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STUDIES ON THE REPRODUCTION AND ARTIFICIAL PROPAGATION OF FRESH-WATER MUSSELS

From BULLETIN OF THE BUREAU OF FISHERIES, Volume XXX, 1910

Document No. 756 . . . . . *Issued May [illegible], 1912*

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STUDIES ON THE REPRODUCTION AND ARTIFICIAL PROPAGATION OF FRESH-WATER MUSSELS

By George Lefevre and Winterton C. Curtis
Professors of Zoology in the University of Missouri

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STUDIES ON THE REPRODUCTION AND ARTIFICIAL PROPAGATION OF FRESH-WATER MUSSELS.

By GEORGE LEFEVRE and WINTERTON C. CURTIS,

Professors of Zoology in the University of Missouri.

INTRODUCTION.

The threatened extinction in the upper Mississippi River and its more important tributaries of those species of the Unionidæ whose shells have been taken in enormous numbers in recent years, both for the manufacture of pearl buttons and for the pearls which they occasionally contain, has led the United States Bureau of Fisheries to undertake an extensive investigation of the possibility of artificially propagating the commercial species and of devising practicable means of restocking depleted waters which present favorable conditions for their maintenance. The general direction of the investigation has been placed in the hands of the writers, who for several years have devoted as much time as their regular duties have allowed to the work, in certain important phases of which, however, many others have collaborated.

It was recognized at the outset that if the investigation was to be of any practical value it must be wide in scope and must extend over a period of at least several years. At that time much remained to be learned concerning the breeding habits and seasons of the commercial species, the biological and physical conditions under which they live, their distribution throughout the Mississippi Valley, and many other essential matters, while it was yet to be discovered whether artificial propagation could be successfully carried out. At the very inception of the work, therefore, a comprehensive plan was outlined which was designed to include every subject that might bear even remotely upon the central problem—the restoration of the exhausted mussel beds—and, although many parts of this program have scarcely been touched, much progress has been made in some of the more important lines.

The plan of work contemplated, besides a thorough investigation of the conditions under which artificial propagation might be possible, a detailed study of the life history and ecology of the Unionidæ, with special reference to the geographical distribution of the group throughout the Mississippi Valley, the breeding seasons and habits, the

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physical conditions of the waters in which different species thrive and attain their maximum growth, food supply, enemies and diseases, rate of growth and the influence of environmental factors upon it, and the behavior of glochidia and fishes as parasites and hosts, respectively.

The results that have already been obtained, although far from complete, will serve as a basis for future investigations, while the lines of attack in the main problems have been definitely indicated. We have proceeded far enough to make it clear that the ultimate end of the investigation is assured, and with adequate facilities for the infection and care of large numbers of fishes and for the maintenance of the young mussels during the early stages of growth following the metamorphosis, the final success of the work can no longer be in doubt. The essential facts in the life history of the Unionidæ are known; the breeding seasons and habits of the commercial species have been sufficiently determined; the general conditions of infection and of the parasitism of the larva have been learned experimentally; and the entire feasibility of artificially propagating at least certain species of fresh-water mussels has been clearly demonstrated; while the requisite conditions for placing artificial propagation on a practical basis are now thoroughly understood.

The writers' personal attention has in the main been directed to a study of the conditions of reproduction in the group and the parasitism of the larva in their bearing upon the problem of artificial infection of fishes with glochidia, while such phases of the investigation as geographical distribution, systematic studies, and a number of special ecological problems have been in the hands of other investigators.

At the recently established biological station of the Bureau of Fisheries at Fairport, Iowa, while construction was still in progress, the work of propagating some of the commercial species was inaugurated, and the excellent facilities of the station, which has been especially designed for the purpose, are now being utilized by members of the staff in attacking fundamental problems of both a scientific and an economic nature.

For the past five summers a number of field parties have been equipped and sent out each year by the Bureau to collect fresh-water mussels and to obtain the fullest

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NOTE.—It is a pleasure to state that a generous grant of money made by the National Association of Pearl Button Manufacturers in the interest of the investigations enabled us to purchase a collection of books and pamphlets, dealing with the literature on the Unionidæ, which has been of invaluable assistance in the course of the work. To individual members of this association, especially to Mr. J. E. Krouse, of Davenport, Iowa, Messrs. W. F. Bishop and Henry Umlandt, of Muscatine, Iowa, and Mr. D. W. MacWillie, of La Crosse, Wis., we are indebted for many courtesies and for shipments of live mussels which they have repeatedly secured for us. Many others have at times assisted us by sending us material, and in this connection we take especial pleasure in thanking Prof. U. O. Cox, of the State Normal School at Terre Haute, Ind., who has kindly furnished us on several occasions with valuable lots of gravid mussels from the Wabash River.

To a number of our students, who in various capacities have been of service to the investigations, we owe much, and among them should be mentioned Miss Daisy Young, Messrs. Howard Welch, F. P. Johnson, W. E. Dandy, L. E. Thatcher, and especially Mr. W. E. Muns, who acted as our assistant in this work for over two years.

Lastly, it is a pleasure to acknowledge our obligation to Mr. G. T. Kline, the biological artist of the University of Missouri, who has contributed much to the value of our work by the beautiful and accurate drawings with which he has illustrated this and previous papers published by us.

By permission of the Commissioner of Fisheries, we have had the privilege of publishing, in advance of this more detailed report, the following papers of a preliminary nature: Experiments in the artificial propagation of fresh-water mussels (Proceedings of the Fourth International Fishery Congress, Bulletin of the Bureau of Fisheries, vol. XXVIII, 1908); The marsupium of the Unionidæ (Biological Bulletin, vol. XIX, no. 1, 1910); Reproduction and parasitism in the Unionidæ (Journal of Experimental Zoology, vol. IX, no. 1, 1910); Metamorphosis without parasitism in the Unionidæ (Science, vol. XXXIII, no. 857, 1911).

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possible data bearing upon their distribution, their habits, and the physical and biological factors of their environment, as well as information concerning the industries which depend upon the mussel. Surveys of this character have now been carried out on the Mississippi River and nearly all of its more important tributaries from Minnesota to Tennessee, and as a result of these investigations an enormous amount of material and information has been collected which, when examined and analyzed, will not only have the greatest economic value, but will constitute one of the most important ecological studies ever made on any group of animals.

I. HISTORICAL.

As has long been known, the Unionidæ carry their young in the gills, which function as brood pouches until the completion of the embryonic development. At the close of this period the larva or so-called glochidium is fully formed and escapes from the egg membrane while still within the gill. In some species the discharge of the glochidia takes place at once, while in others they remain in the brood pouches for several months without further change before being set free into the water.

The glochidium, long thought to be a parasite infesting the gills and known as *Glochidium parasiticum*, was proved by Carus in 1832 to be the larva of the mussel itself, although many years earlier Leeuwenhoek had given it the same correct interpretation. In 1866 Leydig made the important discovery that the glochidium, after leaving the parent, completes its development as a parasite on fishes.

The earliest observations of importance in the development of our knowledge concerning reproduction in the Unionidæ are those of Leeuwenhoek, made about 1695 [a] and recorded in the Arcana Naturæ. During the two preceding centuries the belief had gained ground that the mollusks had sexes like the higher animals, and this no doubt helped to arouse a certain skepticism regarding the existence of any process of spontaneous generation among the representatives of this phylum. The observations of Redi (1668), in disproval of spontaneous generation in insects, furnished collateral evidence and appear to have been the direct incentive for Leeuwenhoek's examination of the reproductive processes in certain mollusks, among others the fresh-water mussels, and the discovery by Leeuwenhoek of eggs and sperm in these mollusks convinced him that their reproduction must be effected by such means rather than by spontaneous generation.

It is surprising to find how accurate were Leeuwenhoek's conclusions regarding the general course of the development as far as the larval stage, later known as the glochidium, and a survey of the subsequent literature shows that not until the work of Carus, in 1832, were there published conclusions more in accord with the facts as now known, nor a better summary of what we now term the embryonic period. The correctness of these early observations, so far as they went, and of the conclusions drawn from them have not been sufficiently recognized in most accounts of the literature, and for this reason an explicit statement of their important features is desirable.

[a] The date of the publication referred to in the literature list is somewhat later, 1722.

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Approaching the subject unhampered by any preconception in favor of the older views, but rather with the belief that the conclusions of Redi would also hold for the bivalves, Leeuwenhoek records, in the 83d and 96th letters of his Arcana Naturæ, the presence of separate sexes in *Anodonta* and *Unio*, as evidenced by the presence of eggs and spermatozoa in separate individuals, and gives some account of the development. That he clearly apprehended the main course of events is evident if we read his description of eggs found floating free in the fluid obtained by puncturing the upper part of the foot upon either side, of similar eggs in more advanced stages within the outer gills, and of various stages in the formation of the glochidial shell. Finally, he observed the snapping of the valves, now so well known as a sign of the last stages in this embryonic development, and upon seeing the rotation of the embryo in the egg membrane he concluded that it must be unattached. He further observed that the individuals, when ready for their egg laying (passage of eggs from ovary to gills), placed themselves in spots where the water was shallow and where they were in direct sunlight—a fact which seems to have been confirmed by other observers of the European species (Schierholz, 1888, p. 8, *Unio* and *Anodonta*). Observing the general similarity between the bivalved larva and the adult, he seems never to have doubted that the glochidia, as they were subsequently called, were the young of the mussel in which they were found and therefore that these mollusks were viviparous, conclusions which so naturally followed from all the facts that it is hard to see how convincing evidence could have been manufactured for any other opinion. Upon removing these fully formed larvæ and setting them aside in dishes of clean water, with a view to observing their further development, Leeuwenhoek met the stumbling block of all observers before the discovery of the parasitism upon the fish was known, for the larvæ lived but a short time, soon becoming infested with a variety of animalcules, which he rightly concluded were the immediate cause of their death.

These conclusions of Leeuwenhoek, so nearly in accord with our present knowledge, were not entirely accepted, because they did not become known to some investigators even a century later and because there was still a considerable recrudescence of the older conception of spontaneous generation. The opinion of Poupart (1706) that these mussels were hermaphroditic gained ground and dominated during the eighteenth century, although the larvæ, when found in the outer gills, were always regarded as the young of the mussel until, in 1797, Rathke offered an entirely different explanation and erected for them a new genus, *Glochidium*, and a species, *parasiticum*. According to this explanation, which came to be known as the Glochidium Theory, it was supposed that these multitudinous larvæ were not the young of the mussels at all, but parasites with which they had become infested. Since Rathke's theory attracted considerable attention at the time and was later supported ardently by Jacobson (1828), and since it has given us the term glochidium, we may note in passing the evidence upon which it was based as stated by its later champion.

1. The form and organization of the little shells is entirely different from that of the adult *Unio* and *Anodonta*.

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2. They are of exactly the same form and size in the two genera and in the individuals of diverse size and age.

3. They are always of the same size and shape when they have reached their complete development.

4. Their valves are of a consistency and hardness in no wise related to their size, as should be the case were they the young of *Unio* and *Anodonta*.

5. Their development is not related to any season of the year nor to a certain age of the animal in which they are found; that is to say, one finds in a single locality at the same time individuals containing eggs, others with little bivalves, and some containing even the fully developed organisms.

6. The enormous numbers which are found at one time in an individual are in no wise proportionate to the number of the adults in any locality.

7. One can not conceive of organs so delicate as the gills being able to serve as a sort of brood pouch, and there is no other example in the animal series of such a condition, although these organs are often the seat of animal parasites.

Jacobson's statement is thus a curious jumble of half truths and of statements which have since been shown to be entirely incorrect.

The importance attached to the dispute thus raised was so great that the Academy of Sciences at Paris appointed two of its members, De Blainville and Dumeril, a committee with instructions to examine into and report upon the whole matter. This report (De Blainville, 1828) presents an exhaustive review of the early literature and details certain experiments performed by the committee with a view to testing the matter by direct observation. These experiments, while tending to confirm the earlier views of Leeuwenhoek, were insufficient for the complete overthrow of Rathke's Glochidium Theory, for although the report was unequivocal in its conclusion that the observations of all previous authors and the evidence advanced by Rathke himself did not justify the Glochidium Theory, its lack of evidence from original observations rendered it not entirely conclusive. Viewed in the light of our present knowledge, its skillful and logical arraignment of Rathke's conclusions shows clearly the scant foundation upon which the Glochidium Theory rested, but it was not until the work of Carus (1832) that the question was finally set at rest. This author was able, in the brightly colored eggs of *Unio littoralis*, to see the passage of the eggs from the ovary to the external gills and their development there to the mature glochidia, and thus to prove beyond any doubt that the innumerable larvæ which crowded the outer gills were the young of the mussels in which they were found.

The paper by von Baer (1830) anticipated some of the points which Carus made the more clear, and from this time on the serious difficulty for students of the embryology was found in the failure to secure, either within the gills of the mussel, or upon removal of the embryos to water, any developmental stages beyond the glochidium.

The period from Carus's paper (1832) to the date of the discovery by Leydig (1866) of glochidia embedded upon the fins of fishes shows little progress toward a more complete account of even the embryonic stages. De Quatrefages, who in 1836 described

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the glochidium as having a very complex structure and possessing many of the organs of the adult mussel, made a distinctly backward step; and his account of hearts, stomachs, livers, intestines, and aortas, all highly developed and double in each individual, reminds one of the description of elaborate systems of organs in the infusoria as given by Ehrenberg in his monograph published during the same year. Pfeiffer (1821, taf. II, fig. E) was the first to observe the minute outline of the glochidium at the umbo of a young shell—a fact which, had it become generally known, would have saved Jacobson his defense of the Glochidium Theory. There remained, however, the unexplained gap between the glochidium and such a stage of the young mussel, and this was filled only by Leydig's discovery of the parasitism. With the clue thus given, the stages by which the glochidium becomes the miniature adult, during the course of its parasitism, were studied by Braun (1878), Schmidt (1885), Schierholz (1878 and 1888), and more recently by Harms (1907–1909). All of these investigators obtained their material in great abundance by the artificial infection of fish with the glochidia, and in their several accounts the structure of the glochidium and the organogeny of the common European species will be found very completely given.

The embryonic stages attracted new attention with the rise of cytological studies, and the paper of Flemming (1875) was exhaustive for the period in which it was written, although Lillie's more detailed and modern account (1895) of the cell lineage and the formation of the glochidium in *Unio complanatus* and *Anodonta cataracta* has rendered Flemming's paper of historical interest only, and has apparently left undone nothing of importance in a description of the early stages in these species.

Further reference to the literature will be made as the several stages of the development are discussed in the species we have followed. Since an excellent summary of the literature, particularly that published since the paper by Carus (1832), may be found in the work of Harms (1909), we omit further elaboration here. The report to the Paris Academy (De Blainville, 1828) gives a good account of the literature for the earlier period, and from this we have obtained a summary of the facts in such early papers as have not been accessible.

II. REPRODUCTION.

The sexes are normally separate in the Unionidæ, but in *Anodonta imbecillis* and in a few other species of this genus the occurrence of hermaphroditism has been occasionally recorded (cf. Sterki, 1898; Ortmann, 1911). Although in the majority of the genera of the Unionidæ the sexes are indistinguishable externally, in a few, notably in *Lampsilis*, the shell of the female differs from that of the male in its greater convexity in front of the posterior ridge and in more or less well-marked differences in the posterior outline of the shell. In such cases the males and females may be readily assorted without recourse to an examination of the soft parts.

At ovulation the eggs pass from the oviducts to the cloaca, and thence back into the suprabranchial chambers, in which they are probably fertilized by spermatozoa brought in by the respiratory current of water. From the suprabranchial chambers they are conducted directly into those portions of the gills in which they are to remain.

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Observations on the passage of the eggs from the ovaries to the gills are extremely meager, and further information is needed concerning the factors involved in directing the stream of eggs from the openings of the oviducts to their final resting place in the water tubes of those regions of the gills which function as brood chambers. We owe to Latter (1891, 1904) the most detailed account of this process which we have, and, in lieu of any direct observations of our own on the subject, we may quote his interesting description (1891) which is based upon *Anodonta:*

If a female be taken from the shell at this season (the spawning season) the eggs may be seen through the transparent wall of the oviduct passing singly, but in a steady stream, to the genital aperture. Their motion is due partly to "labour contractions" of the intrinsic muscles of the foot and partly to the ciliated lining of the oviduct itself. One by one the eggs issue from the genital aperture, whence they are conveyed backwards by the abundant cilia which clothe the external surface of the nephridium. Along the middle line of this surface there is a belt of especially long cilia which appear to be devoted to the transit of the eggs; those dorsal and ventral to the belt work obliquely so as to keep the eggs in contact with it. It is probable that the free dorsal border of the inner lamella of the inner gill plate is, under normal conditions, applied to the visceral mass in this region so as to inclose a temporary tube, one of whose walls is formed by the above-mentioned belt of specialized cilia.[a] In the course of about 50 seconds an egg is thus swept back to the slit between the protractor muscle of the shell and the point of fusion of the right and left inner gill lamellæ; here they meet the stream of ova from the other side of the body and so reach the exhalent current and the cloaca.

The process goes on for some 10 days or more in each individual and the number of eggs is immense * * * probably half a million may be taken as a fair average. On reaching the cloaca * * * their direction is reversed and they pass forward into the cavities of the right and left gill plates, which serve as brood pouches. The method by which this change of direction is accomplished is not quite clear. * * * I have, however, observed on several occasions a violent and sudden reversion of the water currents such as would certainly be fully capable of carrying the eggs forward and into the latticed recesses of the outer gills. This reversion is caused by the animal, firstly, closing all the ventral border of the shell by means of the free edges of the mantle assisted by the flexible, uncalcified rim of periostracum and leaving the siphons alone open, and, secondly, relaxing the adductor muscles so as to allow the elastic ligament to make the valves gape apart. These actions cause the hydrostatic pressure within the shell to be less than that of the water without and consequently there ensues a rush of water into the shell through the open siphons. The whole procedure may be likened to a gulp and is achieved by precisely similar physical forces.

This may possibly be the correct interpretation of the process, but additional observations and experiments should be made for verification. Latter also attempts to account for the fact that the eggs in *Anodonta* pass into the outer gill and not into the inner, but his explanation is unsatisfactory and inadequate. It would be a matter of the greatest interest to discover the mechanism which directs the eggs in the different types of the marsupium into certain water tubes of the gills and not into others. Special structural modifications must be correlated with the particular type as the fundamental cause of these differences, and a very pretty problem is here presented in the determination of such correlations. Since in the genus *Quadrula* all four gills

[a] It is to be remembered that this description is based upon the conditions as they occur in *Anodonta*, in which the inner lamella of the inner gill is not fused to the visceral mass, and the inner suprabranchial chamber is consequently freely open to the mantle chamber; in those forms, however, in which this lamella is fused for a part or all of its length, the eggs are received into the anterior end of the inner suprabranchial chamber, into which the genital apertures open directly, and pass back through this chamber to the cloaca.

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become filled with eggs, a directive mechanism is probably absent in this genus, and a careful comparison of the conditions in *Quadrula* with the structure of the gills in those genera in which only a portion of the gills is utilized as a brood chamber might well furnish the clue to the discovery of a special mechanism in the latter.

While as a rule the great majority of the eggs, when a gravid gill is examined, are found to be fertilized, different species differ markedly in the percentage of unfertilized eggs present, and, in fact, a large proportion of the latter seems to be characteristic of certain genera. In *Lampsilis*, *Symphynota*, *Anodonta*, and a number of other genera it has been very unusual in our experience to encounter any considerable number of unfertilized eggs, while, on the contrary, in *Quadrula*, *Pleurobema*, and in some species of *Unio* it is often true that even a majority of the eggs in a gravid female have failed of fertilization; in fact, in these genera one expects to find a large percentage of such eggs as the usual thing.

The entire embryonic development takes place in the gills of the female, and at the close of this period the larva or glochidium is fully formed. The differences in the length of time the glochidia are retained in the gills will be discussed later, but after their liberation the completion of their development occurs while they are living as parasites on the fish in all of the Unionidæ, so far as known, except in the genus *Strophitus*, whose glochidium, we have recently discovered, undergoes the metamorphosis in the entire absence of a parasitic stage. This extraordinary case will be referred to later.

As the embryology of the Unionidæ has been described by Lillie (1895) in great detail, and as Harms (1909) still more recently has published an excellent account of the post-embryonic development, we shall omit all reference to the actual developmental events, and confine ourselves to a discussion of those phases of the reproduction and parasitism of the Unionidæ in which we have been especially interested in connection with the problem of artificial propagation.

THE MARSUPIUM.

The term marsupium has been generally used to indicate those portions of the mussel's gills into which the eggs are received from the suprabranchial chambers after ovulation and which serve as brood pouches for the retention and nurture of embryos and glochidia until the discharge of the latter. As no better name seems to be available, we shall employ it in this paper.

USE OF THE MARSUPIUM IN CLASSIFICATION.

Since the extent to which the gills are specialized for this purpose varies in different groups of the Unionidæ, Simpson (1900), in his "Synopsis of the Naiades," has made use of the marsupium as the chief diagnostic character on which his classification is based. Those groups in which the marsupium comprises the outer or all four gills he designates as the Exorbranchiæ, while those in which the inner gills alone receive the eggs are distinguished as the Endorbranchiæ. All of the European and North Ameri-

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can species belong to the former group, while the latter contains forms that are found chiefly in Asia, Australia, Africa, Central America, and South America.[a]

As our observations have been confined to the Exobranchiæ, reference will be made only to this group, the following subdivisions of which are recognized by Simpson, each distinguished by special marsupial characters:

Tetragenæ: Marsupium occupying all four gills.

Homogenæ: Marsupium occupying entire outer gills.

Diagenæ: Marsupium occupying entire outer gills, but differing from that of the Homogenæ in that the egg masses lie transversely in the gills.

Heterogenæ: Marsupium occupying only posterior end of outer gills.

Mesogenæ: Marsupium occupying a specialized portion in the middle region of outer gills.

Ptychogenæ: Marsupium occupying entire lower border of outer gills which is thrown into a series of peculiar folds.

Eschatigenæ: Marsupium occupying the lower border only of outer gills, but not folded.

Simpson has established another group, the Digenæ, for the genus *Tritogonia*, but since its marsupium is constituted by all four gills (Sterki 1907), it should at least be included in the Tetragenæ, if not in the genus *Quadrula*, as Ortmann maintains (1909, 1911). For a complete list of the genera occurring in each of Simpson's groups, reference may be had to his Synopsis (op. cit., p. 514–515).

These groups constitute Simpson's subfamily, Unioninæ, his other subfamily, Hyrianæ (Hyriinæ), coinciding with the Endobranchiæ or those Unionidæ whose marsupium occupies the inner gills only. In all of the Unioninæ except the Heterogenæ and Digenæ (*Tritogonia*), according to Simpson, the sexes are indistinguishable externally.

It will be seen from the above classification that three general conditions exist in the Unioninæ, namely, one in which the marsupial adaptation involves all four gills; one in which the entire outer gills only are utilized; and, lastly, one in which some differentiated portion of the outer gills constitutes the marsupial region. It would, accordingly, be a more logical procedure to make these general marsupial conditions the basis of the classification and to recognize only three main groups corresponding to the three general types of marsupium, to which the names Tetragenæ, Homogenæ, and Heterogenæ might be applied; and since all of the remaining forms have a marsupium which may be readily regarded as a secondary modification of one or another of the three types, they could be arranged in appropriate subgroups. If this were done, the Diagenæ would obviously fall within the Homogenæ, while the Mesogenæ, Ptychogenæ, and Eschatigenæ would be placed under the Heterogenæ, as in all of the latter forms the marsupium is some specialized portion of the outer gills.

[a] Besides the Unionidæ, a second family, the Mutelidæ, is recognized by Simpson in his classification of the Naiades or pearly fresh-water mussels. In these forms, which belong to Africa and South America, the marsupium is the inner gills only, and the larva is not a glochidium but the so-called lasidium. The genera embraced in this family are not considered in the present account.

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Quite recently Ortmann (1910a, 1911) has proposed an entirely different arrangement of the Naiades which is based upon a study of the anatomy and the larval characters of the fresh-water mussels of Pennsylvania. His system also lays especial stress on the marsupial differentiations, but it involves a number of important modifications in Simpson's classification which he maintains must be radically recast, in the light of the facts which he has discovered, if it is to represent the natural affinities of the group.

It is not our purpose to present a critical discussion of the relative merits of the two systems, as our only interest in this connection is concerned with the marsupium as an accessory organ of reproduction, but as Ortmann has added a number of important facts to our knowledge of this structure, it is necessary to state briefly the basis of his classification so far as it has to do with the several marsupial modifications. In addition to the marsupial structure, he makes use in his arrangement of families, subfamilies, and genera of a number of other characters which he considers of systematic value; for example, the degree of fusion of the inner lamella of the inner gill with the visceral mass; the dorsal aperture (supra-anal opening); the siphons; the differentiations of the mantle edge; the structure of the glochidium; and shell characters. In contrasting his arrangement with that of Simpson, however, reference will be made only to the marsupium.

Confining himself to North American forms, he divides the Naiades into two families, the Margaritanidæ and the Unionidæ. His discovery that in *Margaritana margaritifera* there are no distinct interlamellar junctions in the gills, but only scattered interlamellar connections, and consequently no definite water tubes, he considers of sufficient importance to warrant him in creating a new family for this genus, Margaritanidæ, which he has thus sharply set apart from the remaining genera grouped under the Unionidæ, a procedure of doubtful wisdom.[a] The fact that complete interlamellar junctions are absent in *Margaritana*, which is further characterized by certain other apparently primitive features, is of the greatest interest, but that these differences are of sufficient significance to justify a separate family for *Margaritana* is not at all clear.

The Unionidæ, after the removal of *Margaritana*, he divides into three subfamilies, distinguished as seen below by definite marsupial characters:

1. Unioninæ. "Marsupium formed by all four gills, or by the outer gills only; edge of marsupium always sharp and not distending; water tubes not divided in the gravid female."

This subfamily includes the following genera, which, however, he has recast to a considerable extent by subtractions and additions of species: *Quadrula* Rafinesque (including *Tritogonia tuberculata*); *Rotundaria* Rafinesque (established for *Quadrula tuberculata*); *Pleurobema* Rafinesque (including *Q. coccinea, pyramidata, obliqua, cooperi-*

[a] The condition described by Ortmann for *Margaritana* is quite similar to that which is found in the gills of *Mytilus* (cf. Peck, 1877), in which complete interlamellar junctions are absent and the inner and outer lamellæ are connected only by scattered strands of subfilamentar tissue passing across the interlamellar space. This similarity in gill structure would argue strongly for the primitive position of *Margaritana* among the Unionidæ. In *Lucina* these interfilamentar junctions are larger and are provided with blood vessels, while in *Mytilus* they are non-vascular. Ortmann does not state whether or not they contain blood vessels in *Margaritana*.

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ana); *Elliptio* Rafinesque (established for the North American species of *Unio* to distinguish them from the European).

2. Anodontinæ. "Marsupium formed by the outer gills in their whole length, distending when charged, and the thickened tissue at the edge capable of stretching out in a direction transverse to the gill, but not beyond the edge (or only slightly so); water tubes in the gravid female divided longitudinally into three tubes, with only the one in the middle used as an ovisac, and closed at the base of the gill."

The following genera are grouped under this subfamily: *Alasmidonta* Say, *Strophitus* Rafinesque, *Symphynota* Lea, *Anodontoides* Simpson, *Anodonta* Lamarck.

3. Lampsilinæ. "Marsupium rarely formed by the whole outer gill, generally only by or within the posterior part of the outer gill; edge of marsupium, when charged, distending, and bulging out beyond the original edge of the gill, generally assuming a beaded appearance; water tubes simple in the gravid female."

The following genera are grouped together under this subfamily: *Ptychobranchus* Simpson, *Obliquaria* Rafinesque, *Cyprogenia* Agassiz, *Obovaria* Rafinesque (including *Lampsilis ligamentina*), *Plagiola* Rafinesque, *Paraptera* gen. nov. (established for *Lampsilis gracilis*), *Proptera* Rafinesque (established for *Lampsilis alata, purpurata, lævissima*), *Lampsilis* Rafinesque (including *Micromya fabalis*), *Truncilla* Rafinesque.

It will be seen by a comparison of the genera which Ortmann assigns to his three subfamilies with the several groups of Simpson, that the most significant change introduced by the former arrangement is the disruption of Simpson's Homogenæ and a redistribution of its genera and those of the Digenæ, Diagenæ, and Tetragenæ among the subfamilies Unioninæ and Anodontinæ, the former receiving all of the genera considered by Ortmann, except *Alasmidonta, Strophitus, Symphynota, Anodontoides*, and *Anodonta*, which, by reason of the peculiar secondary division of the water tubes of the gravid female in all of these genera, he insists should be placed in a subfamily by themselves. Apparently his grounds for the rearrangement are sound. In the Lampsilinæ are included all of the genera of Simpson's Heterogenæ, together with those of the Mesogenæ, Ptychogenæ, and presumably the Eschatigenæ—a procedure which is in harmony with the suggestion made above that the genera in which a differentiated portion only of the outer gill functions as a marsupium should be grouped together.

The reader is referred to Ortmann's monograph for further details and for the considerations which have led him to shift a number of species from one genus to another and to establish certain new genera, while renaming others.

This system has the merit of being based upon a careful study of the anatomy of the species with which he has been concerned, and he has clearly demonstrated the fact that shell characters alone are not sufficient for a determination of true relationships. To what extent his classification will replace Simpson's remains of course to be seen, but in any future discussion of the matter the new facts brought to light by Ortmann in his study of the structural modifications of the marsupium must be reckoned with.

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GENERAL STRUCTURE OF THE MARSUPIUM.

In connection with our investigations on fresh-water mussels we have had occasion to give quite a little attention to the anatomical and histological structure of the marsupium in a number of genera, and, furthermore, we have been particularly interested in the changes that occur in the gills during the period of gravidity. We have already published a brief account (1910b) of some of our observations on the marsupium, with illustrations of the more important types, but, as Ortmann has since added a number of new facts to the subject, it is advisable to present our results in greater detail and with additional illustrations. For this purpose it will be more convenient to follow Simpson's arrangement, and we shall refer to the species examined by us under the several groups established by him. It will also be convenient in connection with the description of the marsupium to refer somewhat incidentally to certain observations on breeding habits, characteristics of the embryos, and related matters. The finer structure of the marsupium is reserved for a subsequent section of this report.

Tetragenæ.—The marsupium in these forms comprises all four gills, a condition which is undoubtedly the most primitive one among the Exobranchiæ. It is the condition occurring in the genus *Quadrula*, in which, following Ortmann, we include *Tritogonia*. We have encountered it in the following species: *ebena* Lea, *heros* Say, *lachrymosa* Lea, *metanevra* Rafinesque, *obliqua* Lamarck,[a] *plicata* Say, *pustulosa* Lea, *trigona* Lea, *tuberculata* Barnes (*Tritogonia tuberculata*), and *undulata* Barnes.

No special structural modifications are present beyond the usual glandular folded epithelium covering the surface of the interlamellar junctions which, as has been known since the work of Peck (1877), are closer together in the marsupial than in the purely respiratory gill. The gills when gravid, although somewhat distended and padlike in appearance, never become swollen to the extent that is seen in many other genera. Figure 5, plate VII, which is drawn from a gravid female of *Quadrula ebena*, illustrates the typical appearance of the marsupium in this group, although the gills shown in the figure are not as fully distended as is frequently the case.

In *ebena* and *trigona* the ovarian eggs and the embryos are frequently brilliantly colored red or pink and when the marsupium is charged the color shows through the colorless transparent walls of the gills, which present a striking appearance on removing the shell. In all of the other species of *Quadrula* observed by us the pigmentation is absent, but in *ebena* and *trigona* the color is found in a majority of the gravid females, the number of such cases being somewhat greater in *trigona* (over two-thirds of all gravid females examined in this species) than in *ebena*. The red pigment, however, whenever it occurs, does not persist, but on the contrary totally disappears in the later stages of embryonic development, and by the time the glochidia are fully formed no trace of it is left. We have never seen a single case of a red or pink glochidium either in these two species of *Quadrula* or in any other genus in which pigmented eggs and embryos occur. It is true

[a] Ortmann (op. cit., 1911, p. 330) states that only the outer gills serve as the marsupium in *obliqua*, and on this ground he has removed the species to *Pleurobema*. If we have made no mistake in the identification of our specimens, our observations on this species are not in accord with his.

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that the marsupium may still be more or less deeply tinged with red, even when it contains fully developed glochidia, but this is due to its containing a variable number of unfertilized eggs, which do not lose the color, and not to the glochidia which are always, as stated, entirely colorless.

The occurrence of unfertilized eggs is very common in all of the species of *Quadrula* which have come under our observation, and their presence is more characteristic of certain species than of others. They are quite rare in *plicata* and *pustulosa*, for example, less so in *metanevra*, common in *ebena*, while in *trigona*, in which they occur more frequently than in any other species of *Quadrula*, they were found in a large majority of cases. The number of unfertilized eggs in different females of a given species varied from cases in which only a few such eggs were scattered among normal embryos all the way to cases in which the marsupium contained no normal eggs or embryos at all. Eggs which have not been fertilized, after remaining in the marsupium, become swollen and stratified (see below), frequently forming exovates and undergoing fragmentation before final disintegration.

There seems to be a definite correlation between the presence of unfertilized eggs in the marsupium and the occurrence of trematode parasites in the testis of the male; in species like *plicata*, in which unfertilized eggs were rare, only occasionally were the testes infested with worms, but in *trigona*, for example, the trematodes were found in a large number of males. It is not at all improbable that the amount of sperm available in a given locality is greatly reduced as a result of the castration of males by this testis infesting parasite.

The abortion of embryos and glochidia, which is so characteristic of the genus *Quadrula*, and the significance of this peculiarity will be referred to later on.

Homogenæ.—The condition in which the entire outer gills only are utilized as a marsupium is present in 16 genera, according to Simpson.[a] We have verified its occurrence in *Alasmidonta truncata* Wright; *Anodonta cataracta* Say, *grandis* Say, *implicata* Say; *Arcidens confragosus* Say; *Pleurobema æsopus* Green; *Symphynota complanata* Barnes, *costata* Rafinesque; and in *Unio complanatus* Dillwyn and *gibbosus* Barnes.

As has already been stated, Ortmann has disrupted the group, placing *Pleurobema* and *Unio* in his subfamily Unioninæ, while segregating *Alasmidonta*, *Anodonta*, and *Symphynota* in his Anodontinæ. This he has done chiefly because of a differentiation of the ventral border of the marsupium and of a secondary division of the water tubes of the marsupium in those genera included in the Anodontinæ. These differences will be referred to below.

The marsupium when filled with embryos or glochidia may be greatly distended beyond its normal dimensions, and in this condition is an enormously swollen padlike structure, with a smooth surface, filling a large portion of the mantle chamber. Figure 3, plate VI, represents the gravid marsupium of *Symphynota complanata*, which may be taken as typical of the Homogenæ, although in *Pleurobema* and *Unio* the distension is not so great.

[a] *Margaritana* is placed in this group by Simpson, but as it utilizes all four gills as the marsupium it should be included with the Tetragenæ.

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In *Pleurobema æsopus* the eggs and embryos, like those of *Quadrula ebena* and *trigona*, are usually, but not always, colored red or pink, but the glochidia are invariably unpigmented. Unfertilized eggs in varying proportions are frequently found in this species either mixed in with embryos at all stages of development or occurring alone; such eggs always show a definite stratification of the egg substances.

Diagenæ.—This group was established by Simpson to receive the genus *Strophitus*, in which the marsupium occupies the entire outer gill and in external appearance is similar to that of the Homogenæ. But it is unique among the Unionidæ in that the embryos and glochidia are embedded in gelatinous cords (called "placentæ" by Sterki, "placentulæ" by Ortmann), which lie transversely in the gills, whereas in all other cases the egg masses are placed vertically, each one occupying an entire water tube. In *Strophitus*, on the other hand, the cords are packed closely together, like chalk crayons in a box, a variable number being contained in a single water tube, while the blunt ends of the cords are distinctly seen through the transparent external lamella of the outer gill. It should be stated that Ortmann (1910b, 1911) has found that the discharge of the cords is not through the lamellæ of the gills, as Simpson (1900) has maintained, but that it occurs in the usual manner through the supra-branchial chambers. A description of the unique cords and the extraordinarily interesting life history of *Strophitus* is reserved for a special section.

Heterogenæ.—In this group the marsupium occupies only the posterior portion of each outer gill, varying in extent from about one-third to two-thirds of the entire length of the latter. In young females the marsupium is shorter and not so fully distended as in older ones. In fact, it is true of all Unionidæ that the marsupium is less heavily charged when the female is young. The differentiation of the posterior region is very conspicuous even in the non-gravid female, as the marsupium is sharply marked off either by a distinct fold or a notch from the anterior respiratory part, and, since it is much deeper dorso-ventrally than the latter, it projects farther down into the mantle-chamber. Its walls are also more membranous in appearance than are those of the respiratory region, and after the discharge of the glochidia it is seen as a flabby collapsed pouch.

When gravid, the marsupium may be enormously swollen, the expansion being greater along the ventral border than above, where, owing to its fixed position, it is incapable of stretching. This greater ventral extension often causes the marsupium not only to assume a fan-shaped form, which is so characteristic an appearance in *Lampsilis*, but also to project forward under the respiratory portion, which in consequence becomes sharply folded over on the outer surface of the marsupium. Not only is the marsupium as a whole expanded in the way described, but each of its swollen water tubes is distended distally beyond the lower extremity of the interlamellar junctions so that the ventral border becomes fluted or corrugated, as shown in figure 2, plate VI. This figure, which illustrates the typical condition in the genus *Lampsilis*, is drawn from a gravid female of *L. subrostrata* when fully charged with glochidia. The folded respiratory portion of the gill, the fan-like expansion of the marsupium, and the corrugated border are all clearly seen.

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When the marsupium is less heavily charged, as in young females, the ventral expansion may not be great enough to cause the conspicuous fold just described, and in cases like this the marsupium, which may then appear kidney-shaped, is marked off from the respiratory end merely by a notch by reason of its greater depth. Such a case is seen in figure 6, plate VII which is taken from a gravid female of *L. recta*.

Simpson has included 14 genera in the Heterogenæ, only three of which, however, have come under our observation, namely, *Lampsilis* (including *Proptera*), *Obovaria*, and *Plagiola*. We have recorded this type of marsupium in *Lampsilis alata* Say, *anodontoides* Lea, *gracilis* Barnes, *higginsii* Lea, *lævissima* Lea, *ligamentina* Lamarck, *luteola* Lamarck, *recta* Lamarck, *subrostrata* Say, and *ventricosa* Barnes; in *Obovaria ellipsis* Lea; and in *Plagiola elegans* Lea and *securis* Lea.

No case of pigmented eggs has been encountered by us in this group, and unfertilized eggs in the marsupium are exceedingly rare.

Mesogenæ.—This group is so designated by Simpson to include the genera *Cyprogenia* and *Obliquaria*, in which a variable number of enlarged water tubes in the middle region of the outer gill are specialized as the marsupium, a larger anterior and a shorter posterior portion of the gill retaining the ordinary respiratory character. We have studied the condition in *Obliquaria reflexa* Rafinesque and also in *Cyprogenia irrorata* Lea, in which the structure of the marsupium is essentially the same, although the two cases differ strikingly in general appearance.

The marsupium of *Obliquaria reflexa* is shown in figure 7, plate VII. Here the modified water tubes, which project far down below the border of the rest of the gill, appear enormously swollen when gravid and show a tendency to curve backward, the degree of curvature becoming progressively greater in the tubes from the anterior to the posterior end of the marsupium. A gradual decrease in the length of the tubes takes place in the same direction. The tubes are slightly larger at their distal ends, so that their form is somewhat club-shaped; this is seen more clearly in the shape of the egg masses which form perfect casts of the cavities of the tubes (fig. 42, pl. XI). The corrugation of the lower border of the marsupium is very conspicuous in the figure. The number of water tubes comprising the marsupium in this species is not at all constant, but on the contrary varies in the individuals examined by us from two to eight; according to Simpson, they range from four to seven. During the breeding season each tube is entirely filled with embryos or glochidia which adhere so firmly together that they form a mass of tenacious consistency.

In *Cyprogenia*, the only other genus included in the group, the marsupium may be regarded as a further development of the condition seen in *Obliquaria*. We have observed it in but a single individual of *C. irrorata*, which was kindly sent to us by Dr. R. E. Coker. This specimen, which contained fully formed glochidia, was collected in the Cumberland River, Kentucky, in November, 1910. The tubes of the marsupium, which present a most striking and unusual appearance, spring from near the middle of the outer gill, are enormously elongated, and curved backward into a close coil, a part of the coil passing under the posterior unmodified portion of the gill, as the tubes

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are turned slightly inward toward the median plane. The marsupium is well shown in figure 8, plate VII. The distension of the marsupial water tubes begins at quite a distance above the ventral border of the rest of the gill, as is seen in the figure. The anterior respiratory portion is sharply separated from the rest of the gill by a cleft which extends almost up to the level of the suprabranchial chamber. At first this was supposed to be an artificial split, but as it occurs on both sides and its edges are perfectly smooth and show no indication of injury, we have concluded that it must be a normal condition. Unfortunately we have had no other specimens with which to compare it.

In our specimen the marsupium is slightly tinged with pink, the color being due to unfertilized pigmented eggs which are scattered among the glochidia. Simpson speaks of the marsupium as being purple.

The unusual form of the marsupium in *Cyprogenia* was originally described by Lea (1827) in *C. irrorata*, but curiously enough he reversed the direction of the coil in his figure, which appears to have been drawn from memory, as such a mistake could hardly have been possible if he had had a specimen before him.[a]

Call (1887) many years later described a similar marsupium in *C. aberti* Conrad, which he very crudely figured. It is strange that, although he reproduces Lea's original figure of *irrorata* by the side of his own, he makes no mention of the error in it. Judging from Call's figure, the number of tubes in the marsupium of *aberti* is much larger than in *irrorata*. He shows about 20, while Lea states that there are 7 or 8 in the latter, and in our specimen there are 7. Simpson gives the number for the genus as 7–23.

Ptychogenæ.—This group contains a single genus, *Ptychobranchus*. The marsupium occupies the lower half of the entire outer gill and is thrown into a series of folds, from 6 to 20 in number, according to Simpson. Each water tube of the marsupium is inflated at its distal extremity to form a globular enlargement projecting beyond the interlamellar junctions—a condition which gives to the free edge of the gill the beaded appearance so characteristic of the genus. This marsupium is well illustrated in figure 1, plate VI, which is drawn from a gravid female of *P. phaseolus* Hildreth. Seventeen conspicuous folds, sharply demarked from each other, are shown in the figure, in which the beaded border of the gill is also clearly seen.

Eschatigenæ.—Simpson has established this group to receive the genus *Dromus* in which the marsupium occupies the ventral half of the outer gill throughout the greater portion of its length. We are indebted to Dr. R. E. Coker for several specimens of *Dromus dromus* Lea, obtained from the Cumberland River in Kentucky in November, 1910, which have furnished the material for our study of this type of marsupium. Three gravid females, all containing glochidia, were included in the lot.

As seen in figure 4, plate VII, the line of demarcation between the dorsal respiratory portion and the ventral marsupial region is quite sharp and regular, owing to a constriction of the gill where the two regions join. Below this line the gill is swollen to an extent varying with the degree to which it is charged with glochidia. The anterior end of the gill is not included in the marsupium and is sharply folded over on the outside of the

[a] We are indebted to Mr. Bryant Walker, of Detroit, for having called our attention to this error in Lea's figure.

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marsupium in this region. The depth of this fold varies with the fullness of the marsupium, as the greater is the distension of the latter the farther forward it is tucked under the anterior respiratory region. Posteriorly the two portions of the gill are sharply defined by a deep cleft, as shown in the figure. The surface of the marsupium is thrown into an irregular series of low undulating folds which are more prominent in the more heavily charged females. In two of the females the marsupium is a salmon pink, while the third is colorless, but here, as in the other cases described in which glochidia are present, the color is due to unfertilized eggs.

The record in our notes of the three females is as follows:

No. 1, small specimen, 44 by 39 mm. Marsupium colorless, only slightly distended and not thrown into folds or undulations; no anterior fold, merely a notch; glochidia colorless.

No. 2, larger specimen, 57 by 52 mm. Marsupium salmon pink, much fuller than no. 1, and thrown into distinct folds; deep anterior fold; glochidia colorless, but many pigmented unfertilized eggs and abnormal embryos mixed with them. (This is the specimen from which the figure was drawn.)

No. 3, largest specimen, 58 by 55 mm. Marsupium with just a tinge of pink, more heavily charged than either of the others and showing prominent folds or undulations; deep anterior fold; glochidia colorless, and a few pigmented unfertilized eggs and abnormal embryos present.

It is evident from this comparison that the smaller, and therefore presumably the younger, females are less heavily charged than the larger and older ones; and, furthermore, that those changes in the gill which are the mechanical effects of gravidity, like the folds, vary directly with the degree of distension of the marsupium. This conclusion holds good for all the Unionidæ which we have had an opportunity of examining, and also applies to the experience of other observers.

The glochidia of *Dromus dromus*, which are excessively minute and of unusual form, being kidney shaped, are referred to later.

INTERNAL STRUCTURE OF THE MARSUPIUM.

The marsupium of the Unionidæ furnishes a beautiful illustration of a remarkable diversity of form in the adaptation of an organ for a specialized function. One can not study this structure in the North American Unionidæ without being forcibly impressed with the great variety of detail which one and the same general adaptation is capable of exhibiting. But whatever be the special direction which the modification has taken, even in the most bizarre forms of the marsupium, like that of *Cyprogenia*, there is never any doubt as to the relation between the structural specialization and the function which it is adapted to perform. The structural basis of the marsupium—one might almost say the unit of structure—is the water tube, and it is from an investigation of its finer structure and its relation to other tubes, similarly modified, that an understanding of the unionid marsupium is gained. The fundamental adaptation is a series of compartments in the interior of the gills provided with a specialized glandular

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epithelium lining the cavity and also with a mechanism in its walls which allows of distension, often to an extraordinary degree.

The various types of marsupium are to be referred to differences in the manner in which these compartments are associated to constitute the marsupium; to different degrees to which the compartments are developed; to differences in the modification of the walls for the purpose of distension; and also to the development of special adaptations in certain forms for increased aeration of the marsupium. Whether in the last specialization the better aeration is needed for the gravid mussel, whose respiration must be considerably interfered with when the entire outer gills are gorged with embryos, as in *Anodonta* and *Symphynota*, or for the embryos themselves, is a question that is discussed later, but from a comparison of the conditions existing in the different types of marsupium it would seem that the respiratory modifications are primarily for the adult and not for the embryos. The reasons for this conclusion should be reserved until the internal structure of the marsupium has been described.

It is chiefly to Peck (1877) that we owe a correct interpretation of the structure of the lamellibranch gill. It was he who first showed that the plate-like gills of the higher forms, consisting each of an outer and an inner lamella, are formed by a series of juxtaposed independent filaments, a fact that was essential to the later recognition of a perfectly regular series of gradations throughout the lamellibranchs from the simple ctenidium of the primitive *Nucula* to the complex double gill of the Unionidæ. In the least modified forms the filaments are straight, either plate-like or filamentous, but in forms above these each filament becomes greatly elongated and bent upon itself to form a compressed U or V, consisting of an inner and an outer limb. One limb, the inner in the outer gill and the outer in the inner gill, is fixed above to the body wall, while the other limb is free in the lower groups (*Arca*, *Mytilus*), fixed in the higher (Unionidæ), although the inner limbs, forming the inner lamella of the inner gill, may not all be fused to the body wall. The filaments constituting a lamella are interlocked either by cilia or by interfilamentar junctions, and the gill may be further strengthened by interlamellar junctions, which are either simple bars (*Mytilus*, *Margaritana*) or continuous septa (Unionidæ, except *Margaritana*).

In his study of the lamellibranch gill Peck described in much detail and with great accuracy the structure of the gills of the Unionidæ, and his account has furnished the basis of all subsequent descriptions. The typical structure of the unionid gill is well known. Each gill consists of two lamellæ, an outer and an inner, composed of series of juxtaposed filaments supported by chitinous rods and fused by the interfilamentar junctions except where the inhalent ostia open into the interlamellar space for the entrance of water. The dorsal edge of the inner lamella of the outer gill and of the outer lamella of the inner gill is fixed to the body wall, while the outer lamella of the outer gill is fused to the mantle (in *Margaritana* it is free posteriorly), and the inner lamella of the inner gill is either free or more or less attached to the visceral mass (cf. Ortmann, 1911). The two lamellæ are continuous along the free ventral borders, and thus form a flattened sac whose cavity opens above throughout its entire length into

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the suprabranchial chamber; the four suprabranchial chambers lead posteriorly into the cloaca, which in turn opens to the outside water through the exhalent siphon. The entire gill is subdivided by a series of close-set septa, the interlamellar junctions (except in *Margaritana*) which separate the interlamellar space into a series of so-called water tubes. Water in the mantle chamber is driven by the cilia guarding the ostia through the lamellæ into the water tubes, whence it passes into the suprabranchial chambers and out through the exhalent siphon. The walls of the gill are traversed by blood vessels and lacunar blood spaces, and the current of water which passes through the gill is a respiratory current.

The water tubes are lined by an epithelium which is ciliated, at least in some species, on the inner faces of the lamellæ, while it assumes a characteristic glandular nature on the inner faces of the interlamellar junctions. The lamellæ and the interlamellar junctions are richly supplied with elastic and smooth muscle fibers, which are especially highly developed in the junctions of the marsupial gills of the female—evidently in adaptation to the great distensibility of which the latter are capable. In fact, the purely respiratory and the marsupial gills exhibit a number of structural differences, most of which were recognized by Peck (op. cit.) and which are undoubtedly to be accounted for on the ground of the difference in function between the two kinds of gills. Peck clearly described and figured the anatomical differentiation between the respiratory and the marsupial gill in *Anodonta*, and pointed out, among other distinguishing marks, the fact that the interlamellar junctions in the latter are not only thicker and wider and are covered by a peculiar folded epithelium, but that they are set much closer together. It will be well here to quote his description (op. cit., p. 59–60):

> The interlamellar junctions in the outer gill plate (the marsupial gill) are, like the vertical vessels, more numerous than those of the inner plate, occurring at intervals of seven filaments. They are long ridges of dense lacunar tissue, running vertically from base to apex of the gill plate, and have a much greater size, measuring more from one lamella to the other than those of the inner gill plate. In fact, they are capable of very great extension, which takes place when the outer gill plate has its interlamellar space occupied by the glochidian young of Anodon (pl. v, fig. 4). This great depth of the interlamellar functions of the outer gill plate is their most remarkable feature, as compared with those of the inner plate. It is accompanied by a different disposition of the vertical vascular trunks; for, whilst these in the inner gill plate lie *in* the interlamellar junctions, in the outer gill plate they lie in the subfilamentar mass of concreted tissue at the line of origin of the great ridges which act as interlamellar junctions. In consequence of this arrangement there are *two* vertical vessels in the outer gill plate to each interlamellar junction, whereas there is only one to each junction in the inner plate. The arrangement of these parts in the outer gill plate is no doubt correlated with its function as a brood pouch. * * * The difference just noted between the outer and inner gill plates, due to the frequency of interlamellar junctions and their relation to the vertical vessels, is accompanied by a further difference of form, which is obvious when the sections given in plate v, figures 2 and 3, are compared. In the outer gill plate the two lamellæ are parallel to one another and of equal thickness. In the inner gill plate the outer lamella is thicker than the inner, and its surface is thrown into a series of folds.

He figures very clearly the conditions described in both a non-gravid and a gravid outer gill and also in the purely respiratory inner gill, and it is clear from his description that the peculiarities of the outer gill of the female are permanent differentiations and are not merely present during gravidity. We have repeatedly observed the same

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differences as described by Peck, not only in *Anodonta* but in a number of other genera, and have also determined that the gills of the male are like the inner gill of the female with respect to the frequency of the interlamellar junctions and the character of their epithelium.

Peck's description has formed the basis of all of the textbook accounts of the structure of the unionid gill, and two of his figures, showing the differences between the inner and outer gills of *Anodonta*, are reproduced in Parker and Haswell's Textbook of Zoology, volume I, page 638.

Ortmann (1911) was evidently unacquainted with Peck's work, as he describes essentially the same differences between the marsupial and respiratory gills but without reference to Peck. He is the first, however, to show that the same differentiation holds good throughout a wide range of genera. In this connection he states that he "made a very important discovery, namely, that *in all our Unionidæ the anatomical structure of the gills, which serve as marsupia, is permanently differentiated*" (op. cit., p. 283). He then describes in detail the points of difference, showing that in the marsupial gill of the non-gravid female the interlamellar junctions, besides being more numerous, are thicker and wider and are covered by an epithelium which is folded and thrown into wrinkles, often of considerable proportions, whereas in the male and in the respiratory gill of the female the epithelium is simple and unfolded (cf. Peck). "There is no question," he says, "that this peculiar structure of the septa of the marsupial gills is an adaptation to their function"—a conclusion long ago arrived at by Peck. It should be stated that Ortmann has discovered another differentiating character between the inner and outer gill, namely, a longitudinal furrow along the ventral border of the inner gill which is entirely absent in the outer. This furrow is present in both males and females. A similar furrow is figured by Peck for the gill of *Mytilus*, but the figure in which it is shown is stated to be from the outer gill (op. cit., pl. IV, fig. 10).

Ortmann, in his careful study of the structure of the marsupium, has described a number of constant differentiations, hitherto unrecognized, which distinguish the several groups established by him in his system of classification. We are relieved, therefore, of the necessity of a detailed description in this place, and reference may be had to his interesting paper. It should also be stated that one of our former students, Mr. J. L. Carter, is now engaged in making a comparative study of the unionid marsupium in a large number of genera, and his investigation, which was undertaken primarily for the purpose of following the changes, both anatomical and histological, occurring in the gill from the pre-gravid to the post-gravid condition, is now well under way. Although Ortmann's work has, in part, rendered this investigation unnecessary, nevertheless Mr. Carter's study will contribute a number of facts, especially facts of a histological character, which are not included in Ortmann's observations.

Only a brief reference here to the internal structure of the marsupium is called for under the circumstances, and, since we shall need to compare our observations with those of Ortmann, it will be a matter of convenience to refer to them under the three subfamilies which he has distinguished. As we have not had an opportunity of exam-

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ining the marsupium of *Margaritana*, we have nothing to add to Ortmann's description of this genus, and shall confine ourselves to the Unionidæ as restricted by him.

Unioninæ.—In this group there is, as Ortmann has shown, the least amount of differentiation and the structure of the marsupium most closely approaches that of the respiratory gill. Aside from the usual permanent differences, namely, the greater frequency of the interlamellar junctions, their increased thickness and width, and the folding of the glandular epithelium, there is little else to distinguish the marsupial from the respiratory gills in this subfamily. Figure 50, plate XIII, which shows a cross section of two water tubes (w. t.) from the gravid outer gill of *Quadrula ebena*, represents the typical appearance in the genera embraced in this subfamily. Only two embryos are drawn in the figure, although actually the water tubes are filled with them. The interlamellar junctions (i. j.) are set very close together, at intervals of about five filaments, and the marsupium is capable of only moderate distention. The epithelium covering the inner surface of the lamellæ is low and ciliated, while that of the interlamellar junctions is high and glandular and exhibits irregular ridges and furrows. The folds of the epithelium are always of course far more pronounced in the non-gravid gill, as in this condition the interlamellar junctions are not stretched as they are when the gill is charged with embryos. The throwing of the epithelium into folds and the bending and crumpling of the septa themselves, when not under tension, is undoubtedly due to the elastic fibers which are wavy and wrinkled in the non-gravid gill, while they are drawn out nearly straight when the marsupium is full.

When highly magnified, as in figure 64, plate XV, the epithelium, resting upon a base of connective tissue and smooth muscle fibers and elastic fibers, is seen to be composed chiefly of greatly swollen cells, whose vacuoles are filled with a clear mucus-like colorless fluid. Scattered among these gland cells and seemingly often lying within the vacuoles are seen several smaller and darker nuclei which are the nuclei of leucocytes (l). In fact, there can be no doubt that the epithelium becomes infiltrated with wandering blood cells from the underlying blood sinuses in the interlamellar junctions, and many indications are present that seem to show that these cells actually wander through the epithelium into the cavities of the water tubes, but what their ultimate fate is, if this be the case, we are as yet unable to say. There is some evidence that they are ingested by the mantle cells of the glochidia in species that carry the larvæ over the winter, like *Lampsilis*, but of this we can not be certain.

The above description of the epithelium of the interlamellar junctions will apply in essential respects to the marsupium of all of the Unionidæ that we have examined, for the same characteristic histological structure is present everywhere.

Anodontinæ.—Ortmann has discovered in the genera which he places in this subfamily a most remarkable differentiation which is evidently an adaptation for increased aeration during the period of gravidity, as it totally disappears after the glochidia are discharged and does not reappear until the onset of the next period. He describes the condition as follows (1911, p. 324, 325):

Here each ovisac of the gravid female is not formed by a whole water tube, but only by a part of it, the middle one, which is separated from two lateral canals by a folding up of the epithelium of the

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septa (interlamellar junctions). In addition, the ovisacs are closed above at the base of the marsupial gill, thus forming a completely closed sac within each water tube. In one case (*Strophitus*) this sac is again divided into secondary compartments. * * * This peculiar structure of the marsupial gill is developed only in the gravid female, and is absent in the sterile (nongravid) female. These characters are apparently connected with the prolonged breeding season, and the peculiar secondary water tubes serve for the aeration of the embryos in the marsupium.

In a preliminary announcement of his new system of the Unionidæ (1910a) he briefly stated this discovery in the following words:

Water tubes in the gravid female *divided longitudinally into three tubes*, one lying toward each face of the gill, the third in the middle; only the latter contains eggs or embryos, and is much larger than the other tubes. This division into three parts is not present in the sterile female.

The statement of the presence of these lateral compartments of the water tubes of the gravid female, made in this brief form and without illustrations, misled us and seemed at that time not to be in accord with our own observations on the marsupium of *Alasmidonta*, *Anodonta*, and *Symphynota*, three of the genera included by Ortmann in the Anodontinæ. We had, it is true, seen narrow slit-like spaces lying opposite the outer and inner faces of the water tubes, which were evidently not blood vessels, as the ostia opened freely into them. We interpreted them as differentiations within the lamellæ themselves and supposed that they were merely collecting canals into which the ostia opened from the outside and which led by irregular apertures on the other side into the water tubes, as our sections showed here and there interruptions (now known to have a different significance) in the inner wall of these canals. It did not occur to us that these might be the lateral divisions referred to by Ortmann, as, in the sections of the marsupium in which we had seen them, they appeared so evidently to lie wholly within the lamellæ.

We were, however, in error, and our failure to recognize that these were really divisions of the water tubes was due to the fact that the sections studied by us were taken from near the ventral border of the gill, where the spaces are much narrower and more slit-like, and also to the fact that at that time we had not happened to see the lateral divisions in the process of being cut off from the water tubes during the early stages of gravidity. Thinking that Ortmann had made some mistake in his observations, we unfortunately published a note (Lefevre and Curtis, 1910a) to this effect and stated that no such division of the water tubes in the three genera referred to was present. A more careful examination of our material, however, and a study of marsupia at different stages of gravidity showed us that Ortmann was entirely correct, and we wish to express our regret at the overhasty publication of our note. The true facts of the case are as Ortmann has stated them to be, although he has only very briefly described the method of formation of the secondary septa which divide the lateral compartments from the central portion of the water tube in which the embryos are confined. Speaking of the origin of the septa, he says (1911, p. 293):

In specimens where the eggs begin to go into the gills, this structure (the lateral divisions of the water tubes) is sometimes not developed, but it appears soon, and the epithelial folds, which form the secondary septa within the water tubes, begin to grow into the lumen of the water tubes, and the folds of the opposing faces of the two septa finally unite in the middle. The point of union (cross section of the line of union) is often distinctly seen in sections.

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At the outset of gravidity, vertical septa begin to grow out in all of the water tubes of the marsupium from the surfaces of the interlamellar junctions close to the inner and the outer lamellæ of the gill. On each side of the gill one septum projects posteriorly, while the other extends anteriorly, and the two meet halfway across the cavity of the water tube. The free edges of each pair of opposed septa then fuse along their entire extent from the ventral border of the gill to the supra-branchial chamber. Specialized elongated epithelial cells forming a serrated border cover the free edge of each septum, and, when the two edges meet, these cells interlock and fuse (fig. 56, pl. XIV). In this way a space, quite narrow and slit-like below, but expanding gradually toward the supra-branchial chamber, is cut off from the water tube on either side, lying between the lamella and the large median division of the tube. As the septa unite, the eggs become confined entirely within the large central space of the original water tube, as Ortmann has stated, and it is this median division alone that functions as the marsupial cavity. We shall speak of the lateral spaces as the respiratory canals, as their function is undoubtedly to conduct a respiratory current of water to the supra-branchial chambers.

In figure 57, plate XIV, one side of a water tube, with the adjacent portion of the lamella, taken from a gravid marsupium of *Anodonta cataracta*, is shown in horizontal section. The gill contains eggs in an early cleavage stage, only four of which, however, are represented in the figure. The septa (s) are seen approaching each other, having not yet quite met. In figure 51, plate XIII, taken from the same species but not so highly magnified as the last figure, the septa have fused and the respiratory canals (r. c.) are completely shut off from the marsupial space (m. s.). In both of these figures the sections were taken near the ventral border of the gill; had they been cut at a higher level, the canals would be seen as much larger spaces. As is clearly shown in figures 56 and 57, plate XIV, the ostia open freely into the respiratory canals, and water must therefore enter the latter directly from the mantle chamber. The condition here should be contrasted with that seen in figures 50 and 53, plate XIII, which show water tubes from the marsupia of *Quadrula* and *Lampsilis*, representatives of Ortmann's Unioninæ and Lampsilinæ; here the ostia lead directly into the cavity of the tubes (w. t.) which are not subdivided and the whole of which becomes filled with eggs. Although it is not shown in figures 51, plate XIII, and 57, plate XIV, the epithelium covering the outer wall of the canals, which is of course the lining of the lamellæ, bears cilia which probably aid in conducting the current of water toward the suprabranchial chamber. Below, the canals are closed, and, since they are shut off from the marsupial cavity after the fusion of the septa, but open freely above into the suprabranchial chamber, there is but one course for the water to take—it must pass upward and enter the suprabranchial chamber. The transition from the more or less flattened epithelium lining the outer and inner walls of the respiratory canals to the large columnar cells on the anterior and posterior surfaces is clearly seen in figure 57.

The same condition appears in figure 58, plate XIV, which shows one end of a canal (the end marked X in the preceding figure) and the adjacent tissues, but under a higher magnification. Among the columnar cells are seen numerous swollen mucus cells,

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which are similar to those occurring on the interlamellar junctions farther in. The respiratory canals must be capable of expansion and contraction to a considerable degree, as a rich supply of smooth muscle fibers, passing in both a vertical and a horizontal direction, may be seen underlying the epithelium of the canals everywhere except in the septum (fig. 58, pl. XIV). Large blood sinuses (b. s.) are found in the lamellæ just outside of the canals, as seen in this figure, which shows how close the blood must come to the water within the canals (r. c.). There can be no doubt that the water passing through the canals is a respiratory current.

Although the respiratory canals open dorsally into the suprabranchial chambers, the marsupial division of the water tubes is completely closed off from the latter, as Ortmann has stated, by a roof which is developed in connection with the septa forming the respiratory canals. The dorsal free border of each interlamellar junction at the level of the suprabranchial chamber expands both anteriorly and posteriorly, but only over the marsupial division of the tube. The anterior and posterior edges of these umbrella-like expansions fuse with each other in exactly the same way as do the septa already described, and, since they also become continuous laterally with the vertical septa which separate the respiratory canals from the marsupial spaces, the latter thereby come to be completely roofed over and do not open at all into the suprabranchial chambers, unless the covering is broken. Of course, the formation of the roofing membrane does not take place until after the marsupium is fully charged with eggs. Owing to the gorged condition of the marsupium in these genera, the egg masses cause the roof to bulge up into the suprabranchial chamber over the marsupial division of each water tube, and on exposing the chambers the upper ends of the egg masses, covered, however, by the delicate transparent roofing membrane, are seen protruding beyond the dorsal boundary of the gill. In the drawing of *Symphynota complanata* (fig. 3, pl. VI), in which a portion of the suprabranchial chamber is exposed, the condition just described is distinctly shown.

As Ortmann has described, the secondary division of the water tubes entirely disappears after the discharge of the glochidia. The dorsal expansions of the interlamellar junctions, which united to form the roof, give way along the original sutures, and the glochidia are enabled to pass out; the septa separate in a similar manner, and are gradually retracted, and when the marsupium returns to the resting condition no trace of these structures is to be seen.

We have confirmed Ortmann's discovery of the respiratory canals in *Alasmidonta*, *Anodonta*, *Strophitus*, and *Symphynota*. Figures representing the marsupial structure in *Anodonta cataracta* (fig. 51, pl. XIII; 57, 58, pl. XIV) have already been referred to. Figure 56, plate XIV, is a section taken from near the ventral end of a water tube in the gravid marsupium of *Alasmidonta truncata;* the young embryos with which the marsupium is filled are not shown. The respiratory canal (r. c.) at this level is quite small and less slit-like than in *Anodonta*, but it widens out toward its dorsal end. The nuclei of the interlocking cells where the edges of the opposite septa have fused are quite distinct in the section. Figure 52, plate XIII, shows a horizontal section from the gravid mar-

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supium of *Symphynota complanata* at a stage when the glochidia are fully formed. In this species, when the marsupium is fully charged, the interlamellar junctions are so stretched that they become greatly reduced in thickness and appear quite membranous. Figures 49 to 53, plate XIII, showing a gravid water tube in *Alasmidonta*, *Quadrula*, *Anodonta*, *Symphynota*, and *Lampsilis*, respectively, are all drawn under the same magnification, and should be compared in order to observe the relative sizes of the tubes in section in the several cases, as well as the different intervals between the interlamellar junctions as shown by the number of intervening filaments in the lamellæ.

Ortmann interprets the respiratory canals of the Anodontinæ as an adaptation for the better aeration of the embryos in the marsupium (1911, p. 325). They are unquestionably a respiratory device, but for many reasons it would seem clear that they serve primarily for the aeration of the blood of the gravid female and not of the embryos. It is difficult to see how a membrane which shuts the embryos off from the water could *increase* the facilities for aeration or why such a condition should be an improvement, as far as the embryos are concerned, over the marsupium in those genera where there are no respiratory canals and the water comes into direct contact with the embryos. In some of the species of *Lampsilis* (*ligamentina*, for example) the marsupium is as heavily charged as in many of the Anodontinæ, and the glochidia are also carried over the winter, yet the respiratory canals are not present. In either case the embryos probably receive an adequate amount of oxygen. But, on the other hand, it is not difficult to see that the respiration of the gravid female might be seriously interfered with, when the entire outer gill is gorged and swollen with glochidia and these same glochidia must remain in the marsupium for months. In the Unioninæ (Ortmann) the marsupium is gravid for only a few weeks at the longest, and, furthermore, the gills are not so heavily charged, while in the *Lampsilinæ* only a differentiated portion of the outer gill receives the embryos and, although the marsupium may be heavily loaded and remain gravid over the winter, the encroachment of the marsupial upon the respiratory function is not so extensive. In these two subfamilies the need of a special respiratory device is, therefore, not as great as in the Anodontinæ. The close association of the maternal blood with the current of water in the respiratory canals, as shown in figure 58, plate XIV, would add further evidence for the view that the secondary division of the water tubes is an adaptation for the better aeration of the blood of the gravid female, in correlation with the prolonged period of gravidity and the interference with respiration by the excessive crowding of the entire outer gill.

Reference should be made to the special conditions existing in *Strophitus*. Aside from the formation of the respiratory canals in the manner peculiar to the Anodontinæ, Ortmann has briefly described a division of the marsupial cavity of each water tube by the outgrowth of horizontal septa from the interlamellar junctions to form separate closed spaces each one of which incloses a single "placentula." Referring to the peculiar position of the "placentulæ," which lie crosswise in the gill, he says (1911, p. 294):

> This arrangement is brought about by further outgrowths of the epithelial layers of the septa (interlamellar junctions), which fill the spaces between two septa, or rather only the middle part, the ovisac,

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and thus the simple ovisac of *Anodonta* and other genera is here divided into a number of swollen, *secondary ovisacs*, running transversely across the gill, each of which contains a short, more or less cylindrical mass of eggs or embryos. * * * Also in *Strophitus* these structures are not present in sterile females, and after the discharge of the glochidia they soon disappear.

We have observed this secondary division of the marsupial spaces in *Strophitus edentulus*.

We have not studied in detail the histological structure involved in the peculiar differentiation of the ventral border of the marsupium of the Anodontinæ and have, therefore, nothing to add to Ortmann's account (1911, p. 295) of the development of elastic tissue in this region, which allows of the enormous stretching of the gill in these genera when gravid. The lamellæ appear to separate along the mid-ventral border, especially in the middle portion of the gill, but are here connected by an elastic membrane which closes the bottom of the water tubes, with the result that "the edge of the marsupium in these forms *does not appear sharp* as in the *Unio* group, *but blunt, rounded off, or truncated*." This distension of the ventral edge, which is much more conspicuous in some genera than in others, is evidently a device to allow of a greater expansion of the marsupium.

Lampsilinæ.—It will be recalled that Ortmann includes in this subfamily Simpson's Heterogenæ, Mesogenæ, Ptychogenæ, and, although he does not refer to the genus *Dromus*, he would probably also place the Eschatigenæ here. We have already spoken of the general external characteristics which distinguish the marsupia in these groups. A great diversity of form is exhibited by the marsupium, but in all of the genera here concerned certain features, which have been referred to, are possessed in common.

In all of the groups here considered the marsupium is formed by a varying number of specialized water tubes in the outer gill, which are modified in different ways. In most, the water tubes are utilized throughout their entire length, as in *Lampsilis* and *Obliquaria*, but in other genera (*Cyprogenia*, *Ptychobranchus* and *Dromus* for example) it is only the ventral portion of the tubes which retain the embryos.

The respiratory canals, which are present during gravidity in the Anodontinæ, are absent in the Lampsilinæ, and the entire cavity of the water tubes in the marsupial region becomes filled with eggs (fig. 53, pl. XIII). The marsupium may show a high degree of distension when charged, as is seen in many species of *Lampsilis*. It is in the Lampsilinæ that we encounter the most capacious marsupial water tubes, the enlargement reaching the maximum size in *Obliquaria* (fig. 7, pl. VII). In figure 53, plate XIII, which is drawn from a gravid marsupium of *Lampsilis ligamentina*, the characteristic appearance of the water tubes in this genus is shown. The great antero-posterior diameter of the tube (w. t.) is very noticeable, as the interlamellar junctions are repeated at intervals of about a dozen filaments; the relatively large size of the tubes may be readily appreciated by a comparison of this figure with figures 49–52, plate XIII. The interlamellar junctions, when the gill is fully charged, are stretched into thin membranous septa (i. j.).

The dorsal free borders of the interlamellar junctions, while not forming a closed roof over the water tubes as they do in the *Anodontinæ*, in *Lampsilis* at least become

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distended into rather conspicuous bulb-like expansions which greatly diminish the openings of the tubes into the suprabranchial chamber, although their edges do not fuse.

As the histological details of the structure of the marsupia in several genera belonging to the Lampsilinæ have been studied by Mr. Carter and will be described in his forthcoming paper, a further account may be omitted here.

PHYLOGENY OF THE MARSUPIUM.

It is not without justification that a phylogenetic significance should have been attached to the several types of the marsupium which occur in the Unionidæ, for it would seem clear that those forms in which the structure characteristic of the respiratory gill is least modified, as in *Quadrula*, are more primitive than those in which the specialization of the marsupium has gone much farther, as in *Anodonta*, *Lampsilis*, and many other genera.

Simpson (1900) has considered these facts in some detail and concludes that the oldest type of marsupium phylogenetically is that occurring in the Endobranchiæ in which the inner gills alone are used as brood chambers. It is a slight transition from this condition to that presented by the Tetragenæ with all four gills functioning for this purpose. Basing his supposition largely upon shell characters and geographical distribution, he further concludes that the Homogenæ marked the next step in marsupial differentiation, while the Heterogenæ and all other groups in which a portion only of the outer gills is modified for receiving the eggs are the latest product of the evolution of the Unionidæ.

That this series correctly represents the phylogenetic sequence in the appearance of the marsupial modifications would seem to be borne out by the structural conditions existing in the several types so far as we have examined them, provided that we assume, with respect to the Homogenæ, that genera like *Pleurobema* and *Unio*, in which the marsupium is less specialized, are more primitive and therefore stand nearer the *Tetragenæ* than such genera as *Anodonta*, *Symphynota*, and others, which, as Ortmann has shown, exhibit certain modifications evidently in advance over the marsupium of the former.

Ortmann (1911), although he does not consider the Endobranchiæ, has arrived at conclusions essentially similar to the above. He points out, however, that the absence of complete interlamellar junctions in the gills of *Margaritana* would indicate that the new family which he has created for this genus, Margaritanidæ, is the most primitive group of the Naiades, and this inference, as was indicated above, is further strengthened by the fact that the simple gill structure of *Margaritana* is apparently similar to that of *Mytilus*, which belongs to a lower group of lamellibranchs than the fresh-water mussels.

His conclusions concerning the sequence of his three subfamilies of the Unionidæ may be quoted (p. 328):

> Of the *Unionidæ*, the *Unioninæ* are certainly more primitive than the other two subfamilies, as is evidenced by the simple character of the structure of the marsupial gills. The *Anodontinæ* and *Lamp-*

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silinæ are more advanced, but they have advanced in different directions, and each has developed special features of the sexual apparatus. Generally speaking, the *Lampsilinæ* contain the most highly advanced types, as is shown by the restriction of the marsupium to a part of the outer gill, and by the strong expression of the sexual differentiation in the outer shell. Yet there are forms among the *Anodontinæ* which show extremely complex structures (*Strophitus*) unparalleled in any other genus, and the peculiar glochidia of the *Anodontinæ* surely mark a high stage of development.

It is not necessary for our purpose to enter into a further discussion of the subject in this place.

CONGLUTINATION OF THE EMBRYOS.

After extrusion of the eggs from the genital apertures, they are received into the suprabranchial chambers, and thence pass, as has already been described, into the water tubes of the gills, eventually filling up those portions which function as the marsupium. In a short time after entering the latter the eggs usually become conglutinated into masses which are molded into the exact shape of the cavity of each marsupial water tube (Lefevre and Curtis, 1910b). The masses are of course separated from each other by the intervening interlamellar junctions of the gills.

Since it is a matter of convenience to have a word to apply to these compact masses in which the eggs or embryos are held together, whether they be plate-like, club-shaped, cylindrical, or of some other form, we shall employ the term conglutinate in referring to them. Ortmann (1911) has proposed the word placenta, which was introduced by Sterki (1898) for the peculiar cords of *Strophitus*, but this is obviously misleading, as there is no connection whatever between the masses and the maternal tissues. The conglutinates vary greatly in different species in size and shape, and, since each is a cast of the cavity of its water tube, they conform to the special conditions existing in the several types of marsupium. The commonest form is that of a flat plate, either elliptical or lanceolate, being usually slightly blunter and thicker above and more pointed and thinner below. Since we have already seen that the antero-posterior diameter of the marsupial water tubes varies very much in different species, the thickness of the conglutinates must vary to the same extent. In *Quadrula* and *Unio*, for example, in which the interlamellar junctions are set close together, the conglutinates are very thin, being not more than twice the diameter of an egg in thickness; whereas in *Lampsilis*, with its much more capacious tubes, they may be three or four times as thick. In other words, just as many eggs will lie abreast in a horizontal section of the marsupium as the antero-posterior diameter of the water tube will allow.

This commoner lanceolate form of the conglutinate, differing, however, in size and thickness, may be seen in the species of *Quadrula*, *Pleurobema*, *Unio*, and *Lampsilis*. In figure 41, plate XI, two conglutinates of *Lampsilis ligamentina* are represented, one from the flat side, the other on edge. An unusual form of conglutinate has been observed by us in *Quadrula metanevra;* it is bifurcated and consists of two flat lanceolate masses which are united for the upper third of their length, but free below. In those genera, however, in which the form of the water tubes of the marsupium departs more widely from the

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usual condition, the conglutinates are similarly modified. In *Obliquaria reflexa*, for example, in which the marsupium consists of several elongated and distended water tubes of tubular form, the conglutinates are large, slightly curved cylindrical masses of nearly uniform diameter and generally blunt at each end. Three of them are shown in figure 42, plate XI; the one on the right was taken from the most posterior water tube of the marsupium, which is not as long as the rest, and its conglutinate is correspondingly shorter. The relation will be understood by reference to the figure of the marsupium of this species (fig. 7, pl. VII).

There seem to be two methods by which the embryos are bound together to form conglutinates—they may either be attached more or less firmly to each other by their egg membranes, which are in this case of an adhesive nature, or they may be embedded in a mucilaginous matrix of varying consistency. The former is by far the commoner condition and is seen in figure 17, plate VIII, which is a detail drawn from one of the conglutinates of *Obliquaria reflexa* shown in figure 42, plate XI; the immature glochidia with their valves open are still contained within the membranes, which are closely adhering and by mutual pressure are squeezed into a polyhedral form. In cases like this it is difficult to determine whether there is a glutinous matrix between the embryos or not, but if any is present, it must be in very small amount, since the embryos seem to be held together solely by the adhesive surfaces of their membranes. In those cases, however, in which a matrix is evident (*Lampsilis*), the embryos are not so closely appressed and are embedded, more or less loosely, in a glutinous binding substance. This condition is illustrated in figure 16, plate VIII, which is a portion of a conglutinate of *Lampsilis ligamentina* seen under higher magnification; as the matrix is transparent, it can not be shown in the figure.

The conglutinates differ markedly in tenacity, for, whereas in some cases the mutual adhesion is not strong and the masses consequently break up readily (*Quadrula*, *Pleurobema*, *Unio*, *Lampsilis*), in others (notably in *Obliquaria*) the embryos adhere so firmly that they may be separated only with difficulty by teasing.

In still other species the embryos can not be said to form conglutinates at all, as they are merely suspended in a slimy mucus which is not of such a consistency as to enable the mass to maintain a definite form when removed from the gill. We have observed this condition in *Alasmidonta*, *Anodonta*, and *Symphynota*, and Ortmann (1911) states that it also occurs in *Anodontoides*.

In most species (*Quadrula*, *Unio*, *Lampsilis*, *Dromus*) in which the conglutinates are found, the adhesion exists only during the embryonic development and by the time the glochidia are fully formed they are found to be free but for the mucus which holds them more or less loosely together. In *Obliquaria reflexa*, however, the conglutination persists, and the fully developed glochidia, still tenaciously adhering, are discharged from the marsupium in the cylindrical masses already described (fig. 42, pl. XI); even after lying in the water for some time they do not separate, and it has perplexed us to understand how the glochidia of this species ultimately become attached to fish, if they pass through a subsequent parasitic stage. Can it be that parasitism has been

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lost in *Obliquaria* as it has been in *Strophitus*, and that the metamorphosis takes place while the glochidia are in the conglutinates? We have not yet had the material by which to answer this question.

The relation of the embryos and glochidia of *Strophitus* to each other is so unusual that its description is reserved for a special section (see below).

STRATIFICATION OF UNFERTILIZED EGGS.

It has already been pointed out that not infrequently eggs pass into the marsupium without being fertilized and remain there throughout the period of embryonic development, as one may find them in the same gill with fully formed glochidia. In some individuals we have found every egg in the marsupium in this condition. Such eggs have been encountered chiefly in summer-breeding species, and they seem to be especially common in *Pleurobema* and *Quadrula*, nearly every gravid female of which has been found to contain at least some unfertilized eggs. After remaining in the marsupium for a time such eggs generally become swollen and stratified into three distinct layers, a heavier, often pigmented, mass at one pole, a clear or hyaline intermediate zone, and a small granular cap at the lighter pole. As the eggs lie in a constant position in the gills, which are placed vertically in the normal position of the animal, it can not be doubted that the stratification is produced by gravity. It has not yet been determined whether the substances which occur in these layers are the same as would be separated out by centrifuging or not, but this is not at all unlikely. As many of the species of mussels in which we have seen this condition, for example, *Quadrula ebena*, *Q. trigona*, and *Pleurobema æsopus*, have brightly colored red or pink eggs, the stratification is quite striking, the pigment being always at the heavier pole, as it is invariably directed toward the lower border of the gill.

ABORTION OF EMBRYOS AND GLOCHIDIA.

There has been a certain amount of discussion among the conchologists as to whether or not the functioning of all four gills as a marsupium is a constant character in *Quadrula*, and observations have been to a certain extent conflicting. Since Simpson has made use of this feature in characterizing the group Tetragenæ, some importance has been attached to the apparent discrepancy in observations.

While examining mussels on the upper Mississippi River in the summer of 1908, we observed a peculiarity of behavior in all of the species of *Quadrula* collected which may account for the conflicting descriptions of the marsupium in this genus, and also for the fact that in some species gravid females have never been observed at all. Every species of *Quadrula* that came into our hands exhibited to a greater or less degree the habit of aborting embryos and glochidia when taken out of the river, and if they were not opened and examined at once upon capture they were generally found shortly afterwards to be either partially or entirely empty. Some individuals discharged the contents of their gills more readily and completely than others, the abortion involving

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either all four gills or only the inner or outer ones, or, again, only a portion merely of one or more gills. In the pre-glochidial stages, when the embryos are conglutinated, the entire masses were discharged, while individuals were frequently seen in the act of aborting their embryos or glochidia which were often expelled with considerable force through the exhalent siphon.

This behavior was so characteristic of the genus that, in order to make a correct determination of the condition of the marsupium, it was necessary to open quadrulas immediately after taking them from the water. When this was done, all four gills were invariably found to be charged on opening females which contained embryos in pre-glochidial stages—that is, at any time before normal spawning had occurred. The habit of readily aborting embryos when disturbed has also been observed by us in *Unio complanatus*, which has been repeatedly seen in the act of discharging the contents of the marsupium shortly after being placed in aquaria. In all likelihood it occurs in other species of *Unio*, and it may possibly be characteristic of all forms in which there is but little structural differentiation of the marsupium. We have, however, also observed the discharge of embryos in *Lampsilis ligamentina*, but only after the gravid females have been kept in the laboratory for some time. This species is apparently very much less sensitive with respect to abortion than the quadrulas and *Unio complanatus* and only frees its gills of the conglutinates after long exposure to artificial conditions. The premature extrusion is probably due to imperfect aeration of the water and results from an effort on the part of the female to secure more oxygen; if this be true, one would not expect to find it occurring so readily in those forms which have a differentiated marsupium, like the Heterogenæ, since here the respiratory and marsupial functions of the gills are not so intimately associated.

Both Schierholz (1888) and Latter (1891) have referred to the occurrence of abortion in *Anodonta*, but according to our experience it has never been encountered in a single instance in either *Anodonta* or *Symphynota*, although gravid females have been kept in tanks in the laboratory for weeks or even months. The presence of the respiratory canals, which have been described as occurring in these genera during gravidity, as well as the temporary membrane which roofs over the marsupial division of the water tubes, might well account for the absence of abortion, or at least its rare occurrence, in the forms in which these special conditions exist. The respiratory canals doubtless lessen the evil effects of poor aeration, while the roofing membrane of the water tubes would certainly offer some obstruction, as long as it was present, to a liberation of the embryos.

BREEDING SEASONS.

In connection with our study of artificial propagation of fresh-water mussels, we have found it necessary to collect data bearing upon the breeding seasons of a fairly wide range of species, since the records of previous observers, for North American Unionidæ at least, have been insufficient to enable us to determine the full extent of the seasons, especially in the case of some of the more important commercial species.

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Although our observations have been largely confined to species occurring in the upper Mississippi Valley and have been concerned primarily with species of commercial value, we have continuous records throughout the entire year for a number of important genera, and in every case the exact stage of development of the embryos has been determined by microscopic examination. Many thousands of such observations have been made, so that we are now in possession of detailed information dealing with the duration and progress of the periods of gravidity obtaining in over a dozen genera of the Unionidæ.

We have fully confirmed the conclusion reached by Sterki (1895) that the North American Unionidæ, with respect to their breeding seasons, fall into two classes, the so-called "summer breeders" and "winter breeders" — a distinction, however, which had previously been pointed out by Schierholz (1888) for European forms and frequently recorded by later observers. The designation "winter breeders," however, is not strictly appropriate, for in the species which belong to this group the eggs are fertilized during the latter half of the summer and the glochidia, which are carried in a fully developed condition in the marsupium throughout the winter, are not discharged until the following spring and summer. In the case of the summer breeders, the eggs are fertilized during late spring and summer and spawning as a rule is over by the end of August.

In view of these facts, it would seem to accord better with the actual conditions to separate the species with respect to the length of time that the glochidia remain in the marsupium, designating them as those that have a "short period" and those with a "long period" of gravidity, rather than to distinguish them as "summer breeders" and "winter breeders," respectively, for with respect to the latter neither ovulation nor discharge of the glochidia takes place in winter. This suggestion was made by us in an earlier paper (1910b), and subsequently Ortmann (1911) proposed the somewhat awkward terms *tachytictic* and *bradytictic* (meaning quick-breeding and slow-breeding) for Sterki's "summer breeders" and "winter breeders," respectively.

The breeding seasons as here defined are based upon data collected in the middle and northern sections of the United States, and in the absence of adequate records from higher and lower latitudes, it is impossible to say to what extent a colder or warmer climate might affect the period of gravidity. That it would have some influence can hardly be doubted, although a distinction between a long and a short season will probably be found to hold true in general.

The breeding season is a generic character, for so far as our observations have gone all of the species belonging to a given genus have essentially the same period of gravidity. The prolonged period, furthermore, is correlated with the more pronounced structural modifications of the marsupium which have been described above.

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LONG PERIOD OF GRAVIDITY.

In the forms which fall into this category the eggs are fertilized, as has been stated, during the latter half of the summer, from the middle of July to the middle of August, and the glochidia, instead of being discharged when fully formed, are carried in the marsupium until the following spring or early summer. In fact, in some cases the close of one breeding period may overlap on the beginning of the next, as one may still find in late July a few straggling females gravid with glochidia formed in the previous autumn, while in other individuals of the species at the same time and in the same locality the eggs are passing into the gills for the next season. This seems to be true of several species of *Lampsilis*. We have encountered it in *ligamentina*, Conner (1909) records it for *radiata* and *nasuta*, while Ortmann (1909) states that his observations make it probable for *ventricosa* and *luteola*. Yet, as Ortmann observes, it is generally true that an interval exists between the close of one period and the beginning of the next. This interval, however, varies in length in different species, in some extending from late spring until August, whereas in others it is of much shorter duration. It is also to be noted that the discharge of glochidia does not take place in all of the individuals of a species at the same time, but on the contrary, spawning may extend over a considerable period throughout the spring and early summer (cf. Ortmann, op. cit.).

All of the genera included in Simpson's Heterogenæ, Ptychogenæ, Eschatigenæ, and Diagenæ have the long period of gravidity, as do also a number of genera of the Homogenæ (*Alasmidonta*, *Anodonta*, *Anodontoides*, *Arcidens*, *Symphynota*), while the Mesogenæ are represented in this group by *Cyprogenia*. These genera are embraced in Ortmann's subfamilies *Anodontinæ* and *Lampsilinæ*, and it should be noticed that in all the gills show a high degree of specialization in adaptation to the marsupial function, a specialization which is undoubtedly correlated with the habit of retaining the glochidia over a period of several months.

In the following list are given the species in which we have determined the long period of gravidity:

Alasmidonta truncata.
Anodonta cataracta.
Anodonta grandis.
Anodonta implicata.
Arcidens confragosus.
Cyprogenia irrorata.
Dromus dromus.
Lampsilis (Proptera) alata.
Lampsilis (Proptera) lævissima.
Lampsilis anodontoides.
Lampsilis gracilis.
Lampsilis higginsii.
Lampsilis ligamentina.
Lampsilis luteola.
Lampsilis recta.
Lampsilis subrostrata.
Lampsilis ventricosa.
Obovaria ellipsis.
Plagiola elegans.
Plagiola securis.
Strophitus edentulus.
Symphynota complanata.
Symphynota costata.

Ortmann (1909) has published some observations on the breeding seasons of the Unionidæ of Pennsylvania, supplemented by data from Lea and Sterki; his results in

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all essential points agree closely with ours. He includes among "winter breeders" several genera which we have not had under observation, namely, *Truncilla*, *Micromya*, *Ptychobranchus*, and *Anodontoides*, while *Arcidens*, which we have recorded, does not appear in his list.

There is given below a brief summary of our breeding records for the genera here concerned. Although in many species we have examined hundreds of individuals and have had them under observation continuously throughout the year, in others the material has been more or less meager and observations scattered, but in most of the forms the records have been adequate for a determination of the general limits of the breeding season.

Alasmidonta.—Embryos from latter part of July to middle of August. No fully formed glochidia have been seen, as gravid females have not been secured after August.

Anodonta.—Embryos from the middle of August to September; ripe glochidia from early October to first of July. A distinct interim exists between close of one period and beginning of next. According to Harms (1909), in European species of *Anodonta* the eggs are fertilized about the middle of August, all of the individuals entering upon the breeding season at nearly the same time, and by the middle of October almost all of the females are gravid with glochidia.

Arcidens.—Glochidia in winter months. Only a few individuals secured.

Cyprogenia.—Glochidia in November.

Dromus.—Glochidia in November.

Lampsilis.—Embryos from first of August to late September; glochidia from late September to first of August. Our most complete record concerns this genus, several species of which (*anodontoides*, *ligamentina*, *recta*, *subrostrata*, *ventricosa*) we have repeatedly had under observation continuously throughout the year. The gravid period seems to be more extended in *Lampsilis* than in any other genus, for, although June is apparently the month when the liberation of glochidia is at its height, some females bearing glochidia may still be found, but in diminishing numbers of course, until the beginning of August, a time when the next season is just setting in. Since ripe glochidia may be obtained in abundance from October to July, inclusive, and since *Lampsilis* furnishes several species of commercial value, the extended period of gravidity in this genus becomes of the greatest importance in artificial propagation, as material is available for the infection of fish throughout the greater part of the year.

Obovaria.—Glochidia during the fall, winter, and spring months. Spawning must occur before June, as no glochidia have been encountered in June, July, and August, although a number of females have been obtained during these months.

Plagiola.—Ripe glochidia during the winter and as late as the end of July; no embryos have been obtained.

Strophitus.—Embryos from late July to middle of August; glochidia from November to middle of July. The interval between the seasons is very short, much shorter than that observed by Ortmann (1909), who records an interim from May 22 to July 11.

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Symphynota.—Embryos during August; ripe glochidia from late September to late June. *S. complanata* is a species which we have had on hand constantly for several years, and we have followed it continuously through the year. Spawning is most active in June.

SHORT PERIOD OF GRAVIDITY.

In the species having the short period of gravidity the entire breeding season is confined to about four months, as it extends only from about the end of April to the middle of August, and the glochidia are discharged as soon as they are fully developed. It is highly probable, however, that the beginning of the breeding season is influenced to a certain extent by temperature, for it would seem that ovulation may be postponed for some weeks by cold weather at this time of the year. It was first pointed out by Sterki (1895) that these summer-breeding forms are confined to a limited group of genera, and Ortmann (1911) has emphasized the fact that it is only the genera having the least specialized marsupia that possess this apparently more primitive breeding season; these are the genera which constitute his subfamily Unioninæ. *Margaritana*, unquestionably a primitive form, likewise breeds only in the summer. In all of these genera the structure of the marsupium approaches most closely that of the respiratory gills; none of the special modifications, so prominent a feature of the marsupium of other genera, is present. There is apparently, however, one exception, for, as will be shown below, our records indicate clearly that *Obliquaria*, which has a highly specialized marsupium, is a summer breeder.

The following are the species which we have observed to have the restricted breeding season:

Obliquaria reflexa.	Quadrula plicata.
Pleurobema æsopus.	Quadrula pustulosa.
Quadrula ebena.	Quadrula trigona.
Quadrula heros.	Quadrula (Tritogonia) tuberculata.
Quadrula lachrymosa.	Quadrula undulata.
Quadrula metanevra.	Unio complanatus.
Quadrula obliqua.	Unio gibbosus.

The following species, which do not appear above, have been determined by Ortmann (1909) to be summer breeders: *Unio crassidens; Pleurobema clava* and *coccinea; Quadrula kirtlandiana, rubiginosa*, and *subrotunda*. Our list, on the other hand, supplements his by the addition of several species of *Quadrula*, for which data have previously been either entirely wanting or quite meager.

Obliquaria.—Since all of the forms which carry the glochidia over the winter have a highly specialized marsupium, we should expect that *Obliquaria*, whose marsupium is of such a nature, would also have the long gravid period. This expectation would be further strengthened by the fact that the very closely related genus *Cyprogenia* belongs in the former group, as has been seen. It was therefore with some surprise that we found *O. reflexa* breeding during the summer. Our record is as follows: Embryos from

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the latter part of May to July 9; glochidia from June 20 to August 8. This is a typical record for a summer breeder, and there can be little doubt that the species must be placed in this group. On the other hand, Sterki (1898, 1903) states that all forms which have a differentiated marsupium carry their glochidia over the winter, and Ortmann (1911) includes *Obliquaria* in his *Lampsilinæ*, all of which he says are "bradytictic," although specific reference to the breeding season of this genus is not made. Since, however, we have not had an opportunity of observing the species during the fall and winter, it is possible that it has the long period, although, if such is the case, its season begins two months earlier than that of any other species in this class—a quite improbable supposition. For the present, at all events, we must consider it a summer breeder.

Pleurobema.—Embryos from early June to early August; glochidia during July.

Quadrula.—Embryos from late May to middle of August; glochidia from early June to middle of August. Hundreds of females belonging to different species of this genus have been examined throughout the rest of the year, but gravid individuals have never been encountered except during the months indicated.

It should be mentioned that in the case of *Q. heros* Frierson (1904) has not found this species gravid in Louisiana until October, when embryos were found. Young embryos were again encountered in November and immature glochidia in January. He concludes that *heros* is an exception in the genus and is not a summer breeder. Our observations on this species are very meager, but since we have found it bearing young embryos in the latter part of May, they would seem not to be in accord with those of Frierson.

According to Harms (1909), *Margaritana*, which breeds in Europe in July and August, produces two successive broods during that time, from sixteen days to four weeks, according to temperature, being required for the development of each. Although we have not determined it beyond all doubt, our records strongly indicate that the species of *Quadrula* also spawn twice during the season, first in June and July and again in July and August. This, however, could not be definitely proven without a most extended series of observations, and possibly not unless individual females were kept in aquaria under close observation throughout the breeding season.

Unio.—Embryos from early June to early August; glochidia from middle of June to middle of August. Conner (1907) records *U. complanatus* as beginning its breeding season in April, and Lea (1863) found it gravid in May; but we have not had an opportunity of examining any species of the genus during these months. According to Harms (1909) the breeding season of *Unio* in Europe begins early in March, or, if the weather is cold, not until the end of May.

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III. THE LARVA.

STRUCTURE OF THE GLOCHIDIUM.

As has long been known, two well-marked types of glochidia are found in the Unionidæ; one provided with a strong shell bearing a single stout hook at the ventral margin of each triangular valve; the other with no such hooks and a more delicate shell, the valves of which are shaped like the bowl of a very blunt spoon.

A possible third type, which appears to be a derivative of the second, is seen in the "axe-head" glochidium, originally described and figured by Lea (1858, 1863, and 1874) in *Lampsilis* (*Proptera*) *alata*, *lævissima*, and *purpurata*.

The first type is characteristically parasitic upon the fins and other external parts of fishes from which scales are absent, the second upon the gill filaments. The occurrence of these types in the genera which we have examined is shown by the following list:

Hooked glochidia:	Hookless glochidia:	Axe-head glochidium:
Anodonta.	Cyprogenia.	Lampsilis (Proptera) alata.
Strophitus.	Dromus.	Lampsilis (Proptera) lævissima.
Symphynota.	Lampsilis (majority of species).	Lampsilis (Proptera) purpurata.
	Obliquaria.	Lampsilis capax.
	Obovaria.	
	Plagiola.	
	Pleurobema.	
	Quadrula.	
	Tritogonia.	
	Unio.	

The axe-head glochidium occurs, so far as known, in only a few closely related species which were generally included in the genus *Lampsilis*, but which, after being first placed in the subgenus *Proptera* by Simpson (1900), have been elevated to the genus *Proptera* by Sterki (1895 and 1903), a change which has recently been approved by Ortmann (1911). The species long known to possess this axe-head glochidium are *Lampsilis* (*Proptera*) *alata*, *lævissima*, and *purpurata*, and recently Coker and Surber (1911) have described it for *Lampsilis capax*.

There is considerable diversity in size among glochidia even from the same genus, as represented by the outlines in text figure 1 (A–O), all of which are drawn to the same scale, the most striking cases being the difference between the two species of *Plagiola* (G and H), and that between *Lampsilis recta* and *gracilis* (K and L). Harms (1909), who has studied the exceedingly minute glochidia of *Margaritana margaritifera*, finds that they are exclusively gill parasites, because their small size makes attachment elsewhere impossible.

The type of glochidium is constant for the genus, so far as our observations go, save in the case of *Lampsilis*, as has just been mentioned. In some cases the shape is also characteristic, as shown by *Symphynota* and *Anodonta* (A, B, and C), in which the shell outline is a distinguishing feature.

In *Dromus dromus* the glochidium, which is of the hookless type (text fig. 1, M), is greatly elongated antero-posteriorly thus presenting an interesting modification.

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THE HOOKLESS TYPE.

Since the greater part of our experimental infections with glochidia of the hookless type have been made with our common species of *Lampsilis*, we have examined the glochidia in this genus more extensively than any others and shall describe, as representative of what has been observed, the hookless glochidium of *Lampsilis subrostrata* which is shown in figures 13, 14, and 15, plate VIII; and, since it is often necessary in the practical work of infection to examine the glochidia alive in water and to determine the exact stage of their development, we shall first speak of their appearance when in this condition.

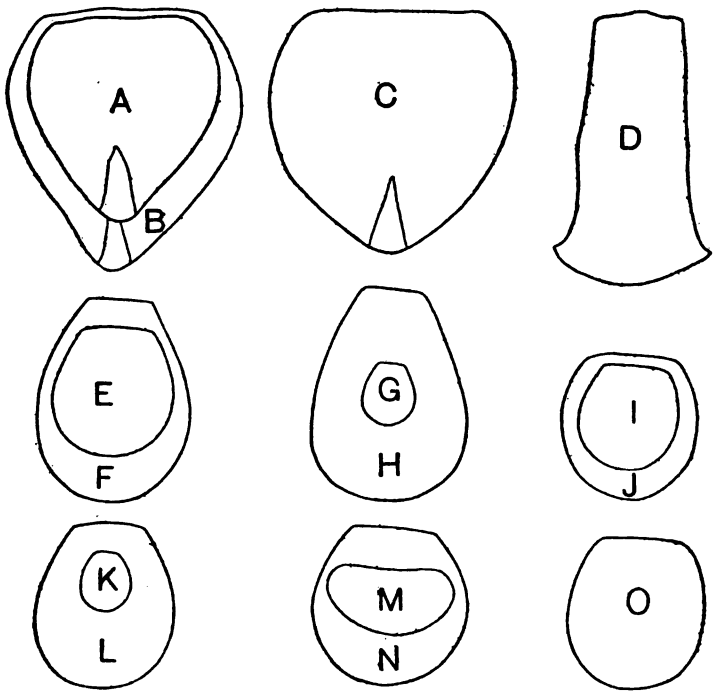

FIG. 1.—Figures showing relative sizes and shapes of the shells of a series of glochidia, belonging to the following species: A, *Symphynota complanata*, 0.30 × 0.29 mm.; B, *S. costata*, 0.39 × 0.35 mm.; C, *Anodonta cataracta*, 0.36 × 0.37 mm.; D, *Lampsilis (Propiera) alata*, 0.41 × 0.23 mm.; E, *Quadrula metanevra*, 0.19 × 0.18 mm.; F, *Q. pustulosa*, 0.30 × 0.23 mm.; G, *Plagiola elegans*, 0.09 × 0.075 mm.; H, *P. securis*, 0.31 × 0.23 mm.; I, *Quadrula ebena*, 0.15 × 0.14 mm.; J, *Q. plicata*, 0.21 × 0.20 mm.; K, *Lampsilis gracilis*, 0.085 × 0.075 mm.; L, *L. recta*, 0.24 × 0.20 mm.; M, *Dromus dromus*, 0.19 × 0.10 mm.; N, *Obliquaria reflexa*, 0.23 × 0.225 mm.; O, *Unio gibbosus*, 0.22 × 0.19 mm.

When examined alive (fig. 13, pl. VIII), this glochidium exhibits a shell which is comparatively firm in structure and which may remain unchanged by the water even many days after its living contents have been destroyed. Evidence of the shell's strength is shown by the fact that its shape remains unchanged after the glochidial muscle has caused the lips of the shell to bite deeply into a host's tissue, and by the fact that it is not easily broken by rough handling, as when the glochidia are tumbled in and out of a pipette during the process of breaking up the conglutinated masses. This strength is due to the carbonate of lime already laid down in the shell and not to the cuticle, which is often referred to by investigators as though it were the sole constituent of the shell of the glochidium; for when the carbonate of lime is dissolved by acid the cuticle becomes wrinkled and the shell partially collapsed. Viewed from the outside and closed (fig. 13, pl. VIII), this shell of the living glochidium exhibits a fine granulation over its entire surface and a distinct border

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around the free margin. At the hinge margin two denser areas may be observed, which, when examined from the inner face of the valve, are found to be continuous with the border around the free margin (fig. 13, pl. VIII). The test with acid shows that this entire border is calciferous and that there is a thinner layer of carbonate of lime over the whole surface and beneath the cuticle. This layer is often cracked, as one might break the shell of a hen's egg, when preserved specimens are slightly crushed under a cover glass, and it is then seen to be distinct from the cuticle which may wrinkle but does not break. Upon the loss of the lime, the cuticle is no longer firm enough to preserve the shape of the shell and successful permanent mounts must therefore avoid acids at any stage of the preparation.

Along the ventral border of the shell is a flange, formed of cuticle only, and so transparent that it is easily overlooked in a ventral view of the open glochidium (fig. 15, pl. VIII). Viewed laterally (fig. 14, pl. VIII), this flange has at a certain focus the appearance of a hook and may easily be mistaken for one when seen under a low magnification. It is, however, a continuous flange, as shown in the figures, and not a hook; and since its edge is very fine it must, when the glochidium closes its valves, cut into and hold to a delicate tissue like that of the gill filament, thus performing much the same function as the hook in the other type of glochidium. The general spoon-like character of the valves is shown clearly by the figures. The adductor muscle is well seen in the living specimen, being a conspicuous object from whatever angle it is examined. Viewed laterally (fig. 13, pl. VIII), or from the ventral aspect (fig. 15, pl. VIII), the adductor is seen to lie nearer the shell margin at one end of the hinge than at the other, a fact which enables one to recognize at a glance the future anterior border of the shell. There is also in this glochidium of *Lampsilis subrostrata* a slight difference in outline by which these anterior and posterior borders of the shell may be distinguished (fig. 13, pl. VIII), while in the hooked type of glochidium (fig. 10, pl. VIII, and text fig. 1, A, B, and C) this difference is even more pronounced and one recognizes the anterior border of the future adult by its slightly greater length.

The two outer pairs of sensory cells with their fine projections (fig. 14 and 15, pl. VIII) are readily seen in the living glochidium; the two inner pairs, in which the cells project but a short distance from the surface, are more easily found in specimens which have been properly preserved and stained. The position of the two outer pairs may also be seen in the closed glochidium (fig. 13, pl. VIII). Little can be seen of the rudiments of the various organs of the adult without the careful staining of well fixed material. In the living glochidium they appear as a slightly denser area on either side of the median line and posteriorly to the adductor muscle (fig. 13, pl. VIII). The cells of the larval mantle (fig. 15, pl. VIII), which occupy the greater part of the surface exposed within the valves, appear in the living glochidium as a dense mass in which cell outlines can not be recognized.

Further details in the structure of this glochidium can only be studied in specimens which have been properly fixed and stained. After trying various reagents, we have found that they may be stupefied in a few moments by the addition of several small crystals of hydrochlorate of cocaine to the water in a watch glass, after which they

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may be fixed with no serious shrinkage by using the solution of plain corrosive sublimate obtained by diluting a saturated solution two or three times with water. Acids should be avoided throughout the whole process. Alum cochineal, Delafield's hæmatoxylin, and borax carmine, alone or with Lyon's blue, have been used as stains, each being more suitable for the demonstration of certain structures. In this stained material the shell shows a slight wrinkling of its ventral flange and is the only part not shown to better advantage than in the living specimens.

The lateral pairs of sensory cells (fig. 14 and 15, pl. VIII) are tall chimney-like structures expanded at the base and terminated by several very fine motionless processes. A denser border where these processes are inserted in the cell is presumably due to their continuation within the cytoplasm which has been observed in sections of these and other glochidia. The two median pairs of sensory cells (fig. 14, pl. VIII) project only a short distance and have short processes. The anterior pair is located ventral to the median portion of the larval adductor muscle, the posterior pair near the outer ends of the rudiments of the adult organs (fig. 15, pl. VIII). The designation of these cells as "sensory" by all writers rests upon their structural features as described by the earlier investigators, and upon the fact, recorded by Lillie (1895), of their staining reaction with methylene blue. The actual connection of the cells with the larval muscle fibers has been sought for by investigators, but never discovered. We have not attempted a further demonstration of the function of these cells by the methods practiced in recent experimentation upon the protozoa and other minute organisms, although such a study might yield some interesting results.

Lining the greater part of the surface between the valves, are the large cells composing the larval mantle (fig. 15, pl. VIII). They are filled with fine granules, which, since these cells actually digest the tissue of the host during the early stages of the parasitism, are probably the zymogen granules from which the digestive enzymes originate. The absence of these cells over the area of flexure ventral to the adductor muscle will be noted in figure 15. In this area the ectoderm is thinner and there is no granulation. The adductor muscle is composed of fibers having elongated nuclei and often seen to branch toward the ends where they are attached to the valves. In a glochidium of *Lampsilis subrostrata*, which has been carried over the winter in the parent gills and which has therefore reached the highest stage of differentiation possible for this glochidium, we can identify the rudiments of foot, stomodæum and enteron, and of the heart, pericardium, and kidney, as described by Harms (1909) in his accounts of the structure and organogeny in the hookless type of glochidium. Reference to figure 15, plate VIII, will make clear the following account of these rudiments.

In the median region, just posterior to the adductor, is a triangular area, the oral plate; behind this a narrow band of closely set nuclei extending well out into the valves, where it becomes wider. The ectoderm in the median part of this area becomes the covering of the foot, while the deeper part of the area is endoderm, the rudiment of the enteron. The lateral expansions of this general mass are mesodermal cells which are closely applied to the endoderm and in which are found the rudiments of the kidney,

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heart, and pericardium. A backward curve in the posterior outline at either side of this mass appears to represent imperfectly developed lateral pits, from the outer borders of which Schierholz (1888), Schmidt (1885), and Harms (1909) agree that the first rudiments of the gills originate, and which are very conspicuous structures in the glochidia of the hooked type. We have never observed any structure resembling the larval thread or its rudiment in the fully formed glochidia of species of the genera *Lampsilis* and *Quadrula*, the glochidia of which we have studied most extensively; and the larval thread is not present in functional condition in any of the species we have studied from the genera listed on page 145, with the exception of *Anodonta* and *Unio*. A discussion of this organ, which has heretofore been assumed to occur in all glochidia, is given after the account of the hooked glochidium which follows.

THE HOOKED TYPE.

Our first infections were performed with the hooked glochidium of *Anodonta cataracta*, which is essentially like the *Anodonta* type of glochidium described for European species, and which has been described in a detailed manner by Lillie (1895). Our later work has been with the young of *Symphynota complanata* and *S. costata*, the glochidia of which resemble one another in structure, as shown by their outlines in text figure 1, A and B, and figures 9 and 10, plate VIII; so that here, as elsewhere noted in the case of hookless glochidia, the outline appears to be a characteristic of the genus, which enables one at once to distinguish the glochidia of *Anodonta* from those of *Symphynota*. There is, however, a marked size difference between the glochidia of these two species of *Symphynota* (text fig. 1, A and B).

In both *Anodonta* and *Symphynota* glochidia, the slightly greater length of one border of the valve between hook and hinge is indicative of the future anterior region. In most hookless glochidia there is a similar slight difference in the anterior and posterior marginal outlines (fig. 13, pl. VIII), but it is more difficult to detect, and in any case the safest guide is the larval adductor muscle, which is always recognizably nearer the anterior end, a position to be correlated with the location of the rudiments of the adult organs in the posterior region. In the living glochidium of *S. complanata* the shell shows calcification beneath the cuticle and is marked as though the calcareous layer were porous.

The external appearance of these hooked glochidia is like that shown for *S. costata* in figure 10, plate VIII. The hooks, with their spines, the fibers of the larval adductor, and the sensory cells are seen when turned in profile view (fig. 9, pl. VIII); but the cellular structure is so obscure in living specimens that the rudiments appear only as a denser area and even the fibers of the adductor muscle are not very distinct. There is no sign of a larval thread or a thread gland, nor do sections of preserved glochidia reveal such a structure. A conspicuous feature of the whole mass of glochidia in *Symphynota*, as taken from the gill of the parent, is the thick, ropy mucus in which they are embedded. This holds them so firmly together that when stirred up in a dish they remain suspended and quite evenly distributed through the water, settling to the bottom only very slowly

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over a period of four or five minutes. During this suspension in the water the sucking of a pipette will draw in glochidia over a wide area, as they are pulled by the invisible strands into which the mucus has been divided. The significance of this mucus and the absence of the thread gland are discussed under another heading of this paper. The mucus is dissolved by the water in a short time, so that after 24 hours the glochidia are found entirely free and snapping actively upon the bottom. We find that these glochidia can be freed from the mucus by repeated washing, and that it is desirable to do this at once if one wishes to keep them alive for the maximum period. When thus set aside it is possible for them to remain alive for as long a time as two or three weeks.

In killing this glochidium we have used successfully crystals of chloral hydrate or hydrochlorate of cocaine added to the water of a watch glass containing the glochidia, and fixation with Merkel's fluid, or with weak corrosive sublimate, as described for the hookless type.

Stained specimens show the same rudiments of stomodæum, enteron, and mesodermal structures, as described by Lillie (1895) and Harms (1909) for the glochidium of *Anodonta*. The lateral pits are conspicuous and the cells of the larval mantle are well developed laterally, though thinning out over the median part of the larval adductor, where their boundaries are not clear and only a few nuclei are discernible. Sections show two kinds of granules within the larval mantle cells, one staining deeply with iron hæmatoxylin and the other with acid-fuchsin. Near each corner of each valve is a cell which stains deeper than the rest and seems to contain more of the granules. The significance of these six cells we can not determine. The sensory cells (fig. 9, pl. VIII) are slightly different in position from those in *Anodonta*. Lying along a line drawn across from hook to hook are three large cells in line beneath the hooks and a smaller one on either side between the larval adductor and the lateral pit.

THE PROPTERA OR AXE-HEAD TYPE.

This glochidium possesses hooks which are not homologous with those of the *Anodonta* type and is to be regarded as more nearly related to the hookless forms, an interpretation which is borne out by the fact that the "axe-head" can be readily imagined as a modification of the glochidial outline seen in some species of *Lampsilis*, the glochidia of which, like those of *subrostrata* (fig. 13, pl. VIII), show some approach to a rectangular form. Its four hooks are so arranged that those of one valve pass inside the opposite ones, thus bringing the ventral margins close together and giving a very firm hold upon the host's tissue. In other respects it does not show marked differences from the hookless type, and the few experiments we have made with it indicate its attachment to the gills rather than to the fins.

Recently Coker and Surber (1911) have observed "an almost exactly similar glochidium" in *Lampsilis capax*, while in *Lampsilis* (*Proptera*) *lævissima* they find an axe-head glochidium which is of a somewhat different outline and lacks the hooks. They point out the fact that in *Lampsilis gracilis*, a species which in its adult features (form of shell) seems almost to intergrade with *lævissima*, the glochidium is of the ordinary

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hookless type, although the outlines of the two glochidia are very similar when seen on edge, as in their figures 1a and 2a of plate I. With respect to the significance of these facts when applied "to a relationship between *lævissima* and *capax*," they conclude that "there would be strong corroborative evidence in adult characters alone" for the closer union of these three species, and this "in spite of the fact that *lævissima* and *capax* are the two extremes in the degree of inflation." The similar degree of inflation of *capax* and *ventricosa* offers, they believe, "only a striking instance of convergence in one character."

THE LARVAL THREAD.

Our observations upon the occurrence of the larval thread (formerly erroneously termed the byssus) are of importance, since the current accounts in textbooks and literature lead one to believe that this structure is a conspicuous feature of all glochidia. Such an assumption is natural because the organ is conspicuous in the European anodontas and unios and in the American species of these genera examined by Lillie (1895).

We find the larval thread present in the species of *Unio* and *Anodonta* which we have been able to examine with care, and the thread is undoubtedly a characteristic of these genera. We have never seen any sign of such a structure in the ripe glochidia of the other genera, above listed, which possess hookless glochidia, nor in the hooked forms of the genus *Symphynota*. Lillie (1895, p. 52) considers the thread a condensed excretory product, which, accepting the account of Schierholz (1888), he thinks has also become an organ which is of use in bringing the glochidium in contact with the fish. This latter function is the one commonly ascribed to the thread. We have not studied the pre-glochidial stages in the development of those species which show no thread-gland in the mature glochidium, although it is important that this should be done with a view to determining whether a homologue of the thread gland is present at any time. We have, however, made repeated examinations of glochidia, either ripe or well along in their development, in several species of *Lampsilis*, particularly in *ligamentina*, *recta*, *anodontoides*, *ventricosa*, *luteola*, and *subrostrata*, and to a lesser extent in species of the other genera mentioned, without finding any trace of the thread which is so conspicuous a feature of the glochidium of *Unio complanatus*.

We have also examined the glochidia of *Symphynota complanata* many times with the same negative results, and a smaller number of observations confirm this for *S. costata*. Since many species thus have no thread in any way functional for attachment to the fish, the question arises whether the thread when present has as important a function in this respect as has been supposed. Our observations upon the glochidia of *Anodonta cataracta* confirm the descriptions of Schierholz (1888) and others who have studied the European species of *Anodonta* as to the tangling of the glochidia into masses by means of their extruded threads, and in this genus the threads do seem effective in drawing other glochidia into contact with the fish when a single one has become attached. This is not, however, effective during the greater part of the period in which the glochidium may remain alive upon the bottom, for the threads are dissolved within a day or

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two and the glochidia then become entirely free from one another. When taken from the parent gill the glochidia of *Symphynota* are entangled in a ropy mucus, and this acts in a manner similar to the threads of *Anodonta*, but it is usually dissolved after a few hours in the water. In the ripe glochidium of *U. complanatus* the threads are extruded immediately after the glochidia are removed from the parent and placed in water, and, according to Harms (1907b, p. 819), the minute glochidia of *Margaritana margaritifera* extrude their threads while still within the egg capsule.

When this extrusion has taken place in *Unio complanatus* the glochidia and broken egg membranes become united into globular masses from which it is difficult to separate individual specimens, and from observing such glochidia in contact with the fish we are forced to conclude that they are not so likely to become attached to the gills or fins as they are later, when they have been separated by the disintegration of the threads. The glochidia of *Lampsilis*, which when fully ripe fall apart into masses of entirely unconnected individuals, appear much better able to attach to the gills of fishes immediately after their discharge from the parent. We believe, therefore, that the thread is something to be gotten rid of rather than an organ of great importance in the attachment to fish, and this is in agreement with Lillie's interpretation of this organ as an excretory product. It is possible that some homologue of the thread exists in these threadless glochidia, and a comparative study of the pre-glochidial stages might yield material for interesting comparisons.

BEHAVIOR AND REACTIONS OF GLOCHIDIA.

At the time of spawning the glochidia, already freed from the egg membranes, and usually held together in slimy strings, are discharged at irregular intervals. Being heavier than water, they sink rapidly to the bottom, coming to rest with the outer surface of the shell directed downward and the valves gaping widely apart. The belief was formerly general that they "swim" about by rapidly opening and closing the valves, after the manner of *Pecten*, and, in spite of frequent denials by Schierholz (1888), Latter (1891), and others, the same statement is still occasionally encountered. In the recent volume on Mollusca in the Treatise on Zoology, edited by Lankester, this inexcusable error is repeated. "The glochidia," we are again informed, "swim actively by clapping together the valves of the shell" (p. 250). They are, on the contrary, as is now well known, entirely incapable of locomotion and remain in the spot where they happen to fall, although it is true that they may exhibit from time to time spasmodic contractions of the adductor muscle, which cause the valves to snap or wink, each contraction being immediately followed by relaxation and opening of the shell. These movements of the valves, however, are never so vigorous as to cause the glochidium to move from place to place in the water.

The glochidia remain in this helpless situation until they die, unless they happen to come in contact with the host on which they pass through the post-embryonic development as parasites. The stimulus which causes the contraction of the muscle and results in attachment to the host is, in the case of hookless glochidia, usually a chemical one,

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but in that of the hooked forms it is mechanical. The latter may be readily imitated and glochidia of this type made to grasp firmly the point of a needle or the edge of a piece of paper by simply touching them between the open valves. When once closed in this manner they do not relax, but remain attached to the object until they die.

The following statement made by Latter (op. cit., p. 56) has been frequently quoted, especially in textbooks, but it has apparently never been verified or disproved.

> The Glochidia are evidently peculiarly sensitive to the odor (?) [sic] of fish. The tail of a recently killed Stickleback thrust into a watch glass containing Glochidia throws them all into the wildest agitation for a few seconds; the valves are violently closed and again opened with astonishing rapidity for 15–25 seconds, and the animals appear exhausted and lie placid with widely gaping shells, unless they chance to have closed upon any object in the water (*e. g.*, another Glochidium), in which case the valves remain firmly closed.

Although it is not stated that the tail which caused such a commotion among the glochidia had been cut off from the fish, it is probable that such was the case. We have repeatedly tested glochidia in the same manner both with fins and gills of different fishes, and, providing that a bleeding surface is not brought in contact with the water containing the glochidia, absolutely no response on the part of the latter takes place. The result, however, is much as Latter describes if a little of the fish's blood gets into the water in the neighborhood of the glochidia, except that our experience has shown that after snapping for a few seconds they come to rest in permanent closure. It therefore seems possible that the contractions seen by Latter were due to the introduction of some blood with the tail of the fish, as otherwise agitation of the glochidia under similar conditions has not been observed by us.

Since the hooked and hookless glochidia, whose reactions to blood and to certain salts we have studied, show important differences in their behavior, they are referred to separately below.

REACTIONS OF HOOKLESS GLOCHIDIA.

It was first observed that glochidia of the hookless type, in marked contrast with the hooked forms, only occasionally exhibit spontaneous contractions and respond either not at all or only sluggishly to tactile stimuli, and the question at once arose as to what causes their closure when they become attached to fish. If the stimulus which brings about a contraction of the adductor muscle in attachment is not a mechanical one, it presumably is chemical in nature, but we were completely in the dark in the matter until it was cleared up by the following experiments, the first of which were made with the glochidia of *Unio complanatus* at Woods Hole, Mass.

When a small drop of blood of either the killifish, *Fundulus diaphanus*, or the white perch, *Morone americana*, was placed over the glochidia contained in a small amount of water in a watch glass, the effect was immediate and very striking. Every glochidium was thrown into rapid and violent contractions, alternating with relaxations, the edges of the valves either quite or nearly touching with each snap. Where the stimulus was strongest—that is, immediately under the drop of blood—the glochidia exhibited two or three strong contractions and then remained closed, but, proceeding outward to zones

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of diminishing intensity, the snapping occurred intermittently for from 10 to 50 seconds. Here the contractions were quite rapid at first, one or two every second, but soon the intervals became longer, until finally the activity was ended by the closure of the valves. In some cases it was observed that after the first few snaps the muscle did not completely relax, and each subsequent contraction caused the valves to describe a shorter arc. This experiment was repeated time and time again, with invariably the same result, and it was astonishing to see what a small quantity of the fish's blood was required to produce the reaction. It should be emphasized, furthermore, that after the stimulus had caused the final contraction of the muscle the valves remained permanently closed.

The experiment was later performed a great many times with the glochidia of *Lampsilis ligamentina* and *subrostrata*, and identically the same reaction was obtained with the blood of several different fishes and that of the frog, *Necturus*, and man.

Since the hookless glochidia, which are essentially gill parasites and, when taken into the mouth of the fish lodge among the gill filaments, produce abrasions of the delicate epithelium covering the latter, a more or less extensive hemorrhage from the blood capillaries occurs, as may be readily seen from a microscopic examination. It is therefore evident that blood exuding from the gill filaments in the immediate neighborhood of the glochidia must have the same effect as in our experiments, and, by exciting vigorous contractions of the adductor muscle, furnish an efficient stimulus in bringing about a firm and permanent attachment to the filaments. It is true that hookless glochidia will occasionally secure an attachment to the edge of the fins and other external parts of the fish, but it is quite evident that they are not adapted to such locations, as they rarely succeed in remaining there. It is possible that when they do become attached to the fins the closure of the valves is due to the presence of blood on the latter; but, since hookless glochidia occasionally close when touched repeatedly, the attachment in these situations is probably brought about by a sluggish response to contact with the edges of the fins. Their characteristic place of attachment, however, is the gill filaments, and this definite reaction to the fish's blood constitutes a most striking functional adaptation to the special habit of hookless glochidia as gill parasites.

Although the matter has not been exhaustively studied, it is in all probability the salts of the blood that are responsible for these reactions. A series of experiments, however, has been undertaken for the purpose of determining the reactions of glochidia of this type to solutions of several different salts, and, although the investigation has not yet been completed, a brief statement may be made here. Diluted sea water and solutions varying in strength from 0.5 to 1 per cent of NaCl, KCl, KNO_3, and NH_4Cl have exactly the same effect as fish's blood, although the intensity of the reaction varies somewhat in certain cases. Weak solutions of $MgCl_2$ and $MgSO_4$, however, as would be expected, inhibit contractions, and glochidia, after treatment with these salts, may be killed in an expanded condition, if allowed to remain in the solutions for a sufficient length of time.

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REACTIONS OF HOOKED GLOCHIDIA.

The larvæ of *Symphynota complanata*, which are provided with stout hooks and as a rule find permanent lodgment only on the fins and other external parts of the fish, were used in studying the reactions of the hooked type of glochidium. In several respects they differ from the hookless forms. When removed from the marsupium and placed in water, they exhibit spontaneous contractions which occur at irregular and rather long intervals, and this irritability may continue in the laboratory for a day or two, or until the glochidia begin to disintegrate. Under such conditions the valves are only partially closed at each contraction of the muscle, which, moreover, is never strong enough to bring the points of the hooks into contact. It is followed at once by relaxation of the muscle and the shell remains widely open until the next snap occurs.

Hooked glochidia, in striking contrast with the behavior of the hookless forms, respond very actively to tactile stimuli, and, as has been stated, close completely and immediately when touched with any object. This reaction must be the main factor in bringing about their attachment to the fish's fins, when they are brushed over by the latter while lying on the bottom. With glochidia like those of *Symphynota complanata* the mere contact is sufficient to produce complete closure of the valves, and, whether they are exposed to the fish's blood or not, attachment is possible as a result of the tactile stimulus alone. They do react to blood, however, and exhibit a few successive contractions, from 5 to 15, before final closure, but the way in which the response occurs is quite different from that shown by hookless glochidia under similar conditions. Instead of being thrown into violent and rapid snapping, the valves closing and opening alternately, there is only partial recovery after each contraction, while the valves are brought closer and closer together by a series of short jerks. The final act of closing is interesting. As soon as the points of the hooks touch, the contraction of the adductor muscle becomes continuous and the hooks are slowly bent inward against each other. Under the steady pressure exerted by the muscle, aided probably by the action of the myocytes, which have been described by Schmidt (1885b), the spines on the outer surface are apposed and the hooks turned in completely between the valves, the margins of which are brought together, if no object intervenes. It will be readily understood that, owing to the turning in of the hooks, the spines are pressed into the fish's tissues, when attachment to the host takes place, and a firm hold is thereby secured.

When the glochidia of *Symphynota complanata* were exposed to salt solutions, the contractions produced were of the kind just described. KCl, KNO_3, and NH_4Cl in solutions of 0.5 to 1 per cent caused a few successive jerks, the contractions being more vigorous and closure occurring sooner with the stronger solutions. NaCl and $Na_2C_2O_4$ in the same strength acted less energetically, and it was necessary to use a 2 per cent solution to produce the same effect as was obtained with the weaker solutions of potassium and ammonium salts. A 0.5 per cent solution of $CaCl_2$ produced no contractions, while a 1 per cent solution after a latent period of 15 minutes caused either partial or complete closure of the valves. $MgCl_2$ and $MgSO_4$, in solutions of 0.5 and 1 per cent,

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inhibited contractions, and when the glochidia were allowed to remain in them they finally died in the expanded condition. When the Mg salts, however, were used in stronger solutions, closure of the valves occurred after a few spasmodic contractions.

IV. THE PARASITISM.

ARTIFICIAL INFECTION OF FISH.

In any investigation which attempts to ascertain the facts of most importance for the artificial propagation of a species, attention is at once directed to those points in the life history where wholesale destruction of the individuals is most likely to occur. These points of wholesale waste are usually to be found in the earlier part of the individual's existence rather than during its adult life and are often preventable by artificial means. In common with other animals which must overcome the chances of parasitism, the Unionidæ produce enormous numbers of eggs, the great majority of which are by virtue of the brooding habit of the female mussel carried safely through their embryonic period and discharged as glochidia. We have not attempted to estimate the numbers of glochidia carried by full-grown adult females, but anyone who has seen them taken from the gills knows that they must be numbered by the hundreds of thousands, or even millions, and had these glochidia any great chance of survival and development to the adult stage the supply of mussels would far exceed anything which has ever been known in nature. When, however, the next stage of the larval history is sought for in nature, it becomes apparent that we have reached a point in the life cycle where the destruction and waste of individuals is wholesale and probably in excess of that which occurs at any other stage. There is no evidence, save in the case of the species *Strophitus edentulus*, the metamorphosis of which we have discussed under another heading of this paper, that any one of the Unionidæ can pass beyond the glochidial stage without becoming a parasite upon some fish, for the failure of glochidia to develop when left in water has been observed by all investigators since Leeuwenhoek.

The large element of chance involved in this shift from parent to fish, which has already been emphasized in our discussion of the glochidium, is again apparent when fish are examined in nature with a view to determining the abundance of the parasitic larvæ under the conditions of natural infection, for all investigators agree that the parasites exist in numbers which are insignificant when compared with the masses of glochidia which occur in the parent mussels. Only an occasional fish is found to be infected and it thus becomes clear that the purely accidental nature of the infection makes necessary the production of glochidia in such abundance as to overcome by sheer force of numbers the chances of destruction. Fish become infected in nature by occasional glochidia, but the chance that any fish will carry under natural conditions the number of glochidia which our experiments have shown that individual fish are capable of carrying, when artificially infected, is a negligible quantity. Here, then, we have the point of greatest destruction in the life cycle of the Unionidæ; and the point of attack for artificial propagation is clear. The fish must be made to carry more glochidia. Under experimental

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laboratory conditions it is found that a given fish may carry successfully a load of glochidia so much in excess of what the same fish would ever be likely to carry in nature that there is no reason why a single fish should not be made, under the conditions of artificial infection, to do the work which a thousand fish perhaps could not do in the state of nature. This has been from the first our main point of attack, and, with this in view, we have studied the parasitism, first, by the infection of small lots of fish in aquaria and, later, by the infection of fish in larger numbers in a hatchery. Other points in the life cycle, as for example the stage immediately following the parasitism, may be found by later work to be places of wholesale destruction; we are convinced, however, that there can be no other where the mortality reaches such proportions as it does when the countless glochidia are spread upon the bottom and left to the chance that will bring them in contact with the parts of a fish's body suitable for their parasitism.

Throughout our experimental infections we have made use of small fish, usually those under 6 inches in length, because such fish are more easily collected in numbers and because we have not had proper facilities for the keeping of larger individuals. Where small numbers of fish are used and each individual can be carefully watched, the attainment of what may be termed an "optimum" infection in every case may be secured with no great difficulty, and by following the methods practised by various investigators ever since Braun (1878) and Schmidt (1885), we have obtained unlimited material whenever necessary. If the glochidia are placed in shallow dishes and in water just deep enough to cover all parts of the fish, the latter will usually keep the water sufficiently agitated to insure a proper suspension of the glochidia and tolerably constant results will follow.

It is very necessary that the glochidia be so distributed in the water as to come in contact with the proper parts of the fish, and, in most cases, to guard against over rather than under infection. Active fish, such as the rock bass (*Ambloplites rupestris*), and the large-mouthed black bass (*Micropterus salmoides*), are very favorable for gill infections, since they keep the water so well agitated that the glochidia hardly settle to the bottom at all, while their strong respiratory movements draw the suspended glochidia continually against the gills. With fish like the crappie (*Pomoxis annularis*), which when undisturbed move about quietly and whose respiratory movements are less vigorous, the water must be stirred to keep the glochidia suspended, or be so shallow that the fish are always near the bottom. The smaller gill slit of the crappie is another factor which makes for a very light infection in fish under 2 inches in length, since the glochidia reach the gills by way of the mouth and not from the opposite direction. For fin infections, sluggish fish like the German carp (*Cyprinus carpio*) need little attention, and the darters (*Etheostoma cœruleum spectabile*), which habitually rest upon the bottom for considerable periods, become quickly loaded with glochidia upon both fins and gills; although, as we shall see, the latter fish appears to be particularly adapted for ridding itself of the entire infection.

In the account which follows, we are discussing the results obtained from the infection of fish in small numbers and kept under careful observation in the laboratory.

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There is no reason for believing that larger numbers of fish would present any more serious difficulties than are to be expected in the keeping of any fish in large numbers within a restricted space; and, if one could insure as uniform and careful an infection of the larger numbers, we have every reason to believe that such infections would prove as successful as those here described.

INFECTIONS WITH HOOKED GLOCHIDIA.

For the infections with hooked glochidia, we have used principally *Anodonta cataracta* from Falmouth, Mass., the species studied by Lillie (1895). With these we infected German carp under 6 inches in length and, unless otherwise stated, the following account refers to this combination which gives typical results. A smaller number of infections, made with *Symphynota complanata* and *S. costata* upon carp and other fishes, are referred to in a supplementary manner. The glochidia of *A. cataracta* become attached in large numbers to the fins (fig. 19–25, pl. IX and X) and gills of the carp. They are also found upon the other external parts which offer the condition of a soft scaleless epithelium like that of the fins; thus, the region about the anus, the edge of the operculum, the lips and in very heavy infections, even the soft area of the ventral surface between the mouth and pectoral fins may become heavily loaded. Within the mouth cavity, the gill filaments and also the gill bars and rakers become well covered. The glochidia which attach to these mouth parts do not remain, for, although the fish may be carrying many of their fellows upon its external parts, in about one week after the infection all glochidia have disappeared from the gill filaments, which then become as clean as though never infected. Scattered glochidia may remain upon the other internal mouth parts, for specimens are occasionally seen well embedded and in advanced stages of their metamorphosis, but in the main these parts also will become free of glochidia.

The general distribution upon the individual fins may be seen by reference to figures 19 to 25, plates IX and X, which show how great a proportion of the glochidia become attached to the fin margins. If a fish is carefully watched, as its slight movements stir up the glochidia during the infection, the latter are seen continually falling upon the upper faces of the pectoral and pelvic fins. They may even be collected with a pipette and heaped upon a motionless pectoral fin, remaining there for some minutes without more than an occasional specimen becoming attached. The margin of the fin is so much more favorable for attachment, that it is often thickly set with glochidia, when none are found upon the fin surface, and this despite the fact that glochidia must, during infection, strike against the surface of the fin many times for every time that one of them comes in contact with a fin margin. It is, therefore, the margin of the fin for which this glochidium is best suited, and, once fastened there, it is almost certain to remain and become embedded by the growth of the host's epithelium.

Considered in a more detailed way and with reference to the parts of the glochidium, we may explain this more frequent attachment to the margin as due to the fact that when the glochidium strikes against any flat surface the sensory hairs are not stimulated and the glochidium, which, as we have already shown in the case of the hooked forms,

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responds principally to tactile stimulation, does not receive the stimulus to permanent closure which is given by the presence of any foreign object inserted between the valves. When a specimen does become attached to the surface of a fin, as is sometimes the case (fig. 21 and 22, pl. IX, fig. 25 and 32, pl. X), it presumably gains its hold by catching upon one of the ridges formed by the fin rays, for the hooks could hardly be used upon a perfectly flat surface. Glochidia sometimes hold to the surface of a fin by a shred of tissue, under which their hooks have caught, remaining there after all the neighboring specimens are completely overgrown (fig 25, pl. X), only to be torn off later without having caused any noticeable hypertrophy of the fin tissue. Figures 25 and 32, plate X, show that glochidia may become overgrown either flat against the surface or upon edge, and figure 24, plate IX, shows a young mussel leaving a surface attachment after a parasitism of 74 days.

The behavior and reactions of glochidia are of course significant in connection with the actual attachment when once the glochidium is brought in contact with a suitable part of the fish's body and receives the normal stimulus to close its valves. The bringing of the glochidium against just that part of the fish is a matter of the chance distribution in the water. Hence the distribution of the glochidia to the several fins is determined solely by the number likely to be brought in contact with a given part of the body. Those fins which brush against the bottom are always the more heavily loaded and the numbers elsewhere depend upon the extent to which the glochidia are kept suspended in the water. The importance of the mucus for the glochidia of *Symphynota* and of the larval thread for those of *Anodonta* and *Unio* in tangling the glochidia into masses and drawing others against the fish when a single one has become attached has probably been exaggerated, as explained in the section of this paper which deals with the function of the larval thread.

Optimum infections, as we shall term those which are close upon the limit of the number of glochidia which a fish can safely bring through the metamorphosis, often show the glochidia very closely set one after another, as in figures 22 and 23, plate IX, and figure 25, plate X, and several hundred may be safely carried by a fish 3 or 4 inches in length. Prolonged exposure causes so heavy an infection of the margins (fig. 19 and 20, pl. IX) that the fin tissue appears unable to overgrow the mass of glochidia, and they then remain attached without overgrowth for a week or more.

Figure 19, plate IX shows how on a part of the fin having no overcrowding normal embedding occurred, while in the more crowded areas the glochidia were still uncovered even seven days after infection. In the middle upper margin of this fin it would seem that the overgrowth might well have taken place, for many cases like figure 25, plate X, have been observed in which glochidia as closely set were properly embedded. The failure of overgrowth in this region is probably due to the presence immediately after infection of a greater number of glochidia many of which have since been detached. In all cases of this kind a smaller number will finally become embedded than in an infection where the fin has received more nearly the optimum load (fig. 21, 22, 23, pl. IX, and fig. 25, pl. X), for the great majority drop off when the fin becomes so mutilated

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that bacterial or fungus infection sets in. These over-infections sometimes cause such hypertrophy that the fins become swollen and the rays so drawn together that it is impossible for them to spread out normally. Often the fins are raw and bleeding for some days and show red areas within where the blood vessels have become abnormal. The fish are likely to die from this or from the similar injury to their gills, and these over-infections are unsatisfactory if one wishes to bring through their parasitism the maximum number of glochidia.

The steps in the implantation of the glochidium by an overgrowth of the fish's tissue may be seen in figures 21 and 22, plate IX, and figure 25, plate X. Figures 21, plate IX, and 26, plate X, show the glochidium 3½ hours after attachment to the fish's fin. Most of the glochidia have bitten deep enough in from the margin to have a good hold for their hooks. The beginning of the hypertrophy appears as a faint mass of tissue, seen with its nuclei in the detailed figure 26, plate X. At the end of 12 hours the overgrowth is well advanced and sometimes, as in figure 27, plate X, shows different stages even in neighboring glochidia. The ragged edge of the host's tissue rises up crater-like about the glochidium, meeting above in a delicate mass, the nuclei of which are shown. Figure 22, plate IX, shows that in 24 hours most of the glochidia are more than half covered, whether upon the edge or the surface of the fins. At the end of 36 hours (fig. 25, pl. X) optimum infections of the carp show all the glochidia which have obtained a proper attachment well embedded, and from this time onward the only change which is visible in whole mounts is a slight increase in the opacity of the cyst, which renders the internal structure of the glochidium less distinct (fig. 23, pl. IX). Some of our infections show embedding in as short a time as 6 hours (*Symphynota*), and Harms (1909) gives 10 to 12 hours as the time which he observed in *Anodonta*, so the time given for the figures above referred to is the maximum for hooked glochidia which have been well located. Glochidia upon the fin surface become embedded in a similar manner and are then in a very secure position (fig. 22, pl. IX, fig. 25 and 32, pl. X).

INFECTIONS WITH HOOKLESS GLOCHIDIA.

Our experiments in artificial infection with hookless glochidia have been more extensive because this is the type of glochidium found in the species of mussels which are of commercial importance. Species of the genus *Lampsilis* (*ligamentina*, *recta*, *anodontoides*, *ventricosa*, *subrostrata*, and *luteola*) have been the most frequently used, but infections have also been made with several species of *Quadrula* and one of *Unio*. The list of fishes employed as hosts for hookless glochidia is also more extensive and we are, therefore, able to make statements which we know to be of wider application than those made for the hooked glochidia.

When the same fish is used, the results for the several species of *Lampsilis* are very uniform and we can thus discuss the parasitism of this genus as a whole; but we do not find the same mussel giving uniform results with all species of fish. The glochidia of this genus have been used successfully for the infection of blue-gill sunfish (*Lepomis pallidus*), yellow perch (*Perca flavescens*), crappie, large-mouth black bass, rock bass,

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the red-spotted sunfish (*Lepomis humilis*), and the green sunfish (*Apomotis cyanellus*). As with the hooked glochidia, the infections have all been made upon fish under 6 inches in length, upon which these glochidia remain in numbers only on the gill filaments, although during infection some may become attached to and even embedded upon fins and other external parts. Harms (1908) concludes that the hookless type persists in much greater numbers on the fins of small than of large fish, and that the hooked type will survive upon the gills if large fish are used. It is doubtless true that the size of the gills and fins is an important factor in determining the place of attachment for each type, since the hookless form is better adapted for holding to a delicate surface like a gill filament or a fine fin, while the hooked type seems likely to be easily torn from such a surface. When the hookless form does once become established upon an

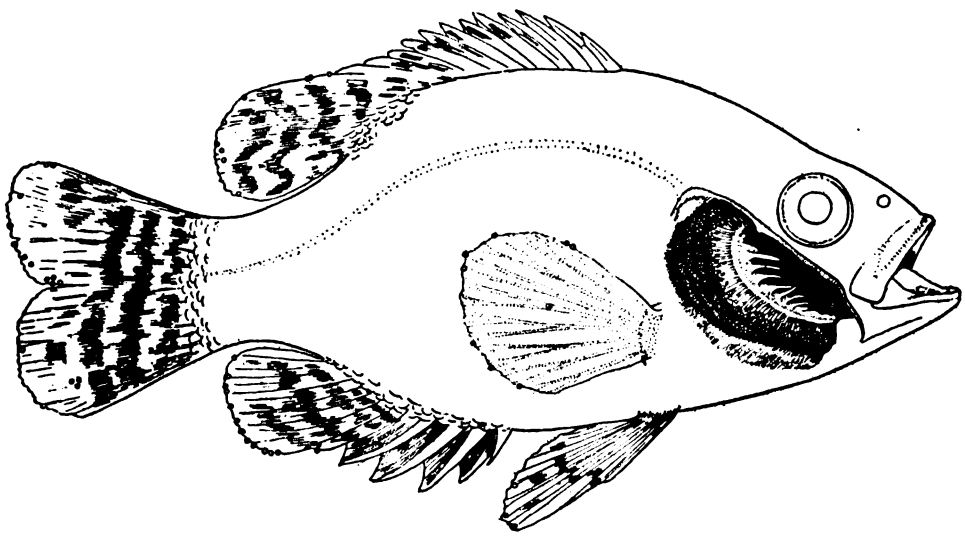

FIG. 2.—Rock-bass (*Ambloplites rupestris*) infected with glochidia of *Lampsilis ligamentina*. About 2,500 were successfully carried through the metamorphosis by each fish in this infection. Note the large number on the gills.

external part, it will develop there without mishap, as shown by the figure of a hooked and a hookless glochidium developing side by side upon the margin of a fin (fig. 29, pl. x). Within the mouth cavity these glochidia become attached to the gill bars and rakers, if these parts are covered by a sufficiently delicate epithelium, though they are always found in the greatest numbers upon the gill filaments. In most of our infections the filaments are more heavily infected toward their outer ends (fig. 43, pl. xi), the distribution varying somewhat with the species of fish. For example, successful infections of rock bass with *Lampsilis ligamentina* show about seven glochidia upon the distal third of the filament to one upon the proximal two-thirds; of large-mouth black bass about 3 to 1, and of yellow perch about 1½ to 1—differences which are probably due to some particular configuration of the mouth parts, which causes the glochidia to fall more upon one region of the filaments than another.

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In a fish which will carry a given glochidium successfully, over-infection of the gills is easily accomplished and easily fatal, although species of fish differ greatly in the amount of infection they are able to withstand without serious mortality. In one of our most successful combinations (rock bass infected with *Lampsilis ligamentina*), fish 4 inches in length were estimated to be carrying in the neighborhood of 2,500 glochidia, an average of more than two for every filament of the gills and yet there was almost no mortality among the fish. A rock bass from this infection is shown in text figure 2, which also illustrates the distribution of the glochidia on a single fish. In this case the success of so heavy an infection is perhaps explained by the distribution of the glochidia upon the gill filaments, for we found by count that there were about seven near the tips to one on the proximal two-thirds of the filament, and thus the greater part of every filament was left unchanged and in full functional condition, while in other infections (large-mouth black bass with *L. ligamentina*), where a much greater proportion of the glochidia were upon the sides of the filaments, the mortality of the fish was heavy, although the amount of infection was much less. A gill of the latter fish infected with these glochidia is shown in figure 39, plate XI. The number estimated for this fish, which was 4 inches in length, being only 450, is less than the optimum.

Implantation upon the filaments occurs in a manner similar to that of the hooked glochidia upon the external parts, but much more rapidly. Figures 35, 36, 37, and 38, plate XI, show the appearance at 15 minutes, 30 minutes, 1 hour, and 3 hours, respectively, after infection, and our observations, showing that the cyst is completed within from 2 to 4 hours, agree with what Harms (1909) has found for gill infections. The proliferation will even continue after the gill has been cut from the fish and placed in a watch glass for observation under the microscope (fig. 54 and 55, pl. XIII). An immediate result of the cyst formation is the obliteration of the lamellæ upon either side of the gill filament, which thus becomes smooth and slightly swollen in the vicinity of the glochidium (fig. 43, pl. XI). Figures 34 and 43, plate XI, show the general and detailed appearance of the cysts and the diversity in the angles at which the glochidia are attached.

The older statement that the hooked glochidia are fin and the hookless gill parasites finds, therefore, confirmation from our work, although it would be better to say that the hooked attach most successfully to large strong margins like those of the fins, and the hookless to soft and fine filamentous structures like the gills in fish of moderate size. The reactions of the two types of glochidia to mechanical and chemical stimuli, with respect to the part they play in attachment, have already been discussed.

SUSCEPTIBILITY OF FISHES TO INFECTION.

The susceptibility of different fishes to infection is a matter which has not been sufficiently considered by any previous investigators. We have evidence that some species are much less susceptible than others to one or the other type of glochidium, and that in these cases any considerable infection is an impossibility. The most striking instances of this are the German carp, certain minnows, and the darters.

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In the case of the carp, while the fish is admirably suited to carrying the hooked glochidia of *Anodonta* and *Symphynota*, we have never been able to secure a successful infection of the gills with the hookless glochidia of the genus *Lampsilis*. The disappearance of the hooked glochidia of *Anodonta* and *Symphynota* from the gills of the carp may be due to the pulling away of these large and heavy glochidia from the delicate gill filaments, as suggested in our consideration of the survival of the two types of glochidia upon fins and gills, respectively. The disappearance of the hookless glochidia of *Lampsilis* from both gills and fins of the carp can not be explained in this manner; it suggests rather that there may be some reaction of the host's tissues comparable to the processes which confer immunity against parasitic bacteria in higher vertebrates. With minnows (*Notropis cayuga* and *N. lutrensis*) 2 to 4 inches in length, we have not been able to secure any considerable infection with the glochidia of *Symphynota complanata*, for, although they will attach in large numbers during infection, they all drop from the fins and gills within a few days. The fins of these minnows are much more delicate than those of the carp, and the explanation is perhaps that so large a glochidium is easily torn away; but the large-mouth black bass has hardly a delicate fin, and for this fish we have records of infections where no glochidia of *S. complanata* became attached during an exposure sufficient for the attachment of many to the gills. In this latter case, the extreme activity of the fish must be considered as a factor which might keep the hooked glochidia from attachment to the fins.

Darters (*Etheostoma cœruleum spectabile*) 1½ to 2 inches in length can not be infected successfully with the glochidia of *Lampsilis*, for although they may fasten so thickly to the fins that many fish die during the first day after their exposure, the surviving fish will slough off considerable portions of the fins and within a week show only the healed and regenerating parts as an indication of their recent experience. The gill slits were so small in these fish that only an occasional glochidium was found upon them.

Such cases as these are of great importance and should be followed up to determine whether the simple mechanical conditions like over-infection, delicacy of fin, or configuration of the mouth parts can give a satisfactory explanation; or whether the histological changes of which the fish is capable, under stimulation by the glochidium, must be regarded as the cause of its immunity. We have not carried out a sufficient number of experiments to feel sure that the simpler explanations can be excluded. In any case, it is interesting that fish like the minnows and darters, which live close to the bottom, are not likely to become heavily infected by some of our most common glochidia.

BEHAVIOR OF FISHES DURING INFECTION.

The behavior of the fish during infection is a matter of some importance and has been already mentioned in an incidental manner. The rock bass, large-mouth black bass, and blue-gill sunfish, which are very active and which consequently exhibit powerful respiratory movements, are well adapted to artificial infection, and the proper suspension of the glochidia in the water is secured by the movements of the fish alone. The crappie, which are sluggish and easily killed by handling, require some special device to

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insure the optimum infection and are not well suited for work on a large scale because of their behavior during infection. Fish which rest upon the bottom are sometimes not so favorable as they might seem because they do not move about enough to keep the glochidia in motion. While other features may be of greater importance, the behavior of the fish as affecting the distribution of the glochidia in the water should always be considered in deciding how useful any fish may be for purposes of infection.

INFECTION OF FISH IN LARGE NUMBERS.

The infection of fish in large numbers has been attempted with a view to determining the feasibility of extending the methods described above to wholesale infections of fish in a hatchery. As a result of two such attempts, we have no doubt that the successful development of the methods needed for infection in connection with the artificial propagation of mussels is only a matter of a little study in a properly equipped station. In December, 1907, about 25,000 small fish, under 6 inches in length, were placed at our disposal at the substation of the Bureau at La Crosse, Wis., and we were able on this occasion to infect by wholesale methods about 12,000 blue-gill sunfish, 3,700 yellow perch, 7,000 catfish, 2,000 crappie, 150 rock bass, 150 carp, and 100 roach. The greater number of these fish were infected with the glochidia of *Lampsilis ligamentina*, and, considering the fact that this was our first experience with so large a number of fish, the results were satisfactory. Smaller lots were infected with the glochidia of *L. anodontoides* and *L. recta*, the results giving every indication that these two species are essentially like *L. ligamentina* in the conditions of their development. The most successful infections were obtained by placing from 100 to 200 fish in a common galvanized iron washtub about two-thirds full of water. It was found that by adding to this body of water the glochidia obtained from two or three specimens of *Lampsilis*, and, when it seemed necessary, stirring the water by hand, tolerably constant results could be secured. Our difficulties were with over- rather than with under-infection. It was also possible to use the same tub a number of times without changing the water or adding to the stock of glochidia. Infection was also attempted by lowering the water in the large retaining tanks of the station to a depth of 4 inches and confining the whole number of fish which had been held in the full tank to this much smaller body of water. This method was found, in the absence of any attempt to keep the glochidia properly distributed through the water, quite inadequate and it became necessary to reinfect these fish in the tubs.

The mortality of the fish in these experiments was decidedly in excess of what one might expect for uninfected fish kept under similar conditions, a result clearly due to the over-infection which is the one thing most to be guarded against. At the end of six weeks some of the remaining fish were liberated in the west channel of the Mississippi River at La Crosse, a locality which we then believed might be suitable for this species of *Lampsilis*.

These infections were made under conditions of limited time and equipment and were wholly tentative, the aim being to make a test of our methods on a large scale. We revisited La Crosse a month after the infection, making careful examinations of the

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fish and by shipping several hundred to Columbia were able to follow the development of the glochidia under the conditions in our laboratory. The results were probably as favorable as could have been expected under the circumstances.

In December 1908 a similar infection was attempted with about 6,200 large-mouth black bass and 3,800 crappie in the station of the Bureau at Manchester, Iowa. Upon this occasion the glochidia of *Lampsilis ligamentina* were again used in a majority of the infections, similar results being obtained with *L. anodontoides*, *recta*, and *ventricosa*, which were used for the minor infections. The black bass took the glochidia very readily and, having had only a limited experience with this species of fish, we gave them an amount of infection equal to that which had been carried successfully by the rock bass infected at La Crosse in the previous experiments. The infection was estimated at from 2,000 to 2,500 glochidia to a fish 4 or 5 inches in length. This proved entirely too heavy for the large-mouth black bass and the mortality among them amounted to about 55 per cent in the 30 days they were under observation. By the third day after the infection the hypertrophy of the gill tissue was so great as to be at once noticeable to the eye, and this was clearly the cause of death. An infection of not more than 1,000 glochidia per fish would have been more nearly the optimum load.

The crappie did not take the infection well despite longer exposure, the reason for this being the size of their gill slits and their behavior as already discussed, and we do not consider small fish of this species favorable for infection with any of the glochidia from mussels which are of commercial importance.

Thirty days after these infections the surviving fish were liberated in the Maquoketa River near Manchester, in a situation where the conditions were favorable for mussels and where the presence of a dam below the point of liberation, together with the absence of mussels of this species, made it seem possible that at some later period their appearance in this locality might be traced to this experiment. We have never made any subsequent examination of this stretch of the river with this in view, a thing which should be done by one of the parties engaged in the field work of the mussel investigation.

These two experiments in the wholesale infection of fish, while disappointing in some respects, give no indication of any insurmountable difficulties. It is fair to conclude that a little experimentation under hatchery conditions will make it as easy to carry the glochidia through their metamorphosis in large numbers as we have found it in small lots of fish kept in aquaria. The high mortality of the fish, being so clearly a matter of over-infection, is a thing which can be guarded against without reducing too greatly the load of glochidia which the fish may carry. It is then only a matter of discovering the most suitable species of fish and finding out how best to handle them in large numbers.

One thing which seems necessary for the rapid and uniform infection of fish in large numbers is a device which will bring about a uniform distribution of the glochidia in the water during the whole period of the fishes' exposure. Without something of the sort it will hardly be possible to handle large numbers of fish with constant and uniform results. We have tried, though not very extensively, two means of effecting

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this. The first consisted of a two-bladed propeller fastened in the middle of the bottom of a tub and rotated slowly, there being enough space in the water above the blades to allow the fish room to escape the stroke. This device was not very satisfactory, but as it was operated by hand and the blades roughly constructed, effective use might be made of a more carefully adjusted mechanism of this type. A second and more promising device consists of a branched system of iron pipes bored with many small holes (text fig. 3), through which fine jets of water are forced out at the bottom of a tank. The amount of pressure in these fine jets can be easily regulated from the main supply pipe, and the height to which the glochidia will be driven from the bottom is thus controlled. The tank may be allowed to overflow at the top and the glochidia

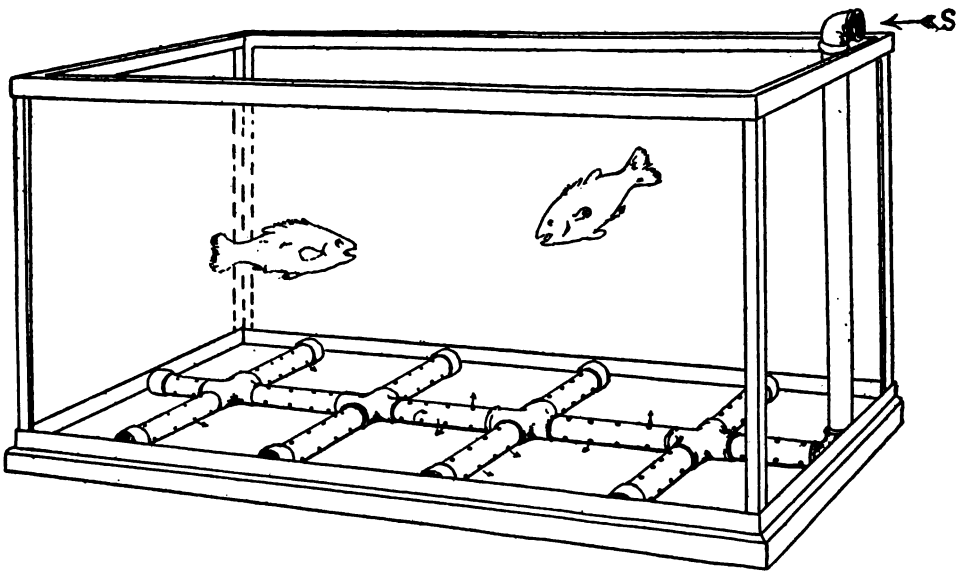

FIG. 3.—Apparatus for keeping glochidia suspended in water while fish are being exposed to them for gill-infections. Tap water entering at S issues in fine jets through the very small holes placed along the top and sides of the pipes on the bottom of the aquarium, and an even distribution of glochidia throughout the water is thereby maintained. By regulating the force of the water entering the pipes at S the glochidia are prevented from rising to the top of the aquarium and escaping with the overflow.

prevented from being carried off in the overflow by so adjusting the force of the jets that the glochidia will not rise quite to the surface. This device keeps the glochidia suspended in a very uniform way, and it may prove to be just what is needed for the uniform infection of large numbers of fish.

CONDITIONS NECESSARY FOR SUCCESSFUL INFECTION.

Three factors should be considered in attempting the infection of any species of fish with glochidia, namely, the uniform suspension of the glochidia in the water, the reaction of the glochidia when stimulated by mechanical or chemical contact with the fish, and the reaction of the fish's tissues after the glochidium has become attached.

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In any attempted infection of fish in large numbers, careful tests should first be made upon a few fish in small dishes, with microscopic examination of the infected parts from fish killed during the time of infection and for several days following, or until it is clear that the glochidia have become safely established in their host's tissues. After even limited experience one learns approximately the number of glochidia needed and can determine roughly their suspension in the water by taking samples at random in a pipette, which when held against the light shows clearly the individual glochidia. During infection it is possible to pick out individual specimens and by lifting up the operculum of the living fish, examine the gills with a hand lens. The glochidia are then seen individually and the progress of the infection can be watched. Fin-infecting glochidia may be seen individually if a fish is placed in a small dish against a black background.

It is not difficult to determine by these means the optimum time for the exposure. When 100 fish 5 to 6 inches in length are taken and the contents of a single marsupium of a large *Lampsilis* is placed in an ordinary washtub, infections may be obtained somewhat as follows: Rock bass, exposed 30 to 40 minutes, 2,000 to 2,500 glochidia on gills of each fish; large-mouth black bass, exposed 15 to 20 minutes, 500 to 1,000 glochidia on gills; crappie, exposed 20 to 30 minutes, 200 to 400 glochidia on gills; yellow perch, exposed 20 minutes, 400 to 600 on gills; German carp (with *Anodonta*), exposed 30 to 40 minutes, 200 to 500 on fins. These figures are given as starting points for anyone attempting artificial infections and can not be taken as representing the results of precise determinations of optimum infections for the fish in question, because the means for determining the numbers and distribution of the glochidia have been only approximate. It will probably always be necessary, in the practice of artificial infection on a large scale, to have the fish examined microscopically by a properly trained observer, and this will be particularly true in the beginning of this work in hatching establishments, because the practical details of artificial infection on a large scale have yet to be solved.

DURATION OF THE PARASITIC PERIOD.

According to the experience of previous observers, the duration of the parasitic period varies inversely with the temperature of the water (Schierholz, 1888; Harms, 1907–1909). Although we have found this to be true in general, our experiments have not shown so definite a relation between temperature and parasitism as has been described by Harms, for example, and it is quite possible that other factors, which are obscure, exert a modifying influence upon the length of time the glochidia remain on the fish. Harms found that the glochidia of *Anodonta* completed the metamorphosis in 80 days at a temperature of 8° to 10° C; in 21 days at 16° to 18°; and in 12 days at 20°; while in the case of the hookless glochidia of *Unio* (which are gill parasites) the period was 26 to 28 days at a temperature of 16° to 17°. He is inclined to attribute the somewhat longer time required for the metamorphosis of *Unio* to the fact that the glochidia in this genus when discharged are in a less advanced stage of development than are those of *Anodonta*—a difference that exists between all hookless and hooked glochidia.

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A few typical cases, selected from our records of infections are given in the accompanying table, which illustrates the far greater variability in the parasitic period than that observed by Harms.

TABLE SHOWING INFECTIONS WITH GLOCHIDIA.

Experiment.	Date.	Mussel.	Fish.	Exposure.	Young mussels liberated.	Duration of parasitism.	Av. temp. during parasitism.
HOOKED GLOCHIDIA.							
				Min.		*Days.*	°*C.*
1	Dec. 3, 1909	Symphynota complanata.	Apomotis cyanellus	15	Dec. 17–19	14–16	16.0
2	Dec. 17, 1909	do	do	15	Jan. 1–4	15–18	16.3
3	Jan. 7, 1910	do	Pomoxis annularis. Apomotis cyanellus. Pomoxis annularis.	12	Jan. 18–21	11–14	16.0
4	Apr. 5, 1910	do	Apomotis cyanellus	30	Apr. 14–18	9–13	17 8
HOOKLESS GLOCHIDIA.							
5	Feb. 19, 1910	Lampsilis ligamentina	Apomotis cyanellus	9	Mar. 5–12	14–21	17.8
6	Mar. 6, 1909	do	do. Micropterus salmoides.	10–15	Apr. 7–11	32–36	19.1
7	Apr. 8, 1909	do	Apomotis cyanellus. Micropterus salmoides.	10–15	Apr. 27–May 1	19–23	20.3
8	Apr. 13, 1910	Lampsilis subrostrata	Apomotis cyanellus	8–15	May 2–8	19–25	18.1
9	May 2, 1910	Lampsilis ligamentina	do. Micropterus salmoides.	7–10	May 15–26	13–24	18.1
10	May 3, 1910	Lampsilis subrostrata	Apomotis cyanellus	50	May 17–25	14–22	18.1
11	July 29, 1909	Unio complanatus	Perca flavescens	7–14	Aug. 12–14	14–16	23.0
12	Aug. 5, 1908	Quadrula plicata	Micropterus salmoides	30	Aug. 17	12	24.4

In the case of *Symphynota complanata*, which has hooked glochidia essentially like those of *Anodonta*, the period varied from 9 to 18 days at average temperatures of 17.8° to 16° C., as compared with Harms's 21 days at practically the same temperature. At lower temperatures, about 10°, we have recorded a period of 74 days for *S. costata*.

The absence of a close correspondence between the temperature and the duration of the parasitism has been much more conspicuous in the case of hookless glochidia, which have shown not only a remarkable range in the period but a considerable irregularity in different experiments made at about the same temperature. The shortest period recorded by us was seven days in an infection of black bass with the glochidia of *Lampsilis subrostrata* and *L. recta* in April when the average temperature during the parasitism was 20.5°, but this unusual time was only observed in this one instance. A still more remarkable case, but at the opposite extreme, was an infection of black bass and crappie with the glochidia of *L. ligamentina* and *L. recta* which remained on the fish for 13 to 16 weeks. The infection was made in November and the young mussels were liberated during a period of about three weeks in the following February and March; during the parasitism the temperature varied from about 16° to 18.° The cause of the extreme duration in this case is not known, for in no other experiment at the same temperature has the parasitism lasted for more than 25 days.

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As may be seen in the table, with hookless glochidia (aside from the extreme cases mentioned) the variation in the period has been from 12 to 36 days at average temperatures ranging from 24.4° to 17.8°; but even at practically the same temperature the difference may be quite marked, as in experiments no. 8 and no. 9. Experiment no. 6 should be noticed as being a case in which, contrary to expectation, quite a long period (32 to 36 days) was recorded at 19.1°, whereas in other experiments (no. 5 for example) the time was only 14 to 21 days at the lower temperature of 17.8°.

It would seem clear that, although within certain wide limits the duration of the parasitism is dependent upon the temperature of the water, nevertheless other factors may enter into the case to either accelerate the metamorphosis or prolong it over a period which is much longer than the usual duration of the parasitism. These factors would seem to be associated with individual physiological differences in the interaction between the fish and the parasite and are probably nutritive in nature, for on one and the same fish some glochidia may remain several days longer than others.

As may be seen from an examination of the table, in which the period of liberation is given in each experiment, not all of the young mussels leave the fish at the same time, but, on the contrary, the liberation may occupy a week or more. Harms found that it required from 5 to 6 days, the greater number leaving the fish during the middle of the period. Our experience has usually been in accord with these observations, but we have found the period to be somewhat more variable, from 2 to 11 days, or even much longer.

IMPLANTATION AND CYST FORMATION.

As has been described, the glochidium attaches itself to the fish by closing its shell firmly over some projecting region which can be grasped between the valves, like the free border of a fin or a gill filament. In so doing, a portion of the epithelium and underlying tissue, including blood vessels and lymphatics and varying in amount with the extent of the "bite," becomes inclosed within the mantle space of the glochidium. This tissue early disintegrates into its cellular constituents, which are taken up by the pseudopodial processes of the larval mantle cells, and, as Faussek (1895) has described, are utilized as food during the early stages of metamorphosis. In figure 60, plate xv, drawn from a glochidium six hours after attachment to a fin, the disintegrated tissue, consisting of loose epithelial cells, blood corpuscles, and fibers which lie scattered in the mantle cavity, is seen in the process of being ingested by the mantle cells. Figure 61, plate xv, shows a later stage, 24 hours after attachment, in which the detritus has been entirely taken up, and the mantle cells are now heavily charged with food material.

Almost immediately after attachment proliferation of the epithelium begins as the initial step in the formation of the cyst which eventually incloses the entire glochidium. The overgrowth of the larva has been described by Faussek (1895) and Harms (1907–1909) as a healing process on the part of the fish's tissues, resulting from the irritation caused by the wound. The proliferation starts around the line of constriction produced by the pressure of the edges of the valves on the epithelium, and, since the glochidium lies between and prevents the immediate closure of the lips of the wound, the extending

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epithelium is forced to slide up over the surface of the shell on all sides, until the free margins meet and fuse over the back of the larva, as may be understood by reference to figures 59 to 61, plate XV, and 35 to 38, plate XI.

So rapid is the overgrowth, especially in the case of implantation on the gills, that it would seem that something more than the mere mechanical irritation produced by the glochidium is concerned in causing the proliferation of the epithelium. We have, therefore, carried out a series of experiments with a view to determining whether or not a chemical stimulus is provided by the larva, and by using various methods have studied the action of glochidial extracts on the epithelium of both fins and gills. The results have been entirely negative, although the question has by no means been settled by the experiments which have been thus far attempted. By further improvements in the technique, some of the difficulties involved in the investigation, which is still in progress, may be overcome.

The process of implantation and cyst formation may be readily observed on the filaments of an excised gill, which under favorable conditions will live long enough in a dish of water to enable one to see the glochidium completely covered by the proliferated epithelium. Figure 54, plate XIII, drawn from the living excised gill, shows the distal end of a single filament bearing a glochidium of *Unio complanatus* which has become nearly covered by the walls of the cyst. In this case the gill was cut from the fish two hours after the infection and the drawing was made an hour later; immediately after the excision of the gill this particular glochidium was hardly half covered. The same glochidium was kept under observation, and two hours later (five hours after the infection) the sketch was made which is reproduced in figure 55, plate XIII. By this time the cyst, which is seen to have very thick walls, was completed, and formed a prominent mass near the end of the filament. Shortly afterwards the tissues of the gill began to disintegrate, but for at least three hours they remained alive and the proliferation of the epithelial cells proceeded rapidly, the entire process of cyst formation taking place in a perfectly normal manner.

The histological changes which the epithelium undergoes in the formation of the cyst have been studied in this laboratory by Miss Daisy Young, and, as her results will soon be published in detail, only a brief reference will be made in this place to the essential points involved in the cellular changes occurring during implantation of the glochidium.

Figure 59, plate XV, shows a very early stage, 15 minutes after attachment, in the formation of the cyst on the fin of a fish which had been infected with the glochidia of *Symphynota complanata*. The section is taken transversely through the glochidium and the free border of the fin on which the parasite has a firm grip. The mass of tissue, consisting of epithelial cells, connective tissue, and blood vessels in the mantle chamber of the glochidium, is the edge of the fin which was inclosed between the valves when attachment was effected. Already the proliferation of the epithelium is beginning in the neighborhood of the constriction, where two mitoses may be seen on the right in the figure. At the edges of the wound caused by the closure of the shell some of the

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epithelial cells are undergoing degeneration, while on the left of the section quite a patch of these cells is sloughing off, a not infrequent occurrence. The region of most active growth and multiplication of cells is just below the line of constriction, and, as the cells at this level increase in number, they appear to push those lying above them up over the outside of the shell, so that the actual covering of the glochidium is due largely to this mechanical gliding of the epithelium over its surface. Sections give no conclusive evidence of amitotic division, while mitoses are generally abundant in the region of active proliferation. An intermediate step in the process of implantation is illustrated in figure 60, plate XV, less highly magnified than the last figure, which shows a glochidium about half covered in six hours after attachment. The free edges of the cyst wall eventually meet over the dorsal side of the glochidium, where they then fuse. Figure 61, plate XV, shows a case of complete implantation on a fin at the end of 24 hours; now the epithelial covering is continuous and the glochidium entirely inclosed. The wall of the cyst is seen at this time to be quite thick, but it usually becomes thinner later on as the cells composing it flatten down. In the last two figures the mantle cells of the larva clearly show epithelial nuclei and cell detritus which have been ingested.

In figures 62 and 63, plate XV, two stages are represented in the formation of the cyst on gill filaments, taken at one hour and three hours, respectively, after attachment. The glochidia are those of *Lampsilis ligamentina*. In figure 62, plate XV, the proliferation has made some progress, especially on one side, and three or four mitotic figures are seen just below the glochidium and near the raw edge of the constricted epithelium. A large mass of the tissues of the filament is also shown in the figure inclosed within the mantle chamber of the glochidium. Figure 63, plate XV, represents a stage when the process is nearly completed and the edges of the epithelial covering have met but not yet quite fused. The cyst wall in this case is much thinner than that shown in figure 61, plate XV, but its thickness is quite variable.

In about one week after attachment, as a rule, the wall of the cyst begins to assume a looser texture, the intercellular spaces becoming infiltrated with lymph, and from this time on to the end of the parasitic period there is little further change in its structure.

Before liberation of the young mussel, the valves open from time to time and the foot is extended. By the movements of the latter the cyst is eventually ruptured, its walls gradually slough away, and the mussel thus freed falls to the bottom.

Portions of the wall of the cyst often adhere to the shell after liberation, while, if the young mussel has hooks, it may hang for a time by shreds of the fin in which the hooks are embedded, as seen in figure 24, plate IX.

METAMORPHOSIS WITHOUT PARASITISM IN STROPHITUS.

In a brief paper (1911) we have recently announced the discovery that in the genus *Strophitus* Rafinesque the metamorphosis takes place in the entire absence of parasitism, and, since the life history of this form is without a parallel in the Unionidæ, so far as is known, reference may be made again to the interesting conditions which obtain in its development.

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It has been known for a long time that in *Strophitus* the embryos and glochidia are embedded in short cylindrical cords which are composed of a semitranslucent, gelatinous substance, and that these cords, which are closely packed together, like chalk crayons in a box, lie transversely in the water tubes of the marsupium. The blunt ends of the cords are seen through the thin lamella of the outer gill, which in this genus, as in *Anodonta* and others, constitutes the marsupium. The position of the masses of embryos, while contained within the gill, is so unusual that Simpson in his "Synopsis of the Naiades" established a special group, the Diagenæ, for *Strophitus*—the only genus of the family in which this peculiarity exists. In other genera the embryos are conglutinated more or less closely to form flat plates or cylindrical masses, each one of which is contained in a separate water tube and lies vertically in the marsupium.

So far as we are aware, Isaac Lea (1838) was the first to observe this interesting arrangement which he described and figured, rather crudely to be sure, in *Strophitus undulatus* (*Anodonta undulata*). In several subsequent communications (1858, 1863) he added further details and illustrations, and also mentioned the occurrence of the transversely placed cords, or "sacks," as he called them, in *S. edentulus*. He recorded the former species as being gravid from September until March, and described the extrusion of the cords from the female, as well as the remarkable emergence of the glochidia from the interior of the cords after the latter have been discharged.

> The sacks were discharged into the water by the parent from day to day, for about a month in the middle of winter. Eight or ten young were generally in each sack, but some were so short as only to have room for one or two. Immediately when the sacks came out from between the valves of the parent, most of the young were seen to be attached by the dorsal margin to the outer portion of the sack, as if it were a placenta.

The essential points in these observations have since been verified by other investigators. Sterki (1898), following the suggestion of Lea, has called the cords, which differ strikingly from the conglutinated masses of *Unio* and other genera, "placentæ," thus indicating that he considered them to have a nutritive function. He also described the extrusion of the glochidia, when placed in water, and their attachment to the cord "by a short byssus thread whose proximal end is attached to the soft parts of the young." He further states that the glochidia are inclosed in the placentæ when the latter are first discharged, and that after their extrusion they remain attached for some time.

Strophitus edentulus, which Ortmann (1909) regards as identical with *undulatus*, is a rare species in all of the localities in which we have collected mussels, and, until recently, our only observations on this form were made upon a few gravid individuals which were taken in the Mississippi River near La Crosse, Wis., during the summer of 1908. Mention has already been made of our records with reference to the breeding season of *Strophitus*.

After verifying the main observations of Lea and Sterki, so far as was possible at that season of the year, we examined the glochidia carefully with a view to determining whether their subsequent life history would exhibit any peculiarities, as might be suspected from their relation to the cords. At that time we did not observe the normal

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discharge of the cords by the female; but we removed them from the marsupium, placed them in water, and, after the glochidia had emerged (fig. 46, pl. XII), employed various means to bring about their attachment to fish. None of these attempts, however, was successful, although the fish were left in small dishes containing many cords for as long a time as 12 hours. In the light of these results, which indicated the inability of this glochidium to attach itself to fish, and in view of the fact that the cords so evidently seemed to be a nutritive device, we felt it to be highly probable that in this species the metamorphosis would be found to occur in the absence of parasitism—a prediction which has been recently verified.

On February 6, 1911, a single female of *Strophitus edentulus*, which had been kept in the laboratory since the preceding November, was seen discharging its cords from the exhalent siphon. The discharge continued until March 25, and during that time the cords were thrown out in varying numbers from day to day. They measured from 2 to 10 mm. in length and about 1 mm. in diameter, although they became more or less swollen after lying in the water for a time. Each cord contained from 10 to 24 glochidia arranged in an irregular row. In many cases the glochidia emerged from the cords in a few minutes after the latter were discharged, and then usually remained attached by the thread in essentially the same manner as has been described by Lea and Sterki (fig. 46, pl. XII). The thread, which is apparently a modified larval thread, is continuous at its distal end with the egg membrane, which generally remains embedded in the cord; so intimate, in fact, is the union between the two that at times the membrane, adhering to the thread, is dragged out of the cord when the glochidium is extruded, in which case, of course, the glochidium becomes entirely detached from the cord.

All attempts to infect fish with these fully formed glochidia were again unsuccessful, even when the exposure was of long duration. Within a few days the extruded glochidia died in spite of every effort to provide the most favorable conditions for their maintenance.

When the cords first began to be discharged, one of our students, Miss Daisy Young, happened to notice that not all of the larvæ were extruded, and that among those which remained in the cords some had lost the larval adductor muscle, possessed a protrusible foot, and showed other signs of having undergone the metamorphosis. Upon careful examination this was found to be true, and it was discovered that these young mussels—for such they undoubtedly are—are subsequently liberated by the disintegration of the cord *after having passed through the metamorphosis in the entire absence of a parasitic period.* We, therefore, have concluded that the emergence from the cords in the glochidial stage is premature, due possibly to some change which has taken place in the gelatinous substance surrounding them as a result of free contact with the water, or to release from the pressure to which they are subjected while in the marsupium. It is perfectly evident that these glochidia neither become attached to fish nor undergo any further development; they have simply come out too soon and are lost.

The young mussels, on the other hand, which have developed inside the cords, when liberated by the disintegration of the latter or removed directly by teasing, are found to

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have reached as advanced a stage of development as is attained by any unionid at the time it leaves the fish. They closely resemble the young of *Anodonta* at the close of the parasitic period, and upon examination have been found to possess the following structures: The anterior and posterior adductor muscles; the ciliated foot; two gill buds on each side; a completely differentiated digestive tract, including mouth, esophagus, stomach intestine, and anus; liver; the cerebral, pedal, and visceral ganglia; otocysts; the rudiments of the kidneys, heart, and pericardium; while they also show a slight growth of the permanent shell around the margin of the shell of the glochidium (fig. 45, pl. XII). The larval muscle has completely disappeared, although some of the mantle cells of the glochidium, as well as the hooks of the shell, are still present. They crawl slowly on the bottom of the dish by the characteristic jerking movements of the foot, after the manner of the young of other species at a corresponding stage, although the valves of the shell gape more widely apart and the foot is shorter and less extensible. We have not succeeded as yet in keeping them alive for more than 10 days, but it is difficult in the case of any species to maintain young mussels of this age under laboratory conditions.

One of these young mussels after removal from the cord is shown in figure 45, plate XII, in which many of the organs of the adult or their rudiments are clearly indicated. A comparison will show that it is essentially as advanced in its development as the young of *Anodonta* when it is liberated from the fish (cf. Harms's figures, 1909, and also our fig. 47, pl. XII, of *Symphynota costata*).

The conclusion is inevitable that we have here to do with a species which has no parasitism in its life history, although the presence of hooks and other typical glochidial structures would indicate that it has originated from ancestors which possessed the parasitic stage like other fresh-water mussels. The cord is undoubtedly to be interpreted as a nutritive adaptation which arises in the marsupium during the early stages of gravidity, since the young embryos are at first contained in an unformed viscid matrix and the cords are a later product.

The whole history of this exceptional species warrants a more detailed study, and Miss Young is now engaged in such an investigation. When her work is completed we hope that it may include the entire course of development, the method of formation of the cords, and the rearing of the young mussels during a much longer period than has thus far been possible.

V. ATTEMPT TO REAR GLOCHIDIA IN CULTURE MEDIA.

Since the relation of the glochidium to the fish is essentially a nutritive one, it seemed to us that it should be possible to rear the larvæ through the metamorphosis artificially, provided a suitable nutritive medium could be found, and accordingly a series of experiments, with this object in view, were undertaken at our suggestion by one of our students, Mr. L. E. Thatcher. Although the result has thus far been entirely negative, we have not despaired of ultimate success, and, since the experiments are to be continued, a brief mention of the methods employed may be made in this place.

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It was natural to suppose that the blood of the fish would offer the most favorable nutritive conditions for the development of the glochidia, and hence it has been used in most of the experiments, which, moreover, have been made in the spring, when the water in the laboratory was comparatively warm and the metamorphosis, if it had occurred, would have taken place as rapidly as possible.

The glochidia of *Lampsilis ligamentina* and *L. subrostrata* were carefully removed from the marsupium with a sterilized pipette and then repeatedly washed in distilled water in order to obtain them as free as possible from bacteria and other organisms. A drop of blood was next taken from a fish's heart and placed on a cover glass and a few glochidia immediately introduced into it. The cover glass was then inverted over a hollow slide containing a moist piece of filter paper, and the chamber sealed with vaseline. Every precaution was taken to avoid contamination by bacteria. As soon as the glochidia came into contact with the blood, of course they snapped shut in the manner already described and in doing so inclosed some of the corpuscles, which it was to be presumed would be ingested by the mantle cells. Although in some cases bacteria and infusoria, probably introduced with the glochidia, appeared, in a majority of the cases the cultures remained free from foreign organisms. In the latter event the glochidia lived for a few days, but finally died without showing any indication of further development. Experiments were tried with the blood of the frog and of *Necturus*, and also with extracts of fish's tissues, bouillon and other nutritive media. In all, however, the results were negative. The failure may possibly have been due to insufficient aeration, and experiments are now being devised in which oxygen is to be introduced into the moist chambers, and it is hoped that we shall yet succeed in rearing the glochidia in nutritive media through the metamorphosis.

VI. POST-LARVAL STAGES.

BEGINNING OF THE GROWTH PERIOD AND LIFE ON THE BOTTOM.

The changes occurring during the parasitism and by means of which the glochidium becomes transformed into the young mussel, ready for life on the bottom, are more properly described by the term development than by the word growth. The latter process becomes the conspicuous feature only when the miniature mussel has left the fish. From this time onward there are very few changes to which the term development may be strictly applied; for, with the exception of the outer gill, all the important organs of the animal have been laid down and have assumed something of their definitive structure (fig. 47, pl. XII).

As soon as they are liberated from the fish the young mussels become quite active and move about on the bottom of a dish by means of the foot (fig. 18, pl. VIII, and fig. 48, pl. XII), securing a hold by flattening the ciliated distal end against the bottom, and then drawing up the body after the characteristic fashion of lamellibranchs. In these movements the cilia of the foot play an active part; they beat vigorously while the foot is being extended, and apparently are effective in part at least in causing the protrusion. When

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the foot reaches its limit of extension, the cilia stop abruptly and remain quiet while the forward movement of the body is taking place, only to resume their activity when the extension begins again. Figure 18, plate VIII, furnishes an excellent illustration of the various positions assumed as the young mussels crawl about in their twisting, jerking movements, and also shows the extent to which the shell has grown beyond the limits of the glochidial valves by the end of the first week of free life.

In the great majority of forms, as appears from the work of other investigators and our own observations, the mussel leaves the fish with only a very narrow margin of adult shell protruding beyond the glochidial outline. The shape is still that of the glochidium, although all other resemblances to this larval stage have disappeared. In the larva of *Symphynota costata* this margin of the adult shell is so narrow, even after some days upon the bottom (fig. 47, pl. XII), as not to protrude beyond the glochidial outline when the young mussel is slightly contracted. Exceptions to this supposedly universal condition have been observed by Coker and Surber (1911) in the young of *Plagiola donaciformis* and *Lampsilis* (*Proptera*) *lævissima*—forms in which there is a considerable growth of the definitive shell and presumably of the other organs during the parasitic period. These cases are unique so far as known, but in view of the small number of species which have been observed at all during this period of their existence other such exceptions may be looked for. No data bearing upon the duration or other conditions of the parasitic life are given in the paper in question, since the material studied was from the gills of a fish which had been preserved after its infection under natural conditions.

These stages immediately following the parasitism and until the mussels are about 20 mm. in length are less known than any others. They have seldom been found by collectors, and the reasons for this are made clear by the work of Isely (1911), to which we shall presently refer. Pfeiffer first observed and figured in 1821 a small shell having the glochidial outline still visible at its umbo, and other cases have been recorded, notably by Schierholz (1888). Such specimens were taken from nature and not from mussels artificially reared. Indeed, no one has yet succeeded in following individual specimens for more than a few weeks beyond the beginning of life on the bottom. Recently Harms (1907, 1908, and 1909) has obtained these stages, by rearing, more extensively than his predecessors and has figured (1907a, p. 811) the young of *Anodonta* with a very substantial increase in size at an age of six weeks after the parasitism, beyond which they could not be reared because of their destruction by small Crustacea. He concludes that the latter constitute a serious danger to the life of the young mussel.

In our own work repeated attempts have been made to rear these stages to a size which can be more easily handled, but without success. Specimens of *Symphynota costata* (fig. 47, pl. XII) and of *Anodonta cataracta* have been kept alive in small dishes containing green plants for a period of from one to two weeks after they had left the fish, and *Lampsilis ligamentina* and *subrostrata* for a period of six weeks. Little or no growth was observed after the first week. The two species of *Lampsilis* formed a conspicuous border of new shell during the first few days of bottom life (fig. 18, pl. VIII, and fig. 48,

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pl. XII) and then ceased growing although they continued to move actively about. This would indicate that the difficulty lies in the lack of a suitable food supply. Crustacea were not observed to play an important rôle, though we do not doubt the correctness of Harms's observations in this respect.

Figures 18, plate VIII, 47 and 48, plate XII, will illustrate the appearance of the young mussels at this period and an examination of figure 47 will show how extensively the organs of the future adult have been laid down. Nothing remains to suggest the glochidium save the shell, and structure and habit alike indicate that the organism is now ready for a life on the bottom essentially like that of the adult.

JUVENILE STAGES AND THE ORIGIN OF MUSSEL BEDS.

For the sake of completeness, we shall discuss briefly at this point the present state of our knowledge regarding the stages between the one last mentioned and that represented by the young mussels over 20 mm. in length, which are often found upon the natural beds. In common with the experience of other collectors, we have seldom found mussels under 20 mm. It would therefore seem clear that these early stages are not at all common in localities where the slightly later stages and the adults are found. Isely (1911) has published a preliminary note upon his study of this "juvenile" period. We shall refer to his results rather fully, since there are no other recorded observations which deal with these stages save in the way of incidental reference to single specimens. This author states the problem by saying (p. 77) that: "Much difficulty was experienced in finding young mussels for study and experimentation. I have collected many specimens from the size of a nickel (20 mm.) to a quarter (24 mm.), but mussels under the size of a dime (17 mm.) have been rare." The latter he terms the "early juvenile" stages, including in this "the period following the time when the mussel completes the parasitic stage and leaves the fish to lead an independent life until it is about 15 mm. in length. This would cover, in most species, approximately the first year of independent existence. Other periods may be designated as later juvenile and adult life." He then reports the finding of 32 specimens in this early juvenile stage representing four genera and nine species, as follows: (1) *Lampsilis luteola*, two; (2) *Lampsilis fallaciosa*, one; (3) *Lampsilis parva*, four; (4) *Lampsilis gracilis*, three; (5) *Plagiola elegans*, one; (6) *Plagiola donaciformis*, sixteen; (7) *Anodonta imbecillis*, two; (8) *Ptychobranchus phaseolus*, two; (9) unnamed species, one.

All these specimens were found in places where the water was fairly swift, from 1 to 2 feet in depth; and on a bottom of coarse gravel, the particles of which were 10 to 25 mm. in diameter. They were anchored by the threads of a byssus gland "strong enough to support the mussel in a rapid current" and capable of sustaining "the weight of a number of small pebbles without breaking."

Here then, as Isely concludes, we have the clue to the habits and ecology of these so little-known stages. The finding of representatives from so many genera and species, both heavy and light shelled, under identical environmental conditions and the presence of the functional byssus in all cases is pretty good evidence that this is the normal

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condition for early juvenile life in a wide range of forms. It is, moreover, interesting to find in the Unionidæ, as in many other lamellibranchs (e. g., *Mya* and *Pecten*) a functional byssus in the early stages, though there is no such organ in the adult.

As these results are very important and of convenience for reference in this paper we may here quote Isely's conclusions in full.

The facts noted above are closely related, not only to the ecology of the juvenile mussel, but also to the ecology of the adult.

1. They indicate the conditions essential for the most successful growth and early development of the Unionidæ. This kind of an environment gives a constant supply of oxygen and sufficient food; is frequented by suitable fish; is free from shifting sand and silt accumulation. Those mussels that drop from the fish in these favorable situations develop in large numbers, while the less fortunate, that drop in shifting sand and silt, die early.

2. In the study of the ecological factors that are inimical to mussel life more attention should be given to the consideration of the juvenile habitat. Absence of gravel bars and stony situations may sometimes explain the scarcity of the Unionidæ in certain streams and lakes where frequently water content has been thought the chief unfavorable factor.

3. It is a well-known fact that in many streams certain stretches of mud bottom are found loaded with mussels, while other areas, in the same stream, equally favorable from the standpoint of the habitat of the adult mussels, have only scattering specimens.

This distribution of the adults may be explained by the assumption (which is fairly well established by experimental study and will be discussed in a later paper) that the average mussel seldom travels far up or down the stream from the place where it begins successful development. Stretches favorable for juvenile development thus come to be the centers of dispersal in the streams where they occur. As a result, areas of mud bottom near these favorable habitats become loaded with mussels by migration.

4. In the study of the life history of the Unionidæ we may consider the embryonic, the glochidial, the parasitic, the early juvenile, and the adult as distinct periods for separate and special study.

These results of Isely's are clearly of very great importance in the problem of artificial propagation and it is to be hoped that his observations may be greatly extended in the near future. The number of different species which he has found is a most promising sign that he is on the right track, and we may hope that we shall soon reach a satisfactory understanding of this stage of the life cycle hitherto so little known.

At this point a word regarding the formation of beds may be opportune. It is a familiar fact that many species are most likely to be found congregated in beds which in some of the larger streams must have contained, before the shells came into commercial use, numbers of mussels which are hardly conceivable. Elsewhere in the stream the mussels are found scattered and wandering over the bottom. In the absence of any indication that the individuals of a species are in some manner attracted to one another, the simplest explanation of the formation of beds would be the same as that given in other cases of this sort. The conditions of food supply, current, character of bottom, etc., must differ considerably, and we may reasonably suppose that some places present the optimum conditions over an extended area and that in such a place a bed may be formed. As the mussels wander over the bottom they may by chance enter such an area of optimum conditions and will then move about less actively or come to rest, because in the absence of unfavorable conditions there is no stimulus to continued locomotion. The result is that individuals which enter are likely to remain and more keep

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coming in. This kind of an explanation has been offered, by the students of animal behavior in recent years, to account for the formation of aggregates in a great variety of the lower organisms; and it appears the most reasonable one in such cases as the one in hand, where there is no evidence that the gregariousness is due to a definite recognition of the presence of other individuals.

RATE OF GROWTH.

It has been quite generally believed, by those investigators who have given their attention to this matter, that the mussel shell grows during the warmer months of the year and that in winter there is no appreciable addition to its margin. When growth begins again in the spring, the winter's rest has left a mark which appears as a dark line on light-colored shells or as a deeper groove in others where the color is not so conspicuous. Finer lines may be found between these rings of growth, but the latter, like the rings of a tree, mark the years. It is certain that these more conspicuous lines or "rings," as we may term them, indicate an alternation of growing and resting periods in the formation of the shell. It is not entirely certain that a single growth period must always correspond to a single year; for, when any lot of shells is carefully examined, some will be found in which the "rings" are distinct and strongly suggestive of an annual increment, while others of the same size may not show these rings in any such distinct fashion, and one is forced to conclude either that the annual rings, if such they be, are not always clearly to be seen or that some mussels may grow at a very different rate from others. The examination of any considerable number of shells leads to the belief that even if the annual-ring theory can be proved conclusively the rings are often not sufficiently distinct from the intervening lines to give an unquestionable record of the age.

Assuming that these rings, when clearly seen, do represent years, it would seem that the shell grows very rapidly during the first few years of the mussel's life and after that much more slowly. To judge from the lines alone, we should say that many of the large *Quadrula* shells had reached one-half their size in ten or a dozen years and then taken forty or fifty for the remainder, so closely set are their later rings of growth; and that shells of these species can not reach the most desirable commercial size in a less period than twenty or thirty years. Since these are regarded as the best of all button shells, the outlook may seem discouraging, because, like hardwood timber, the best shells take too long to grow.

The "ring theory" if proved would not, however, make the situation so discouraging as might seem from the species of *Quadrula;* for we have in some members of the genus *Lampsilis* shells which are almost if not equally desirable, and such evidence as we have from the rings indicates that shells like these may reach a commercial size in a very few years and that even forms like the quadrulas may become marketable within a period of four or five years.

In a recent paper, Israël (1911) has reported his conclusion that there is no winter-rest period and that more than one ring may be formed in a single year. This statement

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is based upon the examination of the shell margin in mussels collected at various seasons of the year and of mussels which had been placed in wire inclosures on the bottom of the stream after having been accurately measured. The results from these plantings were fragmentary because of the accidental destruction of most of the inclosures. In one case, however, he found specimens which "when placed in the inclosure in August, 1909, and measuring 18 mm. in length, had reached, at the time of their examination in June, 1910, a length of 26 mm." He reports that other similar investigations are in progress, the results of which we shall await with interest.

Since no accurate observations on the rate of growth of fresh-water mussels have ever been made, we have attempted to secure definite data bearing upon this problem. The data obtained are derived from two entirely different lines of observation, as indicated by the headings of the sections which follow, and although meager they show that with better facilities it should not be difficult to follow individual mussels from the juvenile to the adult stages, and thus to determine their rate of growth in an accurate manner.

GROWTH OF MUSSELS IN WIRE CAGES.

While engaged in mussel investigations at La Crosse, Wis., during the summer of 1908, we collected a number of young clams (fig. 68, pl. XVII) belonging to 16 different species, and after weighing and measuring them accurately they were distributed in wire cages, which were then anchored by long wires in midstream to the piers of a bridge over the west channel of the Mississippi River opposite La Crosse. One hundred and sixty-three small mussels, belonging to the following genera and representing both thin and thick shelled forms, were planted out in this manner: *Alasmidonta*, *Anodonta*, *Lampsilis*, *Obliquaria*, *Obovaria*, *Plagiola*, *Quadrula*, and *Unio*.

Some of the cages contained only a single specimen of each species represented in it, in which case an absolute identification would be possible, should the cage be recovered later, while, if two or more individuals of a species were put in a cage together, only specimens of practically the same size were selected. In the latter case it would of course be impossible to subsequently distinguish an individual mussel, and only the average rate of growth could be determined for the individuals present. It was assumed that mussels of the same size and under the same conditions would grow at practically the same rate.

These plantings were made at intervals from June 29 to August 10, 1908. An opportunity did not present itself to make an attempt to recover the cages for over two years, but in November, 1910, Dr. R. E. Coker, who knew of the experiment, made a search while on a visit to La Crosse and was fortunate enough to find 2 of the 11 cages planted by us in 1908. One of the cages was deeply buried in the mud and all of the mussels in it were dead; as they showed little or no growth, they were evidently killed shortly after the planting. In the other cage, however, 6 living mussels were found, as follows: 3 *Lampsilis ventricosa*, 1 *Obovaria ellipsis*, 1 *Quadrula solida*, 1 *Anodonta imbecillis*. These 6 mussels, with the exception of the specimen of *Obovaria ellipsis*, were readily referred to definite individuals as recorded at the time the cage was set out. The comparative measurements and weights are given below.

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	June 29, 1908.	November 15, 1910.
Lampsilis ventricosa:		
(1)	45 by 30 mm., 16 grams	85 by 65 mm., 129.85 grams.
(2)	47 by 32 mm., 15 grams	81 by 57 mm., 115.5 grams.
(3)	47 by 30 mm., 16.5 grams	96 by 67 mm., 145.2 grams.
Obovaria ellipsis:		
(1)	52 by 52 mm., 59.1 grams	57 by 55 mm., 74.6 grams.
	(The identification of this specimen is somewhat uncertain.)	
Quadrula solida:		
(1)	35 by 36 mm., 27 grams	45 by 46 mm., 46.3 grams.
Anodonta imbecillis:		
(1)	30 by 25 mm., 8 grams	61 by 28 mm., 13.3 grams.

In each case, the first measurement is the greatest antero-posterior length of the shell, and the second the distance from the top of the umbo to the ventral margin taken approximately at right angles to the lines of growth. An interesting and important feature of these specimens is the fact that the original margin is clearly indicated by a conspicuous line on the shell of each, and as the measurements within this line correspond with the original measurements, the identification is made sure for each individual.

We quote below an analysis of the results sent us by Dr. Coker, who made the second series of measurements after the recovery of the cages:

Lampsilis ventricosa.—They have increased in length by 34 to 39 mm. and in height by 25 to 37 mm., and they now weigh approximately 7, 8 and 9 times as much, respectively, as when first put out. Furthermore, the added area of shell is divided by a conspicuous dark ring and a less distinct ring which, one is tempted to assume, represent the periods of cessation of growth during the two winters. If such an interpretation is made, the growth was accomplished chiefly during 1908 and 1909, while during the present year (1910), the mussel having reached adult size, the growth has been considerably less.

Increase in size stated by percentage (present measurements compared with original measurements). Period, June 29, 1908, to November 15, 1910, 2 years, 4½ months:

		Length.	Height.	Weight.
Specimen no. 1	per cent	188	217	812
Specimen no. 2	do	172	178	770
Specimen no. 3	do	204	223	880

The proportion of increase is slightly greater in height than in length, and the coefficient of increase in weight is, as might be expected, something like the cube of the coefficient of increase in either dimension.

Obovaria ellipsis.—The specimen has probably gained very little in length or height but materially in weight. It was nearer its adult size, is doubtless a slower growing species, and has probably gained in weight by increase of thickness of shell. But we are not so sure of the identity of this specimen.

Quadrula solida.—Has gained nearly 30 per cent in length and height and 70 per cent in weight.

Anodonta imbecillis.—Has more than doubled in length, with negligible increase in height, while it has increased 66 per cent in weight. This is particularly interesting as showing a marked change in form from the young to the adult.

Text figure 4, A and B, represents outline sketches of two of the three specimens of *L. ventricosa* described above, showing the exact size of each after the completion of the growth in the fall of 1910; the line marked *a* is the margin of the shell at the time the planting was made in 1908; while lines *b* and *c* are the two successive rings indicating cessation of growth. The two areas inclosed between these lines, representing the two chief periods of growth which have occurred, are not of equal extent in the three speci-

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mens. In A they are of about equal width, while in B the second area is much greater than the first. The area between line *c* and the margin of the shell is in all three cases very narrow, showing that, as the mussel approaches the adult size, further increase in the shell must take place very slowly. The recovered specimen of *Q. solida* shows only one broad area of growth, and a very narrow one around the margin. This mussel was relatively much nearer adult size when put in the cage than the specimens of *ventricosa*.

Dr. Coker comes to the following conclusion with respect to the age of the specimens of *L. ventricosa:*

> They are very significant, as they show clearly that growth is much more rapid than is generally suspected. Considering what the growth has been since the cages were put out, it is fair to assume that the specimens had only one year's growth at that time. That is to say, they were glochidia in the spring of 1907, and, since they must have been carried in the gills of the mother over the preceding winter, their complete age at this time (Nov. 15, 1910) is a little over four years.

Their age since the metamorphosis would therefore be about three years. Their probable history, on the above assumption, is as follows:

1. Eggs fertilized in August, 1906.
2. Glochidia discharged in spring or early summer, 1907.
3. Liberated from fish in summer, 1907.
4. Collected at age (since metamorphosis) of about one year and placed in cages June 29, 1908.
5. Recovered and remeasured, November 15, 1910.

The rate of growth of these individuals is probably typical of the genus *Lampsilis*, and the experiment indicates at least that commercial mussels may reach a marketable size in three years from the time they leave the fish. With the heavier shelled species (those of *Quadrula*, for example) the rate of growth is probably slower and a longer time must elapse before they are large enough for commercial use.

These experiments, meager as they are, are quite significant and furnish the first definite data, so far as we know, relating to the rate of growth of fresh-water mussels. With the proper facilities and the opportunity of examining the mussels at closer intervals, similar plantings could readily be made and exact information obtained on the growth of all the important species. To prevent the cages from being buried in the sand or mud would seem to be the chief precaution that should be taken in future experiments of this kind.

AN ARTIFICIALLY REARED MUSSEL.

Another experiment, although it does not throw light upon the question of the rate of growth in nature, might be mentioned in this connection on account of its significance for the problem of artificial propagation. A lot of black bass which had been infected with the glochidia of *Lampsilis ligamentina*, *ventricosa*, and *recta* at Manchester, Iowa, on December 2, 1908, were brought to Columbia, Mo., and placed in a large tank containing sand. The fish were left in the tank, where the young clams were allowed to fall off in the hope that some would survive and be later recovered. The sand was examined at intervals thereafter but never thoroughly, as the chance seemed very slight that any of the young clams were still living. On December 26, 1910, however, a single

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small individual of *Lampsilis ventricosa* was found alive and active in the sand of the same tank. There can be no doubt that it was derived from the infection referred to, as no young clams of this species had ever been in the laboratory, and no subsequent infections were made in that tank. The exact size of this young mussel was 41 by 30 mm. on December 26, 1910. It is still alive, but as late as June, 1911, it was practically of the same size. Since it is over two years old, it is evident that it is quite a dwarf, and, had it been reared under favorable conditions, it undoubtedly would have been much larger by this time. The tank in which it has spent all of its life is supplied with tap water, which is obtained from deep wells and contains little that a mussel could utilize as food, and its small size is undoubtedly due to the fact that it has been underfed from the beginning. The shell shows no indication whatever of lines of interrupted growth, but this is only what might have been expected, as the mussel has never been exposed to low temperatures. It is evident, therefore, that it has been growing continuously, but very slowly, throughout its entire life.

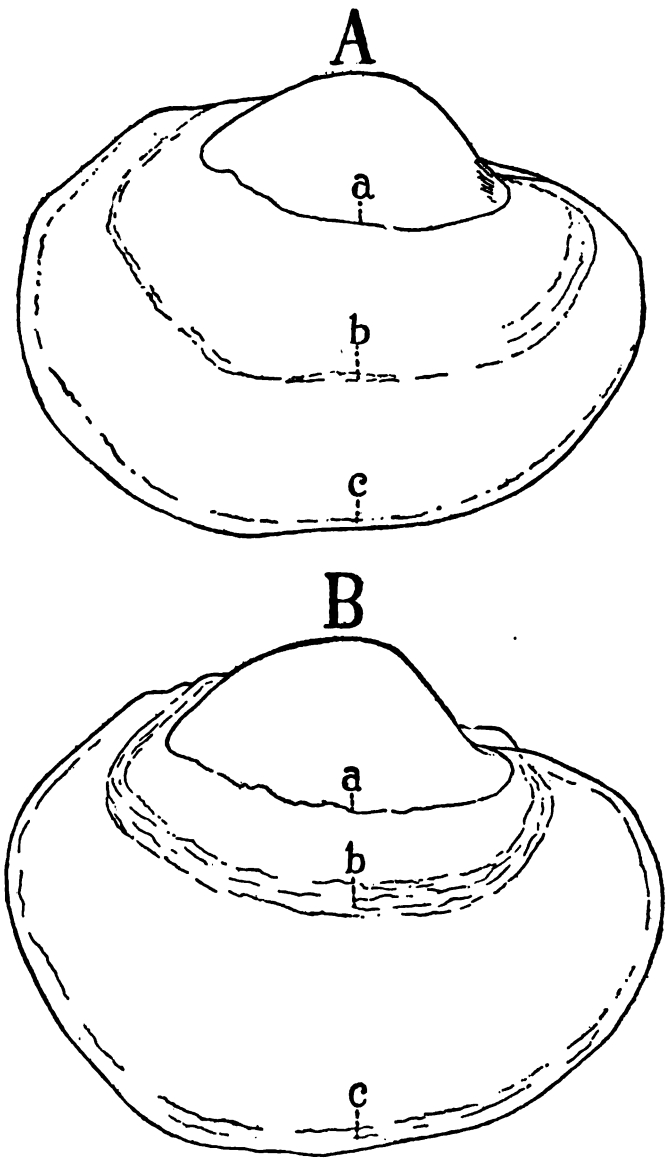

FIG. 4.—Two individuals of *Lampsilis ventricosa* recovered on November 15, 1910, after having been confined in a wire cage in the Mississippi River for two years and four and a half months. The line *a* is the original margin of the shell at the time of planting, June 29, 1908, and the lines *b* and *c* represent the "rings" which are due to the periods of cessation of growth. Natural size.

This individual, however, is of no little interest, as it is the first fresh-water mussel actually reared artificially from the glochidium, and in a sense

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furnishes a demonstration of the feasibility of artificial propagation. Had the food supply in the tank been adequate, it would now be a mussel of about two-thirds the adult size.

THE ORIGIN AND AGE OF MUSSELS IN ARTIFICIAL PONDS.

A second line of evidence bearing upon the rate of growth has been obtained in connection with an examination of certain artificial ponds in the vicinity of Columbia, Mo. In this region it is customary for the farmers to construct, for the watering of cattle, ponds in which water is held the year round by the impervious clay soil. We have examined many of these small bodies of water and have records of the approximate, if not the exact, dates of their construction. In 12 of these ponds, the ages of which are from 5 to 40 years, we have found specimens of *Lampsilis subrostrata* and *Unio tetralasmus* in some numbers, and in two of the ponds the mussels are present in very great numbers.

The occurrence of the mussels in the different ponds has been considered, first, with a view to the question of their original introduction into a given pond, and, second, their rate of growth. The first of these two considerations will be discussed here as a matter of convenience, although it should more properly be considered in a section dealing with the introduction of mussels into favorable localities.

As to their origin in the ponds, we find the facts interesting because it is quite clear that a majority, if not all of the ponds, must have been stocked with mussels which were first introduced as parasites upon fish. The significant facts in this connection are: That we have never found a pond containing mussels but no fish, although there are a number of ponds containing fish in which we have thus far failed to discover any mussels, and that none of the ponds have outlets or other immediate connections with streams in which the mussels occur, but are situated, for the most part, on high ground far from the watercourses, making it impossible that the mussels could have worked their way into these bodies of water by any ordinary process of migration. Since it is very unlikely that persons have introduced adult mussels into so many places by intent or accident, the mussels must have appeared in these ponds by natural means and the most probable of these is their introduction while parasites upon the fish with which the ponds were stocked. The transportation of small individuals attached to the mud on the feet of birds or of terrestrial animals, so often suggested as a means of dispersal in a case like this, is a possible mode of origin, although it seems hardly a probable one in view of the excellent chance the mussels would have of being introduced while still parasites.

One of the above ponds, which is about 40 by 60 feet in area and 10 feet in depth, is particularly interesting since it contains great numbers of *Lampsilis subrostrata* and also of the sunfishes (*Lepomis humilis* and *Apomotis cyanellus*), which we have found in our laboratory experiments to be very favorable hosts for the glochidia of this mussel. The mussels are of all sizes and the pond has existed for many years. We do not know its exact age nor how long ago fish were introduced. The mussels were first discovered in 1907 and have ever since been found in abundance. Their success is doubtless due,

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in large part, to the abundance of a fish favorable for their parasitism. Nothing in these specimens, nor in what we know of the history of this pond, gives a clue to the age of the mussels.

Another pond has great numbers of *Unio tetralasmus*. This pond was constructed in 1901 and during the first year was stocked with fish (the exact species unknown). In 1907 it contained a great many mussels as long as 4 inches, and since that year the largest individuals have slightly exceeded this size, which is near the maximum as we know it for this species. It is inconceivable that these unios were introduced as adults, for they are present in great numbers, and the farmer who owned the land was astonished to find them there four or five years after the pond was established, because it was near the entrance to his dooryard and he knew that no one had introduced mussels in any such numbers and that there was no watercourse connecting the pond with any creek in which mussels occurred. These mussels evidently came as parasites upon the fish with which this pond was stocked during the first year and they had reached a length of 4 inches in a period of five years. The abundance of the adults when the pond was six years old and the presence of some smaller specimens made it seem that more than one generation was represented, and hence some may have reached this size in a shorter time. The shell of *Unio tetralasmus* is light and is by no means a good button shell. Still it is not an impossibility, commercially speaking, for we have been assured by one of the leading button manufacturers, Mr. J. E. Krouse, of Davenport, Iowa, to whom we sent shells from which buttons were cut, that a marketable button could be made from them and would be made if there were no other shells available.

The appearance of *Lampsilis subrostrata* and *Unio tetralasmus* and no other species in all the ponds examined suggests the question, why have these two species and no others become established? If they were introduced as glochidia infecting fish, is it likely that the different lots of fish placed in so many ponds were infected solely with the glochidia of these two species? It seems much more probable that other mussels were introduced in the parasitic stages and that they were not able to survive long upon the bottom of these ponds. We have introduced large adult specimens of *Quadrula metanevra* and *Symphynota complanata* into one of the ponds in question and found some of them still alive after two years. This pond had a very soft mud bottom well covered with a layer of black muck filled with the soft coal soot from the smoke of a neighboring power-house chimney and seemed unsuitable for any variety of mussel. It had become, in spite of this, well stocked with *Lampsilis subrostrata* and is the pond referred to in detail in a previous paragraph. The survival here of these specimens of heavy shelled mussels for a period of two years shows that the adults are not at once killed even by unfavorable conditions, and we are therefore inclined to believe that when these species are introduced into the ponds on fish their destruction occurs in the early juvenile stages.

If a small body of water can be so fully stocked by the scant infection of glochidia obtained by fish in nature, we should be able to introduce mussels like these into a pond far more effectively by the use of fish which had been artificially infected and to rear

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them to adult size within a short term of years. Accordingly, we have attempted the introduction of *Lampsilis ligamentina* into one of the ponds where no mussels had ever been found by placing in the pond several hundred fish well infected with the glochidia of this species; but several examinations of the mud and silt from the bottom, made during the 18 months following, have failed to show anything as a result of the experiment.

The conclusions drawn from these observations are encouraging because they indicate, first, that other species, like those of the genus *Lampsilis*, whose shells are of excellent quality for the best of buttons, may be reared to commercial size in about the same length of time, and, second, that restricted localities can be stocked with mussels by the introduction of fish infected with glochidia. The members of the genus *Lampsilis* have shells which are evidently not much heavier than the shell of *Unio tetralasmus*, a fact which better fits them for life upon soft bottoms where there is little current, and in such localities they often occur. They move about more actively than the heavier shelled species and this, doubtless, enables them readily to seek out the most favorable food conditions in any body of water, instead of remaining long in one place where the conditions are very stable, as do the heavier shelled species. The study of any mussel which can live in small ponds like those in question and from which button shells can be obtained should be followed up with care, since the extensive culture of mussels would be a far simpler matter in ponds than in any stream where high and low water and the shifting of the bottom might so largely interfere with the most carefully located beds. For this purpose the species of *Lampsilis* which give good button shells would seem the most desirable, because they are better adapted for the conditions and because our planting experiments indicate that they reach a marketable size in a shorter time than the quadrulas.

We feel that there is nothing discouraging in what is at present known regarding the rate of growth under the average natural conditions. Moreover, it should be remembered that in most invertebrates where the growth rate has been studied this may be modified to an astonishing degree by the food supply and that the actual size of an individual furnishes no trustworthy clue to its age. It is not at all unlikely that proper study of the food and other conditions necessary for the maximum rate of growth will enable us to obtain shells of commercial size in even slow-growing varieties within a reasonable number of years. To judge from the supposed annual rings of specimens taken in nature, *Quadrula ebena* may take from 20 to 30 years to reach, under natural conditions, the size which is most desirable. The question whether this is a necessity, or only a result of the poverty of food conditions which most mussels meet in nature, is one which must wait upon the proper scientific analysis of the mussel's food and rate of growth in this and other species, and there is no problem in connection with the attempted artificial propagation which has more pressing importance.

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VII. INVESTIGATIONS ON THE UPPER MISSISSIPPI RIVER.

A brief reference may here be made to certain field studies which were carried on in connection with our mussel investigations during the months of June, July, and August, in 1908, on the upper Mississippi River. The Bureau of Fisheries put at our disposal for this purpose its substation, a small building provided with tanks and running water, at La Crosse, Wis., and also its steamboat, the *Curlew*, which not only furnished us with living quarters, but was of invaluable service for transportation from place to place on the river (fig. 65, pl. XVI). The boat, which is ordinarily used in the work of reclaiming young fish from the overflow of the river during the floods which occur in the spring and early summer, is equipped with aerated tanks, seines, and other apparatus and provided us with what was essentially a floating laboratory. With these facilities much was accomplished that would have otherwise been impossible. In addition to the usual crew of the *Curlew*, the party consisted, besides ourselves, of Messrs. W. E. Muns, Howard Welch, F. P. Johnson, and W. E. Dandy, students in the University of Missouri, who served as assistants.

The primary object of the expedition was a determination of the breeding seasons of the commercial species of mussels as far as possible at that time of the year and an examination of the depleted mussel beds in the upper Mississippi River, which have been all but destroyed as a result of the ravages of the mussel fisheries.

With a clamming outfit of our own (fig. 69, pl. XVII), consisting of a flat-bottomed skiff and "crow-foot" dredges—the usual apparatus employed by the mussel fishermen—we were able to secure thousands of mussels, which were examined microscopically for the purpose of determining their sex and the stage of development of the embryos. The data thus obtained furnished a mass of detailed information, especially with respect to those species which breed in the summer, but as they are incorporated in the account already given of the breeding seasons, there is no need to refer to the subject again.

The planting of young mussels in cages for a determination of the rate of growth was also made during this summer, with the result as described in a preceding section.

Some attempts were made to infect fish with glochidia, but this phase of the work was greatly interfered with by the high water of the river, which remained at flood stage unusually late in the summer of 1908 and made the seining of fish very difficult. Some infections, however, were carried out with the glochidia of a few summer-breeding species, the fish being retained in the tanks at the La Crosse station throughout the parasitic period and the duration of the parasitism determined.

A thorough survey of the mussel beds from Winona, Minn., to Lansing, Iowa, was made, and records taken at each locality where mussels were collected. No large beds at all were discovered, and in every instance where mussels were found indications of the ravages worked by the clammers were apparent. An account of the distribution of the species throughout this section of the Mississippi River and their relative abundance is not presented here, as the results of our observations in these respects will be incorporated in the work of the several field parties which have been engaged in the study of

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the geographical distribution of the Unionidæ throughout the Mississippi Valley under the direction of the Bureau of Fisheries during the past four or five years.

While working in the neighborhood of La Crosse, we made a careful investigation of the west channel of the river at this locality, with a view to determining whether places of this nature presented favorable conditions for experimental rearing of young mussels. As is usually the case with the accessory channels of the river in this region, the west channel at La Crosse is dammed across its head for the purpose of confining the water in the main channel, and, although at high-water stages of the river the dam is submerged, during the greater part of the year the volume of water in the channel is greatly reduced and the current retarded. These dams, however, are never tight, and a greater or less quantity of water constantly seeps through them. A thorough study of this channel showed that it contained very few mussels indeed, and of those species that were found living in small numbers under these conditions, the majority belonged to *Lampsilis*, *ventricosa* being by far the most abundant form. Whenever a channel of the river is dammed, the slackening of the current causes an enormous sedimentation to take place, and in these "sloughs," as such obstructed channels are called, sand and mud bars and shoals have been formed to an extent varying with the length of time since the dam above them was built. The more sluggish species of mussels, like the quadrulas, are especially ill adapted to these conditions and are frequently buried and destroyed by the deposits of silt in the river, an occurrence of which we found abundant evidence. With the more actively moving and burrowing species, as those of *Lampsilis*, the case is different, for apparently they may adjust themselves more readily and by their far greater ability to move from place to place they may avoid the danger of being buried. We found little evidence that the quadrulas, for example, move about at all, while, on the contrary, the tracks of slowly wandering individuals belonging to the species of *Lampsilis* were everywhere conspicuous on the sandy bottoms of the shallow sloughs.

An interesting case of the destruction of mussel beds *in situ* by sedimentation is shown in figure 70, plate XVII, which is a photograph taken on the bank of a slough, near Muscatine, Iowa, which was exposed by a gully washed out by rains and cut directly through an extinct mussel bed. The photograph shows the surface of the cut where the mussels are exposed as they lie embedded in the muddy bank. The bed is buried under about a foot of mud, and it is interesting to note that the valves of the mussels are closed and lying together in pairs. The latter fact proves conclusively that this is not an old shell heap, for the valves of the shells would be found scattered and separated in that event, but a mussel bed which had once existed in the river near the bank. It was probably buried under the deposits of sand and mud which followed the building of the dam across the head of the slough. An investigation of the species represented in the bed showed that they all belonged to *Quadrula*, being chiefly *ebena*, *pustulosa*, and *trigona*, while not a single individual belonging to *Lampsilis* could be found in it. It is probable, as already stated, that it is the sluggish species, like those of *Quadrula*, that are the principal sufferers in catastrophies of this nature, and are caught and smothered in the process of sedimentation, while the propensity to wander possessed by the more active species

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enables them to move out into deeper water when the deposit of silt becomes a menace.

The result of our study of the conditions obtaining in sloughs like the west channel at La Crosse, which are closed by dams at their heads, proves conclusively that such waters afford a very unfavorable habitat for mussels, and that therefore they are not adapted to experimental uses.

VIII. ECONOMIC APPLICATIONS.

It may not be inadvisable to discuss briefly certain applications of the results obtained in the foregoing investigations to the practical work of artificially propagating fresh-water mussels on a commercial basis. It must be emphasized at the outset that the ultimate object of the investigations—the restocking of depleted waters with commercial species of mussels—is not dependent for its realization solely upon the success of rearing mussels artificially from the glochidia, but that other methods of attaining the same end may be employed which are of equal, if not greater, importance.

PROTECTIVE LAWS.

Much can undoubtedly be done by securing the passage of laws by State legislatures for the closing of certain streams or sections of streams against all clamming for a period of years of sufficient length to allow of a natural increase of the mussels; by laws prohibiting the use of the ordinary "crow-foot" dredge, which takes immature and adult individuals indiscriminately,[a] and by laws prohibiting the discharge of sewage and factory refuse in the neighborhood of mussel beds. By these and other protective measures of a legal nature, a great deal might be accomplished in the way of conserving the supply of mussels in the more important waters, but, since in the case of many rivers the control is in the hands of two or more States, the passage of such laws would require, to be effective, similar action on the part of several legislatures, and such cooperation might not be obtained without the greatest difficulty.

The utter futility of laws which would establish a closed season of the year against clamming is apparent in the light of our knowledge of the breeding seasons of the Unionidæ. We have already seen that there is no month in the year when some species are not bearing embryos or glochidia, and as species of commercial value are found in both groups—those with the long and those with the short period of gravidity—a closed season at any time would be of little or no avail. Several species of *Lampsilis*, for example, which bear embryos or glochidia from August to July, furnish valuable shells for the pearl-button industry, while the species of *Quadrula* and other summer breeders, gravid from May to August, supply shells of the best quality. Any law then, designed to relieve the situation, which prohibits the taking of mussels during a supposed breeding season is based on ignorance of the facts, for the entire year is the breed-

[a] Mussels caught on a hook of the "crow-foot" are generally so badly injured internally in the process that, even if they are afterwards thrown back into the river, the majority probably die. A special form of hook has been devised by Mr. J. F. Boepple which is so constructed that small mussels can not be caught by it. The use of some such selective apparatus should be required by law.

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ing time of the Unionidæ. A law, however, which would close a river or large section of a river for a period of five years or more would be most beneficial, for in that time much could be accomplished both by artificial and by natural means to restore normal conditions. Even artificial propagation, unaided by certain protective measures, could hardly become effective on however extensive a basis it might be carried on, for unless some means can be devised for saving the young mussels it is difficult to see how much headway could be made against the destruction of the supply. It therefore becomes of vital importance not only to make illegal the use of any apparatus which will catch or injure young mussels, but to see that the law is rigidly enforced.

Certain requisite conditions for the artificial culture of fresh-water mussels, based upon our knowledge of their life history and habits, may now be briefly referred to.

SELECTION AND MAINTENANCE OF A FISH SUPPLY.

Although only a comparatively few kinds of fishes have been thus far used in our experimental infections, and doubtless as our experience widens many more will be found to be favorable for the purpose, success has been attained chiefly with the black basses, rock bass, and the sunfishes. All of these fishes have proved to be extremely resistant to the injurious effects of gill infections (practically all of the commercial species of mussels have hookless glochidia, which are gill parasites); to be able to carry large numbers of glochidia through the parasitic period; and to be easily kept in confinement—three necessary conditions for the success of propagation. It is to be hoped that other fishes will be found to be equally useful, but at present those just mentioned afford the most promising material for the work. As has already been shown, some species of fishes are very easily killed even by light gill infections, while others, according to our experience, have resisted all attempts to bring about permanent implantation of glochidia on their gills. The latter is particularly true of German carp and catfishes.

Fortunately, the basses and sunfishes can be obtained in large quantities without serious difficulty. In the reclamation work conducted by the Bureau of Fisheries along the upper Mississippi River, immense numbers of young bass are annually seined from the sloughs and "lakes" into which they are carried when the river rises over its banks during the flood stages of early summer. When the water recedes these young fish are caught outside the banks of the river, and only the small fraction of them which is reclaimed in the seining operations is saved from the wholesale destruction (fig. 67, pl. XVI). There is no limit to this supply of material for the work of mussel culture, and doubtless extensive use will be made of it at the Fairport station.

Even more valuable for the purpose are the species of sunfishes which we have used (probably other species of the same group are equally good), for, besides being just as resistant and as readily infected as the black bass, they are more easily kept and are less subject to disease in confinement. An adequate number of breeding ponds, in which sunfishes could be left to multiply naturally, would insure a large and constant supply of these fish for artificial infections.

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THE BEST SEASONS FOR INFECTIONS.

It has already been stated that the duration of the parasitic period of the mussel is inversely proportional to the temperature of the water. This fact is obviously important for mussel culture, since the longer the fish have to be kept while carrying the glochidia the greater is the loss from disease and other causes. The loss not only involves the fish but the potential mussels which they are nourishing as well. It therefore becomes desirable to reduce, as far as possible, the length of time that the infected fish must be retained, and this we have seen depends upon the temperature. Late spring and summer, consequently, are the seasons when the maximum efficiency from artificial infections should be obtained, for in the warmer water at that time the duration of the parasitism will be at the minimum—about two weeks or even less. The glochidia of *Lampsilis* are available all through the spring and as late as July, while those of *Quadrula* can be obtained during the summer months, and most of the commercial species of mussels fall in these two genera. Of course infections can successfully be made in the fall and winter and the duration of the parasitism reduced by keeping the water artificially warmed, but the difficulty of maintaining the fish alive under these conditions is greatly increased.

THE MUSSEL SUPPLY.

By far the greater number of species of commercial value, as has already been stated, belong to the genera *Lampsilis* and *Quadrula*, and, as both of these genera are widely distributed, practically all of the mussel-bearing streams of the Mississippi Valley may be drawn upon for a supply of material for cultural purposes. We have found that living mussels may be shipped even long distances with little or no mortality, especially in cool weather, and it is therefore possible to obtain breeding material from places at quite a distance from the station where the infections are to be made, should the local supply be inadequate. We have had on several occasions large numbers of gravid mussels shipped from Terre Haute, Ind., to La Crosse, Wis., to Manchester, Iowa, and to Columbia, Mo., with scarcely the loss of an individual, and have successfully used the glochidia obtained from them in infecting thousands of fishes.

According to our experience mussels thrive very well in confinement, in small ponds and laboratory tanks, and that without any special attention to a food supply. We have for years been keeping both pond and river forms alive in the laboratory for months at a time in tanks containing a few inches of sand on the bottom and supplied by tap water. Under such conditions mussels have frequently been retained in the laboratory from the fall to the following summer. It should therefore be an easy matter to keep mussels for breeding purposes in ponds with natural bottoms in any quantity desired, and, if the ponds are fed with river water, a natural food supply should be present in abundance.

Since, as has been pointed out above, the species of *Quadrula*, *Unio*, and other summer breeders abort their embryos and glochidia with astonishing ease when disturbed, it will be necessary, when making infections with the glochidia of forms exhibiting this peculiarity, to collect the material at a time prior to the fertilization of the eggs and to

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allow them to enter upon the breeding season after being placed in the ponds of the station. We have had females of different species of *Quadrula* become gravid in the tanks of the laboratory after they had been held in confinement for weeks or even months, and therefore no difficulty should be encountered in obtaining a supply of glochidia from these forms under the conditions mentioned.

REARING AND DISTRIBUTING YOUNG MUSSELS.

After the fish have been infected, one of two things may be done in distributing the young mussels resulting therefrom: Either the fish, after having been retained in tanks or ponds until near the end of the parasitism, may be taken to the stream which is to be restocked and the clams allowed to drop off there, or the liberation may take place in ponds where the young mussels may be reared until they are of considerable size, say until they are a year old, and then distributed as desired. Both methods might be used successfully, but in the first case it is to be supposed that only a very small proportion of individuals thus liberated would succeed in reaching maturity, as they would be exposed to the same destructive agencies as are encountered under natural conditions. The difficulty and expense of transporting the infected fish, the mortality among the fish themselves resulting from shipment, and the subsequent loss of large numbers of the young mussels are considerations which lead one to regard this method as not an efficient one. It should be stated, however, that in using this method of distribution it would not be necessary to liberate the fish and thus lose them for subsequent infections, for they could be confined in wire-bottomed fish cars set out in the streams, and after the mussels had all fallen off and dropped through the bottoms of the cars the fish could be returned to the station. This would of course involve a very large amount of labor and much expense.

It would, therefore, seem to be a far more effective practice to retain the young clams in ponds with natural bottoms until they could with safety be liberated in the streams. After infection, in this event, the fish could be set free in these ponds at once, and allowed to remain there throughout the parasitism of the glochidia, at the close of which they could be seined out and made to do service again. Supplied with river water, the ponds should furnish an adequate amount of food for a practically normal rate of growth of the young mussels, which at the end of a year at latest should be of sufficient size to be placed in favorable localities in the rivers. When ready for distribution, the water in the ponds could be drawn off and the juvenile mussels raked carefully from the sand or mud. If properly packed, it should be possible to ship them in large numbers to considerable distances. It is only reasonable to suppose that a large proportion of the mussels thus reared would reach maturity after distribution, and it is certain that the number coming through would be far greater than would be the case if the first method should be pursued.

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IX. CONCLUSION.

Of course, many practical details essential to success will have to be worked out before the artificial propagation of fresh-water mussels will have passed beyond the experimental stage, for the efficiency of the work from an economic point of view will doubtless depend upon the satisfactory solution of certain problems in technique, which, although secondary in character, are nevertheless a prerequisite of success.

However much is yet to be done—and it should be clear that the work is far from completion—the entire feasibility of artificial propagation has been demonstrated beyond the shadow of doubt. Besides filling in the gaps, some of them important ones, in the results already obtained, certain fundamental phases of the mussel investigations remain practically untouched. Chief among these is an exhaustive study of the physical conditions of the waters as affecting the growth of mussels: The relation between the mineral content of the water and shell formation; the relation between the character of the bottom, whether rocky, sandy, or muddy, to the habits of different species; and the relation between the rapidity of current to the life of the mussel and the kind of shell which it secretes. These and many other interesting problems of a similar nature await solution.

The immense mass of data that have been collected by the Bureau of Fisheries with respect to geographical distribution of species and their relative abundance throughout the Mississippi Valley has not been digested, yet the results which will be derived from a careful analysis of this information will have a fundamental economic bearing upon mussel culture. It is essential to know the centers and limits of distribution of at least the more valuable commercial species for the purpose of effectively conducting the operations in restocking streams and of avoiding useless labor in attempting to establish a species where the chances of its survival would be slight.

The whole problem of the food of mussels is as yet untouched. Not only are we ignorant of the specific food forms among the micro-organisms upon which mussels depend, but we do not know whether different species, or rather species living under different physical conditions and species possessing different habits, utilize different food forms. The possibility of artificially rearing cultures of the unicellular organisms used as food—when we know what these forms are—for enriching the water in which young mussels are retained before distribution should be determined, for it is undoubtedly true that results of the greatest practical importance and interest would be derived from such an investigation.

Very little is known at present respecting the enemies and diseases of fresh-water mussels, yet the importance of information of this nature can not be overestimated. Especially should we know the relative susceptibility of different species to parasitic diseases, and whether certain species are immune against the invasion of parasites which in the case of other forms constitute serious enemies.

A most fascinating and valuable field of investigation lies open in the study of the causes of pearl formations, for since these concretions are due, in part at least, to the

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presence of parasites, the possibility of producing them at will offers an interesting opportunity for experimental study.

The Unionidæ, in short, are a group of animals which, for the great variety of problems, both scientific and economic, presented in their unique life history, their structure, functions, and habits, their many interesting adaptations, and in their economic relations, is scarcely excelled by any other invertebrates except the insects. At present we may be said to possess only an introduction to a knowledge of the family, and the writers of this paper will feel amply repaid for their labor if they have succeeded in exposing some of the problems which here lie open for investigation and at the same time in laying the foundation for the artificial culture of fresh-water mussels.

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EXPLANATION OF PLATES.

[Drawings by G. T. Kline.]

PLATE VI.

FIG. 1. Gravid female of *Ptychobranchus phaseolus*. Actual length 96 mm.
FIG. 2. Gravid female of *Lampsilis subrostrata*. Actual length 50 mm.
FIG. 3. Gravid female of *Symphynota complanata*. Actual length 170 mm.

PLATE VII.

FIG. 4. Gravid female of *Dromus dromus*. Actual length 57 mm.
FIG. 5. Gravid female of *Quadrula ebena*. Actual length 98 mm.
FIG. 6. Gravid female of *Lampsilis recta*. Actual length 122 mm.
FIG. 7. Gravid female of *Obliquaria reflexa*. Actual length 55 mm.
FIG. 8. Gravid female of *Cyprogenia irrorata*. Actual length 38 mm.

PLATE VIII.

FIG. 9. Hooked glochidium of *Symphynota costata*, anterior end view. For measurements see text figure 1.

FIG. 10. Hooked glochidium, as above. Lateral view of living specimen.

FIG. 11. Axe-head glochidium of *Lampsilis* (*Proptera*) *alata*, anterior end view. For measurements see text figure 1.

FIG. 12. Axe-head glochidium, as above. Lateral view.

FIG. 13. Hookless glochidium of *Lampsilis subrostrata*, lateral view. For measurements see text figure 1.

FIG. 14. Hookless glochidium, as above. Posterior end view.

FIG. 15. Hookless glochidium, as above. Ventral view.

FIG. 16. Detail of a conglutinate of *Lampsilis ligamentina*. The glochidia, still inclosed in the membranes, are less crowded together than those of figure 17, and are embedded in a mucilaginous matrix.

FIG. 17. Detail of a conglutinate of *Obliquaria reflexa*, showing the membranes closely pressed and adhering together.

FIG. 18. Young mussels (*Lampsilis ligamentina*) one week after liberation from the fish, showing various positions assumed in crawling, the ciliation of the foot, and the new growth of shell.

PLATE IX.

FIG. 19. Fin of a carp about 3 inches long, 7 days after infection with glochidia of *Anodonta cataracta*, showing complete failure of the overgrowth of fin tissue in all places where the glochidia are greatly crowded. See explanation in the text, p. 159, of the conditions along the upper margin.

FIG. 20. Tip of an over-infected fin, as above, 12 hours after infection, showing no appreciable overgrowth because of the crowding. The shadows represent glochidia upon the under surface.

FIG. 21. Pectoral fin of a carp, as above, 3½ hours after infection; an optimum infection.

FIG. 22. Ventral half of caudal fin of a carp, as above, 24 hours after infection; an optimum infection.

FIG. 23. Tip of fin, as above, 32 days after infection. The shadows represent glochidia upon the under surface. The infection is less than the optimum. The glochidia were well overgrown and all alive when the fish was killed.

FIG. 24. Young *Symphynota costata*, attached by only a shred of tissue and about to drop from the fin after a parasitism of 74 days.

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PLATE X.

FIG. 25. Fin, as above, 36 hours after infection with glochidia of *Anodonta cataracta*, showing complete overgrowth of all glochidia which have become properly attached.

FIG. 26. Glochidium of *A. cataracta* upon fin margin of carp, 3½ hours after infection. Proliferation of cyst just beginning.

FIG. 27. Glochidia, as above, upon fin margin of carp, showing different stages of cyst proliferation, even in neighboring glochidia.

FIG. 28. Glochidia, as above, 24 hours after infection.

FIG. 29. Hooked and hookless glochidia (*A. grandis* and *L. recta*) embedded and developing on a fin margin.

FIG. 30. Glochidia of *A. cataracta* upon fin of carp, 3 days after infection, showing the cyst completely formed.

FIG. 31. Glochidium of *A. cataracta* upon fin of carp, developing normally after a shift of 90 degrees from the position first taken.

FIG. 32. Two glochidia of *A. cataracta*, overgrown after 36 hours upon surface of a carp's fin.

FIG. 33. Glochidium of *A. cataracta* 35 days after infection. The metamorphosis is more advanced than in figure 30 and the rudiments of the foot and other organs have assumed greater size.

PLATE XI.

FIG. 34. Three gill filaments of the rock bass infected with glochidia of *Lampsilis ligamentina*. The metamorphosis of the glochidia has hardly begun, although they have been attached for 28 days.

FIG. 35, 36, 37, and 38. Stages in the formation of the cyst surrounding a hookless glochidium (*Lampsilis ligamentina*) upon a gill filament of the black bass. Taken at 15 minutes, 30 minutes, 1 hour, and 3 hours, respectively, after infection. The transverse lines on the filaments indicate the lamellæ.

FIG. 39. Anterior gill of a black bass infected with glochidia of *L. ligamentina*, showing distribution upon the gill as a whole and the appearance of the cysts.

FIG. 40. Gill of yellow perch, as above.

FIG. 41. Two conglutinates of *Lampsilis ligamentina* removed from the marsupium. One is shown from the flat surface, the other on edge. Actual length 17 mm.

FIG. 42. Three conglutinates of *Obliquaria reflexa* removed from the marsupium. Actual length 17 mm.

FIG. 43. Part of a gill of black bass infected with glochidia of *L. ligamentina*, showing the distribution and orientation of the glochidia in an infection above the optimum for this fish. Only the row of filaments toward the observer is shown.

PLATE XII.

FIG. 44. *Symphynota costata*, dissected from fin of carp 47 days after infection. The anterior end is to the left. Rudiments of foot, digestive tract, liver diverticula, and the first gill buds are recognizable; also the hooks and the degenerating adductor of the glochidium. Compare with figure 47. Actual size, 0.39 by 0.35 mm.

FIG. 45. *Strophitus edentulus*, from a living specimen which had completed its metamorphosis without parasitism and which was actively crawling about on the bottom. Seen from the ventral side. The anterior and posterior adductors are well developed and within the foot the pedal ganglia and lithocysts may be seen. Two gill buds are found on either side. Sections show that the internal organization is as far advanced as that of the young mussels shown in figures 47 and 48. ×106.

FIG. 46. A single cord discharged from the marsupium of *Strophitus edentulus*, showing glochidia extruded and others still within the cord. ×13.5.

FIG. 47. *Symphynota costata*, a young mussel which had been crawling upon the bottom for 6 days after a parasitism of 68 days. The very narrow margin of the adult shell has been drawn within the

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valves. The glochidial shell and its hooks are still in evidence. In other respects the young mussel shows most of the features which are characteristic of the adult. The anterior end is to the right. Anterior and posterior abductors, anterior and posterior retractors, digestive tract divided into esophagus, intestine and stomach with its large diverticula, cerebral, pedal, and visceral ganglia, lithocysts, three gill buds, palp rudiments, the heart and pericardium will be recognized by their resemblance to the adult organs. Sections show the rudiments of the kidneys. From a stained and decalcified specimen. Actual size, 0.39 by 0.35 mm.

FIG. 48. *Lampsilis ligamentina*, a young mussel 1 week after the close of the parasitic period. The margin of the shell is extended well beyond the glochidial outline and shows the first lines of growth. More calcification has rendered the shell so opaque that the internal organs are no longer visible without decalcification. Stained specimens and sections show about the same degree of organization as in the *Symphynota* larva of figure 47. The foot with its cilia is shown extended and attached to the bottom preparatory to drawing the mussel forward. From a living specimen. Actual size, 23 by 20 mm.

PLATE XIII.

FIG. 49. *Alasmidonta truncata*. Horizontal section of a water tube of gravid marsupium, taken near ventral border of gill. The respiratory canals (r. c.) are small clefts, indistinctly shown under this magnification (cf. fig. 56); the marsupial space contains young embryos.

FIG. 50. *Quadrula ebena*. Horizontal section of two adjacent water tubes (w. t.) of gravid marsupium containing young embryos.

FIG. 51. *Anodonta cataracta*. Horizontal section of a water tube of gravid marsupium, showing respiratory canals (r. c.) and marsupial space (m. s.); the latter contains young embryos.

FIG. 52. *Symphynota complanata*. Horizontal section of a water tube of gravid marsupium, showing respiratory canals and marsupial space; the latter contains glochidia. Note the thin, stretched interlamellar junctions.

FIG. 53. *Lampsilis ligamentina*. Horizontal section of a water tube (w. t.) of gravid marsupium containing young embryos. Note the thin, stretched interlamellar junctions (i. j.).

FIGS. 54–55. Two stages showing process of implantation of a glochidium of *Unio complanatus* on a filament of a gill excised 2 hours after infection. Figure 54 is taken 3 hours after attachment, while 55 is the same glochidium drawn 2 hours later. The greater part of the cyst was formed while the gill was in a watch glass.

PLATE XIV.

FIG. 56. *Alasmidonta truncata*. Horizontal section through portion of lamella and water tube of gravid marsupium, showing respiratory canals (r. c.) near ventral border of gill; each canal is separated from the marsupial space by a septum (s). The interlocking cells, forming the suture in the septum, are clearly seen.

FIG. 57. *Anodonta cataracta*. Section similar to last, but taken before fusion of folds (s), which are seen not quite touching. The septum is formed by their fusion. Eggs contained in the marsupial space are in an early cleavage stage.

FIG. 58. *Anodonta cataracta*. Region marked X in last figure, highly magnified, showing glandular epithelium of respiratory canals (r. c.), adjacent blood sinus (b. s.), and histological structure of surrounding tissues. Note the muscle fibers.

PLATE XV.

FIGS. 59–61. Transverse sections of glochidia of *Symphynota complanata*, taken 15 minutes, 6 hours, and 24 hours, respectively, after attachment to edge of fish's fin, showing three stages in formation of cyst. In 59 proliferation of epidermis is just beginning; in 60 glochidium is about half embedded; while in 61 formation of cyst is completed. In 59, which is more highly magnified than the other two, and in 60 several mitoses are shown in region of proliferation. In 60 cellular detritus from enclosed edge of fin is being ingested by mantle cells of glochidium.

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FIGS. 62–63. Transverse sections of glochidia of *Lampsilis ligamentina*, taken 30 minutes and 3 hours, respectively, after attachment to gill filament. In 62 the development of cyst has made considerable progress, while in 63 the cyst wall is practically completed. In 62 several mitotic figures are seen in the epidermis where multiplication of cells is taking place.

FIG. 64. Highly magnified section of a portion of the glandular epithelium of an interlamellar junction in the gravid marsupium of *Quadrula ebena*, showing the large mucus cells and the nuclei of several leucocytes (l) with which the epithelium has become infiltrated.

PLATE XVI.

FIG. 65. Station of the Bureau of Fisheries at North La Crosse, Wis., and steamer *Curlew*, used in mussel investigations during summer of 1908.

FIG. 66. Interior of station at North La Crosse, equipped as a laboratory.

FIG. 67. Seining young black bass near La Crosse in a "lake" which had been filled by the overflow of the Mississippi River during the early summer. The fish thus obtained were artificially infected with glochidia.

PLATE XVII.

FIG. 68. Dredging for young mussels in a slough near La Crosse.

FIG. 69. The clamming outfit used in the mussel work on the Upper Mississippi River. The two "crow-foot" dredges, with the mussels still clinging to the hooks just after a haul, are seen resting on the stanchions.

FIG. 70. An old mussel bed near Muscatine, Iowa, buried under a foot or more of sand and mud but exposed in cross section by a gully washed out by rains. The mussels are seen *in situ* embedded in the wall of the gully.

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FIG. 1.

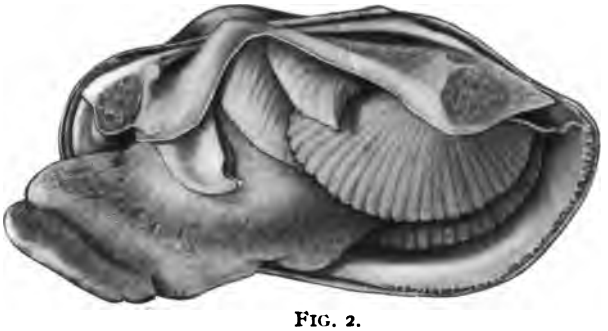

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FIG. 4.

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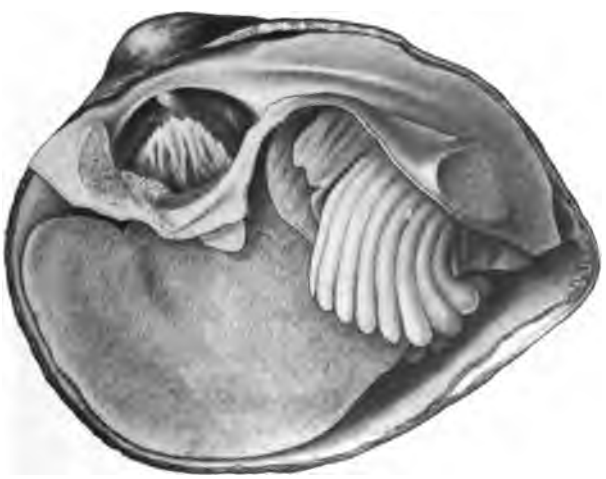

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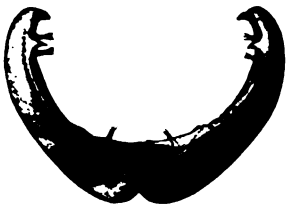

FIG. 11.

FIG. 13.

FIG. 10.

FIG. 14.

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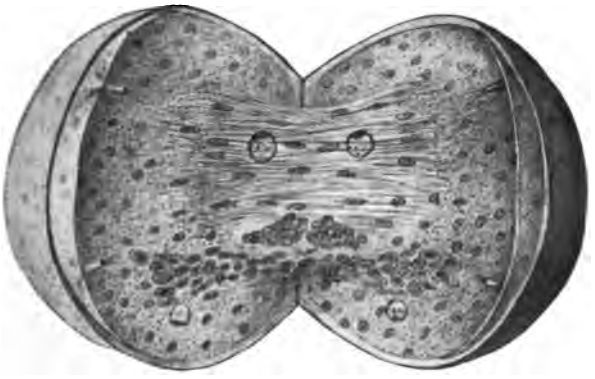
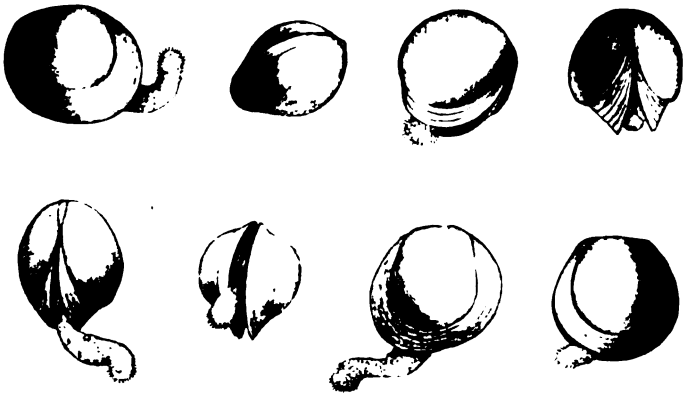


FIG. 15.

FIG. 16.



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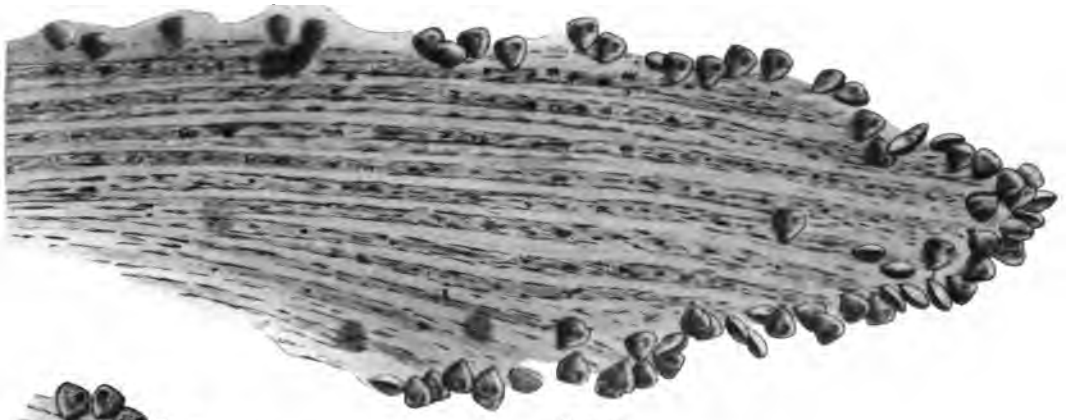

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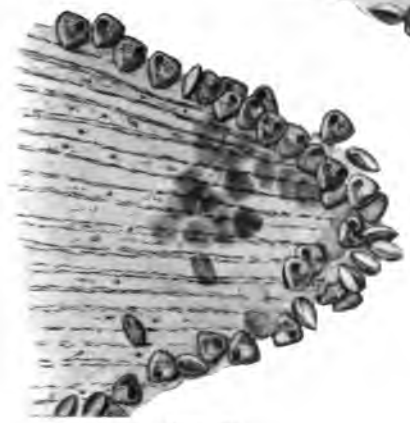

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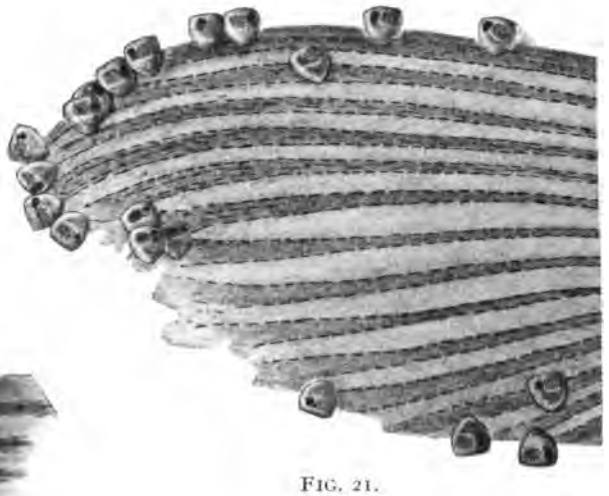

FIG. 21.

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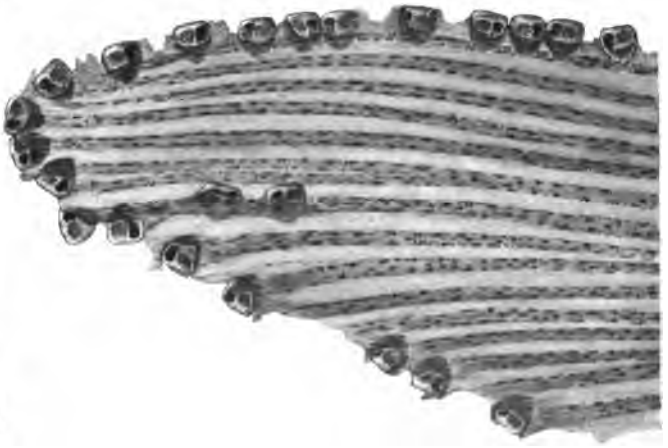

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FIG. 25.

FIG. 26.

FIG. 27.

FIG. 28.

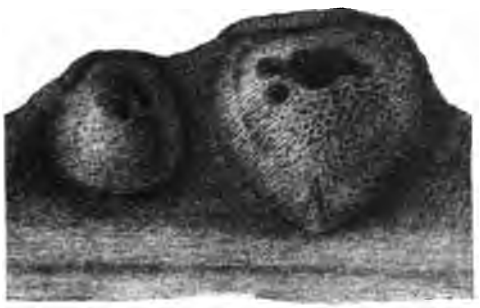

FIG. 29.

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FIG. 34.

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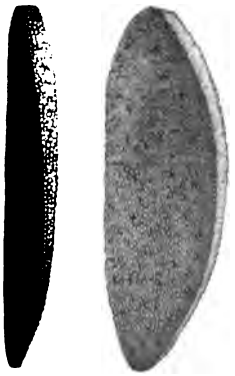

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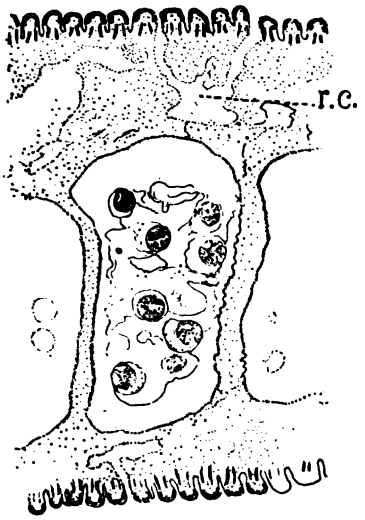

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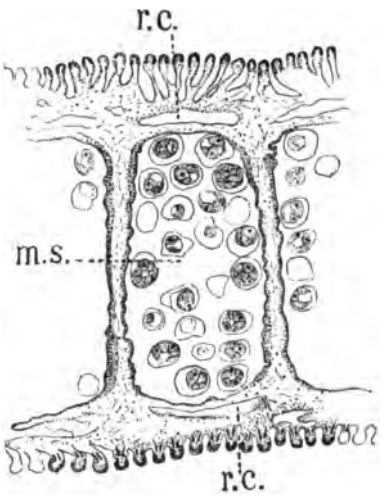
FIG. 50.



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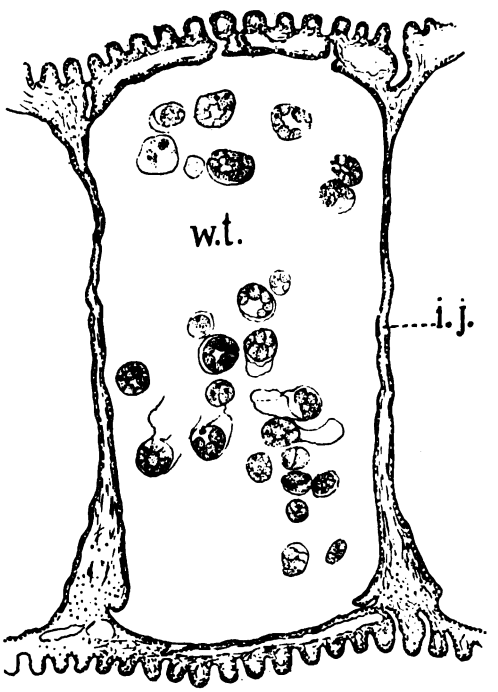

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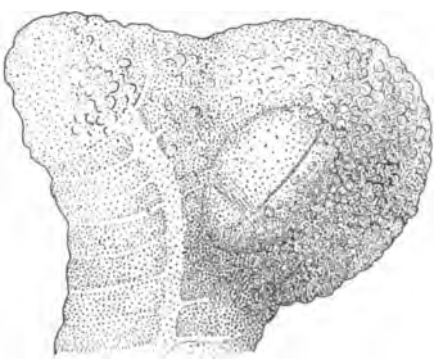

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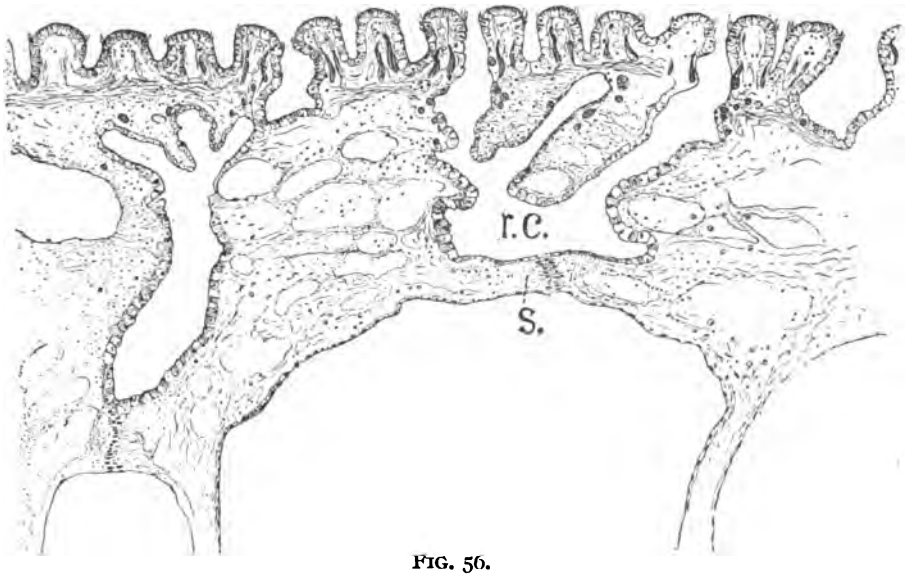

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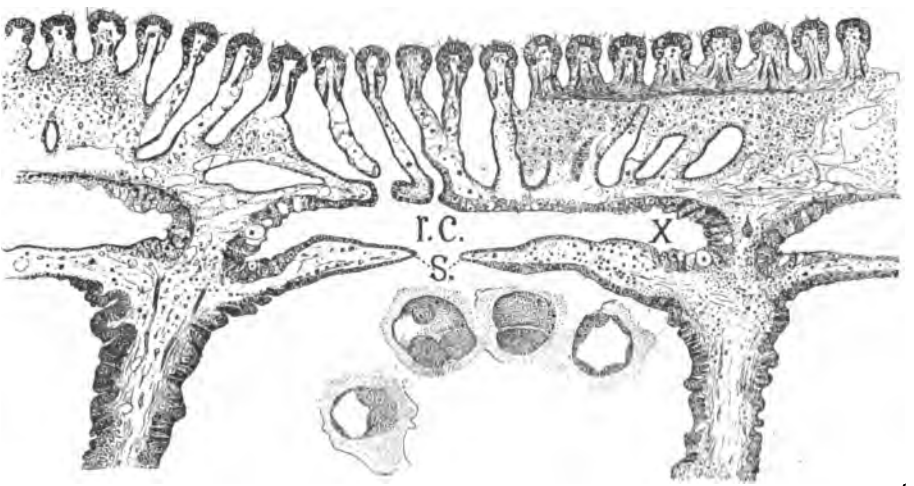

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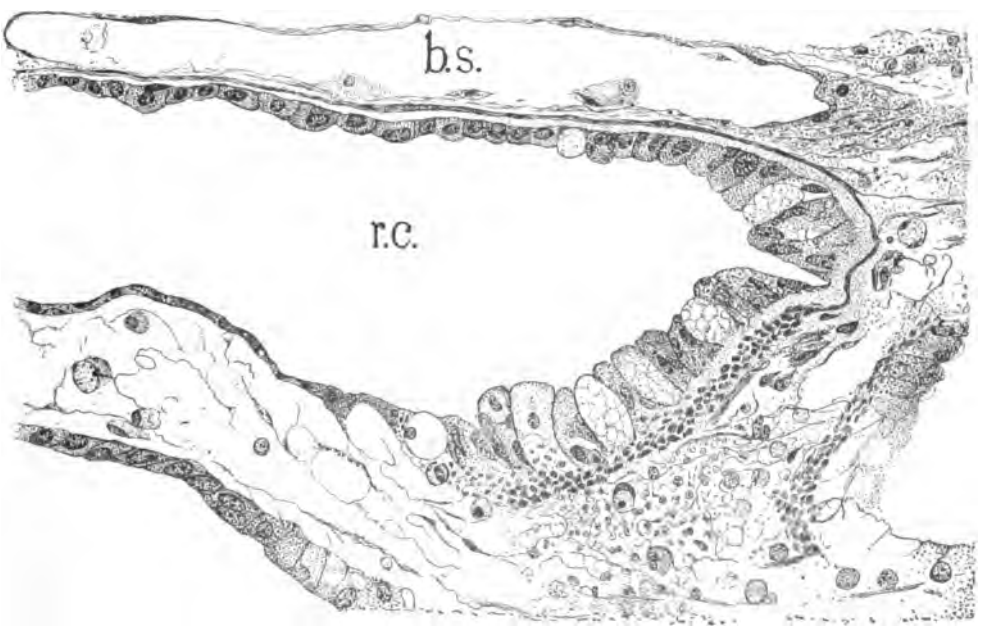

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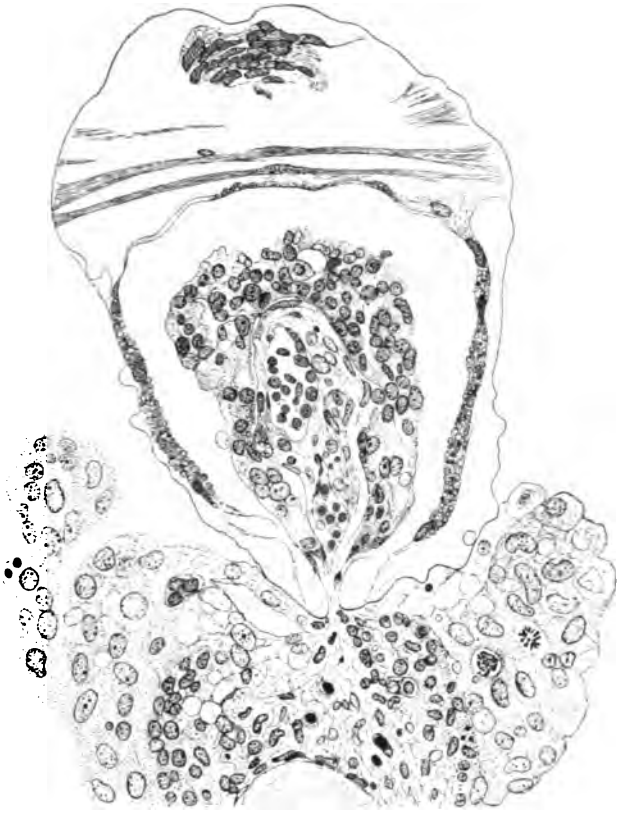

FIG. 59.

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