AMERICAN MUSEUM NOVITATES

Number 347 | Published by THE AMERICAN MUSEUM OF NATURAL HISTORY New York City | April 27, 1929

59.79L:15.6

THE BREEDING HABITS OF TWO SALAMANDERS

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In September, 1927, we visited the Ozark Mountains of southwestern Missouri to study *Typhlotriton spelæus* in the field and to collect a series for an experimental investigation of the causes of eye degeneration in this species. The field work was continued by one of us, Marshall, throughout a part of 1928. In the course of the work Marshall made observations on the breeding habits of two other species of urodeles. The observations on one species, *Ambystoma annulatum*, are different in nearly every detail from the facts previously published, while no information concerning the breeding habits of the other species, *Plethodon glutinosus*, has been published.

Ambystoma annulatum Cope

On the afternoon of September 30, 1927, Marshall, with our guide, John Powell, investigated the report that great numbers of strange animals had invaded a cistern in a sweet potato patch owned by Jess Sims, between Reed's Spring and Marvel Cave, Stone Co., Missouri. The month of September had been fairly dry but it had rained the same morning. In the cistern were found ten adult specimens of *Ambystoma annulatum* swimming near the surface and these were readily caught with a long-handled dip-net. The salamanders must have burrowed into the cistern from under the curbing as the top of the cistern was tightly covered and further, the curbing was approximately two feet high. The owner of the cistern was unable to offer any other explanation as to how the salamanders might have gained entrance to the water. The sweet potato patch was about fifteen yards from the cistern. Some of the sweet potatoes had been dug up and the vines left in piles on the ground. Under these withering vines several more adult specimens were found. In a few instances four or five salamanders were huddled together under one shelter. Approximately thirty yards below the sweet potato patch is a small body of water known as Ghost Pond. Along the edge of the pond a few more salamanders were found under the débris of

brush or leaves. Eggs were everywhere abundant in the pond. Some were attached to water-plants but the greater number were indiscriminately spread over the whole bottom of the pond. The eggs were found to be in different stages of development but no larvæ free from the egg-capsules were obtained even after considerable scooping with the dip-net. Two or three adult *A. annulatum* were brought up in the net, with the eggs. The pond was round, four or five hundred feet in diameter and approximately waist-deep in the center. It must have contained many thousand eggs.

Although no salamanders were seen to lay the eggs, there can be little doubt but that they were laid by this colony of *A. annulatum*. No eggs were found on land where the salamanders were hiding. The pond was low, for there had been little rain and certainly not sufficient that morning to have caused a sudden rise. It was impossible for the eggs to have been laid on land and later flooded or to have been washed into the pond from the bank. Their great number, their wide distribution over the bottom of the pond and the few attached to water-weed definitely established this fact.

There are few ponds in the southern part of Stone County. The region is largely limestone and the rain-water which falls quickly rnns off into the subterranean water-channels. There is, nevertheless, a more or less permanent body of water near Marvel Cave on the Lynch property. During September the pond was about two hundred feet wide. We collected along its boggy shores on several occasions during September without finding any salamanders. On October 8 a very thorough search of this pond was made but not a single egg or salamander of any species was found.

Our previous knowledge of the breeding of *Ambystoma annulatum* rests entirely on the observations of Combs as reported by Strecker (1908). "One night in March after a severe rainstorm" Combs visited a log near Hot Springs, Arkansas, under which he had previously collected two adult *A. annulatum*. On turning the log over he found two additional adults, one of which had already deposited thirty-five eggs in the mud under the log. The salamanders were placed in a jar "half full of mud and water." In twelve hours they had deposited a total of one hundred and fifty eggs. "On the fifth day the eggs began to shrivel up" and when Combs poured more water in the box they "resumed their normal condition." On the tenth day the eggs began to hatch.

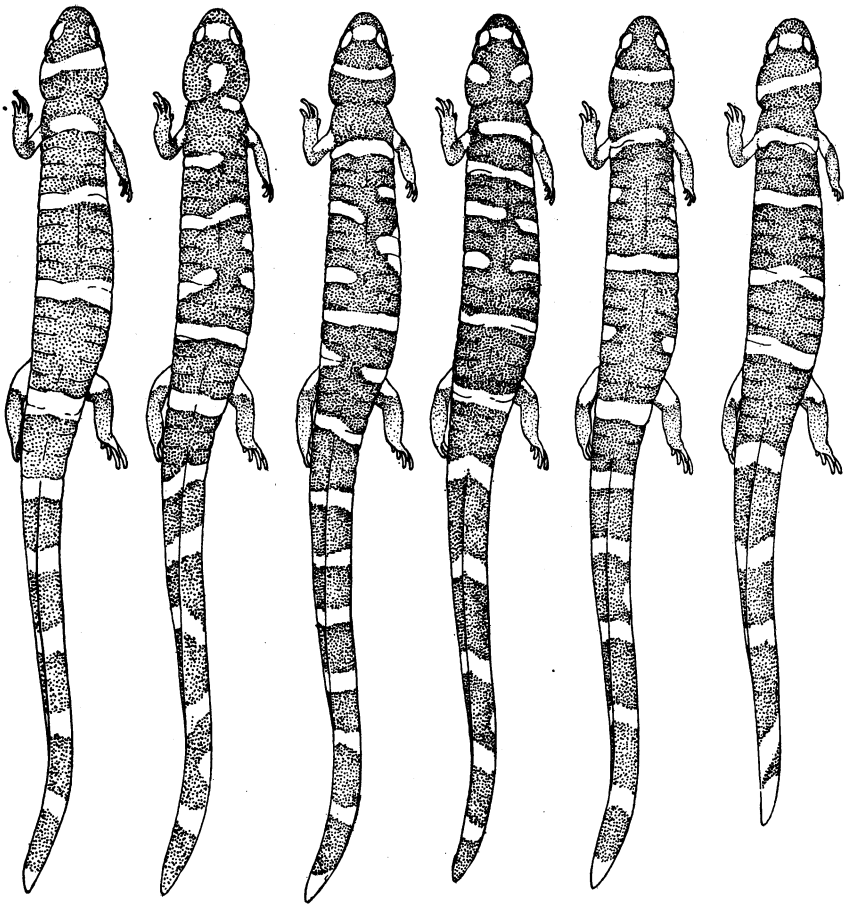

Fig. 1. Color variation in *Ambystoma annulatum*.

From this data Strecker (1908) concludes in part, in regard to *A. annulatum:*

(1) These salamanders inhabit the deserted burrows of crayfish and only come to the surface when the ground is moist.

(2) They deposit their eggs on the ground, under logs or among masses of decayed wood.

(3) The female keeps her eggs supplied with moisture until they are hatched. I have no doubt but what she carries them down into the ground, to the line of moisture, in exceedingly dry weather.

(4) The eggs are never in strings, but are always separate, and as many as 150 may be deposited by the same female.

These conclusions are so completely at variance with our data given above that it would seem that the species considered must be different. We have before us a large series of specimens collected from the Jess Sims farm and have compared them with the type, U. S. N. M. No. 11564. In this specimen there are five cross-bands or rings between the anterior and posterior pair of legs and the first and third rings are incomplete dorsally. In our series there are several specimens identical with the type but others show a wide range of variation. In figure 1 we have shown the range of variation exhibited by the males. The females were equally variable and showed no constant color difference from the males. In no specimen were

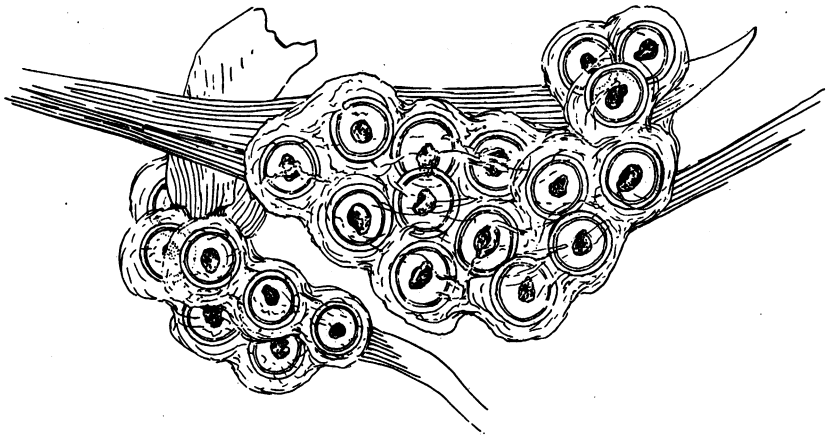

Fig. 2. The eggs of *Ambystoma annulatum*.

all the cross-bands divided into blotches. The least-banded specimens were a pair of opposite sex, each specimen having only two complete and one incomplete cross-bands between the anterior and posterior legs. It is clear that the species is more variable in color pattern than previously assumed.

The eggs of *Ambystoma annulatum* were preserved in jars of formol and no detailed observations made concerning them until later in the laboratory. Some of the egg-masses were probably broken in shipment. All the eggs except one are in bunches. These vary considerably in the number of eggs they include. Of the series preserved in the American Museum there are three bunches of two eggs each, five of three, three of four, one of five, one of five attached by a single egg

to another of five, one of six, two of seven, one of seven attached by a small strand to a bunch of twenty-six, one of ten, one of eleven, one of eleven attached to a bunch of twenty-one eggs, one of eleven attached to thirteen, two of twelve, one of thirteen, three of fourteen, one of fifteen, two of sixteen, one of twenty-one, and one of forty-five eggs. From these data it may be concluded that the eggs of *A. annulatum* are usually laid in bunches of at least ten eggs but usually of not over forty-five.

As shown in figure 2, each bunch is enclosed in a single gelatinous capsule which is so thin that the contour of each egg is more or less preserved. Exclusive of this common capsule each egg is surrounded by two capsules and a thin adherent vitelline membrane. The inner of the capsules is clearly differentiated, for in the preserved specimens both its inner and outer surfaces are finely crenulated and slightly discolored. The common capsule covering an average egg is 8 mm. in diameter, the outer of the two egg-capsules 5 mm. in diameter, the inner 4.4 mm., while the egg itself is 2 mm.

During the late blastula stage a third of the egg of *A. annulatum* is heavily pigmented. As gastrulation proceeds the color becomes a paler brown more uniformly distributed over the embryo. The latest stage in the preserved series in the American Museum is that of an embryo with the branchial and hyoid arches marked out as feeble ridges. A prominent protuberance, the rudiment of the balancer, is present on each mandibular arch. At this stage the embryo is nearly uniform brown both above and below, 7 mm. in total length and with a tail a trifle less than a millimeter long.

The eggs and embryos of *A. annulatum* as described above agree most closely with those of *A. jeffersonianum* as detailed by Piersol (1910) and Smith (1911). The points of agreement are:

(1) Eggs small. They average smaller than those of *A. jeffersonianum* which are smaller than eggs of *A. maculatum* and of *A. tigriuum.*

(2) Eggs well pigmented. They are not as dark as those of *A. jeffersonianum,* but darker than preserved eggs of *A. tigrinum.*

(3) Common envelope of egg-cluster thin, the contour of the individual eggs distinct. This is perhaps the most conspicuous point of agreement with *A. jeffersonianum.*

(4) Egg-clusters small and scattered, the eggs not forming a compact group.

(5) Number of eggs to cluster small. *A. jeffersonianum* averages twenty (Piersol) or fourteen (Smith) to a cluster and this agrees very well with *A. annulatum.*

(6) A balancer is present as in *A. jeffersonianum* and *A. maculatum.* It is usually absent in *A. tigrinum.*

The chief point of disagreement between the breeding process in *A. annulatum* and in *A. jeffersonianum* is that the latter species breeds in the early spring and the former, according to our observations but not those of Combs, after rains in the fall. We shall discuss this discrepancy in greater detail below.

Plethodon glutinosus (Green)

The eggs of the slimy salamander have not been previously described although the species is one of the more common forms of eastern United States. Okey (1916) briefly mentioned the eggs and larvæ of the species, but as larvæ later sent to one of us from the laboratory where Okey studied proved to be not *P. glutinosus* as labeled but *Ambystoma microstomum* we may safely assume that Okey's material was also referable to the latter species.

On August 17, 1928, Marshall found for the first time the eggs of *P. glutinosus*. They were taken in Sheridan Cave, near Mountain Home, Arkansas. The eggs were hanging suspended from a perpendicular wall forming the back of a pocket in the cave wall about four feet above the floor of the cave. The pocket was approximately eight inches long by six inches high and six inches deep. It was near the entrance to the cave. The eggs which were in the cleavage stages were accompanied by a large female, 162 mm. in total length and 75 mm. head and body-length. The female was resting on the floor of the pocket under the eggs. The cave was thoroughly searched but yielded in addition only two immature *P. glutinosus* and three adult *Eurycea melanopleura*.

On September 3, of the same year, an additional set of eggs of *P. glutinosus* was found. These were discovered in Indian Cave, near Bella Vista, Arkansas. They were lying in the bottom of a small crevice in the wall of the cave about three feet above the floor and two hundred feet from the entrance of the cave. The eggs contained well-developed embryos. No adult was found with them but two immature *P. glutinosus* were found in other parts of the cave. The only other salamander found in the cave was a larva of *Typhlotriton spelæus*.

There are eighteen eggs in the first bunch collected and only ten in the second. The eggs are not stalked but held together in a single gelatinous envelope. This common envelope is clearly shown in both bunches of eggs but particularly in the earlier set where the eggs have been little damaged in transportation. We include a figure of two of

the eggs from the older series (Fig. 3) which shows the outer envelope as well as the underlying inner egg-capsules.

Except for the absence of a stalk, the eggs of *P. glutinosus* agree closely with those of *P. cinereus* as described by Piersol (1910*a*). There are two clusters of eggs of *P. cinereus* in the collections of the American Museum. One includes seven and the other eight eggs. Neither shows the egg-membranes clearly and we quote from Piersol (p. 475) concerning these structures in *P. cinereus:*

In their natural condition the number of these is rather difficult to determine but after soaking a few minutes in water they swell somewhat and the following is plainly seen:—an innermost sphere very close to the surface of the egg; a second

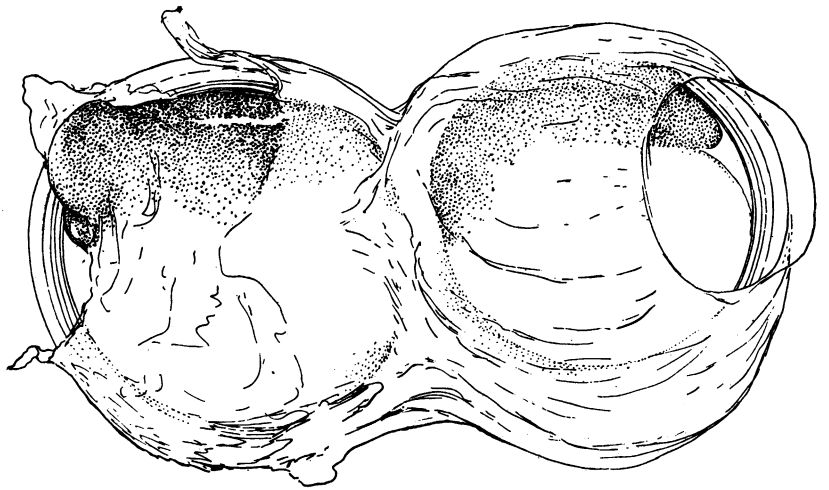

Fig. 3. Two eggs of *Plethodon glutinosus*, the outer capsule broken at two points showing the inner egg-capsules.

enclosing this but separated from it by a greater interval than that between the innermost sphere and the egg; occasionally this sphere is represented by two, one of them fitted very closely around the other. The outermost sphere—usually the third—fuses with the outermost spheres of neighboring eggs at all points of contact. On its surface are threads and bands of a milky white mucus which seem tougher than the rest, which is transparent; these are especially numerous between eggs and at the upper part of the bunch where several uniting form the stalk by which the cluster is suspended.

In both sets of *P. glutinosus* eggs the same envelopes may be recognized. There is a thin adherent vitelline membrane, and a rather thick egg-capsule surrounding it. This egg-capsule may be divided into several layers. In the earlier and better preserved eggs

it is divided into an inner and an outer capsule, the latter not as thick as the former. In the older and in some of the younger eggs the inner of these two capsules may be divided again into several layers. We have figured a case where the inner capsule has three distinct layers (Fig. 3). It is not unlikely that fresh eggs will be found to have only two layers to this capsule, as in *P. cinereus* but, as most descriptions are based on formalin preserved eggs it is important to stress this tendency of the inner egg-capsule to be subdivided in preserved material. Surrounding the outer egg-capsule is the common envelope described by Piersol (1915) as the outermost sphere in *P. cinereus*. Piersol's terminology has in its favor his observations that each egg is laid separately and later the outer sphere fuses to that of the neighboring eggs. In preserved material of *P. glutinosus* the outer spheres of the eggs are completely fused and form a common outer envelope as in *Ambystoma annulatum*.

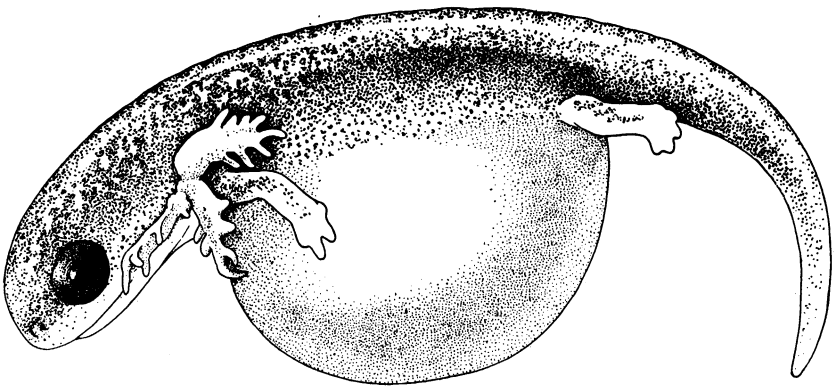

Fig. 4. Embryo of *Plethodon glutinosus*.

An average egg of *P. glutinosus* measures 5.5 mm. in diameter. It is creamy white in color. Its vitelline membrane measures only a fraction of a millimeter more than the diameter of the egg. The inner of the two surrounding egg-capsules measures 6 mm. in diameter, the outer 6.5 mm. The common envelope or outer sphere of Piersol measures 7.5 mm. in diameter in this egg but it is somewhat variable in thickness about the other eggs.

The form of the embryos in the second set of eggs is shown in a figure of one of them (Fig. 4). The antler-like gills are joined at their bases. The embryos are well pigmented above with stellate melano-

phores. These are most numerous along the sides of the body. A narrow vertebral line and a number of small spots scattered over head and back are for the most part devoid of pigment. A great many of the melanophores on either side of the pigmentless vertebral line have their processes extended transversely to the axis of the body, while on other parts of the embryo they exhibit an irregular radiate form. This suggests that these dorsal melanophores are migrating towards the sides of the body where the pigmentation is already greatest. Further, there would seem to be some mechanical obstruction underlying the epidermis to prevent their reaching the midline of the body or sending their processes across the small white spots. A histological examination would reveal the nature of these obstructions. It may be developing glands, rudimentary lateral line organs or other modifications of the integument. Such an examination might reveal the reason for the accumulation of pigment along the sides of the body. This region might be the region of maximum cutaneous respiration, of maximum skin growth or of both. It is idle to speculate as to the causes of color pattern in Amphibia until more facts have been presented. In the meantime it is interesting to note that *P. glutinosus* during its development should exhibit a striped pattern which is later obscured in this species but not in certain other species of the genus.

One of the embryos extended measures 16 mm. in total length. The yolk at this stage is 6.5 mm. in greatest length, and 5 mm. in width. Hence it has either swollen in fixation or during growth. The largest gill is 2.2 mm. long. The proportions of fingers, toes and other structures are shown in the figure of a second embryo (Fig. 4).

DISCUSSION

Our observations on *Plethodon glutinosus* are in accord with a thesis previously advocated (Noble, 1927) that the mode of life-history of an amphibian is usually good evidence of its relationships. *Plethodon glutinosus* is larger and more primitive than *P. cinereus*. Most large species have more eggs than their near relatives of smaller size. Piersol (1910*a*) gives the egg-number of *P. cinereus* as from three to twelve. Our egg-clusters of *P. glutinosus* included ten and eighteen eggs respectively. As the former cluster of *P. glutinosus* lacked a guarding female it is barely possible that some animal may have destroyed the female and devoured a few of the eggs. Their position on the floor of the crevice would support this view although Piersol (1915) has recorded a case of unattached eggs in *P. cinereus*. The most distinctive feature

of the larger cluster of eggs was the absence of a stalk of attachment. Hence it is possible that *P. glutinosus* might not always attach its eggs to an overlying surface. Piersol (1915) has sketched an evolutionary series in the egg-capsules of urodeles. We can not agree with him that egg-capsules of *Autodax* have evolved directly from those of a *Eurycea* type. As pointed out elsewhere (Noble, 1927), *Autodax* (=*Aneides*) has evolved from *Plethodon* and it has redeveloped the individual stalks for each egg. But these stalks are not attached to the underside of a rock as in *Eurycea*. The egg-cluster is laid on land as in *Plethodon*, and although the form of the capsule is more specialized than in that genus it may be derived from the *Plethodon* type by assuming that the gelatinous egg-capsule material was secreted between the times during which any two eggs were laid.

In concluding this discussion of *Plethodon glutinosus* the question might be raised as to where one might expect to find the eggs of the species in regions where no caves occur. In the immediate vicinity of Imboden, Arkansas, no caves are found. Very young individuals (the smallest 32 mm. total length) of *P. glutinosus* have been taken among the moist gravel of springs. A young individual 66 mm. in total length was taken under a stone in a spring outlet near Plainfield, New Jersey. Larger specimens of *P. glutinosus* are usually not found in such situations either at Imboden or Plainfield. Hence, it seems to us possible that the adults penetrate into the ground for some depth to lay their eggs and the young, in seeking the outer world, follow crevices such as those formed by ground-waters and make their exit on occasions near springs.

Turning to *Ambystoma annulatum* it may be asked, how may we account for the great discrepancy between the description of the eggs of *Ambystoma annulatum* given by Combs and that recorded above? Strecker's (1908) report of Combs' description is so detailed that we believe there is little chance of Combs having made an error. Rather it seems to us that we have in *Ambystoma annulatum* a species which usually lays its eggs in the water and conforms to the breeding process characterizing most species of the genus. Under some circumstances it is able to lay its eggs on land and if the eggs are given sufficient moisture development will proceed. This suggestion is rendered plausible by the observations of Brimley (1921) on *Ambystoma maculatum*. It is well known that the species usually lays its eggs in the ponds in the spring. Brimley recorded one case of eggs of this species laid in February "among wet dead leaves lying just above the level of the

water beneath a dead log which lay part in and part out of the water. The albumen around these eggs had swollen up into an irregular mass around each egg, but each egg was distinct and not united to any other, thus the group presented an intermediate stage between the normal condition of the eggs of *maculatum* and that of *opacum*."

In this connection it would be interesting to know if *A. opacum* might not under certain circumstances deposit its eggs in the water. This flexibility of choice of the breeding site, if it could be established, would be detrimental in no way to the thesis advocated as to the stability of the mode of life-history. As previously pointed out (Noble, 1927, p. 45), *A. opacum* agrees essentially in its mode of development with other species of *Ambystoma*. The observations on *A. annulatum* are of interest in showing how flexible the choice of egg-laying site may be in a species which has no terrestrial adaptations in its larval organization.

CONCLUSIONS

(1) *Ambystoma annulatum* lays its eggs in water after rains in late September.

(2) The eggs and embryos agree essentially with those of *A. jeffersonianum*.

(3) The previous report of *A. annulatum* laying eggs on land suggests that the species is flexible in the choice of an egg-laying site.

(4) *Plethodon glutinosus* lays its eggs in late summer in caves in northern Arkansas. The eggs and embryos agree essentially with those of *P. cinereus* except that there is no stalk of attachment for the egg-cluster and the egg-number averages greater.

(5) The egg-capsule number exhibits some variation in preserved material. At least two egg-capsules and a vitelline membrane are present. The inner egg-capsule is normally subdivided into two and frequently into more capsules.

(6) *P. glutinosus* passes through a poorly defined striped pattern during its ontogeny.

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