THE REPRODUCTIVE ECOLOGY AND POPULATION ANALYSIS

OF THE SALAMANDER PLETHODON YONAHLOSSEE

A Thesis

by

GREGORY ARLEDGE HARRIS

Submitted to the Graduate School

Appalachian State University

in partial fulfillment of the requirements for the degree of

MASTER OF ARTS

December 1981

Major Department: Biology

182-1
10

THE REPRODUCTIVE ECOLOGY AND POPULATION ANALYSIS

OF THE SALAMANDER PLETHODON YONAHLOSSEE

A Thesis

by

Gregory Arledge Harris

December 1981

APPROVED BY:

Robert Wayne Van Devender
Chairman, Thesis Committee

Richard N. Henson
Member, Thesis Committee

Mary U. Connell
Member, Thesis Committee

Edgar D. Greene
Member, Thesis Committee

Jeffrey A. Butts
Chairman, Department of Biology

Joyce V. Lawrence
Dean of the Graduate School

ABSTRACT

THE REPRODUCTIVE ECOLOGY AND POPULATION ANALYSIS
OF THE SALAMANDER PLETHODON YONAHLOSSEE
(December 1981)

Gregory Arledge Harris, B. A., Warren Wilson College
M. A., Appalachian State University
Thesis Director: Robert Wayne Van Devender

To describe the reproductive cycle of the salamander *P. yonahlossee*, a total of 420 specimens were obtained in monthly samples from late April to November, 1980. All animals were killed in chloretone, fixed in formalin, and stored in ethyl alcohol. Body length (SVL) in mm was measured, sex was determined, and specimens with differentiated gonads were used to determine size and age at maturity, reproductive cycle and sex ratios. Size ranged from 22 to 92mm SVL.

Neonates first enter the sampled population from July through November near the end of their first year of life. Sexual differentiation takes place at the end of the second year. Maturity is reached after three years of growth and reproduction can begin in the fourth year of life.

Size ranged from 49-92mm SVL in females, but only females 70mm SVL or larger were reproductively active. These were at least four years old. Clutch size ($\overline{x}$=17) was not correlated with SVL (n=10). Inseminated females were found from August 16 to October 17. Clutches were produced at most every two years, with oviposition probably between late fall and early spring.

Minimum size at maturity for males was 65mm SVL at three years of age. Adult males did not reach the same maximum SVL as did females. The overall sex ratio was 1:1, but the ratio changed through the year. The change seems to vary with activity differences between the sexes. Testis weight changed seasonally and was probably related to sperm production. Spermatogenesis occurs from late April through July. Sperm began migration into the vasa deferentia in June, and the vasa deferentia were packed with sperm from July through October. The mental gland was most conspicuous from August through October and corresponded well with presence of sperm in the vas deferens.

In order to estimate population sizes for *P. yonahlossee*, a mark-recapture study of a free-living population was carried out from July 22 to October 16, 1980. Although recaptures were few, population density was estimated to be 2,272 *P. yonahlossee* per hectare.

ACKNOWLEDGEMENTS

I would like to express my gratitude to my thesis advisor, Dr. Wayne Van Devender for his guidance and valuable help during the course of this study. Dr. Ed Greene helped to decipher yards of computer printouts which had me baffled, and Dr. Mary Connell provided a listening ear when it was needed.

I am grateful to Jeanette Tarr for her support, and Van Bullman for aid in laying out the population study area. Steve "Swamp" Morrow provided his keen botanical eye in identifying confusing plant species, and Drs. Ruth and Bill Dewel invested much time and effort in the microscopical work. I would also like to thank Ed Hardy and Maggie McMahon for the use of their property, which provided "prime *yonahlossee* territory." Janice Ashley typed the beautiful final draft of this thesis.

I would like to acknowledge the intellectual stimulation of Judy Williamsen, Dr. Bill Hubbard, and Dr. Richard Henson, which left me in awe.

The value of my wife Karen can never be fully expressed. Her love, dedication, and patience during

the past two years kept my head above water. She supported me, carried Ben, and typed like a champion all at the same time.

Computer time was supplied by the Appalachian State University Computer Center and the Appalachian State University Department of Biology.

DEDICATION

To Dr. and Mrs. Stanley Atkins, who gave a young boy the opportunity and encouragement to explore the natural world without hindrance. Lacking the excitement generated beneath that first rock in Ammons Branch, this work may well have never occurred.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure

Figure

Figure

Figure

LIST OF APPENDICES

Appendix

INTRODUCTION

Plethodon is the largest genus of salamanders in North America with about 24 species (Behler and King, 1979). It is surprising, therefore, to discover that little is known about the life history of most members of the genus. At present, only the most widespread species have been studied in any detail. These include the slimy salamander, *P. glutinosus* (Highton, 1956, 1962a), and the red-backed salamander, *P. cinereus* (Sayler, 1966). It is particularly unusual that one of the largest and most striking members of the genus, *P. yonahlossee*, remains essentially a mystery.

Of the 25 genera in the family Plethodontidae, *Plethodon* is considered to be one of the most successful (Highton, 1962b). This is due in part to the fact that all members of the genus are completely terrestrial, mating and laying their eggs on land. This freedom from water eliminates exposure of adults and young to aquatic predators, and the need to migrate to water to reproduce. Moreover, the eggs and young are not as likely to be threatened by periods of drought. Although these woodland salamanders do mate on land, moisture is still critical for successful development of the eggs. They must seek suitable areas with high humidity and cool, stable temperatures for egg survival and hatching. In most cases such areas are subterranean, and are inaccessible for life history studies.

Even though *Plethodon yonahlossee* is considered by some to be the largest species in the genus (Highton, 1972), many aspects of its biology remain unknown, including population densities, home range, mating habits, ovoposition sites, reproductive seasons, relationship of female body size to clutch size, and age of maturity. The most extensive study of *P. yonahlossee* was carried out by Pope (1950). Lack of long-term investigation has left many questions to be answered.

The purpose of this investigation was: 1) to describe the reproductive cycle of the species, including mating habits, sex ratio, reproductive season, relationship of female body size to clutch size, and age at maturity; and 2) to estimate population sizes, density and home range size for one locality.

REVIEW OF THE LITERATURE

Type Locality

Plethodon yonahlossee was described by Dunn (1917) from near the Old Yonahlossee Road about 1.5 miles northeast of Linville, Avery County, North Carolina at an altitude of 4,200 feet (1280mm). The type locality was reevaluated by Gordon et al. (1962).

Phylogeny

There is some disagreement among experts on the Genus *Plethodon* as to the phylogenetic position of *P. yonahlossee*. Highton (1962b) considered *P. yonahlossee* to be one of the most advanced members of the genus while Dunn (1926), Hairston and Pope (1948), Hairston (1949), and Thurow (1968) thought *P. yonahlossee* to be the most primitive and ancestral to other large plethodons.

Adler and Dennis (1962) described a separate population, *Plethodon longicrus*, just south of the range of *P. yonahlossee*. This closely related form is thought by many to be conspecific with *P. yonahlossee* (Pope, 1965; Highton, 1972; Guttman et al., 1978).

There are still many unanswered questions to be dealt with concerning the relationship between these two forms. For the purpose of this work, they will be considered separate species.

Distribution

Plethodon yonahlossee inhabits the south-central portion of the Blue Ridge Province northeast of the French Broad River as far north as Kibler Park, Patrick County, Virginia (Pope, 1965; Newman, 1954). The known vertical distribution of *P. yonahlossee* is between 2,500 and 5,700 feet (762-1737m) (Dunn, 1926; Pope, 1950; 1965).

Habitat

Plethodon yonahlossee inhabits wooded hills, valleys, and ravines according to Pope (1950). Dunn (1926) remarked on their lengthy burrows under logs, stones, and other debris; but Pope (1950) stated clearly that this salamander does not usually construct its own burrows but uses existing openings in the ground. According to Gordon et al. (1962), they are usually found under logs greater than ten inches (25cm) in diameter with no more than one to three inches (7.5cm) of the log

underground. Hairston (1949) reported that *P. yonahlossee* is restricted to a zone within 100 feet of streams but Pope (1950) and Gordon et al. (1962) have shown that the species can actually occupy a variety of different habitats well away from streams.

Food Habits

Pope (1950) commented on the ability of *Plethodon yonahlossee* to consume organisms with tough exoskeletons, stings, etc. and considered it an important vertebrate predator on the invertebrates of the forest floor. The most important food items by volume were found to be ants (Formicidae), catepillars (Lepidoptera), millipedes (Diplopoda), mites (Acarina), and earthworms (Oligochaeta) (Pope, 1950; Rubin, 1969).

Interspecific Competition

Two other species of large plethodons, *Plethodon glutinosus* and *P. jordani*, usually occupy the same habitat as *P. yonahlossee*. Many believe these species to be in direct competition with *P. yonahlossee*. Hairston (1949) believed *P. yonahlossee* is facing extinction because of the supposedly better-adapted *P. glutinosus*. Highton (1972), however, noted that *P. yonahlossee* can compete with *P. glutinosus* and

P. jordani wherever it is found. *P. yonahlossee* appears to be active later at night (Dunn, 1926; Bailey, 1937), which may help reduce food competition with *P. glutinosus* (Gordon et al., 1962).

Reproductive Cycle

Some work has been done on the reproductive cycle of *Plethodon yonahlossee*. Pope (1950) observed that only males over 56 millimeters in snout-vent length (SVL) have cloacal gland papillae. He also stated that mental glands are never seen in males less than 56mm SVL. These characteristics were used as indicators of sexual maturity. The minimum size for sexually mature females has not been reported. Pope (1950) suggested that mating and egg laying probably takes place sometime during July or August but was not fully convinced because of insufficient data. He also noted the number of ova in three females and concluded that *P. yonahlossee* probably matures in three years and lays eggs in the fourth year.

Work done on other members of the genus may aid in the analysis of *Plethodon yonahlossee*. Highton (1956, 1962a), in his work on the reproductive cycle of *P. glutinosus*, the slimy salamander, showed that individuals mature in two years in Florida or in four years in northern populations. He found that

spermatogenesis in *P. glutinosus* begins in April in Pennsylvania and New Jersey. He suggested that mating probably occurs in the fall and spring in the north with egg laying in late spring. Females remain with the eggs until hatching. In Florida, spermatogenesis begins in August. Presumably, mating takes place a year later in July or August. Egg laying occurs in late August or early September.

Sayler (1966) studied the reproductive ecology of the red-backed salamander, *Plethodon cinereus* in Maryland. She noted abundant sperm in mature testes from June until December. She suggested that mating probably takes place from April to June and that eggs are deposited in June. The young become sexually mature by the end of the second year of growth.

Angle (1969) carried out a similar study on the reproductive cycle of the ravine salamander, *Plethodon richmondi* in Pennsylvania and Maryland. He found the cycle to be very similar to that of the northern *P. glutinosus* and *P. cinereus*, with all three species possessing a biennial cycle requiring two years for females to produce a clutch of eggs.

Plethodon yonahlossee is an enigma with many important questions to be answered: At what age and size do they reach maturity? When do the males produce sperm?

When does mating occur? When are eggs laid and how many does an individual female lay? How often do females reproduce? What is the ratio between the sexes? How dense are the populations? How much do they move and how large is their home range? Finally, how does this species compare in all aspects with other members of its genus?

METHODS AND MATERIALS

Several different methods were used to try to resolve the unanswered questions concerning *Plethodon yonahlossee*. These can be conveniently divided into collecting methods, laboratory procedures for the destructive collection and a population study as described below.

Collecting Methods

Data on the reproductive cycle of *Plethodon yonahlossee* were obtained from collections totaling 420 specimens made at approximately monthly intervals from late April to November, 1980. An attempt was made to capture a minimum of 40 individuals per month to obtain reliable samples. Although several animals were collected during the daytime by searching under rocks and logs, most were found while active on the surface on moist evenings and on roads on rainy nights, usually well after 10:30 p.m. Specimens were collected from seven different locations in Watauga County, North Carolina (Figure 1, Table 1) and are a part of the permanent collection of Appalachian State University (ASU #'s 5402-5816). Collection site location (U.S.G.S. Boone and Deep Gap 7.5 minute

FIGURE 1. Collection site locations near Boone, Watauga County, North Carolina. For site elevations and exact locations, see also Table 1 and Appendix A.

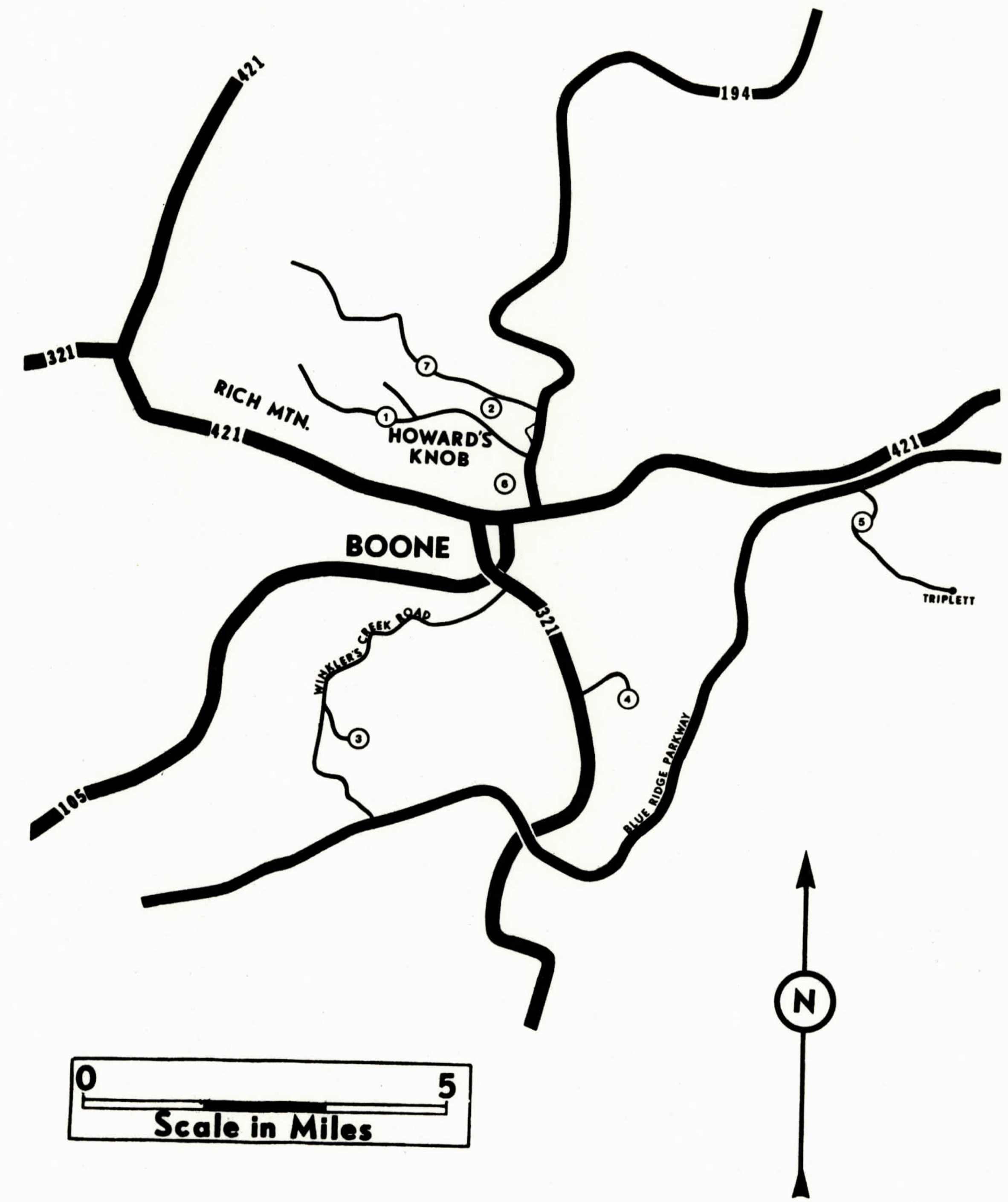


TABLE 1. Collection locations for *P. yonahlossee*. Complete location information is listed in Appendix A. Latitude and longitude were obtained from U. S. Geological Topographic Maps: Boone and Deep Gap Quadrangles, 7.5 minute series.

Site #	Location	Elevation (feet)	Elevation (meters)	Latitude	Longitude
A.	Destructive sites:				
1	North slope Howard's Knob	3600-3840	1097-1170	36°14'23"N	81°41'20"W
2	Straight Ridge	3400-3520	1036-1073	36°14'32"N	81°40'21"W
3	Rainbow Mtn.	3520-3700	1073-1128	36°11'02"N	81°41'04"W
4	Niley Cook Rd.	3520-3600	1073-1097	36°10'54"N	81°38'18"W
5	Triplett Rd.	2850-3140	869- 957	36°13'25"N	81°34'34"W
6	Hidden Valley Circle	3200-3600	975-1097	36°13'25"N	81°40'04"W
7	Howard's Creek Rd.	3160-3200	963-975	36°14'35"N	81°39'57"W
B.	Population sampling site:				
	Rainbow Mtn.	3625-3725	1105-1135	36°11'04"N	81°41'05"W

Quadrangle Topographic Maps) and specimen information are listed in Table 1 and Appendices A and B. Within three days of capture, all specimens were killed in chloretone, fixed in 10% formalin. Later, they were transferred to 70% ethyl alcohol for storage. Altitudes were determined with an aneroid altimeter.

Laboratory Procedures

Body length (SVL) was measured in millimeters from the tip of the snout to the posterior angle of the vent. Sex was determined by macroscopic examination of the gonads. Specimens with undifferentiated gonads were used to determine the size and age at maturity and the annual reproductive cycle (Table 2). In females, relative body mass was measured to the nearest 0.1g with an Ohaus electric gram balance. Mature females were separated from immature females on the basis of the condition of the oviduct. The oviduct of immature females appeared thin and flat while that of mature females were enlarged and thick. Gravid females were separated from nonreproductive mature females on the basis of ovarian egg size equal to or greater than 2.0mm in diameter. Ovarian eggs of reproductive females were counted and measured to the nearest 0.5mm. Time of mating was determined by

TABLE 2. Data recorded for each destructive specimen.

Males	Females	Unsexable juveniles
SVL	SVL	SVL
Testis	Ovaries	
length	egg number	
mass	egg size	
pigmentation		
sperm production		
Vas deferens	Cloacae	
pigmentation	spermatophore	
convolution	sperm	
sperm presence		
Mental Gland Condition	Spermathecae	
	sperm presence	
	Body Mass	

examining the cloaca for the presence of a spermatophore and by preparing histological sections of the spermatheca. Spermathecae of formalin-preserved females were embedded in Spurr's Epoxy Resin, sectioned at 1-2 microns and stained in Toluidine Blue. Sections were examined at 250x, and the presence or absence of sperm was noted. Microscope slides of selected tissue sections were photographed with a Leitz Ortholux 2 Photomicroscope on Kodak Technical Pan Film 2415. Slides were deposited in the biology department at Appalachian State University.

The following criteria were used to indicate the size at maturity and to determine monthly stages of the spermatogenic wave in males: Length of the left testis was measured in mm, and its mass in mg determined on a Roller Smith Precision Balance; additional data recorded were degree of pigmentation of testis and vas deferens, degree of convolution of the vas deferens, and condition of the mental gland which was classified using the term "Mental Gland Score." The score for each individual was either 0 for inconspicuous, 1 for conspicuous but not thickened, or 2 for conspicuous and thickened. Finally, the testis and vas deferens of each male were crushed separately and examined microscopically under phase contrast for sperm.

Population Study

A mark-recapture study of a free-living population of *Plethodon yonahlossee* was carried out at Rainbow Mountain Overlook from July 22 to October 16, 1980. A square plot of wooded land 50m on each side ($2{,}500m^2$) was gridded using red flags spaced at 10m intervals. The 25 $100m^2$ squares were named using letters A through Y. Overstory vegetation was analyzed for dominance by sampling each $100m^2$ square. Diameter at breast height was measured with tree calipers for all trees one inch (2.5cm) or greater in diameter. Plant species in the herbaceous layer were identified and recorded. All species were identified using Radford et al. (1968), Cocke (1974) and Strausbaugh and Core (1978).

Sampling of *Plethodon yonahlossee* was done at night. To minimize habitat destruction, only those animals near their burrows or those found moving about on the ground were captured. After each animal was captured, it was placed in a large petri dish so

that its ventral surface was flat against the bottom of the plate. A piece of foam rubber was placed over the animal and the lid was put on the petri dish. This immobilized the animal while a line from the tip of snout to posterior angle of the vent was traced onto the bottom of the petri plate using a pen with indelible ink. The length of each animal was later recorded from its individual tracing on the petri plate. The sex, whenever apparent, was recorded. A unique combination of toes was clipped to identify each animal and the location of the individual within the grid was recorded on a map.

Three population samples were taken from July 22 to October 16, 1980. During each sampling, individuals were captured, marked, and released at site of capture. During the last two sampling periods, all marked and unmarked individuals were recorded. New animals were marked and released.

Information obtained from the population sampling was analyzed using the "Schnabel Method" (Tanner, 1978). In this repeated sample method, the number of marked individuals is cumulative from previous samples as more individuals are marked each time. The equation for estimation of the population size, N_i, is $N_i = \frac{(M_i C_i)}{R_i}$; where M_i is the number of marked individuals in the population and equals the sum of all individuals previously marked and released. C_i is the number of animals captured in sample i, and R_i is the number of marked individuals in sample i.

Individuals less than 25mm SVL were not marked since their digits were too small to clip reliably. Their location and markings, however, were recorded. Also, location of individuals that were missed in a capture attempt was noted. Approximate numbers of other salamander species found on the forest floor were also noted. This was done by keeping a running tally for each species during each collecting session. Weather and forest floor conditions (i.e., wet, damp, dry, etc.) and the time of sampling were recorded. Rainfall data were obtained from the National Weather Service and through the computerized weather file at Appalachian State University. Principal statistical sources were BMDP programs (Dixon and Brown, 1979). Computer time was donated by the ASU Computer Center and the Department of Biology.

Abbreviations used in this work are Snout vent length (SVL), feet (ft.), meters (m), square meters (m^2), centimeters (cm), millimeters (mm), grams (g), and milligrams (mg). Times used were in Eastern Standard Time (E.S.T.).

RESULTS

Monthly Samples

Plethodon yonahlossee were collected at seven sites (Table 1, Figure 1, Appendices A and B). Numbers of specimens collected at each site in the monthly samples are summarized in Table 3. Samples ranged from 8 animals in November to 119 in August. Most of the *P. yonahlossee* were collected at Site 1 (Table 3). Of the 420 animals collected, 356 (84.8%) came from this study area. This site was located along a little used woodland road on the north slope of Howard's Knob (see Figure 2). Approximately 0.8 mi (1.2 km) of this road was used for collecting. The road followed the contour of the north slope of the ridge and wound its way in and out of several deep coves. In each of these coves was a bold woodland stream cascading down a steep slope. The slopes adjacent to the road seemed to be consolidated talus, with an overstory of red maple (*Acer rubrum*), tulip poplar (*Liriodendron tulipifera*), chestnut oak (*Quercus prinus*), and northern red oak (*Quercus rubra*). The ground was covered with large chestnut

TABLE 3. Destructive collection site summary. Site numbers refer to localities listed in Appendix A, Table 1 and shown on Figure 1.

Month	Destructive Collection Site							Total
	1	2	3	4	5	6	7	
May	22	12	11	0	4	0	2	51
June	23	4	0	2	9	0	0	38
July	27	0	0	0	0	16	0	43
August	119	0	0	0	0	0	0	119
September	62	0	0	4	0	0	0	66
October	95	0	0	0	0	0	0	95
November	8	0	0	0	0	0	0	8
							TOTAL	420

FIGURE 2. Summer aspect of destructive site #1, north slope of Howard's Knob. Observer is facing north-northwest. Altitude is 3,600 feet (1,097m). Road is approximately 4 meters wide.

logs, occasional rhododendron thickets and a diverse herbaceous layer. Most specimens came from the road itself or the banks adjacent to the road. Several animals were also found many feet above the forest floor in large rock crevices like those shown in Figure 3. Usually, these were large adults. *P. yonahlossee* became the most conspicuous and abundant salamander on the forest floor after about 11:30 p.m. It remained active usually until well after 1:30-2:30 a.m.

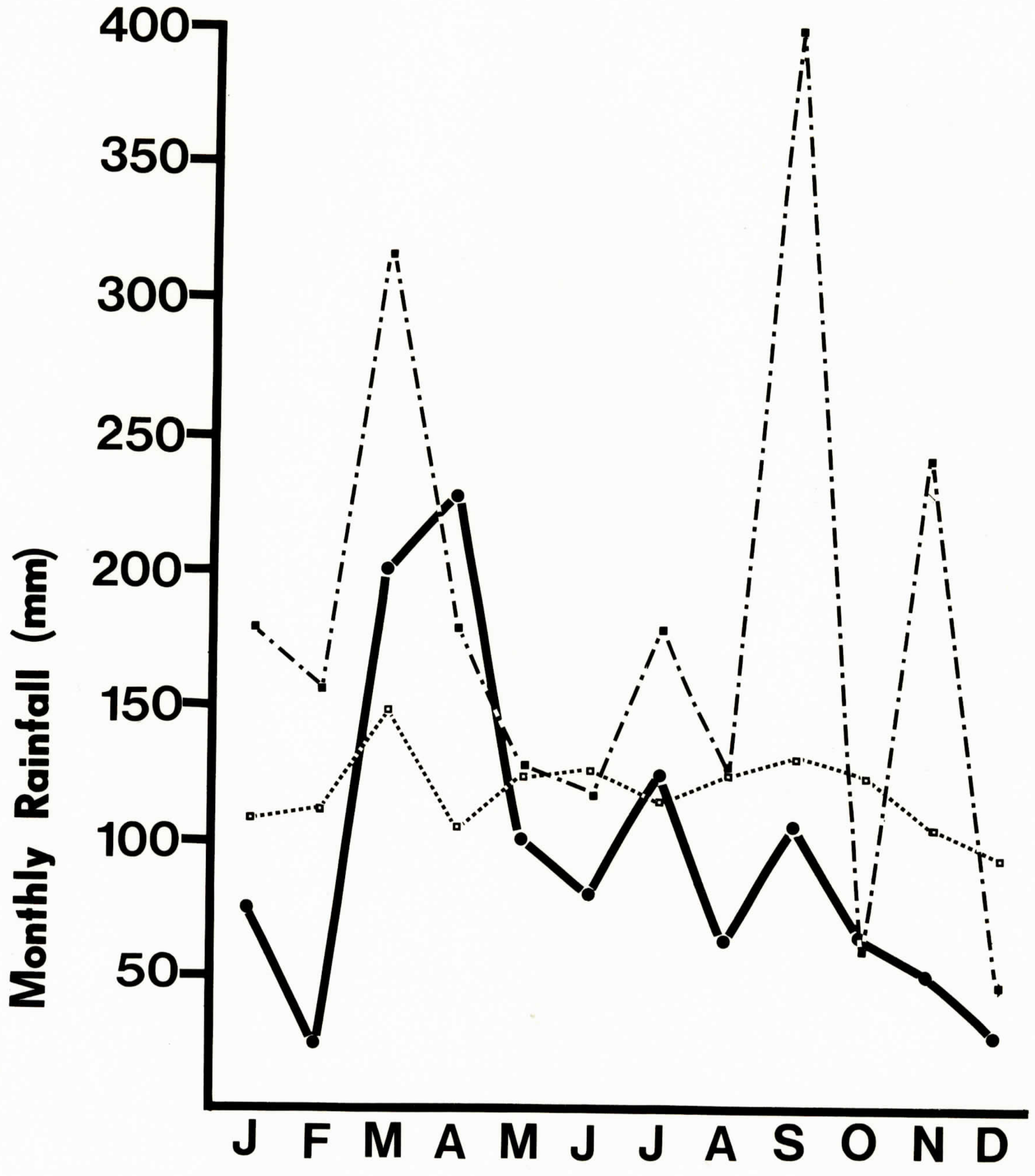
Other salamander species seen during the collecting sessions were *Desmognathus monticola*, *D. quadramaculatus*, *D. ochrophaeus*, *Gyrinophilus porphyriticus*, *Plethodon cinereus*, *P. glutinosus*, *P. jordani* and *P. richmondi*. Most of these were most obvious from dusk until approximately 10:30-11:30 p.m.

At times, few individuals are available; but even brief intervals of precipitation, stimulated surface activity of large numbers of *P. yonahlossee*. This behavior was evidenced by the collection of 119 animals in August in four hours time.

Compared with the previous 20 years, 1980 precipitation in Boone (Figure 4) was sporadic and generally very low. Precipitation in 1979 on the

FIGURE 3. Exposed rock outcropping site #1, north slope Howard's Knob. Elevation is 3,700 feet (1,128m). Rock outcropping is approximately 3 meters high. Crevices extend several centimeters. Several specimens were found along outer perimeter of crevices at night.

FIGURE 4. Monthly rainfall for Boone, Watauga County, North Carolina. Rainfall for 1960-1979------ is an average of each month over the 20 year period. Rainfall for 1979.-.-. and 1980——— is the total rainfall for each month. Rainfall data were obtained from the National Weather Service and through the computerized weather file at Appalachian State University.



other hand was unusually high. Infrequent precipitation in 1980 probably affected the effectiveness of both destructive sampling and population sampling.

Size and Age at Maturity

Data for monthly samples of *Plethodon yonahlossee* are listed in Tables 3 and 4. Data for monthly samples were used to construct size-frequency histograms. These histograms (Figures 5-11) graphically illustrate the relationship between size and age as classes of individuals within a sample of a population. For example, it sometimes is difficult to determine size classes for juveniles because of small sample sizes in some samples. The August sample (Figure 8) most clearly illustrated juvenile age classes. Two distinct classes of unsexable juveniles were apparent. The first group included salamanders 22 to 30mm SVL which were in their first year of growth. The second class contained individuals 35 to 49mm SVL that were in their second year of growth.

Another less distinct group of individuals included juveniles from 50 to 65mm SVL. These salamanders were presumably in their third year of growth. Individuals 65mm SVL and above seemed to mature at four or more years of age. These four age classes

TABLE 4. Monthly body size characteristics for *P. yonahlossee* samples.

Snout-Vent Length in Millimeters

Month	Males				Females				Unsexable juveniles			
	n	$\bar{x}$	Range	s^2	n	$\bar{x}$	Range	s^2	n	$\bar{x}$	Range	s^2
May	15	70.0	58-80	41.4	32	72.5	55-87	83.4	4	36.5	34-40	6.3
June	15	72.1	53-85	94.4	20	74.1	52-85	127.3	3	42.3	35-50	56.3
July	14	67.6	53-82	63.5	14	69.4	50-85	121.5	15	37.1	25-46	36.0
August	45	71.3	49-83	58.6	47	70.3	50-86	121.7	27	35.6	22-49	69.2
September	26	66.0	43-78	94.6	28	68.4	49-91	168.3	12	35.1	25-44	42.3
October	52	71.9	48-82	61.5	32	79.5	52-92	51.7	11	32.1	26-41	17.0
November	4	70.8	61-77	48.3	3	74.7	54-85	320.3	1	25.0	NA	NA
TOTALS	171	70.3	43-85	69.2	176	72.5	49-92	122.7	73	35.5	22-50	48.5

FIGURE 5. Size-frequency distribution for *P. yonahlossee* collected during May 1980 (n=51). Unsexable juveniles are indicated by hatching.

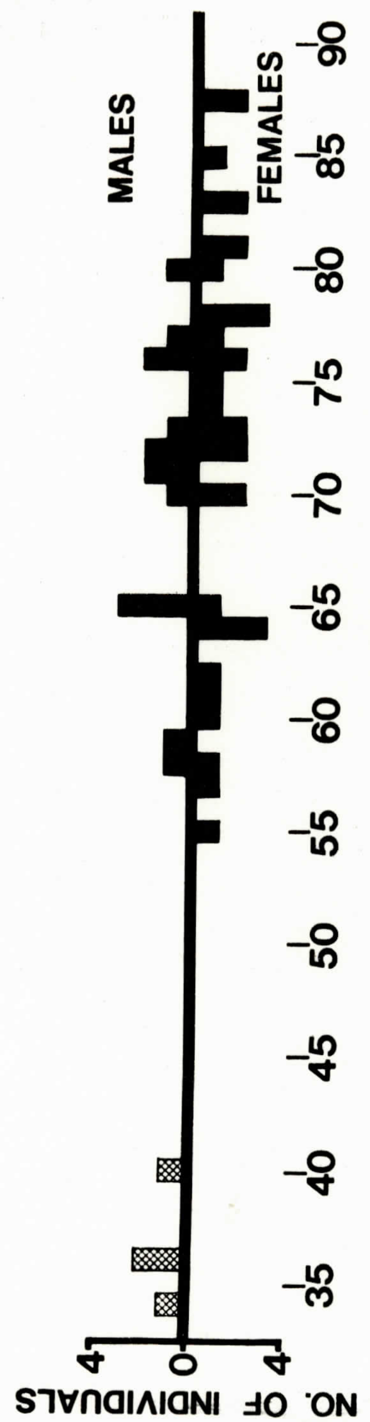


FIGURE 6. Size-frequency distribution for *P. yonahlossee* collected during June 1980 (n=38). Unsexable juveniles are indicated by hatching.

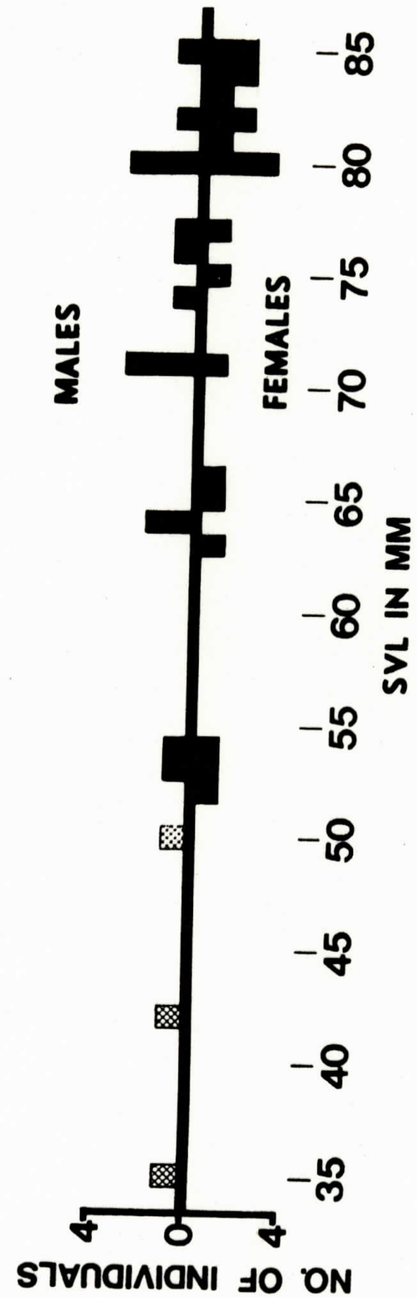


FIGURE 7. Size-frequency distribution for *P. yonahlossee* collected during July 1980 (n=43). Unsexable juveniles are indicated by hatching.

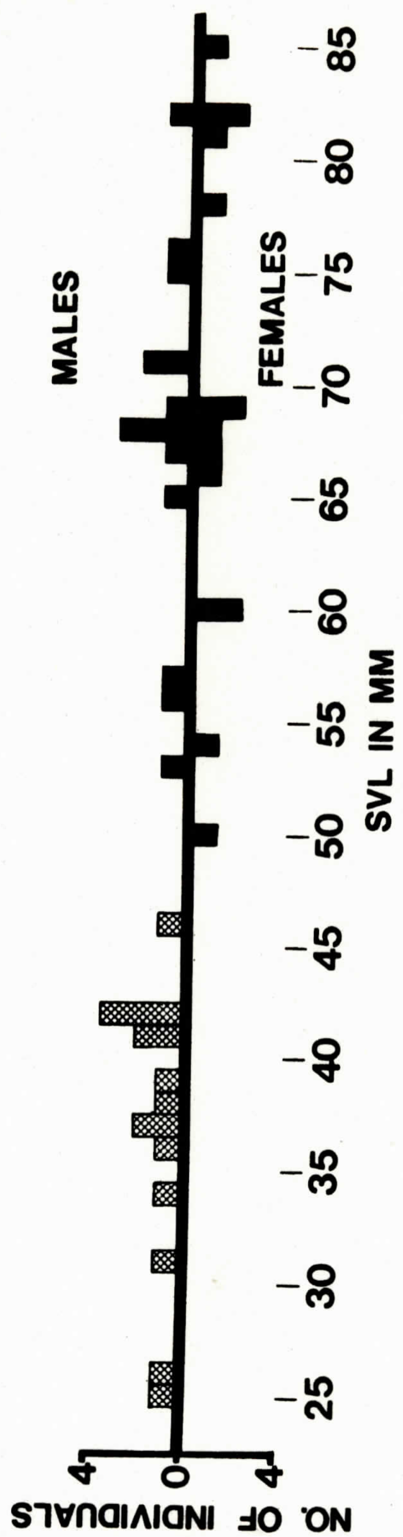


FIGURE 8. Size-frequency distribution for *P. yonahlossee* collected during August 1980 (n=119). Unsexable juveniles are indicated by hatching.

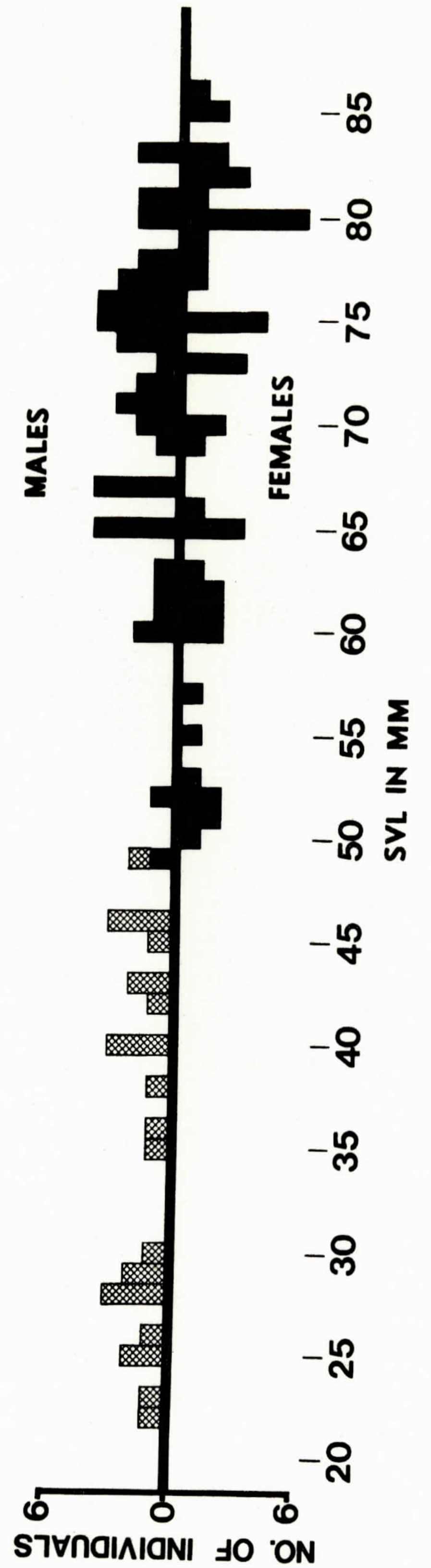


FIGURE 9. Size-frequency distribution for *P. yonahlossee* collected during September 1980 (n=66). Unsexable juveniles are indicated by hatching.

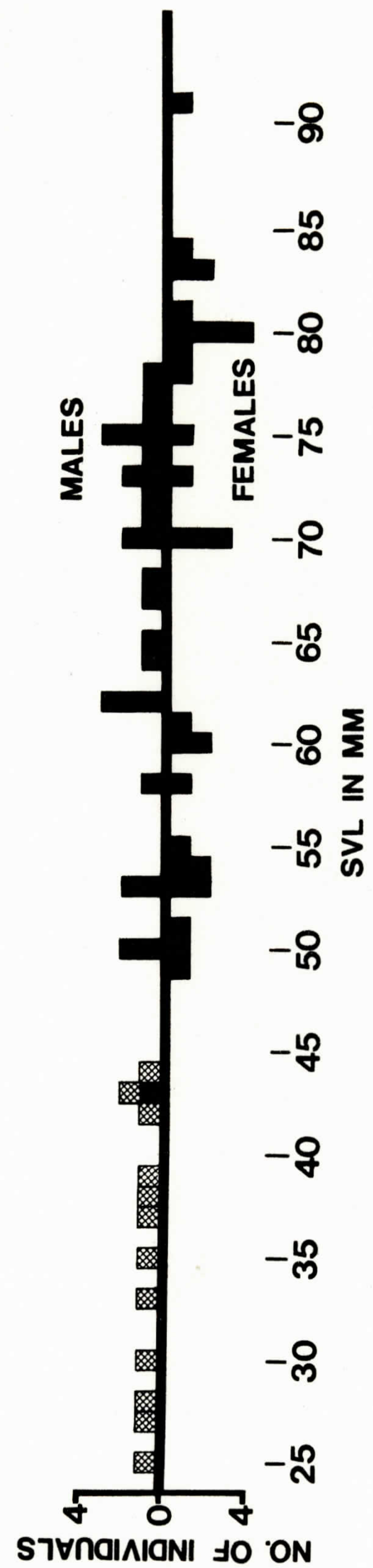


FIGURE 10. Size-frequency distribution for *P. yonahlossee* collected during October 1980 (n=95). Unsexable juveniles are indicated by hatching.

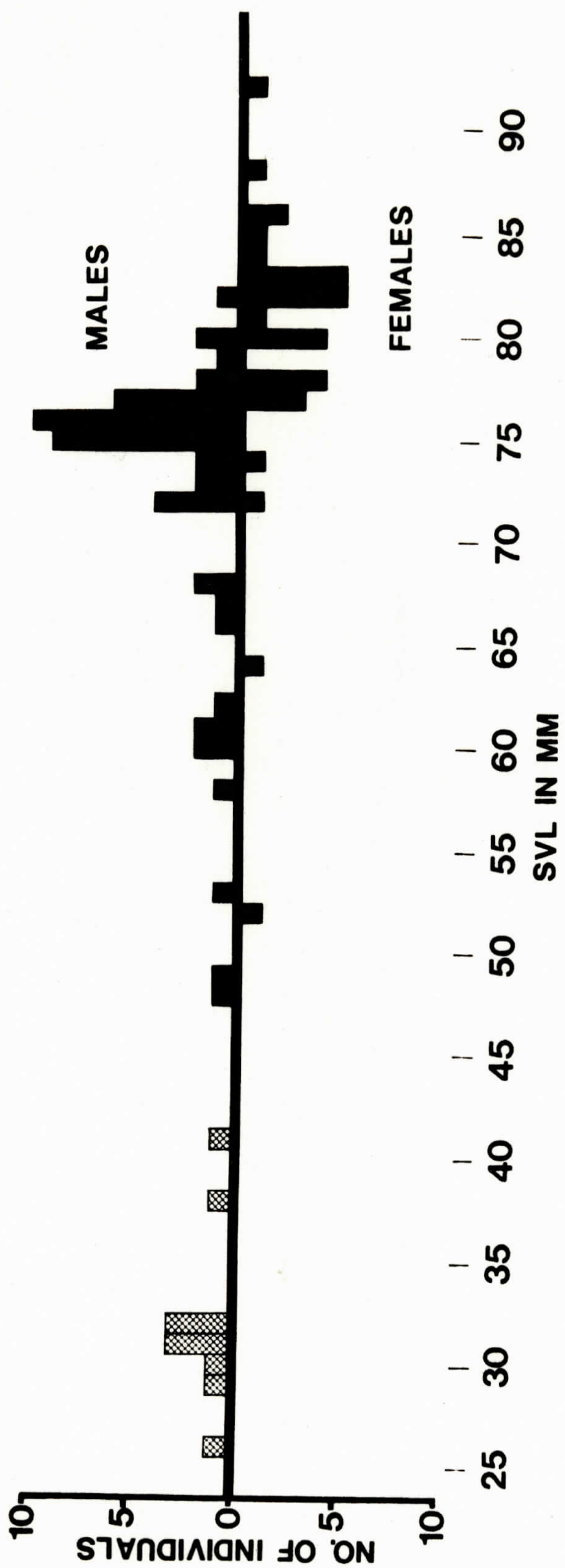
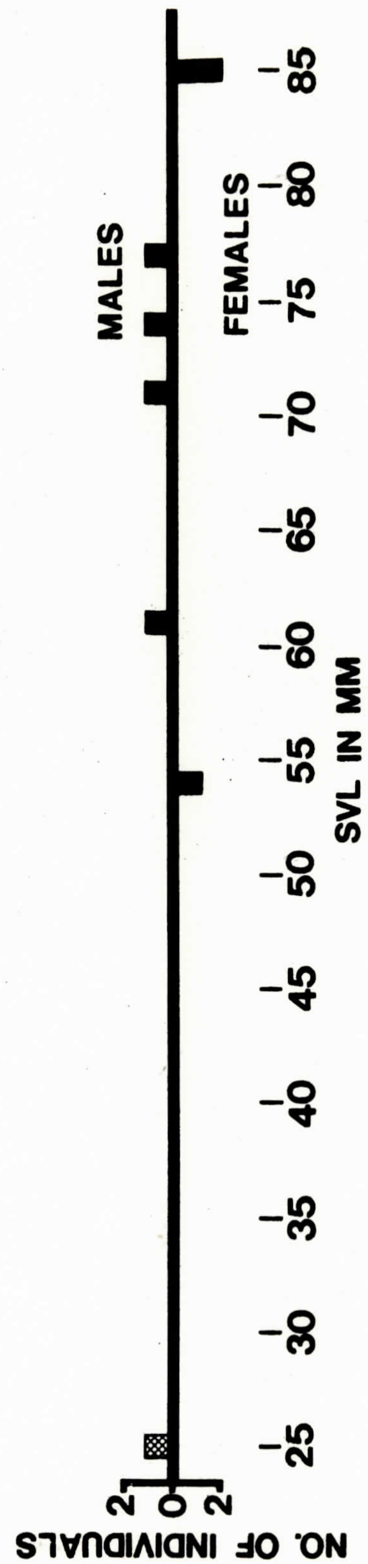


FIGURE 11. Size-frequency distribution for *P. yonahlossee* collected during November 1980 (n=8). Unsexable juveniles are indicated by hatching.



were observed in the other samples but were not so obvious because of smaller sample sizes. When each size-frequency histogram was considered, some size classes were more easily interpreted than were others. It is obvious when observing size-frequency histograms that some size classes can be easily interpreted and some cannot. For more detailed information on interpreting size-frequency histograms, see Bruce (1980).

In the May sample (Figure 5), the only size class of unsexable juveniles ranged in size from 34 to 40mm SVL. A second size class of small salamanders ranged from 55 to 65mm SVL. A third class of males ranged from 70 to 80mm SVL, while the third class of females ranged in size from 70 to 87mm SVL.

The June sample (Figure 6) was similar to the May sample although interpretation was more difficult. The unsexable juveniles (35-50mm) were represented by only three individuals. Five animals between 50 and 55mm SVL may have been a part of this group undergoing sexual differentiation. Animals between 63 and 65mm SVL were probably a second size class while those from 71 to 85mm SVL represented a third class of individuals.

In the July sample (Figure 7), the youngest juveniles (25-34mm SVL and born the previous season) entered the active population for the first time. The second group of animals (36-46mm SVL) was in the second year of growth. Animals from 50 to 65mm SVL represented the third year class while those from 66 to 85mm SVL were a fourth class of older animals.

In the September sample (Figure 9), the two unsexable age classes were less distinct than those in the August sample; however, the sexable male at 43mm SVL and that male in the August sample at 49mm SVL indicated that sex begins to become evident in the second year after hatching. As in previous samples, animals from 49 to about 65mm SVL represented the third age class and those from 67 to 91mm represented a fourth size class.

The first size class of unsexable juveniles in the October sample (Figure 10) ranged from 24 to 32mm SVL. The second size class was represented by two unsexable juveniles from 38-41mm SVL and two sexable males from 48 to 49mm SVL that had probably just undergone differentiation of the gonads. The third class was represented by animals from 52 to 64mm SVL. The fourth class was represented by animals ranging

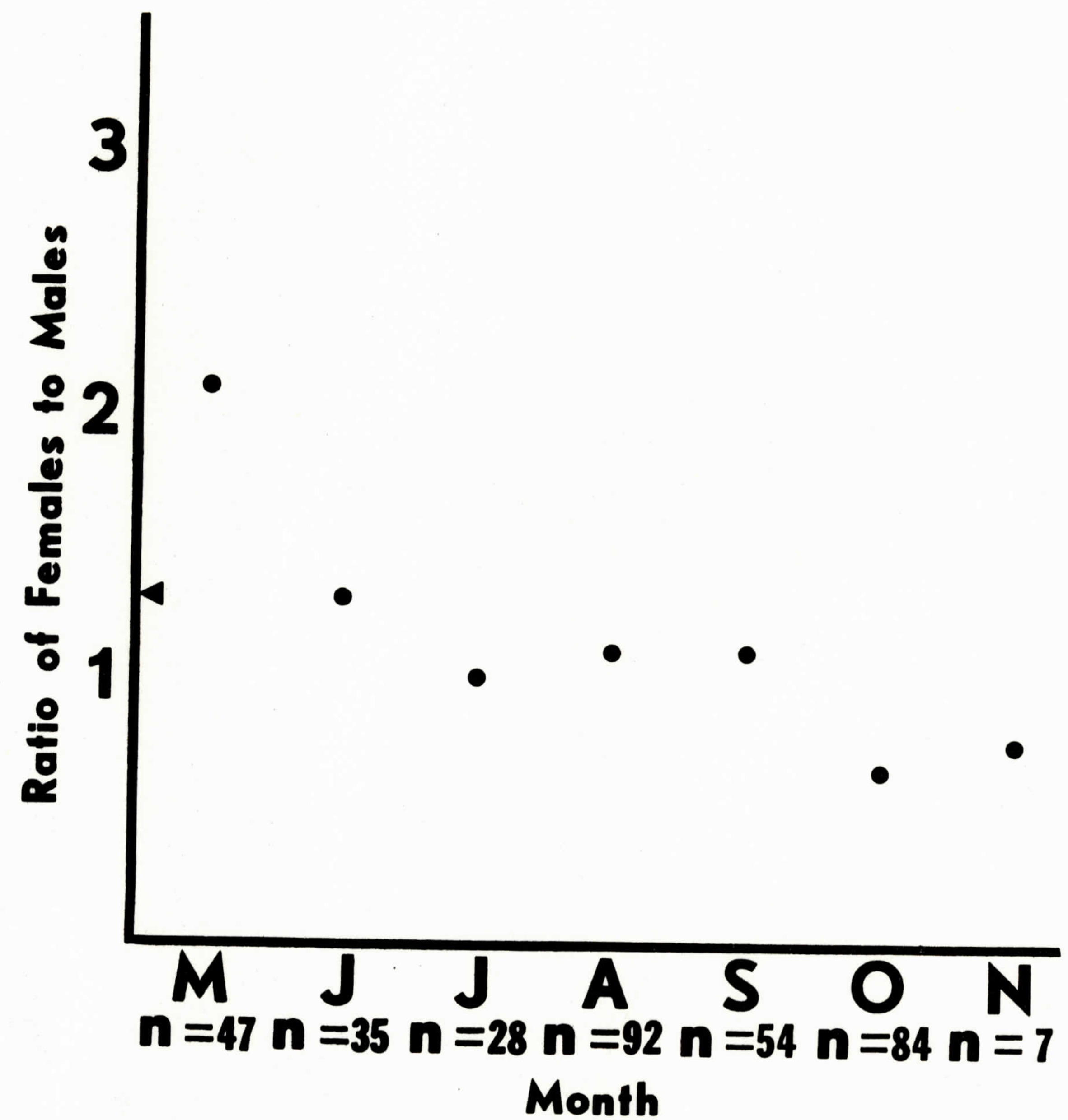
from 66 to 92mm SVL. The size difference between adult males and females was best represented by the October sample. The sample size for November (Figure 11) was so small that no definite conclusions could be drawn.

Gonad differentiation usually occurred between 40 and 50mm SVL, but there was some variation as in any population. In the June sample (Figure 6), there was an unsexable juvenile at 50mm SVL, while there was an obvious male at 43mm SVL in the September sample (Figure 9). Gonad differentiation appeared to occur at about the same size in both sexes.

Sex Ratio and Size Differences

As can be seen in Figure 12, there was a definite pattern in the ratio of females to males from May through November. In May and June, females were more abundant than males. Females were about as common as males from July to September. During October and November, females were less common in the samples. The ratios of females to males were grouped according to the pattern shown in Figure 12 and analyzed statistically using Chi Square. In the May and June samples considered as a group, the 52 females and 30

FIGURE 12. Monthly sex ratios of *P. yonahlossee* collected during 1980. Each point on the graph represents the result of dividing the number of females by the number of males. The arrow indicates the overall ratio of all females to all males for 1980.



males deviated significantly from an expected 1:1 sex ratio ($\chi2(1)=5.9$, $P < .05$). In the July, August and September samples considered as a group, the 89 females and 87 males did not deviate significantly from an expected 1:1 sex ratio ($\chi2(1)=.025$, $P > .05$). The October and November group of 35 females and 56 males did vary significantly from an expected 1:1 sex ratio ($\chi2(1)=4.48$, $P < .05$). The number of females compared with males in the total sample from May through November was as would be expected. Females (n=176) made up 50.7%, while males (n=171) constituted 49.3% of the total sample, excluding unsexable juveniles.

There was a size difference between adult females and males. The ten largest individuals of each sex from the overall 1980 sample were compared statistically using the student's one-tailed t-test ($t(18)=6.35$; $P < 0.001$). Adult females reached significantly larger size than adult males. This size difference was most apparent in the October sample (Figure 10).

Male Reproductive Cycle

Males (n=171) ranged in size from 43-85mm SVL with a mean SVL of 70.3. Males were mature when 65mm SVL or greater (Table 5). A mature male *Plethodon yonahlossee* (70mm SVL) is pictured in Figure 13.

TABLE 5. Body statistics for adult male *Plethodon yonahlossee**

Month	n	Mean SVL(mm)	Mean testis length(mm)	Mean testis weight(mg)	Average testis pigment score(1)	Average vas deferens pigment score (2)	% with convoluted vas deferens	% with sperm in testis	% with sperm in vas deferens	Average mental gland score (3)
May	13	71.8	15.4	38.3	2.4	1.46	77	69	0	.85
June	11	77.0	14.6	43.6	2.1	1.45	100	91	9	.91
July	11	70.9	13.8	34.6	1.8	1.18	100	100	100	1.00
August	38	73.7	12.2	16.2	2.7	1.26	100	97	100	1.87
Sept.	16	72.4	13.9	15.0	2.9	1.56	100	13	94	1.81
Oct.	43	75.0	14.1	18.8	2.6	1.60	98	0	65	1.72
Nov.	4	74.0	13.0	16.0	2.7	2.00	100	0	33	.33

*All males greater than or equal to 65mm SVL.

(1) 1:0: weak pigmentation, 2.0: well pigmented, 3:0: darkly pigmented

(2) 0.0: no pigmentation, 2.0: well pigmented, 2.0: darkly pigmented

(3) 0:0: mental gland not conspicuous, 1.0: mental gland conspicuous, 2.0: mental gland conspicuous and thickened

FIGURE 13. Mature male *P. yonahlossee* collected October 1980. Snout-vent length equals 70 millimeters.

In plethodontid salamanders, the spermatogenic wave begins in the posterior part of the testis and gradually moves forward. Mature spermatozoa are then transported to the vasa deferentia for storage (Noble, 1931). In this study, sperm production was well underway by the first sample in late April and early May when 69% of males showed spermatogenesis (Figure 14). At the same time, vasa deferentia were empty. Percentage of males producing sperm increased to 100% in July, remained high in August, then fell dramatically to 0% in October and November. The pattern of sperm presence in vasa deferentia was similar to that in testes but delayed. Sperm first appeared in vasa deferentia in June at 9%, peaked at 100% in July and August, then gradually declined to 33% in November.

Testis structure correlated well with the presence of sperm in testis smears. Histological sections of testes from May and October are shown in Figure 15. Almost mature spermatozoa were obvious in the seminiferous tubules of a male collected in May 1981 (Figure 15-A). Sperm heads stained darkly while the long tails were thin and clustered. Several seminiferous tubules were seen in a section through

FIGURE 14. Sperm and mental gland cycle. Presence of sperm in the testis and vas deferens and the appearance of the mental gland for all mature *P. yonahlossee* males (SVL equal to or greater than 65mm) collected during 1980 is indicated. Percents for sperm were computed for each monthly sample. Mental Gland Score is the mean of all mature individuals for each month. Scores used were 0 for conspicuous, 1 for conspicuous but not thickened, and 2 for conspicuous and thickened. See also Table 5.

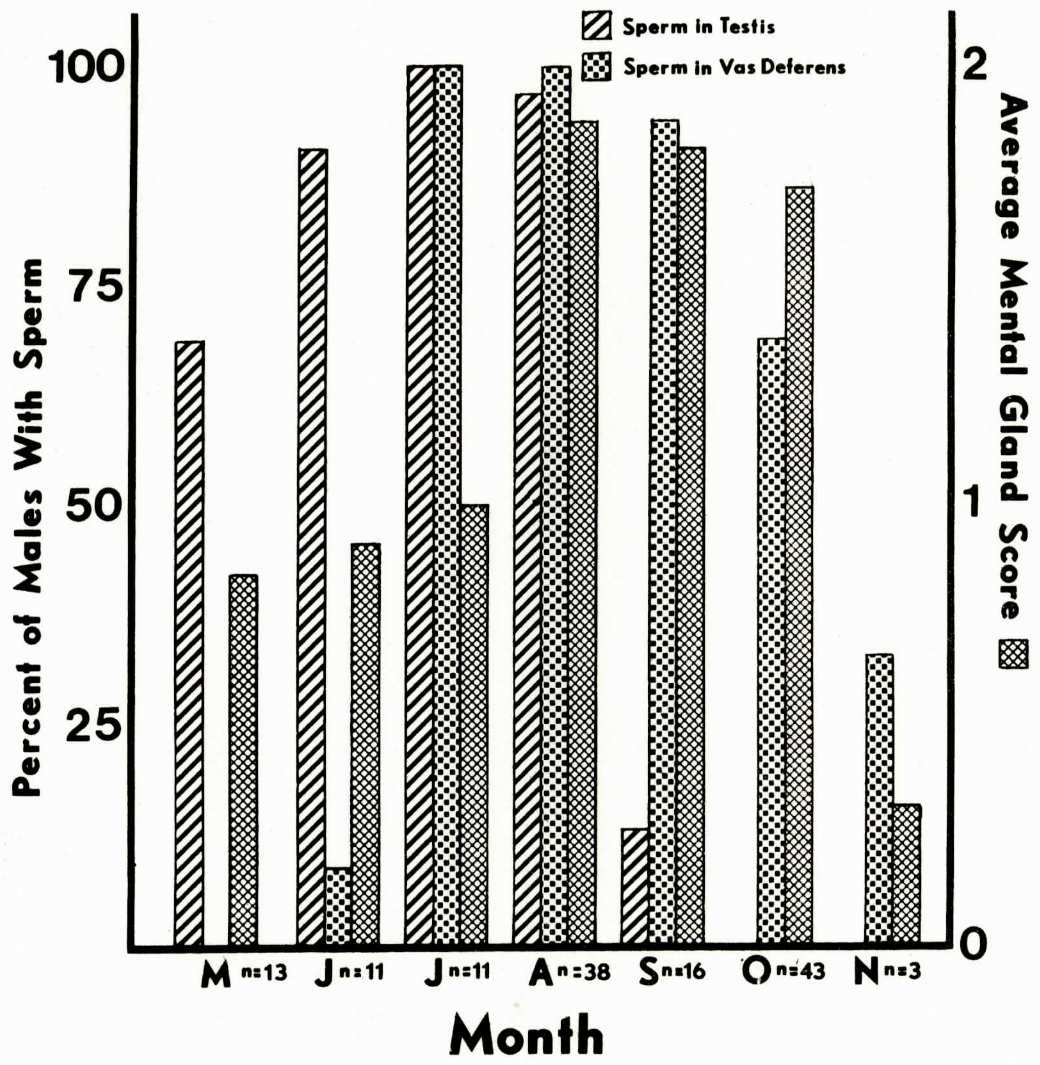
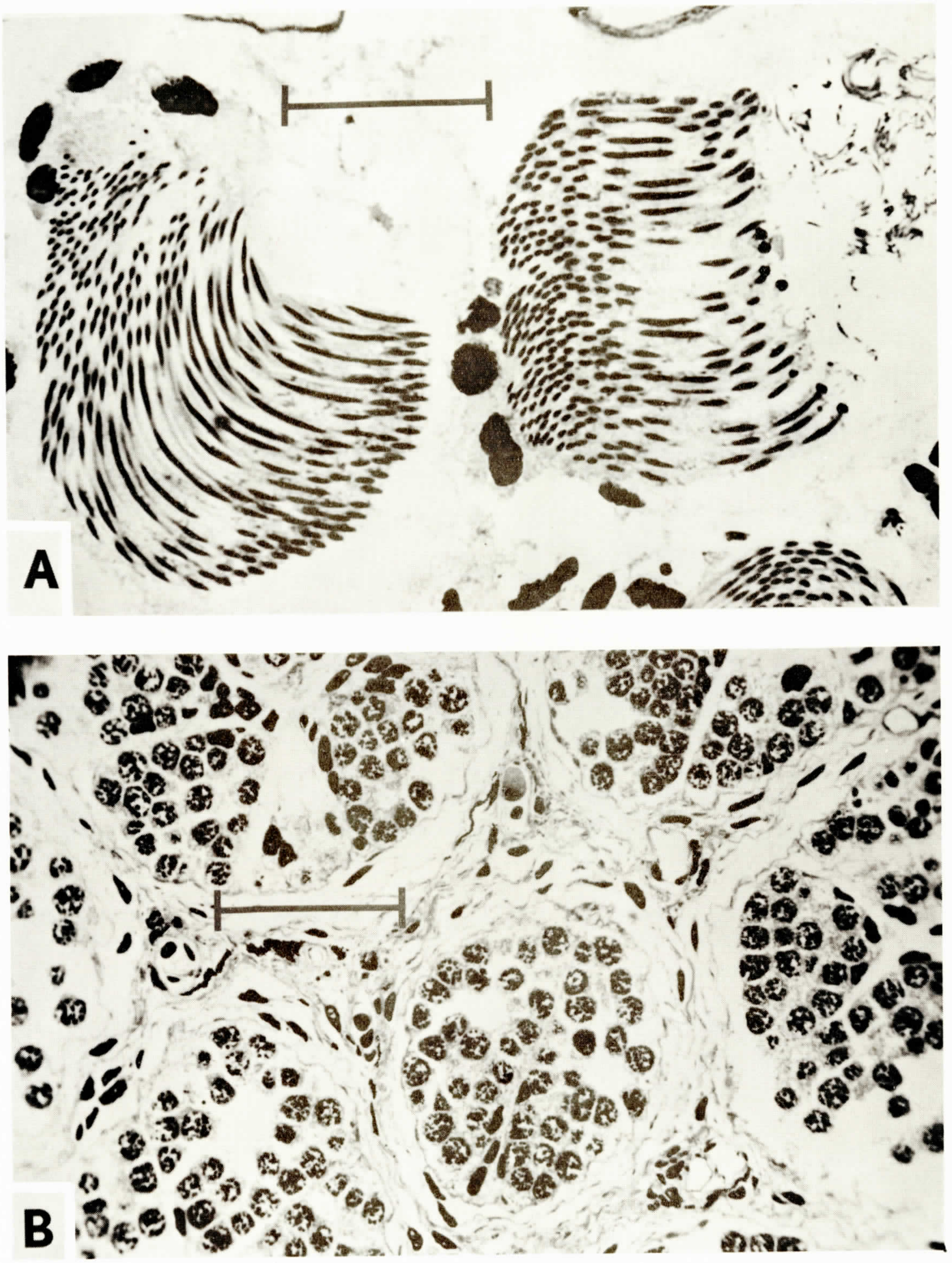


FIGURE 15. Seasonal variation of sperm in the testis of *P. yonahlossee* males.

A. Cross section of testis of male *P. yonahlossee* collected in May 1980. Bar equals 100 microns. Mature sperm can be seen in lumen of seminiferous tubule.

B. Cross section through the testis of a male *P. yonahlossee* collected in October 1980. Bar equals 50 microns. Note lack of mature sperm.



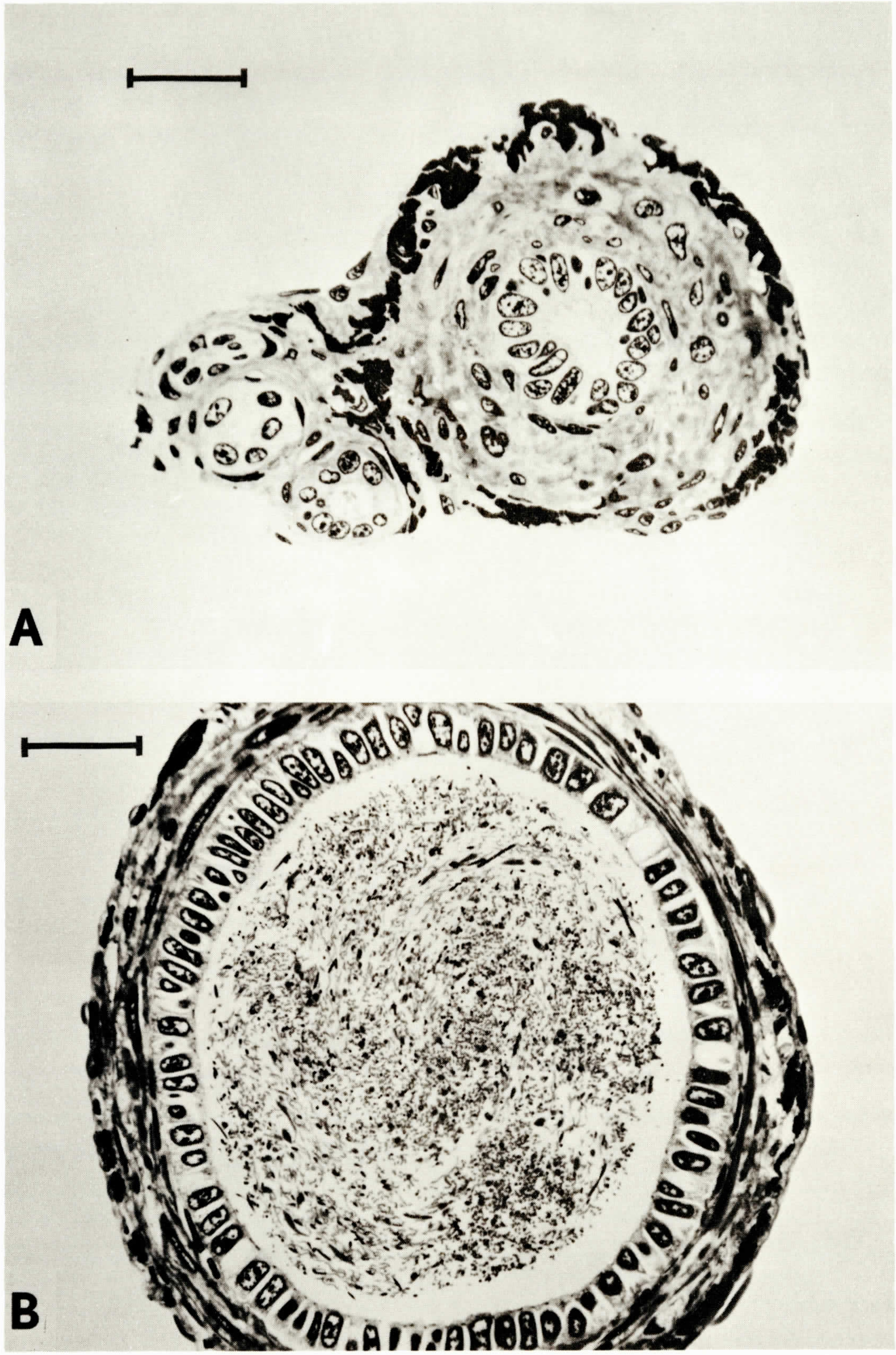
the testis of an October male (Figure 15-A). Each tubule contained clusters of what appeared to be spermatogonia or spermatocytes. There was no evidence of spermatogenesis in this specimen. Sperm in the vas deferens are pictured in Figure 16 for May and August males. Vasa deferentia were empty in May (Figure 16-A) before sperm migrated into them. The walls of the vasa at that time were thickened with few inner epithelial cells and a small lumen devoid of sperm. By October the walls of the vas deferens had expanded greatly and become thinner (Figure 16-B). The epithelial cells lining the interior of the tubule were more numerous due to the large expansion, and the lumen was packed with mature sperm. The vas deferens had increased greatly in size from May to August. In both specimens, melanophores were abundant.

The external appearance of the vas deferens corresponded well with its internal activity over the season. Early in the year, vasa deferentia lacked spermatozoa and were darkly pigmented and small in diameter. From July through October, vasa deferentia were filled with spermatozoa, lightly pigmented and enlarged. There was also a strong relationship

FIGURE 16. Seasonal variation of sperm presence in the vas deferens of mature *P. yonahlossee* males.

A. Cross section through vas deferens of a male collected in May 1980. Bar represents 50 microns. Note the small diameter of the inner canal, the thickness of the surrounding wall, and the absence of sperm in the lumen.

B. Cross section of vas deferens of a male collected in August 1980. Note the large expansion of the lumen and walls of the vas deferens. The lumen is packed with mature sperm. Bar represents 50 microns.



between presence of sperm in the testis and testis weight. Testis weight of mature males increased early in the season, to a maximum in June. After July, testes became markedly smaller and lighter (Table 5).

The mental gland in males was also a good indicator of reproductive activity (Figure 17). Males in May had a smaller mental gland that was not conspicuously thickened (Figure 17-A). In August males had larger and thicker mental glands (Figure 17-B). The naso-labial groove was also enlarged in August. Average Mental Gland Scores increased from May to an August peak score of 1.87 (Figure 14). Several factors, therefore, suggested the peak time for mating activity in males. Mental glands reached their maximal development in August when vasa deferentia were full of sperm. By November, mental glands were smaller and vasa deferentia empty.

Female Reproductive Cycle

A total of 176 identifiable females were collected in 1980. These ranged from 49 to 92mm SVL, with a mean of 72.5 (Figure 18). Examination of oviducts and ovaries suggested that only females equal to or

FIGURE 17. Seasonal variation in the mental gland of *P. yonahlossee* males.

A. Male specimen collected in May 1980. The mental gland is small in diameter and not conspicuously thickened.

B. Male specimen collected in August 1980. Mental gland is large in diameter, thickened and conspicuous. Note also enlargement of naso-labial groove.

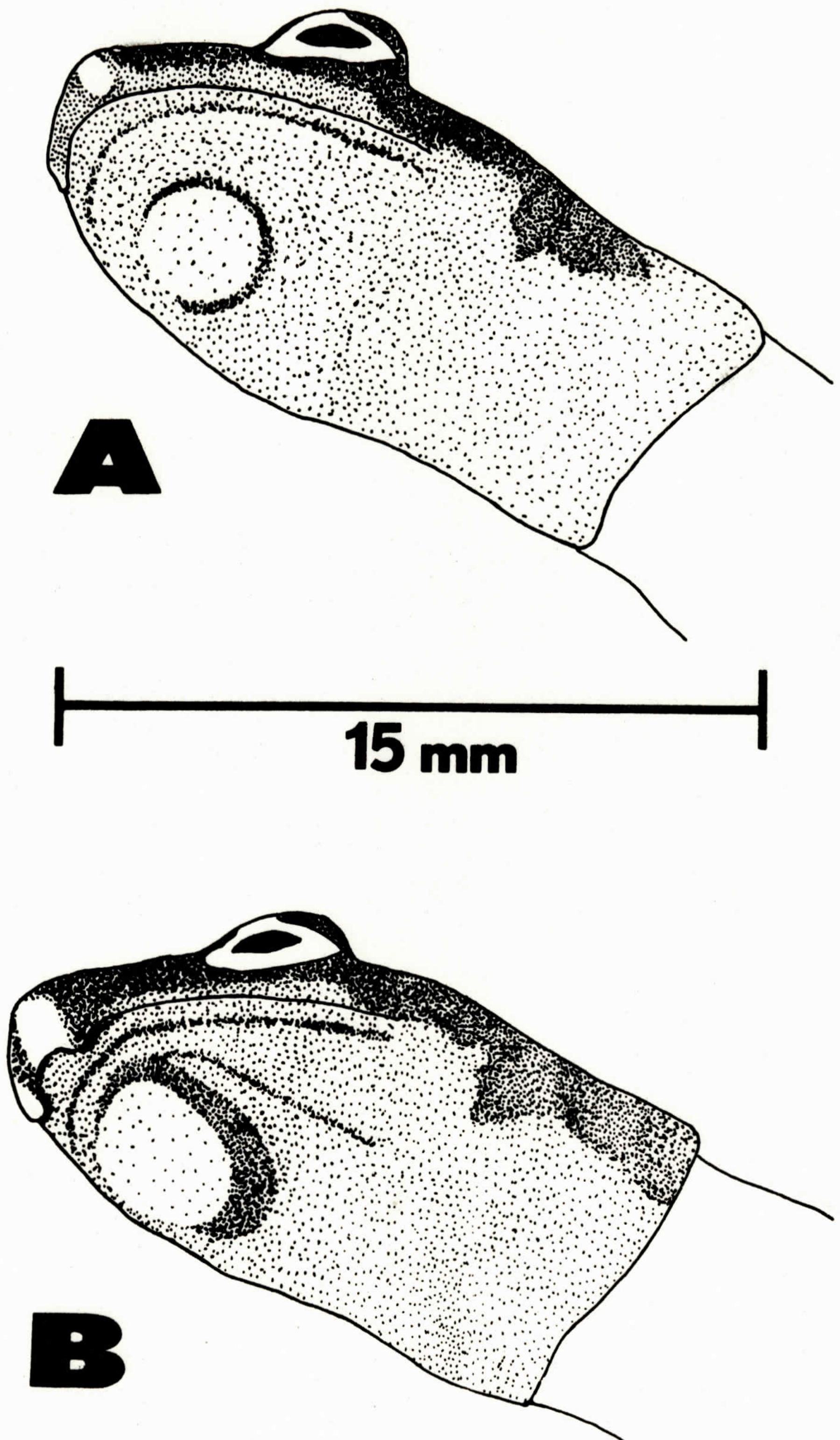
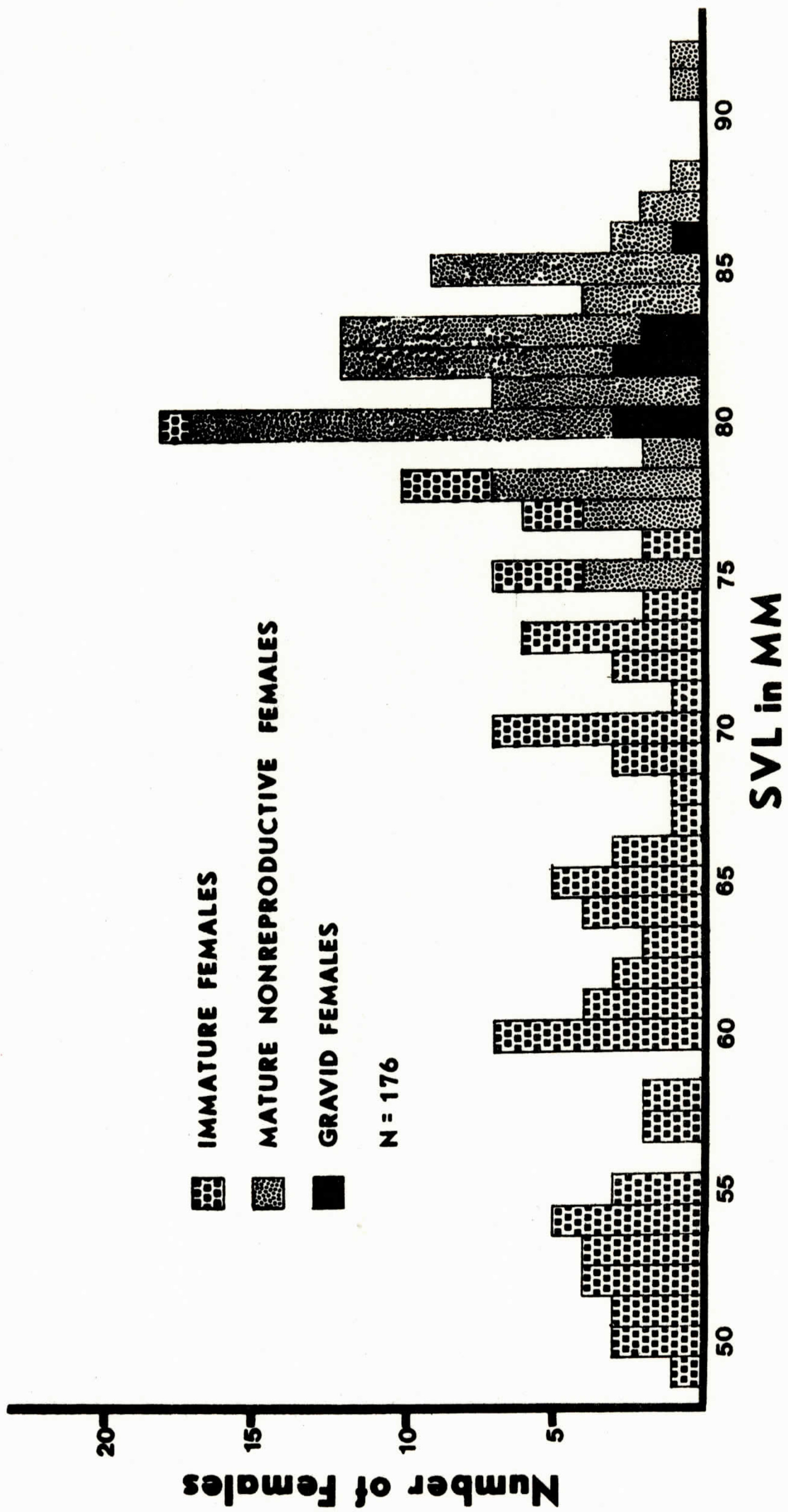


FIGURE 18. Size distribution for *P. yonahlossee* females collected in 1980 (n=176). There were 86 adult females and nine gravid females.



greater than 75mm SVL were mature. Mean SVL for mature females was 81.7mm (n=86). Only nine females were found to be gravid with eggs large enough for deposition in the 1980 season (egg diameter greater than or equal to 2.0mm). An additional specimen, ASU #3070, collected in July 1980 was included with other gravid females in Table 6. All gravid females had SVL greater than or equal to 80mm, and were collected from mid-July through mid-October. Other mature females (n=77) had eggs too small to be deposited in 1980.

After mating, sperm are stored in the female in a modified gland called the spermatheca, which is located in the anterior end of the dorsal roof of the cloaca. For more detailed information concerning female cloacal anatomy, see Sever (1978). Histological examination of spermathecae of gravid females indicated that eight of nine females collected after July had mated. A longitudinal section through the spermatheca of a gravid female collected in July (Figure 19-A) showed no sperm in either the entrance or tubules of the branched spermatheca. Since there was no spermatophore in the cloaca of this female, she had not yet mated. Sections through spermathecae of gravid

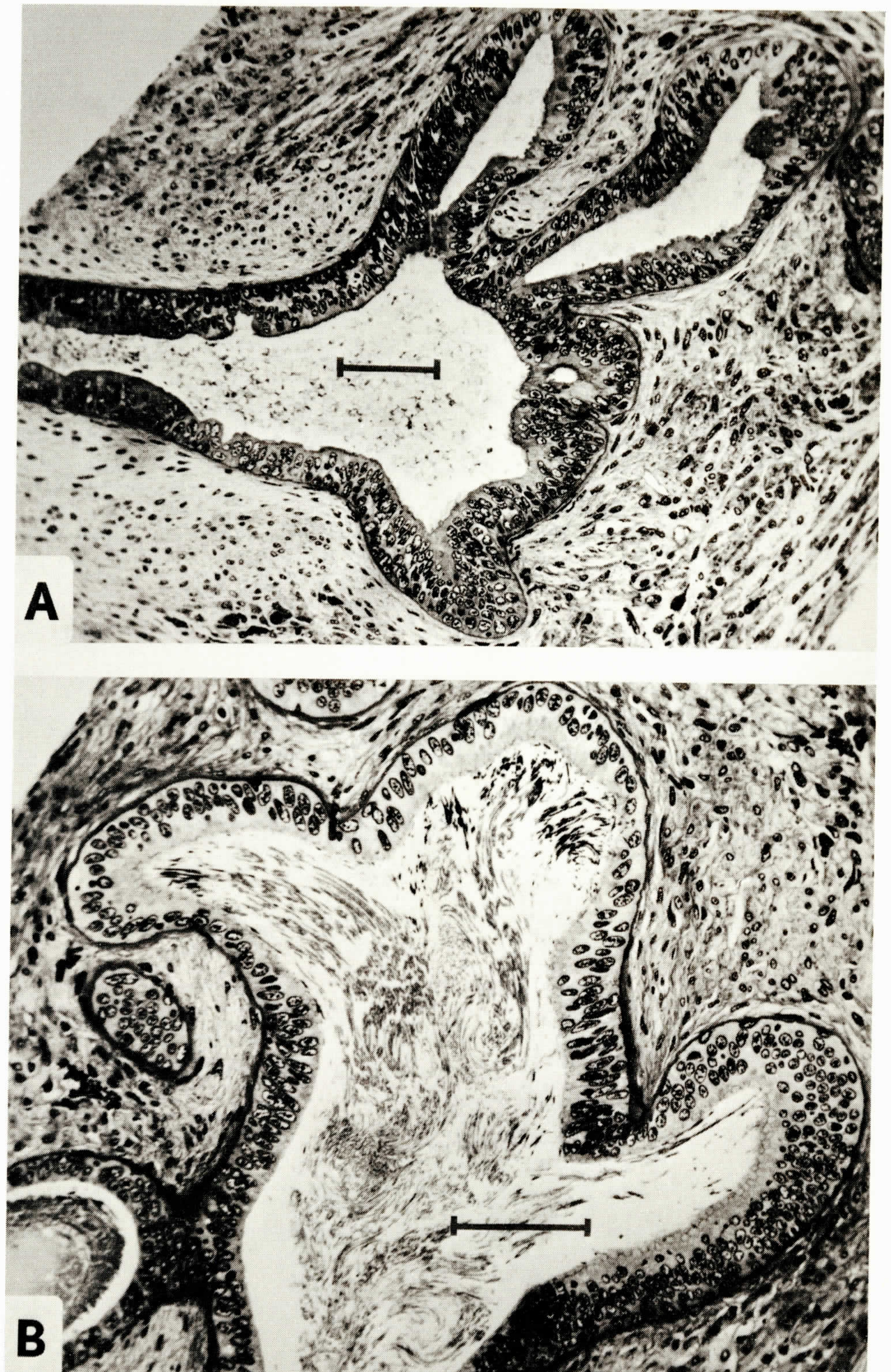
TABLE 6. Summary of data for gravid females

ASU#	Date	SVL	Clutch Size	Mean Egg Size (mm)	Spermatophore	Sperm in spermatheca
3007	13-14 July	82	16	2.0	no	no
5574	16 Aug	82	21	3.0	yes	yes
5604	16 Aug	83	21	2.5	no	no
5661	22 Sept	80	18	4.5	no	yes
5677	22 Sept	83	18	4.0	no	yes
5689	22 Sept	80	17	4.0	yes	yes
5731	17 Oct	82	16	4.0	no	yes
5740	17 Oct	82	19	4.0	no	yes
5742	17 Oct	80	11	3.0	no	yes
5779	17 Oct	86	17	3.0	yes	yes

FIGURE 19. Variation of sperm presence in the spermatheca of *P. yonahlossee* females.

A. Longitudinal section through the spermatheca of a female *P. yonahlossee* collected in July 1980. Bar represents length of 100 microns. Spermathecal duct is lined with a thick layer of epithelial cells. Note the absence of sperm in the neck and arms of the spermatheca.

B. Longitudinal section through the spermatheca of a female *P. yonahlossee* collected in October 1980. Bar represents 100 microns. Note the presence of numerous sperm in the neck and arms of the spermatheca.



females collected in October (Figure 19-B) revealed that both entrance and tubules of the spermatheca were packed with sperm (Figure 20). Mature sperm were oriented in one general direction within the spermathecae heads against the epithelial lining of the tubule. Three gravid females had spermatophores in the cloacal lips as well as sperm in the spermatheca. Non-gravid females did not possess sperm.

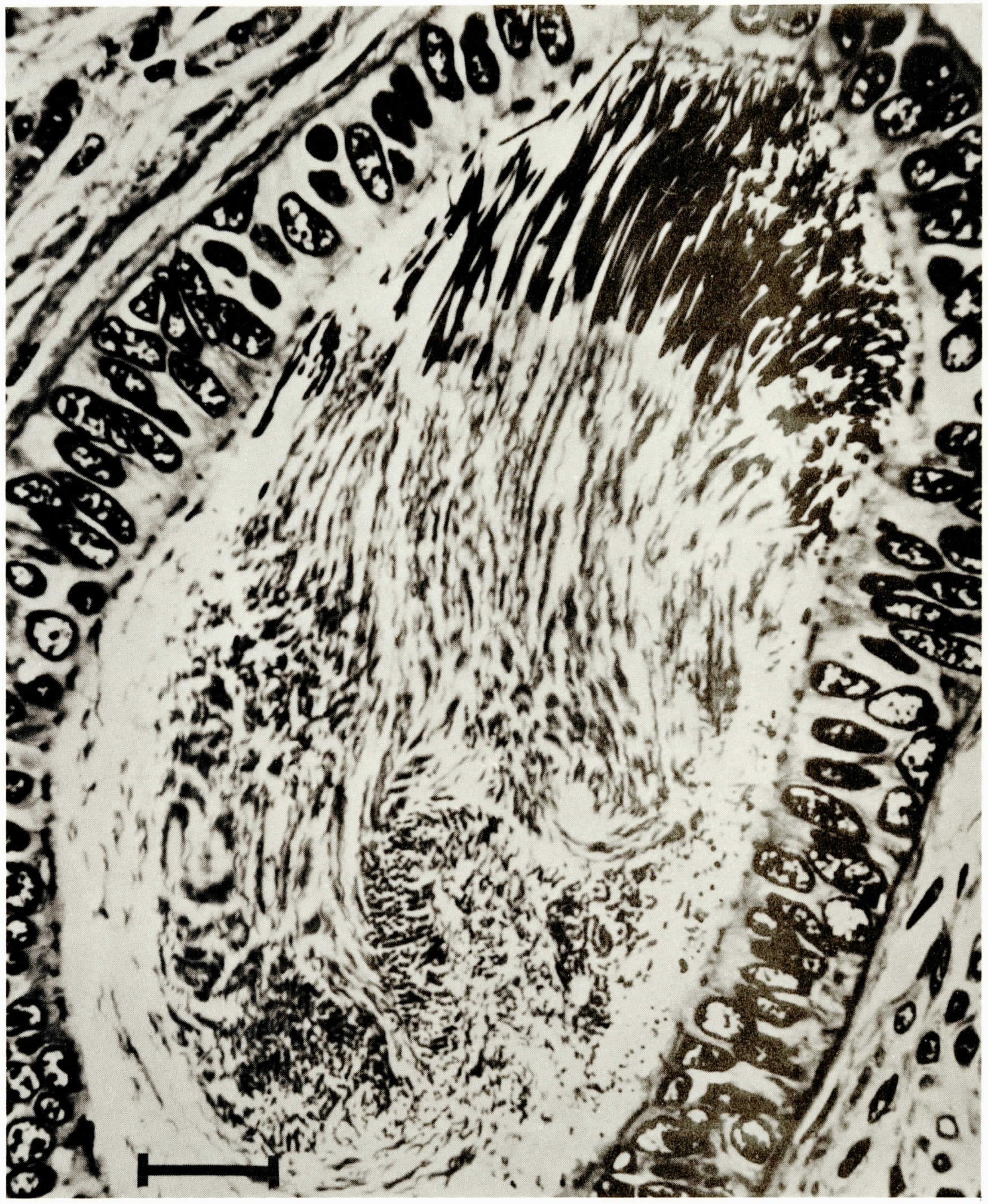
The size range for gravid females during this study was 80-86mm SVL; however, seven females (70-80mm SVL) collected in August, 1981 were all found to be gravid. The average clutch size for 1980 females was 17 with no correlation between clutch size and SVL (Table 5). A spent female (92mm SVL) from a separate collection in May was found to have corpra lutea in the ovaries which indicated the recent laying of eggs.

Mark-Recapture Study

Sampling Area

The study area was located on a south-southwest facing slope atop Rainbow Mountain Overlook (see Table 1 and Appendix A). It started at the crest of the slope at 3,725 feet (1135m) and descended down the incline to an approximate elevation of 3,625 feet (1105m). An exposed rock fault about half way down

FIGURE 20. Enlarged cross section of spermathecal tubule of a female *P. yonahlossee* collected in August 1980. Bar represents 50 microns. Note the orientation of the mature spermatozoa in one direction. The long heads and the frilled, wavy tails can easily be distinguished.



the slope varying from two feet to nine feet in height ran southeast-northwest through the study area. The slope was relatively dry and covered by a second-growth forest (Figure 21) characterized by red oak (*Quercus rubra)*, chestnut oak (*Quercus prinus)*, red maple (*Acer rubrum)*, flowering dogwood (*Cornus florida)*, striped maple (*Acer pennsylvanica)*, and hop hornbeam (*Ostrya virginiana)*, in order of importance value (Appendix C). The study area illustrated in Figure 22 was littered with chestnut logs and stumps. These stumps and logs (Figure 23) provided favorable habitat for *Plethodon yonahlossee* and indicated an earlier Oak-Chestnut association. This has been replaced by an Oak-Maple association.

A diverse herbaceous layer (Appendix D) covered most of the forest floor during the summer months. The northern corner of the study area was covered by a dense carpet of galax. Big rhododendron, (*Rhododendron maximum)* was scattered throughout with two dense thickets in squares K and S. The chestnut logs and stumps (many well-rotted), the rock fault with its accumulated talus, the herbaceous layer, the Rhododendron thickets, and the shady overstory provided a variety of habitats suitable for a salamander population.

FIGURE 21. Winter aspect of population sampling site, Rainbow Mountain Overlook. Elevation is 3,625 feet (1,105m). Observer is facing northwest. See also Table 1, Appendix A.

FIGURE 22. Population study area, Rainbow Mountain Overlook, Watauga County, North Carolina. Each side is 50 meters. Total area is approximately 2,500 square meters. Elongated structures are logs, open circles are largest trees or large stumps. Shaded areas are scattered rocks and larger shaded areas are rock fault. Rhododendron thickets are 6-9 meters in diameter. Points indicate captured sites for marked individuals. Star in Square B is an individual that was marked and recaptured in same location. Star and arrow in Square F indicates a recaptured individual that had moved approximately one meter.

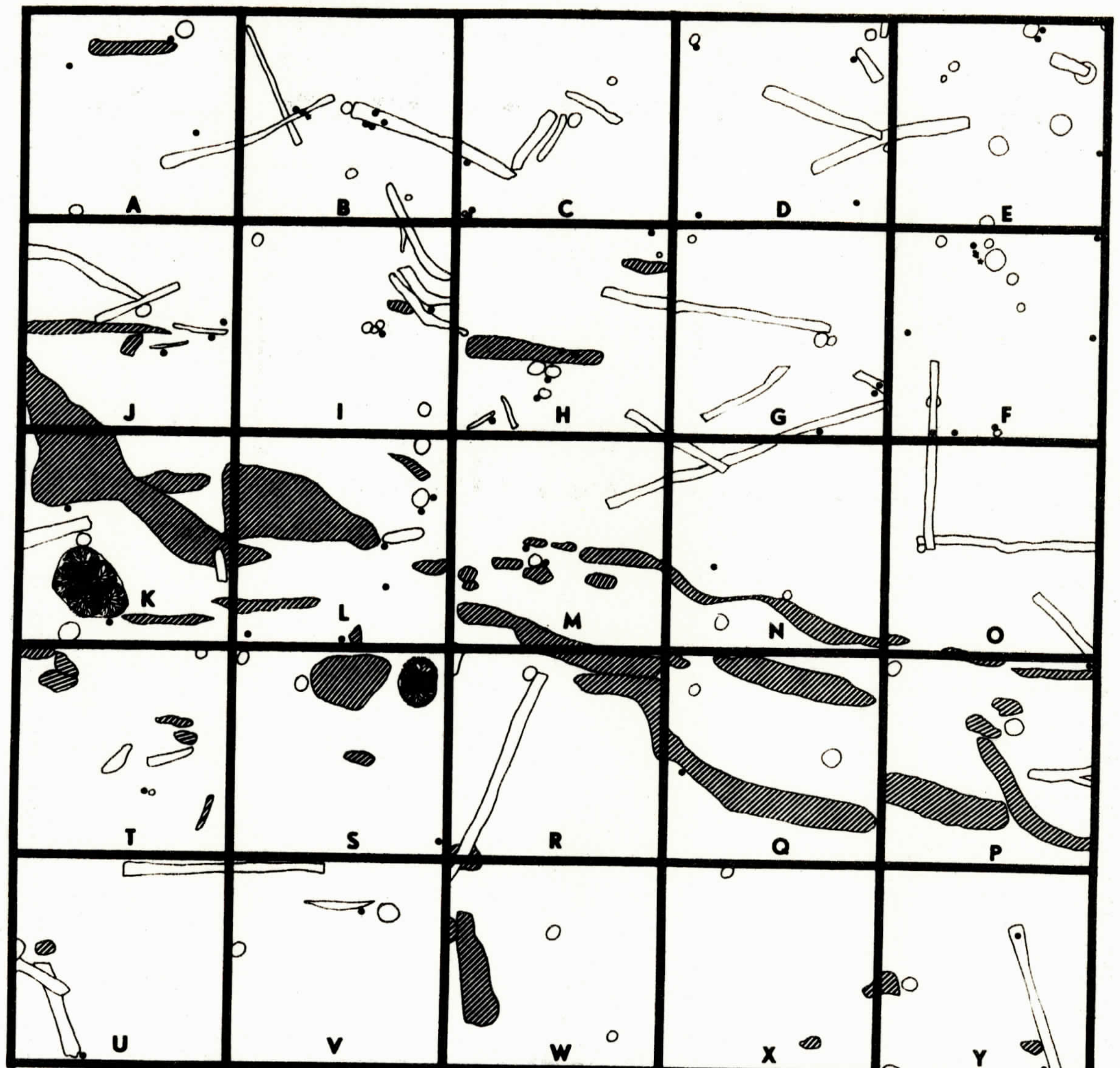


FIGURE 23. Winter aspect of Rainbow Mountain Overlook showing typical microhabitat of *P. yonahlossee*. Typically, several individuals will be found in burrows at the base of a chestnut stump such as this one.

The Population

Data were obtained from three population samples (Table 7). Schnabel estimates for population size were 704 individuals in sample two and 424 in sample three. The mean of these estimates was 564. Since there was only one recapture in each sampling interval, the estimates were only indicative. The location of the two recaptured individuals (Figure 21) indicated very little movement over a period of 2-3 months. However, one animal marked in square E (Figure 22) was found more than five meters away after only a few days. The population density estimate for *Plethodon yonahlossee* for the first sample was 704 individuals per 2,500 square meters or one individual per every 3.6 square meters and 2,778 animals per hectare. For the third sampling period, the density estimate was 424 individuals per 2,500 square meters or one individual every 5.9 square meters and 1,695 animals per hectare. The mean estimate indicated a density of 564 individuals per 2,500 square meters or one individual per every 4.4 square meters. This was equivalent to 2,272 salamanders per hectare.

TABLE 7. Population Sampling Data for *P. yonahlossee*

Sample #	Sample Period	# Captured	# Marked	Recaptures	Cum. Recaptures	Cum. Marked	Unmarked Juveniles	Missed	*P. jordani*(1)	*P. glutonosis*(1)	*D. ocrophaeus*(1)
1	22 July-3 August	32	32	0	0	32	4	7	87	11	8
2	26 August-8 September	22	21	1	1	53	6	5	217	24	7
3	26 September-16 October	8	7	1	2	60	2	4	68	12	2

(1) Numbers shown are estimates based on a running tally kept during each collecting session for each species

The small number of animals recaptured and their lack of activity prevented calculation of home ranges. Several other salamanders were also seen in the study area: *Desmognathus ochrophaeus*, *Plethodon richmondi*, *P. glutinosus* and *P. jordani*. A tally of the three most common species seen was kept during the mark-recapture study (Table 7). In general, *P. jordani* was more common than all other species, including *P. yonahlossee*, with approximately 372 individuals seen within the study area. *P. jordani* appeared to be the most active salamander on the forest floor in the early part of the evening, while *P. yonahlossee* usually did not appear until well after 10:30 p.m. E.S.T.

DISCUSSION

For an understanding of the biology of *Plethodon yonahlossee* it is important to integrate as many aspects of its life history as possible, especially since so many areas of its existence are still unknown. In this discussion, the following subjects will be covered: habitats utilized by *P. yonahlossee;* the life cycle of *P. yonahlossee* including egg laying, size and age at maturity covering when juveniles enter the population sample, juvenile age classes and differentiation of the gonads; sex ratios; adult reproductive cycle including reproductive potential; and finally, population estimates. This material will then be compared with previously available literature. Suggestions for further study will be covered, and finally, a point by point summary of the information gathered in this paper will be presented.

Habitat Utilization

During the course of this study, *Plethodon yonahlossee* was found in a variety of habitats. Collecting sites 1 and 3 (Figure 1, Table 1) are good examples of *P. yonahlossee* habitats.

Site 1 was located on a north-facing slope well below the ridge top, with several deep coves, each containing a stream, while site 3 was a south-southwest-facing slope near the ridgetop, with the nearest stream several hundred yards away. Both areas, however, did have one thing in common, the presence of assorted stumps and logs, especially those of the american chestnut, *Castanea dentanta.*

When the population study area at site 3 was first surveyed in April, all *Plethodon yonahlossee* were discovered under rocks in direct contact with mineral soil. The first specimen at site 1 was also found in late April under a rock. By July, most animals found during the mark-recapture survey were on or near large logs and stumps (Figures 22 and 23). This concentration of individuals near logs may have resulted from poor and inconsistent rainfall during 1980 (Figure 4). It became so dry in summer and early fall at site 3 that many plants in the herbaceous layer wilted. The topsoil layer was only 4-6 inches deep with bedrock below.

This study has also shown that *Plethodon yonahlossee* is quite active and is not restricted to movement on the ground. It can be found high above the ground in rock crevices as was discovered several times at site 1.

Plethodon yonahlossee probably spends the winter months in deep underground rock areas where the temperature remains well above the freezing point and the humidity is near 100%. As spring approaches, individuals make their way to the surface and remain close to burrows under rocks until stable weather conditions permit safe movement on the ground. As summer approaches, they take up residence in and near logs and stumps (especially during periods of drought), where maximum protection against dessication is afforded and food is readily available. Later in the season they apparently return to their subterranean retreats.

Moisture requirements associated with logs and stumps may be more important to *Plethodon yonahlossee* than to other closely associated and related species such as *P. glutinosus* or *P. jordani*. The close proximity of *P. yonahlossee* to lengthy burrows would not only provide a quick escape route but also allow individuals to choose the optimum moisture conditions they require by simple movement from one place to another.

Too much moisture can be as detrimental to *Plethodon yonahlossee* as too little moisture. Captured individuals kept too wet were inclined to form blisters or pustules on the skin. Observations made during the course of this study also showed that during heavy rainfall at night, active *P. yonahlossee* sought cover from direct rainfall.

Life Cycle

Although it is difficult to say when females lay eggs it is proposed here that *Plethodon yonahlossee* eggs are deposited sometime between late fall and early spring. The spent female found in May with corpra lutea in the ovaries helps to formulate a better idea of when eggs are deposited. The presence of corpra lutea in ovaries devoid of eggs indicates the recent exit of eggs from the animal. If *P. yonahlossee* females stay with their eggs until hatching, as do other members of the genus, this female must have deposited her eggs well before March. Eggs are probably deposited in areas similar to the hibernation areas mentioned above.

Two distinct size classes of unsexable juveniles were found in samples collected after June. The size class (22-35mm SVL) contains the youngest individuals which are in their first year of growth. These individuals are presumably the hatchlings of late 1979-early 1980. The second size class of young salamanders (35-50mm SVL) are in their second year of growth and are hatchlings of late 1978-early 1979. These larger individuals undergo sexual differentiation near the end of their second year of growth at about 50mm SVL.

Individuals between 50 and 65mm SVL comprise a less distinct size class of juveniles in their third year of life, and are identifiable by sex. Animals above 65mm SVL are generally considered mature, and are four or more years old.

It is concluded, therefore, that *Plethodon yonahlossee* first enters the sampled population during the latter months of its first year of life. It then undergoes sexual differentiation at the end of its second year, reaches maturity after three years of growth, and begins reproduction in the following year of life.

The minimum size for maturity in males is 65mm SVL. The data on sexual maturity in females indicated that the minimum size for maturity was 75mm SVL in this study. However, seven gravid females collected

in August, 1981 indicated that the minimum observed size for mature female *P. yonahlossee* was 70mm SVL. There seems to be year to year size differences in gravid females.

A significant size difference was found in maximum size between males and females of *P. yonahlossee*. Adult males were smaller than adult females. The overall ratio of females to males in this study showed equal representation of the sexes in the samples.

There is a strong seasonal shift in the ratio of the sexes with females common early in the season and becoming uncommon after September. This pattern seems to reflect activity differences between the sexes. Females might be more actively foraging in the spring, especially if they have been with eggs all winter. As the mating season approaches, males and females would be equally common in the population. If the females lay eggs very late in the season, as is suspected, their numbers should be lower later in the fall. Females would drop out of the active population to search for ovoposition sites.

The spermatogenic wave of male *Plethodon yonahlossee* is well under way by late April. Testis weight increases through June and decreases thereafter. Maximum sperm production early in the season would account for the large testis weight. With the large amount of sperm being produced, seminiferous tubules would be filled to capacity. As the season progresses, sperm migrate from the testes into the vasa deferentia causing the overall weight of the testis to decrease until they are empty of mature sperm late in the season.

Sperm begin migration into the vasa deferentia in June. The vasa deferentia are packed with sperm from July through October. The external appearance of the vas deferens indicates its internal activity. When vasa deferentia are full of sperm, they are thicker and less darkly pigmented. The presence of sperm in the vas deferens correlates well with the appearance of the mental gland, which is also conspicuous and enlarged from August through October.

The 176 *P. yonahlossee* females collected during 1980 present a confusing picture of the female reproductive cycle. Because of the low number of gravid females collected in 1980, it is suggested that at least two years exist between successive egg clutches in *P. yonahlossee*. The year prior to this study, 1979, was a very wet year in Boone, North Carolina. If reproduction by females corresponds to favorable (i.e. wet) weather

conditions, a majority of females may have mated and deposited eggs in 1979. This phenomenon would be expressed in dry 1980 as a large number of spent females. Female reproductive potential is no doubt related to several factors including present weather conditions, the previous year's weather, present and past food supply, and population density.

There seems to be no relationship between the size of females and their egg clutch size. Mating takes place from August through October.

Population Estimates

The small number of samples taken during the population analysis makes it difficult to interpret the data. Average density of *Plethodon yonahlossee* computed from the two recapture periods was 2,272 animals per hectare. The samples could have been more comprehensive, but there were few wet nights during the sampling intervals. Work on dry evenings resulted in few captures.

Regardless of the number of *Plethodon yonahlossee* found during the course of the population sample, large numbers of *P. jordani* were always seen during collecting sessions, even during the driest months (Table 6). It seemed that *P. jordani* was less sensitive to changing moisture conditions than was *P. yonahlossee*.

The two individuals recaptured had moved one meter or less from where they were marked. This might suggest that *Plethodon yonahlossee* has a rather limited home range, but collections made for the destructive sample indicate otherwise. Many individuals of *P. yonahlossee* of all sizes and sexes were commonly collected in the Boone vicinity during wet rainy nights on paved roads and highways. They were obviously far from burrows and other refugia. During drought or other unfavorable conditions, individuals do not move about. For example, during several nights for a month, an attempt was made to remove a single individual from its burrow next to a stump with no success. The animal was never observed to extend anything more than its nose out of its burrow. Presumably, it had not moved about to feed since it snapped several times at a moving blade of grass which was being used to coax it out of its hole.

Comparison With Other Literature

The variety of habitats where *Plethodon yonahlossee* was collected are consistent with those described by Pope (1950) and Gordon et al. (1962). The relationship between *P. yonahlossee* and the american chestnut

(Castanea dentata), was commonly observed in this study. This observation agreed with Gordon et al. (1962) and seemed to be important to the ecology of *P. yonahlossee*. Pope (1950) reported commonly finding *P. yonahlossee* under rocks. This occurrence was not common during the course of this study. The difference could be due to variant weather conditions at the time of collecting. In this study, *P. yonahlossee* was commonly found several hundred yards away from the nearest stream. This observation, of course, conflicted with Hairston's (1949) statement concerning the association of *P. yonahlossee* with streams. Gordon et al. (1962) remarked on the harmful effects of excessive moisture on captive *P. yonahlossee*. Similar observations were made during the course of this study.

Plethodon yonahlossee neonates first enter the sampled population between July and November. Highton (1956, 1962a) reported that the smallest young of *P. glutinosus* enter the population in Maryland and Pennsylvania in the spring, while those in Florida were first discovered at the end of December. Angle (1969) reported that the smallest young of *P. richmondi* were first collected in March and April in Maryland and Pennsylvania, while Sayler (1966) indicated that the young of *P. cinereus* enter the population between September and November.

Plethodon yonahlossee was found in this study to mature after three years of growth, with reproduction possible in its fourth year. Pope (1950) also concluded that *P. yonahlossee* matured in three years. Highton (1962a) determined that *P. glutinosus* in Maryland, Pennsylvania, and southwestern Virginia, mature in four years, with breeding probably taking place in the fifth year.

Pope (1950) reported a minimum size for mature male *Plethodon yonahlossee* of 56mm SVL. The criterion for making that determination was the number of cloacal gland papillae. The present study, using several other indicators, concluded that minimum size at maturity for *P. yonahlossee* males is 65mm SVL. However, Pope's measurement of SVL was different from this study. Individuals in this study were measured to the posterior angle of the vent, while measurement in Pope's research was taken to the anterior angle of the vent. This difference would result in SVL measurements two to five mm smaller than those used in the present study.

Work in the Boone vicinity has shown a significant difference in size between mature males and females. Pope (1950) found a small difference between male and female size for *P. yonahlossee*. Saylor (1966) found no significant difference between the length of male and female *P. cinereus*.

The overall ratio between the sexes was found to be 1:1. Pope's (1950) study did not show this relationship possibly because of the smaller number of individuals collected and the fact that they were all sampled in July and August. He did argue, however, that the scarcity of females late in the season might be due to their guarding of eggs.

Pope (1950) reported a good spermatogenic wave in the anterior third of the testis of a *Plethodon yonahlossee* male collected in late May. His findings are compatible with those in this study. The spermatogenic wave through the testis has been noted in several other members of the genus (Highton 1956, 1962a; Sayler 1966; Angle 1969) and occurs essentially in much the same way as in *P. yonahlossee*.

The mental gland as reported in the present study was most conspicuous from August through October. Pope (1950) found conspicuous mental glands in male *P. yonahlossee* during mid-August and suggested that mating activity was occurring.

Highton (1962a), Sayler (1966), and Angle (1969) each suggested that females of northeastern *P. glutinosus*, *P. cinereus*, and *P. richmondi*, respectively, practice a two year egg-laying cycle with eggs deposited every other year. Sayler (1966) pointed out that, given the alternate year cycle, about half of the females would be expected to deposit eggs each year. This study found a very low number of gravid females much less than half of the female population, suggesting at least 2 years between egg clutches in mature females.

This study indicated that there was no relationship between egg clutch size and adult female body size. Pope's (1950) data was similar to that found here with clutch sizes in three gravid *P. yonahlossee* females (77-78mm SVL) to be 27, 19, and 24.

Results showed that population density of *Plethodon yonahlossee* at site 3 was 2,272 individuals per hectare. No other estimates for population size and density for *P. yonahlossee* are known. Merchant (1972) followed populations of *P. jordani* and *P. glutinosus* for three seasons in the Great Smoky Mountains National Park. He found population densities for *P. jordani* to be 8,621 animals per hectare and for *P. glutinosus* to be 2,325 animals per hectare.

Suggestions For Further Study

Much more attention should be given to *Plethodon yonahlossee*. Long term studies involving as many consecutive monthly samples as possible need to be

done to refute or confirm the data presented here. There appears to be several populations in Watauga County worthy of further study.

It is also suggested that a population estimation study to be carried out on *P. yonahlossee* over at least a two year period to acquire better information about the activity of that population. This study should begin as early in the spring as possible.

It is hoped that this fascinating species will be closely studied so that more aspects of its biology can be learned.

Summary

The following statements were suggested by this study:

1. Eggs of *Plethodon yonahlossee* are probably deposited between late fall and early spring.

2. Youngest individuals first enter the surface population from July through November in the latter half of their first year of life.

3. Sexual differentiation takes place at the end of the second year.

4. Maturity is reached after three years of growth and reproduction can begin in the fourth year of life.

5. The minimum size for maturity in males is 65mm SVL.

6. The known minimum size for maturity in females is 70mm SVL.

7. Adult males are statistically smaller than adult females.

8. The overall ratio of females to males in this study shows equal representation of both sexes.

9. There is a definite seasonal pattern in the ratio of the sexes with females most common in spring and becoming uncommon after September. The pattern seems to be related to activity differences between the sexes.

10. Spermatogenesis occurs at least from late April through August.

11. Testis weight changes seasonally and is probably related to sperm production.

12. Sperm begin migration into the vasa deferentia in June, and the vasa deferentia are packed with sperm from July through October.

13. The mental gland in males is most conspicuous from August through October and corresponds well with the presence of sperm in the vas deferens.

14. At least two years probably pass between successive egg clutches.

15. No relationship exists between female body size and clutch size.

16. Presence of sperm in gravid females indicated that mating takes place from August through October.

17. Population estimates from one location for *P. yonahlossee* indicated a population density of 2,272 animals per hectare.

LITERATURE CITED

LITERATURE CITED

Adler, K. K. and D. M. Dennis. 1962. *Plethodon longicrus*, a new salamander (Amphibia: Plethodontidae) from North Carolina. Spec. Publ. Ohio Herpetol. Soc. 4: 1-14.

Angle, J. P. 1969. The reproductive cycle of the northern ravine salamander, *Plethodon richmondi richmondi*, in the valley and ridge province of Pennsylvania and Maryland. J. Wash. Acad. Sci. 59: 192-202.

Bailey, J. R. 1937. Notes on plethodont salamanders of the southeastern United States. Occ. Papers Mus. Zool. Univ. Mich. 364: 1-10.

Behler, J. L. and F. W. King. 1979. The Audubon Society field guide to North American reptiles and amphibians. Alfred A Knopf, Inc. New York.

Bruce, R. C. 1980. A model of the larval period of the spring salamander, *Gyrinophilus porphyriticus*, based on size-frequency distributions. Herpetologica 36: 78-86.

Cocke, E. C. 1974. Trees and shrubs of North Carolina. Edwards Bros. Ann Arbor.

Dixon, W. J. and M. B. Brown (eds.). 1979. BMDP Biomedical computer programs, P-series manual. Univ. of California Press. Berkeley.

Dunn, E. R. 1917. Reptile and amphibian collections from the North Carolina mountains with special reference to salamanders. Bull. Amer. Mus. Nat. Hist. 37: 593-634.

_____. 1926. The salamanders of the family Plethodontidae. Smith College 50th Aniv. Publ., Northampton, Mass.

Gordon, R. E., J. A. MacMahon, and D. B. Wake. 1962. Relative abundance, microhabitat and behavior of some southern Appalachian salamanders. Zoologica 47: 9-14.

Guttman, S. I., A. A. Karlin, and G. M. Labanick. 1978. A biochemical and morphological analysis of the relationship between *Plethodon longicrus* and *Plethodon yonahlossee* (Amphibia, Urodela, Plethodontidae). J. Herpetol. 12: 445-454.

Hairston, N. G. 1949. The local distribution and ecology of the plethodontid salamanders of the southern Appalachians. Ecol. Monogr. 19: 47-73.

_____ and C. H. Pope. 1948. Geographic variation and speciation in Appalachian salamanders (*Plethodon jordani* group). Evolution 3: 266-278.

Highton, R. 1956. The life history of the slimy salamander, *Plethodon glutinosus* in Florida. Copeia 1956: 75-93.

_____. 1962a. Geographic variation in the life history of the slimy salamander. Copeia 1962: 597-613.

_____. 1962b. Revision of North American salamanders of the genus *Plethodon*. Bull. Florida State Mus. 6: 235-367.

_____. 1972. Distributional interactions among eastern North American salamanders of the genus *Plethodon*. In P. C. Holt (ed.), The distributional history of the biota of the southern Appalachians. Research Div. Monograph 4. Blacksburg, Va.: Virginia Polytechnic Inst. 139-188.

Merchant, H. 1972. Estimated population size and home range of the salamanders *Plethodon jordani* and *Plethodon glutinosus*. J. Wash. Acad. Sci. 62: 248-257.

Newman, W. B. 1954. A new plethodontid salamander from southwestern Virginia. Herpetologica 10: 9-14.

Noble, G. K. 1931. The biology of the amphibia. McGraw Hill Book Co., New York.

Pope, C. H. 1950. A statistical and ecological study of the salamander *Plethodon yonahlossee*. Bull. Chicago Acad. Sci. 9: 79-106.

_____. 1965. *Plethodon yonahlossee*. In W. J. Riemer (ed.), Catalogue of American amphibians and reptiles. Am. Soc. Ichthyologists and Herpetologists. Kensington. p. 15.

Radford, A. E., H. E. Ahles, and C. R. Bell. 1968. Manual of the vascular flora of the Carolinas. Univ. of North Carolina Press. Chapel Hill.

Rubin, D. 1969. Food habits of *Plethodon longicrus* (Adler and Dennis). Herpetologica 25: 102-105.

Sayler, A. 1966. The reproductive ecology of the red-backed salamander, *Plethodon cinereus*, in Maryland. Copeia 1966: 183-193.

Sever, D. M. 1978. Female cloacal anatomy of *Plethodon cinereus* and *Plethodon dorsalis* (Amphibia, Urodela, Plethodontidae). J. Herpetol. 12: 397-406.

Strausbaugh, P. D. and E. L. Core. 1978. Flora of West Virginia, Second edition. Seneca Books, Inc. Grantsville.

Tanner, J. T. 1978. Guide to the study of animal populations. Univ. Tennessee Press, Knoxville.

Thurow, G. R. 1968. On the small black plethodon problem. Series in the biological sciences. Western Illinois Univ. no. 6.

APPENDICES

APPENDIX A

Location Data for Destructive

and Population Samples

of

Plethodon yonahlossee

APPENDIX A

Location Data for

Destructive and Population

Samples of

Plethodon yonahlossee

Site #1

Secondary Road #1318 1.6 miles west of intersection with N.C. 194. Northeast-facing slope of Howard's Knob.

Site #2

Clint Norris Road (Secondary Road #1319) 0.4 miles west of intersection with N.C. 194. North-facing slope of "Straight Ridge" of Howard's Knob adjacent to Appalachian State University Reservoir.

Site #3 and Population Sample

Winkler's Creek Road (Secondary Road #1549) 3.9 miles south of intersection with U.S. 321. West-southwest slope of Rainbow Mountain Overlook at top of Pine Ridge.

Site #4

Niley Cook Road (Secondary Road #1532) 1.1 miles west of intersection with U.S. 321. South-facing slope below Mountain Ridge Apartments.

Site #5

Triplett Road (Secondary Road #1508) 0 to 0.8 miles southwest of intersection with U.S. 421 5 miles east of Boone.

Site #6

Ridge north and east of Hidden Valley Circle off of Chestnut Drive 0.5 miles north of intersection with U. S. 421 on the south-facing slope of Howard's Knob.

Site #7

Howard's Creek Road (Secondary Road #1319) 2.3-3.7 miles west of intersection with N.C. 194.

APPENDIX B

Destructive Collection Sampling

Data for *Plethodon yonahlossee*

From April 16 - November 15, 1980

APPENDIX B

Destructive Collection Sampling

Data for *Plethodon yonahlossee* from

April 16 - November 15, 1980

May Sample

Collection Number	
1.	26 April 1980. Site 1: 1 Adult, under rock, ASU #5409.
2.	27 April 1980. Site 2: 7 Individuals under logs, 1 under rock, ASU #'s 5401-5408.
3.	2 May 1980. Site 2: 4 Individuals under logs, ASU #'s 5410-5413.
4.	17 May 1980. Site 7: 2 Individuals out on road, ASU #'s 5429-5430.
5.	18 May 1980. Site 3: 11 Individuals out on ground, ASU #'s 5414-5424.
6.	18 May 1980. Site 5: 4 Individuals out on road, ASU #'s 5425-5428.
7.	19 May 1980. Site 1: 21 Individuals out on ground, ASU #'s 5431-5451.

June Sample

8.	16 June 1980. Site 4: 2 Individuals out on ground, ASU #'s 5452-5453.
9.	18 June 1980. Site 1: 23 Individuals out on ground, ASU #'s 5454-5476.
10.	23 June 1980. Site 2: 4 Individuals out on ground, ASU #'s 5477-5480.

Appendix II - continued

Collection Number

11. 24 June 1980. Site 5: 6 Individuals out on road, ASU #'s 5481-5486.

12. 25 June 1980. Site 5: 3 Individuals out on road, ASU #'s 5487-5489.

July Sample

13. 16 July 1980. Site 6: 16 Individuals under logs, ASU #'s 5490-5505.

14. 18 July 1980. Site 1: 27 Individuals out on ground, ASU #'s 5506-5530 and 5819-5820.

August Sample

15. 16 August 1980. Site 1: 119 Individuals out on ground, ASU #'s 5531-5649.

September Sample

16. 10 September 1980. Site 4: 4 Individuals out on ground, ASU #'s 5650-5653.

17. 22 September 1980. Site 1: 62 Individuals out on ground, ASU #'s 5654-5715.

October Sample

18. 17 October 1980. Site 1: 95 Individuals out on ground, ASU #'s 5716-5810.

November Sample

19. 15 November 1980. Site 1: 8 Individuals out on ground, ASU #'s 5811-5818.

APPENDIX C

Importance Values for the Woody Overstory of Rainbow Mountain Population Study Area

APPENDIX C

Importance Values for the Woody Overstory of Rainbow Mountain Population Study Area

SPECIES	IMPORTANCE VALUE
Quercus rubra	55.01
Quercus prinus	50.36
Acer rubrum	40.44
Cornus florida	34.92
Acer pennsylvanica	18.46
Ostrya virginiana	12.08
Carya ovata	10.87
Betula lenta	10.34
Amelanchier canadensis	9.50
Carya ovalis	7.88
Rhododendron maximum	7.67
Nyssa sylvatica	6.21
Robinia pseudoacacia	6.05
Prunus serotina	5.28
Quercus alba	4.47
Liriodendron tulipifera	4.32
Carya glabra	4.18
Fraxinus americana	3.78
Magnolia acuminata	3.65

Appendix C - continued

SPECIES	IMPORTANCE VALUE
Hamamelis virginiana	1.67
Ilex ambigua var. *montana*	.86
Rhododendron calendulaceum	.68
Sassafras albidum	.68
Prunus pennsylvanica	.80

APPENDIX D

Plant Species of the Herbaceous Layer

Rainbow Mountain Overlook

Population Study Area

APPENDIX D

Plant Species of the Herbaceous Layer
Rainbow Mountain Overlook

Population Study Area

Acer pennsylvanicum	Striped Maple
Acer rubrum	Red Maple
Actaea pachypoda	Baneberry
Amerlanchier canadensis	Serviceberry
Aquilegia canadensis	Columbine
Aralia nudicaulus	Wild Sarsaparilla
Arisaema triphyllum	Jack-In-The-Pulpit
Aster acuminatus	Mountain Aster
Aster cordifolius	Blue Wood Aster
Aster divaricatus	White Wood Aster
Aster macrophyllus	Bigleaf Aster
Aster solidagineus	White-Topped Aster
Aureolaria flava	Smooth Yellow Foxglove
Betula lenta	Sweet Birch
Botrychium dissectum	Common Grapefern
Campanula diverticata	Southern Bellflower
Carya sp.	Hickory
Castanea dentata	American Chestnut
Clintonia umbellulata	Speckled Wood-Lily
Conpholis americana	Cancer Root

Appendix D - continued

Cornus alternifolia	Alternate-Leaved Dogwood
Dennstaedtia punctilobula	Hay-Scented Fern
Dioscorea villosa	Wild Yam
Dryopteris marginalis	Marginal Shield Fern
Eupatorium rugosum	White Snakeroot
Fraxinus americana	White Ash
Galax aphylla	Galax
Gentiana quinquefolia	Aque-Weed, Stiff Gentian
Goodyera pubescens	Downy Rattlesnake Plantain
Hamemelis virginiana	Witch Hazel
Heuchera villosa	Alumroot
Houstonia purpurea	Large Summer Bluet
Ilex ambigua var. *montana*	Mountain Holly
Impatiens capensis	Jewelweed
Iris crestata	Crested Dwarf Iris
Laportea canadensis	Wood-nettle
Lilium michauxii	Turk's Cap Lily
Lyonia ligustrina	Male Blueberry
Lysimachia quadrifolia	Whorled Loosestrife
Magnolia acuminata	Cucumber Tree
Medeola virginiana	Indian Cucumber Root
Monarda clinopodia	Basil Balm
Ostrya virginiana	Hop Hornbeam
Parthenocissus quinquefolia	Virginia Creeper

Appendix D - continued

Polygonatum biflorum	Solomon's Seal
Polystictícum acrostichoides	Christmas Fern
Potentilla canadensis	Five Finger
Prenanthes altissima	Tall White Lettuce, Rattlesnake Root
Prunus serotina	Black Cherry
Quercus prinus	Chestnut Oak
Quercus rubra	Northern Red Oak
Rhododendron calendulaceum	Flame Azalea
Rhododendron maximum	Rosebay Rhododendron
Ribes glandulosum	Gooseberry
Robinia pseudoacacia	Black Locust
Rubus allegheniensis	Allegheny Blackberry
Sanguinara canadensis	Bloodroot
Sassafras albidum	Sassafras
Smilacina racemosa	False Solomon's Seal
Smilax rotundifolia	Common Greenbrier
Solidago curtisii	Curtis' Goldenrod
Solidago glomerata	Goldenrod
Stellaria pubera	Chickweed
Streptopus roseus	Twisted Stalk
Thalictrum dioicum	Meadow Rue
Thaspium trifoliatum	Meadow Parsnip

Appendix D - continued

Tsuga canadensis	Canadian Hemlock
Uvularia sp.	Bellwort
Vaccinium constablei	Blueberry
Vaccinium stamineum	Buckberry
Veratrum parviflorum	False Hellebore
Viburnum acerifolium	Maple-leaved Viburnum
Viola hastata	Halberd-leaved Violet
Viola rotundifolia	Round-leaved Violet
Viola sp.	Purple Violet
Vitis sp.	Wild Grape

VITA

Gregory Arledge Harris was born in Asheville, North Carolina on July 15, 1953. His parents are Mr. and Mrs. E. B. Harris, also of Asheville, North Carolina. He attended A. C. Reynolds High School in Asheville, North Carolina from 1967 to 1971 and graduated from there in 1971. He attended the University of North Carolina at Asheville from 1971 to 1972. In the fall of 1972 he transferred to Warren Wilson College in Swannanoa, North Carolina and graduated from there in 1976 with a B. A. degree in Biology. He taught Physical Science at Brevard High School in Brevard, North Carolina from 1976 to 1979. In 1979 he entered Appalachian State University and began graduate work towards a Master's degree. This degree was awarded in December 1981 in the field of Biology. His permanent mailing address is 661 1/2 Fairview Road, Asheville, North Carolina 28803. He was married to the former Karen Lynne Sutton of Clearwater, Florida in 1977. They have one son, Benjamin Sutton Harris, born September 22, 1981.