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Filling in the Gaps in Phenology and Life History of the Cumberland Plateau Salamander (Plethodon kentucki)

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Filling in the Gaps in Phenology and Life History of the Cumberland Plateau Salamander (*Plethodon kentucki*)

Thesis submitted to
the Graduate College of
Marshall University

In partial fulfillment of
the requirements for the degree of
Master of Science
in Biology

By

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ABSTRACT

Filling in the Gaps in Population Size, Phenology, and Life History of the Cumberland Plateau Salamander *(Plethodon kentucki)*

The Cumberland Plateau Salamander, *Plethodon kentucki*, is a member of the *Plethodon glutinosus* complex comprising 16 sibling species, which are best differentiated by range. Few studies have been conducted to gain information on the natural history of *P. kentucki.* To alleviate this, two sites at Beech Fork State Park in Wayne County, West Virginia were used to study the salamander's general life history with emphasis on reproduction, phenology, and population size. At each site, three 20m x 20m sample plots were arranged based on viability of the habitat for *P. kentucki.* Ground searches of all cover objects within the plots were conducted once every two to three weeks, preferably on or within 24 hours of rainy nights, between 7:00 and 23:00. Searching under cover objects, which included rocks, logs, and heavy leaf litter, was the best method for finding individuals. Upon capture, salamander sex, age class, and reproductive status were determined. Before releasing specimens, photographs were taken of the lateral surfaces of the animal to be used as a marking technique for recaptures. Environmental variables collected included soil moisture, soil pH, soil temperature, air moisture, air temperature, and precipitation. Environmental data were compared to total captures of *P. kentucki* to determine correlation between phenology and environmental conditions.

ACKNOWLEDGMENTS

First and foremost I have to thank Dr. Tom Pauley. He has been an excellent advisor, a wonderful mentor, and an admirable fishing opponent. Without him I never would have been able to indulge my passion for organismal biology that had lain dormant since my youth. He allowed me the freedom and gave me the support to grow as a student of higher learning and I know I'm better for it. I can't thank him enough for sticking with me, even when time got tight and pressure mounted. He is honestly one of the kindest and best men I know.

When I finally started graduate school I got to study the subjects I wanted instead of the checklist in the infamous Marshall Plan. The first class I knew I wanted to take was Animal Physiology with Dr. David Mallory. I had heard a wide array of horror stories and praise for Dr. Mallory's prowess in the classroom. I knew from the first day when he strolled into class with just a dry-erase marker and began rattling off diseases, structures, and functions I'd never heard of that I wanted more. His class ultimately helped me decide to pursue medical school, so I thank him for his guidance and for asking the really tough questions.

I would also like to thank Dr. Frank Gilliam. Without the use of his lab space and equipment it would not have been possible for me to analyze my soil samples when I needed to. He was always available to read over (and shred to pieces) any piece of writing I sent his direction. If I had a complete statistical meltdown, he was there to make sense of the chaos.

Dr. Jayme Waldron was the single most pivotal asset who helped me tame the hailstorm that was my thesis into the finished product. She was so patient with me. She didn't mind to fully break down a concept after I stared blankly at her following a ten minute explanation. Without her I would never have completed this work, so I thank her so much for her assistance.

I would also like to thank Beech Fork State Park for the use of their wildlife management area as one of my study sites. Mrs. Summers was very helpful in assisting me with the appropriate permits and paperwork.

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I would be remiss if I didn't thank my parents, Rob and Tressa, for their constant support in everything I've ever pursued. They have supported me in every possible facet and driven me to become the person I am today. All of this work is essentially their responsibility for always urging me to succeed and strive for bigger and better things.

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Office of Research Integrity

July 2, 2013

Robert C. Bowers
1841 Sunset View
Milton, WV 25541

Dear Mr. Bowers:

This letter is in response to the submitted thesis abstract titled "A Natural History Study of *Plethodon kentucki* Within Southwestern West Virginia." After assessing the abstract it has been deemed not to be human subject research and therefore exempt from oversight of the Marshall University Institutional Review Board (IRB). The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved the study under protocol #497. The applicable human and animal federal regulations have set forth the criteria utilized in making this determination. If there are any changes to the abstract you provided then you would need to resubmit that information to the Office of Research Integrity for review and a determination.

I appreciate your willingness to submit the abstract for determination. Please feel free to contact the Office of Research Integrity if you have any questions regarding future protocols that may require IRB review.

Sincerely,

Bruce F. Day, ThD, CIP
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Table of Contents

Page #

List of Tables

List of Figures

INTRODUCTION

Global climate change along with habitat fragmentation, degradation, and destruction for urbanization and agricultural use pose a distinct threat to biodiversity. This threat has been especially pronounced for herpetofauna (Alford & Richards, 1999). For nearly 20 years, evidence of amphibian declines, as well as developmental malformations, has shown that amphibians are excellent biological indicators, and their declines are evidence of an unsuitable environment (Lannoo, 2005). Successfully monitoring the status of particular amphibians, our planet's "hidden biodiversity," can be achieved through appropriately observing and analyzing the health of a species (Gibbons, 1983). This does not include only threatened or endangered amphibians, but also the common species. These common species often decline simply because they are not threatened or endangered; hence they are overlooked as possible species of concern. It is difficult to cultivate interest or funding for research of a common species, but in many cases they are the least understood because of apathy and lack of attention. Such is the case with the Cumberland Plateau Salamander, *Plethodon kentucki* (Mittleman, 1951). There are enormous gaps in this salamander's natural history that would not exist if it received the same attention as an endangered species, but these gaps still exist because the species is common. These glaring absences in data in its natural history make *P. kentucki* harder to study in conservationist contexts. To ameliorate some of these deficiencies, this study will focus on the phenology of the animal as well as possible new methodologies that will make studying it easier. Common field-capture techniques and environmental data combined with new photographic mark-recapture techniques and advanced multivariable data analysis will assist in answering some of these questions.

DESCRIPTION OF THE SPECIES

The Cumberland Plateau Salamander, *Plethodon kentucki,* is a member of the family Plethodontidae. Members of this family are commonly referred to as lungless salamanders because they lack lungs and their respiration is primarily cutaneous gas exchange. The genus *Plethodon* is commonly called the woodland salamanders. Members of this genus are so termed because of their highly terrestrial ecology. They have almost entirely abandoned the larval life stage and undergo it while still in the egg. The genus is frequently separated into two groups, large and small species, for ease of classification. *Plethodon kentucki* is one of the five large *Plethodon* that inhabit West Virginia: *P. glutinosus, P. kentucki, P. cylindraceus, P. wehrlei,* and *P. punctatus*. All five species are similar in appearance, but do have slight morphological differences that enable the distinction between them. *Plethodon kentucki* most closely resembles *Plethodon glutinosus* (Figure 1).

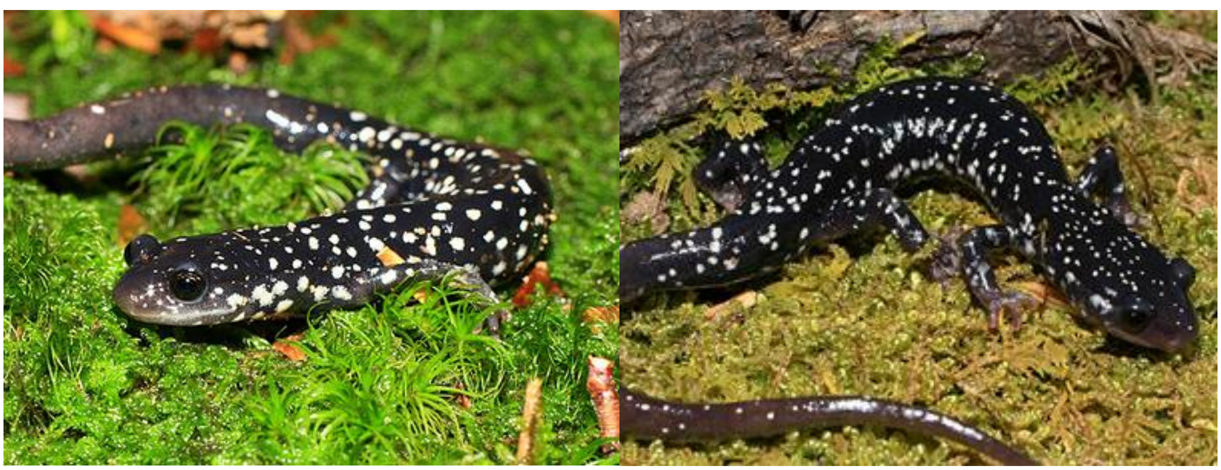

Figure 1. The similarity between *Plethodon kentucki* (right) and *Plethodon glutinosus* (left). Photos courtesy of Kevin Messenger.

The major differences between the two include size, patterning, coloration, and body shape. Adult *P. glutinosus* are slightly larger reaching snout-vent lengths (SVL) of 80mm, whereas *P. kentucki* range from 60 to 77mm (Highton & MacGregor, 1983). The dorsum color ranges from shiny black to dark charcoal with scattered white spots. The white dorsal spots are large in *P. glutinosus,* but can range from extremely small to totally absent in *P. kentucki* (Figure 1). From personal observation of the two species, in most cases, *P. kentucki* has many more spots confined to its lateral surfaces than *P. glutinosus*, which makes its patterning somewhat similar to *Plethodon wehrlei*, another of the large *Plethodon* in West Virginia (Figure 2). Another variation in the patterning of *P. kentucki* is the brassy flecking within the white spots that gives them an off white hue (Figure 2).

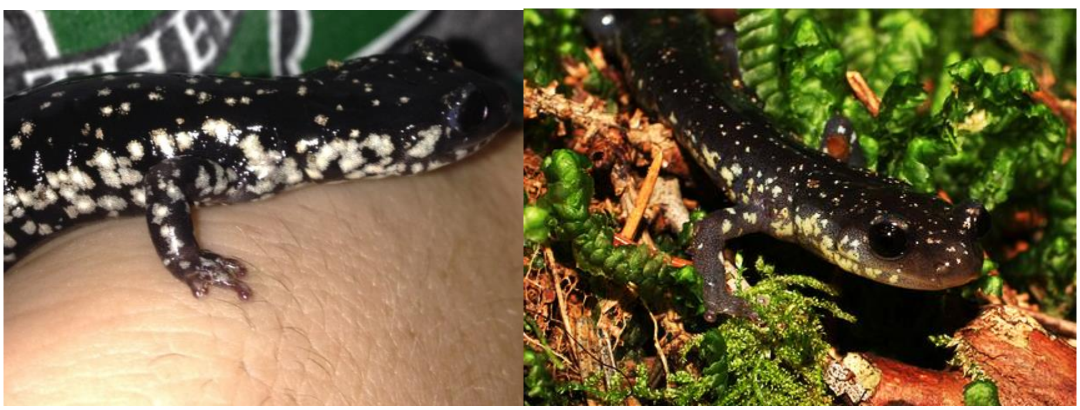

Figure 2. Lateral patterning of *Plethodon kentucki* (left) compared to *Plethodon wehrlei* (right). *P. wehrlei* photo Courtesy of Keven Messenger.

The ventral surface ranges from gray-white to black. A distinctive feature for *P. kentucki* is the lighter coloration of the chin/lower jaw region as opposed to the rest of the venter (Highton & MacGregor, 1983). Because of size constraints, neonatal individuals were only photographed on their dorsal surface (Figure 3).

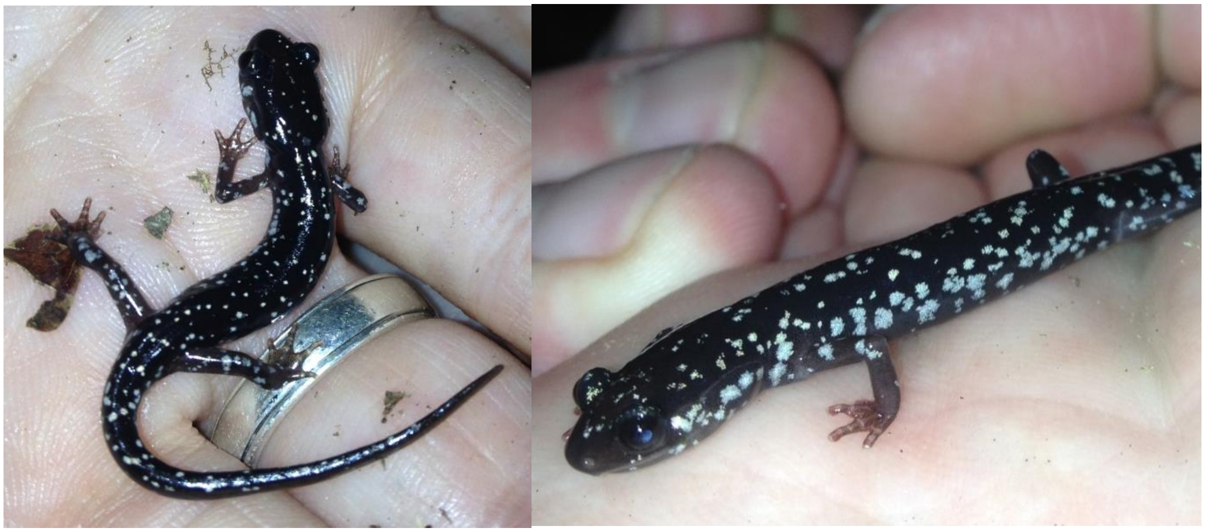

Figure 3. Late neonate *Plethodon kentucki* photography (left) compared to juvenile/adult (right)

Plethodon kentucki was first described as a new species in 1951 by Mittleman. His data were based on morphological differences including pattern, size, vomerine tooth count, and coloration. Highton and and MacGregor (1983) went on to confirm the species' legitimacy through electrophoretic analysis. Although genetically *P. kentucki* was found to be most similar to *P. jordani*, its coloration made it nearly indistinguishable from *Plethodon glutinosus*, which Highton found to be its second closest sibling (Highton & MacGregor, 1983). A second study, Maha and Maxson (1983), focused on determining *P. kentucki's* species status using immunological comparisons of albumin among several species. Both studies deemed *P. kentucki* a new, distinct species from *P. glutinosus*, not just a regional phenomenon. Another study by Highton, Maha, and Maxson (1989) went on to separate what was originally a single species, *P. glutinosus*, into 16 morphologically similar species, which included *P. kentucki*, called the *P. glutinosus* complex. Bailey (1992), a former graduate student at Marshall University, did an ecological study on *P. kentucki*. The major focus of his study was to determine the factors limiting *P. kentucki* to the southwestern portion of West Virginia along with assessing the surface density of the animal within the state (Bailey, 1992). Bailey used the most common mark-recapture methods for salamanders, toe clipping and fluorescent elastomer injections; both of which are invasive techniques for the salamanders (Figure 4). Although he was unable to recapture enough individuals during his study to confidently make an estimate of the total population of *P. kentucki*, Bailey gathered useful data regarding general diel and seasonal activity of the species. Bailey recorded a variety of environmental factors that could have affected the seasonal activity of *P. kentucki* including air temperature, soil temperature, relative humidity, soil pH, and soil moisture. The most influential of these environmental factors was determined to be soil moisture. This study used these same environmental variables, but different analyses to observe interactions among variables; something not possible in Bailey.

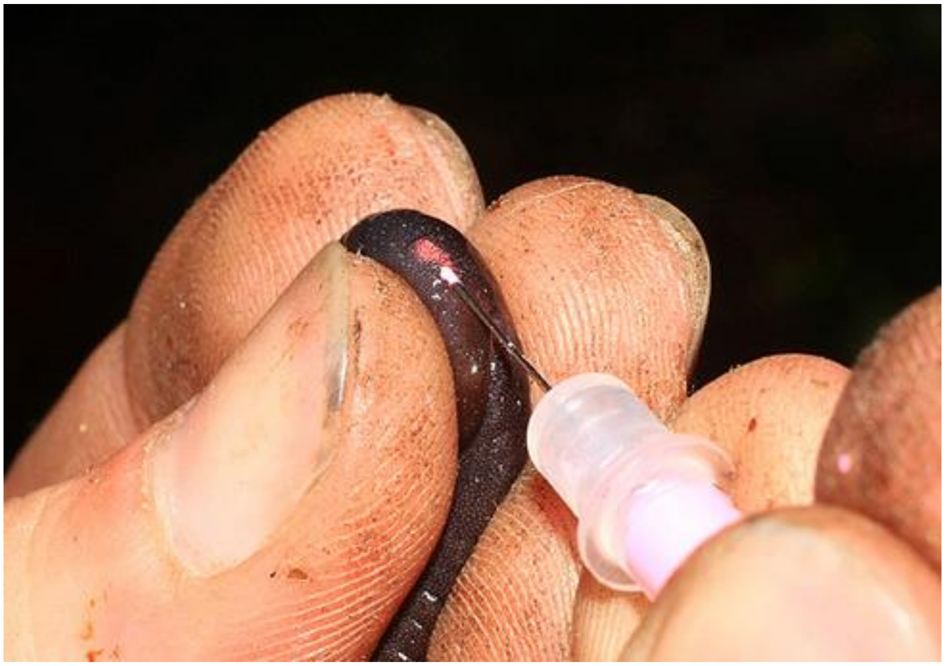

Figure 4. Elastomer injection technique for salamanders. Photo courtesy of Kevin Messenger.

One thorough study by Marvin (1996) established excellent ground work to fill in the gaps in *P. kentucki*'s life history. He conducted a three-year life history and population characteristics study in Harlan County, Kentucky in which he used captive females to gather nesting data. Because he was unable to find nests in the field, Marvin placed gravid female *P. kentucki* into brooding chambers that he subsequently buried at his field site. He had three of his seven gravid females deposit eggs in their brooding chambers, a fact that suggests subterranean environments are likely locations for *P. kentucki* nests. Common hand check techniques used in the study of woodland salamanders would leave underground nests undiscovered, so it is understandable that a nest site has not yet been found. Marvin determined that these animals are biennial breeders, so not every sexually mature female was sexually active every year. Marvin's clutch data was derived from those three buried brooding chambers, so much is still to be desired with regards to wild clutch data. *Plethodon kentucki*'s sibling species, *P. glutinosus*, has been found to nest in rotting logs (Fowler, 1940; Rubin, 1965) and under rocks (Rubin, 1965) while nests of the closest relative, *P. jordani*, are yet to be discovered, but believed to be in underground cavities (Beamer and Lannoo, 2005). It was a goal of this study to discover a wild *P. kentucki* nest, but even with excavation of a known burrow no nests were found.

Chapter 1

Environmental Factors Affecting the Activity of *Plethodon kentucki*

Introduction

In organismal biology, understanding the phenology of a particular species can provide the basic knowledge necessary to understand many other aspects of the organism's life history, including seasonal time and duration of active periods. It is a well-established concept among herpetologists that environmental variables place limitations on the activity levels of many species of plethodontid salamanders, and that, because of their water-permeable skin and proclivity to desiccation, moisture and temperature variables are the major predictors of those constraints (Bogert, 1952; Heatwole, 1962, Pauley, 1978). The activity patterns of *Plethodon cinereus*, a small eastern *Plethodon,* are dictated largely by soil moisture (Heatwole, 1962). Bogert (1952) and Shelford (1914) suggest that moisture and humidity gradients dictate salamander activity. Pauley (1978) found that environmental moisture regimes dictated the partitioning of sympatric species of *P. cinereus* and *P. wehrlei*. Hairston (1949) hypothesized that the distribution of *P. glutinosus* was attributable to atmospheric moisture.

While all of these studies provided valuable insight into the relationship between the surrounding environment and the natural history of several eastern plethodontids, there is a gap in our knowledge of the influence of environmental factors and the natural history of *P. kentucki*. Most of the focus on *P. kentucki* was to determine its legitimacy as a full species (Clay et al. 1955; Highton and MacGregor, 1983; Maha and Maxson, 1983; Highton et al. 1989), There is information available detailing much of the reproductive life history for *P.kentucki* as well as population characteristics (Marvin, 1996). Nevertheless, the driving forces behind the species' phenology are largely unknown. Attempting to address this issue, Bailey (1992), focused on the seasonal and diel activity of *P. kentucki*. He compared capture rates directly to individual environmental variables, such as soil temperature, soil moisture, soil pH, air temperature, and air humidity to observe any significant correlation between those variables and the surface abundance of *P. kentucki*. He found that only soil moisture had any significant correlation to surface density of the salamanders (Bailey, 1992).

The purpose of this study was to reassess the effect of environmental variables on the activity patterns of *P. kentucki*. Of particular importance was determining which of these variables are the most important in dictating activity.

Methods and Materials

Description of the Study Area

The selection of field sites for this study involved a half-year of random site evaluation preceding the study. Throughout the summer, fall, and early winter of 2011 various sites were evaluated for the presence of a viable *P. kentucki* population. The first study site that emerged as ideal habitat was in Wayne County, WV. Coincidentally, it was the same site utilized by Bailey (1992) 20 years earlier. The site is located at Beech Fork State Park (0.8 km east on Beech Fork Road and 0.48 km north on Butler Adkin's Branch Road) on the Campground side of Beech Fork Lake within the Wildlife Management Area (Figure 5). This site was selected because of the high density of *P. kentucki* and because it coincided with Bailey's site. It is a west-facing slope with vegetation consisting predominantly of Oak-Beech mixed deciduous forest (Bailey, 1992). The site's topography consists of steep wooded hillside, bare rock outcrops, a mid-slope plateau, and exposed rock faces. Because *P. kentucki* is the most common species on this hillside, it suggests that the environmental conditions present there are ideal habitat for the salamander, and therefore any data collected would reflect these ideal conditions.

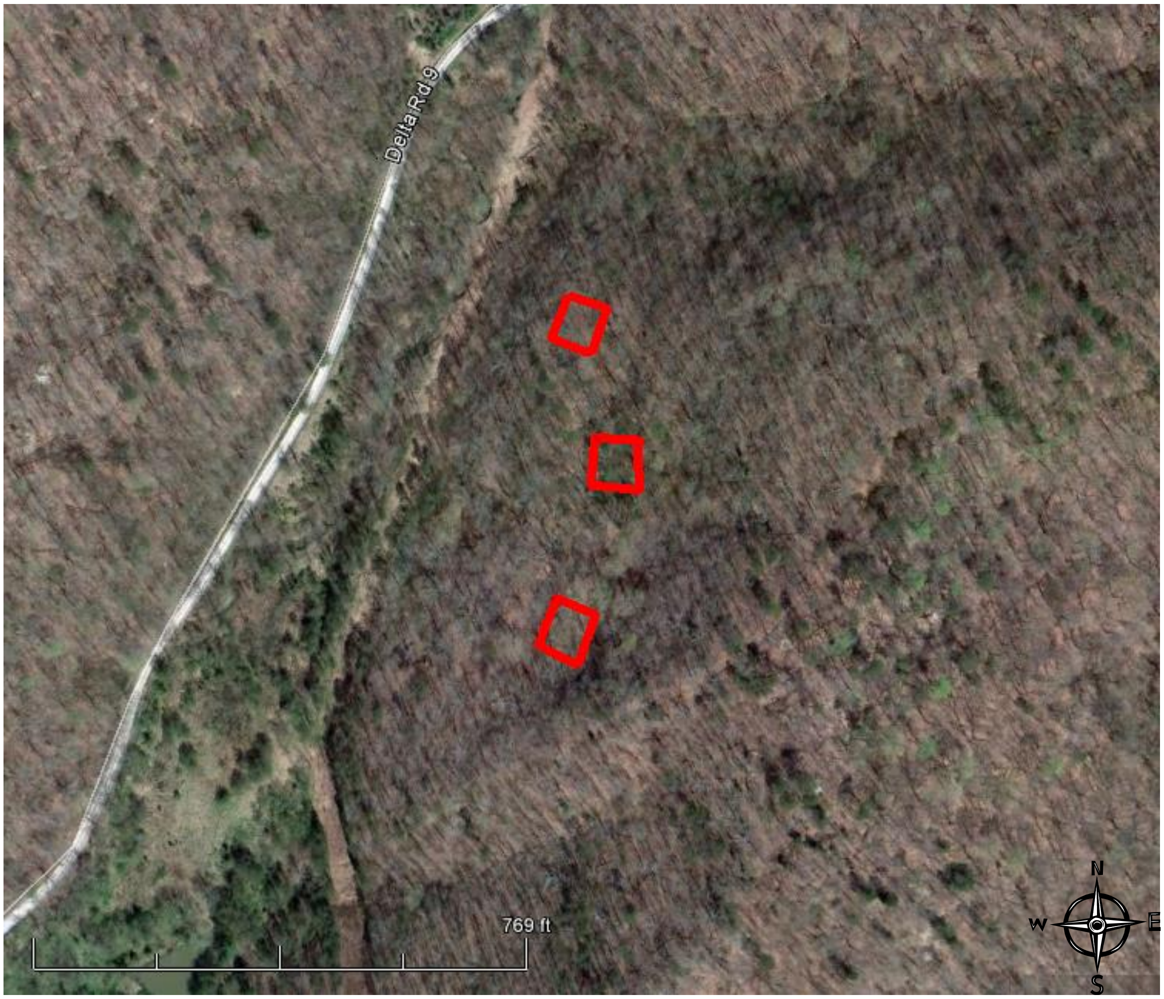

Figure 5. The "Campground" Site at Beech Fork Wildlife Management Area. The red squares represent the placements of study plots as confirmed by GPS coordinates. Photo courtesy of Google Earth.

The second field site is also located at Beech Fork State Park (0.65 km on Falls Branch Road and 0.32 km on County Road 15/2) on the Beech Fork Dam side of Beech Fork Lake (Figure 6). This site was selected because *P. kentucki* was the predominant species on the hillside, and because of the population density of the salamander at this site. It is a north-facing slope with vegetation consisting predominantly of sugar maple-buckeye mixed deciduous forest (Gilliam at al., 2014). The site topography included steep wooded hillside, mossy rock outcrops, a mid-slope plateau, exposed rock faces, and an ephemeral stream. Again, because *P. kentucki* was the most common species at this site, it was inferred that the environmental conditions were favorable for the salamander and environmental data collected from the site would represent the ideal environment for the species as a whole.

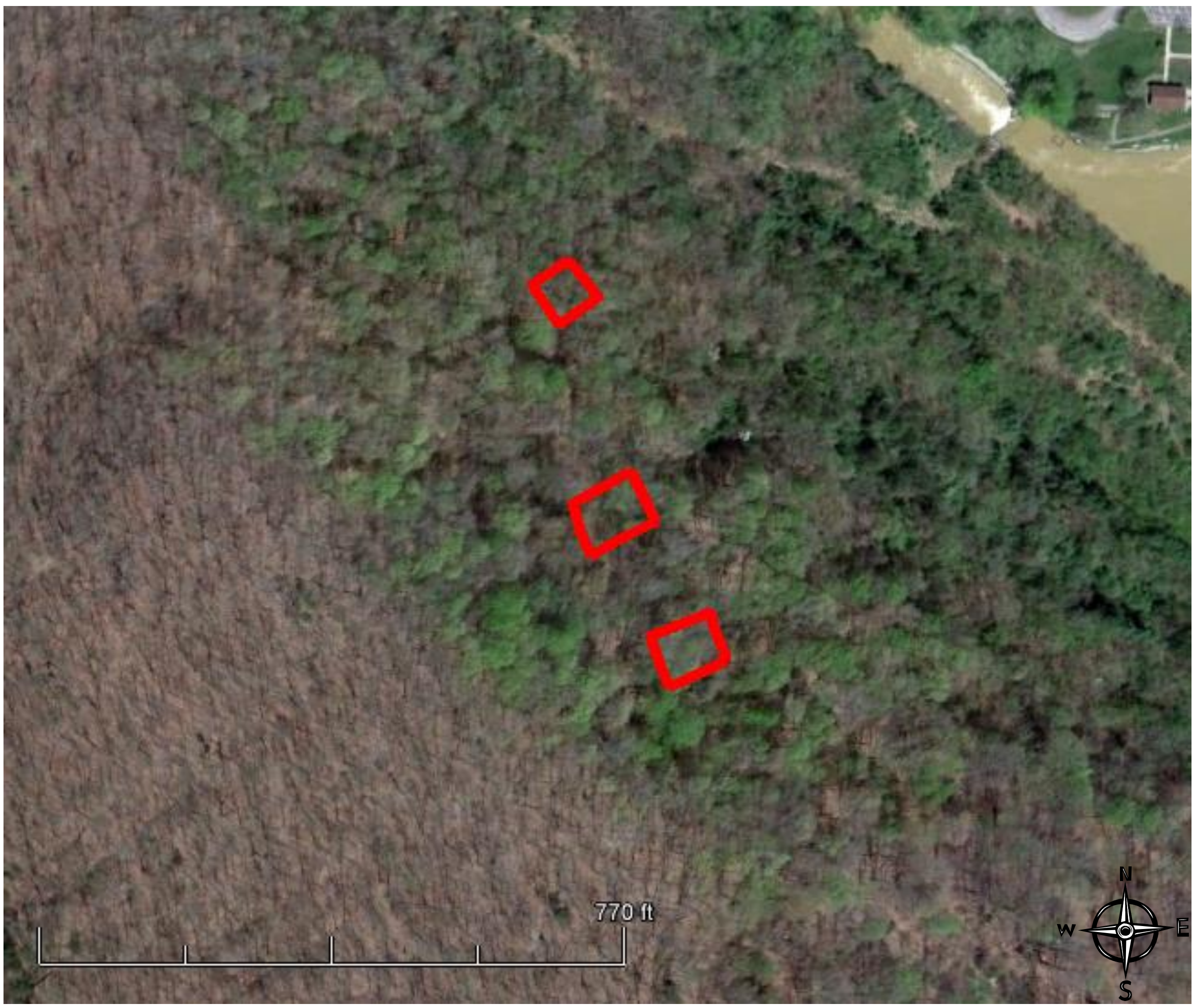

Figure 6. The "Dam" Site at Beech Fork Lake Dam. The red squares represent the placements of study plots as confirmed by GPS coordinates. Photo courtesy of Google Earth.

At each of the two study sites, three 20x20 m square plots were placed on the hillsides. These plots were arranged based on viability of the habitat to support *P. kentucki*, and the suitability for *P. kentucki* nest sites. This has been deemed an acceptable method of gathering data for these salamanders (Heyer et al., 1994). Originally, there were four plots at each site, but heavy June storms downed multiple trees and eliminated one plot from each site. This was deemed to be a confounding factor that could not be avoided, and, because there was still an even number of plots between sites, no new plots were added. Data collected from plot one at the campground site and plot four at the dam site (the two destroyed plots) were therefore not included in this study. Plots were marked as waypoints in a handheld GPS. All plots contained rocky outcrops, downed logs, leaf litter, and living root systems, which have all been deemed suitable habitat for *P. kentucki* (Bailey, 1992; Marvin 1996; Lannoo, 2005) to survive. Based on Bailey's (1993) dietary study, the macro-invertebrates used as food by the salamander were present within all 6 plots.

Sampling Techniques

Standard ground search methods were conducted for each of the three study plots at each site. Ground searches involved turning over all moveable cover objects such as downed logs, moveable rocks, sifting through leaf litter, and searching live root systems at the bases of trees for *P. kentucki* (Bailey, 1992; Marvin, 1996). Sampling occurred from 7 p.m. to 11 p.m. as dictated by the highest diel activity observed by Bailey. Rainy nights were preferentially used as sampling nights. If sampling occurred on a dry night and a rainy night followed within the same sampling week a second sample was taken for that week, provided the second sample was not taken within two days of the first. At least two days were allowed between samples to ensure as little stress on the animals as possible.

Environmental Data Collection

On the night of sampling, environmental variables were recorded for each plot at four random positions and averaged. Soil temperature was recorded using a Taylor® Switchable Digital Pocket Thermometer. Air temperature and relative air humidity were recorded on a Traceable®Thermohygrometer. A substrate/litter sample was collected in a Ziploc bag and returned to the lab. Precipitation data was gathered for the night of sampling from the National Oceanic and Atmospheric Administration (NOAA) website. Soil samples were randomly collected from locations within study plots and across the study site as a whole. These, along with soil and litter samples collected from salamander capture sites were analyzed in the lab. Soil pH was determined using a Thermo Orion® 3 Star pH meter and an Orion® ROSS Ultra combination pH electrode and according to techniques outlined in "Field Sampling Manual" (Pauley, 1999). Percent soil moisture was recorded by weighing soil samples, drying them in an oven, and reweighing them. Study sites were sampled at least once monthly.

Environmental Data Analysis

It was the goal of this study to reexamine the effects of environmental factors on *Plethodon kentucki* activity by employing the same variables defined by Bailey (1992) and using newer Generalized Linear Mixed Modeling (GLMM) techniques to analyze those effects. Generalized Linear Mixed Modeling was an ideal method for predicting *P. kentucki* activity patterns in this study because the measure of activity is based on surface counts, a form of nonnormal data (Bolker, 2009). Classic Gaussian based ANOVA methods would, therefore, not be an effective means of analyzing the relationships between activity and environmental data. The predictive model that was used was the GLIMMIX procedure in SAS (Waldron, 2013). This procedure uses Laplace approximation to determine the likelihood that the random response variable, counts, was actually affected by the covariate/s, the environmental data included in each model. The models were run using both Poisson and Negative-Binomial distributions. Using ĉ values as measures of the goodness of fit for each distribution, the models and distributions with the best fit were selected (Burnham and Anderson, 2002). When determining which models would be used for inference, AIC_C values were used to account for the small sample size and only models with $\Delta AIC_C \leq 2.00$ were supported. To assess the importance of each model, model weights were adjusted across all models and 95% confidence intervals of model-specific (β) and model-averaged beta estimates (β) were used to examine covariate effects (Waldron, 2013).

Results

The two sites were visited 13 times throughout the course of the study. A total of 131 salamanders were captured. Using multiple combinations of environmental variables (Table 1) as predictors of *Plethodon kentucki* activity (measured as surface abundance i.e., counts), three candidate models received support (Table 2). The supported models included soil moisture and soil temperature as functions of activity. Soil moisture significantly affected abundance (F = 11.46, P = 0.0019; from model: Soil temp + moist). Abundance had a positive relationship with moisture ($\hat{\beta}$ = 0.9440 ± 0.3260, 95% CI: 0.3051 to 1.5829). Type 3 Fixed Effects indicated soil temperature (F = 22.12, P = < .0001; from model: Soil temp + moist) was negatively associated with abundance ($\hat{\beta}$ = -1.7213 ± 0.3664, 95% CI: -2.4394 to -1.0031, Table 3).

Table 1

Candidate activity level models derived using environmental data, ranked in order of support. ΔAIC_C = the difference between the model with the lowest AIC_C score and the present model, *w* = adjusted model weights, *K* = number of parameters, temp = soil temperature, moist = soil moisture, Precip = precipitation, RH = relative humidity, pH = soil pH.

Model	*K*	*AICc*	*ΔAICc*	*w*
Soil temp + moist + pH[a]	4	136.87	0.00	0.40
Soil temp + moist[a]	3	137.21	0.34	0.34
Soil temp + moist + pH + Precip[a]	5	138.70	1.83	0.16
Soil temp + moist + pH + RH	5	139.67	2.80	0.10
Soil temperature	2	151.79	14.92	0.00
Soil moist + RH	3	159.51	22.64	4.87E-06
Soil ph	2	160.45	23.58	3.04E-06
RH	2	163.46	26.59	6.75E-07
Soil moist	2	165.37	28.5	2.60E-07
Soil moist + Precip	3	167.72	30.85	8.03E-08
Precip	2	168.85	31.98	4.56E-08

[a] Model used for inference ($\Delta AIC_C \leq 2.00$)

Soil pH was included in two supported models (Table 1), suggesting it had an additive or interactive effect. Soil pH appeared to have a marginally positive interaction with activity (β = 0.5215 ± 0.2817, 95% CI: -0.0307 to 1.0736), but significant effects could not be determined because confidence intervals included zero (i.e., beta coefficients were not significant). Precipitation was included in only the lowest of the supported models (Table 2) and was slightly positively associated with abundance (β = 0.2097 ± 0.2173, 95% CI: -0.2162 to 0.6356), but the confidence intervals included zero.

Table 2

Coefficients from supported (i.e., ($\Delta AIC_C \leq 2.00$) activity level models. Models are listed in order of support according to Akaike's Information Criterion adjusted for small sample size (AIC_C). Confidence intervals (95%) are represented by LCL (lower) and UCL (upper).

Model	Parameter	β	SE	LCL	UCL
Soil temp + moist + pH					
	moisture	0.8471	0.3235	0.2130	1.4812
	temperature	-1.6426	0.3506	-2.3298	-0.9554
	ph	0.5150	0.2834	-0.0405	1.0705
Soil temp + moist					
	moisture	1.1421	0.3373	0.4810	1.8032
	temperature	-1.8014	0.3830	-2.5521	-1.0507
Soil temp + moist + pH + Precip					
	moisture	0.8427	0.3171	0.2212	1.4642
	temperature	-1.7198	0.3656	-2.4364	-1.0032
	ph	0.5279	0.2800	-0.0209	1.0767
	Precipitation	0.2097	0.2173	-0.2162	0.6356

Table 3

Coefficients for model averages of covariates found only in supported models. Covariates are listed in order of importance according to summed adjusted model weights across all activity level models.

Parameter	β	SE	LCL	UCL
Temperature	-1.7213	0.3664	-2.4394	-1.0031
Moisture	0.9440	0.3260	0.3051	1.5829
pH	0.5215	0.2817	-0.0307	1.0736
precipitation	0.2097	0.2173	-0.2162	0.6356

Discussion

Data from this study suggests that soil moisture and temperature drive *P. kentucki* activity. This species is highly fossorial, as such; a major component of their environment is the soil they inhabit, so it is not surprising that the conditions of the soil play such a pivotal role in determining their activity levels (Marvin, 1996). *Plethodon kentucki* activity was observed to be highest when soil conditions were cool and moist. As soil temperatures rose and soil moisture dropped during the hot summer months surface activity dropped to 0 (i.e., no individuals captured June-August 2012). The relationship between activity levels and soil moisture and temperature regimes is logical when cutaneous gas exchange and the threat of desiccation are considered (Pauley, 1978).

There were two distinct peaks in activity in this study which coincided directly with previously observed spring/fall breeding periods (Bailey and Pauley, 1993). There is no evidence to support a significant relationship between soil regimes and sexual activity levels, but it is notable that there was sexual activity during a period of heavy surface activity driven by soil regimes. Soil moisture and temperature were determining factors for surface activity in April and May, 2012 (i.e., the spring breeding period) when two male *P. kentucki* were captured with distinct mental glands present suggesting they were sexually active at that time. It is important to note that although activity in September and October, 2012 did coincide with the fall breeding period, no sexually active males or gravid females were captured at either site, suggesting that soil moisture and temperature were only driving surface activity at this time, not sexual activity. It is well documented that environmental variables, including soil moisture and temperature, can effect multiple aspects of plethodontid life history (Pauley, 1978), so it is likely that those variables were effecting sexual activity levels, but further research would have to be performed to confirm this theory.

The predictive models that were developed for this study will be useful for forecasting activity levels in future studies. The use of these models to accurately predict future surface activity of *P. kentucki* at a given location has not been tested in a practical setting, but theoretically the models should be an excellent tool for researchers to maximize the efficacy of field sampling efforts by only sampling during periods of ideal environmental conditions. The predictive environmental variable models combined with known diel activity profiles (Bailey, 1992) and detailed phenology patterns should make *P. kentucki* much easier to study in the future.

Chapter 2

Viability of Photographic Mark-Recapture Techniques for *Plethodon kentucki*

Introduction

Classic methods of marking salamanders for mark-recapture studies are highly invasive to the animals, time consuming, and often ineffective. The most invasive of these methods for salamanders is PIT (Passive Integrated Transponder) tagging. The use of PIT tags first emerged as a tool for biologists in the mid-1980s (Gibbons, 2004). Often size limitations of various species of salamander dictate that PIT tags be surgically implanted rather than simply injected sub-dermally, as is often the case with larger animals. Even if the PIT tag can be injected there is still the possibility of injury occurring at the site of injection due to the small size of the animals (Gibbons, 2004). There is also the problem of PIT tags falling out or migrating out of the body cavity through natural physiological processes. All of these issues combined with the cost of procuring small enough PIT tags to mark upwards of 100 individuals in a single study make PIT tagging a less than ideal technique for salamander mark-recapture research.

The oldest technique for marking small amphibians for recapture is toe clipping. As the name implies, the toes of the individual are clipped off in a specific order such that thousands of individuals can be marked by removing one or more toes from one or all of the four feet. There are several problems that arise from this technique. Amphibians, especially salamanders, can often regrow appendages (e.g., losing their tail to a predator and regenerating it) and therefore alter their marking and subsequent identification by regenerating clipped toes (Davis and Ovaska, 2001). It is also believed that toe clipping can alter the life history, survivorship, and agility of several species of amphibians (Davis and Ovaska, 2001; Clarke, 1972). There is also the problem of inflicting pain and causing physical stress to study specimens by clipping their toes which can elicit unwanted behaviors from those specimens. Although toe clipping is one of the more common techniques for marking salamanders, it is still very invasive and traumatic to the study specimens.

The use of subcutaneous fluorescent elastomer injections has become another popular method for marking amphibians for recapture studies. Trace amounts of the fluorescent dye are injected under the skin of individuals in specific patterns and colors unique to that individual so they can be identified upon recapture. This technique also has problems. It requires the use of a tiny hypodermic needle to inject the individual, which has the possibility of becoming infected like any injection site does. Often the fluorescent dye will migrate under the individual’s skin or break apart into several different spots (Davis, 2001), which can lead to misidentification of the individual. It is also a painstaking process to inject the dye at the correct depth within the skin. If the elastomer is injected too deep it is impossible to see it upon recapture. This process is also very invasive for the individuals and can cause stress on them.

The advancement of technology, digital photography specifically, has afforded researchers the ability to record and analyze data much more efficiently. Some of the earliest digital cameras were clunky, cumbersome tools that took poor photos and had little storage capacity. The technology fueling digital photography has advanced so far in two decades that the simple cameras integrated into smartphones today have higher resolutions and greater storage capacity than early cameras. Because *P. kentucki* is a salamander with an easily distinguishable and unique spot pattern, it was hypothesized that digital photographs of individuals would be sufficient data to re-identify them upon recapture. This technique was successful on other salamanders with similar markings (Waldron & Pauley, 2007; Flint & Harris, 2005). It was the focus of this study to use these technological advancements as a means of streamlining the process of marking *P. kentucki* for recapture, and eliminating the invasive, time-consuming procedures so often used in standard amphibian mark-recapture research.

Methods

Laboratory Trials

Before attempting to photographically record individual *P. kentucki* in this study, a laboratory trial was performed. Twenty preserved *P. kentucki* specimens were selected from the Marshall University Herpetology Museum collection. Although the collection is very large only a few of the specimens remained preserved well enough to see traces of their pigmentation. The dorsal pigmentation had completely faded away on most specimens. Only the highly pronounced lateral pigmentation signature of *P. kentucki* remained visible, so the lateral patterning, specifically around the head and front legs, was utilized for identification in the lab trials. Photos of all 20 individuals were taken. A salamander was then chosen at random from a larger group of 50 salamanders, which included the 20 photographed individuals. The salamander was then compared to the photographs to determine if it was one the recorded individuals or a new un-recorded specimen.

Field Data Collection

The selection of field sites for this study involved a half-year of random site evaluation preceding the study. Throughout the summer, fall, and early winter of 2011 we evaluated various sites for the presence of a viable *P. kentucki* population. The first study site that emerged as ideal habitat was in Wayne County, WV, the same site utilized by Bailey (1992) twenty years earlier. The site is located at Beech Fork State Park (0.5 mi east on Beech Fork Road and 0.3 mi north on Butler Adkin's Branch Road) on the campground side of Beech Fork Lake within the Wildlife Management Area (Figure 5). We selected this site because of the high density of *P. kentucki* and because it coincided with Bailey's site, which would provide continuity between the two studies. It is a west-facing slope with vegetation consisting predominantly of Oak-Beech mixed deciduous forest. The site's topography consisted of steep wooded hillside, bare rock outcrops, a mid-slope plateau, and exposed rock faces. Because *P. kentucki* is the most common species of salamander on this hillside, it suggests that the environmental conditions present there are ideal habitat for this species, and therefore any data collected would reflect these ideal conditions.

The second field site is also located at Beech Fork State Park (0.4 mi on Falls Branch Road and 0.2 mi on County Road 15/2) on the Beech Fork Dam side of Beech Fork Lake (Figure 6). This site was selected because *P. kentucki* was the predominant species on the hillside, and because of the population density of the salamander at this site. It is a north-facing slope with vegetation consisting predominantly of Sugar Maple-Buckeye mixed deciduous forest (Gilliam et al. 2014). The site topography includes a steep wooded hillside, mossy rock outcrops, a mid-slope plateau, exposed rock faces, and an ephemeral stream. Again, because *P. kentucki* was the most common species at this site, it was inferred that the environmental conditions were favorable for the salamander and environmental data collected from the site would represent the ideal environment for the species as a whole.

At each of the two study sites, 3 20x20 m square plots were placed on the hillsides. These plots were arranged based on viability of the habitat to support *P. kentucki*, and the suitability for *P. kentucki* nest sites. This is an acceptable method of gathering data for these salamanders (Heyer, et al., 1994). Plots were marked as waypoints in a handheld GPS. All plots contained rocky outcrops, downed logs, leaf litter, and living root systems, which are all suitable habitat for *P. kentucki* (Bailey, 1992; Marvin 1996; Pauley and Watson, 2005) to survive. Based on Bailey's dietary study, the macro-invertebrates utilized as food by the salamander were present within all 6 plots (Bailey, 1992).

Study sites were sampled at least once a month. Standard ground search methods were conducted for each of the three study plots at each site. Ground searches involved turning over all moveable cover objects such as downed logs, moveable rocks, sifting through leaf litter, and searching live root systems at the bases of trees for *P. kentucki* (Bailey, 1992; Marvin, 1996). Sampling occurred from 7:00 to 23:00 as dictated by the highest diel activity observed by Bailey (1992). Rainy nights were preferentially used as sampling nights. If sampling occurred on a dry night and a rainy night followed within the same sampling week a second sample was taken for that week, provided the second sample was not taken within two days of the first. We allowed two days minimum between samples to ensure as little stress on the salamanders as possible. Flags were initially placed at the sites of capture locations during April and May, but this was discontinued in September and October as captures were often made too quickly in succession to flag every location.

Individual data collected upon capturing a salamander included the sex, placing the specimen in an age class based on size, and photographs of the specimen. The gender of the salamander was determined by observing the chin region of the head for a round hedonic mental gland (Figure 7). Only sexually active mature males have this gland. The exterior of the cloaca of these males was examined for the presence of spermatophores. Only sexually active males will have spermatophores present in the cloaca. If the salamander was a female, her cloaca and the surrounding region were examined for the presence of eggs. Only sexually mature females will have the development of eggs. Sexually immature juveniles were recorded as juveniles. There are no current field techniques for determining their sexes. We recorded neonatal individuals as neonates, and their gender was also undeterminable. Because of the success and ease of identification in laboratory trials, the lateral surfaces, specifically the head region, of adults and juveniles were photographed in detail to record their individual spot pattern. Only the dorsal surface for neonates was photographed because of their small size. Marvin (1998) used sketches of individual spot patterns in the head and sacral area as a means of capture-recapture identification, but there was no assesment of the accuracy of his recognition technique (Marvin, 1998). Flint and Harris (2005) successfully utilized a variation of

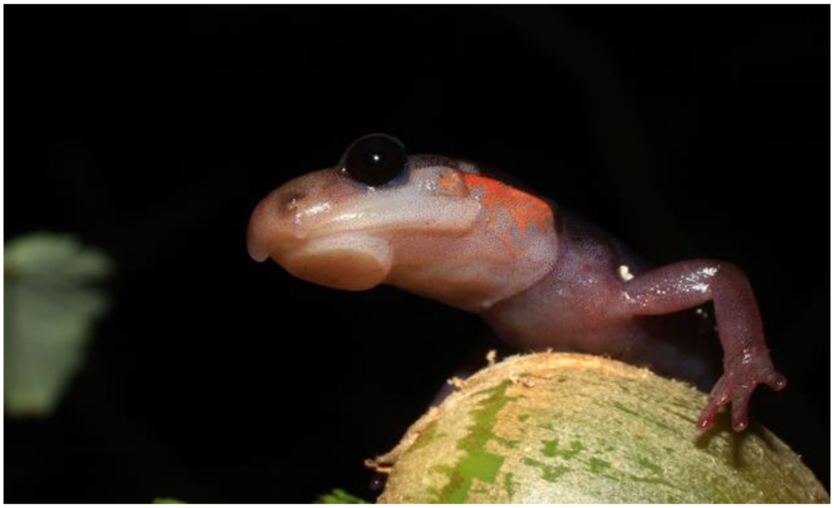

Figure 7. Example mental gland. Species shown is *P. jordani.* Photo courtesy of Kevin Messenger.

this method of noninvasive mark-recapture that involved photography in a study on *Plethodon punctatus*, a salamander in the *P. wehrlei* complex with a pattern similar to *P. kentucki*.

Field Data Analysis

The photographs from each site were sorted into individual digital files. Because the photos were taken using an iPhone 4 ios™, a GPS way-point was recorded for each photo taken. These way-points were used to determine in which study plot at each site the photo was taken. Once the photos were sorted into plots they were further sorted by date taken. This allowed for comparison of photos from one sampling event to another for the same plot. The photos were then organized into age classes. A pair of photos corresponding to one individual were opened and compared to every other photo of individuals matching the plot and age class from later samples. Photos were compared using the naked eye. Pattern recognition software was available which could have compared the photos but was not used due to excessive amounts of glare caused by the cutaneous mucous *P. kentucki* excretes when handled. All photos were compared for evidence of proof of concept as well as recaptures in three separate trials.

Results

Laboratory Results

In three separate photo-comparison trials all 20 individuals were correctly identified from the larger group of 50. A different individual conducted each of the three trials to ensure the researcher did not become familiar with specific salamanders and recognize them from the group. This demonstrated the concept of photographic marking of individuals in a laboratory setting; however, the application and success of this technique in the field could not be confirmed through the laboratory trials.

Field Data Results

We photographed 131 *P. kentucki* throughout the study. Of the 96 individuals placed into the mature age class, only 2 could be positively identified as males. There were 25 individuals placed into the juvenile age class and 10 individuals placed into the neonatal age class. After comparing the 131 salamander photographs within their specified classes, only one individual was recaptured throughout the study (Figure 8). The individual could only be categorized as an adult *P. kentucki* that was captured initially in the root system of a tree and recaptured under a rock in close proximity to the previous capture site. These capture sites were among those marked by flags initially in the study.

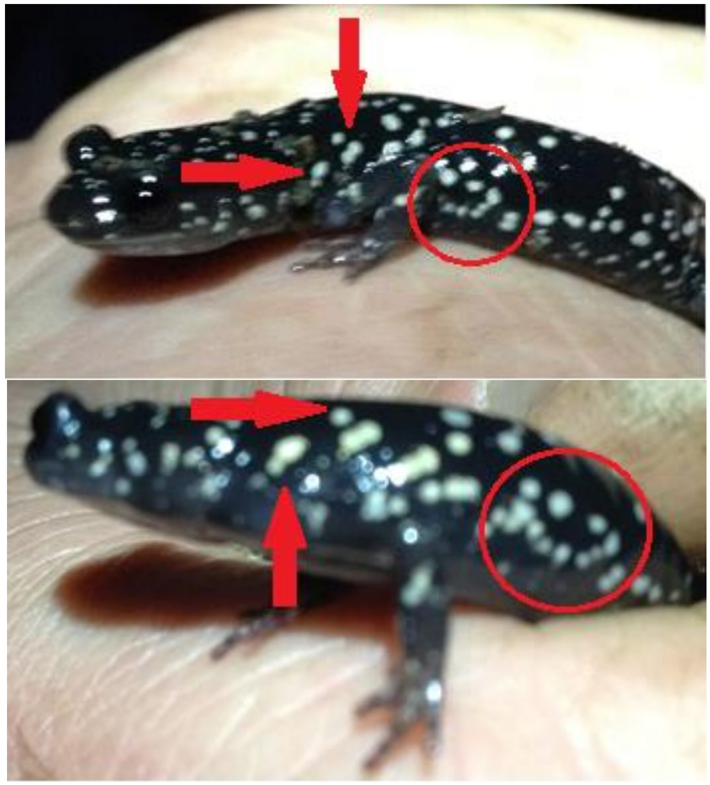

Figure 8. This adult *Plethodon kentucki*, initially captured on April 30 at the "Dam" site was found, upon analysis of the photo records, to be recaptured on May 14.

Discussion

It was found that digital photography as a technique for mark-recapture studies in *P. kentucki* was a viable and useful method of non-invasive markings. It was shown that the technique worked theoretically in the laboratory through multiple trials, but this study has confirmed it as an acceptable field technique as well. In addition to being a non-invasive marking technique, it was also efficient. Handling time of individuals was limited to determination of sex and age class and capturing photos of both lateral surfaces. This minimalized stress and desiccation inflicted on the animals by excessive handling.

Comparing the photographs without the use of pattern recognition software was a simple procedure. When comparing photographs, the first step was to identify any distinguishable marks directly behind the eyes. If the area of patterning behind the eye was questionably the same the patterning surrounding the front legs was used. Often there would be either no patterning at all or solid white surrounding the legs. If both the patterning behind the eyes and around the front legs seemed to match, we examined the lateral patterning on the costal grooves for noticeable differences. Few individuals looked similar enough to move past the second stage of identification.

The broader impacts of this study confirming photographic mark-recapture as a viable methodology include utilizing it for sibling species. There are 15 other species of plethodontid salamanders in the *P. glutinosus* complex that could potentially be studied using this technique due to their similar patterning (Highton, 1989). There is evidence that multiple plethodontid species, including *P. kentucki* across the eastern United States are in decline, so any methods that make studying and preserving these creatures easier is a huge asset to conservation biology (Highton, 2005).

Chapter 3

Field Notes for Beech Fork State Park

Aestivation and Reemergence

This study was conducted between April and October in 2012. After sampling on May 14, *P. kentucki* were not found again at either of the study sites in Beech Fork State Park until September 3 during a heavy rain. These individuals were not included in the study because they were not found within the boundaries of the three designated study plots for each site. As detailed in previous chapters, plot placement was determined before the study began by assessing the surface abundance of *P. kentucki* throughout each site and subsequently placing plots in areas with the highest densities. These assessments were conducted in the fall of 2011 and early spring of 2012. During the course of the study no individuals were found on the surface or under cover objects from late May until early September. Apart from the high levels of activity in May, the seasonal activity pattern observed in this study was similar to the pattern seen in Bailey (1992). Summer aestivation explains the absence of activity during the hot, dry summer months (Bailey and Pauley, 1993). It was discovered that when *P. kentucki* were again found at each study site they were not within the plot boundaries, but much farther up the slope.

At both sites there were mid-slope benches where high densities of *P. kentucki* were found based on initial assessments, and subsequently where plots were set. Above the mid-slope benches there were steep inclines that exposed large rock and boulder fields. When *P. kentucki* became active again in September, all individuals were located on or near these moss covered boulders and rock faces. The suggested reason for the location of the salamanders higher up on the slope and near the rocks is ease of access to cool moist soils via cracks and gaps in the rock faces and boulders that run deep into the ground. The higher levels of salamander activity in cool soils with high moisture content suggest a natural preference for these environmental conditions. It is possible that during periods of harsh environmental conditions, such as low soil and atmospheric moisture and high soil and air temperatures, *P. kentucki* is retreating to these boulder refuges deep within the soil strata where those conditions are much less variable and it is easier to avoid desiccation. Further research is required to confirm these assertions.

Biennial Breeding

Plethodon kentucki breeds biennially, if not less frequently (Marvin, 1996). During assessments of the viability of research sites in November, 2011, 13 male *P. kentucki* were observed to have a well-developed and distinguishable mental gland present indicating they were sexually active and attempting to reproduce. The full courtship of a mating pair of *P. kentucki* was witnessed on November 12th during a site assessment in a ravine just north of the campground site. This confirmed the assumption that the presence of mental glands suggested sexual activity. There were two males with the presence of mental glands in April, which suggests sexual activity during the spring of 2012, but there were no individuals with the presence of mental glands found during the fall of 2012. The absence of these glands was observed at both selected sites which are over 8 km apart. For both study populations there was an unknown, common factor dictating that fall 2012 was not a suitable breeding year for *P. kentucki*. Further research is necessary to understand what factors determine mating activity annually for *P. kentucki*.

Sympatric Species

There were several other amphibian and reptilian species found while sampling for *P. kentucki* at Beech Fork State Park. Among the sympatric salamander species found within the study plots were the Southern Two-lined Salamander (*Eurycea cirrigera*), the Southern Ravine Salamander (*Plethodon richmondi*), the Eastern Red-spotted newt (*Notophthalmus viridescens viridescens*), the Marbled Salamander (*Ambystoma opacum*), and the Spotted Salamander (*Ambystoma maculatum*). The Frog species found included the Wood Frog (*Lithobates sylvatica*), the Spring Peeper (*Pseudacris crucifer*), the Mountain Chorus Frog (*Pseudacris brachyphona*), the American Toad (*Anaxyrus americanus*), Fowler's Toad (*Anaxyrus fowleri*), and Cope's Gray Treefrog (*Hyla chrysoscelis*). Only one snake species was observed at the campground site; a single Northern ring-necked snake (*Diadophis punctatus edwardsii*) that stayed in a rocky outcrop at the edge of plot two. One species of turtle, the Eastern Box Turtle (*Terrapene c. carolina*), was found within the study area. There were no lizards observed at either site during the course of this study.

Literature Cited

Alford, R.A. and S.J. Richards. 1999. Global amphibian declines: a problem in applied ecology. Annual Review of Ecology and Systematics 30:133-165.

Bailey, J.E. 1992. An ecological study of the Cumberland Plateau Salamander, *Plethodon kentucki* Mittleman, in West Virginia. Master's thesis. Marshall University, Huntington, West Virginia.

Bailey, J.E. and T.K. Pauley. 1993. Aspects of the natural history of the Cumberland Plateau Salamander, *Plethodon kentucki,* in West Virginia. Association of Southeastern Biologists Bulletin 40:133.

Beamer, D.A. and M.J. Lannoo. 2005. Jordan's Salamander (Plethodon jordani). Pp. 816-818. In Lannoo, M.J. (Ed.), Amphibian Declines: The Conservation Status of United States Species. University of California Press, Berkeley, California.

Bogert, C.M. 1952. Relative Abundance, Habitats, and Normal Thermal Levels of Some Virginian Salamanders. Ecology. Vol. 33, No.1. pp. 16-30.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology and Evolution. 24:127–135.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer

Clarke, R.D. 1972. The effect of toe clipping on survival in Fowler's toad (*Bufo woodhousei fowleri*). Copeia 1972.1:182-185.

Clay, W. M., R.B. Case, and R. Cunningham. 1955. On the taxonomic status of the slimy salamander, *Plethodon glutinosus* (Green), in southeastern Kentucky. *Trans. Kentucky Acad. Sci*, *16*, 57-65

Davis, T.M. and K. Ovaska. 2001. Individual Recognition of Amphibians: Effects of Toe Clipping and Fluorescent Tagging on the Salamander *Plethodon vehiculum*. Journal of Herpetology 35.2: 217-225.

Flint, W.D. and R.N. Harris. 2005. The efficacy of visual encounter surveys for population monitoring of *Plethodon punctatus*(Caudata:Plethodontidae). Journal of Herpetology 39:578-584.

Fowler, J.A. 1940. A note on the eggs of *Plethodon glutinosus*. Copeia 1940:133.

Gibbons, J. W. and K.M. Andrews. 2004. PIT tagging: simple technology at its best. Bioscience. 54(5), 447-454.

Gibbons, J.W. 1983. Their blood runs cold. The University of Alabama Press, Tuscaloosa, Alabama.

Gilliam FS, R Hédl, M Chudomelová, RL McCulley, and JA Nelson. 2014. Variation in vegetation and microbial linkages with slope aspect in a montane temperate hardwood forest. *Ecosphere* submitted.

Hairston, N. G. 1949. The local distribution and ecology of the plethodontid salamanders of the southern Appalachians. Ecological Monographs. 19(1), 47-73.

Heatwole, H. 1962. Environmental Factors Influencing Local Distribution and Activity of the Salamander, *Plethodon cinereus*. Ecology, Vol. 43, No. 3 (Jul., 1962), pp. 460-472.

Heyer, W.R. et al. 1994. Measuring and monitoring biological diversity. Standard methods for amphibians. Smithsonian Institution Press, Washington D.C.

Highton, R. 2005. Declines of eastern North American Woodland Salamanders (*Plethodon*). *in* Lanoo, M. 2005. Amphibian declines: The conservation status of United States species. University of California Press, Berkeley and Los Angeles, CA.

Highton, R. and J.R. MacGregor. 1983. *Plethodon kentucki* Mittleman: A valid species of Cumberland Plateau Woodland Salamander. Herpetologica 39:189-200.

Highton, R., G.C. Maha, and L.R. Maxson. 1989. Biochemical evolution in the Slimy Salamanders of the *Plethodon glutinosus* complex in the eastern United States. University of Illinois Press, Chicago, Illinois.

Lannoo, M. 2005. Amphibian declines: The conservation status of United States species. University of California Press, Berkeley and Los Angeles, CA.

Maha, G.C., and L. R. Maxson. 1983. Immunological evidence for the validity of *Plethodon kentucki.* J. Herpetology. 17: 398-400.

Marvin, G.A. 1996. Life history and population characteristics of the salamander *Plethodon kentucki* with a review of *Plethodon* life histories. American Midland Naturalist. 136:385-400.

Marvin, G.A. 1998. Territorial behavior of the plethodontid salamander *Plethodon kentucki*: influence of habitat structure and population density. Oecologia. Volume 114, Issue 1, pp. 133-144.

Marvin, G.A. and V.H. Hutchinson. 1996. Courtship behavior of the Cumberland Plateau woodland salamander, *Plethodon kentucki* (Amphibia:Plethodontidae), with a review of courtship in the genus *Plethodon*. Ethology 102:285-303.

Mittleman, M.B. 1951. Two new salamanders of the genus *Plethodon*. Herpetologica 7:105-112.

Pauley, T. K. 1978. Moisture as a factor regulating habitat partitioning between two sympatric Plethodon (*Amphibia, Urodela, Plethodontidae*) species. Journal of Herpetology. 491-493.

Pauley, T.K. 1999. Protocols for Long-term Monitoring Projects. Department of Biological Sciences, Marshall University. Unpublished manual.

Pauley, T.K. and M.B. Watson. 2005. Plethodon kentucki. In Amphibian Declines: The Conservation Status of the United States Species. M.J. Lannoo, Editor. University of California Press, Berkeley, California. Pp. 818-820.

Rubin, D. 1965. Amphibians and reptiles of Vigo County, Indiana. Master's thesis. Indiana State University, Terre Haute, Indiana.

Shelford, V.E. 1914. Modification of the behavior of land animals by contact with air of high evaporation power. Journal of Animal Behavior. 4:31-49.

Waldron, J. L., S.M. Welch, S.H. Bennett, W.G. Kalinowsky, and T.A. Mousseau. 2013. Life history constraints contribute to the vulnerability of a declining North American rattlesnake. *Biological Conservation*, *159*, 530-538.

Waldron, J.L. and T.K. Pauley. 2007. Green Salamander (*Aneides Aeneus*) Growth and Age at Reproductive Maturity. Journal of Herpetology 41(4): 638-644.

Waldron, J.L. et al. in press. The Herpetofauna of the Bluestone National Wild and Scenic River. Proceedings of the West Virginia Academy of Science. in press.