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# COMPARISONS OF MORPHOLOGY AND REPRODUCTIVE STATUS OF PLETHODON GLUTINOSUS AT HIGH, MIDDLE, AND LOW ELEVATIONS IN WEST VIRGINIA

Thesis submitted to The Graduate College of Marshall University

In partial fulfillment of the Requirements for the degree of Master of Science Biology

by

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May 2005

## ABSTRACT

## "COMPARISONS OF MORPHOLOGY AND REPRODUCTIVE STATUS OF PLETHODON GLUTINOSUS AT HIGH, MIDDLE, AND LOW ELEVATIONS IN WEST VIRGINIA"

#### by Cynthia F. Lucas

Plethodon glutinosus populations in West Virginia were looked at in three different elevations. Objectives were to determine if there is enough difference in elevation to cause different populations to vary in morphology and reproduction. Nine morphological measurements were taken to represent both size and proportional shape. Sexual dimorphism was found in all measurements only in low elevations, males being larger (p<0.001-p=0.041). Mean measurements between the three elevations showed significant differences (p<0.005) in all measurements excluding head width, tail length, and 3<sup>rd</sup> digit length. Salamanders were dissected; testes and vasa deferentia were removed from males and ovaries with follicles were removed from females. Results showed males in lower and middle elevations deposit spermatophores May-July. Low sample sizes in higher elevations provided inconclusive results. Females of lower elevations lay eggs early-mid summer. Middle and higher elevations results revealed a likely spring oviposition, or possibly a fall oviposition.

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ABSTRACT	ii	
AKNOWLEDGEMENTSiii		
TABLE OF CONTENTS	iv	
LIST OF FIGURES	V	
LIST OF TABLES	vii	
CHAPTER I	1	
REVIEW OF LITERATURE	1	
Introduction	1	
Morphology of <i>Plethodon glutinosus</i>	3	
Reproduction of <i>Plethodon glutinosus</i>	5	
Conclusion	8	
CHAPTER II	9	
MATERIALS AND METHODS	9	
Salamander Collection	9	
Morphology	10	
Reproductive Status	11	
Reproductive Status Males	11	
Reproductive Status Females	11	
Data Analysis	12	
CHAPTER III	13	
RESULTS	13	
Morphology	13	
Reproductive Status Males	15	
Reproductive Status Females	15	
CHAPTER 1V	18	
DISCUSSION	18	
Morphology	18	
Reproductive Status Males	19	
Reproductive Status Females	20	
Sources of Error	22	
Conclusion	22	
LITERATURE CITED	23	
FIGURES	27	
TABLES	47	
APPENDIX I-morphological data	<u>5</u> 3	
APPENDIX II-reproductive data, males		
APPENDIX III-reproductive data, females		
CURRICULUM VITAE	65	

# **TABLE OF CONTENTS**

## **LIST OF FIGURES**

- Figure 1.1 *Plethodon glutinosus* in its natural habitat.
- Figure 1.2 Total range of *Plethodon glutinosus*.
- Figure 1.3 West Virginia counties represented in this study.
- Figure 2.1 Mental gland on a sexually mature male.
- Figure 2.2 Testes and vas deferentia of a sexually mature male.
- Figure 2.3 Ovaries and follicles of a sexually mature female.
- Figure 3.1 Snout-vent lengths (mm) from each elevation.
- Figure 3.2 Head widths (mm) from each elevation.
- Figure 3.3 Tail lengths (mm) from each elevation.
- Figure 3.4 Tail circumferences (mm) from each elevation.
- Figure 3.5 Head depths (mm) from each elevation.
- Figure 3.6 Axilla to groin lengths (mm) from each elevation.
- Figure 3.7 Eye to nostril lengths (mm) from each elevation.
- Figure 3.8 First digit lengths (mm) from each elevation.
- Figure 3.9 Third digit lengths (mm) from each elevation.
- Figure 3.10 Sperm observed in the testes.
- Figure 3.11 Sperm found in the vas deferens.
- Figure 3.12 Sperm wave from low elevations.
- Figure 3.13 Sperm wave from middle elevations.
- Figure 3.14 Sperm wave from high elevations.
- Figure 3.15 Average number of parts containing sperm between low, middle, and high elevations.

Figure 3.16	Follicle volumes (ml) from low elevations.
Figure 3.17	Follicle volumes (ml) from middle elevations.
Figure 3.18	Follicle volumes (ml) from high elevations.
Figure 3.19	Average follicle volumes (ml) at low, middle, and high elevations.
Figure 3.20	Number of mature follicles present in all gravid specimens studied.
Figure 3.21	Average diameter (mm) of mature follicles in all gravid specimens studied.
Figure 3.22	Snout-vent length (mm) compared to follicle volume (ml) in all three elevations.
Figure 3.23	Snout-vent length (mm) compared to follicle volume (ml) of gravid females in all three elevations.
Figure 3.24	Snout-vent length (mm) compared to the number of eggs in gravid females from all three elevations.

# LIST OF TABLES

Table 2.1	Number of <i>Plethodon glutinosus</i> used for the morphology section of the study.
Table 2.2	Number of <i>Plethodon glutinosus</i> used in the reproductive part of the study.
Table 3.1	Comparisons of mean measurements between females and males from low elevations.
Table 3.2	Comparisons of mean measurements between females and males from middle elevations.
Table 3.3	Comparisons of mean measurements between females and males from high elevations.
Table 3.4	Comparisons of mean measurements between females and males from all elevations.
Table 3.5	Comparisons of mean measurements between the three elevations by using the Kruskal-Wallis One Way Analysis of Variance on Ranks followed by the Dunn's test.
Table 3.6	Total number of non-gravid and gravid females.
Table 3.7	Linear Regression results comparing SVL to follicle volume in all females.
Table 3.8	Linear regression results comparing SVL to follicle volume in gravid females.
Table 3.9	Linear regression results comparing SVL to the number of eggs each gravid female was carrying.

#### **CHAPTER 1**

#### **Review of Literature**

#### Introduction

West Virginia has five major provinces: (1) Allegheny Plateau, (2) Allegheny Mountains, (3) Ridge and Valley, (4) Great Appalachian Valley, and (5) Blue Ridge Mountains. The elevation ranges from 1,482 meters at Spruce Knob in Pendleton County to 73 meters at Harper's Ferry in Jefferson County, with a mean elevation of 504 meters (Green and Pauley, 1987). Temperature and precipitation are affected by these different elevations. Grafton and Dickerson (1969) stated that the annual average precipitation increases by 152 millimeters as the elevation increases from 610 to 914 meters on the west side of the mountains and decreases by 229 millimeters as elevation decreases from 914 to 610 meters east of the mountains. Lee (1969) determined that the mean annual temperature decreases by -16°C for each 305 meter increase in elevation. These precipitation and temperature differences can alter the habitats and the way of life for species living at a lower elevation versus the same species living at a higher elevation. This study will test the effects of 3 different elevations in West Virginia on morphological characteristics, sexual dimorphism, and reproductive status of *Plethodon glutinosus*, the Northern Slimy Salamander.

*Plethodon glutinosus* is a large terrestrial salamander in the family Plethodontidae. The family Plethodontidae is the largest family of salamanders and contains 27 genera and about 240 species (Petranka, 1998), of which 7 genera and 21 species are found in West Virginia (Green and Pauley, 1987). The scientific name of this genus refers to the large number of teeth that these salamanders have, but the prefix *pleth* 

also describes the population densities of the salamanders (Highton, 1995).

Plethodontids are all lungless and use their skin and the lining of their mouth and throat for gas exchange. One hypothesis suggests that an advantage to being lungless is the specialization of the hyoid apparatus in the throat which may be used as a mechanism for projecting the tongue to capture prey (Pough *et al.*, 1998). They also possess a nasolabial groove from each nostril to the upper lip. This is said to help keep the nostril clean and free of water and may also transfer olfactory sense data to the nose (Goin and Goin, 1962).

*Plethodon glutinosus* is named for its ability to produce skin secretions when roughly handled to deter predators. The "slime" becomes sticky as it dries, is difficult to remove, and is irritating to mucous membranes. Adult salamanders are black with small silvery white or metallic gold flecks. The amount of flecking varies and can be a distinguishing characteristic of a specific population (Figure 1.1). The underside is a lighter grayish-black. There are 16 costal grooves and the tail is rounded in a cross section (Green and Pauley, 1987; Petranka, 1998). Hatchlings are gray to black and lack pigmentation on their underside. They have pigment-free areas that appear to be scattered light spots (Wells and Gordon, 1958).

The range of the Northern Slimy Salamander, shown in Figure 1.2, is from central New York to central Florida and from central Missouri to central Texas. There are a few geographic isolates in New Hampshire, Louisiana, Arkansas, and eastern Texas (Petranka, 1998). In West Virginia, *Plethodon glutinosus* are found statewide (Green and Pauley, 1987). In this study, salamanders were used from 45 of the 55 counties in West Virginia (Figure 1.3).

Northern Slimy Salamanders are associated with eastern deciduous forests and live in moist habitats. These terrestrial habitats include: Flood plains, leaf piles, under logs and stones, in crevices of shale banks, and along the sides of gullies and ravines (Grobman, 1944). This species has been found to inhabit caves to oviposit (Highton, 1962a), and during droughts (Humphries, 1956). They are most likely to be collected by flipping rocks or logs during the day and searching the forest floor at night. They surface from underground in late February or early April, depending on elevation. At lower elevations, Northern Slimy Salamanders retreat underground during hotter summer months, and at higher elevations they are active all summer (Green and Pauley, 1987).

#### Morphology of *Plethodon glutinosus*

The size of the hatchlings can vary among populations. The snout-vent length (SVL), the length between the tip of the snout to the posterior end of the cloaca, has been reported to be 12-15 mm (Highton, 1956) and 31 mm (Wells and Gordon, 1958). Tail length (TL), the length from the posterior end of the cloaca to the tip of the tail, has been reported to be 20-26 mm (Highton, 1956), 31 mm (Wells and Gordon, 1958), and 18-26 mm (Minton 1972). Most females become sexually mature when they measure 46-56 mm SVL, and most males at 40-53 mm SVL (Petranka, 1998).

Morphological studies in northern states (Maryland and Pennsylvania), and southern states (Florida) show that the Northern Slimy Salamander has much variation within its own species. Highton (1962b) showed that the average SVL of salamanders of 1 year was 40 mm in Florida and 30 mm in Maryland and Pennsylvania. He also found that the maximum size of males in Florida was 65 mm, and in Pennsylvania and Maryland 82 mm. Females were 69 mm in Florida and 85 mm in Maryland and

Pennsylvania. These differences were due to temperature variations and the length of the hibernation periods at each location.

Carr (1996) studied the geographic variation of 14 of 16 species in the *Plethodon glutinosus* complex. He used 9 different morphological measurements to determine if each species of the *P. glutinosus* complex could be separated by these measurements. Using principle component analysis (PCA), *Plethodon glutinosus* separated out from the other 13 species, however *Plethodon glutinosus* intraspecies variation. For example, within the 5 sample areas (NY, OH, PA-1, PA-2, and IN), SVL varied from 61.8 mm (PA-1) to 71.2 mm (OH) and TL varied from 61.5 mm (PA-1) to 73.7 mm (NY). He concluded that 48-90% of adult salamanders from a single sample of each species could be allocated correctly to its population.

Two populations of *Plethodon glutinosus* were studied by Semlitsch (1980) at Cunningham Falls State Park, Frederick County, Maryland, and 3 populations near Centerville, Bedford County, Pennsylvania. His results showed that the mean body size of adults and the minimum size at first reproduction were the most significant size variables among the populations. Elevation, season, and time of collection were constant, showing that these salamanders can differ morphologically even in similar environments.

The growth rate in the northern states is slower than in the south because of a longer hibernation period, which decreases activity. However, it was found that the northern populations attain a larger adult size. One hypothesis to explain this is that northern populations have genetically adapted to permitting larger broods per female to compensate for a biennial reproductive cycle (Highton, 1962a).

Sexual dimorphism in most salamanders is very slight, with females being larger than the males. Larger females are thought to be related to egg-carrying capacity, because there is a strong correlation between body size and clutch size (Duellman and Trueb, 1986). In few species, males are larger than females. This is an advantage during combat between males (Shine, 1979). Northern Slimy Salamanders have a slight sexual dimorphism in SVL. Females are on average 0-6% larger in SVL than males (Highton, 1956; Pfingsten, 1989; Pope and Pope, 1949; Semlitsch, 1980).

As elevation increases, summer shortens and many environmental factors change. This could change the abundance or availability of food for the Northern Slimy Salamanders (Powders and Tietjen, 1974). Temperature and water have an effect on habitat selection and niche segregation. To survive in a habitat, species must be able to obtain energy for growth, maintenance, and reproduction. With shorter summers and longer winters *Plethodon glutinosus* may be smaller at higher elevations due to the limited amount of prey. However, when temperatures are higher and the area is drier for longer periods of time, the surface activity for the salamander is limited. This means if they obtain most of their food from the surface, a time will come when energy requirements will exceed energy intake (Spotila, 1971). As a result, *Plethodon glutinosus* may be smaller at lower elevations.

#### Reproduction of *Plethodon glutinosus*

Oogenesis in females occurs in the ovaries. The ovaries are thin-walled sacs surrounded by germ cells. The germ cells divide and produce ova (eggs). Follicle cells encase the ova for nourishment and support. Many follicles (an ovum and follicle cells) will develop and form the ovaries. Oviducts are tubes on each side of the dorsal body

wall, lateral to the ovaries. The anterior ends remain open, and as ova are shed into the body they move into the oviducts. Ova move to the posterior ends of the oviducts by way of cilia. The posterior ends expand into an ovisac which empties into the cloaca where ova can become fertilized (Noble, 1931; Zug *et al.*, 2001).

Testes of males are a mass of seminiferous tubules in a thin-walled sac. Spermatozoa, sperm, are produced by spermatogonia in the seminiferous tubules during spermatogenesis. The posterior part of the testes fill with sperm first. The anterior end will then begin to fill and will greatly increase the size of the testes. As sperm leaves the testes, secondary spermatogonia for the next year's cycle are slowly proliferated. The sperm then flows into the vasa deferentia. When the vasa deferentia are swollen and packed with sperm, the salamander is ready to mate (Burger, 1937). Males deposit a gelatinous packet, the spermatophore, which contains sperm. Females pick up the spermatophore and the gelatinous cap will dissolve in the cloaca to release the sperm (Goin and Goin, 1962). Each sperm has a head, a midpiece, and a filamentous tail. The head contains the nucleus and an acrosome. The acrosome has enzymes that digests the egg capsule of a female and allows for penetration (Zug *et al.*, 2001).

In breeding, the male begin by putting his nasolabial grooves and mental gland (a circular gland under the chin, present in sexually mature male) in contact with the head, body, or tail of the female. The male begins a foot-dance by raising and lowering his hind limbs either separately or simultaneously. As the dance progresses, both the hind and front limbs are raised and lowered. The male holds his mental gland against the female's body, occasionally raising his head. He rubs the female with his nasolabial grooves and gently grasps her with his mouth and then releases. He pushes his head

under her chin and passes beneath her; maintaining contact. The female straddles the male's tail and they move forward. This is called the tail-straddle walk. The male stops and begins rocking his sacral region as the female moves with him. He lowers his vent into the substrate to deposit a spermatophore. When finished he moves his tail to the side, and the female still remains in contact. They then move forward and the female picks up the sperm cap with her cloacal lips. Sperm are stored in the spermatheca and eggs are fertilized inside the female's cloaca as they pass by (Organ, 1960; Petranka, 1998).

Females have been shown to reproduce annually in southern states and biannually in northern states. This may be a result of ova not being able to reach maturity during a long hibernation period. Oviposition has to occur during late summer in southern populations and in late spring or early summer in northern populations (Highton, 1962a). Females oviposit in many types of habitats including: under decaying logs and stumps, in rocks and crevices, and in caves (Petranka, 1998). They lay a cluster of eggs surrounded by two jelly envelopes (Noble and Marshall, 1929; Wood and Rageot, 1955). The embryonic period lasts around 2 to 3 months, depending on latitude. Hatchlings have been found in Alabama during November and December (Highton, 1962a), during October in Indiana (Minton, 1972), and in early August through September in Mississippi (Brode and Gunter, 1958). Females guard their eggs and remain with their hatchlings for 1-2 weeks (Pentranka, 1998).

The sexual cycle in males is annual, and there is no inactive period between cycles (Burger, 1937). Breeding times vary geographically depending on latitude. Males in Florida begin spermatogenesis in August and transfer sperm from the testes to the vasa deferentia December through March, whereas in Maryland and Pennsylvania, this

transfer of sperm occurs September through April (Highton, 1962a). Males in Alabama begin mating in March (Trauth, 1984), whereas in the northern states, mating peaks in September and October (Bishop, 1941a; Highton, 1962a).

#### Conclusion

*Plethodon glutinosus* is a common and well studied salamander. A lot is known about their morphology and reproduction status in northern and southern populations. This study, however, will look at different *Plethodon glutinosus* populations in one area, West Virginia, at three different elevations. The objective is to determine if there is enough difference in elevation to cause different populations to vary in morphology and reproduction.

#### **CHAPTER 2**

#### **Materials and Methods**

#### Salamander Collection

*Plethodon glutinosus* from the West Virginia Biological Survey Museum, Huntington, West Virginia were used for this study. Salamanders used were collected from 1935 to 2001 by various collectors. They were grouped into three elevations: high (914 meters and above), middle (457-914 meters.), and low (457 meters and below). To determine this range, the highest elevation in the state, 1482 meters, was subtracted from the lowest elevation, 73 meters, and was divided by three, which roughly equaled 457 meters (Green, 1987). Also as elevation increases, the vegetation changes. Higher elevations contain mainly red spruce trees as opposed to northern hardwoods such as sugar maple, beech, and yellow birch trees. This could have an effect on a salamander's habitat and prey availability (Strausbaugh and Core, 2003). The location of each salamander used in the study was available in the museum data base. By using Map Tech Terrain Navigator (1998) and the Report of the Upland Vertebrates in the New River Gorge National River Volumes 1, 2, and 3, (Pauley, 1991) the elevation of each salamander was determined.

Salamanders in each elevation were selected by the month that they were collected and by their size. The time that they were collected was relevant for the reproductive part of the study, and the size determined the salamander's maturity. Only sexually mature salamanders were used, therefore, most salamanders were 45.0 mm in SVL or larger. This size was chosen because most females become sexually mature when they measure 46.0-56.0 mm SVL, and most males become sexually mature when

they reach 40.0-53.0 SVL (Petranka, 1998). Gender was determined by the presence of a circular mental gland, present in the sexually mature males (Figure 2.1); however females were distinguished from immature males only after dissecting them.

#### Morphology

Measurements were taken to represent both size and proportional shape. Nine measurements were selected for this representation (Carr, 1996): 1. snout-vent length (SVL), 2. head width (HW), 3. tail length (TL), 4. tail circumference (TC), 5. head depth (HD), 6. axilla to groin length (A-G), 7. eye to nostril length (E-N) 8. length of the 1<sup>st</sup> digit, and 9. length of the  $3^{rd}$  digit. SVL is a measurement of the length between the tip of the snout to the posterior end of the cloaca. TL is the length from the posterior end of the cloaca to the tip of the tail. HW is the width of the head starting just behind the eyes and HD is the depth of the head, also taken just behind the eyes. TC is a measurement taken by wrapping a string around the tail just below its base, and then measuring the length of the string. A-G is a measurement from the "armpit" to the posterior end of the cloaca, and E-N is the distance between the anterior angle of the orbit and the nostril. Lengths of both the digits were measurements starting at the base of the digit through the tip of the digit. Two hundred salamanders were studied: 63 from low elevation (40 females, 23 males), 78 from middle elevation (38 females, 40 males), and 59 from high elevation (27 females, 32 males) (Table 2.1). Each was measured to the nearest 0.1mm by a dialMax Swiss Precision caliper and recorded into a Microsoft Excel spreadsheet. Salamanders with large outliers were re-measured for precision.

#### **Reproductive Status**

One hundred and fifty six salamanders were studied: 63 in low elevation (40 females, 23 males), 65 in middle elevation (35 females, 30 males), and 28 in high elevation (12 females, 16 males) (Table 2.2). All 200 salamanders used for the morphology studies could not be used because in the reproductive studies because their reproductive tracts had been removed during a previous study. The results were recorded in a Microsoft Excel spreadsheet.

#### <u>Males</u>

To determine the reproductive status in males, the testes and vasa deferentia were removed (Figure 2.2). Anterior and posterior, left and right halves of the testes and anterior and posterior, left and right halves of the vasa deferentia were placed on a microscope slide with a drop of Wright's stain, making a total of eight slides per salamander. Each reproductive part on the slide was teased apart with a dissecting needle and a cover slip was placed on the slide to compress the tissue. The sperm wave was determined by examining the presence of sperm in the tissue and the number of reproductive parts containing sperm, by examining each part under a compound microscope (40X) (Pauley, 1980).

#### Females

In the females, protocol was similar to that of Canterbury and Pauley (1994) when they examined reproduction of *Aneides aeneus*, the Green Salamander. The total volume of both ovaries with follicles was measured (Figure 2.3). This volume was determined by immersing the ovaries with the follicles into 5.0 ml of water in a 10.0 ml graduated flask and noting the amount of displaced water. The number of the follicles and the average

follicle diameter in the sexually mature females were counted and measured. The diameter was taken by averaging the diameter of five different follicles.

#### Data Analysis

In the morphology section, all the statistics were done using Sigma Stat version 2.0 and SAS. Box and whisker plots were done to show the graphical display of the center and variation of the male and female data sets at each elevation. They were also used to check the assumption of same shape distribution for the nonparametric tests. To statistically compare the 2 groups (male vs. female) t-tests or Mann-Whitney Rank Sum Tests were performed depending on whether the data met the required assumptions. Kruskal-Wallis One Way Analysis of Variance on Ranks followed by the Dunn's test (if necessary) was done to compare the data of the 3 elevations.

The data in the reproduction section were displayed by scatter graphs made in Microsoft Excel. Linear regressions were used to determine associations in females between SVL and follicle volume, and SVL and the number of eggs.

#### **CHAPTER 3**

#### Results

#### <u>Morphology</u>

The specimen number, date, county, elevation, sex, and all the morphological data are shown in appendix I. Sexual dimorphism of low elevation individuals showed there was a significant difference in all measurements. Males were larger in all measurements than females (Table 3.1). For example, mean snout-vent length (SVL) in males was 14% larger than the females, males had 18% larger mean head width, and mean tail length (TL) was 16% larger in the males.

Sexual dimorphism in middle and high elevations showed few significant differences (Table 3.2). In middle elevations, the significant measurement was the eye to nostril length (E-N) measurement. Males were larger than females, 3.3 mm to 3.0 mm (p=0.049). However, mean measurements of males were not always larger than those of females. Although not significant, mean values for females were larger for TL, tail circumference (TC), axilla to groin length (A-G), and 3<sup>rd</sup> digit length. In high elevations, there were no measurements to support sexual dimorphism, and again, larger measurements varied between the males and females (Table 3.3).

Over all sexual dimorphism existed in few measurements. Table 3.4 demonstrates that there was a significant sexual dimorphism in head width, (HW), head depth (HD), E-N, and 3<sup>rd</sup> digit length; the males being larger once again. Mean HW was 7% larger in males; mean HD was 14% larger in males; mean E-N in males was 14% larger than in females; mean 3<sup>rd</sup> digit length in males was 3% larger than in females .The other means were larger in males although the difference was not significant.

Investigating general morphological differences in low, middle, and high elevations, revealed no significant differences in HW, TL, or 3<sup>rd</sup> digit length (Table 3.5). In mean SVL, salamanders in middle elevations was 7% larger than in high elevations, and 2% larger in middle elevations compared to low elevations. Mean TC had a significant difference (p < 0.05) in low and high, and middle and high elevations. Salamanders in low elevations had a mean TC of 8% larger than those in high elevations, and salamanders in middle elevation were 18% larger than those in high elevations. Mean HD had a significant difference (p < 0.05) between low (4.5 mm) and middle (4.6 mm), low and high (3.9 mm) elevations, and middle and high elevations. Mean A-G was significant between middle and high elevations, middle elevations being 7% larger, and low and middle elevations, with middle elevations being 6% larger. Mean E-N length was 2.9 mm in low elevations, 3.2 mm in middle elevations, and 2.9 mm in high elevations. The significant difference was between low and middle elevations, and middle and high elevations (p < 0.05). Mean 1<sup>st</sup> digit length was significantly different in middle and high elevations, with middle elevations being 14% larger.

Results above can be seen graphically in figures 3.1-3.9. They are represented in box and whisker plots, which show the variations of all measurements in each group. There were a few groups that had several outliers in some of the measurements, for example, in figures 3.7-3.9.

#### **Reproductive Status**

#### Males

The results of the male reproductive studies are shown in appendix II. Figure 3.10 is an enlarged view of the sperm found in the testes, and figure 3.11 is an enlarged view of sperm found in a vas deferentia. In low elevations, sperm cells were found in more parts of the reproductive tract (all halves of the testes, and all halves of the vasa deferentia) filled with sperm in early spring, March-May. The number of reproductive parts containing sperm decreased in June and July, and the sperm started to increase again in August and September (Figure 3.12). In middle elevations, the sperm wave started out with more parts containing sperm in March, decreasing through July, and increasing again August-December (Figure 3.13). High elevations showed a small number of parts containing sperm in April and May, increasing in June-November (Figure 3.14). (Keep in mind, this elevation had a low sample size, N=16). Figure 3.15 shows the average parts containing sperm in all three elevations. High elevations peak first, with the most parts containing sperm, followed by middle and low elevations. High and middle elevations show a second peak, whereas low elevations level off.

#### <u>Females</u>

Appendix III shows the results of the female reproductive studies. In low elevations, the females had a larger follicle volume in April and May. This volume decreased and there was no sign of a second reproductive cycle (Figure 3.16). Middle elevations showed one female having a peak follicle volume in May. The other female's follicle volumes were small and increased in August and September (Figure 3.17). In high elevations, the peak follicle volumes were in July and August (Figure 3.18). Figure

3.19 shows the average follicle volumes in the three different elevations. Again, low elevations had one peak in early spring. Middle elevations had two peaks; however, the first peak is by only one salamander. High elevations peaked in July, but again high elevation was represented by a low sample size, N=12.

Another measurement made was the number and size of follicles in the gravid females. There were 13 gravid females; 5 in low elevations, 6 in middle elevations, and 2 in high elevations (Table 3.6). Figure 3.20 shows the number of follicles in each gravid female in each elevation. In low elevations, gravid females were collected in March-May. The follicle number ranged from 25 follicles to 32 follicles. In middle elevations there were two gravid females in May, two in August, and two in September. The follicle number ranged from 10 to 37. There were two gravid female in high elevations, one in July with 31 follicles and one in August with 23 follicles. Figure 3.21 shows the average diameter of each follicle in the gravid females.

SVL was compared to follicle volume to determine if larger females had larger follicle volumes. This was done on all the females collected to get an overview of the data, and then done again on just the gravid females (Figure 3.22-3.23). The two variables in low and middle elevations showed a significant relationship, when SVL increases, follicle volume increases (low p<0.001, middle p=0.039) (R<sup>2</sup>low=0.333, R<sup>2</sup>middle=0.123) (Table 3.7). Gravid females showed no relationship between follicle volume and SVL in low and middle elevations (p=0.721, p=0.086), and there are not enough specimens available to test in high elevations. The R<sup>2</sup> values are relatively low (low = 0.0355, middle=0.562) (Table 3.8).

Figure 3.24 shows SVL compared to the total number of eggs found in the gravid females. There was no association found in low or middle elevations (p=0.120, p=0.233) (R<sup>2</sup>low=0.608, R<sup>2</sup>middle=0.330), and there is not enough data available to test in high elevations (Table 3.9).

#### **CHAPTER 4**

#### Discussion

#### <u>Morphology</u>

Sexual dimorphism was found in the lower elevations, with males being larger than females. Middle and higher elevations showed little to no sexual dimorphism. When comparing all females to all males, there were some sexual dimorphic characteristics that were significant, however male measurements were always on average larger than female measurements. This is the opposite of the findings of Highton (1956), Pfingsten (1989a), Pope and Pope (1949), and Semlitsch (1980b) who found that the females are on average 0-6% larger than males. This may support the idea of Shine (1979) that it is an advantage for males to be larger for male-male combat. Male-male aggression is documented for *Plethodon glutinosus* (Organ, 1960; Thurow, 1976) and has also been found in other species including *Plethodon jordani*, the Appalachian Woodland Salamander (Arnold, 1977). For example, Hairston (1949, 1951) proposes that altitudinal distribution of *Plethodon glutinosus* is limited by competition with *Plethodon jordani*. Plethodon glutinosus burrows under logs, rocks, and other covers; Plethodon jordani also retreats to burrows during the day. The inability for *Plethodon glutinosus* to use the subterranean portion of a certain habitat may put it at a competitive disadvantage. Males may also be larger to compete for a female during breeding season.

However, since the measurements were found to be opposite of most findings, there could be other explanations that are due to sampling bias. Salamanders collected for this study were collected from the 1930's to the present. Some collectors may have chosen larger salamanders to put in the database for ideal specimens. The ratios of males

to females in each elevation also varied. In low elevation, where the males were significantly larger than the females, there were fewer males sampled than females, 26 to 50 respectively. Middle and high elevation ratios varied but only by a few specimens. This large difference in sample size in low elevations may have made a difference in the results.

When looking at all 3 elevations, salamanders in middle elevations had the largest measurements followed by low elevations, and higher elevations had the smallest measurements. Population densities are dependent on many factors such as climate, available resources, or variation in predator pressure. Spotlia (1971) suggests that widespread success of *Plethodon glutinosus* is due to its ability to survive in unsuitable microhabitats where conditions do not exceed its tolerance limits. This may be the case in the middle elevations.

### **Reproductive Status**

#### <u>Males</u>

Highton, (1962a) concluded that spermatogenesis begins during August in Florida and April in Maryland and Pennsylvania. Transfer of sperm from testes to vasa deferentia occurred December to March in Florida and September to April in Maryland and Pennsylvania. In West Virginia, the same reproductive pattern occurred in low and middle elevations but due to few salamanders studied in higher elevations, the results were inconclusive. Reproductive tracts of salamanders in lower elevations contained sperm in March through May and sperm occurrence declined in June, July, and August. This shows that males deposit spermatophores in June and/or July. Salamanders in middle elevations had sperm cells throughout the reproductive tract from March and

April, and declined in May through July. This may support mating seasons in late spring, early summer. In higher elevations, there were not many salamanders to study because most of the reproductive tracts had already been removed from them during a previous study. There is a high number of sperm cells in the reproductive tract starting in June and continues through November, however there were no samples taken in September or October. This high amount of sperms cells could remain in the reproductive tract over the winter until mating in April and May. There were no salamanders studied over the winter months so it is possible that mating could occur March.

#### <u>Females</u>

In low elevations, follicle volumes were greatest during March, April, and May. Volumes then decreased, which indicated oviposition during early to mid summer. In middle elevations, one salamander had a large peak volume in May; however, other salamanders during this month had low follicle volumes. These low volumes made it difficult to predict when oviposition occurred. Salamanders of this elevation group could oviposit in June through July; however, there is also a decreased follicle volume starting in October, possibly indicating oviposition in the fall. Highton (1962a) described oviposition in Florida being in late August and early September, so it is possible for a fall oviposition. However, being that Florida is considered lower elevation, it is more likely that these salamanders oviposit in June through July. Another possibility is that these salamanders may have a prolonged egg laying season that starts in June or July and ends in September or October. Salamanders from higher elevations had a low follicle volume in May, increasing through August, and decreasing slightly in September. With

lack of data in months before May and after September, there is no sure way to tell if they oviposit in early spring, or in the fall.

Highton (1962a) concluded that salamanders oviposit in late spring in Maryland and Pennsylvania and in late August to early September in Florida. Highton (1962b) also described egg-laying in West Virginia and found that oviposition occurs in late spring through midsummer. He reported a gravid female that was collected at 3,800 feet in West Virginia that was brooding a clutch in August, as well as one at 2,000 feet brooding a clutch in July. The results of salamanders in lower elevations do agree with Highton's work; however due to small sample sizes, more data is needed in middle and higher elevations to determine exact times of oviposition.

The number of mature follicles measured in each gravid female from all elevations ranged from 10 to 37. Reported mean values for average clutch size include 16 in Florida (Highton, 1962b), 25 in southeastern Kentucky (Bush, 1959), 17 in Pennsylvania (Highton, 1962b), 26 in Maryland (Highton, 1962), 17-24 in New York (Bishop, 1941), and 23 in Virginia (Pope and Pope, 1949).

The average diameter of a freshly laid egg is usually between 3.5-5.5 mm (Noble and Marshall, 1929; Wood and Rageot, 1955). Mature follicles measured in this study ranged between 1.6 to 4.6 mm. Once deposited, average egg diameters would probably fall in this expected range.

In this study, a relationship was found in low and middle elevations between snout-vent length and follicle volume. As snout-vent length increased, follicle volume increased as well. Generally larger body size in females has been thought to be related to egg-carrying capacity (Duellman, 1986).

#### Sources of Error Encountered During the Study

There were 2 major problems that occurred during this study. One was that many salamanders used for the study did not have tails. This decreased the amount of data used for the morphology section. If all of the salamanders would have had tails, this would have provided more data, and would have allowed a combined analysis of salamanders with tails and salamanders without tails instead of having to subgroup according to presence of a tail.

The second major problem was the lack of preserved salamanders that had reproductive data in the higher elevations. This made the sample sizes very small for both males and female, and there was not any reproductive data for certain months of the year.

To perfect this study, it would have been ideal to have equal sample sizes and salamanders collected throughout the entire year. It may have also been beneficial if temperature and precipitation data were available to determine whether or not these variables had an effect on morphology and reproduction.

#### Conclusion

This study shows that a difference in elevation in West Virginia does cause a distinction in morphology and reproductive status of *Plethodon glutinosus*. Each elevation provides different abiotic and possibly biotic factors that will have an effect on both morphology and reproduction. Further research is needed to fully determine the effects that elevation has on *Plethodon glutinosus* overall.

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# FIGURES



Figure 1.1. *Plethodon glutinosus* in its natural habitat. Picture taken by Robert Makowsky.



Figure 1.2. Total range of *Plethodon glutinosus* (Petranka, 1998).


Figure 1.3. West Virginia counties represented in this study; low (457 m and below), middle (457-914 m), and high (914 m and above) elevations.



Figure 2.1. Mental gland on a sexually mature male.



Figure 2.2. Testes (white arrow) and vas deferentia (blue arrow) of a sexually mature male.



Figure 2.3. Ovaries and follicles of a sexually mature female.



Figure 3.1. Snout-vent lengths (mm) from each elevation, L-Low, M-Middle, H-High.



Female-L Male-L Female-M Male-M Female-H Male-H

Figure 3.2. Head widths (mm) from each elevation.





Figure 3.3. Tail lengths (mm) from each elevation.



Figure 3.4. Tail circumferences (mm) from each elevation.



Female-L Male-L Female-M Male-M Female-H Male-H

Figure 3.5. Head depths (mm) from each elevation.



Female-L Male-L Female-M Male-M Female-H Male-H

Figure 3.6. Axilla to groin lengths (mm) from each elevation.



Figure 3.7. Eye to nostril lengths (mm) from each elevation.



Female-L Male-L Female-M Male-M Female-H Male-H

Figure 3.8. First toe lengths (mm) from each elevation.



Female-L Male-L Female-M Male-M Female-H Male-H

Figure 3.9. Third toe lengths (mm) from each elevation.



Figure 3.10. Sperm observed in the testes (Scale: 63X). The sperm resemble tiny threads of string.



Figure 3.11. Sperm found in the vas deferens (Scale: 63X). The sperm resemble tiny threads of string.



Figure 3.12. Sperm wave from low elevations. Y axis represents number of reproductive parts containing sperm. The sample number is given when more than one salamander occupies a single plot, (n=23).



Figure 3.13. Sperm wave from middle elevations. Y axis represents number of reproductive parts containing sperm. The sample number is given when more than one salamander occupies a single plot, (n=30).



Figure 3.14. Sperm wave from high elevations. Y axis represents number of reproductive parts containing sperm. The sample number is given when more than one salamander occupies a single plot, (n=16).



Figure 3.15. Average number of parts containing sperm between low, middle, and high elevations. Y axis represents average number of reproductive parts containing sperm.



Figure 3.16. Follicle volumes (ml) from low elevations. The y axis represents follicle volume in ml. The sample number is given when more than one salamander occupies a single plot, (n=40).



Figure 3.17. Follicle volumes (ml) from middle elevations. The y axis represents follicle volume in ml. The sample number is given when more than one salamander occupies a single plot, (n=35).



Figure 3.18. Follicle volumes (ml) from high elevations. The y axis represents follicle volume in ml. The sample number is given when more than one salamander occupies a single plot, (n=12).



Figure 3.19. Average follicle volumes (ml) at low, middle, and high elevations. The y axis represents the average follicle volume in ml.



Figure 3.20. Number of mature follicles present in all gravid specimens studied.



Figure 3.21. Average diameter (mm) of mature follicles collected in all gravid specimens studied.



Figure 3.22. Snout-vent length (mm) compared to follicle volume (ml) in all three elevations.



Figure 3.23. Snout-vent length (mm) compared to follicle volume (ml) of gravid females in all three elevations.



Figure 3.24. Snout-vent length (mm) compared to the number of eggs in gravid females from all three elevations.

## TABLES

Table 2.1. Number of *Plethodon glutinosus* used for the morphology section of the study. The numbers in parentheses represent the salamanders without tail measurements.

	Female	Male	Total
Low	40 (10)	23 (3)	63 (13)
Middle	38 (7)	40 (12)	78 (19)
High	27 (18)	32 (18)	59 (36)
Total	105 (35)	95 (33)	200 (68)

Table 2.2. Number of Themouon grannosus used in the reproductive part of the study	Table 2.2. Numb	per of <i>Plethodon</i> glutinos	sus used in the reproductive	part of the study.
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	Female	Male	Total
Low	40	23	63
Middle	35	30	65
High	12	16	28
Total	87	69	156

Table 3.1. Comparisons of mean measurements between females and males from low elevations. P values from t-test (T) (normal distribution) or Mann-Whitney Rank Sum Test (MW) (not normal distribution) are given.

	n	SVL	HW	TL (n=30F n=20M)	TC (n=30F n=20M)	HD	A-G	E-N	1 <sup>st</sup> Toe	3 <sup>rd</sup> Toe
Female	40	61.2	8.9	61.6	17.5	4.3	31.2	2.6	0.8	3.4
Male	23	69.9	10.5	72.0	22.3	5.0	35.8	3.3	1.0	4.0
p value		0.002 (MW)	<0.001 (MW)	<b>0.041</b> (T)	<0.001 (T)	0.002 (T)	0.006 (T)	<0.001 (T)	0.021 (T)	<0.001 (T)

Table 3.2. Comparisons of mean measurements between females and males from middle elevations. P values from t-test (T) (normal distribution) or Mann-Whitney Rank Sum Test (WM) (not normal distribution) are given.

	n	SVL	HW	TL (n=31F n=28M)	TC (n=31F n=28M)	HD	A-G	E-N	1 <sup>st</sup> Toe	3 <sup>rd</sup> Toe
Female	38	67.4	9.9	67.8	22.0	4.4	35.0	3.0	0.8	3.6
Male	40	67.6	10.0	62.2	20.6	4.7	34.7	3.3	0.9	3.5
p value		0.745 (MW)	0.579 (T)	0.149 (T)	0.174 (T)	0.164 (T)	0.292 (MW)	0.049 (T)	0.388 (T)	0.434 (T)

Table 3.3. Comparisons of mean measurements between females and males from high elevations. P values from t-test (T) (normal distribution) or Mann-Whitney Rank Sum Test (MW) (not normal distribution) are given

	n	SVL	HW	TL (n=9F n=14M)	TC (n=9F n=14M)	HD	A-G	E-N	1 <sup>st</sup> Toe	3 <sup>rd</sup> Toe
Female	27	63.2	9.6	61.6	17.5	4.0	33.0	2.8	0.7	3.4
Male	32	63.1	9.9	62.5	18.3	3.9	32.2	2.9	0.7	3.5
p value		0.928 (T)	0.233 (T)	0.866 (T)	0.461 (T)	0.619 (T)	0.483 (T)	0.232 (T)	0.579 (MW)	0.626 (T)

Table 3.4. Comparisons of mean measurements between females and males from all elevations. P values from t-test (normal distribution) or Mann-Whitney Rank Sum Test (not normal distribution) are given.

	n	SVL	HW	TL (n=70F n=62M)	TC (n=70F n=62M)	HD	A-G	E-N	1 <sup>st</sup> Toe	3 <sup>rd</sup> Toe
Female	105	64.0	9.4	64.4	19.5	4.3	33.0	2.8	0.8	3.5
Male	95	66.6	10.1	65.4	20.6	4.9	33.1	3.2	0.8	3.6
p value		0.69 (MW)	<0.001 (T)	0.702 (T)	0.132 (T)	0.032 (T)	0.266 (MW)	<0.001 (T)	0.085 (MW)	0.048 (T)

Table 3.5. Comparisons of mean measurements between the three elevations by using the Kruskal-Wallis One Way Analysis of Variance on Ranks followed by the Dunn's test.

	SVL	HW	TL	TC	HD	A-G	E-N	1 <sup>st</sup> Toe	3 <sup>rd</sup> Toe
Low	64.4	9.5	65.8	19.4	4.5	32.9	2.9	0.8	3.6
Middle	67.5	9.9	65.1	21.3	4.6	34.8	3.2	0.8	3.6
High	63.1	9.8	62.1	18.0	4.0	32.6	2.9	0.7	3.4
Significant Difference p<0.05	M-H M-L	None	None	L-H M-H	L-H M-H L-M	M-H L-M	L-H M-H	M-H	None

	Non-gravid Females	Gravid Females
Low Female	35	5
Middle Female	29	6
High Female	10	2

Table 3.6. Total number of non-gravid and gravid females.

Table 3.7. Linear Regression results comparing SVL to follicle volume in all females.

	p Value	R Squared Value
Low	<0.001	0.333
Middle	0.039	0.123
High	0.074	0.074

Table 3.8. Linear regression results comparing SVL to follicle volume in gravid females. The dashes for high elevation mean that the sample size was too low to test.

	p Value	R Squared Value
Low	0.721	0.0355
Middle	0.086	0.562
High		

Table 3.9. Linear regression results comparing SVL to the number of eggs each gravid female was carrying. The dashes for high elevation mean that the sample size was too low to test.

	p Value	R Squared Value
Low	0.120	0.608
Middle	0.233	0.330
High		

## **APPENDIX I**

Species number, date collected, county, elevation (meters), sex, and morphological measurements (mm) collected. "X" indicates that the measurement could not be taken because of a broken tail. (SVL=snout-vent length, HW=head width, TL=tail length, TC=tail circumference, A-G=axilla to groin length, E-N=eye to nostril length, length of 1<sup>st</sup> toe, length of 3<sup>rd</sup> toe)

												1 <sup>st</sup>	3 <sup>rd</sup>
#	Date	County	Elev.	Sex	SVL	HW	TL	тс	HD	A-G	E-N	Тое	Тое
4789	2/0/87	Kanawha	324	F	46.4	7.1	44.7	11.7	2.5	22.9	1.8	0.4	3.0
6772	2/29/41	Doddridge	366	F	57.0	8.1	52.1	10.9	4.8	30.1	2.5	0.8	2.5
6989	3/10/1992	Raleigh	451	F	80.8	11.1	97.5	21.6	4.4	42.7	4.1	0.8	3.9
3248	3/10/1966	Wirt	208	М	72.3	10.8	82.2	22.1	4.6	35.4	3.5	1.0	5.6
3292	3/13/1966	Wood	243	М	81.0	12.0	89.6	26.2	5.8	43.6	3.6	1.4	4.6
2869	3/17/1961	Putnam	205	F	78.4	11.3	97.1	25.3	3.2	21.0	2.6	0.2	2.4
3892	3/20/1968	Harrison	365	М	57.7	8.6	57.9	17.4	4.1	29.2	2.6	1.0	3.7
3893	3/20/1968	Harrison	365	М	58.6	8.5	40.7	18.1	5.1	28.1	2.6	0.9	3.5
3865	3/27/1968	Doddridge	364	F	68.2	9.5	74.6	21.7	5.1	35.6	3.0	0.7	2.8
2614	3/28/1953	Logan	204	F	54.4	8.5	Х	Х	3.2	27.0	2.2	1.1	3.8
3321	4/1/1966	Lewis	264	F	55.1	8.3	35.1	15.0	3.7	28.6	2.0	0.5	3.1
3129	4/5/1963	Jackson	183	F	79.7	11.5	82.9	23.1	5.2	39.5	3.0	1.0	3.6
3130	4/5/1963	Jackson	183	М	77.4	12.2	89.0	21.9	5.9	40.5	4.4	0.9	3.7
2868	4/7/1961	Putnam	205	F	69.6	8.7	59.3	21.9	5.4	37.1	3.1	1.1	3.9
2297	4/11/1948	Brooke	197	F	69.9	9.5	77.1	27.1	5.3	37.9	2.5	0.9	3.9
3048	4/15/1963	Roane	283	М	76.3	11.1	84.0	22.8	5.3	37.5	3.9	0.9	3.6
3111	4/15/1963	Wirt	288	М	72.9	11.5	79.9	18.8	4.7	36.7	3.6	0.7	4.0
3194	4/17/1965	Clay	243	М	73.1	10.2	77.6	19.7	5.0	37.0	3.0	1.0	3.9
2100	4/18/1948	Monongalia	294	М	77.2	11.7	73.4	28.2	5.7	40.9	3.4	2.0	5.0
3213	5/2/1965	Roane	214	F	84.0	12.0	98.1	29.1	6.2	48.1	3.1	1.2	3.9
2505	5/5/1948	Ohio	318	F	53.0	8.0	56.1	16.0	4.0	28.4	2.4	1.2	4.1
2319	5/7/1950	Gilmer	306	М	80.8	12.2	80.9	28.8	6.2	43.2	3.7	1.3	4.4
4059	5/10/1969	Kanawha	171	F	76.9	10.3	75.7	19.2	4.9	41.8	2.8	0.9	3.9
3103	5/16/1963	Ritchie	304	М	74.7	11.4	Х	Х	5.5	39.5	3.5	1.1	4.0
3096	5/16/1963	Roane	183	М	78.1	13.1	89.2	21.8	6.0	40.1	3.5	0.8	4.9
3097	5/16/1963	Roane	183	М	65.7	9.7	64.9	24.0	5.0	27.8	3.0	0.9	3.1
1563	5/18/1941	Marshall	342	F	65.3	9.2	Х	Х	4.0	33.6	3.3	0.7	3.7
1564	5/18/1941	Marshall	342	F	51.5	7.5	35.4	16.9	3.4	28.9	2.7	0.6	2.8
3574	5/21/1967	Taylor	402	М	71.1	10.1	72.0	19.0	4.7	37.3	2.8	0.9	4.3
3806	6/1/1968	Berkeley	360	М	62.3	8.8	48.8	21.1	4.6	31.8	3.4	0.9	3.7
3807	6/1/1968	Berkeley	360	F	39.6	6.3	32.2	11.2	2.5	18.7	1.7	0.6	2.1
7729	6/10/1992	Fayette	305	F	77.7	11.9	64.5	23.2	5.2	41.5	3.5	0.9	4.8
2943	6/23/1961	Wetzel	356	F	62.6	9.3	70.5	19.1	4.4	32.3	2.9	0.6	3.4
6556	6/24/1991	Monongalia	407	F	51.0	7.6	40.4	16.1	3.6	25.7	2.1	0.7	3.5
6557	6/24/1991	Monongalia	407	М	67.3	9.9	76.9	18.5	4.2	39.1	2.8	0.7	3.6
1102	6/29/1939	Cabell	179	F	49.6	6.7	49.2	7.4	2.6	24.8	2.1	0.4	2.6

#	Data	County	Elou	Sav	e\//	ЦМ	ті	то	μг		EN	1" Too	3'" T
#	Date	County	Elev.	Sex	SVL	HW			HD	A-G	E-N	10e	100
198	7/12/1935	Kanawna	183	IVI	66.9	10.0	65.8	24.9	5.1	34.7	3.4	0.7	3.3
378G	7/6/1937	Morgan	193	M	65.5	9.6	68.7	21.8	3.9	33.7	3.2	0.5	3.8
138	7/18/1935	Braxton	376	F	45.3	6.6	45.0	13.0	2.6	21.6	1.8	0.3	2.8
139	7/18/1935	Braxton	376	F	62.5	8.6	Х	Х	3.4	31.5	2.7	0.3	3.2
2373	7/21/1945	Harrison	423	М	64.3	10.3	59.0	19.7	4.4	32.2	3.3	0.6	3.3
2548	7/25/1947	Harrison	357	F	72.0	9.8	78.9	19.1	5.2	40.6	2.9	0.8	4.(
2759	7/25/1956	Pleasants	243	F	45.8	7.4	Х	Х	3.7	23.3	2.1	0.8	2.9
479A	7/28/1937	Taylor	340	F	70.5	11.1	Х	Х	5.2	39.4	2.9	0.8	2.
2724	8/8/1948	Tyler	241	Μ	62.6	9.9	60.9	24.6	4.3	32.2	3.0	0.6	3.8
2676	8/8/1956	Wood	243	F	52.1	7.7	Х	Х	4.2	25.9	2.0	0.6	3.4
502	8/9/1937	Boone	212	F	59.5	8.5	54.6	20.1	4.5	30.0	2.7	0.6	3.6
631	8/14/1938	Lincoln	197	М	63.7	9.8	Х	Х	4.6	33.0	3.3	0.7	3.
6496	8/20/1991	Fayette	378	F	56.1	9.3	42.8	19.6	4.3	28.2	2.0	1.1	4.(
639	8/22/1938	Logan	303	F	74.6	10.5	Х	Х	4.8	38.1	3.2	1.6	3.
6115	8/23/1991	Fayette	354	F	56.6	8.4	Х	Х	4.9	29.0	2.3	0.8	2.9
2419	9/0/50	Kanawha	305	F	66.6	9.8	Х	Х	4.8	33.2	2.6	0.7	3.
2678	9/13/1956	Mingo	243	М	68.1	10.2	Х	Х	5.2	34.4	3.5	1.3	4.
6745	9/15/1991	Monongalia	372	F	54.7	8.5	Х	Х	4.7	29.3	2.5	0.6	3.
6751	9/28/1991	Wayne	215	М	69.1	9.9	78.0	26.5	4.5	35.3	2.9	1.1	4.
13969	9/29/1991	Mason	182	F	44.2	6.8	45.0	16.8	3.5	22.7	2.2	0.3	2.
13974	9/29/1991	Mason	182	F	62.4	9.1	80.2	7.0	4.7	31.0	3.1	1.1	3.
13976	9/29/1991	Mason	182	F	56.2	8.1	58.0	15.3	4.2	28.4	2.3	0.6	2.9
2186	10/1/1949	Harrison	366	F	57.7	8.8	55.0	16.8	4.0	29.4	3.1	0.6	3
4554	10/10/1981	Gilmer	318	F	53.2	8.2	60.8	15.5	4.0	27.7	2.2	0.5	3
4555	10/10/1981	Gilmer	318	F	69.4	10.2	69.5	16.6	53	37.6	2.2	13	3.
4556	10/10/1981	Gilmer	318	F	61.3	0.1	41.2	11.6	5.0	30.1	2.0	0.6	3
5252	10/10/1981	Eavotto	205		56.0	9.1	74.4	10.2	1.0	25.7	2.5	1.0	2.
224h	0/22/1909	Broston	505	M	64.2	0.5	76.0	10.0	4.0	20.7	2.1	0.5	3.
760	0/20/1935	Presion	000		74.6	9.7	70.9	23.0 V	4.9	34.2	3.2	0.5	4.0
760	12/14/1930	Randolph	694		74.0	10.8	^	A	5.0	39.0	3.7	0.7	4.
1206	4/27/1941	Upsnur	587		77.1	10.3	84.4	18.9	4.5	39.4	3.8	0.6	4.
1207	4/27/1941	Upshur	587		55.5	7.9	58.4	18.0	3.7	27.9	2.6	0.8	3.
1303	9/17/1941	Hardy	470	F _	70.2	10.2	50.6	21.0	4.8	35.9	3.8	1.0	2.9
2788	4/25/1945	Mercer	728	F	59.4	9.4	65.9	22.5	4.8	31.2	2.8	0.6	3.
2499	4/29/1948	Wyoming	481	F	63.1	9.0	68.3	26.9	4.3	32.9	2.6	0.7	3.
2777	6/28/1949	Monroe	904	М	59.4	8.8	51.2	19.2	3.7	34.7	3.0	0.6	2.
2776	6/28/1949	Monroe	700	М	63.6	9.9	53.0	22.2	4.0	30.5	2.8	0.9	3.
2817	4/18/1959	Fayette	245	Μ	74.9	11.8	36.3	25.0	5.5	39.9	3.4	1.2	4.
12918	5/20/1999	Pocahontas	756- 805	F	48.6	7.8	х	х	3.5	23.1	1.8	0.7	3.
4832	4/2/1989	Fayette	465	F	73.6	10.3	71.7	20.8	4.3	37.5	3.4	1.0	4.
4980	4/16/1989	Fayette	549	М	68.8	10.4	72.3	24.7	5.5	34.4	3.5	1.0	3.
5009	4/22/1989	Summers	626	М	77.0	11.6	65.0	22.0	4.7	39.0	3.9	0.9	3.
5145	7/23/1989	Raleigh	732	F	70.7	11.0	73.0	22.8	4.4	36.0	2.8	0.4	4.
5169	7/30/1989	Fayette	488	F	80.9	10.8	Х	Х	4.0	42.6	3.7	0.9	4.
5242	8/20/1989	Fayette	561	М	66.7	9.4	76.3	22.6	4.4	32.8	3.5	1.0	2.
5255	9/2/1989	Raleigh	680	F	71.5	10.2	77.0	21.6	4.3	37.8	3.0	0.8	3.
		<b>–</b>	E 40	M	60.7	11.0		- -	57	26.2	25		4

щ	Deta	Country	Elevi	<b>6</b>	0//	1114/		<b>T</b> 0			<b>E</b> N	1 <sup>st</sup>	3
#	Date	County	Elev.	Sex	SVL	HW			HD	A-G	E-N	10e	
5415	10/1/1989	Fayette	605		50.1	7.6	65.0	18.2	4.1	29.2	2.5	0.5	4
8783	8/26/1990		811		73.1	10.5	77.7	28.6	5.3	37.8	3.0	0.7	
8785	8/26/1990		811		67.0	11.0	71.4	26.1	5.1	38.8	3.0	0.7	
8823	9/16/1990		811	F	65.8	10.0	57.6	25.8	5.3	36.9	3.3	1.0	4
8822	9/16/1990	Tucker	811	F	62.5	10.3	42.8	23.6	4.9	29.3	3.2	0.9	-
6530	5/15/1991	Fayette	494	M	73.8	10.6	84.3	24.0	4.8	38.6	3.3	1.2	
6661	5/21/1991	Fayette	457	F	67.4	10.0	81.6	19.4	3.8	38.9	3.5	0.9	
5761	8/20/1991	Fayette	686	M	69.0	11.0	70.6	20.6	4.6	35.5	3.7	1.0	2
5762	8/20/1991	Fayette	686	М	68.5	11.7	78.6	23.9	5.5	36.8	3.8	1.5	
6690	8/23/1991	Fayette	488	F	78.2	10.6	82.8	24.9	5.2	41.0	3.4	0.9	
6694	8/23/1991	Fayette	488	F	64.8	9.0	60.2	20.8	4.5	34.0	2.8	0.6	:
6696	8/23/1991	Fayette	488	М	69.4	9.7	77.6	24.1	5.2	35.6	3.5	0.8	1
6020	9/11/1991	Fayette	600	М	84.8	11.9	53.7	24.0	7.4	42.0	4.7	1.2	4
5861	10/24/1991	Fayette	550	F	80.9	11.4	83.1	20.9	5.3	40.1	3.7	1.6	
6021	11/11/1991	Fayette	600	F	67.3	9.0	72.5	21.4	4.4	32.6	3.1	0.7	
6357	12/6/1991	Fayette	578	F	57.4	8.7	58.7	18.6	3.8	30.6	2.7	1.0	:
6360	12/6/1991	Fayette	578	М	66.9	10.2	77.5	23.4	5.4	35.0	3.2	1.0	
6959	3/10/1992	Summers	507	F	72.2	8.8	81.8	18.8	4.4	39.1	3.5	0.8	
6961	3/10/1992	Summers	507	F	77.5	10.7	84.2	26.6	4.8	39.7	3.5	1.0	
6966	3/10/1992	Summers	507	F	67.9	8.9	76.2	16.4	3.8	34.2	2.8	0.8	
6958	3/10/1992	Summers	507	F	76.9	11.2	97.4	22.5	5.4	41.0	3.4	1.1	
6915	3/10/1992	Fayette	487	М	68.2	9.7	40.3	21.4	4.5	36.0	3.1	1.4	
6916	3/10/1992	Fayette	487	М	61.1	8.2	38.6	17.3	3.3	33.1	2.9	0.9	
6917	3/10/1992	Fayette	487	М	66.7	8.7	71.9	18.8	4.8	35.6	3.2	1.0	
6962	3/10/1992	Summers	507	М	73.5	9.7	42.7	20.2	4.2	36.7	3.3	0.9	
6964	3/10/1992	Summers	507	М	64.2	9.4	76.4	7.2	4.0	34.1	3.1	0.7	
7189	5/19/1992	Raleigh	732	F	66.8	10.4	59.3	24.6	5.3	33.7	2.8	0.9	4
7212	5/19/1992	Raleigh	732	F	66.2	9.8	43.3	16.9	4.1	35.1	2.8	0.5	
7148	5/19/1992	Raleigh	732	М	74.7	11.4	67.0	22.0	5.0	37.5	3.2	1.0	
7204	5/19/1992	Raleigh	732	М	65.3	9.5	54.8	19.6	3.9	33.7	2.7	0.7	
7169	5/20/1992	Raleigh	732	М	68.1	11.0	37.5	22.0	4.2	33.8	3.3	0.7	
7205	5/20/1992	Raleigh	732	М	63.3	8.9	68.3	17.0	3.7	32.2	3.1	1.0	
7459	6/9/1992	Favette	503	F	65.1	10.4	63.6	22.7	3.9	33.2	2.9	0.6	
7460	6/9/1992	Fayette	503	F	68.7	11.1	50.0	22.3	4.0	36.2	3.1	1.0	
7462	6/9/1992	Favette	503	М	68.4	10.8	46.1	23.3	4.9	34.5	3.3	1.0	
7705	6/10/1992	Favette	488	F	67.5	9.7	Х	Х	4.4	32.7	3.0	0.9	
7704	6/10/1992	Favette	488	M	70.9	10.9	67.1	23.7	5.8	35.2	3.7	0.8	
7108	6/11/1992	Favette	533	F	64.9	10.2	48.0	25.3	4.3	36.7	2.5	0.8	t.
7877	12/31/1002	Raleigh	732	F	45.6	6.8	30.1	15 3	34	21.1	23	0.6	
9069	6/10/1002	Preston	518	F	65.3	10.0	60.3	19.5	42	30.6	2.0	0.5	
101/0	11/20/1002	Ralaich	720		50.0	87	67 4	22.4	∠ ⊿ ∩	20.0	2.0	0.0	
11746	6/11/1000	Randolph	201	M	69.0	10.7	52.2	10.0	ч.Э Л F	23.9	3.2	0.0	
12045	6/17/1998	Bondlatar	094		64.0	10.3	5Z.Z	19.9	4.5	33.0	3.3	0.9	
13045	7/40/4000	Pendleton	695	IVI	01.3	0.Ŏ	04.3	9.7	4.3	32.0	2.1	0.7	$\vdash$
13025	7/16/1998	ivionroe	578 756-		70.2	δ.5 10.5	74.6 V	17.3 V	4.2	35.4	3.2	0.8	
12735	7/17/1999	Pocahontas	805		70.5	10.5	~	~	4/			0.9	

Appendix I	Continued												
#	Date	County	Elev.	Sex	SVL	нw	TL	тс	HD	A-G	E-N	1 <sup>st</sup> Toe	3 <sup>ra</sup> Toe
12679	7/17/1999	Pocahontas	756- 805	М	65.9	10.4	х	х	5.1	33.8	3.4	1.0	3.9
12653	9/10/1999	Pocahontas	756- 805	М	70.6	10.9	х	х	4.3	35.3	3.5	0.8	3.7
13289	7/2/2000	Pocahontas	835- 853	м	65.5	11.1	х	х	4.4	36.3	3.9	0.8	3.1
13313	7/4/2000	Pocahontas	756- 805	М	69.2	10.2	х	Х	4.1	36.3	3.5	0.5	2.8
13268	9/2/2000	Pocahontas	850- 853	М	67.9	9.5	х	х	5.4	32.0	3.0	0.7	3.4
13346	9/2/2000	Pocahontas	850- 853	М	67.7	10.1	х	Х	5.7	35.5	2.7	0.6	3.2
13589	10/6/2000	Pocahontas	756- 805	М	61.8	9.4	х	х	3.7	27.4	3.2	0.6	3.5
13594	10/6/2000	Pocahontas	756- 805	М	60.1	9.3	х	х	4.8	31.9	2.5	0.6	3.1
14357	5/11/2001	Fayette	508	F	82.0	11.6	93.6	24.8	5.1	45.1	3.2	1.2	4.3
14194	10/13/2001	Pocahontas	756- 805	М	67.4	9.2	х	Х	4.0	31.5	3.0	0.6	3.3
14239	10/13/2001	Pocahontas	835- 853	М	57.9	8.3	х	х	3.2	30.4	2.1	0.5	3.4
484	7/12-18/37	Grant	843	F	71.5	10.5	Х	Х	5.1	38.7	3.0	0.6	3.4
653	9/20-22/38	Upshur	540	F	49.4	7.7	Х	Х	3.0	24.9	1.9	0.4	2.9
5324	4/14/1943	Pocahontas	1000	М	61.5	10.0	Х	Х	3.7	29.9	3.5	0.7	2.9
13547	5/9/2000	Pocahontas	975- 1036	F	63.4	8.9	х	Х	3.5	35.3	2.7	0.7	3.4
13335	5/9/2000	Pocahontas	1186- 1244	F	61.7	9.0	х	х	3.7	31.0	3.1	0.6	3.3
13311	5/10/2000	Pocahontas	902- 1183	М	65.1	9.6	х	х	4.4	38.0	2.7	0.7	3.1
9862	5/20/1993	Pocahontas	1264	М	67.4	10.3	64.3	16.4	4.0	30.2	3.4	0.9	3.9
13272	5/26/2000	Pocahontas	902- 1183	F	68.2	9.8	х	х	5.5	37.0	2.9	0.7	3.5
10971	5/27/1993	Randolph	990	F	67.5	10.6	68.8	18.5	3.9	33.2	3.1	0.8	3.3
10973	5/27/1993	Randolph	990	F	53.7	8.4	42.3	17.5	3.4	28.0	1.9	0.6	3.1
14179	5/28/2001	Pocahontas	1186- 1244	М	54.8	7.2	х	х	3.5	25.7	2.7	0.6	3.1
14224	5/28/2001	Pocahontas	902- 1183	М	67.4	11.6	х	х	3.9	36.1	3.7	0.7	3.4
14242	5/28/2001	Pocahontas	1186- 1244	М	61.6	11.3	х	х	3.7	31.1	2.5	0.7	3.6
9299	6/10/1993	Preston	1219	М	65.0	9.7	72.0	15.9	3.6	29.9	2.9	0.7	3.6
9300	6/10/1993	Preston	1219	М	63.2	9.5	70.6	17.9	3.3	30.4	2.6	0.6	3.6
9301	6/10/1993	Preston	1219	М	60.8	9.8	62.7	17.7	3.6	29.9	2.5	0.8	3.8
9304	6/10/1993	Preston	1219	М	54.5	9.9	32.3	16.2	4.3	31.7	3.1	0.8	3.5
9306	6/10/1993	Preston	1219	М	59.4	9.4	55.8	20.8	4.1	30.4	2.6	0.8	3.5
9307	6/10/1993	Preston	1219	F	61.4	9.0	56.9	11.6	3.2	31.2	2.7	0.5	3.0
9308	6/10/1993	Preston	1219	М	62.0	9.4	69.0	14.1	3.4	31.0	3.2	1.0	3.1
9436	6/16/1993	Webster	1250- 1280	F	66.3	9.3	71.4	15.2	3.7	34.4	2.5	0.4	4.0
9437	6/16/1993	Webster	1250- 1280	F	66.7	9.3	71.9	15.0	4.0	39.8	2.9	0.7	2.8
9438	6/16/1993	Webster	1250- 1280	М	66.7	10.4	40.1	17.0	3.8	34.6	2.6	0.6	2.6

#	Date	County	Elev.	Sex	SVL	нw	TL	тс	HD	A-G	E-N	1 <sup>st</sup> Toe	
0.400	C/4C/4000	) Mahatan	1250-	_	74.0	0.0	50.0	47.0	4.0	24.0	2.0	0.7	
9439	6/16/1993	vvebster	1280		71.2	9.9	56.8	17.9	4.3	34.6	3.0	0.7	┢
116	6/27/1935	Pocanontas	945 975-	IVI	64.2	9.3	73.2	22.3	4.4	32.4	3.2	0.9	┢
13259	7/3/2000	Pocahontas	1036	F	61.6	9.0	Х	Х	4.3	32.2	2.6	0.8	
13230	7/5/2000	Pocahontas	1036- 1112	F	64.4	9.8	х	х	3.9	35.7	3.2	1.0	
12825	7/8/1999	Pocahontas	1186- 1244	М	64.0	11.9	х	х	4	34.4	2.8	0.7	
12876	7/10/1999	Pocahontas	975- 1036	F	60.9	9.0	х	Х	4.3	31.0	2.6	0.7	
12831	7/11/1999	Pocahontas	975- 1036	м	67.5	10.3	х	х	3.8	35.3	3.1	1.0	
12720	7/18/1999	Pocahontas	902- 1183	М	65.7	10.1	х	х	4.0	36.5	3.0	0.7	
12881	7/18/1999	Pocahontas	902- 1183	F	62.4	10.8	х	х	3.8	34.0	3.0	0.7	Γ
9401	7/23/1993	Pocahontas	1244- 1259	м	69.1	11.3	49.8	20.7	4.4	36.4	3.0	0.7	Ī
9403	7/23/1993	Pocahontas	1244- 1259	F	69.0	10.5	62.0	21.4	3.8	37.7	2.6	0.5	Ī
14255	7/26/2001	Pocahontas	1036- 1113	м	64.2	10.0	х	х	4.0	34.7	2.4	0.5	Ī
14295	7/26/2001	Pocahontas	1036- 1113	м	64.6	10.4	х	х	4.5	33.6	3.4	0.6	
9145	8/17/1993	Pocahontas	975- 1134	F	62.2	9.9	69.4	20.0	4.4	33.7	2.7	0.6	
9146	8/17/1993	Pocahontas	975- 1134	М	66.1	10.0	71.1	20.6	4.1	32.6	3.1	0.6	
9147	8/17/1993	Pocahontas	975- 1134	F	68.8	10.6	54.7	20.2	3.8	37.0	3.2	1.1	
9599	8/17/1993	Pocahontas	1364	М	47.1	7.0	Х	Х	3.3	22.6	1.9	0.5	
1957	8/27/1947	Tucker	975	F	60.3	9.0	Х	Х	4.5	30.6	2.9	0.7	
13203	9/2/2000	Pocahontas	902- 1183	F	54.5	9.0	х	х	3.5	28.5	2.7	0.6	
13236	9/2/2000	Pocahontas	902- 1183	F	73.1	10.6	х	х	4.3	37.3	2.8	0.7	
13257	9/2/2000	Pocahontas	902- 1183	F	74.0	11.5	х	х	4.0	35.7	3.2	0.3	L
13325	9/2/2000	Pocahontas	902- 1183	М	63.5	10	х	х	4.3	33.9	2.4	0.5	
13383	9/2/2000	Pocahontas	902- 1183	F	70.2	9.9	х	х	5.1	39.3	3.4	0.6	
14290	9/8/2001	Pocahontas	1036- 1113	М	59.5	8.9	x	x	3.7	27.1	3.3	0.6	
12676	9/11/1999	Pocahontas	1186- 1244	М	68.2	11.5	x	x	5.1	36.3	3.5	0.9	
12725	9/11/1999	Pocahontas	902- 1183	М	67.4	10.5	х	х	4.0	32.8	3.2	0.6	
12824	9/11/1999	Pocahontas	902- 1183	F	55.4	8.3	х	х	2.7	26.8	2.4	1.0	ſ
13656	10/3/1998	Pocahontas	902- 1183	М	60.6	9.4	х	х	3.4	32.2	2.5	0.7	ſ
			002-	1									T

Appendix I	Continued												
#	Date	County	Elev.	Sex	SVL	нพ	TL	тс	HD	A-G	E-N	1 <sup>st</sup> Toe	3 <sup>rd</sup> Toe
13687	10/3/1998	Pocahontas	902- 1183	F	61.9	9.8	х	х	4.3	30.6	2.99	0.7	3.6
13712	10/3/1998	Pocahontas	902- 1183	F	63.1	9.9	х	х	4.2	31.8	3.0	0.6	3.0
13231	10/6/2000	Pocahontas	1036- 1113	F	46.6	7.8	х	х	2.9	20.2	2.3	0.6	2.4
13316	10/6/2000	Pocahontas	1036- 1113	М	58.5	9.2	х	х	4.1	26.6	2.7	0.6	3.3
13701	10/10/1998	Pocahontas	975- 1036	F	57.7	9.6	х	Х	4.9	34.6	2.4	1.2	3.5
14187	10/13/2001	Pocahontas	1036- 1113	М	62.5	9.6	х	х	4.1	38.3	3.4	0.7	3.4
9166	11/23/1993	Pocahontas	1341- 1433	М	69.3	10.4	77.0	20	3.5	35.1	3.2	0.8	4.7
9167	11/23/1993	Pocahontas	1341- 1433	М	70.7	10.0	76.8	20	4.3	34.8	2.9	0.9	3.0

## **APPENDIX II**

Reproductive	data	of all	males.
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Species #	Date	Elev. (meters)	Left Anterior Testies	Left Anterior Vas Def	Right Anterior Testies	Right Anterior Vas Def	Left Posterior Testies	Left Posterior Vas Def	Right Posterior Testies	Right Posterior Vas Def
3248	3/10/1966	273	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm
3292	3/13/1966	240	Sperm	No Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	Sperm
3892	3/20/1968	362	No Sperm	Sperm	Sperm	No Sperm	Sperm	Sperm	Sperm	Sperm
3893	3/20/1968	362	Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	Sperm	Sperm
3130	4/5/1963	247	Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
3048	4/15/1963	281	No Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
3111	4/15/1963	273	Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
3194	4/17/1965	357	Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
2100	4/18/1948	401	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm
2319	5/7/1950	306	Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
3103	5/16/1963	295	No Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
3096	5/16/1963	281	Sperm	Sperm	No Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm
3097	5/16/1963	281	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm
3574	5/21/1967	408	No Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	Sperm	Sperm
3806	6/1/1968	197	No Sperm	No Sperm	No Sperm	No Sperm	No Sperm	No Sperm	No Sperm	Sperm
6557	6/24/1991	401	No Sperm	Sperm	No Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm
198	7/12/1935	321	No Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
378G	7/6/1937	283	Sperm	No Sperm	No Sperm	Sperm	No Sperm	Sperm	No Sperm	No Sperm
2373	7/21/1945	362	No Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm	Sperm	No Sperm
2724	8/8/1948	287	No Sperm	Sperm	No Sperm	No Sperm	Sperm	No Sperm	Sperm	Sperm
631	8/14/1938	285	No Sperm	No Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	No Sperm
2678	9/13/1956	402	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm
6751	9/28/1991	264	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm
6915	3/10/1992	605	No Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
6916	3/10/1992	605	Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	Sperm	Sperm
6917	3/10/1992	605	Sperm	Sperm	No Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm
6962	3/10/1992	665	No Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
6964	3/10/1992	665	No Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
4980	4/16/1989	549	No Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
2817	4/18/1959	605	Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
5009	4/22/1989	665	No Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm

Continued					-				
Date	Elev. (meters)	Left Anterior Testies	Left Anterior Vas Def	Right Anterior Testies	Right Anterior Vas Def	Left Posterior Testies	Left Posterior Vas Def	Right Posterior Testies	Right Posterior Vas Def
5/15/1991	494	No Sperm	No Sperm	Sperm	Sperm	No Sperm	Sperm	Sperm	Sperm
5/19/1992	732	No Sperm	No Sperm	No Sperm	No Sperm	No Sperm	No Sperm	No Sperm	No Sperm
5/19/1992	732	No Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm	Sperm	No Sperm
5/20/1992	732	No Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
5/20/1992	732	No Sperm	Sperm	No Sperm	No Sperm	No Sperm	Sperm	No Sperm	Sperm
6/9/1992	503	Sperm	No Sperm	No Sperm	No Sperm	No Sperm	No Sperm	Sperm	No Sperm
6/10/1992	605	Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm	Sperm	Sperm
6/11/1998	894	Sperm	No Sperm	No Sperm	No Sperm	No Sperm	No Sperm	No Sperm	No Sperm
6/28/1949	708	Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm	Sperm	Sperm
6/28/1949	708	Sperm	Sperm	No Sperm	Sperm	Sperm	Sperm	No Sperm	No Sperm
6/17/1998	773	Sperm	No Sperm	No Sperm	Sperm	No Sperm	No Sperm	No Sperm	Sperm
7/16/1998	708	Sperm	No Sperm	No Sperm	No Sperm	No Sperm	Sperm	No Sperm	No Sperm
8/20/1989	561	Sperm	Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	No Sperm
8/20/1991	686	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm
8/20/1991	686	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm
8/23/1991	605	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm
8/28/1935	627	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm
9/11/1991	605	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm
10/1/1989	549	Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	Sperm	Sperm
10/1/1989	605	Sperm	No Sperm	Sperm	Sperm	No Sperm	Sperm	Sperm	No Sperm
12/6/1991	605	Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
12/14/1936	894	Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
4/14/1943	967	No Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
5/20/1993	967	No Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm	No Sperm	No Sperm
6/10/1993	1219	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm
6/10/1993	1219	No Sperm	No Sperm	No Sperm	No Sperm	Sperm	Sperm	No Sperm	Sperm
6/10/1993	1219	No Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm	No Sperm	Sperm
6/10/1993	1219	Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm	Sperm	Sperm
6/10/1993	1219	No Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	No Sperm	Sperm
6/10/1993	1219	Sperm	Sperm	Sperm	No Sperm	Sperm	Sperm	Sperm	Sperm
6/16/1993	1245- 1280	No Sperm	Sperm	Sperm	Sperm	No Sperm	No Sperm	Sperm	No Sperm
6/16/1993	1250- 1280	No Sperm	No Sperm	No Sperm	Sperm	Sperm	Sperm	Sperm	Sperm
	Continued Date 5/15/1991 5/19/1992 5/20/1992 5/20/1992 6/9/1992 6/10/1993 6/10/1993 6/28/1949 6/28/1949 6/28/1949 6/28/1949 6/28/1949 8/20/1981 8/20/1981 8/20/1981 8/20/1981 8/20/1981 8/20/1981 8/20/1981 10/1/1989 10/1/1989 10/1/1989 10/1/1989 10/1/1989 10/1/1989 10/1/1989 10/1/1989 10/1/1989 10/1/1989 10/1/1989 10/1/1983 6/10/1993 6/10/1993 6/10/1993 6/10/1993	Continued   Date Elev. (meters)   5/15/1991 494   5/19/1992 732   5/20/1992 732   5/20/1992 732   5/20/1992 732   5/20/1992 732   6/9/1992 503   6/10/1992 605   6/11/1998 894   6/28/1949 708   6/28/1949 708   6/28/1949 708   6/28/1949 708   8/20/1988 561   8/20/1989 561   8/20/1991 686   8/20/1991 686   8/20/1991 605   8/20/1991 605   8/20/1991 605   8/20/1991 605   9/11/1989 605   10/11989 549   10/11989 605   12/6/1991 605   12/14/1936 894   4/14/1943 967   5/20/1993 1219   6/10/1993 12	ContinuedContinuedLeft Anterior TestiesDateNo5/15/1991494Sperm5/15/1992732Sperm5/19/1992732Sperm5/19/1992732Sperm5/20/1992732Sperm5/20/1992732Sperm5/20/1992732Sperm5/20/1992732Sperm6/9/1992503Sperm6/10/1992605Sperm6/11/1998894Sperm6/28/1949708Sperm6/28/1949708Sperm6/28/1949708Sperm6/28/1949708Sperm6/28/1949708Sperm7/16/1998708Sperm8/20/1989561Sperm8/20/1989561Sperm8/20/1989561Sperm8/20/1989561Sperm8/20/1989605Sperm8/20/1989605Sperm8/20/1989605Sperm8/20/1991605Sperm10/1/1989605Sperm10/1/1989605Sperm10/1/1989605Sperm10/1/19891219Sperm6/10/19931219Sperm6/10/19931219Sperm6/10/19931219Sperm6/10/19931219Sperm6/10/19931219Sperm6/10/19931219Sperm6/10/19931219Sperm <td>ContinuedLeft Anterior TestiesLeft Anterior Vas DefDateRo SpermNo Sperm5/15/1991494Sperm5/15/1992732Sperm5/19/1992732Sperm5/19/1992732Sperm5/19/1992732Sperm5/20/1992732Sperm5/20/1992732Sperm5/20/1992732Sperm5/20/1992732Sperm6/9/1992503Sperm6/10/1992605Sperm6/10/1992605Sperm6/11/1998894Sperm6/28/1949708Sperm6/28/1949708Sperm6/17/1998773Sperm6/17/1998708Sperm7/16/1998708Sperm8/20/1989561Sperm8/20/1989561Sperm8/20/1989561Sperm8/20/1989561Sperm8/20/1989561Sperm8/20/1989561Sperm8/20/19895627Sperm8/21/19106055Sperm8/21/19116055Sperm8/21/1912SpermSperm10/1/1989784Sperm10/1/1980627Sperm10/1/1981605Sperm10/1/1982SpermSperm10/1/19831219Sperm6/10/19931219Sperm6/10/19931219Sperm<td< td=""><td>Continued Elev. (meters) Left Anterior Testies Left Anterior Vas Def Right Anterior Testies   5/15/1991 494 Sperm No Sperm Sperm   5/19/1992 732 Sperm No Sperm No Sperm   5/19/1992 732 Sperm No Sperm No Sperm   5/20/1992 732 Sperm Sperm No Sperm   5/20/1992 732 Sperm No Sperm No Sperm   6/9/1992 733 Sperm No Sperm No Sperm   6/9/1992 503 Sperm No Sperm No Sperm   6/10/1992 605 Sperm No Sperm No Sperm   6/11/1998 894 Sperm No Sperm No Sperm   6/28/1949 708 Sperm No Sperm No Sperm   6/11/1998 7073 Sperm No Sperm No Sperm   6/28/1949 708 Sperm No Sperm No Sperm   6/11/1998 6761 Sperm Sperm Sperm</td><td>Continued Left Anterior Left Anterior Right Anterior Right Anterior Right Anterior   5/15/1919 494 Spern No Spern Spern Spern Spern Spern Spern Spern Spern No Spern Spern No Spern Spern Spern Spern No Spern No Spern No Spern No Spern Spern Spern No Spern Spern</br></td><td>Continued Left Anterior Testies Left Anterior Testies Right Anterior Testies Right Anterior Testies Left Anterior Testies   5/15/1991 494 No No Sperm Sperm No Sperm   5/15/1992 732 Sperm No Sperm No Sperm No Sperm   5/19/1992 732 Sperm Sperm No Sperm No Sperm No Sperm   5/20/1992 732 Sperm Sperm No Sperm No Sperm No Sperm   5/20/1992 732 Sperm Sperm No Sperm No Sperm No Sperm   6/9/1992 605 Sperm Sperm No Sperm No Sperm No Sperm   6/10/1992 605 Sperm No Sperm No Sperm No Sperm No Sperm   6/28/1949 708 Sperm No Sperm No Sperm No Sperm No Sperm   6/28/1949 708 Sperm Sperm No Sperm No Sperm Sperm   6/11/1998 6/12 Sperm Sperm</td><td>Continued Continued Left Pate Left Anterior (meters) Left Anterior Testics Right Anterior Vas Def Right Posterior Left Posterior <thleft Posterior Left Posterior <th< td=""><td>Continued Left (meters) Left Anterior Testics Right Anterior Vas Def Right Anterior Vas Def Right Posterior Left Posterior Right Posterior   5/15/1991 494 Sperm No Sperm Sperm No Sperm Sperm No Sperm</td></th<></thleft </td></td<></td>	ContinuedLeft Anterior TestiesLeft Anterior Vas DefDateRo SpermNo Sperm5/15/1991494Sperm5/15/1992732Sperm5/19/1992732Sperm5/19/1992732Sperm5/19/1992732Sperm5/20/1992732Sperm5/20/1992732Sperm5/20/1992732Sperm5/20/1992732Sperm6/9/1992503Sperm6/10/1992605Sperm6/10/1992605Sperm6/11/1998894Sperm6/28/1949708Sperm6/28/1949708Sperm6/17/1998773Sperm6/17/1998708Sperm7/16/1998708Sperm8/20/1989561Sperm8/20/1989561Sperm8/20/1989561Sperm8/20/1989561Sperm8/20/1989561Sperm8/20/1989561Sperm8/20/19895627Sperm8/21/19106055Sperm8/21/19116055Sperm8/21/1912SpermSperm10/1/1989784Sperm10/1/1980627Sperm10/1/1981605Sperm10/1/1982SpermSperm10/1/19831219Sperm6/10/19931219Sperm6/10/19931219Sperm <td< td=""><td>Continued Elev. (meters) Left Anterior Testies Left Anterior Vas Def Right Anterior Testies   5/15/1991 494 Sperm No Sperm Sperm   5/19/1992 732 Sperm No Sperm No Sperm   5/19/1992 732 Sperm No Sperm No Sperm   5/20/1992 732 Sperm Sperm No Sperm   5/20/1992 732 Sperm No Sperm No Sperm   6/9/1992 733 Sperm No Sperm No Sperm   6/9/1992 503 Sperm No Sperm No Sperm   6/10/1992 605 Sperm No Sperm No Sperm   6/11/1998 894 Sperm No Sperm No Sperm   6/28/1949 708 Sperm No Sperm No Sperm   6/11/1998 7073 Sperm No Sperm No Sperm   6/28/1949 708 Sperm No Sperm No Sperm   6/11/1998 6761 Sperm Sperm Sperm</td><td>Continued Left Anterior Left Anterior Right Anterior Right Anterior Right Anterior   5/15/1919 494 Spern No Spern Spern Spern Spern Spern Spern Spern Spern No Spern Spern No Spern Spern Spern Spern No Spern No Spern No Spern No Spern Spern Spern No Spern Spern</br></td><td>Continued Left Anterior Testies Left Anterior Testies Right Anterior Testies Right Anterior Testies Left Anterior Testies   5/15/1991 494 No No Sperm Sperm No Sperm   5/15/1992 732 Sperm No Sperm No Sperm No Sperm   5/19/1992 732 Sperm Sperm No Sperm No Sperm No Sperm   5/20/1992 732 Sperm Sperm No Sperm No Sperm No Sperm   5/20/1992 732 Sperm Sperm No Sperm No Sperm No Sperm   6/9/1992 605 Sperm Sperm No Sperm No Sperm No Sperm   6/10/1992 605 Sperm No Sperm No Sperm No Sperm No Sperm   6/28/1949 708 Sperm No Sperm No Sperm No Sperm No Sperm   6/28/1949 708 Sperm Sperm No Sperm No Sperm Sperm   6/11/1998 6/12 Sperm Sperm</td><td>Continued Continued Left Pate Left Anterior (meters) Left Anterior Testics Right Anterior Vas Def Right Posterior Left Posterior <thleft Posterior Left Posterior <th< td=""><td>Continued Left (meters) Left Anterior Testics Right Anterior Vas Def Right Anterior Vas Def Right Posterior Left Posterior Right Posterior   5/15/1991 494 Sperm No Sperm Sperm No Sperm Sperm No Sperm</td></th<></thleft </td></td<>	Continued Elev. (meters) Left Anterior Testies Left Anterior Vas Def Right Anterior Testies   5/15/1991 494 Sperm No Sperm Sperm   5/19/1992 732 Sperm No Sperm No Sperm   5/19/1992 732 Sperm No Sperm No Sperm   5/20/1992 732 Sperm Sperm No Sperm   5/20/1992 732 Sperm No Sperm No Sperm   6/9/1992 733 Sperm No Sperm No Sperm   6/9/1992 503 Sperm No Sperm No Sperm   6/10/1992 605 Sperm No Sperm No Sperm   6/11/1998 894 Sperm No Sperm No Sperm   6/28/1949 708 Sperm No Sperm No Sperm   6/11/1998 7073 Sperm No Sperm No Sperm   6/28/1949 708 Sperm No Sperm No Sperm   6/11/1998 6761 Sperm Sperm Sperm	Continued Left Anterior Left Anterior Right 	Continued Left Anterior Testies Left Anterior Testies Right Anterior Testies Right Anterior Testies Left Anterior Testies   5/15/1991 494 No No Sperm Sperm No Sperm   5/15/1992 732 Sperm No Sperm No Sperm No Sperm   5/19/1992 732 Sperm Sperm No Sperm No Sperm No Sperm   5/20/1992 732 Sperm Sperm No Sperm No Sperm No Sperm   5/20/1992 732 Sperm Sperm No Sperm No Sperm No Sperm   6/9/1992 605 Sperm Sperm No Sperm No Sperm No Sperm   6/10/1992 605 Sperm No Sperm No Sperm No Sperm No Sperm   6/28/1949 708 Sperm No Sperm No Sperm No Sperm No Sperm   6/28/1949 708 Sperm Sperm No Sperm No Sperm Sperm   6/11/1998 6/12 Sperm Sperm	Continued Continued Left Pate Left Anterior (meters) Left Anterior Testics Right Anterior Vas Def Right Posterior Left Posterior <thleft Posterior Left Posterior <th< td=""><td>Continued Left (meters) Left Anterior Testics Right Anterior Vas Def Right Anterior Vas Def Right Posterior Left Posterior Right Posterior   5/15/1991 494 Sperm No Sperm Sperm No Sperm Sperm No Sperm</td></th<></thleft 	Continued Left (meters) Left Anterior Testics Right Anterior Vas Def Right Anterior Vas Def Right Posterior Left Posterior Right Posterior   5/15/1991 494 Sperm No Sperm Sperm No Sperm Sperm No Sperm

Appendix II	Continued									
Species #	Date	Elev. (meters)	Left Anterior Testies	Left Anterior Vas Def	Right Anterior Testies	Right Anterior Vas Def	Left Posterior Testies	Left Posterior Vas Def	Right Posterior Testies	Right Posterior Vas Def
116	6/27/1935	967	Sperm	Sperm	Sperm	No Sperm	Sperm	Sperm	No Sperm	No Sperm
12825	7/8/1999	1186- 1244	No Sperm	Sperm	No Sperm	No Sperm	Sperm	No Sperm	Sperm	Sperm
9401	7/23/1993	1244- 1259	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	No Sperm
9146	8/17/1993	975-1134	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm
9166	11/23/1993	1341- 1433	Sperm	No Sperm	Sperm	No Sperm	Sperm	Sperm	Sperm	Sperm
9167	11/23/1993	1341- 1433	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm	Sperm

## **APPENDIX III**

Reproductive data of all females.

Species #	Date	Elev. (meters)	SV L (mm)	Volume (ml)	# of Follicles	Average Diameter (mm)
4789	2/0/87	321	46.4	0.1		<i>,</i>
6772	2/29/41	325	57.03	0.4		
6989	3/10/1992	451	80.79	2.5	32	3.5
2869	3/17/1961	248	78.41	0.2		
3865	3/27/1968	325	68.21	1		
2614	3/28/1953	432	54.41	0.3		
3321	4/1/1966	369	55.14	0.6		
3129	4/5/1963	247	79.72	3.2	29	2.1
2868	4/7/1961	248	69.61	2.8	27	1.8
2297	4/11/1948	313	69.92	7	25	3.4
3213	5/2/1965	281	84.03	8.8	29	3.4
2505	5/5/1948	318	53.03	0.2		
4059	5/10/1969	321	76.92	4.6	gravid eggs all smashed	
1563	5/18/1941	342	65.3	0.2		
1564	5/18/1941	342	51.53	1		
3807	6/1/1968	197	39.64	0.1		
7729	6/10/1992	305	77.71	0.8		
2943	6/23/1961	356	62.58	1.8		
6556	6/24/1991	401	51.04	1.5		
1102	6/29/1939	229	49.57	0.2		
138	7/18/1935	373	45.25	0.5		
139	7/18/1935	376	62.52	0.6		
2548	7/25/1947	362	72.02	2		
2759	7/25/1956	279	45.84	0.2		
479A	7/28/1937	408	70.54	0.4		
2676	8/8/1956	240	52.12	1.1		
502	8/9/1937	394	59.47	2.1		
6496	8/20/1991	378	56.14	0.8		
639	8/22/1938	432	74.62	1.6		
6115	8/23/1991	354	56.61	1		
2419	9/0/50	321	66.59	1.8		
6745	9/15/1991	401	54.68	1.8		
13969	9/29/1991	218	44.21	0.6		
13974	9/29/1991	218	62.36	1.8		
13976	9/29/1991	218	56.22	0.2		
2186	10/1/1949	362	57.73	2		

Appendix II	Continued					
Species #	Date	Elev. (meters)	SV L (mm)	Volume (ml)	# of Follicles	Average Diameter (mm)
4554	10/10/1981	306	53.18	0.2		
4555	10/10/1981	306	69.43	2		
4556	10/10/1981	306	61.25	0.8		
5352	10/22/1989	305	56.89	1.2		
6959	3/10/1992	665	72.21	1		
6961	3/10/1992	665	77.47	2.1		
6966	3/10/1992	665	67.94	1.7		
6958	3/10/1992	665	76.91	2		
4832	4/2/1989	605	73.59	1		
2788	4/25/1945	775	59.44	1.2		
1206	4/27/1941	559	77.13	2		
2499	4/29/1948	566	63.08	1.7		
14357	5/11/2001	605	82.01	16.5	31	4.6
7189	5/19/1992	732	66.81	1.4		
7212	5/19/1992	732	66.15	1.9		
6661	5/21/1991	605	67.44	1.8	10	3.1
7459	6/9/1992	503	65.13	1.5		
7460	6/9/1992	503	68.65	0.5		
7705	6/10/1992	605	67.52	1.8		
9069	6/10/1993	627	65.28	0.8		
7108	6/11/1992	533	64.9	1.8		
484	7/12-18/37	642	71.52	2		
12735	7/17/1999	756-805	70.25	1.4		
12779	7/17/1999	835-853	70.66	1.6		
5145	7/23/1989	732	70.72	1.6		
5169	7/30/1989	488	80.85	2.1		
6690	8/23/1991	605	78.21	1.8		
8783	8/26/1990	830	73.08	2.1	37	3.3
8785	8/26/1990	830	67.03	2.8	18	2.4
8823	9/16/1990	830	65.78	2.8	28	2.4
8822	9/16/1990	830	62.52	5.8	19	3.0
653	9/20-22/38	559	49.42	1.6		
5861	10/24/1991	605	80.91	1.8		
6021	11/11/1991	605	67.33	1.2		
10149	11/29/1993	732	58.98	0.5		
6357	12/6/1991	605	57.39	0.4		
7877	12/31/1992	732	45.62	0.2		
6694	8/23/1991	605	64.84	1		
1207	4/27/1941	559	55.47	1.6		
10971	5/27/1993	1014	67.52	0.8		
10973	5/27/1993	1014	53.72	0.2		

Appendix III Continued						
Species #	Date	Elev. (meters)	SV L (mm)	Volume (ml)	# of Follicles	Average Diameter (mm)
9307	6/10/1993	1219	61.35	1		
9436	6/16/1993	1250- 1280	66.3	0.4		
9437	6/16/1993	1250- 1280	66.68	1.2		
9439	6/16/1993	1250- 1280	71.18	0.5		
9403	7/23/1993	1244- 1259	68.99	2	31	1.6
9145	8/17/1993	976-1134	62.21	1.2		
9147	8/17/1993	976-1134	68.82	2	23	2.2
1957	8/27/1947	975	60.28	0.8		
13257	9/2/2000	902-1183	73.98	1.5		
13383	9/2/2000	902-1183	70.19	1.6		
# **Curriculum Vitae**

### **Cynthia Faye Lucas**

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### Summary

Highly motivated worker with top performing skills. My interests and abilities as a Master's of Science in Biology graduate have allowed me to gain the experience necessary to pursue a career in the scientific field. Reputation for dedicated teamwork, driven to learn and utilize in-depth scientific content and a commitment to meeting department needs. Able to communicate effectively and highly organized.

#### **Education:**

8/2002-5/2005 M.S., Biological Sciences, **Marshall University**, Huntington, WV Thesis: Comparisons of Morphology and Reproductive Status of *Plethodon glutinosus* at High, Middle, and Low Elevations in West Virginia

9/2001-5/2002 Biology, Glenville State College, Glenville, WV

8/1996-5/2000 B.S. Biological Sciences, Fairmont State College, Fairmont, WV

#### **Personal Experience:**

- Cared for and trained animals, as well as interacted with the public to further educate them on the animals at the zoo.
- Completed a rotation in the Animal Nutrition Center, and the Animal Health Clinic.
- Conducted presentations, group presentations, journal club, and poster presentations in biological sciences.
- Prepared education materials and instructed and tutored students in introductory biology, general ecology, and genetics laboratories.
- Maintained science laboratories by monitoring and distributing supplies.
- Assisted in sales of drug products at a community pharmacy.
- Monitored drug prescriptions at a pharmacy to insure appropriate drug product distribution to customers.
- Received pharmaceutical orders, assured their accuracy and documented overstock/outdates of prescriptions and over the counter medications.

## **Employment History:**

9/2004-present Children's Zoo Keeper, Birmingham Zoo, Birmingham, AL

- 8/2002-5/2004 **Teaching Assistant,** Department of Biology, Marshall University, Huntington, WV
- 9/2000-4/2002 Pharmacy Technician, Kroger Pharmacy, Weston, WV

6/1997-8/1997 **Park Counselor,** Elkins Parks and Recreation Commission, Elkins, WV

### **Poster Presentation:**

Status of the West Virginia State Collection of Amphibians and Reptiles. 65<sup>th</sup> Annual Association of Southeastern Biologists Meeting. 2004.

### **Computer Skills:**

- Microsoft Word
- Microsoft Excel
- Power Point
- SigmaStat

### Achievements and Activities:

- Dean's List, Fairmont State College, Department of Biology
- Advanced Open Water and Rescue SCUBA Diver
- Emergency First Responder Certification
- NAAMP (North American Amphibian Monitoring Program) Volunteer