Neves

Allen
1921

RICHARD J. NEVES

AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
THE BIBLIOGRAPHIC SERVICE, MARCH 14, 1921.

STUDIES OF THE BIOLOGY OF FRESHWATER MUSSELS.[1]

EXPERIMENTAL STUDIES OF THE FOOD RELATIONS OF CERTAIN UNIONIDÆ.

WILLIAM RAY ALLEN.

CONTENTS.

1. INTRODUCTION.

The writer, while a member of the Indiana University Biological Station at Winona Lake, Indiana, devoted several summers to ecological studies of the freshwater mussels. The work was done under the direction of Dr. Will Scott, and formed a part of his general limnological program. A first paper on the feeding habits of the Unionidæ appeared in 1914. The present paper has expanded the former through the use of experiment

---

[1] Contribution from the Zoölogical Laboratory of Indiana University, No. 174; submitted as a partial fulfillment of the requirements for the degree of Doctor of Philosophy.

methods, and approaches the study of the crystalline style from the physiological standpoint. In subsequent papers I hope to present further studies along the lines of reactions to stimuli and distribution.

For the study of the smaller glacial lakes and their inhabitants the region is especially favorable. Winona Lake has a maximum length of two miles, and being of the kettle-hole type, is deep. Its greatest depth is eighty feet. The bivalve population is limited to a shelf about its margin. Three small creeks drain into the lake. Their course follows the flat, marshy land between moraines, and their volume is small and comparatively constant. The Unionids have not extended up into the creeks. The lake forms the upper limit of their distribution in the Winona drainage system.

Eight species of mussels have been recorded from this lake: *Lampsilis luteolus, Anodonta grandis, A. edentula, Quadrula rubiginosa*, and *Lampsilis subrostratus* are common, the first two being very abundant. *Lampsilis glans, Micromya fabalis*, and *Margaritana marginata* are rare. I have found a single specimen of a ninth species, *Quadrula undulata*. All these mussels are of the "lake" type, as contrasted with "river" mussels.

For most of the following experimental studies *Lampsilis luteolus* and *Anodonta grandis* have been employed. For work during the winter, and for comparison with the above mentioned lacustrine forms, mussels from White River were used. These include *Quadrula heros, Q. pustulosa, Lampsilis anodontoides, L. ligamentinus*, etc. They were collected from the east fork of White River, near Shoals, Indiana, and from the west fork of the same river near, Gosport, Indiana.

2. CONSTANCY OF THE FEEDING ACTIVITY.

In a previous paper I stated that the lake mussel continues feeding at nearly all times, when under normal conditions (Allen, '14). Virtually all the observations made since the publication of that paper have been of a confirmatory nature. Rarely is a freshly collected mussel found to be without food material in its alimentary tract, often much of it wholly undigested. When brought into captivity defecation usually continues to occur even after several hours, and indicates that feeding has at no time been long suspended. The normal position of the palps, gills, and mantle with respect to each other is favorable to ingestion. The structure of the mussel is such that it requires a greater effort to refrain from feeding than to continue feeding. The ciliary apparatus is in constant activity, regardless of the presence or absence of food. The gills are at all times siphoning water. Particles suspended in the water are at all times being sifted out and caught upon the mucous secretions of the gills. The gills have no way of rejecting such collections. They are always passed on by the fixed ciliary tracts of the gills to a given point on the lower margin of the inner gill which hangs between the labial palps. Without an adverse stimulus and an avoiding movement of the palps the collections pass on to their apposed surfaces. Thence it is but a short distance to the mouth.

If, despite the structure of the mechanism of ingestion, one might still doubt that feeding is a constant process, he is forced to grant that feeding is speedily resumed by mussels artificially starved. Those starved a sufficient length of time to get rid of the crystalline style, when placed again in lake or stream, begin the renewal of the style within fifteen to thirty minutes.

(a) *The Feeding Posture.*

To what extent feeding is conditioned by the position of the mantle, palps, and gills the following experiment will show:

Some *Lampsilis* and *Anodonta* were starved several days to insure the disappearance of the crystalline styles. The renewal of these organs was then taken as an index of the feeding activities. Various individuals were placed in the lake, in lake water, and in Pocahontas creek. Some were partially inserted in sand in the normal posture, others with the siphons turned upward, others laid upon the right or left side, and still others upon the hinge and having the gape of the shell uppermost.

At intervals some from each situation were examined. The styles were found to be reformed in all of them at about the same rate. The position of the ciliated parts, with relation to one another and to gravity, was obviously of no consequence. Food could be passed from the gills to the palps as readily upward as downward or horizontally. Due to the buoyancy of the mucus in which food material is gathered and passed to the stomach, so near the specific gravity of the surrounding water, cilia need exert only a very slight traction upon the food masses to move them in any direction.

No Unionids have acquired an asymmetry like that of the oyster, but some species, particularly Quadrulæ, are always found lying upon one side or the other. They feed as readily upon one side as upon the other. Neither the right side nor the left is favored. There is no exceptional arrangement of ciliated parts within the mantle chamber for countering the unsymmetrical pull of gravity, unless the unusual size of the labial palps of some species may be interpreted as a means of preventing the loss of food between gills and palps.

No matter, then, in what position the gills and palps may be, food is readily passed mouthward, and the process of food collecting goes on constantly in the absence of adverse stimuli. Only with the closure of the siphons is the streaming of fresh material interrupted. Only in case of powerful stimulus are the palps caused to move out of line, away from the inner gill, thus refusing food masses entirely. It has been difficult to demonstrate the constancy of the food stream upon the contiguous faces of the labial palps. The ciliated furrows (p. 234) have the functions of accepting or rejecting material, but to what extent the latter function is exercised under normal circumstances it is difficult to determine directly. When fed in high concentrations certain specific substances were rejected entirely, others taken readily, *e.g.*, starch grains were never recovered in the alimentary tract, while *Glœocapsa* passed through in great numbers (p. 227). It is permissible, therefore, to postulate that the labial palps behave in harmony with the rest of the food-gathering apparatus.

(*b*) *The Degree of Digestion a Response to Tissue Demands.*

The cilia of the alimentary tract are virtually in constant movement. (Nelson, '18, has reported the partial suspension of the cilia of the style sac.) It involves little, if any, additional energy to keep a stream of water coursing through it. That much food material passes through without being digested is shown both by the mass color of the feces and by the appearance of diatom and other algal cells from the feces, when examined microscopically. It has been argued that diatoms are accidental and not the real food. Empty diatom tests are found in considerable quantity in fecal matter, and there is no doubt that the contents have been digested out of, at least, some of them. Furthermore, the freshly formed style, in a mussel which has been starved and then fed with diatomaceous matter, has the amber color of diatomin. In this case digestion is prompt and rapid. The style tends to become colorless as soon as the streaming of food through the style sac is checked by the growing style.

Since the ciliary mechanism is functioning constantly, and since some material is digested and some is not, it may be concluded that the demand for food on the part of the tissues affects rather the secretion of digestive fluids and ferments than the control of ingestion.

On several grounds it is seen, then, that a given particle may or may not be digested on its passage through the alimentary tract. Nelson's suggestion ('18), that the style sac serves as a means of returning undigested particles to the stomach where they may be exposed again to the digestive secretions, is very plausible. The morphology of the structure fits such a function to a nicety. Moreover, while a starved mussel is renewing its style much green material is being threaded into its core. The fact that some diatoms are digested while others are not might at first thought find a sufficient explanation in Nelson's view. For some particles would be diverted into the style sac and others continue on undigested. This would perhaps as readily explain the presence of both normal diatoms and empty tests in the rectum as my suggestion of uninterrupted feeding. There is no doubt that Nelson's explanation is entirely adequate for *Ostræa* and *Modiolus,* for these forms interrupt siphoning and feeding with every tide. Their styles are absorbed and renewed regularly with the ebb and flood. Hence twice each day the style sac is thrown open to the passage of food; and for this reason, if for no other, one should find a mingling of digested with undigested food in the rectum. In the Unionidæ this mingling does not have an adequate explanation in the style sac. For there is no loss of crystalline style except with starvation. No normal mussel is found without it. Under normal conditions the style has no color, with a very slight core of green. The amount of food which passes through the style or style sac under ordinary conditions is very slight. Hence most of the digested diatoms whose tests are found in the rectum have been through the stomach but once.

Résumé.—In the rectum and feces the simultaneous occurrence of green diatoms and empty tests shows that part, but not all, of such material is digested. At any rate it is usable as food. Ingestion is continuous, but digestion is discontinuous, and dependent upon demand for nutrition on the part of the tissues. In the Unionidæ the return of material from the intestine to the stomach through the style sac does not account for much of the food actually digested, for such a transference of food is possible only when the style sac is empty. Since digestion is a chemical reaction the contents of the stomach should be affected alike, and not some wholly digested and some not at all, when equally digestible.

3. THE UTILIZATION OF NANNOPLANKTON AND MEGALOPLANKTON.

The materials recovered from the rectum grade down from the largest phytoplankton to very small species. While stomach and rectal contents vary, the larger particles are obviously oftener found undigested than the smaller. This suggests that the smaller are more easily digested; that everything else being equal, digestibility is inversely proportional to size. The question arises: does the nannoplankton constitute an important, though less conspicuous element of the food of a mussel?

Juday (see Ward and Whipple, '18) has shown that the nannoplankton content of a lake may exist in vastly greater number and in a much greater volume than does the net plankton. Even though most of such organisms should escape through the gills of the mussel, the recovery of only a fraction of them is sufficient in some lakes to equal or surpass the total volume of larger organisms utilized.

(a) *Experiments in Selective Feeding.*

A few experiments were made to separate the nannoplankton from the grosser, in order to feed them separately.

There is no hard and fast line by which the nannoplankton may be distinguished, except Lohmann's ('11) arbitrary size limit of 25 microns. Stomach and intestinal contents were examined for evidence of a predominance of either larger or smaller forms. So far as the diatoms are concerned such evidence is not very conclusive, though on the whole the smaller organisms apparently have the better of the argument. As for Flagellata, they were commonly seen in the rectal contents, but more often in the stomach. This is as we should expect, for some of them do not possess so resistant a test as the diatoms.

The fact that even a few of the smaller flagellates and diatoms are found demonstrates that the gill-meshes are fine enough to accomplish something with the nannoplankton, while the diminished number of flagellates in the rectum implies the more complete digestion of that group. Certain flagellates are more resistant to digestion than others, *e.g., Peridinium*; some are doubtless of more frequent occurrence in the alimentary tract on account of their colonial form and greater bulk, *e.g., Pandorina.*

We have no very efficient mechanical means for separating plankton according to size. The nets of finest bolting silk allow nearly all flagellates and all but the large or colonial diatoms to pass through. It was found that water, poured through a net suspended in the air, forces more large organisms through the meshes than in the case when the net is suspended in water, and the water containing plankton poured through slowly.

A concentration of net plankton actually took place in the plankton bucket. A complete separation of coarse from fine plankton is not claimed, nor was it necessary to the experiment. Undoubtedly some of the grosser forms passed through the silk. But these were never in sufficient quantity to be detected in the intestines of mussels feeding in the escaping water. One complete experiment follows:

Water from the creek or lake was first poured through a copper gauze in order to remove such debris as plant fragments and flakes of limy incrustation. From these it passed into a conical plankton-net of No. 20 bolting-silk, terminating in a detachable Birge bucket. The lower portion of the cone and the bucket were suspended in a jar of water. Measured quantities of the natural water, varying from 50 to 250 liters in the several experiments, were passed through. From time to time the meshes of the bucket became choked and the process was slowed down. This was especially true in the lake following high winds, when there was considerable turbidity. At intervals, therefore, the bucket was removed, rinsed out into a container, and the gross filtrate brought into contact with a group of mussels which had been starved for a few days in order to cause the disappearance of the crystalline style. The overflow water which had passed through the silk was allowed to siphon over into another jar in which were kept another group of starved mussels. They were thus given opportunity to feed upon nannoplankton almost solely.

In order to demonstrate that any regeneration of the crystalline style that might occur could not be ascribed to the chemical or physical character of the water itself, a quantity of the strained water was also siphoned over into a sheet of filter paper and mussels placed in the jar beneath. The mussels fed upon filtered water in no instance showed the least evidence of re-forming the style. That many organisms passed through the plankton net is well shown by the amber-green coating which soon formed on the filter paper. Upon these organisms the second group of mussels had opportunity to feed, provided the gills might be a sufficiently effective mechanism to entrap particles which had passed through the silk.

During the progress of the experiment, usually about four hours, checks were kept in the lake or creek, near the experiment. They also had been starved for the same length of time, in tap water.

The water was taken from a depth of two to three feet in the lake, along the east shore, where it is open to wind and wave action, but has the shelter of a broad zone of *Potamogeton*. The creek water was from the mouth of Pocahontas creek, having a depth from a few inches up to more than a foot, and a bottom of gravel and rubble. A drain from a large septic tank enters 500 feet upstream. In its upper course it receives the water of drainage ditches in swamp land, and has a small, though constant, volume throughout summer. Its flow is rapid, and the character of the bottom such that mussels have not ascended it. The water has a slightly brown color due to its swampy course (Rice, '16).

In this experiment two mussels were used as checks, three kept in filtered water, ten placed in the overflow from the plankton net, and ten were fed upon the gross concentrate given them in tap water.

Of the two checks, one developed a well formed crystalline style, the other none, but it had a large amount of green material in the intestine, showing that it also had resumed feeding.

Of the ten fed upon net plankton, seven renewed the style more or less completely, three not at all; of the nannoplankton fed eight had formed a style and two had not. These data are not full, yet they demonstrate the ability of the respective food materials to renew the style.

It will be seen that there is little difference in the power to renew the style between the larger plankton which remained, and the smaller which went through the silk. It is certain that the larger forms do have a food value, for there were few of the smaller which remained in the plankton bucket, and the residue of grosser material alone was put into the aquarium with the first group of mussels. It is possibly not quite so well demonstrated that the other eight renewed the styles in response to the ingestion of nannoplankton solely, but such is a reasonable supposition. Two things at least cannot be gainsaid: (1) there was a significant increase in the ratio of megaloplankton to nannoplankton within the bucket, and of course a reversal of this ratio outside, without greatly affecting the crystalline style renewal in either case; (2) no net plankton was recognized in the intestines of the second group of experimental animals, although conspicuous in those used as a check.

The above facts do not argue a greater digestibility of the smaller organisms. But when the matter of dilution or concentration is taken into account, we have such an argument. The greatly concentrated net-plankton was fed in a container of water which was changed but slightly throughout the experiment. The nannoplankton, on the other hand, was caused to stream over the second group of mussels in the original water, and with the original concentration. If it had been concentrated as much as the net plankton a much more marked ingestion would probably have been obtained. It is hoped that in the future a method for concentrating nannoplankton in quantity may be applied to the solution of this question.

An experiment similar to the foregoing consisted in placing starved mussels in the creek, enclosed in a tight metal container, whose two ends were then closed with bolting-silk. The creek was dammed on either side of the container, so as to raise the level slightly and maintain a flow of water through it upon the mussels. The stopping of the meshes of the silk was expected to interfere with the flow after a few hours. But, as a matter of fact, there was sufficient eddy and overflow to keep the silk fairly well washed, so that at the end of the experiment a slight current might still be detected.

Experimental mussels and checks were placed both above and below the sewer outlet, where they were allowed to feed for twenty-four hours. Small numbers were used here—four mussels in each situation. So far as the results derived are trustworthy, they show a utilization of nannoplankton as food, and corroborate those of the previous experiment.

The mussels from the three situations showed a well-defined gradation in the reconstruction of the crystalline style: (1) those from experimental conditions showed only a partial renewal; (2) the checks nearby had virtually completed the renewal; (3) below the sewer outlet the checks had well formed and entirely hyaline crystalline styles. Presumably they had an additional source of food—the sewer.

The mussels used as a check, and whose styles had grown large and hyaline, accumulated considerable masses of green in the intestine during the twenty-four hours. Examination showed this material to comprise, among other forms, *Navicula, Oscil-*

latoria (small amount), *Scenedesmus, Synedra,* and *Tabellaria.* Considerable debris, both organic and inorganic, was found, and some fragments of considerable length.

In the case of those fed upon "bolted" water considerable amounts of green were found in the intestine, and the styles were flaccid and green. No large particles were obtained. *Tabellaria Diatoma,* and *Navicula* occurred, but it was only very small species in each case, and no coherent members of colonies. Very little inorganic stuff was found, but many organic fragments, some of them partially digested. The smaller flagellates were proportionately more numerous than in the checks. Naturally the diatoms were of the solider, creek type, and none of the graceful lake forms adapted for flotation.

(*b*) *The Feeding of Sewage.*

The presence of Oscillatoria may be taken as an index of the amount of sewage in the food. It also shows a well-defined gradation in the several feeding stations: (1) no *Oscillatoria* was recorded from the experimental animals above the sewer outlet; (2) very little appeared in the checks kept in the unscreened stream at this point; (3) somewhat more *Oscillatoria,* consisting of very incomplete filaments, was seen in the experimental animals below the sewer; and (4) the checks below the sewer contained numerous large fragments of *Oscillatoria,* and many relatively large bits of debris never met with elsewhere.

Mussels which had been kept in the mouth of the creek for several weeeks prior to these experiments were opened at the same time. The styles were well formed and without color. On various occasions the styles of the experimental animals reacted differently. At one time all of the styles were whitish when partially renewed, having a distinct white, spiral core. In those most perfectly formed the white color was disappearing, and the entire mass was becoming more solid and more hyaline. On other occasions the newly formed styles were of the typical amber hue which suggested diatomin. Subsequently mussels opened here contained sometimes whitish, sometimes colorless styles. At times freshly formed styles were found which were green in the stomach and becoming white in the style sac, indicating a change from one color to the other.

Only one explanation of the above differences offers itself—namely the effect of bacteria. These, through a mass effect or through the breaking down of the style substance itself, are probably responsible for the white color. Rice (*l.c.*) has shown that the abundance of bacteria and of nitrates is here subject to profound variation, on account of the periodic discharge from the septic tank mentioned above. Whatever the cause of the white color it might have been expected to be mingled with green from the normal food brought down from above. That green is actually present in concealment is shown by the boiling of such styles (p. 226). The white style pertains mostly to the creek. In the lake a white style is observed only during low water in mussels which have been feeding near the creek outlet. During freshets and great dilution of the bacteria it does not occur even in the creek. These observations may be taken as a further indication of the direct dependence of the crystalline style upon the character of the food.

(*c*) *The Feeding of Infusions.*

Previously I had observed the renewal of the style and the accumulation of material in the alimentary tract of animals fed with hay infusions rich in ciliates (Allen, *l.c.*). The unmistakable finding of protozoan fragments in the stomach showed that some such material is ingested, and I was satisfied that the style renewal was due to their presence. The above nannoplankton studies suggested that there might also be food value in the bacteria and flagellates which are present in such concentration in infusions. The former experiments upon the feeding of infusions were repeated with the same results. Again, white crystalline styles appeared (p. 220). However, in order to determine if the bacteria and flagellates present may be responsible for the style renewal an attempt was made to separate them from the large ciliates. The above method for the separation of plankton was applied here. It was possible, at any rate, to dispose of most of the bacteria by washing. The process resulted in the death of many ciliates due to crushing and to the change into fresher water. However, it was possible to accumulate a considerable mass of living and fragmented infusoria. Starved mussels fed upon this concentrate did not renew the style. This negative result must not be trusted too implicitly, considering that, as reported above, ciliate material is sometimes found in the stomach. Yet the present experiment points toward a greater utilization of the smaller organisms of infusions than of relatively large ciliates. Aside from the ciliated protozoa the infusorian population consisted mostly of extremely minute forms. The maceration experiments described elsewhere (p. 228) show conclusively that the Unionid gill is capable of intercepting very small material indeed—even the pyrenoids of algæ.

It may be questioned whether the formation of a crystalline style is a reliable index of the feeding activity. In my opinion it is as reliable as a direct examination of the alimentary tract. On some occasions food may be found in the intestine before regeneration of the style is perceptible. But, on the contrary, there are as many occasions when a starved mussel recently fed is seen to have the beginnings of a style before anything is readily recoverable from the intestine. In far the greater number of cases examined the synchronism between style renewal and the presence of food is exact. The bearings of this upon the significance of the style are discussed below (p. 229).

Résumé.—The more minute plankton organisms are of as great nutrient value as the more conspicuous, often undigested matter commonly listed from rectal or fecal examinations. However the net plankton is shown to have a food value as well. Experiments which more or less perfectly separated the net- from nannoplankton show that both are capable of re-forming the crystalline styles of starved mussels. The minute flagellates sometimes exceed the volume of net plankton in lakes many fold. Since it is certain that they can be entrapped by the gills of the mussel and can be ingested, it is likely that their rarity in the rectum is due to the fact that most of them have been digested. Infusions which have nothing of food value except ciliates and minute organisms renew the style.

4. The Physiology of the Crystalline Style.

Everyone, myself included, who has dealt with the crystalline style during the past decade, has made apology for adding to the bulky list of the things not certainly known concerning that organ.

(*a*) *Recent Studies.*

The most thoroughgoing and satisfactory account of the crystalline style is that of Nelson ('18). He has assembled and organized the literature on the subject to the minutest detail, has very effectualy eliminated most of the groundless speculations, and has sifted out the truth contained in the rest. Nelson's work on the morphology is of a sort which virtually closes that subject. All future studies of the crystalline style may well make Nelson's work the point of departure.[2] There is no occasion to review the literature here or to describe either the style itself or the associated portions of the alimentary tract. I shall be content to record the physiological data which have accumulated during the intermittent observations made over a period of some six years.

Most of the writers, with the exception of Mitra ('01), and Nelson (*l.c.*), have taken a viewpoint which has been fatally erroneous, it seems to me. Despite all the divergent speculations which observers have permitted themselves to make (a point well reviewed by Nelson), they have really been looking for *one* explanation—the most plausible function that this organ might be supposed to perform. Few have granted the probability that two or more uses of the style might exist concurrently.

---

[2] The preceding sentence, when written, was prophetic. For just as this paper is about ready for the press, Edmondson's timely account of the crystalline style in *Mya arenaria* has appeared ('20). Like Nelson, he has devoted considerable study to the morphology, but has centered his attention upon the renewal of the style. It is gratifying to find others interested in the physiological study of the style, for, aside from its chemistry, most work has been done from the viewpoint of structure.

This author's account of *Mya arenaria* shows the style to have diverged very far, indeed, from its homologue in the Unionidæ. It lies in a distinct cæcum. Operative methods instead of starvation were necessary to remove it. It is a very solid structure, nearly insoluble, and nearly devoid of albuminoids. Its regeneration in *Mya* precedes rather than follows resumption of feeding. Seventy-four days were required for its reformation in *Mya*, while one day more or less suffices in the Unionidæ.

Mitra (*l.c.*) was the first to recognize clearly that the crystalline style may meet several needs. The fact that it is dissolved when food is wanting, and that in solution it may be taken up by the blood, leads to the conclusion that it is (so far as it goes) a reserve of nutriment. Furthermore, the work of Mitra and others has shown that it bears enzymes capable of furthering starch digestion. Dr. Scott Edwards has kindly checked over this matter for me, with confirmatory results. Insofar as suitable food is brought into contact with it, it is a means of supplying digestive ferments.

Nelson (*l.c.*) has shown very well that the rotation of the crystalline style against the "gastric shield" in the stomach shreds the dissolving end so as to form a brush. The rotation of this brush sweeps the food into the proper ciliated channels, aids in dissolving the food out of the mucous masses in which it reaches the stomach, and acts as a substitute for peristalsis in mingling food with the digestive fluids from the liver, etc. It might have been pointed out that these movements afford a ready means of bringing the contained enzymes of the style into thorough contact with the food.

Since the style actually accomplishes all these things, we can not choose any one of them as the function for which it was designed. We cannot assert that the style is exactly adapted to perform any one of them, or that it is the function which it has always performed in ancestral forms. If such were the case one might expect to find somewhere in the more primitive existing species a style little changed from the ancestral condition. But the Najades have taken to fresh water, and as a result have become profoundly modified in life history, ontogeny, and structure. While the crystalline style has shown as little structural change as any organ, it is not improbable that its relation to the organism as a whole, to metabolism, has suffered changes of which we know nothing.

Nelson suggests a further function of the style sac, that of returning undigested material from the intestine to the stomach, to prevent the loss of food. In *Modiolus,* which undergoes a periodic cessation of feeding and dissolution of the style, the cilia of the sac periodically carry a stream of food from the intestine through it into the stomach. In common with all ciliated epithelia this organ raises the very interesting and equally difficult problem of accounting for the present direction of the beat of the cilia. In the simpler ancestral forms the beat most probably was in the opposite direction.

The style sac of *Modiolus* contains more food in winter than in summer. It is difficult to see why the structure in *Modiolus* should be (as Nelson reports) more effective in winter, when the metabolism is low and the food requirement slight, than in summer, when the demand for food is greater. In winter the secretion of the style substance is slowed down by the temperature to such an extent that the organ is not promptly re-formed with each feeding period (p. 229). The style sac therefore contains no style and may be utilized to reconvey food from the intestine to the stomach. In the Unionidæ it is certain that the return of particles through the style sac is a phenomenon which takes place normally only after starvation. Of the many hundreds of specimens examined when taken from the water, not half a dozen were ever found in which the style was lacking.

Usually the newly formed style has an abnormally large core of plankton. This indicates that the first undigested or partially digested material which streams into the intestine is diverted at the posterior end of the style sac and carried forward again into the stomach. In the meantime the glands of the typhlosole (Nelson, *l.c.*) begin secreting and wrapping the spiral of the style substance about this core. The streaming in of materials from the intestine is limited more and more as the style more and more completely fills the lumen of the sac. After the style moves forward and has been dissolved its entire length, the newer portion, with a diminished core, has entirely replaced the original portion with its loose structure and large core of food. This takes place twice daily in *Modiolus* or *Ostræa,* and the newly regenerated style is thus oftener encountered. In *Lampsilis* or *Anodonta* under normal circumstances, it is a very infrequent occurrence. My observation has been to a very great extent upon lake forms, which are not subject to many vicissitudes. It is presumable that river forms, if taken at the right times, demonstrate a more periodic activity in response to the rise and fall of the current, the degree of turbidity, etc.

Nelson is probably in accord with these reservations concerning the Unionidæ, for he agrees (*l.c.*, p. 100) that the formation of the style is directly dependent upon the food supply. In the marine forms the return of food through the style sac to the stomach is considerable. In the Unionids the return of food through the style sac has become reduced because the sac is usually occupied by the style.

The spiral character of the style, caused, as demonstrated by Nelson, by its axial rotation, has been brought out nicely in three ways in my observation:

(*a*) A regenerated style in one starved *Anodonta* was found to have a great deal of green matter throughout, and but a very slight amount of style substance. It had grown to at least normal diameter. When kept for a time the secreted portion dissolved out, leaving the green cohering portion wrapped about the core like the threads of a screw, or a lathe shaving.

(*b*) A few whitish styles were boiled for a short time in strong sodium hydroxide. They were much reduced, and the residue was in the form of a close rope-like spiral.[3] The white appearance had given place to the amber-green color characteristic of most newly regenerated styles.

(*c*) The actual rotation of the style has often been observed.

Examinations were frequently made of the food substances which could be recovered from the core of more or less completely regenerated styles. The food never showed a perceptibly greater degree of digestion at the stomach end than posteriorly. It is not likely, therefore, that the digestive processes are much furthered during progress through the style or style sac toward the stomach.

(*b*) *The Feeding of Specific Substances.*

A series of experiments in feeding certain substances, and in forced feeding by injection into the stomach, were undertaken

---

[3] Edmondson's ('20) figures of partially formed styles very well represent these.

to throw light upon the actual stimulus which initiates the renewal of the style. The stimuli may be either mechanical or chemical.

Carborundum, carmine, starch, etc., of varying fineness, were introduced into the incurrent siphon with the streaming water. Such organic or inorganic material, however neutral, of whatsoever dilution, or however administered through the respiratory water, were never found subsequently in the alimentary canal. Nor did the molluscs ever display any indication of style renewal in response to these things. The experiment has a further significance to be discussed on page 229.

A culture of *Glœocapsa* was looked over carefully and found to have very few organisms of the size of *Glœocapsa,* but much coarse debris. This material was washed into a jar with active, starved mussels, and agitated from time to time to prevent its settling. After eighteen hours the mussels were examined. The crystalline styles were partially renewed in all cases. In others kept in jars of *Glœocapsa* not stirred frequently the styles failed to show any indications of re-forming, even after three or four days. Evidently not enough food to stimulate style formation had been taken into the siphons. In all, small masses of *Glœocapsa* were encountered in the rectum, in the stomach, and in clots of mucus upon the gills and palps. The clots were almost pure *Glœocapsa.* The stomach and intestine contained minute fragments of green, partially digested individuals, and sometimes *Glœocapsa* cells without the capsule. There is thus no doubt but that a pretty rigid selection of the alga from the coarser matter with it was taking place, and that the alga was being digested.

The frequent occurrence of the *Glœocapsa* in the rectal contents, still wrapped in mucus, shows the effect of the want of the style. Had that organ been present the mucus masses would probably have been torn up, the alga freed in the stomach and exposed to digestive action. It appears that the process of digestion does not function perfectly, even prior to the formation of a style, and not even a hungry mussel exposes all particles equally well to the digestive fluids.[4]

---

[4] Edmondson finds the alimentary tract of *Mya arenaria* empty of food until the style is partially replaced.

A quantity of *Spirogyra* and other filamentous algæ was cut as finely as possible with scissors, then macerated with a pestle. Examined microscopically there were found fragments of cell-wall varying in size, fragmented chloroplasts, and pyrenoids. Without screening, this material was fed, in considerable quantity to starved mussels. The water was agitated occasionally so as to keep some of the macerated material in suspension. One mass of alga had been taken from a dish in which decomposition had gone far. Although the mussels held the siphons nearly closed in the decomposing culture, they nevertheless ingested sufficient alga to answer the purpose of the experiment. Feeding the decomposed alga is comparable to the feeding of infusions, and the animals reacted similarly. In all cases of feeding infusion, decaying alga, and the feeding of mussels below the sewer outlet in Pocahontas creek, the regenerated crystalline styles had the same milk-white appearance. Thus there is no doubt that in all cases the color was due to bacteria. Mussels fed upon macerated fresh *Zygnema* had clear, and colorless or green, styles.

In one mussel fed upon a maceration of *Spirogyra* the partially regenerated style had a large core, and only two or three thin layers of style substance. This gave it the proportions and appearance of a rubber tube. When stretched out in a watch crystal and placed under the weight of a cover glass, the contents slowly oozed out at a broken point. The core was then seen to consist almost entirely of pyrenoids (or like bodies) closely packed, and in very great quantity. Their mass had a gray-yellow color. Only here and there was there a minute spot of green. Not a trace of cell walls or of a spiral fragment of chloroplast was found here or in the stomach. We have then another case of the rigid selection of food particles, and a little suggestion of the character of the materials which are capable of inciting the secretion of a new style. Again we have evidence that the gill is capable of taking very small particles from the water.

In a decaying macerated *Spirogyra* culture several starved mussels kept the siphons almost entirely closed. When a few c.c. of alcohol were added they shortly began and continued siphoning vigorously. Yet the food sorting mechanism functioned normally, for no stomachs nor intestines were found to contain larger fragments than usually occur there.

(c) *Forced Feeding.*

From the above experiments, and on grounds discussed elsewhere (p. 227), it is seen that the ingesting apparatus exercises considerable choice, and that (at least under experimental conditions) only certain sorts of material are admitted to the stomach.

An attempt was made to introduce distasteful matter into a starved mussel with the food. When fed alone, carmine had never been ingested. It was therefore administered with *Glœocapsa* and macerated *Spirogyra*. In no case was it found in the alimentary canal. Very little of the food entered, for that matter. The presence of the carmine caused a rejection of most of the food as well.

In order to ascertain if substances rejected by the mouth might yet have the power to stimulate the secretion of the crystalline style, these were introduced little by little through a fine pipette directly into the stomach. Fine carborundum (120–180 gauge) carmine, and starch were tried. In none of these cases was any trace of a style to be found later. It should be explained that the shock of operation was not alone responsible for this failure, for when *Glœocapsa* was fed to the animals in the same way, it was capable of renewing the style to a slight degree. There is sufficient ground for the conclusion that mere mechanical stimulation of the intestine or style sac on the part of fine particles is not sufficient to initiate the formation of the style. There must be a stimulus of a chemical nature as well. A reaction to the feeding activity might have incited secretion through reflexes from the palps. Yet this is inadequate to account for the renewal of the style when *Glœocapsa* was administered through the stomach wall.

(d) *The Effect of Temperature on Style Renewal.*

With the approach of winter it becomes more and more difficult to secure a prompt renewal of the crystalline style on the resumption of feeding. Where experiments are made in water of quite low temperature the same behavior is observed. Riddle, ('09), Krogh ('14) and others have shown that the rate of metabolic processes is related to body temperature. In this case the decrease in temperature probably directly affects the rate of secretion of the typhlosole glands. It is not improbable that a seasonal metabolic rhythm exists.[5]

In order to test the effect of temperature alone in this matter and to eliminate the other possible elements, the following experiment was carried out:

Checks were kept at room temperature. In each repetition of the experiment one jar was placed in the cold water of the outlet of an artesian well. When the temperature was sufficiently reduced the experimental starved animals were introduced into their respective jars. Meantime concentrations of lake plankton were made by filtering through fine bolting-silk, and the plankton remaining in the bucket washed out into the check and experimental jars. Each mussel had a jar to itself. The water in all had a decided green tint when agitated, for the concentration was in all cases from 100 volumes to 1. The jars were well shaded to eliminate the possible action of sunlight in orienting the planktonts, and thus keeping the food equally available to all. The amount of water was equalized, and the mussels so placed that the exhalent stream played obliquely upon the sides of the jar and maintained an eddy to keep the planktonts in circulation.

The material was collected at about 10:00 A.M., and about three hours were allowed for the temperature adjustment. The experiments began at about 1:00 P.M., and continued from three to four and one-half hours, usually four. The average temperature of the experimental jars at the beginning of the experiments was 13.4° C., varying between 12.6° C. and 14.0° C. The average of the same jars at the end of the experiments was 13.1° C., varying between 12.0° C. and 14.4° C. There was an average loss in temperature of 0.3° in these jars. The checks gave an average at the beginning of 26.0° C., varying between 23.6° C. and 30.0°; at the end an average of 27.2° C., and a variation between 24.0° and 29.6° C. The average rise in temperature of

---

[5] The crystalline style of *Mya arenaria* is reformed more rapidly during summer than winter. Edmondson (*l. c.*) ascribes this to the increased metabolism of the approaching breeding season.

the checks was 1.2° C. At the beginning the checks averaged 12.6° higher than the others, and at the end averaged 14.1° higher. Nearly ten degrees (9.6) separated the lowest check from the highest temperature in an experimental jar. As was to be expected the atmospheric temperatures created considerable variation in the checks (6.4°) and much less in the cooled jars (2.4° C.)

Of the twenty animals used only ten showed partially renewed styles. Of these ten only two occurred at the reduced temperature, and eight in the checks. The two which appeared in the low temperature were smaller than any of the eight formed in the checks. Moreover, the two were both in *Anodonta*, and *Anodonta* has shown a greater response always than *Lampsilis*, never failing to show some renewal. *Lampsilis* more slowly loses and more slowly regains its style than *Anodonta*.

While the number used is small, exact quantitative results are here unnecessary, and there is sufficient demonstration of the qualitative effect of temperature upon style formation. This effect may be partially explainable through the effect of temperature upon the cilia and the rate of ingestion. But the reason mentioned above is probably more pertinent, for the intestine was usually found to contain food in the experimental animals.

So long as the quality of the ingested material is right, the quantity required to initiate the formation of the style is very small. At times a single battery jar of water dipped at random from the surface of the littoral has contained sufficient food to restore it, in part. Held to the light the water had given no hint of green. But after it had been siphoned from one to two hours, the resulting thread-like crystalline style contained a conspicuous core of green.

Where food is abundant the length of time needed to renew secretion after the beginning of feeding is very short. A fair beginning may sometimes be observed within fifteen to thirty minutes. Large well-formed styles are sometimes secreted in four hours or less. The time depends largely upon the degree of starvation. More often twenty-four hours, at least, are required. On the whole it is a much more deliberate process than in some tidal forms, where the breaking down and renewal of the style occur rhythmically.

Of passing interest is the observation that small, newly formed styles sometimes may be seen coiled up in the stomach, where they have pushed forth more rapidly than they could be broken up and dissolved against the gastric shield.

Résumé.—It is here contended that the crystalline style accomplishes a number of purposes, for none of which it is entirely indispensable, nor entirely a perfect adaptation; that it is no longer performing an identical, single, primitive function traceable to a primitive Lamellibranch ancestor. The response of this organ to similar conditions is much the same in various bivalves; but the tranquil life of the lake has stabilized the feeding activity and the style formation in the Unionids, while the styles of some species inhabiting the marine littoral are profoundly affected by the tidal phenomena.

The formation and dissolution of the crystalline style goes on in the same way that a paper, candle-lighter might, if extended, to an indefinite length by rolling up a sheet of paper of indefinite length, and burning off the free end as rapidly as new paper is added at the other.

There is no evidence that digestion is furthered during the passage of food through the style or style sac.

The feeding of inert substances, both normally and through the stomach wall, indicates that the mechanical stimulation of the wall of the enteron is not alone the cause of the secretion of a new style. The rate of formation of the style is shown to depend in part upon temperature. Little food and little time are required to set the process going.

5. The Mechanism of Ingestion.

It has long been known that the gills with their great multiplication of surface are responsible for the movement of respiratory water, and for the concentration of food material from the water. It is surprising to encounter in the work of so eminent a student as Simpson, written only a score of years ago ('99), a statement that the siphoning is due to the waving of the palps.

It was shown by Posner ('75), Wallengren ('05), and others, against the contention of M'Alpine ('88), that the collections of food are transmitted to the labial palps, and by their cilia to the mouth. The writer shows (*l.c.*) that the ciliary streams of the upper portion of the mantle chamber all tend toward the mouth; while those of the lower portion of the visceral mass and mantle lead away from the mouth. The latter accomplish the duty ascribed by M'Alpine *to all* the cilia, that of freeing the mantle chamber of heavier materials and rejected food clots.

It was stated by the writer that the food material is subject to rejection at four points: (1) the siphons, (2) the point on gills and mantle where the food stream passes to the palps, (3) the furrowed surfaces of the palps, and (4) the lips, at the mouth. More recent observations have all corroborated this. Perhaps the fact has not been sufficiently stressed that only an unusual chemical or tactual stimulus results in the closure of the siphons or lips. The palps somewhat oftener refuse masses from the gills and mantle by turning aside. The greater number of reactions occur as the food stream passes between the contiguous palp surfaces.

The work of Wallengren ('05), and others, has demonstrated the action of the labial palps, the ridges of which are capable of reversing the food stream. Near the distal margin of each ridge of the palp surface the beat of the cilia is toward the apex, both in front and behind. When the ridges are inclined forward, the effective beat of the cilia is forward; when the ridges alter their axis, the backward-beating cilia are brought into play and the others turned under.

The course of the ciliary streams at the bottoms of the furrows between the ridges is much more difficult to observe. Wallengren believes that the cilia strike downwards to the edge of the palps, and that the resulting streams belong to the excurrent system. Siebert ('13), working on *Anodonta cellensis,* says they strike in the opposite direction—upward to the apex of the inverted V formed by the two palps, thence forward to the lips and mouth.

(a) *The Rôle of the Labial Palps.*

The writer has checked the matter as carefully as possible, and believes that there is ground for the views of both Wallengren and Siebert. The details of arrangement may not correspond exactly in the several species. On the lower half of the palps the cilia under consideration usually strike downward, and those of the upper half strike upward. Thus the lighter and finer particles tend to be drawn upward and forward as food, while the heavier, coarser, materials are more likely to be carried downward. The differentiation of the mechanism corresponds pretty well to that of the upper and lower portions of the ciliated surfaces of the mantle chamber as a whole. It is impossible to make direct observation of the streaming on any one ridge of the palps. But where substances of varying fineness are placed together on the palps, such as carborundum dust and carmine, there is a tendency to assort them. The carborundum particles move along the apices of the ridges and are carried nearly lengthwise of the palps. The carmine gravitates farther into the furrows between the ridges. Near the lower margin of the palps carmine is carried obliquely downward and forward, and on the average reaches the lower edge of the palps before the carborundum. When placed on the upper portion of the contiguous palp surface, carmine is drawn upward and forward to a greater extent than the carborundum, then forward toward the mouth.

The respective upward or downward pull upon the carmine may be accentuated by stretching the palps lengthwise, thus drawing the ridges farther apart and exposing the cilia of the furrows to a greater extent.

Attempts were made to effect a reversal of the ciliary currents of the furrowed surface of the palps by injections of curari, strychnin, atropin, pilocarpine, and by electric stimulation. The injections were made through the body wall into the sinuses near the base of the palps. Observations were made at various intervals from ten minutes up to several hours after injection. It was never found possible to control the reaction. There was a perceptible response to none of the several drugs except strychnin. This sometimes caused a contraction, at other times a relaxation of the palps. When the palps were in a state of contraction, most of the streaming carmine and carborundum were drawn to the edge and into the mantle chamber. These meager results tend to corroborate previous observations on the reaction of the palps—that food may be rejected through a greater or less erection of the ridges. Negative results prove nothing, while ever so slight positive evidence may be taken as an indication that a reversal of the ciliary streams can, and actually does, take place, through the bringing of another set of cilia into play.

The above shows clearly that the palps bear two sets of cilia working at right angles to each other. Due to their interaction materials often travel obliquely downward or obliquely upward. The former materials are eliminated at the lower edge of the palps, the latter reach the mouth.

The effectiveness of the assorting mechanism is well brought out by the measurements of the ingested particles. The largest fragment I have ever secured from the enteron was found in the intestine—a pinnately branched alga 3.3 mm. in length, probably *Myxonema*. The second largest particle was a bit of *Oscillatoria*, 1.5 mm. in length. It is unusual in Winona Lake mussels to encounter fragments of greater length than 500 microns. Starved individuals, probably experiencing a sensation akin to hunger, are observed to ingest freely much more large material than under normal circumstances.

Nelson ('18) has described the action of the food sorting cæcum of *Modiolus*, a diverticulum of the stomach. Since *Modiolus* ingests large quantities of sand in its periodic feeding, *Nelson's cæcum* affords a means of separating food from sand. It has been shown elsewhere in this paper that feeding in the Unionidæ is a more constant function, and that little sand and mud are taken into the stomach. The gills and labial palps are an entirely sufficient assorting mechanism.

(*b*) *The Gills as an Assorting Mechanism.*

Little attention has been given to the gills as having a possible food-sorting function. I find that they play no small part. Clots of mucus taken from various parts of the gills and palps have been examined, and often contain little but the finest ingestible material. This was well shown in the case of a *Glœocapsa* culture fed to a starved mussel. The culture was very pure except for numerous fragments much coarser than the *Glœocapsa* itself. There was an almost complete separation of *Glœocapsa* from the other material by the gills themselves. Almost all the larger fragments had been separated out by the gills themselves before the finer had been agglutinated in mucus. Little of the former was found in the masses present in the alimentary tract.

The marsupial function of the gravid gill of the female interferes somewhat with its respiratory and food collecting functions. Ortmann ('12) has shown that secondary water tubes appear, in which water circulates about the egg masses, and accomplishes the aeration of the eggs and glochidia. Yet the volume of water siphoned is much less than in the case of the non-marsupial gills. This is well brought out by the fact that the gravid females almost invariably regenerate the crystalline style much more slowly than others. When kept under artificial conditions for some time the gill-masses are usually aborted, another indication that the gravid gills are unable to meet all the demand upon them. The greater remoteness of the marsupial gill has suggested that it has become differentiated for the storage of the eggs and has lost its food collecting function. This notion is pretty well refuted by the facts mentioned above concerning the regeneration of the style in gravid females as compared with non-gravid females. It has also been suggested that the mantle has taken over much of the respiratory duty of the gills. If this were true the gravid female should be under no special respiratory difficulty. When first brought into captivity these females die at a much greater rate than others. The accessory water-tubes seem to be a very imperfect makeshift, sufficient perhaps for the glochidia, but affording the mother little aid.

Since in the gills and palps there exists a mechanism well adapted for the sorting of food; since both observationally and experimentally this mechanism is shown to accomplish a concentration of food; and since the contents of the alimentary canal have a decided green or brown color due to such concentration, we may feel safe in the reiterated conclusion that the Unionids exercise choice in the ingestion of materials. As stated by Zacharias ('07), Petersen ('11), the writer ('14), Baker ('16), and others, considerable quantities of inorganic and organic debris are carried into the stomach with food. Probably much of the stuff which Evermann and Clark ('17) call "mud" is organic. The fact that neither they nor other writers list *sand* in the stomach contents is further evidence of a selection of food material, and that river species are not an exception. Starved mussels were placed in the lake in two localities—(1) an open leeward shore in clear water; (2) near the outlet of Pocahontas creek, in muddy water following a rainstorm. The mussels of the first situation reformed the crystalline styles within a few hours. The others contained great quantities of muddy mucus, and did not have well renewed styles until the following day. The slow renewal of the style may be accounted for in part by the dilution of the food. But the presence of mud must be held partially accountable, for immediately after the subsidence and clearing of the water it was always found to contain ample food material to renew the style promptly.

In most species the position of the siphons at some distance above the substratum tends to keep out most of the grosser particles, admitting little but plankton and other materials in suspension.

In the discussion of the crystalline style (p. 227) the feeding of specific inert substances were recounted. When such materials which were readily identifiable were admitted with the incurrent water they were in no case found in the alimentary tract. As far as size is concerned these particles could very readily have entered the mouth. Since all were rigidly excluded we cannot doubt that sense organs exist for their detection, and that the assorting mechanism is a fairly effective one. Of the materials mentioned only starch might be expected to have a food value, though we cannot assume that it is in acceptable form. As a matter of fact the rejecting reactions were more vigorous in response to starch than to the other substances.

In the above experiments on the crystalline style neutral substances were introduced through the body wall into the stomach. At later periods the intestine and rectum were opened. Carmine and starch grains were recognized throughout the length of the alimentary canal. Only a few carborundum flakes were found in the intestine, and the rest were not carried out of the stomach. It is thus shown that the ciliary streams of the intestine are capable of manipulating only minute particles. The cilia are too small or too sparse to take care of the 120-gauge carborundum even in suspension in the liquid of the gut. Thus they must be altogether inadequate to keep a stream of sand in motion, if sand were ingested, unless it were of extremely fine grade.

(*c*) *The Marly Incrustation of the Shell.*

Since the dense, limy incrustation deposited on the exposed portions of the shells of lake mussels is the site of the active proliferation of diatoms, I suggested (*l.c.*) that this might be a source of food. In order to test its food value the following simple experiment was made:

A number of freshly collected mussels were placed in an aquarium; an equal number, having the incrustation scraped and washed off, acted as controls. During intervals, covering several days the animals were opened and the condition of the crystalline styles noted. The experimental animals were found to contain a trace of the style up to the fifth day, while the checks had virtually lost it by the end of the second day and entirely lost it on the third. Of course the crowding created rather special conditions, unlike those of the lake. The conclusion is that the incrustation contains considerable food.

6. SUMMARY AND CONCLUSIONS.

1. Feeding in the Najades is a nearly constant function under normal conditions. The presence of much undigested and sometimes living matter in the rectum and feces shows that there is a greater fluctuation in the degree of digestion than in the rate of ingestion.

2. The posture of a mussel has no effect upon the continuity of the feeding process, a further indication that under normal

circumstances ingestion may go on with less effort than an interruption of feeding.

3. The return of undigested material from the intestine through the style sac to the stomach is an unusual occurrence in the Najades, which takes place only after periods of starvation, and which is interrupted with the reformation of the style. It is a function much less significant in the Najades than in the tidal forms.

4. Experiments in feeding relatively finer and coarser plankton show that both are capable at least of stimulating the renewal of the crystalline style. Both have food value. It is probable that the nannoplankton furnishes a much greater part of a mussel's food than has been suspected. The studies of intestinal contents of the Unionids have not demonstrated what the *actual* food is, but rather the undigested residue. Experiment here has shown, however, that the organisms undigested in the feces are sometimes digested, under another set of conditions.

5. Starved animals fed in Pocahontas creek below the outlet of a sewer showed the following peculiarities in intestinal contents: (1) the occurrence of many relatively large organic fragments; (2) abundance of minute flagellates; and (3) great quantities of *Oscillatoria* filament.

6. The regeneration of the crystalline style is in response to the ingestion of food, and not due to the physico-chemical character of the water.

7. Creek-fed mussels show a variation in the color of the style through hyaline, amber, and milky. The apparently rhythmic character of this variation corresponds roughly to the variation of sewage discharged from a septic tank. The milky color is accounted for by the presence of bacteria.

8. A repetition of experiments in feeding infusions indicates that flagellates (and bacteria) present are responsible to a greater extent for the renewal of the style than are the bulkier ciliates.

9. The reformation of the crystalline style is a satisfactory index of the renewal of the feeding activity.

10. The function of the crystalline style varies. It may more or less imperfectly perform several functions at once. The

rhythmic loss and renewal of the style in tidal forms has no parallel in freshwater species.

11. The feeding of specific substances in high concentration never produces a renewal of the crystalline style unless such substances have a food value. No indication was observed that the stimulus for its secretion is a mechanical one.

12. The style is much less readily formed in autumn or winter. That temperature is responsible is shown by experiments in which starved mussels were fed a concentration of plankton in water of high and low temperature, respectively.

13. A very small amount of plankton is sufficient to stimulate style formation. Also only a short time is required.

14. The labial palps are the primary assorting mechanism. The gills are of considerable importance in this matter also.

15. Sand is never ingested, at least by lake forms, and mud but slightly. Much less inorganic debris finds its way into the stomach than would be the case if selection were not exercised by the gills and palps.

16. The cilia of the alimentary canal are unable to move coarse materials, or to maintain a stream of sand or heavy mud.

17. Gravidity of the gills is a serious hindrance to the respiratory and alimentary activities.

LITERATURE CITED.

Allen, W. R.
'14 The Food and Feeding Habits of Freshwater Mussels. BIOL. BULL., Vol. 27, pp. 127–139.

Baker, F. C.
'16 The Relation of Mollusks to Fish in Oneida Lake. Tech. Pub. no. 4, N. Y. State Coll. Forestry, pp. 1–366.

Baker, F. C.
'18 The Productivity of Invertebrate Fish Food on the Bottom of Oneida Lake, with Special Reference to Mollusks. Tech. Pub. no. 9, N. Y. State Coll. Forestry, pp. 1–264.

Edmondson, C. H.
'20 The Reformation of the Crystalline Style in *Mya arenaria* after Extraction. Jour. Exp. Zoöl., Vol. 30, pp. 259–291.

Ege, R., and Krogh, A.
'14 On the Relation between the Temperature and the Respiratory Exchange in Fishes. Int. Rev. ges. Hydrobiol., Bd. 7, pp. 48–55.

Evermann, B. W., and Clark, H. W.
'17 The Unionidæ of Lake Maxinkuckee. Proc. Ind. Acad. Sci., pp. 251–285.

Headlee, T. J., and Simonton, J.
'03 Ecological Notes on the Mussels of Winona Lake. Proc. Ind. Acad. Sci., pp. 173–179.

Lohmann, H.
'11 Über das Nannoplankton und die Zentrifugierung kleinster Wasserproben zur Gewinnung desselben in Lebenden Zustande. Int. Revue ges. Hydrobiol., Bd. 4, pp. 1–38.

Nelson, T. C.
'18 On the Origin, Nature, and Function of the Crystalline Style of Lamellibranchs. Jour. Morph., Vol. 31, pp. 53–111. Has an exhaustive bibliography on the crystalline style.

Ortmann, A. E.
'12 Notes upon the Families and Genera of the Najades. An. Carnegie Mus., Vol. 8, pp. 221–365.

Petersen, C. G. J., and Boysen-Jensen, P.
'11 The Valuation of the Sea. Rept. Dan. Biol. Sta., Vol. 20.

Rice, T. B.
'16 A Study of the Relations between Plant Growth and Combined Nitrogen in Winona Lake. Proc. Ind. Acad. Sci., pp. 333–362.

Riddle, O.
'09 The Rate of Digestion in Cold-blooded Vertebrates—the Influence of Season and Temperature. Am. Jour. Physiol., Vol. 24, No. 5.

Simpson, C. T.
'00 Synopsis of the Naiades, or Pearly Fresh-water Mussels. Proc. U. S. Nat. Mus., Vol. 22, pp. 501–1044.

Ward, H. B., and Whipple, G. C.
'18 Freshwater Biology. New York, 1111 pp., and 1547 figs.

Zacharias, O.
'07 Planktonalgen als Molluskennahrung. Archiv f. Hydrobiologie u. Planktonkunde Bd. 2, pp. 358–361.