4.—THE FOOD OF THE OYSTER, CLAM, AND RIBBED MUSSEL.

By John P. Lotsy, Ph. D.

During a stay on the James River, Virginia, in the summer of 1892, I had hoped to study the food supply of both the young (embryonic) and the adult oyster, but as the season was too far advanced to allow the collection of any embryos only the latter part of the investigation proved feasible.

Collections were made at many places on both sides of the James River from Newport News to Old Point Comfort, specimens being obtained from both natural and cultivated beds, from muddy and sandy bottom, and from piles and stones, especially around Fort Wool on the Ripraps. They were taken from various depths, some being gathered on a bottom left exposed at low tide; others were obtained which did not grow on the bottom, but which were, so to speak, suspended in the water near the surface, attached to piles and rocks, also exposed during low tide; still others were collected from deeper places, never uncovered by the tide, growing either on the bottom or on permanently submerged stones and piles. To determine whether any changes in the food supply were dependent upon the season of the year, material was obtained daily from the beginning of June until the end of September, and whenever an opportunity offered shipments brought from farther up the river were examined to see if the greater amount of fresh water there present had any influence on the character of their food.

Before entering further into details it is necessary to note that the oyster is constantly ingesting a stream of water, which, passing the mouth, brings near and into this always opened organ all the objects of greater or less size coming within the influence of this stream. The mere presence, therefore, of particles of various organic matter in its stomach, even in great quantities, does not indicate that the oyster uses them as food, but only proves that these particles were present in the surrounding water at the time of ingestion. This is a consideration too often overlooked. If an animal of the structure of an oyster be placed in a bucket of water in which is suspended a great number of carmine granules, these granules will doubtless be found in the stomach of the animal after a certain length of time, yet nobody would claim that they were the food of the oyster. A similar thing occurs in nature. In the many oysters which I have opened and of which I investigated the stomach contents I never failed to find numerous particles of sand. The finding of some animal or plant or other object in the stomach of the oyster does not prove that it composes any part of its food supply, no matter how numerous the form may be in individuals, but it can only offer a suggestion for further investigation. In order to demonstrate which of these various objects serve as food, it is necessary to prove not only that they are ingested, showing a possible source of nutriment, but also that having passed through the digestive tract only the indigestible parts remain. To conclude from the fact alone of the occurrence of any animal or vegetable in the stomach of an oyster that it forms a part of its food is no more justifiable than to affirm that the fishes brought up by a water-wheel are food for the wheel.

The method followed by me in studying the stomach contents of the oyster was as follows: The oyster was carefully opened, guarding against any injury from the knife except the separation of the muscle which connects the two valves of the shell. The oysters were, as a rule, examined immediately after being taken from the water, usually within fifteen minutes, and very rarely after as long an interval as two hours. After separating the gills at the oral extremity with a scalpel, so that the opening of the mouth was exposed, the tip of a finely drawn-out glass tube having a rubber ball at the other end was introduced into the stomach. The contents of the stomach were now sucked out by removing the hand from the hitherto compressed bulb.

The contents of the stomach of an oyster which has recently fed—in other words, of every oyster collected when the shells are open—present a beautiful dark-golden color. A drop of this material obtained in the manner described above and placed under the microscope for examination shows that the stomach of the adult oyster contains a large number of diatoms, embracing a great many species. The constant occurrence of these forms in great quantities suggested the possibility of their serving as food. In addition to the diatoms a quantity of decaying organic matter at least equal in amount, and also of some of the lower algæ, besides sand, etc., were often found. Rhizopods, a few euglenas, an occasional foraminiferum, and other animals of lower grade were seen, but only once was a copepod found; in fact, animal life was practically absent.

An idea which early occurred to me was the importance of examining simultaneously the stomachs of the other common bivalve mollusks of the James River, to see if any uniformity in the nature of the food in this natural group could be detected. With this object the stomachs of the hard clam or quahog (*Mercenaria mercenaria*), of the soft clam (*Mya arenaria*), and of the ribbed mussel (*Modiola plicatula*) were also examined. In all of these species the contents of the stomach were found to be the same as in the oyster.

The first question to be settled was whether or not the oyster and these other mollusks actually digested the diatoms found in their stomachs; and, second, what part of the additional decaying organic matter was digested. It was possible, for example, that the diatoms, if abundant in the surrounding water, were merely ingested and would pass the intestinal canal unchanged, while the decaying organic matter might be digested. In order to settle this point, several oysters and clams were placed in separate glass dishes, their shells being previously carefully cleaned with a brush. The sea water in these dishes was either naturally very pure or strained through filter paper; after a few hours a considerable quantity of fæces was deposited in the dishes. The excrements of the oyster, as well as those of the mussel and soft clam, are well formed, consisting of a hollow tube or of a solid rod of excrementitious substance; the amount of sand in them is enormous, forming by far the greatest bulk.

The cell walls of the diatoms, on account of the silica which they contain, are indigestible; for this reason it was easy to determine with accuracy whether digestion of the diatoms actually took place, as it had been previously ascertained that very few empty shells of diatoms were present in the stomach, by far the greater number being in fresh condition. The examination of the excrements under the microscope showed that the decaying organic matter had passed through the alimentary canal entirely unchanged. At first it seemed as if the diatoms also were very imperfectly digested, but soon it became evident that this was an error based on superficial examination, since the undigested diatoms were more conspicuous on account of their coloring matter, while the delicate transparent shells of those which had been digested escaped observation. To avoid this error the following method was adopted: The excrements of a certain number of oysters or clams were collected, broken up in water, and well mixed. From this average sample two preparations were made and in each of these twenty-five fields selected at random were counted. I have tabulated below the results of the examination of two such samples. The great difference in the number of diatoms present in each field is due to the fact that the samples were very differently diluted with water.

In Column I, under "dead," is recorded the total number of dead diatoms observed. The letter *d* following a number indicates that not all the diatoms were completely digested, although by far the most were nearly so, only a little of the coloring matter remaining.

Column II shows the number not entirely digested, and the difference between the numbers in the two columns indicates in each instance the number in which only the clean silica skeleton remained.

In none of these cases was the additional decaying organic matter digested. The numbers of individuals examined being very different, having been taken from different localities and representing different genera, the fact that the results coincided so closely in the proportionate number of digested and undigested diatoms seems to indicate a very complete digestion of the ingested diatoms in this group.

Average sample of the excrements of 21 oysters, collected on plants of Mr. Cock, in shallow water, Hampton Creek, Va.

Field No.	Preparation No. 1. Living.	Preparation No. 1. Dead. Col. I.	Preparation No. 1. Dead. Col. II.	Preparation No. 2. Living.	Preparation No. 2. Dead. Col. I.	Preparation No. 2. Dead. Col. II.
1	0	7	1 *d*	0	12	1 *d*
2	2	13	6 *d*	0	21	3 *d*
3	1	4	....	0	16	2 *d*
4	0	8	....	0	20	....
5	1	8 *d*	1 *d*	1	17	1 *d*
6	0	10	....	0	20	....
7	0	7	....	0	22	3 *d*
8	0	13	3 *d*	0	28	4 *d*
9	0	4	1 *d*	0	29	3 *d*
10	0	12	....	0	27	4 *d*
11	0	14	4 *d*	1	20	2 *d*
12	0	16	4 *d*	0	8	1 *d*
13	0	14	1 *d*	0	22	2 *d*
14	0	12	3 *d*	0	18	3 *d*
15	0	15	....	0	10	1 *d*
16	0	13	1 *d*	0	15	2 *d*
17	0	16	1 *d*	0	12	4 *d*
18	0	15	3 *d*	2	13	3 *d*
19	1	35	2 *d*	0	38	3 *d*
20	0	20	1 *d*	0	14	7 *d*
21	0	11	2 *d*	0	23	3 *d*
22	0	17	2 *d*	0	24	4 *d*
23	0	14	2 *d*	0	12	2 *d*
24	0	13	....	0	11	2 *d*
25	0	11	....	1	23	3 *d*
	*5 Living, 1.5 per cent.	322 = 284+38 Entirely digested, 87 per cent. Nearly digested, 12 per cent.		5	475 = 412+63	

Results.

Results of Preparation No. 1:

	Per ct.
Living	1.5
Nearly digested	12
Entirely digested	†87
Total	100

Results of Preparation No. 2:

Living	1
Dead	99
Entirely digested	85
Nearly digested	14
Living	1
Total	100

Average from the two preparations:

Digested		99
Entirely	86	
Nearly	13	
Living		1
Total		100

*These few living diatoms might have been derived from the sea water used for breaking up the excrements. All five belonged to the same species, and as diatoms have a motion of their own it is possible that the same individual figured in each case.

†The smaller species were all entirely digested, so that they seem to offer the best food for the oyster.

Average sample of the excrements of 17 oysters, collected by Mr. R. Armstrong 10 miles up the James River from Newport News, Va.; deep water.

Field No.	Preparation No. 1. Living.	Preparation No. 1. Dead. Col. I.	Preparation No. 1. Dead. Col. II.	Preparation No. 2. Living.	Preparation No. 2. Dead. Col. I.	Preparation No. 2. Dead. Col. II.
1	0	3	....	0	1	....
2	0	4	....	0	8	1 *d*
3	0	5	1 *d*	0	2	3 *d*
4	0	2	....	0	3	....
5	0	3	....	0	3	1 *d*
6	1	2	....	0	2	....
7	0	6	3 *d*	0	1	....
8	0	2	....	0	4	....
9	0	2	....	0	2	2 *d*
10	0	5	....	0	2	....
11	0	4	....	0	2	....
12	0	4	2 *d*	0	2	....
13	0	5	2 *d*	0	3	1 *d*
14	0	3	....	0	3	....
15	0	2	1 *d*	0	2	....
16	0	2	....	0	3	....
17	0	4	....	0	1	....
18	0	3	1 *d*	0	3	....
19	0	2	....	0	2	1 *d*
20	0	1	....	0	2	....
21	0	4	....	0	1	....
22	0	6	1 *d*	0	2	....
23	0	3	1 *d*	0	3	....
24	0	4	....	0	3	....
25	0	1	....	0	2	....
	1	82=70+12		0	63=54+9*	

Results.

Results from Preparation No. 1:

	Per ct.
Entirely digested	85
Nearly digested	14
Living	1
Total	100

*Results from Preparation No. 2:

Entirely digested	86
Nearly digested	14
Living	0
Total	100

Average from the two preparations:

Digested		99
Entirely	85.5	
Nearly	14	
Living		0.5
Total		100—

*Enormous quantity of sand and many big species of diatoms.

Average sample of the excrements of 4 clams, collected on the flats at the Soldiers' Home, Hampton, Va.

Field No.	Preparation No. 1.			Preparation No. 2.			Results.
	Living.	Dead.		Living.	Dead.		
		Col. I.	Col. II.		Col. I.	Col. II.	
1	1	7	2 *d*	0	2	....	Results of Preparation No. 1:
2	0	3	....	0	1	....	*Per ct.*
3	0	2	....	0	3	....	Living 2
4	0	0	....	0	0	....	Entirely digested 83
5	0	3	1 *d*	0	1	....	Nearly digested 15
6	0	3	2 *d*	0	1	....	
7	0	2	....	0	2	1 *d*	Total 100
8	0	3	1 *d*	0	1	....	
9	0	3	1 *d*	0	1	....	Results of Preparation No. 2:
10	0	0	....	0	4	....	Living 0
11	0	2	....	0	4	....	Entirely digested 86
12	0	1	....	0	1	....	Nearly digested 14
13	0	3	1 *d*	0	0	....	
14	0	0	....	0	1	1 *d*	Total 100
15	0	3	....	0	1	....	
16	0	5	....	0	5	....	Average:
17	0	2	1 *d*	0	2	....	Digested 99
18	0	3	....	0	4	2 *d*	Entirely 84.5
19	0	2	....	0	2	1 *d*	Nearly 14.5
20	0	1	....	0	0	....	Living 1
21	0	1	....	0	1	....	
22	0	1	....	0	1	....	Total 100
23	0	1	....	0	2	....	
24	0	2	....	0	1	1 *d*	
25	0	2	....	0	2	....	
	1 *	55 = 46 + 9		0	43 = 37 + 6		

* Probably derived from the sea water used to break up the excrements.

There was another possible kind of food yet to be considered, namely, such substances as might be in solution in the water in which the oyster lives. The oysters attached to the stones of the Ripraps, which also showed their stomachs full of diatoms, are surrounded by perfectly clear water, but from a large number of chemical analyses which I have made of similar water on former occasions, after having strained the diatoms, etc., out, I am convinced that hardly a trace of organic matter is to be found dissolved in it, so that this possible source of food can be entirely excluded.

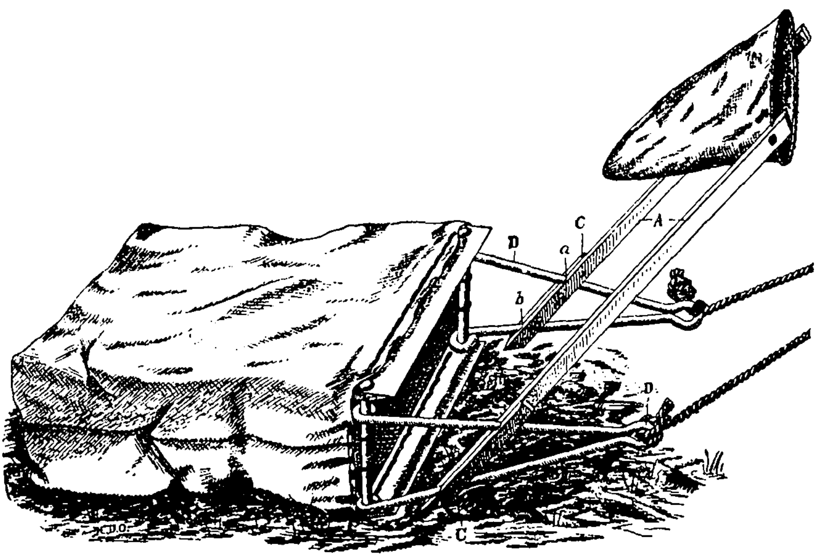
After having determined in this way that the food of the oysters and clams in the James River consists practically of diatoms, the question presented itself, Where do these diatoms come from? The use of the common Müller's pelagic tow net revealed their presence at the surface of the water in enormous quantities, and no difference could be detected in their numbers or distribution during the daytime or nighttime. The occurrence of diatoms in such numbers at the surface explained well their presence in the stomachs of the oysters attached to the stones and piles submerged only a little under water, but this could not account for their presence in those living at the bottom in deeper places, where even at low tide considerable water remained. It was therefore thought advisable to collect at different depths in order to study their perpendicular distribution from the surface to the bottom. The result showed that they occurred in equal quantity at all depths up to 70 feet, which, according to the official maps, is the greatest depth found at the mouth of the James River.

The idea is commonly held that the soft organic mud on the oyster beds stimulates the growth of diatoms, but a microscopic examination of specimens of mud taken from different oyster beds revealed the fact that they were apparently not more numerous in such places than on the sandy bottoms similarly situated, provided that they were continually covered with water. On the other hand, those muddy places which are left dry between the tides were found to be much richer in diatoms than similar sandy places. The species living on the bottom apparently differed from the pelagic ones, but their presence in the stomachs examined indicates that they also served the oyster for food. These observations were made at places where the current was strong. They do not, therefore, by any means preclude the possibility that in stagnant or slowly flowing water muddy banks may form a much better soil for diatoms than sandy ones. The fact that they are more numerous on muddy bottoms near the shore which are left uncovered by the tide even indicates this. We might account for the facts above stated by supposing that the diatoms are swept along so fast by the strong currents that they have not time to settle on these mudbanks, and might perhaps also thus explain the controversy between oyster-growers as to whether muddy or sandy bottoms furnish the most favorable places for planting. That sand is sometimes preferable we learn from the following quotation from Professor Brooks's oyster report. Within the harbor, for instance, considerable "muddy bottom has been utilized by first paving it with coarse beach sand. No spot where there is not a swift current is considered worth this trouble."

This, of course, is in complete harmony with our facts, since the stronger the current the more food that is offered. That there is an abundant food supply for oysters on sandy bottom is proved by the fact that the clams, living upon the same food as the oysters, are often found on pure sand flats.

The fact that the mud bank, on microscopic examination, did not prove to contain more diatoms than the sand did not seem to furnish sufficient evidence on which to base an opinion as to the stimulating power of the mud on the growth of diatoms, as this might possibly be perceptible in the greater quantity of diatoms in the water above. To determine this, diatoms were collected from the water over mud banks and also from over pure sand and the results compared, but no perceptible difference could be detected. The instrument used for this purpose was a Müller's net secured firmly by means of two strong wooden poles to the dredge in such a way that it was immovable. The poles "A" are of strong wood. Two incisions are made in these, extending about half way through the wood at the points *a* and *b* in such a manner that the iron bars D of the dredge fit perfectly in them. A crosspiece C is now screwed on, so as to retain the poles at an angle of about 45° with the bottom when the dredge is lying on it. The net is fastened between the poles near the top, the ring fitting into incisions in either side, enough space being allowed between them and the top for the usual rope of the net to be firmly wound around it in order to keep the latter in position. This arrangement allows the use of both net and dredge separately or together with very little trouble or expense. When the apparatus is in use, the dredge is drawn along on the bottom in the direction of the arrow, while the net is held about a foot above the bottom and a few feet in front of the dredge, so that the mud stirred up by the latter does not interfere with the net, and in the latter only those objects are taken which are normally suspended in the water passing over the mud bank.

In order to study the diatoms over as wide an area as possible, collections were made daily from Newport News down to Hampton, and even from points several miles out in the bay. From these catches



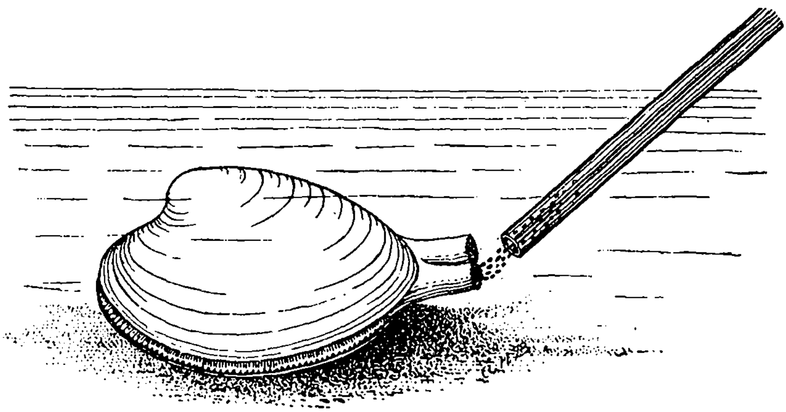
about 50 species of diatoms were drawn on the spot. For staining the diatoms, the lower algæ, and other low forms of life, I employed the method described farther on, which I think offers some advantages worthy of consideration. Several jars full of diatoms were preserved and carried to Baltimore for classification. I have not, however, been able to find a reliable work on the classification of American diatoms, and as specialists assure me that such a one does not exist, this plan had to be abandoned, since the time at my disposal just now does not permit me to undertake it. Nevertheless, such a classification would be of great value, and if the necessary collection of diatoms from different points of the American coast could be obtained to enable such a work to be done on a broad basis, it would also pay from a practical stand-point. It would be of great interest to so determine the habitat of the different species as to ascertain which grow on the bottom and which are freely suspended in the water. At the same time a careful study of the life-history of the diatoms should be made. It does not seem to me that it would be very difficult to fatten oysters by bringing them into ponds in which a large quantity of diatoms had been developed under favorable conditions. To accomplish this satisfactorily, however, a closer study of the life-history of this group would be necessary.

The quantity of diatoms which may be seen on a bottom near the shore, for example, does not in the least furnish us with a basis for measuring the amount of oyster food there present, as many of these forms are firmly fixed to the bottom, and so, of course, are entirely useless for that purpose. Since it is well known that too much fresh water kills the marine diatoms, a careful study of the influence of fresh water upon them would be necessary in order to determine the most promising places for oyster-culture in our rivers. My station last summer, so near the mouth of the river, was not well fitted for this, but I was able to show that oysters coming from 15 miles farther up the river contained in their stomachs the same species of diatoms as those collected around Newport News, or even around Hampton.

As the water surrounding the habitat of the oysters contained, besides diatoms, a great number of copepods, it seems strange that these were not found in the oysters' stomachs also, as the stream of water ingested by the oysters was certainly strong enough to draw the copepods into their mouth along with the other floating particles. The idea naturally suggested itself that perhaps the oyster might possess a power of discrimination between the higher and more active animals, such as copepods and the lower foraminifera, and especially the diatoms, although the fact that its mouth is continuously open does not favor this view. It was thus thought advisable to make some experiments bearing upon this subject. As copepods were not to be obtained easily in pure cultures, it was thought that a substitute for them might be found in finely hashed fish, or, better still, shrimps. It might safely be assumed that if oysters should prove to be able to discriminate between such a food material and diatoms the chances are that they would still more readily distinguish the latter from the actively swimming copepods, since the presence of these would be more readily detected by their movements.

Such a fact, however probable, could not be demonstrated, but the question which could and should be determined by this method was: Do the oyster and the other bivalve mollusks possess in general a power of discriminating between the different kinds of food offered to them? For this purpose it was necessary to obtain, in the first place, cultures of diatoms in which animal life was absent. Since diatoms have never hitherto, so far as I am aware, been obtained in pure culture, some experiments had to be made to accomplish this. I was able to obtain very good cultures, though not pure ones; the latter not being attempted. It seems to me that it would be easy by the method which I employed to obtain cultures of a single species, only contaminated by bacteria. The method was this: Some sea water was placed in an Erlenmeyer flask with a little of the pelagic catch added, in order to give it the necessary elements for the growth of the diatoms. The flask was then plugged with cotton and sterilized by boiling. Afterwards, when it had cooled, a few drops of the pelagic catch containing but a few diatoms were introduced into this sterilized medium. After some days small colonies of diatoms appeared on the wall of the flask, especially on the side turned toward the light. One of these colonies was removed by means of a sterilized platinum needle and introduced into another Erlemeyer flask containing the same medium. This culture was afterwards used for experiments.

The experiments were carried on in the following way: A hash of fish and one of shrimps was suspended in water, the suspension containing particles not larger than a copepod. Clams were first used for the experiments. A culture of diatoms in sufficient quantity to cause a small, well-defined cloud in the water was offered to them by means of a fine glass tube, the end of which was brought close to the ventral opening of the siphon, care being taken (see figure) not to



touch it. The culture was now allowed to flow through, and soon disappeared in the opening of the siphon. Many such cultures were accepted by the clam, but when similar experiments were made with a hash of fish, the result was either that the opening closed as soon as the particles of fish touched it, or the suspension was accepted as before, but almost as soon as taken it was forcibly ejected and often thrown to a distance of six or seven inches. The shrimp hash was rejected in the same manner.

When soft clams were used, the same results were obtained, and when the hash was brought between the open shells of the oyster, the same phenomena were observed, the suspension being rejected and the shells immediately closed, while the diatoms were readily accepted. Though these experiments were repeated over and over again, I always obtained the same results with a single exception in the case of a soft clam, an individual apparently without a discriminating taste, which accepted a great quantity of the hash, but finally rejected it also.

Besides the diatoms some lower algæ were found to be present in the water, especially near the shore, and I have no doubt that in winter and early spring the reproductive spores of the higher algæ growing on oyster beds will prove to be an additional source of oyster food. I therefore made a list of the algæ found during the time of my stay in places where oysters were living in the James River. It should be borne in mind in connection with this list, however, that my visit was made during the hottest months of a very hot summer, a particularly unfavorable season for the growth of algæ. For this reason the small number of species collected is not to be wondered at.*

Algæ collected.

1. Melanophyceæ.
   - Fucus vesiculosus.
2. Chlorophyceæ.
   - Bryopsis plumosa.
   - Ulva lactuca.
   - Ulva clathrata.
   - Ulva hopkirkii.
   - Cladophora, sp.
   - Entocladia viridis.
3. Florideæ.
   - Dasya elegans.
   - Chondria tenuissima.
   - Polysiphonia variegata.
   - Rhabdonia tenera.
   - Ceramium rubrum.
   - Gracelaria compressa.
   - Polysiphonia urceolata.
4. Cyanophyceæ.
   - Lyngbya, sp.
   - Oscillaria, sp.

Looking back on our results, we see that the oyster lives almost exclusively on diatoms, and it will be well to recall the structure and physiological properties of these low plants. The diatoms are small, microscopic plants, surrounded by a firm membrane having a structure of a small box; that is, consisting of the two halves of the cell wall, one fitting over the other as the cover does over a pasteboard box. These cell walls, formed of cellulose, are incrusted with an enormous quantity of silica, often arranged in beautiful and delicate designs, so that after the soft parts have been destroyed by heating to incandescence, the perfectly clean silicious skeleton remains, showing all its delicate detail of structure. Inside of this cell wall the plasmatic body of the diatom, provided with a nucleus, is seen during life. In some species more or less definite portions, in others the whole plasma, is diffusely tinged with a brownish color. This color is of particular interest to us, for just as the trees, by means of their green coloring matter, are able to convert inorganic into organic matter—that is, animal food—so are the diatoms in the same way by means of their brown color substance. Let us see what this teaches us, and first glance at the economic peculiarities of higher animal life.

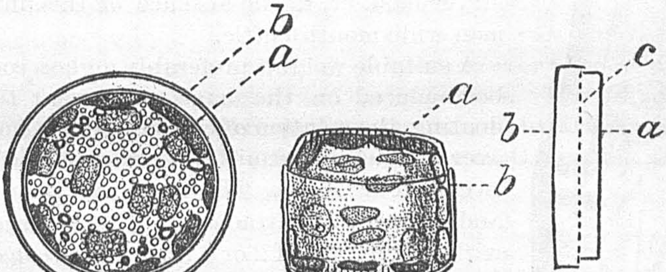
Starting from any animal life, we see that its existence always depends, either directly or indirectly, on the presence of plants, since these alone are able to form organic matter, all animals being destroyers, but never producers, of it. For example, cattle live directly on plants,

*That the flora at Hampton is very much richer is strongly suggested by the fact that in April of this year, during an afternoon walk along the shore, I found in great abundance Phyllitis, Ectocarpus, Pelagella, three genera of which in August no trace was left.

but the lion, devouring the cattle, depends as well on the plants, since without them the existence of his prey would be impossible. Exactly the same thing takes place in the water; the fishes preying on other fishes, these on smaller fishes, these again on other animals. All have to come back finally to animals living on plants. So we see that in the present case our oyster lives directly on plants, and there is no danger, as long as our waters contain the necessary salts for plants to live upon, that the food supply of the oyster will become exhausted, unless, indeed, it should be found that in the embryonic stage the oyster depends upon some more precarious food supply.

One subject of interest remains to be considered, namely, How do diatoms multiply?

This is accomplished as represented in the accompanying figures. The shells *a* and *b* separate as far as possible, so that one fits but slightly over the other. A cross wall *c* is now formed which splits into two, one of these forming the box for each of the two halves. It will be readily understood that in this way every daughter diatom is a little

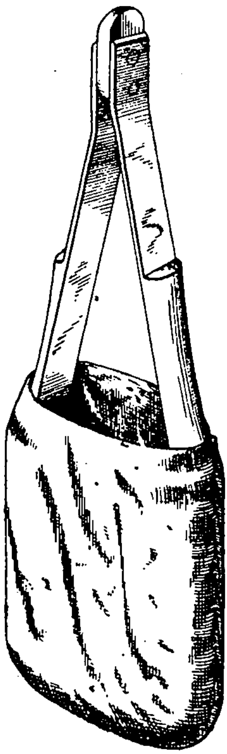


smaller than the mother, since the box of the mother now serves as the cover of the daughter. If this be repeated a certain number of times, the diatoms would finally become too small for existence, but then the small diatom leaves its shell and either simply grows, forming a new cell wall after a certain time, or it finds a mate, the bodies of both merging into one, and in this way the loss of size resulting from this mode of division is compensated.

A SIMPLE METHOD OF STAINING SMALL ORGANISMS.

In the staining of unicellular algæ, diatoms, and the reproductive organs of the higher algæ, as well as many other micro-organisms, the greatest difficulty, as is well known, is encountered in the great loss of specimens entailed by the more or less complicated staining process now in use. The one now commonly employed is as follows: The specimen is hardened in a 1 per cent aqueous solution of chromic acid for twenty-four hours, washed carefully in water until the last trace of the acid is removed, then stained with a solution of carmine. It does not need to be pointed out that by the use of this method it is easy to lose the greater part of the organisms, and the disadvantages of it are increased the more simple the appliances of the laboratory. Hence, during a short stay at the seashore for the purpose of study of these forms, where the equipment of a hastily constructed laboratory is necessarily meager, great inconvenience is experienced.

In order to obviate this difficulty I have used a method which I found both simple and satisfactory. Small bags having the shape represented in the accompanying figure are made of bolting cloth, a fine mesh being used so that the desired organisms can not pass through. The organisms having been removed by means of an ordinary glass tube from the glass dish in which the surface nets were emptied, are now transferred to the bag just described. During this manipulation the little bag is kept open by means of a pair of forceps in the manner indicated, after which the bag is securely closed by tying a string around its mouth. Several bags filled in this way are then placed in an Erlemeyer flask or, in the absence of this, into a common wide-mouth bottle.



A suitable weight, preferably a glass rod, having been placed on the bags to prevent them from floating, the solution of chromic acid is now poured over them and permitted to remain in contact for twenty-four hours. The bags are then removed, and having been attached to a long piece of cord, with an interval of 2 or 3 inches between every two bags, the whole is tied to any convenient object and washed in a stream of water until free from the chromic acid. This usually takes about two hours. The bags are now removed from the water and immersed in the staining fluid for a sufficient time. The excess of stain is washed away in water, and if overstaining has occurred the organisms can be decolorized while still inclosed in the bags by adding a trace of HCl to the water. The bags are now cut open, the stained organisms transferred to a watch glass and mounted. Should they still be overstained they can be further decolorized in the manner stated.