FOOD AND FEEDING IN FRESH-WATER MUSSELS.

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CONTENTS.

INTRODUCTION.

In connection with experiments being carried on by the United States Bureau of Fisheries at its biological station at Fairport, Iowa, relative to the propagation of commercial species of fresh-water mussels, it became important to have as precise information as possible concerning the food requirements and the feeding processes of these mussels. With these considerations in mind, the investigations to be described hereafter were undertaken. Since any attempt at propagation must deal especially with the young mussels, it is very essential to know exactly the feeding reactions of and the food required by the juvenile mussels that are being handled. In these investigations, therefore, especial emphasis has been laid upon the study of the juvenile mussels.

The work was carried on by the senior author during July, 1921, at the United States fisheries biological station, Fairport, Iowa, and during August, 1921, at the Iowa Lakeside Laboratory, Lake Okoboji, Iowa, and by the joint authors [1] during the summer of 1922 at the Fairport laboratory. During the latter season an unparalleled opportunity for the study of juvenile mussels was presented in the use of specimens of *Lampsilis luteola* and *L. ligamentina*, which were being reared in troughs in the highly successful propagation experiments conducted by Dr. A. D. Howard and B. J. Anson, of the Fairport station of the United States Bureau of Fisheries. Any desired quantity of specimens was freely placed at our disposal by these gentlemen, to whom we wish to express our gratitude. Thanks are due H. Walton Clark, also of the Fairport station, who very kindly supplied us with numerous specimens of juvenile mussels of various species which he collected from the river.

The photomicrographs were taken by J. B. Southall, of the Fairport biological station. Figures 1, 2, and 6 were drawn by Edwin Meisenholder, a student of the senior author. Acknowledgment is made to Dean C. P. Lommen, of the department of biology, University of South Dakota, for reading the text and for suggestions.

Opportunity is here taken to thank the United States Bureau of Fisheries for the financial support that rendered the investigations possible and to express our appreciation of the courtesies extended by R. L. Barney, director of the United States fisheries biological station, and by Dr. R. B. Wylie, director of the Iowa Lakeside Laboratory.

HISTORICAL.

An effort has been made to make the following review of the literature as complete as possible so far as food and feeding of fresh-water mussels are concerned. Reference is also made to a number of papers bearing on the same subject in other species of lamellibranchs on account of their bearing upon certain principles involved. For convenience the literature on the fresh-water mussels is dealt with first.

[1] The senior investigator is responsible for the work relating to the anatomical features of the mussels and the process of ingestion, while the junior investigator studied especially the material ingested.

FRESH-WATER MUSSELS.

Posner (1875) concluded that food material is carried to the labial palps and moved forward by their cilia to the mouth. He shows a figure of a longitudinal groove in the lower edge of the inner gill for carrying material forward to the palp. Ortmann's (1911) mention of this groove is not the first, as so considered by Bush (1922).

Simpson (1900) stated that the flapping of the palps swept the material into the mouth, which idea is quite erroneous.

Wallengren (1905) considered that the masses of material were brought to the labial palps and that cilia there swept them along to the mouth. He further stated that the minute particles that fell between the ridges on the palps were carried downward by cilia in the grooves and reached the lower edge of the palp and were thrown off. Another conclusion at which he arrived was that the cilia on the posterior side of the ridges on the palps beat forward and move particles along from crest to crest of the ridges, but that those on the anterior side of the ridges beat backward, and that on occasion the crests of the ridges, which usually lean forward, may be slanted backward. This would bring into play the cilia on their anterior slopes and material might be carried posteriorly off the palps by this means.

Wilson and Clark (1912) mention a few algal forms, the lorica of the rotifer *Keratella* sp., and an Ascaris, that were found among the stomach contents of certain fresh-water mussels.

Clark and Wilson (1912) found in the alimentary tract of a number of mussels "a large mass of muddy matrix" in which were mingled various diatoms, algal forms (Trachelomonas), active "Euglena-like organisms," loricas of rotifers, and fungus spores. Baker (1916) suggests that this "muddy matrix" is probably the kind of material described by Petersen (1911) as "dust-fine detritus."

Siebert (1913), after studying *Anodonta cellensis* Shröt, concluded that the cilia in the bottoms of the furrows on the apposing faces of the palps strike upward instead of downward, his findings being the direct opposite to those of Wallengren.

Allen (1914 and 1921) has made a very thorough investigation of the feeding process in fresh-water mussels. He states that the food particles are carried into the mantle chamber by water currents induced by cilia on the gills and are intercepted on the surface of the gills by cilia there. Mucus is secreted about the particles by certain cells of the gills, so that they are aggregated into clots. The material falling upon the outer surface of the outer gill is carried upward by ciliary action to the dorsal edge of the gill and then forward to the palps in the groove formed by the juxtaposition of the gill and mantle. That striking the inner surface of the outer gill moves upward to the dorsal edge, where it passes to the outer face of the inner gill. The cilia of this gill beat downward, and all material reaching it by any means is carried downward to the ciliated groove on the ventral edge. In this groove it is swept forward to the palps. Allen (1914), on page 130, says:

> Thus particles which find their way between the palps [that is, are brought there by the cilia of the gills] are carried to the mouth. As will soon be seen, very little undesirable matter ever reaches the mouth or palps, but even here Wallengren (1905) has pointed out how selection and rejection may be made.

* * * the inner surfaces of the labial palps, except their outer margins, are made up of minute vertical ridges or furrows. These constitute a quite complex mechanism for the sorting of material. * * *.

Upon the ridges, as elsewhere, occurs a ciliated epithelium; but the ciliary currents are disposed in a unique manner. Upon the anterior slope of each ridge they are directed backward, while those on the posterior slope lead forward. This seeming conflict is not such in fact, because only one set of cilia comes into action at a time. The position of the ridges determines which set shall function at a given moment. Their normal position seems to be * * * a somewhat reclining one, overlapping one another toward the anterior. Thus, the after slopes are ordinarily brought uppermost, the ciliary currents leading to the mouth are upon the surface, while the cilia which lead from the mouth lie somewhat underneath the ridges. So long as no adverse stimuli are received, particles which lie between the palps are thought to be passed on forward from one ridge to another to the lips and mouth.

In the event that distasteful matter reaches the palps a reflex erection of the ridges brings uppermost the cilia leading backward, and such material is returned from summit to summit to the edges of the palps and discharged into the mantle chamber.

It is extremely difficult to observe the cilia which lie at the bottoms of the furrows. Wallengren (1905) ascribes to them the duty of carrying lengthwise of the furrow to the lower margin of the palps the minute particles that may fall between the ridges, but Siebert (1913) thinks they lead in the opposite direction.

The present authors judge from Allen's remarks, just quoted, and from his subsequent statements, that Allen himself did not observe this change of position in the ridges and the passage of material posteriorly across the palp from crest to crest of the ridges. It appears that he is summarizing Wallengren's statements. We are not told that Wallengren states that he actually saw this behavior of the palps, and the fact that Allen says "* * * Wallengren has pointed out how selection and rejection *may* be made," and "So long as no adverse stimuli are received particles which lie between the palps are *thought* to be passed on forward," etc., conveys the impression that Wallengren had set forth a theory rather than observations. Unfortunately the present authors did not have access to Wallengren's paper while writing this report. Allen's own observations are given on page 133 of his 1914 paper:

No one, to my knowledge, has succeeded in inducing a mussel to behave normally after the shock of removing parts of the shell and mantle in order to observe the palps at work; but I have repeatedly obtained the reactions which occur. When the palps lie in contact with either body, mantle, or gill their collections of material pass between the palps and mouthward. Otherwise such material is carried *down* by the several structures and discarded.

As will appear later, these observations agree with those of the present authors. The word "down" is the significant one here; the material leaving the palp passes down off it in the vertical furrows and was never seen to be carried backward across its face from ridge to ridge.

In general, Allen ascribes to the fresh-water mussel a high degree of ability to select the material ingested. This selection may be exercised at the incurrent siphon, the gills, the palps, and the mouth. The palps are, however, most important in this respect. Allen lists in the stomach contents of certain mussels 14 genera of diatoms, 16 of other algae, 9 of desmids, besides débris (organic and inorganic), mold, ova, spermatozoa, and spores. He especially stresses the point that the amount of inorganic débris was quite small, consisting of rather insignificant quantities that had accidentally passed the assorting mechanism. This fact is cited as one proof of his contention that the mussels exercise choice of the material ingested. Another proof, he thinks, is the observation that the mussels with which he worked did not ingest carmine grains. Furthermore, he never found sand in the alimentary tract of the mussels, and states that no one has reported finding it there. He thinks that quite probably "much of the stuff which Evermann and Clark call 'mud' is organic" (Allen, 1921, p. 237). It will be noted below that Coker, Shira, Clark, and Howard (1921) list "silt" in the stomach-contents of certain mussels examined.

The senior author (1915 and 1916) endeavored to test histologically the contention of Pütter (1907 and 1908) that material in solution formed a large part of the food of many aquatic invertebrates and that this was to some extent absorbed directly by the cells of the outer body wall. It was found that fresh-water mussels could make use of nutriment in solution in the water, and that in the case of fat at least, some of this could be absorbed directly by the outer epithelial cells of the body, especially those of the gills.

In natural conditions probably the concentration of dissolved material is not sufficiently great to admit of its forming a very important part of the nutriment of lamellibranchs. Martin's (1923) statement, however, on page 152 of his paper, that "It must be regarded not only as unproved, but as improbable, that dissolved organic substances play an appreciable part in the nutrition of the oyster" is too sweeping, to say the least. Since the only experiments with lamellibranchs (those of Churchill and Mitchell) that put the possibility of using such material to the test gave affirmative evidence, the matter can not be dismissed in favor of the negative with no experimental evidence whatever. While probably such material, as just said, is not a very important item in the dietary of lamellibranchs, it can not be said that it does not "play an *appreciable* part."

Kellogg (1915) worked out with great care the details of the arrangement and movements of the cilia of the mantle cavity, gills, and palps in some 30 species of lamellibranchs, including 2 fresh-water mussels—*Unio* (*Symphynota*) *complanata* and *Anodonta* sp. In regard to the fresh-water mussels he states that material is caught on the gills, carried to the palps (substantially as described by Allen), and is moved across these by forward-beating cilia on the transverse ridges. He states that there is no reversal in the direction of the beating of the cilia, and that "there is no selection or separation of food organisms from other water-borne particles." He found that the cilia in the bottoms of the grooves on the palps beat downward and that on occasions the palps "elongate," thus causing the grooves between the ridges to widen and expose more freely the cilia therein. Material is removed from the palps by these downward-beating cilia. Kellogg concluded that volume alone determined whether or not the "collected foreign matter that reaches the palps shall proceed to the mouth or be removed from the palps" and that "a lamellibranch is able to feed only when waters are comparatively clear." In turbid waters all material passes off the palps and the animal might actually be starving in the midst of plenty. (See Grave and Nelson below). In fresh-water mussels the material thrown from the palps into the lower part of the mantle chamber is moved backward by cilia on the mantles and passed out between the valves at a point below the incurrent siphons in a more or less continuous stream during feeding.[3]

Baker (1916) lists as feeding on detritus and plankton *Lampsilis radiata, L. borealis, L. luteola, L. iris, Anodonta cataracta, A. implicata, A. marginata, A. grandis footiana, Strophitus undulatus* and *Margaritana margaritifera.*

Nelson (1918) found in fresh-water mussels that the esophagus was lined with cilia, which carry the ingested particles to the stomach. He also described the large tufts of cilia on certain cells lining the stomach, which cilia cause to rotate the crystalline style, the anterior end of which projects from the intestine into the stomach. Nelson showed that the style in lamellibranchs possesses at least four functions: (1) Separating foreign particles from the food in the intestine, (2) serving as a substitute for peristalsis, (3) restoring, in some species, to the stomach undigested food particles that have started down the intestine, (4) bearing enzyms. His conclusions relative to the possibilities of a lamellibranch feeding in heavily turbid waters are given below.

Cobb (1918) found that both the attached and the detached palp responded to mechanical, chemical, electrical, thermal, and photic stimuli by curling outward and upward at its free (posterior) tip. Such a reaction would cause the ridges, which would be on the convex side of the curled palp, to be pulled farther apart, allowing the cilia in the grooves to be more exposed and more material to be carried down to the lower edge of the palp. This curling reaction was observed by the present authors in juvenile mussels actually feeding, as will be described later, and fits in well with Kellogg's statements relative to an elongation of the palp to expose the cilia in the grooves; the curling would make the convex side longer and the ridges farther apart.

Evermann and Clark (1920) published extensive lists of material found in the alimentary tract of the fresh-water mussel. These include a wide variety of diatoms and other algæ, Protozoa, and organic and inorganic débris.

Coker, Shira, Clark, and Howard (1921) concluded that there is practically no discrimination in the kind of material ingested in nature, listed a variety of plant and animal forms, laid stress on the quantity of detritus taken, found considerable mud in certain adult mussels, and stated that in 60 juveniles ranging from 5 to 21 mm. long, the contents of the alimentary canal consisted of about 92 per cent "organic remains (principally vegetable matter)," 3 per cent "inorganic remains (silt, etc.)," and about 5 per cent unicellular green algæ and diatoms. Certain feeding experiments were performed, which tended to show that detritus formed the main bulk of the food in nature, although considerable quantities of plant and animal forms were taken. Substances such as fish meat, tadpole tails (macerated), animal fat, fish blood, and fresh vegetable were not taken as readily, or scarcely at all in some cases. Olive oil in the form of an emulsion was ingested and digested by certain of the mussels.

Bush (1922) studied the histology of the groove in the ventral edge of the inner gill and showed that it is lined with ciliated cells by means of which the material that has passed down the gill is carried forward to the palps (fig. 3).

[3] This expulsion has no relation to the occasional ejection of material from the mantle chamber by a spasmodic closing of the valves, such as is often observed.—Authors.

LAMELLIBRANCHS IN GENERAL.

Erman (1833) stated that the food of lamellibranchs was carried forward in currents set up by the action of the cilia and then fanned into the mouth by the palps.

Thiele (1886) considered that the structure and position of the palps showed that their chief function was to transfer to the mouth the food collected by the gills.

MacAlpine (1888), however, from observations made upon detached portions of the palps and gills, concluded that they take no part in the feeding process but carry away foreign material.

Lotsy (1893), working with marine clams and oysters, concluded that they can discriminate between various sorts of food material, but made no statement relative to the mechanism by which this was accomplished.

List (1902) stated that in "die Mytiliden" all foreign bodies that reach the palps are carried into the mouth if they do not exceed a certain size.

Petersen and Jensen (1911) showed that the organic débris resulting from the disintegration of eelgrass, etc., forms a very important item in the food of marine invertebrates.

Blegvad (1914) reached the conclusion that "Detritus forms the principal food of nearly all the invertebrate animals of the sea bottom, next in order of importance being plant food from fresh benthos plants."

Grave (1916), working with oysters, concluded that considerable choice of food material was exercised by means of a reversal in the direction of the beating of certain cilia of the palps. He refers to Parker's (1905) experiments in which a reversal of the cilia was found in Metridium in response to certain stimuli such as crab juice and the like. In this paper Parker calls attention to the observations of Purkinje and Valentin (1835), in which they noted spontaneous reversal of the cilia on the "accessory gills" (Nebenkiemen of Purkinje and Valentin) of the mussel, and to the fact that these observations were confirmed by Engelmann (1868, 1879, and 1898). Grave disagreed emphatically with the conclusion of Kellogg that lamellibranchs do not feed when the water is too full of sediment, offering as proof the results of the examination of the stomach-contents of a number of oysters kept some hours in very turbid water. These oysters had ingested great numbers of food organisms *and also great quantities of sediment* (italics are the authors'). After a period of 14 days the oysters kept in this very turbid water had made "perceptible growth of shell." Grave thought that there was a reversal of the beat in certain cilia on the palps by which sufficient silt was removed from the palps to allow feeding to proceed.

Mitchell (1918) found that oysters kept in solutions of dextrose absorbed this sugar and converted it into glycogen.

Nelson (1920) described the ingestion of great quantities of carmine particles by veligers of oysters. These veligers have no palps, and Nelson stated that this is the reason that the carmine was not discriminated against and separated from the food material. In his 1921 paper he showed by conclusive experiments that oysters "continue to feed in waters bearing as high as 0.4 gram, dry weight, of suspended matter per liter," thus calling into question Kellogg's conclusions in this connection.

Martin (1923) made examinations of the stomach contents of oysters under natural conditions and conducted feeding experiments. He found that they ingested animal and plant forms and artificially ground algal forms. In some cases the particles of ground marsh grass (*Spartina glabra*) were not ingested but found imbedded in mucus at the bottoms of the mantles, which circumstance was taken to indicate that this material had been rejected.

MATERIALS AND METHODS.

The following species of mussels were used in the investigations: *Lampsilis gracilis*, *L. lævissima*, *L. anodontoides*, *L. fallaciosa*, *L. ventricosa*, *L. luteola*, *Obovaria ellipsis*, *Anodonta grandis*, *A. corpulenta*, *A. imbecillis*, *Quadrula pustulosa*, *Q. pustulata*, *Q. undata*, *Q. plicata*, *Obliquaria reflexa*, *Plagiola donaciformis*, and *Sphærium* sp.

The species *Lampsilis luteola* is abundant in Lake Okoboji and furnished the basis for much of the work done there. Juveniles of this species, available in quantities from the propagation experiments as stated above, furnished the basis for a large share of the direct observations made upon the feeding reactions in juvenile mussels.

Owing to the transparency of the valves of the juveniles it was possible to observe directly under the binocular microscope, when light from a 75-watt electric light was reflected up through the mussel, the ciliary action of gills, mantle, and palps, and the ingestion of food. Feeding experiments were also made with both juvenile and adult mussels and the contents of the alimentary canal examined both by means of paraffin sectioning and by dissection. Examination was made of the contents of the stomachs and intestines of mussels taken directly from troughs in which the propagation experiments were being conducted, and also of specimens taken from Lake Okoboji, the Little Sioux River near Lake Okoboji, and the Mississippi River at Fairport.

The anatomy of the palps and gills of juvenile and adult mussels was studied with reference to the feeding process, largely by means of sections stained with hæmatoxylin and eosin.

INGESTION.

To summarize the general process of ingestion, the action of cilia on the gills and in the superbranchial chamber causes a current of water to enter the inhalent siphon, pass through the gills into the superbranchial chamber, and out the exhalent siphon. Cilia upon the gill filaments intercept the particles (animals, plants, débris, and the like) contained in the stream of water. These are entangled in mucus secreted by certain cells of the surface of the gills, and through the concerted action of the cilia the masses of mucus, together with the contained particles, are carried forward on the gills and deposited upon the labial palps. Cilia on these carry the mucus and the particles either to the mouth or to the ventral edge of the palps, where they fall into the mantle chamber. In the latter case the material drops to the edge of the mantle chamber and is carried backward by the cilia near the ven-

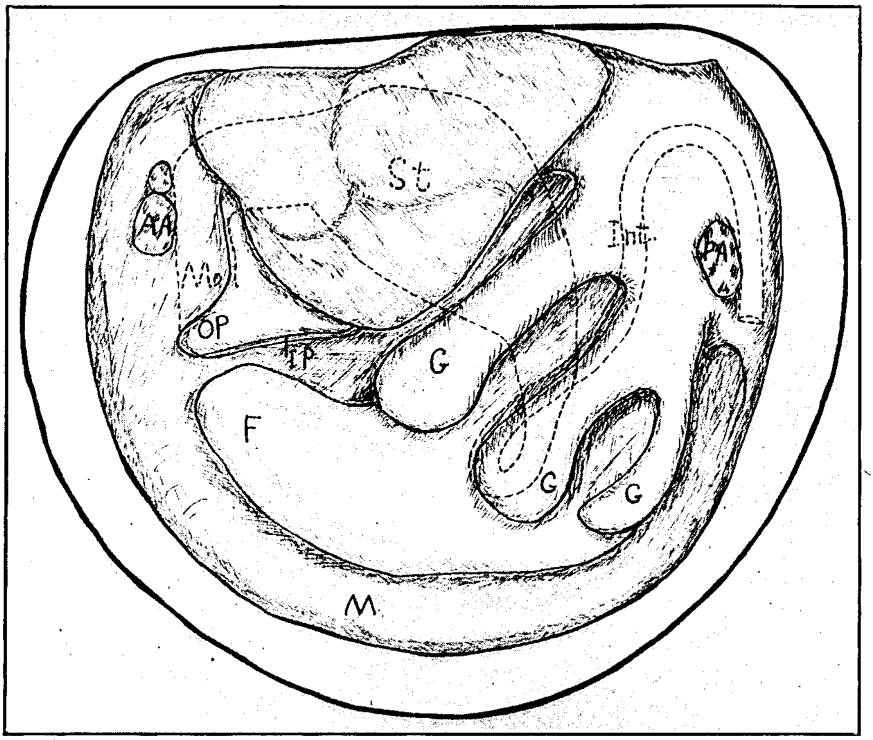


FIG. 1.—Side view of a mussel immediately after leaving the cyst on the gill of the fish. About 0.2 mm. long. *AA*, anterior adductor; *PA*, posterior adductor; *F*, foot; *G*, papillæ of developing inner gill; *M*, mantle; *IP*, inner palp; *OP*, outer palp; *Mo*, mouth; *St*, stomach; *Int*, intestine. (Adapted from Herbers.)

tral margins of the mantles and expelled between the valves just beneath the inhalent siphon. It might appear at first thought that particles suitable for food were carried to the mouth and those unsuitable thrown off the palps and out of the mantle chamber. The matter is, however, not as simple as it would appear to be at first sight, and, as noted above, investigators disagree concerning both the probability of selection or choice of substances ingested and the cause for and the mechanism by which certain material is thrown off the palps. It will be noticed especially that Allen (1914, 1921) and Kellogg (1915) hold diametrically opposed views regarding these points. Allen holds that the mussel exercises considerable choice in the ingestion of material, reversing the streams across the palps by altering the slope of the transverse ridges (Wallergren, 1905). Kellogg maintains that for lamellibranchs, in general, including fresh-water mussels, "there is no selection or separation of food organisms from other water-borne particles."

Kellogg further declares that if too much material is present it is all removed from the palp and nothing enters the mouth, and that the bivalve may actually starve under such conditions. Grave (1916) takes positive exception to this assertion of Kellogg, and states that the lamellibranch, especially the oyster, can select food material from the silt or débris and consequently is able to feed even though the water be heavily loaded.

On account of these divergent views it seemed necessary to investigate again the mechanism of and factors involved in the ingestion of food in fresh-water mussels before proceeding further. Especially did it seem of advantage to undertake this study, in part at least, upon the very early juvenile stages, since the transparency of the valves allowed direct examination to be made of the entire feeding process under the binocular microscope.

OBSERVATIONS AND EXPERIMENTS.

MUSSELS 0.2 MM. LONG.

When the mussel, after metamorphosis, leaves the gill of the fish it measures from 0.2 to 0.25 mm. in length. Its main anatomical features are represented in Figure 1. The gills consist of three papilla-like protuberances on each side, attached at their dorsal ends to the upper part of the visceral mass. These will later elongate and join at the free ends to form the lamellæ of the inner gill, the outer gill developing much later. The outer palp appears as a ridge projecting downward from the side of the visceral mass. The inner palp has not yet begun to develop, and the siphons are not in evidence.

Six specimens of *Lampsilis anondontoides*, which had been off the fish less than 24 hours, were secured from the rearing troughs. They measured about 0.2 mm. in length. Almost no postglochidial shell had been formed. They were not very active. These mussels were placed in a watch glass with a little material from the trough and observed under the binocular microscope, light from a 75-watt electric bulb being reflected up through the culture. Since this treatment did not affect the feeding reactions of the mussels in the least, it was made the usual procedure in the following experiments.

One mussel finally became very active and moved about by means of the ciliated foot. Very fine particles of débris floating on the water could be seen to pass between the edges of the valves into the mantle chamber. The particles were for the most part extremely fine, best seen with the relatively low powers of the binocular by turning the substage mirror so that the particles caught the light and resembled dust motes in a sunbeam. They entered the mantle chamber in front of and at the sides of the foot, not in the siphonal region. Owing to the greater relative convexity of the shell, darker pigmentation of the tissues, and minuteness of the parts of these small mussels it was impossible to follow these particles after they entered the mantle chamber. No particles, however, except discharged feces, were seen to leave the mantle chamber, so it would appear that the material was ingested. Several small flagellates and ciliates were drawn near or approached the gaping valves but darted away before or upon touching them. In fact, most of them were too large to enter, even if they had been inert.

On the next day one of this same lot of mussels (now about 36 hours off the fish) was observed for an hour or two. It steadily took into the mantle chamber fine particles in front of and at the sides of the foot. At times a black mass could be observed whirling about in the stomach. Later on, by observing somewhat larger mussels, this was ascertained to be a mass of ingested material and mucus being rotated by cilia on certain cells of the stomach wall. Powdered carmine was added to the culture. Considerable was drawn into the mantle chamber and soon a red mass could be seen whirling about in the stomach, showing that carmine had been ingested. (Nelson, 1920.)

MUSSELS 0.28 MM. LONG.

The anatomical features at this stage have not progressed much beyond those of the preceding. A specimen of *Lampsilis luteola* was observed as described above. Minute floating particles were being drawn between the valves at the anterior end and on the ventral side. Some very small flagellates were seen to pass in, but none could be observed to pass to the esophagus, all passing off the rudimentary palps. Powdered carmine was put in the culture and the currents ceased, though the valves remained gaping. No action was seen for 10 minutes, when the mussel was removed and placed in a culture free from carmine and the current soon began again.

MUSSELS ABOUT 1 MM. LONG.

At this stage the papillæ of the inner gill have united at the ventral ends, presenting more the appearance of the adult structure. The outer gill is yet undeveloped. The alimentary canal has about the shape shown in Figure 2. The siphons have not yet appeared. There are, however, two palps on each side, the apposing faces of each pair showing transverse ridges (fig. 9, opposite p. 458). The ridges and the furrows are ciliated. When mussels of this size were lying or crawling upon a glass substratum with a relatively small amount of débris about them, the currents of material passed in at points all along the valves from the mid-ventral side to the anterior end, as was true of the smaller forms. An effort was made to learn what happened in case the mussel were upon a substratum in which it might partly bury itself, leaving exposed the posterior end where the siphons later develop. This latter position is, of course, that assumed by the older stages after the byssus has disappeared, and possibly by the very small ones before the development of the byssus. The byssus does not appear until the mussel is over 1 mm. long.

Accordingly, a specimen of *Lampsilis luteola* about 1 mm. in length was placed on a substratum of sand grains about the size of the mussel, into which sand grains it at once crawled. These were then moved with a needle, so that a portion of the anterior end of the mussel was visible through the chinks between them. Fine débris was gently dropped from a pipette upon the sand grains. Particles of the débris could be seen to be sucked down between the grains and into the anterior end of the mantel chamber of the mussel. It would appear that in a mussel of this

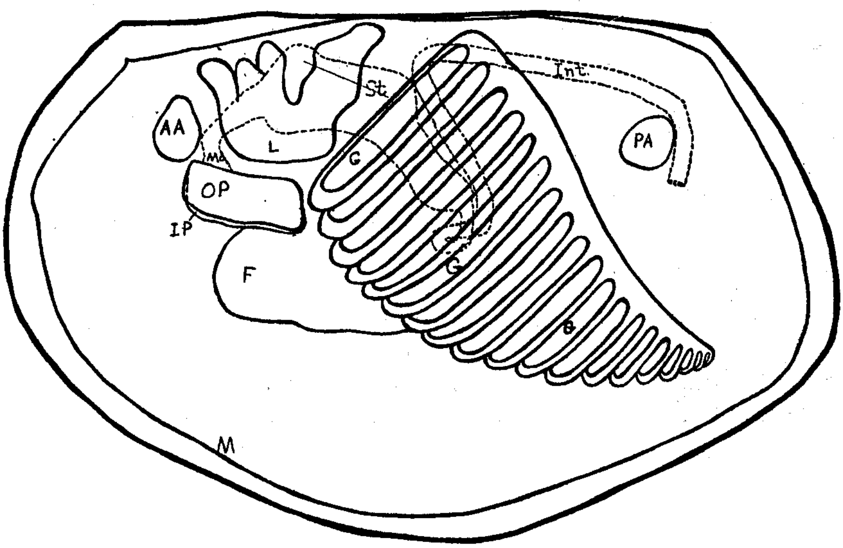


FIG. 2.—Side view of mussel about 2 mm. long. *AA*, anterior adductor; *PA*, posterior adductor; *F*, foot; *G*, gills; *IP*, inner palp; *OP*, outer palp; *L*, liver; *M*, mantle; *Mo*, mouth; *St*, stomach; *Int*, intestine. (Adapted from Herbers.)

size, with no siphons, the currents enter all along from the midventral side to the anterior end.

Several specimens of *Lampsilis luteola* were observed. A current of particles consisting of fine débris passed in around the foot. Part of these were carried forward to the mouth and part passed off the palps, down along the inside of the mantle and back to a point at the posterior end below the place where the inhalent siphon later develops. Here they passed out of the mantle cavity, as stated by Kellogg. No cilia could be seen on the edges of the mantles in the siphonal regions, although cilia were visible elsewhere along the edges of the mantles.

A specimen of *Lampsilis luteola* was placed in a heavy culture of Euglenas measuring about 60 by 18 micra when elongated. Some were rolled up in an approximate spherical shape and measured about 25 micra in diameter. The mussel opened at intervals and took in some Euglenas. In several instances a particular Euglena was observed throughout its entire journey from the outside to the stomach. It was drawn in at the anterior end or ventral side and fell upon the gill (the inner—the outer not being developed yet) and was carried forward to the palp. The outer palp lifted at the posterior end and caught the Euglena under it. Owing to the transparency of the tissues the strong electric light thrown up from below made it possible to see the bulky green Euglena carried forward between the apposing faces of the palps, move slowly behind the anterior adductor muscle, and up the esophagus slowly for about half the way, then with increasing speed until it fairly shot into the stomach. After a time a number of Euglenas could be seen being whirled about in the stomach by the cilia there.

Another specimen of *Lampsilis luteola* was taken from the rearing trough where it had been feeding actively, the intestine being full (as observed through the shell), and put at once into a culture containing carmine grains, Euglenas, and some smaller unidentified organisms. The mussel opened the valves at once and carmine, Euglenas, and the other organisms were all seen to pass up the esophagus. In about 30 minutes the intestine was red back to the anterior end of the rectum, which still contained some of the material previously ingested.

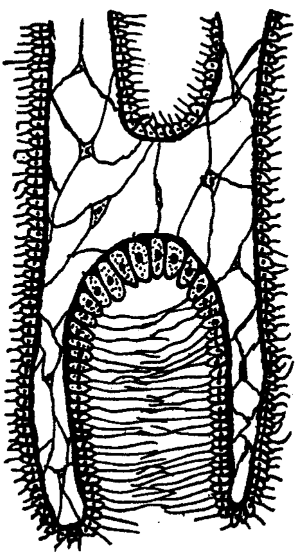


FIG. 3.—Cross section of groove on ventral edge of inner gill. The larger cells in the upper end of the groove bear no cilia and secrete mucus, according to Bush. (After Bush.)

Three specimens of *Lampsilis luteola* were observed while in a suspension of carmine grains. The current of particles entered at the anterior end. The particles could be seen to be carried quickly back past the anterior part of the visceral mass, practically without touching it, and to fall upon the gill (the inner). They passed down the gill, as stated by Allen (1914) and Kellogg (1915), to the lower edge and forward along this to the anterior end. In adult mussels there is a ciliated groove along the lower edge of this gill (fig. 3) through which the food is carried (Posner, 1875, and Bush, 1922). From the gill the carmine particles passed between the apposed faces of the palps. A great deal of it was carried downward and passed off the lower posterior corner of the palp. While in this experiment none was actually seen to enter the mouth, the lower loop of the intestine (fig. 2, p. 449) soon became quite red, showing that carmine had been ingested. After a time the mussel ceased ingesting and closed.

MUSSELS 2 TO 2.5 MM. LONG.

As far as the structures involved in ingestion are concerned, there is no marked advance in a mussel of this size from that of one measuring 1 mm. in length. The rudiments of the siphons are beginning, but there are no tentacles apparent, and the material enters the mantle chamber at various points from just below the region of the inhalent siphon to the anterior end. These points may vary from time to time in the same mussel, or the currents may enter at two or three points at once. In many mussels up to at least 4 or 5 mm. in length it is not at all uncommon to observe particles entering the anterior end and the ventral side simultaneously.

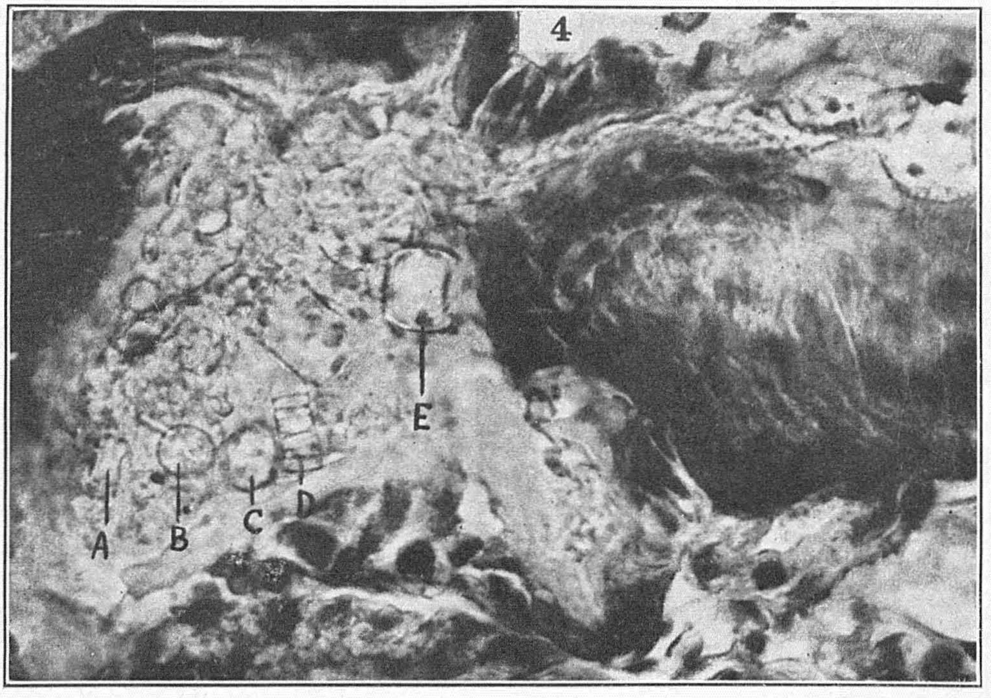


FIG. 4.—Section of stomach of *L. luteola* 1 mm. long. *A*, *B*, and *E*, *Stephanodiscus* sp.; *C*, probably a diatom; *D*, filamentous diatom, probably Odontidium. ×900.

FIG. 5.—Section of stomach of *L. luteola* 2 mm. long, which had been observed ingesting red Euglenas. *A*, Euglenas; *B*, crystalline style; *C*, entrance to intestine. ×430.

This statement applies, of course, only to observations made with the mussel lying on its side on a substratum of glass with usually only a moderate amount of débris about it.

Two specimens of *Lampsilis luteola* were removed from a rearing trough and observed at once in a culture of the material taken directly from the trough. A current of the fine débris in the water was passing in and between the palps. Much of this passed downward and off the palps, but at the identical time some particles moved forward between the palps and up the esophagus. These particles passed between the palps rather near the upper edges. It could not be seen that they differed from those particles passing off the gills. It merely appeared that some of the débris moved across the inner faces of the palps to the mouth while some moved down and off. Both processes were continuing simultaneously. A specimen of *Lampsilis luteola* of this size also ingested carmine grains. The experiment was continued until carmine grains were observed in the feces.

A specimen of *Lampsilis luteola* was placed in a culture containing red Euglenas 160 by 35 micra in size. Some of the Euglenas were ingested, notwithstanding their size. They were apt to assume an approximate spherical shape when being moved in the ciliary currents. In the bright light reflected from the electric bulb up through the mussel red Euglenas were quite conspicuous and could be seen to fall upon the gills and pass down, some falling into the mantle chamber and some passing forward and between the palps. Of these latter some were thrown off and some taken forward to the mouth and up the esophagus to the stomach. In the stomach they were seen as a dark mass, rotating clockwise (viewed from anterior end of the mussel). The Euglenas were whirled about by this and batted, so to speak, into the cæca of the stomach at the sides and below the entrance from the esophagus.

This specimen was fixed and sectioned and a photomicrograph of a section through the stomach is shown in Figure 5. Portions of the Euglenas can be seen, also tufts of long cilia on the sides of the stomach and a cross section of the style, which evidently projects into the stomach, as shown by Nelson (1918) for adult mussels. The style is kept in rotation by the cilia, and it is the movement of the two that causes the ingested food to be whirled about in the stomach as observed in mussels whose valves are sufficiently transparent to allow this movement to be seen. In view of Nelson's (1918) excellent work on the crystalline style and the fact that the style was not the especial object of this study, no further suggestion will be made here concerning its function.

A specimen of *Lampsilis luteola* was observed while in a culture containing some of the diatom *Stephanodiscus*. Some of these were ingested and could be followed as they passed from gill to palps and between these to the stomach. At the same time other specimens of *Stephanodiscus* were being carried down to the lower edges of the palps and thrown off. One or two ciliates larger than the diatoms were also observed during their entire course to the stomach. Their route was that already described. A considerable mass of débris was pushed with a fine glass rod up about the mussel as it lay on its side. Many particles were drawn in all along the edges of the valves from the anterior to the posterior end. Great numbers of these passed between the palps but were carried to the lower edges and thrown off. No attention was given to the esophagus in this instance. Carmine was then put into the culture and a mass of particles pushed against the gaping valves of the mussel. A long stream of red particles shot in through the lower part of the region of the inhalent siphon, swept across the gill and between the upper part of the palps like a flash, and up the esophagus—a bright red stream—into the stomach. The instant the advance end of the stream entered the stomach the valves closed sharply, but the carmine between the palps and in the esophagus passed on into the stomach. The valves remained closed and no more material of any sort entered. In about 10 minutes the loop of the intestine was red in color, showing that the carmine had passed through the stomach.

MUSSELS 3 MM. LONG.

At this size, while there has been as yet no significant structural development, the mussels have by no means remained stationary. Tentacles have developed about the siphons, but when the mussel was lying on its side in a watch glass these were not extended and visible. A specimen of *Lampsilis luteola* was placed in a suspension of carmine grains. At first a few were ingested, appearing very bright and distinct as they passed between the palps. Then the mussel closed and remained so. After 10 minutes the experiment was terminated.

A specimen of *Lampsilis luteola* with the intestine practically empty was placed in a watch glass and some mud from the Mississippi River added to the water. This "mud," upon examination under the microscope, proved to be composed of sand grains measuring from 3 micra to 20 micra across and of organic débris. Some of the latter was as fine as the sand grains; some larger. Streams of this material entered the mantle chamber of the mussel in front and on the ventral side and passed between the palps, as heretofore described. Heavy streams of the material could be seen to pass down the inner face of the outer palp (looking through the palp, of course) and along the lower edge to the lower posterior corner and off. This end of the palp, especially at the lower corner, would curl upward somewhat (Cobb, 1918). A fairly heavy stream or rope of material would be streaming down off this corner all the time if any amount of material were entering between the palps. The streams of material passing down the inner face of the palp were in fixed vertical rows, without doubt corresponding to the positions of the transverse ciliated grooves on the inner face of the palp. At times particles passed along across the inner face of the palp and forward to the mouth and could be watched until they entered the stomach. These particles usually passed across the upper part of the palp, in the apex of the inverted V formed by the apposing faces of the palps. Since the upper part of the palp is more opaque than the rest, only the larger particles could be followed to the mouth, and it is almost impossible to observe fine particles passing along the esophagus unless they are brightly colored. It would seem, however, that many smaller particles must have passed forward and been ingested, since the intestine was filled and feces were being discharged in less than an hour after the beginning of the experiment. The mussel was fixed and sectioned and a photomicrograph of a portion of the intestine is shown in Figure 26 (opposite p. 461). Apparently there was no selection of material, all sorts being ingested.

A specimen of *Lampsilis luteola* was placed in a suspension of carmine. Particles could be seen to pass across the inner face of the outer palp over the dark vertical streaks representing the furrows between the ridges. In their passage these particles were scattered across the upper half of the palp. At the same time other particles were passing down the vertical streaks to the lower edge of the palp and back to the posterior end and off. The mussel soon closed the valves and did not ingest until removed from the carmine.

In all these cases the stream or rope of material mingled with mucus discarded from the lower posterior corner of the palp moved downward and backward along the lower part of the mantle chamber and was pushed out somewhat below the region of the inhalent siphon (fig. 6).

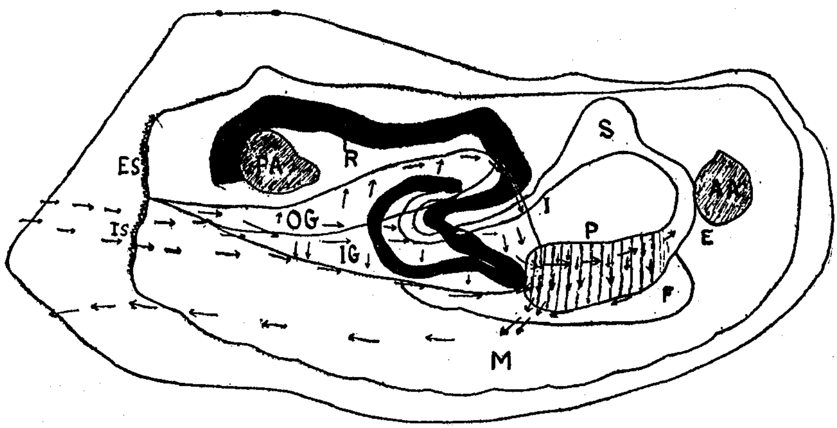


FIG. 6.—Outline of mussel 12 mm. long, showing by arrows the course taken by material in the mantle chamber. *AA*, anterior adductor; *PA*, posterior adductor; *F*, foot; *IS*, inhalent siphon; *ES*, exhalent siphon; *IG*, inner gill; *OG*, outer gill; *P*, palp; *E*, mouth; *S*, stomach; *I*, intestine; *R*, rectum filled with material; *M*, mantle.

MUSSELS FEEDING IN HEAVY SUSPENSIONS.

A specimen of *Lampsilis luteola* about 3 mm. long was placed in a very heavy suspension of débris from the river bottom. Great quantities of material were drawn in around the foot, fell upon the gill (the inner, as yet), and passed forward and between the palps. Heavy streams passed down the inner face of the outer palp, as described above, in fixed vertical rows and were thrown off in a very heavy mucus rope from the posterior corner. A very few particles were seen to pass along the upper part of the inner face of the palp to the mouth. Evidently the mussel could obtain at least a small amount of material even though the water were heavily loaded.

A specimen of *Lampsilis luteola* about 3 mm. long was placed in a watch glass on a bottom of material from a creek bed, consisting of fine sand, débris, and small black particles (probably organic). The mussel crawled into this. The tentacles and siphons were extended then and plainly visible. A current passed in at the inhalent siphon, as in the adult. From 9.20 to 9.50 a. m. the mussel was all hidden but the siphons, and masses of débris were piled over these with a fine glass rod. This débris was drawn in rapidly. At 9.50 a. m. the débris was cleared off the region over the palps, mouth, and esophagus, so that the light could penetrate. Débris was then piled over the inhalent siphon so that a great quantity was passing in and between the palps. Great streams of this passed downward, and a heavy rope of material poured off the posterior corner of the palp. A good many particles and masses of mucus-entangled material passed forward across the upper part of the inner faces of the palps to the mouth. These sometimes stopped along the way for a time as if the palps pressed together and held them. There was no doubt at all that some material was ingested.

Some carmine was mixed with sand and débris, and at 10.15 a. m. the mussel was arranged as before, so that the palps, etc., could be seen. Then the mixture was left piled over the siphon as before. The valves opened and closed a few times and then admitted some material—carmine, sand grains, and débris—some of which was seen to pass between the palps to the esophagus and mouth. A heavy stream passed off the palps, of course, at the same time. Soon, however, the valves closed and the mussel did not reopen them. On being removed to clear water red material could be seen in the stomach and the loop of the intestine. This mussel ingested material, although the water was so heavily laden the siphons were completely buried and a heavy strand was passing off the posterior lower corner of the palp all the time. This strand or mucous rope of material was at least 40 micra wide most of the time.

In the clear water with no carmine, as the foot was stretched out, cilia having begun moving in the stomach, it could be seen that the material in the interior part of the intestine, at least as far down as the loop, was rotating. This was no doubt due to the rotation of the crystalline style.

At 11.15 a. m. red particles began to be observed in the feces; also multitudes of sand grains. In one small mass were 3 sand grains, about 30 by 30 micra, and one 60 by 30, besides more minute ones. In another was a grain 40 by 30 by 25 micra.

Two specimens of *Lampsilis luteola*, one 4 mm. long and one 2½, were kept in clear water a few hours until the alimentary canal was quite empty throughout. They were then placed in such a heavy suspension of débris from the rearing trough that the mussels could not be seen. The suspension was agitated with a fine glass rod, so that the mussles were kept covered during the experiment, which continued from 4.30 to 5.15 p. m. At the end of that time so much material had been ingested that the rectum appeared as a solid black line as far as the anus.

MUSSELS 4 MM. LONG.

The outer gill has not yet developed. A specimen of *Lampsilis luteola* was placed in a suspension of carmine at 9.45 a. m. Carmine began to pass in and accumulate in oval boluses in the esophagus. Each bolus passed into the stomach in a swift gulp, so to speak, several seconds to a half minute between gulps. After about 10 minutes the stomach appeared red from the carmine being rotated there by the cilia. Then the loop of the intestine began to appear red. About this time the mussel ceased ingesting and was removed to clear water. The remainder of the carmine

FIG. 7.—Section of stomach of *L. luteola* about 3 mm. long, which had been kept in a suspension of carmine. *A*, carmine grains; *B*, *Scenedesmus* sp.; *C*, entrance to intestine. ×430.
FIG. 8.—Cross section of intestine of mussel of Figure 7. *A*, epithelium of intestine; *B*, mass of carmine.

passed slowly out of the stomach and accumulated in a mass about 1 millimeter long, which moved slowly along the intestine and was discharged in one mass into the mantle cavity at 11.30 a. m., about one hour from the time it had been ingested. The walls of the alimentary canal retained a pinkish coloration as if the epithelium had been stained by the carmine. In this observation a rotating mass of material could be seen in the loop of the intestine, consisting, no doubt, of particles kept in motion by the crystalline style.

A specimen of *Lampsilis luteola* was observed while in a suspension of débris, particles of which passed to the palps, where some passed down the dark vertical streaks (the grooves) on the inner faces and some passed forward, usually fairly near the top of the palp, to the mouth. The larger share of particles passing off the palp went down the first two or three grooves, but a considerable number reached the middle or even the more anterior grooves before being carried down. This material on reaching the lower edge of the palps moved backward along the edge and merged with the heavier stream passing down the posterior two or three grooves. The posterior end of the palp as far forward as the posterior two or three grooves was often curled up somewhat.

A specimen of *Lampsilis luteola* was allowed to ingest carmine until it became red from stomach to anus. In this, as in all the other cases, no considerable quantity of material accumulated in the stomach. The ciliary movement going on there, and perhaps the action of the style, kept the material passing on into the intestine fairly rapidly, so that the stomach was never packed full, as the intestine usually is. This mussel was fixed and sectioned, the sections being mounted unstained. In Figures 7 and 8 photomicrographs of sections of the stomach and intestine are shown. Particles of carmine were plainly visible in the stomach, and the lumen of the intestine was packed solidly with it. The epithelial cells of the alimentary canal had taken the stain sufficiently to give them a marked pink color.

MUSSELS 12 MM. LONG.

At this size the anatomy of the mussel is substantially that of the adult, except that the outer gill is still less than half its final width (fig. 6, p. 453). For that reason it plays little or no rôle in feeding.

Several specimens of *Anodonta imbecillis* were observed ingesting small particles of débris. This passed in at the inhalent siphon, fell upon the inner gill, passed down to its lower edge, went forward along it (probably a groove there), and entered between the palps. Some particles moved forward across the upper part of the palp to the mouth and accumulated in boluses in the esophagus and were ingested. Other particles passed down the vertical grooves and were thrown off the palp, as previously described (fig. 6). Sometimes the posterior end of the outer palp curled far upward. The inner palp could not be seen clearly in the living mussel, but in some of the sections the posterior end of the inner palp was curled around so it lay next to the visceral mass (fig. 13). Cobb (1918) found that both the attached and detached palp responded to stimuli by curling outward at its posterior tip. This would operate to stretch the inner surface of the palp, thus widening at least the posterior grooves and perhaps all of them. More material would fall into them and be carried downward. The crystalline style was seen to rotate in one specimen, clockwise, viewed from the anterior end.

MUSSELS OVER 12 MM. LONG.

A specimen of *Lampsilis gracilis* 18 mm. in length was placed in a watch glass of water containing various plankton forms and débris and observed with the aid of a binocular microscope for some time. The mussel kept a stream of water passing in at the incurrent siphon during nearly the whole period. Everything carried by the approaching current was swept in except particles so large that they caught against the short tentacles located upon each side of the siphon. Filaments of algæ, as Lyngbya or Spirogyra, four or five times as long as the width of the siphon, were carried in if brought up endwise. Such filaments were usually swept along in endwise fashion, just as a log is carried by the current in a river. Filaments that met the siphon more or less broadside were caught by the tentacles and held outside. Usually very shortly after particles of débris or filaments had lodged upon the tentacles the valves were closed suddenly thus forcing a jet of water out of the mantle cavity and blowing the material off the tentacles. The current of water usually began entering the mussel again after a short interval. The culture contained also the spherical algæ, Pleodorina and Volvox, débris, and some nauplii and copepods. Pleodorina was carried in by the current, as were the filamentous algæ when moving endwise. Some of the Volvox were too large to pass into the siphon. The nauplii, being active, swam out of the current and escaped, being swept into the mantle cavity. After the mussel had been feeding for a time, perhaps 15 minutes, a string of mucus began slowly to emerge from between the valves just beneath the inhalent siphon. This continued to appear slowly and in an almost continuous strand during the whole time that the mussel was siphoning a stream of water through its body. Upon examination the mucus was found to contain quantities of filaments of Lyngbya and Spirogyra and some Pleodorina. In the light of other observations, quite evidently this mucus had been carried from the palps by ciliary action, bringing with it some, at least, of the material that had entered the inhalent siphon and which had failed for some reason to reach, or at least to enter, the mouth.

During the observation some of the feces expelled from the exhalent siphon were examined and found to contain fragments of Pleodorina, diatoms, and a few pieces of filaments of Lyngbya and Spirogyra, showing that some of the Pleodorina were being ingested and that a few, at least, of the filaments of Lyngbya and Spirogyra had been brought up to the mouth in such a position that they could enter.

A specimen of *Lampsilis gracilis* about 15 mm. long was placed in a culture similar to that just described. Even at this size the valves of this species are so thin that they are somewhat transparent, and the course taken by some of the larger particles after they had entered the mantle chamber could be observed with the aid of the binocular microscope and the electric light. The filaments of algæ, being fairly large, could in many cases be observed during almost their entire course through the mantle cavity. Most of the filaments were whipped forward rapidly over the gills and could be seen to pass between the palps. Their movements at the anterior part of the palps and in the mouth region could not be observed, since the body of mussels of this size is too opaque to allow sufficient light to pass through. At frequent intervals rolls or masses of mucus could be seen to move downward across the palp, bringing entangled filaments along. These masses dropped off the ventral edge of the palp into the mantle cavity and were carried back along the ventral side of this and thrown out just below the inhalent siphon, taking the filaments with them.

Some of the filaments that entered the mantle chamber were carried well up to the dorsal side near the umbo and lodged there for a time, apparently out of reach of cilia. Eventually, however, they would be moved downward to the palp. Some filaments were seen to have been carried to a point entirely forward of the palps, dorsal to the anterior part of the foot. Their precise course in reaching this region was not ascertained. These were later carried downward and along the ventral side of the mantle chamber and out. It should be emphasized that in this and the preceding observation the mucus masses were ejected from between the gaping valves below the inhalent siphon fairly steadily, being forced out by the action of cilia located on the inner surface of the mantle, not by spasmodic closings of the valves. The latter remained motionless during the process.

STUDY OF SECTIONS OF THE PALPS.

ADULT MUSSELS.

Prof. H. Walton Clark, of the Fairport staff, removed several specimens of *Anodonta imbecillis* 10 to 12 mm. long from the river and placed them at once in Bouin's fixative. This killed them instantly, and in a few hours the shells were decalcified by the acetic acid in the fixative. The mussels were then run through the alcohols and cleared in cedar oil. Several specimens so prepared were kindly placed at the disposal of the authors by Mr. Clark. One valve and mantle of these were dissected away with needles under a binocular microscope. The intestine and in some cases the stomach contained ingested material; also in many instances masses of material could be seen upon the palps, some in the grooves, some along the upper part, some forward near the mouth, and sometimes a mass in the esophagus.

The region of the crystalline style was clear and colorless except for a little material along the sides of the style, this serving to mark it off and render it more easily discernible. In some cases the head of the style was visible in the stomach. Some of these mussels were lightly stained with borax carmine and photomicrographs were taken, two of which are shown in Figures 22 and 23 (opp. p. 459). One or the other of these figures shows the points just enumerated, and Figure 23 shows also a mass of débris just leaving the lower posterior corner of the palp.

Frontal sections, which would cut the ridges and grooves transversely, show that vertical ciliated ridges and grooves occur on the inner faces of the palps of mussels from at least the length of 1 mm. upward (figs. 9 to 16, inclusive).

Figure 14 is that of a portion of the palp of an adult *Lampsilis luteola* sectioned transversely to the ridges and grooves and shows the ciliation quite clearly. Data were kept so that the anterior and posterior ends of the palps could be recognized in the sections. In this figure the free edges of the ridges lean toward the anterior end of the palp. It will be noted that there is a conspicuous notch or depression on the posterior side of each ridge near the bottom and another, not as large and well defined, on the anterior side and near the top of the ridge. The notches are, of course, cross sections of longitudinal depressions or grooves along the sides of the ridges. The epithelial cells in the bottom of one of these depressions are scarcely half as high as those covering the remainder of the ridge. The cilia there are short. The posterior notches are shown by Allen (1914, fig. 13) but are not commented upon. The anterior notches are not shown. No further reference to these depressions has been found in the literature on the subject.

It can further be seen (figs. 15 and 16) that the epithelial cells and cilia are both relatively long from the posterior notch upward to a point just anterior to the crest of the ridge, and from this point downward to the anterior notch they are shorter. Immediately below the anterior notch is a group of longer cells and cilia, while below this the cells and cilia are short. In the bottom of the furrow between the ridges the cells and cilia are longer again, and from the bottom upward on the posterior side of the ridge to the posterior notch the cells and cilia are as long as at any point on the palp. These structural details may be observed in specimens as small as 7 mm. (figs. 10 and 13 show them to some extent) but are more conspicuous in the adult.

THEORY OF THE FUNCTION OF GROOVES ON RIDGES OF PALPS.

What is the connection between the anatomical features just described and the observations made of the actual process of ingestion, especially those made in the mussel 10 to 12 mm. long wherein these structures are present? By the use of the strong transmitted electric light, as described, particles were seen to pass across the inner face of the outer palp, from ridge to ridge, to the lips and mouth. Other particles were seen to pass down the furrows between the ridges to the ventral edge of the palp. Both activities were usually occurring simultaneously, either one to a greater or less degree according to circumstances. Particles were never seen to pass across the palp in a posterior direction, and particles were never seen to pass up the grooves toward the dorsal edge of the palp (Siebert, 1913, and Wallengren, 1905). There was never the slighest evidence of a reversal in the direction of beat of the cilia, even when carmine grains were touching the mantle edge, palp, mouth, esophagus, or stomach. The valves often closed, apparently to exclude carmine, either at once or after some had been ingested, but no carmine was ever seen to move backward across the palp. Carmine was seen to move down the grooves, as were all sorts of other particles, often at the same time that other carmine grains were moving forward across the palp.

It would seem that the anatomical and behavioristic evidence would support the following theory concerning the action of the cilia. The cilia in the area over the posterior side and crest of the ridge between the posterior and anterior notches always beat forward, moving particles toward the mouth, the ridges leaning well forward. The cells and cilia in most of this area are long. Just above the anterior groove, however, the cilia are short, and these would allow some particles in the process of transfer to the next ridge to fall down between. If the palp were stretched

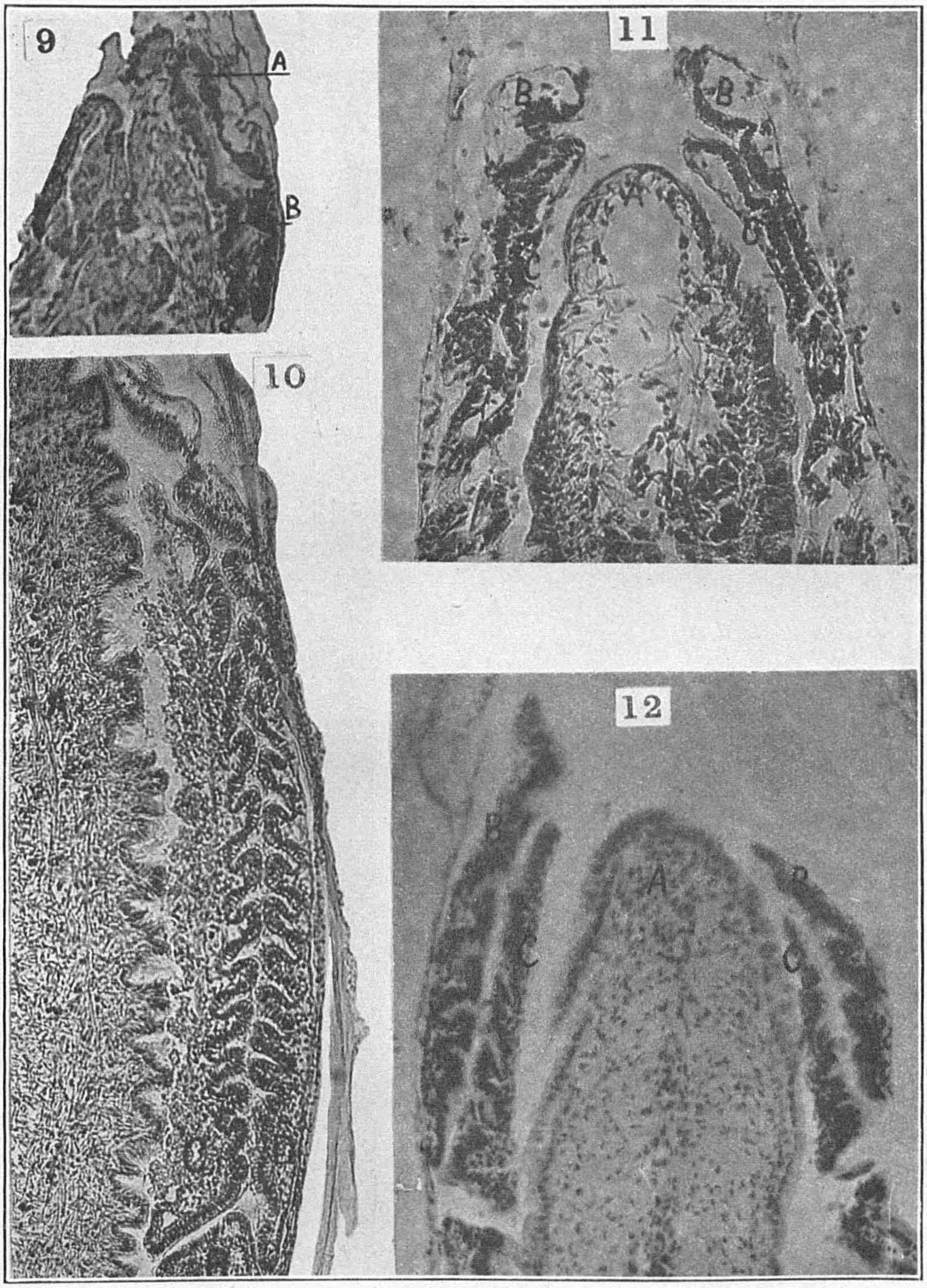
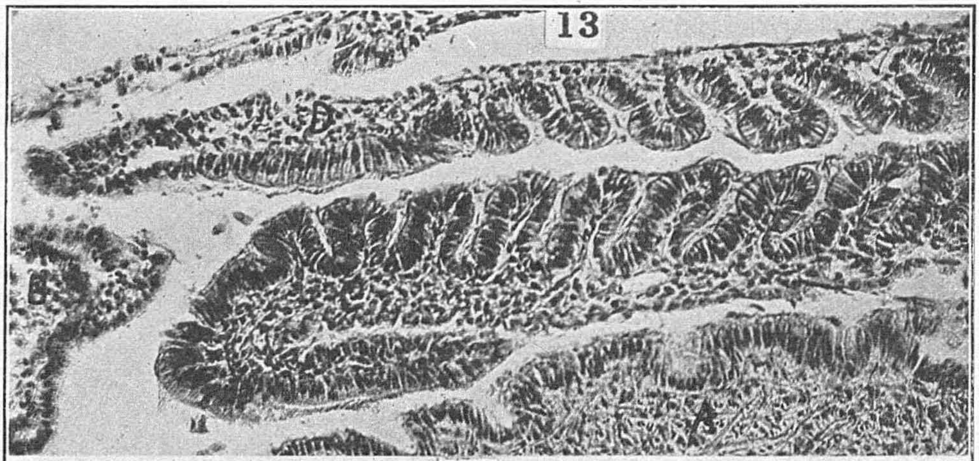
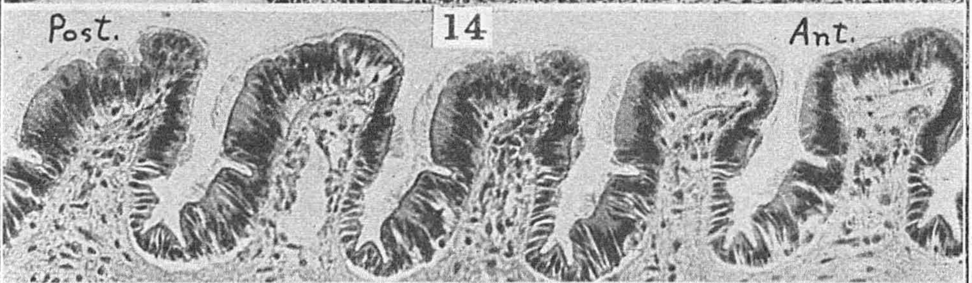
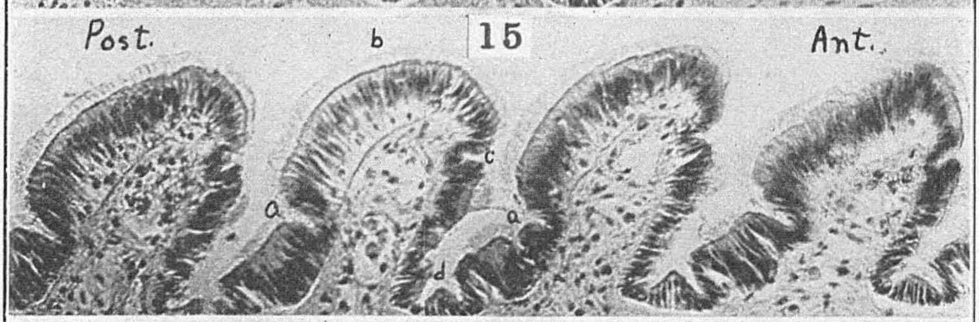


FIG. 9.—Frontal section of *L. luteola* 1 mm. long, showing palps on right side. *A*, mouth; *B*, palps, showing vertical ridges in cross section. ×200.
FIG. 10.—Frontal section of right palps of *L. luteola* 8 mm. long. *A*, foot; *B*, gill; *C* and *D*, palps. ×200.
FIG. 11.—Frontal section of palps of *L. luteola* 3 mm. long. *A*, foot; *B*, outer palps; *C*, inner palps. ×200.
FIG. 12.—Frontal section of palps of *L. luteola* 5 mm. long. Letters as in Figure 11. ×200.







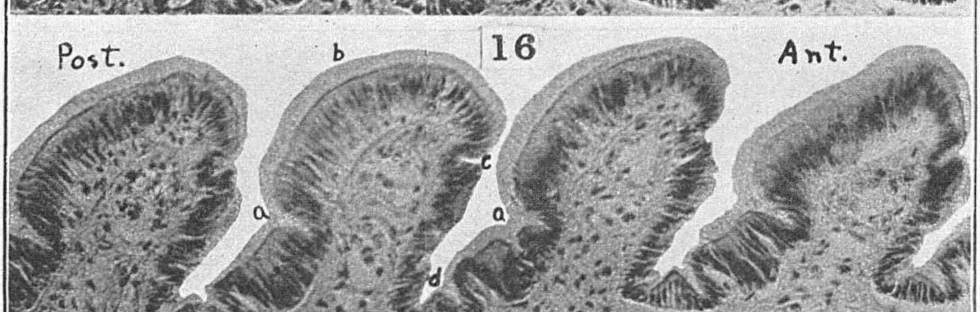
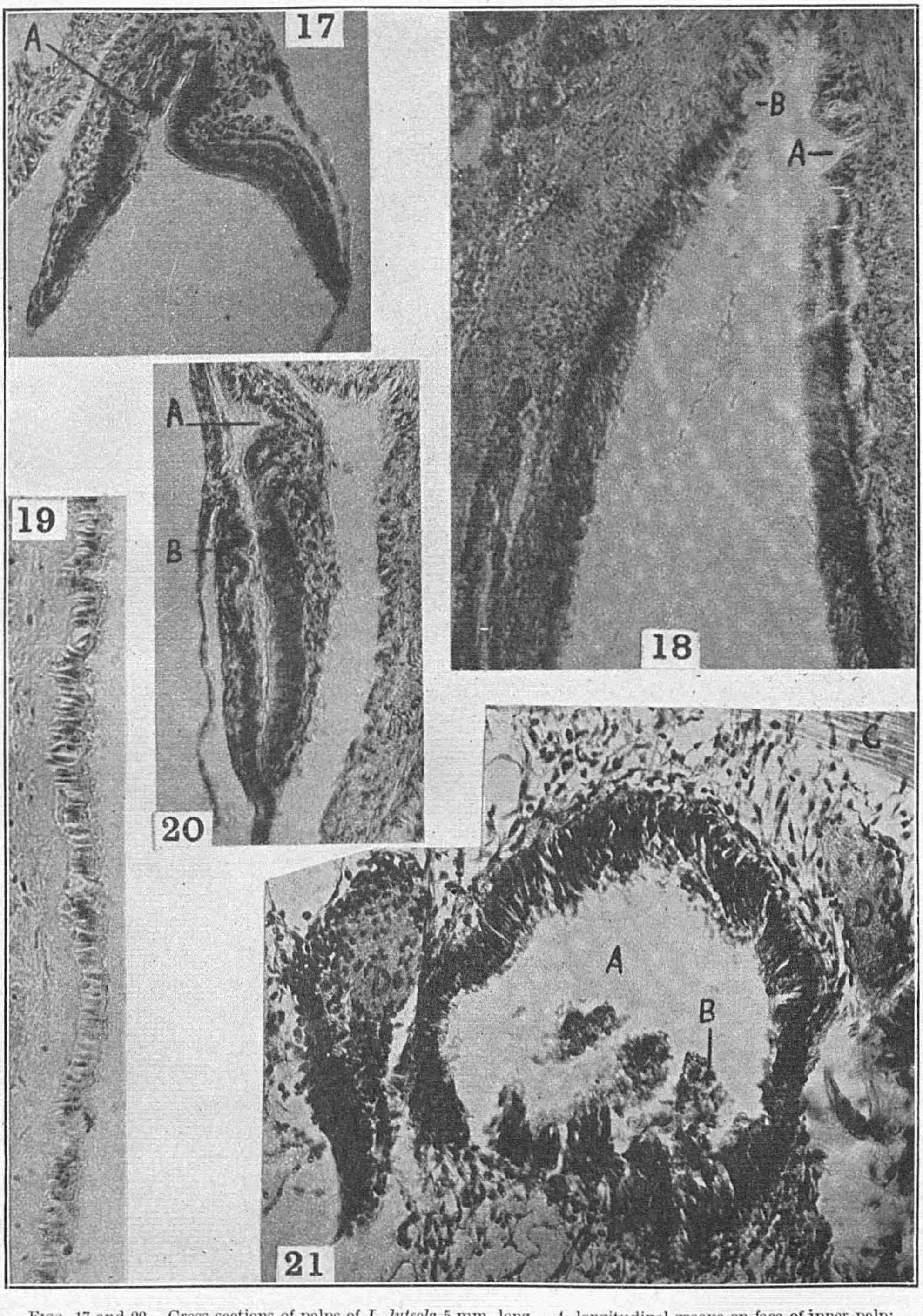


FIG. 13.—Frontal section of posterior ends of palps of *L. luteola* 10 mm. long, showing grooves on sides of ridges and posterior end of inner palp curled about, causing the grooves on the apposing face to be widened. *A*, foot; *B*, gill; *C* and *D*, palps. ×200.

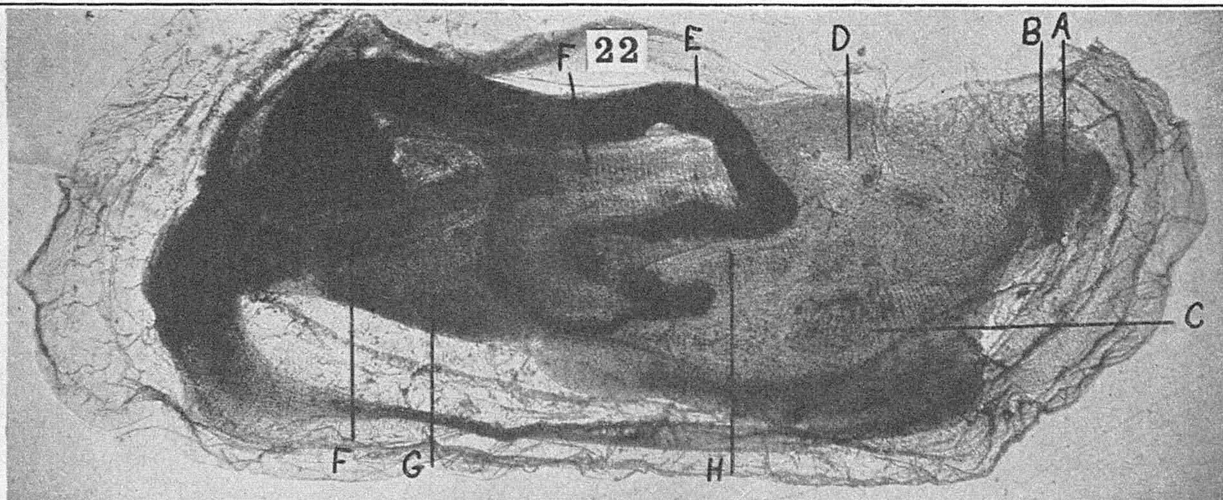
FIG. 14.—Cross section of ridges on inner face of palp of adult *L. luteola*, showing grooves on (Ant.) anterior and (Post.) posterior sides. ×100.

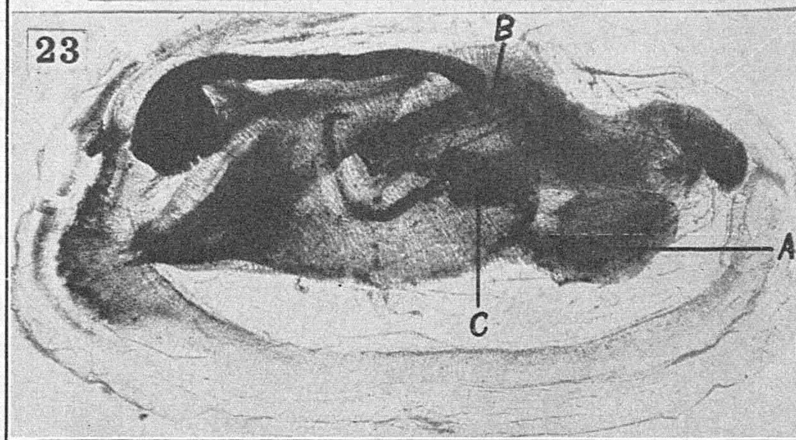
FIG. 15.—Cross section of ridges on inner face of adult *Quadrula pustulosa*, showing *a*, posterior groove, and *c*, anterior groove. The evidence seemed to show that in the area *abc* the cilia beat toward Ant., while in the area *cda* they beat down the groove between the ridges. ×100.

FIG. 16.—The same as Figure 15, except that the background of the negative was painted out before the print was made.



FIGS. 17 and 20.—Cross sections of palps of *L. luteola* 5 mm long. *A*, longitudinal groove on face of inner palp; *B*, groove on outer palp. ×200.
FIG. 18.—Cross section of upper portion of palps of adult *L. luteola*. Letters as in Figure 20. ×125.
FIG. 19.—Portion of ciliated epithelium on outer side of palp. ×100.
FIG. 21.—Cross section of esophagus of *L. luteola* 8 mm. long, showing the ciliated epithelial lining and masses of material in the process of being ingested. *A*, lumen of esophagus; *B*, *Cosmarium* sp.; *C*, portion of anterior adductor muscle; *D*, cerebral ganglia. ×430.





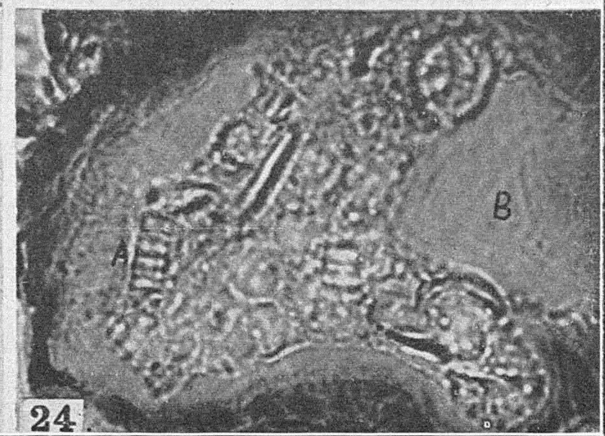


FIG. 22.—View of right side of *Anodonta imbecillis* about 12 mm. long, with right valve and mantle removed. *A*, anterior adductor; *B*, bolus of material in the mouth; *C*, outer palp with material visible through it, some near the upper margin and some in the vertical grooves; *D*, stomach; *E*, rectum filled with material; *F*, outer gill, scarcely one-fourth full width yet; *G*, inner gill; *H*, crystalline style.

FIG. 23.—View of right side of *L. luteola* about 10 mm. long, valve and mantle removed (taken on smaller scale than Fig. 22). *A*, mass of material leaving the palp; *B*, crystalline style; *C*, mass of material caught on the gill (to be disregarded in studying the figure).

FIG. 24.—Section of stomach of *L. luteola* less than 1 mm. long. *A*, filament of a diatom, as was shown by the fine characteristic markings visible under the oil immersion lens; may be Odontidium. ×900.

(as described on p. 444), thus widening the furrows, more particles would fall into them. These would drop upon the large cilia just below the anterior groove, upon those below the posterior groove on the next ridge ahead, or even to the bottom of the furrow, where there are also cilia. The cilia in the furrow between the two notches beat downward and carry particles to the lower edge of the palp, where they pass back to the lower posterior corner and off. To summarize, the cilia shown in Figures 15 and 16 beat forward in the region *a b c* and downward in region *c d a*.

LONGITUDINAL GROOVE ON FACES OF PALPS.

Cross sections were made of both juvenile and adult palps (figs. 17, 18, and 20), these paralleling, of course, the vertical ridges. As will be noted from the figures, there is a well-marked ciliated groove running longitudinally along the inner face (i. e., the side next to the outer palp) of the inner palp immediately below the line of union of the two palps. The groove is well defined in both the juvenile and the adult. There are indications of a smaller, less marked groove at a similar region on the outer palp, but it is much less conspicuous. Recalling the direct observations on ingestion, in numerous cases material was seen to pass forward between the palps quite near the dorsal margins. Apparently the material observed near the dorsal edges was being carried forward in the ciliated grooves described. In the experiments in which the mussels were feeding in a heavy suspension of débris all the material that was ingested passed between the palps in this upper region. The grooves were so widened and filled that the lower part of the palps was occupied mostly by masses of material streaming downward and off. It would seem that the longitudinal grooves, or at least the large one on the inner face of the inner palp, act as an accessory mechanism for carrying material forward. Especially in the case of a heavy suspension of particles in the water, the action of the groove or grooves enables the mussel to obtain some material, although most of the palp is entirely engaged in removing the overabundance of débris.

Figure 19 shows a portion of the outer face of the palp of an adult mussel. It will be seen that it is ciliated. Allen (1914) and Kellogg (1915) agree that the cilia on the outer faces of the palps beat away from the mouth and remove material from the palps. No observations were made on this matter by the present authors.

CONCLUSIONS.

The evidence of direct observation of the process of ingestion does not in the least favor the view that the mussel exercises any selection of ingested particles by means of the gills or palps. The palps act quantitatively, not qualitatively. The palps appear to be a mechanism for reducing the quantity of material to an amount that can be handled by the mouth.

The stretching or lengthening of the palps, as just stated, allows more material to be swept off, so the quantity brought up to the mouth can be regulated to some extent in this manner. The experiments in the ingestion of carmine showed that at times the mussels closed the valves, usually abruptly, and often kept them so as long as carmine grains were present in the water about the siphons or edges of the mantle. It must be assumed that the carmine was, so to speak, distasteful to them, and therefore excluded. This selection was not brought about, however, by the palps, but by closure of the valves, usually only after carmine particles had touched some portion of the alimentary canal.

FOOD.

JUVENILE MUSSELS.

Considerable knowledge of the materials ingested by fresh-water mussels, especially in the juvenile stages, was secured in the course of the observations described above relative to the mechanism of ingestion. A survey of the experiments detailed on the preceding pages shows that, in general, mussels from the very earliest moment at which they fall from the fish begin to ingest particles of débris and both animal and plant organisms. A number of the mussels experimented with were embedded and sectioned and photomicrographs made in certain instances. These will now be discussed in detail.

Figure 4 (opp. p. 451) shows a section across the stomach of a mussel 1 mm. long. Stephanodiscus, probably Odontidium, and other diatoms appear in it. Fragments of other plant forms and débris may also be noted. In other sections through this same stomach there were found, besides débris numerous specimens of Cocconeis, many Stephanodiscus, several filamentous diatoms probably Odontidium, and many cells of green algæ no doubt from colonial forms such as Volvox and Pleodorina. There were also certain yellow bodies, which appeared to be pollen grains of some sort.

Figure 24 (opp. p. 459) is that of a section of the stomach of another mussel 1 mm. long. The head of the style may be observed projecting into the stomach. A portion of a filament of what appeared under the oil immersion lens to be *Odontidium* sp. can be seen, together with débris and other fragments. In other sections through this same stomach the pollen grains of the ragweed (*Ambrosia artemisiifolia*) were identified. This ragweed was abundant around the edges of the ponds that supplied the water for the rearing troughs from which the mussels were taken. Pollen grains of other plants, débris, and fragments of diatoms were also abundant in the sections.

Figure 25 is a section through the stomach of a mussel about 3 mm. long. The anterior end of the style appears. Two or three specimens of Stephanodiscus, two of the desmid Cosmarium, and probably Odontidium may be seen here.

Figure 26 is a section through the intestine of the same specimen. Stephanodiscus, an unrecognizable green alga, some pollen grains, and filaments that may be Odontidium, appear.

In other sections from this specimen, in addition to the forms just mentioned, there were found the large diatom Coscinodiscus, Cocconeis, a partly digested Pandorina colony, Volvox, Scenedesmus, Melosira, and many unrecognizable fragments of filamentous diatoms, cells of green algæ, pollen grains, and débris.

In the stomach contents (obtained by dissection) of a mussel 1 mm. long were found débris, a Navicula 43 micra long, Cocconeis, Cosmarium, and fragments of filamentous diatoms. In other small mussels were found broken or partly digested

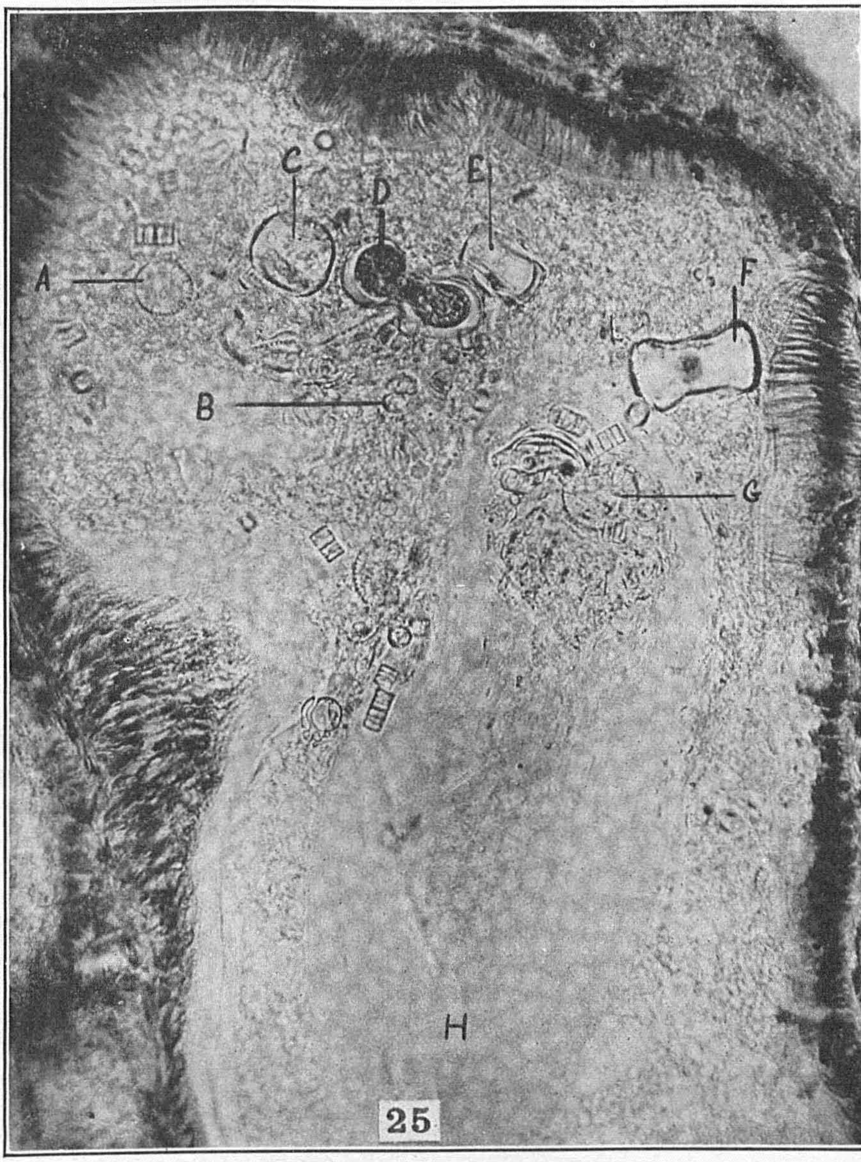


FIG. 25.—Section of stomach of *L. luteola* 3 mm. long. *A*, probably *Stephanodiscus* sp.; *B*, *Cosmarium* sp.; *C*, *E*, and *F*, *Stephanodiscus* sp.; *D*, Cosmarium; *G*, probably *Stephanodiscus* sp. The small filaments are probably *Odontidium* sp.; *H*, crystalline style. ×430.

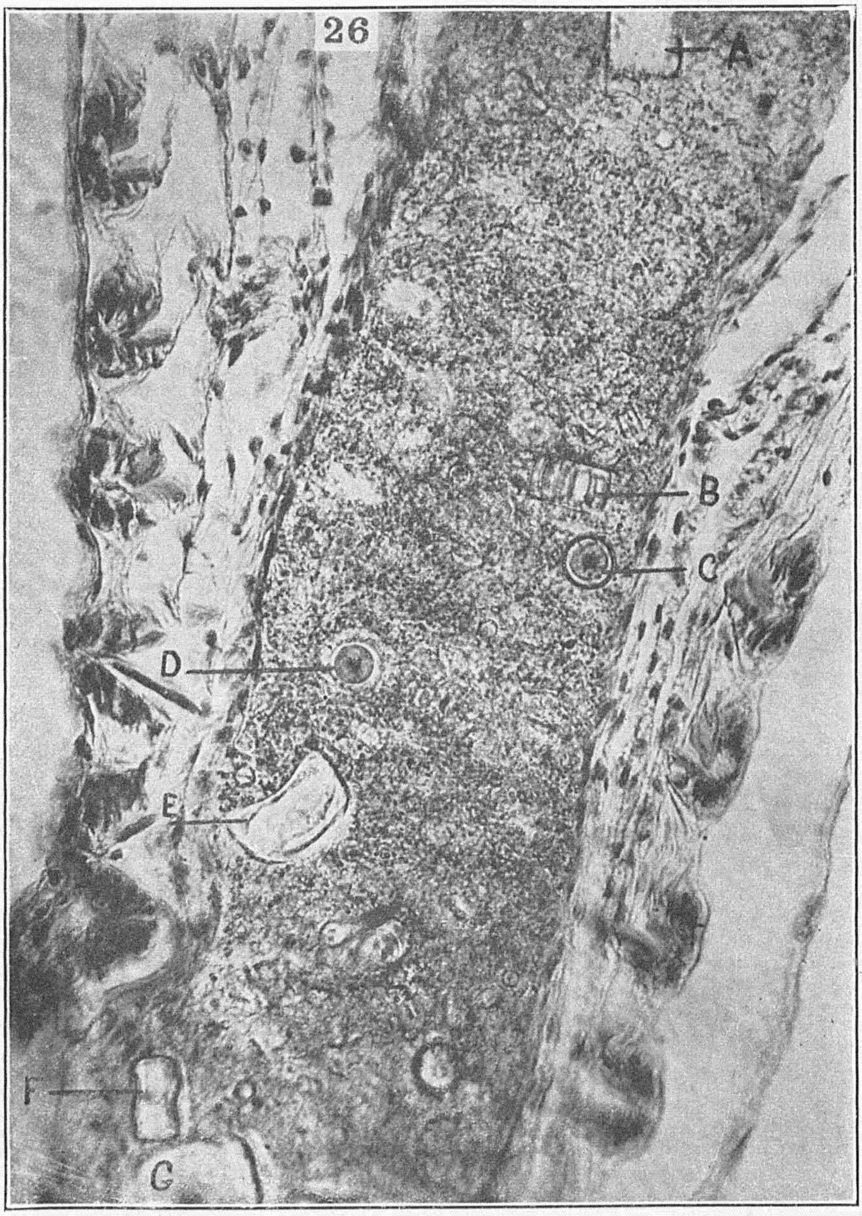


Fig. 26.—Longitudinal section of rectum of *L. luteola* 3 mm. long. *A*, *E*, *F*, and *G*, *Stephanodiscus* sp.; *B*, filamentous diatom, may be Odontidium; *C*, probably a pollen grain; *D*, cell of a green alga. ×430.

colonies of green algæ, the desmid Staurastrum, Scenedesmus, and the diatom Cyclotella, besides much débris.

Examination was made of the contents of portions of the alimentary canal of mussels varying in size from 9 to 15 mm. long, the smaller ones being dissected under the binocular microscope. Some were from the rearing trough, others from the Mississippi River. A list of various organisms and material found in them follows. Greenish-brown masses consisting apparently of mucus, organic débris, and the following entire or fragmentary recognizable forms: Trachelomonas, two or three species, numerous ones living; tests of the rotifer *Keratella cochlearis;* Chlamydomonas, some living; *Monas* sp., one living; *Phacus pleuronectes; P. longicaudus; Peridinium tabulatum; Euglena acus; E. spirogyra; E. deses;* encysted Euglenas; *Kirchneriella* sp.; *Pediastrum duplex; Tribonema* sp.; *Scenedesmus quadricauda; S. acuminatus; S. denticulatus; S. arcuatus; S. dimorphus; S. brasiliensis; S. abundans; S. bijuga; S. arcuatus platydisca; Cœlastrum sphaericum; C. microporum; Crucigenia irregularis; C. quadrata; Chroococcus minutus; Tetraedron caudatum* or *pentaëdricum; Tetrastrum staurogeniæforme; Pandorina morum; Microcystis incerta; Aphanocapsa pulchra; Merismopedia punctata; M. tenuissima; Platydorina caudata; Staurastrum* sp.; *Pediastrum duplex; P. tetras; P. simplex duodenarium; Pandorina* sp.; *Selenastrum westii; Platydorina* sp.; *Microcystis incerta; Staurastrum* sp.; *Pleodorina californica;* Oocystis; *Dinobryon setularia; Tetrædron* sp.; Volvox; numerous green cells of broken-down colonial algæ forms; many species of diatoms, some filamentous; empty tests of diatoms, and cells broken from filaments. Some specimens contained great numbers of empty loricas of Dinobryon, one fragment being made of over 20 united zooids.

ADULT MUSSELS.

The alimentary tracts of some dozen mussels were examined either immediately upon being taken from their natural environment or after having had the adductor muscles cut and being placed in formaldehyde to stop digestion and preserve the intestinal contents. Some of these mussels were taken from the Mississippi River, some from Lake Okoboji, and some from the Little Sioux River (Iowa). The following is a list of the kinds of material found in the alimentary canal:

Greenish mass made up of mucus in which were mingled Difflugia tests; Diatoms, various species; Volvox; Pleodorina, whole and fragments; algæ, whole and fragments; sand grains, one 752 by 400 micra, others 44 by 28 micra, and smaller; Microcystis; fragment, 960 micra long, of cellulose wall of plant; lorica of a cladoceran 720 micra long; loricas of rotifers, probably Anuræa; a flagellate, apparently Lagerheimia; Scenedesmus; Pediastrum; fragments of filamentous algæ; *Staurastrum brevispinum; Cœlastrum sphæricum;* Euglenas, normal and encysted—some living and active—*Euglena viridis, E. deses; E. acus;* fragment 70 micra long of a small crustacean. Débris, organic and inorganic, and sand grains were found in every specimen examined.

Allen says, "As stated by Zacharias, Petersen, the writer [Allen], Baker, and others, considerable quantities of inorganic and organic débris are carried into the stomach with food. Probably much of the stuff which Evermann and Clark call 'mud' is organic. The fact that neither they nor other writers list *sand* [italics are Allen's] in the stomach contents is further evidence of a selection of food material, and that river species are not an exception." In view of this statement it seems important to note that sand was found in the alimentary tract of every mussel examined but one, whether they were taken from a lake or river. The mussels from the Little Sioux River, which is little more than a muddy creek, contained an especially large quantity of sand and mud. Allen further states (1921, p. 227) that powdered carborundum, starch, and carmine were never ingested by the mussels in his experiments, and he concludes that mussels exercise a quite rigid selection of the material ingested. Kellogg, as previously quoted, says "there is no selection or separation of food organisms from other water-borne particles." In view of this diversity of views relative to the matter, further experiments were performed.

FEEDING EXPERIMENTS.

INORGANIC MATERIAL.

On page 454, under the observations on ingestion, an experiment is described in which a small juvenile mussel ingested sand grains in such quantities that the feces were more than half composed of them.

In addition to the use of sand, experiments were made in feeding borax carmine. It had been found in the observations on ingestion that the mussels would take it to some extent. The first lot of mussels used consisted of specimens ranging in length from 12 to 25 mm. taken from the Mississippi River. A suspension of borax carmine in tap water was made and the different mussels kept in it for varying periods and the contents of the alimentary tract then examined microscopically. While some of the borax carmine actually dissolved in the water, a good deal did not, and in the examinations search was made in the alimentary canal for discrete undissolved particles. It is to these that reference is made in the records below.

Lampsilis gracilis—1½ to 2 hours. Intestine filled whole length with carmine as far as the anus.

Obovaria ellipsis—4 specimens; 1½ to 4½ hours. No carmine whatever in the alimentary tract.

Lampsilis gracilis—2 hours. Carmine abundant.

Lampsilis lævissima—2 specimens; 1¼ and 3 hours. Carmine present.

Lampsilis fallaciosa—3 hours. Some carmine.

Obovaria ellipsis—23 hours. Some carmine in intestine.

Quadrula pustulata—20 hours. Much carmine whole length of the intestine.

Anodonta corpulenta—24 hours. Much carmine whole length of the intestine.

Lampsilis gracilis—26 hours. Much carmine the whole length of the intestine. Examined the red material in the posterior part of the rectum and some few particles of carmine were found, which were as large as the small diatoms found intermingled. Most of the carmine, however, consisted of quite fine irregular particles.

Quadrula pustulosa—23 hours. Considerable carmine in the intestine; the style was pink.

Quadrula undata—22 hours. A little carmine in the intestine; the style was faintly pink.

Lampsilis gracilis—3 hours. Considerable carmine in the intestine.

A specimen of *Lampsilis luteola*, 3 mm. long, was kept for a time in a carmine suspension and then sectioned. Particles of carmine were found in the stomach, and the intestine was packed full. A photomicrograph of a section is shown in Figure 7 (opp. p. 455).

Several large or adult mussels were tried in similar suspensions. The results follow.

Plagiola donaciformis—4 hours. No carmine.

Lampsilis ventricosa—5 hours. No carmine.

Sphærium sp.—2 specimens. Much carmine in the intestine.

Lampsilis anodontoides—22 hours. No carmine.

Quadrula plicata—22 hours. No carmine.

Quadrula undata—18 hours. No carmine.

Obovaria ellipsis—19 hours. No carmine.

Obliquaria reflexa—19 hours. Carmine in stomach and rectum. This individual was 1¾ inches long, an adult being somewhat over 2 inches in length.

Lampsilis lævissima—27 hours. No carmine.

Lampsilis lævissima—27 hours. 3 or 4 small particles of carmine found in the rectum.

Lampsilis gracilis—22 hours. No carmine.

The results are summarized in the following table:

TABLE 1.—*Showing results of test in borax carmine suspension.*

Ingested carmine.		Did not ingest carmine.	
Small (25 mm. or less).	Adult.	Small (25 mm. or less).	Adult.
Lampsilis gracilis, 4 specimens.	Sphaerium, over half a dozen.	Obovaria ellipsis, 4 specimens.	Plagiola donaciformis, 1 specimen.
L. lævissima, 3 specimens.	Obliquaria reflexa, 1 specimen.		Lampsilis ventricosa, 1 specimen.
L. fallaciosa, 1 specimen.	Lampsilis lævissima, 1 specimen, a few particles only.		L. anodontoides, 1 specimen.
Obovaria ellipsis, 1 specimen.			Quadrula plicata, 1 specimen.
Quadrula pustulata, 1 specimen.			Q. undata, 1 specimen.
Q. undata, 1 specimen.			Obovaria ellipsis, 1 specimen.
Q. pustulosa, 1 specimen.			Lampsilis gracilis, 1 specimen.
Anodonta corpulenta, 1 specimen.			

It will be noted that none of the adult mussels except the Sphæriums, the *O. reflexa*, and the *L. lævissima* ingested carmine, and that only one of the juveniles, *O. ellipsis*, failed to take any. Except in the cases of *O. ellipsis*, *L. gracilis*, and *O. undata*, the adults were of different species than the juveniles. The fact that carmine was not taken by them may be a question of a species difference and not one of age. From the fact, however, that in three cases it was an age difference, it would seem that the preponderance of evidence is in favor of the theory that as the mussels approach the adult stages they take less carmine. This would, no doubt, account for the differences between our results and those of Allen. From the observations made on the process of ingestion in the smaller mussels it would seem that the adult mussels in the presence of carmine simply close the valves and cease to siphon water through the mantle cavity. Observation of them while in the suspensions substantiated this; they remained closed most of the time. The matter of the ingestion of such inorganic material as sand and the like will receive further attention in connection with the following experiments.

ORGANIC MATERIAL.

In an effort to ascertain more precisely what the food of mussels is and whether or not they exercise any marked degree of choice between different material or between organic and inorganic substances a number of experiments were performed. Organic material alone was used in most cases, but in a few instances sand and mud were mixed in the cultures. In cases where the bottoms of the cultures were of sand or mud, search was made in the alimentary tract for particles of these substances. These experiments were conducted at the Iowa Lakeside Laboratory at Lake Okoboji, Iowa, where the tap water consisted of filtered lake water, which contained, of course, some few food organisms. Mussels kept in it, however, for a day or two were found not to obtain much food, as was shown by an examination of the alimentary tract. Some of the mussels used in the experiments were previously kept in this tap water until the rectum was observed to be empty, and then utilized in the tests. These were small mussels in which examination could be made through the semitransparent valves. The results of the experiments follow.

A specimen of *Anodonta grandis* about 25 millimeters long, the rectum nearly empty, was kept for seven hours in a culture of Lyngbya, Anabæna, and Microcystis. Upon examination the stomach was found to contain considerable material, mainly Microcystis, a few pieces of the filamentous Lyngbya, and Anabæna being included, however. The rectum contained entire and disintegrating Microcystis in abundance and a little of the other two forms. The filaments probably could not be ingested in most cases because of their size and shape, while the rounded Microcystis could enter the mouth. A *Sphærium* sp., half-grown, in similar culture to that just mentioned for 12 hours, gave similar results.

Anodonta grandis, 25 millimeters long, in tap water over a bottom of sand and mud from the Little Sioux River 18 hours, contained in its stomach Euglena, entire; many fine sand and dirt particles; a few fairly large sand grains, one 32 micra across, and some diatoms; rectum, packed full, many particles of sand and dirt, an entire encysted Euglena; a few round algal forms; one or two Scenedesmus.

Anodonta grandis, 25 millimeters long, rectum empty, in culture of material from Spirit Lake, the bottom of which was mud from Little Sioux River (there was a suspension of mud in the water for at least an hour after the beginning of the experiment), after an experiment of six hours contained the following in the rectum: Euglenas, one dozen at least living individuals and numerous partly digested fragments; some encysted Euglenas; Scenedesmus; diatoms, elongate and rounded, some entire; a large entire desmid 460 micra long; bits of Microcystis; one or two fragments of filaments of diatoms; a few small living flagellates, unidentified; débris of plants, sand, and particles of earth; a living Vorticella head; a number of other forms of a nature similar to these mentioned. Three other specimens were kept in similar cultures with about the same results; the mussels took anything present small enough to enter the mouth.

A culture was made up containing a quantity of red Euglenas and Microcystis, which are green in color. Four mussels, ranging from 18 to 25 millimeters long, were kept in this for a time—about 24 hours in most cases. Examination showed that the mussels took some of everything small enough to enter the mouth, including sand and débris. To cite one case in detail, the alimentary canal could be traced from the stomach to the anus by the red color due to the red Euglenas. Upon teasing this red mass apart clumps of the green Microcystis could be found in it. Numbers of other forms and material of different sorts were also found, as sand, earth, loricas of rotifers, the test of a Cladoceran, bits of algæ, and part of the test of a Difflugia. Upon examining the masses in the intestine it appeared as if the coloring matter from the Euglenas was in the form of a liquid, or assumed that form when the Euglenas disintegrated. Many of the loricas of the rotifers, the test of the Cladoceran, and the spaces in the disintegrating algæ previously occupied by chromatophores were filled with what appeared to be lakes of pink or orange-red color. Some of the mucus mingled with the material in the intestine was stained pink. It seemed evident that the coloring was in liquid form, or took that form when the Euglenas disintegrated and spread throughout the mass, collecting in almost any empty space and staining many of the materials a pink or orange-red color. Evidently the red Euglenas were being digested.

BROKEN FILAMENTOUS MATERIAL.

Since the water of the ponds supplying the rearing troughs contained much débris from the disintegration of algæ and filamentous diatoms, it seemed of interest to use such material in direct feeding experiments upon juvenile mussels. Some of the alga Œdogonium was ground up in a mortar and put in tap water to form a culture in which several mussels were kept, most of them some 10 or 12 hours. The intestine was then examined with the following results:

Lampsilis luteola, 10 mm. long. Two pieces were found that were unmistakably portions of Œdogonium, one being a complete cell. There was also a general mass of fine débris that appeared to be largely made up of fragments of the cell wall and pyrenoid bodies from Œdogonium.

L. fallaciosa, 12 mm. long. One almost entire cell and a recognizable portion of another; a general mass of fragments as in the preceding case. Five other mussels were used in the same experiment with practically the same results.

Further proof that fragments of aquatic forms of various sorts are ingested is furnished by observations made upon some specimens of *L. luteola* taken from a certain trough in which the water contained a great abundance of empty loricas of Dinobryon. Quantities of these loricas were found in the alimentary canal of these mussels, sometimes as many as 20 being still united in one fragment, as recorded on page 461.

TIME REQUIRED FOR PASSAGE OF FOOD THROUGH THE ALIMENTARY CANAL.

From experiments previously described (p. 454), material was found to pass through the alimentary canal in somewhat less than 1 hour. In another case (p. 455) carmine grains passed through in an hour and 10 minutes. Approximately the same results were obtained in several experiments with juvenile mussels from 2 to 4 mm. in length.

In larger specimens, even those as long as 25 mm. in certain species, the valves are so thin that one can ascertain by holding the mussel up to the light whether the rectum is filled or empty. If filled, it will appear as a dark brown or black thread extending from the point anterior to the heart where it emerges from the visceral mass to the anus. If empty, it will appear pale or colorless. Specimens may be kept in filtered water until the rectum is seen to be empty and then placed in various cultures containing food or other materials, and almost the precise moment ascertained when the first waste material reaches the anus.

A specimen of *Anodonta grandis* about 15 mm. long, with rectum empty, at 9 a. m. was placed in filtered water in a container the bottom of which was covered with a layer of mud and sand. The water contained in suspension particles from the sand and mud, including probably some organic forms. At 2 p. m., or in five hours, the rectum was filled nearly to the anus with black material, which had been ingested from the suspension. The mussel was then placed in clear filtered water, and at 4.45, or in two and three-fourths hours, the rectum was empty.

A specimen of *A. grandis* 25 mm. long, the rectum empty, at 8.45 a. m. was placed in a culture the bottom of which was composed of river mud. The water was thick with suspended material, part of which was fine particles of mud. The water remained quite turbid all through the experiment. The mussel began crawling at about 9 a. m. At 10.15 nothing could be seen in the rectum, but, through the valves, masses of dark material could be discerned upon the palps. At 11.40 the rectum was filled within about 1 mm. of the anus.

Three mussels from 15 to 20 mm. long were kept in suspensions of borax carmine for periods varying from one and one-half to three hours, in which periods the rectum became completely filled. The entire length of the intestine could be traced without dissection owing to its bright red color. Taken as a whole, the experiments show that in juvenile mussels a particular particle of material passes through the alimentary canal in from one to five hours, varying roughly with the size of the mussel.

GENERAL CONCLUSIONS.

1. In the small juvenile mussel, from 0.2 mm. to perhaps 2 mm. in length, before the siphons have developed, material drawn in by the cilia on the gills passes between the valves at points from the midventral side to the anterior end.

2. Before the outer gill has developed, the material falls upon the inner gill, passes down to the ventral groove, and forward to the palps. After the outer gill has developed the material that falls on the outer side of that gill moves up to the dorsal edge and forward to the palps. That striking the inner side moves up to the dorsal edge, is transferred to the inner gill, and moves down, together with the

material that falls directly upon the inner. These observations confirm those of Allen and Kellogg made upon adult mussels.

3. At the palps the material passes between their apposed faces. Some of it is carried down off the palps by downward-beating cilia in the bottoms of the vertical grooves. The cilia in these grooves beat downward, as stated by Wallengren (1905), and not upward, as thought by Siebert (1913). Some of the material is carried forward to the mouth by the forward-beating cilia on the crests of the ridges between the grooves. These observations also agree with those of Allen and Kellogg.

4. A longitudinal groove was found on the apposing face of each palp immediately ventral to the line of union of the two palps. No previous mention of these grooves has been found in the literature. Some material is carried forward to the mouth by cilia in this groove. This groove functions especially when the mussel is feeding in heavy suspensions.

5. A vertical groove along the posterior side of each ridge, near its base, is described, and another was found along its anterior side near the top. The former is figured by Allen but not discussed; the latter is not mentioned in the literature reviewed by the authors. The theory is here advanced that these grooves mark the boundaries between the forward-beating cilia on the ridge and the downward-beating cilia in the groove.

6. There is never any reversal in the direction of the beating of the cilia. The palps at times curl outward at the posterior ends, thus making their inner sides convex and longer, widening the grooves. More material would therefore fall into them.

7. There is no selection whatever of the kind of material passing down off the palps and that going forward to the mouth. Carmine grains could be seen going both routes at the same time. The mechanism of the palps operates quantitatively only, reducing the amount of material in such a way that it can be handled at the mouth. These observations confirm those of Kellogg, but are opposed to those of Allen.[3]

8. Mussels can and do feed when the water is so heavily loaded with material that the animal is invisible in the suspension. This feeding is accomplished by the action of the longitudinal ciliated grooves near the dorsal side of the inner faces of the palps. Food may pass along this groove while all the lower part of the palp is engaged in removing the heavy accumulation below. This observation disagrees with that of Kellogg, but confirms those of Grave and Nelson. The ingestion is not accomplished, however, at least in the fresh-water mussel, by the selection of food material and the rejection of the silt, etc., but by taking a limited amount of all the

[3] Since this paper went to press there has been received by the authors a preliminary paper by Thurlow C. Nelson entitled "The mechanism of feeding in the oyster" (Proceedings, Society for Experimental Biology and Medicine, Vol. XXI, 1923, pp. 166-168). Nelson studied ingestion in oyster "spat" by direct observation through the transparent shell. He found that the larger particles of material caused more mucus to be secreted than did the smaller, thus increasing the mass. The larger masses were not admitted between the palps, being too large to enter. He also found that the cilia in the more anterior grooves of the palps beat upward and carry small particles up, and that these particles reach the mouth. The present authors found in the fresh-water mussel, as stated in the body of this paper, that the cilia in these grooves beat downward in all cases. Nelson's general conclusion is that there is a "mechanical sorting of the material ingested," apparently on the basis of size, judging from his paper. This agrees with the findings of the present authors in the mussel. Nelson, however, considers that the inorganic material, the nonfood, is mostly larger than the food, and the separation on the basis of size results in a separation at the same time of the two classes of material. As will be seen from the present paper, these conclusions differ from those of the authors.

material present by means of the groove mentioned. Grave states that in one experiment with oysters after three hours the stomach was so filled with "sediment" that he could not estimate the number of diatoms. It would seem that his experiments went to prove that lamellibranchs feed in heavy suspensions of silt, but that they do not accomplish this by a selection of one kind of material from another.

9. In the tests with carmine there was evidence of rejection of material. Sooner or later many of the mussels ceased to ingest carmine. The adults rarely took any. Rejection, however, was not made by any selective action of the palps. From the observations it appeared that the rejection was due to a stimulation (perhaps by the sense of taste) of the alimentary canal by the carmine, followed by a closure of the valves. Material was thus excluded from the mantle chamber.

10. Material passed through the alimentary canal in from one to five hours in mussels from 2 to 25 mm. long, the length of time being roughly proportional to the size of the mussel.

11. In the mussels about 0.2 to 0.25 mm. long, which have just dropped from the fish, the ingested particles are, of course, very small—only a few micra in size. The material is essentially the same, however, as that for the larger juveniles—Protozoa, diatoms, and minute particles of detritus. Mussels of a length of 1 mm. were found to ingest Euglenas measuring about 60 by 18 micra when elongated and about 25 when contracted. Specimens 2 mm. long swallowed red Euglenas 160 by 35 micra. Mussels from 3 to 4 mm. long ingested sand grains as large as 30 micra across.

12. The food of fresh-water mussels from the moment of falling from the host, throughout life, consists (aside from what is probably a relatively small amount of dissolved material) of microscopic animal and plant forms and débris or detritus resulting from the decay and disintegration of such forms. Along with this material everything else small enough to be admitted to the esophagus, not active enough to escape, and not possessing what might perhaps be called a "chemical or disagreeable taste" (as carmine no doubt does), is also ingested. Even these last substances are often ingested as carmine, as stated above. From this heterogeneous material the alimentary canal digests and absorbs what it can and the rest passes on. Entire diatoms with color, contents, and nucleus are found in the rectum (fig. 18) and in the feces. Broken and partly disintegrated plant forms, however, are found in abundance in the alimentary canal. Some of these are of sufficiently fresh appearance to warrant the assumption that they were entire when ingested.

Protozoa, since they lack the cellulose walls, are no doubt more easily handled. In the experiments with the red Euglenas it was shown that these forms disintegrated in the alimentary canal, giving the appearance of having been acted on by the digestive fluids. It would appear that a very important food element is the organic remains in suspension in the water, especially abundant in ponds or rivers in which a luxuriant plant life is constantly going through a process of disintegration.

13. In case it is desired to rear young mussels from the time they drop from the fish it would appear to be necessary, as far as food is concerned, only to arrange ponds, uncontaminated by sewage or stock, and place in them some of the common water plants and algæ. The requisite diatoms, Protozoa, etc., will appear and

flourish there, and these, with the detritus from the decay of all the living forms, will supply food for the juvenile mussels placed in the pond or to which the water from the pond is conveyed. The problem of the rearing of young mussels is made easier by the fact that they will thrive on any of the microscopic animal and plant forms and their detritus, and it is unnecessary to plan any complicated arrangements to provide special food for them.

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