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Status of the Small-mouthed Salamander, Ambystoma texanum (Mathes) in West Virginia

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By

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ABSTRACT

Status of the Small-mouthed Salamander, *Ambystoma texanum* (Mathes) in West Virginia.

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Over the past 60 years little natural history information on *Ambystoma texanum* (Small-mouthed Salamander) has been collected in West Virginia, the eastern most location for the species. Three historical breeding populations of *A. texanum* have been documented in the state, but salamanders from 2 of these locations have not been documented for the past 30 years. Searches of historical sites and favorable habitat in Mason, Jackson, and Wood counties yielded no evidence for the presence of *A. texanum*, except for the known breeding site in Wood County. Information on time of breeding and egg development was collected at the Wood County breeding site. Eggs were in late stages of development with one clutch hatching on April 17, 2001. Six of the 24 captured adult *A. texanum* at the Wood County site showed various limb and eye malformations. Data collection ended when the breeding pool dried up on May 13, 2001.

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CHAPTER I

Introduction

Purpose of Study

Green (1940) first reported the presence of *Ambystoma texanum* in Wayne County for West Virginia. However he stated that all efforts to find more specimens were unsuccessful. *Ambystoma texanum* has been found since 1940 in Mason and Wood counties. Kraus and Petranka (1989) split *A. texanum* into two sibling species, *A. texanum* and *A. barbouri* by their tooth morphology and ecological differences, with *A. barbouri* being a new species. Longbine et al. (1991) found that *A. barbouri* was the species in Wayne County and *A. texanum* in Mason and Wood counties.

Populations of *A. texanum* have been eliminated throughout their range as floodplain forests have been cleared and converted into agricultural fields and other developments (Petranka, 1998). *Ambystoma texanum* is found in West Virginia only in the Ohio River floodplain, which has undergone habitat changes or destruction over the past 100 plus years. *Ambystoma texanum* is currently listed as a S1 species in West Virginia by the Nongame Wildlife and Natural Heritage Program, which means that the species may be critically imperiled in the state. The global rank is a G5, “Demonstrable secure globally” documented by the Natural Heritage Network.

Except for information on the dates of salamanders collected at the known breeding ponds little natural history information has been collected for *A. texanum* in West Virginia. There also has not been a documented report of the *A. texanum* in Mason County for over 30 years. The purpose of this study was to collect natural history information for the species in West Virginia and search for old and new populations.

Distribution

Ambystoma texanum is currently known from Ohio westward to southern Wisconsin, south to eastern Kansas, and Texas, throughout Louisiana, Mississippi, and Alabama (Figure 1.1). Disjunct populations occur in southeastern Indiana, southern Ohio and western West Virginia (Petranka, 1998). Currently the only known breeding population in West Virginia is in Wood County (Figure 1.2).

Description

Ambystoma texanum is a medium sized mole salamander with a small head and mouth (Green and Pauley, 1987; Petranka, 1998). The dorsal color is an olive-brown to dark gray with a speckled or lichen pattern that increases along the sides of the body and down the tail (Figure 1.3). The ventral side has a darker base color with fewer markings. Black immaculate specimens have been recorded (Allyn and Shockley, 1939). Adults range from 110 to 190 mm total length with 14 costal grooves (Smith, 1934; Green and Pauley, 1987; Downs, 1989). Downs (1989), found that females measure 3-11% longer in SVL (snout-vent length) than males. Adpressed limbs are most often separated by two to four intercostals spaces.

Hatchlings are from 7 to 14 mm in length (Liner, 1954; Minton, 1972) depending on stage of development at time of hatching. They are a dark gray-green to brown with 3 to 6 light yellowish to olive green saddles of paired blotches along the midline of the back (Petranka, 1998). The throat is darkly pigmented and the belly has a light coloration (Downs, 1989). Within a few weeks of transformation the juvenile salamanders start to acquire the adult color pattern.

Habitat

The most common habitat of *A. texanum* is mesic deciduous forests. They are also found in poorly drained bottomland floodplains and in drier upland forest with small ponds (Petranka, 1998). Minton (1972) found that *A. texanum* is able to survive in close proximity to human disturbances such as agriculture fields and houses. Within the habitat, they are often found in the tunnels of crayfish and the burrows on moles (Johnson, 2000) and under logs and rocks (Minton, 1972; Williams, 1973).

Study Site

The location used for the natural history study of *A. texanum* is at the Boaz Swamp in Wood County, owned by the Wood County School District (Figure 1.4). The swamp is a riparian wetland of the Ohio River, which holds water year round. There is a large residential community to the southeast above the riparian area (Figure 1.5).

The breeding pool studied was situated parallel to Boaz Swamp at the northeast end of the swamp southeast and immediately adjacent to the CSX Railroad tracks. There are several gas wells in the area along the riparian zone, and the area is also used as pastureland. People visit the swamp regularly to fish and hunt.

Railroad tracks and a small patch of woods border the ephemeral breeding pool (Figure 1.6). The pool appears to have been created by the construction of the railroad. There is no information on the seasonal water level for the pool. During my study period, (January to August 2001) the pool dried up by 13 May. Robert Phipps, a local photographer, reported that the pool had little water in it on the previous Tuesday, May 8, 2001.

The pool measures 88.5 m long and 3.3 m wide at maximum water volume (Figure 1.7). It is positioned 6 m from the center of the railroad tracks and 32 m from the edge of Boaz

Swamp. A small wood lot with a thin canopy is located between the pool and the swamp with *Acer saccharinum*, *A. negundo*, *Platanus occidentalis*, *Ulmus rubra*, as the dominant trees. *Fagus gradifolia* and *Quercus* spp. are also present in the area.

The bottom structure of the pool is comprised of large limestone rocks that measure 50 X 150 cm in length and 20-50 cm in width that are used for the railroad bed. Grasses are the dominant forbs in and around the pool.

CHAPTER II

<u>Natural History</u>

<u>Introduction</u>

On February 25, 1991 24 *A. texanum* (12 males and 12 females) were trapped at Boaz Swamp, Wood County (Pauley, per. comm.). Traps contained spermatophores and egg masses, which would indicate that mating, had occurred on the night of February 24, 1991. Other trappings have been conducted across the state with no new populations found (Pauley, per. comm.). This is the extent of the previous natural history information of *A. texanum* in West Virginia.

<u>Collection of Salamanders</u>

In November 2000, a drift fence with pitfall traps was set at the Boaz Swamp breeding site that Pauley trapped at in 1991 (Figure 2.1). The drift fence encircled the breeding pool and pitfalls were placed on the outside and inside of the fence (see Dodd and Scott, 1994) (Figure 2.2).

Funnel traps (commercial minnow traps) were used to sample ephemeral pools for the presence of adult and larval *Ambystoma* salamanders. Funnel traps were placed at the Boaz Swamp site in all pools (Figure 2.3). Funnel traps were the primary sampling method used at the Point Pleasant and McClintic Wildlife Management Area (WMA) historic sites, along with hand searching.

Dip nets and hand collection of salamanders during the day and night were employed at and around ephemeral pools. Dip nets were used to sample pools for adults and larvae. Hand searches were preformed in breeding pools, to search for larvae and eggs. Searches were conducted under woody debris, rocks and any other refugia.

Searches in ephemeral pools extended from Wood County to southern Mason County. Funnel traps and hand searches were used to sample new sites for adults and eggs during the breeding season. Funnel traps were also used to sample for larvae in new ephemeral pools.

Environment and Breeding Data

Environmental measurements taken at ephemeral pools included pH, water temperature, soil temperature, air temperature, and relative humidity. Water pH was measured with Oakton Instruments Waterproof pHTestr 2. Enviro-Safe armored thermometer was used to take water temperatures. Reotemp stainless steel Bi-metal thermometers were used to record soil temperatures. Air temperature and relative humidity were taken with a Thermo-Hygro digital Max/Min thermohygrometer. All temperatures were recorded in centigrade.

Captured adult *A. texanum* were sexed by examining the cloace. Cloaca of males in the breeding season become highly swollen and provides a simple means of distinguishing the sexes (Downs, 1989). Snout-vent length (SVL) was recorded from the tip of the snout to the posterior edge of the vent. Vent-tail length (VTL) was recorded from the posterior edge of the vent to the tip of the tail. Total-length (TL) was the addition of these 2 measurements. Cranial width (CW) was measured at the widest point on the head, just behind the eyes. All measurements were made in millimeters with a Spi plastic dial caliper. The number of costal grooves (CG) and notes on injuries and or abnormalities were recorded for each individual.

A visible implant fluorescent elastomer from Northwest Marine Technology, Inc was used to make a unique color pattern for 2 adult salamanders. Salamanders were tagged on the dorsal side just above the fore and hind legs. Toe clipping was also used to mark adult salamanders similar to Martof (1953).

Museum specimens from the West Virginia Biological Survey (WVBS) were used for morphometric studies. Necropsies were preformed on museum specimens for stomach contents, development of follicles and sperm-waves. Stomach flushing on wild caught specimens proved to be difficult because of the small gape and large size of the salamanders. Flushing was not preformed due to possible injures.

Five spermatophores were collected during my study. Spermatophores were fixed in 10% formalin as in Labanick and Davis (1976). Measurements were made after fixation of spermatophores. Spermatophores were deposited in the WVBS at Marshall University.

Eggs were collected each time new clutches were observed at the Boaz Swamp site. Eggs were collected from each clutch from March 3, 2001 to March 30, 2001 to measure and stage their development. All but one clutch of 9 eggs was found in funnel traps. Collected eggs were placed in 90% ethanol and measured at a later date. Eggs were fixed in formalin and deposited in the WVBS. Harrison stages of development were used to stage eggs (Feder and Burggren 1992).

On April 8, 2001 3 larvae were collected at the Boaz Swamp site to be staged and measured. Total length of 7 larvae was measured in a plastic bag the field with Spi plastic dial caliper. Photographs of 3 live larvae were also taken to measure total length. Collected larvae were fixed in formalin, placed in 90% ethanol and deposited in the WVBS.

Results and Discussion

On February 9, 2001 I made my first trip to Boaz Swamp to check the drift fence and funnel traps. The 1991 site where Pauley placed funnel traps and I set my drift fence never held water in 2001. This created a large problem in that I would not be able to collect data needed to estimate the population size of *A. texanum* at Boaz Swamp. I also would not able to collected information on the time of larval development and juvenile emigration.

On each trip to Boaz Swamp, pitfall traps at the drift fence and funnel traps were searched. All pools in the area and woods were searched. Two to 3 hours were spent looking for salamanders at Boaz Swamp on each trip. On 2 occasions I found 2 *Plethodon electromorphus* under woody debris.

Environmental measurements were taken at the ephemeral pool that *A. texanum* used as a breeding site. Nine funnel traps were placed in this pool on February 9, 2001. The pH ranged from 6.9 on February 9, 2001 to 7.7 on March 8, 2001. The pH became more consistent in April and was 7.4 when last recorded on April 18, 2001. Water temperature in the breeding pool was 7°C when the first adult *A. texanum* were captured in funnel traps. During my study the water temperature ranged from 2°C to 23°C in the breeding pool and averaged 9.5°C.

Ambystoma texanum was never found moving to or from breeding pools at the Boaz Swamp site. Without the observation of adult movement, I cannot say that *A. texanum* moves when stimulated by moisture and temperature as suggested by Kraus and Petranka (1989) and Petranka (1984). Although the first salamanders captured on February 16, 2001, most likely moved into the breeding pool on the night of February 15 when there was a light rain and the air temperature was 6.5°C. No salamanders were present in the traps on the night of February 14, 2001.

The breeding pool was the only pool that I observed *A. texanum* make use of during my study. Dr. Thomas Jones (per. comm.) at Marshall University found *Lepomis cyanellus* (Green Sunfish) in the main pool of Boaz Swamp during a survey at the site. Kats et al. (1988) found that the pond-breeding *A. texanum* do not respond to the fish chemicals of *L. cyanellus*. Kruse and Francis (1977) found that *A. texanum* were palatable to fish. These findings may indicate the reason for the lack of *A. texanum* eggs or larvae in the main pool at Boaz Swamp. All other pools in the area showed no sign of *A. texanum*.

During my study, 24 adult *A. texanum* were captured; 17 males and 7 females. All salamanders were captured with funnel traps at the breeding pool. There were 13 recaptures, most only once. One male was recaptured 2 times and a second male was recaptured 3 times. All recaptures were from funnel traps except for 2 that were found under woody debris on the bank of the pool.

Elastomer tags were first used to identify individuals in this study. Elastomer tags could not be seen in *A. texanum* when they were placed under a long-wave ultraviolet light. The dorsal color pattern of the salamanders was too dark for the implant to be seen. Toe clipping was then used for identification of individuals. Two toes were removed from each salamander to make a unique pattern (see Martof 1953).

On February 16, 2001 the first *A. texanum* were captured, 2 males in the same trap. All other traps were empty and no salamanders were found in the pool or under nearby debris. Table 1 lists the complete capture records of *A. texanum* at Boaz Swamp.

Table 2.1 Capture records.

DATE	# CAPTURED	# RECAPTURED	MALE	FEMALE
16-Feb	2	0	2	0
17-Feb	11	1	9	2
20-Feb	1	1	1	0
3-Mar	8	5	6	2
19-Mar	12	4	9	3
25-Mar	3	2	1	2
18-Apr	FIRST LARVAE			

Morphometric studies of *A. texanum* included 11 males and 3 females from West Virginia in the WVBS. Eight of the museum salamanders were from Boaz Swamp; the other 6 were from Point Pleasant and McClintic WMA. Both sexes displayed similar color and marking patterns. All captured and museum salamanders had 14 CG.

Sexual dimorphism was shown in the SVL between males and females. SVL ranged from 67.9 mm to 88.9 mm for 28 males and averaged 79.2 mm. Female SVL ranged from 76.7 mm to 95.7 mm for 10 salamanders and averaged 84.8. Female SVL's were significantly larger than male SVL's ($p=0.003$), $\alpha=0.848$ (t-test) (Figure 2.4). Downs (1989) found a similar trend in the SVL of *A. texanum* from Ohio. Necropsies were preformed on 14 *A. texanum* museum specimens to study stomach contents. One salamander contained a piece of an earthworm. All other digestive tracks were empty or contained soil. All salamanders were captured in the breeding season and Petranka (1998) stated that adults feed very little during the breeding season.

Until this study information on the breeding activity of *A. texanum* has not been collected in West Virginia. The 3 female museum specimens were captured in February or March. One female (WVBS 4002) with a SVL of 86.7 mm was captured on March 14, 1969 in McClintic WMA and contained immature follicles (Figure 2.5). This may indicate that females breed every 2 years. Figure 2.6 is a picture of a female with a SVL 82.6 captured on February 25, 1995 (WVBS 11254) at Boaz Swamp that contains mature follicles that would have been deposited that spring.

Sperm-wave analysis was preformed on 11 male museum specimens. All specimens were captured in the spring breeding season. Each male was found to contain mature sperm in its vas deferens.

Spermatophores were first observed on February 17, 2001 (Figure 2.7). Eighty spermatophores were deposited in one trap that also contained 9 males and 2 females. Many spermatophores were deposited atop a previous spermatophore. This is similar to the findings of Arnold (1976) in which he presented a theory for this behavior as "sexual interference". Ten more spermatophores were found in a second trap on March 19, 2001 that contained 2 males and one female. All observed spermatophores were seen in funnel traps.

Spermatophores measured 5.0 to 5.4 mm at the base and 5.0 to 5.2 mm in height. Four lateral horns were present along with a medial groove on the anterior and posterior of the stalk (Labanick and Davis, 1976). See Labanick and Davis (1976) for a complete description of *A. texanum* spermatophore.

Eggs were first observed at Boaz Swamp on March 3, 2001 in 2 different funnel traps along with a female salamander in each (Figure 2.8). No other traps contained female salamanders. A third group of eggs and a female were found on March 19, 2001 also in a funnel trap. On March 25, 2001 eggs were again found in a funnel trap with a female. One clutch of 9 eggs was found outside of a funnel trap, attached to a submerged twig on March 30, 2001. No other eggs were found at Boaz Swamp during my study.

Egg clusters were in small groups that were more elongate than round, "sausage-shaped" (Minton, 1972). A vitelline membrane and 2 outer gelatinous envelopes surround an egg (Downs 1989; Petranka, 1998). The outer jelly layer is flimsy and easily separated. An alga was present in the jelly matrix surrounding the eggs. Freshly laid eggs measured 2.12-2.14 mm in diameter with a dark brown to black animal pole and white vegetal pole that is consistent with Downs (1989) and (Petranka) (1998).

The number of eggs per female was 141, 212, 241, and 172 and averaged 192, which is low compared to the number previously reported in the literature of 550-700 (Figure 2.9) (Smith, 1934; Cagle, 1942; Plummer, 1977; Camper, 1990; Trauth et al., 1990). Eggs from the 4 females averaged 14 eggs per cluster, and ranged from one egg to the largest cluster 35 eggs, which is consistent with the literature. The difference in the number of eggs per female may be low because females might not have deposited all eggs in the traps. I believe there might be a second reason, poor habitat. Time was spent on each trip looking for eggs in the breeding pool with no luck.

Eggs from each trap were collected to be staged on every visit to Boaz Swamp. The first 2 clutches observed were staged to 12 and 13. At 20°C this is 65 to 75 hours of development. The clutch collected on March 19, 2001 was staged to 13 also. On March 25, 2001 eggs were collected and found to be at stage one (single cell), zero hours of development. This would indicate that the eggs were laid on the night of March 24, 2001. Eggs were collected on April 7, 2002 and staged to 36, 180 hours of development (Figure 2.10) and (Figure 2.11).

No new eggs were found after March 30, 2001. On April 8, 2001 while collecting eggs to be staged I observed larvae. Balancers were present on 2 of the larvae, which represent stage 40 (252 hours of development). Complete development is reached at stage 46 (525 hours of development). This indicates that *A. texanum* has plasticity in its hatching time. Such plasticity has been demonstrated in amphibian metamorphosis (Newman, 1988; Crump, 1989; Skelly and Werner, 1990; Warkentin, 1995). The 2 theories are that larvae may hatch early to avoid predation or to escape an unsuitable environment. Hatching response may have been from the collection eggs, a predator response.

Thirteen larvae were measured on site and 3 were collected on April 18, 2001 (Figure 2.12). Larvae averaged 14.0 mm (n=13) with a maximum of 15.5 mm at complete development and hatched in 4 weeks. During the week of May 8, 2001 the breeding pool at Boaz Swamp dried up (Figure 2.13).

Along with the drying of the breeding pool, 6 funnel traps were stolen from the Boaz Swamp site. One of these funnel traps contained the clutch of eggs that were of known date of deposit. These circumstances have had a significant impact on the data that was available.

There were very few species of amphibians and reptiles observed at the Boaz Swamp breeding site during my study (Table 2.2). C*helydra s. serpentina* was also observed in the 1991 breeding pool and was suspected to have impacted the population of *A. texanum* during the breeding season (Pauley, per. com.). Great Blue Herons (*Ardea herodias*) were also observed to feed at the site. I feel the largest threat to *A. texanum* at this site is from continued human impact.

Table 2.2 Amphibians and reptiles observed at Boaz Swamp.

Amphibians	**Reptiles**
Plethodon electromorphus	[1]*Chelydra s. serpentina*
Pseudacris c. crucifer	[1, 2]*Nerodia s. sipedon*
[2]*Rana catesbeiana*	
[2]*R. clamitans melanota*	
[2]*R. palustris*	

1-Reported as predator (Petranka, 1998).
2-Found dead in funnel trap.

CHAPTER III

Malformations

Introduction

In the past few decades the conservation of amphibians has become a major focus of many environmental organizations. Reports of declines in populations of amphibians around the world have fueled the urgency for the need of information on the potential impact that pollutants may have on amphibians (Carey and Bryant, 1995). Abnormalities in amphibians may contribute to global trends in population decline, which could be indicative of environmental threats to human health (Johnson et al., 1999). Recently reports of abnormalities in frogs and toads in West Virginia have been filed with the USGS Northern Prairie Wildlife Research Center (NPWRC).

In May of 2001, in Randolph County, one Eastern American Toad (*Bufo a. americanus*) was found with only one eye, a condition called anophthalmia. One Pickerel Frog, (*Rana palustris*) from Ohio County in April of 1998 was found with an extra hind limb bud, a condition known as polyphalangy. In Mineral County, July 1997, one Northern Green Frog (*R. clamitans melanota*), was also found with two feet on one of its hind limbs. (NPWRC).

Field Studies

During my field collection of breeding adults I captured 3 male and 3 female *A. texanum* at the breeding pool in funnel traps with 3 different types of malformations. These 6 salamanders represent 25% of the 24 individuals that I captured in the 2001 spring breeding season.

Table 3.1. Malformations observed in *Ambystoma texanum*.

Male	02/17/2001	Ectrodactyly
Female	02/17/2001	Ectrodactyly
Male	02/17/2001	Anophthalmia
Female	03/03/2001	Ectrodactyly
Male	03/19/2001	Polyphalangy
Female	03/25/2001	Ectrodactyly

Four salamanders (3 females and one male) were missing whole digits, a condition referred to as ectrodactyly. Three of the 4 salamanders where missing one toe from one of their front feet. The fourth salamander had only 4 toes on its right hind foot. One male captured had 7 toes on his left hind foot, an example of polyphalangy (Figure 3.1). The sixth salamander a male was missing its left eye. In a personal communication with Dr. Thomas Pauley, he could only recall observing one toad with a malformation in the 36 years he has spent researching in West Virginia. To the best of my knowledge these 6 *A. texanum* represent the highest number of malformations in the state. A report was submitted of the malformations on January 22, 2002 to the USGS NPWRC.

Discussion

The highly aqueous skin of amphibians makes them excellent monitors of pollutants. Petroleum products along with, acidification, insecticides, and various metals have been found to lethally impact amphibians (Freda, 1991; Rowe et al., 1992; Sadinski and Dunson, 1992; Berrill et al., 1994; Mahaney, 1994). With the proximity of gas wells to the breeding site of *A. texanum* and runoff from the residential area above, testing of the soil and water needs to be conducted to look for known teratogenic agents. Defoliants may also have been sprayed at the breeding pool by the railroad company.

Malformations in breeding adults are not debilitating and may not have much effect on individuals or the population. If metamorphosing juveniles are found to have a malformation rate of 25%, then this is a high number and would qualify a site as a hotspot by the USGS NPWRC. The frequency of abnormalities in affected populations often exceeds the expected frequency of 0-5% (Johnson et al., 2001). If malformations are found to occur in the larval stage, this would drastically reduce their survivorship, and impact the population. The question that needs to be addressed is what the causes are and when are salamanders exposed to the malforming agents? There are 3 prevailing lines of thought; natural, pseudo-natural, and artificial causes.

Natural causes include failed predation. Predators such as birds, mammals, reptiles, and fish occur at Boaz swamp. It has been well documented that *A. texanum* lives in crayfish tubes and uses them to as refugia when they are moving (Strecker and Williams, 1928; Cagle, 1942; Minton, 1972; Parmelee, 1993; Petranka, 1998;). The loss of an eye, toe or foot could easily be associated to encounters with crayfish. More terrestrial subterranean burrows occupied by small mammals could also account for observed malformations resulting from encounters with shrews or other small mammals. Laboratory examinations (radiographing) will need to be preformed to rule out natural causes.

Pseudo-natural causes like parasites infection could also account for the malformations observed at the Boaz Swamp breeding site. The presence of parasites can cause deformities in limbs, the loss of limb elements or extra elements to grow. It has been found that larvae of parasitic trematodes burrow into developing amphibians, its intermediate host, forming cysts, which cause deformities (Johnson et al., 1999). Johnson et al. (1999) found that community analysis of breeding pools with high rates of abnormal amphibians also support high numbers of aquatic snails, which are the first hosts of trematode parasites. Gardiner and Hoppe (1999) concluded from their research that abnormalities seen at their study pools are similar to the abnormalities that result from exposure of developing vertebrates to exogenous retinoids.

The ecological interaction between parasites that cause malformations and their hosts is not well understood and needs further research (Helgen et al., 1998; Gardiner and Hoppe, 1999; Johnson et al., 1999; Johnson et al., 2001). Live animals are needed to properly identify parasitic cysts (Helgen et al., 1998). This requires putting down the animal to perform a necropsy. With so few *A. texanum* observed a collection of any kind may have a drastic effect on the population.

Artificial causes (pollution) have been well documented to induce malformations in amphibians across North America (Ouellet et al., 1997; Raimondo et al., 1997; Rowe et al., 1998; Helgen et al., 1998; and Hopkins et al., 2000). The position of the breeding pool at Boaz Swamp would lend it to possibly be polluted from the residential area above the swamp, the Ohio River, the railroad, and gas wells in the area. All of these sources have been shown to harbor teratogenic compounds, which causing developmental malformations (Hopkins et al., 2000).

Agricultural and residential runoffs have been found to cause developmental anomalies in metamorphosing amphibians. Ouellet et al. (1997) found that pesticides, fungicides, and fertilizers all contain teratogenic compounds, which could have produced the same anomalies that I observed at Boaz Swamp. There are also the associated pressures of habitat loss from agriculture and new homes.

Many studies in have looked at the effects that coal-burning power plants have on developing amphibians (Raimondo et al., 1997; Rowe et al., 1998; and Hopkins et al., 2000). In polluted sites, 18 to 37% of larvae in pools studied exhibited lateral curvatures of the spine (Hopkins et al., 2000). These percents compare with the 25% malformations in adults observed at Boaz Swamp. Impacts, if any, on larvae at Boaz Swamp from power plant byproducts have not been considered. Power plant byproducts can contain trace elements including As, Cd, Se, Cu, Cr, and V (Hopkins et al., 2000), which may be linked to malformations.

Amphibians may potentially be subjected to more environmental stressors and toxic exposure due to their biphasic life cycles and skin permeability. For these reasons, amphibians are important indicators of general environmental health and may provide early warnings of deteriorating environments. If more animals are found, radiograph and parasite analysis should be preformed along with soil and water tests in the area.

The loss of the 2001 recruitment class of *A. texanum* at the Boaz Swamp breeding site may have more of an impact than can be understood at this time. I was unable to observe if there were malformations in larvae at the breeding pool, which would have the largest impact on the population. Breeding adults observed with malformations may have little if any impact from their malformations, as they have survived to breeding size and were found at the breeding pool.

CHAPTER IV

Status of *Ambystoma texanum*

Introduction

A major focus of this investigation was to assess the status of *A. texanum* in West Virginia. As stated previously the species is ranked G5 and occurs across a large area of the United States and into the territory of Canada. The populations in West Virginia are at the extreme eastern limit of the species. The West Virginia populations may be small but they are as important as any other. Questions remain as to how the species reached West Virginia and how long it has been here. For these reasons it is even more important to protect these genetically distinct populations in West Virginia.

The current laws in West Virginia provide little protection for salamanders of any species. At any time a person may possess 250 specimens in total of all species combined (Level, 1997). For a nominal fee a person can obtain a permit for the collection, sale and possession of any salamander in the state except *P. nettingi*, which requires a state and federal permit because it is listed as a federally threatened species. These regulations do little to protect a species in West Virginia like *A. texanum* or its habitat.

The habitat used by *A. texanum* has also not fared well over the last 40 years. The majority of the Ohio River floodplain has been developed from Pittsburgh to St. Louis. As for *A. texanum,* little undeveloped land is left from Huntington to Parkersburg in West Virginia. The biphasic life style of amphibians requires 2 very different types of habitats to exist for reproduction to be successful. The occurrence of both habitats in close proximity is needed for an amphibian species to survive.

Field Studies

Searches for adults during the breeding season and also for eggs and larvae were conducted at known historic locations. The Point Pleasant and McClintic WMA sites were visited at night during the migration period and during the day to look for the presence of eggs and larvae.

Other locations along the Ohio River floodplain that may support *A. texanum* were searched from Boaz to southern Mason County. Areas that looked appropriate and included terrestrial and aquatic habitats that may support *Ambystoma* salamanders were sampled by hand during day and night, and with funnel traps. Listening for frog species that breed in ephemeral pools was also used to locate sampling sites for *A. texanum*.

Discussion

McClintic WMA in the late 1960’s and early 1970’s was managed for the removal of Sacred Lotus Water Lily (*Nelumbo nucifera*), an invasive plant from Asia. Ponds were drained, soil from the edge of the pond was pushed to the center and allowed to dry over 2-3 years, and then the soil from the pond bottom and soil around the edge of the pond were removed (Robert Phipps, per. com.). This management practice at times combined smaller ponds into larger continuous ponds. Also, many drainage ditches have been created in upland area to dry out the land (Dave McClung WMA manager, per. com.). These practices may have negatively impacted populations of *A. texanum* in the WMA. Salamanders may have been present in the soil at the time of removal. It has been found that adult *A. texanum* remain closer to their breeding ponds than do other *Ambystoma* species (Parmelee, 1993).

The records in the WVBS do not include specific locations of the salamanders collected in the WMA in the 1960's. This creates a second problem in that *A. texanum* may still remain in the WMA in an ambiguous corner. No adults, eggs or larvae of *A. texanum* were found at this site during my study. *Ambystoma maculatum, A. opacum* and *A. jeffersonianum* adults and eggs were fund in the WMA in 3 different breeding pools (Figure 4.1).

The Point Pleasant site from 1960's to present has gone through some changes, (Landowner, per. com.) including more homes being built in the area. The terrestrial area surrounding the breeding pond is quite small and houses with mowed lawns line a majority of the pond's edge. I was unable to find any data on the size of the habitat used by *A. texanum* at this site in the 1960's. Specific location information is also lacking for this site. No adults, eggs or larvae of *A. texanum* were found at this site in my study.

Searches for *A. texanum* along the Ohio River floodplain were not successful. Very little undisturbed land occurs along the river. Much of the floodplain is either developed or is used for pasture and hay fields. There is also a great amount of commercial development along the river, which may lead to problems with pollution. One intact location that I had hoped to survey was the large amount of land owned by Century Aluminum, located just south of Ravenswood in Jackson County. The environment biologist for Century Aluminum did not let me search for *A. texanum* on their land for fears that the species may be found on the land and latter become a protected species in the state. The biologist did not want possible land management conflicts to occur in the future.

For the Boaz Swamp population of *A. texanum*, the health of the population and if the population will be able to sustain itself are key questions. We must consider the idea of metapopulations. Metapopulation is a population of populations. It appears that there may only be one breeding population at Boaz Swamp. If this is the case, then without metapopulation structure it may only a matter of time before that population is extirpated (Petranka, per. com.).

A population may act as a source or as a sink. With zero recruitment in the 2001-year class it would seem that the Boaz Swamp population is acting as a sink.

Amphibians can be especially susceptible to catastrophes and environmental stochasticity (Gibbs, 1998) due to human-caused fragmentation of their habitat. A longterm (5-7 year) study would be needed to assess if the population at Boaz Swamp is a sink population. This is a very important question that should be addressed if the species is to survive in the state. It is unknown what the minimum viable population is for *A. texanum*. The observed malformations at the Boaz Swamp breeding site may also be negatively impacting the population if they are occurring in the larval stage.

Studies to delineate a terrestrial buffer zone for pond breeding species of salamanders have found that a buffer zone of 165 m would be needed to encompass 95% of a population of *Ambystoma* salamander (Semlitsch, 1998). Demaynadier and Hunter (1998) found that edge sensitive species, (*P. cinereus, A. maculatum, A. laterale, and R. sylvatica*) require a buffer of 25-35 m for abundance and distribution of sensitive species to normalize in a forest. The terrestrial habitat at Boaz Swamp is only 35 m wide and bordered by railroad tracks, a road, and a large pond with Green Sunfish (*L. cyanellus*).

A management plan for *A. texanum* must include creating more breeding pools that are fish free. The placement of pools should be in the best available terrestrial habitat. Consideration should be given to placing the pools as far away as possible from the railroad gas wells and other possible pollution sources.

A monitoring program should be set up to track the breeding success of *A. texanum* and to estimate population numbers. The occurrence of further malformations will need to be documented also. As it stands, the population of *A. texanum* at Boaz Swamp does not appear to be in good health. If this is the last population in the state it may be an uphill battle for the species in West Virginia. Egg masses were present in the breeding pool from the 2001 study site on April 7, 2002.

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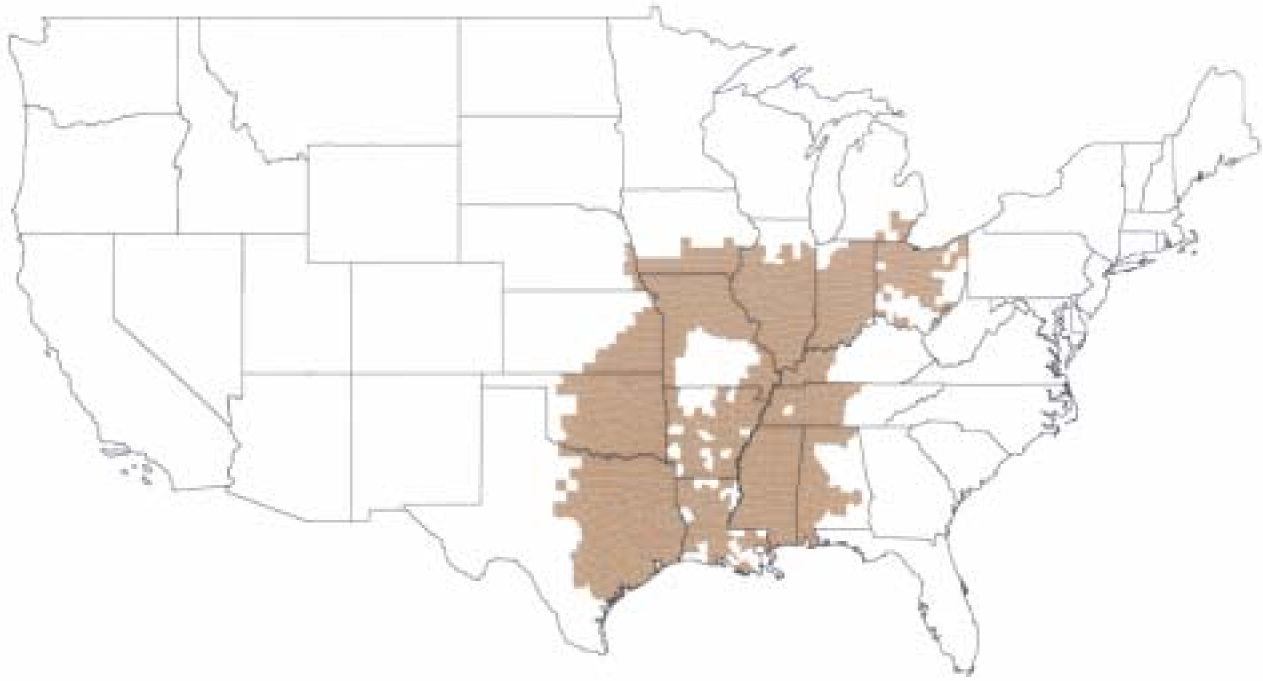

Figure 1.1 Range of *Ambystoma texanum* in the United States.
http://nova.bsuvc.bsu.edu/home/00mjlannoo/Ambystomatidae/ambystomatidae.html

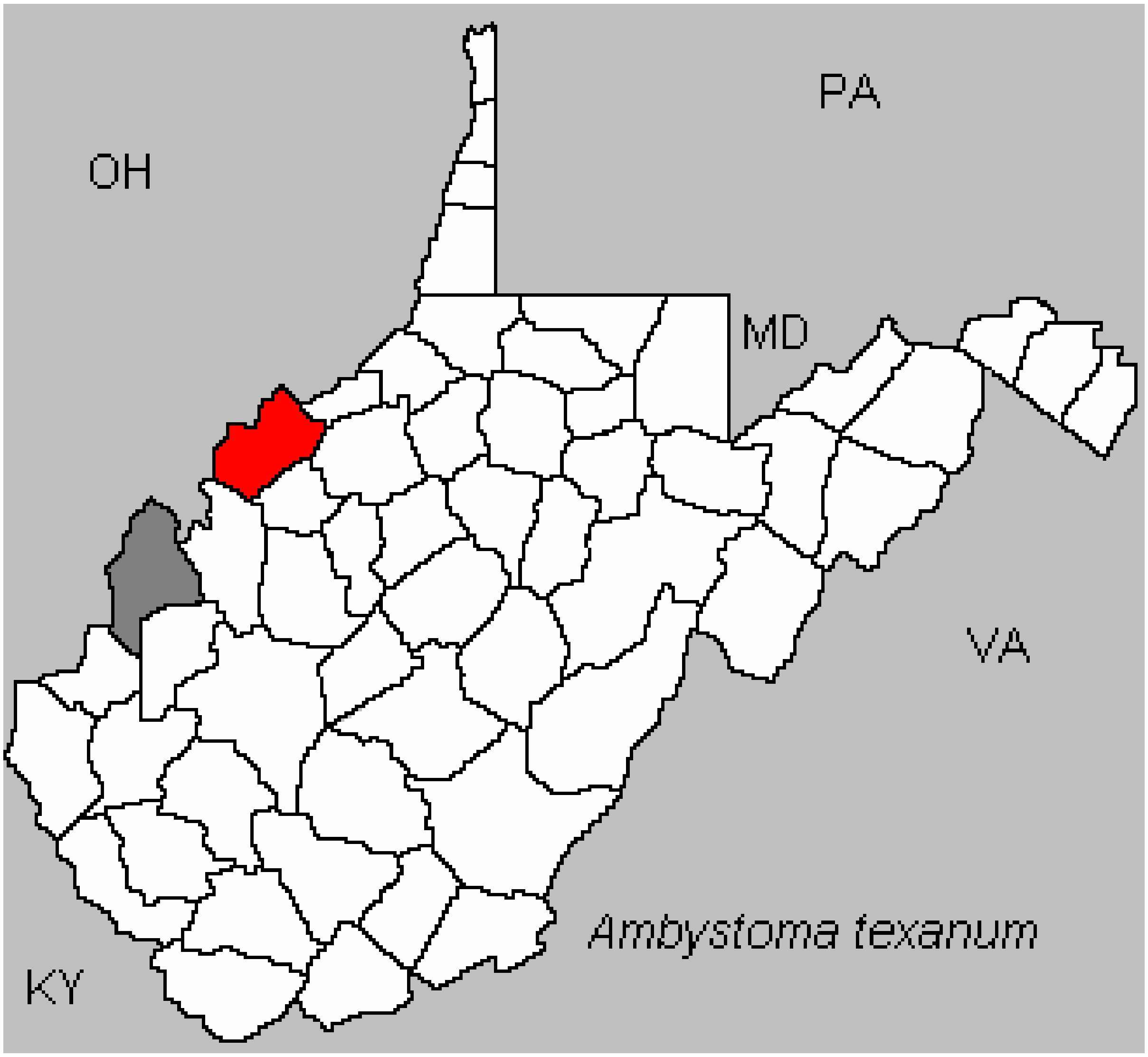

Figure 1.2 Range of *Ambystoma texanum* in West Virginia. Wood County in red and Mason County in gray.

Figure 1.3 Male and female *Ambystoma texanum* from Boaz Swamp.
Photo by Robert Phipps

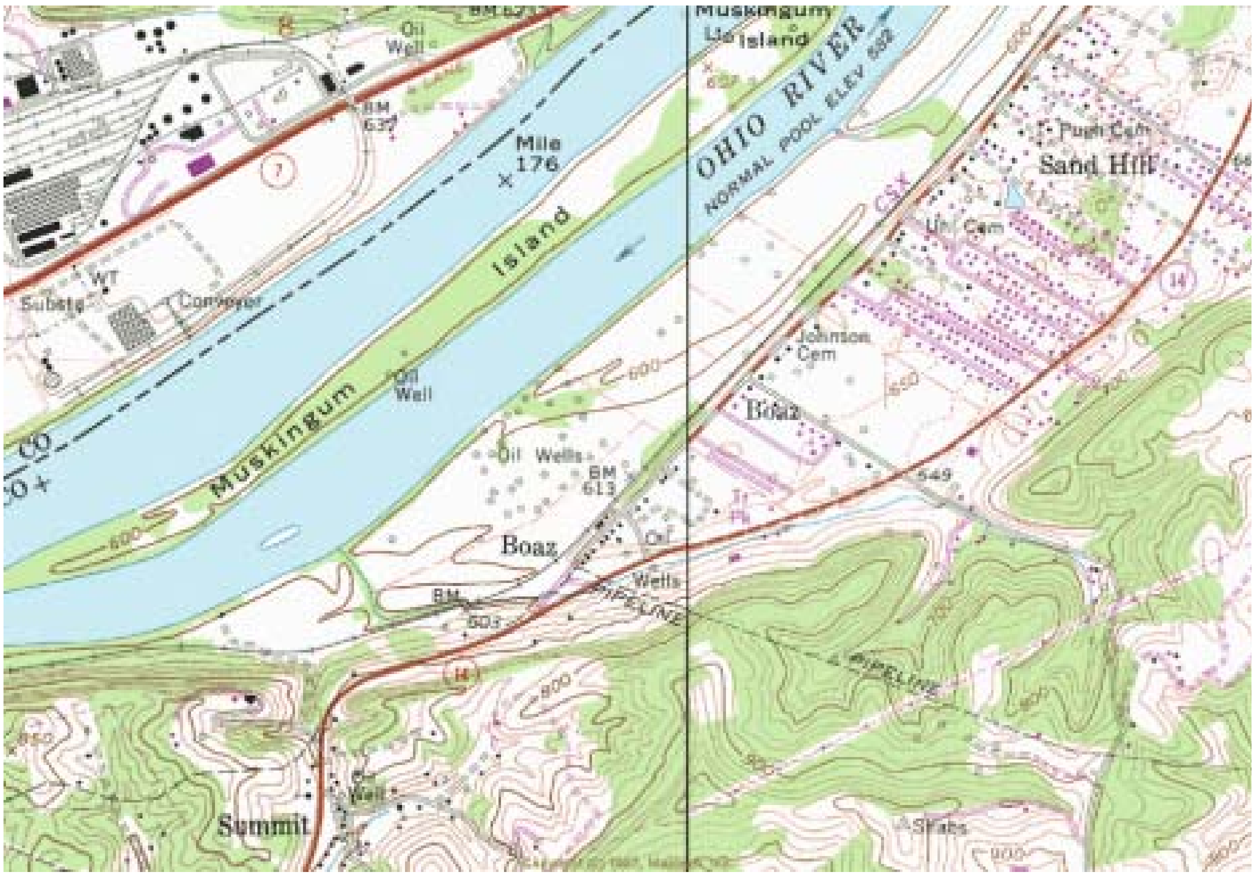

Figure1.4 Map of Boaz West Virginia 1975.

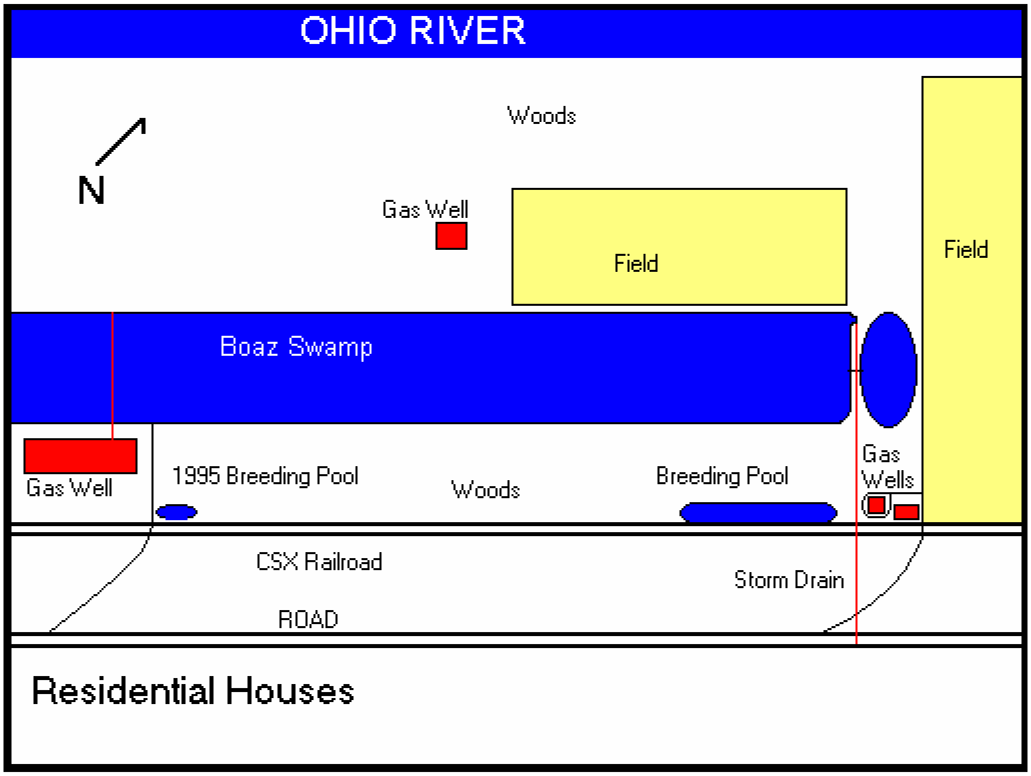

Figure 1.5 Map of Boaz Swamp showing breeding pools.

Figure 1.6 Railroad tracks next to Boaz Swamp breeding pool.

Figure 1.7 Boaz Swamp breeding pool at low water.

Figure 2.1 Drift fence with pitfall traps at Boaz Swamp.

Figure 2.2 Drift fence with covered pitfall trap at Boaz Swamp.

Figure 2.3 Funnel trap at Boaz Swamp.
Photo by Robert Phipps

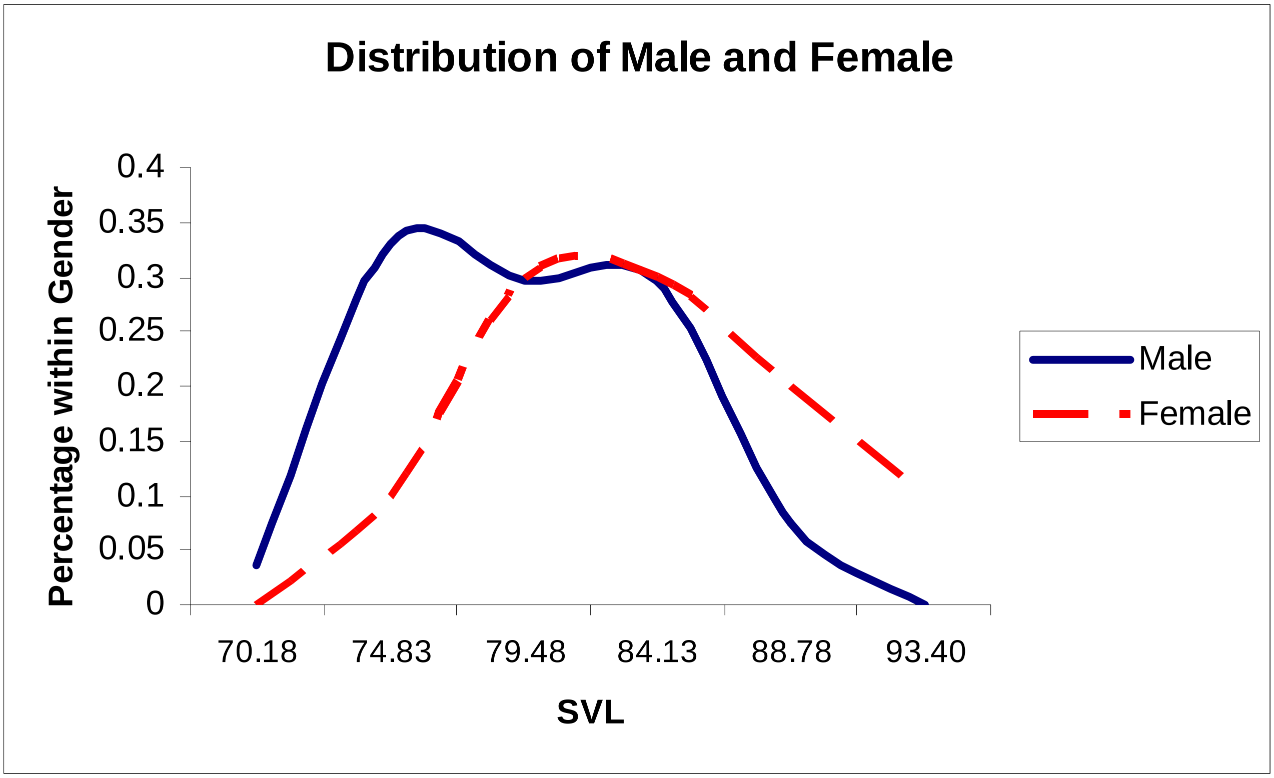

Figure 2.4 Graph of adult *Ambystoma texanum* SVL.

Figure 2.5 Immature follicles of adult *Ambystoma texanum* with SVL of 86.7.
Photo by Mizuki Takahashi

Figure 2.6 Mature follicles of adult *Ambystoma texanum* with SVL of 82.6.
Photo by Mizuki Takahashi

Figure 2.7 Spermatophore of *Ambystoma texanum*.
Photo by Robert Phipps

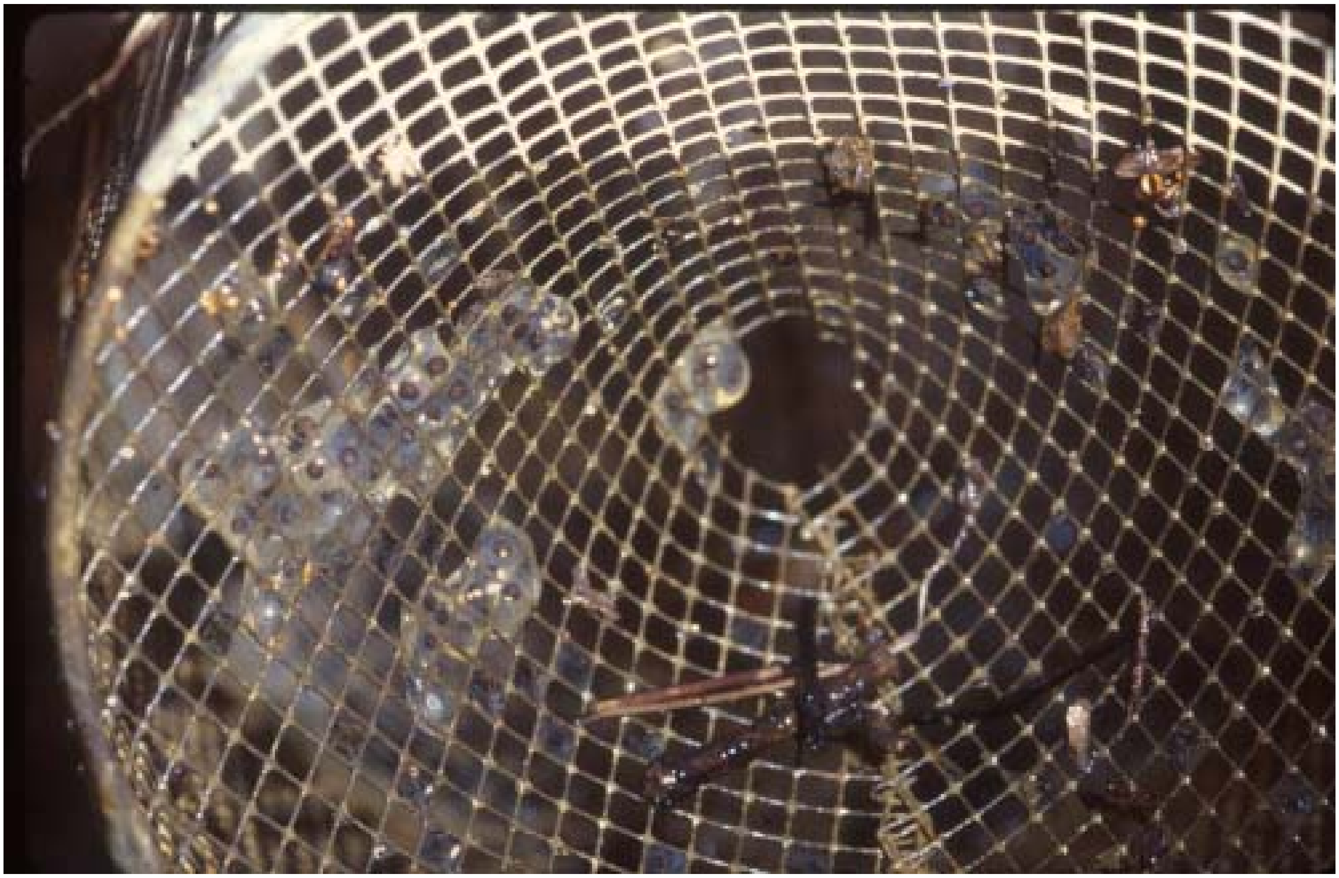

Figure 2.8 *Ambystoma texanum* eggs attached to a funnel trap at Boaz Swamp.
Photo by Robert Phipps

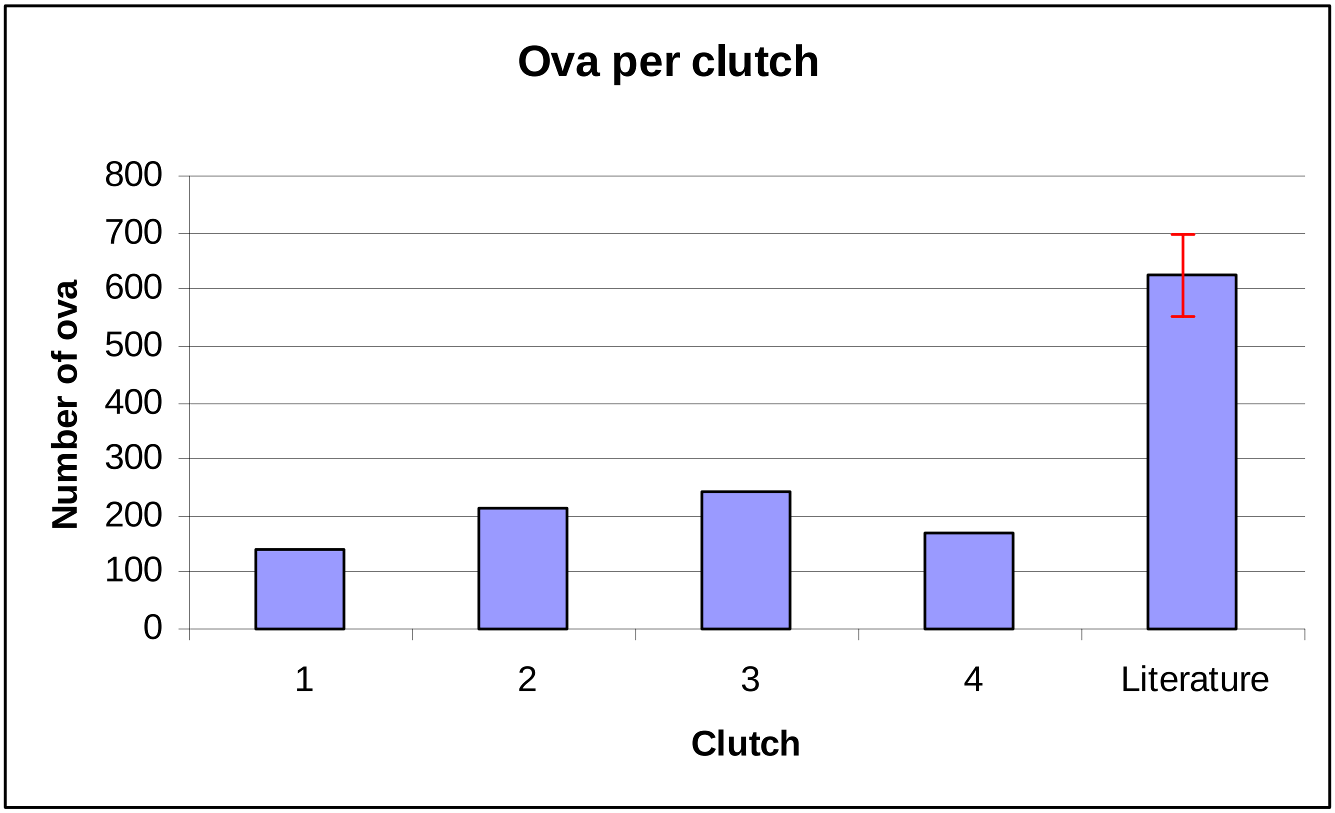

Figure 2.9 Ova per clutch for *Ambystoma texanum* at Boaz Swamp in West Virginia.

Figure 2.10 *Ambystoma texanum* egg mass collected April 7, 2002 at Boaz Swamp.
Photo by Keith Johnson

Figure 2.11 Dorsal view of an embryo of *Ambystoma texanum* collected April 7, 2002 at Boaz Swamp.

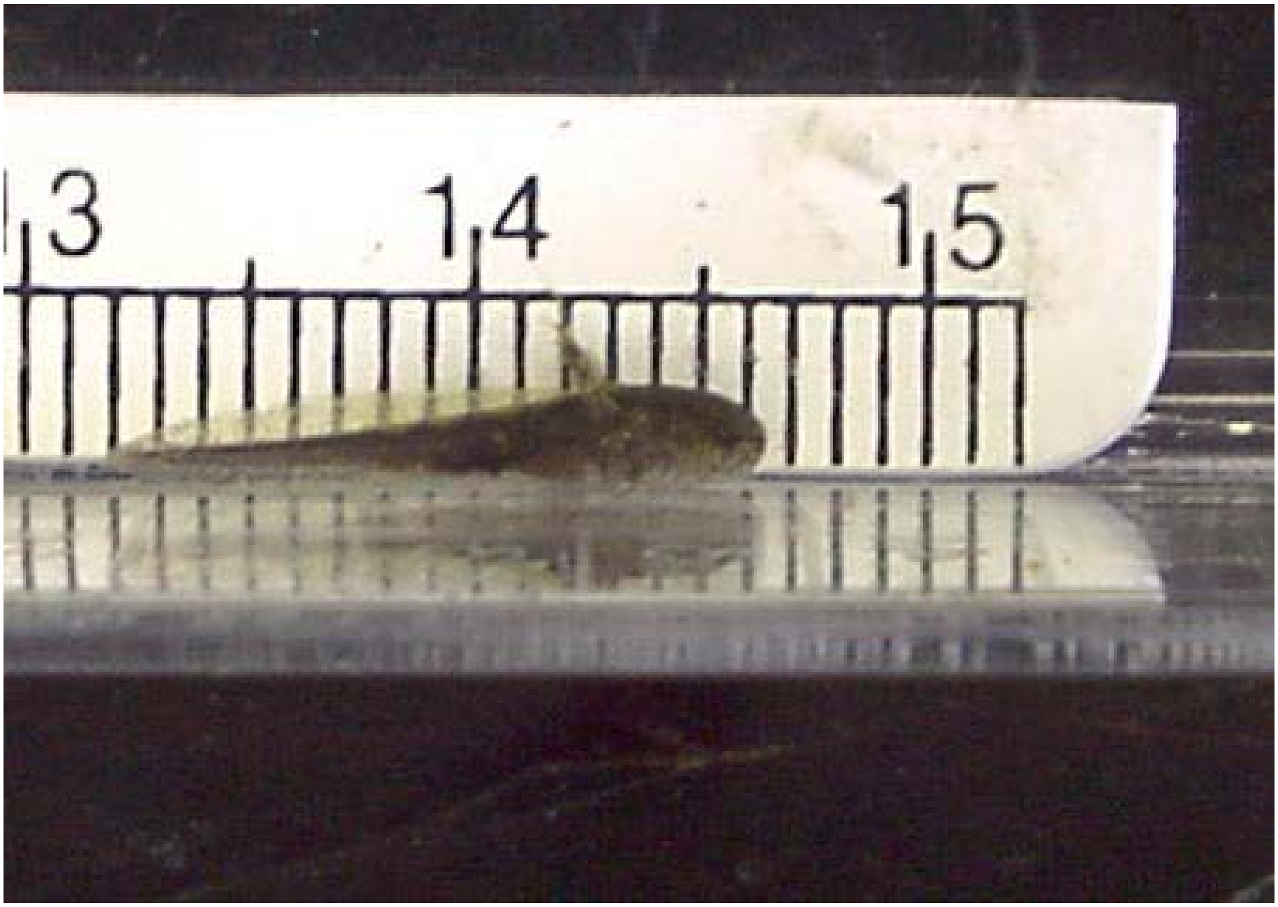

Figure 2.12 Early stage larva of *Ambystoma texanum*.

Figure 2.13 Dry breeding pool at Boaz Swamp on May 13, 2001.

Figure 3.1 *Ambystoma texanum* with 7 toes on right hind foot.
Photo by Robert Phipps

Figure 4.1 Breeding pool at McClintic WMA.

CURRICLUM VITAE

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EDUCATION:

Marshall University, Huntington, WV
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Duties:

•Masters Thesis-Status of the Small-mouthed Salamander (*Ambystoma texanum*) in West Virginia-funded by the NWNHP of WV. Collected natural history information in the field on a species rare to West Virginia over a two year period. Thesis written will provide information to the West Virginia Nongame Program for the development of a management plan for the conservation for the species.

•Nontarget Impacts from Regional Insecticide Applications and Gypsy Moth Defoliation-funded by USDA-Forest Service; Responsible for the collection of field data on salamanders and habitat environment such as temperature and pH. Input data into a spreadsheet, wrote quarterly and annual reports, and presented findings at ASB conferences in 2001.

•Report of Upland Vertebrates in the Bluestone National Wild and Scenic River-funded by National Park Service; collected field data on habitat and location of vertebrates along the river and in the upland adjacent to the river, organized data for the final report.

•Report of Upland Vertebrates in the Gauley River National Recreation Area-funded by National Park Service; Crew leader, responsible for the collection of field data on habitat and location of vertebrates in the park, designed field data sheets, designed database, entered all data into database, prepared reports.

•Timberline Four Season Resort funded by Timberline; collected field data on Federal Endangered Cheat Mountain Salamanders and took environmental measurements.

Fish and Wildlife Technician 1 Seasonal 05/1998-04/2000
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Duties:

•Wildlife Unit-Input data from seasonal duck harvest into a spreadsheet to produce management documents. Trapped Bald Eagles to place GPS tracking unit on birds for movement study. Trapped Wild Turkeys and transported them to Canada. Conducted wildlife observation and counts at Perch River WMA. Implemented management controls on Mute Swan populations at Perch River WMA. Responsible for distribution of live traps to public. Assisted with necropsies, specimen collections, and rehabilitation of wildlife. Prepared specimens for collection and participated in public education exhibitions. Operated incinerator and was responsible for lab equipment and supplies, and equipment maintenance. Public relations, and answer phones. Represented unit at regional outdoor shows.

•Fisheries Unit-Conducted shore based creel census on Lake Ontario, and streamside creel census on Northern New York trout streams. Public relations. Participated in aerial count of boaters fishing. Experience with stream electroshocking (with a back pack unit) and boat electroshocking during day and night. Experience with gill nets, trapnets and seines in ponds, lakes and large rivers. Responsible for the placement of nets and the collection of data. Pressed and read fish scales. Collected water samples and performed basic quality tests, and responsible for equipment maintenance.

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Duties:

•Contracted with New York State Herp Atlas Project to perform regional Herp Atlas Survey. Responsible for the survey of 10 quadrangles in Jefferson County, adjacent to Lake Ontario. Prepared report of my findings at the end of the field season.

Wildlife Technician 06/1997-07/1997
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Duties:

•Assisted Dr. Merritt in collection, recording, and organization of small mammal data. Trapped and marked small mammals to record movement using radio telemetry. Prepared study skins of small mammals. Analyzed data collected.

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SOCIETY FOR THE STUDY OF AMPHIBIANS AND REPTILES
AMERICAN SOCIETY OF ICHTHYOLOGISTS AND HERPETOLOGISTS

GRANTS:

Nongame Wildlife and Natural Heritage Program of West Virginia for research on the status of the Small-mouthed Salamander *Ambystoma texanum* in West Virginia

PUBLICATIONS:

Felix, Z., J. Wooten, N.J. Dickson, R. Fiorentino, A. Breisch, M. Takahashi, and T.K. Pauley. 2001. Nontarget impacts on terrestrial and aquatic salamanders from insecticide applications and gypsy moth defoliation. Association of Southeastern Biologists. 48(2):92-93.

Fiorentino, R.J. 2002. Status of the Small-mouthed Salamander, *Ambystoma texanum* (Mathes) in West Virginia. Proceedings of the West Virginia Academy of Science.