

***Ambystoma jeffersonianum* at Tuscarora Pond, Douthat State Park:
Data from a population at the Southern extent of the species range**

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Introduction

The Jefferson Salamander, *Ambystoma jeffersonianum*, has a range extending from Ontario, Canada and New England down into Virginia and south-central Kentucky (Petranka, 1998). In Virginia, the salamander is found along the western mountains of the Ridge and Valley Physiographic Province. Virginia is on the southern end of the species' range. The Virginia Fish and Wildlife Information Service (VaFWIS) database lists it as known from about twenty counties. In Bath County, it is reported only at Tuscarora Pond, a vernal pool in Douthat State Park; along with *Ambystoma opacum*, *Ambystoma maculatum*, *Lithobates sylvatica* and fairy shrimp, all indicator species for vernal pools (Brown and Jung, 2005). Of the three species of *Ambystoma* found at Tuscarora Pond, the Jefferson Salamander is the least common and is listed in Virginia as a Tier IV species in need of moderate conservation effort. After the 2003 VHS Survey at Douthat State Park (Gibson and Hobson, 2006), the authors took an interest in developing an ongoing study to obtain basic information for one of the species' most southern populations.

Salamanders in the genus *Ambystoma* are known as the mole salamanders because they spend most of their adult life underground. Because of this, they are infrequently observed outside of the breeding ponds. Thus, to study adult populations, most studies have limited surveys to the early spring when adults congregate (March-April in the northern ranges, earlier in southern populations). For *Ambystoma jeffersonianum*, breeding has been reported in late December and early January in southern Indiana (Downs, 1989). Populations in central Kentucky migrate into ponds in late October and November (Douglas and Monroe, 1981), and eggs have been observed as early as 3 January (Smith, 1983). In West Virginia, the initiation of breeding has been reported from early February to early March (Wilson and Friddle, 1950). Records of breeding from Virginia populations are scarce. The first was given by Jopson (1973). He reported adults at a breeding pond in Rockingham County on 24 January 1972. He later reported finding breeding pools on Shenandoah Mountain with eggs observed as early as 29 January (Jopson, 1984) and reported that Amos Showalter had found eggs deposited as early as late December or early January. He also reported that breeding had been observed as late as 9 April. Stevenson et al. (1996) reported that egg masses had been observed on 10 March 1995 and 16 February 1996 in Allegheny County. They also reported adults in breeding ponds at several locations on 23 February 1996.

Breeding typically lasts only days in the north but can last 3-11 weeks in southern populations. These consist of 2-3 major migrations sparked by rains and separated by periods of cold

weather. Males are reported to stay at ponds for 16-30 days compared to 19-21 days for females (Petranka, 1998). After breeding, adults return to their underground forest retreats, often exiting the pond in the same general direction as entering which suggests fidelity to a particular microhabitat (Douglas and Monroe, 1981). Males breed annually but females may skip one or more years between breeding events (Downs, 1989). Females have been found to be 13% longer than males with adults ranging from 65-95 mm snout to vent length (SVL)(Uzzell, 1964).

Females lay eggs within 1-2 days of mating, depositing small masses of eggs on fallen branches and other vegetative structures (Petranka, 1998). Fresh ova are 1-2.5 mm in diameter and are surrounded by jelly layers which absorb water, producing a protective layer. In central Kentucky, egg masses measure 39 x 43 mm and average 23 eggs/mass (range 2-67). In New York, egg masses measure 25 x 50 mm and average 16 eggs/mass (range 7-60). Other egg estimates include a single mass of 35 eggs from West Virginia, 22 eggs/mass (range 8-55) from southern Ohio, and 30-33 eggs/mass in another Ohio population. Females appear to lay several egg masses each as counts of ovarian eggs range from 140-280 (Uzzell, 1964), 183 and 212 from two Ohio populations (Uzzell, 1964), and 210 for four individuals from New York (Bishop, 1941).

The incubation time for eggs is inversely proportional to incubation temperature. Embryos hatch in late April – early May after 30-45 days in New York. They hatch in 28 days in an Ohio Population, and from late March to mid-April after 21-98 days in central Kentucky (summary from Petranka, 1998). Embryonic survival appears to be high. Survival rate was estimated at 87% in Ohio (Brodman, 1995), 60-88% in Massachusetts (Cook, 1983), and 71-96% in Pennsylvania.

Information on larvae is less common. Larvae grow rapidly and are voracious predators. They can be cannibalistic or consume body parts bitten from conspecifics, but the major prey are invertebrates. Metamorphosis has been reported from early July to mid-September after 2-4 months in New York (Bishop, 1941). It has occurred as early as 22 June in Indiana (Minton, 1954), and 66-80 days in Ohio (Downs, 1989). Estimates of larval survival are scarce, but indicate rates are quite low. Estimates for two years in an Ohio population are 0.08 and 0.7% (Downs, 1989) and 0% for a Maryland pond (Thompson et al., 1980). Larger larvae of *Ambystoma opacum* as well as some aquatic insects are known predators of larvae.

This summary of *Ambystoma jeffersonianum* reproduction, taken primarily from Petranka (1998), has no references to data from any Virginia populations. The significance is that Virginia is the southeastern limit of its range. Therefore, the biology of Virginia populations holds special significance and needs to be examined. Tuscarora Pond in Douthat State Park in Bath County holds one of the largest populations in the area. The only larger populations reported in the VaFWIS database are from two ponds west of Harrisonburg in Rockingham County. Populations over 100 individuals were reported in 2003, however, there do not appear to be any continuing studies at these sites. The Tuscarora population is far enough from regularly traveled routes to be safe from most forms of exploitation, and its occurrence within a state park should promote its protection and, therefore, its stability.

Materials and Methods

The site was surveyed in 2006-2009 from December until April, as time and weather permitted. Adults were captured by dip net, examined, measured (from tip of snout to posterior end of vent, weighted on an Acculab V-333 balance with readability to 0.1g), sometimes marked, and then released. In 2007 and 2008 the number of eggs per egg mass, and for a subset of masses the number of live and dead eggs, were recorded. In 2009, four gravid females were captured and placed individually in a five gallon plastic bucket with a male and small branches. On 1 April these adults were released and the number of eggs oviposited in the bucket were counted.

Results

To determine the date of first breeding in an early spring season, we made surveys as time allowed in 2006-2009. In 2006 when the fewest surveys were made, eggs were ready to hatch by mid-February, indicating that breeding occurred in either late December or early January. In 2007, the first breeding occurred after 30 December but before 13 January when 10 adults and multiple egg masses were observed. On 3 February two adults were observed swimming under the ice that covered the pond. On 24 February one adult was observed swimming under the ice. On 3 March four gravid females were captured, measured and released. In 2008 43 adults and 18 egg masses were found on 9 February. The adults were measured, marked and released. Prior to that, the pond was dry. The pond then completely dried by 23 February. There were three adult males in the pond on 8 March and one on 22 March. In 2009 the pond was dry until mid-March. There were three adult males in a few inches of water on 21 March, and eight females on 28 March with more water in the pond.

The number of eggs per egg mass, the percentage of viable eggs and average number of eggs per female is recorded in Table 1.

Table 1. Data on *Ambystoma jeffersonianum* eggs from a population in Bath County Virginia.

	AVER	STD	Range	N
# eggs/mass	39.6	16.4	7-69	26
% eggs viable	94.7		88-100	11
# eggs/♀	175	33.7	133-214	4

In 2007 and 2008 when adults were captured by dip-netting, they were weighed and measured for snout to vent length (SVL). The length and weight data for males, gravid females and nongravid females are presented below in Table 2.

Table 2. Data on *Ambystoma jeffersonianum* adults from a population in Bath County Virginia.

	SVL (mm)	STD	Wt (g)	STD	N
Males	86.1	4.3	11.0	1.6	2
Gravid Females	90.4	5.1	13.4	1.6	25
Nongravid Females	92.1	6.0	12.1	1.4	14

To determine if the size difference between sexes was statistically different, first a Kolmogorov-Smirnov test was done comparing the groups in both years to a normal distribution. The results were not significant ($P=0.18$ in 2007 and $P=0.63$ in 2008) showing that SVL in both sexes was normally distributed. Once it was known that SVL was normally distributed, a t-test was done to test for a significant difference between the sexes for both years. Females were significantly longer than males both years ($P=0.0014$ in 2007 and $P=0.0066$ in 2008)

To determine if there was a significant difference in weight between females which were gravid vs. those which appeared to have already laid their eggs, first an analysis of variance was performed on the distribution of SVL vs. weight for the gravid vs. spent females. When the distributions were found to be non-overlapping (no significant difference between the slopes of the distributions, $P=0.89$), another analysis of variance was performed between the two distributions to compare the average weight for each size. Gravid females were found to be significantly heavier than spent females ($P=0.0015$).

Discussion/Conclusions

The date of first breeding can be difficult to determine when the field site is remote and weather (snow) and work can preclude frequent visits. There were only spotty observations made during the winter and early spring of 2006, but eggs were ready to hatch by mid-February, indicating that breeding had occurred in either late December or early January. In 2007, breeding occurred between 30 December (when there were no eggs) and 13 January (when both adults and eggs were observed), probably on 5 January during a warm rain which occurred then. In 2008, 43 adults and 18 egg masses were found in Tuscarora Pond on 9 February. Prior to that time the pond was dry such that breeding was not possible. Due to drought in 2009 the first breeding was delayed until later March. On 21 March three males and eight females were found. Seven of the females appeared to be gravid, on 28 March. At Tuscarora Pond, the date of first breeding appears to occur in early January if sufficient water is present. Breeding typically occurs with the first warm rains, any time after late December. This puts *Ambystoma jeffersonianum* breeding before *A. maculatum* but after *A. opacum*. The breeding order determines the size of the larvae in the spring, which determines the predation order of these three species, giving an advantage to whichever species breeds first and is largest. The dates found here are consistent with those reported from other Virginia populations with breeding on 24 January (Jopson, 1972), 29 January (Jopson, 1984), and 16 February (Stevenson et al., 1996). Breeding appears to continue through the spring, with varying numbers of adults, until late March when the last adults are found at the breeding site. As one would expect with the direct tie amphibians have to free standing water, breeding is linked to the availability of water. Breeding occurs as water from rain and snow melt fills the pond, and needs to be completed in time for the larvae to undergo metamorphosis prior to the pond drying in the summer.

In 2007 we observed adults in Tuscarora Pond during surveys from January 13 until March 24. In 2008 we attempted to determine the length of time individual animals remained in the pond by individually marking animals by toe clipping and hoping to recapture them during subsequent surveys. On 8 February 2008 we marked and measured 43 adults. However, the pond then completely dried forcing all animals to leave the pond. Surveys in March found only four adults

in the pond, indicating the breeding season had ended for 2008.

The average number of eggs found per egg mass (39.6) is only slightly higher than those reported (16-35) for other populations (Petranka, 1998). However, because females are reported to have 140-280 ovarian eggs (Bishop, 1941; Uzzell, 1964; and Petranka, 1998) this number has questionable value since females must lay more than one egg mass. To address this question, in 2009 we placed four gravid females individually in five gallon buckets with a male. After three days the adults were released and the eggs counted. The average of 175 eggs per female is on the low side when compared to the number of ovarian eggs found, but is probably a more realistic number since not all eggs in the ovaries may mature and be laid that year. This estimate is the first report for a Virginia population. Unfortunately, we did not count the number of egg masses produced by each individual female. However, if one takes the average number of eggs per female and divides by the average number of eggs per egg mass one gets $175/39.6$ or 4.4 egg masses per female. Thus, on average females are producing 4-5 egg masses for their entire clutch of eggs.

Previous reports of embryonic survival are fairly high. Survival rates of 60-96% are reported for more northern populations (Cook, 1983; Brodman, 1995). The report of 95% here is made on egg masses which have not yet hatched, and represent the eggs which have not suffered either early developmental abnormalities or environmental problems such as freezing which would have killed the embryos. It is possible that such problems could have occurred after the counts were made in early March 2008. In fact, breeding in February of 2008 was totally negated by a drought which dried the pond and killed all the eggs laid earlier that year. The estimate of 95% is a best case situation where there is no drought.

There was no attempt to measure larval survival. We did not develop realistic methods for marking larvae until late in the study. However, both *Ambystoma maculatum* and *A. opacum* coexist in Tuscarora Pond as well as *Notophthalmus viridescens*, which would all be significant predators.

Uzzell (1964) found that females were 13% larger than males. The present study found that females were longer than males by an average of 8% in 2007 and 5% in 2008. While our numbers are not as large as previous studies, it shows the same trend, that females are significantly larger than males.

In 2007 the survey where most females were captured and measured appeared to be after a major breeding event. Many of the females did not appear to be gravid, but looked as though they had already laid their eggs. This is in contrast to 2008 when a large number of females appeared gravid, or eggs could be seen through the body wall. When the distribution between size (SVL) vs. weight for the two groups were compared, gravid females were found to be significantly heavier than spent females ($P=0.0015$). The difference in average weight was 1.52 g, which should represent the average weight of the egg mass while still in the body cavity, before it is laid and absorbs water.

These data represent some of the first basic biological information for *Ambystoma jeffersonianum* in Virginia from a population at the most southeastern extent of the species range. While the fossorial nature of the species makes it difficult to study, it is hoped that this report may generate further interest and future studies.

Acknowledgements

This work was performed under Virginia Scientific Collecting Permits 32983 and 34079, and Department of Conservation and Recreation Research and Collecting Permits DO-RCP-031609 and DO-RCP-111207. The authors thank the staff at Douthat State Park for allowing the study and Norman Reichenbach for help with the statistics used. John Orr reviewed the manuscript and made many helpful improvements to the paper.

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