XXIII.—REPORT OF EXPERIMENTS IN THE ARTIFICIAL PROPAGATION OF OYSTERS, CONDUCTED AT BEAUFORT, N. C., AND FAIR HAVEN, CONN., IN 1882.

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The chronicle of the different successful efforts to artificially impregnate the egg of the oyster is short. In 1879, Dr. W. K. Brooks succeeded with the American species (*Ostrea Virginiana*). In 1880, following his methods, I succeeded with the Portuguese (*Ostrea angulata*), one of the European species. During the same season Mr. J. A. Ryder made another attempt, likewise following Brooks's methods, with our domestic oyster, and I have no doubt, though I have seen no published accounts of other experiments, that since the initial trial in 1879 Dr. Brooks has had many followers both in this country and abroad.

The history of these several efforts to raise the oyster from the egg by means of the artificial impregnation of the ova has been before the public for some time. Dr. Brooks' experiments are detailed in the Report of the Maryland Fish Commission for 1880, and in the Studies of the Johns Hopkins Seaside Laboratory for the same year. Mr. Ryder's experiences and my own are published in the Report of the Maryland Fish Commission for 1881. All these are so well known that it is unnecessary to here recapitulate even their principal features; but one point is worthy of notice. Each experiment has attained about the same degree of success, or perhaps it would be better to say has failed at nearly the same point. The egg has been impregnated and the embryo maintained alive for various periods; but beyond a certain stage, neither Dr. Brooks, Mr. Ryder, nor myself have yet succeeded in keeping them.

The success of the initial experiment was so great, and the advance in oyster culture appeared of so much importance, that investigators, myself among the number, were perhaps too sanguine; possibilities appeared probabilities. We expected that as soon as a few changes were made in the apparatus, and methods somewhat more nearly perfect were introduced, we would be able to produce young oysters with the same facility as young shad, and with a greater surety with the former of reaping the reward of our labors, than was possible in the case of the latter. But after my experience of the past spring and summer I am convinced that it will require a series of pains-taking experiments, extending over considerable time and conducted under many dissimilar conditions, before the artificial production and culture of the oyster is made a matter of practical importance.

Both Brooks and Ryder in conducting their experiments naturally considered first the scientific aspects of the case. Their training and profession led them, unintentionally perhaps, to regard the practical application of their discoveries as of less moment; my own feeling was the exact opposite. The great interest on their part centered in the process rather than in the result; on my own, in the result rather than in the process. Their desire was to raise one oyster, mine to raise many. But feeling that they were more competent than myself to accomplish the result they had in view, and knowing that one oyster must be raised before any method of raising many could be perfected, it appeared to me very desirable that I should associate myself with either or both of those gentlemen, that in my effort to obtain results of practical value I might have the assistance of their counsel and the benefit of their larger experience. Accordingly, during the winter and spring I had several consultations with Dr. Brooks and Mr. Ryder, and as the former proposed to continue his experiments with the oyster during the spring at Beaufort, N. C., and as that locality offered facilities for the work not possessed by others, I determined to join Dr. Brooks as early as possible and work in conjunction with him as long as the condition of the oysters permitted, subsequently joining Mr. Ryder in the Chesapeake or at Saint Jerome, should he continue the prosecution of his researches.

The want of success which had attended all previous experiments appeared to be due to the deficient supply of water to the aquaria. Various methods of obviating this difficulty had been devised by Dr. Brooks or Mr. Ryder, and are detailed in their accounts of their experiments, but no method had proved successful. The embryo was too minute to permit the removal of any of the water without carrying along the animal, and consequently the supply was limited to the capacity of the jar or receptacle. Being unable to afford the large quantity of water necessary or apparently necessary to the life of the young oyster, it occurred to Dr. Brooks and myself that we might overcome the difficulty by adding to the water already in the aquaria an inordinate amount of the several constituents of the sea-water which were supposed to be essential to the development of the embryo.

The study of the natural conditions under which the oyster propagates and lives, together with past experience, led us to the conclusion that the principal obstacles would be removed by increasing the supply of oxygen and carbonate of lime, with, perhaps, the addition of artificial currents of air or water through the jars. We were of the opinion that, working under the above conditions, and with care in the manipulation of the eggs, we would make a considerable advance towards the solution of the problem; but a very brief experience convinced us that it was more than probable that the supply of a large amount of food was also an essential factor in the equation, and as we proceeded with the experiments we also ascertained that several conditions which we had supposed were necessary to success might be safely ignored. Probably had the experiments been conducted simply with the view of determining the effect of the various conditions upon the development of the eggs and embryos, we might be able to speak more definitely and with more authority as to the necessity of supplies of oxygen, lime and food; of the necessary temperature and density to be maintained; of the rate and manner of development under different circumstances and of other matters of interest and value. But that end was not our main object; the determination of those questions appeared to be of secondary importance and only incidental to the work. In the order of their relative importance we proposed to accomplish, if possible, the following things.

1st. To raise one oyster up to the time of attachment.

2d. Guided by the experience attained, to perfect a method by which oysters could be raised from the egg in sufficiently large numbers to make the process one of practical value.

3d. To determine the conditions necessary and favorable to growth.

While we were not successful in attaining either of the first two ends, yet some advance has been made and incidentally we have obtained information and gathered experience that throws additional light on the course to be pursued in the future.

I arrived at Beaufort, N. C., on the 23d of May, Dr. Brooks having preceded me some two weeks, and I remained until the 23d of June, when finding that it was difficult to get oysters in proper condition for experiment, and wishing to prolong the season as much as possible, I returned to Washington with the intention of proceeding thence to some point on Long Island Sound and continuing the experiments. I also wished to try the effect of placing a large number of embryo oysters, that had passed through the first swimming stage, on some defined and protected area, such as the private oyster beds of the Connecticut oyster-growers. The plan having met with your approval, I proceeded to New Haven, Conn., and remained in that vicinity about a month, continuing the previous experiments, and at the same time fertilizing as large a number of eggs as possible, and depositing them on one of the beds of H. C. Rowe & Co., of Fair Haven. I have not yet received information which will enable me to speak definitely as to the success of the latter experiment, but such as I have received points in a favorable direction. While at Beaufort and at Fair Haven the experiments were continuous and occupied every day and a good many nights. Every effort was made to solve the problem, and at the close of the season I regret to say I am as unable as at its commencement to state the causes which prevented success or indicate those which must operate to produce favorable results. It is hardly worth while to give a detailed history of a series of experiments that produced negative results only, or to describe at length appliances and apparatus that were chiefly remarkable for failure, though both have their value in tentative work. I shall not therefore burden this report with such accounts, but confine myself to a statement of the methods and apparatus we found of assistance, and of the influence, so far as we were able to observe it, of the various natural conditions affecting the development of the egg.

1. *Selection of the oysters.*—This is a point of more importance than I had supposed. Even in the height of the spawning I found it necessary to use care in selecting the oysters from which I was to derive the generative matter, and it is especially necessary to use this care with regard to the males. At Fair Haven I have frequently hunted over thirty or more oysters before finding a male with the spermatozoa in an entirely satisfactory condition. The superfluous male fluid is difficult to get rid of after the eggs have been impregnated, and this difficulty is much increased when the spermatozoa are dead or immature; but unless removed they soon pollute the water. Bad eggs are of less trouble, but their removal is sufficiently embarrassing to make it desirable to use only those that appear entirely ripe. While experimenting on a large scale, with immense numbers of eggs, as I did at Fair Haven, the temptation is great, on account of the saving of time and labor, to take any and all oysters without exposing the contents of the generative organs to the microscopic examination; but I found by experience that it would not do to trust to subsequent manipulation for the removal of unripe ova and spermatozoa; such a course required in the long run more time and labor than the selection of good oysters would have done in the first place, and was not always entirely efficacious. I think the failure of some of the experiments, both at Beaufort and Fair Haven, was undoubtedly due, or partially due, to neglect of these precautions. At Beaufort we were sometimes compelled to take inferior oysters, but at Fair Haven it was possible at all times, with care, to secure a sufficient number entirely suitable for experiment; and if other investigators in this field are located near any large oysters area they should not experience any great difficulty in obtaining ripe animals throughout the spawning season. It must be remembered that the time of spawning or the ripeness of the oyster in any locality is dependent upon several conditions, the principal being the depth and density of the water, the shallow and brackish water oysters spawning first. I am of the opinion that even those oysters from the same spot will be found to be in various stages of ripeness, such having always been my experience, and, consequently, it is necessary to search carefully for unfit animals, even when the majority of the lot appear in an entirely proper condition. This refers, of course, to an experiment on a large scale, somewhat similar to my own at Fair Haven, where I used the generative matter of several hundred oysters nearly every day. Working on a smaller scale the experimenter would naturally use sufficient caution. Throughout the season, with large and small numbers of eggs under observation,

the large number of eggs which never advanced in development was constantly noticeable. Many had apparently escaped the attack of spermatozoa, and many more only advanced through the very earliest stages of segmentation. This was the case very frequently with the eggs taken from oysters that were, so far as could be judged, perfectly ripe, the eggs under the microscope appearing free from granular matter, well defined and of clean outline, well-shaped, and germinative vesicle obvious. The frequent recurrence of this circumstance leads me to believe that the eggs of even one oyster are not all ripe at the same time, though they may approximate to it. It seems very desirable that, as recommended by Mr. Ryder, the histology of the ovaries should be exhaustively studied, that the matter may be settled; its importance to future experiments and to practical work is too obvious to need comment. One other point may be mentioned in this connection. I found one female oyster at Beaufort and one at Fair Haven with the visceral mass, including the ovaries, filled with *Bucephalus cuculus*. I have not met with any similar case, and consequently, the evil must be exceptional; but that it is possible is certain, and that possibility is an additional reason for using care in selecting the ova for artificial impregnation, as the presence of *bucephalus* as well as infusoria in large numbers in the water of the aquaria will conduce to failure. Dr. Brooks, in his account of the initial experiment, has described the appearance of the generative fluids when in proper condition, and I need not duplicate his work. I only desire to impress my conviction that success or failure of experiments in this field is due, to considerable extent, to the care, or want of it, in selecting the oysters from which are taken the products of generation.

2. *Impregnation of the egg.*—The methods followed and advised by Brooks should be strictly adhered to. The males should be first treated, and care should be taken that not more spermatozoa is used than is necessary. One male will supply sufficient for half a dozen females, but it is better to use a larger number and only partially wash out the spermaries. In both sexes the ripe fluid is most easily removed, and though after the first washing there appears in the crystal or saucer a large amount of generative matter, yet most of it is probably unripe and had better be thrown away. A perusal of Brooks' notes on the structure of the generative organs will be sufficient to convince any one that the course I recommend will have its influence in preventing unripe ova or spermatozoa from getting into the aquarium jars; and, as I have stated, the insurance of this point is a matter of importance. The impression derived from Dr. Brooks' paper has been strengthened by the experience of the past season, and I am of the opinion that in several of the experiments I would have had more swimming embryos had I not had so many eggs; many never developing and only polluting the water.

In removing the generative organs, the time and labor expended in getting rid of the mantle, gills, digestive tract, and fragments of the muscle is well bestowed. There is but little danger of an insufficient number of eggs being secured while the presence of fragments of the organs just mentioned, due to hesitancy in using the scissors and sacrificing large portions of the ovaries or testes, exerts a very deleterious influence upon the future development of the egg.

As soon as the generative organs of the male are removed they should be washed out in salt water. It is not advisable to defer this action for any considerable time and consequently only a few oysters should be treated. The spermatozoa soon dies if left exposed to the air and I account for the large number of males with dead cells which I noticed at Fair Haven, by my non observance on several occasions of this precaution. I was in the habit, at first, of opening thirty or forty oysters and removing the generative organs of all, before washing out the cells. As this required considerable time, and as at first I did not select the males for treatment before removing the eggs from the females, many of the difficulties I experienced in disposing of superfluous spermatozoa, and in securing thorough impregnation of the eggs, are due, probably to the above cause. With the females it is, so far as my experience goes, not necessary to use so much care. The principal precaution to be taken is in bringing the ova and spermatozoa in contact as soon as possible after the former have been exposed to the water. As Dr. Brooks points out, the eggs soon disintegrate after they are placed in the water if they are not attacked by the spermatozoa. To sum up, it is advisable to use a moderate number of oysters of both sexes, not to be over particular in securing the contents of the generative organs, to treat the males first and supply the spermatozoa with water as soon as possible, and to bring the two fluids in contact immediately after the eggs have been washed out of the ovaries. Ten or fifteen minutes should be the maximum time of the operation. Though the observance of these points precludes, to a certain extent, the manipulation of large numbers of oysters by one person, yet, so far as my experience goes, it seems essential to success. In the experiments conducted on a small scale, when I used care in all these particulars, I obtained proportionately much better results than when working on a large scale and using a hundred or more oysters; in the former case to accomplish the object I had in view every care was necessary; in the latter I sacrificed something to the supposed necessity of obtaining a very large number of fertilized eggs every day; but, after considerable experience with both methods, I am now convinced that a few oysters and eggs, carefully treated, will produce a larger number of embryos than when the number of oysters is so great as to preclude the observance of the most minute precautions.

The most satisfactory results were obtained when the two fluids, having been well mixed together and the fragments of mantles, gills, and organs allowed to settle, the contents of the tumbler were poured off, and the sediment at the bottom *thrown away*. There is no other way of satisfactorily getting rid of the fragments of the various organs. I have strained the fluids through fine muslin, have squeezed them out of the generative organs with the hand instead of cutting them up, have even ground up the visceral mass in a coffee-mill; but I found no method so productive of good results as obtained by chopping the reproductive organs into a few pieces and washing them once or twice in a glass of salt water, allowing the glass to stand a few minutes and then pouring off the water containing the eggs and excess of male fluid.

After getting rid of the *debris*, the contents of the glass should be stirred frequently for ten or fifteen minutes and then allowed to stand quiet that the heavy, fertilized eggs may sink to the bottom. Only one precaution is here necessary; too many eggs must not be collected in one glass. The layer on the bottom should not exceed one-eighth of an inch in thickness, and a smaller number of eggs is preferable. I succeeded better when I used a large number of small glasses (tumblers) than when I used one or two large jars (trout hatching jars) for this part of the process, and not only were the results better but there was an appreciable saving in time, with the tumblers. The evils of the large receptacle are the same as those noticed when too many eggs were put in one tumbler. So much time was taken up by the eggs in sinking to the bottom after each renewal of the water, that spermatozoa were carried along with them; and the eggs after reaching the bottom were packed so closely together, and on top of each other, that they resembled a slimy mass of mucous, and could not be readily detached from the glass or from one another. Such conditions obviously hinder development and should be guarded against.

The removal of the excess of male cells and unripe or floating eggs is a simple matter of easy accomplishment. As soon as the eggs are at the bottom of the glass, siphon off the water and refill the tumbler. After the first two or three operations the eggs can be seen as a white cloud sinking through the water; ten minutes rest between the operations is quite sufficient time for the eggs to sink. If they do not descend in a solid mass, but "straggle" to the bottom, it is an indication that there are many but partially ripe or unimpregnated eggs in the lot and that the prospect of a successful issue to the experiment is slight. The water should be changed until, after the eggs have sunk to the bottom, it is perfectly clear. The investigator can then proceed to the next operation.

3. *Care of the eggs during segmentation.*—After the water in the glasses has been cleared, and all deleterious matter disposed of, it is of greatest importance that the eggs should have *room* for development; that is that they should not press upon or in other ways incommode each other. There are a number of other points of importance to be observed but I defer their consideration, for the present confining myself to features of the manipulation that must be common to all experiments.

If the eggs are in a thick layer on the bottom, only a few, comparatively, develop, and this fact consequently necessitates the use of shallow jars, or dishes, having a large bottom area, during this part of the process, or else some other form of apparatus which will produce the same desired result, viz, freedom from pressure. During the past season we accomplished this end, approximately, by placing the eggs when once free of *débris* and unripe eggs or excessive spermatozoa, in soup plates or large platters; at Fair Haven I used the largest dishes I could purchase and with good results. The principal point is not to overcrowd the eggs; it is better to err on the side of too much caution, sacrificing some of the eggs if necessary, rather than in the attempt to save all, run the risk of very many failing to develop. I also devised another method which gave fair results, and which appears susceptible of improvement. It consisted of a small glass funnel holding about a pint, and having a jet of air through a narrow orifice in the bottom. The water containing the eggs was placed in the funnel and a small stream of air, as little as possible, was forced up through the apparatus. The eggs by this means were kept constantly in motion, rising in the middle and sinking along the sides of the funnel. We got very good results from this appliance but I am not prepared to say that it succeeded very much better than the platters. A slight improvement in the form of the funnel would add to its efficiency; the nearer perfectly conical its interior form the better.

We did not find it necessary to add any water during the process of segmentation, but having once placed the eggs in the plates or funnel, we allowed them to remain undisturbed until the first swimming stage was reached. It is very important that when using the plates all the preparations for operations subsequent to the time of arrival at the swimming stage should be completed several hours before that event occurs. The plates must not be moved nor the eggs disturbed, else it will be impossible to avoid carrying into the second receptacle many undeveloped eggs. In using the cone, a rest due to cessation of the air jet for one hour or more was allowed, before any attempt to remove the swimming embryos was made.

Attention is called to Brooks' description of the development of the egg, in order that the significance of this latter point may be appreciated. The embryo swims at the surface but a comparatively short time; very large numbers of eggs never reach this stage and the swimming embryos must be separated from them before the disintegration of the former renders the water so impure as to insure the destruction of the latter. This removal is not always easy of accomplishment, but we succeeded, partially, by using two plates. In one was placed the eggs during segmentation, with water enough to nearly fill it. The second plate was arranged under the first, so that any overflow from the latter would fall into it. After the embryos began to swim the first plate containing them was overflowed, drop by drop, by means of a minute stream of water falling on the edge of the upper plate. Each drop carried over many embryos, and after the lower plate began to fill and inspections of the overflow showed a diminution in the number of embryos, the plate containing the eggs was removed and either the contents thrown away, or a second resting period allowed in order that another lot might develop, in which case the operation was repeated. In using the funnel it was suspended over a jar or large beaker, and after the embryos began to swarm to the surface the air jet was reduced or cut off, and in the latter instance, a minute jet of water substituted. Originally the funnel was only half filled, and the water jet being small occupied one or two hours in filling the remaining space and at the same time kept up a gentle movement among the eggs. Care was necessary that the undeveloped eggs should not be thrown very near the surface so as to interfere with the swimming embryos or become mixed with them. After the funnel filled, the water jet caused a gentle overflow into the beaker, which overflow was continued as long as the number of embryos passing over justified it.

The sketches show the manner in which we arranged this very simple apparatus, though it is hardly of so complex a character as to require illustration; they may, however, be of assistance to others, and perhaps contain the germ of a method or appliance that will produce valuable results.

After the swimming embryos have been separated from the undeveloped eggs, their subsequent treatment involves the consideration of many natural conditions, all of which have more or less influence upon the success of the experiment; but as they also exert an influence upon the development of the eggs during segmentation, and as the life of the embryo does not, apparently, depend upon manipulation, I have considered it best to allude to the effect of varying natural influences, separately from those due merely to management of apparatus or generative fluids.

Influence of temperature.—While I am not prepared to say that the temperature of the water during the development of egg or embryo is the most important consideration, and while I do not wish to be understood as stating these following influences in the order of their relative importance, yet, so far as my experience goes, I am inclined to think that the extent of the influence of temperature is sufficient to cause it to rank at the head of the various causes affecting the success of the experiments. It is scarcely possible in an investigation conducted among so many and various affecting conditions, to eliminate sufficiently the influence of all others so that one may be able to speak definitely as to the remaining cause for success or failure; but after considerable experience I feel justified in coming to the following conclusions with reference to the effect of temperature.

a. The condition of the eggs is not only dependent upon the depth of water (or temperature) from which the parent is derived, originally, but a very short exposure of the animal to water of an increased temperature caused a deterioration of the generative matter. I have tried to fertilize the eggs of numbers of oysters that had lain over night in the Quinnipiac River and invariably failed; the eggs in nearly every case appeared to be over-ripe. Oysters taken from the bed at the same time and from the same locality, but kept in a basket over night, gave good results.

b. The process of segmentation is hastened by a high temperature and retarded by a low. At Beaufort I experimented with several lots of oysters, or eggs rather, exposing them to different degrees of heat. All the eggs came from the same lot of oysters and were fertilized at the same time. They were exposed to a temperature ranging from 70° to 80°, and quite a marked difference was noted in the rate of segmentation. At the same time a lot of eggs under Dr. Brooks's care made no advance in development for several hours (the night was a cool one) until placed by the fire, when the segmentation began and advanced rapidly. I always noticed the retarding or destructive effects of low temperatures, both at Beaufort and Fair Haven.

c. A very high or very low temperature or violent changes of temperature destroys the egg. In the experiment mentioned above, though the eggs exposed to the high temperature (80°) advanced most rapidly, yet but few reached the swimming stage. I noticed subsequently, at Fair Haven, the same circumstance. High temperature also appears to conduce to irregular segmentation, the egg dividing at once into a number of segments, and the distinction between macromere and micromere not being so apparent as under ordinary circumstances; but I made no special study of this matter and cannot state with certainty that the irregularity is due to the high temperature. In 1879 Dr. Brooks noted the destructive effects of low temperature, and though during the past season I made no experiments having especially in view the settlement of this point, yet as the invariable result of a few hours low temperature was the failure of the experiment in raising the egg, and, as there is but a very slight advance, if any, in the development after the low temperature sets in, I think it safe to conclude that low temperatures tend to stop the progress of the egg. Without intending to establish a fixed standard I am of the opinion that the temperature should be between 65° and 75° Fahrenheit, and that the nearer 70° it is, the more likely is the experiment to be successful. Whatever temperature is started with, the changes afterwards, during the segmentation of the egg or development of the embryo, should be gradual. I noticed that after a change of a few degrees, due to exposure to the rays of the sun, or to a cool shower of rain, or a squall of wind, my experiment usually failed. So far as it is possible to judge in the absence of experiments looking directly to the obtainment of information upon this point, I consider a change to low temperature more disastrous than a change to high. How great a range can be safely permitted it is impossible to say.

The remarks upon the effect of the temperature on the development of the egg hold good, to some extent, with regard to the development of the embryo, though I did not make any experiments upon the latter. The range of temperature permissible with the embryo is probably greater than with the egg. At least I noticed that a sudden fall of temperature which would destroy the eggs did not apparently have any effect upon the swimming embryos, certainly not any immediate one.

Density.—Some years ago Count Pourtales called attention to the fact that oysters did not exist in water of a less density than 1.01—1.00 representing the density of distilled water. My investigations in the Chesapeake lead me to the same conclusion, and I also inferred that a violent change of the density of the water surrounding the oysters would not only affect the mature animals, but would influence the formation of the generative fluids, their development, and the different processes by which they were converted into "spat," in a manner somewhat similar to the effect of changes of temperature. My observations during the past season tend to confirm these latter impressions, but the changes of density and temperature are usually so closely correlated that it is hardly possible to eliminate the influence of either.

As I am not here dealing with the mature animal, except so far as is necessary in considering the artificial production of the young, I shall not revert to the effect upon flavor, growth, shell, characteristics, &c., due to dissimilarity in the constituents of the water; how much or little they influence reproduction, it is, in the absence more exhaustive experiments, at present impossible to say; but the following points are of interest: Shoal-water oysters spawn first, and the less the depth of water the less the density. Also, shoal-water oysters generally lie in the neighborhood of fresh-water streams, or in water of low specific gravity. Deep-water oysters, or those exposed to exactly opposite conditions, not only present exactly opposite characteristics to the shoal-water oysters as regards time of spawning, but they also, so far as my observation extends, contain a much smaller amount of generative matter. So many other conditions obviously operate in effecting the foregoing that it is, however, impossible to decide which influence predominates.

That a change from water of considerable density to that of less very soon has an appreciable effect upon the generative matter appears to be settled; that the effect is a deleterious one is not so clear, but in my own opinion it is. It is well known among oystermen that transplanting during the spawning season puts a stop to the reproductive process; or as they express it in the Chesapeake region, "Plants do not spawn" The transplanting there is from deep and dense water to shoal and brackish, and my own experience at Fair Haven under similar conditions leads me to conclude that the oystermen are correct. The cause appears to be, that in the substitution of warmer water of lower specific gravity, not only is the formation and expulsion of the ova and spermatozoa hastened, but the animal becomes diseased, or at least abnormally replete, from the absorption of an inordinate amount of food or the endosmotic action of the fresh water.

In experimenting at Beaufort I used for the impregnated eggs, while segmenting, ordinary sea water, and in other cases water diluted one third with fresh well-water, and met with sufficient success with the latter to impress me with the peculiarity. In one case I divided the lot of eggs immeditely after impregnation, subjected one-half to salt water undiluted and the other half to water diluted one-third, and noticed quite a marked difference in the development of eggs and embryos, those in the brackish water advancing most favorably and living longest; but as I was not able to continue these experiments in a very accurate manner, and as so many other conditions may have influenced the development, it would be rash to decide that a marked reduction of density is necessary to the success of the experiments. On the whole, other things being equal, I should prefer brackish water to pure sea water. It is worthy of note in this connection, that at Fair Haven the experiment failed at an early stage when water from the Sound, which approximates to pure sea water, was used; but as changes of temperature and errors of selection and manipulation were accompanying causes of failure, the influence of the density of the water remains obscure.

As to the influence of the density upon the embryo, I am unable to speak with any authority, owing to the fact that we had swimming embryos under observation for but very short periods, and having in view a particular end to reach, were loth to experiment with those that progressed favorably. Both Dr. Brooks and myself succeeded in raising the embryo to an advanced stage in both salt and brackish water; but we did not experiment with the water after once placing the embryos in it, and consequently I am unable to say what would be the effect of a change of density. The embryos are of so delicate an organization that it seems probable that a change in this or in any particular must affect them disadvantageously.

Currents.—Oysters do not live, obviously cannot live, for any length of time in perfectly quiet water. The currents passing over the beds are necessary to the animals in many ways, but principally in supplying food and in securing the contact of male and female fluids. It is well known among oystermen that there is but a limited reproduction on beds not subjected to the influence of tidal or other currents; and in consideration of that fact I made a few experiments with the intention of ascertaining whether a current had any effect upon reproduction other than the obvious one of securing contact of the generative fluids. I accomplished my end and secured an artificial current, by revolving in the aquarium jar a paddle wheel, composed of oyster shells, but the appliance had no appreciable effect except a deleterious one, and was soon abandoned. Beyond securing more thorough aeration of the water, currents in the aquaria appear to be useless; indeed, the more quiet the embryos were, apparently the more successful was the experiment.

So far as the current acts in keeping the eggs in motion and free from contact, it is beneficial; but this can be accomplished in other ways, and in future experiments I should throw the action of currents out of consideration.

Aeration.—In the early part of the season this was deemed a very important matter, and though our experience failed to confirm the opinion, yet notwithstanding, I am inclined to think that in the future it will be demonstrated that the influence of aeration is considerable, and that it is a necessary factor to success. Certainly the immense numbers of embryos, amounting to many millions in the aquarium jars, cannot, in so confined a space, receive the normal quantity of oxygen, and the fact that constant aeration has been found necessary in all analogous systems of artificial culture makes it probable that it is essential to successful oyster propagation. But, notwithstanding this apparent necessity, my actual experiments, both at Beaufort and Fair Haven, did not appear to be influenced advantageously by artificial aeration, though I gave it a long trial, under various conditions, before abandoning it. Air was forced through the water in large and small bubbles, at the bottom, in the middle, and just below the surface of the water in the jars; the jet was constant at one time, then changed to an intermittent one, and jets were forced over the surface of the water in the jars or shallow dishes; but in no case with any marked advantage. As I have stated in referring to the influence of other conditions, the embryos that were undisturbed advanced most rapidly, and lived longest. As, however, other investigators may wish to experiment in this direction, and as the matter is not conclusively settled in my own mind, I append sketches and descriptions of two forms of air-pump, used by Brooks and myself, which, as the apparatus is easily and cheaply contrived, may be of assistance. In connection with the aeration of the water one point of importance must be noticed, though I have alluded to it already indirectly. The temperature of the air must be, as nearly as possible, that of the water in the jars or plates. This precaution must be observed, even if it is not deemed necessary to the life of the embryo, in order that exact knowledge regarding the effect of aeration may be obtained.

Effect of adding large amounts of lime to the water.—Carbonate of lime is the most important constituent of the shell of an oyster and the probable necessity of increasing the amount available for the formation of the shells of the embryos, early suggested itself to Brooks and myself. Before my arrival at Beaufort Dr. Brooks had made several experiments in supplying inordinate amounts of this constituent with gratifying success; and in the majority of the experiments at Beaufort we attempted to increase the amount by depositing in the bottom of the jars or plates, fragments of the shells of oysters and one of the varieties of echinoidea. The latter is known commonly as the "sand dollar" and being flat, smooth and clean answered the purpose very well.

Dr. Brooks succeeded several times in obtaining embryos in quite an advanced stage, and generally at Beaufort we noticed that though the development did not proceed much, if any, beyond the stages described and figured by Brooks three years before, yet the rate of development was very much increased, the embryo arriving at the most advanced stage in forty-eight hours, while in 1879 it had required some seven and eight days to reach the same point. Dr. Brooks informs me that his earliest experiments, those prior to my arrival, were the most successful, and that he succeeded in maintaining the embryo alive until they had advanced considerably beyond any stage described by him. The experiments subsequent to my arrival were not so successful, except in the respect noted, viz, the increased rate of development, and this appeared to be due to the addition of the lime to the water. A few experiments in which lime was not used gave rather conflicting results, the embryos reaching the advanced stages in nearly the same time, but in much smaller numbers. I should say that fully one hundred lived in the water containing the shells to one that survived in the other.

The rapid advance was so gratifying, and the addition of lime, which was the only essential change from the previous methods of experimenting, seemed so conclusively its cause, that both Dr. Brooks and myself have been, perhaps, too sanguine of success from the use of the shells and, perhaps, too hasty in assigning to them so much influence. At Fair Haven I was unable to obtain nearly so good results though I used besides the shells quantities of precipitated chalk which should have been more easily decomposed than the shells. I found the embryonic shells in this latter case to require from four to six days to develop, which is not a very material advance over Brooks's results in 1879. Viewing that fact together with the results of the experiments at Beaufort, especially those conducted without the assistance of the lime, I am of the opinion that the influence of that factor is not so great by any means as I had supposed, and that the success we met with in the early part of the season must be assigned to other causes, though the supply of lime may have rendered, and probably did render, some assistance.

Presence of Infusoria.—A short time after placing the swimming embryos in new water, even though all care is used and all precautions observed, infusoria will begin to appear and thenceforward will exercise a more or less deleterious influence upon the success of the experiment. It is impossible to obviate this difficulty though care in selecting the oyster and in manipulating the eggs may do much to diminish the evil. Attention should also be given to the water selected, which, so far as my experience goes, should be recently taken from the sea. If shells are used in the jars or plates, they should be thoroughly cleaned and boiled before using them. But notwithstanding all precautions which may be taken, the entire absence of infusoria cannot be secured. Numbers of embryos will die and decompose, and the infusoria resulting will constantly increase.

Their influence seems to be a most disastrous one. They not only consume the oxygen and such small quantities of food as may exist in the water, but appearances indicated a direct attack upon the ciliated, embryo oysters. We frequently observed numbers of embryos, that had developed in a perfectly normal manner, lying at the bottom of the jars and plates, incapable of movement on account of the absence of cilia, and after continued observation of embryos and infusoria in watch crystals, I feel a reasonable certainty that upon the first indication of impaired vitality on the part of the oyster embryo, it is immediately attacked by the infusoria. Of one thing there is no doubt whatever; the destruction of young and the advent of infusoria are coincident, though which is the cause and which the effect is not so clear. Probably each accelerates the other.

Supply of food.—Considering the immense number of embryos collected in a comparatively small receptacle, in these and similar experiments, it is evident that the maintenance of anything like an adequate supply of food is a very difficult matter. Indeed, it is not yet positively ascertained what the food of the embryo is and until that point is settled it is hardly possible to devise any method of supplying the aquaria. The probability is that the bacteria evolved from the decomposing matter in the waters of creeks, rivers, and littoral area, form the principal article of food, but the difficulty of obtaining and affording a sufficient supply to the millions of embryos in a jar or plate seems insurmountable. Brooks tried to produce the bacteria by decomposing starch and then adding the water to the aquaria, but met with no success, and as little attended the use of ordinary ditch water diluted with that from the sea. By collecting water and mud from an oyster bottom lying remote from the dense waters of the Inlet, and adding small quantities to the water already in the plates, we met with a moderate degree of success; the young oyster, as seen under the microscope in a watch crystal, attracting and securing minute particles of animal or vegetable matter, and digesting them. In quite a number of cases the stomach was gorged with food and the sweep of the cilia, continually brought more within reach of the minute animals. Notwithstanding this success, however, the embryo died eventually without making any material advance, and I account for this destruction by the supposition that we were unable to supply sufficient food, each oyster obtaining but a small amount compared with its necessities and normal supply; and also by the fact that after a day or two the amount of sediment carried in with the water containing the food was sufficient to smother the embryo. Those oysters under observation certainly did thrive very well; their shells were large and well defined, and though we saw none that had actually attached to any "cultch," yet Dr. Brooks discovered numbers of shells, which had not been separated, with one of the valves very much larger than the other. As it is well known that immediately after attachment, the upper valve grows much more rapidly than the lower or attached one, it is probable that the empty shells noticed by Brooks had been attached until the oyster died, and the survival of the embryo until that stage was reached (point of attachment) must have been due to the supply of food being approximately sufficient, as I did not notice any embryos in a similar state of development except in those experiments in which we attempted to increase the available amount of food. In future experiments in this direction the attempt must be made to supply bacteria unaccompanied by sediment or large quantities of water. Otherwise there will be but a renewal of our experience of the past season.

Time and place of attachment.—The experiments of the past season have thrown some additional light on this subject, and have confirmed my previous impressions gained during the surveys of the oyster beds of the Chesapeake Bay. There is but little doubt in my mind now that the young fry will prefer the lower or more secluded points or surfaces in selecting a place for permanent fixation. I observed in 1879, in the Chesapeake, that the lower sides of the collectors I placed on the beds secured a much larger "set" of young than the upper. As disturbing influences may have prevented the continued life of the young attached to the upper sides, and as those sides were more accessible to the various enemies of the young, I hesitated in deciding that the large number of oysters discovered on the lower sides was due principally to the natural habits of the embryo; but my observations at Beaufort and Fair Haven indicate that such is really the case. It was uniformly noticed, both by Brooks and myself, that the largest number of embryos was always found on the lower side of the shells placed in the jars and plates, and this difference was quite sufficient to be remarkable, even had not our attention been directed to it primarily.

As to the time of attachment, we were unable to obtain any positive evidence, but incidentally I observed certain peculiarities of attachment both at Beaufort and Fair Haven which are significant. Dr. Brooks states that after the eggs have passed through the various stages of segmentation and have developed cilia, the embryos swarm to the surface, remaining there for a limited period and then swimming about through the entire volume of water. Our observations at Beaufort and my own at Fair Haven confirm the truth of Brooks' statement, and it is hardly necessary to allude to it, but that I wish to call attention to the fact, and to the peculiar movements of the embryos during this stage of their existence. While at Fair Haven I had constantly under observation many different lots of embryos in this stage, and the uniformity of their movements attracted my attention. If a large number are collected in a deep beaker and held up to the light they will be seen as a cloud of dust, permeating the whole volume of water. In a few moments a dense film of embryos will be found at the surface of the water; sometimes this is so thick that it becomes opaque when looking through the bottom of the beaker. If the beaker is jarred so as to make a slight wave motion on the surface, the embryos will be seen descending from that surface in long comet-like streams and these streams invariably tend towards the sides of the glass. The mass of water will present for several minutes a very peculiar, streaked appearance, due to the descent of the embryos. Close observation will discover that after reaching the bottom the embryos rise again through the water (now quiescent) to the surface. If a shell is placed in the bottom of the beaker the tendency of the streams to avoid the edges and pass under the lower side is quite remarkable. Curious whirls and eddies appear to exist, and it is noticeable how closely the embryos follow the lead of the head of the column.

Our observations at Beaufort showed us that after the embryos had once developed the shell to any extent there was but little motion of translation, the animals remaining quietly in one place at the bottom. Indeed their specific gravity at this period, together with their deficient locomotive powers, should prevent any very rapid or extensive movements. Now it has been observed by numbers of persons, indeed every one who has visited oyster regions, that the piles of wharves, the trunks of trees near the water, the abutments of bridges and piers, and many other substances similarly situated, are covered with young oysters and sometimes with old, and that this attachment appears to be greatest between the high and low water marks. Numbers of floating objects have also frequently been covered with the young fry, such as boards, branches of trees, and the bottoms of vessels. Considering the fact that the embryos swarm to the surface immediately upon the development of their swimming powers, that upon any disturbance there they sink or swim towards the bottom, making way diagonally as if in search of some secure place for attachment, that as soon as shells are developed they remain in a comparatively quiescent state at the bottom, and that when it is possible large numbers attach about the surface of the water, I have come to the conclusion that the oyster embryo is predisposed at least to fix itself very soon after the process of segmentation is completed; that as soon as cilia are developed they serve a double purpose, not only affording a means of translation, but also one of fixation. This is a hypothesis, of course, and the postulates upon which it is based are only in a measure sound; but judging by the evident disposition of the embryo with shell to remain at the bottom and nearly in one place, I can account for the attachment at the surface of the water only by assuming that it occurs at some period anterior to the development of the shell. It is not very difficult to understand how the embryos in swarms at the surface may be disturbed by a slight commotion of the waters, and streaming off obliquely to the bottom, may come in contact, and be entangled by the cilia with any of the various objects it is so frequently found attached to; and failing to find such, it will continue its search until it reaches the bottom and then bury itself under or about shells or other exposed "cultch." Failing to attach there, it will rise again to the surface and repeat the process until, the shell having formed and each succeeding ascent being less, the embryo will be unable to overcome its own weight and will remain at the bottom, attached and sure of life if the object be suitable, or unattached and thus certain of a speedy demise. If I am correct in this view, its importance is considerable, especially so to private oyster-culturists. It is evident that if attachment may occur at any time during the free swimming stage, "cultch" or collectors may be exposed, indeed should be, not only at the bottom, but serierally to the surface. The indications are that floating brush collectors, exposed as closely as convenient without offering too great an obstacle to currents and navigation, would give good results, and I regret that I was unable to make an experiment with them during the past summer. If my view is correct, the oyster planters of Long Island Sound and Narragansett Bay lose annually a large number of oysters by neglecting floating collectors. Many of them have tried one or two forms and have met with varying degrees of success or failure, but I do not know of any instance where the failure was due to inherent causes, but rather to defective apparatus or methods. I do not wish to be understood as advocating brush collectors, as they may in certain localities be unsuitable; but I strongly advise floating collectors both for experiment and practical work.

Though my experiments during the past season have not been productive of any positive results, I do not feel by any means discouraged as to the eventual solution of the problem. It still seems possible to artificially produce and raise an oyster, and in course of time I have no doubt that the object I had in view will be achieved either by myself or others. It may be attained by some happy, accidental discovery, or by a patient investigation of all the conditions affecting and favoring the life of the embryo oyster. Perhaps I and others have spent too much time in attempting to reach the desired end at a single leap. It is my own opinion that we have, and that the final result will only be obtained after a careful and pains-taking series of experiments, which will begin as nearly as possible, with the most important influence and determine its extent before attempting to proceed further. In other words I would recommend for the future that experimental work follow a strictly scientific course; that there be a massing of facts and a careful digestion of them; that the hypothesis based upon such digestion be carefully followed out to its legitimate conclusion, and if ending adversely a review of the ground be undertaken. The conditions affecting in more or less degree the life of the oyster and embryo are fairly well known, and in studying the extent of the influence of these various conditions will be found the road which will lead to the attainment of the main object.

I have left until the last the mention of my experiment in placing embryo oysters on the beds, not because it was not of some importance, but because it was an incidental part of the work I had undertaken. After the experience acquired at Beaufort, and after observing the disposition of the embryo, with shell, to remain quietly at the bottom, I came to the conclusion that if the artificially-impregnated eggs could be protected during segmentation, and the resulting embryos maintained alive until the shells were well developed, they might be deposited on the beds with a fair chance of a large number, probably a majority, surviving. To keep the embryos alive until they reached such a stage was not a matter of much difficulty, nor was the deposition on any chosen point of a bed an operation that could not be easily performed. The only question was whether any decisive result would be obtained, or rather whether it would be possible to ascertain positively that the embryos had survived. Considering that there might be a large natural attachment on a bed and that the artificially-raised embryos might be dispersed over a somewhat large area, it appeared very doubtful if any results would be produced upon an inspection of the beds. I thought the chance however, of a successful issue sufficiently good to justify my trying the experiment, and accordingly while at Fair Haven I fertilized as many eggs as possible and deposited the embryos, in various stages of development, on one of the beds owned by H. C. Rowe & Co., of Fair Haven. That the experiment should attain an obvious success evidently depended upon the deposition of a very large number of eggs and it was my endeavor to furnish as many as possible for the purpose; but owing to accidents of weather and apparatus I did not succeed in placing on the bed as many embryo oysters as I wished or had hoped to do. I soon found that in working on so large a scale I lost a proportionately larger number of oysters than when fertilizing the eggs of a few females for experiments in aquaria; but I succeeded in placing at one point some ninety million embryos, of which fully one-half were well advanced in development. They were carried out to the buoy marking the spot, by one of Mr. Rowe's steamers and then lowered in a double-headed can to the bottom; the covers of both ends of the can were then removed and the young fry allowed to wash out. As they were practically on the bottom I hoped they would at once find points for attachmont among the recently spread shells, and that an inspection of the bed in the autumn would show that the attachment at this point was sufficiently great, and above that on other contiguous portions, to justify the assumption that the superiority was due to the attachment of the artificially-raised embryos. Naturally, in order to afford conclusive evidence, the superiority would have to be very great; but even should such not be the case it by no means proves that a majority of the embryos did not attach, as they might have been widely dispersed by some unforeseen cause or accident. If any evidence at all is given, it must be of a favorable nature. The absence of evidence proves the experiment, but not the theory to be at fault.*

*Since writing the above the author has received the results of the examination made by Mr. Rowe, which are summarized as follows: The attachment of spat was general over the whole bed. It was noticeably larger at the point where the artificially-raised embryos were deposited, and was also larger along a line extending N.

In conclusion I have to express my indebtedness to Dr. Brooks for the assistance afforded me at Beaufort, not only in conducting the experiments, but in permitting me to use the steam-launch and employés attached to the Johns Hopkins Laboratory. I am also under great obligations to Mr. H. C. Rowe, of Fair Haven, Conn., who allowed the use of his office and part of his packing-house as a temporary laboratory, supplied me with all the oysters I used, and put his steamers at my service whenever I desired to employ them. I am also indebted to him for making my stay at Fair Haven as agreeable as possible, and giving me all the information in his power regarding the Connecticut oyster fisheries.

EXPLANATION OF THE PLATE.

Fig. 1. *Method of arranging plates so as to separate swimming embryos from dead eggs.*—B is the lower dish, containing oyster shells or carbonate of lime. A is the plate holding eggs in process of segmentation. Plate A is slightly inclined and nearly full of water. When the embryos begin to swim, a minute supply of water is added to A from the jar C, by means of a siphon, D. The supply is so arranged that the drops strike the edge of the plate A, so as to prevent any disturbance of the eggs in the bottom of the plate. Water from A will overflow into B drop by drop, carrying the swimming embryos with it. A piece of paper or card-board (*x*) should be placed so as to prevent any considerable fall from A to B.

Fig. 2. *Method with funnel.*—The figure explains itself sufficiently, B representing the funnel and A the jar which is to contain the embryos. The only point of importance is the necessity of giving the funnel B a slight inclination so that the overflow may be more constant and in one place.

Fig. 3.—B shows the character of funnel used during the past season, *x* being the cork with perforation for glass tube *z*, through which the jet of air or water was forced. The disadvantage of the arrangement consisted in the form of the funnel. The eggs collected in a thick layer on and around the cork, as shown by the shaded parts in the figure; in the middle, the jet kept the majority in suspension. The improved cone or funnel shown in Fig. 4 would, I think, obviate to a great extent this evil, the interior of the funnel being perfectly conical and the tube of rubber conveying the air or water fitting over the apex of the exterior. The channel *yz* should be very small, as only a minute jet of either air or water is necessary or desirable.

Fig. 5 shows an air pump devised by Brooks, which is a convenient arrangement for the use of laboratories situated where there is no great head of water attainable. B is a tube, with funnel at the top (*c*) in-

W. and S. E. from that point. (The usual set of the tide is in that direction.) While the foregoing are favorable indications, they are not considered sufficiently strong to justify an assertion that the experiment was entirely successful. Other attempts may settle the question.

serted in a jar or tank, A, which is hermetically closed. The end of the tube B should pass through the cover *g g*. E is the overflow pipe, fitted with a sliding extension, D, so that the overflow may take place at any desired height. F is the air pipe; C, the stream of water entering the funnel from a small vessel. The apparatus requires but very little water and little or no head to supply all the aeration necessary for laboratory purposes. The water running from the receptacle H, through the nozzle C, is made to impinge against the sides of the funnel so as to give a rotary motion. A small glass ball or vial partially closes the upper end of the tube B, and the column of water in the tube is thus a broken one, the intermediate spaces being filled with the air carried in by the suction. As the tank A fills with water the air is forced out of F. The overflow of superfluous water is regulated for different depths, or rather for different pressures on the end of F, by raising or lowering the movable arm D. The power of the pump is of course proportional to the length of the tube B. Four feet fall of water furnishes a sufficient supply for most laboratory purposes.

A second simple pump is shown in Fig. 6. A A are two casks having stop-cocks at *m*, *n*, *o*, *p*, *s*, *t*. The cocks *s* and *t* are connected by rubber hose; from *m* and *n* hose runs to a point, *k*, whence the main air-supplying hose is led to the aquaria. Both casks are fitted with slings and tackles for raising and lowering them, and the pump is worked in the following manner: The cask *a* being filled with water, the cocks *s* and *m* are closed and the cock *o* opened; the cask is then raised six or eight feet. The cock *p* of cask A′ is closed and cocks *n* and *t* opened. Then, by opening cock *s*, the water flows from *s* through *t* into A′, forces air out of *n* to *k*, and thence to the aquaria. When the upper cask is empty, it is lowered and the other one raised, and the process reversed. A 40-gallon cask will supply air for eight to ten hours, and with a small luff-tackle can be raised easily by two persons.

I have made these sketches and accompanied them with the descriptions not because of any great merit or originality the apparatus possess, but because others may find their use of benefit and be saved some trouble in experimenting with methods and appliances having the same end in view.

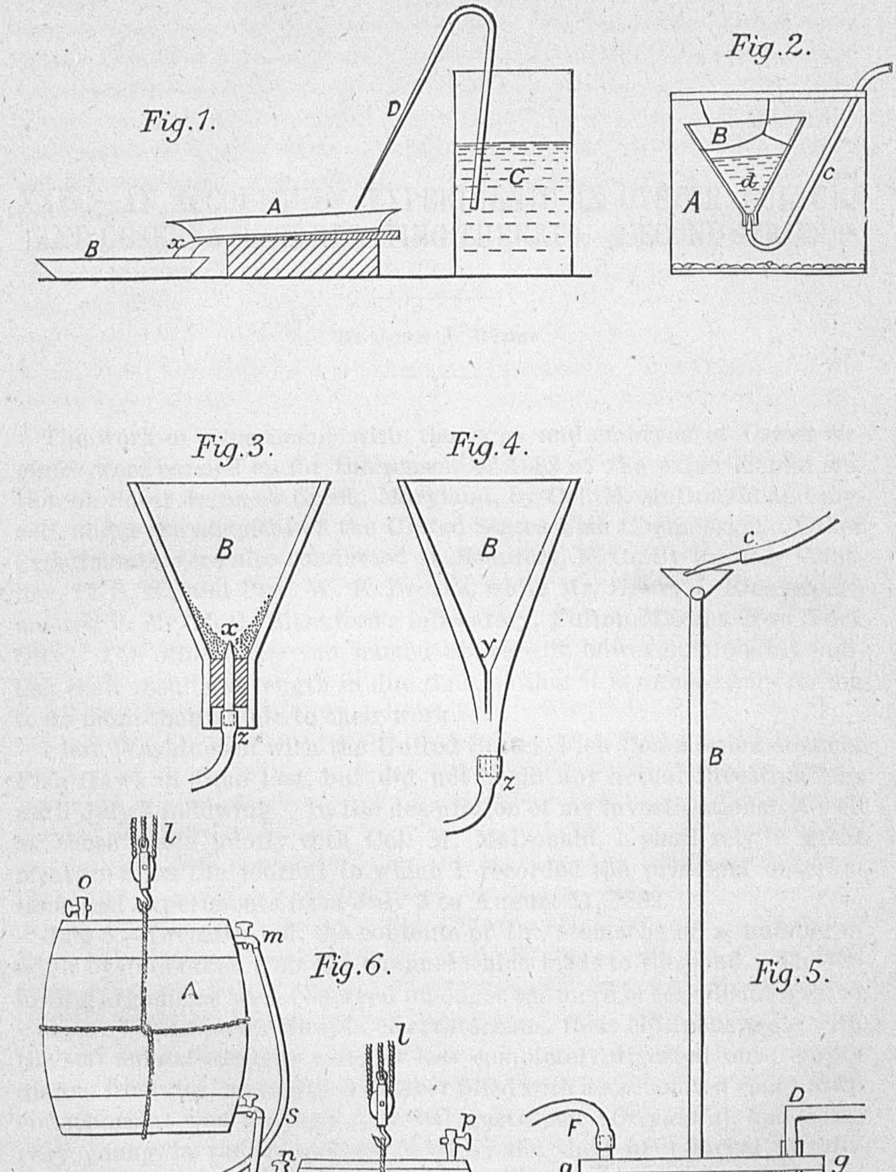
Fig.1.
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Fig.2.
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Fig.6.
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