

UNIVERSITY OF TORONTO  
STUDIES

BIOLOGICAL SERIES

Bio I  
732-738

No. 9: SPAWN AND LARVA OF AMBYSTOMA JEFFER-  
SONIANUM, BY W. H. PIERSOL

(REPRINTED FROM THE AMERICAN NATURALIST, VOL. XLIV)

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[Reprinted from THE AMERICAN NATURALIST, Vol. XLIV., December, 1910.]

## SPAWN AND LARVA OF AMBYSTOMA JEFFERSONIANUM

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### SPAWN

AMONG the various accounts of the habits and spawn of *Ambystoma punctatum* occasional mention may be found of *Ambystoma jeffersonianum* but always in such connections as to suggest that *A. jeffersonianum* is by far the less common species in the locality. This along with the considerable similarity existing between the spawn of the two species may explain why no account of the spawn of *A. jeffersonianum* has as yet appeared. Descriptions of the spawn of *A. tigrinum* sufficient for distinguishing it from that of the other two species is given by B. G. Smith (1907).

In most localities near Toronto *A. punctatum* is a much more common species than *A. jeffersonianum*, however in one piece of woodland that is quite isolated from all the others examined, the former species is rarely to be found, while the latter is very abundant. This woodland contains four pools that last throughout the year, although they become heavily choked by vegetation during the late summer and autumn. The value of these pools as a collecting ground for spawn, *Branchippus*, etc., was discovered some years ago by my colleague, Dr. Huntsman and his observations on the *Ambystoma* spawn suggested to him the possibility of distinguishing in it two kinds. Later the writer also became familiar with this woodland in connection with observations on *Plethodon* and with the consent of Dr. Huntsman undertook also the investigation of the *Ambystoma* spawn of the pools.

The writer first visited these pools in spawning time

three years ago and found a small amount of spawn of a type already familiar to him for some years from its abundance in pools in other localities. But the greater amount was of a type that differed from this in the points detailed below. These two types have proved to be the *punctatum* type and the *jeffersonianum* type, respectively. The predominance of the latter subsequently found its explanation in the fact that 31 of the 33 individuals captured in the woodland since then have been of the latter species. It is impossible to determine accurately the proportions in which the two types of spawn occur, but estimating roughly, the *jeffersonianum* type is at least ten times as abundant as the other.

As will appear below, a small percentage of the eggs of *A. punctatum* will approach in size, or color, or mode of deposition—but rarely in more than one of these points at a time—the eggs of *A. jeffersonianum*. Consequently, the separation of the latter as a type when found in a pool where the *punctatum* spawn greatly predominates, is not an obvious thing. But when the proportions are reversed, as in the special pools mentioned, the distinction is most easily made. Observations in the field have agreed in all four seasons and have been supplemented by the capture of females just previous to egg-laying and comparison of mature ovarian eggs and eggs laid by them in the laboratory, with those obtained in the pools; and finally by the rearing in the laboratory of larvæ from the two types of spawn.

The points of difference in order of constancy are as follows:

1. *Size*.—The eggs of *A. jeffersonianum* are distinctly the smaller, the usual diameter being 2-2.25 mm.

2. *Color*.—The eggs of *A. jeffersonianum* are much the darker, the pigment being but little removed from a true black and covering a much larger proportion of the surface of the egg than in *A. punctatum*; even the lower surface is usually as dark as the upper surface of many of the eggs of the latter species.

3. *Time of Laying*.—The deposition of most of the

spawn by *A. jeffersonianum* precedes that by *A. punctatum* by a few days. It has been impossible to visit daily the pools where the spawn of *A. jeffersonianum* is most abundant, owing to their distance from the university; one pool much nearer has yielded a small amount of it and has provided more accurate although more scanty data. In general the deposition of the bulk of the *jeffersonianum* spawn coincides with that of the first *punctatum* spawn. Variations from this occur—for instance, this year the spawn in the single pool just mentioned followed the above rule, while in the group of four pools nearly all the *jeffersonianum* spawn had been deposited three days before any *punctatum* spawn appeared; and to complete the irregularity the last spawn of all to be deposited was that of *A. jeffersonianum*. It was in small quantity and probably all from one female. (These eggs and the larvæ from them were unusually small, the larvæ seemed vigorous, but could not be kept alive many days after their own supply of yolk was exhausted.) Another check on the time is furnished by the spawning of *Rana sylvatica*. This year—an unusually early season—the writer observed the first deposition of spawn in these pools by the wood-frog. It began at 10.30 A.M., March 31. Spawn of *A. jeffersonianum* had appeared seven days previously.

4. *Spawn-masses*.—The typical spawn mass of *A. jeffersonianum* is a small one, the number of eggs being usually about twenty; the extremes encountered have been small masses of jelly without any eggs and a mass containing forty-one. *A. punctatum* does indeed deposit masses of spawn containing as few eggs as this, but the number is usually much larger. The complement of ripe ovarian eggs carried by two females of average size was 128 and 161. These are probably representative numbers and indicate a rather smaller complement than that possessed by *A. punctatum*—130 to 225—(Wright and Allen, 1908) which in turn is much smaller than that of *A. tigrinum*—1,000 or more (Powers, 1907).

5. Hardly less characteristic than the small masses is

the manner in which they are frequently to be found attached in succession to long slender twigs, each mass being usually in contact with its neighbors. A sentence in one paper on *A. punctatum* (Wright, 1908), "one stem—had within a length of one and a half feet 14 bunches of eggs, 15–20 eggs to the bunch," reads very much like a description of spawn of *A. jeffersonianum*. Many stems so laden have been found each year in the special pools mentioned. The largest piece in Fig. 1 is a portion of one of them. The twigs selected by *A. jeffersonianum* are, as a rule, very slender. *A. punctatum* will make use of both stout and slender twigs indifferently, and no small quantity has been found attached to the margins of leaves and to grass, even in the presence of such twigs as are generally preferred. Eggs of *A. jeffersonianum* have not been found except attached to twigs or stems of water plants.

The low vitality of much of the spawn of *A. jeffersonianum* is a feature that has been noticed in each year. No accurate estimate of the proportion that dies has been made, but judged roughly by the conditions found in the pools it is probably not overstating the loss to say that three fourths of the eggs do not live to begin gastrulation. The same proportion of loss has occurred in spawn reared in the laboratory, while spawn of *A. punctatum* brought from the same pools a little later and kept under the same conditions has suffered practically no loss. The egg does not die, as a whole, but cells here and there precede, the others going on dividing as usual one or more times, only to die at last. The surface view of such an egg when death is complete shows an irregular mingling of minute cells with many others two or three times as great, and at intervals others even up to eight or ten times as great, in diameter. These dead eggs imbibe considerable water, and become very much larger than the living ones and under natural conditions are soon infected by fungi; but in the laboratory they have been kept for weeks and have remained free from it; showing that death has not been caused by a fungus that

only later becomes visible. All the eggs of a mass either die or develop properly; one or two of the eggs may prove exceptions to this, but whatever the defect may be it involves practically all the eggs of a bunch. Whether it may extend to all the eggs of a female it has not been possible to determine. This loss has also been observed in spawn of *A. jeffersonianum* from a second locality and is not likely to be due to any quality of the water, for in the pools of each locality spawn of *A. punctatum* has been found developing with very little loss, and that apparently due to infection by fungus. Neither can it be ascribed to low temperatures from early deposition, for the earliest is no more liable to die than that which comes later along with or after the spawn of *A. punctatum*.

#### LARVA

Spawn of *A. jeffersonianum* brought to the laboratory has been allowed to develop and the larvæ fed until the larger specimens had attained a length of 30-40 mm. In these it has been possible to detect a peculiarity of marking not present in similar larvæ of *A. punctatum*. This peculiarity consists of a massing of dark chromatophores into three or four spots placed in a row along each side of the mid-dorsal line, giving the animal, when viewed from above, the appearance of being banded (Fig. 2). Viewed from the side the same can be detected, but is less conspicuous (Fig. 3). Incipient banding is often indicated as soon as the chromatospheres are well differentiated (Fig. 4).

In looking over a large number of larvæ all gradations will be found between individuals in which the above shows distinctly and those in which it is impossible to detect it. For example, in 115 laboratory-reared larvæ examined at one time, 80 (69 per cent.) showed the distinctive marking. Of the balance, some individuals under different conditions showed it also (either extreme expansion or contraction of the chromatophores obscures the pattern), but some never did. Exact numbers for this division of the 31 per cent. are not available.

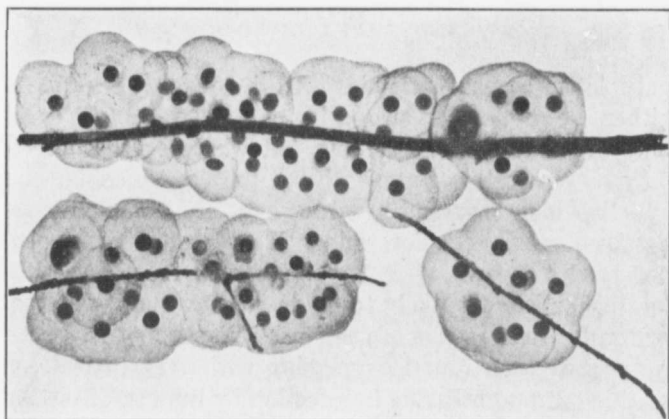


FIG. 1. Spawn of *A. jeffersonianum*. Natural size. The eggs of some of the masses are dead, others are in various stages from blastopore to medullary groove formation.



FIG. 2. Larvæ of *A. jeffersonianum*. Enlarged three diameters. Chromatophores considerably but not extremely contracted.

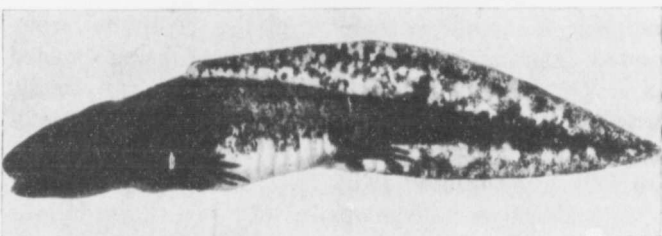


FIG. 3. Larva of *A. jeffersonianum*. Enlarged three diameters. Chromatophores considerably but not extremely expanded. The viscera and right side of the trunk have been dissected away and the photograph taken by both direct and transmitted light.

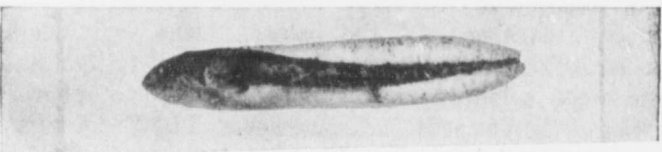


FIG. 4. Larva of *A. jeffersonianum*. Enlarged four diameters.



This year an attempt was made to remove all the spawn of *A. punctatum* from the special pools. In the middle of June larvæ 30–35 mm. long were collected from them and examined as to this pattern; it was found in but 35 per cent. Two causes may have contributed to this—the abundance of brush in the pools may have caused some spawn of *A. punctatum* to be overlooked, and the great expansion of the chromatophores—much greater than ever attained in the laboratory, the pools being very dark, probably disguised it in some cases. It was found impossible to put these larvæ under observation in the laboratory to test this point, for owing to the long journey or to the change of water they invariably died within a few hours.

Little importance would have been attached to a point of coloration so variable as this had it not been found to be uniformly lacking in similar larvæ of *A. punctatum*, whether raised in the laboratory or taken from the pools known to contain little, if any, spawn of *A. jeffersonianum*. In view of the range of coloration for *A. tigrinum* as larvæ (indicated by Powers), the degree of constancy noted is perhaps the most that could be expected.

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