
168	immature	38.1
169	♀	49.9
170	♂	59.0
172	♀	56.3
173	♂	52.6
174	♂	59.9
175	♂	55.4
177	♀	60.3
178	♀	57.8
179	♂	56.1
180	immature	32.2
182	immature	42.0
183	♂	47.5
184	immature	41.6
185	♀	54.4
187	immature	42.0
188	♀	62.0
189	immature	30.6
190	♂	52.5

192	immature	29.9
193	immature	31.6
194	immature	36.3
195	♂	53.2
197	immature	36.9
198	♀	51.4
199	♀	55.8
200	♂	57.6
202	immature	34.5
203	♀	59.9
204	♂	53.6
205	♂	48.5
207	♂	55.9
208	♀	49.8
209	♂	57.7
210	♂	50.1
212	♀	58.4

VITA

Name .... Ronald Allen Canterbury

Parents .... Mack Canterbury
Dollie Canterbury Stover

Wife .... Beverly D. Canterbury

Birthplace .... Cleveland, Ohio

Birthdate .... November 20, 1966

Schools Attended:

Eccles Elementary
Eccles, West Virginia 1972-1978

Trap Hill Middle School
Surveyor, West Virginia 1978-1981

Liberty High School
Glen Daniel, West Virginia 1981-1985

Concord College
Athens, West Virginia 1985-1989

Marshall University
Huntington, West Virginia 1989-1991

Degrees Received:

B. S. in Biology, Concord College ... 1989

BIOLOGY COURSES TAKEN AT CONCORD COLLEGE:

General Biology I&II, Animals as Organisms, Plants as Organisms, Ecology, Herpetology, Ornithology, Introduction to Research, Independent Research, Special Topics (Biology and Cultures of Belize), Entomology, Appalachian Biogeography, Advanced Tropical and Marine Biology, Parasitology, Comparative Vertebrate Anatomy, Biology Seminar I&II, Molecular Biology, Genetics, Biochemistry.

BIOLOGY COURSES TAKEN AT MARSHALL UNIVERSITY:

Special Problems (Upland Vertebrate Study of the New River Gorge National River - 9 hrs), Mammalogy, Ichthyology, Plant Taxonomy, Plant Morphology, Limnology, Animal Ecology, Seminar I&II, Thesis (6 hrs).