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The Ecology and Natural History of the Northern Leopard Frog, Rana pipiens Schreber, in West Virginia

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The Ecology and Natural History of the Northern Leopard Frog, *Rana pipiens* Schreber, in West Virginia

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ABSTRACT

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The purpose of this study was to gather ecological and life history data for the Northern Leopard Frog, *Rana pipiens* Schreber, in West Virginia. In Chapter 1, natural history data, such as morphometrics (larval and adult), dorsal coloration, and emergence time were recorded. In Chapter 2, it was discovered that *Aeromonas hydrophila* and *Pseudomonas ssp.* skin infections affecting *R. pipiens* could be identified using BIOLOG analysis. The scope of Chapter 3 was to analyze habitat partitioning between 3 sympatric anurans (*R. pipiens, R. catesbeiana,* and *R. clamitans melanota*). The following habitat partitioning gradient was discovered. *Rana pipiens* was the most terrestrial species and *R. catesbeiana* was the most aquatic species. *Rana c. melanota* inhabited the transition zone between the terrestrial and aquatic habitats. The purpose of Chapter 4 was to analyze the diet composition of *R. pipiens*. It was discovered that Coleopterans, Annelids, and Hymenopterans comprised 22.9%, 17.3% and 11.9% of the diet, respectively.

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Description of Study Area

This study took place at the Greenbottom Swamp, 17 miles north of Huntington, in northern Cabell County, West Virginia. The Greenbottom Swamp is a 1,100-acre Wildlife Management Area (WMA) bordered by Route 2 on the southern side of the swamp and by the Ohio River on the northern side of the swamp. The actual swamp borders the Powell Wetland on the western side, while the eastern portion of the swamp extends to the Cabell Co./Mason Co. border. The West Virginia Division of Natural Resources created the western end of the swamp as a mitigation project in 1992 (Pauley and Barron, 1995). The eastern end of the swamp starting at the Jenkin's house has existed since the turn of the 20th century.

The Greenbottom WMA consists of a series of permanent ponds and numerous ephemeral wetlands. A majority of this study was completed at one large temporary wetland (Hoeft Marsh) directly adjacent to the first pull-off at the southern end of Greenbottom (Figure 1). The Hoeft Marsh is a long, narrow slough that is approximately 500 m long by 16 m wide. It should be noted that water levels within this marsh change drastically between seasons. The Hoeft Marsh did not dry completely during this study, but in August, water levels were much lower than in spring months. In drought years, it is a good possibility that this marsh could dry completely.

Vegetation surrounding the study site was typical of wetland areas. The terrestrial portion of the marsh was dominated by *Cyperus strigosus* (Gallingale), *Aster pilosus* (White Heath Aster), *Onoclea sensibilis* (Sensitive Fern), *Asclepias syriaca* (Common Milkweed), *Impatiens capensis* (Spotted Touch-me-not), and *Polygonum cuspidatum* (Japanese Knotweed). The vegetation within the marsh was typical of emergent wetland

vegetation. The vegetation dominating the aquatic portion of the swamp was *Jussiaea palustrus* (Marsh Purslane), *Cephalanthus occidentalis* (Buttonbush), *Typha latifolia* (Broad-leaved Cattail), *Hibiscus moscheutos* (Swamp Rose Mallow), *Juncus effusus* (Common Rush), *Polygonum coccineum* (Water Smartweed), *Wolffia punctata* (Water Meal), *Lemna minor* (Least Duckweed), and *Spirodela polyrhiza* (Greater Duckweed).

This study site was utilized in all aspects of my thesis. The only information that was not gathered directly from this portion of the swamp was the data for gut content analysis and the anuran distribution data.

Figure 1: Hoeft Marsh at Greenbottom WMA

CHAPTER ONE: The Natural History of *Rana pipiens* in West Virginia

Introduction:

The status of the Northern Leopard Frog (*Rana pipiens*) has been a topic of controversy for many years. It has been noted that many of the once flourishing populations of *R. pipiens* now seem to be in serious decline (Hine *et al*., 1981). It is apparent that amphibians in most habitats are declining worldwide (Vitt and Caldwell, 1990). However, distinguishing between amphibian declines and natural population fluctuations remains a difficult task, because most amphibian populations go through periods of decline and abundance (Wake, 1991; Pimm and Redfearn, 1988). It has been suggested that the only way to differentiate between declines or periods of low population numbers is through long-term monitoring and natural history studies (Pechmann *et al*., 1991).

As with most amphibian declines, researchers hypothesize that pesticide use, aquatic acidification, habitat destruction, disease, and over collection are having detrimental effects upon populations of *R. pipiens*. Perhaps the most important factor affecting populations of *R. pipiens* is habitat destruction. Leopard Frogs require 3 distinct habitats to complete their life history: a breeding pond (usually a body of water lacking a fish population), summer foraging habitat (large grassy meadows), and overwintering habitat (usually a well oxygenated stream or lake) (Merrell, 1977). It has been reported that destruction of wetlands has resulted in a three-fold decline in native populations of *R. pipiens* in Iowa (Lammoo *et al*., 1994). Preserving terrestrial habitat as well as aquatic habitat is essential to protect remaining populations of *R. pipiens*.

Pesticide use also serves as another potential factor of amphibian decline. It has been reported that there is varied tolerance to pesticides among frogs of the same species and varied tolerance at different life stages (Bridges and Semlitsch, 2001). It was discovered that there are differential toxicities of pesticides within populations of Southern Leopard Frogs (*Rana sphenocephala*) (Bridges and Semlitsch, 2000; Berill *et al*., 1995), and also differential toxicities between different larval anuran species (Sanders, 1970). Bridges (2000) discovered that pesticide exposure upon various life stages of *R. sphenocephala* did not directly affect age at metamorphosis, but greatly reduced size at metamorphosis. Weis (1975) also found that pesticides such as DDT inhibits regrowth of tails in larval *R. pipiens* and American Bullfrogs (*Rana catesbeiana*), which could greatly affect foraging efficiency and the larvae's ability to escape predators.

Additionally, aquatic acidification is a serious problem in wetland areas that lack the buffering qualities to balance the influx of acid rain and other forms of acid precipitation. Frogs stressed by the acidic conditions of the wetlands have reduced immune system function, making them vulnerable to bacterial infections (Brodkin, 1997). Pierce and Wooten (1992) suggest that there is considerable genetic variation to tolerance of low pH conditions in anurans. This is quite significant because populations with low genetic diversity are much more vulnerable to aquatic acidification and less likely to tolerate acidic conditions.

Infections from opportunistic bacteria and fungi serve as another compounding factor in amphibian declines. It is believed that stress created from increased UV-B radiation and aquatic acidification causes increased infections from the fungus *Saprolegnia ferax* (Kiesecker and Blaustein, 1995). This dualistic effect is known as

synergism and is thought to be the cause of increased susceptibility to other opportunistic infections, such as red-leg disease (*Aeromonas hydrophila*) (Hird et. al, 1981).

A majority of North America, excluding most northern Canada, is inhabited by at least one species from the *R. pipiens* complex. In West Virginia, *R. pipiens* probably occurs only in the Appalachian plateau in close proximity to the Ohio River. Because of limited distribution within West Virginia, the natural history of *R. pipiens* in this state is virtually unknown. Natural history studies upon this frog have been well documented in other states, such as Minnesota and Wisconsin (Merrell, 1977; Hine *et al*., 1981). Natural history studies are very important, because they provide preliminary information regarding population structure, habitat requirements, and general ecology. This information is essential if long-term monitoring programs are to be established.

The *R. pipiens* complex is a well represented species complex, with approximately 27 living or recently extinct species groups (Hillis, 1988). Speciation within this complex has been well studied. All members of this species complex possess the typical green to brown dorsal color with different arrangements of black spots upon the dorsal surface. Several studies have identified morphological characters that distinguish species within this complex, such as the appearance of the dorsolateral folds (Pace, 1974; Frost and Bagnara, 1976). Other researchers have studied the interspecific isolating mechanisms, such as calling patterns that have lead to speciation within this complex (Frost and Platz, 1983; Moore, 1946). Additionally, Frost (1983) compared niche partitioning between sympatric species of the *R. pipiens* complex. Many studies have attempted to determine the phylogeny and taxonomy of this diverse group. Using molecular techniques, researchers have attempted to determine the phylogeny of the

Mexican coastal Leopard Frogs (Zaldivar-Riveron, 2004). Aditionally, Pace (1974) analyzed systematics between 4 closely related species of the *R. pipiens* complex and discovered several external characters to differentiate these frogs from one another. Hillis (1988) gives a complete history of research upon taxonomy and systematics within the *R. pipiens* complex.

Species Description

The Northern Leopard Frog (*Rana pipiens)*, pictured in figure 2, is a medium sized frog, with 2 to 3 rows of irregularly arranged black spots. The shape of the spots can range from small circles to large blotches, which are usually encircled in a whitish cream-colored border. The dorsal color of this frog ranges from dark brown to dark green and can vary greatly between frogs (Figure 3). The dorsal color of the frog extends below the dorsolateral folds until it contacts the ventral surface. Green and Pauley (1987) suggest that *R. pipiens* may attain a snout-vent length of 52-102 mm. The dorsolateral folds begin behind the eyes and extend onto the groin. Additional black spots extend alongside below the dorsolateral folds and along the dorsal surface of the front and back legs. Although the dorsal surface of this frog is quite colorful, the belly and undersides of the legs are pale white.

Two other genetic polymorphisms of *R. pipiens* exist throughout its range. The two polymorphisms are known as the Burnsi and Kandiyohi polymorphisms (Merrel and Rodell, 1967). The Burnsi polymorphism results in a phenotype without any of the normal dorsal spotting. The dorsal color also is altered from the normal green or brown color to tan. The Kandiyohi polymorphism results in a phenotype with a very dark dorsal color. The dorsal color may be altered so much that the normal spotting pattern is hard to

Figure 2: Photograph of an Adult Female *R. pipiens*

Figure 3: Photograph of Brown and Green Dorsal Phenotype

see, giving the frog a speckled appearance. Neither of these genetic polymorphisms was seen during this study.

Rana pipiens is most commonly confused with the Pickerel Frog (*Rana palustris*), which has two rows of symmetrically placed rows of dark spots located along the dorsal surface of the frog (Green and Pauley, 1987). The spots are usually square shaped and are not outlined with lighter colors. The undersides of the legs are pigmented with either orange or yellow, which may extend onto the ventral surface of the frog.

Breeding begins in early March in West Virginia and may not start until late March to early April in upper latitudes (Merrel, 1977; Hine *et al*., 1981). Temperature has been cited as the factor most greatly affecting the start of the breeding season. Males usually begin calling as the water temperature approaches 10°C (Merrell, 1977; Hine *et al*., 1981). Females usually emerge from hibernation at the same time as the males, but remain much more secretive during the breeding season. Upon copulation, the female releases an egg mass that has been estimated to contain 300-800 eggs (Zenesik, 1963), 2000-5000 eggs (Merrell, 1977), and 1,937 eggs per mass (Pauley and Barron, 1995). From this, it can be seen that number of eggs per mass is highly variable. The eggs develop and may hatch after 7-10 days, depending on water temperatures (Hine *et al*., 1981).

Larval *R. pipiens* transform upon reaching a length of 2.0-3.0 inches (Green and Pauley, 1987). Larvae develop for about 3 months before transforming (usually around mid-June to early-July). Larvae have a brownish coloration and are identified by a high tail crest and copious flecking upon the lateral surface of the tail (Green and Pauley, 1987). Additionally, larvae have a teeth row arrangement of 2 upper rows and 3 lower

rows (Green and Pauley, 1987). Larvae subsist by feeding upon algae and decaying debris (Altig and Kelly, 1974).

Materials and Methods:

Surveys for Leopard Frogs began in early February and continued until mid-November 2003. Frogs were monitored for one complete season to ensure all stages of the frog's natural history had been adequately investigated.

Capture Techniques

Leopard Frogs can be very difficult to capture at times. The cryptic dorsal coloration along with an incredible jumping ability makes them excellent escape artists. In early spring, frogs were captured in the breeding pools by using a long-handled dip-net. The dip-net had a reinforced metal ring that would resist bending due to forceful swipes through vegetation in pursuit of frogs. The ring was fitted with a deep mesh net that had holes large enough to allow water to run out, but small enough to hold adult frogs and/or larvae. All surveys were completed at nighttime in an attempt to get closer to the frogs without spooking them. A Petzl-Duo headlamp was used to not only locate frogs, but also blind them and allow a more elusive approach. Upon capture, frogs were placed in a large Rubbermaid 5-gallon plastic container until proper data were recorded. Upon completion, the frogs were released at the prior point of capture. In the summer and fall months, frogs were captured by searching large, open fields, typical of summer foraging habitat. All surveys except one were completed at night, for the same reasons listed above. Areas were searched until either movement was detected or a frog was

spotted directly. Upon detection, the basket of the long-handled dip net was cast upon the frog as quickly as possible. Usually, the frightened frog jumped directly into the net and the net was swept upward in one complete motion. Upon capture, the frog was placed into a moist pillowcase that was kept on a belt loop of the researcher. The pillowcase was kept on the belt loop, because it allowed the researcher to have both hands free to capture the frogs; one hand to manipulate the net and one hand to secure the frog upon capture.

Larvae were captured within the breeding pools most usually near the areas where egg deposition had occurred. Larvae were sequestered by sweeps with a long-handled dip-net most usually around aquatic vegetation. Upon capture, larvae were identified and placed in large plastic Ziploc bags. Larvae were identified according to the key to anuran larvae found in Amphibians and Reptiles of West Virginia (Green and Pauley, 1987). The Ziploc bag was filled with water taken directly from the swamp. Surveys began on 4/4/2003 and were completed weekly. Thirty *R. pipiens* larvae were captured each sampling trip, to ensure an adequate sample size. In later samples (May-June), the sample size was dropped to twenty, because larvae were much more difficult to capture, most likely due to predation. The surveys were continued until the first juvenile frogs were found. Digital photographs were taken in sample sizes of ten and each larva was measured with the digital morphometrics program, *Image J*.

Morphometrics

Upon capture, the following set of measurements was taken for each fully metamorphosed frog: snout-vent length (SVL), cranial width (CW), tibia length (Tib),

and thumb width (THW). The following set of measurements was taken for each incompletely metamorphosed frog: SVL, CW, Tib, nares width (NW), eye-snout length (ESL), eye-nose length (ENL), eye width (EW), tail height (TH), and tail width (TW).

Plastic vernier calipers accurate to 0.1 mm were used to obtain precise measurements for each frog. All measurements were recorded in millimeters and were double checked for accuracy upon completion of the measurement.

External characters

During summer surveys, records of dorsal coloration and dorsal spot number were documented. The dorsal color was recorded as either brown or green, regardless of the degree of coloration. Dorsal spot number was determined by counting the number of spots between the dorsolateral folds covering the dorsal surface of the frog from urostyle to snout.

Mark-recapture

A mark-recapture study was completed for 3 consecutive evenings (3/18-3/20) to study population dynamics and migration within established breeding colonies of *R. pipiens*. Two 8 m X 8 m quadrats were flagged off surrounding actively breeding colonies of *R. pipiens*. Each quadrat was surveyed for one hour beginning at 8:00 PM (quad 1) and 9:00 PM (quad 2). The quads were surveyed from the perimeter to avoid disturbing the remaining frogs. Once a frog was spotted, only then did the researcher enter the quadrat to capture the frog. Upon capture, frogs were placed into plastic 5-gallon Rubbermaid containers filled with a small amount of water. Upon completion of

the surveys, frogs were taken away from the swamp until proper markings and measurements were completed. After application of the markings, frogs were taken back to the respective quadrats and released in the middle of the quadrat.

Elastomers were used to mark captured frogs. Frogs were given an evening/quad mark based upon location on the body and color of the marking. Elastomers were applied through a 5 cc medical syringe. To apply the elastomer marking, the syringe was inserted subcutaneously and a minimal amount of elastomer was injected into the frog. Due to the fluorescing nature of the elastomer, a UV flashlight was used to identify previously marked individuals.

The sampling evening/quad marks were designated as such. On evening 1, frogs in quad #1 were given a yellow marking on the ventral side of the left tibia, while frogs captured in quad #2 were given an orange marking on the ventral side of the right tibia. On evening 2, frogs in quad #1 were given a yellow marking on the ventral side of the left humerus, while frogs in quad #2 were given an orange marking on the ventral side of the right humerus. The frogs captured on evening 3 were not given a marking and were just designated as evening 3 captures. The breeding areas were surveyed for 3 consecutive evenings to monitor movements to and from the breeding circles.

Statistical Analysis

In this portion of the study, the following statistical analysis methods were used: linear regression, ANOVA, one-way t-test, and descriptive statistics. *MS Excel* was used to complete the linear regression analysis, while *Sigma Stat* 2.03 was used to complete one-way ANOVA, one-way t-tests, and descriptive statistics.

Environmental Conditions

The following environmental conditions were recorded upon each visit to the research site: air temperature, water temperature, soil temperature, relative humidity, and water pH. An outdoor armored thermometer was used to monitor water temperature and air temperature. A digital soil thermometer was used to monitor soil temperature. All environmental conditions were taken in duplicate to obtain an average reading.

Results:

Breeding cycle

Figure 4 illustrates the influence of water temperature upon the life history of *R. pipiens*. The blue line represents the fluctuations in water temperature. The dashed line indicates the cited water temperature (10°C) required for male *R. pipiens* to initiate calling. The rectangles at the bottom of the graph represent the developmental stages in the life cycle of *R. pipiens* in West Virginia. From the figure, it can be seen that the first day of emergence was 2/03/2003. The frogs began to call on 3/14/2003 and continued to call until 4/7/2003. On the first day of calling, the water temperature was 10°C, which agrees with the cited values (Hind et al., 1981). Egg deposition occurred approximately 2 days after the onset of an established full chorus on the evening of 3/16/2003. Eggs developed for about 1 week, before hatching on 3/26/2003. Tadpoles existed for about 2.5 months, with the first metamorphs emerging on 6/11/2003. From this figure it can be concluded that the life cycle (egg-metamorph) of *R. pipiens* requires approximately 3 months.

Morphometrics

Figure 5 illustrates the relationship between snout-vent length (SVL) and cranial width (CW) in *R. pipiens.* Morphometric data for this graph was obtained from frogs captured from March-August. Data from these seasons was recorded to represent all size classes. Using SVL as the independent variable and CW as the dependent variable, a near linear relationship was established. Linear regression analysis was used to determine an R^2 value of 0.9168. This R^2 value indicates a direct relationship between SVL and CW. The majority of the frogs represented by this graph had SVL lengths between 45.0 mm and 60.0 mm.

Figure 6 illustrates the relationship between SVL and tibia length (Tib L) in *R. pipiens*. As in figure 5, morphometric data was also obtained from frogs captured from March-August. A linear regression was used to analyze the relationship between these two variables. Upon using this test, an R^2 value of 0.9195 was obtained, illustrating a linear relationship. It is obvious from the results that an increase in SVL correlates with a linear increase in Tib L. There are two outliers with SVL of approximately 45.0 mm and 59.0 mm.

Figure 7 is a scatter diagram comparing the relationship between SVL and thumb width (TW) between male and female *R. pipiens*. Morphometric data for this graph was gathered in March at the peak of the breeding season for *R. pipiens* in West Virginia. Data was gathered then, because eagerly calling males were easily distinguished from the more secluded females. A distinct separation between TW and SVL can be seen for male and female *R. pipiens*, indicating the presence of a sexual dimorphism. However, the linear relationship between SVL and TW is not correlated very strongly. Using linear

regression analysis, the R^2 values for male and female *R. pipiens* were 0.2058 and 0.3592, respectively. This graph shows that an increase in SVL, does not equate to an equal increase in TW for male or female *R. pipiens*.

Figure 8 illustrates the mean SVL of *R. pipiens* from metamorphosis (June) until hibernation (mid-November). Morphometrics for this graph were gathered to determine the extent of development in one growing season. From the graph it can be seen that most of the growth occurs in the summer months. Mean SVL growth from June through August was 16.6 mm. Growth slowed down considerably in the fall months, with a mean SVL growth of only 2.8 mm. The mean SVL growth for *R. pipiens* throughout one season was 23.2 mm.

Figure 9 illustrates the maturation rates of larval *R. pipiens* throughout one growing season. From the figure it can be seen that growth quickly picks up after the first month of development. Total average growth after the first four sampling periods was approximately 9 mm. The most considerable growth periods occurred between Apr. 21-Apr.28 and May 25-June 11, with average total growth measurements of 18 mm and 16 mm, respectively. Additionally this graph illustrates that larval *R. pipiens* reach an average total length of 90 mm before the occurrence of metamorphosis.

Figure 10 demonstrates the relationship between total length (TL) and cranial width (CW) for larval *R. pipiens*. From the graph, it can be seen that there is indeed a linear relationship between TL and CW for larval *R. pipiens*, $R^2= 0.9805$. It is obvious that an increase in TL does equate an equivalent increase in CW. Additionally, the data is much more clustered at the 0 mm-20 mm marks than at any other area within the regression.

Table 1 illustrates morphometrics of fully developed larval *R. pipiens*. These frogs had fully developed appendages and partially developed mouthparts. Additionally, most of the frogs had not fully absorbed their tail segment. Measurements taken and their abbreviations are as follows: SVL (snout-vent length), CW (cranial width), TIB (tibia length), NW (nostril width), ENL (eye-nares length), ESL (eye-snout length), EW (eye width), TH (tail height), and TL (tail length). From the table it can be seen that SVL, CW, TIB, NW, ENL, ESL, and EW did not vary considerably among the frogs measured. However, there were considerable standard error values for tail height and tail length, 12.2 mm ± 1.10 and 48.2 mm ± 3.93. The same trend is also seen in the data range for TH and TL, 13.2 mm and 43.9 mm, respectively.

Sexual variation

Table 2 compares the mean SVL of male and female *R. pipiens* captured throughout the study. The mean SVL for males and females was 58.2 mm and 60.3 mm respectively. Although females had a larger mean SVL than males, a one-way t-test was used to show that the difference was not significant ($p= 0.094$).

Table 3 compares the percentage of males and females encountered during the spring and fall. In the spring, males accounted for 96% of the captures, while females accounted for only 4% of the captures. In the fall, percentages changed greatly. Males accounted for 69% of the captures, while females accounted for 31% of the captures. Although sample size in both seasons differed greatly, the change in percentage of males to females is quite drastic.

External Characters

Figure 11 compares the dorsal color variation in *R. pipiens* from July-September. From the graph, it can be seen that green was the more dominant dorsal color. In July and September, the percentage of frogs with the green dorsal color was 66%, while the percentage of frogs with the brown dorsal color was 34%. In August, the percentage of frogs with the green dorsal color was 75%, while only 25% of the frogs encountered had the brown dorsal color.

Figure 12 illustrates the distribution of dorsal spots on male and female *R. pipiens*. All size classes were counted including recently metamorphosed frogs. The graph includes frogs with spot numbers ranging from 10 to 30 dorsal spots. The graph illustrates a definite bell curve with 17-20 dorsal spots being the most common phenotypes. There seems to be an aversion to both extremes (10-12 dorsal spots) and (28-30 dorsal spots). Besides the bars representing 16 and 22 dorsal spots, the graph has a normal bell curve distribution.

Mark-recapture

The data in tables 4 and 5 illustrate results of a three-night mark recapture study for quadrats 1 and 2. The capture results for quadrat #1 for evenings 1, 2, and 3 were 38 captured frogs, 20 captured frogs, and 5 captured frogs, respectively. On evening 2 there were 4 total recaptures, 1 being from quadrat #1 the other 3 from quadrat #2. On evening 3 there were 2 total recaptures, 1 from evening 1 and the other from evening 2. On evening 3 there were no crossover recaptures from quadrat #2.

The capture results for quadrat #2 for evenings 1, 2, and 3 were 52 captured frogs, 7 captured frogs, and 4 captured frogs, respectively. On evening 2 there were 2 recaptures, both being crossover recaptures from quadrat #2. On evening 3 there were no recaptures. All frogs captured this evening were newly encountered frogs that had immigrated into the breeding site.

Discussion:

Breeding cycle

In this section, field notes describing important events of the breeding cycle are included. Please refer to figure 4 to examine the dates explained in the text. As mentioned earlier, the life cycle of *R. pipiens* (egg-metamorph) is completed in 3 months. However, events leading up to breeding and metamorphosis are more complex. In this figure, it can be seen that the first *R. pipiens* were seen on the evening of 2/3/2003. This is the earliest know occurrence for emerging *R. pipiens* ever recorded in WV. While searching the walking path at the first pull-off at Greenbottom, three *R. pipiens* and one *Pseudacris c. crucifer* were discovered. Two of the *R. pipiens* were dead in a road-rut pool, while the third *R. pipiens* was found sitting motionless on the walking path. Post-hibernation frog deaths seem to be fairly common for *R. pipiens* and may have something to do with immunosuppression due to cold hibernating temperatures (Hind *et al*., 1981). On the evening of 2/21/2003 four *R. pipiens* were captured searching the walking path of the lower pull-off at Greenbottom Swamp, WV. Two of these frogs were positively identified as males, one as a female, and the other frog was identified as a juvenile. It is believed that weather conditions on this day influenced the movements of frogs. Air

temperature was 5°C, while water temperature was 4.5°C. It had been raining the entire day, but as the survey was started, the rain switched to a drizzle. On the next evening (2/22/2003), one *R. pipiens* a gravid female, with an SVL of 65.6 mm, was discovered along the same walking path. Daytime temperatures on this day were as high as 25°C. Although temperatures had dropped to 10°C by nighttime, it rained quite heavily throughout the entire evening. Three *Ambystoma maculatum* and one *R. sylvatica* were also seen migrating into the swamp.

The next two surveys began on the evenings of 3/5/2003 and 3/8/2003. On the evening of 3/5, three *R. sylvatica* were heard calling from the mitigated end of the swamp. On the evening of 3/8, a full chorus of *R. sylvatica* was heard calling from the same breeding pool. Three *R. sylvatica* egg masses were discovered in close proximity to the calling males. While searching the breeding pool for additional egg masses, one male *R. pipiens* with an SVL of 57.4 mm was captured. The frog was captured after it submerged into the swamp attempting to escape. Two other male *R. pipiens* were captured with SVL's of 58.1 mm and 67.0 mm. Approximately ten other *R. pipiens* were also observed this evening floating throughout the deeper portions of the swamp, but were spooked very easily.

On the evening of 3/13/2003, one female *R. pipiens* with an SVL of 68.2 mm was captured 1.0 m below the surface. No other frogs were seen during the survey, because air temperatures were only 2.5°C, while the water temperature was 6.5°C. Frogs were probably confined to the water due to the cold air temperatures.

From figure 4, it can be seen that the first full chorus of *R. pipiens* was heard on the evening of 3/14/2003. Two males and one female *R. pipiens* were captured, having

SVL's of 63.3 mm, 66.1 mm, and 69.7 mm, respectively. There was one major breeding area identified, with males actively calling from the surface of the water. As can be seen from figure 4, water temperature had reached 10°C. This is significant, because the cited water temperature for male *R. pipiens* to initiate calling is 10°C (Merrell, 1977; Hine *et al*., 1981). On 3/15, four males with SVL's of 63.1 mm, 61.3 mm, 63.0 mm, and two females with SVL's of 61.4 mm and 63.0 mm were captured. Two additional breeding colonies were identified with actively calling males. The following two different calls were identified: the first being a long extended guttural call and the other being a series of short quick grunts. Noble and Aronson (1942) suggest that the long extended call is the sexual call of the male. It is also cited that males then emit a series of short quick grunts once the male begins to pursue a female.

As can be seen in figure 4, the first egg masses were found during the daytime and evening of 3/17/2003. All egg masses were deposited where males had been actively calling the night before. The egg masses were also deposited within 1 m of each other. Although many egg masses had been laid, males still continued to call. The egg masses were submerged and attached to vegetation directly under the surface. At the first site, 36 egg masses were found, while 19 egg masses were found approximately 3.0 m from the first breeding site. The mean egg mass length and mean egg mass depth was 80.0 mm±1.87 and 34.0 mm±1.11. On this evening, 19 male *R. pipiens* were captured and measured. Mean SVL, cranial width (CW), and thumb width (TW), were as follows: 62.2 mm ± 1.01, 4.99 mm ± 0.95, and 4.35 mm ± 0.121. Males were much more active and were therefore more easily captured. During the survey, two pairs of frogs were seen in amplexus, but were not disturbed. No additional females were seen within the

breeding circle. The females captured the night before seemed to be hiding near the vegetation approximately 5 m out from the actively calling males.

On 3/28/2003, egg masses hatched, producing very small *R. pipiens* larvae approximately 9 mm long. Larvae were mainly concentrated in the areas where males were actively calling a week prior. One large female *R. pipiens* was captured on the evening of 4/2/2003, with an SVL of 77.3 mm. Even though eggs were deposited two weeks prior, several *R. pipiens* were still heard calling from the water.

Morphometrics

As was discussed above, CW and Tib L were highly correlated to SVL. Figures 5 and 6 provide a description of the size distribution throughout a population of *R. pipiens*. A considerable portion of the frogs surveyed had SVL measurements of 40.0 mm to 60.0 mm. Numbers of frogs within the 60.0 mm to 80.0 mm range were much lower, indicating that a majority of a population of *R. pipiens* is composed of frogs in the 40.0 mm to 60.0 mm range. This size range was probably seen because frogs included in this graph were mainly captured during the summer and fall months. A majority of the frogs captured would be this year's emerging frogs, with occasional adults from previous years.

These graphs also provide linear growth models for normal populations of *R. pipiens*. By extrapolating the expected value of a morphometric character from the best-fit trendline, one could predict the presence of growth defects within the population. From these graphs it can be seen that most of the measurements lie within reasonable

distance of the line, indicating normal growth patterns within this population of *R. pipiens*.

Figure 7 not only describes the size distribution of male and female *R. pipiens* captured in early spring, but also compares TW vs. SVL for both sexes. Contrary to the results seen in figures 5 and 6, a majority of the frogs captured had SVL's ranging from 55.0 mm to 68.0 mm. Larger frogs were captured more frequently, because frogs included in this graph were captured during the peak of the breeding season, while males were actively calling. Immature frogs were probably present, but were not surveyed because actively breeding males and females were more prevalent and easier to catch. Immature *R. pipiens* most usually reside on the outer edges of active breeding circles (Merrell, 1977). It can also be seen that minimum SVL of actively breeding males is approximately 55 mm. This is important because males in West Virginia may have the potential to reach sexual maturity in one growing season. Sexually mature females would be much harder to identify without dissection, because they do not vocalize, indicating their readiness to breed. Additionally, mature females are hard to distinguish from immature frogs, because thumb width between these two developmental stages does not differ greatly.

As was stated in the results section, TW was not highly correlated to SVL. This is important because it indicates that a larger SVL does not signify an equal increase in TW. Although a linear correlation was not seen between these morphological characters, this graph does effectively illustrate TW as a sexually dimorphic character between male and female *R. pipiens*. This indicates that TW is an accurate method for sexing breeding *R.*

pipiens. It should be noted that the presence of swollen thumbs in actively breeding males is a characteristic seen within male frogs of the genus *Rana.*

As was presented in the results section, figure 8 estimated the average growth of *R. pipiens* throughout one season. From the results in figure 8 it can be postulated that males have the potential to reach sexual maturity after one growing season. Additionally, it was determined that SVL increased 23.2 mm in one growing season. This is quite important because the cited value for growth of *R. pipiens* in Northern Michigan was approximately 11 mm during the first year after metamophosis (Force, 1933). This author also suggested that males did not reach sexual maturity until the third growing season after metamorphosis. However, it has also been observed for *R. pipiens* that some individuals can grow up to 22 mm in one season (Ryan, 1953). One explanation for discrepancies between times to maturity could be the total lengths of time frogs are active. This particular population of *R. pipiens* is one of the most southern populations in the eastern United States. Thus, this population would be exposed to earlier warm spring temperatures and would be permitted to emerge earlier and hibernate later than populations in Northern Michigan. This would greatly lengthen the growing season for these frogs. Therefore, it is probable that males could reach sexual maturity after the first growing season. In addition, it has been suggested that *R. pipiens* does have the potential to breed the year following transformation (Ryan, 1953).

Additionally, this graph illustrates the effects of seasonality upon growth. Frogs grew much more during the summer months than during the fall months. During warm summer months, frogs were more actively foraging for food. Insect prey was probably obtained more easily, thus allowing frogs to allocate a higher percentage of energy to

growth. As temperatures decreased, the frogs had to allocate more energy to survive colder evenings. Additionally, insect prey was less abundant during cooler fall months preventing frogs from obtaining energy to allocate towards growth.

The results in table 1 indicate that most larvae captured were very close in developmental stages. Most measurements taken had small standard error values, indicating that frogs were probably developing at the same rate. The only measurements that varied considerably were TH and TL. This is because each tadpole may have been in different stages of tail absorption. Additionally, one metamorph had absorbed a considerable portion of its tail, increasing the standard error greatly.

Sexual Variation

As was discussed above, table 2 illustrates that there is variation in average SVL between male and female *R. pipiens*. Although the difference is not significant, there appears to be a noticeable difference in average SVL. It has been discovered that SVL is a sexually dimorphic character for *R. pipiens* (Hind et. al, 1981). This author cited average SVL values of 67 mm and 63.8 mm for females and males, respectively. It is apparent that the cited value for average SVL difference is greater than the values obtained during this study. One explanation for the differences in average SVL is the season frogs were surveyed. The frogs measured during this study were captured during the fall months, while the frogs surveyed by the above listed authors were captured during late spring. Frogs that were captured in the spring would not include young-of-the-year and would most likely include mature, breeding frogs. Frogs captured in the fall

would include young-of-the-year, which would include frogs that would show signs of breeding maturity, but may not be completely mature.

As was discussed above, table 3 illustrates that there are distinct differences in the percentages of males to females during the spring and fall months. During the early spring months, males were much more prevalent than females, 96% and 4% respectively. During the fall surveys, males were still more prevalent than females, but not to the extent observed in the spring (68% males and 32% females). Hind *et al.* (1981) obtained similar results regarding sexual variation during the spring. Of the frogs captured, 86% were males, while 14% were females. The above authors also saw a drastic change in sexual variation during the fall surveys, but their sexual ratio values more closely approached a 1:1 distribution.

The drastic difference in sexual ratios between seasons reveals the behavioral differences exhibited by male and female *R. pipiens* (Hind, *et al.* 1981). Males are much more active during the breeding season than females. Males actively call from open water, while more secretive females hide in vegetation, emerging only to breed. Thus males are much easier to locate and are more likely to catch the researcher's attention. During the summer and fall months, both sexes retreat to summer foraging habitat and exhibit similar behavior. This explains why sexual variation more closely approaches a 1:1 distribution during the summer and fall months. A small sample size (n=62) could have greatly affected sexual variation values, explaining why the presented sexual ratios did not approach a 1:1 distribution for this study.

Larval Morphometrics

As was presented earlier, figure 9 demonstrates the growth pattern of larval *R. pipiens*. As can be seen in the figure, the tadpoles required approximately 2.5 to 3 months to emerge from the water. Different values have been listed for the number of days required for transformation of *R. pipiens* larvae. Ting (1955) suggests that *R. pipiens* larvae transform 62 days after insemination. Taylor and Kollros (1946) suggest that *R. pipiens* larvae require 70 days after hatching to begin metamorphosis. Additionally, the previously cited authors also suggest that the frogs require 90 days to fully complete metamorphosis. The discrepancies in these values may be a result of the rearing conditions. Many factors, such as water quality, water temperature, and larval crowding have drastic effects upon larval growth rates. As can be seen in the graph, the larvae grew extraordinarily fast. This can be attributed to an exceptionally warm and wet spring. Water temperatures were quite high early in spring and with the spring rain; the larvae had plenty of space to develop uninhibited. It is believed these two factors led to an accelerated growth rate of larval *R. pipiens*.

As the larvae emerge, they do not initially move onto land, but reside on the bases of emergent vegetation. Larvae probably remain here until the tail is completely absorbed and adult mouthparts have fully developed. Anuran larvae go through a fasting period that begins with the appearance of forelimbs and probably continues until the complete development of adult mouthparts (Hedeen, 1972; Jenssen, 1967). This fasting period is required, because the digestive tracts of the larval frogs are undergoing massive alterations. After the development of adult mouthparts and absorption of the tail, the young frogs most likely move onto land to begin foraging.

As was seen in figure 10, TL was highly correlated with CW. This is quite important; because it illustrates that the body size of larval *R. pipiens* increases linearly with an increase in total length. This graph is useful, because it can be used as a standard curve to tell if larvae are developing correctly. This curve could be used in studies exploring the effects of toxins, such as pesticides upon larval growth.

Notice that as the larvae become considerably larger, the correlation is not as strong as it is in the 20 mm region of the graph. This difference possibly reveals the effects of microhabitat upon larval growth. Since no two larvae are going to have the same living conditions, the larvae are highly unlikely to develop at the exact rate.

External Characters

As was discussed earlier, figure 11 illustrates that green dorsal color is much more prominent than the brown dorsal color in *R. pipiens*. The Northern Leopard Frog is polymorphic for dorsal color, with the two most common phenotypes being green and brown (Corn, 1981). As can be seen from the figure, green to brown dorsal color distributions for July, August, and September were 2:1, 3:1, and 2:1, respectively. It is apparent that green dorsal color is the more commonly expressed phenotype. Green and brown phenotypes are expressed as a simple Mendelian system of two alleles at one locus, with green being dominant to brown (Fogleman et. al, 1980).

In order for genetic polymorphisms to exist within a population, it must be advantageous to express one phenotype over another. In *R. pipiens*, it was discovered that tadpoles expressing the brown dorsal polymorphism transform up to 5 days earlier than frogs expressing the green dorsal color (Corn, 1981). If this is true, larvae

expressing the brown dorsal phenotype have a selective advantage over larvae expressing the green dorsal phenotype. Larvae expressing the green dorsal phenotype will have to spend additional time in the water, making them more likely to be eaten by fish and other predators. Although the green dorsal pattern is the more dominant phenotype, a selective advantage of the brown dorsal color during the larval stage allows the brown polymorphism to exist.

The results in figure 12 suggest that dorsal spot number is influenced by a selection mechanism. The bell curve is skewed slightly to the left, but the highest frequency lies within the range of 17-21 dorsal spots. Frogs expressing this range of dorsal spots may be more able to camouflage themselves from potential predators. Frogs that express low numbers of dorsal spots (10-12) and high numbers of dorsal spots (28-30) may lose the ability to blend in with the surrounding environment, because they possess too much of one dorsal color. When a grassy area is looked at from above, a variety of dark and light areas are visualized. A typical Leopard Frog has a green to brown dorsal color with a various pattern of dorsal spotting. The dorsal color (green or brown) mimics the surrounding vegetation, while dorsal spotting mimics shading produced by overhanging vegetation. Frogs that have too many or too few dorsal spots might lack the breakup pattern necessary to remain camouflaged from predators.

One problem with the above explanation is the different shapes and sizes of the dorsal spots. Dorsal spots on *R. pipiens* can vary from small circular spots to elongated blotches. It is because of this that the above explanation can only be an assumption until a more detailed analysis has been completed. Only through analysis of total percent

coverage of the dorsal spotting to the area of total dorsal color could a true theory be developed.

Mark-recapture

From the data presented in tables 4 and 5 it is apparent that considerably more frogs were captured on the first evening when compared to evenings 2 and 3. Quads surveyed were well established breeding areas with many actively calling males. This would explain the very high number of frogs collected on the first evening. However, the number of frogs captured on nights 2 and 3 decreased considerably. The decrease in number of frogs was not as severe in quadrat #1 as was seen in quadrat #2. The severe decrease seen in quadrat #2 was due to the extremely long period of time it took to measure and apply elastomer markings to the 91 total frogs captured on evening 1. Upon completion of the surveys and proper workup, frogs captured from quadrat #1 were placed back into the quadrat around 1:00 AM. Frogs within quadrat #2 took much longer to measure and mark and were not replaced back into the quadrat until 5:00 AM. This appears to have had a detrimental effect on the captures in quadrat #2 for evenings 2 and 3. Since sampling was started again the following evening at 8:00 PM, the frogs in quadrat #2 were only given 15 hours to relocate after being disturbed from their normal breeding patterns. Additionally, there were no longer calling males present in either of the quads, and as a result, frogs may have relocated to other areas of actively breeding frogs. This is evident in recapture data presented for quadrat #1. On evening 2, there were 4 recaptures. Three of these recaptures were frogs that were captured the night before in quadrat #2. These frogs are referred to as crossover frogs, because they left

their first breeding circle (quadrat #2) and moved to another established breeding circle (quadrat #1). The quadrat #1 crossover frogs probably relocated because there were no longer any calling frogs in their original breeding circle. Since frogs captured in quadrat #1 were placed back into their original quadrat around 1:00 AM, frogs had considerable time to begin calling again. As frogs were replaced into quadrat #2 around 5:00 AM, they may have been attracted to calling males from quadrat #1 and relocated accordingly.

It should be noted that of the 20 frogs captured in quadrat #1 on evening 2, only 1 was a quadrat #1 recapture. Excluding the 3 additional crossover recaptures, 16 new frogs emigrated into the quadrat from the surrounding swamp. Since no frogs would have been calling from this quadrat for a considerable period of time, these frogs must have been attracted by pheromones released by breeding frogs from the previous evening or some chemical cue produced by females upon oviposition. At the time of the survey, there was a considerable number of egg masses (~20) already deposited in both of the quadrats. Frogs may have sensed previous breeding activity and moved into the area where the egg masses had been deposited.

As for the severe decrease in captures in quadrat #2, the sampling technique seems to have disturbed the breeding patterns of frogs greatly. Although there were 4 recaptures on evening 2, there were no recaptures on evening 3. It appears that a majority of the frogs captured in this quadrat had relocated to other areas of the swamp. Similar capture decreases were seen in quadrat #1, however on evening 3 there were 2 recaptures. These recaptures were original captures from quadrat 1. One was a frog that was captured on evening 1 and one was a frog that was captured on evening 2. These recaptures indicate that not all frogs relocated to other areas upon release. Additionally,

it indicates that 1 frog relocated back into its original breeding site. This is important because it illustrates that the frog was able to relocate to its original breeding area after moving out of the quadrat on evening 2.

The mark-recapture study described above illustrates that high numbers of *R. pipiens* congregate in breeding colonies. However, sampling methods seemed to be influential on the movements of frogs. If this experiment were to be repeated, sampling would be completed every other evening instead of 3 consecutive nights of sampling. Therefore frogs in both quadrats would have adequate time to relocate.

The natural history data gathered from this study will provide biologists with information regarding population structure, life history information, and reproductive data for *R. pipiens*. This information could potentially provide government agencies with data that would aid in mitigation and protection of already existing wetlands.

Figure 4: Influence of Water Temperature upon the Life History of *Rana pipiens*

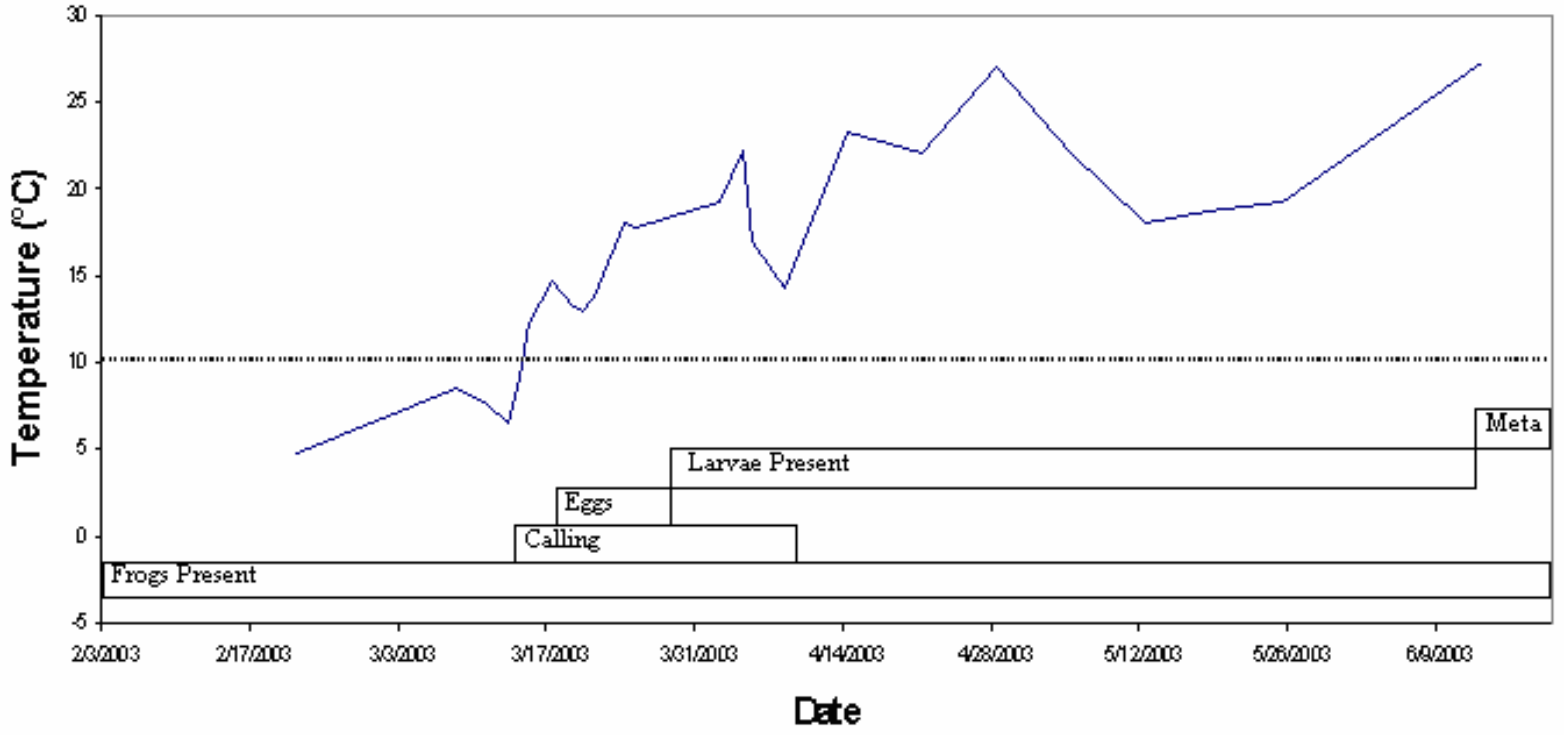


Figure 5: Comparison of Snout-vent Length vs. Cranial Width for *R. pipiens*

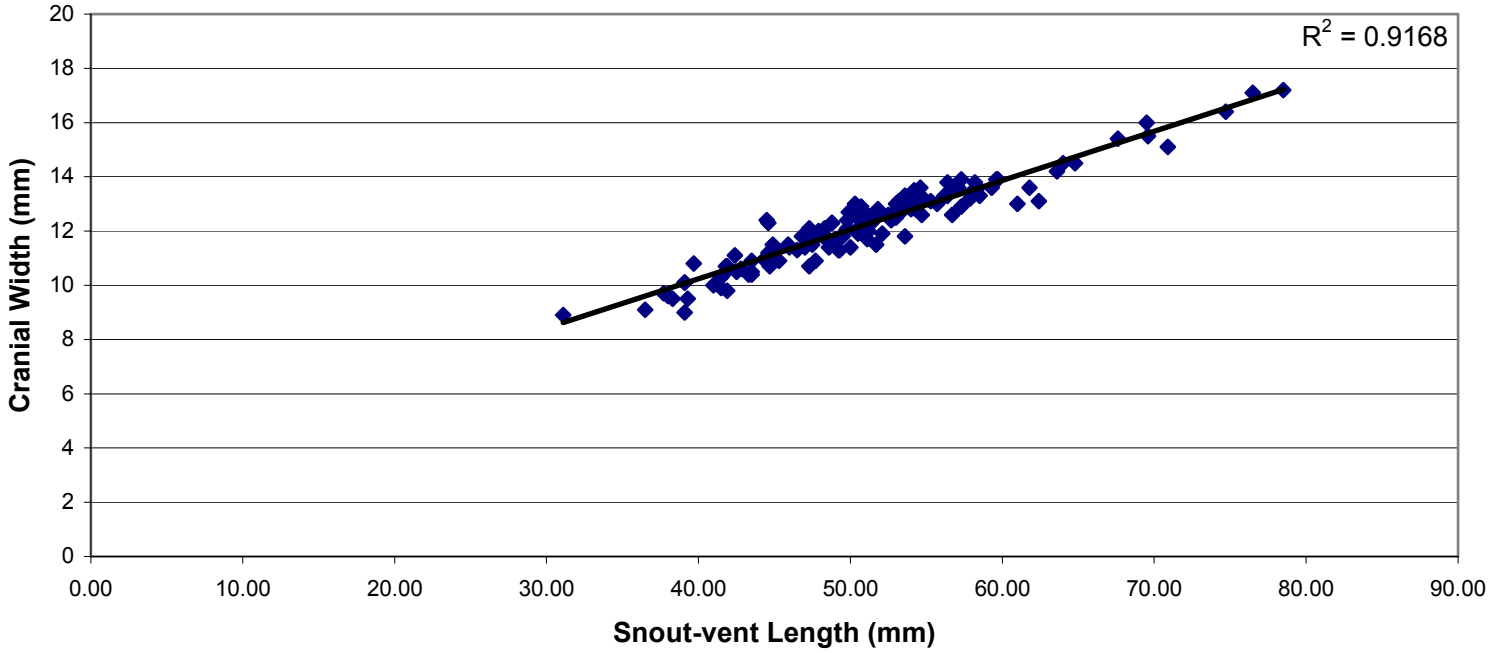


Figure 6: Comparison of Snout-vent Length vs. Tibia Length for *R. pipiens*

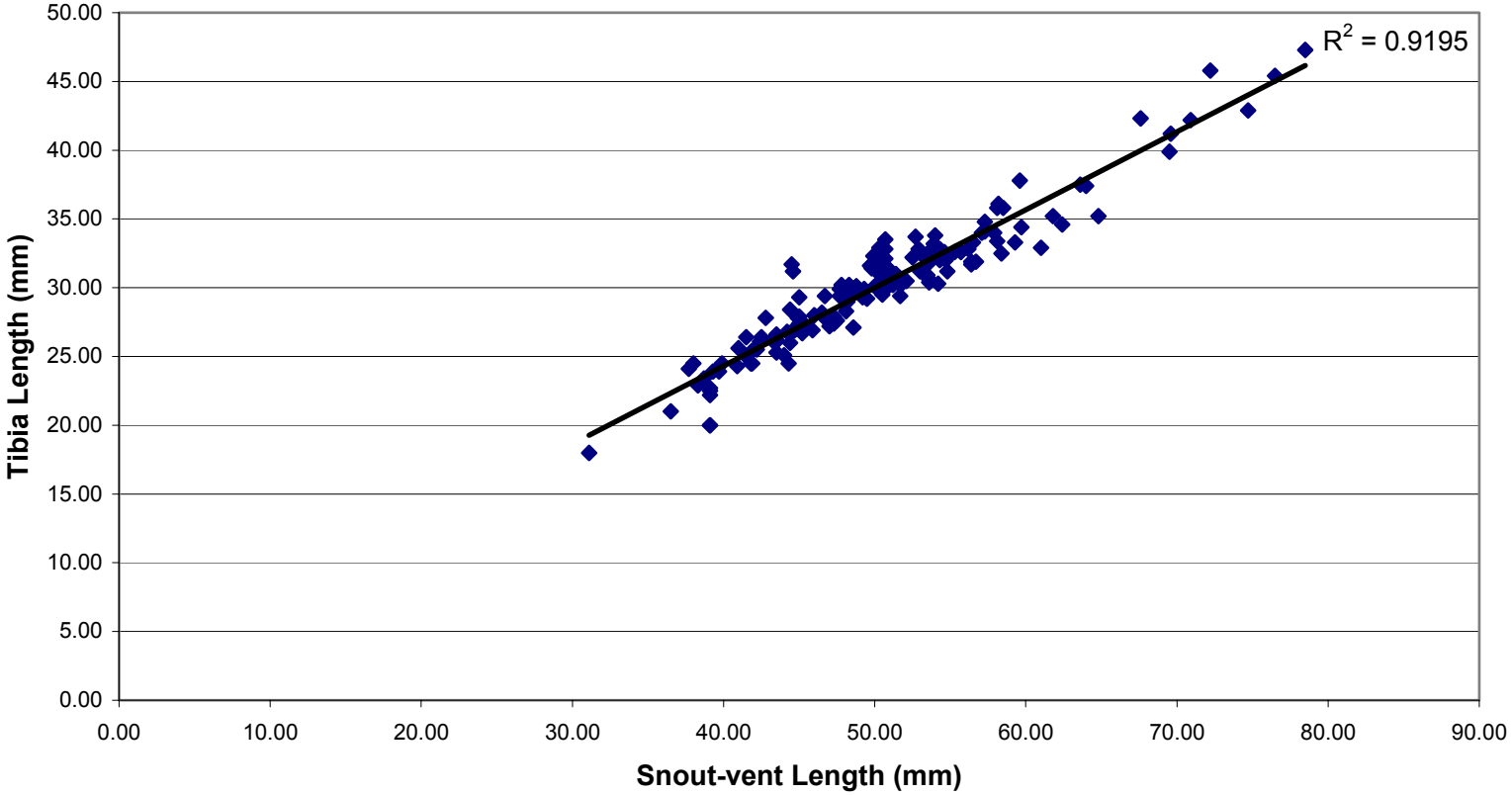


Figure 7: Snout-vent Length vs. Thumb Width in Male and Female *R. pipiens*

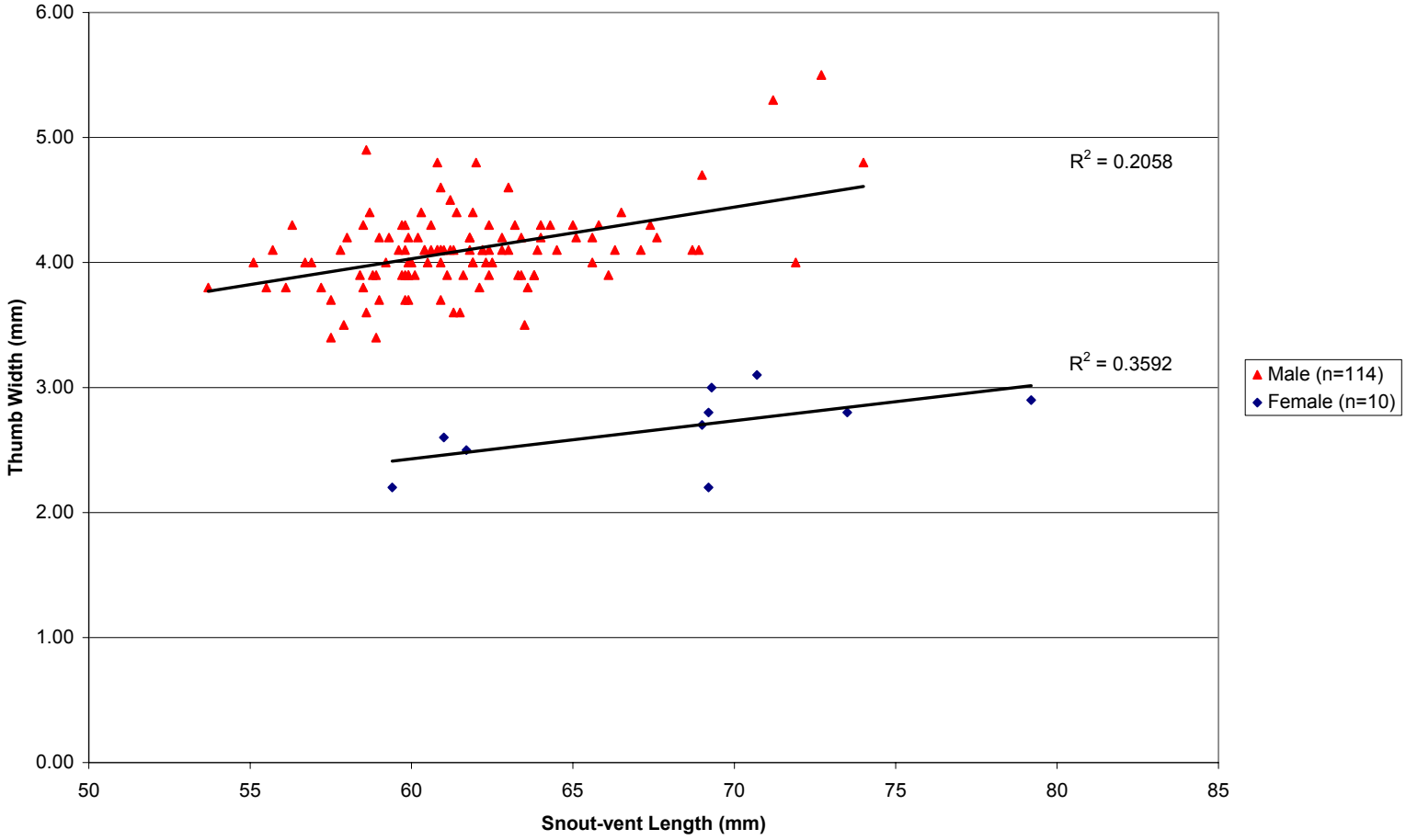


Figure 8: Mean Snout-vent Length of *R. pipiens* from June through September (2003)

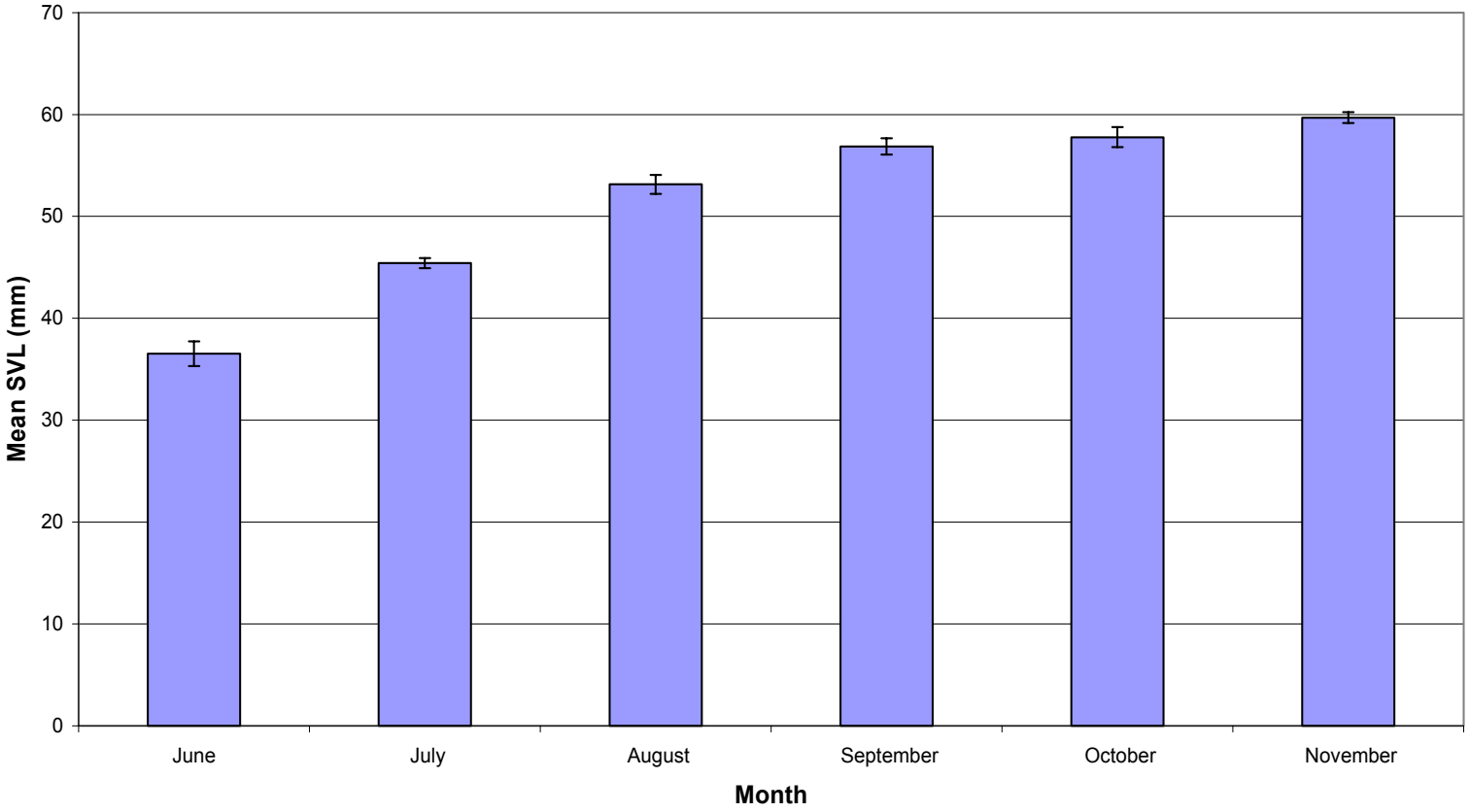


Figure 9: Mean Growth Rates of Larval *R. pipiens* (Spring 2003)

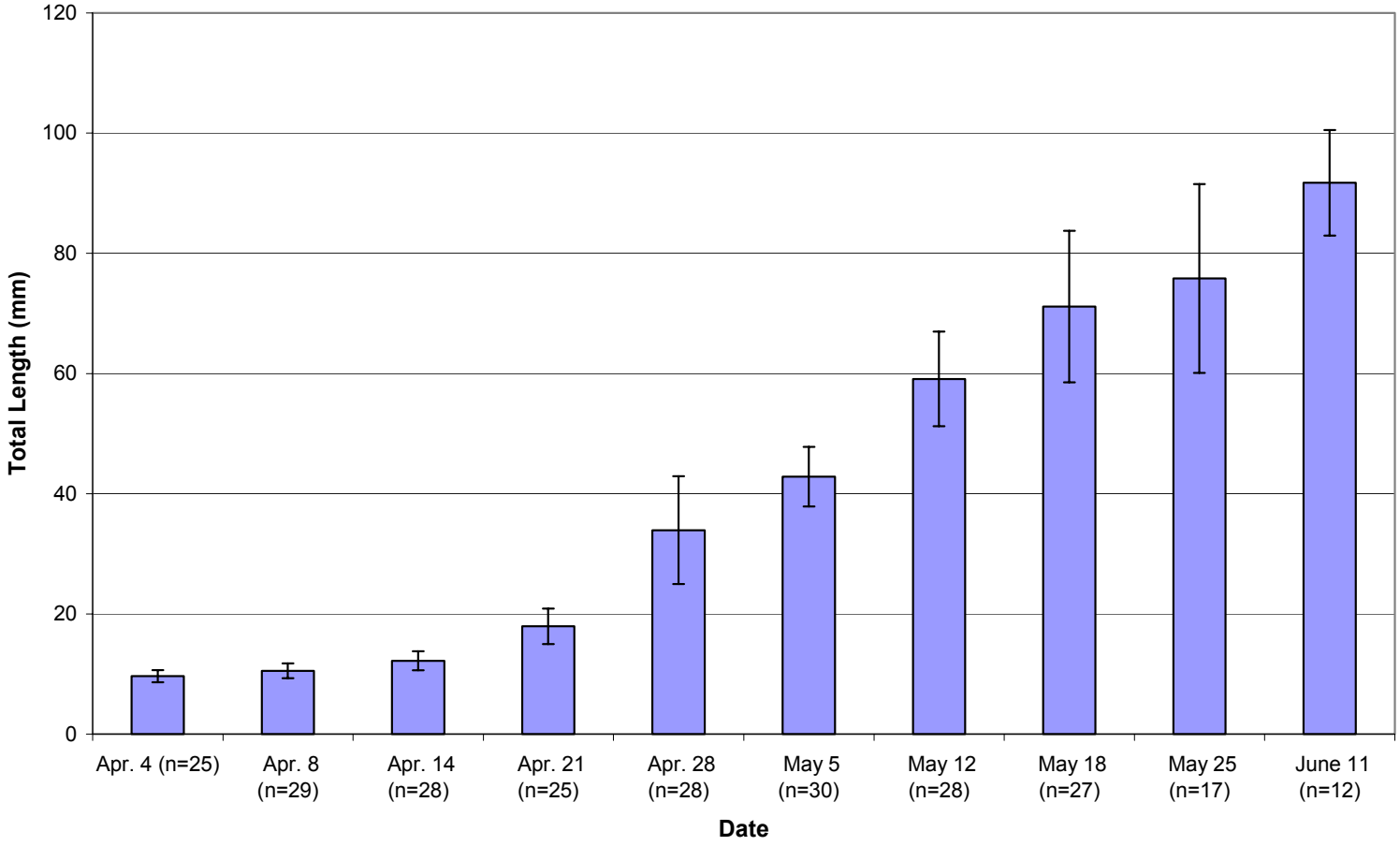


Figure 10: Total Length vs. Cranial Width for Larval *R. pipiens* , Spring 2003 (n=229)

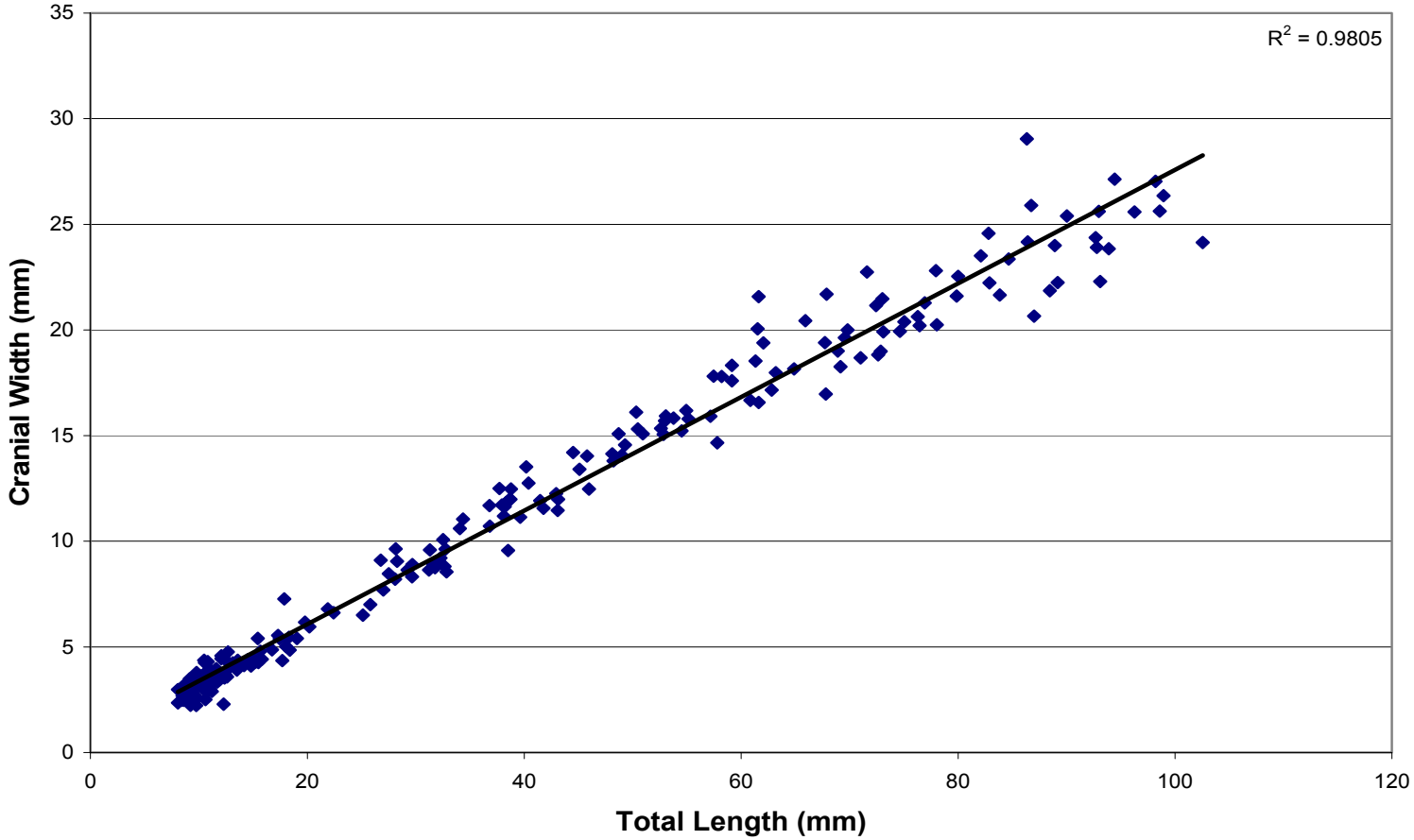


Figure 11: Dorsal Color Variation in *R. pipiens*

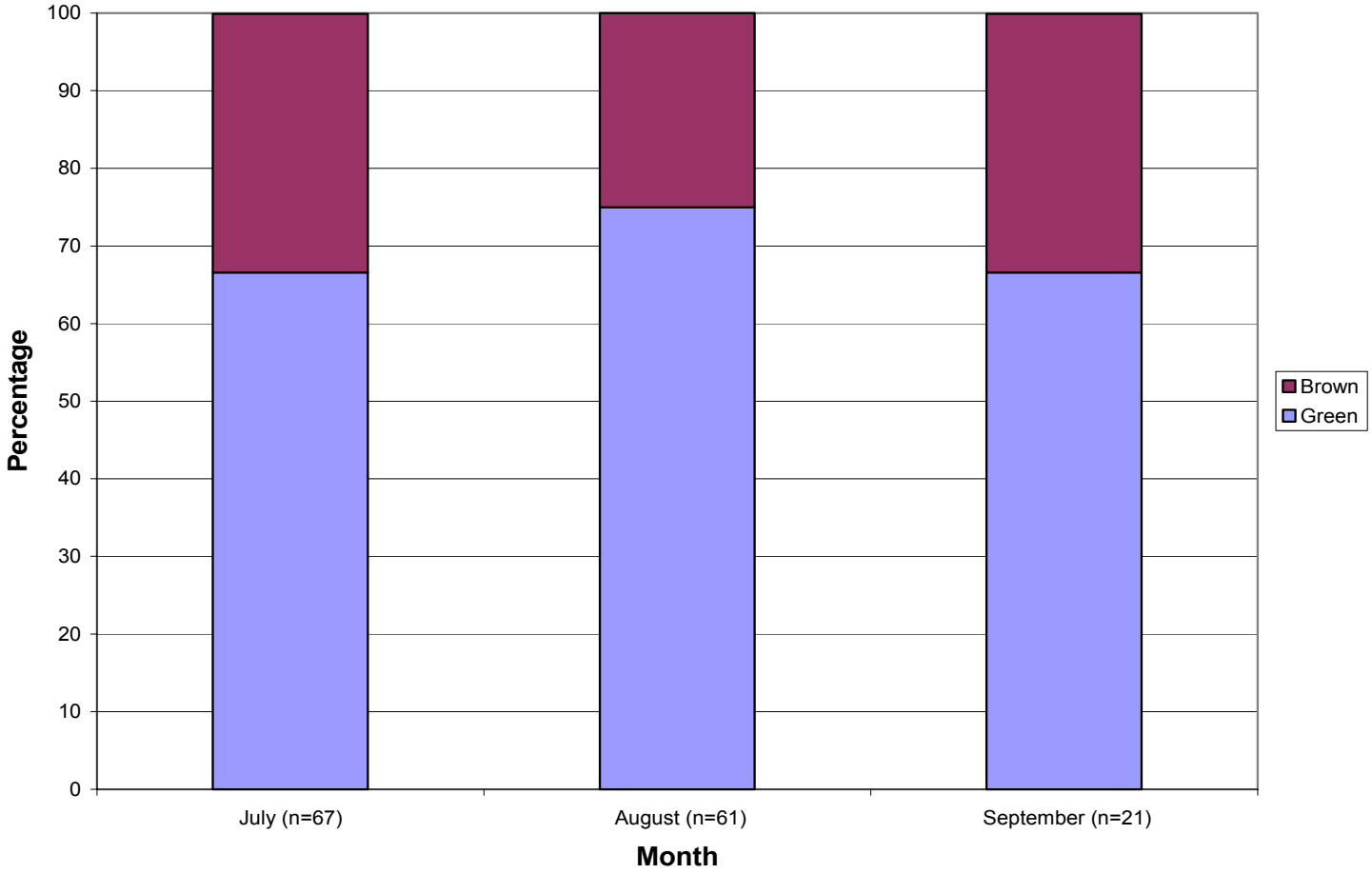


Figure 12: Spot number on dorsum of *R. pipiens* (n=241)

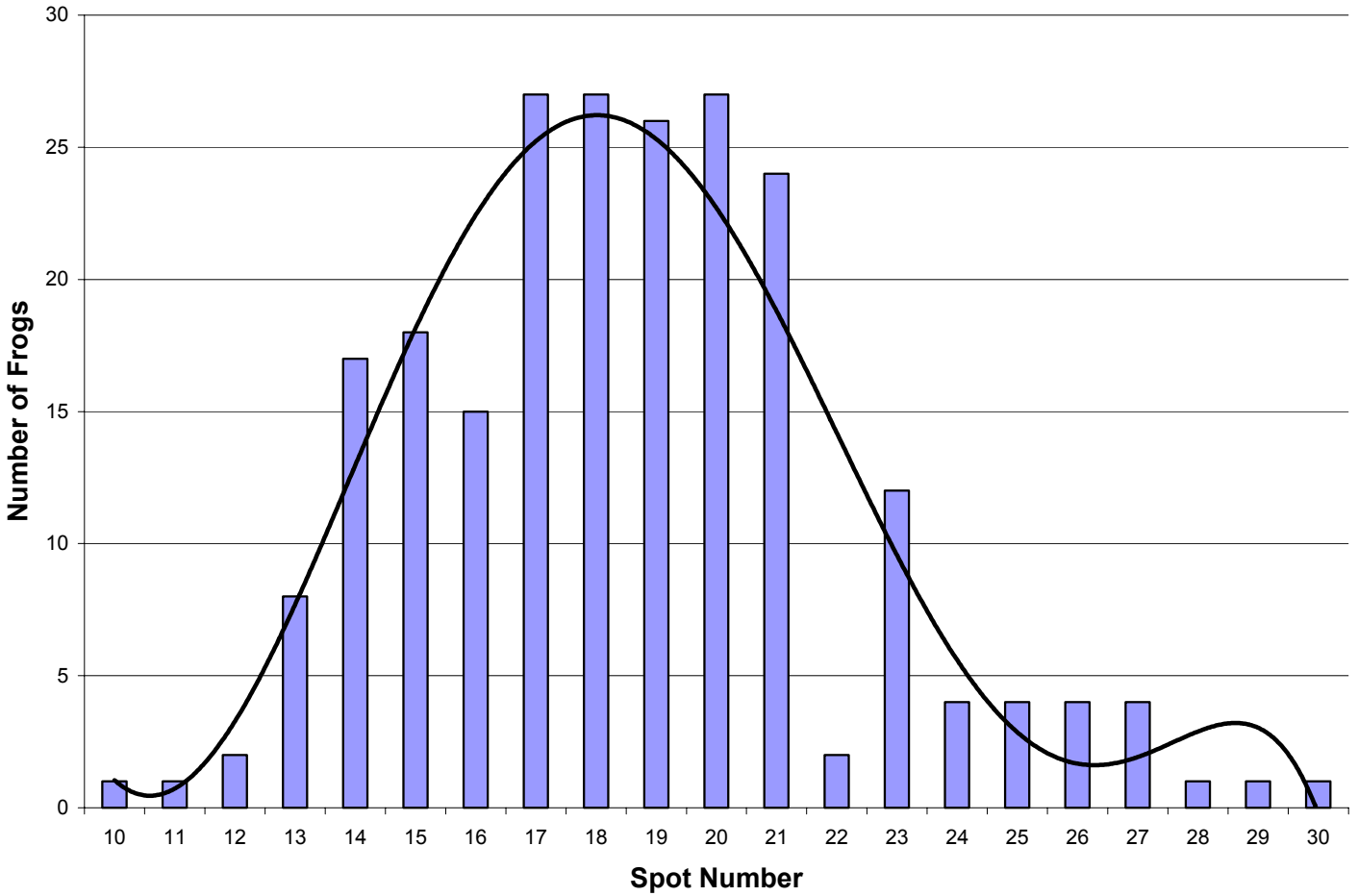


Table 1: Morphometrics of Fully Developed *R. pipiens* Larvae (n=12)

	SVL	***CW***	***TIB***	***NW***	***ENL***	***ESL***	***EW***	***TH***	***TL***
Mean	32.3	9.90	17.0	2.88	2.76	5.40	3.11	12.2	48.2
St. Err.	0.24	0.78	0.35	0.32	0.19	0.25	0.16	1.10	3.93
Range	1.20	2.30	4.00	1.00	2.20	3.20	1.70	13.2	43.9

Table 2: Mean SVL of Male and Female *R. pipiens* (Fall 2003)

Sex	***Number (n)***	***SVL (mm)***
Male	44	58.2±0.6
Female	20	60.3±1.3
		p=0.094

Table 3: Seasonal Sexual Variation of *R. pipiens* (2003)

Season	***Number (n)***	***Male***	***Female***
Spring	127	96.0%	4.0%
Fall	62	68.0%	32.0%

Table 4: Results for Mark-recapture Study of *R. pipiens*, Quadrat #1

	Number (n)	***Total Recap.***	***Night 2 Recap.***	***Night 2/3 Recap.***	***Night 1/3 Recap.***	***Night 1/2/3 Recap.***	***Crossover***
Night #1	38	---	---	---	---	---	---
Night #2	20	4	1	---	---	---	3
Night #3	5	5	---	1	1	0	0

Table 5: Results for Mark-recapture Study of *R. pipiens*, Quadrat #2

	Number (n)	***Total Recap.***	***Night 2 Recap.***	***Night 2/3 Recap.***	***Night 1/3 Recap.***	***Night 1/2/3 Recap.***	***Crossover***
Night #1	52	---	---	---	---	---	---
Night #2	7	4	2	---	---	---	2
Night #3	4	0	---	0	0	0	0

CHAPTER TWO: The Prevalence of *Aeromonas hydrophila* and *Pseudomonas spp.* Skin Infections and other Malformations of *Rana pipiens* in West Virginia

Introduction:

Over the past decade, the population status of many anuran species has come into question, because once flourishing populations of anurans have declined drastically. In addition to the more obvious agents of decline, such as habitat destruction, the effects of deformities and disease upon amphibians have been questioned greatly. There is evidence to suggest that the immune systems of amphibians, normally protecting against infections, are failing to protect frogs from pathogens (Maneiro and Carey, 1997). Additionally, the cited authors suggest that immune systems of amphibians from temperate climates are extremely sensitive to temperatures at or near hibernating temperature. This is important for frogs, such as *R. pipiens* that have their entire range contained in the temperate zone. For frogs affected by bacterial infections, it appears that deaths occurs during cooling in the fall (hibernation) or during the spring thaw when body temperatures rise, because immune function has not been restored (Maniero and Carey, 1997). Although bacterial infections may enter through the skin, it has been suggested that the intestine is a chief entry point for a majority of bacteria, including *Aeromonas hydrophila* (Waaij *et al*., 1974).

There are many accounts describing major amphibian declines caused solely by bacterial agents. Approximately 300 *Bufo americanus* inhabiting 2 ponds in Charleston, WV on March 22, 1948, had all died by March 25, 1948 from the bacterial induced condition known as "red-leg" disease (Dusi, 1949). At the time of its discovery in 1891,

"red-leg" disease was identified as *Bacillus hydrophilus fuscus* (Rusell, 1898). As more information was discovered regarding this disease, the nomenclature was changed to *Pseudomonas hydrophila* (Dusi, 1949). In the most recent publications, the observed nomenclature for this bacterium is *Aeromonas hydrophila*.

Frogs affected by "red-leg" disease usually display the following series of symptoms. Upon infection, the first noticeable sign are lesions, ecchymoses, and ulceration on the legs and face (Emerson and Norris, 1905). In later stages of the disease, infected frogs become unresponsive to any form of stimuli and assume a supine position with the legs sprawled outward (Emerson and Norris, 1905). Before death, some frogs have muscular spasms so severe the hind legs are extended as far forward as the head (Russell, 1898).

The expression "red-leg" disease is quite arbitrary, because there are several bacterial species known to elicit the typical physiological conditions of "red-leg" disease. In addition to *A. hydrophila*, several other bacterial species have been identified as causative agents of "red-leg" disease (Gibbs *et al*., 1966). However, *A. hydrophila* is most commonly referred to as the major causative agent of "red-leg" disease and the major focus of this chapter.

Bacterial Species Description

Aeromonas ssp.

Within the genus *Aeromonas*, the following 4 species are recognized: *A. hydrophila*, *A. caviae*, *A. sobria*, and *A. salmonicida* (Altweg, 1999). Bacteria within this genus are described as gram-negative, oxidase-positive rods expressing both

respiratory and fermentative metabolism (Popoff, 1984). In addition to being oxidase-positive, they are also catalase positive, reduce nitrate to nitrite, and ferment D-glucose to acid or gas (Altweg, 1999). Aeromonad bacteria are most commonly found in aquatic ecosystems worldwide, mainly freshwater. They are occasionally found in marine environments, but seem to be more common in marine watersheds directly associated with freshwater (Altweg, 1999). Cold-blooded animals such as frogs, lizards, and fish are most commonly infected by *Aeromonas* infections and frequently are carriers (Gravenitz, 1987).

Pseudomonas ssp.

Bacteria of the genus *Pseudomonas* are aerobic, non-spore forming, gram-negative rods, with a slightly curved shape (Holt, 1994). Most isolates are oxidase-positive, catalase positive, and appear as lactose nonfermenters (Kiska and Gilligan, 1999). Additionally, most Pseudomonas ssp. degrade glucose oxidatively and convert nitrate to nitrite or nitrogen gas (Kiska and Gilligan, 1999). The various strains of *Pseudomonas spp.* are all nutritionally variable and have distinctive colony morphology that aids in identification (Kiska and Gilligan, 1999). *Pseudomonas spp.* are found worldwide, most usually associated with moist environments. In addition to moist environments, these bacteria are found directly associated with water and soil and plants, namely fruits and vegetables (Kiska and Gilligan, 1999).

Materials and Methods:

Sampling Methods

Frogs were sampled from spring-fall 2003 and were captured in the same manner as explained in chapter one. Information regarding the prevalence of any malformations and/or skin diseases was recorded for each frog. Frogs with malformations and skin infections were taken back to the lab for further analysis.

Identification of malformations

If frogs were discovered with malformations or any other growth defects, the description of the malformation along with where it occurred on the body of the frog was recorded. Malformations that were observed in this study included errors in development (i.e. polydactyly), visible parasites, and external skin growths (tumors). Digital photographs of affected frogs were taken to document the occurrence of the malformations.

Identification of Bacterial Colonies

Frogs displaying symptoms of red-leg infections (legs with red discoloration and visible open wounds) were brought back to the lab for bacterial analysis. Frogs displaying symptoms and the progression of "red-leg" disease are shown in figure 13. A swab from leg wounds of affected frogs were taken and streaked using sterile techniques onto a Hektoen enteric (HKE) and a Tryptic Soy agar (TSA) plate. The HKE plate was used because it only permits colonization by gram-negative bacteria. As mentioned earlier, *Aeromonas hydrophila* is a gram-negative bacterium, so by using the HKE plates,

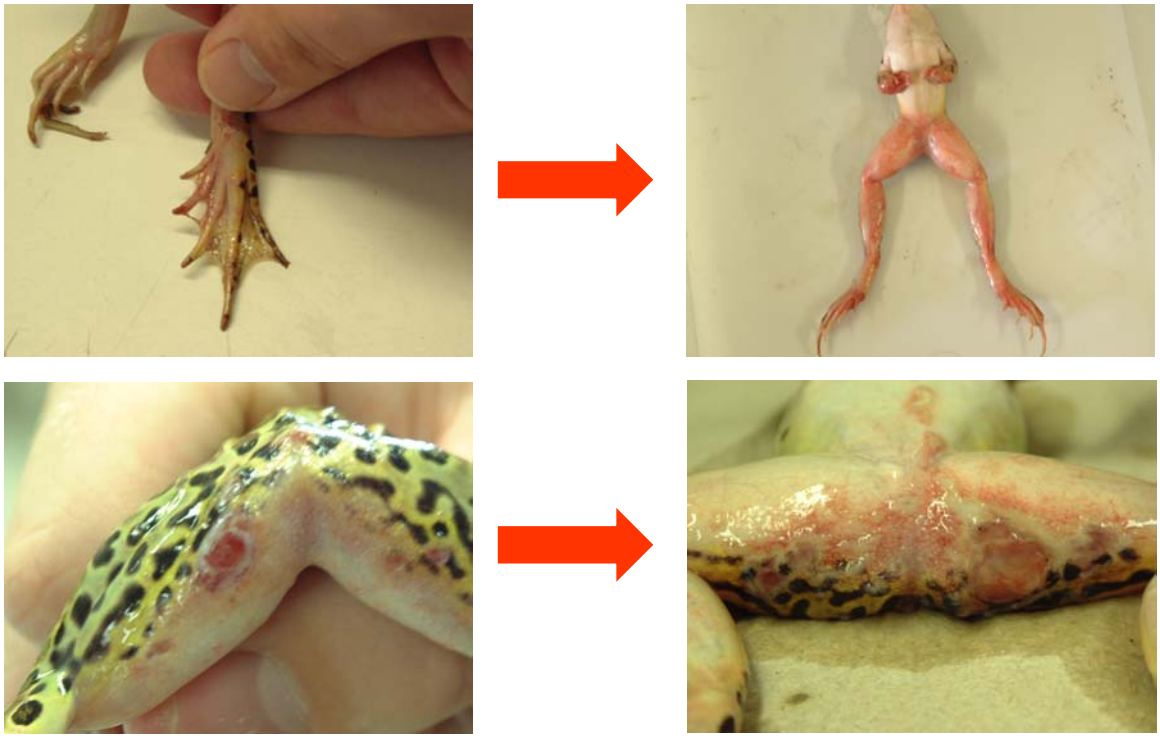


Figure 13: Progression of "Red-leg" Disease in *R. pipiens*

gram-positive bacteria could be eliminated. After a 24-hour incubation period, the plates were analyzed for actively growing cultures of bacteria. The TSA plate showed a lawn of bacteria with many mixed colonies, while the HKE plate showed a series of isolated orange and green colonies. The orange colonies were described as lactose fermenting bacteria (LF), while the green colonies were described as non-lactose fermenting bacteria (NLF). After an oxidase test, both colonies were described as oxidase positive. Five colonies of the lactose fermenting (LF) and 5 colonies of the non-lactose fermenting (NLF) bacteria were each streaked onto both TSA and R2A plates. Streaks were done on both R2A and TSA plates to ensure that each original colony could be isolated onto a new plate successfully. After 48 hours of incubation, an isolated colony from each TSA plate was streaked onto blood agar plates. The isolated colonies from the TSA plates were chosen because bacterial growth upon these plates was more successful. A total of 10 blood agar plates (5 LF and 5 NLF) were streaked and incubated for a period of 24 hours.

After incubating for 24 hours, a sterile swab was used to remove bacteria from the plate. The swab was then immediately immersed into a tube of inoculating fluid. The swab was pressed against the side of the tube to break up any large clumps of cells. The swab was swirled within the inoculating fluid until a homogenous solution was created. Upon achieving a homogenous solution, the optical density of each solution was determined by using a Spectronic Gensys 5 spectrophotometer. For proper BIOLOG analysis, each sample must register an optical density reading of 52% ± 2% transmittance. Transmittance values were increased and decreased by suspending more bacteria and adding more inoculating fluid, respectively. Upon achieving a proper optical

density level, the inoculating fluid was promptly transferred to a GN2 MicroPlate™ by means of a BIOLOG multichannel micropipette. Each Microplate is composed of a series of 96 wells with various metabolites deposited in each well. As the bacteria are inoculated into the plates, the bacteria react with the metabolites producing either a positive (blue) or negative (colorless) reaction. After the Microplates were inoculated, they were wrapped in parafilm and incubated for 24 hours. After incubation, each Microplate was placed into the BIOLOG machine and processed. The BIOLOG machine compared the series of positive and negative reactions to the various bacterial species within the databank. The most closely related species along with the confidence interval were produced upon analysis. A detailed readout describing the sampled bacterial colonies was produced for each Microplate. Sample readouts for the results of *A. hydrophila* and *P. maculicola* identification are shown in figures 14 and 15, respectively.

Results:

Bacterial Identification

Two frogs with symptoms of "red-leg" disease were brought back to the lab for analysis of skin infections. Of the two frogs collected, only one could be sampled because the other expired before bacterial analysis could be performed.

Two different colonies were identified as 3 separate bacterial species using BIOLOG bacterial analysis. Orange colonies were positively identified as *Aeromonas hydrophila*, while green colonies were identified as a mixed colony of *Pseudomonas ssp*. Colonies identified as *A. hydrophila* had an average confidence interval (CI) of 99.2%± and an average similarity value (Sim) of 0.77. The similarity value is an estimate

Figure 14: BIOLOG Readout for Bacterial Identification of *Aeromonas hydrophila*

Program	: Biolog MicroLog3 4.01A
Threshold Mode	: Automatic : 136/222
590/750 Filters Used	: 6 / 5
Read Time	: Oct 03 2003 3:48 PM
Incubation Time	: 16-24
Sample Number	: 2
Plate Type	: GN2
Strain Type	: GN-NENT
Strain Number	: 2
Strain Name	: RAPI
Other	: Orange Colony on Enteric Agar
Data Generation Mode	: Reader
Number +/b/- Reactions	: 36 / 6 / 54
Database To Search	: MicroLog
Progressive ID File Name	: C:\BIOLOG40\GN401.KIC

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	45	<922>	<876>	<402>	<277>	-1+	<768>	-54	<599>	-5	17
B	26	<719>	1	<694>	-23	<859>	-25	-8	-19	<865>	<653>	<743>
C	11	<835>	109+	13	-26	51	<847>	<868>	32	-4	<613>	{142}
D	{181}	<275-	32	124	-35	14	<828>	13	-14	38	32	-35
E	-10	-48	27	37	-20	<715>	45	103	18	-6	-44	<482>
F	{209}	29	-21	{139}	88	<374>	<833>	<518>	<480>	<500>	<442>	<334>
G	{196}	8	6	53	-30	85	16	<674>	<866>	98	22	10
H	-6	<843>	<334>	{166}	-57	<389>	-36	-10	<716>	<351>	<679>	<669>

=> Species ID : AEROMONAS HYDROPHILA DNA GROUP 1 <=

	Species	PROB	SIM	DIST	TYPE
=>1]	AEROMONAS HYDROPHILA DNA GROUP 1	100	0.77	3.51	GN-NENT
2]	AEROMONAS ICHTHIOSMIA	0	0.00	5.90	GN-NENT
3]	AEROMONAS ENCHELEIA	0	0.00	5.96	GN-NENT
4]	AEROMONAS ALLOSACCHAROPHILA	0	0.00	6.18	GN-NENT
5]	AEROMONAS VERONII DNA GROUP 10	0	0.00	6.67	GN-NENT
6]	AEROMONAS HYDROPHILA-LIKE DNA GROUP 2	0	0.00	7.20	GN-NENT
7]	AEROMONAS VERONII/SOBRIA DNA GROUP 8	0	0.00	7.25	GN-NENT
8]	AEROMONAS MEDIA DNA GROUP 5B	0	0.00	7.62	GN-NENT
9]	AEROMONAS EUCRENOPHILA DNA GROUP 6	0	0.00	8.26	GN-NENT
10]	AEROMONAS JANDAEI DNA GROUP 9	0	0.00	8.27	GN-NENT

Figure 15: BIOLOG Readout for Bacterial Identification of *Pseudomonas maculicola*

Program	: Biolog MicroLog3 4.01A
Threshold Mode	: Automatic : 71/156
590/750 Filters Used	: 6 / 5
Read Time	: Oct 03 2003 4:01 PM
Incubation Time	: 16-24
Sample Number	: 7
Plate Type	: GN2
Strain Type	: GN-NENT
Strain Number	: 7
Strain Name	: RAPI
Other	: Green Colony on Enteric Agar
Data Generation Mode	: Reader
Number +/b/- Reactions	: 29 / 15 / 52
Database To Search	: MicroLog
Progressive ID File Name	: C:\BIOLOG40\GN401.KIC

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	1	12	66	{99}	{135}	-9	{144}	-11	13	63	-6
B	-0	{94}	-13	-14	-14	<301>	-12	-16	-21	-11	<198-	<175>
C	-15	-4	33	-15	-13	-15	-18	-16	-24	-19	{143}	62
D	70+	<314>	<400>	{82}	6	-6	<330>	-9	-23	-1	<296>	-9
E	{154}	3	-4	<339>	0	<273>	-7+	<239>	<425>	-9+	-17	<231>
F	<256>	7	-8	{94}	{78}	<212>	{96}	<356>	<618>	<355>	-12	{74}
G	<273>	<366>	{84}	<227>	30	<522>	<322>	{105}	<489>	21	{126}	<324>
H	<281-	<248-	13	-16	<316>	<312>	<283>	-12	{80}	-22	-27	-20

=> Species ID : PSEUDOMONAS MACULICOLA <=

	Species	PROB	SIM	DIST	TYPE
=>1]	PSEUDOMONAS MACULICOLA	98	0.60	5.96	GN-NENT
2]	PSEUDOMONAS FLUORESCENS BIOTYPE C	1	0.01	7.44	GN-NENT
3]	PSEUDOMONAS AERUGINOSA	1	0.00	7.68	GN-NENT
4]	PSEUDOMONAS CITRONELLOLIS	0	0.00	8.24	GN-NENT
5]	PSEUDOMONAS NITROREDUCENS/AZELAICA	0	0.00	8.26	GN-NENT
6]	PSEUDOMONAS AURANTIACA	0	0.00	8.72	GN-NENT
7]	PSEUDOMONAS FULVA	0	0.00	9.21	GN-NENT
8]	PSEUDOMONAS PUTIDA BIOTYPE A	0	0.00	9.42	GN-NENT
9]	PSEUDOMONAS PUTIDA BIOTYPE B	0	0.00	9.54	GN-NENT
10]	PSEUDOMONAS LUNDENSIS	0	0.00	9.59	GN-NENT

of how closely wells of the tested Microplate match records within the database. In order for successful identification, the Sim value must be at least 0.5. Green colonies were identified as *Pseudomonas fulva* (100% CI, 0.62 Sim), *Pseudomonas maculicola* (96.5 CI, 0.65 Sim), and an unidentifiable colony of *Pseudomonas* (0% CI, 0.30 Sim).

Tables 6 and 7 illustrate the species description, CI value, and Sim value for each microplate. In table 6, it can be seen that bacteria tested in plates (1-5) can be confidently identified as *A. hydrophila*. Microplates #2 and #3 both had CI values of 100% and Sim values of 0.77 and 0.82, respectively. Microplates #1 and #5 both had CI values of 99% and Sim values of 0.72 and 0.75, respectively. Microplate #4 had the lowest CI value (98%) and also had a Sim value of 0.77.

From table 7, it can be seen that the bacteria tested in plates (6-10) were identified as multiple *Pseudomonas ssp*. Microplate #6 was identified as *P. aeruginosa*, but it should be noted that no CI was produced (0%) and the Sim value was only 0.30. Thus, the description for this particular Microplate should be interpreted with caution. Microplates #7 and #8 were both identified as *P. maculicola* having CI values and Sim values of 98%, 0.60 and 95%, 0.61, respectively. Microplates #9 and #10 were both identified as *P. fulva*, having CI values and Sim values of 100%, 0.68 and 100%, 0.55, respectively.

Results of Hindlimb malformations

Throughout this study, 3 frogs were discovered having varying forms of hindlimb malformations. Of the 3 frogs affected, 2 were *R. pipiens*, while 1 *R. catesbeiana* was found with hindlimb malformations. The malformation affecting the first *R. pipiens* was

described as Ectrodactly/Brachydactyly. Ectrodactly is a type of malformation that is described as a condition where at least 1 entire digit (phalange and metatarsal) is missing (Meteyer, 2000). In figure 16 it can be seen that the frog is missing 3 complete phalanges on the right hindlimb. The description of Brachydactyly will be discussed below with the next malformation.

The malformation affecting the second *R. pipiens* was described as Brachydactyly. This malformation is described as a shortening of digits caused by a reduction in the number of phalanges (Meteyer, 2000). In figure 17 it can be seen that all 5 digits are greatly reduced in size on the right hindlimb. Besides the reduction in size of the phalanges, the remainder of the hindlimb structure was relatively unaffected. However, the normal pigmentation pattern was altered on the hindlimb of this frog. The normal pattern of dark circular blotches was altered to numerous small black flecks.

The third malformation affecting the *R. catesbeiana* specimen was described as Polymelia. This type of malformation refers to a condition where supernumerary limb segments are present (Meteyer, 2000). In figure 18, an extra set of phalanges can be seen growing out from the ankle of the frog. Additionally, this specimen had a continuous band of skin that connected the hip to the ankle. This was problematic, because it restricted the hindlimb and made it very difficult for this frog to jump.

Results of External Tumors

During this study, 41 external tumors were discovered affecting *R. pipiens*. The frog pictured in figure 19 illustrates the appearance of the external tumors. In figure 20, it can be seen that tumors were found in association with various body parts. A majority

of the tumors (42%) were found to be associated with the mouthparts. The next most commonly affected area was the anus, with 29% of the tumors located in this area. Additionally, 10%, 7%, and 7% of the external tumors were found to be associated with the eyes, back, and snout, respectively. An additional 5% of the external tumors were associated with various body parts, such as the stomach and appendages.

It should also be noted that the number of frogs affected by external tumors was much lower in the fall months than in the earlier summer months. From this, it should be explained that frogs expressing external tumors did not recover, but probably died from detrimental effects of the tumors. This is very important, because many affected frogs were young of the year that would have made up a majority of the breeding population of *R. pipiens* the following spring.

Discussion:

Bacterial Infections

From the above results, it is apparent that *Aeromonas hydrophila* and *Pseudomonas ssp.* colonize skin lesions of *R. pipiens*. These findings are significant, because as discussed earlier, *A. hydrophila* is the major cause of "red-leg" disease in many anuran species. Frogs suspected of being infected with "red-leg" disease had physiological conditions similar to those described in the literature, such as denuded areas around the feet, deterioration of toe webbing, and open lesions on the legs (Emerson and Norris, 1905). Additionally, legs had the characteristic red tinge caused by

Figure 16: Adult *R. pipiens* with Ectrodactly/Brachydactly Rear Limb Malformation

Figure 17: Adult *R. pipiens* with Brachydactly Rear Limb Malformation

Figure 18: Juvenile *R. catesbeiana* with Polymelia and Altered Skin Webbing Rear Limb Malformation

Figure 19: Photograph of *R. pipiens* with an External Tumor Located on the Snout

hemorrhaging of surface capillaries. However, the frogs had not progressed into the later stages of the disease. Frogs experiencing later stages of "red-leg" disease become completely debilitated and show little response to any form of stimulus (Emerson and Norris, 1905; Gibbs et al. 1966).

The occurrence of *A. hydrophila* at Greenbottom Swamp is important, because it indicates that frog populations are being exposed to harmful pathogens. These findings are also very important because this population of *R. pipiens* is not only the largest, but also one of the few stable populations of *R. pipiens* known in West Virginia. If an epidemic of *A. hydrophila* were to infect this population, implications could be serious. However, the presence of *A. hydrophila* does not mean that all frogs possessing this bacterium will become infected. It has been reported that *A. hydrophila* can be isolated from healthy frogs and tadpoles in their natural environment (Hird *et al*., 1981). Additionally, *A. hydrophila* is more commonly associated with the developmental stages associated with water (Hird *et al*., 1981). The cited authors discovered that 32% of adult *R. pipiens* sampled were positive for *A. hydrophila*, while 63% of sampled *R. pipiens* larvae were positive for *A. hydrophila*. The results of the cited experiment are logical, because *A. hydrophila* thrives in aquatic habitats, such as ponds and breeding pools. This discovery could have serious implications, because it suggests that a majority of amphibian declines caused by *A. hydrophila* might occur in the larval stage and as a result may go unnoticed.

The discovery of *Pseudomonas spp.* indicates that these bacteria may be part of the natural flora on the skin of *R. pipiens*. There are no records describing *P. fulva* and *P. maculicola* as bacterial species linked to amphibian declines. It is suggested that *P. fulva*

functions as an ice nucleator in freeze tolerant bivalves (Loomis and Zinser, 2001). Most freeze tolerant organisms use low molecular weight sugars and proteins to function as natural antifreeze, but these particular bivalves lack these proteins and sugars. So it is believed these bivalves use the bacteria as an agent of ice-nucleation to protect against freezing temperatures. Additionally, *Pseudomonas* bacteria active in ice nucleation have also been isolated from freeze tolerant frogs (Lee *et al*., 1991). *Rana pipiens* is not known as a frog with freeze tolerant properties. However, this frog is a very early spring breeder that is exposed to extended periods of freezing temperatures. *Pseudomonas fulva* may provide a small degree of freeze tolerance essential for *R. pipiens* to survive freezing spring temperatures. In addition to *P. fulva* and *P. maculicola*, *P. aeruginosa* was also identified from the infected frog. *Pseudomonas aeruginosa* is notorious for causing major eye and ear infections in humans. It is suggested that *P. aeruginosa* is pathogenic for *R. pipiens* under suboptimal environmental conditions, such as overcrowding and excessive temperatures (Brodkin *et al*., 1992). In this experiment, *P. aeruginosa* was identified on only one Microplate, having a CI value of 0% and a Sim value of 0.30. It is more likely that the Microplate did not react completely and the BIOLOG data most closely resembled the data for *P. aeruginosa*, resulting in a false positive.

External tumors and hindlimb malformations

The discovery of external tumors and hindlimb malformations is important, because it suggests that primary stressors are inducing malformations in these frogs. Primary agents of amphibian declines include habitat degradation and fragmentation, water pollution, acid deposition, and UV-B light. Secondary agents of amphibian

declines include disease, malformations, and tumors. Disease, malformations, and tumors are detrimental to anurans; however it is likely that primary stressors induce these secondary agents. For frogs such as *R. pipiens*, the destruction of habitat is a very detrimental primary stressor. *Rana pipiens* unlike many other frogs requires 3 distinct habitats to complete its life history. It requires breeding habitat (temporary pools), terrestrial foraging habitat, and overwintering habitat (large, well oxygenated lake) (Merrell, 1977). With increasing development, the terrestrial areas surrounding wetlands are usually developed, leaving only the aquatic habitat. For many frogs, the remaining aquatic habitat is enough to complete their life history, but for *R. pipiens*, the terrestrial habitat is just as important as the aquatic habitat. Another important primary stressor is the pollution of water sources from sources, such as agricultural runoff. This is a likely cause for the appearance of the external tumors, because the study site is in close proximity with the Ohio River. Periodically the Ohio River floods its banks and inundates the entire Greenbottom WMA. Runoff from agricultural areas along with harmful chemicals may accompany the floodwater into the swamp. The presence of these harmful substances may lower the immune response of the frogs, allowing aberrant growths such as external tumors to occur more frequently.

Although hindlimb malformations do not seem to affect frogs as adversely as tumors and infections, the frogs discovered represent survivors of hindlimb malformations. More frogs are probably affected by malformations than are known, because many frogs die before the malformations are discovered (Meteyer, 2000). These malformed frogs lack the ability to escape predators and are usually eaten. This fact

suggests that malformations may be just as harmful as other secondary agents of amphibian decline.

From this study it can be concluded that *A. hydrophila* was identified in *R. pipiens* using BIOLOG analysis. Additionally, external tumors and hindlimb malformations also affect frogs inhabiting the Greenbottom WMA. Knowing that these agents are affecting frogs in West Virginia, conservation biologists can look for additional areas of the state where other populations of amphibians are affected by similar factors.

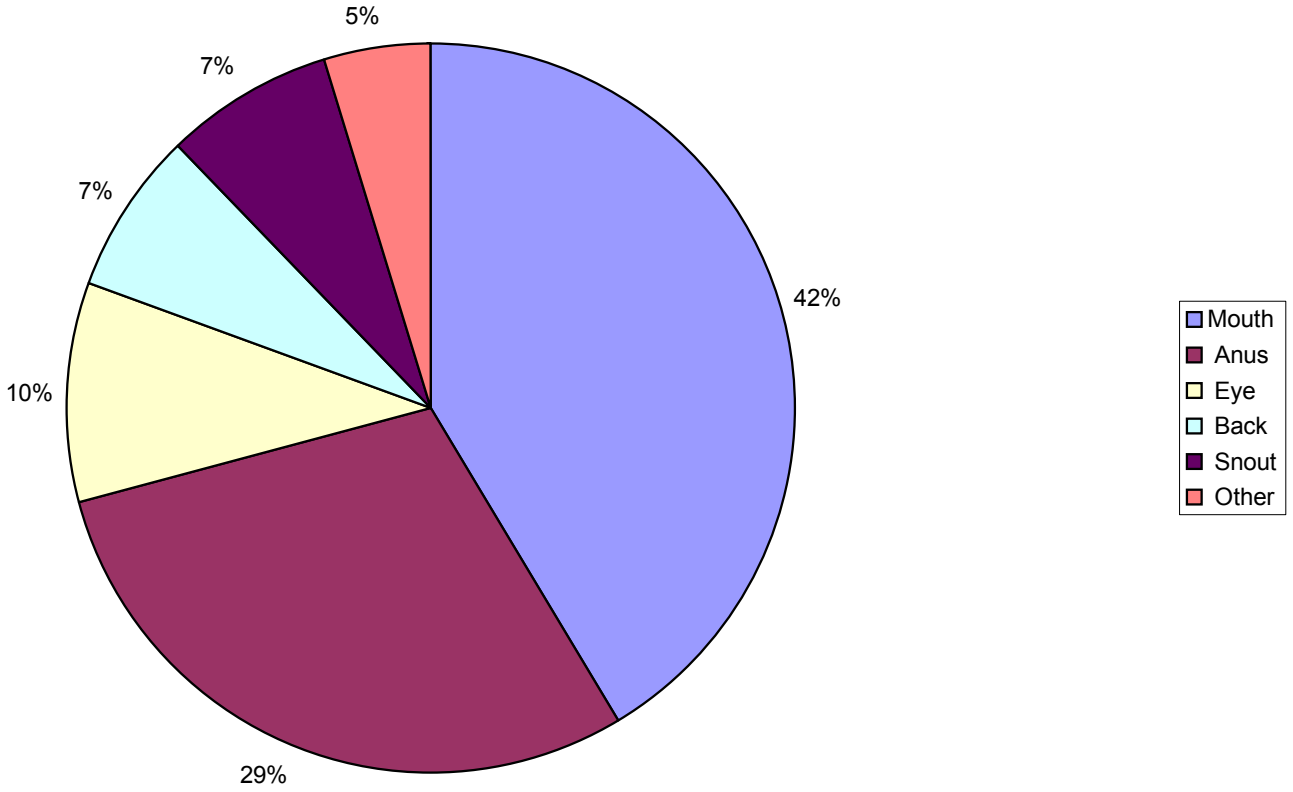
Table 6: Results of Bacterial Identification for Microplates 1-5

	Plate 1	**Plate 2**	**Plate 3**	**Plate 4**	**Plate 5**
Species	*A. hydrophila*	*A. hydrophila*	*A. hydrophila*	*A. hydrophila*	*A. hydrophila*
Colony color	Orange	Orange	Orange	Orange	Orange
CI value	99%	100%	100%	98%	99%
Sim value	0.72	0.77	0.82	0.77	0.75
Transmittance	52.2%	52.6%	52.1%	52.4%	52.3%

Table 7: Results of Bacterial Identification for Microplates 6-10

	Plate 6	**Plate 7**	**Plate 8**	**Plate 9**	**Plate 10**
Species	*P. aeruginosa*	*P. maculicola*	*P. maculicola*	*P. fulva*	*P. fulva*
Colony color	Green	Green	Green	Green	Green
CI value	0%	98%	95%	100%	100%
Sim value	0.30	0.60	0.61	0.68	0.55
Transmittance	51.9%	52.1%	52.3%	52.2%	53.7%

Figure 20: Number and Location of External Tumors for *Rana pipiens* (n=41)



CHAPTER THREE: Analysis of Anuran Assemblage Interactions at Greenbottom WMA

Introduction:

Assemblage level experiments are important because they illustrate niche partitioning between closely related, sympatric species. If the same habitat is to be exploited by several species, niche partitioning is essential to avoid competitive interactions that may be detrimental to the existence of an organism. The goal of these types of studies is to understand how organisms interact, and to determine the role of interspecific interactions in structuring a particular community (Hecnar and M'closkey, 1997). Buskirk (2003) suggests that competition between organisms helps maintain habitat partitioning. Associations of amphibians in aquatic communities are important for the evolution and development of predator-prey associations, competitive interactions, and natural history characteristics (Collins and Wilbur, 1979).

In this experiment, habitat partitioning between the following 3 sympatric anuran species was examined: Northern Leopard Frog (*Rana pipiens*), Green Frog (*Rana clamitans melanota*), and American Bullfrog (*Rana catesbeiana*). The above listed frog species are illustrated in figures 21-23, respectively. *Rana c. melanota* and *R. catesbeiana* commonly inhabit permanent aquatic areas during and after the breeding season (Green and Pauley, 1987). *Rana pipiens* utilizes more temporary wetlands for breeding and forages in terrestrial areas during the summer and fall months (Green and Pauley, 1987). Collins and Wilbur (1979) noted that *R. catesbeiana* and *R. c. melanota*

Figure 21: Photograph of an Adult *R. pipiens*

Figure 22: Photograph of an Adult *R. c. melanota*

Figure 23: Photograph of an Adult *R. catesbeiana*

were sympatric in permanent wetlands, while *R. pipiens* and *R. c. melanota* were sympatric in temporary wetlands during the breeding season.

In order for similar organisms to utilize the same habitat, these organisms must utilize different life history strategies, such as differences in time of breeding, clutch size, and age at maturity. Life history characters provide an organism with a means to cope with selective pressures exhibited by the environment (Collins, 1975).

The purpose of this experiment was to analyze habitat partitioning displayed by the above frog species. Additionally, population demographics and habitat preferences were also observed and recorded.

Materials and Methods:

Description of study site

This study was completed in a temporary wetland located in the Greenbottom WMA. This study took place in a temporary wetland called the Hoeft Marsh, which was located at the western end of the swamp. The Hoeft Marsh is a long, narrow slough that was approximately 500 m long by 16 m wide. For a more detailed description of the Greenbottom WMA, please refer to the study site description on pages 1-3 of this manuscript. The study site was composed of the 3 following habitat regimes: aquatic zone, transition zone, and terrestrial zone. The aquatic zone had a maximum water depth of approximately 1 m with a substantial portion of shallow and wet, grassy areas. The aquatic zone was dominated by the following plant species: *Hibiscus moscheutos*, *Juncus effusus*, *Polygonum coccineum* and *Cephalanthus occidentalis*. The transition zone was described as the area of land between the aquatic zone and the terrestrial zone and had

significantly more vegetation than the aquatic and terrestrial zones. The transition zone was dominated by the following plant species: *Rosa multiflora*, *Typha latifolia* and *Cyperus strigosus*. The terrestrial portion of the marsh was a large open meadow, periodically cut for agricultural purposes. The terrestrial portion of the marsh was dominated by the following plant species: *Asclepias syriaca*, *Impatiens capensis*, and *Polygonum cuspidatum*.

Survey Methods

The surveys began in September and continued into mid-November, with 11 sampling events completed. Surveys were initiated in the fall months to ensure that listed frog species had completed their breeding cycles and had transitioned to the respective habitats utilized during the non-breeding season. The survey area was an aquatic/terrestrial quadrat 60 m long X 34 m wide (Figure 24). The dimensions of the 3 habitat zones were as follows: aquatic zone (16 m), transition zone (2 m), and terrestrial zone (16 m). The quadrat was surveyed for one hour simultaneously by 2 researchers, one in the aquatic zone and one in the terrestrial zone. Additionally, the researcher monitoring the terrestrial zone also monitored the transition zone. All surveys were started at 20:15 hours and were terminated promptly at 21:15 hours. The researcher monitoring the aquatic zone located frogs by inducing eye-shine with a Petzl-Duo headlamp. The aquatic zone was monitored in passes parallel to the length of the quadrat. All open water areas and all emergent vegetation were actively searched for frogs. The researcher monitoring the terrestrial/transition zone searched the area in a series of S-shaped passes parallel with the width of the quadrat, with the distance

between each pass being approximately 1 m. Frogs within the terrestrial/transition zone were located by spotting them directly with a Petzl-Duo headlamp or by flushing them out of grass clumps.

Upon capture, the frogs were removed from the quadrat and a species-specific flag was used to mark the relative position of each frog. The relative position was measured as the distance of the flag from the aquatic/transition zone interface and transition/terrestrial zone interface. Aquatic frogs were given a negative distance reading, while frogs captured in the terrestrial zone were given a positive distance reading. Frogs captured within the transition zone were given a relative distance measurement of 0 m. Maximum distance measurements within the terrestrial and aquatic zones were 8 m, because past this measurement, the frog was closer to the opposing edge of the quadrat. For each frog captured, SVL, sex, and reproductive status were recorded. In addition to population demography data, detailed microhabitat information was recorded for each capture.

To determine significance of habitat partitioning, Kruskal-Wallis one-way ANOVA on ranks with pairwise comparison (Dunn's Method) was used to analyze the data. The Kruskal-Wallis one-way ANOVA is a non-parametric statistical test that analyzes three or more groups of categorical data. The Kruskal-Wallis test is used in place of the more traditional one-way ANOVA when the data does not pass a normality test. For completion of statistical analysis, Sigma Stat version 2.03 was used. Population demographic information was analyzed by using descriptive statistics.

Results:

From the data, it is apparent that there is habitat partitioning among the 3 frog species studied. In figure 25, the following habitat partitioning gradient can be seen: *R. pipiens* (mainly terrestrial and some aquatic), *R. catesbeiana* (completely aquatic), and *R. c. melanota* (terrestrial and aquatic at transition zone). Significant habitat partitioning ($p < 0.05$) was observed between the following groups (*R. catesbeiana* & *R. pipiens*) and (*R. catesbeiana & R. c. melanota*). However, significant habitat partitioning was not observed between *R. pipiens* and *R. c. melanota*. In figure 25 it can be seen that the highest number (16 captures) of *R. catesbeiana* were at the -6 m mark in the aquatic portion of the quadrat. As for *R. pipiens*, the highest number of captures occurred at the 0 m mark and 4 m & 6 m mark, having 8 and 10 captures, respectively. Although the sample size was small, *R. c. melanota* was most commonly seen at the 0 m and –2 m mark, both having 3 captures. It can also be seen that *R. pipiens* and *R. catesbeiana* were the dominant species at this study site, with 82 and 75 captures, respectively. *Rana c. melanota* was the least common species, with only 8 captures.

Additionally, it can be seen in figures 26 and 27 that there are different population demographics between *R. pipiens* and *R. catesbeiana*. Three major SVL groupings resulted for *R. catesbeiana*, while 2 major groupings resulted for *R. pipiens*. The major SVL groups for *R. catesbeiana* were at 40-49 mm, 80-89 mm, and 120-129 mm. The number of representatives for each class decreased as the SVL increased. The 40-49 mm group had the highest number of captures (n=20). The 80-89 mm group had the highest peak in the second grouping (n=16). The 120-129 mm group had the highest number of captures in the third grouping (n=3). The major SVL groups for *R. pipiens* were at

50-59 mm and 60-69 mm. The 50-59 mm group had the highest number of captures (n=44). The 60-69 mm group had the next highest number of captures (n=20). Contrary to the frequency distribution for *R. catesbeiana*, the frequency distribution for *R. pipiens* did not have as many population size groupings and the maximum SVL for *R. pipiens* rarely exceeded 80 mm.

Figure 28 is a pie chart displaying the habitat associations for *R. catesbeiana* observed during the habitat partitioning study. As can be seen from figure 28, 34% of *R. catesbeiana* were captured in close proximity to *Hibiscus moscheutos*. Additionally, 26% of the *R. catesbeiana* were captured in open water, while 20% were captured in association with small grass bed hummocks. Only 11% of *R. catesbeiana* were associated with aquatic grass, while only 7% and 2% of *R. catesbeiana* were associated with *Juncus effusus* and *Polygonum coccineum*, respectively.

Figure 29 is a pie chart displaying the habitat associations for *R. pipiens* observed during the habitat partitioning study. A majority of the frogs captured (77%) were found in association with the terrestrial grass habitat. The next highest habitat association was the open water habitat (8%). Very few *R. pipiens* were found associated with aquatic grass (5%) and small grass bed hummocks (5%). Only 3% and 2% of *R. pipiens* were associated with *H. moscheutos* and *J. effusus*, respectively.

Since the sample size for *R. c. melanota* was small (n=8), habitat preferences for this frog will be presented as anecdotal evidence. During this study, 2 sexually mature females were captured. One was captured in association with open water approximately 2 m from the transition zone. The other female was captured resting out of the water on a *H. moscheutos* shrub approximately 1 m from the transition zone. There were also 4

juvenile *R. c. melanota* captured during this study. Two of which were captured at the junction of the transition zone and aquatic zone. As for the other 2 juvenile frogs, one was captured in open water, while the other frog was captured in association with *J. effusus*. It should be noted that there were not any sexually mature male *R. c. melanota* captured during this study. Additionally, several *R. c. melanota* were captured outside of the study quadrat. All of these frogs were associated with some sort of transition habitat or ephemeral aquatic habitats.

Figure 30 is a frequency histogram illustrating the grass height preferences for *R. pipiens*. It is apparent from the graph that a considerable number (n=22) of frogs were captured in grass with heights ranging from 20-29 cm. The next most frequent number of captured frogs occurred in grass ranging from 10-19 cm high, with 13 captures. It should be noted that only 6 frogs were captured in grass with heights ranging from 0-9 cm.

Discussion:

Data presented in figure 25 indicates there is significant habitat partitioning between the above listed frog species. These findings are important because it illustrates how 3 species of closely related frogs can exist in the same habitat. It is interesting that *R. pipiens* and *R. catesbeiana* were much more numerous than *R. c. melanota*. Collins and Wilbur (1979) noted that ponds where *R. catesbeiana* and *R. c. melanota* were sympatric, *R. catesbeiana* were always the more dominant frog species. It is believed this was due to predation of *R. catesbeiana* upon juvenile and sub-adult *R. c. melanota*. It has also been noted that relative abundances of Green Frogs and Leopard Frogs increased drastically following the extinction of Bullfrogs, indicating that the Bullfrogs had a

negative influence upon other sympatric anuran species (Hecnar and M'closkey, 1997). Interactions between *R. c. melanota* and *R*. catesbeiana are unique, because the nature of these interactions changes as the frogs metamorphose from larvae to adult frogs. As larvae, both frog species compete for microhabitat and food resources. However, as larvae metamorphose into adult frogs, interactions change from competitive to predatory, with *R. catesbeiana* preying heavily upon *R. c. melanota* (Werner et al., 1995). Diet analyses of adult *R. catesbeiana* revealed that emerging juvenile *R. c. melanota* represent a considerable portion of the Bullfrog's diet (Werner et al., 1995).

Additionally, as was demonstrated by this experiment, the available habitat for *R. c. melanota* to inhabit was greatly reduced, because *R. catesbeiana* dominated the aquatic habitat up to the edge of the transition zone, while *R. pipiens* dominated the terrestrial habitat. *Rana pipiens* were also captured in considerable abundance in the aquatic portion of the swamp, indicating that they require not only terrestrial habitat, but also considerable quantities of aquatic habitat to exist. From this, it is apparent that *R. catesbeiana* and *R. pipiens* dominate the aquatic and terrestrial portions of the swamp, respectively. The only remaining habitat for *R. c. melanota* to inhabit was the small area of transition zone. It has been shown in wetlands where *R..catesbeiana* and *R. c. melanota* were sympatric, *R. c. melanota* were always found in close proximity to the shore, while *R. catesbeiana* were distributed throughout the aquatic portions of the wetland (Werner et al., 1995). It should also be noted that *R. c. melanota* were seen throughout the swamp in temporary pools and wet areas away from permanent pools. Werner et al. (1995) observed that *R. c. melanota* commonly inhabit temporary water sources, such as ephemeral breeding pools. It may be that *R. c. melanota* move to these

temporary pools to avoid predation from larger *R. catesbeiana* that dominate the permanent pools. Hecnar and M'closkey (1997) suggest *R. catesbeiana* are important for structuring anuran communities through interspecific and intraspecific interactions.

Results of the habitat analysis also support the habitat partitioning results discussed above (figure 28). As was established, *R. catesbeiana* is a completely aquatic frog. It utilizes aquatic areas not only for breeding, but also for foraging purposes. In figure 28 it was established that 34% of *R. catesbeiana* were associated with *H. moscheutos*. This aquatic shrub is commonly known as Swamp Rose Mallow, a member of the Malvaceae Family. These shrubs function as exceptional habitat, because they provide frogs with excellent protection from potential predators. When the frog becomes frightened, all it has to do is dive under water and hide between roots of the shrubs. These shrubs also have large bases that emerge above the surface of the water. When frogs want to leave the water, they can rest on the bases and forage for flying insects. During this study, several frogs were seen out of water perched on bases of the shrubs.

Additionally, it was established that 22% of *R. catesbeiana* were also found in association with open water. This is very important because it illustrates that not all of the *R. catesbieana* were associated with some sort of cover object. Thus, these frogs probably had not established a territory and were in the open water foraging for aquatic insects.

It was also established that 20% of the Bullfrogs were associated with grass bed hummocks. These frogs associated with this type of habitat were either perched on the hummock out of water or in the water adjacent to the grass bed hummock. In either case, these frogs were probably using the grass bed hummocks as protection and as a place to

forage. This data indicates that while *R. catesbeiana* are entirely aquatic frogs, they utilize many different types of microhabitat, ranging from aquatic grass to emergent shrubs.

Contrary to the results for *R. catesbeiana*, were the habitat preference results for *R. pipiens* (Figure 29). As was established, *R. pipiens* requires 3 distinct habitats to complete its life history. However, during this study, *R. pipiens* mainly utilized terrestrial habitat, most likely for foraging purposes. It is interesting to note that although a majority of the frogs were found in association with terrestrial habitat, a considerable amount of frogs were also captured in the aquatic zone. It is believed that these frogs selected the aquatic habitat to escape freezing ambient temperatures. On evenings when considerable numbers of *R. pipiens* were found in the aquatic zone of the quadrat, air temperatures had dropped to 2˚C. It should also be noted that *R. pipiens* found within the aquatic zone were not associated with a particular type of microhabitat. It is believed that frogs were only in the aquatic zone to avoid freezing air temperatures. The following day, these frogs most likely moved back into the terrestrial zone when air temperatures increased to a favorable level. Thus, these frogs did not need to secure aquatic territories.

From results presented in figure 30, it is apparent that *R. pipiens* has a preferential grass height when foraging. As was presented earlier, the preferential grass height was established as 20-29 cm. In addition, a considerable number of frogs were recorded foraging in grass with heights ranging 10-19 cm. It is believed more frogs were recorded in grass with the above listed heights, due to foraging specifications. If grass becomes too high, frogs may not able to move around very well, hindering foraging efficiency. Additionally, sit-and-wait predators, such as *R. pipiens* rely upon visual cues to locate

and subdue potential prey items. If grass is too high, the frog will not be able to effectively locate prey and as a result will miss opportunities to capture prey items. Conversely, grass that is too short (0-9 cm) may expose frogs too much, allowing predators, such as garter snakes to locate the frogs more easily. Also, insects would be less likely to frequent areas lacking coverage.

From this study, it can be concluded that *R. pipiens* was the most terrestrial frog, while *R. catesbeiana* was the most aquatic frog. It was also established that *R. c. melanota* most usually associates with the transition zone between the aquatic and terrestrial interface. Additionally, detailed habitat data for each of the above listed frog species was obtained.

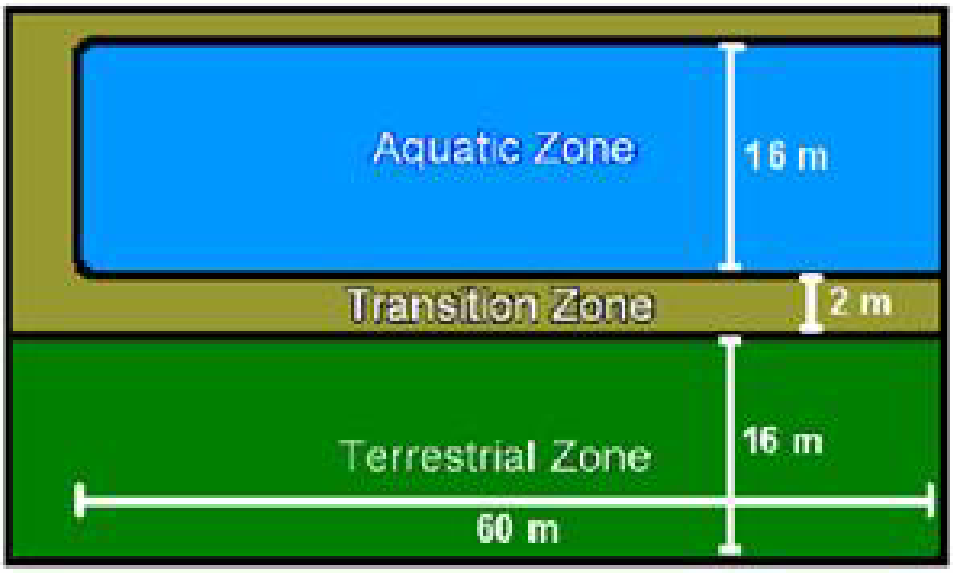


Figure 24: Model of Study Area Analyzed during Habitat Partitioning Survey

Figure 25: Distance from Land/Water Interface for 3 Sympatric Anuran Species

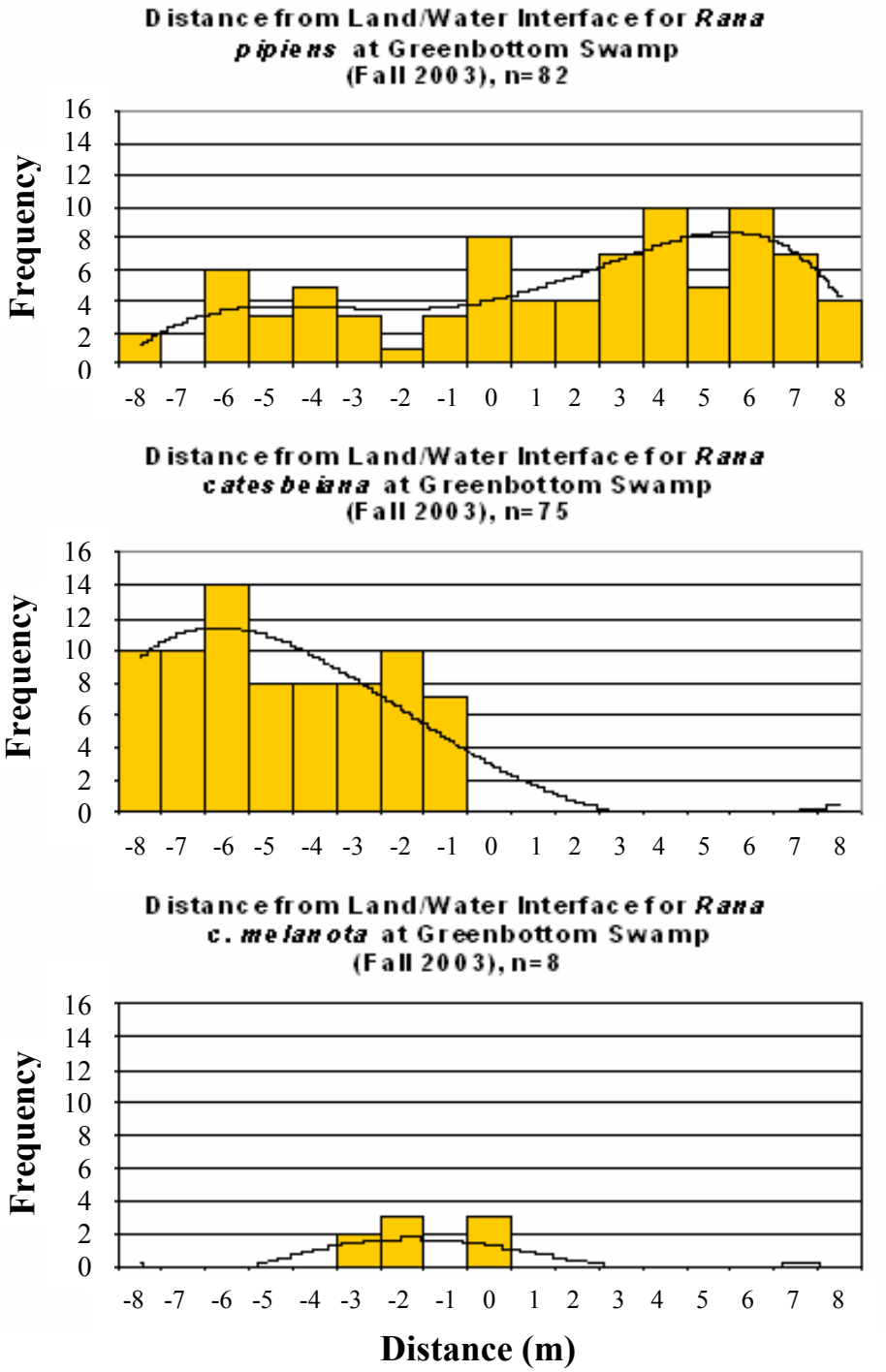


Figure 26: Frequency Distribution of Snout-vent Lengths for *R. pipiens* (Fall 2003), n=71

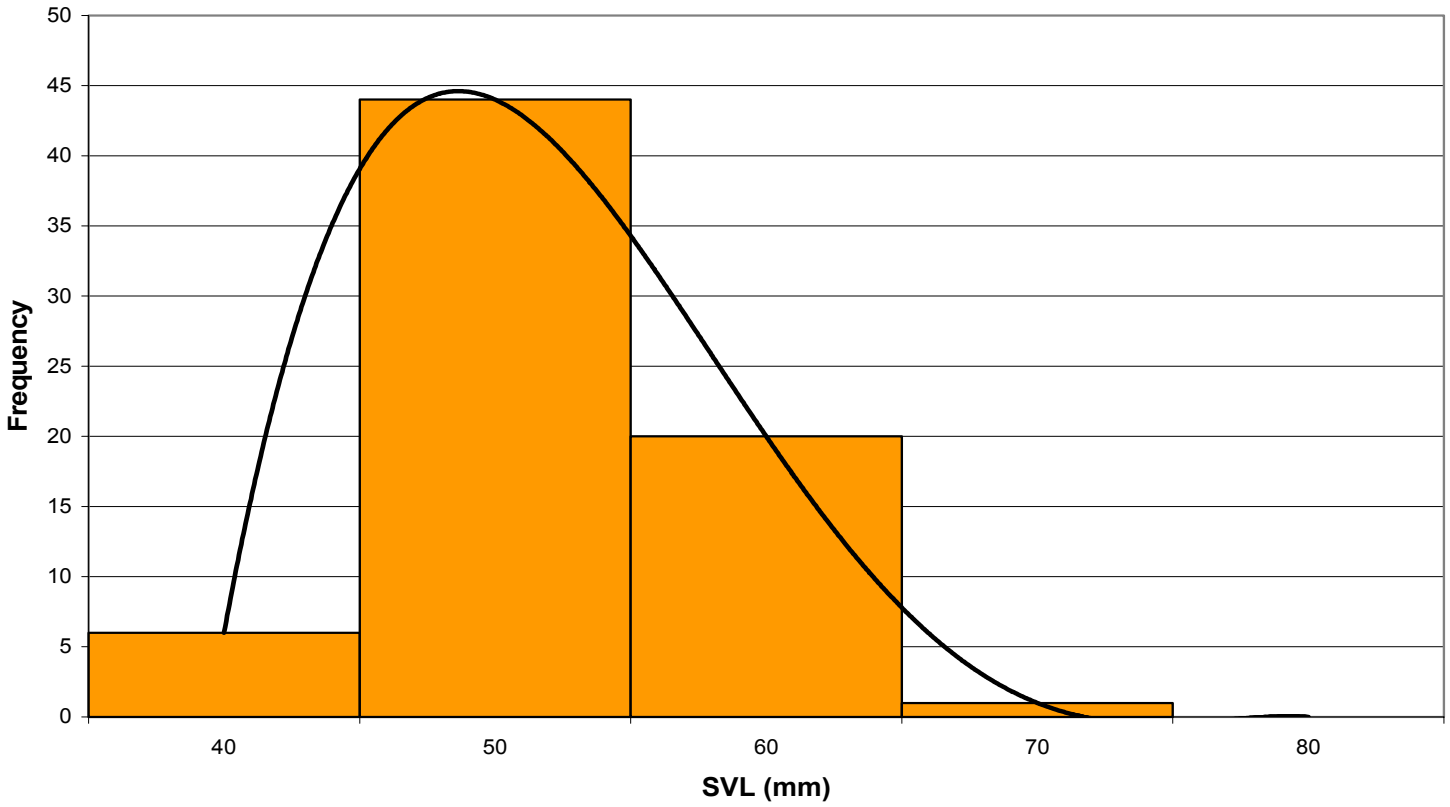


Figure 27: Frequency Distribution of Snout-vent Lengths for *R. catesbeiana* (Fall 2003), n= 65

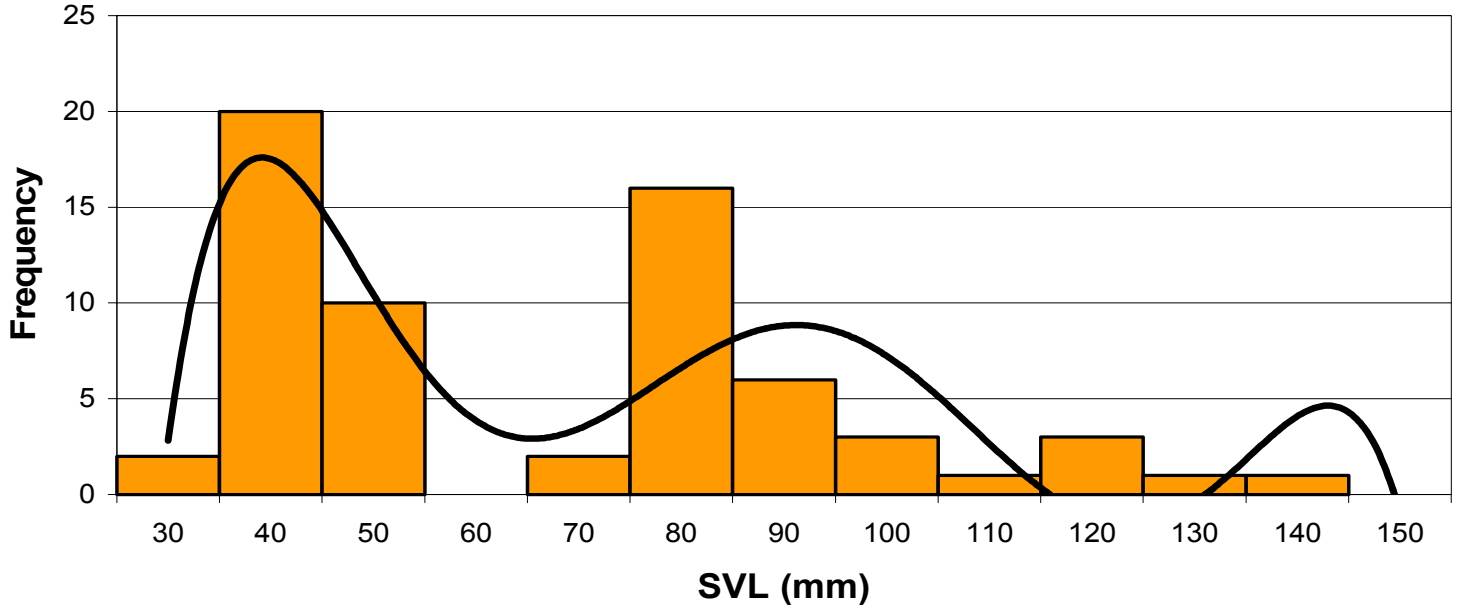


Figure 28: Habitat Associations for *R. catesbeiana* , n=64

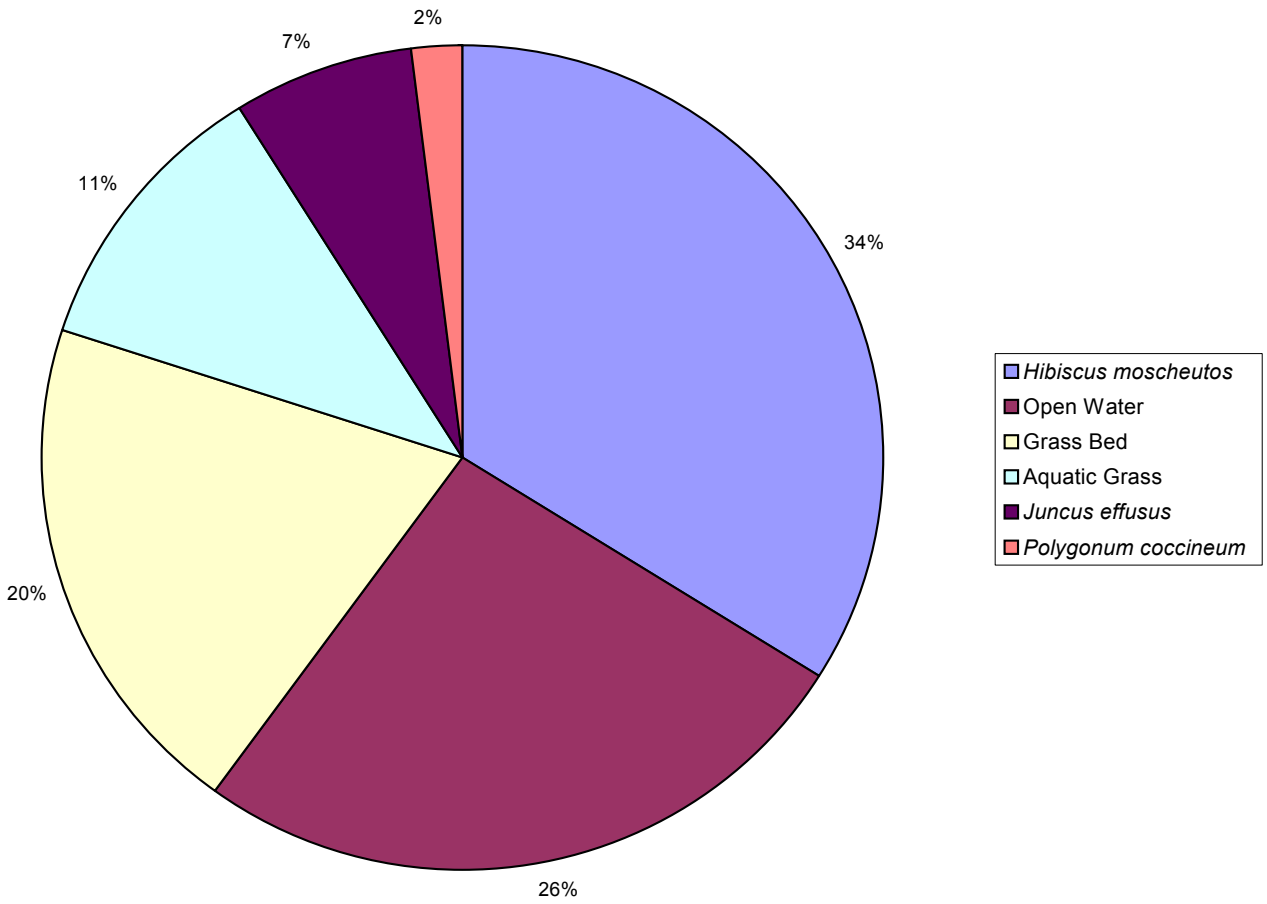


Figure 29: Habitat Associations for *R. pipiens* , n=74

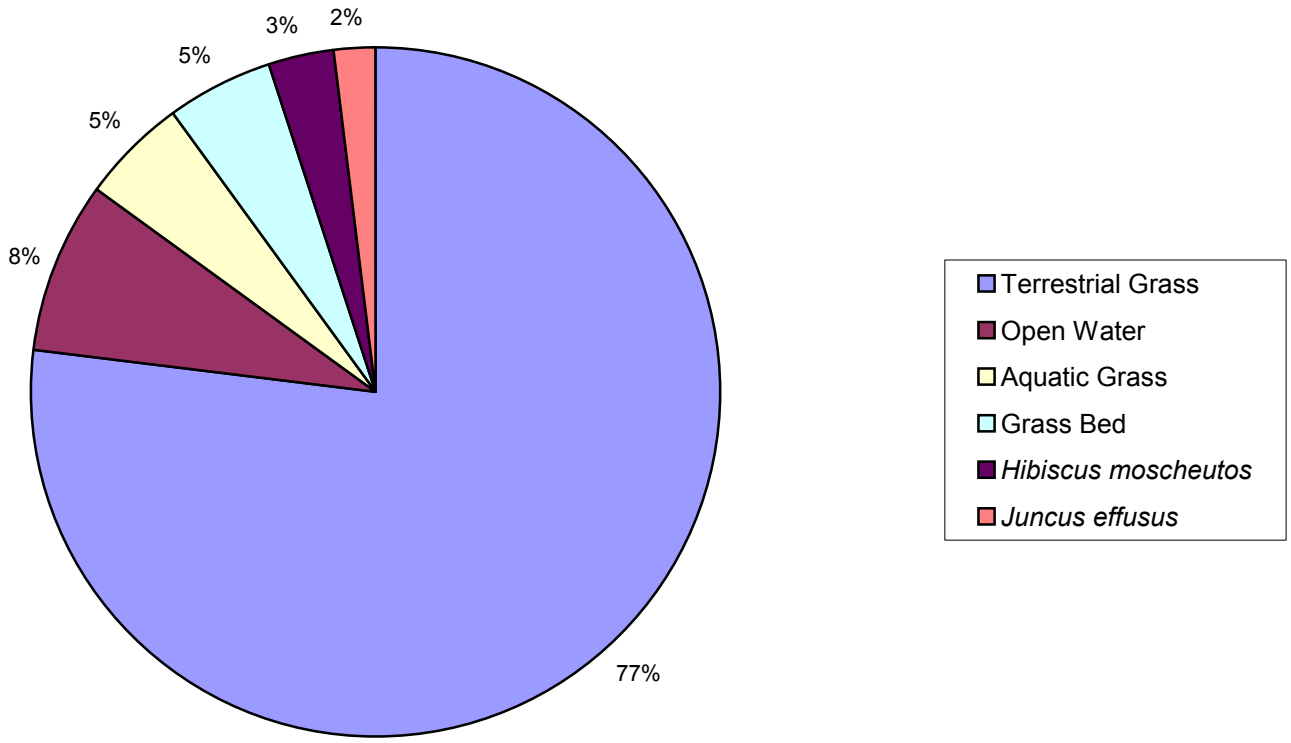
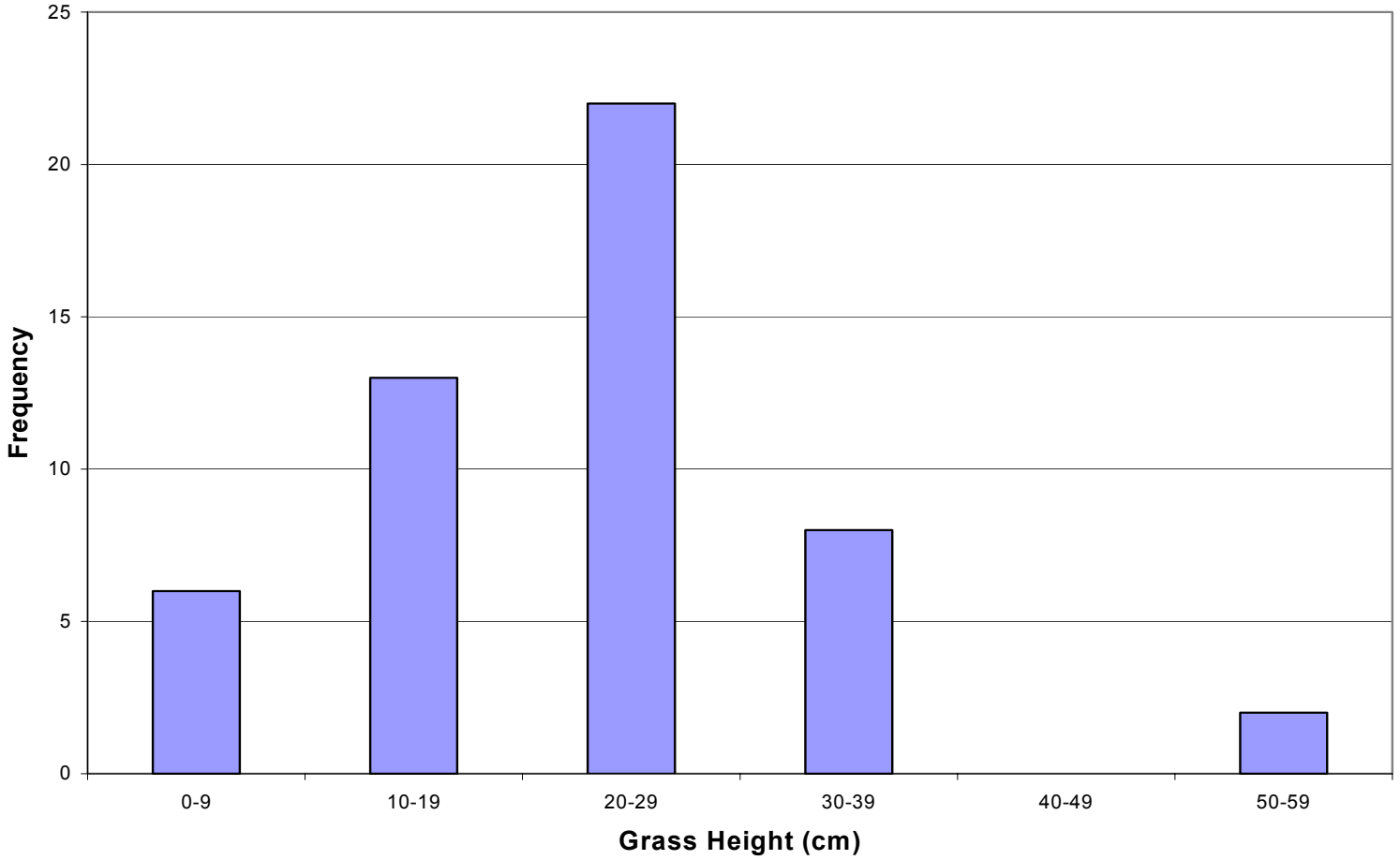


Figure 30: Frequency Distribution of Grass Heights Displaying Microhabitat Preferences for *R. pipiens* , n=51



CHAPTER FOUR: Diet Analysis of Juvenile and Adult *Rana pipiens* in West Virginia

Introduction:

Studies analyzing the stomach contents of anurans are important to determine many aspects of ecology and natural history. Food habit information is important to recognize habitat restrictions and conditions (Parker and Goldstein, 2004). Also, some amphibians may be restricted to certain food types and may feed more readily upon one food source than another. Recognizing these feeding restrictions may aid in protection and procurement of such stenophagic species. Additionally, these studies are important to determine the influence of prey size and type upon body condition and distribution of frog species (Parker and Goldstein, 2004). Food studies are also very helpful to determine differences in feeding ecology in areas where organisms exhibit sympatric distributions. It was revealed by diet analysis that *R. esculenta* and *R. dalmatina*, two sympatric Ranid frogs, fulfill completely different feeding niches (Guidali *et al*., 2000). This example is important, because it illustrates how food studies can reveal niche partitioning and differences in feeding ecology.

In previous diet analysis studies, killing study organisms for gut content analysis was commonplace. More recently, gut flushing has proven to be a more humane and effective way to analyze diet composition in many organisms. Stomach flushing is relatively simple and very useful for analyzing stomach contents and feeding habits of anurans and squamates (Legler and Sullivan, 1979). Additionally, stomach flushing has

been favored over sacrificing study organisms, because it aids in longterm studies and allows diet analysis of protected species (LeClerc and Courtois, 1993).

Feeding ecology and food analysis studies have been completed for a variety of anuran species. Diet analysis has proven useful in studies of natural history (Hamilton, 1948), effect of developmental stage upon diet (Jenssen, 1967), niche overlap (Hedeen, 1972; Forstner *et al*., 1998), diet of sympatric species (Guidali *et al*., 2000), and congenic/conspecific predation (Krupa, 2002). In addition to the above listed studies, there has been considerable research completed analyzing the feeding behavior of the Northern Leopard Frog, *R. pipiens*. Due to its widespread distribution, feeding ecology of *R. pipiens* and related species has been well documented (Knowlton, 1944; Linzey, 1967; Collier *et al*., 1998; Parker and Goldstein, 2004). The compilation of food data for a variety of frog species is important, because it allows comparisons of feeding ecology and potential management options to help preserve the array of anuran species still remaining.

The objectives of this study are as follows: to identify the major prey items of *R. pipiens* in West Virginia and to determine the relationship between frog size (SVL) and number of prey items eaten.

Materials and Methods:

Study Area

The study areas utilized for diet analysis were portions of the Hoeft Marsh and surrounding areas at Greenbottom WMA, in Cabell Co., WV. For a more detailed

description of the swamp and/or study area, please refer to the description of study area on pages 1-3 of this manuscript.

Survey Methods and Gut Flushing Techniques

Surveys for frogs began in late June and were continued until mid-November. Frogs were surveyed at night and were located by visual survey with a Petzl-duo headlamp. Since *R. pipiens* forages in meadows in the summer and fall months, a majority of searches were completed in terrestrial habitat. Frogs were captured after either being spotted directly or after being scared from grass clumps. Upon capture, frogs were secured in a moist pillowcase and carried on the belt-loop of the researcher until processed. Survey periods usually lasted 1-2 hours after which, frogs were taken back to the research vehicle to be processed. Prior to gut flushing, SVL, tibia length, cranial width, dorsal spot number, dorsal phenotype, and number/type of abnormalities were recorded.

To complete stomach flushing, a 50 cc syringe, outfitted with 5 mm plastic tubing, was used. The syringe was filled completely with de-chlorinated water and inserted into the mouth of the frog. If the tubing could not be placed into the mouth, a thin credit card was placed between the upper and lower jaws and gentle pressure was applied until the frog opened its mouth. The tubing was then inserted into the mouth and into the stomach. The frog was then held over a dissecting tray and the water was slowly injected into the stomach of the frog. The water was injected until either the stomach contents had been evacuated or until 50 cc of water had been injected into the frog. If no food items were recovered after the initial attempt, the syringe was re-filled and a second

attempt was made. If food items were not recovered after the second flushing attempt, then it was determined that the frog's stomach was empty.

Upon completion of gut flushing, the stomach contents were placed in a vial containing 70% alcohol and labeled with an evening/frog specific identification tag. The food samples were analyzed in the lab under a dissecting microscope and identified to the lowest taxonomic level possible (Figure 31).

Results:

Table 8 summarizes prey type, number of a specific class/order of prey items, and percentage of total food items the prey grouping represented. From the table, it can be seen that there were 20 distinct food groupings identified. Within these 20 food groupings, there were 227 food items successfully identified. Three main prey groups served as the prey base for *R. pipiens*. These groups were adult Coleoptera (22.9% of total items), Annelida (17.3% of total items), and Hymenoptera (11.9% of total items).

In addition to the food items presented above, there were other interesting food items recovered. Although Order Trichoptera represented only 5.8% of the food items recovered, 11 of the 13 larval Caddisflies recovered came from the stomach contents of one frog. Contrary to the idea that anurans are opportunistic feeders, this particular frog seemed to be specializing on one prey item. Additionally, the remains of one *Pseudacris c. crucifer* (Northern Spring Peeper) were recovered from the stomach contents of one *R. pipiens* having an SVL of only 50.7 mm. The Spring Peeper represents the only vertebrate prey item recovered during this study.

Figure 31 illustrates the relationship between SVL and total number of prey items ingested. From the figure it can be concluded that there is no relationship between these two variables. The R^2 coefficient was only 0.004, indicating that there is not a linear relationship between frog size and number of stomach contents. Notice that frogs with the highest number of stomach contents (14 and 12) had an SVL of 54 mm and 59 mm, respectively, while much larger frogs had considerably lower numbers of stomach contents.

Discussion:

Data presented within table 8 provides an accurate representation of the food items for *R. pipiens* in West Virginia. Data presented support the theory that *R. pipiens*, like many other anurans, are indiscriminate, opportunistic feeders (Forstner *et al*., 1998; Guidali *et al*., 2000; Collier *et al*., 1998). The opportunistic feeding behavior may arise due to several reasons. It has been suggested that anurans forage upon whatever prey items are available in specific habitats rather than foraging upon specific food items (Forstner *et al*., 1998). Additionally, it appears that availability of a particular food item has a much greater influence than specificity in the diet of many anurans (Linzey, 1967). In table 8, it can be seen that adult Coleopterans were the major prey item for *R. pipiens*. This was most likely due to the fact that during the summer and fall months, *R. pipiens* forages in large, grassy meadows. Since a majority of Coleopterans are terrestrial insects and are ubiquitous in most habitats, they would have been the most common and the most frequently encountered prey item. Collier *et al*., (1998) discovered that Coleopterans were the most commonly encountered prey item in a population of *R. pipiens* in

northeastern Ohio. It has been suggested that Coleopterans function as the staple food item for *R. pipiens*. Frogs will feed on other insects when in abundance, but Coleopterans function as a cushion when numbers of these other prey items decline (Linzey, 1967). Additionally, Annelids (earthworms) comprised a large portion of *R. pipiens* diet. On damp and wet evenings, the ground was permeated with emerging earthworms. The availability of this food item explains why they were so frequently encountered in the stomach contents of *R. pipiens*.

As was presented earlier, one *Pseudacris c. crucifer* was found in the stomach contents of one *R. pipiens*. Although congenic and conspecific predation is commonplace in larger frogs, such as *R. catesbeiana*, it is fairly uncommon in frogs such as *R. pipiens*. Knowlton (1944) reported that one mature *R. pipiens* was found to have preyed upon a juvenile conspecific. More commonly, *R. pipiens* is taken as a food item by *R. catesbeiana* and *R. c. melanota* (Krupa, 2002; Hamiton, 1948). Additionally, *R. palustris*, which is approximately the same size as *R. pipiens*, has been recorded feeding upon juvenile *Bufo americanus* (Hamilton, 1948).

Data presented in figure 31 indicate that frog size (SVL) has no influence upon the number of stomach contents. If this were a linear relationship, then frogs with snout-vent lengths of 40 mm-60 mm should have had an average number of food items (4 or 5) instead of the much higher number of food items observed. Also, only 4 food items were recovered from the largest frog sampled (73 mm). It is more likely that frog size has more influence upon total volume of prey items ingested. Werner *et al*. (1995) discovered that total prey volume was highly correlated with SVL. From this, it can be concluded that frog size has no influence upon the total number of food items ingested.

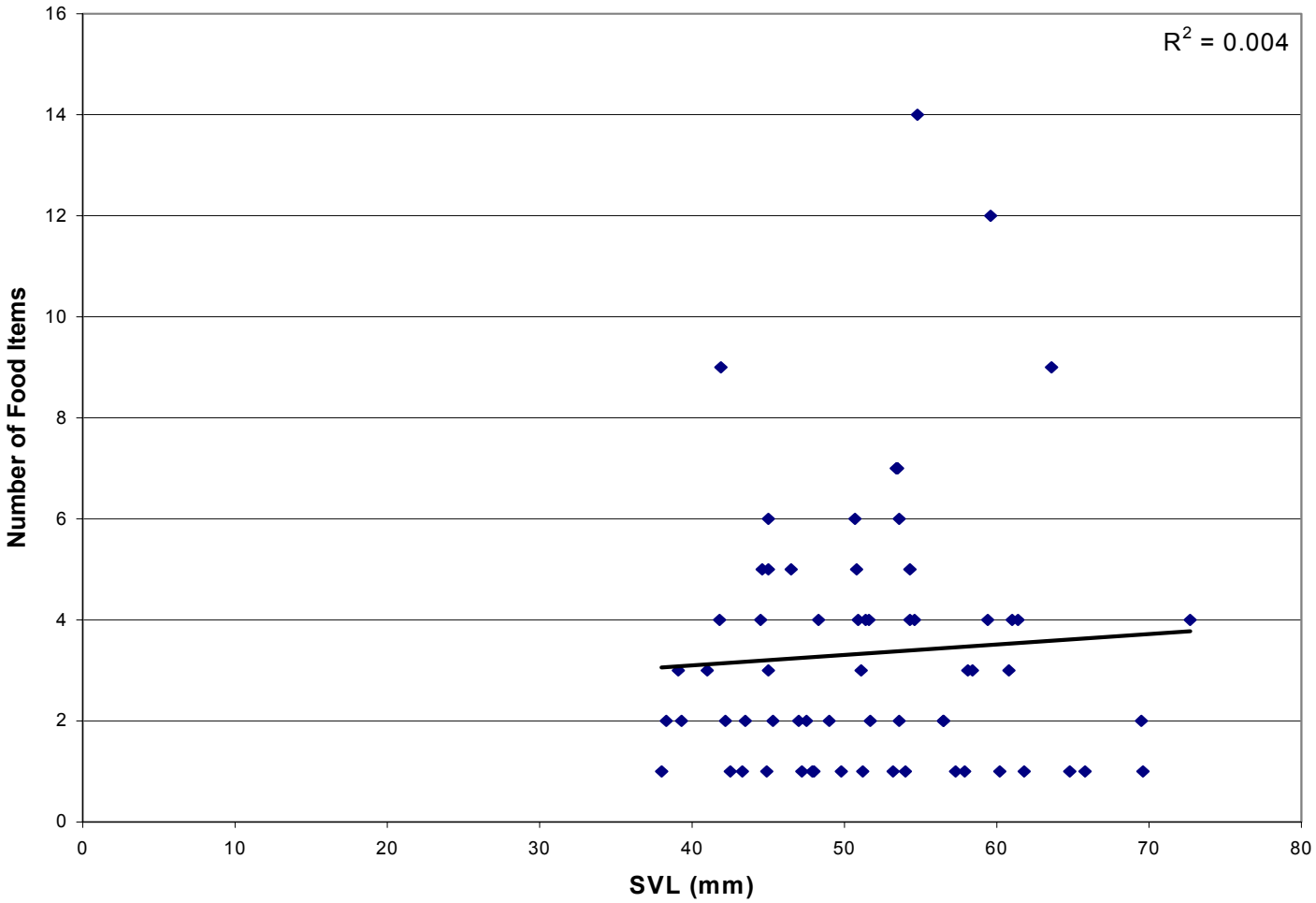
From this study, it can be concluded that *R. pipiens* does feed upon a variety of prey items including congenics, although it is much more likely to feed upon smaller and more abundant prey, such as Coleopterans and Annelids. During this study it was also discovered that SVL does not influence the total prey items ingested, but rather total volume of prey items ingested.

Figure 31: Sample of Gut Contents Obtained from *R. pipiens*

Table 8: Results of Diet Analysis for *R. pipiens* in West Virginia, n=64

Food Type	Number of Food Items	Percentage of Total Items
Annelida	39	17.3%
Anura	1	0.4%
Araneae	11	4.8%
Coleoptera (Adult)	52	22.9%
Coleptera (Larvae)	14	6.2%
Collembola	1	0.4%
Diplopoda	5	2.2%
Diptera (Adult)	11	4.8%
Diptera (Larvae)	5	2.2%
Ephemeroptera	1	0.4%
Gastropoda	17	7.5%
Hemiptera	5	2.2%
Homoptera	7	3.1%
Hymenoptera	27	11.9%
Isopoda	8	3.5%
Lepidoptera (Adult)	2	0.9%
Lepidoptera (Larvae)	5	2.2%
Odonata	2	0.9%
Trichoptera (Larvae)	13	5.8%
Solifugae	1	0.4%
n=20 food groupings	**n=227 total items**	**n=100%**

Figure 32: Scatter Diagram of SVL vs. Number of Stomach Contents per Frog for *R. pipiens* (n=64)



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WILLIAM B. SUTTON

Curriculum Vitae

1019 10th Ave. Huntington, WV 25701
Tel: (304)-457-3282
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EDUCATION:

Master of Science: Biological Sciences
Marshall University, Huntington WV. Expected completion, Summer 2004
GPA: 4.0/4.0
Advisor: Thomas K. Pauley
Thesis: Ecology and Natural History of the Northern Leopard Frog (*Rana pipiens*, in West Virginia

Bachelor of Science: Biological Sciences; Minor: Chemistry
Wheeling Jesuit University, Wheeling WV. May, 18 2002
GPA: 3.82/4.0
Advisor: Dr. Robert Shurina
Thesis: The Effects of the Higher Alcohols Phenylethanol, Tryptophol, and Tyrosol upon a Whitbread Strain of *Saccharomyces cereviseae*

High School Education: Diploma certifying completion
Philip Barbour High School, Philippi WV. May 1998

PUBLISHED ABSTRACTS:

Sutton, W.B. and T.K. Pauley. 2004. Discovery of *Aeromonas hydrophila* and *Pseudomonas spp.* skin infections and other malformations of *Rana pipiens* in West Virginia. Southeastern Biology 51(2): In Press.

Sutton, W.B. and T.K. Pauley. 2004. Analysis of anuran community level interactions at Greenbottom Swamp in Cabell County, WV. Southeastern Biology 51(2): In Press.

ORAL PRESENTATIONS:

Sutton, W.B. and T.K. Pauley. 2004. Discovery of *Aeromonas hydrophila* and *Pseudomonas spp.* skin infections and other malformations of *Rana pipiens* in West Virginia. ASB conference, Memphis TN.

Sutton, W.B. 2004. Speciation of Plethodontid Salamanders in the Eastern United States. Graduate Seminar Presentation.

Sutton, W.B. and R.D. Shurina. 2002. The Effects of the Higher Alcohols Phenylethanol, Tryptophol, and Tyrosol upon a Whitbread Strain of *Saccharomyces cereviseae*. Tri-Beta National Conference, San Antonio, TX.

Sutton, W.B. and R.D. Shurina. 2002. The Effects of the Higher Alcohols Phenylethanol, Tryptophol, and Tyrosol upon a Whitbread Strain of *Saccharomyces cereviseae*. Tri-Beta Regional Conference, Indiana University of Pennsylvania.

Sutton, W.B. and R.D. Shurina. 2002. The Effects of the Higher Alcohols Phenylethanol, Tryptophol, and Tyrosol upon a Whitbread Strain of *Saccharomyces cereviseae*. Wheeling Jesuit Research Symposium.

TECHNICAL REPORTS:

Sutton, W.B., M.B. Watson, and T.K. Pauley. 2004. Long Term Evaluation of the Effects of Bacillus thuringiensis kurstaki, Gypsy Moth Nucleopolyhedrosis Virus Product Gypcheck, Entomophaga maimaiga (Humber, Shimazu, and Soper), and their Interaction on Nontarget Organisms in Mixed Broadleaf-Pine Forests in the Central Appalachians. Final Results, In Press. Prepared for the USDA, funded by the USDA.

Sutton, W.B. 2003. Pitfall Surveys for *Scaphiopus holbrookii* (Eastern Spadefoot) in Chesapeake Ohio. Final report. Prepared for Ecotech, Inc.

Pauley, T.K., M.B. Watson, W.B. Sutton, L.D. Phu, V. Dozeman, M.R. Mann, and A.M. Mann. 2003. Amphibians, Reptiles, and Birds at Sites of Proposed Mud River Alterations, Milton, WV. Final Results. Prepared for the U.S. Army Corps of Engineers, funded by USACE.

RESEARCH GRANTS:

2003 West Virginia Division of Natural Resources Wildlife Diversity Program Grant $6415.69

2003 Marshall University Research Foundation Summer Research Grant $500.00

TEACHING EXPERIENCE:

Herpetology Laboratory Instructor, August 2003-December 2003
Marshall University, Huntington WV

- Instructed weekly laboratory classes assisting students with identification of amphibians and reptiles known to occur in West Virginia
- Reviewed basic biology and taxonomy of amphibians and reptiles

Ornithology Laboratory Instructor, January 2004-May 2004
Marshall University, Huntington WV

- Instructed students to identify West Virginia birds by sight and call
- Reviewed basic biology and taxonomy of birds

GRADUATE RESEARCH EXPERIENCE: Contact info- Dr. Thomas K. Pauley (304)-634-5404

Graduate Research Assistant**,** August 2002- May 2004
Marshall University, Huntington WV- Graduate stipend through WV DNR

Stream Salamander Biomonitoring Study. Funded by USGS/BRD and EPA.

- Used quadrat and transect sampling to conduct stream surveys
- Sampled streams throughout various ecoregions of West Virginia
- Collected stream quality data
- Identified and recorded data upon captured adult and larval stream salamanders
- Utilized GPS to locate potential stream sites

Herpetological and Avian Surveys of the Mud River in Milton, WV. Funded by USACE.

- Initiated frog call surveys to identify frog species inhabiting the area
- Conducted bird surveys through sight and call identification
- Conducted visual searches for amphibian and reptile species
- Conducted aquatic turtle surveys by using hoop traps
- Co-authored the final report for the USACE

Long Term Monitoring of the Effects of Gypsy Moth Pesticide Applications on Salamander Populations in Monongahela National Forest. Funded by USDA.

- Prepared the final report for the USDA
- Prepared Powerpoint presentation detailing the results gathered from this study for USDA meeting in Minnesota
- Responsible for performing statistical analysis of the collected data

West Virginia Atlas of Amphibians and Reptiles. Funded by WV DNR.

- Trapped aquatic turtles with hoop and basking traps
- Collected, measured, and photographed county record amphibians and reptiles
- Transferred encounter records form topographic maps to GIS using Arcview
- Constructed and maintained *MS Access* database for map encounter records

Amphibian and Reptile Inventory of Gauley River National Recreation Area, Harpers Ferry State Park, Catoctin St. Park, and Chesapeake and Ohio Canal. Funded by the National Park Service.

- Surveyed for salamanders, anurans, lizards, and snakes with visual encounter searches and evening call surveys
- Measured captured amphibians and reptiles and recorded locations with a GPS

North American Amphibian Monitoring Program (NAAMP). Funded by the USGS.

- Coordinated 2004 survey for West Virginia as the state regional coordinator
- Entered call data from 2003 survey into NAAMP database

Pitfall Survey for *Scaphiopus holbrookii* (Eastern Spadefoot) in Chesapeake Ohio. Funded by Ecotech, Inc.

- Responsible for checking pitfall traps for amphibian, reptile, and mammal species
- Recorded environmental conditions and data for each captured animal
- Responsible for writing final report

Additional Research Assisted

- Natural history of *Gyrinophilus spp*. (Spring Salamander) in WV caves Greenbrier Co., WV
- Day and night searches for *Cryptobranchus a. alleganiensis* (Eastern Hellbender) in WV
- Surveys for *Plethodon nettingi* (Cheat Mountain Salamander) Grant Co., WV
- Population demography of *Heterodon platirhinos* (Hog-nosed Snake) Raleigh Co., WV
- Amphibian breeding phenology of a farm pond, Wayne Co., WV

Thesis Research, February 2003-May 2004
Marshall University, Huntington, WV

Life History of the Northern Leopard Frog (*Rana pipiens*)

- Observed and recorded field data pertaining to the life history of *Rana pipiens* as it occurs in West Virginia, such as time of egg laying, calling period, length of larval period, and time of metamorphosis

Competition between *Rana pipiens* larvae and *Rana clamitans melanota* larvae

- Established three experimental arenas to study the effects of competition upon growth of *Rana pipiens* larvae
- Used *Image J* to measure larval growth parameters weekly
- Applied ANOVA to analyze competition effects upon larvae

Diet Analysis of *Rana pipiens*

- Used gut flushing techniques for diet analysis
- Identified all food items down to order
- Compared relative size of prey and number of prey items to size of frog

Study of Skin Infections and other Malformations of *Rana pipiens*

- Utilized Biolog (bacterial identification machine) to isolate and identify *Aeromonas hydrophila* and other skin infections
- Recorded all malformations and skin tumors found on frogs during entire research period

Analysis of Anuran Assemblage Level Interactions

- Established a large aquatic/terrestrial quadrat to study habitat partitioning between *Rana pipiens*, *Rana clamitans melanota*, and *Rana catesbeiana*
- Completed 1-hour night searches of the above listed quadrate
- Analyzed population demography of the three frog species listed

Distribution of *Rana pipiens* in West Virginia

- Inventoried wetlands throughout West Virginia
- Compiled records of amphibians recorded from each survey site
- Used GPS to record localities for each amphibian record

PROFESSIONAL EXPERIENCE:

Head Brewer, May-August 2002
River City Ale Works, Wheeling WV

- Responsible for operation of all brewery equipment
- Assisted in formulation of all beer recipes
- Responsible for maintenance of all storage and dispensing equipment

Organic Chemistry Tutor, September 2000-May 2001
Wheeling Jesuit University, Wheeling WV

- Initiated weekly study sessions to lecture on concepts surrounding Organic Chemistry
- Held one on one study sessions to assist students with homework assignments upon request
- Contact info.- Dr. Mary Railing, (WJU) 304-243-2000

General Biology Laboratory Teacher's Assistant, September 2000-May 2001
Wheeling Jesuit University, Wheeling WV

- Responsible for weekly lab preparation
- Provided assistance to students completing laboratory assignments
- Contact info.- Dr. Ken Rastall, (WJU) 304-243-2000

AWARDS AND HONORS:

- Winner of the Frank G. Brooks award at the 2002 national Tri-Beta convention in the paper presentation category (San Antonio, TX)
- Winner of the Frank G. Brooks award at the 2002 regional Tri-Beta convention in the paper presentation category (Indiana University of Pennsylvania)
- Received top honors in the paper presentation category at 2002 Wheeling Jesuit University research symposium
- Member of the Biological Honors Society (Beta Beta Beta), Wheeling Jesuit University
- Member of the Jesuit Honors Society (Alpha Sigma Nu)
- Recipient of the 2002 Betty Thacker Award (top Biology student, Wheeling Jesuit University)
- Collegiate of the year (1999), Wheeling Jesuit University
- Deans List (7/8 semesters), minimum GPA 3.5, Wheeling Jesuit University
- Eagle Scout, Troop #63 (1998), Philippi, WV

INVITED ADDRESSES AND PRESENTATIONS

- Keynote speaker at 2004 Wheeling Jesuit University Research Symposium (April 20, 2004)
- Guest speaker at WV DNR science camp, Ripley West Virginia (May 2004)
- Guest instructor at WV 4-H Camp (Cabell Co.)

RELEVANT COURSEWORK:

Highlands Biological Station: *Biology of Plethodontid Salamanders*

Marshall University: ***Herpetology, Ornithology, Plant Taxonomy, Fish Biology, Biostatistics, Conservation Biology, Evolutionary Biology***

Wheeling Jesuit University: ***Ecology, Physiological Ecology, Vertebrate Biology, Entomology***

FIELDWORK SKILLS:

Herpetology: Telemetry (frog), drift fences, pitfall, funnel traps, call survey, quadrat survey, hoop traps, dip net, road search, acrylic elastomer tags, eye shine location, snorkeling, photography, gut flushing

Ichthyology: Large, moderate, and small body water electroshocking, fish ID (WV)

Ornithology: Visual survey and ID with spotting scope or binoculars, call ID (eastern North America)

Botany: Plant ID (WV), keys, pressing and preparing herbarium specimens

Entomology: Basic insect identification (aquatic and terrestrial)

ADDITIONAL LABORATORY SKILLS: Gel Electrophoresis, PCR, NMR, Gas Chromatography, BIOLOG, and microbiological techniques

COMPUTER SKILLS: *MS Word, MS Excel, MS Access, MS Powerpoint, Sigma Stat, Sigma Plot, Delta Graph, SAS, Image J, TOPO, and very basic knowledge of ArcView*

REFERENCES:

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