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A Natural History Study of Bufo a. americanus, the Eastern American Toad, and the Phenology of Spring Breeders in Southwest West Virginia

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A Natural History Study of *Bufo a. americanus,* the Eastern American Toad, and the Phenology of Spring Breeders in Southwest West Virginia

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By

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ABSTRACT

A Natural History Study of *Bufo a. americanus,* the Eastern American Toad, and the Phenology of Spring Breeders in Southwest West Virginia

By Tomi Maria Bergstrom

Natural history traits have not been thoroughly collected and analyzed on the "common hoptoad," *Bufo a. americanus* (eastern American toad) in West Virginia. Updating natural history information is important to understand the present population status (Jackson, 2001). I chose to have two study sites at opposing physiographic regions to constitute a better understanding of *B. a. americanus* natural history in WV. The study was initiated in spring 2008 at Beech Fork State Park and Green Bottom Swamp Wildlife Refuge (in Mason and Cabell counties) and in 2009 incorporated Canaan Valley of Tucker County. Ten natural history morphometrics and characters were recorded from each individual collected. These morphometrics were compared among males from Cabell and Mason counties (mean = 74.4mm) to Tucker County. Males in the Allegheny Mountains had larger snout-vent lengths than those in the Allegheny Plateau (Mean = 74.4). Morphometrics were compared between sexes and females had the following significantly larger characteristics: Snout-vent lengths ($p<0.001$), tibia lengths ($p<0.001$), eye diameters ($p<0.001$), and tympanic membranes. Three Song Meters™ were positioned: Song Meter™ #1 in a permanent pond at Beech Fork State Park, Song Meter™ #2 at a permanent wetland in Green Bottom Wildlife Refuge, Song Meter™ #3 at an ephemeral wetland in Beech Fork State Park. Seven species were recorded calling that provided valuable information on anuran breeding phenology in West Virginia such as their daily calling activity and optimal calling time. Short-term studies such as these can be revolutionary to updating natural history data for species and improving protocols to monitor anurans.

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Funding was hard to come by, but Marshall University's Research Foundation awarded me with $250 to cover the initial costs of the data loggers purchase. This was a start and encouragement to acquire the instruments that were essential to my thesis. I conducted research on my animals under the Division of Natural Resources scientific collecting permit number 2009.070 and my research methods were approved by the Marshall University Institutional Animal Care and Use Committee (IACUC) under project number 394.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Introduction to eastern American toad and automated recording systems (ARS)

Eastern American Toad Natural History Study

Description

The eastern American toad is taxonomically found in the family *Bufonidae* known as true toads or common hop-toads. More than 480 species are included in this family (Elliott et al., 2009). The eastern American toad was placed in the *Bufo* genus, which contains over 200 species (Green and Pauley, 1987). Recently taxonomists have suggested placing most North American members of the genus *Bufo* in the genus *Anaxyrus* (Frost et al., 2006) as well as changing some members of the genus *Rana* to *Lithobates*. Due to this recent change, Smith and Chiszar (2006) suggested *Anaxyrus americanus americanus* as the proper scientific name for the eastern American toad, but I will refer to it as *B. a. americanus* for the remainder of this paper and refer to all newly modified *Lithobates* species as *Rana*, since it is the most commonly used name found in the literature. Members of this family are found nearly worldwide (Elliot et al., 2009). They are characterized as rough skinned, “warty,” squat-bodied, and short-legged amphibians that most people think of in reference to “toad” (Green and Pauley, 1987). *Bufo a. americanus* has a reddish or rusty (Green and Pauley, 1987) colored dorsum, but can also be tan or grayish (Dorcas and Gibbons, 2008). Females tend to be more patterned (Conant and Collins, 1998), but both sexes can have dark spots that are brown or black, and warts that vary from yellow, orange, or red to dark brown (Conant and Collins, 1998). Some individuals are almost entirely brick red without dark spots on their back (Jensen et al., 2008). A light middorsal stripe (Conant and Collins, 1998) is sometimes present down the center of the back (Dorcas and Gibbons, 2008).

The average adult is 2.0 to 3.5 inches (5.1—8.9cm) (Green and Pauley, 1987), with the largest female 4.25 inches (11cm) long from snout to vent (Dorcas and Gibbons, 2008). Toads are generally sexually dimorphic; females tend to be larger than males (Jensen et al., 2008). Sexes can be easily distinguished in breeding season when males have dark nuptial pads on their thumbs and inner fingers and dark throats that inflate into rounded vocal sacs (Conant and Collins, 1998).

Morphometric notes on the shape and sizes of shoulder (parotoid) glands and cranial ridges, the relative number and prominence of warts, and differences in coloration and pattern (Conant and Collins, 1998) must be compared to differentiate the species of *Bufo*. Differences in cranial crests (ridges behind the eyes) and parotoid gland shapes and positions among various species are shown in Figure 1.0. *Bufo a. americanus* has four distinguishing characteristics: (1) kidney shaped parotoid glands that are not flush against cranial crests (Green and Pauley, 1987; Dorcas and Gibbons, 2008) but may be connected by a short spur (Green and Pauley, 1987), (2) have dark spots present on the back encompassing no more than two warts (Conant and Collins, 1998), (3) have enlarged spiny warts on the tibia (Green and Pauley, 1987), and (4) often have numerous dark spots or other dark markings on their white or cream colored venters (Dorcas and Gibbons, 2008).

Subspecies Differential

The American toad species is divided into two subspecies, *Bufo americanus americanus* (eastern American toad) and *Bufo americanus charlesmithi* (dwarf American toad). *Bufo a. charlesmithi* are often reddish in coloration (Green, 1997) and smaller seldom exceeding 2.5 inches (6.4cm) (Conant and Collins, 1998). Compared to the *B. a. americanus* which can have various degrees of ventral marking (Blair, 1943), the venter of *B. a. charlesmithi* is plain (Smith, 1961) or only faintly spotted (Conant and Collins, 1998). Dorsal spots are small and only include a single wart, if present at all (Conant and Collins, 1998).

The two species of American toads have a significant range in the United States; however, *B. a. americanus* occupies the majority of the area except a small southwest portion. *Bufo a. charlesmithi* resides in a small southwest section of the species' (*Bufo americanus*) range (Green, 1997) and therefore, does not extend into West Virginia. The two subspecies have substantial range overlaps in states where their populations converge; in Missouri among the area that bisects the northern and southern portions (Johnson, 1987), in southwestern Kentucky (Jackson Purchase area) between the Mississippi, Ohio, and Tennessee Rivers (Barbour, 1971), and throughout the southwestern state line of Illinois (Smith, 1961).

Bufo Genus: West Virginia Species Differential and Genetic Comparison

The *Bufo* genus has two species that are inhabitants of West Virginia, *B. a. americanus* and *Bufo fowleri* (Fowler's toad). These *Bufo* species can often be confused; as stated previously, and the position of the cranial crests, presence of spots on abdomen, and the condition of warts on the tibia are often considered when distinguishing between the *Bufo* species.

Bufo a. americanus and *B. fowleri* ranges are sympatric and they have been documented to hybridize in several regions (Allard, 1908; Miller and Chaplin, 1910; Hubbs, 1918; Myers, 1927; Pickens, 1927; Blair, 1941; Volpe, 1952; Cory and Manion, 1955; Zweifel, 1968; Brown, 1970; Jones, 1973; Green, 1982). Hybridization does not always occur since the two species tend to have different temperature tolerances (Frost and Martin, 1971) and habitat preferences. Green and Pauley (1987) state that in West Virginia it is not unusual to encounter toads with a combination of *B. fowleri* and *B. a. americanus* characteristics; however, they are not considered very similar species from a genetic standpoint.

Bufo a. americanus have been studied extensively to determine what species they are most closely related to and additionally if any subspecies were misclassified. Although *B. a. americanus* and *B. fowleri* are commonly recognized to hybridize, they are not as closely related as the *Bufo woodhousii* (Woodhouse's toad). A mitochondria gene sequences comparison by Masta et al. (2002) revealed that *B. woodhousii* and *B. americanus* are more closely related than any other species of toad. The synonymy of *B. a. americanus* and *Bufo hemiophrys* (Canadian toads) was introduced by Cook (1983) based on morphological variation (Green and Pustowka, 1997). The publication of Schmidt's (1953) list clarified the taxonomic difference of the *Bufo americanus* and *Bufo terrestris* (southern toads), the two species were previously known as *B. t. americanus* regarding their synonymy by Nettings and Goin (1946).

Distribution of Bufo a. americanus in the United States of America

Bufo a. americanus has a substantial distribution over the eastern half of the United States (Klemens, 1993) reaching just west of North Dakota and allowing the western state line of Minnesota to approximate the western border (Oldfield and Moriarty, 1994;), relatively following the South Dakota, Iowa, Nebraska, Kansas, and Missouri state boundaries (Collins, 1974, 1982; Seifert, 1978; Cochran, 1986; Olson, 1987; Whiting and Price, 1994). The northern border extends south from the Canadian border (Conant and Collins, 1998), throughout the Great Lakes region (Harding, 2000) east to Maine (Hunter et al., 1992). The eastern boarder runs down the coastal plain of the southeastern states (Klemens, 1993), missing Delaware, and occupying all of Virginia except the southeastern portion (Mitchell and Reay, 1999) to North Carolina

(Miller, 1979; Corin, 1976; Kraus and Schuett, 1982; Cochran, 1986). The southern border is irregular, but *B. a. americanus* occurs in some part of every southeastern state except Florida (Dorcas and Gibbons, 2008). The distribution dips into sections of the southeastern states such as South Carolina, Georgia, Alabama (Mount, 1975), and then encompassing nearly the whole state of Mississippi, pardoning the most southern tip (Whiting and Price, 1994; Conant and Collins, 1998) which is occupied by *B. a. charlesmithi* (Lazell and Mann, 1991; Himes and Bryan, 1998). Mount (1975) remarks “I have been unable to find *B. americanus* in the central or western portions of the Appalachian Plateaus region or in the Tennessee Valley.” Plate 3.0 displays how the distribution of the *B. a. charlesmithi* fits into that of *B. a. americanus* as described by Garman (1892), Mount (1975), Smith (1947, 1961), and Minton (1972). *Bufo a. americanus* encompasses much of the Appalachian Mountain chain and their presence among ranging elevations have been suggested through the studies of several biologists. Throughout the Allegheny Mountains Green and Pauley (1987) found *B. a. americanus* in peaks ≤ 1,200 m, while Huheey and Stupka (1967) previously found them in peaks > 1,524 m in the Great Smoky Mountains and Klemens (1993) cited them at 610 m in the Berkshire Mountains. *Bufo a. americanus* are common in West Virginia and have been documented in all 55 counties (Green and Pauley, 1987). The West Virginia Division of Natural Resources (WVDNR) has this species listed as a common S5 state ranking, which means within the state the species is “very common and demonstrably secure.”

Natural History Traits

The generally nocturnal animal has a home range of 0.16ac or greater as recorded in Kansas by Fitch (1958) and Collins (1974, 1982). Outside of the breeding season, *B. a. americanus* are cited in the literature as a primarily terrestrial amphibian. Green and Pauley (1987) observed that in West Virginia individuals may be found in dense woods, but are more frequently found in open pastures, agricultural areas, and gardens. Smith (1947) also considered *Bufo americanus* to be essentially a prairie species. Kirkland et al. (1996) found *B. americanus* to be the most abundant amphibian captured in a burned deciduous forest in central Appalachian Mountains. Analogously, Blymyer and McGinnes (1977) found them one of the most readily emerging amphibians to recently clear-cut areas. Mount (1975) and Johnson (1987) both describe *B. americanus* as common in and along forest borders, regardless of fragmentation or distribution to a forest. Toads will repeatedly use particular hiding places during the day (Dole 1972), including, but not limited to, under stones, boards, woodpiles, walkways, porches, or other cover (Wright and Wright, 1949) such as loose, damp soil (Fitch 1958). Temperature has played a large role in the life cycle of *Bufo americanus*, since they are not freeze tolerant (Miller, 1909a; Storey and Storey, 1986) and evidently have no mechanism for freeze tolerance (Holzwart and Hall, 1984). During cold winter weather, *Bufo americanus* hibernate terrestrially (Miller, 1909b) by burrowing into soil or a similar site that permits them to burrow below the frost line (Wright and Wright, 1949; Ewert, 1969.) Hibernation begins as the temperature falls below their normal activity minimum of about 9^{o}C, which is usually October in northern U.S. populations (Oldfield and Moriarty, 1994.) Toads move in spring to breeding sites from wherever they hibernate during the winter (Miller, 1909a; Oldham, 1969), both over land and along streams (Maynard, 1934). Fitzgerald and Bider (1974a, b) determined that humidity and rainfall were the primary climatic influences on *B. a. americanus* movements; in rainy spring weather they are known to travel up to distances of $\leq$ 1km (Ewert, 1969).

Males will display breeding site fidelity and with the warming of spring temperatures will orient themselves to breeding sites prior to the arrival of females (Ewert, 1969). Wright and Wright (1949) and Green and Pauley (1987) have recorded breeding habitats to vary from areas such as farm ponds to tire and horse tracks, and roadside ditches to floodplain pools with grassy areas in 5-10cm deep water (Miller, 1909a). Adult males can generally be heard singing their advertisement calls around dusk, but have also been recorded to call on warm humid days during the peak of their breeding season (Aronson, 1944; Brown and Littlejohn, 1972). In West Virginia, toads emerge from hibernation early in March and may be heard calling shortly afterward. Green and Pauley (1987) noted that emergence may be later at higher elevations, and breeding is frequently interrupted by recurring cold weather.

Males call while floating or while sitting near the water's edge (Jensen et al., 2008). In 1852, Henry David Thoreau referred to the *Bufo americanus* call as "the dream of the toad" in his journal entry. It has been described otherwise as a high-pitched musical trill (Green and Pauley 1987), a beautiful melodic trill (Elliott et al., 2009), a long, high-pitched, musical bu-r-r-r-r (Jensen et al., 2008), and as one of the most pleasant sounds of early spring (Conant and Collins, 1998). The call may last between 6-30 seconds with a trill rate of about 30-40 per second (Conant and Collins, 1998). On any given night, a few aquatic males engage in vocalization to attract females, while the majority will actively swim about the pond surface attempting to intercept females or to displace males that have achieved amplexus (Forester and Thompson, 1998). The peak of breeding activity generally last less than 15 days (Hunter et al., 1992), but within the period of 10-14 days (Harding, 2000), although males have been known to remain at a site for over 20 days (Forester and Thompson, 1998). Klemens (1993) reports that in northern localities, it is common to hear single advertisement calls late into May or early June as previously supported by Aronson (1944). Miller (1909b) and Aronson (1944) report that females appear at breeding sites intermittently after males begin singing their advertisement calls. The breeding population size tends to fluctuate from night to night (Collins and Wilbur, 1979) since the females do not persist there for as long as males. Due to the irregular arrival of females, competition results in males breeding choruses (Kruse, 1982; Wells and Taigen, 1984). If a male accidentally clasps another male, a chirp like release call (Dorcas and Gibbons, 2008) is repeatedly vocalized until released. Leary (2001) states that anuran release vocalizations function to prevent prolonged amplexus between males of the same or differing species, whereas advertisement vocalization potentially act as a premating isolation mechanism. In the incident of unwanted clasping, by either male on male or male on a female that has already deposited her eggs, body vibrations are often used to signal release of amplexus (Stebbins and Cohen, 1997). Herpetologists have debated over the process for mate selection and it is still a subject of much debate. Kruse (1981) found that mating success was independent of size, while Licht (1976) suggests that females will select a male that is the most appropriate size for successful amplexus. Females have been found to orient toward conspecific mating calls during a short period that is closely associated with ovulation (Schmidt, 1971) and select mates in regard to individual characteristics (pulse rate, call duration, call frequency) of the male's call that are favorable (Wilbur et al., 1978; Howard and Palmer, 1995) such as low frequency (Howard and Young, 1998). In mating, the male approaches the female from the rear and grasps her behind her forelimbs in a tight clasp (amplexus). Amplexus induces the female to deposit her eggs so the male may fertilize them externally (Harding, 2000).

Eggs are described as long gelatinous strands containing several thousand eggs with a diameter of 1.0-2.0mm (Wright and Wright, 1949) arranged in double strings or tubes which are separated from each other by a partition (Green and Pauley, 1987), usually entwined around vegetation or resting on the pool bottom (Wright and Wright, 1949; Behler and King, 1998). Clutch size records range from 4,000-8,000 eggs (Wright and Wright, 1949) to 2,000-12,000 (Gilhen, 1984) or 2,000-20,000 (Johnson, 1987). Hatch time of eggs depends on environmental factors such as depth of water, over-canopy shading (Werner and Glennemeier, 1999), or water temperature. Hatching has been recorded to occur in 3-12 days after deposition (Smith, 1961; Martof et al., 1980; Vogt, 1981). The larval period lasts 50-60 days, and transformation occurs when tadpoles reach a snout-vent length of 7-12mm (Wright and Wright, 1949). Miller (1909a) notes that metamorphosis occurs rapidly; newly metamorphosed animals will leave water within one day of acquiring their front legs and will have completely reabsorbed their tail a mere two days later. A whole cohort can transform within 3-12 days (Wright and Wright, 1949). During this transitional stage, toad tadpoles are vulnerable to predation from larvae of predaceous diving beetles, (Kruse, 1983), newts, and dragonfly naiads; however, most vertebrate predators find the tadpoles distasteful (Brodie et al., 1978). As in other species of *Bufo*, toxins contained in the skin secretions are also secondarily deposited in the ova (Licht, 1968) and are thought to afford some protection to newly laid, fertilized eggs, which then decreases as the zygotes develop (Phisalix, 1922).

Conservation

Pauley and Green (1987) stated that toads evidently tolerate humans well; this is widely agreed with in the literature. For example, Conant and Collins (1998) refer to *Bufo americanus* as the "common and abundant 'hoptoad' of the Northeast." *Bufo a. americanus* do not appear to be as sensitive to habitat fragmentation as other amphibian species (Hager, 1998; Kolozsvary and

Swihart, 1999; Lehtinen et al., 1999). Moreover, nowhere is this species considered to be under threat of extirpation (Lannoo, 1998); nonetheless, this species is subject to natural fluctuations in population size (Casebere and Taylor, 1976; Christein and Taylor, 1978; Heyer, 1979; Hecnar and M'Closkey, 1996). *Bufo a. americanus* are known to be sensitive to environmental stressors such as lethal chemical contaminants and acid precipitation. According to the literature, out of all environmental stressors, chemical contaminants have the most harmful effects on *B. a. americanus* larvae. At the larval stage *Bufo americanus* have no defense against pesticides such as bezopyrene, methoxychlor, toxaphene and endrin, as well as lead and ammonium nitrate (Clark and Diamond, 1971; Hall and Swineford, 1979, 1981; Bracher and Bider, 1982; Hecnar, 1995; Jofre and Karasov, 1999; Harris et al., 2000). Ouellet et al. (1997) have noted high prevalences of hindlimb deformities, primarily in juveniles, on wild-caught *B. americanus* from agricultural sites exposed to pesticide runoff. Despite the fact that many biotic and abiotic agents are potentially harmful to limb development, agricultural contaminants were suspected as primary aggressors (Ouellet et al. 1997). Carbaryl, an ingredient in commonly used pesticides, has negative effects on toad survival (Boone and James, 2003). Clark (1986) and Wyman (1988) found that acid precipitation has reduced the distribution of *B. a. americanus* in affected regions of their range, due to a sublethal effect on larvae nitrogen balance (Clark and Lazerte, 1985; Dale et al., 1985; Tattersall and Wright, 1996). UV-B radiation, a source of degradation to many anuran clutches, was not found to have substantial detrimental effects upon *B. americanus* eggs or larvae under natural conditions (Grant and Licht, 1995; Crump et al., 1999). Gomez-Mestre et al. (2006) found that if additional stressors were present, such as UV-B radiation or unusually cool conditions that *B. americanus* egg clutches experienced high levels of infection from water mold, a naturally occurring mold (Kiesecker and Blaustein, 1995; Beebee, 1996; Robinson et al, 2003). Water mold infection perpetuates premature hatching, resulting in smaller and less developed hatchlings (Gomez-Mestre et al., 2006). Regardless of these threats and concerns, *B. a. americanus* deposits such a large quantity of eggs that these environmental stressors generally are counteracted; many tadpoles will unquestionably complete metamorphosing.

Monitoring Amphibians

Finding the Appropriate Method to Detect Declines

Declines in amphibian populations have been noted since the 1970s (Gibbs et al., 1971; Hayes and Jennings, 1986; Tyler, 1991; Pounds and Crump, 1994; Bradford et al., 1994; Drost and Fellers, 1996; Green, 1997; Lannoo, 1998; Bury, 1999; Campbell, 1999). In 1989, at the World Congress of Herpetology, informal conversations among scientists led to a concern that amphibian declines were more than local phenomena and may be a global issue (Wake and Morowitz, 1991). Stuart et al. (2009) declared that declines are nonrandom in terms of species' ecological preferences, geographic ranges, and taxonomic associations and the lack of conservation remedies for these poorly understood declines means that hundreds of amphibian species now face extinction.

In an effort to understand amphibian declines, scientists are taking a closer look at monitoring methods. Current studies are demonstrating that underlying causes of amphibian declines may be far more complex than anyone originally imaged (Miller, 2000). It is crucial to know how populations are changing (Beebee, 1996) and these concerns have encouraged the development and standardization of surveying and monitoring methods for amphibians (Heyer et al., 1994). A comprehensive coverage of methods for measuring and monitoring amphibian populations has now been set forth (Heyer et al., 1994).

Some current surveying and monitoring methods for amphibians are nocturnal line-transects, pit-traps, and automated recording systems. To evaluate variation in amphibian populations, numerous monitoring programs based on calling surveys have been employed (Crouch and Paton, 2002; Vandewalle et al., 1996). Because many anurans have well-defined breeding seasons and males produce loud advertisement calls, surveys of breeding choruses may provide relatively a simple means to monitor trends in populations (Scott and Woodward, 1994). A popular monitoring program in the United States, especially on the east coast, is the North American Amphibian Monitoring Program (NAAMP). This program is sponsored by the U.S. Geological Survey and operates with the help of volunteers who visit a designed route to survey for anuran calls at three different specified periods (NAAMP, 2009). There is some debate about the credibility of a volunteer based program such as NAAMP. Although relatively inexpensive and potentially valuable, this method has several limitations, including misidentification by inexperienced volunteers, lack of a permanent sampling record, and disturbance to calling anurans (Bridges and Dorcas, 2000; Peterson and Dorcas, 1994). Devices and methods to monitor anuran calls have been targeted in the past century to develop reliable data under a number of environmental conditions and scenarios.

Automated Recording Devices

Visual encounter and auditory surveys using transects are the most frequent methods used for monitoring amphibians (Crump and Scott, 1994; Zimmerman, 1994; Lips et al., 2001), but these techniques can often be hazardous to the investigator to monitor at night or disturbing to calling animals. In the past decade, automated recording systems (ARS) have been developed to address the limitations associated with manual call surveys (Todd et al., 2003). Bridges and Dorcas (2000) support the idea of automated recording systems to provide the ability of sampling for extended periods of time, thus increasing the probability of detecting a given species; decrease disturbance to calling anurans, thus decreasing the probability of missing easily disturbed species; a permanent sampling record allowing repeated evaluation by multiple investigators; and accurately evaluate interspecific and temporal variation in calling behavior. These systems allow sampling of anuran populations without an observer present (Bridges and Dorcas, 2000; Mohr and Dorcas, 1999; Parris et al., 1999; Peterson and Dorcas, 1992, 1994; Varhegyi et al., 1998), and help develop models to optimize effectiveness of manual call surveys (Bridges and Dorcas, 2000). Automated recording systems can vary as far as auditory equipment specifications are concerned; a microphone, time stamp, and recorder in a waterproof container are the basics needed to construct a system. Most systems are constructed similar to the ARS method first utilized used by Peterson and Dorcas (1994). Peterson and Dorcas' ARS is known as the Frog-logger (www.frogloggers.com). Other recording devices are also available to monitor calling anurans, as well as birds. Song Meter™ (www.wildlifeacoustics.com), the Amphibulator (http://doi.ieeecomputersociety.org/), or the Automated Digital Recording System (ADRS) are all recording systems that are being used in wildlife surveys. The use of an ARS is still being considered in field studies; however, their use is strongly encouraged in regional amphibian monitoring programs to help establish optimal sampling times and for interpreting data (Todd et al., 2003). Solid reliable information on something such as amphibian optimal calling times suggests that a small network of frog-loggers would be a useful tool to track breeding phenology (Corn et al., 2000). I believe that automated recording systems will be able to detect more species than nocturnal line transects and provide key information on anuran breeding phenology. I have two main objectives from this study. I want to collect natural history data of *B. a. americanus* including calling phenology in West Virginia (via Song Meters ™) and similarly compare morphometric data from some of the highest elevations to the lowest in West Virginia; to determine if Song Meters ™ are reliable devices to record spring calling anurans in West Virginia.

Materials and Methods

Study Sites

I conducted a two-year natural history study in West Virginia on *B. a. americanus* populations that inhabited Green Bottom Swamp Wildlife Management Area, Canaan Valley Wildlife Refuge, and Beech Fork State Park. I originally had four sites reserved for natural history study, but lack of funding and allowance of time restricted my sites to these three locations. I chose these locations due to their distance from Marshall University and my home in Randolph County and their ecological differences, such as plant composition, elevation, and habitat for appropriate toad breeding sites.

Green Bottom Swamp Wildlife Management Area

Green Bottom Swamp WMA is located in Cabell and Mason counties (38° 35’01’’N W 82° 14’03’’W). Formerly owned as plantation lands of U.S. Congressman and Confederate General Albert G. Jenkins (Johnson, 1947), it is now owned by West Virginia Division of Natural Resources and United States Army Corps of Engineers and offers 444 ha of wetlands and wildlife habitat (Duda, 2008). The Green Bottom WMA land sets at 164.6 m elevation and is a composition of farmland, mixed hardwood forests, wetlands, and open water for fishing opportunities (Duda, 2008). The Green Bottom Swamp, my area of focus, lays in a 353 ha area in a man-made patchwork of buttonbush swamp and farmland (McCoy, 2009a).

Canaan Valley National Wildlife Refuge

Canaan Valley National Wildlife Refuge, the nation's 500th refuge, is located in Tucker County (39^{o}02'43"N 79^{o}26'47"W). The Refuge is approximately 10,117.1 ha and was dedicated in 1994 to be managed by the United States Fish and Wildlife Service (www.canaanvalley.org). The moist valley provides extensive wetlands covered with shallow and intermittent waters that are some of the largest in the entire central and southern Appalachian region. According to the official Canaan Valley website (www.canaanvalley.org) these 6,000 or so acres of shrub swamp and bog represent approximately 40% of the wetland found in West Virginia. I choose this site primarily for its noteworthy elevation within the Allegheny Mountains. Setting at about 975.4 m the aggregation of unique habitat varies in elevation plus or minus 300 m according to your position on ridge or in valley. Since *B. a. americanus* is found from the lowest elevations to the highest elevations in West Virginia (Green and Pauley, 1987) my Canaan Valley study site was an important counterpart to the low elevation sites for this natural history study.

Beech Fork State Park

Beech Fork State Park is located in Cabell and Wayne counties (38^{o}18'36"N 82^{o}20'52"W) and is owned and managed by U.S. Army Corps of Engineers and West Virginia Division of Natural Resources. Beech Fork encompasses 1272 ha at 220.1 m average elevation (McCoy, 2009b).

Beech Fork Lake alleviates the pressure from the Ohio River during flooding events and consequently has many areas that are in floodplain territory. Spring showers allow for the formation of many ephemeral pools that serve as breeding habitat for *B. a. americanus*. A particularly large and remote ephemeral pool was the primary study site for my natural history study, and to document the breeding call phenology of spring anurans.

Primary Site for Natural History Study

This study was first initiated in the spring of 2008 in Beech Fork State Park and Green Bottom Swamp. Because of the abundance of *B. a. americanus* toads found at Beech Fork State Park, it became my primary site to gather natural history data. Sites were periodically visited beginning in early February when air temperatures were around 15^{o}C and after heavy rain. Changes observed at the sites, such as unusually low or high water levels, or emergences of vegetation were recorded. Calling amphibians and discovered egg clutches from other amphibian species were also identified and noted.

Natural History Study

When *B. a. americanus* were spotted, they were caught by hand; a dip net was used in deeper water or when the animal was out of arm's reach. Once an animal was in hand, it was checked for deformities and sexed (see Plate 1.0). This information as well as morphometrics was recorded. Calipers (model Spi 2000) were used to make the following measurements to the nearest .2mm: 1) Snout-to-vent length (SVL), 2) tibia length, 3) eye diameter, 4) tympanic membrane diameter, 5) parotoid gland area, 6) snout length. Four additional observational measurements were also noted: 7) warts per black spot, 8) cranial ridge position (connected by spur or separated to paratoid gland), 9) condition of warts on tibia (spiny or not), and 10) percentage of spots on chest (as shown in Plate 1.1, they fall into one of these four ranges: 0-25%, 26-50%, 51-75%, 76-100%). Abiotic environmental factors were recorded each time a field survey was conducted. Air temperatures (recorded in degrees Celsius) and percent relative humidity were recorded with a hand held Thermo-Hygrometer that ranged from 0^{o}C –50^{o}C air temperature and 2% - 98% relative humidity. These measurements were made by laying the device as close as possible to the water surface level as to not bias the reading of the animal's environmental influences. Water temperature was measured with a simple aquatic thermometer. Two thermometers were placed in the water, one along the edge in shallow water and one in a deeper area towards the middle of the ephemeral pool, for a minimum of 60 seconds. From the two thermometer readings the mean was mentally calculated for the water temperature and recorded in degrees Celsius.

A plant survey was conducted with the help of Marshall University's resident botanist, Dr. Dan Evans, in mid-March to determine the primary herbaceous species found in the breeding habitat area; trees and shrubs were also noted. Most plants could be identified to species, although it was still too early for some sedges and grasses to produce flowers that are essential for species identification. This plant survey was used to compile and create a diagram (Figure 6.0) of the ephemeral wetland at Beech Fork State Park permanent pond site (#2) with a legend marking the occurrences of herbaceous plant species. This was also useful to determine egg placement in relation to vegetation, as well as, to note possible preferences for egg deposition.

When eggs were observed being deposited, sites were flagged and numbered with orange marking tape with the following information: date clutch was first observed or date when clutch was deposited, if the eggs were intertwined around vegetation or debris, and the approximate number of strings of eggs laid. Egg clutches *were not* contained for observation. Flagged sites were visited 1-3 times weekly. Observational data such as depth of water, beginning time of hatching, and damaged or infected clutches were noted on each visit. Eggs were staged with a magnifying glass to determine a Gosner (1960) stage number. Photographs were taken with a Kodak 10x optical zoom to help visually document habitat changes, life stages, amplexus, and deformities.

Anuran Breeding Phenology

I used three automated recording system known as Song Meters ™ to monitor spring breeding calls. Song Meter ™ is a product offered by Wildlife Acoustics, Inc. Song Meter ™ specifications include an 8.4” x 7.1” x 2.4” weatherproof enclosure with operating temperatures from –20^{o}C to +70^{o}C, built-in stereo omnidirectional microphones that have a sensitivity of –35+/- 4dB (0dB=1V/pa@1KHz), a frequency response of 20Hz – 20,000Hz (same as the human ear), and a signal-to-Noise Ratio of >62dB. Song Meter ™ is a cost-effective digital audio recorder specifically designed to monitor wildlife populations, such as birds and frogs, in harsh outdoor environments over extended periods of time (Agranat, 2008). Song Meter ™ is programmed to record automatically on a scheduled time. I set them to record for a minute every half hour, starting at 1630 and stopping at 0900. A few weeks into the recording it was decided to make this a daily 24-hour recording session after *B. a. americanus* was heard calling in full chorus at noon.

Song Meter ™ has the battery life and memory capacity to record for almost 100 hours spread out through several weeks, or even months (Agranat, 2008). The device was powered by four “D” cells (NiMH or Alkaline); recordings were stored on 8GB PNY Optima SD cards that use

"SPI mode" for storage in 16-bit PCM (.wav) digital format. Once recordings were obtained and uploaded with a MobileMate™ SD Memory Card Reader, they were analyzed by Song Scope ™ software. This software offers a visual detection on spectrographs and the option to build the program to analyze recordings automatically and thus detect species automatically. I did not have the program analyze recordings automatically, but listened to each one individually with the spectrograph playing for visual detection to establish the density of calling species. All calls were identified to species and calling activities were quantified according to the numerical classification scheme recommended by NAAMP (U.S. Geological Survey, North American Amphibian Monitoring Program): 0 = Species not detected; 1 = Individuals can be counted; there is space between calls; 2 = Calls of individuals can be distinguished but there is some overlapping; 3 = Full chorus, calls are constant, continuous and overlapping. Each Song Meter ™ was attached to a wooden board with four woodscrews, one in each corner (after mounting model used by USGS National Wetlands Research Center) which was recommended because it does not degrade the weatherproofed case (see Plate 2.0). Then each board was attached with woodscrews, with a cordless screwdriver, and into a tree at a suitable location in my study site, i.e., near toad breeding habitat (see Plate 2.1). One was mounted in Green Bottom Swamp at a permanent wetland pool and two were mounted in Beech Fork State Park, the first at an ephemeral wetland and the second at a permanent pond.

Song Meter ™ #1 was placed in Beech Fork State Park at a permanent pond; recordings were initiated on March 27, 2009 at 12:00AM and terminated on May 3, 2009 at 1700. From March 27 through April 8, the Song Meter ™ recordings were programmed to record from 1900 to 0900 h. On April 9 this was expanded to 1630 to 0900 h, and finally on April 10 the recorder was programmed to collect data a full 24-hour period. Song Meter ™ #2 was installed in a permanent wetland pool at Green Bottom Swamp; recordings began on March 30, 2009 at 0600 h and terminated on May 2, 2009 at 1600 h. The Song Meter TM was programmed to record from 0900 to 1800 h. Data analysis was halted after April 10, 2009 due to technical difficulties experienced by the meter's expose to high-voltage power lines. Song Meter TM #3 was mounted in an ephemeral pool in an open field at Beech Fork State Park; recordings were commenced on April 5, 2009 on midnight and terminated on May 3, 2009 at 1900 h. From April 5, 2009 through April 8, 2009 the Song Meter TM was programmed to record from 0900 to 1700 h; on April 9, 2009 0900 through 1500 h; on April 10, 2009 to May 3, 2009 a full 24-hour period. To assist conclusions concerning calling activity levels, environmental data loggers were adhered to each Song Meter TM.

I used temperature loggers produced by Nexsens Technology, Inc. The micro-T temperature logger is based on iButton TM technology. The Nexsens website describes micro-T as "a self-contained, self-powered, and field-rugged temperature data logging system." The logger is just under 1.5 cm in diameter and consists of a computer chip, temperature sensor, and battery enclosed in a 16mm thick stainless steel can. Given its small size it was easily mounted to the bottom of the Song Meter TM box with adhesive pads. Micro-T temperature loggers measure air temperature from –20 to 85 degrees Celsius; the percent relative humidity loggers measure relative humidity from 0 to 100%. Additionally, the loggers have the following specifications: Typical life is 1-3 years, 2,048 samples measurements can be recorded, each that are accurate +/- 0.5 C; +/-5% RH, with a resolution of 0.0625 C; 0.04% RH. Loggers were programmed to record air temperature and percent relative humidity every 30 minutes, starting on the hour, 24 hours a day. This environmental information was correlated with calling activity levels collected from the Song Meters TM to determine spring breeding anuran calling phenology. Common applications listed from the Nexsens website for these loggers include water temperature profiling, cargo transportation monitoring, ambient air monitoring, animal roosting behavior studies, new product research and development, wide-area temperature networks and much more.

Statistical Analysis Programs and Applications

The statistical program SigmaStat ™ was used to determine p-values, mean values, and standard deviations from T-tests calculations. I used SigmaStat ™ to compare the morphometric differences between male toads in higher elevations (site in Canaan Valley National Wildlife Refuge) to male toads in lower elevations (sites in Beech Fork State Park) and between all males and females measured in the duration of my natural history study at Green Bottom Swamp, Canaan Valley National Wildlife Refuge, and Beech Fork State Park sites (see Table 1.0). Microsoft Excel™ was used to create graphs and figures to show correlations in breeding phenology and electronically organize data sets into tables.

Results

Natural History Study

Elevation Differential

Pair-wise comparisons of six morphological characters between two populations were calculated with SigmaStat ™ and are summarized in Table 1.1. Only two variables, snout-vent length and parotoid gland, passed the normality test and equal variance test, and therefore were evaluated with t-tests. Both snout-vent length and parotoid gland area were larger among the males at the high elevation site in Canaan Valley both were $p < 0.001$. Since the remaining four morphometrics did not meet the assumptions of a t-test, they were analyzed using the non-parametric Mann-Whitney Rank Sum Test. Tibia length of toads in Canaan Valley was significantly longer ($p<0.0001$), eye diameter was significantly wider ($p= 0.067$), and tympanic membrane diameter was wider ($p<0.001$) as compared to Cabell and Mason county sites. At the lower elevation sites in Cabell and Mason counties, snout length was longer ($p=0.004$).

Sex Differential

Pair-wise comparisons of the same six morphological characteristics were also calculated between sexes with SigmaStat ™ and a summary of the result's are in Table 1.2. Two of the six variables, snout-vent length and parotoid gland area, passed the normality test and equal variance test, and therefore were evaluated with t-tests. Females have a significantly longer ($p< 0.001$) snout-vent length and parotoid gland area than males. Because the remaining four characteristics lack normality and equal variance, non-parametric Mann-Whitney Rank Sum test was used. Three of the four measurements were found to be significant; female tibia lengths were larger ($p<0.001$), female eye diameters were larger ($p<0.001$), and female tympanic membrane diameters were wider ($p=0.005$). Snout length differences in the median values between the sexes were not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a significant difference ($p=0.456$).

Hybridization Occurrence

Morphometric notes on hybridization are summarized in Table 1.3 and abdominal spot percentage is displayed in Figure 1.1. The highest percentage of abdominal mottling was found in the 1-25% range, at 43% of the whole. Only 5% of the total number of animals measured (99) were found to have clear abdomens, i.e., without mottling. Seven of 99 toads measured did not have spiny tibias, 21 of the 99 had more than 2 warts per black spot on their dorsal, and two lacked a single spur or a spur touching the parotoid gland. Two toads displayed two of these characteristics, thus suggesting some hybridization between *B. fowleri* and *B. a. americanus*. The first from Beech Fork State Park had at least one spot with 3 warts and lacked abdominal mottling. The second was from Canaan Valley and lacked abdominal mottling as well as a spur touching the cranial ridge. These results were not statistically quantified due to the lack of supporting information of hybridization. Tadpole data are quantified in Table 1.4. Eggs strands were partitioned (*Bufo fowleri* eggs are not) and deposited in close relation to other clutches in shallow water ranging from 1 mm to 120 mm. Generally, eggs were found at a depth of 80 mm or less, due to being attached to vegetation (*Juncus* or *Carex*) or floating plant debris from last year's cattail (*Typha latifolia*) growth. Numerous clutches were infected with water mold. Hatched tadpoles were generally found in black mats by the thousands in close aggregations feeding on beds of algae.

Anuran Calling Phenology

Song Meter™ #1

Data sets were collected from three Song Meters™. The species detected each day of the 36-day study are graphed in Figure 2.5 and included *Bufo a. americanus*, *Pseudacris c. crucifer*, *Rana palustris*, *Rana clamitans melanota*, and *Rana catesbeiana*. Data summaries of optimal calling time, temperature, and percent relative humidity can be found in Table 2.0; daily calling activity of each detected species is graphed in figures 2.0 through 2.4. Note, these single exerts (figures 2.0 through 2.4) were selected with regards to the best representation of the species optimal calling activity. Enough information was collected to determine optimal calling times for *B. a. americanus*

(2000 through 2100 h), *P. c. crucifer* (1900 through 2000 h), and *R. palustris* (2000 through 0100 h).

Song Meter ™ #2

The species recorded calling during the 12-day study are graphed in Figure 3.3, this includes *B. a. americanus, P.c. crucifer,* and *R. pipiens.* Their optimal calling time was determined to be 1900 to 2100 h, 1900 to 2200 h, and 0000 to 0100 h, respectively. Data summaries of optimal calling time, temperature, and percent relative humidity can be found in Table 2.1; a single example of daily calling activity is graphed in figures 3.0 through 3.2 of each detected species, it is typical of their optimal calling times.

Song Meter ™ #3

Recorded species from this 29-day study are graphed in Figure 4.6. Data were collected on eight species (*B. a. americanus*, *P. c. crucifer*, *R. palustris*, *R. clamitans melanota*, *R. catesbeiana*, *H. chrysoscelis*, *S. holbrookii*, and *R. sylvatica*), two species (*S. holbrookii* and *R. sylvatica)* were only recorded once throughout the whole study. Optimal calling times were as follows: *B. a. americanus* (2100 through 2300 h), *P. c. crucifer* (1900 through 2000 h), *R. palustris* (2200 through 0200 h), *R. clamitans melanota* (2000 through 0500 h), *R. catesbeiana* (0500 h), and *H. chrysoscelis* (2000 through 2200 h). Corresponding data analysis regarding temperature and percent relative humidity can be reviewed in Table 2.2; daily calling activity of each detected species is graphed in figures 4.0-4.5, these figures are selected exerts of the animal's optimal calling time.

Discussion

Importance of Baseline Studies

Amphibian Declines

Amphibian populations are known to fluctuate naturally and in response to a number of biotic and abiotic factors; therefore, long-term studies are needed to have reliable conclusions on amphibians. Much effort is directed at conserving individual species, and considerable thought has been directed at developing criteria for categorizing endangered and threatened species (e.g., Mace and Lande, 1991); however, Dodd and Franz (1993) point out that few authors have discussed common species. The definition of a common species is not only debatable in literature, but also among the general public. When people think of a common species, they think of the species being widespread, ordinary, general, or even a nuisance. A wide spread and abundant species could suffer a 50% population decline, yet it still might be perceived as common to many people (Dodd and Franz, 1993). It is clear that no single common cause underlies amphibian declines around the world (Griffiths and Beebee, 1992) and that the only profitable way to study these declines is to carry out detailed investigations of individual cases, such as natural history studies. The lack of historic population data makes it difficult to establish the causes of population declines, interpret them (Pechmann et al., 1991), or recommend management-related research (Dodd and Franz, 1993). Short-term studies could be helpful in updating this baseline data on threatened species as well as common species. Short-term studies are criticized for lacking substantial evidence that any one population is declining, however, significant information can be gathered and collaborated to establish trends and update baseline data. Dodd and Franz (1993) state that with relatively small amounts of habitat protected and most funding directed at task-oriented research on a few species, the biological status of apparently common species may be overlooked. Although most common species have been studied in the area of their range it is important to update this information; rather than ignore common species until problems become glaringly apparent (Dodd and Franz, 1993).

Importance of Updating Natural History Information

Natural history studies allow herpetologists to understand how animals operate in daily activity and specifically during breeding seasons, and they also establish monitoring protocols. Lack of creditable baseline data can negatively impact implementations on fauna and breeding habitat. If a habitat manager has preconceived ideas of what are or are not common species they may initiate management programs that actually lead to the inadvertent loss of a possibly declining species (Dodd and Franz, 1993). Thus, it is proposed that the word "common" may be used as a general observation of relative abundance but it should not be used in a conservation context unless a specific definition is provided (Dodd and Franz, 1993). Jackson (2001) stresses that updating natural history information is important to understanding the present population status, and that the present population status is not always the key to understanding the past or the future status. If a species was presumably common 30 years ago and lacks long-term population data, would it still be perceived as common? This train of thought could familiarize biologists and the public to be more knowledgeable about animals that are assumed "abundant" or "common" to their region.

Natural History Study

Elevation Differential

Bufo a. americanus are found throughout much of the eastern United States (Dorcas and Gibbons, 2008) and live in the highest elevations to the lowest elevations in West Virginia (Pauley, 2001). They are found abundantly in many different physiographic regions throughout their range (Wright and Wright, 1949). Green and Pauley (1987) describe 5 physiologic provinces: (1) the *Allegheny Plateau,* an area of rolling foothills that extends from the western border of the state to the Allegheny Mountains; (2) the *Allegheny Mountains*, a chain of the Appalachian Mountains that reaches heights of over 1,219 m; (3) the *Ridge and Valley*, an area east of the Allegheny Front (the eastward-facing edge of the Allegheny Mountains) that is composed of a series of steep-created ridges and wide valley; (4) the *Great Appalachian Valley*, a wide valley east of the Ridge and Valley province; and (5) the *Blue Ridge Mountains*, a mountain range at the tip of the Eastern Panhandle. I chose my study sites to be in two of the largest physiographic regions found in West Virginia, the Allegheny Mountains and Allegheny Plateau. I thought having these opposing study sites would help constitute a better understanding of *B. a. americanus* natural history in West Virginia.

The Allegheny Plateau and the Allegheny Mountains have differences in elevation of 900m or greater. Differences in elevation can affect many natural history traits and strategies, such as time of emergence from torpor, quantity of eggs laid, and foraging activity. Higher elevations have cooler environments and shorter seasons. These cooler areas can lead to smaller bodied animals yielding a higher fat content at high elevations than that at low elevations. Takahashi and Pauley (2010) suggest this is due to a lower level of resource acquisition in high elevations. Amphibians are generally thought to be smaller due to lower temperature, which often limits their food source; however, there is general support for the rule of larger body at lower temperature or higher altitude and latitude both in mammals (Ashton et al., 2000) and ectotherms (Atkinson, 1994; Atkinson and Sibly, 1997). In amphibian systems, many previous studies support this general rule (Hairston, 1949; Martof and Rose, 1963; Pettus and Angleton, 1967; Tilley, 1973; Smith-Gill and Berven, 1979; Berven, 1982). Differences in elevation among my study sites provided an area to test this hypothesis of morphometric body differences in *B.*

a. americanus. In my comparative study of males from Canaan Valley State Park (Allegheny Mountains) to males from Beech Fork State Park (Allegheny Plateau), I found significant differences between snout-vent length, tibia length, tympanic membrane diameter, parotoid gland size, and snout length. Males in the Allegheny Mountains (mean = 67.9mm) were larger in regards to snout-vent length compared to the Allegheny Plateau (mean = 74.4mm). The smaller body in higher elevation rule is not always followed and recent studies have explored more complex mechanistic understandings of body size variation by incorporating not only temperature, but also other extrinsic factors such as productivity in larval habitats and resource availability (Bernardo, 1994; Bernardo and Reagon-Wallin, 2002; Bernardo and Agosta, 2003). In my study, *B. a. americanus* is ostensibly one of these exceptions. The difference in snout-vent length could also relate to foraging activity and prey selection and availability. Snout length was found to be significantly larger ($p = 0.004$) in the Allegheny Plateau (mean = 7.0 mm) compared to the Allegheny Mountains (mean = 5.6 mm). Whether the snout-vent length is due to fat storage, temperature, or other extrinsic factors, *B. a. americanus* did exhibit significant size differences, as shown in Table 1.1, between the Allegheny Mountains and Allegheny Plateau regions of West Virginia. Owen (1989) hypothesized that animals with stable climates should permit a more constant influx of resources into an assemblage and thus allow for a finer degree of food resource exploitation and the evolution of greater niche specialization. A study analyzing stomach contents would provide more conclusive reasons to interpret this life history trait. I would predict that they feed on very different prey items or the group that I sampled in the Allegheny Mountains was an older population. A detailed study on this subject is needed to state anything with certainty.

Sex Differential and Natural History Traits

The same six morphometrics were compared among sexes from all sites and were statistically analyzed (t-test); the results have been summarized in Table 1.2. Sexual dimorphism is something that is often noted in the animal kingdom; in most *Bufonidae* species, females grow larger than males (Conant and Collins, 1998). I compared six measurements between sexes and found females to be significantly larger for the following five characteristics: Snout-vent length ($p<0.001$), tibia length ($p< 0.001$), eye diameter ($p<0.001$), tympanic membrane diameter ($p = 0.005$), and parotoid gland area ($p<0.001$). Snout length was not significantly different between sexes.

Females had a larger mean snout-vent length (76.3mm) compared to males (69.6mm). This was expected since it is reported in the literature and supported by my visual observations in the field. Since females (mean = 6.6 mm) have been recorded to select mates (mean = 6.0 mm) due to their calling (Schmidt, 1971), it was not surprising that tympanic membrane diameter was found to be significantly different. Females (mean = 113.8 mm) were found to have a significantly larger parotoid gland area than males (mean = 90.8 mm). I expected females to have larger parotoid glands due to the quantity of bufotoxin that would be incorporated into the egg embryos as a natural protection and deterrent to predators (Licht, 1968). Perhaps the protocol to sample parotoid glands on females should be conducted after egg deposition has occurred to avoid measuring females with a lower bufotoxin reservoir.

Natural Predators and Infections

Although common amphibians are found in abundance, they are still subject to predation and fatal natural occurrences, especially during their breeding season. Males generally congregate at a breeding site; this visual as well as auditory queue creates a target for blue heron, snakes, or a variety of scavengers such as raccoons. Many organisms experience high mortality rates in their early life stages, because of predation, infection, and harsh abiotic conditions (Wilbur, 1980; Gosselin and Qian, 1997). Some predations I observed included the remains of more than a dozen toad carcasses still clasping in amplexus or depositing eggs, many nests had been disturbed (most likely by the snapping turtle observed at the site) and perhaps the most noteworthy and lethal natural occurrence was the discovery of water mold on egg clutches, shown in Plate 1.2. Infections from water mold (*Saprolegnia ferax*) have contributed to amphibian declines via embryo mortality in the Pacific northwestern United States (Blaustein et al, 1994; Kiesecker et al, 2001).

Water molds of the family *Saprolegniaceae* cause substantial mortality for aquatic eggs of a wide range of fish and amphibian species throughout the temperate world (Blaustein et al., 1994; Czeczuga et al., 1998; Kiesecker et al., 2001). Water molds spread from egg to egg; therefore, communal egg masses are at a greater risk of infection than clutches laid singly (Kiesecker and Blaustein, 1997; Green 1999). Eggs hatch early when infected, yielding smaller and less developed hatchlings (Gomez-Mestre et al., 2006). Water molds attack aquatic eggs worldwide and have been associated with major mortality events in some cases, but typically are only found in association with additional stressors (Gomez-Mestre et al., 2006). Major outbreaks of water mold infection of amphibian eggs have only been reported to occur when mold acted synergistically with other environmental stressors, such as UV-B radiation or unusually cool conditions (Kiesecker and Blaustein, 1995; Beebe, 1996; Robinson et al., 2003). Water mold was most prominent at the ephemeral pool in Beech Fork State Park, where nearly every egg mass of *B. a. americanus* was eventually infected with the mold. *Ambystoma jeffersonianum* (Jefferson salamander) eggs deposited in the ephemeral pool at Beech Fork State Park were also noted to have the mold present on several clutches of eggs. A study in Lynn Woods Reservation, Massachusetts in spring 2005 determined that *B. a. americanus* eggs experienced the highest level of infection among other anurans (Gomez-Mestre et al., 2006). Additionally, this experiment concluded that the mean mortality of *B. a. americanus* infected clutches was only 25%, suggesting that most embryos were able to escape before being killed by the pathogen. In my field study, I noted that toad embryos with water mold were alive although they were free floating and not yet fully developed. Gomez-Mestre et al. (2006) also observed early *B. americanus* hatchlings in ponds survive infection, develop to the free-feeding larval stage, and feed on the water mold hyphae that had consumed part of their own clutch. *Rana sylvatica* were also observed eating mold hyphae on infected *B. a. americanus* eggs, but were never observed eating toad eggs themselves. Furthermore, *B. a. americanus* embryo survival was reduced by mold unless *R. sylvatica* was also present, seemingly rescuing toad eggs from the effects of mold by feeding on the pathogenic infection (Gomez-Mestre et al., 2006). I found this to be an important natural history trait that was only briefly mentioned in the literature. Most field studies usually report *R. sylvatica* predating on *B. a. americanus* eggs (Petranka et al., 1994), causing severe mortality. Amphibians provide a model system for studying the effects of predation on life history shifts (e.g. Werner, 1986). Salamander eggs will hatch later and at a more developed stage when predators, such as flatworms are in direct contact. By delaying hatching, salamanders reach a developmental stage where they are less susceptible to predation by flatworms (Sih and Moore, 1993). Given that predaceous diving beetles were often noted in association with *B. a. americanus* clutches and during several nights temperatures dropped below freezing, it could be suggested that the additional stressors propagated the water mold infection through the ephemeral pool at Beech Fork State Park. With climate changes in force, water mold infections could propagate. Even though it is a naturally occurring infection and many anurans have seemingly adapted to the infection, information regarding its presence should be more common in the literature.

Calling Phenology of Bufo a. americanus and Other Spring Anurans

Bufo a. americanus emerged from torpor in late March and could be found migrating to breeding spots on rainy warm nights; however, these toads were rarely observed calling. Call surveys for amphibians are a popular technique for monitoring and determining population density. Because many anurans have well-defined breeding season and males produce loud advertisement calls, surveys of breeding choruses may provide a relatively simple means of monitoring trends in populations (Scott and Woodward, 1994). Several problems and biases associated with call surveys are found throughout the literature. They include a lack of roads and wetlands; species with audible calls in western North American; inter-observer bias; and problems associated with recruiting and training a large pool of volunteers (Corn et al., 2000). Others argue that call surveys are not a sufficient method to monitor amphibians since only males call. Perhaps most relevant, if a species is not sampled during its optimum calling time results may show false negatives. False negatives occur when an area is sampled and a species is not detected and therefore assumed to be absent from that site, when it could be detected if sampling periods were two hours later in the day. Discovering the optimum calling time for each species of interest will certainly make results more creditable and helpful for monitoring programs. This is where automated recording devices become a useful field tool. When breeding sites are hard to gain access to, a person can make one-day trip to the site and install a recording device to do the job for them. The most positive attribute of automated recording devices is that they can record 24 hours a day, although this may not be necessary once optimum calling density is established; it can help determine the optimal calling time and provide precise monitoring protocols to assist in recording an animal that only calls on a single night out of a month, such as *Scaphiopus holbrookii*. As Peterson and Dorcas (1992) have pointed out, an advantage of an automatic recording system is the detection of infrequently calling species, such as *Rana catesbeiana*, which was only recorded twice during their 46-day study. Although Corn et al., (2000) recorded automated recording systems to be the highest cost out of call surveys; they collect a surplus of valuable information. Automated recording devices are promising tools for detection of rare species, and they provide data on phenology and behavior related to environmental conditions (Peterson and Dorcas 1994; Varhegyi et al., 1998) that are difficult to obtain by other means and provide a permanent record that can be revisited and reevaluated if needed. They could help detect rare species, quantify anurans in any breeding habitat, and improve the methods we rely on to monitor amphibians on a global scale.

Song Meter ™#1

Song Meter™ #1 was installed at a permanent pond in Beech Fork State Park. Song Meter™ #1, as well as Song Meter ™#2 and Song Meter ™#3, obtained recordings of *B. a. americanus* and *P. c. crucifer*. Phenology data for *R. palustris, R. clamitans melanota,* and *R. catesbeiana* was also recorded on Song Meter™ #1. Figures 2.0 - 2.4 show the daily calling activity of the animals detected. *Pseudacris c. crucifer* had an optimal calling time from 1900 to 2000 h, *B. a. americanus* from 2000 to 2100 h, *R. palustris* from 2000 to 2200 h, and *R. clamitans melanota* from 1900 to 2200 h. In this timeframe (1900 to 2200), these animals would be recorded if surveyed according to the NAAMP protocol. Data on *R. catesbeiana* calls did not supply enough information to determine an optimal calling time; this is most likely because the population was not copious enough.

Although Song Meter ™#1 was situated at a permanent pond it also had an ephemeral pool which was created in a shallow outlet as the water level of the pond increased. Most amphibians were located within the ephemeral pool since the attached permanent pond inhabited fish communities. For this reason, large populations of breeding amphibians may have avoided this site since fish generally prey on tadpoles, amphibian egg clutches, and adults. If a larger population had been present at this breeding site more phenology information could have been concluded. An interesting natural history note is that *B. a. americanus* only called in full chorus for 9 days at this site, from April 17, 2009 to April 26, 2009. *Bufo a. americanus* is labeled as an explosive breeder, with short breeding periods (Oseen and Wassersug, 2002), my findings support this also. There were few disturbances or background noise at this site and calls of most anurans were easily detected and determined (see Figures 5.0 and 5.2). Every species recorded in my field notebook for this site was also recorded on the Song Meter™.

Song Meter ™ #2

Song Meter ™ #2 was installed at a permanent wetland pool in Green Bottom Swamp. The recording device was previously mounted under high-voltage electrical wires and it unknowingly damaged the equipment so that all recordings had noticeable background static. In addition to the static on the spectrograms (see Figure 5.1), high quantities of traffic were also problematic as well as train and airplane noise. I do not feel confident making assumptions that an animal was recorded unless I could see its call in addition to hearing it on the Song Meter ™ recording;

therefore, only three anurans were recorded and analyzed with confidence, *B. a. americanus, P. c. crucifer,* and *R. pipiens.*

The calls of *B. a. americanus, P. c. crucifer,* and *R. pipiens* allowed them to be recognized on the spectrograph, despite high levels of background static and interference. On Figure 5.1 the advertisement call as well as the trill of *P. c. crucifer* is distinguishable through the static and interference.

The 10-day breeding period was also short, but with high calling densities (See Appendix). *Pseudacris c. crucifer* was the most aggressive caller at this site and often eliminated the possibility of hearing or seeing other anuran calls on the spectrographs. *Pseudacris c. crucifer* would generally start to call around 1900 h and maintain a high calling density until 2200 h, in which it would intermittently call until 0500 h. *Rana pipiens*, a species of concern that is being tracked by the WVDNR Heritage Program, was recorded on Song Meter TM #2. Although this information could prove significant for monitoring this animal, one must consider the possibility of collecting false negatives due to the static and interference. With regards to the previous statement, *R. pipiens* was found to have an optimum calling time from 2300 to 0000 h. The likelihood of the NAAMP protocol (surveying one hour after sunset) detecting this animal is low, since it does not call in abundance until after 2300. This would be something to consider if conducting a population density study on *R. pipiens. Rana catesbeiana* was expected to have high calling densities at this site, since they are restricted to open canopy ponds with permanent water (Skelly et al., 1999). *Rana catesbeiana* advertisement call is a loud bass note (Dorcas and Gibbons, 2008), which I would prefer to confirm visually on a spectrogram. The low-pitched call was not distinguishable with the presence of static and background noise on the spectrograms. This impeded their call from being confirmed visually; therefore, I did not consider this animal because the data may have contained false positives. An example of a false positive would be assuming *R. catesbeiana* was present on a spectrogram, when it was actually a mallard duck call contorted by static and a semi-truck passing by.

Song Meter ™ #3

Song Meter ™ #3 was stationed in Beech Fork State Park at a large ephemeral wetland. This meter recorded the highest number of species (8). Since this was the most remote site I surveyed, it had minimal background noise and disturbances to the calling choruses. Among *B. a. americanus* and *P. c. crucife*r, other calls included *R. palustris*, *R. clamitans melanota*, *R. catesbeiana*, *H. chrysoscelis*, *S. holbrookii*, and *R. sylvatica. Scaphiopus holbrookii* and *R. sylvatica* were each recorded briefly on a single segment on May 2, 2009 and April 5, 2009, respectively.

Green and Pauley (1987) have the earliest recorded date for *R. clamitans melanota* calling as April 25 in West Virginia, but the recordings from Song Meter ™ #3 identify *R. clamitans melanota* with a single call on April 5, 2009, having overlapping calls on April 12, 2009, and in full chorus on April 17, 2009. It is very fortunate that *S. holbrookii* was recorded even once, when considering that they call at any time after heavy rains in the spring, summer, or fall (Pauley, 2001). Song Meter ™ #3 recorded them calling at the Beech Fork State Park ephemeral pool site on a single clip during a series of warm nights (15-25°C), but with only occasional rainfall (.33in). Populations have been observed at Beech Fork State Park (Green and Pauley, 1987), although hearing their call can be a rare occurrence.

If the Song Meter ™ had been placed at the site earlier in the spring, more data may have been collected on *R. sylvatica*. I did note hearing them several nights before the Song Meters™ were installed. *Rana sylvatica* is an explosive breeder (Dorcas and Gibbons, 2008), since it will generally complete its breeding cycle in one week's time. Song Meter ™ #3 picked up the end of their breeding cycle. It would be interesting to put a Song Meter ™ out at the first of the year to determine when *R. sylvatica* emerges from hibernation since they are one of the earliest breeders, known to breed when ice can still be found on the water's surface (Dorcas and Gibbons, 2008; Green and Pauley, 1987).

Comparative Studies with ARS

In a study conducted in South Carolina by Bridges and Dorcas (2000), daily calling patterns for *H. chrysoscelis*, *R. clamitans melanota*, and *R. catesbeiana* were determined. *Hyla chrysoscelis* showed peaks from sunset until about midnight, I found its optimal calling time to be from 2000 through 2200 h, which would agree with Bridges and Dorcas's data. *Rana clamitans melanota* called throughout the day but peak calling occurred after midnight, between 0100 and 0530 h; I found the optimal calling time to be anywhere from 2000 to 0500 h. *Rana catesbeiana* called throughout the day, peak-calling activity occurred between 0200 and 0600 h; 0500 h was the determined optimal calling time from my study.

Effects of Environmental Factors on Anuran Calling

Influence of environmental factors, such as temperature and percent relative humidity on calling activity of anurans has been debated throughout the literature. In my study air temperature and percent relative humidity data loggers were adhered to each Song Meter ™ to observe if these environmental factors had a consistent influence on anuran calling densities. A study conducted by

Oseen and Wassersug (2002) concluded that calls of spring breeding species *R. sylvatica*, *P. c. crucifer*, and *B. a. americanus* were most associated with the time of day (i.e. they called primarily at night), while summer breeding species, *R. clamitans melanota* and *R. catesbeiana*, were associated primarily with high water temperature. As determined by Oseen and Wassersug (2002) explosive breeders, such as *R. sylvatica* and *B. a. americanus,* have relatively short breeding periods and were determined to respond to fewer environmental variables (air and water temperature, rainfall, barometric pressure, relative humidity, and wind velocity) than species with prolonged breeding periods (*P. c. crucifer*, *R. clamitans melanota*, and *R. catesbeiana*). Water temperature was the single environmental variable, which predicted calling activity for most species (Oseen and Wassersug, 2002). It is suggested that future studies with ARS include water temperature data loggers to find evidence to support this theory in West Virginia.

Notes on Bufonidae Calling Phenology

Bufo a. americanus had a long breeding phase at Song Meter^{TM} #3 with calls recorded from April 5, 2009 to April 21, 2009 with high call indexes; single calls were recorded up until May 1, 2009. Even though the breeding season for *B. fowleri* is reported to be in mid April in lower elevations of West Virginia (Green and Pauley, 1987), their call was not identified on any of the three Song Meters^{TM} recordings. The most likely breeding site for *B. fowleri* would have been at Green Bottom Swamp (Song Meter #2). Green and Pauley (1987) state that Fowler's toad breeds in more permanent bodies of water such as streams and along lakeshores. Although some people may confuse the key characteristics used to separate *B. a. americanus* from *B. fowleri,* their advertisement calls are unmistakably different. Whereas the call of *B. a. americanus* is a long high-pitched musical trill, *B. fowleri's* call is a 1-4 second "*waaahh*" or nonmusical buzzing (Dorcas and Gibbons, 2008). Since the spring of 2009 was unusually cool this lowered my expectations of hearing overlapping calls among the *Bufo* species at my study sites.

Automated Recording Systems in West Virginia

West Virginia provides a diversity of breeding habitats for amphibians to complete their breeding cycles. More often than not, the best breeding sites are found in remote areas of the state. One of the main reasons these areas are termed as "good breeding spots" is due to the low levels of disturbances. In an effort to survey theses areas, yet not distress or impair the breeding choruses, an automated recording system is clearly a well-founded option. As presented by Dorcas and Foltz (1991) and Peterson and Dorcas (1992, 1994), the systems allow for extended sample periods, minimal disturbance to calling anurans, and provide a permanent sampling recorded allowing repeated evaluation by multiple investigators. In the past decade, ARS have been used to evaluate variation in amphibian populations and have been implemented in numerous monitoring programs that are based on calling surveys (Crouch and Paton, 2002; Vandewalle et al., 1996). Bridges and Dorcas (2000) state that perhaps the most important factor automated recording systems can offer is data can be used to develop models to optimize effectiveness of manual call surveys. If breeding choruses are not surveyed appropriately, temporal and spatial variation in calling patterns can result in failure to detect certain species, thus underestimating the number of species calling and overestimating population fluctuations (Crouch and Paton, 2002; Mohr and Dorcas, 1999; Peterson and Dorcas, 1992).

Proposed ARS Protocol for West Virginia

One of the main objectives of this study was to determine if automated recording systems were reliable devices to record spring calling anurans in West Virginia. After comparing my field notes on calling anurans to the number of species detected by the Song Meter ™, I encourage the use of automated recording systems to detect spring anurans in West Virginia. The most significant finding from the recording systems is that an appropriate surveying protocol for spring anurans of West Virginia can be established. From my study, I can suggest the following recommendations: Automated recording systems should be positioned at anuran breeding sites as early as the first of February; recorders should be placed away from roads, electric lines, or other background noises; the user friendly systems such Song Meter ™ by Wildlife Acoustics is particularly effective because of its operating temperatures (-20°C to +70°C), durability and clear recordings in harsh weather. Recording intervals, at a minimal, should be at least 30 seconds in every hour to avoid collecting partial calls from species such as *R. pipiens,* (*Rana palustris* has a similar call, but the two can be easily distinguished by grunting at the end of *R. pipiens* call) and recording intervals should not exceed one minute every half hour. If using a Song Meter ™ recorder, the recommended method to analyze data is Song Scope ™ Bioacoustics Software. Although recording systems are vulnerable to theft, vandalism, and damage by wildlife, they are still excellent tools to use to establish monitor protocols. They are especially suited for remote mountainous areas such as Canaan Valley. Overall, this study has revisited the natural history information on a common species that is abundant in West Virginia and created a protocol that may be followed for using automated recording systems in West Virginia. The proposition that amphibian populations are undergoing a worldwide decline in numbers is somewhat weakened by the absence of long-term information on "typical" or "natural" patterns of numerical fluctuation in such populations (Blaustein et al., 1994). Long-term studies focusing on natural history characteristics are becoming less common, and such baseline information is essential to an objective evaluation of the hypothesis of global amphibian decline (Blaustein et al., 1994). These studies are becoming rare, especially in graduate programs, but the effort is needed in a time of global amphibian declines.

APPENDIX Song Meter ™ #2

Time	*B. a. americanus*	*P. c. crucifer*	*R. pipiens*	Date	Temp	% RH
6:00	0	0	0	03/30/09	4	81
6:30	0	0	0	03/30/09	3.5	77
7:00	0	0	0	03/30/09	3.5	74
7:30	0	0	0	03/30/09	3.5	72
8:00	0	0	0	03/30/09	3.5	72
8:30	0	0	0	03/30/09	5	69
9:00	0	0	0	03/30/09	5.5	65
18:00	0	1	0	03/30/09	18.5	28
18:30	0	3	0	03/30/09	19	24
19:00	0	3	0	03/30/09	19	22
19:30	0	3	0	03/30/09	14.5	33
20:00	0	3	0	03/30/09	10.5	45
20:30	0	3	0	03/30/09	9	56
21:00	0	3	0	03/30/09	7.5	59
21:30	0	3	0	03/30/09	6.5	69
22:00	0	3	0	03/30/09	6	68
22:30	0	3	0	03/30/09	5.5	70
23:00	0	3	3	03/30/09	4.5	77
23:30	0	3	3	03/30/09	4.5	75
0:00	0	3	3	03/30/09	3.5	81
0:30	0	2	2	03/31/09	2.5	81
1:00	0	3	3	03/31/09	2.5	83
1:30	0	2	2	03/31/09	1.5	88
2:00	0	2	1	03/31/09	1	87
2:30	0	2	2	03/31/09	0.5	88
3:00	0	2	1	03/31/09	0.5	91
3:30	0	2	1	03/31/09	0.5	91
4:00	0	3	1	03/31/09	0	93
4:30	0	2	0	03/31/09	0	92
5:00	0	2	0	03/31/09	-0.5	93
5:30	0	2	0	03/31/09	-0.5	98
6:00	0	0	0	03/31/09	-1	96
6:30	0	0	0	03/31/09	-1	94
7:00	0	0	0	03/31/09	-1.5	96
7:30	0	0	0	03/31/09	-1.5	98
8:00	0	0	0	03/31/09	-0.5	97
8:30	0	0	0	03/31/09	1.5	97
9:00	0	0	0	03/31/09	5.5	79
18:00	0	1	0	03/31/09	20	19
18:30	0	2	0	03/31/09	19	23
19:00	0	3	0	03/31/09	17	38
19:30	0	3	0	03/31/09	15.5	58
20:00	0	3	0	03/31/09	14.5	61
20:30	0	3	0	03/31/09	13	64
21:00	2	3	0	03/31/09	12.5	67
21:30	0	3	0	03/31/09	11	73
22:00	0	3	0	03/31/09	10.5	75
22:30	0	3	0	03/31/09	10	75
23:00	1	3	3	03/31/09	9.5	77
23:30	0	3	2	03/31/09	10.5	66
0:00	0	3	3	03/31/09	13	49
0:30	0	2	3	04/01/09	11	66
1:00	0	3	3	04/01/09	10.5	68
1:30	0	3	3	04/01/09	10.5	68
2:00	0	2	3	04/01/09	11.5	61
2:30	0	3	3	04/01/09	9.5	73

3:00	0	3	3	04/01/09	9.5	75
3:30	0	3	2	04/01/09	9.5	77
4:00	0	3	3	04/01/09	10	76
4:30	0	3	3	04/01/09	10	82
5:00	0	2	2	04/01/09	10	90
5:30	0	0	0	04/01/09	10	90
6:00	0	2	0	04/01/09	10	95
6:30	0	0	0	04/01/09	10	93
7:00	0	1	0	04/01/09	10.5	96
7:30	0	0	0	04/01/09	10.5	95
8:00	0	0	0	04/01/09	10.5	97
8:30	?	?	?	04/01/09	11	97
9:00	0	1	0	04/01/09	11	96
18:00	0	1	0	04/01/09	20	19
18:30	0	1	0	04/01/09	21	17
19:00	0	3	0	04/01/09	20.5	22
19:30	0	3	0	04/01/09	19	21
20:00	0	3	0	04/01/09	13	40
20:30	0	3	0	04/01/09	11	47
21:00	0	3	0	04/01/09	9.5	52
21:30	0	3	0	04/01/09	8	61
22:00	0	3	0	04/01/09	7.5	70
22:30	0	3	2	04/01/09	6	68
23:00	0	3	2	04/01/09	5	77
23:30	0	3	3	04/01/09	4.5	80
0:00	0	2	3	04/01/09	3.5	82
0:30	0	3	3	04/02/09	3	85
1:00	0	2	1	04/02/09	3	86
1:30	0	2	0	04/02/09	2.5	91
2:00	0	2	0	04/02/09	1.5	90
2:30	0	2	0	04/02/09	1.5	92
3:00	0	2	0	04/02/09	1	91
3:30	0	2	0	04/02/09	1.5	97
4:00	0	2	0	04/02/09	1	97
4:30	0	1	0	04/02/09	1	95
5:00	0	1	0	04/02/09	1	97
5:30	0	3	0	04/02/09	1	96
6:00	0	1	0	04/02/09	0.5	97
6:30	0	0	0	04/02/09	1	98
7:00	0	0	0	04/02/09	1	102
7:30	0	0	0	04/02/09	1.5	99
8:00	0	0	0	04/02/09	2.5	98
8:30	0	0	0	04/02/09	7	84
9:00	0	0	0	04/02/09	11.5	63
18:00	0	0	0	04/02/09	23.5	37
18:30	0	1	0	04/02/09	22.5	39
19:00	0	3	0	04/02/09	21	43
19:30	0	3	0	04/02/09	19.5	49
20:00	0	3	0	04/02/09	19	51
20:30	0	3	3	04/02/09	18.5	53
21:00	1	3	2	04/02/09	18	54
21:30	2	3	3	04/02/09	17.5	57
22:00	2	3	3	04/02/09	17	60
22:30	3	3	3	04/02/09	16	65
23:00	2	3	2	04/02/09	16	65
23:30	1	3	3	04/02/09	15.5	67
0:00	2	2	3	04/02/09	15.5	69
0:30	2	3	3	04/03/09	15	72
1:00	3	3	3	04/03/09	15	74
1:30	2	3	1	04/03/09	14	84
2:00	2	2	2	04/03/09	14	88
2:30	1	2	2	04/03/09	13.5	93
3:00	2	2	3	04/03/09	13.5	93
3:30	2	2	3	04/03/09	13.5	93
4:00	3	3	3	04/03/09	13	96
4:30	3	2	2	04/03/09	13	95
5:00	3	2	2	04/03/09	13	95
5:30	3	3	3	04/03/09	13.5	93

6:00	1	1	0	04/03/09	14	88
6:30	0	2	0	04/03/09	14	82
7:00	2	0	0	04/03/09	13	84
7:30	0	0	0	04/03/09	13	84
8:00	0	0	0	04/03/09	13	84
8:30	1	0	0	04/03/09	13	88
9:00	2	1	0	04/03/09	13	90
18:00	0	0	0	04/03/09	10.5	74
18:30	1	0	0	04/03/09	10.5	74
19:00	0	3	0	04/03/09	11	70
19:30	1	3	0	04/03/09	10.5	69
20:00	2	3	0	04/03/09	10	69
20:30	2	3	0	04/03/09	10	68
21:00	3	3	0	04/03/09	10.5	68
21:30	3	3	0	04/03/09	10	70
22:00	1	3	0	04/03/09	9.5	72
22:30	3	3	0	04/03/09	9	77
23:00	1	3	0	04/03/09	9	71
23:30	0	3	0	04/03/09	8.5	67
0:00	1	3	3	04/03/09	8	67
0:30	1	3	2	04/04/09	7.5	68
1:00	1	3	2	04/04/09	7	65
1:30	1	2	0	04/04/09	7	63
2:00	1	3	1	04/04/09	7	64
2:30	1	3	3	04/04/09	6.5	65
3:00	1	2	0	04/04/09	6.5	67
3:30	1	2	0	04/04/09	6.5	68
4:00	0	3	0	04/04/09	6.5	68
4:30	0	3	0	04/04/09	6	76
5:00	0	2	0	04/04/09	4.5	80
5:30	0	1	0	04/04/09	3.5	86
6:00	0	0	0	04/04/09	2	86
6:30	0	0	0	04/04/09	1.5	90
7:00	0	0	0	04/04/09	1	92

7:30	0	0	0	04/04/09	1	96
8:00	0	0	0	04/04/09	1.5	97
8:30	0	0	0	04/04/09	4.5	97
9:00	0	0	0	04/04/09	7.5	72
18:00	0	0	0	04/04/09	19.5	26
18:30	0	0	0	04/04/09	19.5	23
19:00	1	3	1	04/04/09	19	27
19:30	3	3	0	04/04/09	18.5	29
20:00	3	3	0	04/04/09	12.5	42
20:30	3	3	0	04/04/09	10	51
21:00	3	3	0	04/04/09	8.5	59
21:30	2	3	0	04/04/09	7.5	65
22:00	2	3	3	04/04/09	7	67
22:30	1	3	1	04/04/09	6	72
23:00	1	1	1	04/04/09	4.5	77
23:30	1	2	1	04/04/09	4	82
0:00	3	2	2	04/04/09	3.5	83
0:30	2	3	2	04/05/09	3	86
1:00	1	1	0	04/05/09	3	88
1:30	2	3	0	04/05/09	2.5	88
2:00	3	2	0	04/05/09	2	90
2:30	2	3	0	04/05/09	2	91
3:00	2	3	0	04/05/09	1.5	92
3:30	0	3	0	04/05/09	1	93
4:00	2	3	1	04/05/09	1	95
4:30	0	3	1	04/05/09	1	95
5:00	0	2	0	04/05/09	2	98
5:30	0	0	0	04/05/09	1.5	93
6:00	0	0	0	04/05/09	1.5	97
6:30	0	0	0	04/05/09	0.5	96
7:00	0	0	0	04/05/09	0.5	98
7:30	0	0	0	04/05/09	0.5	97
8:00	0	0	0	04/05/09	2.5	98
8:30	0	0	0	04/05/09	4	94

9:00	0	0	0	04/05/09	7	82
18:00	3	0	0	04/05/09	27	20
18:30	0	0	0	04/05/09	25	26
19:00	3	3	0	04/05/09	24	36
19:30	3	3	0	04/05/09	20.5	49
20:00	3	3	3	04/05/09	19	47
20:30	3	3	3	04/05/09	18	51
21:00	3	3	3	04/05/09	17.5	53
21:30	3	3	2	04/05/09	20	31
22:00	3	3	0	04/05/09	22.5	31
22:30	3	3	0	04/05/09	22	33
23:00	3	3	0	04/05/09	21	45
23:30	3	3	0	04/05/09	15.5	87
0:00	3	3	1	04/05/09	15	81
0:30	3	3	3	04/06/09	14	74
1:00	3	2	1	04/06/09	14	72
1:30	3	3	0	04/06/09	13	77
2:00	3	3	0	04/06/09	13	82
2:30	3	2	1	04/06/09	12.5	83
3:00	3	2	1	04/06/09	12.5	84
3:30	3	3	1	04/06/09	11.5	87
4:00	3	3	2	04/06/09	12	88
4:30	3	3	0	04/06/09	12	88
5:00	3	3	0	04/06/09	12	87
5:30	3	3	0	04/06/09	12	82
6:00	3	1	0	04/06/09	11.5	72
6:30	3	1	0	04/06/09	11.5	70
7:00	3	0	0	04/06/09	11	70
7:30	3	0	0	04/06/09	10.5	75
8:00	2	0	0	04/06/09	10.5	79
8:30	0	0	0	04/06/09	9.5	78
9:00	0	0	0	04/06/09	8.5	83
18:00	2	1	0	04/06/09	6.5	84
18:30	2	3	0	04/06/09	5	86

19:00	2	3	0	04/06/09	5	85
19:30	3	3	0	04/06/09	4.5	87
20:00	1	3	0	04/06/09	4	86
20:30	1	3	0	04/06/09	4	86
21:00	2	3	0	04/06/09	4	86
21:30	2	3	0	04/06/09	3.5	83
22:00	2	3	1	04/06/09	3.5	82
22:30	2	3	0	04/06/09	3.5	84
23:00	1	3	1	04/06/09	3	84
23:30	2	3	0	04/06/09	3	86
0:00	3	3	0	04/06/09	3	86
0:30	1	3	0	04/07/09	3	87
1:00	0	3	0	04/07/09	3	86
1:30	0	3	0	04/07/09	3	77
2:00	0	3	0	04/07/09	2	64
2:30	1	3	0	04/07/09	1.5	67
3:00	0	2	0	04/07/09	1.5	69
3:30	0	2	0	04/07/09	1.5	71
4:00	0	1	0	04/07/09	1.5	73
4:30	0	2	0	04/07/09	1.5	72
5:00	0	2	0	04/07/09	1.5	73
5:30	0	2	0	04/07/09	1	73
6:00	0	0	0	04/07/09	0	72
6:30	0	0	0	04/07/09	0.5	73
7:00	0	0	0	04/07/09	1	74
7:30	0	0	0	04/07/09	1	74
8:00	0	0	0	04/07/09	1	71
8:30	0	0	0	04/07/09	1	71
9:00	0	0	0	04/07/09	1.5	70
18:00	0	0	0	04/07/09	5	56
18:30	0	0	0	04/07/09	5	57
19:00	0	0	0	04/07/09	5.5	54
19:30	3	3	0	04/07/09	4	57
20:00	0	3	0	04/07/09	3.5	59

20:30	0	3	0	04/07/09	3.5	60	22:00	0	3	0	04/08/09	3.5	70
21:00	0	3	0	04/07/09	4	59	22:30	0	2	0	04/08/09	3	74
21:30	0	3	0	04/07/09	4	63	23:00	0	3	0	04/08/09	2	75
22:00	0	2	0	04/07/09	4	68	23:30	0	2	0	04/08/09	1.5	80
22:30	0	3	0	04/07/09	4	71	0:00	0	2	1	04/08/09	1	84
23:00	0	2	0	04/07/09	3.5	72	0:30	0	2	0	04/09/09	2	85
23:30	0	1	0	04/07/09	3	71	1:00	0	3	0	04/09/09	2	87
0:00	0	2	0	04/07/09	3	66	1:30	0	2	0	04/09/09	2	87
0:30	0	2	0	04/08/09	3.5	61	2:00	0	2	0	04/09/09	2.5	87
1:00	0	2	0	04/08/09	4	64	2:30	0	0	0	04/09/09	2.5	86
1:30	0	2	0	04/08/09	3	67	3:00	0	3	0	04/09/09	2	88
2:00	0	2	0	04/08/09	1.5	72	3:30	0	0	0	04/09/09	1	87
2:30	0	3	0	04/08/09	1	75	4:00	0	2	0	04/09/09	0.5	91
3:00	0	2	0	04/08/09	2.5	67	4:30	0	1	0	04/09/09	0	90
3:30	0	2	0	04/08/09	2.5	62	5:00	0	2	0	04/09/09	-0.5	92
4:00	0	1	0	04/08/09	2.5	63	5:30	0	3	0	04/09/09	-0.5	95
4:30	0	2	0	04/08/09	2	65	6:00	0	0	0	04/09/09	-1	93
5:00	0	0	0	04/08/09	1.5	65	6:30	0	0	0	04/09/09	-1	94
5:30	0	0	0	04/08/09	1.5	67	7:00	0	0	0	04/09/09	-1.5	95
6:00	0	0	0	04/08/09	1	67	7:30	0	0	0	04/09/09	-1	95
6:30	0	0	0	04/08/09	1.5	70	8:00	0	0	0	04/09/09	-0.5	95
7:00	0	0	0	04/08/09	1	69	8:30	0	0	0	04/09/09	3.5	91
7:30	0	0	0	04/08/09	1	72	9:00	0	0	0	04/09/09	6	79
8:00	0	0	0	04/08/09	2	71	18:00	0	0	0	04/09/09	21	19
8:30	0	0	0	04/08/09	4.5	62	18:30	0	2	0	04/09/09	19.5	29
9:00	0	0	0	04/08/09	5.5	56	19:00	0	2	0	04/09/09	18	24
18:00	0	0	0	04/08/09	13	29	19:30	3	3	0	04/09/09	16.5	26
18:30	0	3	0	04/08/09	13	29	20:00	2	3	0	04/09/09	13	40
19:00	0	2	0	04/08/09	12	28	20:30	2	3	0	04/09/09	12	49
19:30	2	3	0	04/08/09	11.5	31	21:00	2	3	0	04/09/09	10.5	51
20:00	2	3	0	04/08/09	10.5	34	21:30	1	3	0	04/09/09	10	62
20:30	0	3	0	04/08/09	7.5	49	22:00	2	3	0	04/09/09	9.5	61
21:00	0	3	0	04/08/09	5.5	61	22:30	2	3	0	04/09/09	9.5	67
21:30	1	3	0	04/08/09	4.5	65	23:00	3	3	0	04/09/09	9.5	72

23:30	2	2	0	04/09/09	9.5	67
0:00	2	2	0	04/09/09	9.5	76
0:30	1	3	0	04/10/09	9.5	71
1:00	3	3	0	04/10/09	9	75
1:30	2	3	0	04/10/09	9	73
2:00	2	3	0	04/10/09	8	76
2:30	0	3	0	04/10/09	7.5	78
3:00	1	3	0	04/10/09	7	81
3:30	2	3	0	04/10/09	7	82
4:00	0	2	0	04/10/09	6.5	80
4:30	0	2	0	04/10/09	7	81
5:00	0	0	0	04/10/09	8	74
5:30	1	3	0	04/10/09	8	83
6:00	0	0	0	04/10/09	8	84
6:30	0	0	0	04/10/09	7.5	81
7:00	0	0	0	04/10/09	7.5	83
7:30	0	0	0	04/10/09	7.5	83
8:00	0	0	0	04/10/09	8	84
8:30	0	0	0	04/10/09	10.5	73

9:00	0	0	0	04/10/09	11.5	61
18:00	0	0	0	04/10/09	14.5	87
18:30	2	2	0	04/10/09	14	90
19:00	3	3	0	04/10/09	13.5	92
19:30	2	3	0	04/10/09	13	90
20:00	3	3	0	04/10/09	13	92
20:30	3	2	0	04/10/09	12.5	94
21:00	2	3	0	04/10/09	12	93
21:30	3	3	0	04/10/09	12	95
22:00	1	3	0	04/10/09	12	96
22:30	1	3	0	04/10/09	11.5	97
23:00	0	3	0	04/10/09	11.5	97
23:30	0	3	0	04/10/09	11.5	97
0:00	0	3	0	04/10/09	11.5	97
0:30	0	3	0	04/11/09	11.5	98
1:00	0	3	0	04/11/09	11.5	97
1:30	0	2	0	04/11/09	11	94
2:00	0	3	0	04/11/09	10.5	95
2:30	0	3	0	04/11/09	10.5	94

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TABLES

Table 1.0 GPS coordinates of *Bufo a. americanus* sampling of 2008 through 2009.

Location	GPS Coordinates	Density Sampled
Beech Fork State Park, Ephemeral Wetland	N 38° 18.322' W 082° 20.280'	High
Beech Fork State Park, Permanent Pond	N 38° 18.269' W 082° 20.516'	Moderate
Mill Creek Wildlife Refuge, Ephemeral Pools	N 38° 29.495' W 082° 07.111'	Low
Green Bottom Wildlife Refuge, Permanent Wetland	N 38° 35.019' W 082° 14.037'	Low
Canaan Valley State Park, Permanent Pond	N 39° 00.482' W 079° 26.328'	Low
Canaan Valley State Park, Permanent Pond	N 39° 02.606' W 079° 24.352'	Low
Timberline Resort, Permanent Pond	N 39° 02.606' W 07° 23.971'	High
Teter Farm, Ephemeral Pool	N 38° 58.066' W 79° 29.341'	Low

Table 1.1 Summary of pair-wise comparisons of six morphological characters between two populations of *Bufo a. americanus* in Cabell and Mason counties (L) and Canaan Valley (H).

T-Test Results					
Characteristic	Mean	Std. Deviation	SEM	T Value	P Value
Snout-vent Length	67.906 (L)	5.253 (L)	0.743 (L)	-4.967	<0.001
	74.411 (H)	3.572 (H)	0.820 (H)		
Parotoid Gland Area	85.586 (L)	15.452 (L)	2.185 (L)	-4.315	<0.001
	104.695 (H)	18.842 (H)	4.323 (L)		
Mann-Whitney Rank Sum Test Results					
Characteristic	Median	25%	75%	T Value	P Value
Tibia Length	27.100 (L)	26.000 (L)	28.200 (L)	1018.500	<0.001
	30.400 (H)	29.400 (H)	32.000 (H)		
Eye Diameter	8.400 (L)	8.000 (L)	9.000 (L)	802.000	0.067
	8.800 (H)	8.800 (H)	9.000 (H)		
T. Membrane Diameter	8.400 (L)	8.000 (L)	9.000 (L)	190.000	<0.001
	6.100 (H)	6.000 (H)	6.600 (H)		
Snout Length	7.000 (L)	5.600 (L)	8.200 (L)	451.000	0.004
	5.600 (H)	5.250 (H)	6.000 (H)		

Table 1.2 Summary of pair-wise comparisons of six morphological characters between male (M) and female (F) *Bufo a. americanus* at all GPS coordinates found in Table 1.0.

T-Test Results

Characteristic	Mean	Std. Deviation	SEM	T Value	P Value
Snout-vent Length	69.687 (M)	5.642 (M)	0.679 (M)	5.042	<0.001
	76.360 (F)	6.892 (F)	1.258 (F)		
Parotoid Gland Area	90.848 (M)	18.438 (M)	2.220 (M)	-5.652	<0.001
	113.883 (F)	19.098 (F)	3.487 (F)		

Mann-Whitney Rank Sum Test Results

Characteristic	Median	25%	75%	T Value	P Value
Tibia Length	27.400 (M)	26.400(M)	30.000 (M)	1962.500	<0.001
	30.100(F)	28.800 (F)	31.400 (F)		
Eye Diameter	8.800 (M)	8.000 (M)	9.000 (M)	2230.500	<0.001
	9.700 (F)	9.000 (F)	10.400 (F)		
T. Membrane Diameter	6.000 (M)	5.400 (M)	6.600 (M)	1868.000	0.005
	6.600 (F)	6.000 (F)	7.400 (F)		
Snout Length	6.200 (M)	5.400 (M)	7.450 (M)	1598.500	0.456
	6.400 (F)	5.800 (F)	7.200 (F)		

Table 1.3 Morphometric notes on hybridization, a summary of observed key characteristics of *Bufo a. americanus.*

Characteristic	Detail	All Animals Measured	Low Elevation	High Elevation
Maximum Number of Warts per Spot	2 or Less	78.8%	73.4%	96%
	3 or More	21.2%	26.6%	4%
Condition of Warts on Tibia	Spiny	94%	99%	79.1%
	Not Spiny	6%	1%	20.8%
Abdominal Spot Percentage	0-25	47%	48%	37.5%
	26-50	20%	24%	12.5%
	51-75	13%	14.6%	12.5%
	76-100	19%	13.4%	37.5%
Cranial Ridge Position	97% had 1-2 spurs from the cranial ridge were touching the parotoid gland, 3 animals from Canaan Valley had no spurs touching the parotoid gland			

Table 1.4 Summary of *Bufo a. americanus* egg strand and tadpole data.

Clutch Number Location	Associated with vegetation?	Depth of Egg Deposition	Disturbed Egg Strand?	Mean		Observed	
				Water Temp	RH	Dates	Gosner Stage
#1, Roadside Ditch at Beech Fork State Park	NO	N/A	NO	20	N/A	4/21/08	20
#2, Ephemeral Pool at Beech Fork State park	NO	Not greater than 80mm	Yes, mosquito larvae and water mold observed on 4/9/09	24-29	21%	4/5/09-	16-17;
#3, Ephemeral Pool at Beech Fork State Park	NO	0-25mm	Yes, by animal and water mold observed on 4/5/09	26.5	49.5%	4/5/09; 4/29/09	20-22; 33-34
#4, Ephemeral Pool at Beech Fork State park	YES	N/A	Yes, by water mold observed on 4/9/09	27	37	4/6/09	10
#5, Permanent Pond at Beech Fork State Park	YES, *Juncus*	0-30mm	Yes, severe water decrease on 4/29/09	24.5	70	4/27/09; 4/29/09	24-25; 24-25

Table 2.0 Data summary of Song Meter™#1, permanent pond at Beech Fork State Park.									
Species Recorded	Began Calling	Stopped Calling	**Optimal Calling Time (h)**	Low Temp. (°C)	High Temp. (°C)	**Mean Temp. (°C)**	Low %RH	High %RH	**Mean %RH**
B. a. americanus	4/2	4/26	**2000-2100**	6.5	40	**16**	7	100	**72.7**
P. c. crucifer	3/27	5/3	**1900-2000**	5	39	**15**	12	104	**65.8**
R. palustris	3/27	5/3	**2000-0200**	6	19.5	**12**	28	93	**60**
R. clamitans melanota	4/9	4/29	**1900-2200**	9.5	31.5	**29**	12	91	**34**
R. catesbeiana	4/24	5/2	**N/A**	11	51.5	**27**	8	93	**50**

Table 2.1 Data summary of Song Meter™#2, permanent wetland at Green Bottom Wildlife Refuge.									
Species Recorded	Began Calling	Stopped Calling	**Optimal Calling Time (h)**	Low Temp. (°C)	High Temp. (°C)	**Mean Temp. (°C)**	Low %RH	High %RH	**Mean %RH**
B. a. americanus	3/31	4/10	**1900-2100**	2	24	**13**	20	96	**69**
P. c. crucifer	3/30	4/11	**1900-2200**	-.05	22.5	**9.5**	21	98	**67**
R. pipiens	3/30	4/6	**0000-0100**	2.5	19	**11**	47	96	**71.5**

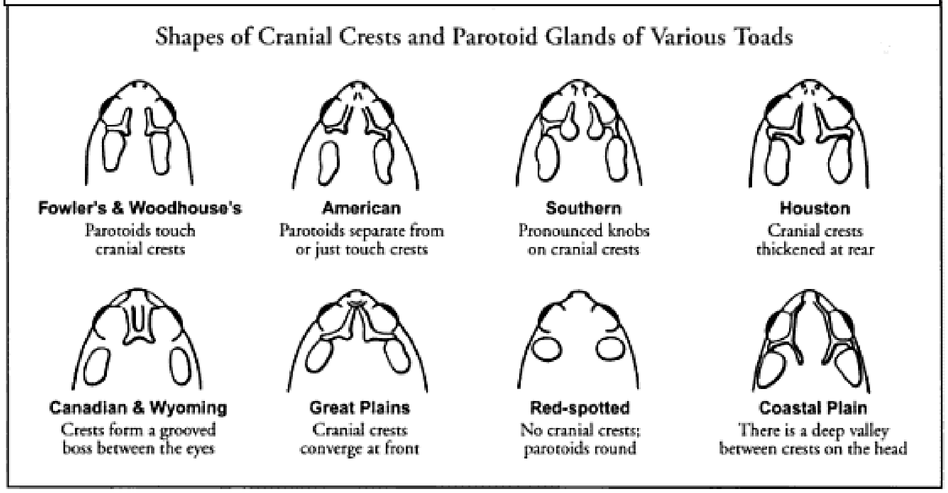
Table 2.2 Data summary of Song Meter™#3, ephemeral wetland at Beech Fork State Park.

Species Recorded	Began Calling	Stopped Calling	**Optimal Calling Time (h)**	Low Temp. (°C)	High Temp. (°C)	**Mean Temp.**	Low %RH	High %RH	**Mean %RH**
B. a. americanus	4/5	4/21	**2100-2300**	0.5	30	**16.3**	10	97	**46.5**
P. c. crucifer	4/5	5/2	**1900-2000**	-2.5	31.5	**12**	5	101	**61.5**
R. palustris	4/8	4/30	**2200-0200**	-.05	31.5	**10.5**	10	100	**50**
R. clamitans melanota	4/12	5/3	**2000-0500**	2.5	35	**22**	14	98	**37**
R. catesbeiana*	4/22	5/1	**0500**	10.5	35	**23**	13	95	**54**
R. chrysoscelis	4/22	5/1	**2000-2200**	6	29	**20**	29	91	**52**
R. holbrookii*	5/3	5/3	**0030**	N/A	N/A	**22**	N/A	N/A	**68**
R. sylvatica*	4/5	4/5	**1700**	N/A	N/A	**9**	N/A	N/A	**72**

Note: *Only one instance of this species calling was recorded. **This species only reached a calling density of overlapping calls (2), not full chorus (3).

FIGURES

Figure 1.0 Comparison of cranial crests and parotoid gland positions on various toads.



Illustrations from the *Peterson Field Guide to Reptiles and Amphibians of Eastern and Central North America* by Roger Conant and Joseph T. Collins © 1998 by Houghton Mifflin Company

Figure 1.1 Abdmoninal spot percentage of *Bufo a. americanus* in West Virginia from all GPS coordinates listed in Table 1.0.

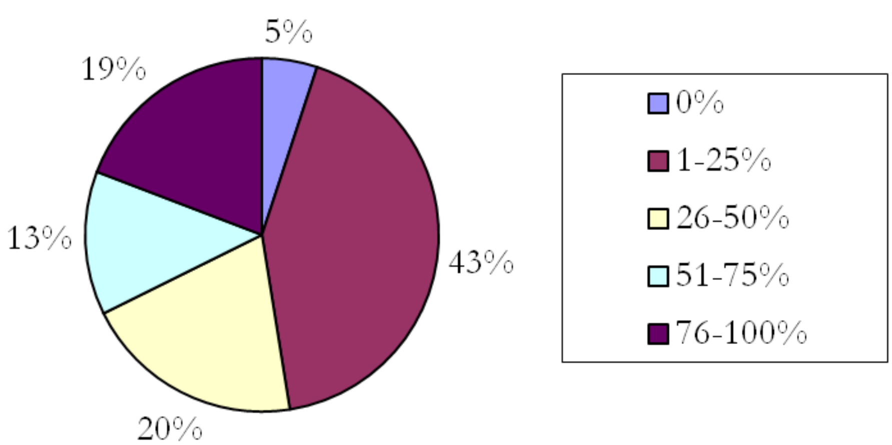


Figure 2.0 Daily calling activity of *Bufo a. americanus* from a permanent pond at Beech Fork State Park.

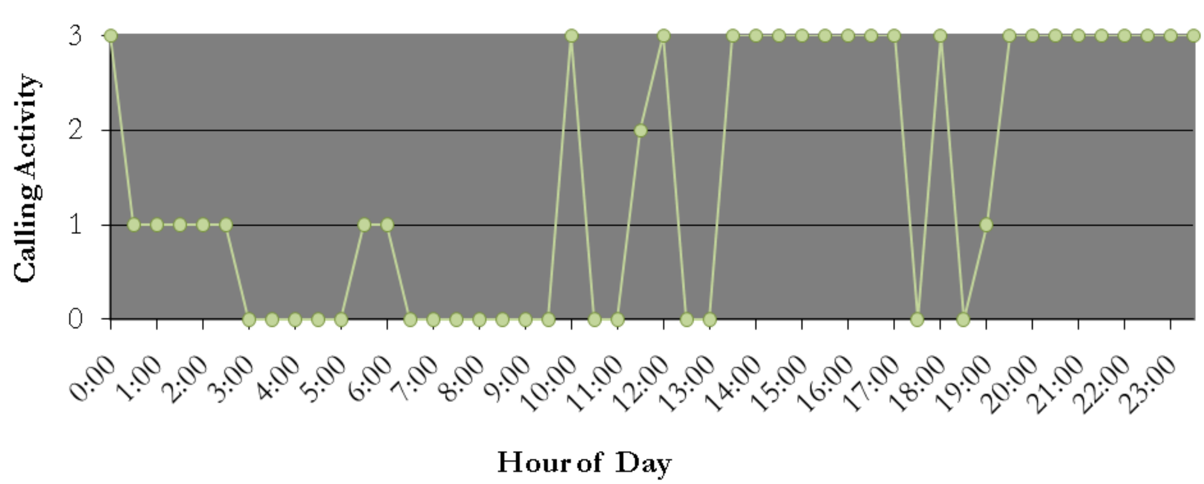


Figure 2.1 Daily calling activity of *Pseudacris c. crucifer* from a permanent pond at Beech Fork State Park.

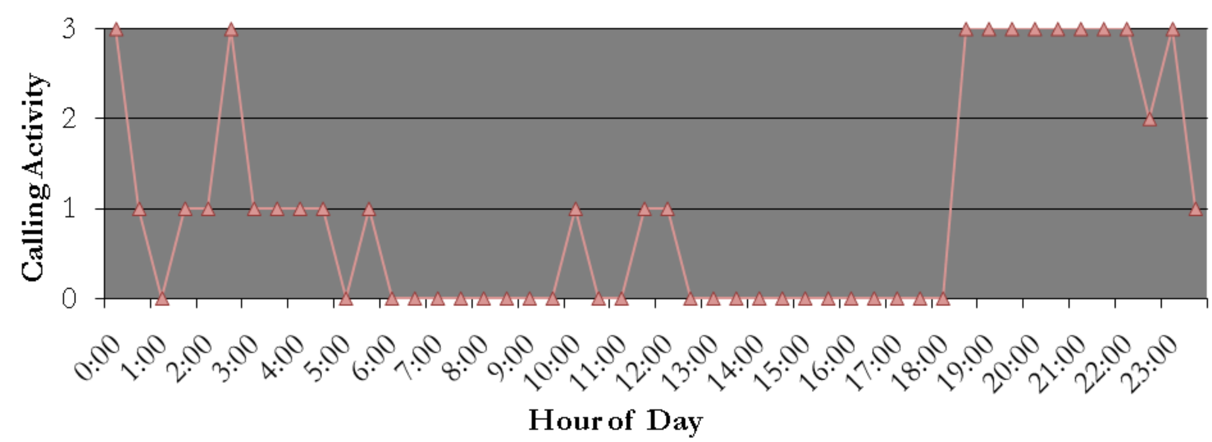


Figure 2.2 Daily calling activity of *Rana palustris* from a permanent pond at Beech Fork State Park.

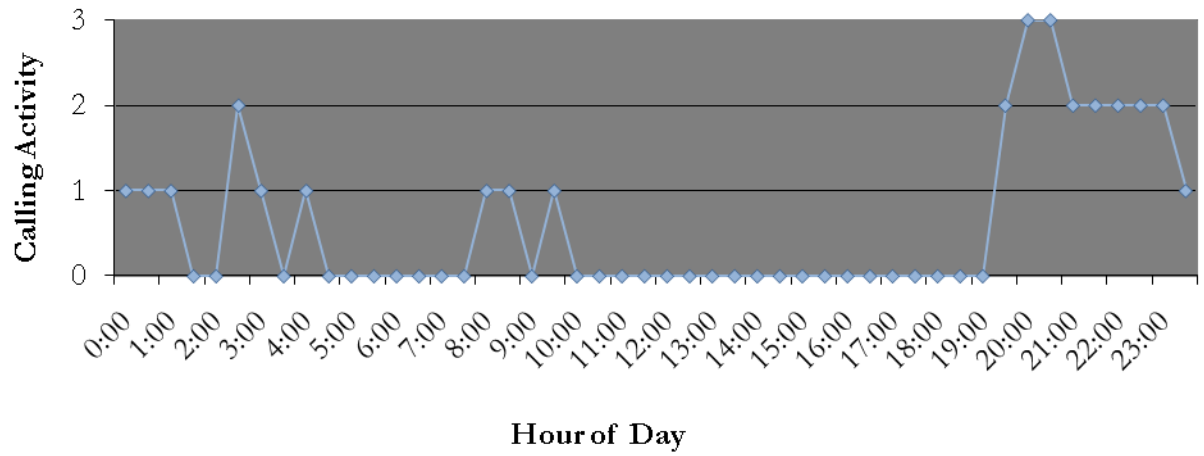
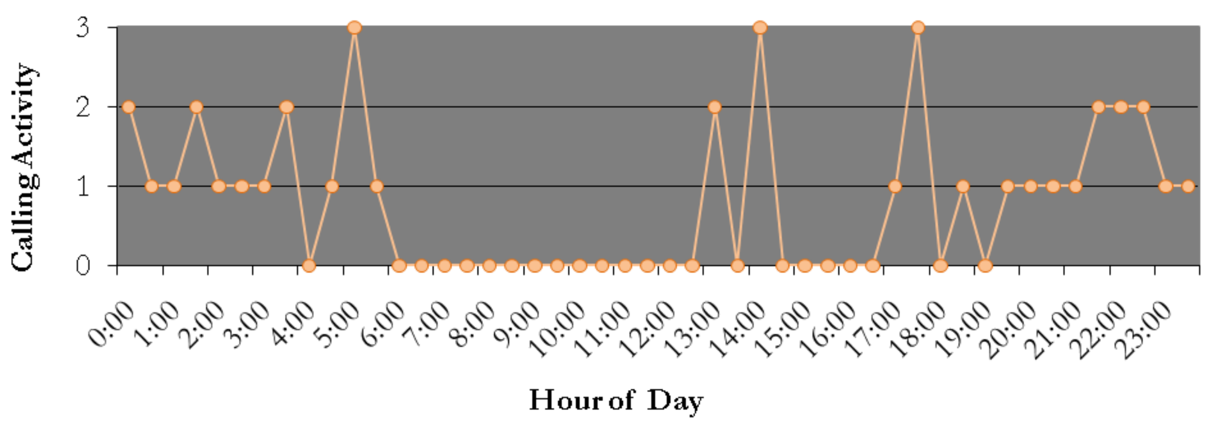


Figure 2.3 Daily calling activity of *Rana clamitans melanota* from a permanent pond at Beech Fork State Park.



2.4 Daily calling activity of *Rana catesbeiana* from a permanent pond at Beech Fork State Park.

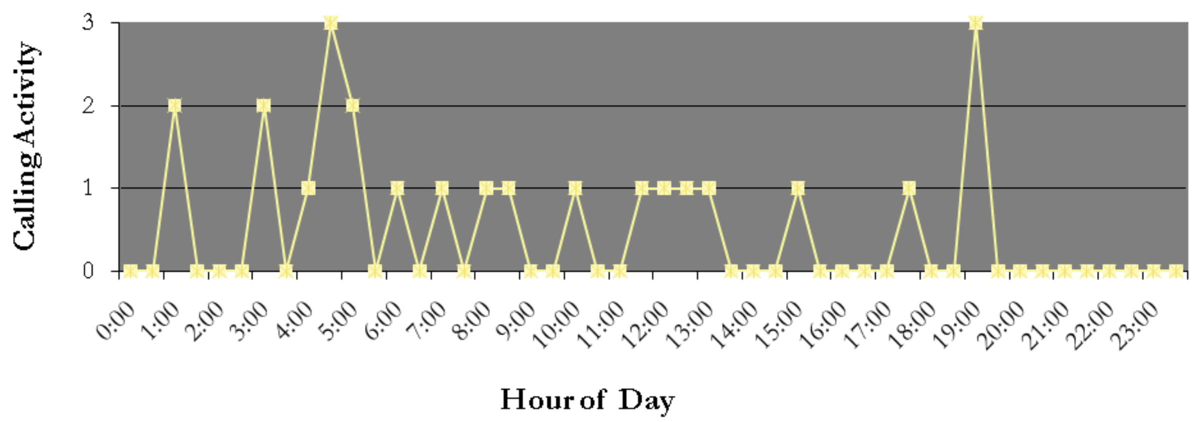


Figure 2.5 Calling anuran species recorded over a 36-day study at a permanent pond in Beech Fork State Park.

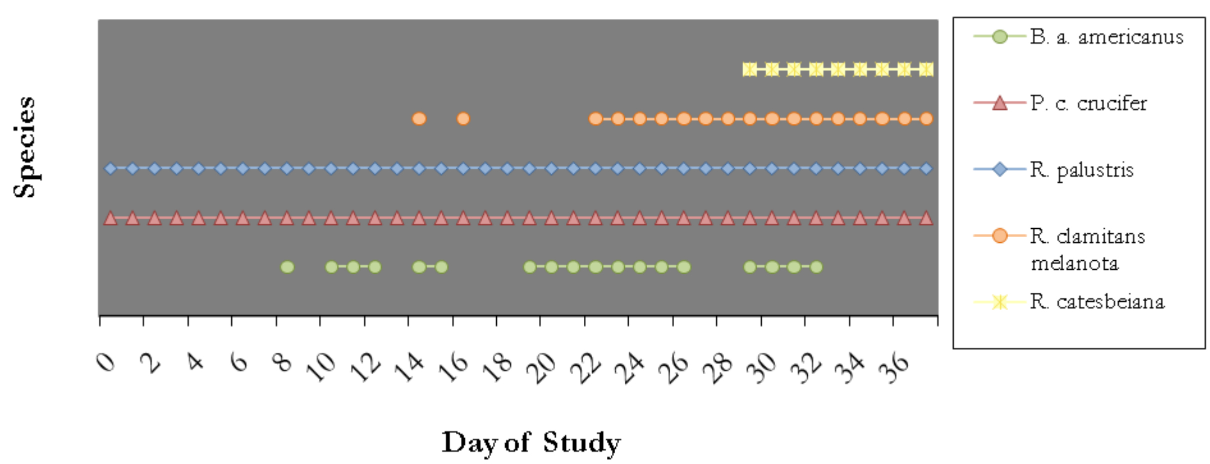


Figure 3.0 Daily calling activity of *Bufo a. americanus* from a permanet wetland in Green Bottom Wildlife Refuge.

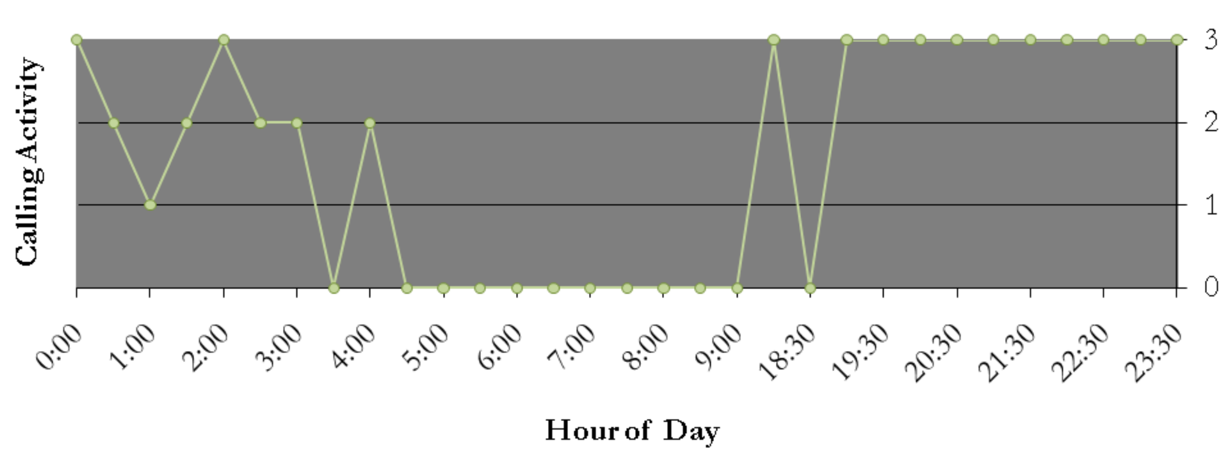


Figure 3.1 Daily calling activity of *Pseudacris c. crucifer* from a permanet wetland in Green Bottom Wildlife Refuge.

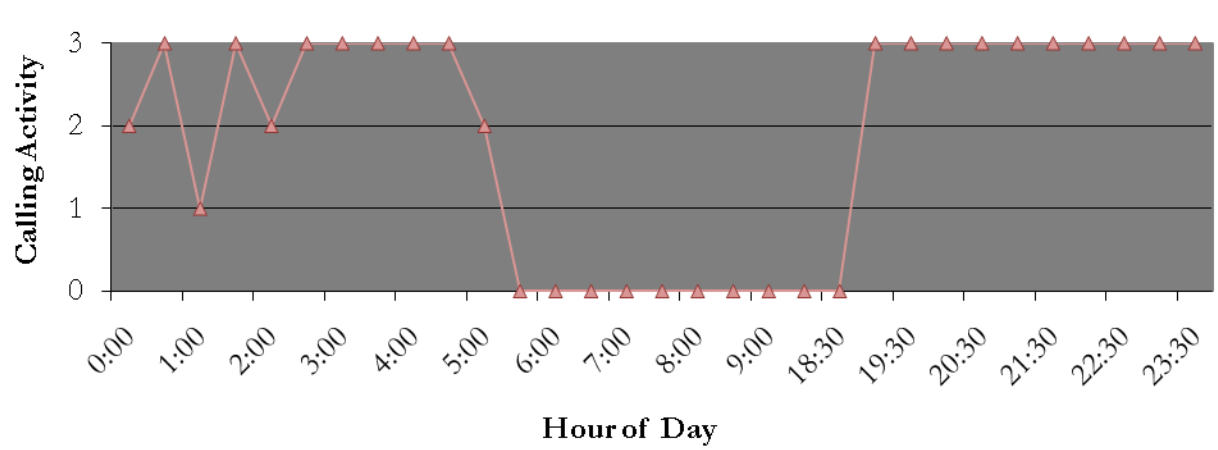


Figure 3.2 Daily calling activity of *Rana pipiens* from a permanet wetland in Green Bottom Wildlife Refuge.

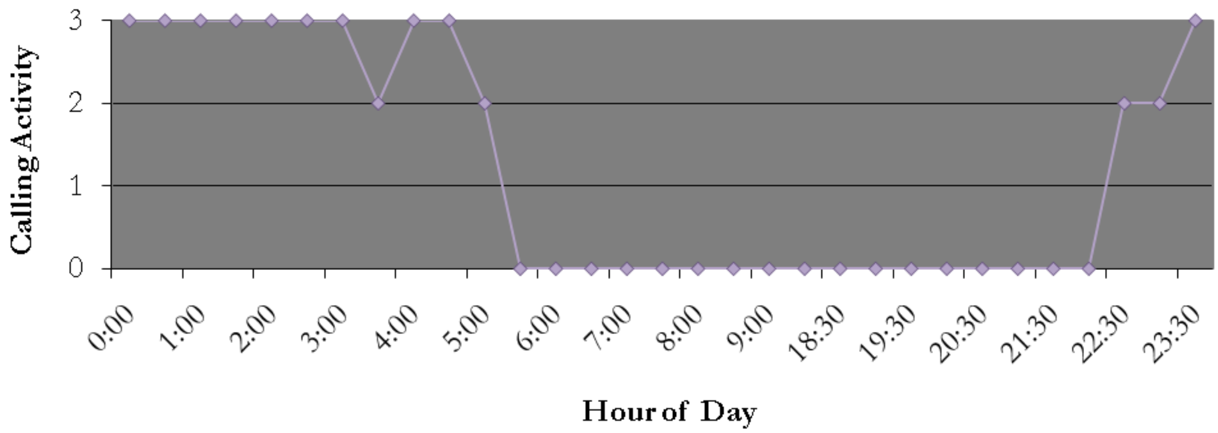


Figure 3.3 Calling anuran species recorded over the 12-day study at Green Bottom Wildlife Refuge.

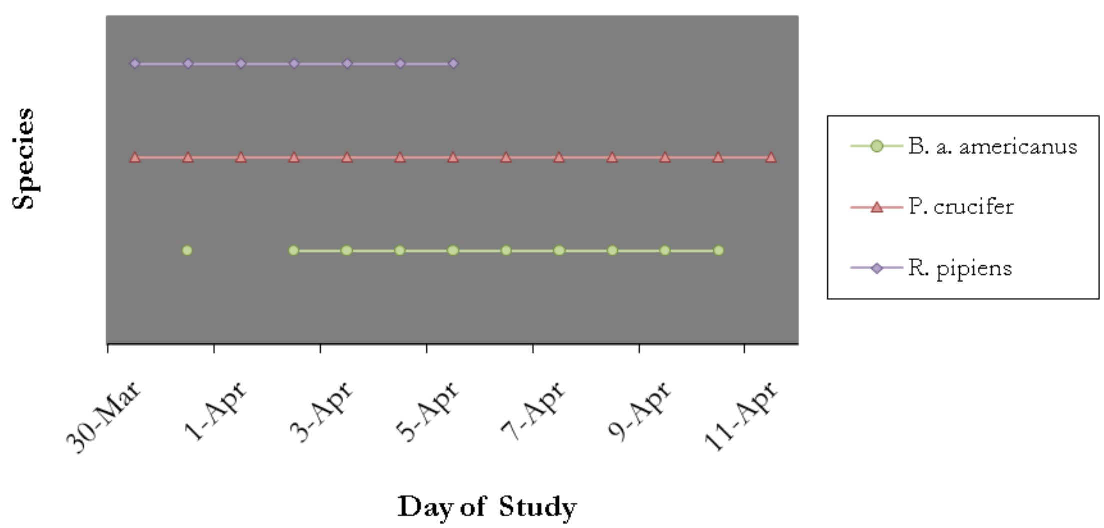


Figure 4.0 Daily calling pattern of *Bufo a. americanus* in Beech Fork State Park at an ephemeral pool.

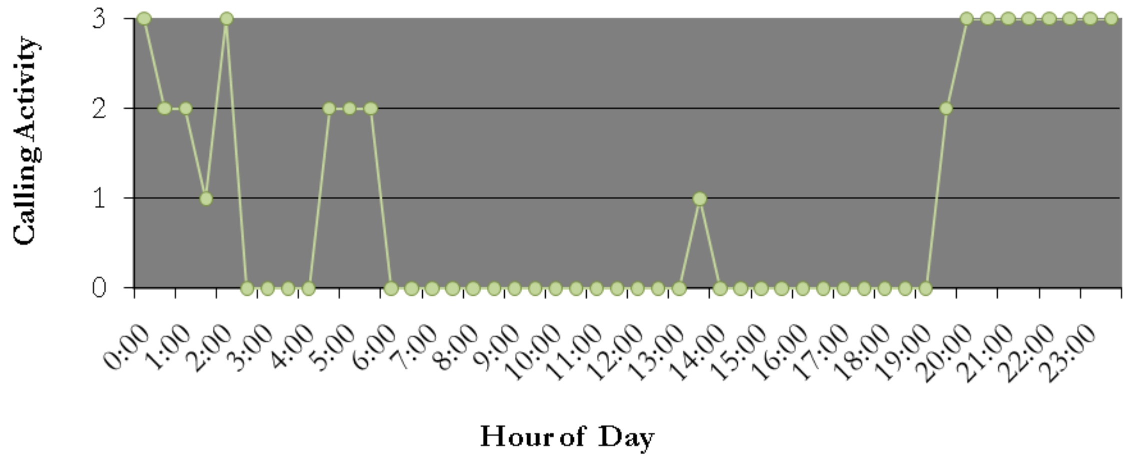


Figure 4.1 Daily calling pattern of *Pseudacris c. crucifer* in Beech Fork State Park at an ephemeral pool.

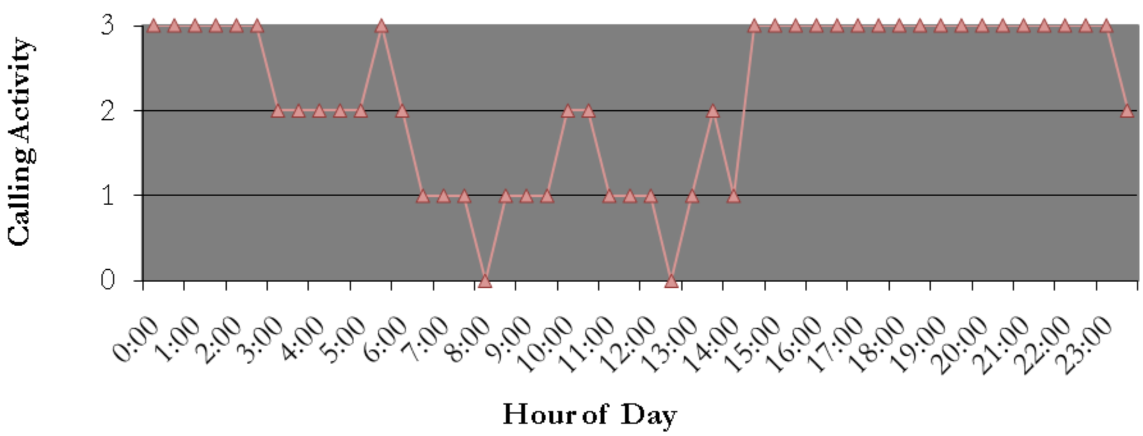


Figure 4.2 Daily calling pattern of *Rana palustris* in Beech Fork State Park at an ephemeral pool.

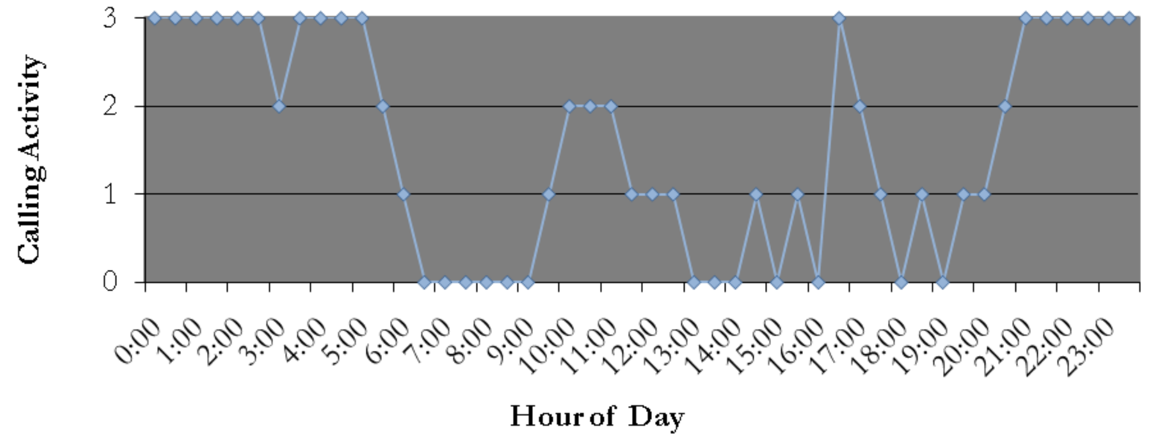


Figure 4.3 Daily calling pattern of *Rana catesbeiana* in Beech Fork State Park at an ephemeral pool.

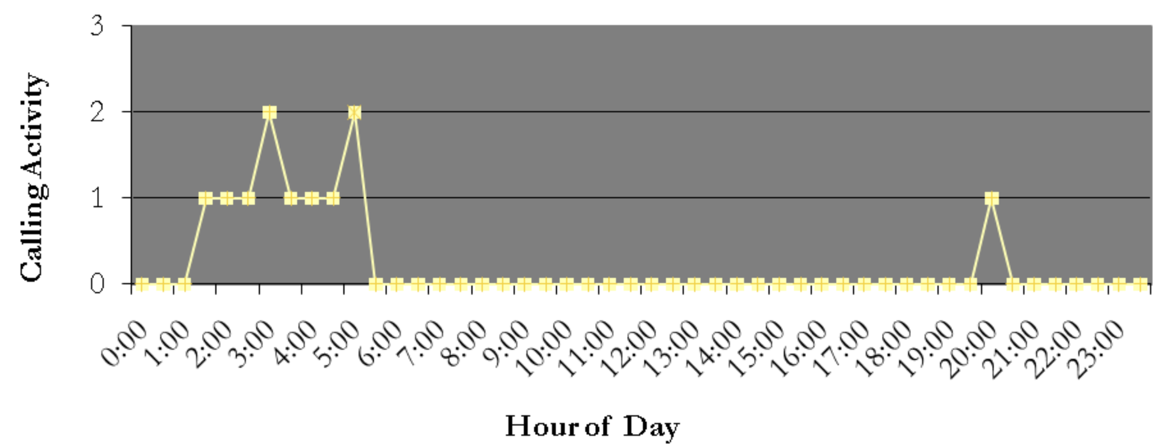


Figure 4.4 Daily calling pattern of *Rana clamitans melanota* in Beech Fork State Park at an ephemeral pool.

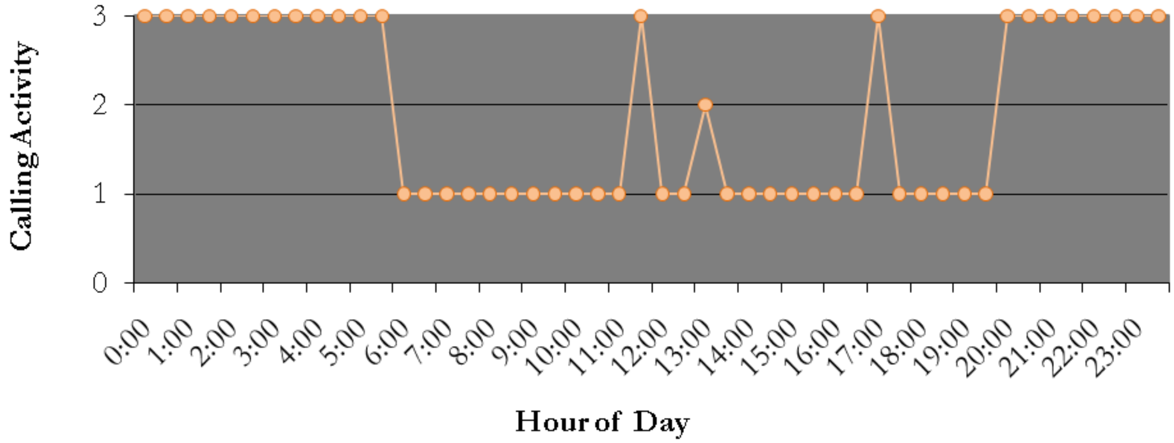


Figure 4.5 Daily calling pattern of *Hyla chrysoscelis* in Beech Fork State Park at an ephemeral pool.

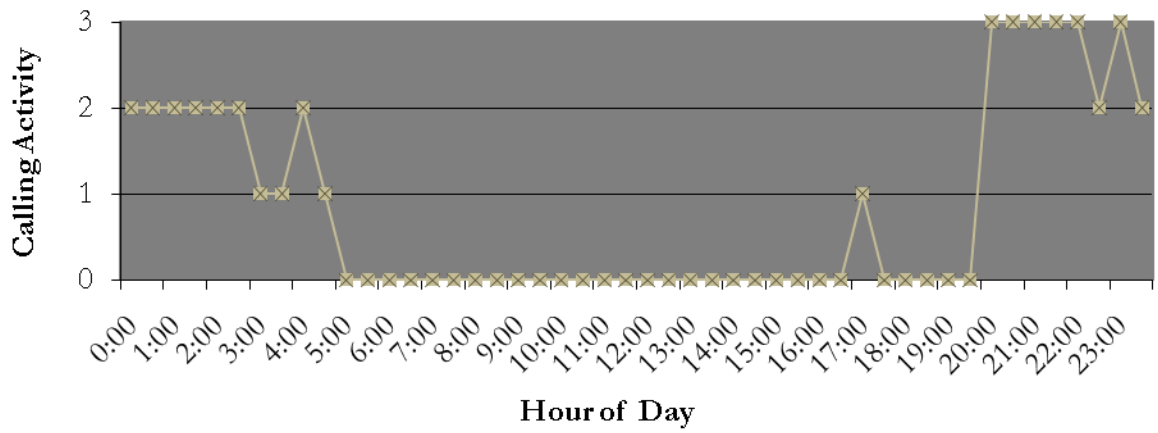
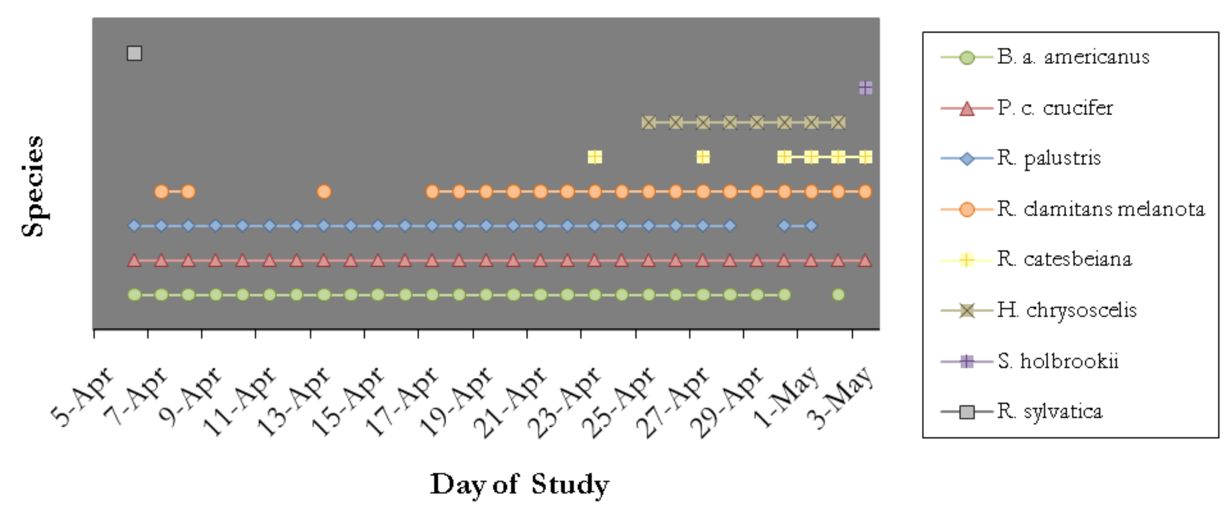


Figure 4.6 Calling anuran species recorded over the 29-day study at an ephemeral wetland in Beech Fork State Park.



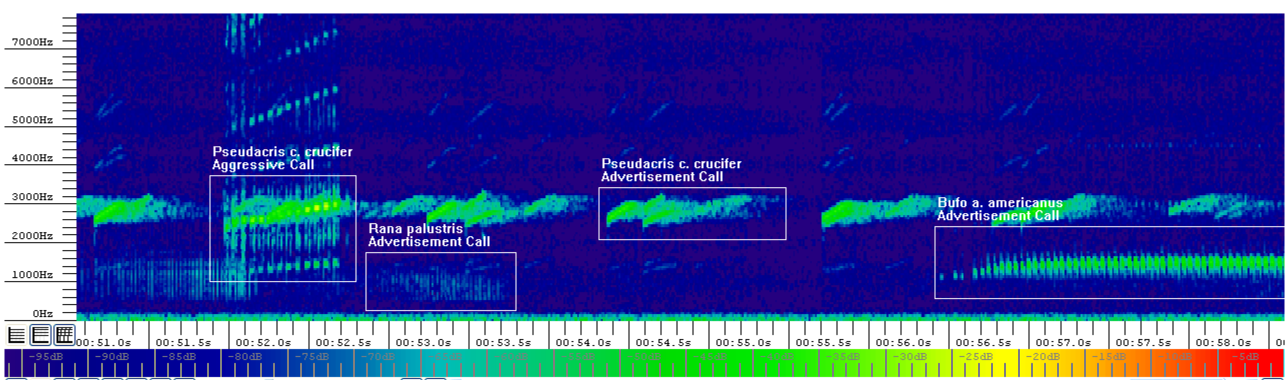


Figure 5.0 Excerpt from a Song Meter™ recording at an ephemeral wetland in Beech Fork State Park, note the difference in *Pseudacris c. crucifer* aggressive and advertisement call. Anuran advertisement calls are labeled for example to display the differences in shape, time, and hertz range.

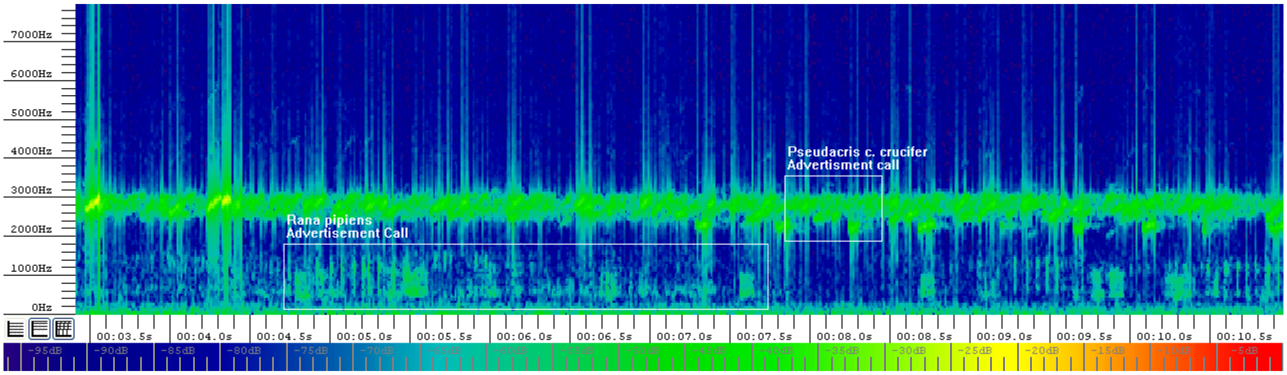


Figure 5.1 Excerpt from a Song Meter™ recording at a permanent wetland in Green Bottom Swamp; note how the static has distorted the animal's calls in comparison to other recording excerpts.

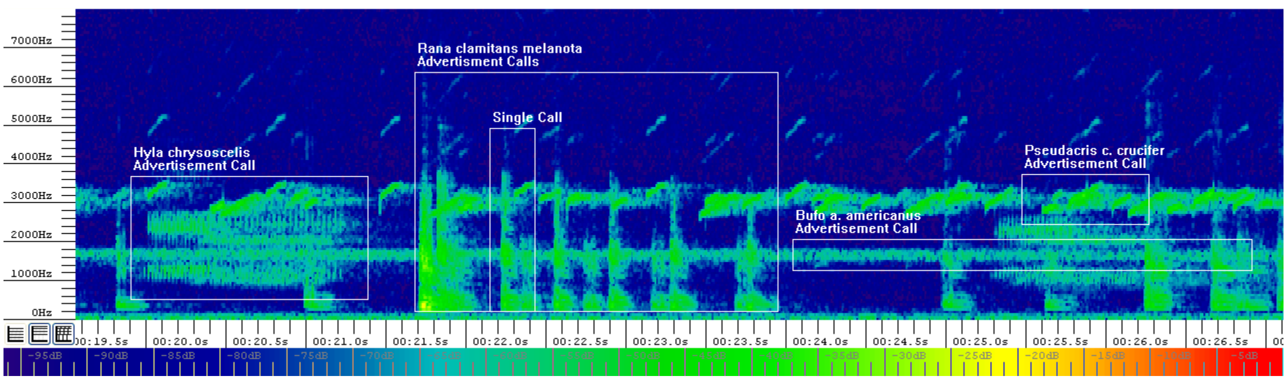


Figure 5.2 Excerpt from a Song Meter™ at an ephemeral wetland in Beech Fork State Park; note the overlapping of species calls. In this excerpt *R. clamitans melanota* was rated with a call index of 3 (full chorus).

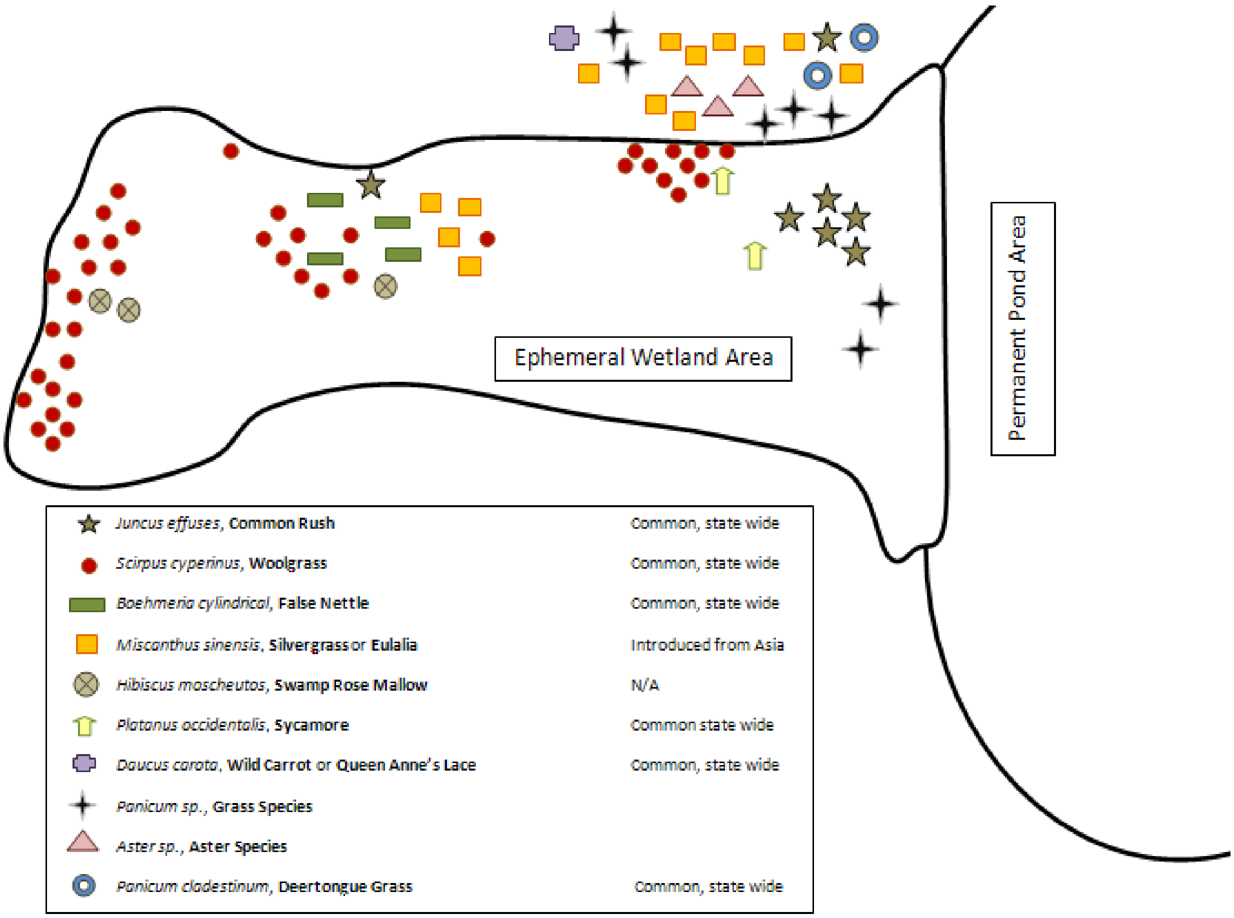


Figure 6.0 Vegetation summary of ephemeral wetland area at site #3, Beech Fork State Park permanent pond. Several clutches of *B. a. americanus* eggs were discovered in the ephemeral wetland area in late April intertwined around the vegetation.

PLATES

(All photos were taken by Tomi M. Bergstrom)

Plate 1.0 Example of sexual dimorphism between *B. a. americanus* sexes; female on the left and male on the right.

Plate 1.1 Example of range of abdominal spotting on *B. a. americanus*; 26-50% pictured on the left and 76-100% pictured on the right.

Plate 1.2 Presence of water mold (white irregular embryos) on *B. a. americanus* egg strand (left photo) and evidence of egg deposition disturbance (bottom photo) showing a deceased adult and eggs infected with water mold.

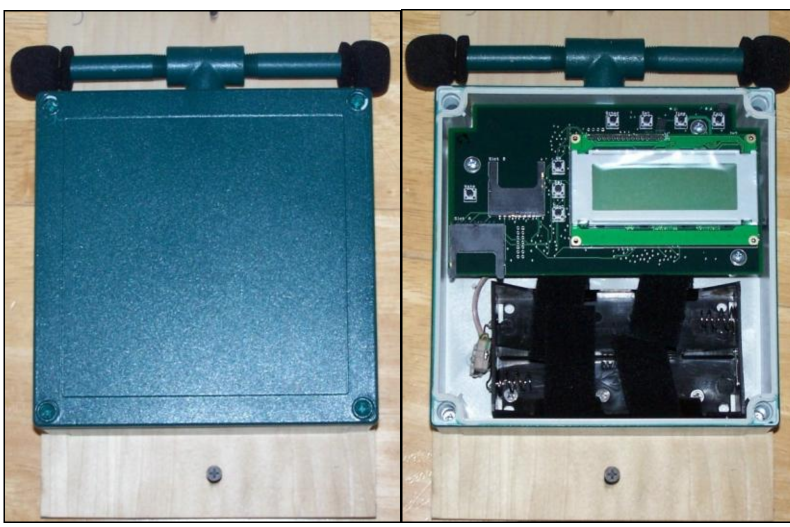


Plate 2.0 Song Meter™ closed (left) and opened (right). When the case is closed it is weatherproofed from the elements. Each meter was attached to a wooden board that would then be mounted to a tree at the field site.

Plate 2.1 Song Meter^{TM}#2 mounted at Green Bottom Swamp Wildlife Refuge. Meters were mounted after the model used by USGS National Wetlands Research Center.

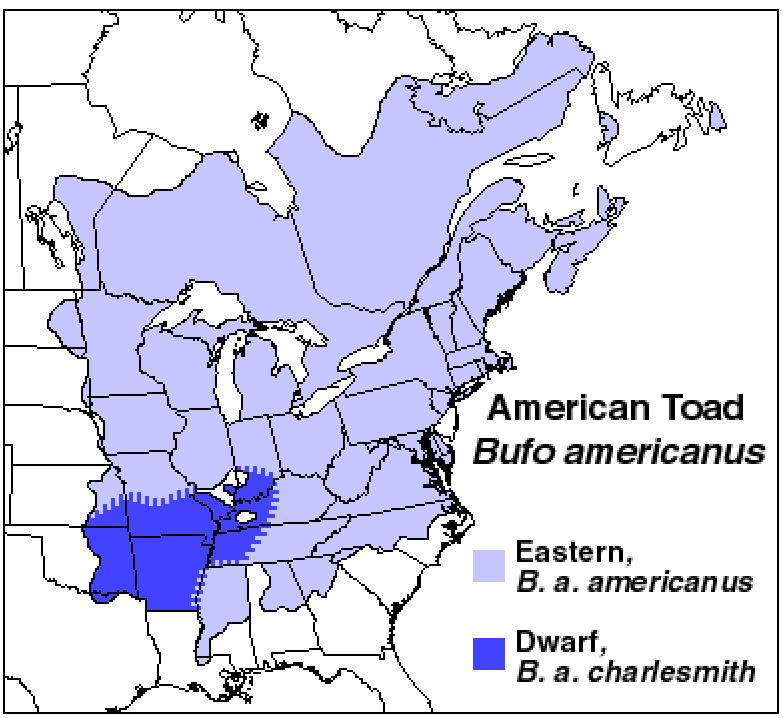


Plate 3.0 Distribution map of American toad subspecies, map adopted by the USGS: http://www.npwrc.usgs.gov/narcam/idguide/american.htm.