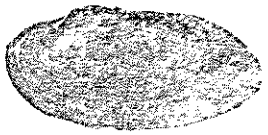
Vol 12



PROPAGATION OF FRESH-WATER MUSSELS

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IN 1695, Leeuwenhoek, the Dutch microscopist, studied the anatomy and reproductive processes of fresh-water mussels. He learned that the eggs pass from the ovaries to the gill passages, where embryonic development is completed. His work was lost to science for about a hundred and fifty years, however, and the general belief was that the organisms found in a mussel's gills were parasites preying upon the mussel. The organisms were given the name, Glochidium parasiticum.

Carus, in 1832, concluded that these tiny living bivalves were the larvae of the adult mussel which bore them, but he was unable to bring about continued development after they were separated from the parent mussel.

It was not until 1866 that Leydig discovered that the larval mussels became parasites on fishes and that parasitism was essential to their development. Little was known of their life history or ecology until a considerably later date. The discovery in the latter years of the nineteenth century that the shells of fresh-water mussels could be used in the manufacture of buttons led to the invention and improvement of cutting and finishing machines and the opening of mussel fisheries in the upper Mississippi River and its tributaries.

Intensive harvesting of mussels in these streams caused fear that a valuable natural resource would be depleted, and in 1894 the United States Bureau of Fisheries became actively interested in gaining knowledge of mussels and in developing means of artificially propagating them in order to insure a continued supply to meet commercial demands.

A biological station was built at Fairport, Iowa, on the Mississippi River, for the primary purpose of studying mussels and developing methods of propagating them.

Prior to 1910, George Lefevre and Winterton C. Curtis worked there intensively on reproduction and artificial propagation. Their studies were continued at the University of Missouri. Much of their work was of a pioneer nature.

Dr. Robert E. Coker, who was in charge of the Fairport Biological Station for some time, made extensive studies of mussel populations and advocated conservation measures for the protection of mussels. Dr. Coker, with the collaboration of Dr. A. F. Shira, Dr. H. W. Clark, and Dr. A. D. Howard, made intensive studies of the natural history of fresh-water mussels and of methods of propagation.

By the efforts of workers at the Fairport Station, many attempts were made to rear mussels in ponds and troughs and in enclosures in the river.

Propagation was undertaken successfully by means of artificially infecting the gills of fish with glochidia. Most of this work was conducted in the Lake Pepin area of the Mississippi River. Experimental work led to the determination of the species of fishes required as hosts to the various species of mussels and provided more accurate knowledge of the life history and ecology of mussels.

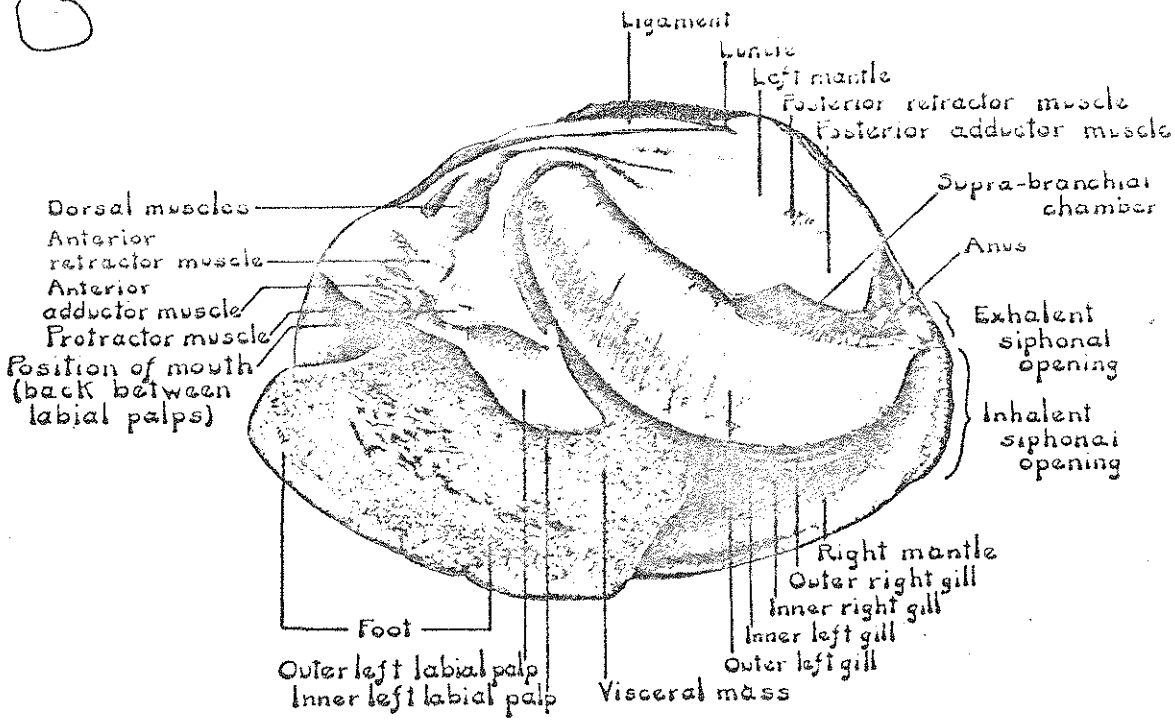


FIG. 1.—The structure of a fresh-water mussel. (Based upon drawing of white heel-splitter, *Symphynota complanata*, from Lefevre and Curtis, 1912.)

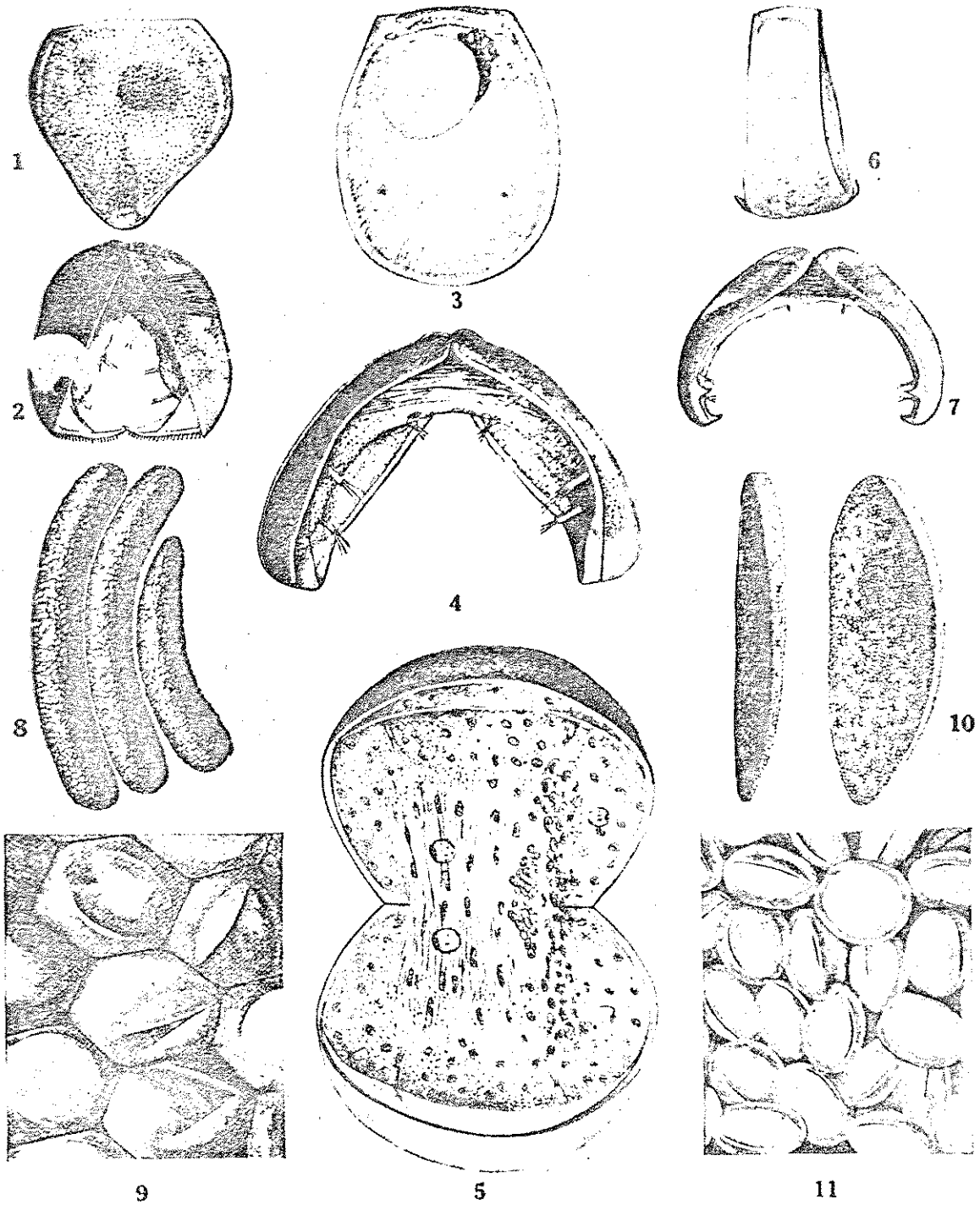
The efforts of these workers gave us the first complete picture of the life history of fresh-water mussels.

LIFE HISTORY

In the so-called long-term or winter breeders, fertilization takes place in late July and August. This group of breeders includes yellow sand-shells and muckets. Embryonic development occurs within a few weeks, and the young mussels are carried as mature glochidia until the following spring or early summer, when the gravid females move into shoal water and spawning occurs. The eggs of short-term or summer breeders are fertilized early in spring, and spawning takes place in the fall. This latter group includes pig-toes, niggerheads, pimple-backs, and butterflies.

Sexes are separate, and spermatozoa are drawn from the male mussel through the incurrent siphon of the female to fertilize the eggs. Some investigators believe that actual fertilization takes place while the eggs are migrating from the ovaries to the gills, which swell to accommodate an estimated 2 million eggs. Each gill chamber holds a mass of eggs in a jellylike matrix called a conglutinate. As embryonic development progresses, the eggs become separate in the brood pouch and shell membranes disappear. At the end of this embryonic phase, the larval mussel is a minute bivalve, 0.4 to 0.5 millimeter in longest dimension, possessing a thin tissue of mantle on the inner surface, a cross band of muscle tissue between the valves, and fine hairlike projections believed to be tactile in function. Germ plasm is, of course, present in the living tissue. In this latter phase the larvae are referred to as glochidia.

In natural reproduction the glochidia are dispersed into the water through the excurrent siphon of the parent mussel and are carried in suspension by water currents. As the water bearing them passes through the gill chamber of a fish,



[Figures from Lefevre and Curtis, 1912.]

FIGS. 1 and 2.—Hooked glochidium of *Symphynota costata*.

FIGS. 3, 4, and 5.—Hookless glochidium of *Lampsilis subrostrata*.

FIGS. 6 and 7.—Ax-head glochidium of *Lampsilis (Proptera) alata*.

FIG. 8.—Conglutinates (masses of glochidia) from the three-horned warty back, *Obliquaria reflexa*.

FIG. 9.—Portion of conglutinate of *Obliquaria reflexa*, magnified. Glochidia still within egg membranes which are closely pressed and adhering together.

FIG. 10.—Conglutinates (masses of glochidia) from the mucket, *Lampsilis leptodon*.

FIG. 11.—Portion of conglutinate of *Lampsilis ventricosa*, magnified. Glochidia inclose fluid membranes and are imbedded in a mucilaginous matrix.

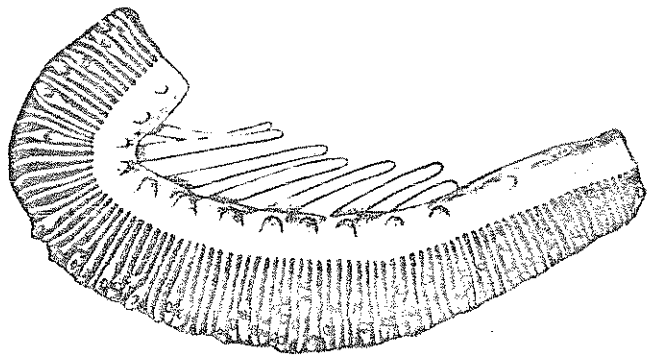


FIG. 1.—Gill of a black bass infected with glochidia of mucket, *Lampsilis luteola*.

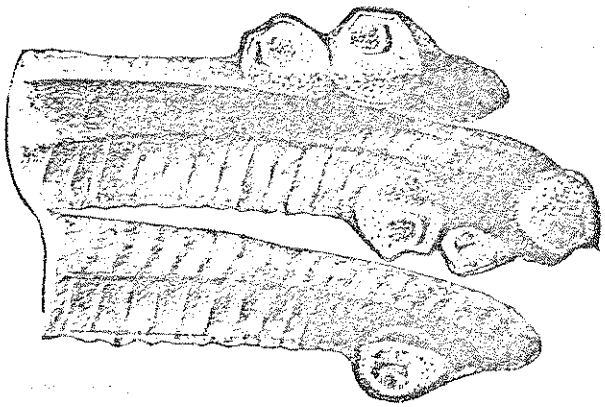


FIG. 3.—Three gill filaments of rock bass, with glochidia of mucket.

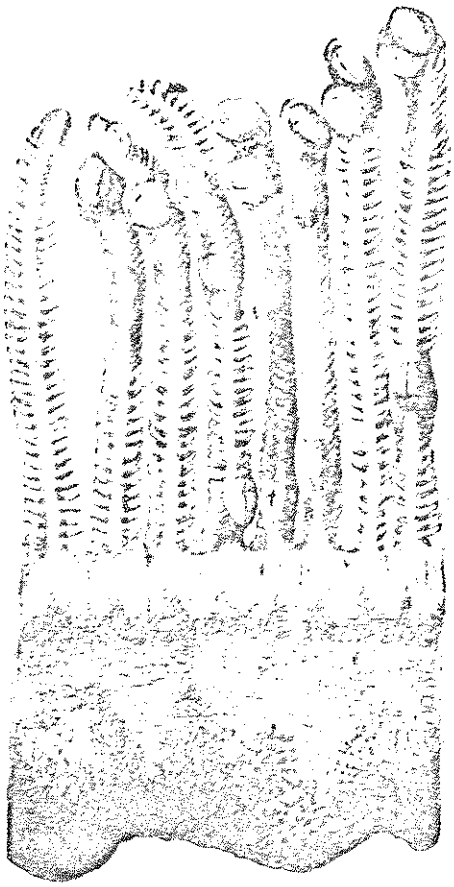


FIG. 2.—Part of fig. 1, enlarged.

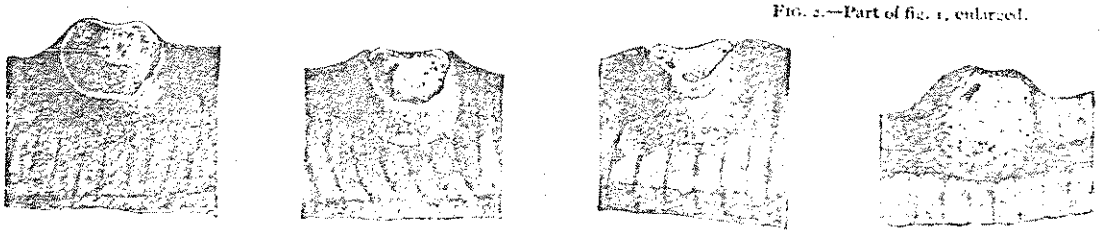


FIG. 4.—Stages in formation of cyst surrounding a glochidium of the mucket. Taken at 1 minutes, 15 minutes, 1 hour, and 3 hours, respectively, after infection.

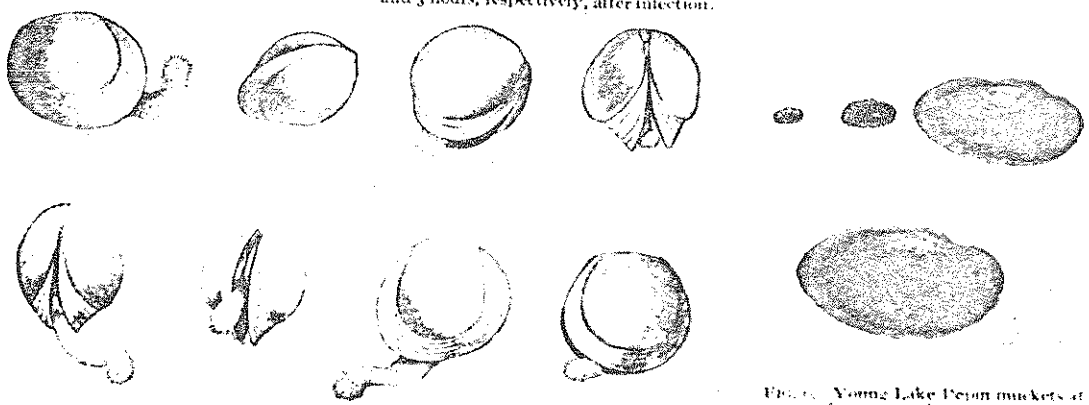


FIG. [illegible] Young muckets, one week after liberation from the fish, showing new growth of shell, cilia on foot, and positions assumed in crawling. Enlarged.

FIG. [illegible] Young Lake Pepin muckets at ages of [illegible], 6, and 5 months, respectively. Natural size.

[Figs. 1–5 after Lefevre and Curtis.]

the glochidia attach themselves to the fish's gill by closing on the tissue. A cyst is formed by the proliferation of cells from the fish's gill. The formation of this protective cyst begins almost at once, and the glochidium is usually completely enclosed within 24 hours. The attachment to the tissue of the fish marks the beginning of the parasitic phase. Glochidia are nourished through this phase by the blood of the fish. They do not increase in size but develop parts similar to those of an adult mussel. The time they remain on the fish is governed by the water temperature and varies from 10 to 30 days in summer conditions. It may last until the following spring if the attachment takes place late in the fall.

At the end of the parasitic phase, the glochidium is equipped with a protractile foot. This larval foot has a hardened terminal portion. Movement of the foot effects the release of the glochidium from its cyst. Chemical change may aid in the break-down of the cyst.

After release from the host fish, the glochidium falls to the bottom. A byssus gland secretes a long sticky strand which is quite strong and may serve as an anchor on swift rocky bottoms. Young mussels from the end of the parasitic phase until they reach a length of 20 millimeters are referred to as juvenile mussels.

FORMER METHODS OF PROPAGATION

In 1930, Dr. M. M. Ellis of the U. S. Bureau of Fisheries announced that it was possible, by laboratory methods and the use of nutrient solution, to bring young mussels from the glochidial to the juvenile stage without the use of a host fish. This method, although successful in the laboratory, did not prove to be practical for handling the large quantities of glochidia needed in a rehabilitation program.

As a result of early experiments at the Fairport Station and in the interest of economy, the Bureau of Fisheries inaugurated a program of mussel planting in which the fish-rescue crews regularly employed on the upper Mississippi during the summer infected the rescued fish with glochidia before releasing the fish into the parent stream. The fish crews obtained gravid female mussels of the desired species, either from the stream where rescued fish were being liberated or from selected locations where more desirable specimens were available. In the latter instance, the gravid mussels were packed in ice and shipped to the site of operations, where, after tempering, they were held in running water until needed. Gravid mussels were carried by fish crews in pails or tubs of water and used as required. Host fish were placed in a tub containing a small amount of river water. Glochidia were then added to the water by opening one or two gravid mussels and pressing out the glochidial masses. After waiting a few minutes for the glochidia to become fixed, the workers emptied the tub into the stream and then repeated the process as necessary. This fish-infection program was carried on until 1930. By that time, the changes in habitat caused by silting and pollution had seriously diminished the mussel-producing potential of the upper Mississippi River and many of its tributaries.

Conversations with commercial users who had knowledge of this work and who were familiar with the yearly harvest of commercial mussels, disclosed the prevailing opinion that increased mussel production was evident in the areas where such infection work had been done.

Although the method used produced results in Lake Pepin and other areas, and although it was performed economically by combining fish infection with fish rescue work, later investigation and testing of the method showed that infection thus obtained gave inconstant results as to the numbers of glochidia fixed on the fish's gills. (Unpublished reports, Ellis, Westfall, and others.) Further experiments demonstrated that the excitation of the fish during seining and handling and the anoxia caused by crowding in tubs accelerated liberation of carbon dioxide and kidney wastes into the water, and that these substances reached concentration levels which caused premature closing of glochidia. Mucous secretion from gills and body surface greatly increases when fish are excited. This is particularly true of gar. Strands of

mucus from excited fish in the tub entangled particles of debris, forming slime strings which, in turn, enmeshed glochidia and thus contributed to the conditions which tended to prevent successful attachment of glochidia. Thick coatings of mucus on the gills interfered with closing action of glochidia to such extent that many were sloughed off within 24 hours after attachment.

As these conditions became apparent, a method of fish infection was developed in which the objectionable factors could be controlled or eliminated (Ellis, Westfall, and Ellis, unpublished report). This method was tested and proved successful by Dr. B. A. Westfall on the White and St. Francis Rivers in Arkansas. In 1936 plantings were made with gar confined in enclosures. When the areas were examined in 1937 and 1938, thick beds of juvenile sand-shells were found. Similar success was achieved with plantings of muckets in Illinois. The same method was used in intensive plantings in Arkansas and Indiana during the summer of 1941. Comparison of tonnage purchased from the planted areas in 1945 with the tonnage of 1941 showed a marked increase.

This method is referred to as the modified artificial infection method and is employed in the present mussel planting program.

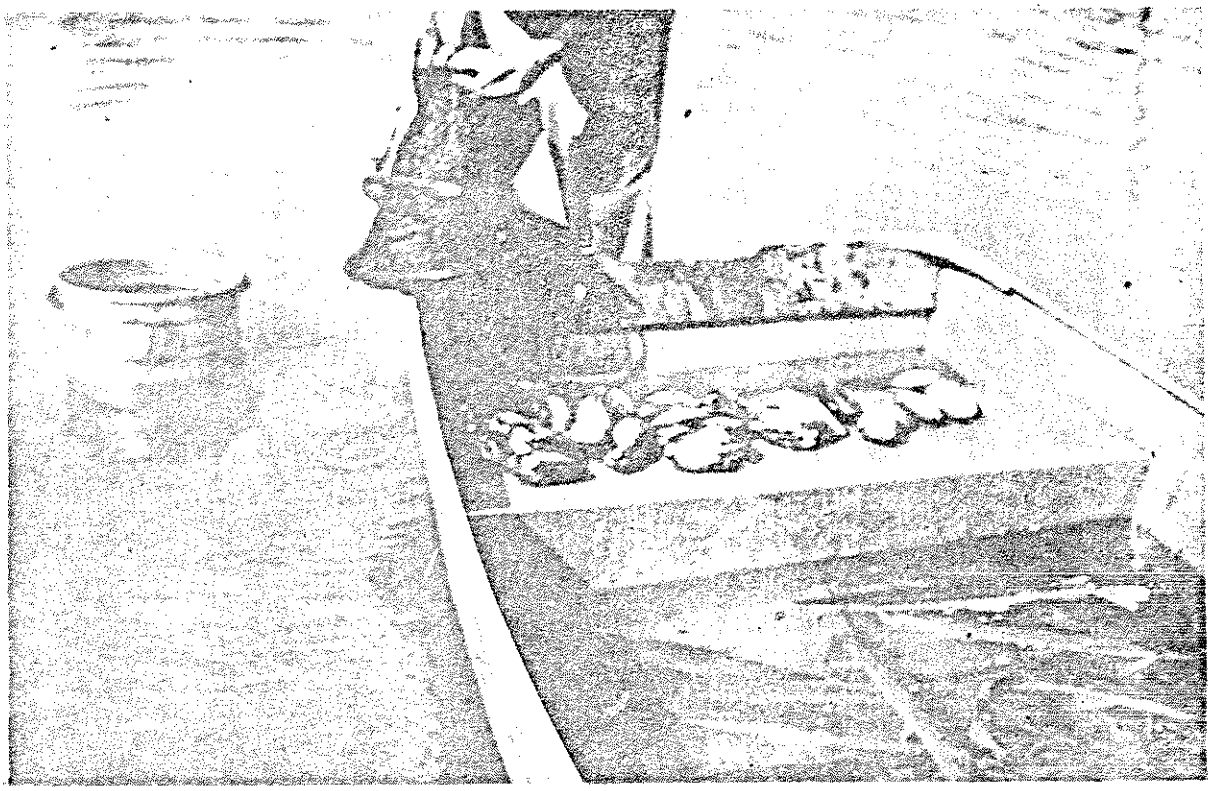
MODIFIED ARTIFICIAL INFECTION METHOD

Selection of Glochidia

Only fresh glochidia are used, and the infection is made as soon as possible after gravid mussels are collected. Gravid female mussels are selected by examining freshly caught mussels. Preliminary examination is made by inserting a thin-edged "clam opener" between the valves at the ventral edge and prying the valves apart only enough to see into the interior. The modified gills, or marsupia, are quite plump in the "ripe" mussel. If all gills are flat, the specimen is either a male or a very "green" female. Different numbers and/or portions of the gills may serve as brood pouches. In the sand-shell the outer gill of each pair carries the glochidia. In the ripe yellow sand-shell the gill is full an rounded along its entire length and ha black beading on the distal edge.

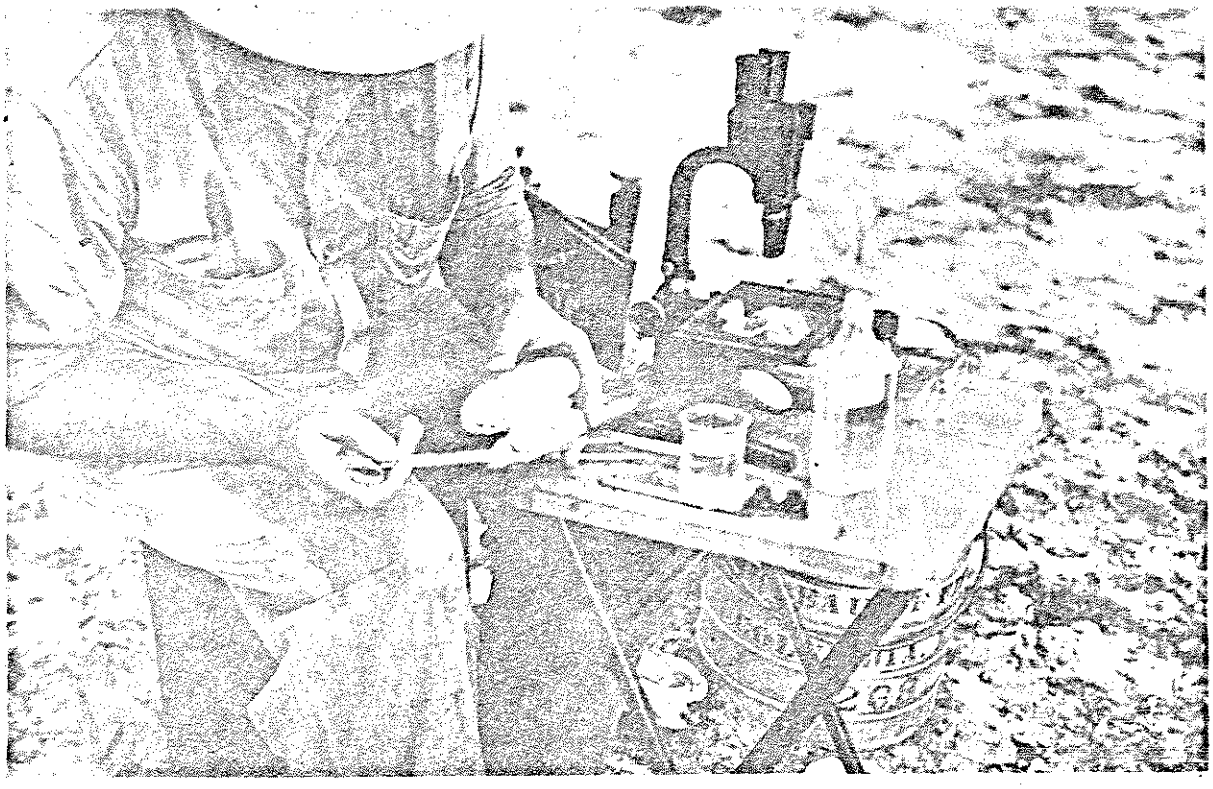
Males and females of some species o mussels may be distinguished by differ ences in the contour of the shells. Th female yellow sand-shell has a swellin near the posterior which gives the ventra line of the shell a slightly emarginat appearance. This swelling coincides wit the position of the modified or marsupia gills.

When a number of gravid mussels have been found and segregated, they are place in flowing stream water to be convenient for use. The final selection is ther made by taking a sample of glochidia fro each mussel. The valves are pried apart only far enough to admit the end of a pipette. Caution is used in opening, as the mussel, if not yet ready, may be returned to the stream for later use, and adductor muscles torn by too much stretching will cause the mussel to die. After the valves are slightly separated, a small wooden wedge is placed between them to hold them apart. The point of a sharp scalpel is then inserted between the valves to make a slit at the end of one marsupial chamber. A bulb pipette with a small amount of water at the tip is then touched to the slit in the gill. Water is forced from the pipette. If the glochidia are free from the agglutination, they will be carried out with the drop of water and may then be drawn into the pipette. The pipette is held vertically until the glochidia have settled to the tip. They are then placed on a watch glass or slide and examined under a low-powered lens or microscope. Healthy glochidia which are ready for parasitism will be in a gaping position. The valves may usually be seen to move slightly in and out; they occasionally snap together but immediately open. Under higher magnification the valves have a pearly, slightly granular appearance when they are fully mature. Sometimes a marsupium may have the appearance of containing fully matured glochidia. Examination will show them to be still within the egg membrane. Fully matured glochidia always separate easily and are readily removed from the chambers. Often they will burst forth from the stretched marsupium when it is touched



Sorting mussels from holding cage. The gar will serve as host fish.

Indicating location of glochidia in ripe mussel.



Washing glochidia.

Tanks (see text) used while inoculating host fish with glochidia.

by the point of a scalpel. If the valves of the glochidia are opened wide, i.e., flattened to 180°, they are dead. This is the condition found when bacterial or protozoan parasites have consumed the living tissue.

The means used to determine the suitability of glochidia for use in infection is to test the closing time. A number of glochidia are placed in a drop of water under a low-powered microscope. A drop of 0.5-percent NaCl is introduced into the drop and its effect observed. The salt solution should cause the closing reaction. The closing of about 80 percent of the glochidia in a period of 60 seconds is considered good. Observation has shown that glochidia reach the optimum of closing response when they have been removed from the brood pouch and held in fresh water for about 30 minutes.

Washing Glochidia

Washing in a flow of filtered stream water has been shown to be effective in cleaning glochidia and selecting by relative density those that are living and free of infection.

Further tests will be made to demonstrate the efficacy of the washing procedure. The present method makes use of the flow of filtered stream water siphoned through a rubber tube with screw clamp to control flow rate. Water is placed in a beaker of fairly large size (500 to 800 cubic centimeters), and glochidia from several mussels (previously tested and approved) are put into the water. The siphon hose is introduced into the beaker with the opening near the bottom and at an angle which will cause centrifugation. Flow is regulated so that the glochidia are kept in motion and the lighter ones are carried away as the beaker overflows. Dead glochidia are readily removed in this way. This season a settling-out process is to be tried as a method of selecting the more desirable glochidia.

Washing the glochidia is not imperative to obtaining fairly heavy infections, but it is believed that a higher percent of survival may be obtained by washing.

Transportation of Gravid Mussels

Gravid mussels should be transported only when it is impossible to obtain desirable specimens near the site of operations.

Short trips are possible with mussels placed in pails or tubs of water when the temperature can be kept reasonably low and the time involved is not more than 2 to 3 hours. Express shipments have been made successfully when mussels were placed in pails and interspersed with cracked ice. The writer has found that when such shipments were made over long periods, the mortality following shipment was high.

Our present practice when required transportation involves a relatively long time, is to use auto ice-boxes in which bottom drains have been drilled. When transported by this method, each mussel is individually wrapped in cheesecloth. The cheesecloth is cut into pieces about 10 by 18 inches. Each shell is then wrapped in several layers of cloth, each piece being stretched tightly at right angles to the ventral or opening edge so as to make sure that the valves are tightly closed. Layers of wrapped shells are alternated with layers of cracked ice in the ice-box. If the trip is long, it may be necessary to renew the ice occasionally. Keeping mussels alive and in good condition by this method requires two things: (1) shells must not be allowed to open; (2) water from melting ice must not be allowed to accumulate to such extent as to immerse the mussels. In either case, high mortality results. The writer has not studied the physiology of this, but the supposition may be that when the mussel opens in the absence of water, non-replaced body fluids are lost and death ensues. When water immerses the mussel, it may be possible for the mussel to resume respiratory activity and subsequently death results from anoxia. It has been demonstrated that mussels exposed to air but having the valves closely bound together so that no living tissue is exposed, will remain alive for long periods. An adequate buffering system, probably drawing on carbondate from the shell, will maintain blood pH at the normal level

until just an hour or two before death. The survival time of mussels forced to remain closed has in some instances been as much as 30 days. High temperatures, naturally, will decrease the survival time.

The handling of brood mussels should be kept at a minimum. Prolonged storage in confinement and repeated handling often bring on abortion. Summer breeding mussels are more likely to abort than winter breeders.

Handling of Host Fish

Host fish are used as soon as possible after capture. The earliest seining is done near the site of operation. Fish are held in a temporary pen made by staking out a live net made of seine webbing if they are to be held only a short time or of hardware cloth if they are too numerous or must be held overnight. Gar (Lepisosteus osseus or platostomus) are hosts to the yellow sand-shell, the species most frequently planted at present. Gar are not so tenacious of life as is generally supposed, and great care must be used in order to prevent mortality among infected fish. Experience has shown that in holding gar overnight (which should be done only when daylight fishing will not meet the need) mesh or web too small for bills to penetrate should be used, and that an area of moderate current should be chosen. Heavy losses may occur either in swift water or in static water--in the latter particularly if the fish are crowded. Gar are surprisingly sensitive to bruising. Rolling in the seine should be avoided, and the number of times the fish are handled should be kept at a minimum. Repeated handlings cause bleeding between scales; and our experience has been that when this bleeding occurs, the fish almost invariably die within 2 weeks. All species should, of course, be handled with care.

Equipment and Procedure

Four metal tanks are arranged near the river. In hot weather shade is essential to prevent increase of the temperature of static water in tanks. If trees are not conveniently located, a canvas fly may be used. The tanks are painted with asphaltum varnish. They are 18 inches wide, 22 inches deep, and 54 inches long. Each tank has a threaded drain opening very near the bottom in one end, and a nipple and faucet may be screwed into the opening so that the outflow can be controlled. The four tanks are placed parallel to each other with the drain ends toward the river, and there is sufficient space so that operators may walk between the tanks.

A portable gasoline motor and pump unit with fittings adapted to 3/4-inch garden hose is used to supply running water for the first tank. The intake end of the hose is screened against debris and placed well below the surface and upstream from the zone of activity. The delivery end of the hose is covered with several thicknesses of cheesecloth to filter out suspended matter. The outlet faucet is adjusted to balance the inflow and maintain a constant level about 10 inches below the rim of the tank.

The second tank is used for gill disinfection and is filled with filtered water to a marked level which will give a known volume. An amount of neutral acriflavine which will give a solution of 1 part per million is added. The number of cubic inches of water divided by 61 gives the number of milligrams to be used to make a solution of 1 part per million. As this solution must be renewed after every four or five lots of fish, it is impractical to make analytical weights for each batch of solution. Standard No. 3 gelatine capsules are filled with acriflavine powder. A capsule is filled by holding the lower (inside) portion with a forceps and dipping into a beaker of the powder. The powder is slightly compressed and leveled by pressing the open end against the side of the beaker, and the cap is then put in place. Dosage is standardized by weighing five of the filled capsules, averaging their weights, and subtracting the average weight of five empty capsules which have been dried in a desiccator. This weight may then be used in calculating the level to calibrate the tank in order to obtain a solution of 1 part per million. Capsules are thereafter filled in the same manner without further analytical weighing. This

COMMERCIAL MUSSELS AND THEIR HOSTS

/Table from Coker, Shira, Clark and Howard/

Mussels		Hosts																												
Scientific name	Common name	A. melas, bullhead	A. nebulosus, bullhead	A. calvus, bowfin	A. chrysopa, eel	A. grunniens, sheepshead	D. cepedianum, gizzard shad	E. lucius, pike	E. gibbosus, red-ear sunfish	H. tergisus, mooneye	I. punctatus, spotted cat	L. osseus, long-nosed gar	L. platostomus, short-nosed gar	L. tristoechus, alligator gar	L. cyanellus, blue-spotted sunfish	L. auryorus, sunfish	L. humilis, orange-spotted sunfish	L. pallidus, bluegill	L. olivaris, yellow cat	M. dolomieu, smallmouth black bass	M. salmoides, largemouth black bass	N. maculosus, mud puppy	P. chrysochloris, river herring	P. flavescens, yellow perch	P. annularis, white crappie	P. sparoides, black crappie	R. chrysops, striped bass	S. platorhynchus, sand sturgeon	S. gyrinus, madtom	S. canadense, sauger	S. vitreum, walleye
Lampsilis anodontoides	Yellow sand-shell	o	o	a	-	o	-	-	-	-	o	AN	A	A	no	-	n	o	-	-	na	-	-	o	no	no	a	o	-	o	-
Lampsilis fallaciosa	Slough sand-shell	-	-	-	-	-	-	-	-	-	o	-	NA	-	-	o	-	-	o	-	o	-	-	-	n	o	-	n	-	-	-
Lampsilis higginsii	Higgin's eye	-	-	-	-	n	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-
Lampsilis ligamentina	Mucket	o	o	o	n	o	-	a	-	-	o	-	a	-	N	-	-	aN	-	N	AN	-	-	AN	NA	A	AN	o	n	A	-
Lampsilis luteola	Fat mucket	o	o	-	-	o	-	-	-	o	o	-	o	-	-	-	-	NA	o	A	A	-	-	AN	A	A	A	o	n	A	NA
Lampsilis recta	Black sand-shell	-	-	-	n	-	-	-	-	-	o	-	-	-	a	-	-	aN	-	-	-	-	-	-	N	-	-	-	-	-	-
Lampsilis ventricosa	Pocketbook	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	A	A	-	-	A	AN	-	-	-	-	N	-
Obovaria ellipsis	Missouri niggerhead	-	-	-	-	o	-	-	-	-	o	-	-	-	n	-	-	o	-	-	o	-	-	o	o	o	-	NA	-	-	-
Plagiola securis	Butterfly	-	-	-	-	NA	-	-	-	-	-	-	-	-	-	-	-	o	-	-	o	-	-	-	o	o	-	-	-	-	-
Quadrula ebenus	Niggerhead	o	o	o	-	o	a	-	-	o	o	-	-	-	o	-	-	o	o	-	o	-	TN	o	o	o	-	o	-	-	-
Quadrula heros	Washboard	A	A	-	N	Af Nf	Nf	-	-	-	Af	-	-	-	A	-	-	NA	AN	-	o	n	n	-	NA	A	N	-	Nf	-	-
Quadrula metanevra	Monkey-face	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	-	on	-	-	o	-	-	-	-	-	-	-	-	N	-
Quadrula plicata	Blue-point	a	a	-	-	-	-	N	N	-	o	-	A	-	-	A	-	a	o	-	AN	-	-	A	Nf AN	A	n	-	-	N	-
Quadrula pustulata	Warty-back	-	-	-	-	-	-	-	-	-	N	-	-	-	-	-	-	-	n	-	-	-	-	-	n	-	-	-	-	-	-
Quadrula pustulosa	do.	NA	A	-	-	-	-	-	-	-	AN	-	o	-	-	-	-	o	an	-	o	-	-	-	on	o	-	n	-	-	-
Quadrula solida	do.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	-	-	-	-	-	-	-	-	-	-	-	-	-
Quadrula undata	Pig-toe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	n	n	-	-	-	-	-

Explanation of table:

- N -- Found on the gills in natural infection.
- Nf -- Found on the fins in natural infection.
- n -- Record of natural infection but of doubtful significance.
- A -- Carried through on gills after artificial infection.
- Af -- Carried through on fins after artificial infection.
- a -- Results of artificial infection unsatisfactory or not uniform.
- o -- Tested and found unsuitable.
- T -- Tested; development occurred; host perhaps suitable, but experiment not carried to conclusion.

method is sufficiently accurate, as some variation in the strength of the solution is permissible, but solutions should never contain less than 0.75 or more than 2 parts per million. When a new stock of acriflavine is obtained, new average weights should be taken, as the fineness of the commercial powder may vary and cause an appreciable difference in the weight contained in each capsule. It is important that the acriflavine powder be kept dry at all times. When capsules are taken into the field, they are stored in small glass bottles.

The third tank is used only as a rinse to prevent the transfer of antiseptic solution to the infection tank. It is filled to a suitable level with filtered stream water and changed when the temperature rises or after 50 fish have been rinsed.

The fourth tank is used as the infection tank. Filtered water is used in a quantity commensurate with the size and number of fish to be infected, but this is always a relatively small amount. Better infections are obtained by thus concentrating the glochidia. This is particularly true of Centrarchids. The water in this tank should be watched carefully, and it should be discarded and renewed at once if it becomes warm or if mucous strings appear.

The procedure in handling the fish through the series of tanks is as follows:

Fish (a few at a time) are brought from the holding enclosures and put into the wash tank. They are given sufficient time in this tank to recover from excitement and for waste products and mucus to wash away. The usual time is from 15 to 30 minutes. After sufficient time in the wash tank the fish are transferred, one at a time, to the disinfecting tank. The transfer is made by hand and as gently and quickly as possible. Workers accustomed to handling fish can accomplish this with a minimum of disturbance. Small fish must be handled with a dip-net. As soon as all are transferred, another lot is placed in the wash tank.

The acriflavine solution cleans the gills of mucus. It also has a bacteriostatic effect and temporarily arrests the flow of mucus. The time required in this bath is from 5 to 15 minutes. The solution should be renewed after four or five lots of fish have been treated.

One or two minutes are sufficient in the rinse tank. After being rinsed, the fish are transferred to the infection tank, which has previously been charged with washed glochidia. Motion of the water caused by respiratory and swimming movements of the fish aids in keeping the glochidia suspended, but the occasional stirring of the water is advisable. Gills are examined at intervals to observe the progress of infection. Examination is facilitated by the use of a hand magnifying lens. Fifteen minutes will usually be sufficient for good infections. Infections are considered adequate when the glochidia are fixed thickly enough to appear as a white rim on the edges of the gills. Infections heavy enough to injure the fish are not likely with gar but may occasionally occur with Centrarchids. Each fish is examined. When satisfactory infections are apparent, the fish are placed in a separate holding pen for distribution.

Infected fish are carried in the tanks for distribution. They are carried by boat or truck to selected points for release. In the interest of returning fish to the stream in the best possible condition, fish are rarely carried more than 10 miles from the site of infection.

BIBLIOGRAPHY

Allen, W. R.

1914. Food and feeding habits of fresh-water mussels. Bull. Marine Biol. Lab. 27.

1921. Studies of the biology of fresh-water mussels. Bull. Marine Biol. Lab. 40.

Churchill, E. P., Jr.

1915. The absorption of fat by fresh-water mussels. Bull. Marine Biol. Lab. 29.

1916. The absorption of nutriment from solution by fresh-water mussels. Jour. Exp. Zool. 21 (3): 403-429.

------ and Lewis, S. I.
1923-24. Food and feeding of fresh-water mussels. Bull. U. S. Bur. Fish. 39: 439-471.

Coker, R. E.; Shira, A. F.; Clark, H. W.; and Howard, A. D.
1919-20. Natural history and propagation of fresh-water mussels. Bull. U. S. Bur. Fish. 37: 79-181.

Corwin, R. S.
1920. Raising fresh-water mussels in enclosures. Trans. Am. Fish. Soc. 49: 81-84.

Howard, A. D.
1912. Catfish as a host for fresh-water mussels. Trans. Am. Fish. Soc. 42: 65-71.

1914. Propagation of fresh-water mussels. Trans. Am. Fish. Soc. 44.

Howard, A. D. (continued)
Parasitism among fresh-water mussels. Trans. Am. Fish. Soc. 44.

1917. Artificial infection of river herring with fresh-water mussels. Trans. Am. Fish. Soc. 46.

Artificially reared second generation of fresh-water mussels. Trans. Am. Fish. Soc. 46.

Lefevre, George, and Curtis. W. C.
1912. Studies on the reproduction and artificial propagation of fresh-water mussels. Bull. U. S. Bur. Fish. 30: 105-201.

Reuling, F. H.
1920. Artificial rearing of fresh-water mussels in troughs. Trans. Am. Fish. Soc. 49: 153-155.

More than forty million fish from hatcheries of the U. S. Fish and Wildlife Service were stocked in 16,455 farm ponds during 1948. The Federal Government places no restrictions on fishing the farm ponds so stocked, but the farmers must observe State laws in regard to licensing and the opening of their ponds to the public.

There are only two occasions in American life when people have a regard for one's privacy. One is when you are at prayer; the other when you go fishing. At both, you are able to be by yourself.

--Herbert Hoover.
