

OBSERVATIONS ON THE EGGS OF *AMBYSTOMA MACULATUM*
WITH ESPECIAL REFERENCE TO THE GREEN ALGAE
FOUND WITHIN THE EGG ENVELOPES

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Those who have observed large numbers of eggs of the spotted salamander (*Ambystoma maculatum*) have undoubtedly seen many masses colored a delicate or even a vivid green. The color of these egg masses is due to unicellular green algae which thrive in the egg envelopes, eventually giving to the jelly a green appearance. Often the algal growth, especially within the innermost jelly envelope, is so dense that it is difficult to see the developing embryo.

Published accounts of this phenomenon appeared first in the writings of Orr ('88) who noted the occurrence of unicellular globular green algae in the inner envelope of *Ambystoma maculatum* eggs. Concerning the significance of this association he wrote: "I have not discovered how the Algae enter the membrane, nor what physiological effect they have on the respiration of the embryo, but it seems probable that in this latter respect they may have an important influence." Eycleshymer ('95) noted "numbers of minute algae" in the outermost envelope of *Ambystoma* eggs but failed to find them in the innermost envelope. A rather vague reference to the occurrence of algae in amphibian egg envelopes was made by Lambert ('10). He observed that the "gelatinous substance surrounding frog's eggs which were developing in a laboratory aquarium . . . showed a characteristic green color. This was due to the presence of certain unicellular algae, familiar to me by reason of several seasons' observations." Professor Gilbert M. Smith of Stanford University informed me that Lambert gave the name *Oophila amblystomatis* to certain unicellular green algae found growing in the egg envelopes of *Ambystoma*, but a de-

scription of this alga has never been published nor has the name been recognized by algalogists.

Storer ('25) devoted considerable space in his "Synopsis of the Amphibia of California" to a description of another algae-amphibian egg association. He noted that the inside of each capsule of *Dicamptodon ensatus* eggs was "lined with a coating of small rounded green algae, of sufficient density to be seen easily with the unaided eye." Storer was unable to identify the algae nor did he attempt to find out how the algae entered the egg. He did speculate on this latter point, however, as well as on the possibility of a symbiotic relationship. Recently Henry and Twitty ('40) have indicated that the algae-inhabited eggs Storer was dealing with might actually have been those of *Ambystoma gracile* rather than *Dicamptodon*.

Breder ('27) noted that *Ambystoma maculatum* eggs "containing algae would not hatch in the dark while those without it [algae] readily did so." She concluded that the algae probably "helps provide oxygen for the developing embryos in the presence of light and robs them of it in darkness." Richards ('40) noted that while the capsular fluid of *Amblystoma punctatum*¹ eggs does not support bacterial growth, "occasionally an alga may grow within the capsule." Showalter ('40) found non-motile unicellular green algae in salamander eggs taken from a mountain lagoon near Madison College, Harrisonburg, Virginia. He did not identify the eggs but made the observation that the larvae, a few days after hatching, ate the algae. Bishop ('41) observed: "In the eggs of both *A. jeffersonianum*

¹The names *Ambystoma maculatum* and *Amblystoma punctatum* are synonymous.

and *A. maculatum* the second envelope, that is, the inner one if the vitellus is disregarded, supports an algal growth which often almost obscures the embryo. This alga was identified by Dr. Gilbert M. Smith of the Department of Botany, Stanford University, as a species, probably new, of *Palmodactylon*."

The investigators above have done little more than to note that unicellular green algae do inhabit the jelly envelopes of certain amphibian eggs. There is no agreement as to which egg capsules are inhabited. The nature of the green alga itself has never been established nor has a description of it ever appeared in the literature. Where the alga comes from and how it enters the egg capsules has never been determined. Though several workers suggest that the algae affect the rate of development of the eggs, no experimental proof has been offered demonstrating this point. It is hoped that the present paper will throw some light on these problems.

ACKNOWLEDGMENTS

The writer is indebted to Professor C. McC. Mottley (Cornell University) for first arousing interest in this problem and to Professor A. H. Wright (Cornell University) for valuable criticisms and suggestions during the course of the study. Professor Gilbert M. Smith (Stanford University) kindly examined the algae and was helpful in determining its approximate taxonomic position while Professor W. C. Muenscher (Cornell University) rendered helpful assistance also in this connection. Professor W. W. Ballard (Dartmouth College) and Professor W. T. Dempster (University of Michigan) generously sent me egg masses from Hanover, New Hampshire, and Ann Arbor, Michigan, respectively. Mr. Arthur Smith made the photographs of the eggs and algae.

NAME OF THE ALGA

The alga found in the egg capsules of various species of *Ambystoma* is a uni-

cellular green type belonging to the Chlorophyceae. When first collected it was thought to be *Chlorella* since this alga is commonly associated with animal tissues but measurements and further observations revealed that it did not belong to this genus. Subsequently Dr. Gilbert Smith kindly examined some of the algae and wrote to me as follows: "Upon examining them I rejected the idea that they were a *Chorella* since their size and general appearance resembles much more closely *Chlorochytrium*, an endophytic alga of plant tissues but one, so far as I am aware, which has not been recorded from animals. In looking through Print's revision of the Chlorophyceae . . . I find that he includes a genus *Oophila* among the questionable genera. Lambert, an American, distributed dried specimens of a new genus and species that he called *Oophila amblystomatis*. This was found in the egg membrane of *Amblystoma punctatum*. From all that I can gather this seems to be identical with your organism but there is a nice legal quibble as to whether this name is valid or not since Lambert did not publish a description but did distribute specimens in a regularly distributed exsiccata." It is possible that a second genus of green alga also inhabits the egg membranes of *Ambystoma* since *A. jeffersonianum* and *A. maculatum* eggs collected by Bishop ('41) contained algae which Smith identified as *Palmodactylon*.

NATURE OF THE ALGA

The writer has not yet completely worked out the life cycle of the alga which appears in the jelly envelopes. It seems desirable to present the data thus far collected, however, reserving a formal description of the alga for a later date. The green alga is unicellular and may be spheroidal or ovoid in shape (Plate II, figures 10, 11, 13, 14). Both spheroidal and ovoid forms may be motile or non-motile. Motile algae may have two or four polar flagellae. Probably the biflagellate ones are gametes while the large quadriflagellate algae are either zygotes

or autospores. Spheroidal forms vary in diameter from 6–30 μ while ovoid ones vary in length from 8–30 μ . The chloroplast of the larger algae is very granular while that of the smaller sizes contains few granules; the granules are probably starch particles. The largest spheroidal algae (25–30 μ diameter) are invariably non-motile and have a dense granular chloroplast surrounded by a thick capsular wall.

In addition to the spherical and ovoid stages described above, a third narrow, elongate, biflagellate type (Plate II, figure 12) commonly appears. This latter type generally varies in length from 15–25 μ , while the flagellae appear to be about as long again. Whether or not this is a stage in the life cycle of the spheroidal and ovoid algae has not yet been definitely determined. It is significant, however, that whenever the latter types of cells are found, so also is found this narrow, elongate type.

NATURE OF THE EGGS OF *Ambystoma maculatum*

A single egg of *Ambystoma maculatum* is surrounded by three gelatinous envelopes. The relative thickness of these envelopes after they have taken on their full quota of water is shown in figure 1. These envelopes have been carefully described by Piersol ('29) and since the nature of each envelope undoubtedly has

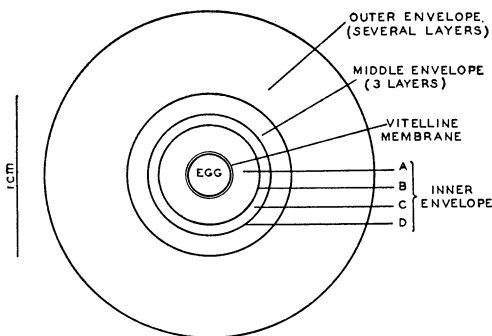


FIG. 1. Diagram showing the relative thickness of the three egg envelopes of *Ambystoma maculatum*.

something to do with the algal population it harbors, the following summary of Piersol's findings is herewith presented.

Inner envelope

- (a) A soft gelatinous material next to the vitelline membrane.
- (b) An extremely thin pearly layer scarcely more than outer surface for the material above but not gelatinous.
- (c) A thicker layer, quite tough, perfectly transparent, firmly attached to
- (d) A thin very pearly layer.

Middle envelope

- (a) A soft gelatinous layer adhering to the inner envelope and to
- (b) A thick tough layer, very pearly. It consists of a variable number of minor layers (usually 5 to 7) clinging closely together. At the interfaces are numerous fine whitish granules which give the whole layer its pearly lustre.
- (c) A much thinner but still tougher layer, its outer surface slightly pearly.

Outer envelope

- (a) This consists of several layers, from within outward growing thicker, less tough and less clearly marked off from one another by very slightly pearly interfaces. There is irregularity in thickness in different parts of the same layer, and it is difficult to decide whether what appear concentric layers may not be all one spiral wrapping. The amount of pearly material is usually noticeable only on careful examination. But it may be so extremely abundant that only the outer eggs of a mass can be obscurely seen through it.

The writer has noted that as the egg develops, the space within the inner envelope becomes relatively larger, while the

middle and outer envelopes become relatively thinner (Plate I, figure 9). Also the original pearly lustre in the middle envelope disappears during later developmental stages.

Under normal conditions the eggs of *Ambystoma maculatum* are not deposited singly but in a mass containing as many as 140 eggs (Wright and Allen, '09). When the eggs are thus deposited, the outer gelatinous envelope of one egg appears to be continuous with neighboring outer envelopes, thus enclosing the eggs with their two remaining envelopes in a compact mass of jelly (Plate I, figure 6).

DISPOSITION OF THE ALGAE

It has already been noted that Orr ('88) observed the green algae to be in the innermost envelope of *Ambystoma maculatum* eggs while Eycleshymer ('95) noted the algae in the outermost envelope and failed to find them in the inner envelope. Actually the algae occur in all three envelopes. In the outermost envelope they are usually not numerous enough to be visible macroscopically. In the middle envelope they are sufficiently dense to impart a light green color to this envelope while within the inner envelope the algae flourish, literally paving area b (figure 1) with large spherical non-motile unicellular bodies 25 to 30 μ in diameter. Only eggs at late stages of development show all three envelopes inhabited.

ALGAL ENTRANCE INTO THE EGG ENVELOPES

When the writer first noticed algae-infested eggs of *Ambystoma maculatum* in 1937, the inner envelopes were observed to be inhabited with a dense algal flora while the middle and outer envelopes were relatively translucent. This led him to suspect that the algae in some way lodged on the egg in the genital tract of the female before the jelly envelopes were laid down around the egg. Accordingly oviducts of several ripe females were washed out with Ringer's solution and

the washings carefully examined but no trace of the alga was found. These washings were subsequently placed in a sterile media in which the alga was known to flourish but no algae developed. Thus it was concluded that the algae did not enter the genital tract of the female, adhering to the eggs as they passed down the oviduct or through the uterus. The problem therefore resolved itself into demonstrating that the algae entered the egg envelopes from the water after the eggs had been laid. An examination of debris on the bottom of the pools in which the eggs were laid revealed the presence of numerous free-living unicellular motile and non-motile green algae which corresponded in size, shape, and character to those found within the jelly envelopes on previous occasions. An experiment was devised to determine whether or not these algae were capable of entering the jelly envelopes and multiplying in this new medium.

On the evening of April 16, 1941, the writer collected a pair of *Ambystoma maculatum* in a small pond in the vicinity of Ithaca, New York, and placed them in a laboratory aquarium filled with algae-infested pond water. The following morning (April 17) two somewhat mashed spermatophores containing large clumps of inactive sperm were found on the bottom of the aquarium. The female had laid two eggs, apparently quite recently, for the envelopes had not swollen very much. With a pipette a small amount of fluid from the cloaca of the female was withdrawn. Eight inactive sperm were found but no algae in this cloacal sample. The male was removed from the aquarium, a few twigs were placed in the water and within one hour and a half about twenty-five eggs were deposited, some in clusters on the twigs, others singly on the bottom of the aquarium. At that time the female was carefully rinsed several times in algae-free water and placed in an aquarium partly filled with tap water. The following morning (April 18) she had deposited about sixty eggs in the tap water. Thus the same

female deposited about 25 fertile eggs in algae-inhabited pond water and about 60 fertile eggs in algae-free tap water. Six hours after the last mass was deposited a few eggs from each aquarium were examined. Using a magnification of 200 diameters, no algae were visible in the jelly envelopes of the tap water mass. In the pond water mass one spheroidal unicellular green alga about $20\ \mu$ in diameter was seen in the outer jelly and a much smaller spheroidal alga ($7\ \mu$ dia.) was observed in the middle jelly envelope. It appeared that the envelopes of those eggs laid in pond water had been penetrated by the algae while the envelopes of those laid in tap water had not. Since these tiny cells might readily have been overlooked when not present in sufficient numbers in the jelly envelopes, part of each egg mass was placed in a separate container filled with flowing tap water. To accelerate the growth of any algae which may have been present in the jelly envelopes, the masses of eggs were lighted continuously. One week later (Apr. 25) no algae were to be found in the envelopes of the eggs laid in tap water while those laid in pond water exhibited several spheroidal and ovoid unicellular green algae in the outer jelly envelopes. These latter algae were not yet numerous enough to be visible macroscopically. At this time part of the algae-free mass of eggs was removed to the pond from which the adult salamanders were originally taken. These eggs were carefully examined before being placed in the pond water and their envelopes were definitely algae-free. One week later (May 2) these same eggs were examined. Macroscopically they appeared algae-free. Microscopic examination, however, revealed algae in both outer and middle envelopes. These cells were mostly of the spheroidal and ovoid motile and non-motile types and were most numerous in the outer jelly. Two motile cells of the narrow elongate type (about $15\ \mu$ long) were also observed.

In regard to the laboratory eggs which remained in running tap water, it should be stated that though these eggs remained lighted for a period of over four weeks, the jelly envelopes of the eggs laid originally in tap water showed no sign of the algae. The eggs which were originally laid in pond water and subsequently removed to running tap water at the end of one week, as stated above, exhibited spheroidal- and ovoid-shaped algae in the outer jelly envelope. At the end of two weeks the outer envelope of these same eggs contained whole clusters of small unicellular green algae. Each cell had a diameter of approximately $9\ \mu$ and as many as nine or ten cells constituted a cluster. Probably these clusters represented the offspring or part of the offspring of a single original cell. As yet (at end of 2 weeks) but few algae had appeared in the middle envelope, and none at all were seen in the jelly of the inner envelope. At the end of one month the egg envelopes had taken on a delicate green color and microscopic examination revealed algae in all three egg envelopes.

Eggs which were laid about the same time in the pond and left there by now were hatching or had hatched and their inner envelopes were literally paved with large non-motile, spheroidal, unicellular green algae, while about the embryos in the unhatched eggs swarmed countless hundreds of motile spheroidal and ovoid-shaped algae. Higher water temperatures in the pond had caused a more rapid development of the eggs and had likewise probably speeded up the rate of penetration and reproduction of the algae.

Thus it was concluded that the algae-free envelopes of *Ambystoma* eggs, placed in pond water containing the unicellular green algae discussed above, become penetrated within six hours by these plants. These algae may penetrate the jelly envelopes either at the time the eggs are laid or at a much later date as in the case of the laboratory eggs which had remained algae-free for one week before being removed to the pond, there to be-

come algae-inhabited. The unicellular green alga which characteristically inhabits the eggs of *Ambystoma* passes through the outer jelly envelope relatively soon and multiplies rapidly within the jelly of the middle envelope. Cells from this envelope later penetrate the inner envelope and here, especially in the fluid

latter envelope however which, shortly before the eggs hatch, contains the densest concentration of algae—a population so rich that oftentimes the developing embryo is completely concealed by its verdant blanket. Though the rate of algal penetration varies with light and temperature, nevertheless the sequence of penetration as described above is always the same.

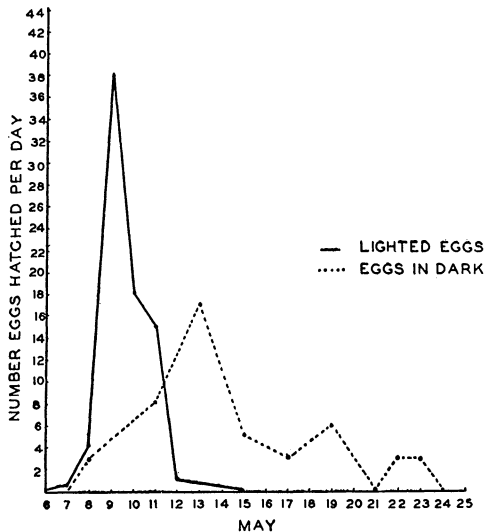


FIG. 2. The hatching rate for algae-free (eggs in dark) and algae-inhabited (lighted) eggs of *Ambystoma maculatum* mass 1.

(area a of figure 1) about the developing embryo, the motile algae increase their numbers enormously. As the motile cells ($10\ \mu$ – $20\ \mu$ diameter) increase in size they lose their flagellae and come to rest against the inside of the inner envelope (area b of figure 1) as large spheroidal ($30\ \mu$ diameter) green bodies, each with a highly granular chloroplast surrounded by a thick capsular wall. When the salamander eggs are about ready to hatch, the algae in the outer envelope may or may not be dense enough to impart a green color to it. Usually the middle envelope harbors the algae in sufficient numbers so that it appears macroscopically a delicate light green. At the time the middle envelope first exhibits a greenish tinge, the inner envelope still appears uncolored and transparent. It is this

FACTORS INFLUENCING THE DISTRIBUTION OF ALGAE WITHIN THE EGG CAPSULES

As the temperature of the water increases the rate of development of the embryo salamander is speeded up. Meanwhile the jelly envelopes about the embryo become less and less viscous until at the time the egg hatches the new born larva is capable of wriggling its way through the now watery jelly which previously insulated it. The writer believes that as these envelopes become less and

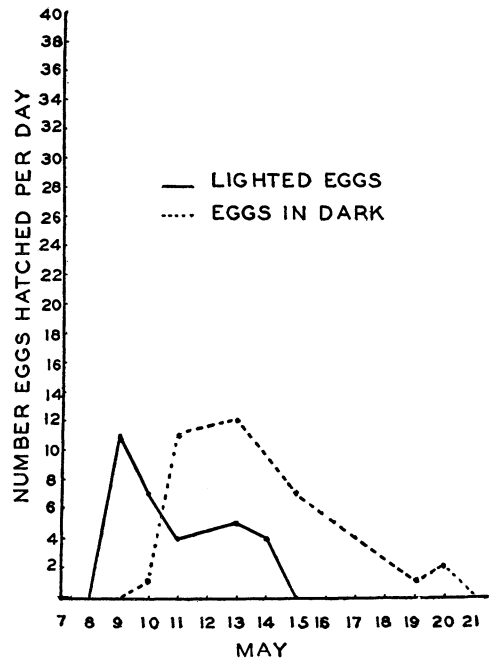


FIG. 3. The hatching rate for algae-free (eggs in dark) and algae-inhabited (lighted) eggs of *Ambystoma maculatum* mass 4.

less viscous, motile cells may pass through and multiply in the jelly more readily; hence the concentration of algae increases, with later stages of development, in the outer and middle envelopes. A soft gelatinous, almost fluid material is normally found within the inner envelope about the developing embryo and once the algae reach this area they reproduce rapidly. Motile cells (both biflagellate and quadriflagellate) swarm in enormous numbers about the developing embryo. On several occasions dense concentrations of these motile cells have been observed about the proctodaeum of the embryo, possibly attracted to nitrogenous excretions from this region. If the egg was shaken, the algae scattered, only to quickly resume their position in the region of the proctodaeum.

IS THE ALGAE-EGG RELATIONSHIP A SYMBIOTIC ONE?

While it has been generally assumed by certain observers (Orr, '88; Storer, '25; Breder, '27) that the relationship between the eggs and the algae is a symbiotic one, no one has as yet presented any experimental evidence to justify this conclusion. It is apparent that the algae flourish within the jelly envelopes of the *Ambystoma* eggs examined. The algae within the innermost envelope, next the developing embryo, thrive and multiply especially well. This is probably due both to the fluid nature of the medium as well as to the presence of CO₂ and nitrogenous wastes given off by the embryo. It can

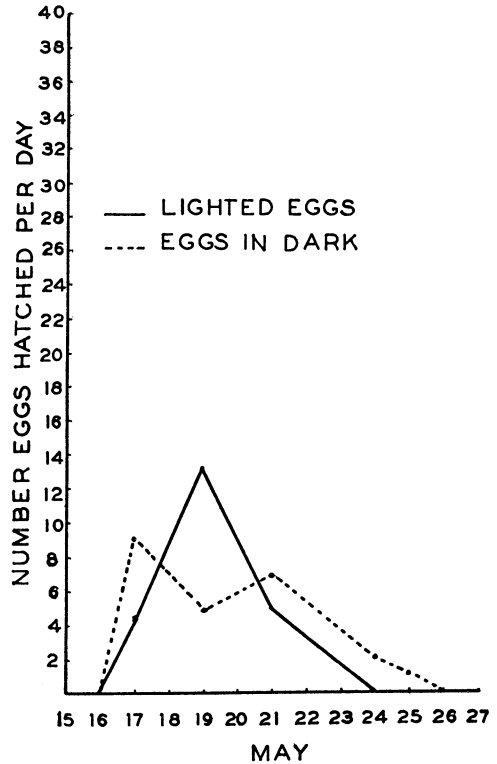


FIG. 4. The hatching rate for algae-free (eggs in dark) and algae-inhabited (lighted) eggs of *Ambystoma maculatum* mass 7.

hardly be denied that the algae at least profit by this association. The question now is: is there any change in the developmental rate of the embryos which might be attributable to the algae? It is hoped that the following experimental data will partially clarify this latter point.

Several masses of algae-inhabited *Ambystoma* eggs were halved, one-half being

TABLE I. History of certain egg masses

Mass no.	Eggs of	Placed in lighted and unlighted jars on:	Stage when placed in jars	Lighted			Unlighted		
				Begin. and end of hatching	No. days elapsed	Total no. eggs hatched	Begin. and end of hatching	No. days elapsed	Total no. eggs hatched
1	<i>A. maculatum</i>	April 11, 1939 Ithaca	yolk plug	May 7-15	8	78	May 8-24	16	48
4	"	April 14, 1939 Ithaca	crenate groove	May 9-15	6	31	May 10-21	11	46
7	"	April 29, 1939 Ithaca	neural groove	May 17-24	7	29	May 17-26	9	25
3	<i>A. jeffersonianum</i>	April 14, 1939 Ithaca	medullary plate	Apr. 28-May 5	7	20	May 3-5	2	6

placed in a lighted battery jar filled with running tap water and the other half placed in a battery jar from which all light was excluded, also filled with running tap

water. Those eggs which remained lighted developed a rich algal flora within their envelopes (especially the innermost one) while the algae failed to multiply within the envelopes of the egg masses placed in the dark. The eggs were examined once a day and a record was made of the current stage of egg development and time and rate of hatching for each of the masses. Water temperatures in the lighted and unlighted jars were checked twice a day and did not vary from each other more than $\pm 0.5^\circ \text{C}$. at any one time during the course of the experiment. Nearly always the temperatures in the two jars were exactly the same. The history of some of the egg masses thus observed is tabulated in table I.

The above tabulation indicates that in general those eggs which had remained lighted and developed the algae hatched earlier and over a shorter period of time than did those eggs which were placed in the dark and failed appreciably to develop the algae. The rate at which both the algae-free and algae-inhabited eggs hatched for each of the four masses is shown graphically in figures 3-6. In all cases the unlighted algae-free eggs either began hatching later or hatched over a longer period of time or both than did the lighted algae-inhabited eggs.

Observations made on the growth of *A. maculatum* embryos selected at random in the algae-free (unlighted) and algae-

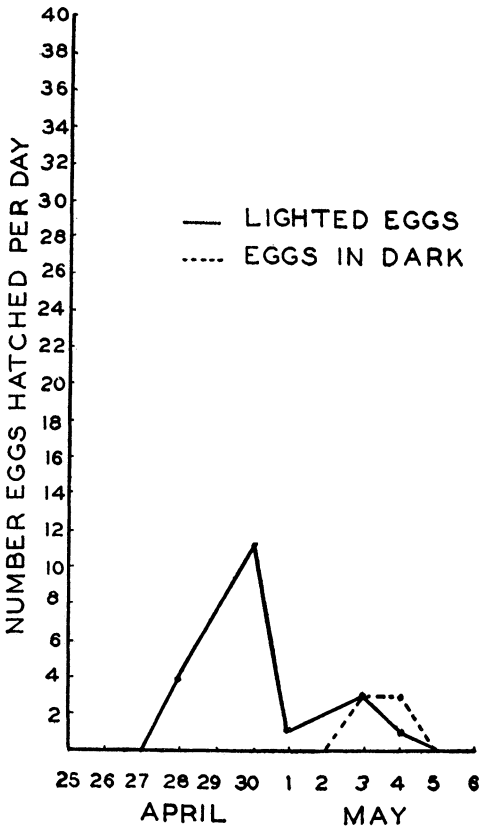


FIG. 5. The hatching rate for algae-free (eggs in dark) and algae-inhabited (lighted) eggs of *Ambystoma jeffersonianum* mass 3.

PLATE I

Explanation of figures

FIG. 6. Normal egg mass of *A. maculatum*, shortly after being laid. No algae visible as yet. Approximately $\times \frac{2}{3}$.

FIG. 7. Eggs of *A. maculatum* at late stage of development. The embryos are partially concealed by the rich population of unicellular green algae within the inner envelopes. Approximately $\times \frac{2}{3}$.

FIG. 8. The *A. maculatum* larvae have emerged, leaving the egg envelopes behind. Note the inner envelopes colored by the unicellular green algae. Approximately $\times \frac{2}{3}$.

FIG. 9. Two eggs of *A. maculatum* showing relative size of the envelopes. One egg (above) recently laid and the other egg (below) at later stage. As development proceeds the diameter of the inner envelope increases and the pearly lustre of the middle envelope is gradually lost. Approximately $\times 1\frac{1}{2}$.

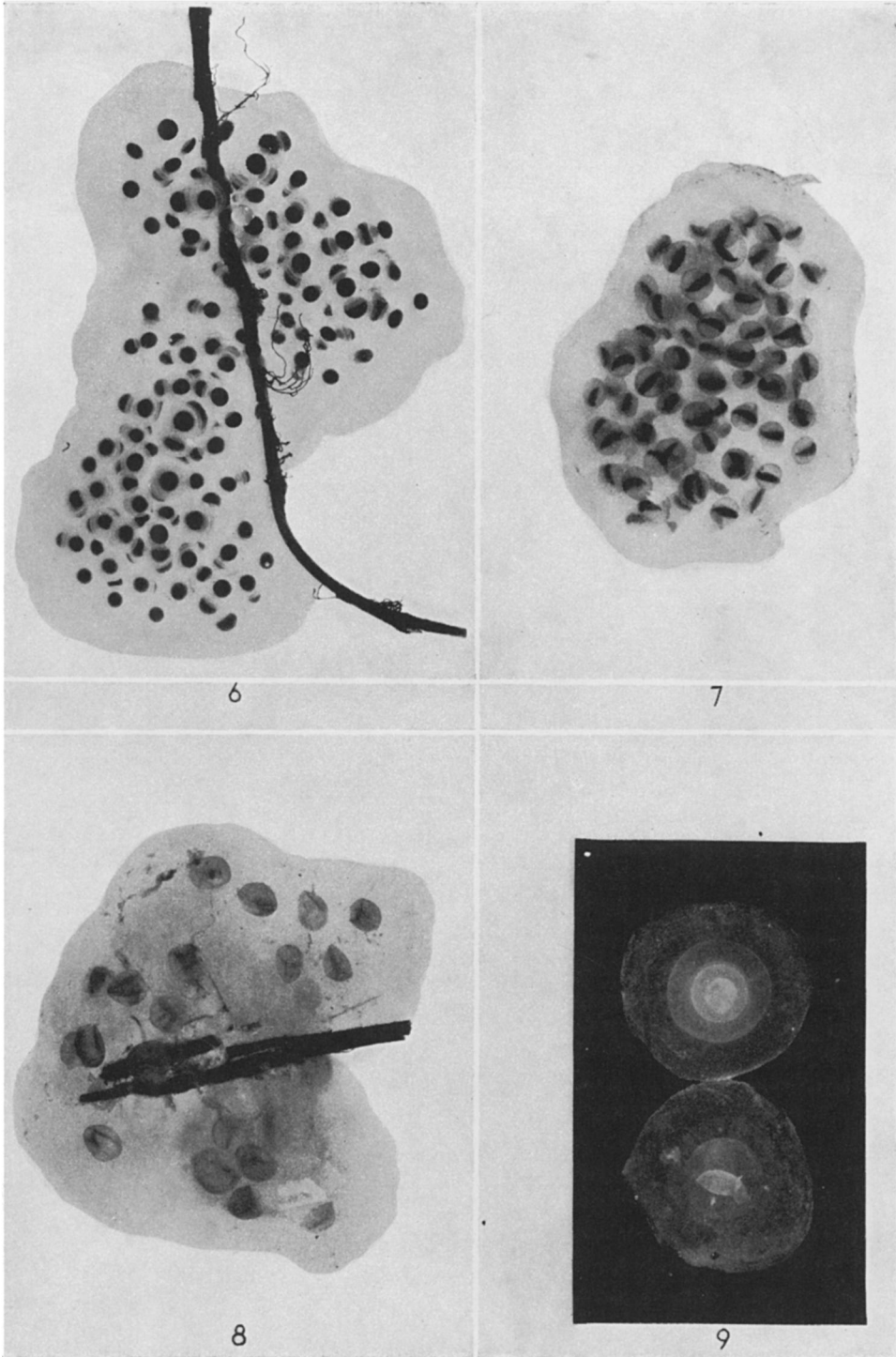


PLATE I

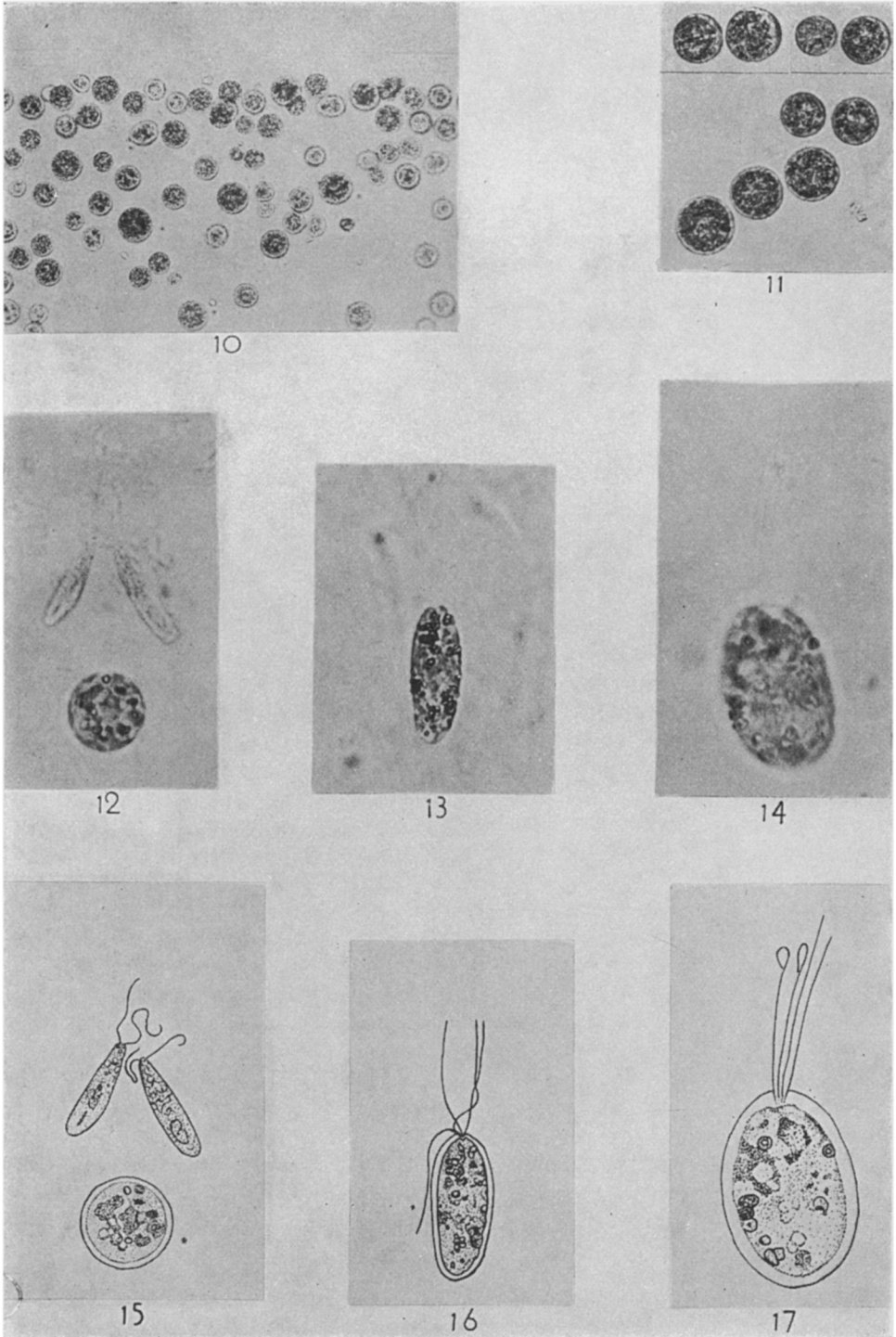


PLATE II

inhabited (lighted) egg masses referred to above revealed the following:

Mass no.	Date embryos examined	Harrison stage (lighted mass)	No. examined	Harrison stage (unlighted mass)	No. examined
1	May 9, 1939	43, 43	2	41, 41	2
4	May 9, 1939	43, 43	2	40, 40	2
7	May 15, 1939	40, 40	2	37, 37, 39	3

The table shows that at approximately the time the first embryos hatched from the lighted masses, two embryos selected at random from the algae-inhabited were one to three developmental stages (Harrison stages) in advance of embryos similarly selected from the algae-free masses. The number of embryos selected is definitely too small to permit a valid conclusion. The data suggest, however, the possibility that embryos in algae-inhabited masses develop more rapidly than those in algae-free masses; hence the earlier average hatching rate for the embryos in the algae-inhabited masses due possibly to the earlier appearance of functional hatching glands on the heads of the embryos.²

² Noble and Brady ('30) have demonstrated that shortly before emergence from the egg membranes, a series of unicellular hatching glands appear on the snout of *Ambystoma opacum* and probably all urodele larvae (Noble, '31). These glands produce a secretion which digests the egg capsules and liberates the embryo.

The question which might appropriately be raised at this time is: could not this variation in time and rate of egg development and hatching be due to the presence or absence of light rather than to the presence or absence of algae? The logical experiment to conduct to determine this point would be to secure an algae-free mass of eggs and place half the mass in a lighted jar of flowing water and the other half in a darkened jar of flowing water of the same temperature and then note the rate of egg development. Because of the difficulty in securing algae-free eggs however, this experiment has not yet been tried. Of the hundreds of *Ambystoma* egg masses observed by the writer during the past four years, but one has been algae-free and that was used for another type of experiment. It is generally admitted, however, that light does not have an appreciable influence on the rate of development of animal (including amphibian) eggs. Laurens ('33), after reviewing this subject quite thoroughly, concludes: "Although light is one of the most striking attributes of the environment of living organisms, the results of practically all of the experiments designed to show its effect on the normal growth processes of animals have been negative." Thus it appears probable that the more rapid rate of development and earlier and shorter period of hatching of the algae-inhabited eggs as compared with

PLATE II

Explanation of figures

FIG. 10. General view of the non-motile green algae which line the inner envelope of *A. maculatum* eggs. Note that the chloroplast is slightly granular in the smaller algae and densely granular in the larger ones. Approximately $\times 250$.

FIG. 11. Spheroidal non-motile green algae which inhabit all three egg envelopes of *A. maculatum*. Approximately $\times 470$.

FIG. 12. Narrow elongate biflagellate (above) and small spheroidal (below) types of green algae which inhabit all three egg envelopes of *A. maculatum*. Approximately $\times 1200$.

FIG. 13. Ellipsoidal quadriflagellate type of green alga found in all three egg envelopes of *A. maculatum*. Approximately $\times 600$.

FIG. 14. Ovoid quadriflagellate type of green alga found in all three egg envelopes of *A. maculatum*. Approximately $\times 1200$.

FIG. 15. Tracing of figure 12.

FIG. 16. Tracing of figure 13.

FIG. 17. Tracing of figure 14.

the algae-free eggs was due to the presence of the algae rather than to the presence of light. It would be worthwhile now to follow up the larvae after hatching and determine whether those from algae-inhabited eggs are in general hardier and metamorphose earlier than those from algae-free eggs. In this connection W. W. Ballard (in litteris) has observed that those eggs of *Ambystoma* surrounded by a rich growth of algae make safer and hardier experimental material than those without it.

EXTENT OF THIS PHENOMENON

Observations over the past four years have led the writer to believe that this association of unicellular green algae with the eggs of *Ambystoma maculatum* is very widespread in northeastern United States. Out of hundreds of egg masses observed about Ithaca, but one has been found which did not harbor these algae. This mass came from a small isolated temporary pool formed by the spring rains. Since this pool was located on a section of old washed stream bed, it is possible that the water did not contain the unicellular green algae which characteristically inhabit *Ambystoma* eggs. During the past few years the writer has received several masses of eggs from Ann Arbor, Michigan, and Hanover, New Hampshire. Though these eggs appeared to be algae-free when they first arrived, microscopic examination revealed their envelopes to be algae-inhabited and they all subsequently developed a rich green algal flora.

OTHER AMPHIBIAN EGGS WITH WHICH THE ALGA IS ASSOCIATED

The unicellular green alga found so commonly within the egg envelopes of *Ambystoma maculatum* has also been noted in all three egg envelopes of *Ambystoma jeffersonianum*. Unlike *A. maculatum*, the alga does not flourish sufficiently within the innermost envelope to impart a deep green color to this area. The egg envelopes of the wood frog

(*Rana sylvatica*) have also been observed to harbor this alga. The particular wood frog eggs (a hundred or more masses within an area of five square feet) the writer examined were just hatching and the little tadpoles could scarcely be seen through the dense green algal flora within their egg envelopes. Nearby eggs of the leopard frog (*Rana pipiens*) exhibited no such algal flora neither macroscopically nor microscopically. Possibly the chemical composition of wood frog egg envelopes is more nearly like that of *Ambystoma*, hence supplying a favorable site in which the unicellular green algae may flourish. The eggs of the American toad (*Bufo americanus*) have been examined but no algae have been found. Probably this is because the eggs hatch too quickly (usually two days or less after laying) and the jelly disintegrates before the algae can become established.

OTHER ORGANISMS INHABITING THE EGG ENVELOPES OF *Ambystoma maculatum*

At early stages of egg development a holotrich protozoan, probably *Holophrya*, is found commonly associated with the algae within the jelly envelopes of *Ambystoma* eggs. This protozoan has a flexible cell wall and moves about in an almost amoeboid manner at times, gorging itself on the unicellular green algae until its cytoplasm looks like one large chloroplast. The periphery of an *Ambystoma* egg mass soon after deposition becomes covered with diatoms, principally *Synedra*. These flourish sufficiently to soon impart a brownish tinge to the outer surface of the jelly. Frequently another diatom, *Navicula*, is also found but never in abundance. By the time the eggs are ready to hatch the jelly envelopes have become quite watery, permitting the entrance of a host of different protozoans, nematodes and rotifers.

SUMMARY

1. A unicellular green alga, as yet undescribed but closely related to the genus

Chlorochytrium, is commonly found within the egg envelopes of *A. maculatum* and *A. jeffersonianum*. It has also been noted in the egg envelopes of the wood frog, *Rana sylvatica*.

2. This alga may be spheroidal or ovoid in shape, may vary from 6 μ to 30 μ in diameter and possesses both motile and non-motile stages.

3. The motile stages of the alga may be biflagellate (probably gametes) or quadriflagellate (probably zygotes or autospores). The flagellae are polar and are as long or longer than the cell body.

4. The alga possesses a single chloroplast which is slightly granular in small cells and densely granular in large ones. The largest cells are spheroidal, non-motile and 25 μ to 30 μ in diameter.

5. These unicellular green algae enter the egg envelopes from the water after the eggs have been deposited. Eggs deposited in algae-free water do not harbor the algae.

6. The algae are found in all three egg envelopes of *Ambystoma* at late stages of egg development; they are least numerous in the outer and most numerous within the inner envelope.

7. Assuming that the presence or absence of light neither appreciably speeds up nor retards the developmental rate and time of hatching of *Ambystoma* eggs, the evidence at hand suggests the possibility that the relationship between algae and developing egg is a symbiotic one.

8. It appears that this association of unicellular green algae with the eggs of *Ambystoma maculatum* is not an occasional nor a local one for all the egg masses of this salamander in central New York, with one exception, have been found to harbor the algae. All the egg masses from Michigan and New Hampshire which have been examined have

likewise been inhabited by the same unicellular green algae.

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