## STUDIES OF THE BIOLOGY OF FRESHWATER MUSSELS.

### II. THE NATURE AND DEGREE OF RESPONSE TO CERTAIN PHYSICAL AND CHEMICAL STIMULI.*

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CONTENTS.

### 1. INTRODUCTION.

During the progress of studies upon the nature of the food of the Unionidæ (Allen, '14) it became important to know what power of food selection is exercised. Such selection involves the possession of sensory nerve endings in the epithelia. The writer has attempted to demonstrate the nerve endings in the various epithelia by two methods— histological and experimental. Histological methods have so far been almost wholly unsuccessful, insofar as revealing the qualitative arrangement of the several types of nerve endings

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is concerned. The experimental method as applied to the study of the control of ingestion has been much more profitable (Allen '21, 1). The present paper embodies the observations made during the same time upon the effects of stimulation of the several regions of the mussel epithelium which are normally exposed.

The mussels used in this work were the species commonest in Winona Lake, Indiana, and in the White River at Gosport and Shoals, Indiana. From the lake were obtained *Anodonta grandis* and *Lampsilis luteolus;* from White River, *Quadrula heros, Q. pustulosa, Q. lachrymosa, Q. undulata, Unio crassidens, U. gibbosus, Lampsilis ligamentinus, L. alatus, Plagiola elegans,* and others.

The methods of study will be outlined under each respective heading in the body of the paper. For convenience they will be grouped first as to their chemical or physical nature.

## 2. The Sensory Epithelia.

The exposed soft parts of an extended mussel are the foot, mantle edge, and siphons. That they bear more highly sensory epithelia than those enclosed within the shell is easily shown by experiment.

The *foot* is of course largely muscular. Numerous blood sinuses lie beneath its epithelium and cover the muscular portion. The sinuses diminish toward the ventral margin of the foot, and where they occur the foot is most sensitive to tactile stimuli.

With the gaping of the shell to allow for the protraction of the foot, open spaces anterior and posterior to the foot would be formed were it not that the *mantle* is adapted for closing the apertures. It is inserted into the shell parallel to its margin, in the pallial line. The edge of the mantle distad to this line is muscular, and the muscular zone of the right and left mantle lobes respectively are projected beyond the edge of the valves until they meet in the median line. The deep-burrowing sedentary *Lampsilis anodontoides* produces furthermore a considerable overgrowth of periostracum which answers the same purpose when the mantle is retracted.

The *siphons* are by no means so well developed nor so deserving of the name as in some of their marine relatives.

Compared with the general mantle margin, they are more muscular, slightly more protractile, and possess a greater innervation. In many marine species the mantle edges are sufficiently united to constitute closed tubes. But the fresh-water forms (Sphaeriidæ excepted) have the mantle edges united only at one point—i. e., their junction with the supra-branchial septum, thus separating the dorsal and ventral siphons.

The siphons differ from the subjacent general mantle edge in the possession of papillæ. The dorsal siphon tends to have a single row of small papillæ, while the ventral has two or three rows of much larger ones. By means of these the sensory surface is much increased.

### 3. PHYSICAL STIMULI.

Many details of the following experiments will be omitted, for the sake of summarizing numerous experiments in few pages, and to secure continuity and clearness.

### (a) *Mechanical, or Tactual, Stimuli.*

The effects of contact of the siphons, mantle edge, and foot with objects of various character were noted. A dissecting needle, camel's hair brush, and wooden spatula were used for their varying size, hardness and sharpness. They were brought into contact with a greater or less surface, with greater or less impact, and for greater or less lengths of time.

### (1) *The Foot.*

Upon the muscular portion of the foot a needle-point produces an unappreciable local effect, simply a depression of the area surrounding the point touched. At times of low activity the foot may even be pricked without a very con-siderable retraction. If a long time (30 seconds or more) elapses between stimuli, the same point may be touched repeatedly without additional result. However, when the same point or adjacent points are repeatedly stimulated at rapid intervals, the foot may be withdrawn to a considerable extent. This again depends upon temperature, oxygen supply, etc. Those individuals which are well fed, which have been living in well ærated water, and in water of 18° to 25° C. react **more readily to all stimuli than do those under other con-**

ditions. Extremely unfavorable state of the water may result in a mussel's tendency to lie prone with the foot extended and mantle gaping.

The camel's hair brush is incapable of initiating more than local movements. The spatula produces a wider response than does the needle point. The larger, then, the surface stimulated the more profound is the response. Repeatedly touching with the spatula soon causes the foot to retract, sometimes entirely within the shell.

The response to two needle points simultaneously is greater when some distance apart, but not so great as that produced by the spatula.

### (2) *The Mantle Edge.*

The mantle margin is more sensitive than the foot. A gradation from the middle, both forward and backward, is perceptible. That is the poorest innervation occurs near the middle of the mantle in the portion near the foot. Toward the two extremities it becomes more sensitive.

The camel's hair brush, needle, etc. produce a marked response when touched to the mantle edge, and the spatula still greater effect. Not so many repetitions of a stimulus or so vigorous contact is required as upon the foot. The stimulus of one contact is held over for a longer time than on the foot, while a second or third increases the effect. Repeated or vigorous stimulation causes a discharge of water from the mantle chamber by adduction of the shell, and when kept up may incite burrowing.

### (3) *The Siphons.*

A minimum of stimulation produces a maximum of response when applied to the siphons. The camel's hair brush is as effective as the needle point. Contact with a single papilla results usually in the retraction of the papilla alone. A repetition at intervals may cause the adjacent region of the siphon to draw back. Only a considerable impact upon a single papilla can cause the siphons to close or cause a discharge of water from the mantle chamber. Of course in this case the thrust is transmitted through the papilla to the surrounding mantle, and other nerve endings are affected.

The result is greatly amplified when two or three papillæ are stimulated together or in close succession. The nearby portion of the siphon is drawn back sharply, and repetition may cause the discharge of water. Often also there is a readjustment of posture, and more or less burrowing may follow.

If the contact is sufficiently vigorous upon either ventral or dorsal siphon, both close. Yet a slighter pressure upon the ventral siphon is required than upon the dorsal to cause the closure of both. This is as might be expected. The ventral opening is the larger and it receives the incurrent stream. For both reasons it should be well guarded. Mussels are often seen to hold the tips of the opposing rows of papillæ together in the median line, thus testing the entering water more thoroughly than when the siphons are wide open. There is less likelihood of attack through the excurrent opening against the streaming water.

We have reason to conclude that there exist no local reflexes by which the muscles of one siphon are caused to react to a stimulus from receptors of the other. Such stimuli probably must pass through the visceral ganglion, for it innervates the two siphons through distinct tracts by way of the pallial nerve.

Furthermore, it is easily seen that the two lips of either siphon are without a nerve commissure connecting right with left. When a sufficiently slight tactual or chemical stimulus is applied to one side of a siphon, that side alone gives a response. If the stimulus is increased in magnitude it reaches the visceral ganglion and is transmitted also to the other side of the siphon. This involves of course the pallial nerves of both mantles, both hemispheres of the visceral ganglion, and their commissure. Local reflexes govern only the muscles of the respective halves of either siphon. The fusion of the two siphons with the supra-branchial septum and with each other is very superficial, and has not affected the nerve supply. The siphons are not units in a complete morphological sense, but only somewhat differentiated areas of the mantle edge, left and right.

The two siphons are, then, differentiated primarily for the admittance and discharge of water. In the exercise of these functions they have acquired a further specialization—the

ventral in a sensory direction, the dorsal in a motor, for the control of the flow of water. The ventral is often observed to stand open, while the dorsal is to a greater or less extent closed. Surprisingly the sense organs of the ventral siphon exercise a more profound and lasting effect upon the dorsal siphon than upon the muscles of the ventral itself; and as a corollary a more profound effect than is produced by the sense organs of the dorsal itself. The following sequence of reactions has been secured many times:

1. Ventral strongly stimulated. Both siphons close vigorously. Both reopen together.

2. Stimulus repeated soon. Both siphons close. Ventral reopens, then dorsal.

3. Stimulus again repeated. Dorsal siphon closes. Ventral not entirely closed, and reopens at once.

Thenceforward there is a greater effect upon the dorsal than upon the ventral. The former has the manner of opening cautiously, tentatively. If the dorsal siphon is similarly irritated, there comes at last to be some response from the ventral, and a more local, temporary one from the dorsal itself.

The parts of the ventral siphon were shown not to be equally sensitive. The inner margin, next the lumen, is most responsive. The papillæ of the inner circle are longer and larger than the outer ones. The latter may sometimes be touched with only a local, temporary effect, while an equal contact with the inner or marginal papillæ results in a general and vigorous response. This should be expected since the marginal papillæ are nearest the inflowing water. The dorsal does not show such unequal distribution of the tactile organs.

In studying the method of burrowing in *Quadrula undulata* it was observed that the animal employed the papillæ to gauge its depth in the substratum. When pulling itself further into the sand, the mantle and siphons were withdrawn more or less out of the way and the valves as closely appressed as possible. Alternating with this movement the siphons were extended and the papillæ spread out, some of them upon the sand. Without doubt the relation of the papillæ to the substratum, and to the free water above, provide the necessary orientation of the burrowing reflexes.

The still more deeply burrowing forms, such as *Plagiola elegans*, withdraw wholly into the sand. A little funnel-shaped crater affords communication with the water above. The papillæ of the siphons are spread out radially upon the sand at the bottom of this crater. They not only aid in holding back the sand, but also warn the animal when it accumulates. The response is an adduction of the shell, perhaps repeated, which sweeps out with a jet of water the excess sand and restores its proper equilibrium.

### (b)  *Thigmotropism and Rheotropism.*

At this point mention should be made of the mechanical effects of obstructions in the substratum and the thrust of the current.

The movements of a mussel, by alternate distension and retraction of the foot and the flow of blood in the serous spaces, suggest amoeboid locomotion. Observation has shown also that there is no little resemblance to the thigmotropic reactions of *Amoeba* when obstructions are met with. Under conditions in which gravity and current may be disregarded it is learned that a mussel, diverted from its course by a stone or bit of wood, does not revert to the old course, but continues in the new.

This avoiding reaction is sometimes the result of inability to draw the shell past the barrier, and sometimes can be explained as an unequal tactual stimulation of the surfaces of the burrowing foot. As soon as equilibrium of friction is restored the animal proceeds again, in a new straight line.

In running water, if not of too great velocity, mussels often travel in a direct line transversely or obliquely to the current. When turned aside they often revert to the former course as soon as an obstruction is passed. The angle which their course makes with the current seems somewhat dependent upon the stream velocity. It is difficult to determine whether the orientation to the current is due to pressure upon the shell or to friction upon the siphon region.

After checking the movements of many individuals in streams it seems very probable that rheotropic reactions may account in part for the grouping of mussels in shoals, as well as for their orientation upon the shoals.

A state of approximate equilibrium in the substratum is preferred in streams. Where there is too great current eddies tend to develop around the shells of the more emergent species, washing the sand away and loosening their hold.

The importance of the above factors in local distribution has been discussed in the third paper of this series (Allen, '21, 2).

### (c)  *Light and Phototropic Behavior.*

The siphon not only has a better innervation, but is also more deeply pigmented than any other part of the mantle. This at once suggests light reception as one of its functions. Such differentiation of mantle finds its greatest expression, of course, in the more or less complex eyes of the Arcidæ and Pectinidæ. Hecht ('21) has defined the physiological aspects of photoreception in *Mya arenaria* and *Ciona intestinalis*.

### (1)  *Light Intensity.*

Three sets of experiments were carried out simultaneously in bright sunlight, subdued sunlight, and artificial light respectively. The animals were placed either in tubs or aquaria in which water was kept level by siphons, and ærated by dripping tap water. A sufficient depth of clean sand was used to allow entire freedom of movement. The experimental animals were periodically re-oriented with respect to the sources of light and their new positions and tracks noted— usually once each day. In the first experiment aquaria were used, at a south window. In the second the tubs stood just far enough from a south window to be out of direct light. In the third a 40-watt lamp was lowered into the center of a tub by means of an anchored museum jar of small diameter.

*Quadrula heros* was most useful in these experiments, due to its more frequent and more prompt response. This response is in part owing, no doubt, to its habit of turning and resting upon one side. Thus when originally placed erect on the sand it has this additional stimulus to incite movement. The species has an exceptionally heavy shell. When standing erect, hinge upward, it is in rather unstable equilibrium and tends through sheer weight to turn upon one side or the other.

It might be suspected that the results were due to the animals' seeking their reclining posture. That the stimulus to turn is not wholly due to this factor, but that light also

initiates movement is shown in the first experiment by the fact that the response is very much less on cloudy days, and that they did not follow the law of chance in the direction of movement.

### EXPERIMENT 1.   DIRECT SUNLIGHT.

Single *Quadrula heros* were set in the center of an aquarium upon clean sand, beneath three or four inches of water, after previously allowing them to become adjusted to that depth. They were placed at a south window in direct sunlight throughout the day. They were put into an erect, unnatural posture, like that of *Lampsilis* or *Anodonta*. The siphons were turned toward the south.

The following table is a check upon the several successive occupants of an aquarium for three weeks.   Possibly on account of shell weight, none failed to burrow after each forced change of posture, although some time always elapsed during which they remained motionless.

| Date | Amount of turning of siphons in degrees from south | Remarks |
|------|------|------|
| May  8 | 90 | |
| "   9 | 180 | |
| "  10 | 45 | |
| "  10 | 45 | Cloudy. |
| "  13 | 180 | Changed in morning; siphons turned eastward. |
| "  15 | 00 | Very warm, partly cloudy. |
| "  16 | 00 | Very warm, partly cloudy. |
| "  17 | 00 | Very warm, partly cloudy. |
| "  19 | 90 | Change in morning; siphons turned eastward. |
| "  20 | 90 | |
| "  21 | 90 | |
| "  23 | 90 | |
| "  25 | 90 | |
| "  27 | 00 | |
| "  28 | 30 | Eastward; cloudy forenoon. |
| "  29 | 90 | |

Total avoiding light.....................11
Total turning toward light............... 0
Failure to respond to light.............. 4

After recording each observation the experimental animal was set back to its original position, or another of the same species substituted.   The reactions of May 15–17 were probably vitiated by the unusually high temperatures, although the water was changed daily.

The large number of quarter turns is significant. It is especially so since the animals usually turned clockwise, and were found with the siphons pointing westward. This is easily explained as an avoidance of the forenoon sun. Each experiment began in the evening, too late for the same day's sun to be effective. During the night the animal was becoming adjusted to its new position, and remained inactive. Not much movement took place until the following morning. Consequently the usual turning to the east with the siphons to the west during the forenoon accomplished as great avoidance of the sun as possible. During this rotation and following there was also a lateral burrowing movement which resulted in the animal's lying on the left valve, its foot parallel to the surface, and hinge turning northward. In this species the siphon is never extended far beyond the shell, and is usually wholly within the shelter of the valves. In this position they received the maximum amount of shade in the experimental animals.

In order to demonstrate that there is no preference for lying on the left valve, a series of similar experiments were made in the dark. The response was much less prompt in darkness. Fifteen finally assumed a position on the left side and sixteen on the right.

The degree of torsion is seen to be much reduced by cloudy weather. The above expression in degrees is of course approximate, for with the accompanying lateral movement exact measurement is difficult. Nor is it important.

## EXPERIMENT 2. SUBDUED SUNLIGHT.

In each of two tubs placed in indirect sunlight near the south windows six *Quadrula heros* were stationed. The windows of the room were such that the brightest light came into the tubs from the southwest, the least light from the dark southeast corner. There was considerable reflected light from the northeast. From still greater illumination at the west and northwest the light shaded off toward the east and northeast.

Running tap water kept the animals in vigorous condition. They were checked up and reset at frequent intervals. Those in "tub 1" were placed with the siphons toward the maximum light of the southwest, those of "tub 2" the reverse, with the

siphons pointing away from the maximum light, but not toward the minimum of the southeast. The following table indicates the reactions over a short period, though the experiment was kept up with modifications from January to the end of May.

| DATE | TUB 1 | | | TUB 2 | | |
|---|---|---|---|---|---|---|
| | Remain toward light (Positive) | Turn to south (Negative) | Turn to north (Negative) | Remain toward light (Less Positive) | Turn to south (Negative) | Turn to north (Most Positive) |
| April 11..... | 1 | 3 | 2 | 1 | 5 | 0 |
| " 17..... | 0 | 4 | 2 | 4 | 2 | 0 |
| " 23..... | 1 | 5 | 0 | 3 | 2 | 1 |
| " 26..... | 3 | 2 | 1 | 2 | 3 | 1 |
| " 29..... | 2 | 4 | 0 | 1 | 5 | 0 |
| May 2..... | 3 | 3 | 0 | 2 | 3 | 1 |
| " 7..... | 4 | 2 | 0 | 1 | 4 | 1 |
| Totals...... | 14 | 23 | 5 | 14 | 24 | 4 |
| Another experiment using more intense light | 4 | | | 13 | | |

Total remaining toward light of greatest intensity, but not direct.............. 4
Total remaining toward light of second order of intensity.....................14
Total remaining toward light of third order of intensity.....................27
Total reacting positively to light............................................ 4
Total reacting negatively to light...........................................52
Of the last total, turning to north from southwest............................ 5
Of the last total, turning to south (darkest corner)..........................47

During the winter months the reactions were very slow and much less definite than in spring, though qualitatively similar. While it appears probable that there is a sort of hibernation, or seasonal rhythm in metabolism, aside from the effects of the immediate physical surroundings, yet there is no doubt that temperature is largely responsible for the increased activity of April and May.

.From the above table it will be seen that nearly every day's record shows the directive agency of light. Usually a greater number of *Quadrula* reacted negatively to light than the number which failed to move at all. A still smaller number turned toward light of greatest intensity. It will be noted

that the reaction time was much greater in subdued sunlight than in direct. Given more time the above predominance of negative reactions would probably have been greater. Not only is it significant that there was an avoidance of southwest light, but that nearly all such cases turned the siphons toward the darkest corner of the room—the southeast—and few to the somewhat greater light of the north. Of course those which turned from the east to the north were reacting positively, while those which turned from the west to the north were changing the siphons into light of less intensity. The supplementary experiment at the end of the above table was an attempt to ascertain the result of using light of an intensity intermediate between Experiments 1 and 2. Equal numbers were used in tubs 1 and 2. In the former only four remained exposed to the greater light intensity, while thirteen remained passively siphoning toward the northeast in the second tub. In the totals it may seem inconsistent that the same number (fourteen) should have remained facing either direction. This is explained by the fact that the shock of handling or inertia is with difficulty overcome at first. Of more significance is the undoubted subsequent orientation with respect to light. The original inertia is seen to be overcome most rapidly by light of the greatest intensities.

In order to make certain that *Quadrulœ* do not have a preference for lying on the right or left side, a part of the experiment was repeated in darkness. The results were: Remaining erect, 13; on right side, 13; left side, 14. None were ever observed to change from one side to the other, either after assuming their position voluntarily, or after having been placed arbitrarily. Large numbers of observations verify this point. It is certain that no other element than light is responsible for influencing the direction of movement in the above experiments.

### EXPERIMENT 3. ARTIFICIAL LIGHT.

In the tub kept in darkness and illuminated by a 40-watt lamp eight mussels of as many species were kept and checked from April 25 to May 27.

During the progress of the experiment the mussels were placed variously, having the siphons toward the light, away from it, and at various distances from it of one inch up to about eight inches. They were observed and reoriented twenty

times in the thirty-two days. Being 45° apart in the tub there was room for each to move without interference. There being twenty records for each individual, we have a total of 160 records. The total number actually changing posture was 61 out of the possible 160. There remain 99 negative results, a ratio of about three to two more or less positive results. The following species participated. The number of times each moved is indicated. *Lampsilis alatus*, 1; *L. ligamentinus*, 18; *Quadrula lachrymosa*, 0; *Q. pustulosa*, 1; *Q. heros*, 9; *Unio crassidens*, 14; *U. gibbosus*, 10; *Plagiola elegans*, 8. Average 7.6 out of a possible 20.

The above record is not significant, as an analysis of the behavior of the respective species, perhaps, on account of the small number used. But we do have a fair test of the average reaction in light of low intensity. The fact that *Quadrula heros* only changed position 9 times out of 20; 56 times out of 84 in Experiment 2; and 11 times out of 15 in Experiment 1, show a responsiveness to light that is in the inverse ratio of its intensity.

Temperature may be in part responsible. It varied between 12° C. and 16° C., averaging about 14° C. in the third experiment. In the second it averaged about 16° C. and ranged between 13° C. and 17.5° C. In the first experiment temperatures were still a little higher, but on the warmest days, which were also very cloudy, there was little response to be seen. Thus light was shown to be more pertinent than temperature.

Although only 61 out of 160 moved at all in this series of observations, yet only 11 of the 61 turned either toward or away from the light. The remaining 50 burrowed directly downward without a reorientation. Light evidently had little to do with these movements. Even when placed nearly in contact with the source of light there was not a perceptibly greater turning away from it than when separated by the radius of the tub. When tried four days in darkness 12 moved and 20 failed to do so, a not very different ratio. The above-mentioned reactions of *Quadrula heros* gave 13 out of 40 failing to burrow in darkness.

There are other evidences (Allen, '21, 2) that the optimum conditions for mussel life include a moderate amount of light, rather than extreme amounts. Light itself has little importance in the life of the organism—its significance lies in the accompanying complex of conditions.

### (2) *Responses of the Siphons to Rapid Light Changes.*

When the mussel has been siphoning undisturbed the siphons respond to changes in light intensity. The magnitude of the response is more or less proportionate to the change in light. (See also Hecht, '21). If an object is interposed between the sun and the siphons, changing them from a condition of maximum illumination to deep shadow, there occurs a very prompt and complete closing of the siphons. A considerable time is then necessary to accustom the siphons to the lower light intensity. They slowly and tentatively reopen in the shadow. If the shadow is withdrawn, the direct sunlight again causes a closure, and a slow renewal of siphoning again takes place. By rapid and repeated changes between sunlight and shadow the siphons are first caused to close, then to draw into the shell, and finally comes an adduction of the valves and spurting of water from the siphons. Then normal siphoning is renewed. This reaction may be produced several times in succession. Usually a burrowing movement, or at least a change of posture, takes place.

The same reactions may be gotten in indirect sunlight, especially at a north window, or wherever most of the light comes from a single direction. At noon on bright days the response at a north window is not of much less magnitude than in direct sunlight. After four o'clock the reactions diminish in magnitude and reach the vanishing point before twilight. Very faint shadows elicit no response.

In these experiments we again get the maximum of response from mussels which have been kept in running water. In standing water not frequently changed the closure of the siphons is not so rapid in bright light, so easily produced in subdued light, nor so quickly repeated on second stimulation. The siphons do not close so completely, and reopen more quickly. A certain indifference to stimuli, not unlike fatigue, is seen when the water is very impure.

As shown above (Experiment 1) the Unionids kept at south windows very promptly take to burrowing. This is well brought out in the case of *Quadrula heros*. *Lampsilis ligamentinus* and others are very alert. A similar reaction can be obtained at north windows. But it is much slower and does not always occur. The degree of turning is usually less, and the direction of movement less uniform.

Light might be expected to affect the tonus of the siphons, thus determining the size of the apertures, and aiding in the regulation of the flow of water.   But such does not prove to be the case.   When other conditions are made as nearly equal as possible, it is found that a mussel maintains the same siphon aperture whether in direct sunlight, shadow, or darkness. When carefully changed from one light intensity to another, or when masked and unmasked, there is a brief closure.   But the animal sooner or later becomes adjusted to the changed intensity and reopens the siphons to about the same extent as before.

### (3) *Phototropic Reactions in Nature.†*

(a) Light *initiates* movement, (b) *orients* movement, and (c) rapid changes in light *affect the siphonal aperture*.   These conclusions have been derived experimentally, and under controlled conditions.   In the more complex life of a lake or stream the reactions are less easily predictable.

A mussel in too shallow water is exposed to unusual illumination.   Light may be a constant stimulus to locomotion until deeper water is reached.   Thus the first conclusion is in harmony with the mussel's requirements in nature.   The trails of moving mussels show more or less trial and error in the finding of deeper water.   If light were the sole directive agency you might expect to find all the mussels of a level stretch of shallow bottom to be moving in the same direction. That such is not the case is doubtless due to the other physical, chemical, and physiological conditions.

It is improbable that light is the sole factor even in the rare cases where a well-defined light gradient exists.   Abrupt light gradients seldom occur except accompanied by sharp gradients in depth or turbidity.   In the former case pressure enters as an important factor.   Only in turbid water or in the presence of shadows is it likely that the conditions of the experiments are approximated closely enough to obtain pure phototropic reactions.

†The recent paper of Coker, Shira, Clark, and Howard (1921) has come to hand just as the present manuscript is ready for publication.   Their observations have led them to the same conclusions as my own, except that they prefer to regard the depopulation of shaded areas as positive reactions to light of moderate intensity.

The bivalve is vulnerable only where not covered by the shell.   It is particularly important to guard the siphons against invasion.   Hence their prompt closure even with the passing of a shadow.   No doubt enemies and large objects are excluded in this way.   The spurting of water is the means of driving away a persistent animal or bulky floating object.

It is possible that the comparatively sparse population of *Potamogeton* beds is due to the checkered light on the bottom. Of course the shadows of surface objects are much diffused at the bottom.   The *Potamogeton* bed is more difficult to traverse than open bottom or *Chara*.   Yet it is conceivable that the flickering light may keep a mussel more or less in motion until more evenly illuminated bottom is reached.   At any rate the interiors of many beds of plant are devoid of mussels, and the margins more populous than the interior.   An experiment (Allen, '21, 2) in which a lot of forty marked mussels all escaped after being planted in a *Potamogeton* bed at a depth of three and one-half feet, may be explained thus.   Several areas of Winona Lake have during recent years shown great increase in *Potamogeton*.   Invariably the number of mussels is correspondingly decreased.   Some beds are virtually abandoned.

### (d) *Hydrostatic Pressure.*

Mussels were kept in aquaria and tubs in their normal posture upon sand, and otherwise behaving normally.   Then the water was gradually siphoned away so as to reduce its depth several inches.   Numerous individuals were incited to spurt water and burrow.   The reaction has been seen with such frequency wherever mussels are handled as to leave no doubt that there is a pressure sense, and that a mussel tends to seek its optimum depth—at least the depth to which it is temporarily adjusted.

During the early summer of 1919 Winona Lake was deeper than in many previous summers—perhaps two feet higher than its observed previous minimum depth—due to rains and to the construction of a dam at its outlet.   The mussel population of its shallow wave-cut terrace was therefore subjected to an increase of pressure of from 10 to 100%.   Many of them had moved toward shore, as was evidenced by the long, zig-zag tracks, and the presence of the animals at the shoreward end. Two weeks later the lake had gone down more than a foot.

Many new tracks were then traced, and the makers found at the deeper end.   Evermann and Clark ('17) make brief reference to this behavior as of seasonal occurrence in Lake Maxinkuckee.

The matter was checked up experimentally by planting mussels well inshore.  Those facing the lake sooner or later moved out.  Others facing the shore at first moved inward, but finally turned and went out.

On the low gradients of the wave-made terraces the above responses were slow, and often more or less random.  On the very level areas no preponderance of mussels pointing in either direction was discernible.  It was desirable to repeat upon a sharp gradient.  The sand bank of the Sugar Creek delta has such a gradient—about 30°, more or less.  Mussels were planted at a depth of two feet in normal posture, and faced both toward and away from shore.  The outcome was much the same as above, but the response took place within a few days.  There was much less random movement on the part of those facing shore; they turned very sharply toward deeper water, usually describing an arc of about a foot in radius to make the turn.

This bit of shore is well exposed to wave action—the only other element except pressure or light which might have oriented the movements.  That it was not wave action is amply shown by many cases where the same reaction took place in sheltered coves.  Light is eliminated by the fact that in this case the siphons were oriented toward the meridian sun in the act of turning toward deep water, a result which is the exact reverse of that in Experiment 1.  This and many other observations indicate that where light and pressure are opposed, the latter proves the more potent stimulus.

The forty marked mussels mentioned in the above section may have been incited to movement by changes in depth.

### (e) *Equilibrium, Shell Weight, and Posture.*

That bivalves possess an organ of the lithocyst type for maintaining an orientation with respect to gravity has long been known.  The stereotyped conception of this function represents the animal as lying on its side, putting its foot out laterally, securing a leverage on the substratum, and pulling itself erect.  This is accurate enough in the main, but does not take all cases into account.  The posture and manner of

burrowing vary considerably. Thus the organ of equilibrium serves to keep one species perpendicular, and another horizontal or oblique.

The *Lampsilis* and *Unio* species usually have the erect position, while the *Quadrulæ* tend to lie prone. (1) Variation in posture is closely associated with (2) shell weight, (3) with mobility, and (4) with metabolic activity. At one extreme we have the very heavy shell of *Quadrula*, at the other the very light one of *Anodonta*. *Quadrulæ* are extremely sedentary, *Anodontæ* active. *Quadrulæ* nearly submerge themselves in the substratum, *Anodontæ* sit high in the water, the shell sinking only a little way. *Anodonta* exposes its siphons boldly, is the most sensitive of all the Najades to stimulation upon siphon and mantle edge, and of all the Najades is the only one which exhibits a sensitiveness upon the entire mantle surface through the shell. *Quadrula*, on the other hand, does not extend the siphons beyond the shell, does not have so well-developed siphonal papillæ, and reacts very slowly to stimuli. *Anodonta* recovers from stimuli and resumes siphoning most promptly, while *Quadrula*, when caused to close the shell, may remain sealed up tightly for hours. *Anodontæ* kept in aquaria, die most quickly of all mussels when conditions are not kept right. In fact when miscellaneous mussels are kept indoors, even under the most favorable conditions possible, the *Quadrulæ* survive most of the others.

The thin, translucent shell of *Anodonta* is accompanied by a more widely distributed light-sensitiveness. Experiments such as the above show a certain avoidance of the light, but have a less marked orientation than *Q. heros*. The thick shell and well-shaded siphons of the latter admit light from virtually a single direction, making it perhaps the most favorable for such studies of light orientation.

The above points of structure mark the *Anodonta* as a lake dweller, *par excellence*, and *Quadrula* as a river form. *Unio* and *Lampsilis*, of more generalized type, are more readily adaptable to either situation. Representatives of the several genera have taken on divergent habits, however. *Q. rubiginosa* inhabits lakes, and affects the upright position. Yet it has a rather heavy shell and is quite sedentary. When planted experimentally it does not move far. In aquaria movements of the several species accord somewhat with the surroundings, and somewhat with the habits of the species.

In moving water there may be less need for locomotion than in lakes, and less movement actually seems to take place. The constant change of water is the equivalent of changing locality. Isely's observations ('13) have shown that a mussel may remain upon the same spot for long periods of time, in streams.

Deep burrowing forms are much commoner in streams. The principal activity necessary to river species is to keep the siphons at the right distance from the substratum despite shifting bottom, etc. *Plagiola elegans* submerges entirely beneath the surface of the sand. Marine clams, Sphaeriidæ, etc., in like situation elongate the siphons to reach the surface of the substratum. This species, like the ant lion, maintains a little crater whose center is at the siphons, and whose shape is dependent on the angle of rest of the sand. Silt and rolling sand are kept out by spurts of water from the mantle chamber. *Lampsilis anodontoides* is of a more generalized genus which has taken on a form comparable to *Anodonta*, except for its shell thickness. This is considerable and marks the species a river mussel, as well as assigning to it the deep burrowing habit. It also communicates with the water above through a crater-shaped pit. The siphonal papillæ radiate out from the center and rest upon the floor of the crater. In this position they perform their sensory functions and at the same time hold back the sand from the siphons. With the shell thickness and burrowing habit of this species, as well as the *Quadrulæ*, may be associated the less delicate perception of stimuli. *L. anodontoides* accepts much more carmine into the mantle cavity than do other *Lampsiles*. Burrowing species less promptly renew the crystalline style after starvation (Allen, '21, 1).

## Résumé.

The foot, mantle, and siphons bear tactile organs, whose abundance is in the reverse of the order named. Their principal functions are the orientation of movements with respect to the substratum and avoidance of enemies. Certain local reflexes exist which do not pass beyond the immediate stimulated region. The innervation of each half of each siphon is independent, and from the splanchnic ganglion. The ventral siphon is the more sensory; the dorsal is the motor termination of many reflexes which have their receptors in the ventral siphon (and in the osphradium). The dorsal has more to do with the regulation of water flow. The

papillæ of the siphon margin are the most highly sensory of all areas.    They are employed variously—in guarding the siphon, in gauging the depth while burrowing, and in holding the sand back from the siphon.

There is often an orientation in response to current that holds the bivalve to a definite course.

The mantle, and especially the siphons are pigmented and light sensitive.   Experiments show clearly a negative phototropism in bright light, and an orientation of posture and movement with respect to light.   Low intensities of light may incite movement without orienting it.   The reaction to moving shadows is obviously one of avoidance of enemies and debris.

The magnitude of reactions is dependent in part upon temperature and oxygen.   Light does not determine the permanent aperture of the siphons.

A sense of pressure exists by which the optimum depth adjustment is made.   When conflicting with other senses, that of pressure is shown to predominate.   Reactions to gravity vary in the species in harmony with the posture habits.

### 4.   Chemical Stimuli.

Experiments were made to ascertain the reactions to varying strengths of several acids and bases.   No attempt to determine the differential effects of the respective reagents was made, but only the types of reaction and location of the receptors.

The reagents were brought into contact with the epithelia through a finely drawn pipette.   The latter was manipulated very cautiously so as to eliminate possible mechanical stimuli through pipette or solution.   The mantle edge and both siphons were tested; other epithelia by causing the reagents to enter with the incurrent water by carefully introducing the pipette through the center of the siphon far enough to prevent contact with the siphons themselves.

The best series of reactions was obtained by the use of acetic acid, ammonium hydroxide, carmine, and foul water from decaying Spirogyra, and hay infusions.

Drops of 1.0% acetic acid were applied at several points on the outside of the respective siphons and mantle edge beneath the siphons.   In all cases there was a rapid closing of both siphons and a rapid and violent discharge of water.

When siphoning was begun again, the point which had been touched with acid was found to be drawn back sharply—even sometimes the entire half siphon, and this effect was perceptible after several hours.

If while still under this initial stimulus 0.1% to 1.0% acid was administered, the animal still responded, but much less promptly. The effect of the 0.1% acid was of much less duration, but at first of equal magnitude.

Very few animals gave any response to the acid of 0.01% more or less, when applied to either mantle or siphons.

By the use of ammonium hydroxide a similar scale of responses was obtained; 0.5% evoked a very rapid and profound response—even in mussels that had been very inactive. The effect passed much more rapidly than in the case of the 1.0% acid. 0.1% ammonium hydroxide had a great effect upon the same individuals just recovering from the stronger alkali, but more effect on fresh animals. 0.01% obtained a very slight and tardy response. When given in water of low temperature—12° to 14° C.—0.01% gave no reaction whatever.

From the above it is clear that the mantle edge and siphons are provided with organs of chemical sense, which are keenly sensitive to acid and alkaline substances of high concentration. The response was equal in the case of the ventral siphon and of the subjacent mantle edge, but noticeably less in the dorsal siphon.

Carmine and foul water elicit no reaction from siphons or mantle edge, as was true of all very low concentrations.

The osphradium has been known as an organ of chemical sense, largely from its structure. Before water may be tested chemically it must first pass through the ventral siphon, the mantle chamber, the gill chambers, and the suprabranchial chamber. At first thought the position of the osphradium under the visceral ganglion seems remote and futile, since the character of the water may not be known until just before leaving the animal. Conversely it may be of some advantage to have all the water pass a common point such as this. However it is probable that the osphradium is phylogenetically a more ancient structure than the siphons, and long associated with the visceral ganglion—hence its position there rather than upon the neogenetic mantle structures. The mussels of lake or stream seldom encounter solutions of great concentration.

When dilute, their diffusion is sufficient to cause a closure of the shell before much harmful matter can enter.

The following tests of the osphradium were made to determine whether it possesses chemo-receptors, and if so their relative sensitiveness, compared with those of the mantle and siphons.

The finest carmine, pipetted from the upper levels of a carmine suspension which had settled for several days, was introduced into the ventral siphon. There was no response from the siphons. Just before the carmine could be seen to have reached the dorsal siphon, there occurred a quick closure of the ventral siphon, followed by that of the dorsal, and a prompt reopening of both. Thus it is seen that the carmine had not affected any sense organs until it had nearly reached the dorsal siphon—the location of the osphradium.

Stale water and infusions were never found to affect the chemo-receptors of the siphon and brought about no reaction until they reached the osphradium.

There was still the slight possibility that the gills or mantle might possess organs of chemical sense. Therefore to produce a more marked effect upon the osphradium alone than is possible by introducing reagents into the siphon with respiratory water, the following experiment was tried.

A few drops of 1.0% to 10.0% acetic acid were drawn into a fine pipette, the latter inserted into the dorsal siphon just far enough to reach the osphradium, the acid discharged and instantly drawn back again into the pipette with considerable water from the suprabranchial chamber. Thus it was made sure that the concentrated acid affected the osphradium region only, and that only very dilute acid if any. reached the dorsal siphon. The following results were noted:

(1) Neither siphon fully opened within a week or more, though the water was purposely allowed to grow stale sometimes.

(2) The papillæ of both siphons remained in contraction.

(3) Vigorous burrowing, and frequent spurting.

(4) Both siphons still very sensitive to dilute acid.

(5) The osphradium was still sensitive to dilute solutions; not all its receptors had been destroyed or paralyzed.

Another test consisted in isolating the osphradium from the outgoing current. This was accomplished by lowering the water level to a point just sufficient to flood the floor of

the suprabranchial, and so as to leave the osphradium above the surface.  As soon as the animal had ceased spurting and grown accustomed to the water level, and accustomed to having the posterior end out of water, the fine carmine suspension previously mentioned was introduced into the still submerged ventral siphon.

A stream of carmine could thus be kept passing through a mussel for an indefinite length of time.  The siphons were not adjusted to register so nearly neutral a reagent.  When the water level was raised again slightly and the osphradium flooded the usual response to carmine again took place.

These reactions verify the usual conception of the function of the osphradium, and show something of its range.

## 5.  TONUS OF THE SIPHONS.

It has been seen that mechanical stimulation and light exert no permanent influence upon the muscle tonus of the siphons and regulation of the streaming.  Chemical stimuli do have this function.  Mussels placed in water containing little oxygen maintain always a much wider aperture.  As noted previously this is the more pronounced in the case of the dorsal siphon.

When the water is greatly fouled by material in decay, the siphons (especially the dorsal) more or less completely close.  Entire closure may result in such inactive mussels as *Quadrula heros*, but rarely so in the more active *Anodontæ* or *Lampsiles*.

## 6.  ACCESSORY SIPHONS.

When under conditions of deficient oxygen not only do the siphons widen to their greatest extent to bring in more water, but also additional spaces between the mantle edges are thrown open.  In most species there occurs a very definite accessory dorsal siphon, the *supra-anals* of Ortmann ('12) above the dorsal.  It is not provided with papillæ, and is not so sensitive to the various stimuli enumerated above.  It is only opened in emergencies; uncommonly under normal conditions.  In the several species the supra-anal varies in its length, and in its distance from the dorsal siphon.  Likewise there is a variation in the extent of the cloaca above the suprabranchial channel.  It reaches its maximum dimensions in

*Quadrula heros* or *Lampsilis alatus*, whose mantle extends dorsad to the full extent of the *ala*. The ciliary movement of the cloacal portion of the mantle is ventrad and centers at the dorsal siphon—not the supra-anal. The higher the shell and mantle extend above the siphons, the longer is the accessory siphon of the several species.

Ventral to the incurrent siphon other temporary openings may appear between the mantle edges. Rarely (in extreme exhaustion) this opening extends the full length of the mantle. When the siphons have been injured or over-stimulated, and when they lie above the surface of the water, the mantle is allowed to stand open along its ventral margin.

### Résumé.

It was shown by experiment that the siphons and mantle margin are subject to chemical stimulation only of considerable concentration. Lower concentrations take effect through the more sensitive osphradium. Since high concentrations do not ordinarily concern the animal in its normal habitat, the chief sensory functions of the siphon are those of physical character. By means of the osphradium the mussel reacts to very small changes in the chemical nature of the water. The muscular tonus of the siphon and mantle is under control of the chemo-receptors of the osphradium. Through the resulting apertures of the siphons, and occasional accessory openings of the mantle, the regulation of water flow is accomplished. Normally the ventral siphon has a more constant aperture, while the streaming is controlled by the anal.

### 7.　Concluding Remarks on the Nervous System.

The siphons, due to their exposure, and to the intake of water, have attracted a large share of mantle musculature, and with it the richest share of motor and sensory nerve endings. These center at the splanchnic or visceral ganglion. So far as the "conscious life" of the mussel has come to center about the siphons and osphradium, the visceral ganglion is physiologically the brain. In the sense that cephalization is usually accompanied by corresponding development of a cephalic ganglion and cephalic sense organs, the visceral ganglion has largely taken over these functions in the mussel which communicates with the outside through posterior siphons. To be sure the cephalic ganglion still retains two pairs of commissures in

communication with the other ganglia, but its sensory functions are of a less peripheral character than those of the visceral.

By virtue of homology and position at the anterior extremity of the body the cephalic ganglia are entitled to their name. They possess little analogy to a true cephalic organ.

That the mantle and visceral innervation of the mussel is divided about equally between the cerebral and visceral ganglia, and that the two ends of the animal are rather independent of each other, is shown by the following experiment:

The two valves were wedged one-fourth inch apart by a fulcrum of wood at the center of the ventral margin. The point was selected where the pull of the two adductors just balanced each other. Then the two ends of the mantle and visceral mass were prodded by turns. By turns there was a greater adduction at the stimulated end. This was sufficient to overcome the already vigorous contraction of the other adductor, and caused the relaxing end to gape more widely. Additional stimulus at either end overcame the balance. The magnitude of the response decreased progressively as the stimulus was applied nearer and nearer the block, where the branches of the pallial nerves overlap and anastomose. The narrowness of the mollusk's existence is much expatiated upon. The principal reactions of the bivalve when summed up include only: Protraction and retraction of the foot, adduction of the shell, protraction and retraction of the mantle margin and siphons. The movements of locomotion and burrowing, respiration, waste disposal, and driving away of intruders are chiefly made up of combinations of these five reactions, supplemented by ciliary activity. It is as though the whole existence of a mammal were made up of stretching, yawning, and sneezing. Usually more than one of these reactions follows a considerable stimulus. After many observations one can become more or less skilled in predicting from the manner of a movement just what will follow it. For instance, in the way of closing the shell one can foretell that burrowing will ensue. When the siphons are opened in a less cautious way, one may predict that the animal is about to "sneeze"—i. e., spurt water through the siphonal region.

In many of the activities of the mussel cumulative movements are seen. The initial stimulus is insufficient to incite the entire series of movements directly. Only a small portion of the foot or siphon may be set in motion at first, then in wave-

like fashion the contraction passes on and engages all parts of the organ. Local reflexes are probably responsible for this behavior, for it is observable even in a foot or siphon which is dissected out and not in communication with the central nervous system. The contraction of one region stimulates the adjacent areas, and they in turn by contracting pass the movement onward.

Three elements at least have contributed to the degenerate character of the bivalve nervous system:

(1) The aquatic life; comparative uniformity of the physical and chemical environment. New and difficult conditions have not often confronted them. The advent of the industrial period, and stream pollution have found them unprepared except as to great fecundity.

(2) The nearly sedentary life, passive feeding habits, and slight need of locomotion.

(3) The sheltered existence within the shell, and passive immunity from attack by enemies.

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