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Natural History of the Marbled Salamander, Ambystoma opacum (Gravenhorst), in West Virginia, with Special Notes on Reproduction and Larval Development

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Natural history of the marbled salamander, *Ambystoma opacum* (Gravenhorst), in West Virginia, with special notes on reproduction and larval development

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by

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Abstract

This study observed a population of *Ambystoma opacum* in Kanawha State Forest, Kanawha County, five miles south of Charleston, West Virginia. Data were collected from September 1995 to November 1997. Reproductive biology and salamander development were examined. Predation of *A. opacum* and potential predators were also noted.

Like other species in the genus *Ambystoma*, adult *A. opacum* are mostly fossorial and rarely seen above ground outside of the breeding season. During the breeding season, adults make their way to the breeding pools. Males ventured to the breeding pool first in early September and ranged in size from 49 to 75mm snout-vent length (SVL). Females arrive a few days later and ranged in size from 59 to 77mm SVL. In this study, two females were found over 160 meters from the breeding pool.

During courtship a male deposits a spermatophore on the substrate and the female clips the sperm packet off the top with the lips of her cloaca. Two spermatophores were collected and described. Successful courtship can occur at the breeding pond, or during the migration to the pond.

Ambystoma opacum differs from other West Virginia ambystomatids in three ways: 1) breeding occurs in the fall, 2) the female lays eggs in a terrestrial habitat and 3) females generally brood the eggs for some time, but will abandon the nest if disturbed. This habit of brooding may increase viability of the eggs by deterring potential predators and decreasing the chance of fungal growth on the eggs.

A clutch of eggs is comprised of eggs laid singly in a crudely excavated nest found mostly under logs in this study. Clutch size ranged from 61 to 113 eggs. Eggs were laid directly on the soil in most nests. *Ambystoma opacum* eggs are well adapted to a terrestrial habitat and readily dehydrate/hydrate while the embryo develops inside. This was witnessed on numerous occasions. Eggs hatch when the nest is inundated with water and will do so while the embryo is at various stages of development; from early stages with a great deal of the yolk sac remaining to well-developed, late-stage larvae.

In this population, newly hatched larvae possessed well-developed forelimbs and were approximately 17.0mm total length. Once hatched,

larvae are mostly nocturnal, but could be observed on cloudy, overcast days. Stratification of larvae was not witnessed. Larvae attained total lengths of over 45mm by mid-May but were not observed to transform.

Literature reports numerous predators of *A. opacum* eggs and larvae, many of which were observed at the study site. Predation was observed by rusty blackbirds. Larvae at this time were approximately 30mm TL. Although no oophagy was witnessed, a red-spotted newt was observed on an unbrooded nest.

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Introduction

Purpose of study

The purpose of this study was to determine the natural history of *Ambystoma opacum*, marbled salamander, in West Virginia. Life history of this mole salamander had not previously been studied in West Virginia.

This study was conducted in Kanawha State Forest, Kanawha County, approximately five miles south of Charleston, West Virginia. Data were collected on reproductive biology and salamander development.

Description of species

Ambystoma opacum is a medium-sized salamander that can attain a total length (TL) of about thirteen centimeters. It is a stout salamander with a black base color and white crossbands across its dorsum. Generally, crossbands are hourglass-shaped laterally, broader on the sides of the salamander and more slender along the longitudinal midline of the body (Fig. 1). There is, however, great variation in this pattern. This gives the salamander a marbled appearance, hence its common name. Male *A. opacum* are generally smaller than the females and the white on their dorsum is brighter and often in greater abundance (Fig. 2). The remainder of the body of this salamander is dark gray to black. There are twelve coastal grooves.

Ambystoma opacum, like other salamanders in the genus *Ambystoma*, are mostly fossorial and rarely seen above ground when not in the breeding season (Semlitsch, 1981; Williams, 1973). During the late summer/early fall breeding season, *A. opacum* migrate to dried, temporary ponds or pools with reduced water levels, usually on rainy nights (Marangio and Anderson, 1977). *Ambystoma opacum* is different from other West Virginia *Ambystomas* in three aspects: 1) breeding occurs in the fall; 2) mating occurs on land, which includes a short courtship, spermatophore deposition and reception of the spermatophore by the female (Krenz and Scott, 1994; Lantz, 1930) and 3) clutches of eggs are deposited in a nest in a terrestrial habitat rather than in an aquatic environment (Brandon, 1961; Dunn, 1917; Noble and Brady, 1933). The female often stays with the eggs until the nest is inundated with water (Fig. 3) (Jackson et al., 1989; Kaplan and Crump, 1978; McAtee, 1933). Once the nest is inundated, the eggs hatch (Doody, 1996; Petranka et al., 1982).

Studies by Petranka and Petranka (1980) and Bishop (1941) show that the larval period varies and can range from three to nine months. Total length of newly transformed salamanders range from 41mm (Stewart, 1956) to 71.5mm (Bishop, 1941) when they make their way from the breeding pool to the forest. Variation of TL of newly transformed salamanders and length of larval period are common (Smith, 1990) and are dependent on various pressures to metamorphose such as high conspecific density (Stenhouse, 1985a) or the drying of the breeding pool (Worthington, 1968).

Juveniles may return to the breeding pool to mate their first year, but some may not become sexually mature until they reach six years of age, with an average of 3.3 and 4.0 years for males and females, respectively (Scott, 1994). Graham (1971) and Stenhouse (1985b) reported *A. opacum* are usually loyal to the pond where they first breed and new breeding pools are usually colonized by salamanders that have not yet mated.

Taxonomy & distribution

Ambystoma opacum belongs to the mostly fossorial group of mole salamanders in the Family Ambystomatidae. This group of salamanders spends most of its time beneath the ground and surfaces only during the mating season. The genus *Ambystoma* has been found in Oligocene and Pleistocene deposits dating back approximately 30 million years. *Ambystoma* *opacum* fossils have been found from the Pleistocene and Pliocene epochs (Holman, 1968; Tihen 1955, 1969). *Ambystoma opacum* has been well studied and its nomenclature has been established for decades (Petranka, 1998). Color variations of this species appear in all populations and there are no recognized subspecies.

Ambystoma opacum occurs throughout most of the eastern United States from New England south to northern Florida and west to Illinois and eastern Texas (Fig. 4). It most likely occurs in every county of West Virginia, although specimens have not been collected from roughly half of the state (Fig. 5)(Green and Pauley, 1987).

Description of habitat

Outside the breeding season, this species lives in a variety of habitats from eastern deciduous forests to coastal plains to tallgrass prairies and from moist, sandy areas to dry hillsides. *Ambystoma opacum* has the least affinity to water of the members of the genus *Ambystoma* (Noble and Brady, 1933). Bishop (1941) reported that *A. opacum* was often found in drier habitats than its cogeners.

Description of study site

This study was conducted in a pool adjacent to the Spotted Salamander Trail in Kanawha State Forest (Figs. 6 and 7). The trail is one of the most traveled in the forest because of its proximity to the main forest road. Also, it is paved and cabled for handicapped access and has numerous plaques around the path describing the immediate flora and fauna. In spite of all the human activity, the study site supports the largest known *A. opacum* and *A. maculatum* (the latter of which is the trail's namesake) populations in the forest. The elevation is 800 feet.

The southern end of the pool is fed by an underground water source (Fig. 8). On the northeastern end there is an overflow that allows water to flow into Davis Creek whenever the water level becomes too high for the confines of the pool. Water level of the breeding pool fluctuates with the seasons but did not completely dry up during the study, except for the end of the summer of 1998. Often as water declines in the summer, the pool is divided into two separate smaller pools. The southern section consisted of the water inflow, the northern section, the outflow. During the study, the pool's lowest levels occurred during the time of *A. opacum* migration.

The perimeter of the pool was mostly mossy roots and grass clumps. Near the middle of the pool was a small "island," the perimeter of which was composed of grass and moss similar to the perimeter of the pool.

Materials and Methods

Salamander Procuring

Collection by hand

Many of the adult *A. opacum* were captured by hand, the majority during the migratory period, when large numbers of adults were encountered.

Dip nets

Dip nets were used to collect free-swimming larvae. Different size nets and handles, from long-handled D-frame nets to small, home-aquarium types, were used depending on the situation. During the breeding season, dip nets were also used to collect adults in the pool.

Funnel traps

I did not initially plan to use funnel traps in this study because of their conspicuous attributes; however, another graduate student briefly worked in the same breeding pool and used funnel traps in his study. As previously stated, dip nets were used the majority of the time for collecting larvae, but if nets were not producing, I did inspect the funnel traps of my colleague for *A. opacum* which usually contained a small number of larvae. Funnel traps were used only briefly as they proved to be too vulnerable to human disturbance in this highly traveled area.

Pitfall traps and drift fences

The majority of the adult salamanders in the study were caught with pitfall traps. Some of the pitfalls were placed along natural obstructions such as logs or rocks. The majority, however, were used in conjunction with drift fences. Fourteen pitfalls were used during this study. They ranged from 6 to 57 meters from the breeding pool (Fig. 8).

Pitfall traps were green buckets 19cm deep, with a 17.8cm opening and a volume of 4.25 liters. Pitfalls were buried so the rim was flush with the ground (Fig. 9). This sized trap minimized the capture of non-target species because most anurans, small mammals and snakes were able to escape from the relatively shallow traps. Similar to Stenhouse (1985b), two damp sponges were placed in the bottom of the pitfalls for moisture and shelter for the captured salamanders. The sponges would also float if the trap flooded, giving trapped animals some form of buoyancy.

Drift fences used in this study were brown gutter guards that were 16cm in height. When secured on the forest floor, approximately 14cm remained above the substrate (Fig. 9). The brown color, holes and short height of the drift fence made them inconspicuous in this highly traveled area of Kanawha State Forest. Their low height, as well as making them inconspicuous, allowed larger animals, or animals with the ability to jump, to cross the fence. Gibbons and Semlitsch (1981) state that for pitfalls to be effective one must match the fence and trap to the species that you are attempting to capture.

Breeding Data

Water

Water temperature was taken with a plastic armored thermometer. Water pH was measured with a Waterproof pHep 1 pH Tester (Fig. 10).

Adults

When adults were collected, the gender was determined by examining the cloaca, dorsal coloration and body shape, similar to the method described by Graham (1971). Snout-vent length (SVL) was recorded. Snout-vent length was measured from the tip of the snout to the posterior edge of the vent. All adults collected in 1995 and 1996 were measured. In 1997, however, during peak migration, many captured *A. opacum* were only sexed and the SVL was not recorded due to the large numbers encountered. A two-tailed t-test was run to determine if there was significant difference in SVL between the male and females measured.

Spermatophore

Two spermatophores were collected during the study. These were deposited on a sponge by a male *A. opacum* caught in a pitfall trap with two females. The spermatophores were placed in 90 percent ethanol for roughly 48 hours. After slides were taken of the spermatophores, they were fixed in formalin and placed in the Marshall University Vertebrate Museum collection.

Nests

Soil pH and moisture was measured using an E.W. System Soil Tester (Fig. 10). Soil temperature was measured beneath the egg masses with a Reotemp Stainless Steel Bi-Metal thermometer.

Nest dimensions were measured with a ruler to the nearest 1.0mm. Nest temperature was measured with a thermometer placed within the mass of eggs for at least five minutes. The thermometer used for this was the plastic-armored thermometer used to measure air and water temperatures.

Brooding Females and Fecundity

Eggs were counted in nests in 1995. Snout-vent length of the brooding females, if present, was measured. A Pearson-r correlation was run to determine whether there was a correlation between female SVL and clutch size. In later years, nests were merely located. Nests were not measured and the eggs were not counted to keep disturbance of the brooding females and nests to a minimum. This allowed me to measure the length of time females brood their eggs.

Eggs and Larvae

Eggs were collected from six selected nests on a weekly basis beginning 9 October 1995. Eggs were collected from fewer nests each week as nests were inundated by water and eggs hatched. Eggs collected were placed in 90 percent ethanol for later examination. Eggs were weighed and diameter measured. Larvae inside selected eggs were removed and TL measured. The larvae were patted dry and weighed. Harrison stages of development were determined according to the methods by Rugh (1948). Weighing was done on a Allied Fisher Scientific Model 7301A balance scale to 0.01g. A Pearson-r correlation was run to determine if there was any correlation between larva size and egg diameter. Larvae were placed back into 90 percent ethanol and transported to Marshall University, fixed in formalin and placed in the Marshall University Vertebrate Museum collection.

Free-swimming larvae were collected monthly beginning 15 November 1995. An attempt was made to collect five larvae during each visit to the study site. Collected larvae were placed in 90 percent ethanol for later examination. Larvae were measured and developmental status noted. Snout-vent length was measured for all free-swimming larvae and TL measurements were made of those larvae in satisfactory enough condition to do so. Larvae were deposited in the Marshall University Vertebrate Museum collection.

Results and Discussion

Water

On 6 October 1995, the section of the study pool with the water inflow was 21.0°C and pH 6.3. The northern section of the pool which had no inflow of water was 25.5°C and pH 5.8. When autumn rains started and began to fill the pool, the two sections became one as the water level rose and the temperature and pH became more consistent throughout the pool.

Adults

During the study, 152 adult *A. opacum* were captured; 80 males and 72 females. Males and females of *A. opacum* show sexual dimorphism in both size and coloration. During this study, SVL of 55 *A. opacum* adults was measured, 24 males and 31 females. Snout-vent length of males ranged from 49 to 75mm with a mean of 62.2mm. Females were significantly larger with a SVL range of 59 to 77mm and a mean of 70.0mm ($p < 0.05$).

Adults captured during this study showed extensive variation in dorsal coloration. Males were consistently brighter and had a greater abundance of white markings. During the night of peak migration in 1997, one male *A. opacum* was captured that was almost completely white-backed. Conversely, on the same evening a nearly all black female was observed.

When most adult *A. opacum* were discovered during the breeding season on the roads of the study area, touching the tail of the salamander would cause the animal to "sprint" for 1 to 1.5 meters. Another touch to the tail would set the reaction off again. Salamanders moved a lesser distance when they found cover. Other adults would simply "sprint" until cover was found.

When a salamander was uncovered or encountered in/on leaf litter, its reaction to touch and light were very different. Generally the salamander would touch its snout to the ground and loosely coil. This behavior was also reported by Ducey (1989). Noble and Brady (1933) reported that the animal would hold this posture until a means of escape was found. I found that the salamander only needed to find a leaf to hide its head. Brodie (1977) reported that when disturbed, *A. opacum* elevates its tail and will then lash its tail toward the danger. Occasionally in my study, a salamander reacted this way when disturbed, but I found the animals I encountered stopped the posture after raising the tail. In only a couple of instances did the animals lash their tails towards me.

When observing adult *A. opacum* in the water, the salamanders appeared uncomfortable and ill-adapted. They swam quite slowly and were usually in shallow areas of the pool close to some structure that was near, or protruded from, the water surface, such as a rock or a log. They behaved very differently from *A. maculatum* which appeared more adapted in the water and were very quick swimmers. Noble and Brady (1933) observed this behavior and reported that *A. opacum* are much more terrestrial than their cogeners. In their laboratory experiment, when *A. opacum* adults were placed in a tank with 2cm of water, they would attempt escape. At 3cm, they increased their attempt to escape and would rest on a stone in the middle of the tank. At 15cm of water, two of the six *A. opacum* drowned. When in the tanks, *A. opacum* refused to eat. In the same experiment, *A. maculatum* swam around in the tank comfortably and ate regularly. In the field, they found that if *A. opacum* was picked up and placed in water a few centimeters deep the salamander would swim to the surface and climb out of the water onto an object, rather than swimming into deeper water and hiding under the leaf litter as *A. maculatum* will do.

During 1997, when I witnessed the most active migration period, I first caught *A. opacum* in my pitfalls on 7 September. On this day, only males were captured (n=6). Two days later 69 adults were captured, 38 males and 31 females. The majority of the males were already around the water, while the majority of the females were still making their way to the pool. There were exceptions to this in both sexes, but reports in the literature suggest that adult males migrate to the breeding ground first and are later joined by the females (Noble and Brady, 1933).

More recently it has been shown that many females arrive at the breeding grounds already inseminated suggesting that they were successfully courted during migration (Krenz and Scott, 1994; Sever et al., 1995). On 9 September 1997, in a pitfall trap, there were two spermatophores deposited on the sponge. When I discovered this, there were two females and one male caught in the trap. They were still 23 meters from the breeding pond, yet the male deposited the spermatophores. The sperm caps had already been removed. This spermatophore deposition and cap removal could be taken as evidence that *A. opacum* will mate before reaching the breeding grounds, or could also be because of the circumstances of being trapped in such a small area with two receptive females at this time of year. Because of the large number of salamanders migrating this evening, I checked my trap line once every 15 to 20 minutes, so at the most the three salamanders were in the pit for 20 minutes.

Male *A. opacum* leave the breeding pool first while females remain to brood their nests (Noble and Brady, 1933; Petranka, 1998). My findings agreed. After the peak migratory night, very few *A. opacum* were caught in the pits until 20 September, when 21 males were captured, most of which were moving away from the breeding ground. Seven roaming females were also observed, five around the boundaries of the breeding area and two moving towards the pool.

Between running the pitfall trap-line during peak migration in 1997, I ventured away from the pool to attempt to observe adults still migrating to the breeding site. The two salamanders captured the greatest distance from the breeding site were females. One was approximately 170 meters from the pool, the other 165 meters. There may have been some individuals even farther away, but I did not want to wander too far because of the need to empty my pitfall traps. These distances fall well within those reported in the literature. Williams (1973) reported adult *A. opacum* maintain home ranges 0 to 450m from their breeding ponds. Most of the migrating salamanders in this study were coming from the east across the forest road from the Polly Hollow area (Fig. 6). One other pool very close to the study site and the slower sections of two nearby streams were also surveyed for *A. opacum*, but no adults were encountered in these areas.

The latest a male was observed was 12 October 1996. The latest a female was observed was 8 November 1996.

Spermatophore

The spermatophore of *A. opacum* (Fig. 11) is deposited on terrestrial substrate. They were approximately 5mm tall, 3mm wide at the head and 6mm wide at the base. The base of the spermatophore is gelatinous and nearly transparent (Fig. 12). The head of the spermatophore is truncate, roughly rectangular with rounded protuberances at the corners and slightly concave (Fig. 13). The white sperm packet rests on the top of the spermatophore and is positioned so the female can easily grasp it with her cloaca (The two recovered in this study had already had their sperm caps removed.). This description is similar to those given by Lantz (1930) and

Noble and Brady (1933).

Spermatophores found in this study were first placed in 90 percent ethanol until photographs were taken before being placed in formalin. Labanick And Davis (1978) found with small-mouthed salamanders (*A. texanum*), there was no difference in spermatophore morphology between spermatophores placed in alcohol and those immediately fixed in formalin.

Nests

In 1995, eighteen *A. opacum* nests were discovered at 16 different locations in the study area. Fourteen nests were single female nests. One nest was a confirmed double nest (Fig. 14), and another was an assumed double nest based on the way the eggs were spread out over a large area. It is common for this species to have communal nests (Graham, 1971; King, 1935; Noble and Brady, 1933). Petranka (1990) stated that communal nests could be determined by the shape of the nests, with double nests resembling the shape of a dumbbell. Although the only communal nests discovered in this study were doubles, communal nests of up to seven females have been reported (Graham, 1971). No nests were found in the adjacent pool or nearby streams.

Dimensions of thirteen measured single nests ranged from 6.6 x 5.5cm (total nest area = 36.3cm^2) to 17.8 x 6.4cm (113.9cm^2) and depths from 0.5 to 4.8cm (Table 1). Dimensions of the two double nests were 19.1 x 5.2cm (99.3cm^2) and 20.6 x 7.2cm (148.3cm^2) (Table 2). Depths for the two double nest were 1.5 to 2cm and 0.5 to 1cm, respectively. Mean nest area for the single nests was 66.1cm^2. When including all nests the mean nest area was 73.8cm^2.

Table 1. Single nest dimensions and clutch size.

Nest	Dimensions (cm)	Nest area (cm^2)	Nest depth (cm)	Clutch size
1	6.6 x 5.5	36.3	2.2	78
2	7.9 x 6.4	50.6	4.2	83
3	12.1 x 6.3	76.2	3.0	61
4	8.4 x 6.3	52.9	3.5	73
5	8.8 x 11	96.8	4.8	77
6	6.3 x 7.4	46.6	1.8	88
7	13.2 x 9.3	122.8	3.6	72
8	7.5 x 7.1	53.3	2.3	87
9	17.8 x 6.4	113.9	1.8	129
10	12.2 x 5.3	64.7	1.3	113
11	6.8 x 5.5	37.4	3.8	97

When the nests were first located the temperature of the soil around the eggs and the egg mass was measured (Table 3). Soil temperature ranged from 5.5 to 8.8°C with a mean temperature of 7.2°C. Temperature of the egg mass ranged from 6.8 to 13.5°C, with a mean of 9.4°C. This is noticeably cooler than the 16 to 18°C nest temperature that Moore (1939) reported, but well within the 7 to 10°C range that Noble and Brady (1933) used to keep eggs viable in the laboratory. Air temperature on the day measurements were taken was 22.5°C. Soil beneath four of the nests was also measured for pH and percent moisture on 15 October 1995 (Table 4). Only four nests were measured because the water level had risen above the other nests by the time I was able to procure the measuring device. Mean pH of the nests was 5.9, and the pH ranged 5.7 to 6.6. Soil percent moisture ranged from 52 to 60 percent, with a mean of 56.8 percent.

Table 2. Double nest dimensions and clutch size.

Nest	Dimensions (cm)	Nest area (cm^2)	Nest depth (cm)	Clutch size
1	19.1 x 5.2	99.3	1.8	144
2	20.6 x 7.2	148.3	0.8	114

Table 3. Nest temperatures.

Nest	Egg mass Temperature (°C)	Nest substrate Temperature (°C)
1	10.0	8.0
2	11.5	8.0
3	12.0	7.0
4/5	10.5	8.5
6	10.0	9.0
7	9.0	6.5
8	7.5	6.5
9/10	7.0	5.5
11	8.0	5.5
12	7.0	6.0
13	7.5	7.0
14	8.0	6.5
15	---	8.0
16	14.0	8.0
17	10.5	8.0
18	9.0	8.5

Table 4. Nest pH and nest substrate % moisture.

Nest	Soil pH	Soil % moisture
1	5.8	60
2	5.7	52
3	6.6	55
4	5.7	60

When nests were first discovered on 6 October 1995, they were various distances from the edge of the water. The distances ranged from 0.7 to 6.0m. Elevation of the nests above the waterline was also variable and ranged from roughly 2 to 7 cm. Reports in the literature indicate that lower/intermediate depths are used for nests because those that are on the pool bottom may become inundated too soon with a chance of the pool evaporating and those placed too far from the pool bottom may never be inundated (Jackson et al., 1989; Petranka and Petranka, 1981). Either of these scenarios would be fatal to *A. opacum* larvae.

Nests were found under various structures. Cover analyses of eighteen nests are reported here. The majority (n=12) were found under logs. This differs from the findings of Petranka (1990) and Figiel and Semlitsch (1995) who found nests to be built primarily under leaf litter and grass clumps, respectively. Other nests were either under rocks (n=3) or leaf litter (n=3). Most nests were below at least a thin layer of leaf litter resting directly on the muddy substrate as observed by Brimley (1920). Some nests were not excavated all the way to the substrate and the eggs were laid on a thin layer of leaves. Two nests found under rocks also had very little soil and the majority of eggs were lying directly on a rock surface.

Brooding Female and Fecundity

The number of eggs per nest was quite variable and ranged from 61 to 129 eggs in single nests with a mean of 86.4 eggs (n=13) (Table 1). Large range in clutch size is consistent with what is reported in the literature (Dunn, 1917; Noble and Brady, 1933; Petranka, 1990; Scott, 1990). Double nests had 114 and 144 eggs each (Table 2).

Literature reports suggest clutch size is directly correlated to the SVL of the brooding female (Petranka, 1990; Walls and Altig, 1986). In single nests located in 1995 that had a brooding female present (n=9) this correlation was not observed (Table 5, Fig. 15). Pearson-r correlation coefficient was -0.26 and was not significant at the 0.05 level. Snout-vent lengths ranged from 69 to 75mm for the brooding females and clutch sizes ranged from 61 to 129. The smallest female had a clutch of 83 eggs while the largest had a clutch size of 97. The largest clutch size of 129 and the smallest of 61 were both brooded by 71mm SVL females.

Brooding of the eggs by the female most likely reduces predation, egg desiccation, egg clumping and fungal growth on the eggs (Jackson et al., 1989; Salthe and Mecham, 1974; Petranka, 1990). On 22 October 1998, a non-brooded nest was located. The eggs appeared in poor condition and stuck together in a loose mass rather than the usual distinct, individual eggs. In addition, there was a powdery mildew type fungus growing on the surface of the majority of the eggs. As reports in the literature imply, this fungal growth may have been present due to the fact that the female had left the nest and was not stirring the eggs with her movements. On the other hand, the poor condition and/or fungal growth on the eggs may have been the cause for nest abandonment by the female rather than the effect.

Table 5. Female snout-vent length versus clutch size.

SVL (mm)	Clutch Size
69	83
70	78
70	113
71	61
71	129
72	77
73	73
74	72
75	97

In 1995, eighteen *A. opacum* nests were found. Of these 18 nests, 15 were being brooded by females. The first nests were found on 6 October and 11 brooding females were found. On 7 October, I returned to the study site to search for more nests and record nest data. Two of the females had abandoned their nests, most likely due to the disturbance of locating and uncovering the nests (King, 1935; Noble and Brady, 1933). After collecting nest data, I returned to check on the remaining females on 9 October. Only three females remained on their nests. On 15 October, only one female remained. By 22 October, the last female had left her nest even though the water did not reach the nest until 29 October.

King (1935) found individual females react differently to nest disruption. After initial disruption (6 October), King reported that four of seven females continued brooding. One month later (6 November) one female was still on her nest, but left within another month (6 December) even though the water had not yet reached the nest. In another case, King reported that one female remained with her nest for another two weeks after being removed twice for photographs.

In 1997, disturbance was kept to a minimum to determine how long the females would brood their nests. I located females on nests on 11 October, but all were gone by 18 October even though the nests had not yet been inundated with water. I later discovered that West Virginia Division of Natural Resources personnel had been in the area on 15 October with a biology class and had disturbed the site extensively while looking for amphibians. No *A. opacum* were seen after this disturbance.

The latest a female was observed brooding a nest in this study was 1 November 1996. Water was still approximately 1m from the nest. By 5 November 1996 this female had left before the water inundated the nest.

Eggs and Larval Development in the Egg

Eggs of *A. opacum* are spherical, rugged structures well adapted to temporary, yet prolonged, exposure in a terrestrial habitat. They readily pick-up pieces of substrate (sand and soil) while in the nest which camouflages the eggs and adds to their toughness. The majority of the eggs observed were covered with substrate (Fig. 16), but the rare egg was virtually transparent and the developing larvae inside could be observed (Fig. 17).

Employing the amphibian egg morphology nomenclature of Duellman and Trueb (1986), the outer capsule of the egg was tough. Substantial pressure with a scalpel was needed to break through this first layer. The inner capsule of the egg is a very distinctive enclosure (Fig. 18). The diameter of the inner capsule was 5.8mm in a complete egg that had a diameter of 7.3mm. More pressure with a scalpel, albeit not as much pressure as with the outer capsule, was needed to get into the inner capsule and to the vitelline membrane of the egg to free the larva.

Eggs regularly contract and expand due to the relative humidity of the nest area (Noble and Brady, 1933) while the larva continues to develop inside the egg. I also found this to be the case. Eggs with smaller diameters than those collected a week earlier contained larvae that were more advanced in their stage of development (Table 6, Fig. 19). The largest average egg diameter was 7.4mm, collected on 15/17 October 1995. The smallest average diameter (6.0mm) was observed on 22 October, one week after collecting the eggs with the largest diameter. Average TL of the larvae in the 15/17 October eggs was 11.8mm, while the average larval length in the 22 October eggs was 13.2mm. An average growth of 1.4mm was observed even though the eggs had shrunken by almost 1.5mm. The largest average TL of 17.0mm were larvae from eggs collected 12 November just prior to hatching. Diameters of these eggs averaged 6.5mm, 0.9mm less than the largest egg diameter seen collected a month earlier. There was no correlation found between the diameter of the egg and the length of the developing embryo. The Pearson-r correlation coefficient was zero.

Table 6. Egg diameter versus larval total length.

Collection date	Egg diameter avg. (n)	Larva TL avg. (n)
10/9/95	6.5 (20)	10.6 (7)
10/15&17/95	7.4 (12)	11.8 (3)
10/22/95	6.0 (11)	13.2 (9)
10/29/95	6.4 (7)	15.6 (4)
11/5/95	6.8 (5)	15.5 (5)
11/12/95	6.5 (4)	17.0 (2)

Another example of the ability of the egg to contract and expand while the larva, unaffected, develops inside was observed within eggs collected from the same nest on the same date. On 12 November, the largest egg had a diameter of 7.0mm and weighed 0.11g. The smallest had a diameter of 5.5mm and weighed 0.05g (Fig. 20). When the larvae were released from each egg, both had a TL of 17.0mm (Fig. 21). The larva from the smaller egg was actually in an advanced stage of development compared to the larva released from the larger egg. The Harrison Stage for the smaller egg was 46 (balancers lost) while that of the larger was 45 (balancers intact). The larva of the smaller egg was difficult to work with due to its tightly coiled body. These were the last eggs found and according to Walls and Altig (1986), yolk reserves in larvae at this stage are depleted and the larvae will start to search for prey items.

Eggs hatch when inundated with water. Newly hatched larvae may be as small as 11mm, still carrying a large amount of yolk, up to a well-developed 18mm (McAtee, 1933). Anderson (1972) stated the time of hatching is independent of the time of egg deposition, and that inundation at a pre-hatching stage is less detrimental to the survival of larvae than prolonged terrestrial exposure. One nest I had been collecting eggs from was partially inundated with water on 29 October 1995. The half that was covered with water was crowded with free-swimming larvae. The other half of the nest was still "dry" and contained unhatched eggs. Studies reported in the literature suggest that once larvae have reached a certain stage of development, hatching can be quite rapid when conditions are suitable

(Brimley, 1920; Noble and Brady, 1933).

King (1935) reported *A. opacum* eggs will hatch if they are only very moist and that larvae will quickly perish if there is not enough water to swim or support their bodies. A hatching situation I observed on two separate occasions illustrated this point. On 15 October 1995, a nest was inundated, yet there was not a path of water to the main pool. The eggs had hatched and the larvae were packed tightly in the muddy water. The water level of the pool eventually rose and connected the nest to the rest of the pool allowing the larvae to survive. On 15 November 1995, another nest was in the same situation, but when I discovered it the larvae had already perished in the isolated, thick, muddy water.

Free-swimming Larval Development

Free-swimming larvae that were used in this study were usually captured during brief diurnal trips to the study site. Larvae were usually caught with a dip net near or at the perimeter of the pond as Hassinger et al. (1970) reported. On two occasions I had to break through ice that had formed on the pool to collect larvae. On these occasions, larvae were not hiding in the substrate, but were swimming about similar to their nocturnal activities. This behavior may be because of the lower light conditions in the water caused by the ice cover and has been documented in other studies (Anderson and Graham, 1967). Anderson and Graham (1967) reported *A. opacum* larvae stratifying during low-light times, whether caused naturally by diel cycle or artificially by cloud or ice cover. This behavior has been hypothesized to be the result of larvae following food sources (Petranka and Petranka, 1980). Stratification has been observed predominantly in young larvae, but not in newly hatched or the pre-metamorphic larvae. In this study, larvae were more active at night, but no distinct stratification was observed. This agrees with the observations of Branch and Altig (1981). I found all aged larvae avoided light, either that of my lamps or natural light. This is contrary to what Marangio (1975) reported. He found that only large and transforming larvae avoided light while small larvae were attracted to it.

Free-swimming *A. opacum* were first collected on 15 November 1995 and were then collected at least once a month until May. Deckert (1916) reported hatchling *A. opacum* had fully developed front legs, while Dunn (1917) reported hatchlings with only forelimb buds that still had their balancers. Hatching of *A. opacum* is predominantly dependent on the inundation of the nest. Timing of nest inundation in these two papers support the discrepancy in developmental levels of the newly hatched larvae.

Average SVL's and TL's (when measured) of collected larvae are presented in Table 7. The first larvae collected had an average SVL of 10.0mm and forelimbs with three moderately well-developed digits (Fig. 22). There was just a slight indication of a rear limb beginning to develop (Fig. 23). By the end of December, the rear limbs were pronounced nubs (Fig. 24). Average SVL had increased to 13.7mm. Although rear digit differentiation begins to be seen in larvae collected on 26 January 1996, true digits were not observed until the 8 March 1996 collection (Fig. 25). Average SVL's of larvae were 16.0mm and 17.3mm for January and March larvae, respectively. Larvae collected on 26 January had well-developed forelimbs with four digits obviously present (Fig. 26).

Larvae had a steady growth rate throughout the collection period, except for the 26 January and 23 February 1996 collections (Table 7; Figs. 27 and 28). These collection dates showed larvae at nearly identical lengths. Doody (1996) found that *A. opacum* larvae growth rates were slow in the colder winter months and increase markedly with the hatching of *A.*

texanum. Doody described the *A. texanum* larvae as a "high-quality prey." A similar situation may have occurred in this study.

Table 7. Average snout-vent and total lengths of collected larvae.

Date	SVL Avg. (mm)	TL Avg. (mm)
11/15/95	10.0	---
12/29/95	13.7	27.5
1/26/96	16.0	28.2
2/23/96	15.8	29.1
3/8/96	17.3	32.0
3/24/96	18.1	33.3
4/5/96	21.6	36.7
4/20/96	25.5	43.0
5/19/96	26.8	45.5

Ambystoma opacum larvae collected on 20 April 1995 had well-developed rear limbs (Fig 29). Also, a fifth digit starting to develop on the rear limb was first observed distinctly on larvae collected on 20 April 1996. A well-developed fifth digit was observed on larvae collected 19 May 1996 (Fig. 30).

Transformed *A. opacum* salamanders were not observed in this study. The last *A. opacum* larvae collected were on 19 May 1996. These had an average SVL of 26.8mm and average TL of 45.5mm. Many sources report larger lengths for newly transformed individuals (Bishop, 1941; Keen, 1975; Petranka and Petranka, 1980; Worthington, 1969). Brandon (1961), however, reported that *A. opacum* larvae begin transformation at TL's of 37 to 50mm and Stewart (1956) reported 41 to 63mm . Larvae in this study fall into this range.

Ambystoma opacum larvae that are within a few days of transformation are usually seen at the surface of the water during darker hours "exercising" their developing lungs (Petranka, pers. comm.). This was not observed in this study after numerous nocturnal visits to the study area. The last *A. opacum* larvae I collected still had dorsal fins and gills (Fig. 31). Walls (1990) and Petranka (pers. comm.) reported that the gills and dorsal fin are absorbed by the time larvae leave the breeding pools. After this, small, white, uniformly-scattered flecks appear on the dorsum before they become more concentrated into the characteristic banding pattern (Brandon, 1961). Newly transformed larvae leave the pool at night during rain, or directly after rain (Murphy, 1963; Pechmann, 1995; Stenhouse, 1987).

Something discovered by accident was the amount of teeth that *A.*

opacum larvae possess. While taking larvae out of their vials for pictures, I neglected to put the larvae collected on 29 December 1995 into a Petri dish with enough ethanol. When I returned much later I found the larvae completely dehydrated. The skin had drawn up over the body and in the mouth area. This exposed many teeth when viewed under high magnification (Fig. 32). I was able to rehydrate the specimens by soaking them in distilled water for a couple of hours.

Predation on and by *Ambystoma opacum*

I witnessed predation on *A. opacum* one time during the study. On 23 February 1996, a rusty blackbird walking the perimeter of the pool preyed on an *A. opacum* larva, that were approximately 30mm TL, on two separate occasions.

On 9 October 1995, a red-spotted newt (*Notophthalmus viridescens*) was seen on an unbrooded nest. Although I did not witness the newt eating eggs, there are literature reports that this species is a significant egg predator of *A. opacum.* In the laboratory, newts have been observed preying on *A. opacum* larvae up to 40mm TL (Scott, 1990; Walters, 1975). Noble and Brady (1933) found dusky salamanders (*Desmognathus fuscus*), two-lined salamanders (*Eurycea bislineata*) and green frogs (*Rana clamitans*) to prey upon *A. opacum* eggs, but only in unbrooded nests. These three species were all found in my study area. In the case of the dusky salamander, larval *A. opacum* were also found in the stomach contents. The following species found in my study area were reported by Hassinger et al. (1970) as potential predators of *A. opacum*: common snapping turtle (*Chelydra serpentina*), northern water snake (*Nerodia sipedon*), and eastern garter snake (*Thamnophis sirtalis*).

Ambystoma opacum larvae are voracious predators of other larval herpetofauna. Of the sympatric amphibian species in this study, *A. opacum* will feed on larval spotted salamanders (*A. maculatum*) (Stewart, 1956; Stenhouse et al., 1983), larval American toads (*Bufo americanus*), and larval spring peepers (*Pseudacris crucifer*) (Stenhouse, 1985b). Walters (1975) showed in the laboratory that *A. opacum* larvae will feed on red-spotted newt eggs and hatchlings; American toad eggs and hatchlings; green frog eggs, small larvae and larvae up to 9mm in length and gray treefrog hatchlings and small larvae. Cannibalism is not common in this species and is usually density related and consists of feeding on gills, fins, tails and limbs

(Petranka, 1989; Polis and Myers, 1985; Smith, 1988).

Other herpetofauna seen in the study area in addition to *A. opacum* included ten species of salamanders, six species of toads and frogs, two turtles, two lizards and five species of snakes (Tables 8 and 9).

Table 8. Other amphibians observed during study

Ambystoma maculatum[2]	Spotted salamander
Notophthalmus v. viridescens[1, 2]	Red spotted newt, red eft
Desmognathus f. fuscus[1]	Northern dusky salamander
Desmognathus monticola	Seal salamander
Plethodon cinereus	Red-backed salamander
Hemidactylium scutatum	Four-toed salamander
Plethodon g. glutinosis	Slimy salamander
Pseudotriton r. ruber	Northern red salamander
Eurycea b. cirrigera[1]	Southern two-lined salamander
Eurycea l. longicauda	Longtail salamander
Bufo a. americanus[2]	Eastern American toad
Pseudacris c. crucifer[2]	Northern spring peeper
Hyla chrysoscelis	Gray treefrog
Rana catesbieana	Bullfrog
Rana clamitans melanota[1, 2]	Green frog
Rana sylvatica	Wood frog
[1] Reported as predator in literature	[2] Reported as prey (in field and/or laboratory) conditions in literature.

Table 9. Reptiles observed during study.

Chelydra s. serpentina[1]	Common snapping turtle
Terrapene c. carolina	Eastern box turtle
Sceloporus undulatus hyacinthinus	Northern fence lizard
Eumeces fasciatus	Five-lined skink
Nerodia s. sipedon[1]	Northern water snake
Thamnophis s. sirtalis[1]	Eastern garter snake
Diadophis punctatus edwardsii	Northern ringneck snake
Elaphe o. obsoleta	Black rat snake
Agkistrodon contortrix mokasen	Northern copperhead

[1] Reported as predator or potential predator in literature.

Conclusion

This population of *A. opacum* studied in West Virginia was similar in some aspects and dissimilar in others to those reported in the literature. The study site is comparable to breeding areas reported in the literature. Adults migrated in early September and left the study area by mid-November. The two females found over 160m from the breeding pond are similar to distances previously reported. Adult SVL's ranged 49 to 75mm for males and 59 to 77mm for females. Spermatophores collected were similar in size and shape to those described in the literature. Clutch sizes ranged 61 to 129 eggs from female *A. opacum* 69 to 75mm SVL. There was no correlation between SVL of the brooding females and fecundity. Eggs showed the ability to hydrate and rehydrate dependent upon the nest conditions while the embryos continued their development apparently unaffected. Eggs hatched when the nest was inundated with water. Newly hatched larvae averaged approximately 17mm TL and had moderately well-developed forelimbs. Development of larvae was similar to that reported in other studies. By mid-May larvae were over 45mm TL, which was within the range of transforming larvae reported in the literature. These were the most advanced *A. opacum* larvae observed, yet still possessed dorsal fins and gills. Newly transformed juveniles were not observed.

Stratification of larvae was not witnessed. All larvae were more active during darker hours and all avoided light. During the day, larvae were most often observed near the perimeter of the pool.

Many predators of *A. opacum* reported in the literature were observed at the study site. The only predation witnessed was a rusty blackbird feeding on larvae approximately 16mm in length on the perimeter of the pool in February. A red-spotted newt, reported as a predator in the literature, was seen on an unbrooded nest, but oophagy was not witnessed.

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Figure 1

Ambystoma opacum adult

Figure 2

Ambystoma opacum sexual dimorphism

female (above), male (below)

Photos by J.J. Watkins

Figure 3

Ambystoma opacum female on nest

Photo by J.A. Watkins

Figure 4

United States distribution of *Ambystoma opacum*

(From Petranka, 1998)

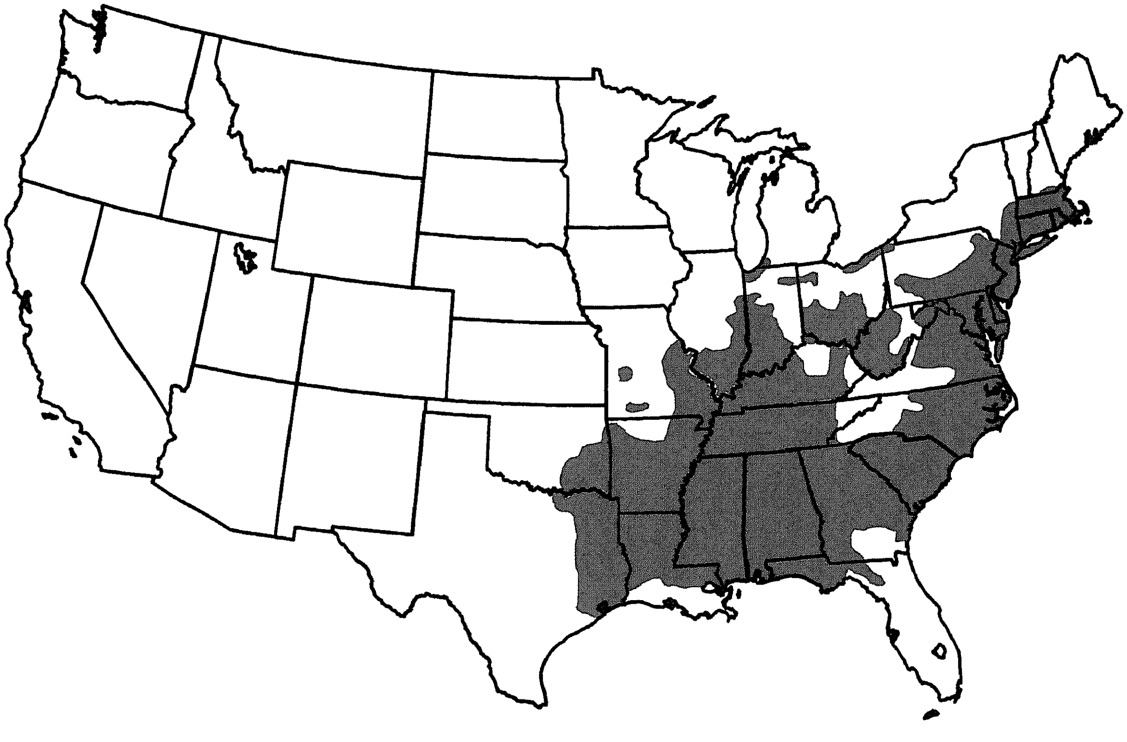


Figure 5

West Virginia records of *Ambystoma opacum*

(From Green and Pauley, 1986)

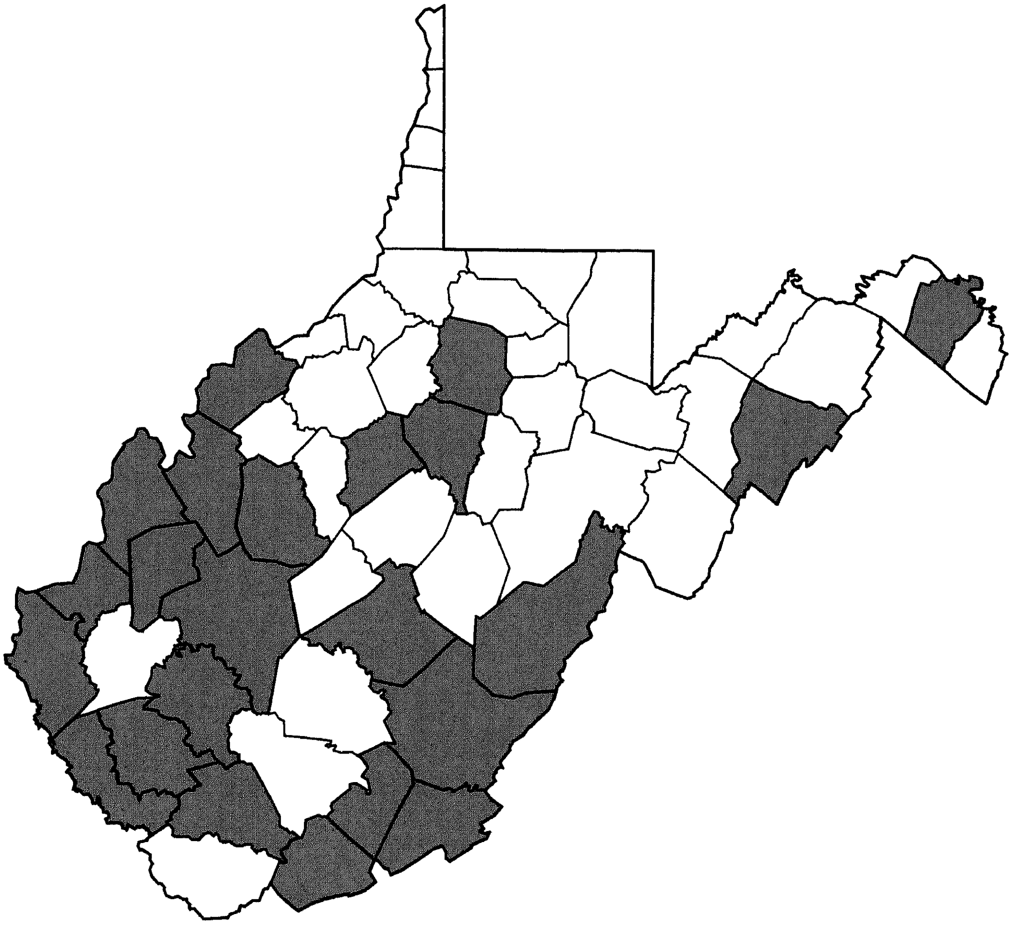
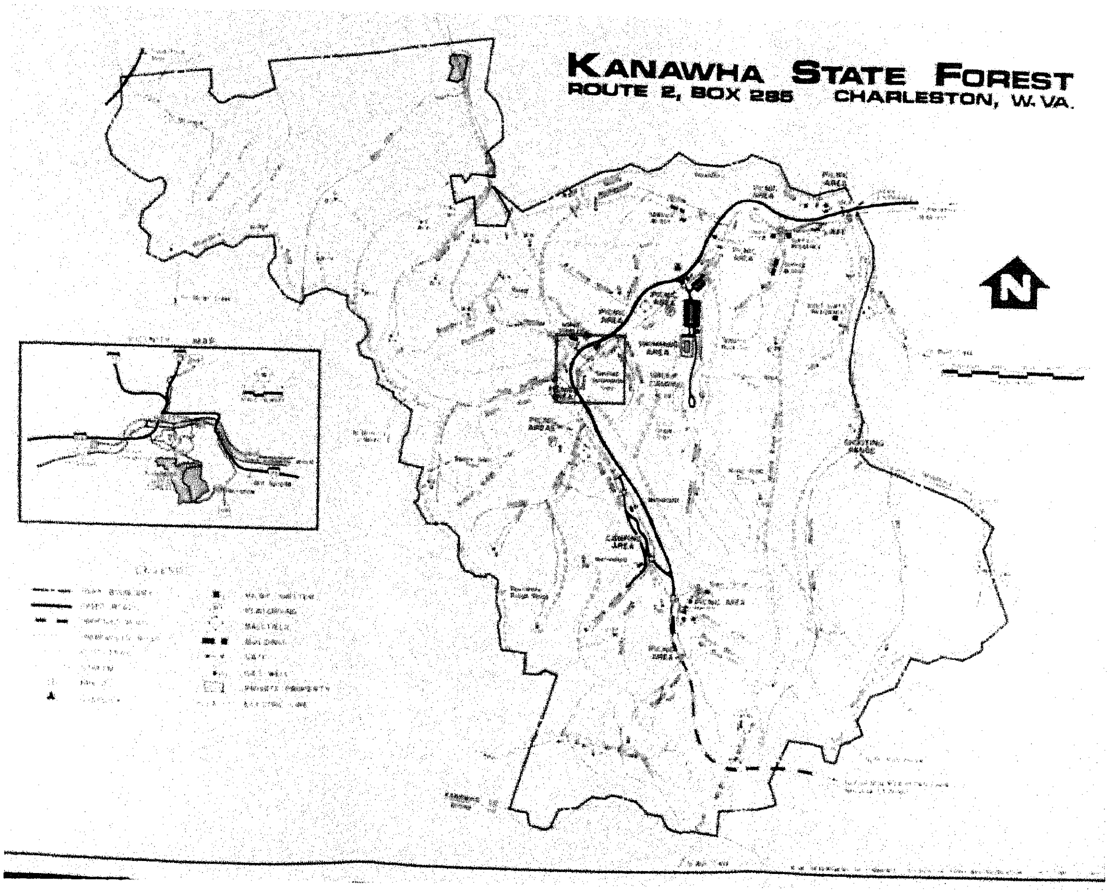
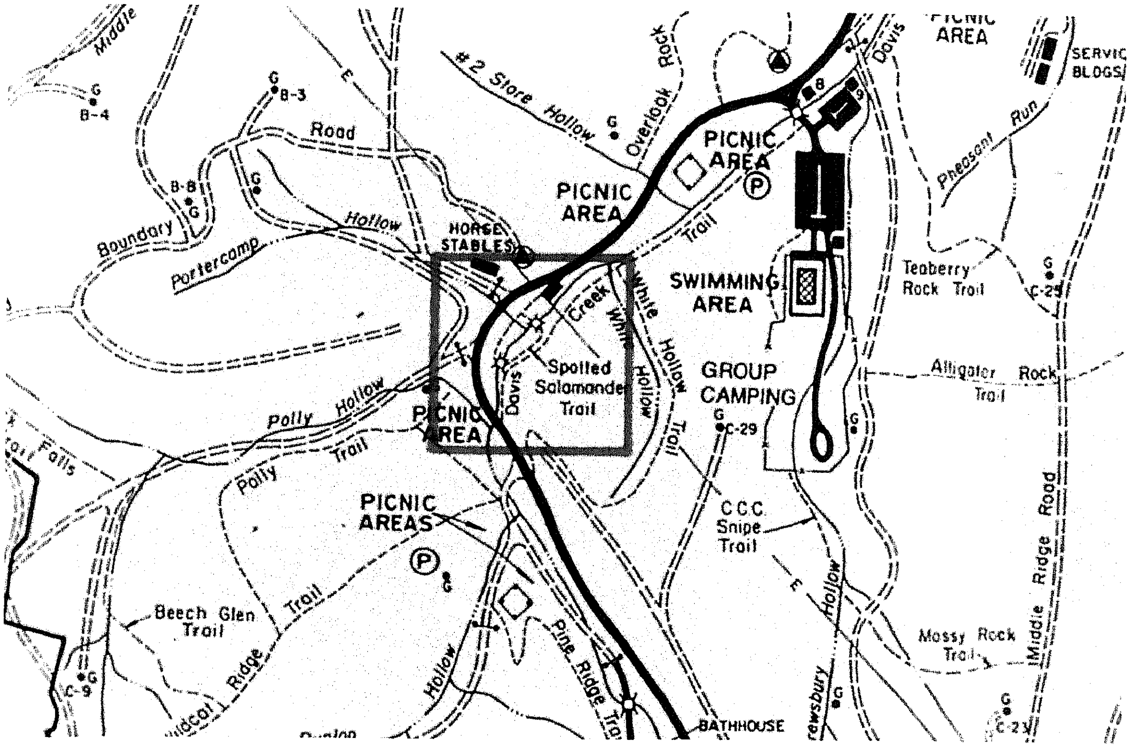


Figure 6

Study site on Kanawha State Forest map

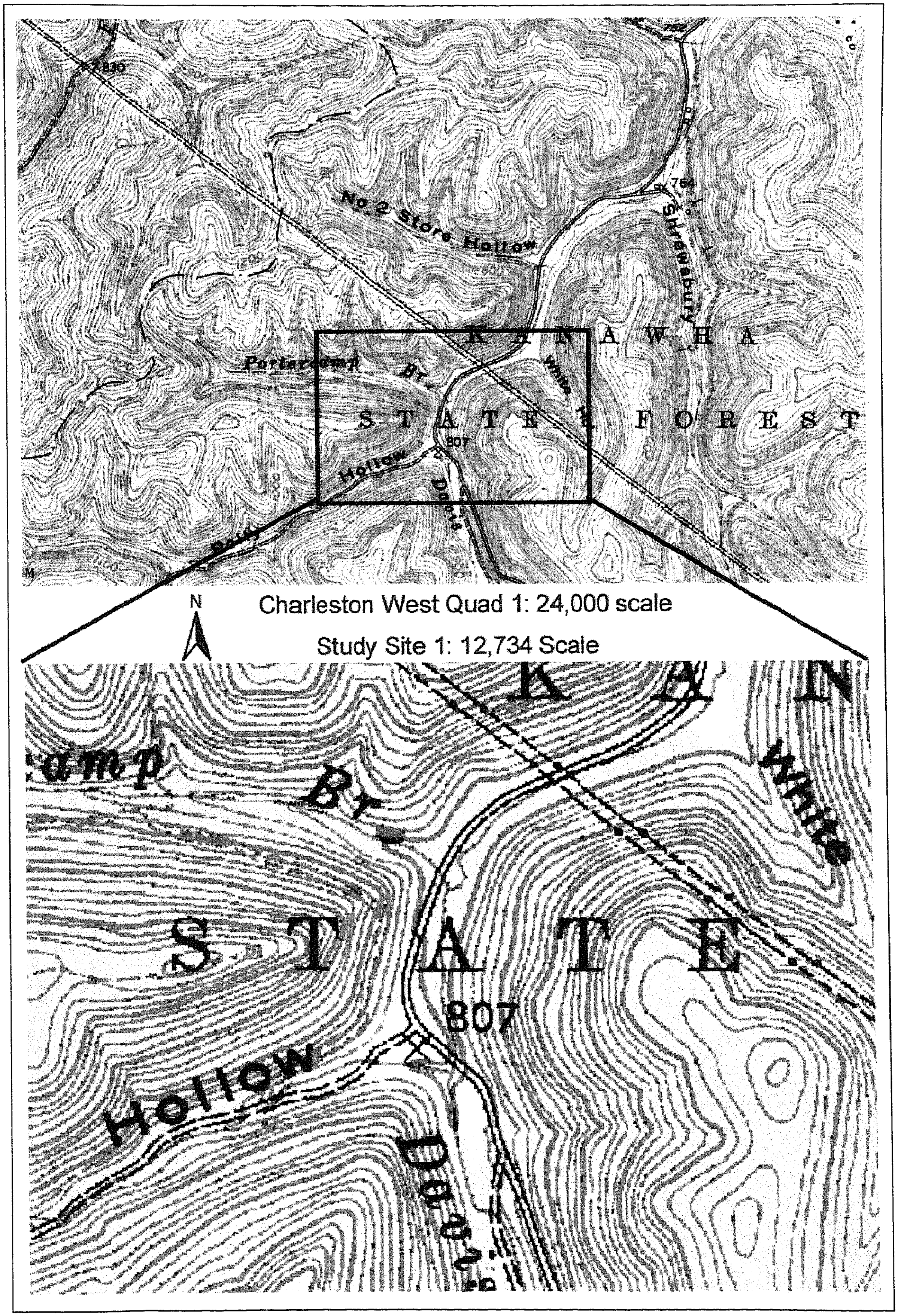




Photos by S.C. Haynes

Figure 7

Study site on USGS topographical map



No. 2 Store Hollow
Shrewsbury
KANAWHA
STATE FOREST
Porterscamp
Br.
White
Hollow
Davis
830
764
752
807
N
Charleston West Quad 1: 24,000 scale
Study Site 1: 12,734 Scale
camp
Br.
White
KAN
STATE
807
Hollow
Davis

Figure 8

Diagram of study site breeding pool and

pitfall trap placement

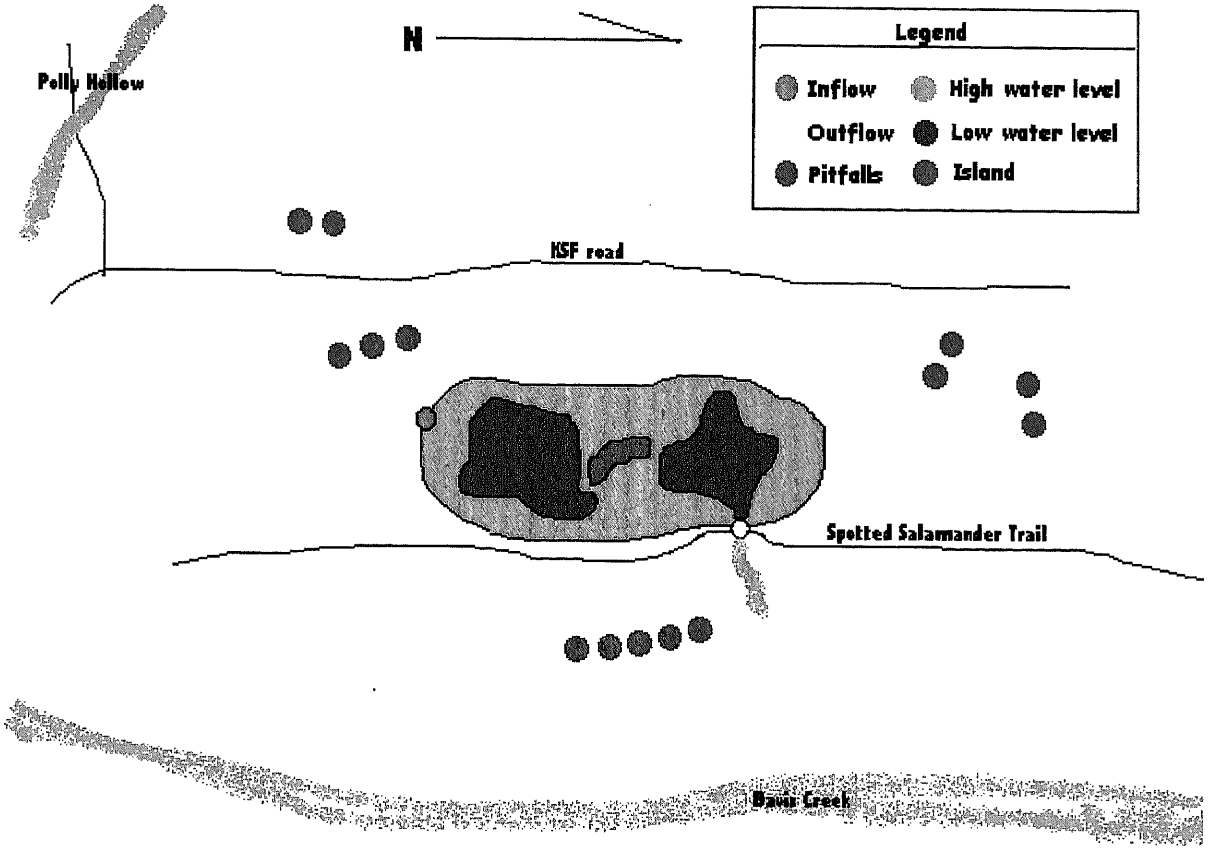

N
Legend
Inflow
High water level
Outflow
Low water level
Pitfalls
Island
Polly Hollow
KSF road
Spotted Salamander Trail
Davis Creek

Figure 9

Pitfalls traps and drift fence in field

Figure 10

Waterproof pHep 1 Tester (left) and

E.W. System Soil Tester (right)

Photo by J.J. Watkins

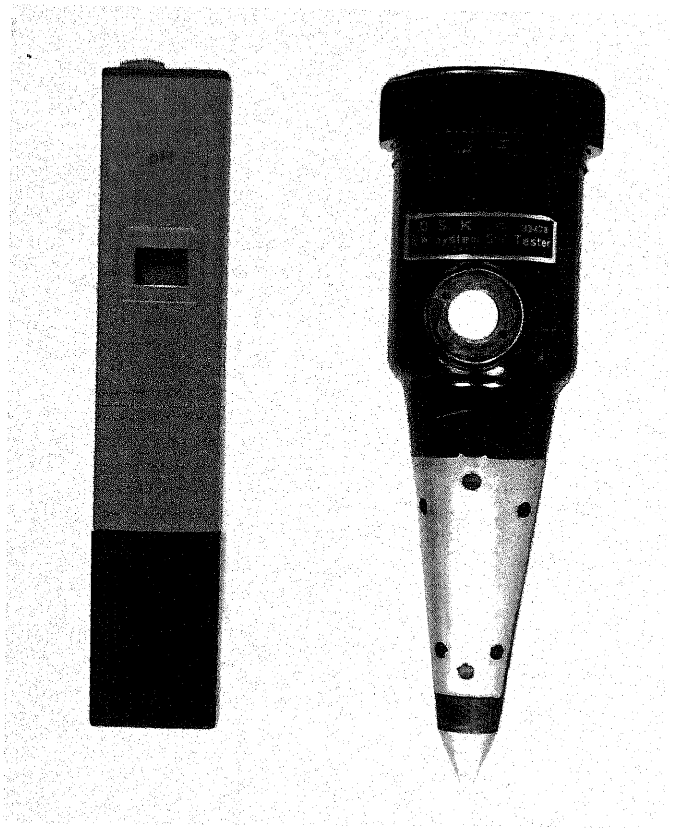


Photo by S.C. Haynes

Figure 11

Ambystoma opacum spermatophore

Figure 12

Ambystoma opacum spermatophore (close-up)

(8X)

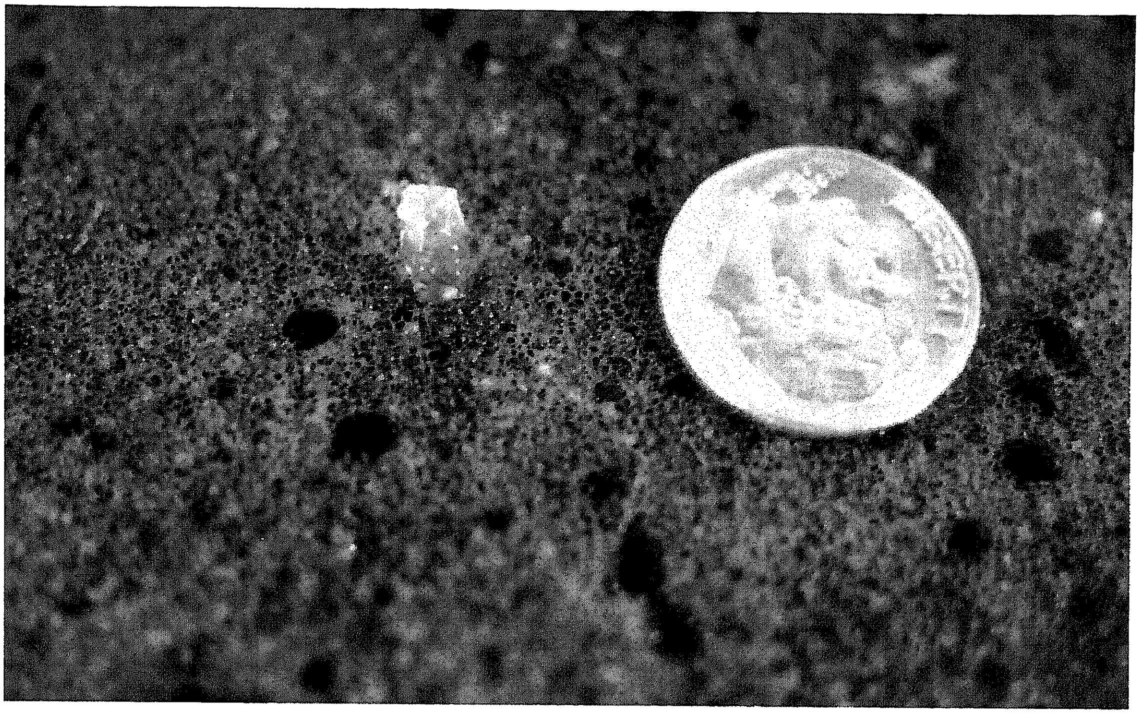


Photo by S.C. Haynes

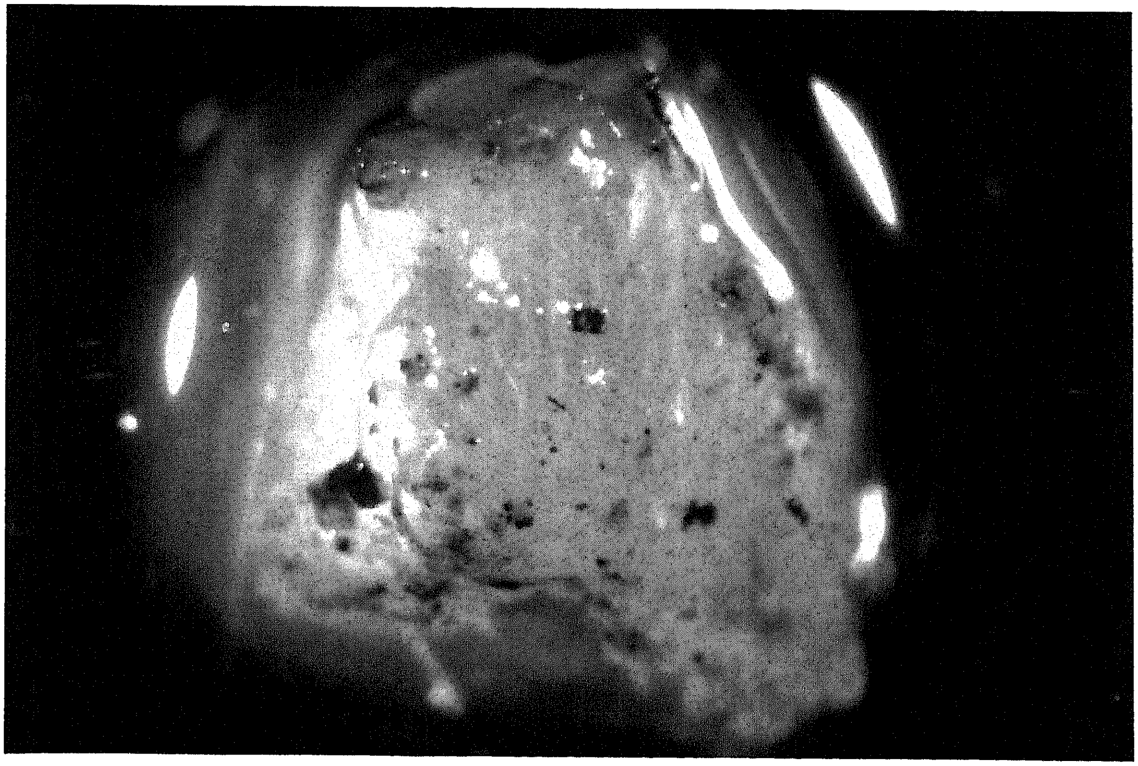


Photo by J.J. Watkins

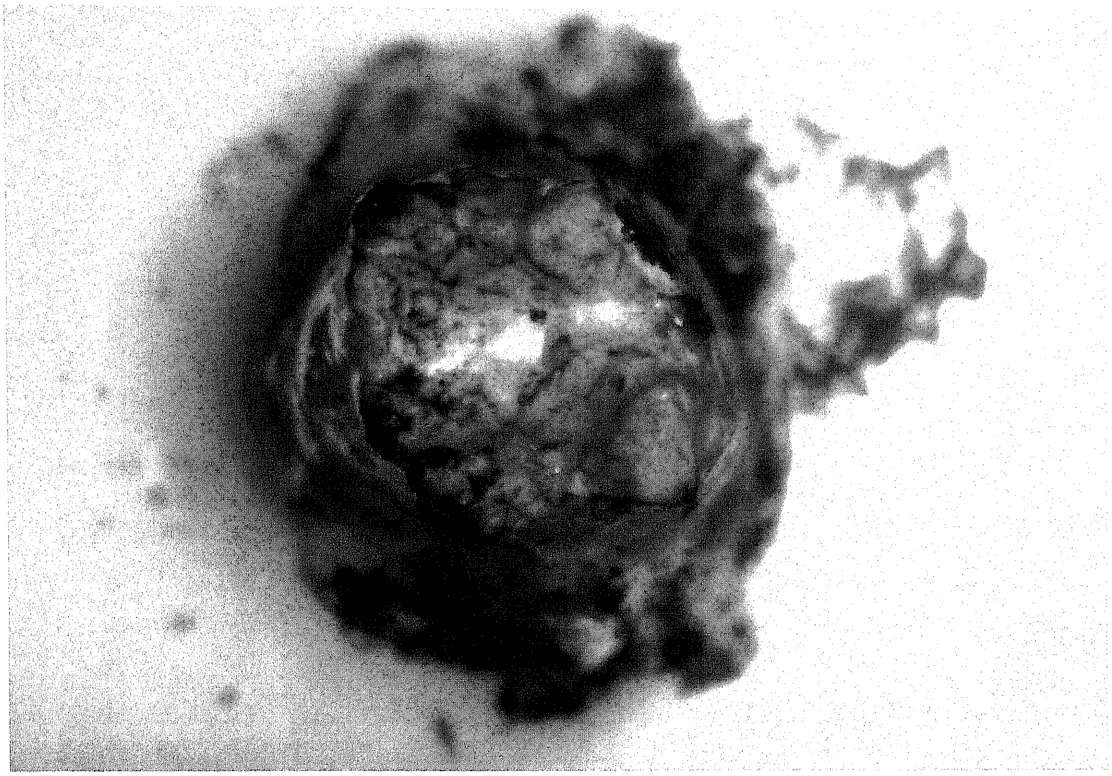
Figure 13

Ambystoma opacum spermatophore (close-up of cap)

(9X)

Figure 14

Communal double nest with a third in close proximity



Photos by J.J. Watkins

Figure 15

Female snout-vent lengths versus clutch size

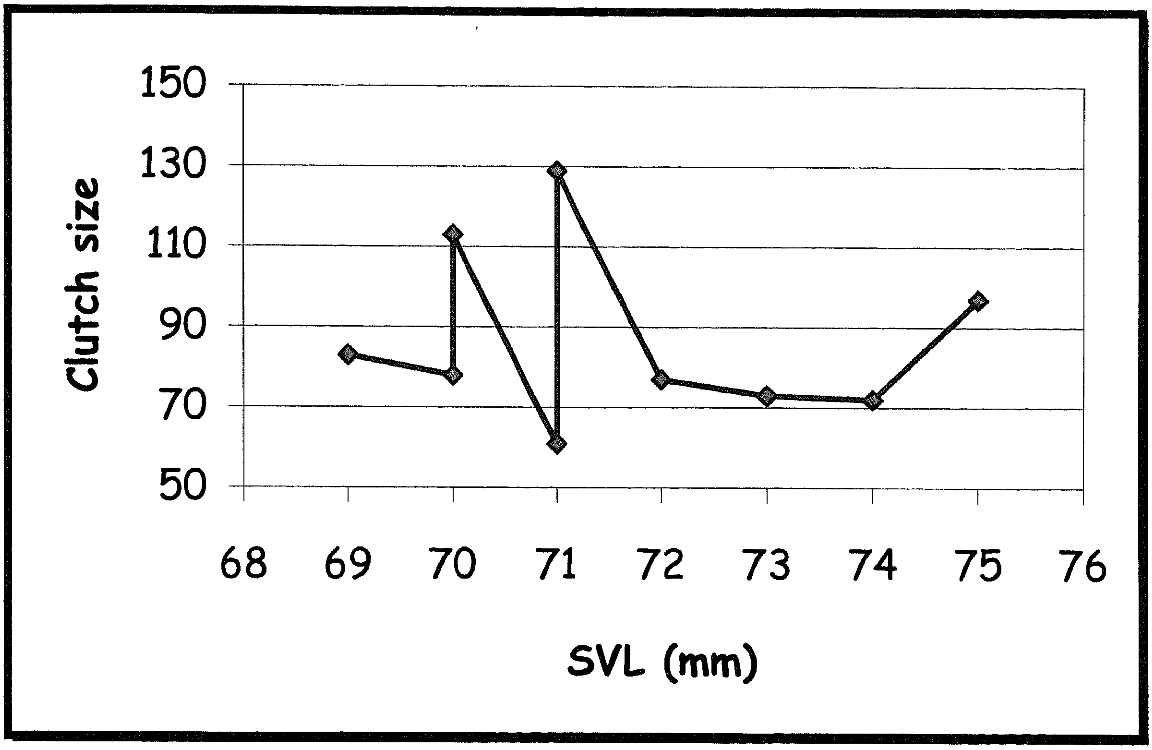

Clutch size
150
130
110
90
70
50
68
69
70
71
72
73
74
75
76
SVL (mm)

Figure 16

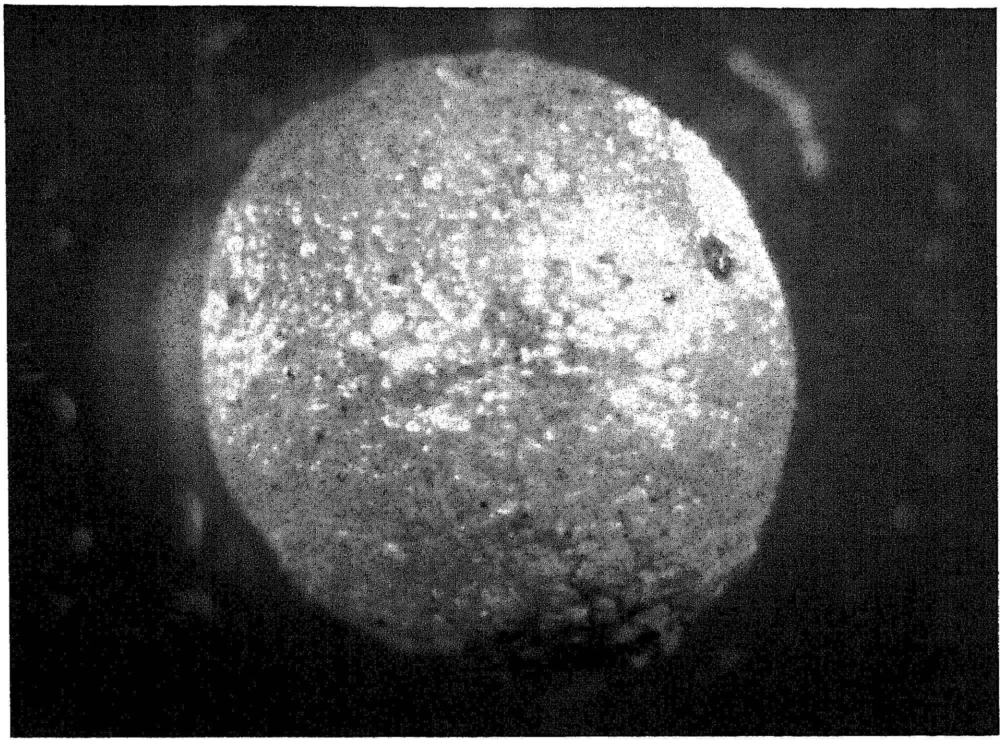
Ambystoma opacum egg

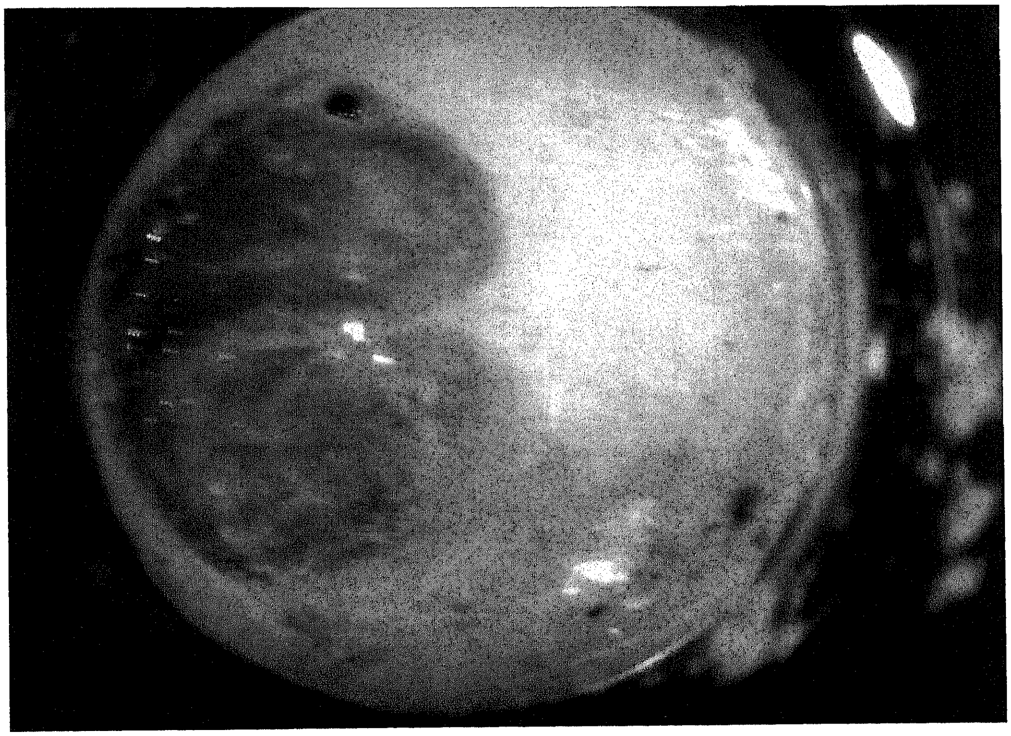
(7X)

Figure 17

Ambystoma opacum egg (uncharacteristically clean)

(7X)





Photos by J.J. Watkins

Figure 18

Inner capsule of egg

(10X)

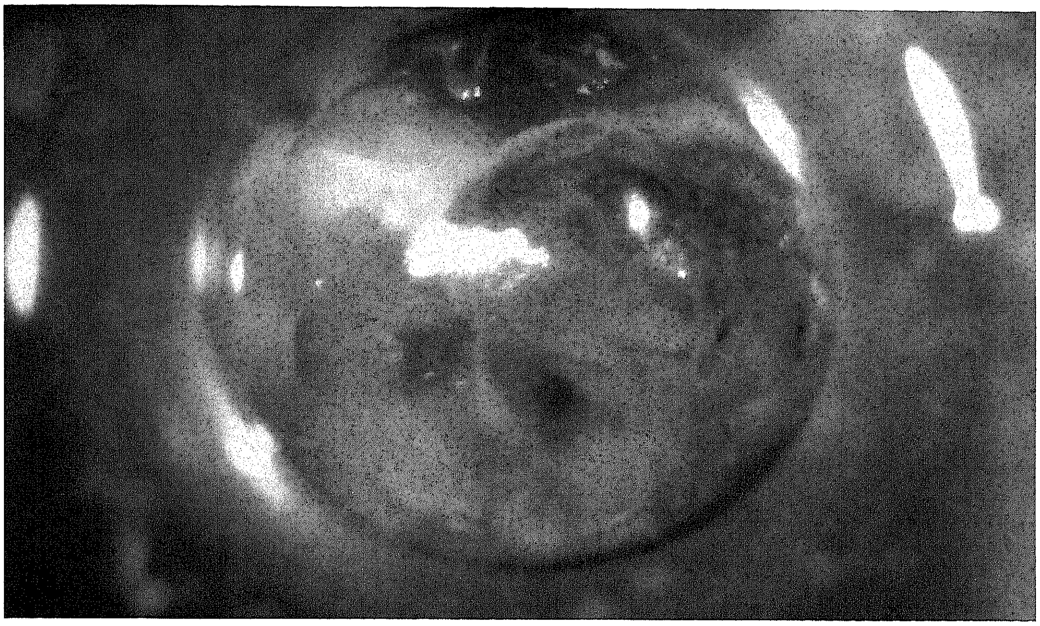


Photo by J.J. Watkins

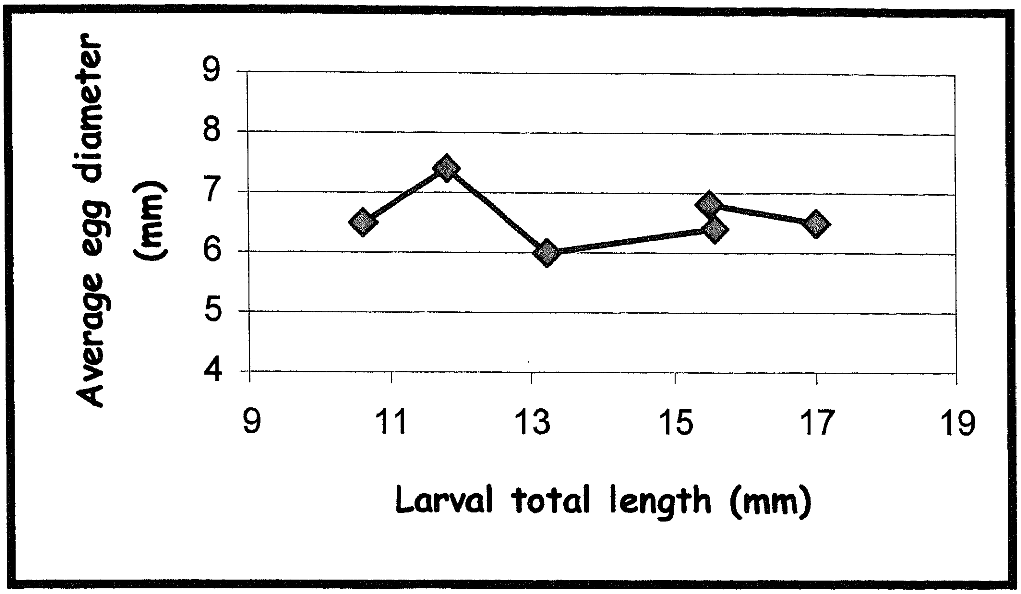
Figure 19

Egg diameter versus

larval total length in 1996 (top)

Egg diameter and larval total length

each collection period (bottom)


Average egg diameter (mm)
9
8
7
6
5
4
9
11
13
15
17
19
Larval total length (mm)

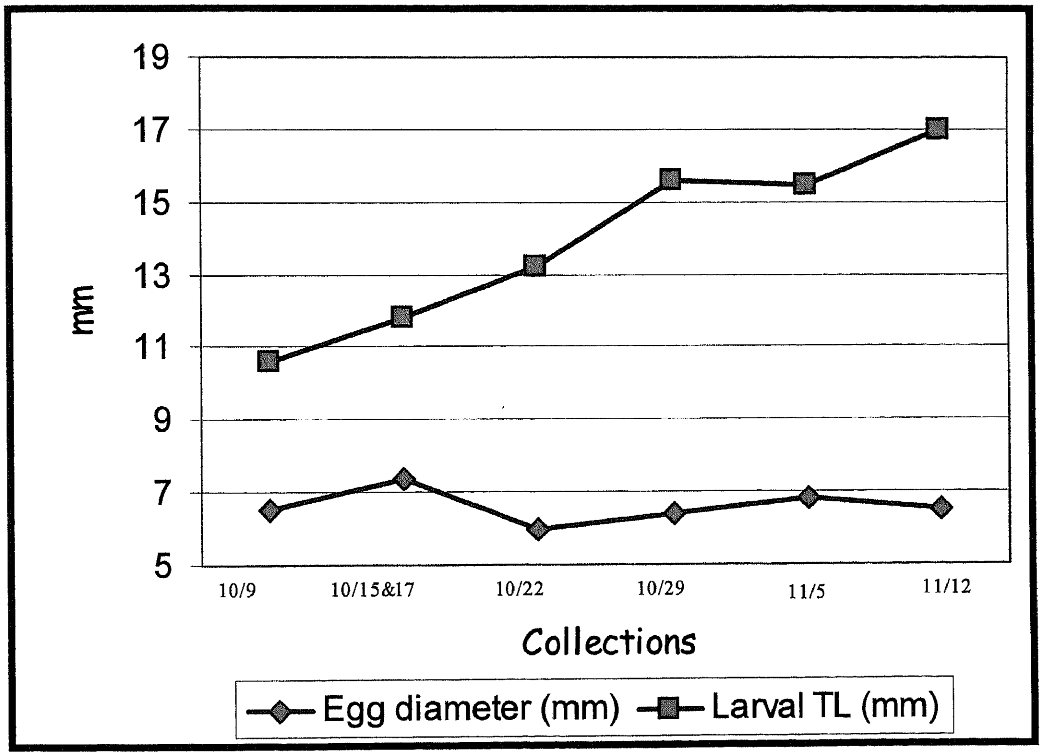

mm
19
17
15
13
11
9
7
5
10/9
10/15&17
10/22
10/29
11/5
11/12
Collections
Egg diameter (mm)
Larval TL (mm)

Figure 20

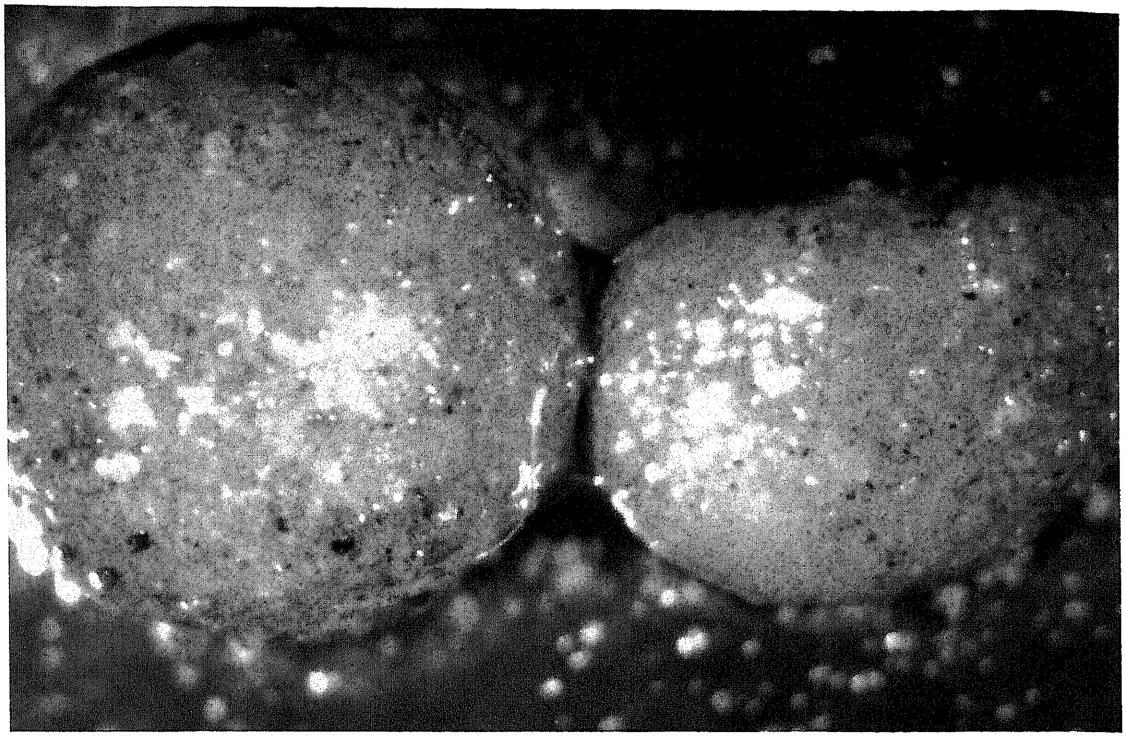
Hydrated and partially dehydrated eggs from same nest

(7X)

Figure 21

Larvae released from eggs pictured in Figure 20

(7X)



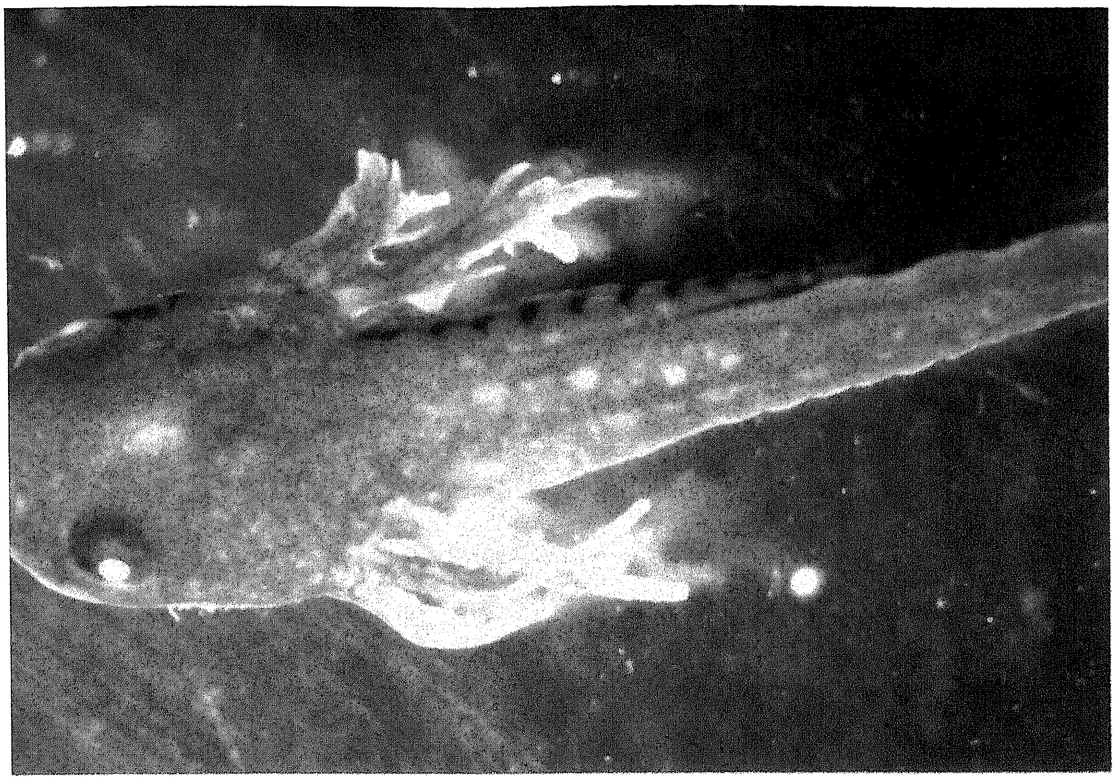
Photos by J.J. Watkins

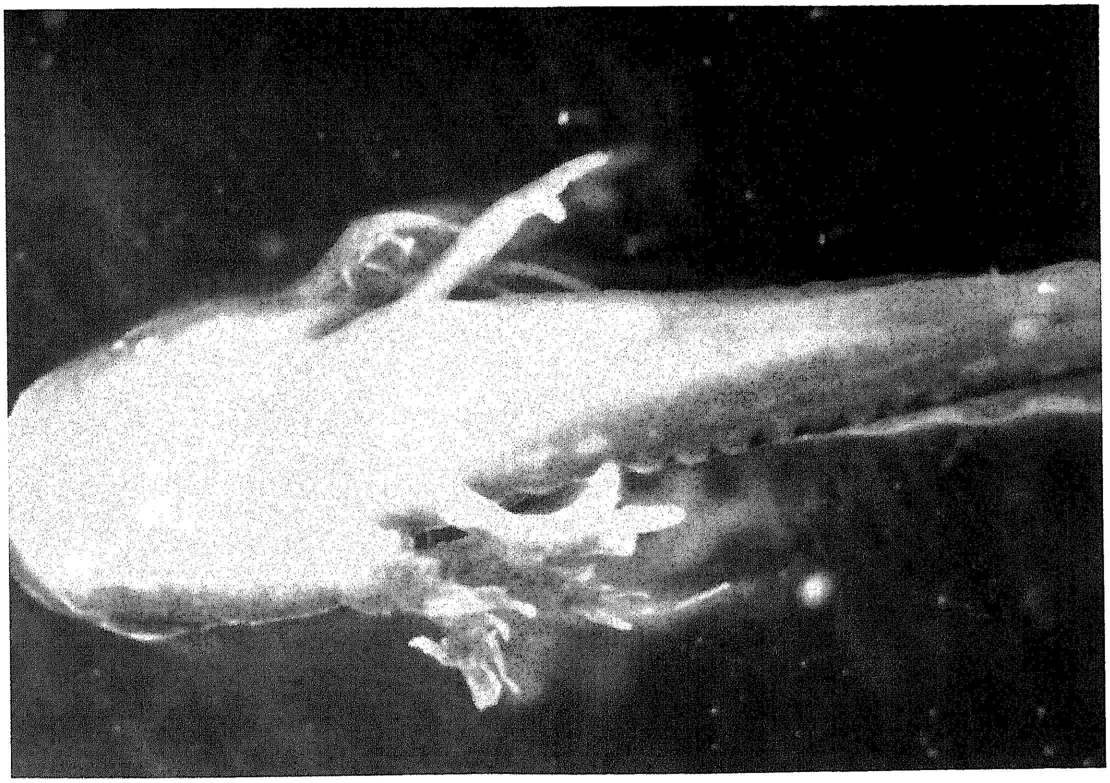
Figure 22

Ambystoma opacum larvae collected 15 November 1995.

Dorsal (top) and ventral (bottom) views

(7X)





Photos by J.J. Watkins

Figure 23

Ambystoma opacum larva collected 15 November 1995.

Rear limb just developing

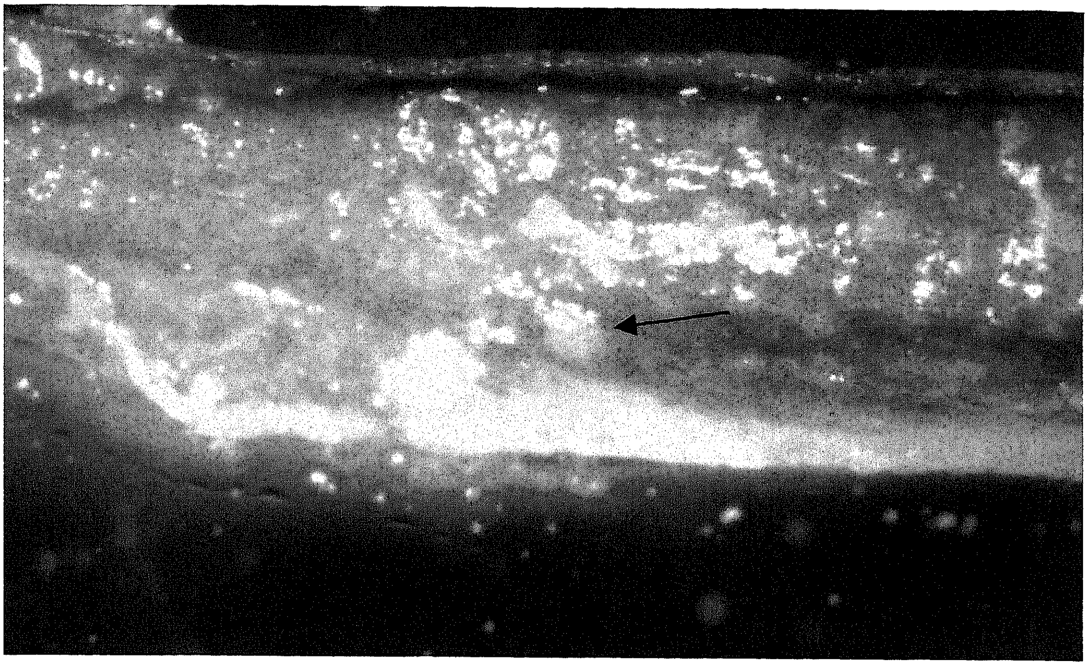
(15X)

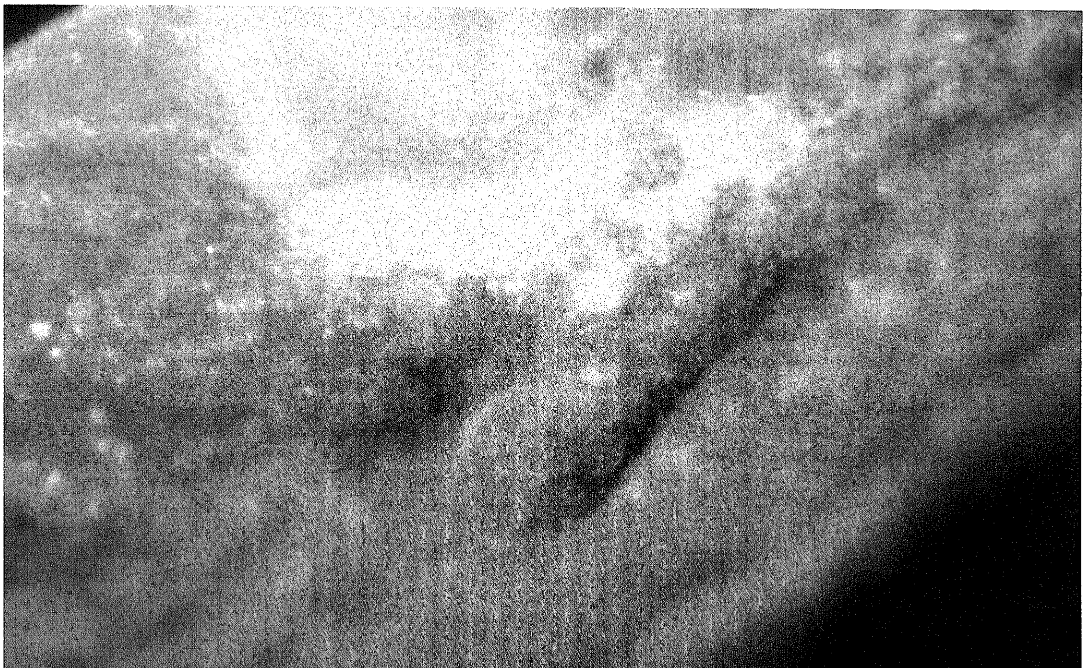
Figure 24

Ambystoma opacum larvae collected 29 December 1995.

Rear limb developing

(41X)

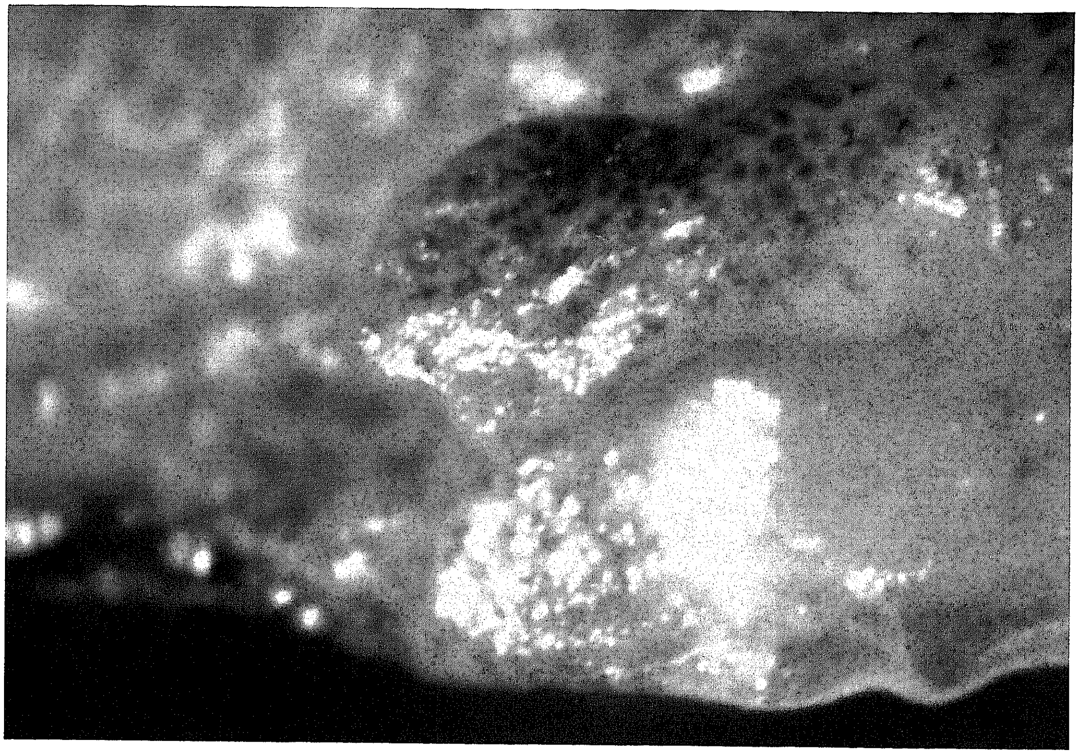


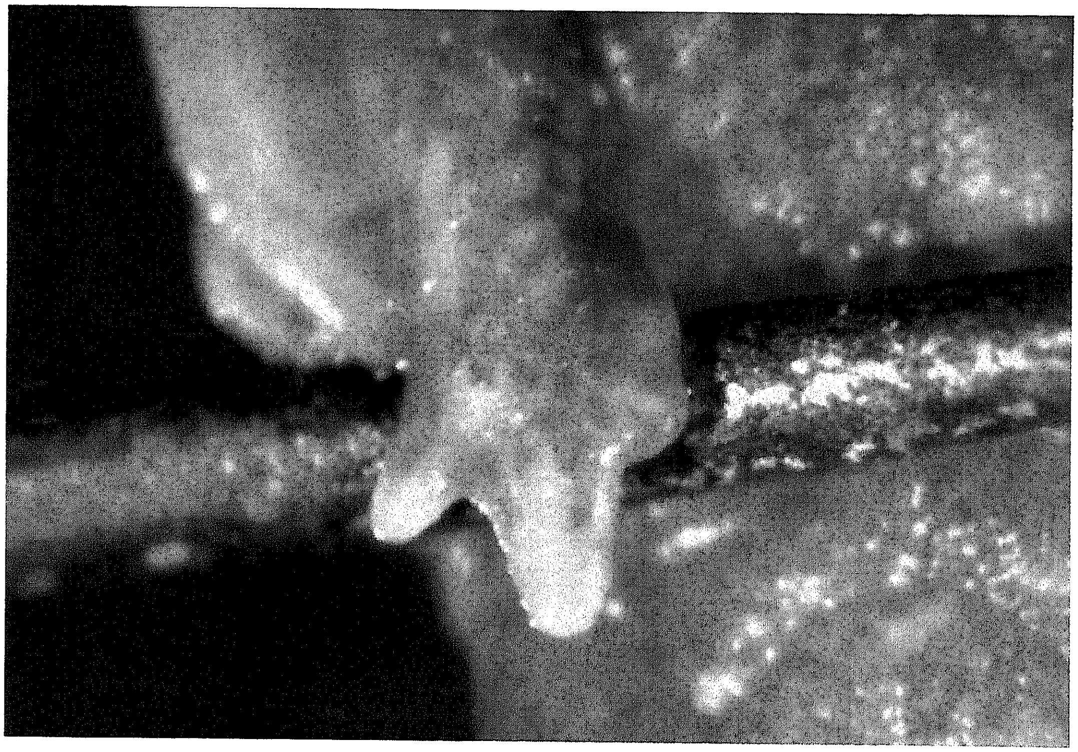


Photos by J.J. Watkins

Figure 25

Ambystoma opacum larvae collected 26 January 1996 (top) (30X) and 8 March 1996 (bottom) (25X) showing rear-limb digit development





Photos by J.J. Watkins

Figure 26

Ambystoma opacum larvae collected 26 January 1996 showing

well-developed forelimb digits

(20X)

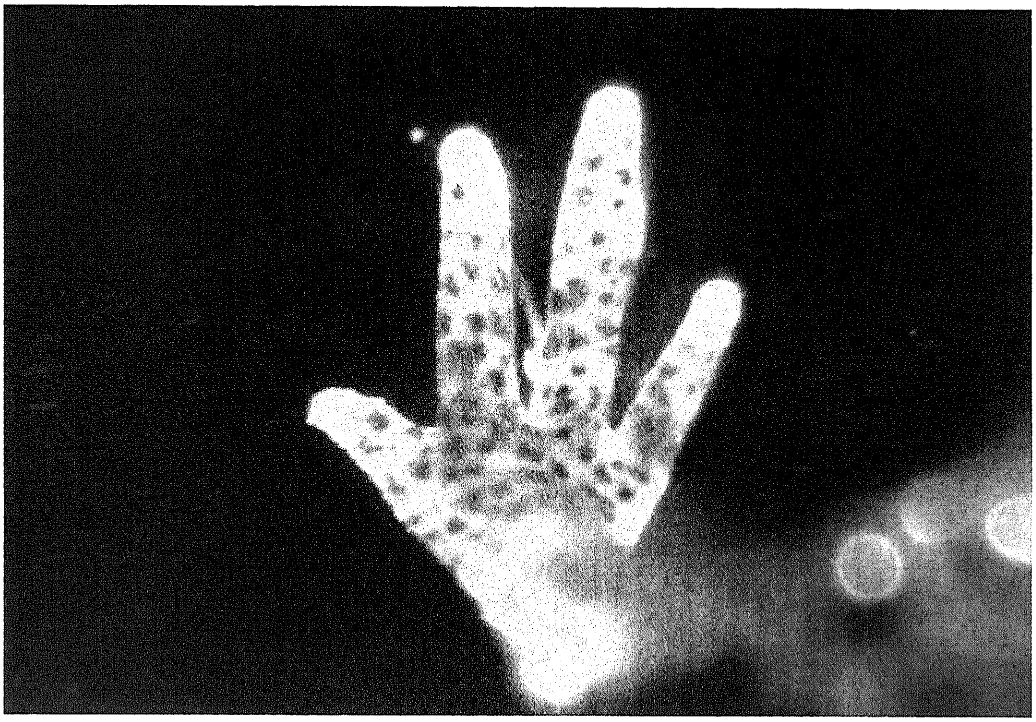


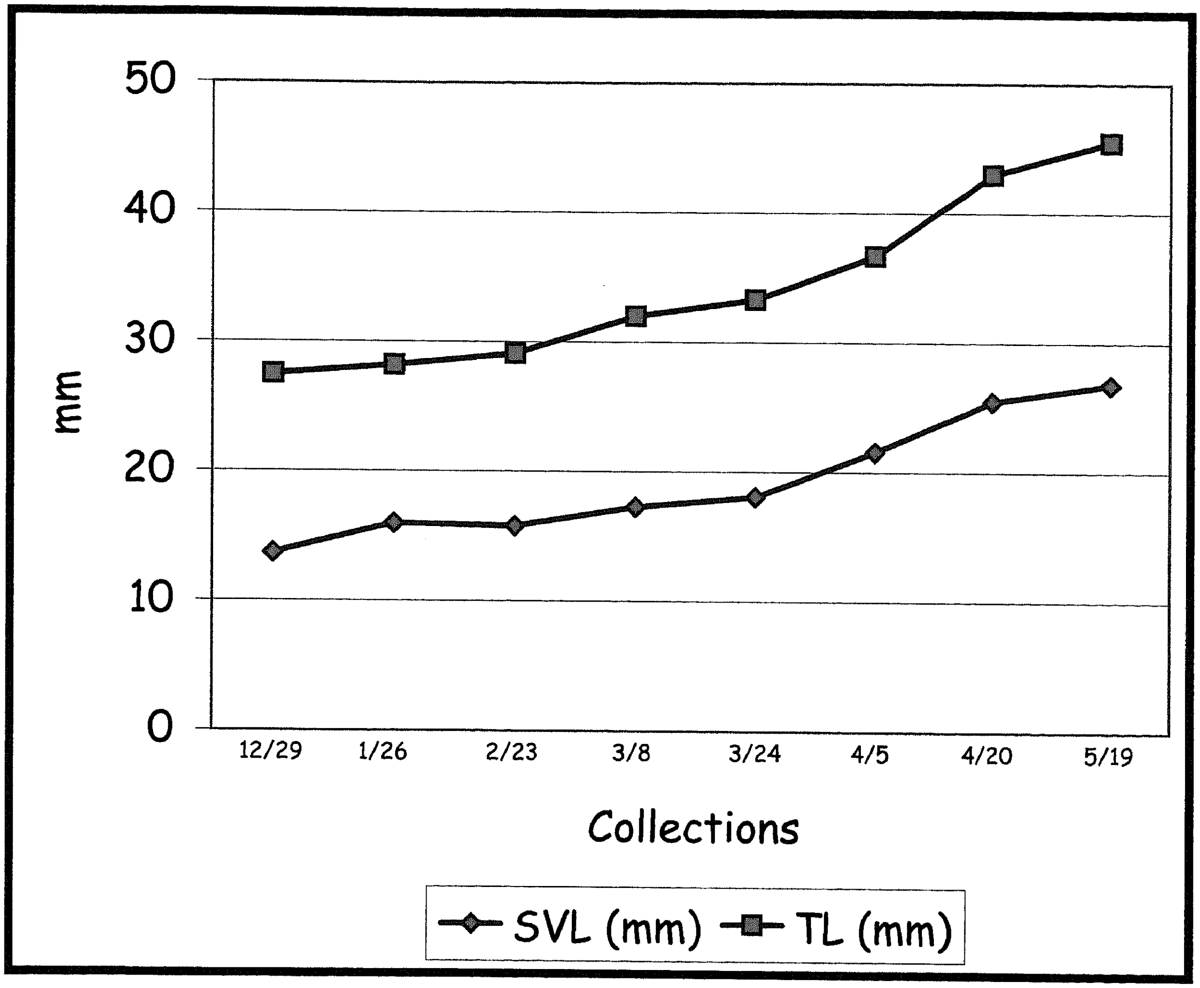
Photo by J.J. Watkins

Figure 27

Snout-vent and total lengths of collected *Ambystoma opacum*

larvae collected in 1995-96

(15 November collection could not be measured for TL)



50
40
30
20
10
0
mm
12/29
1/26
2/23
3/8
3/24
4/5
4/20
5/19
Collections
SVL (mm)
TL (mm)

Figure 28

Free-swimming *Ambystoma opacum* larvae

collected in 1995-96

Figure 29

Ambystoma opacum larva collected 20 April 1996 showing

rear limb development

(12X)

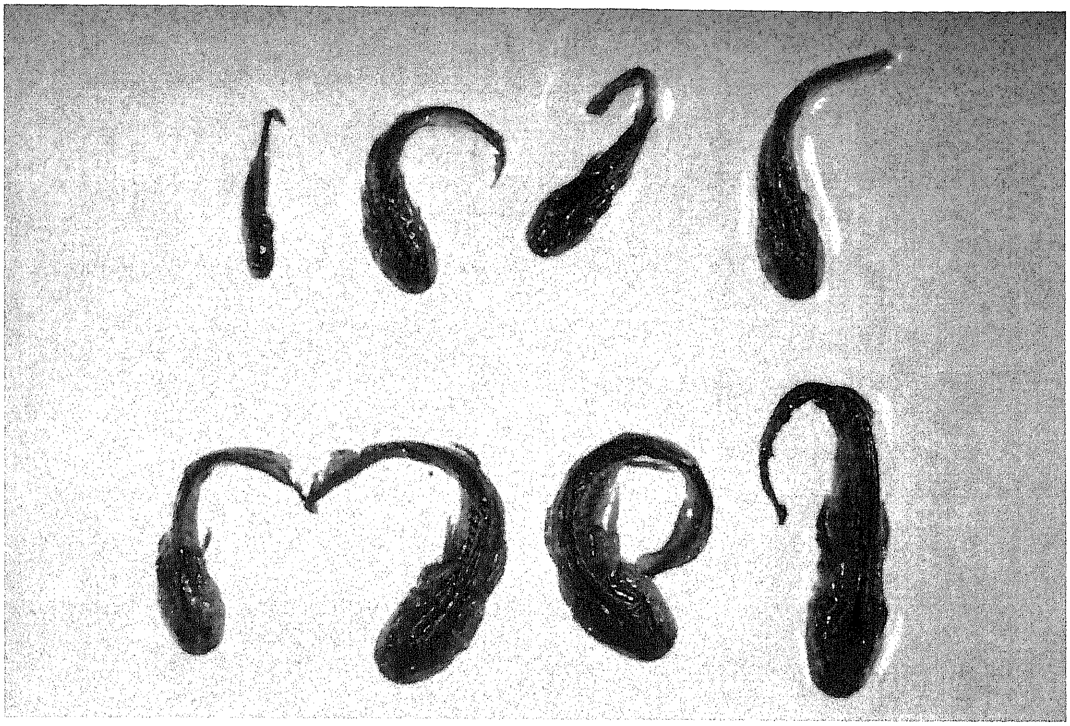


Photo by S.C. Haynes

Photo by J.J. Watkins

Figure 30

Ambystoma opacum larvae collected 20 April 1996 (top) (10X) and 19 May 1996 (bottom) (7X) showing rear-limb digit development

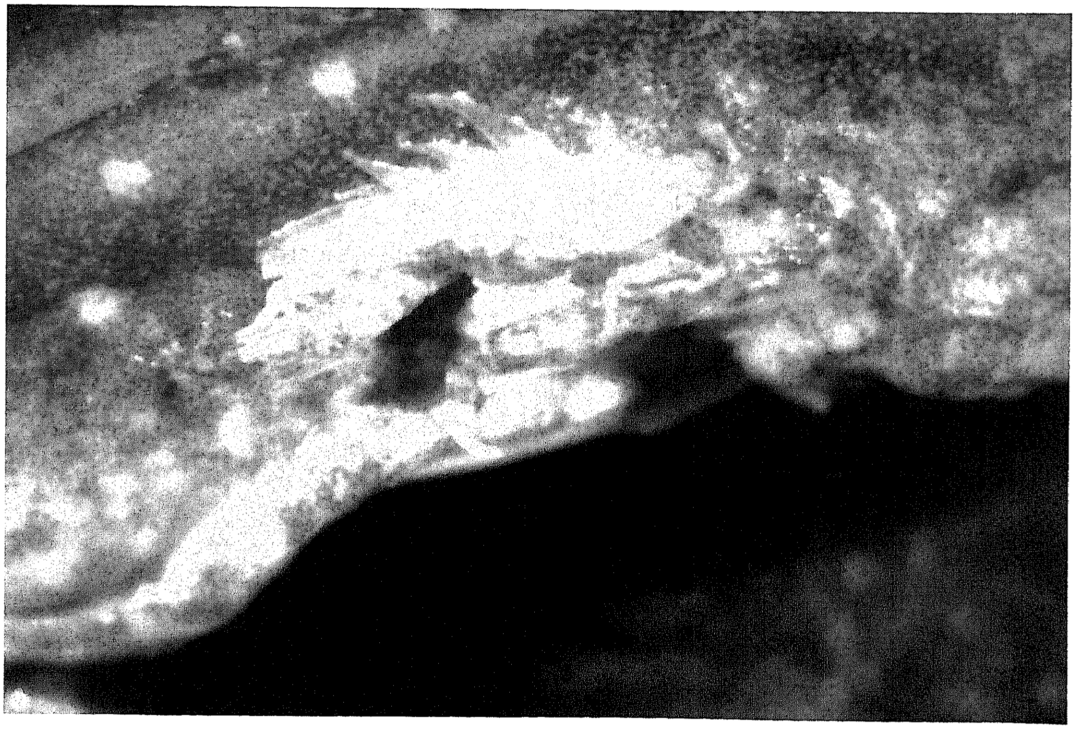
Photos by J.J. Watkins

Figure 31

Ambystoma opacum larva collected 19 May 1996 showing

dorsal fin and gills

(7X)



Photos by J.J. Watkins

Figure 32

Teeth of larval *Ambystoma opacum* (dehydrated specimen)

(40X)

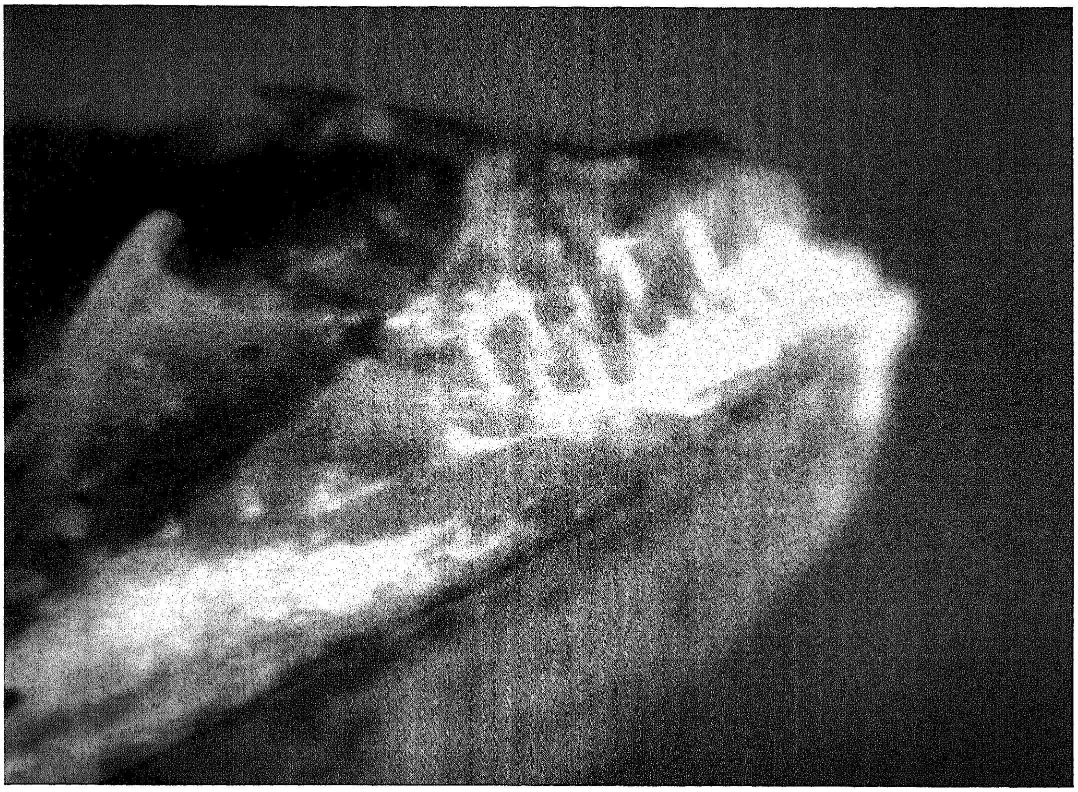


Photo by J.J. Watkins