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Amphibians Among Road-Rut Pools in West Virginia

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AMPHIBIANS AMONG ROAD-RUT POOLS IN WEST VIRGINIA

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APPROVAL OF THESIS

We, the faculty supervising the work of Abby Sinclair, affirm that the thesis, *Amphibians Among Road-Rut Pools in West Virginia,* meets the high academic standards for original scholarship and creative work established by the Biological Sciences Program and College of Science. This work also conforms to the editorial standards of our discipline and the Graduate College of Marshall University. With our signatures, we approve the manuscript for publication.

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ABSTRACT

As anthropogenic environmental stressors such as urbanization continue to increase, it is necessary to understand their impact on amphibian ecology. Due largely to their biphasic life history amphibians are negatively impacted by the destruction of lotic habitats i.e., wetlands for the creation of roads and urban centers. I examined the effects of three human-made road rut pools on the reproductive success and species richness of amphibians in southwest West Virginia from May to October 2012. I employed three detection techniques: drift fence pitfall trap arrays, funnel traps and dipnet sampling. Additionally, habitat covariates were taken (i.e., water depth) at each sampling. A total of nine species were detected at various life stages (Larval, Juvenile, and Adult). Larvae were the most commonly detected stage. All larvae were aged according to the Gosner and Harrison staging charts. While the objectives of this study were addressed, there was not enough data to make any statistically significant conclusions. However, this study does allow for several anecdotal observations to be made which are important for future studies.

CHAPTER 1

OBSERVING AMPHIBIAN SPECIES AND HABITAT PARAMETERS IN ROAD-RUT POOLS IN WEST VIRGINIA

INTRODUCTION

There is an urgency to effectively understand the ecology of amphibian taxa due to rapid decline worldwide (Alford and Richards, 1999) as urbanization is increasing. Urbanization is the growth in population of humans in an area. Urban sprawl encroaches on natural habitat and affects the natural biota. Before considering the issue of how to manage human activity with wildlife activity, it is important to understand the interaction between the two. Urban environments influence reptiles and amphibians in various ways. Amphibians are negatively affected by introduced chemicals, habitat alteration, and roads (Mitchell et al., 2008; Knutson et al., 1999; Price et al., 2006; Semlitsch and Bodie, 2003). Roads disturb amphibians in direct and indirect ways. When habitat is altered, important features such as hydroperiod and species composition are changed. Amphibians are also bioindicators, making them excellent monitors of ecosystem health due to their permeable skin and in many cases, biphasic life cycle. More studies are being conducted in order to understand the relationship between urbanization and the effect to natural areas vital to amphibians such as forests and wetlands. As urbanization increases, some amphibians could adapt to the environmental changes and it is imperative to understand these changing, new relationships.

Some species thrive in urban environments while others find it hard to survive (Rodda and Tyrrell, 2008). Some native species are considered invasive because they outcompete other natives when the habitat is altered. Specialist species are easily outcompeted once a new species, especially a generalist, shares and dominates the niche once held by the specialist. Generalist species tend to adapt better to urbanized environments than specialists species. For example, Red-eared Sliders (*Trachemyes scripta elegans*) are a native species that has the ability to become invasive and outcompete other native species (Pearson et al., 2015). *Trachemyes scripta elegans* is a generalist omnivore which allows them to proficiently adapt to urbanized areas more successfully than other native reptiles (Ernst et al., 1994; Plummer et al, 2008). The Blue Tongue Lizard (*Tiliqua Scincoides*) is proficient at adapting to suburban environments (i.e., lawns and subdivisions), where it feeds on pests and domesticated pet food outcompeting other lizards that are unable to adequately adapt to developed areas (Koenig et al., 2001; Rodda and Tyrrell, 2008). Additionally, exotic invasive species, those not native to the area, often colonize and outcompete native species. When an exotic species is introduced to an unoccupied niche, it will rapidly colonize the area and become invasive (Rodda and Tyrrell, 2008). The United States has been affected by several non-native invasive species in recent years such as the Cuban tree frog (*Osteopilus septentrionalis*), Cane toad (*Rhinella marina*) and American bullfrog (*Rana catesbianus*). Human made alterations in the environment cause changes in species composition and interaction.

Amphibians are particularly susceptible to pollution and environmental changes due to a number of physiological traits including highly permeable skin (including cutaneous respiration in some species) and an aquatic larval stage. Amphibian eggs are gelatinous which exposes them to deleterious environmental conditions. In contrast, the increased protection of the calcified exterior of an amniotic egg, as observed in reptiles and birds, is less susceptible to desiccation and environmental toxins. The permeability of amphibian skin is highly affected by environmental pollutants such as vehicles, road and agriculture runoff (Mitchell and Brown, 2008; Collins and Russell, 2009; Rohr and McCoy, 2010). Amphibians undergo a biphasic life cycle enhancing their function as bioindicators. Unlike fish who spend their whole lives in water, amphibians are exposed to both aquatic and terrestrial habitats throughout their life. Hayes et al. (2006) and Rohr et al. (2006) suggest chemicals, such as atrazine, lower an amphibian's survival and reproductive success by means of mortality and hindering gonadal development (Hayes et al., 2006, Rohr et al. 2006). Impervious surfaces typical of urban settings accumulate pollutants and greatly alter hydrology affecting the habitat availability and habitat quality for amphibians (Snodgrass et al. 2008). During rain events impervious surfaces prevent groundwater recharge and increase surface runoff to water sources (i.e., streams and ponds) that many amphibians inhabit (Dunne and Leopold, 1978). Exposure to ultraviolet radiation and pesticides can result in direct amphibian mortality (Blaustein et al., 2003; Carey and Bryant, 1995). Non-lethal pesticides and heavy metals have been shown to slow development and cause physical abnormalities (Bridges & Semlitsch, 2000). The effect of harmful chemicals is apparent years after the amphibian was exposed. Russell et al. (1995) found pesticides in the tissues of Spring Peepers (*Pseudemys crucifer*) 26 years after pesticides were discontinued in an area in southern Ontario. A synergistic negative effect of pH and UV radiation has been observed in Pickerel Frogs (*Rana pipiens*) resulting in decreased embryonic development (Long et al., 1995). Blaustein et al. (2003) noted that nearly all amphibians are vulnerable to toxicological threats which have the ability to cause direct mortality or negative developmental effects.

Amphibians are impacted by the construction, maintenance, and presence of roads. While essential to urban development, roads act as barriers and are often avoided by animals further disturbing migration routes and subdividing species populations (Forman and Deblinger, 2000). Direct road mortality is a factor of amphibian decline, particularly near wetlands. Species of salamanders migrate simultaneously to breeding habitats that when intersected by roads can be subject to increased mortality (Semlitsch et al., 2007). Increased mortality occurs due to the rain prompting mass movements in amphibians (Duellman, 1954). Amphibians are not fast movers; consequently the probability of mortality is higher than faster moving vertebrates, such as a hares (Hels and Buchwald, 2001). Gravid salamanders are known to be slower than males or postgravid females (Finkler et al., 2003). One solution to overcome road mortalities is through the creation of crossing structures (Andrews et al., 2008). Crossing structures are characterized by a barrier wall leading animals to a tunnel or underground road passage. Florida Department of Transportation (FDOT) constructed a barrier wall-culvert in response to a high number of road kills on U.S. Highway 441 which resulted in a significant decrease in road mortality (Dodd et al., 2004).

The indirect effect of roads on amphibian survival can be harder to quantify than direct effects (Mitchell et al., 2008). Roads fragment, destroy, and degrade natural habitat. Habitat fragmentation and degradation caused by urban land use can negatively affect the distribution, abundance, and diversity of amphibians (Knutson et al., 1999; Andrews et al., 2008). When habitats are altered, the composition of the original environment changes making it unsuitable for amphibians. Habitat degradation changes temperature and moisture of an environment which are variables crucial to the success of amphibians. Moisture is crucial to amphibians because the permeable nature of their skin and eggs makes them extremely sensitive to changes in the environment. Cover objects which are important in providing cover and temperature regulation are often moved or altered in the creation of roads. Temperature regulation is necessary because amphibians can easily overheat or desiccate in direct exposure to sunlight. Roadway construction is also a major factor in destruction or modification of water bodies (permanent and ephemeral) that are crucial in the lifecycle of amphibians. Additionally, roads can impact the natural flora and fauna of the habitat. Plants alongside the road are mostly exotic and are generally more resilient than native species (Cale et al., 1991; Tyser and Worley, 1992). When these exotic species out-compete native species it reduces the survival success of the native flora (Gelbard and Belnap, 2003). Roads also cause an edge effect indicating the area surrounding the road is affected ecologically (Forman and Alexander, 1998). For instance, when a road bisects a forest, another edge is created which changes the composition of the forest by moving the edge closer to the center of the forest. As the distance away from forest edge increases, humidity and litter moisture increases; whereas, temperature and shrub cover decreases (Matlack, 1993). Amphibians are moisture and temperature dependent; therefore the increased creation of edges as a result of roadways creates a less suitable habitat for amphibians and potential for decreased survivorship.

Wetlands are distinct ecosystems characterized by hydric soils and often hydrophyte vegetation. When wetlands are altered or destroyed the hydroperiod is affected. Hydroperiod is the length of time water is present in an area. The length of the hydroperiod, or how long the wetland is saturated, is crucial to the reproductive success of amphibians. Many amphibians require lentic habitats such as ephemeral pools for reproduction. Therefore wetland management is particularly important. Road-rut pools are believed to mimic some of the same habitat characteristics of ephemeral pools. It is essential to understand which conditions road-rut pools and ephemeral pools exhibit to maximize amphibian survivorship. One reason amphibians inhabit ephemeral pools is the exclusion of predators such as fish (Willson and Hopkins, 2013; Hazell et al., 2004; Porej and Hetherington, 2005). Ecological trade-offs such as water bodies with longer hydroperiods but predator presence are important occurrences to recognize when habitat changes because there will be different species compositions in habitats with different hydroperiods. *Rana catesbeianus* are more likely to be found in habitat with longer hydroperiods than a species like *Hyla chrysoscelis*, which has a shorter larval period. Cromer et al. (2002) found that some disturbed habitat including skidder-ruts are known to hold water year round which provides habitat for aquatic amphibian species such as Ranid frog larvae. He also found that skid trails supported more species abundances and richness than natural wetland sites. Furthermore, species composition differs within different hydroperiod lengths. Shorter-hydroperiod wetlands such as road-rut pools are likely to support species absent in longer-hydroperiod wetlands. Habitat quality can be accessed by observing species richness or number of different species found in a water body. Species richness did not have a relationship with wetland size but richness does positively correlate with hydroperiod (Snodgrass et al., 2000). Smaller wetland habitats, such as ephemeral pools, are equally important as larger wetlands because ephemeral wetlands contain species unique to the habitat. Educated management strategies can be created by observing species presence and recording the environmental conditions associated with successful wetland communities. Gorman et al. (2009) suggest employing growing season prescribed fires as a management tactic to encourage growth of herbaceous vegetation and reduced canopy cover in habitat known to the endangered Flatwoods Salamander. Further investigation is needed to determine the long term effects of wetland destruction and alteration and how this affects amphibians.

It is still not completely understood how well amphibians use urban water habitats. Brand and Snodgrass (2010) carried out a study focusing on pond-breeding amphibians' use of stormwater ponds and other available wetlands in suburban and forested areas. The results of Brand and Snodgrass (2010) contrasted the results of prior studies, leading to the promotion of conserving anthropogenic wetlands for pond-breeding amphibians. Additionally, hydroperiods of natural wetlands were found to be shorter than artificial habitats, making them less suitable as they did not allow enough time for amphibians to metamorphose successfully. Delis et al. (1996) found higher species abundance for select species in stormwater ponds. Stormwater ponds in urbanized areas provided suitable habitat while sustaining species diversity and richness for amphibians in western Washington (Ostergaard et al., 2008). If urban, created habitats, such as stormwater ponds are used successfully by amphibians, then other human-made sources should be examined to determine rates of reproductive success. Further studies are needed to investigate the suitability of urban habitat.

A variety of human-made habitats are used by amphibians including road-rut pools. Road-rut pools are indentions in the ground that are filled with ground or rain water. Road-ruts are created by trucks, ATVs or any other vehicle capable of causing "ruts." During the current study, ruts in unpaved roads were the focus; such roads as those used for logging, gas wells, or trail access. Few studies have examined the use of road-rut pools or roadside ditches by amphibians but are often mentioned in the literature (Zhen-xing et al., 2010). An inventory by the National Parks Service at Petersburg battlefield determined that small depressions in the landscape such as road-rut pools are considered one of the most valuable habitat types for amphibians (Mitchell, 2007). The 1993 study by Adam and Lacki (1993) concluded that road-rut pools were observed as important structures for amphibians in Daniel Boone National Forest. Surface area and depth were particularly important variables associated with amphibians' selection of road-ruts (Adam and Lacki, 1993). More studies are needed to sufficiently interpret how this type of habitat is used by amphibians and whether it aids to amphibian survival.

The goal of my study was to collect baseline data and determine the use of amphibians in road-rut pools using Northern Green Frogs (*Rana clamitans*) as the model species. My objectives were to 1) ascertain what species use road-rut pools, 2) observe what environmental conditions regulate the activities of these species and 3) anecdotally have an understanding of amphibians' purpose in road-ruts.

Amphibians are in decline due to various reasons including pollutants, habitat alteration, and road creation and urbanization. It is essential to study amphibian responses to changing environmental conditions and habitat encroachment. Observing species presence also facilitates the understanding of amphibian relationships to these factors. In order to evaluate decisions that influence herpetofauna and their environment, biologists must consider the species' life histories and various strategies for conservation.

METHODS

Study Sites

My study contained two site locations. The first site was located at Chief Cornstalk Wildlife Management Area (WMA). Chief Cornstock WMA is located in Mason County, West Virginia approximately 67 kilometers northeast of Huntington, WV. Chief Cornstalk consisted of 4,763 hectares of oak-hickory hardwood forest. Recreation in the WMA consisted of lake fishing, a shooting range, and permitted hunting. The road-rut pools within Chief Cornstalk were located on County Route 42/3. The two road-ruts observed for this study were named Chief Cornstalk Road-rut One (CCRR1) and Chief Cornstalk Road-rut Two (CCRR2). The geographic coordinates for CCRR1 were N38°43'8.78" W82° 2'26.46" and for CCRR2 were N38°43'8.02" W82° 2'25.10". Both Chief Cornstalk sites had an elevation of 291 meters.

The second study site was located at Beech Fork Wildlife Management Area (WMA) in Wayne County, WV, with an elevation of 193 meters. The WMA contained 1,233 hectares of oak, hickory, and pine forest. Beech Fork WMA had a campground as well as recreation areas for baseball and a swimming pool. My study site was on the campground side of the lake off Hughes Branch Rd/Fisher Bowen Branch Rd/Co Rd 17. The one road-rut studied at Beech Fork was close to Beech Fork Creek. The geographic coordinates for the Beech Fork road-rut (BFRR) were N 38°01.518’ W 081°30.484’. The Beech Fork Road-rut was near a human-made pond. The pond was observed full of water during the preceding winter (2011/2012) but had been drained during the 2012 summer of this study.

Collection Methods

Road-rut pools were chosen based on the following variables. Pools needed to have a longer hydroperiod than other road-ruts in order to study them for the multiple months. Sampling was preformed weekly from mid-May through October, 2012. Ponds needed to be located close enough for me to collect data each week.

Pitfall traps and drift fences were the primary methods of collection. Both methods are commonly used to inventory amphibian populations of a given area (Corn and Bury, 1990; Heyer et al., 1994). I encircled road-rut pools with drift fences and pitfall traps (Figure 1B). Pitfalls were constructed by placing 18 liter buckets in the ground to the point where the top of the bucket is level with the surface of the ground and spaced approximately 10 meters away from the next pitfall. Pitfalls were located on both sides of the fence to determine directionality of amphibian movement. The drift fence was constructed of silt fencing measuring 60 centimeters high at the lowest points and was secured with wooden stakes. The bottom of the silt fencing was tucked into the ground (Figure 2). Fencing acted as a barrier to the animals and led them to the pitfalls in the ground where they were collected weekly. Pitfalls were never left opened and unchecked longer than a day. Pitfalls were covered with bucket lids when not in use to prevent accidental trapping between sampling. Traps were opened on the initial trip to the study site and checked the following day for amphibians. The species, sex, stage class, and morphological data was recorded when individuals were collected. Individuals were then released on the opposite side of the fence in the direction they were heading.

Other methods of collection during the course of the study include dipnets and aquatic funnel traps. Dipnetting with a D-shaped net was performed every other month over the course of the summer at each road-rut location for a total of 3 times. Dipnet sampling was not used every week as to minimize habitat disturbance because dipnetting disrupts the vegetation and increases the turbidity in the water. In order to determine the number of dipnet sweeps, the surface area of the road-rut was approximated by using the equation of an ellipse. An ellipse was chosen as it most closely represented the shape of the road-ruts in this study. The area of an ellipse is determined by measuring the two different radii lengths multiplied by Pi (Figure 3). Once the surface area was determined, a number of dipnet sweeps was decided equal to the square meters of each given road-rut. For instance, if the road-rut's area was approximately four square meters, four dipnet sweeps were conducted. If a surface area was less than halfway to the next integer, then the number of dipnet sweeps was rounded up.

Larval stages were determined when larvae were collected. The Gosner and Harrison larval staging charts were used to determine life stages; The Harrison chart was used for larval salamanders and the Gosner chart for larval anurans.

Additionally, aquatic funnel traps were used as a method of capture. Two funnel traps were deployed on the same schedule as the pitfall traps. Traps were set and checked the following day. Detected species were documented and covariates were taken before the animal was released. Funnel traps were removed when the pitfall traps were closed.

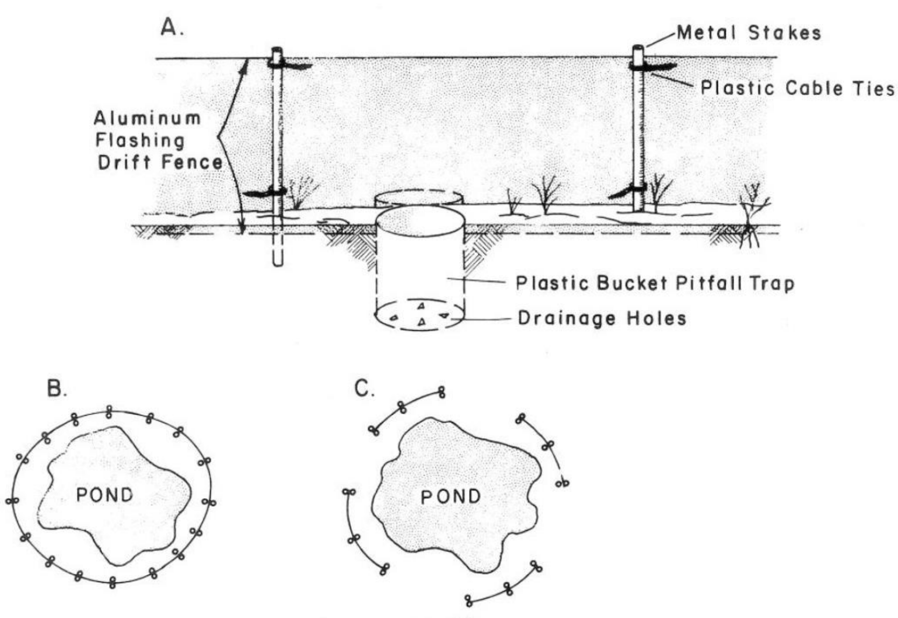


Figure 1. Figure procured from Gibbons and Semlitsch, 1981

A) Basic layout of a drift fence and pitfall trap. B) Drift fence encircling breeding site. C) Partial drift fence.

Figure 2. Drift fence encircling CCRR1

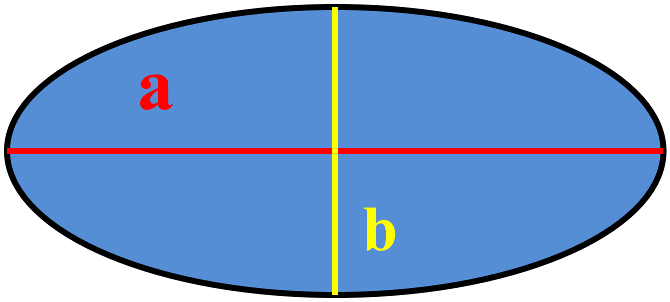


Figure 3. Area of an ellipse = ∏(a)(b)

I collected various other environmental data during each sampling period. Digital Max/Min thermohygrometers were used to measure air temperature and relative humidity. Water temperature of each pool was recorded with metal encased thermometers. I recorded water at each pool using an Oakton handheld pH meter. Water depth was monitored from May through October. I measured water depths at multiple points in the pool with a meter stick recording the maximum and average depth. The longest length and width of the pool were measured. The HACH test kit model OX-2P was used to determine dissolved oxygen. The Ammonia Mid-Range HACH test kit model NI-8 was used to record ammonia level in the water.

Morphometrics

I recorded snout-urostyle length (SUL) and tail length (TL) for all captured salamanders. For captured frogs and toads, I recorded the following measurements: snout-vent length (SVL), eye diameter (ED), tympanum diameter (TD), thigh length (THL), tibia length (TIL), and foot length (FL). A dial caliper was used to make these measurements.

RESULTS

Environmental Data

The average water depth of each road-rut pool fluctuated weekly over the course of the 2012 field season (Figure 4). The only road-rut pool that completely dried during my study was CCRR1. The maximum water depth (Figure 5) was 18 cm at Beech Fork (BFRR), 28 cm at Chief Cornstalk One (CCRR1), and 31 cm at Chief Cornstalk Two (CCRR2).

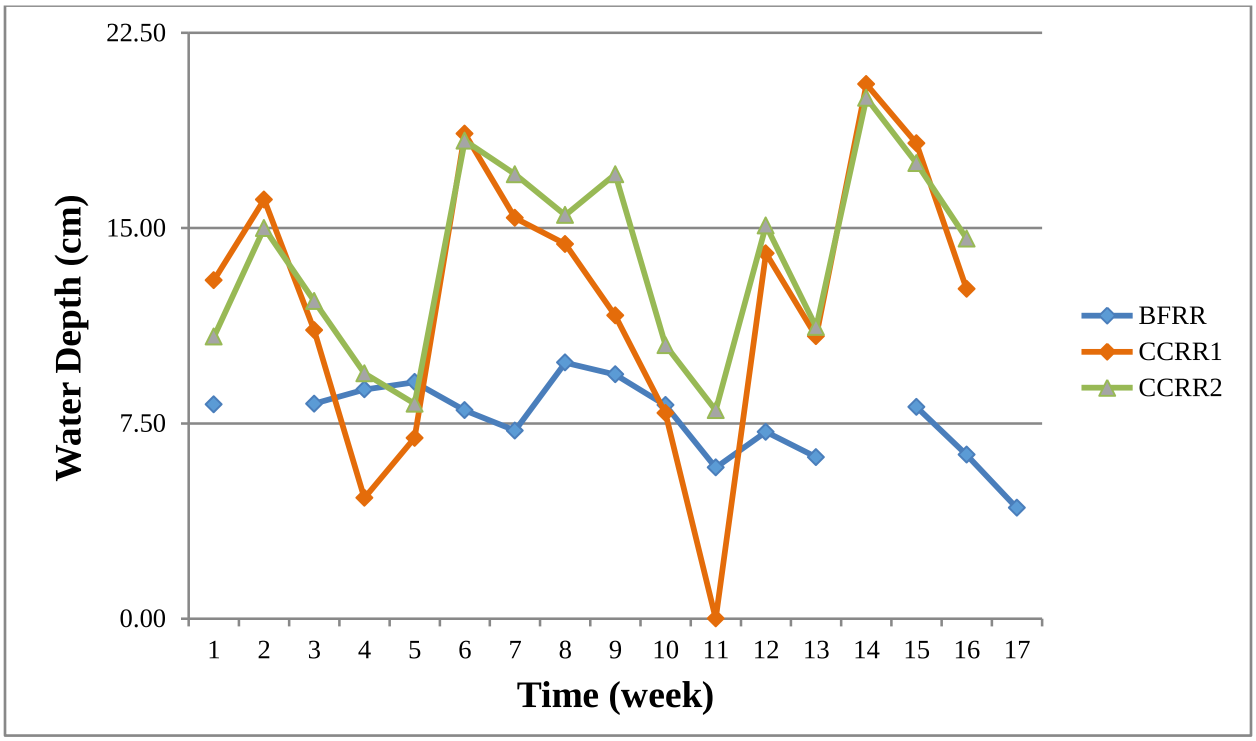


Figure 4. Average water depths of road-rut pools over time

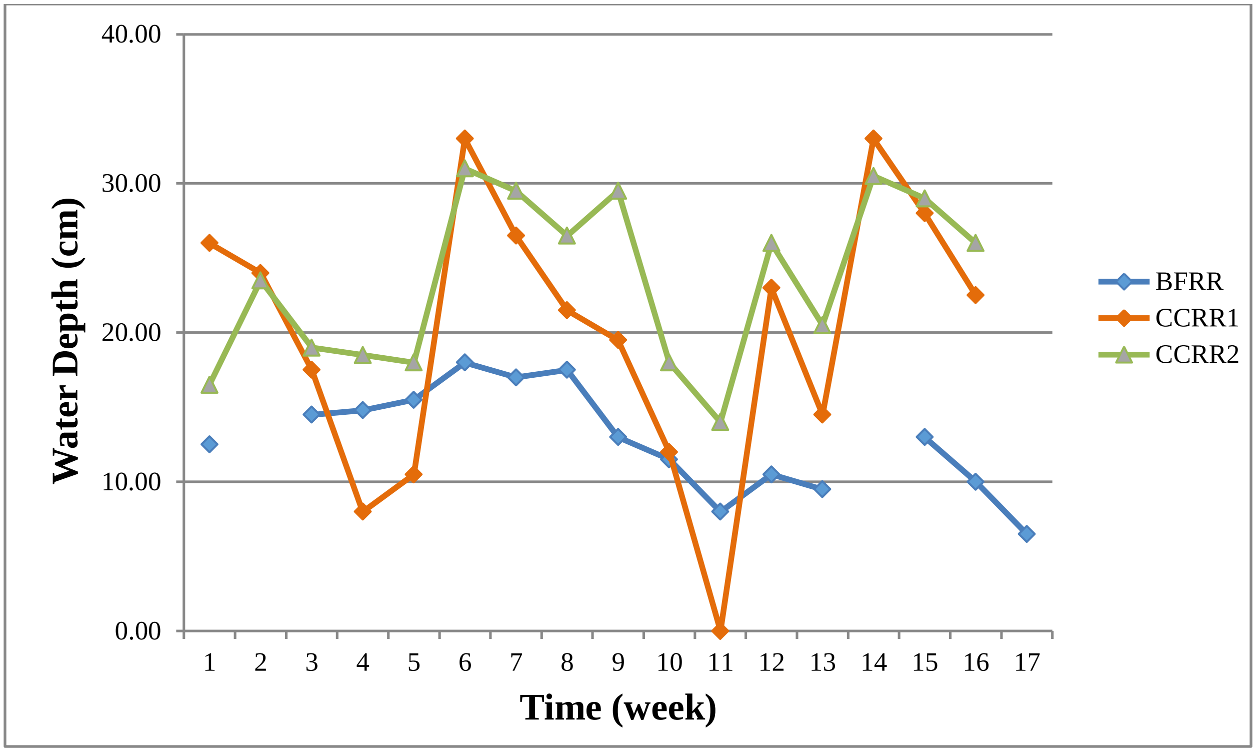


Figure 5. Maximum water depths of road-rut pools over time

Environmental parameters collected at CCRR1, CCRR2, and BFRR each week showed no significant fluctuations (Table 1). Data collected includes pH of water, air temperature, relative humidity, dissolved oxygen (DO) and ammonia.

Table 1. Environmental parameters recorded at each road-rut pool study site

Date	Site	PH	Air Temp (C°)	Relative humidity (%)	DO (mg/L)	Ammonia (mg/L)
6/15/2012	BFRR	8	24.3	78.50	.	.
6/28/2012	BFRR	7.8	25.4	55.50	.	.
7/6/2012	BFRR	7.5	31.0	75.50	.	.
7/12/2012	BFRR	7.5	28.3	63.50	.	.
7/22/2012	BFRR	7.5	32.1	67.50	9.0	0.2
8/3/2012	BFRR	7.4	30.2	78.50	7.0	0.2
8/10/2012	BFRR	7.5	38.8	44.50	10.0	0.2

8/16/2012	BFRR	7.7	17.5	91.50	5.0	0.2
8/22/2012	BFRR	7.7	22.4	82.00	6.0	0.2
8/31/2012	BFRR	7.7	22.6	87.00	2.2	0.4
9/7/2012	BFRR	7.3	27.4	80.50	0.8	0.6
9/15/2012	BFRR	7.4	22.5	32.00	4.0	0.4
10/5/2012	BFRR	7.2	29.8	40.50	1.6	0.4
10/12/2012	BFRR	7.5	10.2	70.00	6.0	0.2
10/25/2012	BFRR	7.4	26.5	41.50	0.8	0.4
5/19/2012	CCRR1	.	.	.	.	.
5/20/2012	CCRR1	7	.	.	.	.
6/14/2012	CCRR1	7.9	54.5	48.50	.	.
6/21/2012	CCRR1	8	51.7	21.50	.	.
6/27/2012	CCRR1	11.3	43.8	12.50	.	.
6/27/2012	CCRR1	9.3	38.0	21.00	.	.
7/3/2012	CCRR1	8.8	40.2	39.50	.	.
7/14/2012	CCRR1	7.6	33.3	63.00	.	.
7/21/2012	CCRR1	6.8	26.2	92.00	5.0	0.2
8/9/2012	CCRR1	7.6	35.1	53.00	9.0	0.2
8/15/2012	CCRR1	7.6	32.0	65.00	9.0	0.2
8/20/2012	CCRR1	9	27.9	73.00	13.0	0.2
8/30/2012	CCRR1	.	26.7	60.00	.	.
9/8/2012	CCRR1	6.8	18.8	95.00	2.4	0.4
9/14/2012	CCRR1	7.1	31.1	38.00	10.0	0.2
9/20/2012	CCRR1	6.9	17.5	60.00	6.0	0.4
10/4/2012	CCRR1	7.5	29.3	42.00	9.0	0.2
10/11/2012	CCRR1	7.5	13.0	56.50	10.0	0.2

8/3/2012	CCRR1	7	29.0	57.50	6.0	0.2
5/19/2012	CCRR2	.	.	.	.	.
5/20/2012	CCRR2	7	.	.	.	.
6/14/2012	CCRR2	10.8	26.3	71.00	.	.
6/21/2012	CCRR2	10.5	36.1	43.00	.	.
7/3/2012	CCRR2	9.9	36.0	57.00	.	.
7/14/2012	CCRR2	9.5	37.3	57.50	.	.
7/21/2012	CCRR2	6.8	31.9	71.00	4.0	0.2
8/3/2012	CCRR2	9.2	39.6	41.50	16.0	0.2
8/9/2012	CCRR2	.	34.8	57.50	11.0	0.2
8/15/2012	CCRR2	6.9	25.2	73.00	3.0	0.4
8/20/2012	CCRR2	7.2	31.9	48.00	8.0	0.2
8/30/2012	CCRR2	9	28.5	52.50	13.0	0.4
9/8/2012	CCRR2	6.8	19.4	94.50	4.0	0.2
9/14/2012	CCRR2	7.1	29.8	44.50	7.0	0.2
9/20/2012	CCRR2	6.8	15.0	63.00	4.0	0.2
10/4/2012	CCRR2	7.6	27.6	44.50	10.0	0.4
10/11/2012	CCRR2	7.4	15.7	59.50	9.0	0.1

Species Composition

Individuals mature enough to identify sex by secondary sex characteristics in all road-rut pools were recorded (Table 2). No male *Rana catesbeianus* were detected in any of the road-rut pools. The ratio closest to 1:1 was *Rana clamitans* with a 5:6 female to male ratio.

Table 2. Amphibian sex ratios in road-rut pools

Rana clamitans		*Notophthalmus v. viridescens*		*Rana catesbeianus*		*Hyla chrysoscelis*	
F	M	F	M	F	M	F	M
5	6	15	5	13	0	4	2

Species richness for BFRR was 4, for CCRR1 was 6, and for CCRR2 was 5 (Table 3). Beech Fork Road-rut (BFRR) contained the following species: *Hyla chrysoscelis* (Cope's Gray Treefrog), *Rana clamitans* (Northern Green Frog), *Ambystoma maculatum* (Spotted Salamander), *Rana sylvaticus* (Wood Frog); Chief Cornstalk Road-rut One (CCRR1) contained: *Rana catesbeianus* (American Bullfrog), *Notophthalmus v. viridescens* (Red-spotted Newt), *Hyla chrysoscelis* (Cope's Gray Treefrog), *Rana clamitans* (Northern Green Frog), *Pseudacris c. crucifer* (Spring Peeper), *Anaxyrus americanus* (American Toad); and Chief Cornstalk Road-rut Two (CCRR2) contained: *Rana catesbeianus* (American Bullfrog), *Notophthalmus v. viridescens* (Red-spotted Newt), *Hyla chrysoscelis* (Cope's Gray Treefrog), *Rana clamitans* (Northern Green Frog), *Ambystoma jeffersonianum* (Jefferson Salamander).

Table 3. Species Richness of Road-rut Pools

Site	Species Richness
BFRR	4
CCRR1	6
CCRR2	5

Amphibian species caught at each study site were recorded according to life cycle stages (larval (L), juvenile (J), and adult (A)) and displayed in Table 4. The most frequently detected larva at any of the road-rut pools was *H. chrysoscelis* (N=1,159). Of the 1,159 *H. chrysoscelis* larvae caught, 843 were at CCRR1 and 316 at CCRR2. No *H. chrysoscelis* larvae were caught at

BFRR but 1 juvenile was found. The next most frequent larvae caught were 221 *R. sylvaticus* all of which were captured at BFRR. The third most detected larvae were *R. clamitans.* All *R. clamitans* larva were caught at Chief Cornstalk with 73 found in CCRR2 and 4 found in CCRR1.

Juvenile individuals consisted of newly metamorphed frogs to young frogs without secondary sex characteristics. The most abundant juveniles were *R. clamitans* (N=38). Of these, three were found at BFRR, 15 at CCRR1, and 20 found at CCRR2. There were 24 juvenile *R. catesbeianus* caught; 19 at CCRR1 and five at CCRR2. All 12 juvenile *R. sylvaticus* were caught at BFRR. Fewer adults were detected. Total adults found consisted of: 13 *R. catesbeianus*, 12 *Notophthalmus v. viridescens*, seven H. *chrysoscelis*, 12 *R. clamitans*, and one *Ambystoma jeffersonianum*.

Table 4. Amphibian species caught at each study site by life cycle stages
L stands for larvae, J for juveniles and A for adults.

Site	*Rana catesbeianus*			*Notophthalmus v. viridescens*			*Hyla chrysoscelis*		
	L	J	A	L	J	A	L	J	A
BFRR	0	0	0	0	0	0	0	1	0
CCRR1	0	19	10	7	0	4	843	0	6
CCRR2	0	5	3	12	0	8	316	0	1
Totals	0	24	13	19	0	12	1159	1	7
	Rana clamitans			*Pseudacris c. crucifer*			*Anaxyrus americanus*		
	L	J	A	L	J	A	L	J	A
BFRR	0	3	3	0	0	0	0	0	0
CCRR1	4	15	6	1	1	0	0	3	0
CCRR2	73	20	3	0	0	0	0	0	0
Totals	77	38	12	1	1	0	0	3	0
	Ambystoma maculatum			*Rana sylvatica*			*Ambystoma jeffersonianum*		
	L	J	A	L	J	A	L	J	A
BFRR	27	0	0	221	12	0	0	0	0
CCRR1	0	0	0	0	0	0	0	0	0
CCRR2	0	0	0	0	0	0	1	0	1
Totals	27	0	0	221	12	0	1	0	1

Larval Stages

Larvae were put into developmental stages according to the Gosner and Harrison staging charts. *Hyla chrysoscelis*, *Rana sylvaticus*, and *Rana clamitans* had the highest detection success. The most abundant stage for *H. chrysoscelis* was 25 (n=102). Stage 25 was also the most abundant stage for *Rana sylvaticus* (n=221) and *R. clamitans* (n=77). Figures 6, 7, and 8 show the distribution of the Gosner stages among *Hyla chrysoscelis*, *R. sylvaticus*, and *R. clamitans*.

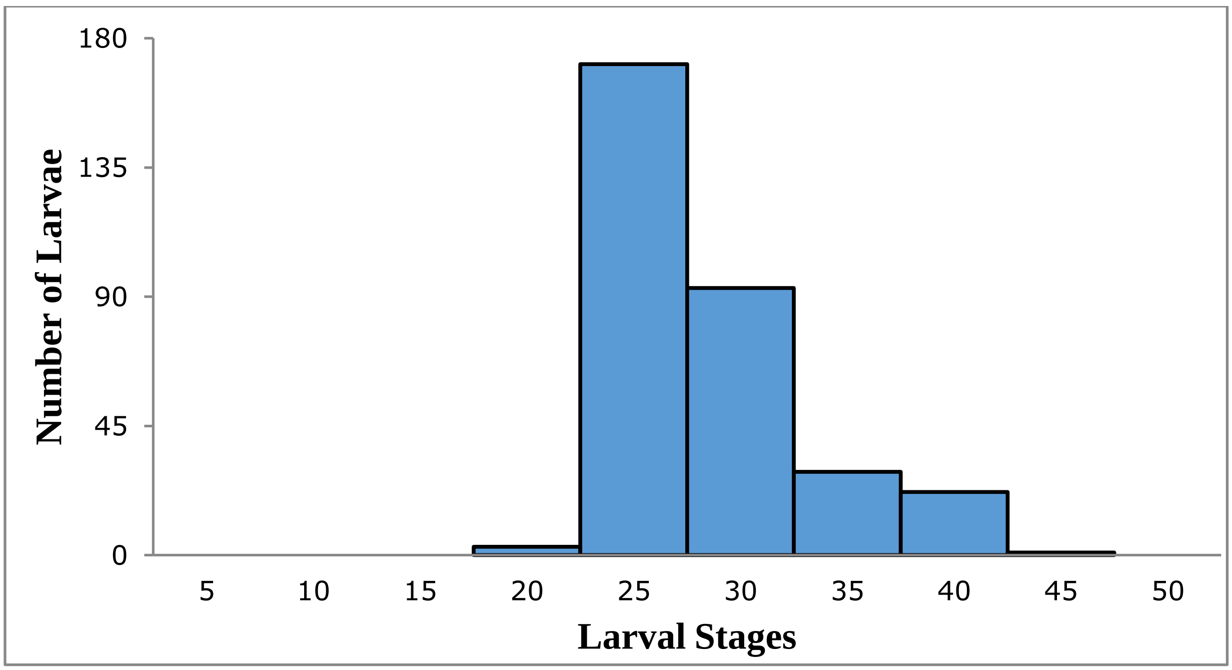


Figure 6. Histogram of *Hyla chrysoscelis* Gosner larval stages

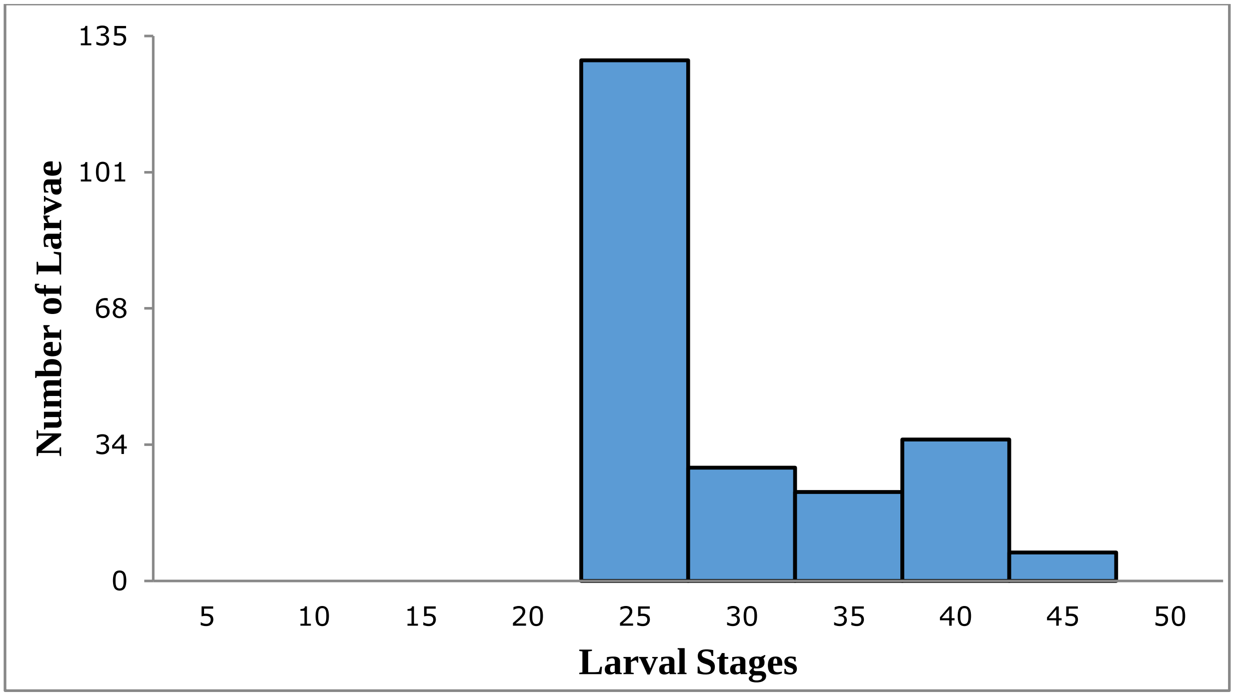


Figure 7. Histogram of *Rana sylvatica* Gosner larval stages

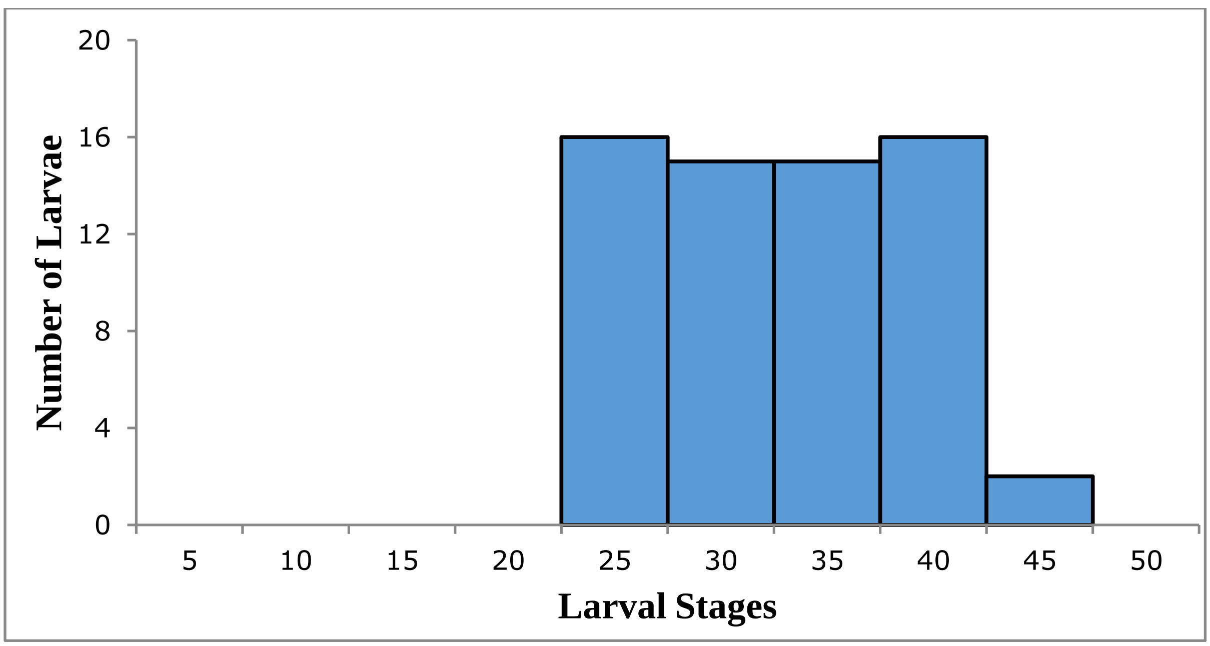
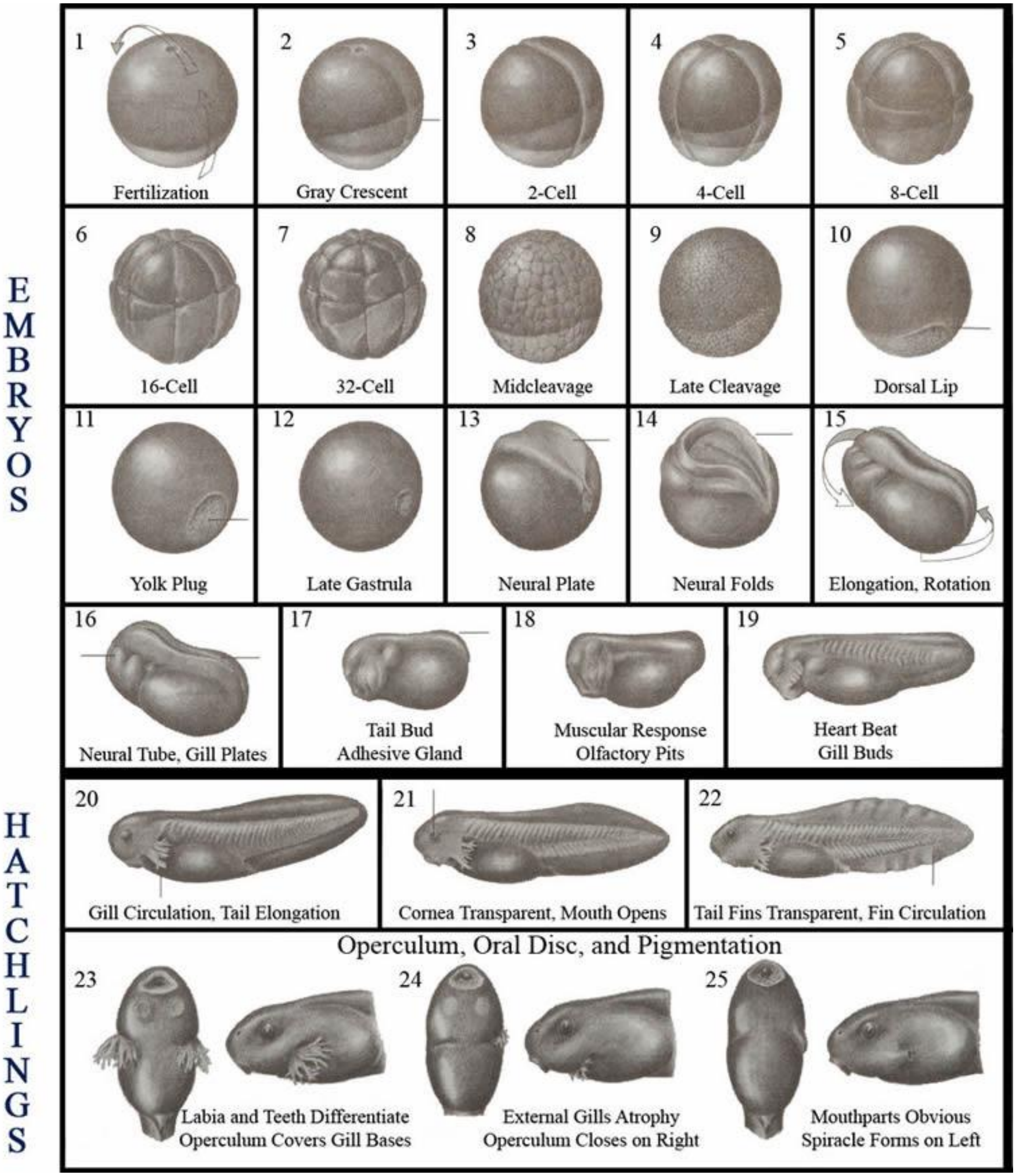


Figure 8. Histogram of *Rana clamitans* Gosner larval stages


EMBRYOS
HATCHLINGS
1 Fertilization
2 Gray Crescent
3 2-Cell
4 4-Cell
5 8-Cell
6 16-Cell
7 32-Cell
8 Midcleavage
9 Late Cleavage
10 Dorsal Lip
11 Yolk Plug
12 Late Gastrula
13 Neural Plate
14 Neural Folds
15 Elongation, Rotation
16 Neural Tube, Gill Plates
17 Tail Bud
Adhesive Gland
18 Muscular Response
Olfactory Pits
19 Heart Beat
Gill Buds
20 Gill Circulation, Tail Elongation
21 Cornea Transparent, Mouth Opens
22 Tail Fins Transparent, Fin Circulation
Operculum, Oral Disc, and Pigmentation
23 Labia and Teeth Differentiate
Operculum Covers Gill Bases
24 External Gills Atrophy
Operculum Closes on Right
25 Mouthparts Obvious
Spiracle Forms on Left

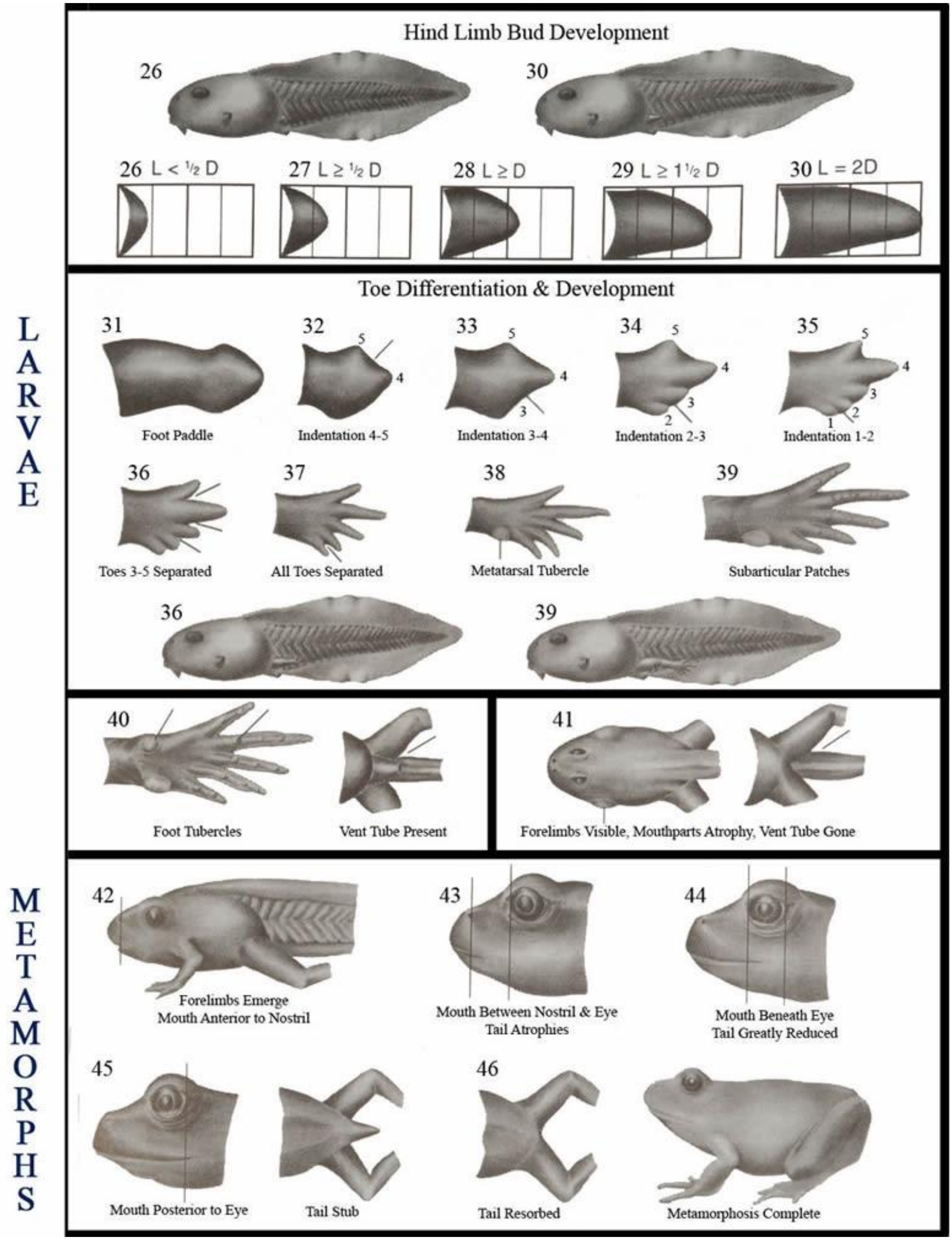


Figure 9. Gosner anuran larval stages key

Captures by Season

Collection did not begin until late spring as the target species, *R. clamitans*, does not breed until approximately May. *Rana clamitans* was seperated and categorized by cohort (larvae, juveniles, and adults) (Figure 10). The highest count of all three cohorts of *R. clamitans* peaked during the summer. The number of captured specimens decreased as age of specimen increased (e.g. larvae had the highest capture success and adults had the lowest incidence of capture).

The phenology of each species caught was collected (Figure 11). *Hyla chrysoscelis* and *Rana sylvaticus* had the highest capture success compared to other documented species. *Rana clamitans* was the next highest capture (Figure 12) peaking during the summer months of July and August.

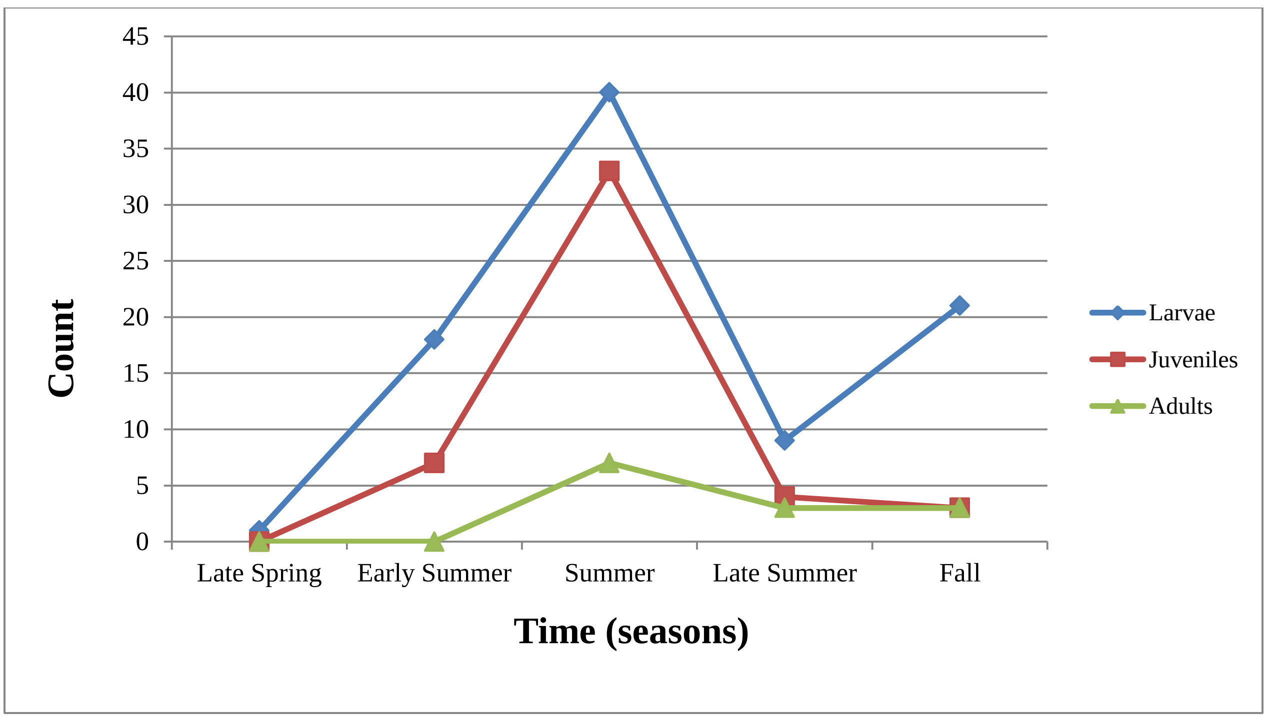


Figure 10. *Rana clamitans* divided by age cohort over time by seasons

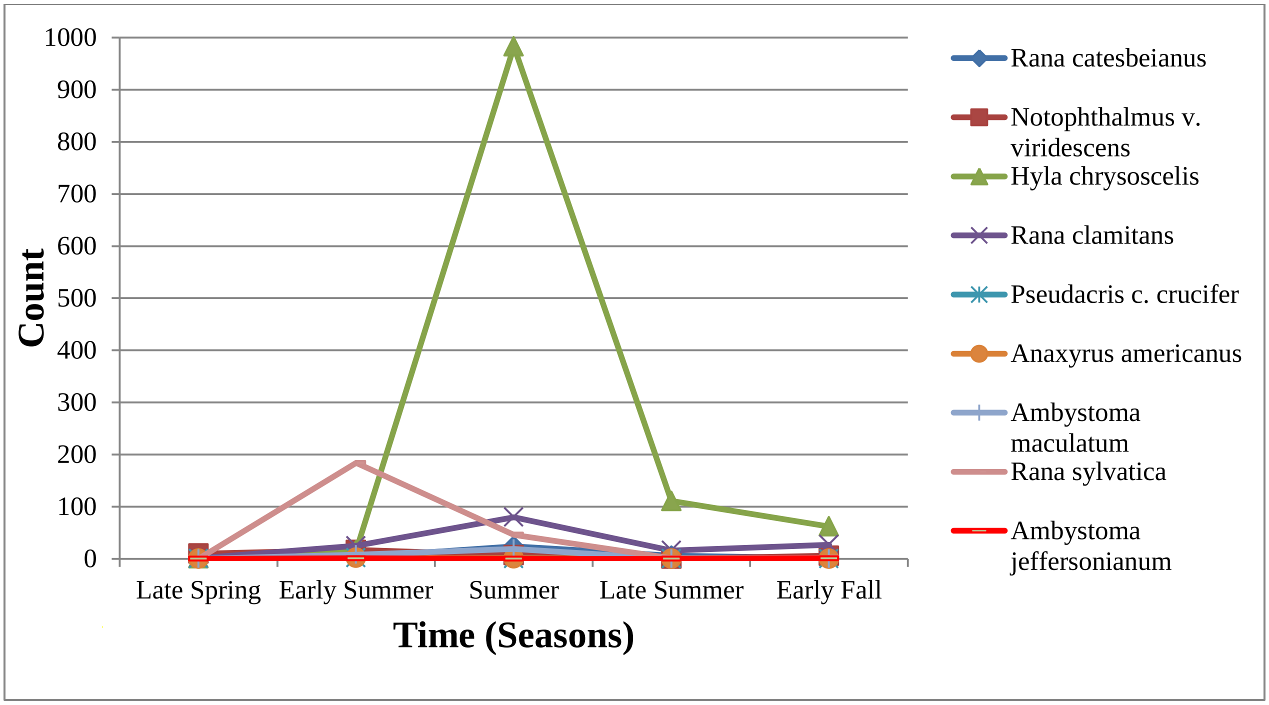


Figure 11. The total number of species found in the road-rut pools over time divided into seasons

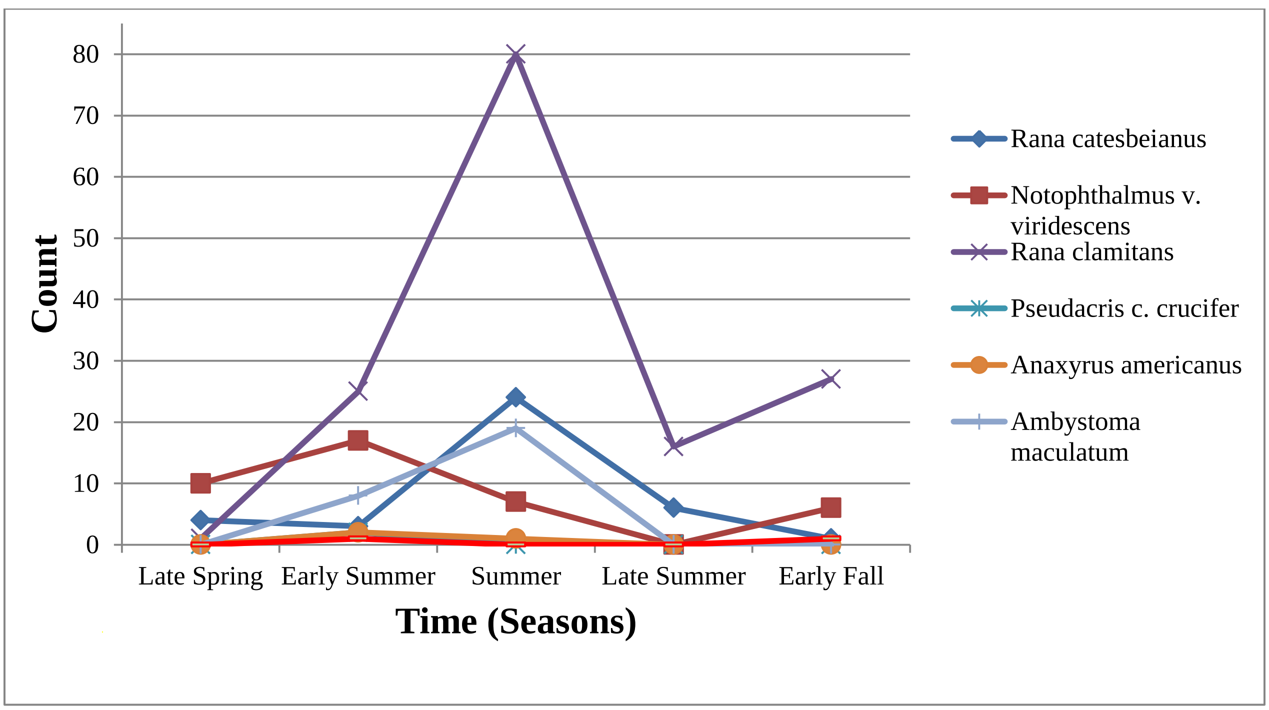


Figure 12. The total count of species observed (omitting *H. chrysoscelis* and *R. sylvatica*) in the road-rut pools over time divided into seasons

Morphometrics

Morphological measurements were recorded for juveniles and adults. The morphological measurements of *R. clamitans* (Table 5) includes: mean, median, mode, standard deviation, variance, and range. Each measurement data has a high variance and has a positive skew to the left which conveys the mean is larger than the median.

Table 5: Descriptive statistics of *Rana clamitans* morphometrics
Mass was measured in grams. All other morphological measurements were millimeters. The following notation: SUL=snout-urostyle length, ED=eye diameter, THL=thigh length, TIL=tibia length, and FL=foot length

MASS (g)	
Mean: 11.60	Std. Deviation: 19.09
Median: 2.00	Variance: 364.34
Mode: 1.00	Range: 84.10
SUL (mm)	
Mean: 39.90	Std. Deviation: 20.52
Median: 29.50	Variance: 420.91
Mode: 29.10	Range: 80.50
ED (mm)	
Mean: 4.65	Std. Deviation: 2.01
Median: 3.90	Variance: 4.03
Mode: 3.00	Range: 7.60
TD (mm)	
Mean: 3.89	Std. Deviation: 2.50
Median: 2.70	Variance: 6.26
Mode: 2.30	Range: 9.80
THL (mm)	
Mean: 19.24	Std. Deviation: 9.83

Median: 14.40	Variance: 96.62
Mode: 12.70	Range: 35.90
TIL (mm)	
Mean: 19.48	Std. Deviation: 10.80
Median: 14.45	Variance: 116.61
Mode: 10.30	Range: 38.60
FL (mm)	
Mean: 28.00	Std. Deviation: 16.06
Median: 20.50	Variance: 257.81
Mode: 15.30	Range: 62.60

DISCUSSION

Road-rut pools are indentions made in the ground by various vehicles that are filled with water. I studied road-rut pools as they are common types of human-made habitats commonly used by amphibians and their role in amphibian survivorship is not well understood (Mitchell, 2007; Adam and Lacki, 1993). The objectives of this study were to ascertain what species use road-rut pools, determine what environmental conditions regulate the presence of amphibians in road-ruts, and to better understand amphibians' use of road-rut pools in West Virginia. An important question to ask when considering the utility of road rut pools in West Virginia is whether they serve as a "sink" or a "source." A "sink" has a short hydroperiod and water will disappear before the larvae can fully develop resulting in mortality. A "source" has a longer hydroperiod and allows successful survivorship and eventual recruitment (Pulliam, 1988; Willson and Hopkins, 2013). The objectives of this study were addressed; however there was not enough data to make any statistically significant conclusions. This study does allow for several anecdotal observations to be made which are important for future studies.

The most abundant life stage detected was larvae, followed by juveniles, and the least abundant life stage detected was adults. This large production of offspring that decreases number with each increasing age cohort is reflective of amphibians' R-selected reproductive strategy. R-selected species produce a great number of offspring quickly to ensure some survive to adulthood. Larvae of *Hyla chrysoscelis*, *Rana sylvaticus*, and *Rana clamitans* were abundantly observed indicating that these species use road-ruts for breeding. Although the hydroperiods of the road-rut pools used in this study did not support all species of amphibian reproduction, they served other purposes. Road rut pools, regardless of the length of hydroperiod, provided a water source which prevented desiccation for migration to breeding ponds (Martof, 1953; Muths, 2003; Semlitsch, 1981). Road-rut pools serve as a microhabitat to feed and rest in preparation of breeding events in larger aquatic habitats nearby. Road-rut pools are absent of fish and other predators, which increases the chance of survival among amphibians (Kats et al., 1988; Walston and Mullin, 2007).

Additionally, road-rut pools may also serve as a less competitive habitat than larger breeding ponds. The temporal arrivals of breeding events among interspecifics affect growth and metamorphose time among species such as Hylids (Lawler and Morin, 1993). Competition affects survival, species evenness, and the size the frog is when metamorphosis occurs (Purrenhage and Boone, 2009). I believe competition is the reason *R. clamitans* juveniles dominated the road-rut pools studied and that it is a contributing factor for the success of the species in West Virginia. I think juvenile *Rana clamitans* use road-ruts to avoid competition of other bigger, and more established *Rana clamitans.*

Observing species richness is an efficient approach to determine the composition in road-rut pools. Five prominent species observed in this study were: *Rana clamitans, Rana catesbeianus, Hyla chrysoscelis, Rana sylvaticus,* and *Notophthalmus v. viridescens.* Although I observed numerous Hylid egg masses, adults were rarely caught in pitfall traps. When adults were captured it was during amplexus (Willson and Gibbons, 2010). Pitfall traps do not serve as a good capture method for tree frogs because they can climb out of the traps evading detection. For future studies, I recommend dipnet surveys, funnel traps and visual encounter surveys (VES). I believe these methods would be more efficient and economic when sampling amphibians in road-rut pools. I believe these methodologies will increase the statistical power of future studies on road rut pools. Dipnets and funnel traps are much easier to transport and require less of a time commitment to install than pitfall trap surveys. Funnel traps are a more passive sampling technique and are less destructive to the amphibian’s habitat. Egg mass surveys would also provide a better outlook on what species is reproducing in road-ruts. Using a variety of methodologies to capture all life stages would offer a clearer understanding of how amphibians use road-rut pools. Also, more study sites could be included using dipnets, funnel traps, and VES instead of pitfall traps increasing the statistical power of future studies.

CHAPTER 2

CAPTURE SUCCESS OF *RANA CLAMITANS* WITH METHOD AS A PREDICTOR

INTRODUCTION

Inventory and monitoring of wildlife is the foundation for other studies and analyses. Species richness can be a revealing variable in different habitats. Inventory and monitoring data are often overlooked and are not considered for publication (Heyer et al., 1994) but data are essential for scientists and managers, especially when making management and conservation decisions.

Presence absence data is a commonly used data tool in wildlife ecology (Gu and Swihart, 2004). In order to detect all the species in a community several sampling techniques must be implemented (Hutchens and DePerno, 2009). Studying different sampling methods in attempt to determine which yields the highest detection can make future studies and sampling efforts more efficient.

Accurate sampling techniques are essential to managing amphibian success. It is important to choose capture methodology that yields accurate results. In ecology, detection is rarely constant due to many parameters and this is referred to as imperfect detection (Kellner and Swihart, 2014). Species may be present but not detected by the observer which results in false negatives. Knowing what the best sampling methods for the species of focus will help offer the most accurate picture of abundance, population distribution, and dynamics; this aids overseers to make better management decisions.

Several different species are found in road-rut pools but the Green Frog (*Rana clamitans*) is an anuran species most commonly seen in road-rut pools in West Virginia (Thomas Pauley, personal conversation, December 2018). *Rana clamitans* starts breeding in April and continues throughout the summer. Eggs are generally laid in permanent bodies of water and once the tadpoles metamorphose into froglets they disperse to temporary pools.

Sampling techniques can vary according to the space, time, and money available. Depending on the target species' life history traits some sampling methods will be more suited than others. Passive sampling techniques such as pitfall traps and funnel traps lead the animal into the trap space where they cannot escape. Dipnetting and visual encounter surveys are active sampling techniques because the study organism is captured by the investigator directly. There were three different sampling methods used to collect both adults and larvae *R. clamitans* to determine which method resulted in the highest capture.

METHODS

The primary habitat in this study was road-rut pools so collection methods were chosen to best fit small water types closest to road-rut pools. Road-rut pools are indentions in the ground from vehicles and ATVs that are then filled up with rain water. This study occurred at Chief Cornstalk Wildlife Management Area and Beech Fork Wildlife Management Area during the summer months of 2012.

The life history of *R. clamitans* was also documented. For this study, three standard techniques were used to collect amphibians, specifically *R. clamitans*, including drift fences with pitfall traps, aquatic funnel traps, and dipnetting. The pitfall traps were targeted to collect the froglets and adults. The funnel traps and dipnetting were used to catch both adults and larvae.

Three collecting methods were used to detect *Rana clamitans* in selected road-rut pools: pitfall traps-drift fence arrays, aquatic funnel traps, and dipnetting. Dipnetting was performed three times every other month over the course of the summer at each road-rut location from May to October. This method was not used every week in attempt to minimize disturbance. Two funnel traps were placed in each road-rut at the same time the pitfalls were opened. The following day the funnel traps were removed when the pitfalls were closed.

I used two different analyses for adult and larvae data. First, I used a conditional logistic regression to examine adult capture probability as a function of method. A binary response was used to model the capture probability. I stratified site to account for the lack of independence among study sites. Next, I used a conditional logistic regression to examine tadpole capture probability as a function of method excluding pitfall traps. Pitfall traps were not configured in the tests for tadpoles because it was not a method targeting that cohort.

Secondly, I used the glimmix procedure in SAS 9.2 using count data of both adult amphibians and larvae. I analyzed capture success with method as the predictor. This analysis was to examine if one capture method caught more *R. clamitans* than another method. All analyses were achieved using SAS.

RESULTS

During the study season of 2012 48 larvae and 76 adult *R. clamitans* were observed at two different wildlife management areas with three different trapping methods. Method significantly affected adult capture probability (X^2 = 13.20, df = 2, P = 0.0014). I was more likely to capture adults in pitfalls as compared to dipnets (estimate = 1.2633 ± 0.4295, X^2 = 8.65, P = 0.0033). I failed to detect an effect of adult capture probability between funnel traps as compared to dipnets (estimate = -0.5150 ± 0.4670, X^2 = 1.2161, P = 0.2701). I failed to detect an effect of method on tadpole capture probability (X^2 = 0.048, df = 1, P = 0.8267). Adult captures differed significantly by method ($F_{2,107}$ =6.82, P = 0.0016). I failed to detect an effect of pitfalls (method 1) on adult capture probability (estimate 2.0250 ± 1.0757; t_{107} = 1.88; P = 0.0625). I failed to detect an effect of funnel traps (method 2) on adult probability (estimate 0.5965 ± 1.1161; t_{107} = 0.53; P = 0.5942). Tadpole counts did not differ by method ($F_{1,57}$ =0.21, P = 0.6496).

Table 6. Solutions for Fixed Effects

Effect	Method	Estimate	Standard Error	DF	t Value	Pr>|t|
Intercept		-2.4283	1.0967	2	-2.21	0.1572
Method	1	2.0250	1.0757	107	1.88	0.0625
Method	2	0.5965	1.1161	107	0.53	0.5942
Method	3	0	.	.	.	.

DISCUSSION

It is important to know the most effective capture methods for amphibians in order to study them accurately. Amphibians are declining worldwide (Alford and Richards, 1999; Stuart et al., 2004); therefore it is necessary to understand amphibian ecology and its interactions with the changing environment. In my study pitfall traps were the most successful method to capture adult *R. clamitans* ($P = 0.0033$). I used additional techniques including funnel traps and dipnets; however, those techniques showed no statistical significance ($P=0.2701$). Although funnel traps have been successful in detecting amphibians in previous studies (Wilson and Pearman, 2000; Fronzuto and Verrell, 2000), it may not have been successful in this study due to the decreased volume and depth of water in road-rut pools. Road-rut pools have shorter hydroperiods than larger aquatic habitats such as ponds, limiting the effectiveness of funnel traps during droughts or periods of minimal precipitation. The use of glow sticks in funnel traps has been shown to increase detection (Bennett et. al, 2012) when the water level is high enough. In this study more larval amphibians than adults were detected using funnel traps. The limited hydroperiods of road-ruts may not be able to support the growth period of larval amphibians (Snodgrass et. al, 2000; Pechmann et. al, 1989).

I used funnel traps and dipnets to detect larval *R. clamitans* as terrestrial trapping methods i.e., pitfall traps are not adequate for detection of larval life stages. There were no significant results pertaining to the best capture method for tadpoles in this study. The presence of larvae indicates what species are breeding in the road-rut pools. Often, larvae are convenient to study due to their density in one area, and are more easily accessible than adults. Although necessary for population assessments, some sampling techniques for larval amphibians are known to be damaging to habitat and larval stages. Dipnetting can disturb vegetation and increase turbidity which can alter habitat for amphibians (Bennett et. al, 2012). However, dipnetting may be the best option for sampling road-rut pools because only one researcher is needed and it is an economical option compared to pitfall traps and drift fence arrays. For future studies I recommend sampling multiple road-rut pools instead of focusing on a limited number as done in this study. Dipnetting coupled with visual encounter surveys (VES) is suggested to capture more specimens and provide additional power to the statistical analysis. Also, pitfall traps can be costly and time consuming (Enge, K.M., 2001). The combination of dipnettng and VES could provide more insight on amphibians' use of road-rut pools.

Green frogs are a good model species as they share life stages with many species of amphibians. Methodology is important to monitoring both common species and species of concern. All rare species were once common. Concepts discovered from studying common species also create valuable baseline questions to apply to other species including rare ones. Additional sampling methods and efforts are needed to decipher the best capture method for both tadpoles and adult amphibians. The results from this study can be applied in further studies for both common and rare anurans as they share common life history traits.

It is beneficial to use a generalist species to test the best collection method. A generalist has loose parameters on where it occurs (Steen et al., 2010). Once the method best suited to detect common or generalist species is determined, it can then be applied to imperiled or rare species. The generalist species, the Green Frog (*Rana clamitans)*, is believed to be the most abundant *Ranidae* species found in West Virginia (Thomas Pauley, personal conversation, December 2018). *Rana clamitans* uses both small aquatic habitats i.e., road-ruts and larger aquatic habitat i.e., ponds. The green frog (*Rana clamitans)* starts breeding in April and continues throughout the summer. Eggs are primarily laid in permanent bodies of water. Once the tadpoles metamorphose into froglets they disperse to temporary pools. I believe *Rana clamitans* use road-rut pools to avoid competition from other anuran species, as well as, other *R. clamitans* as there is less competition of resources (i.e., mate selection, territory and food allocation) in using a smaller waterbody. Studying unique habitats will benefit management decisions for those habitats and wildlife in the future. The way humans manage lands and waters affects the species that live in them.

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APPENDIX A

MARSHALL UNIVERSITY
Joan C. Edwards School of Medicine
www.musom.marshall.edu

Animal Resource Facility

Dear Sir/Madam:

The following application and protocol to use laboratory animals at Marshall University was reviewed and received final approval by the Institutional Animal Care and Use Committee (IACUC) on July 23, 2012

Title of application: "Use of Roadside Ditches and Puddles in West Virginia"

IACUC Project No.: 504-1-15

Expiration Date: December 1, 2012

Name of Principal Investigator: Thomas Pauley

Co-Investigator: Abby Sinclair

As a condition of approval, the Institutional Animal Care and Use Committee required the following modifications to the above-referenced application:

None

Monica A. Valentovic, Ph.D.
Chairperson, IACUC

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APPENDIX B

SPECIES ACCOUNTS

Rana clamitans

Rana clamitans (Northern Green Frog) is distributed widely along the eastern United States. Reproduction is aquatic and the breeding season of the Northern Green Frog begins around April and extends throughout the summer. The reproduction stage of the Green Frog occurs aquatically in which eggs are deposited in a foamy surface film in shallow water among emergent vegetation (Green and Pauley, 1987). The number of eggs produced can range from 1,000-7,000 in each clutch. Eggs are ~1.5mm in length and have a coloration of white on the bottom, changing to black near the top (Green and Pauley, 1987). Hatching takes only a few days, while the larval stage can range between a few months to an entire year. This widespread duration can occasionally lead to larvae to overwinter. Once metamorphosed, frogs disperse from water in search for suitable habitat. Schroeder (1976) discovered that major dispersal routes of newly transformed frogs favored drainage ditches or temporary pools. Adult Green Frogs are known to occur in permanent aquatic habitats but juveniles are observed to be commonly found in road-rut ponds (Pauley and Lannoo, 2005). Green Frogs are opportunistic feeders, meaning prey items are determined by what is present in their habitat. There are different predators at different life stages ranging from turtles eating eggs, aquatic insects consuming larvae, to adults being eaten by various avian species (Pauley and Lannoo, 2005).

Notophthalmus viridescens (Eastern Newt)

Notophthalmus viridescens (Eastern Newt) is another common species of its range throughout the eastern part of the United States. *Notophtalmus v. viridescens* (Eastern Red-spotted Newt) is a subspecies of the Eastern Newt with the largest distribution (Hunsinger and Lannoo, 2005) of the four subspecies in addition to the Broken-striped Newt (*N. b. dorsalis*), the Central Newt (*N. v. louisianensis*), and the Peninsula Newt (*N. d. piaropicola*). Furthermore, the Red-spotted Newt is the only subspecies found in West Virginia. Reproduction occurs aquatically, depositing eggs singly on submerged vegetation. Courtship and amplexus occurs in shallow water (Hunsinger and Lannoo, 2005). Amplexus is the mating posture where the male grabs the female to encourage egg deposition. Eggs are deposited between spring and early summer and range between 200-375 eggs dispersed throughout the entire breeding season (Hunsinger and Lannoo, 2005). The Red-Spotted Newt goes through different morphological stages including larvae, efts, and fully transformed aquatic adults. The larval stage will last about two to three months before metamorphosing into the eft stage. The eft stage is unique because the Red-spotted Newt is fully terrestrial but not yet sexually reproductive. The terrestrial eft stage is known to last from about two years up to seven. Efts establish home ranges that increase in size each year, possessing an average of 266.9 m^2 during the first year (Hunsinger and Lannoo, 2005). Adult newts usually live in permanent pools of water. In some areas, the Eastern Newt undergoes a fourth morphological stage, producing a neotenic adult. A neotenic adult is one that has bypassed the eft stage and transforms to an adult directly from larval stage. In doing so, the adult retains larval characteristics such as external gills (Hunsinger and Lannoo, 2005). Fauth and Resetarits (1991) discovered that newts are good colonizers. Additionally, predators are dependent upon the density of newts, naming them keystone predators. Red-spotted Newts are carnivorous and opportunistic feeders in all life stages (Hunsinger and Lannoo, 2005). Predators of the Red-spotted Newt are limited due to their skin containing tetrodotoxin which is a neurotoxin that causes harm and often death if consumed (Hunsinger and Lannoo, 2005). Although this Red-spotted Newt's toxic skin deters many predators, it is still subject to predation by various other animals, including some other salamanders, American bullfrogs, and raccoons (Hunsinger and Lannoo, 2005). Eastern newts have an average adult age of 3-8 years old and a maximum age varying from 9-15 years (Petranka, 1998).

***Rana catesbianus* (American Bullfrog)**

Rana catesbianus (American Bullfrog) has one of the largest distributions of any North American amphibian (Bury and Whelan, 1984). Although it has always had a large distribution, the original range is blurred by the introductions of the *R. catesbianus* in other areas especially in the western regions of the United States. *R. catesbianus* is usually common in most of its range but there are some implications of decline caused by many disturbances including habitat degradation and loss, water pollution, and over-harvesting (Casper and Hendricks, 2005). These frogs breed in the spring and early summer in permanent bodies of water (Casper and Hendricks, 2005). Reproductive males call from territories which measure around 2-5 meters in diameter (Harding, 1997). The eggs of *R. clamitans* are small averaging only about 0.13cm in diameter but they are fruitful, covering the top of the water in a circular mass around 2 feet or more (Green and Pauley, 1987). Larvae will hatch between 3 to 5 days (Bury and Whelan, 1984). Larval period is the longest of any West Virginia anuran being 12 to 14 months. Although *Rana clamitans* larvae may also overwinter, they generally metamorphose earlier than *R. catesbianus* and often times are able to metamorphose in the same season as when they hatched. Adult *R. catesbianus* habitat is associated with warmer lentic waters which consist of examples such as ponds, marshes, and reservoirs (Casper and Hendricks, 2005). Unlike other frogs, *R. catesbianus* can coexist with predatory fishes (Hecnar and M'Closkey, 1997). When winter comes the frogs go into torpor, or hibernation, by burying themselves in mud at the bottom of ponds and streams (Green and Pauley, 1987). Sexual maturity usually occurs at 1-2 years for males and 2-3 years for females (Howard, 1981). *R. catesbianus* are opportunistic and rapacious feeders preying on anything they can physically consume from insects to mice to even other frogs. There are also many different taxa that feed on different life stages of the bullfrog; those include such animals as raccoons, insects, spiders, fish, salamanders, frogs, turtles, alligators, snakes, birds and mammals (Casper and Hendricks, 2005). Pollutants and habitat destruction are known to negatively affect the abundance of the bullfrog. In regions and habitats in which it is native *R. catesbianus* should be conserved, but in areas such as the western United States where the *R. catesbianus* is not native it should be eradicated.

***Hyla chrysoscelis* (Cope's Gray Treefrog)**

The appearance of *Hyla chrysoscelis* (Cope's Gray Treefrog) is made up of a cryptic pattern consisting of greens, grays, and browns but the venter is white. It also has noticeable toe pads which is a characteristic of being in the *Hyla* family. The distribution of this frog throughout the United States is as west as eastern Texas and Minnesota and as south as Louisiana stretching on to cover most of the eastern United States reaching as northeast as West Virginia and parts of Maryland (Cline, 2005). Breeding occurs during mid-April till mid-July and *H. chrysoscelis* males may still call throughout the summer (Green and Pauley, 1987). Calling is centered near ponds, ephemeral pools, and ditches (Fellers, 1979; Godwin and Roble, 1983; Conant and Collins, 1998). Females lay up to 1,800 eggs on the surface of shallow water in patches of 30-40 (Green and Pauley 1987). Once the eggs hatch in 4-5 days they take about 2 months or 60 days to metamorphose (Green and Pauley 1987). Tadpoles generally filter feed, while the adult form of Gray Treefrogs eats insects (Cline, 2005). *Hyla chrysoscelis* is nearly impossible to distinguish physically from *Hyla versicolor* but the mating calls of the males slightly differ. *H. versicolor* has a slightly slower trill in their call than does *H. chrysoscelis. H. chrysoscelis* is also a diploid which means it has 2 sets of chromosomes per cell whereas *H. versicolor* is a tetraploid and contains 4 sets of chromosomes per cell. *H. chrysoscelis* is listed as Endangered in New Jersey (Cline, 2005) but otherwise does well in the rest of its range and is actually considered a colonizing species.