XXIV.—AN ACCOUNT OF EXPERIMENTS IN OYSTER CULTURE AND OBSERVATIONS RELATING THERETO. (SECOND SERIES.)*

By John A. Ryder.

The work of experiment with the eggs and embryos of *Ostrea virginica* were carried on for the season of 1882 at the experimental station on Saint Jerome's Creek, Maryland, by Col. M. McDonald and myself, under the auspices of the United States Fish Commission. Other experiments were also conducted at Beaufort, N. C., by Francis Winslow, U. S. N., and Prof. W. K. Brooks, while Mr. Henry J. Rice experimented in Mr. E. G. Blackford's laboratory, Fulton Market, New York City. The other observers named above will, however, probably publish their results at length in due time, so that it is unnecessary for me to do more than allude to their work.

I left Washington with the United States Fish Commission steamer Fish Hawk in June last, but did not begin any actual investigations until July 3 following. In the description of my investigations, as well as those made jointly with Col. M. McDonald, I shall rely in great measure upon the journal in which I recorded the principal observations and experiments from July 3 to August 11, 1882.

July 3.—Investigated the contents of the stomachs of a number of adult oysters taken from the channel which leads to the pond. The following organisms were observed amongst the more or less disintegrated "chyme" examined: Nauplii of crustaceans, their chitinous tests with the soft animal contents more or less completely digested out; empty diatom frustules, as well as a number filled with a vacuolated rich-brown endochorme; one shell of a larval gastropod (*Crepidula*), and some very young larvæ of nudibranchiates; the shell of a larval lamellibranch, not identified, with the valves still adhering together. Mature zoöids of *Pedicellina americana* Leidy were also noticed, and in the posterior portion or pyloric end of the stomach vast numbers of vibrios were noticed, which I identify as a form generically identical with *Spir-*

* The first of this series has already been published in the report of the Maryland Commissioner for 1881, embracing my work for that year. The present paper was prepared some time in September, 1882.

illum.* The filaments are capable of straightening out and contorting themselves into a great variety of forms. The usual shape of these organisms is that of an apparently spiral thread of extreme tenuity, which exhibits lively movements of progression in right lines, like the *Oscillatoriæ*, *Diatoms*, and lower *Algæ* in general. In consequence of the power which the filaments have of bending themselves, they may also exhibit vermicular movements. This organism is sometimes found in prodigious numbers in the vicinity of the crystalline style. Sewage is not the source of it, because this *Spirillum* [*Trypanosoma*] is found equally abundant in the stomachs of oysters from muddy or from clean bottoms, from deep or shallow water, and far from any possible source of sewage contamination. I also found the tests of a Difflugian in another instance, with the sarcode digested out; the species appears to be *Difflugia acuminata* Shr.† The cephalula stage of development of some worm, species not identified, a mass of vivid green algous cells, stellate hairs from the leaves of a neighboring exogenous tree, filamentous algæ and desmids, completes the list of organisms and organic remains encountered in the gastric contents of the oyster when examined microscopically. Many more might doubtless have been found, had one taken the trouble to spend time in the search.

To-day, at 2.50 p. m., I fertilized a lot of oyster ova; examined about fifty adults in full spawning condition; but in consequence of the fact that the water-supply fixtures were not yet in working order, I gave up experimenting for the present.

The interim from the 3d to the 10th of July was employed in getting our equipment into shape for the work.

July 10.—Impregnated a lot of eggs of the oyster at 3 p. m. to-day; not a very good lot. Had some difficulty in finding a ripe male; but the second lot fertilized at 3.30 p. m. came out much better and began to segment normally within an hour after the time of impregnation.

July 11.—Best lot of yesterday at 3.30 p. m. had the velum distinctly developed to-day, with the shell-gland formed or forming. Tempera-

[* I leave my original description of this organism as I wrote it in 1882. M. A. Certes, in his "*Note sur les parasites et les commensaux de l'huitre*," Bull. de la Soc. Zoologique de France, 1882, describes and figures what is evidently the same organism under the name of *Trypanosoma Balbianii*, and shows that, instead of being spiral as I have described, it is really provided with an extremely thin spiral frill wound around the very slender fusiform body, the frill being the locomotive apparatus of the organism. It measures about $\frac{1}{1000}$th of an inch in length. From M. Certes' description, which I have since verified, it is evident that I am in error in regarding it as a *Spirillum*, and that it is, consequently, probably not to be considered as belonging to the group of *Schizomycetes* at all. (January 3, 1884.)]

[†This may have been the test of a species of *Tintinnus*, a peritrichous infusorian, some of the species of which build a chitinous case covered with grains of sand very like the tests of *Difflugia acuminata*. For further facts regarding *Tintinnus* as food for the oyster, see my paper entitled "Rearing oysters from artificially fertilized eggs, together with notes on pond culture." Bull. U. S. Fish Commission, III, 1883, p. 293. (January 4, 1884.)]

ture of water ranged to-day from 87° to 78° Fahr. The apparatus for blowing air upon the surface of the water in the glass hatching-dishes was applied to-day; it seemed to help to keep the water aerated and cooler by 3 or 4 degrees than in a vessel over which the air was not blown. Added a little, not over a tablespoonful, of a saturated calcic hydrate solution [lime-water] to the water in which the embryos were developing at 3.30 p. m. This was probably soon after converted into calcic carbonate by combination with the free carbonic dioxide in solution in the water.

I fertilized a fine series of eggs to-day at 12 m. and 12.20 p. m., which were developing finely at 3.30 p. m. Added a little lime-water or calcic hydrate to the water in which these last were developing, as soon as they were placed in the hatching-house.

In order to test the possibility of changing the water on the eggs, I devised a simple filtering apparatus, constructed as follows: Over one end of a straight-glass argand lamp-chimney I secured a diaphragm of filtering paper between single thicknesses of light muslin or cheese-cloth, the whole held to the chimney by a stout rubber band, which bound down the free overlapping edges of the cloth and paper to the chimney all around. This apparatus was found to answer to a certain extent, but, like all the filters hitherto tried, was found to clog up and finally become impervious. The chimneys were suspended with a peculiarly arranged wire ring, which it is unnecessary to describe, depending for about two-thirds of their length into shallow glass bottles with wide necks. The fresh water was poured into the upper open ends of the chimneys from time to time by hand, and allowed to percolate through the diaphragm below into the bottle, overflow from the latter around the chimneys, and run off. This arrangement would work for a while only; the diaphragm would finally clog altogether, and, if the number of embryos in one of the chimneys or cylinders was too great, putrescence was soon established, when our experiments would come to an end. It was also found that the chimneys were too deep; their great depth, as compared with their width, would force the eggs to settle on the small area on the diaphragm at the bottom, tending to suffocate the ova, arrest their development, and kill them. In order to change the water, I then resorted to common glass funnels and filtering paper, with indifferent success.

I to-day examined some of the oysters one year and eleven months old, which had caught on the collectors put into the creek in August and September, 1880, by my party, under the auspices of the Maryland Commissioner. The largest specimen measured 3¾ inches in length and 2⅞ inches in width. Another smaller specimen was found to measure 2 inches in width and 2¼ inches and a quarter in length. These specimens were found to have the reproductive organs developed and contained ripe spawn. This showed how rapidly oysters which were started from the egg would develop in the course of twenty-three months.

July 12.—Eggs of the 10th, at 9 a. m. to-day, so diminished in numbers as to be hard to find. Those still alive have velum developed and are swimming about actively. Infusoria are developing rapidly and in large numbers; of these there were large numbers of holotrichous species, besides very small monads, which were by far the most numerous. I have about concluded that we put entirely too many eggs into a given volume of water, thus increasing the chances of putrefaction. I do not see, however, that the protozoa are destructive; none that I have seen appear to be capable of destroying an oyster embryo. Some vibrios which have made their appearance indicate a more alarming condition of affairs. Eggs of the 10th were practically dead to-day, though a few embryos might still be found after much searching.

Eggs of the 11th were not as completely freed from milt as they should have been. The water in the hatching-dishes is putrescent this morning, with teeming hosts of putrefactive organisms. Zoöglœar membranes or pellicles are visible on some of the dishes. Heat has been greater to-day than yesterday; last night was cooler than previous one. Thermometer 85° in the air at 9 a. m.; water in the dishes 84° at the same hour. Quite a number of embryos are still alive however; the last lot more developed than the first at the same relative age. Many with the shell-gland developing. Some were also seen to disintegrate while under observation. Some had a slimy filament attached to them which impeded their progress in swimming. These phenomena may explain Davaine's statement regarding the detachment of the velum; in other words what he saw was probably simply a putrefactive process involving the incipient disorganization of the embryos.

At 2 p. m. I transferred the embryos of the 11th into a 2-gallon glass aquarium, and then filtered off most of the water through a cotton-wool filter, which seemed to work pretty well, separating the most of the eggs from the water which runs through quite rapidly. The putrescent odor after this operation was not now so apparent.

The cotton-wool filter was constructed precisely as the one in which filtering paper was used, only instead of the latter I used a thick pad of raw cotton saturated with water, varying from one-fourth to three-eighths of an inch in thickness. This contrivance, for the construction of which I had received my first hint from the experiments of Pasteur and Tyndall, allows the water to pass through rapidly, but is very effective for a long time, as it clogs very slowly. I have great hopes of the performances of this last form of filter.

Meanwhile the putrescent action in the aquarium has apparently exhausted itself. I have had the air-blast blowing on the surface of the water, and have also immersed one blast-nozzle so as to cause the air to bubble up through the water in the aquarium.

July 13.—Putrescence has been to some extent impeded by the air-blast, eggs of the 10th July swimming about at a lively rate and in the condition of Brooks's Fig. 38. There are, however, but very few sur-

vivors now remaining, and if one is careful to examine the *débris* and sediment at the bottom of the aquarium a few dead shells of embryos may be detected with all of the soft parts gone. The most important step in advance to-day has been a thorough test of the cotton-wool filters, which will hold the eggs, but which will lodge in the meshes of the filter, which is a serious drawback. This requires that after using one of the filters for a short time, in order to change the water on oyster embryos, its action must be reversed; that is to say, one must let fresh water pass through the contrary way in order to wash out those embryos which have lodged in the meshes of the cotton wool.

In consequence of the air-blast blowing continually over the surface of the water in the hatching-dishes, there has been considerable evaporation going on, so as to raise the specific gravity of the water in the dishes considerably. This does not seem to affect the health of the oyster larvæ which are still alive.

I fertilized a lot of ova to-day, with very unsatisfactory results; the impregnation was not at all successful. Ten adult oysters were used in the operation—3 males and 7 females. The males were plentier than on previous days. Temperature of the water to-day ranged from 80° F. to 85° F.

June 14.—Cotton-wool filter impracticable for use with a continuous flow of water, but may be useful in the course of other experiments for the renewal of the water on eggs and embryos. This was fully tested by using a series of McDonald jars, connected together with rubber hose somewhat like a series of Wolff's bottles. The exit-pipe of each jar was filled with a cotton-wool filter, so that the water in the third and last jar had undergone three distinct filtrations, the result of which was that the water had become exceedingly clear and free from foreign particles, in fact had been more effectually cleansed than by the use of any other filter I ever had seen tried. Theoretically this apparatus, through which the water ran in a stream about as thick as a crow's quill, ought to have retained the eggs and embryos of the oyster, even though these were only one five-hundredth of an inch in diameter. The result of an experimental test showed that such was the fact; that the eggs and embryos would be retained, but that they would lodge in the meshes of the filters, where they would finally be covered by other sedimentary organic and inorganic matter. The result of this experiment showed us clearly that this method of incubation would have to be abandoned for something which would meet and satisfy the conditions of our problem more completely. A poor lot of ova were used in testing this apparatus, and after its unfavorable performance was made apparent, it was not thought advisable to waste any more eggs in its use. The prevailing temperature of the water to-day was from 82° F. to 87° F.

The embryos of previous lots which had been incubating in glass dishes and aquaria had not been amounting to anything up to this time; they were therefore abandoned after a few had reached the age of from

four to five days and then died. Many, in fact the majority, of the survivors were more or less diseased, showing vesicular protuberances from the surface of the body and slow and abnormal movements of the cilia, with a tendency to develop and trail a slimy thread-like appendage after them to which various foreign bodies would adhere and impede the free movements of the infantile oysters. This slimy thread I regarded as a product of retrogressive development, perhaps, indeed, of incipient putrefactive or disorganizing changes. The 15th and 16th days of July were employed in following up the development of the lots of eggs which had been fertilized before those dates.

July 17.—Another lot of eggs were impregnated this day at 10.30 a. m., an entirely new method being employed in the operation. The eggs and spermatozoa were in fact squeezed from the animals with the end of a smooth, slightly curved pipette; the latter, which was provided with a collapsible rubber bulb at top, was also used to lift up the generative products and transfer them to the dishes in which they were fertilized. The pressure of the side of the pipette was applied progressively along the oviducts, which open and pour out their contents uniformly at one point on either side of the body. In this way I find that I get quite as many eggs as by chopping up the visceral mass, and without contaminating the emulsion of eggs and spermatozoa with fragments of the other tissues of the body. Temperature of the water to-day fell from 84° F. to 76° F.

My success in taking the eggs and spermatozoa by pressure upon the generative organs and ducts led me to think of applying a similar method of investigation to the removal of the contents of the stomach. A short pipette or medicine dropper with a collapsible bulb compressible between the thumb and forefinger was used. The nozzle of the pipette was inserted into the mouth and through the gullet into the stomach, when the contents of the latter may be drawn into the pipette by relaxing the pressure of the thumb and finger upon the bulb. If carefully done no extraneous matters will be taken into the pipette; absolutely nothing except the contents of the stomach will be removed in the operation just described. In a lot examined this morning, I find grains of pine pollen from the neighboring trees, empty frustules of diatoms of various species, and a considerable number of large, brown, boat-shaped ones with the brown endochrome still in them. Many of these were still alive and exhibited their singular and characteristic movement in right lines. A great amount of organic slime and *débris* of organisms was also noticed, but these fragments of the soft parts of organic bodies in most cases were not in a sufficiently good state of preservation to enable one to identify them. As a whole, the slimy contents of the stomach were greenish, the color being due to at least two causes—the color of the biliary secretion, and the microscopic particles of food.

The sexes of the oyster may be readily made out by the peculiar characters of diffusibility proper to each kind of product when dropped

into the water. I find that the masses of eggs when squeezed from the oviducts and dropped into clear water immediately dissociate and diffuse themselves as a uniformly granular cloud if the eggs are perfectly ripe. If the eggs are not perfectly ripe, they do not separate so readily, but tend to adhere together in masses. This accordingly becomes a most excellent test to determine the degree of maturity of the ova; a very important practical point in the artificial method of culture yet to be developed.

The milt or semen of the oyster is stringy and flocculent when dropped into clear water. If stirred the masses break up into wisps and stringy clouds before mixing intimately with the water. When the admixture is complete the water charged with milt assumes an opalescent or bluish-white tint. In practice it is found best to use a very dilute mixture of water and milt for purposes of artificial fertilization, the philosophy of which is this: One spermatic particle only is needed to fertilize an embryo; the spermatozoa are vastly—a thousand fold—more numerous than the ova. A superabundance of spermatozoa used in the process of fertilization simply causes the eggs to be covered with them. The ineffectual ones on the outside of the egg eventually die and putrefy and needlessly pollute the water in which the eggs undergo their development.

The lot of oyster ova impregnated at 10.30 a. m. to-day are already swimming, and have reached the stage at which the micromeres have included the macromeres. The development attained so for has required five hours.

July 18.—Temperature of water to-day ranged from 75° F. to 84° F. Another lot of eggs were impregnated this morning. Embryos of the 17th not doing so well at 3 p. m. to-day. Was probably not careful enough to get rid of superfluous milt. Filters still hold the embryos, but many of them have threads of slimy matter hanging to them, with blister-like protuberances, which are abnormal, due probably to imperfect renovation, aeration and purification of the water, and accumulation of slimy sediment in the jars and aquaria. I fertilized another large lot at 12 m. to-day, using nineteen adults in the operation.

July 19.—Oyster embryos of 17th and 18th diminishing in numbers rapidly. Amœbæ and Infusoria are beginning to make their appearance in the aquaria. The mortality of the embryos is surprising, and as yet I see no sure way which promises much to prevent it; the embryonic shells have been forming, and there is every reason to believe that, could we prevent the initial putrescence and mortality, we could carry them along much further than has yet been done. The temperature of the water has varied from 76° F. to 85° F. to-day.

July 20.—The embryos are hard to find this morning in the jars containing the lots of July 17 and 18, although the men employed in filtering off the water and renewing it have worked most conscientiously both night and day. *Amœba proteus* I find to be abundant in the sedi-

ment, besides numerous hypotrichous infusorians. These are not chargeable with killing the embryos, but probably appropriate their dead bodies after they have fallen to the bottom and begun to disintegrate. Temperature of water ranged from 84° F. to 86° to-day.

July 22.—The remarkable set of experiments in the incubation of the ova of the oyster instituted to-day by Colonel McDonald led to a new series of experimental results as singular as they are contradictory. The above-named gentleman with characteristic ingenuity arranged a series of his hatching-jars so as to form what he called a *closed circuit.* The first element of the apparatus was a cylindrical glass aquarium, about 14 inches in height, placed about 4 feet above the level of the floor of the hatching-house. This was connected by means of a siphon tube of rubber to one of his glass hatching-jars, such as are used in hatching shad ova; a glass tube passing through the cork formed the inlet connection, and a similar tube reaching nearly to the bottom of the jar was joined to a rubber tube outside to form the outlet. Then followed a second jar connected to the first, with similar pipe connections, except that it discharged into a glass aquarium set at a still lower level, the bottom of which was covered with pebbles, to which some living seaweeds were attached and in growing condition. The water then passed through a rubber siphon tube from this second aquarium to two more closed hatching-jars placed at a lower level and arranged just as the first pair; the discharge-pipe of the last jar then carried the water into an aquarium, which rested on the floor. In order to maintain a circulation in this apparatus it was necessary to keep dipping up the water very carefully, so as not to injure the embryos, from the aquarium resting on the floor into the one standing 4 feet above that level. In order to supply lime to the embryos the two pairs of closed hatching-jars had been about one-third filled with clean sun-bleached oyster shells, and the purpose to be served by the living *Laminariæ* placed in the middle aquarium was to supply oxygen to the embryos and absorb the carbonic dioxide thrown off by these and other organisms in the jars and aquaria. The water with which the apparatus was charged was carefully filtered through a cotton-wool filter so as to free it from sediment and objectionable organic matter. After the apparatus was filled no attempt was made to change the water, as it was soon discovered that the water would remain perfectly sweet without renewal. Theoretically, this contrivance appeared to satisfy all the conditions of the problem which had been placed before us for solution.

A lot of eggs were placed in this contrivance at 11 a. m. I was particular to get as fine a lot of ova as possible, so as to test the matter fairly. In order to do this the eggs were expressed from the adults by the new method already alluded to, and fertilized so successfully that I am convinced fully ninety per centum were developing normally when put into the apparatus. My delight and astonishment the next morning at finding that many of the embryos had apparently attached them-

selves to the sides of the jars and the surface of the oyster shells were great indeed. The valves to our surprise were found remarkably well developed and had already grown so as to cover the sides of the embryos, and from between the edges of the valves the velum could be seen to protrude and be retracted at intervals. In fact within the short space of twenty hours we had embryos farther advanced in development than any figured by Professor Brooks in his admirable work on the development of the oyster, published in 1880.

The oyster shells in the jars in the McDonald apparatus may have helped to supply the necessary carbonate of lime for the rapid growth of the valves of the larval oysters. It will be desirable to put this matter to the test. The mode of fixation is still problematical. I even doubt whether the objects which I see fixed to the inner surface of the jars and aquaria by the help of a good triplet are oysters at all; keep vacillating between one and the other opinion all day, because of the imperfect means at my disposal to determine their true nature with certainty. Unfortunately I had not thought of putting small transparent slips of mica or glass in the aquaria in order to afford surfaces for fixation, which could be transferred to the stage of the microscope, without injury to the minute and delicate embryos. The temperature of the water in the apparatus has ranged to-day from 73° to 80° Fahr.

July 23.—The young oysters detected for the first time fixed to the sides of the jars this morning must be examined by such means as will certainly establish their true nature. In order to accomplish this I had our carpenter, Mr. Tolbert, make me a stand of wood to hold the draw-tube objectives and eye-pieces of my microscope in a horizontal position so as to view the embryos in their natural relations on the inside of the glass vessels undisturbed. I find at last, by this means, that the little objects fast to the inner surface of the glass are truly oysters. The pouring and lifting of the water from the lowermost to the uppermost aquarium does not seem to interfere with the attachments of the embryos or their development. Their mode of attachment is still a puzzle. I cannot clearly discern even with a magnification of 150 times—the highest I can use under the circumstances—how the attachment is made.* The positions of the individuals vary; some adhere by the side to the glass and display only a profile view of themselves; others are apparently swayed more or less by the current of water flowing

[*This point I have since settled approximately in my paper entitled, "On the mode of fixation of the fry of the oyster," Bull. United States Fish Commission, II, 1882, pp. 383–387. Unfortunately the figures are reversed in the plate which accompanies that paper through, an unintentional oversight. Figs. 3, 4, 5, 6, and 7 viewed from above should have the tips of the umbos directed towards the left instead of to the right. Fig. 8, viewed from below, should have the beak or umbo of the larval shell directed to the top of the page instead of to the bottom for a like reason. The direction of the umbos or beaks of the larval valves of young oysters is invariably the same, or to the left as viewed from above. The larval valves as well as those of the spat stage are also glued to the surface of attachment, so that the existence of a byssus seems doubtful. (January 4, 1884.)]

through the jar. Some are visible with the edges turned towards the observer and open and close their valves at intervals. Even the ciliary action and protrusion and retraction of the velum is often apparent. The attempt to discover a byssal attachment was fruitless. I usually found such an amount of sediment and slimy matter in the vicinity of the embryos as to prevent me from making out anything of a definite and positive character in regard to this point. The water still remains pure however; no signs of any putrescent action are yet apparent. The cotton filter has been of great use to us. In order to discover whether the attachment of the embryos was accidental or weak, Colonel McDonald detached one of the jars from the circuit and caused a continuous current of water to flow through it. This showed that the attachment was pretty firm; the current so established through the jar did not detach the embryos, as we could see by examining the inner surface of the glass jar with our microscope extemporized for that purpose. The temperature of the water to-day has ranged from 74° to 88° Fahr.

July 24.—Colonel McDonald has arranged a second set of jars and aquaria similar to the first, with the exception that the oyster shells have been purposely omitted in order to learn whether their presence tends to favor the formation of the valves of the young embryos. At 4 p. m. to-day another lot were placed in the second McDonald apparatus. At 4 p. m. the next day shells begin to be developed, but are not so far advanced in this respect as the first lot in the first closed circuit apparatus, and it must also be borne in mind that the temperature of the water is now rising. Temperature of the water to-day has ranged from 79° to 88° Fahr.

July 25.—The lot of embryos which were fertilized on the 22d are still developing finely, and we find that the continuous current of water passing through the jars taken from the closed circuit yesterday does not detach the affixed fry, as revealed by microscopic examination. Allowing a stream of water to pour over the shells to which the fry has attached itself, does not detach the latter, nor does a lively movement through the water of shells, to the surface of which fry has attached itself, produce any detachment of the latter. The entire animal is now covered by the valves; the velum is, however, still protruded. The protrusion and retraction of the velum is evidently effected by the relaxation and contraction of the minute pallial muscles of the embryo. The fixation of the first lot of the 22d must have taken place about twenty hours after the ova were fertilized and began to develop. Strangely enough the embryos fertilized yesterday at 4 p. m. as yet show no signs of having attached themselves to the inside of the jars and aquaria comprising apparatus No. 2. The temperature of the water in the apparatus to-day ranged from 80° to 84° Fahr.

In order to study the fry which was put into the McDonald apparatus No. 1 on the 22d more narrowly, we removed some of the shells from the bottom of one of the hatching jars and scraped the surface with a sharp scalpel. Examining the sediment removed in this way, under high powers of the microscope, we very readily discovered some

of the fry which had been attached to the surface of the shells. We could not discover that it had increased perceptibly beyond the original volume of the egg but could readily make out the course of the intestine, the liver, and stomach, in the cavity of which we could perceive the particles of food which had been swallowed in active rotary movement, impelled to rotate within the alimentary cavity by the numerous vibrating cils or filaments with which its inner surface is clothed. The presence of a body cavity was very evident, but no evidence of a pulsatile movement or the presence of a heart was evident. The hinge border of the valves of these embryos was truncate and linear, as in the valves of the embryos of the European oyster. No evidence of an umbonal prominence with a spiral tendency was apparent, such as may be perceived in the valves of the larval shell when it is over four times as large across, or of the size when it is suddenly converted into that of the spat, as I have described elsewhere.

July 26.—I fertilized a very fine lot of eggs to-day at 10 a. m., which began to swim about at 2.30 p. m. These were placed in apparatus No. 1, in which the old oyster shells had been introduced, as already described. They may, however, be a little too numerous for the volume of water, which we estimate in these latter experiments should equal about 200 times the bulk of that in which the eggs were originally impregnated. This dilution, we find, prevents the development of any putrescent action, but does not arrest the development of hypotrichous and peritrichous infusorians in vast numbers in all of our apparatus. I am latterly inclined to regard the infusorians as a necessary evil not to be gotten rid of. In fact, I doubt if they do any damage further than to act as scavengers. I often noticed, even when the water in our apparatus was perfectly sweet, that the amoebæ, infusorians, both stalked and free-swimming species, vastly exceeded in numbers the oyster embryos present in the incubating apparatus.

I introduced a simple and effective floating collecting apparatus into the aquaria to-day. I took some corks and cut into them some distance with a knife, and then took slips of mica and pressed them into these incisions edgewise in various positions. Having arranged my slips of mica on the corks so that they could not be readily detached, the whole was placed in the aquarium so that the plates of mica would be wholly immersed. These were my collectors upon which the fry could attach itself. When fixation had been accomplished it was an easy matter to transfer these slips of mica, with the fry attached, to the stage of the microscope for more critical and exact inspection. But, alas! my expectations were not realized; I did not succeed in having any embryos attach themselves to the slips of mica in the course of further experiments. Collectors of this kind were now introduced into both apparatus No. 1 and No. 2. The temperature of the water to-day ranged from 83° to 90° F., a perceptible rise since the 22d and 23d.

July 27.—I could find to-day but very few of the embryos which were fertilized yesterday, and I sought in vain also for those of the 22d, which

would have been five days old to-day had they survived. These were found to have attained their climax of development on the 25th, after which they began to die rapidly. To-day all traces of our promising brood of the 22d have vanished from their attachments.

This ill-fortune we have supposed to be due to the high temperature of the water prevailing since the 23d. To correct this supposed unfavorable condition, Colonel McDonald has arranged for a current of well water to flow around the jars in the closed circuit to lower the temperature in the latter and maintain it at an approximately uniform point.

July 28.—I again overhauled the slips of mica forming my miniature collecting apparatus in the incubating apparatus, as well as the shells, with the result that no adherent oyster fry was discovered. The abundance of compound stalked infusorians as well as test-building species on the surface of the mica collectors was remarkable. The temperature of the water to-day ranged from 82° to 88° F.; that of the air was 96° F.

July 29.—I re-examined the collectors put into the incubating apparatus of the 22d; like the search of yesterday it resulted in discovering nothing, although the second lot of ova put in the apparatus No. 1 on the 26th ought to be now pretty well developed. The contrivance for reducing the temperature and rendering it constant in the incubator works very well; the thermometer in apparatus No. 1 indicates a practically uniform temperature ranging from 79° to 80° F. To test this last modification of the incubating apparatus, I introduced a lot of fertilized ova at 2.30 p. m. to-day.

July 30.—I find all of the eggs of yesterday dead. Examined the mica, shells, sides of the jars, and sediment, and failed to find any live embryos remaining. The temperature in the jars of the closed circuit with cooling attachment has been maintained uniformly at 78° F. all day.

July 31.—Search for embryos of the 29th repeated, with no indications of any live ones. I fertilized another lot to-day at 3 p. m. Wind very high, water in the bay very boisterous and muddy; placed a part of the eggs above mentioned in an aquarium without any provision for changing or aerating the water. I find in fact, by increasing the volume of fresh, pure water in the aquarium to about 200 times the bulk of the water used to fertilize the eggs, that I have no further trouble with putrescent action, as was shown by the subsequent behavior of this aquarium. This last lot of ova was not put into the aquarium until a decided advance in their development had been noted.

August 1.—Embryos fertilized at 3 p. m. July 31 still alive to-day at 11 a. m. in the aquarium, into which I had put a few thousands yesterday at 4 p. m. At 4.30 p. m. to-day I put in another lot of ova, which had been fertilized at 3.15 p. m. The impregnation, conducted according to my new method, was very successful. Some of this new lot were put into the closed circuit and a part into an aquarium with plants. I put none into my plain aquarium, in which I did not change the water or supply with plants.

I examined some very minute fry and spat obtained from old oysters tonged up in the deeper waters some distance off shore to-day. I removed them from their attachments as carefully as I could and placed the living translucid spat, measuring from a sixteenth to an eighth of an inch in diameter, under a compressor for more critical examination under the microscope. I counted the heart beats by my watch in these young oysters, the cardiac contractions being visible through the shell. The heart I found to pulsate at the rate of eight to twelve times per minute, and appeared to be somewhat irregular in its action.

August 2.—Examining the embryos placed in the closed circuit and aquarium yesterday, I find that they are for the most part dead, even under the most favorable conditions. The temperature in the closed circuit or McDonald apparatus with the cooling attachment remains quite constant and stands at about what it did at first. Not yet content with my results I fertilized another lot of eggs at 3 p. m. to-day.

August 3.—I fertilized another lot of eggs to-day at 12 m. The lot of the previous day appear to be dead; cannot find any alive in the apparatus.

August 4.—Eggs and embryos of yesterday have disappeared. The dead embryos and unimpregnated eggs appear to have become the prey of large numbers of *Actinophrys sol*, a sun animalcule, which has multiplied rapidly in the closed circuit and aquaria. I find in fact some of these animalcules embracing and enveloping dead oyster eggs, which they appear to grasp and surround bodily with their own living sarcode. They appear to me to be really scavengers, and not at all destructive to living oyster embryos which have begun to swim. In fact the *Actinophrys* cannot pursue its prey, being very slow in its movements, and progresses with a very deliberate rolling motion; not swift enough to follow a healthy young oyster provided with a well developed velum.

August 5.—The density of the water in St. Jerome's Creek ranges from 1.007 to 1.0095 according to the standard hydrometers used by the United States Coast Survey. At the beach opposite the barges the density was found to be 1.01 and a quarter of a mile off shore it was found to be about the same. For the determination of the density off shore I am indebted to the kindness of Dr. J. Alban Kite, of the steamer Fish Hawk. The specific gravities indicated above are greatly below the average of the ocean, as appears upon comparison with a table given by Young, in his Physical Geography, where it is stated that the specific gravity of the water of the ocean ranges from 1.02548 to 1.02919. The waters of the Chesapeake do not appear to have a density much above that of the Black Sea, even near their confluence with the Atlantic at Hampton Roads, where the specific gravity is 1.014. As one recedes from the mouth of the bay to the north the density diminishes, so that over a large part of its area it is practically little more than brackish. Along shore and in the estuarine prolongations of the bay inland is where the oyster of the Chesapeake is at home. From their prolonged stay generation after generation in water of approximately the same specific

gravity they have become adapted to it, as would appear by the facts to be cited in the sequel.

I impregnated two lots of eggs in water of specific gravity 1.009, and in water of specific gravity 1.021 respectively, the latter having been prepared artificially from fresh water to which sea salt had been added. This experiment showed us that the Saint Jeromes oysters could not be impregnated in water as dense as the last mentioned. The milt was killed by increasing the specific gravity even to a comparatively moderate degree. The eggs sank in water of the greater as well as lesser specific gravity. The spermatozoa became immobile in the denser water almost immediately, or in that which was abnormal to them. This experiment was repeated, in order to verify my conclusions, with the same result as before. A specific gravity of 1.013 was the limit of change which appeared to be endurable by the spermatozoa. These are singular facts, and show how nicely the vital conditions of the oyster have been gradually adapted to the environment. These facts appear to indicate that the characters of the protoplasm of a species themselves become specific in consequence of such adaptations.

The embryos of day before yesterday I find to be dead. Both lots of to-days' eggs poor; spawn is apparently getting scarce. Myriads of spat, I find, have caught on the shells in the natural beds out in the bay off the barges. This young spat measures from $\frac{1}{2}$ millimeter up to a quarter of an inch in diameter. It must have caught for the most part within the last two weeks.

August 6.—I found young oysters to-day on the shells of the old ones measuring only one-ninetieth of an inch in their shortest diameter. This is about the size which was asserted by me to be the limit of the dimensions of the fry two years ago.* The shell of the spat develops continuously from the rim of that of the fry, but presents a totally different microscopic character. Both dissolve readily in acetic acid under the microscope, leaving the organic matrix of conchioline behind. The fry shell has well-marked lines of growth like that of the spat, but homogeneously transparent, and, unlike the shell of the spat, does not have its carbonate of lime arranged prismatically in an organic matrix. It looks like a *Pisidium* when viewed from the side, and like a *Cardium* when looked at from the end, being very ventricose and perfectly symmetrical. After decalcification with acid the line of demarcation between the spat shell and that of the fry remains indistinctly marked in the matrix. The horny membranous matrix afterwards dissolves very slowly in caustic potash. It has been very hot to-day, the air being 93° Fahr. in the shade. The temperature of both air and water are

* See first series of these investigations, in Appendix A, to Report of T. B. Ferguson, A Commissioner of Fisheries of Maryland, Hagerstown, 1881, pp. 59, figures 6 and 7, in Report entitled "An account of experiments in oyster culture and observations relating thereto, made at Saint Jerome's Creek, Maryland, during the summer of 1880," by John A. Ryder.

unfavorable for our work. Embryonized ova in aquaria all have died and begun to disintegrate.

August 7.—I had a large lot of oysters opened to-day; some of them from the deeper water of the bay. The spawn appears scarce and poor in quality, judging by the physical tests already described. I fertilized another lot of eggs at 11 a. m. Temperature of the water to-day has ranged from 87–90° Fahr.

August 8.—Embryos of yesterday's lot mostly dead. A lot impregnated to-day at 2 p. m., which were apparently in very fine condition at 3 p. m., in water of specific gravity 1.009, or that usual in Saint Jerome's Creek. Put aquarium and closed circuit in operation with water of a specific gravity of 1.013. Temperature, 80° Fahr.

August 10.—Upon making an examination of the aquaria and closed circuits to-day I find a very few embryos of the 8th still alive, but weakly, with vesicular watery prominences apparent on their surfaces. Vast numbers of a brownish diatom have now made their appearance, especially in one of the aquaria, in association with *Amœba radiosa*.

August 11.—I made the last examination of the apparatus to-day. The diatoms which were observed yesterday are now more numerous than ever in the incubating apparatus. They are brownish, with a boat-shaped central portion, with a styliform prolongation at either end. It is the same type of diatom, the silicious tests of which I have found more abundantly than any other in the intestine of the adult oyster. They have multiplied with enormous rapidity, showing that it was not the purity of the water which was lacking to favor the growth of the oyster embryos. The latter have entirely disappeared, in spite of the careful search instituted for the purpose of finding them. The fine lot of eggs fertilized on the 8th instant have amounted to nothing.

In order to see if I could catch some natural spat in the oyster pond I had a frame constructed to hold a number of panes of ordinary window glass. The frame was arranged to float, and had the bottom closed with a coarse wire screen. Upon this wire screen I placed a number of spawning oysters, then arranged the glass plates above them and left the structure floating from a temporary anchorage provided for the purpose and arranged for the convenience of the observer in making the examination of the collector. I found, however, that a large amount of gelatinous, transparent, slimy matter soon covered the plates of glass. This slimy matter I found, as I had previously learned, was largely composed of the lowest and most destructive vegetable organisms—*Vibriones*, *Bacteria*, and *Micrococci*—which I did not determine with precision. After failing, during several examinations, to find any young oyster fry or spat, I abandoned the project for the time being, with the hope that I might resume the investigation at another time. My object was to get the fry to adhere to some transparent basis upon which I might examine it with the microscope in a natural and undisturbed condition.

Our observations closed with this date. We left the station on the next day—the 12th of August.

Recapitulating, we have learned that it is easy to keep the water in the incubators pure and sweet. We found that it was not safe to alter the specific gravity of the water which was normal to the eggs and spermatozoa. We learned, too, that with the highest temperatures we did not get the best results. Our experience with the fixation of the fry was very unsatisfactory, but we have observed enough to lead us to the conclusion that the fixation may occur very early or within twenty hours of the time of fertilization. We have found that we were previously in the habit of using too much milt; that this tended to establish putrescent action in the water in which the embryos were developing. The air-blast did not prevent the putrescent action alluded to when too much milt was used; in fact we abandoned it after discovering that we got just as good results without its use. Every adverse condition which we could think of was met by us with some proviso in the arrangement of our apparatus, yet we cannot claim a full measure of success. We have materially improved the extraction of the eggs and milt from the adults, and made the methods of fertilization more simple and effective by using less milt. After dealing with many millions of ova, under a great variety of conditions, it is natural that we should have made some progress, as indicated by the foregoing recital of our experiences. The effect of free caustic lime added in solution to the water in the incubators was not clearly of any advantage, nor was the presence of oyster-shells of apparently any more importance, as was shown by our final experiments. The amount of carbonate of lime in the water necessary to the oyster is necessarily very minute. On the chalky coast of the English Channel lime is found only in the proportion of .0057 parts to 100 of water, and the Rhine at Bonn, it has been estimated by Bischof, would supply lime enough to form a mass of oysters covering four square miles to a depth of a foot. It must also be borne in mind that the great tributaries of the Chesapeake are constantly carrying vast supplies of lime into the bay in an imperceptible form. That artificial supplies of lime will determine the more rapid development of the shell of the embryo oyster appears to me not yet very clearly demonstrated. The difficulties in our way are probably not as great as we suppose, and the simple and practical manner in which some one will one day solve the question may very probably surprise all of us.

[Since the foregoing was written M. Brandely has published the results of his work upon the Portuguese oyster, upon which he was engaged at the very time we were conducting the experiments above described. An even simpler method than that devised by M. Brandely was found to give very promising results at the hands of the writer at Stockton, Md., on the premises of Messrs. Shepard and Pierce; and by that method, devised and put into practice within the next twelve months, a very important advance was made in the practical culture of the American oyster, as already very fully described in a paper, by the author, cited in the first part of this article.]