UNITED STATES DEPARTMENT OF THE INTERIOR, J. A. KRUG; Secretary
FISH AND WILDLIFE SERVICE, ALBERT M. DAY, Director

EFFECT OF DIFFERENT CONCENTRATIONS OF MICRO-ORGANISMS ON THE FEEDING OF OYSTERS (*O. VIRGINICA*)

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Fishery Bulletin 42
From Fishery Bulletin of the Fish and Wildlife Service
VOLUME 51

UNITED STATES GOVERNMENT PRINTING OFFICE • WASHINGTON : 1947
For sale by the Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C. Price 20 cents

ABSTRACT

These studies were conducted to learn what effect different concentrations of micro-organisms in sea water may have on the feeding activities of oysters. The micro-organisms were those which are considered as oyster food. Of these, *Chlorella* sp., *Nitzschia closterium* and *Euglena viridis* were most often used. It was found that heavy concentrations of micro-organisms interfered with the feeding of oysters by reducing the rate at which the oysters filtered the water through their gills. The type of shell movement was usually changed when the oysters were kept in water rich in micro-organisms. In very heavy concentrations pumping, and therefore feeding, ceased entirely. A correlation between the density of micro-organisms and the rate of feeding was often noticed. In light concentrations the rate of feeding and shell movements of the oysters remained normal and sometimes the presence of small quantities of plankton in sea water stimulated the pumping activities.

The size of the micro-organisms was found important because a much larger number of small cells, such as *Chlorella*, was needed to produce the same effect as caused by a smaller number of larger cells. Both the cells of the micro-organisms and the products which they released in the water affected the oysters. The cells interfered with the normal function of the gills, while the products which they released contained substances inhibiting the oysters.

The quantities of pseudo feces were usually roughly proportional to the quantities of food cells present in the water, whereas a reverse relationship existed in the formation of true feces. The presence of large quantities of pseudo feces usually indicated that feeding proceeded under the unfavorable condition caused by a heavy concentration of food cells in the water.

The results of the experiments showed that oysters feed efficiently only if the water contains small quantities of suspended matter. Oysters can also feed in water containing a relatively large number of micro-organisms, but under such conditions the rate of feeding is decreased.

EFFECT OF DIFFERENT CONCENTRATIONS OF MICRO-ORGANISMS ON THE FEEDING OF OYSTERS (*O. VIRGINICA*)[1]

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Since the middle of the last century, when cultivation of oysters became widely practiced in Europe and in this country, many interesting problems on the ecology and physiology of these mollusks have attracted the attention and efforts of biologists. The questions which from the very beginning aroused widespread interest were those dealing with the food and feeding of oysters. Numerous articles, discussions, and reviews of literature on these subjects have been since offered by many workers.[2]

Regardless of extensive studies there still remain several unsolved basic problems on the feeding of oysters. Of these, the one which deals with the relative efficiency of oysters feeding in clear or turbid water caused a strong controversy, but no conclusive evidence has been presented so far to uphold any of the theories advanced.[3] Also, in referring to turbidity of water it was not made clear whether it was due to the presence of rich plankton or to suspended particles of detritus and silt. Neither were any critical experiments made to determine whether there may be a correlation between the density of plankton population and the quantities of water pumped by the oysters. To clarify at least certain aspects of this complex problem we made a study of the effects of different concentration of micro-organisms upon the rate of pumping and, therefore, feeding of oysters and on the shell movements of these mollusks. While this will remain the chief theme, certain related problems will also be included in the scope of our discussion.

The oyster obtains its food by straining water through its gills and retaining the food particles, which are later directed toward the mouth. Thus, in addition to the respiratory and excretory functions the gills of the oysters also play a very important part in feeding. The complex structure and the functions of the oyster gills have been well described by a number of investigators.[4] An oyster can feed only if its shells are open and the entrance to the branchial cavity is not obstructed by the edges of the mantle. The rate at which the water passes through the oyster is controlled by the beat frequency of the lateral cilia of the gills, by the expansion and contraction of the gill ostia, the position of the edges of the mantle, and by the activity of the adductor muscle. The lateral cilia, which form rows along the filaments of the gills, propel the water into the passages of the gill lamellae. The water is then passed through the supra-branchial chambers and finally reaches the cloacal or promyal chamber from which it is dis-

[1] Approved for publication November 7, 1946. Fishery Bulletin 42.

[2] Martin 1923; Churchill and Lewis 1924; Savage 1925; Yonge 1926; Nelson 1938.

[3] Kellogg 1915; Grave 1916.

[4] Kellogg 1915; Yonge 1926; Nelson 1938.

NOTE.—We wish to express our thanks to Miss Frances Tommers and Messrs. William Arcisz and Charles Nomejko for their assistance during these studies and for analysis of the experimental data.

charged. The quantity of water leaving the oyster can be measured by the method described in this paper, thus the rate of pumping and, therefore, feeding can be easily estimated.

Biologists disagree about the effect of different quantities of material suspended in water upon the efficiency of feeding of oysters and related species. Kellogg (1915), after extensive studies of the arrangement and movements of the cilia of the gills, palps, and mantle cavities of a large number of lamellibranchs, concluded that these animals are able to feed only when the water is comparatively clear. He wrote, "It is my belief, after a good many years of observation, that lamellibranchs are able to feed only when the surrounding water is relatively free from solid particles; just how free, in a given case, I am not able to say, and the difficulties in determining the matter are great if not insurmountable." Kellogg concluded that the volume of food organisms and other particles suspended in the water determines whether the "collected foreign matter that reaches the palps shall proceed to the mouth or be removed from the palps." Grave (1916), on the other hand, disagreed with Kellogg's conclusions offering evidence that some food was found in the stomachs of oysters that were kept in very turbid water. Nelson (1921), Churchill and Lewis (1924), and later Yonge (1926) upheld Grave's opinion.

Yonge (1936) in his paper on the evolution of the swimming habits of lamellibranchs suggested that oysters evolved from ancestors that lived in very clear water and that the descent of some forms of lamellibranchs into more turbid waters was made possible by the evolutionary development of a more efficient cleansing mechanism involving not only the gills, palps, and mantle, but also the adductor and velum. He thinks that the possession of such efficient mechanisms made it possible for *O. virginica* and *O. angulata* to extend into muddier waters than *O. edulis*. Elsey (1935) found that *O. gigas* withstands existence in turbid water much better than *O. lurida*, which closely resembles *O. edulis*.

Dodgson (1928) working in England with *Mytilus edulis* also arrived at a conclusion directly opposite to that of Kellogg (1915). He maintained that mussels will feed and pass true feces when immersed in water so turbid that the animals are invisible at the depth of 2 to 3 inches. Fox, Sverdrup, and Cunningham (1937) in studying the rate of pumping by the California mussel, *M. californianus*, found that this animal also was capable of swallowing large amounts of calcareous mud from water containing a very heavy suspension of this material.

Churchill and Lewis (1924) studying very young fresh-water mussels, *Lampsilis luteola*, also noted that they could ingest particles of food and debris even when kept in very muddy water. These investigators think that mussels can and do feed when the water is heavily loaded with suspended material. More recently, however, Ellis (1936) showed that fresh-water mussels are at a distinct disadvantage if exposed to silt-laden waters. In his experiments, mussels retained in silt-free water kept their shells closed less than 50 percent of the time, while those in turbid water remained closed from 75 to 95 percent of the time.

The question of whether or not oysters and other lamellibranchs exercise selective power in ingesting plankton and small particles of detritus, or silt, is also a subject of controversy. Kellogg (1915) was one of the first to point out that these mollusks ingest small particles, regardless of their food value. Yonge (1923), Churchill and Lewis (1924), and Nelson (1924) expressed opinions which, in general, support Kellogg's contention. In the latter paper on this subject Yonge (1926) states that "Nothing but a purely mechanical or quantitative selection has been found in the oysters." In the same article Yonge says, however, that some lamellibranchs, such as *Syndosmya*, *Tellina*, or *Gari*, may exercise a certain qualitative selection of their food. Some protobranchs may do the same. Yonge points out, however, that in both cases such a qualitative selection, if occurring, takes place outside the mantle cavity.

The opposing theory, namely, that oysters and mussels possess the ability to select those particles which have definite food value, was advanced by Lotsy (1895), Allen (1914), and especially by Grave (1916), who maintained that oysters exercise a considerable choice in food material by reversing the direction of the beating of certain groups of cilia of the palps.

MATERIALS AND METHODS

The animal used in our work was the common American oyster, *Ostrea virginica*, of Long Island Sound. Only healthy individuals, varying from 4 to 6 years of age, were selected.

The micro-organisms, the effect of which we have studied, were (a) a green alga—*Chlorella* sp.; (b) a diatom—*Nitzschia closterium;* (c) a dinoflagellate—*Prorocentrum triangulatum;* and (d) a euglenoid—*Euglena viridis* (Loosanoff and Engle, 1942, 1944).[5] The forms selected were representative of four very large groups of organisms, which are regarded as constituting the bulk of the oyster food. Furthermore, each of the above-mentioned organisms had been considered by at least one author as playing an important part in the oyster diet. For example, Martin (1928) used *Chlorella* and *Nitzschia* in his experiments on the feeding of oysters, regarding them as very important sources of oyster food. He (Martin 1929) also found that *Prorocentrum triangulatum* is extremely common in Barnegat Bay, Delaware Bay, and Chesapeake Bay, where it is sometimes the most abundant organism in the stomach of the oyster. In still another article Martin (1927) stated that *Euglena* is digested by oysters.

The size of the forms used in our experiments varied from 5 microns (*Chlorella* sp.), to 60 microns (*Euglena viridis*). This difference helped to judge whether or not the size of the food organisms may have a certain effect, or effects, on the rate of pumping.

A method of cultivating micro-organisms, which we recently developed (Loosanoff and Engle 1942a), permitted us to grow the large quantities of plankton necessary for these studies. In some of our experiments as much as 100 gallons of culture were used daily. Because of the large quantities of food material needed it was impossible to keep the cultures quite bacteria-free, or even single-specied. Nevertheless, after some experience, we found it comparatively easy to grow mass cultures which were composed of practically one species.

We determined the number of organisms in the cultures with the Yoe photoelectric colorimeter. A detailed description of this apparatus and its uses was given by its designers[6] and, therefore, need not be repeated here. It will suffice to say that since in our determinations micro cells of 5.0 cc. capacity were used, the reading of the blank on the microammeter was set, as recommended by the designers, on the 25 mark instead of the 50.

[5] These forms were identified by Dr. James B. Lackey of the United States Public Health Service, to whom we wish to express our appreciation.

[6] Yoe and Crumpler 1935.

Before using the colorimeter in our experiments it was necessary to construct the curves for the conversion of the microammeter readings into the numbers of cells per cubic centimeter. We counted the number of cells per cc. of strong cultures and many of their dilutions using the Sedgwick-Rafter cell for *Nitzschia* and *Euglena*, and the Hellige haemocytometer for *Chlorella*. At least 10 counts were made for each dilution. Simultaneously with each count the reading was taken from the microammeter of the colorimeter. Finally, enough data were obtained to construct a curve by means of which the microammeter readings could be converted into the number of cells per cc. This method proved very rapid and accurate, extremely simplifying the work of determining the number of micro-organisms in the samples. Since our studies were largely confined to *Chlorella* sp., a curve used for the conversion of the microammeter readings into the numbers of *Chlorella* cells per cc. is given in figure 1.

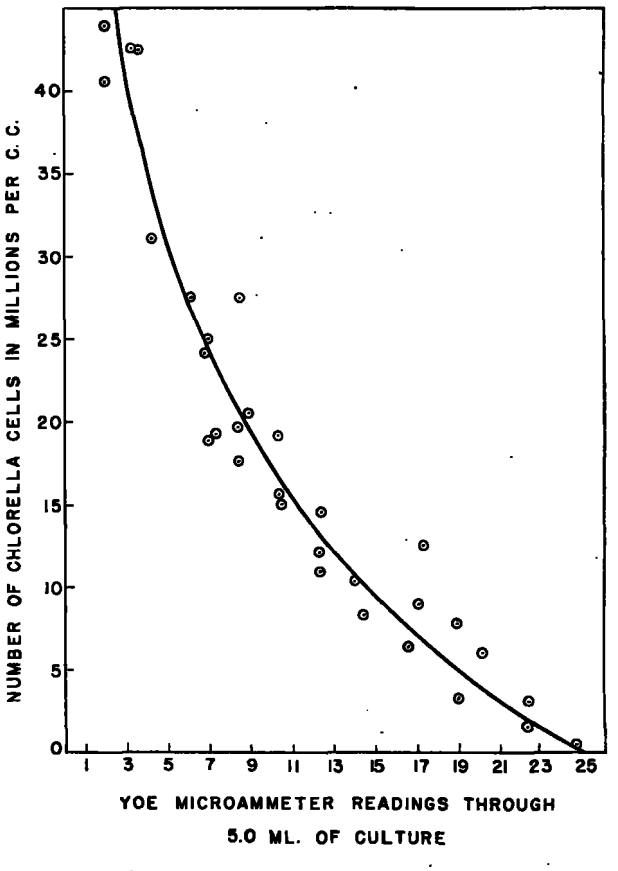

FIGURE 1.—Curve for the conversion of the microammeter readings into the number of *Chlorella* cells per cc.

Because only a few experiments were carried on with *P. triangulatum* no curve was constructed for this species. In using this form the relative density of the population of its cultures was determined with the colorimeter. Cultures, the microammeter readings of which were above 23, were considered as light, while those below that mark were regarded as medium and heavy.

As the success of our studies depended upon the employment of proper experimental methods, we give here a detailed description of the series of apparatus used. We do not claim credit for originality in devising all the apparatus, because several were made in accordance with well known mechanical principles, or represented modified and improved types of the models employed by other investigators. In some instances prototypes of apparatus have been used for many years. For example, the oyster chamber was first used by Galtsoff, while use of the apron was suggested by Moore (1908) and later employed by Nelson (1936). We would like to mention, however, that the series of apparatus, description of which we give, was used in our laboratory since 1937. Much of our apparatus, such as the constant-level chamber, which we devised, and the tripping vessel, which represents considerable improvement over the old types was made here and often loaned to other investigators working on problems, such as the reaction of oysters to chlorination and the various aspects of feeding of oysters.

The entire series of the apparatus is shown diagrammatically in figure 2. A group of six 15-gallon aquaria, *A*, connected with each other by

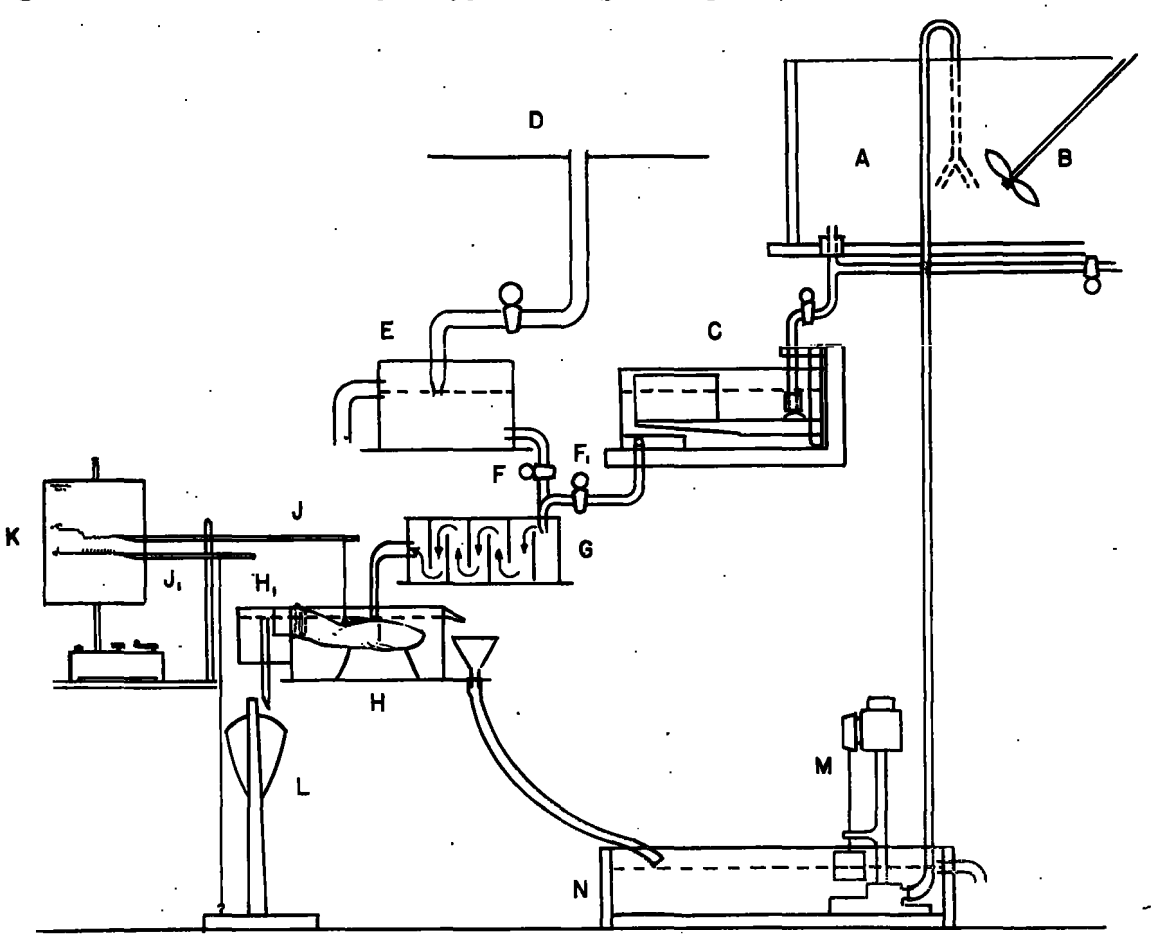

FIGURE 2.—Diagram of the series of apparatus employed to record the rate of water pumping and shell movements of the oyster. *A*—aquarium; *B*—stirrer; *C*—constant level unit; *D*—sea water storage tank; *E*—overflow chamber; *F* and F_1—flow adjusting valves; *G*—mixing trough; *H* and H_1—two compartments of the oyster chamber; *I*—oyster; *J*—lever recording shell movements of the oyster; J_1—lever recording movements of the tripping vessel *L*; *K*—kymograph; *L*—tripping vessel; *M*—pump; *N*—basin.

rubber tubes, was used as the reservoir for food cultures. By manipulating the clamps, or stop cocks, the aquaria could be used singly or in groups. When working with the organisms which had a tendency to settle on the bottom, the content of the aquaria was agitated by an electrical stirrer, *B*. Culture from the aquaria flowed into the constant-level unit, *C*, provided with an automatic float-control valve. This unit, which we devised, was so constructed that a steady level of liquid was constantly maintained within it. This device eliminated any waste of culture material, which would have occurred if a simple constant head overflow chamber had been used.

Sea water, pumped into a 5,000-gallon storage tank, *D*, located in the attic of the laboratory building, flowed into the overflow chamber, *E*, which was provided with an outlet, and thus also maintained a constant level. A uniform flow of culture or sea water, or any combination of the two, could be obtained by adjusting valves *F* and F_1. The fluids then entered the first compartment of the mixing trough, *G*, in which they were homogenized. We made the mixing trough in accordance with the principle commonly employed for the mixing and aeration of water flowing through the trough used in hatching salmon or trout eggs. The trough was divided into compartments by several dams which were so arranged as to cause the mixture to flow over the top of the first dam, then under the next dam, and again over the succeeding dam, repeating this process throughout the entire length of the trough. We found our trough much more satisfactory than other types, where stratification of liquids was often noted. The mixture finally entered the oyster chamber, *H*, containing the experimental oyster, *I*, the excurrent side of which was covered with a rubber cone-shaped apron that led the water pumped by the oyster into the smaller chamber, H_1. A silk string glued to the shell of the oyster, *I*, was attached to the counter balanced lever, *J*, which recorded every movement of the valve on the kymograph, *K*.

The water pumped by the oyster into chamber H_1, overflowed through the glass tube standpipe into the tripping vessel, *L*, of known capacity. When the vessel was filled with the water pumped by the oyster it tripped over, emptying its content, and at the same time striking a string attached to the lever, J_1, which touched the kymograph, *K*. Thus, each tripping was recorded, and because the capacity of the vessel was known, the quantity of water pumped by the oyster during certain intervals could be easily determined.

On rare occasions, when the quantity of culture was limited and it was necessary to save the overflow from the oyster chamber, *H*, an automatic sump pump, *M*, placed in a basin, *N*, designed to catch the overflow, returned the liquid to the culture reservoir, *A*.

Following is a more detailed description of some of the units that were briefly outlined above:

The constant level unit (*C* in fig. 2) is shown in detail in figure 3. The fluid from the culture reservoir flows through the intake tube, *A*, into the main chamber, *B*. As the level of the liquid in the

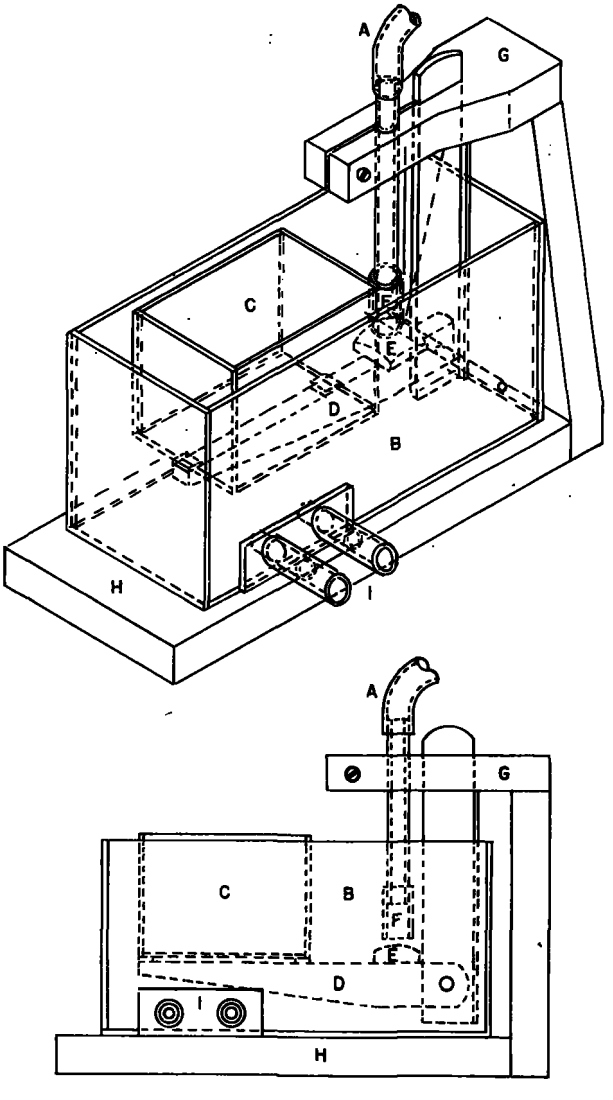

FIGURE 3.—Constant-level chamber. Description in text.

chamber rises, the float, C, attached to the hinged lever, D, also rises and finally, when a certain level is reached, pulls the valve seat, E, tightly against the rubber valve, F, which is then closed and the flow of liquid stopped. The position of the valve is adjustable by moving the intake tube up or down and by holding it in the desired position by a clamp, G, which is part of the base that holds the whole unit. The liquid is drawn from the chamber through the outlet tubes, I, to the mixing trough (G in fig. 2).

The details of the oyster chamber are shown in figure 4. The fluid from the mixing trough flows

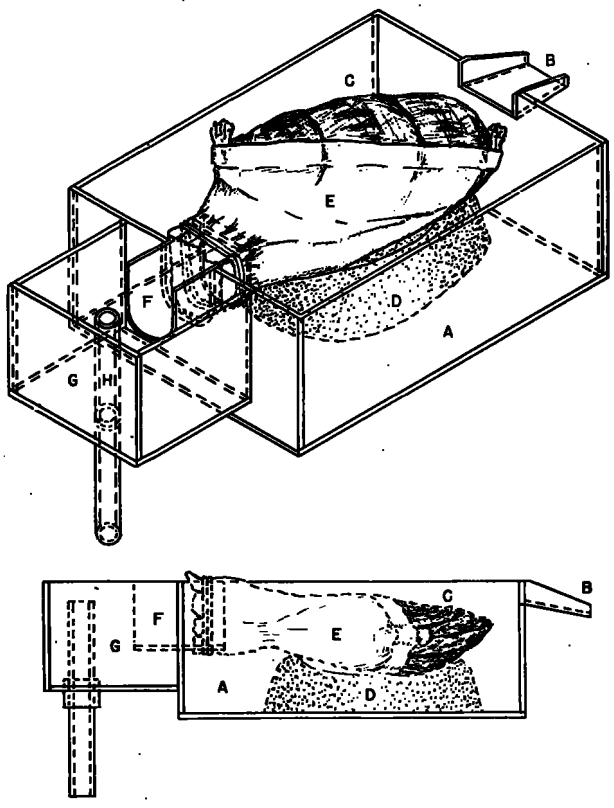

FIGURE 4.—Oyster chamber. Description in text.

into the oyster chamber, A. The excess of fluid not utilized by the oyster leaves the chamber through the opening, B. The oyster, C, lying with its flat valve uppermost on the concrete base, D, has its excurrent side enclosed in a rubber cone or apron, E, that is tightly attached to the trough, F, which leads the water pumped by the oyster into the overflow chamber, G. A standpipe, H, is adjusted in height to a level slightly above that of level, B, of the oyster chamber. The proper adjustment of the height of the standpipe is very important to prevent a syphoning effect from the oyster chamber to the overflow chamber. The sensitivity of the standpipe must be such that a few drops of water added to the chamber, G, will immediately start an overflow through the pipe.

In measuring the rate of water flow the correct results depended upon the principle that the flow of water between the oyster chamber, A, and the overflow chamber, G, could take place only through the interior of the oyster and be caused only by the pumping activities of the animal. Therefore, we took every precaution to avoid leakage of water from one chamber to the other by properly attaching the apron, E, to the trough, F, and to the oyster. The water-tight connection between the apron and the trough was easily made by using a heavy rubber band, which presses the apron to the sides of the funnel connecting the two chambers. Attachment of the apron to the oyster was, however, more complicated and, therefore, requires a more detailed description. We offer the steps of this operation below:

1. The surface of the shell was scrubbed clean.
2. When dry the shell was washed with alcohol, and again allowed to dry. Care was taken to keep the alcohol from entering the interior of the oyster.
3. A layer of liquid rubber cement was spread on the shell along the line where the apron was to be attached. This line extended around the long axis of the oyster.
4. A strip of one-half-inch rubber tape was pressed firmly against the shell along the line of the applied rubber cement, except at the points where the tape made the turns around the hinge and bill of the oyster.
5. Cotton plugs were inserted between the bill of the oystershell and the tape, and between the hinge and the tape. This arrangement allowed for freedom of shell movement and yet maintained a leak-proof joint.
6. When the rubber tape was glued tightly to the shell a layer of rubber cement was put on its upper surface. Then a piece of dental-rubber dam, cut long enough to fit completely around the oyster with a little overlapping for joining the ends to form the cone, was glued to the rubber tape. The rubber cone covered only the excurrent part of the oyster, while the incurrent side was left free. The small end of the rubber cone was attached and by a rubber band was held tightly to the trough connecting the oyster and the overflow chambers.

Our type of tripping vessel (*L* in fig. 2) is shown in figure 5. The liquid from the overflow chamber (*G* in fig. 4) is led by the standpipe (*H* in fig. 4) to flow into the main receptacle, *A*, of the tripping vessel. As the liquid rises, a float, *B*, also rises until the arm, *C*, clears the notch in the side of the main receptacle and the top-heavy vessel trips over spilling out its water content. The vessel is returned to the normal upright position by the pull of the counter weight, *D*. As the vessel goes through the partial revolution which empties it, the silk string, *E*, is touched thus making a mark on the kymograph record. The vessel is suspended over the base, *G*, by two adjusting screws, *F*.

The sea water overflow chamber is of relatively simple construction (fig. 6). The water flows in the chamber, *B*, through the spigot, *A*, and is fed to the experimental animals through the outlets, *D*. The excess water overflows through the pipe, *C*.

We made all the apparatus from sheets of cellulose acetate, commonly known as plastocoele. The shavings of this material dissolved in acetone served as glue.

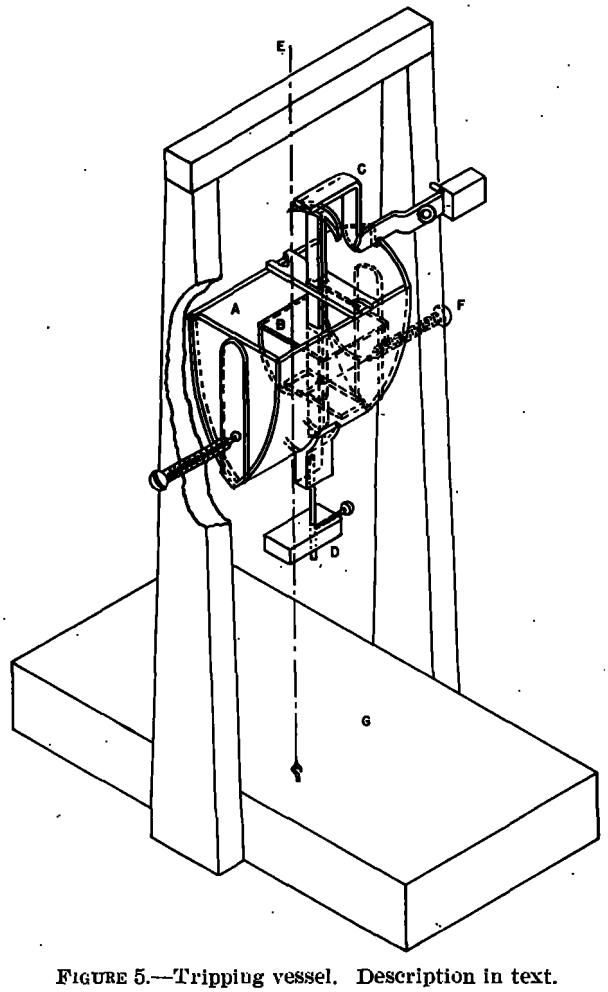

FIGURE 5.—Tripping vessel. Description in text.

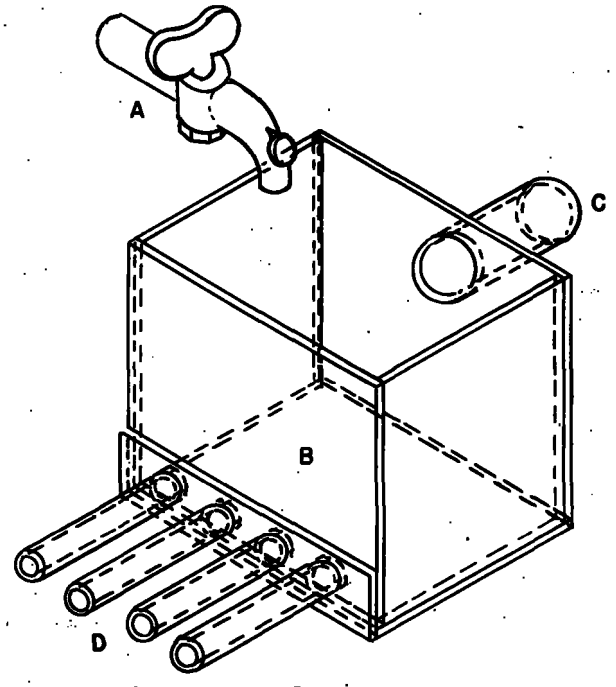

FIGURE 6.—Sea-water overflow chamber. Description in text.

The kymograph drums usually rotated at the rate of 1¼ inches per hour. When necessary, an interval timing device was also connected to the kymograph. The records obtained provided information regarding the shell movement of the oysters and the rate at which the animals pumped the water. A section of such a record is given in figure 7.

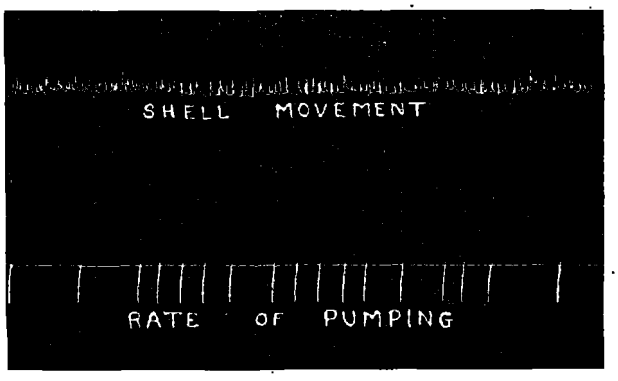

FIGURE 7.—Section of kymograph record. Upper line shows the shell movements of the oyster, and the lower indicates the rate of pumping. Each vertical line of the lower record designates the emptying of the tripping vessel of 239 cc. capacity.

EXPERIMENTS AND OBSERVATIONS

PRELIMINARY EXPERIMENTS

At the beginning of this work it was our opinion that oysters kept under favorable conditions would "fatten" if given large quantities of food. Accordingly, we placed oysters in large, outdoor, tide-filled tanks to which rich, mixed cultures of micro-organisms were added. Regardless of such presumably favorable conditions, the oysters failed to show improvement and usually became poorer than the control animal kept in the tank with ordinary sea water. Sometimes, when very large quantities of food organisms were added to the water, the oysters became sick and many died. Examination showed that the stomachs of the oysters that were still alive were empty and that the crystalline style was absent.

At first we could not suggest the direct cause of the poor condition of the oysters but, as our studies progressed, we began to suspect that large quantities of micro-organisms in the water were interfering with the normal feeding behavior of the mollusks. To verify this suspicion we made a preliminary series of experiments to determine the effect of micro-organisms of various types and in different concentrations upon the oysters.

In all our experiments we kept the salinity and temperature of the cultures equal to or closely approaching those of sea water. In some cases it was also found necessary to control the pH of the cultures. This was easily accomplished by regulating the intensity of the light striking the glass walls of the aquaria containing the cultures. By placing the culture in direct sunlight its pH would be quickly increased, whereas by shading the aquaria the pH could be reduced. As a rule, however, the pH of the cultures ranged from 7.8 to 8.2, thus closely resembling that of the sea water used in the experiments.

In the first, rather exploratory series of experiments, we exposed the oysters for periods ranging from 20 to 52 hours to a constant flow of sea water containing *Chlorella* cells in different concentrations. Before that, however, we kept the oysters for at least 12 hours in running sea water to obtain a record of their activities under normal conditions. The change from ordinary sea water to that containing the cultures was always accomplished by simultaneously shutting off the flow of one and turning on the other. Thus the change was accomplished without disturbing the experimental oysters.

The numbers of the *Chlorella* to which the oysters were subjected ranged from about 1½ to 5½ million cells per cc. of sea water. We noticed that in the concentrations of approximately 2.0 million cells per cc. and heavier the oysters became affected (fig. 8). The rate of pumping, i. e., feeding

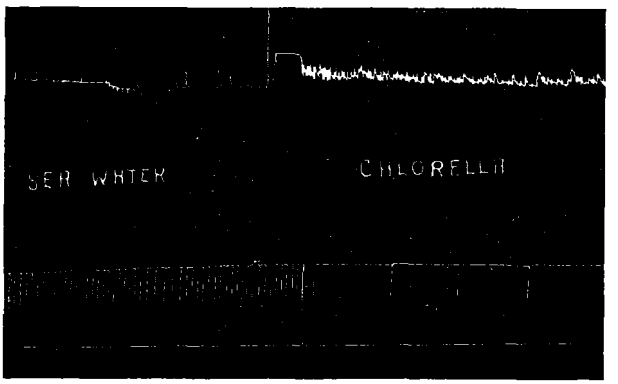

FIGURE 8.—Changes in shell movements and in the rate of pumping of the oyster when ordinary sea water was substituted with that containing approximately 5 million *Chlorella* cells per cc. Each vertical line of the lower record represents 244 cc. of water pumped by the oyster.

markedly decreased, as compared with the rate of pumping of the same oysters before they were exposed to *Chlorella*. This decrease varied from 69 to 90 percent in concentrations of about 5½ million cells per cc., and in somewhat lighter concentrations, between 2 and 3 million cells per cc., it ranged from 19 to 56 percent.

The kymograph records of the shell movements of the oysters showed that exposure to a concentration of 5½ million cells of *Chlorella* per cc. did not decrease the time the oysters kept their shells open. They indicated, nevertheless, that the type of shell movement during the exposure noticeably differed from that observed while the oysters were kept in running sea water (fig. 8).

During the periods when the oysters exposed to the concentrations of *Chlorella* showed a decrease in the rate of flow and a change in the type of shell movement, the control oysters kept in running sea water functioned normally.

After observing that the strong concentrations of *Chlorella* depressed the feeding activities of the oysters and changed the character of their shell movements we decided to determine whether com-

paratively light concentrations would have the same effect. We subjected 10 oysters first to a flow of sea water and then to a weak culture of *Chlorella* of approximately 500,000 cells per cc. We found that in about half the cases the rate of pumping somewhat increased when the oysters were kept in weak *Chlorella* culture, but in the others a slight decrease was seen. Some oysters showed no appreciable change in the rate of pumping when sea water was substituted with a weak *Chlorella* culture (fig. 9). The change from one condition to another did not change the type of the shell movements of the oysters. The behavior of the control oysters was not basically different from that of the experimental ones. Therefore, we concluded that light concentrations of *Chlorella*, such as 500,000 cells per cc., did not affect the oysters unfavorably.

FIGURE 9.—Kymograph record showing that no appreciable change in the shell movements and the rate of pumping of the oyster occurred when the flow of sea water was substituted with that of weak *Chlorella* of 500,000 cells per cc. Each vertical line of the lower record represents the emptying of the tripping vessel of 265 cc. capacity.

Practically the same results were obtained when the oysters were exposed to light concentrations of *Nitzschia* of 20 to 30 thousand cells per cc., and to *Prorocentrum triangulatum*.

EXPOSURE OF OYSTERS TO INCREASING AND DECREASING CONCENTRATIONS OF MICRO-ORGANISMS

In the next series of experiments we subjected the oysters to gradually increasing concentrations of *Chlorella*. As usual, to obtain a record of the normal behavior of the oysters they were first exposed to a flow of sea water. The average quantity of water pumped hourly by each oyster during that period was found and accepted as normal, or 100 percent. Any deviations in the rate of pumping in subsequent exposures were estimated in relation to that figure. After the exposure to sea water the oysters were subjected for hourly periods to increasing concentrations of *Chlorella* ranging from approximately 2 to 13 million cells per cc. (fig. 10). The different concentrations were

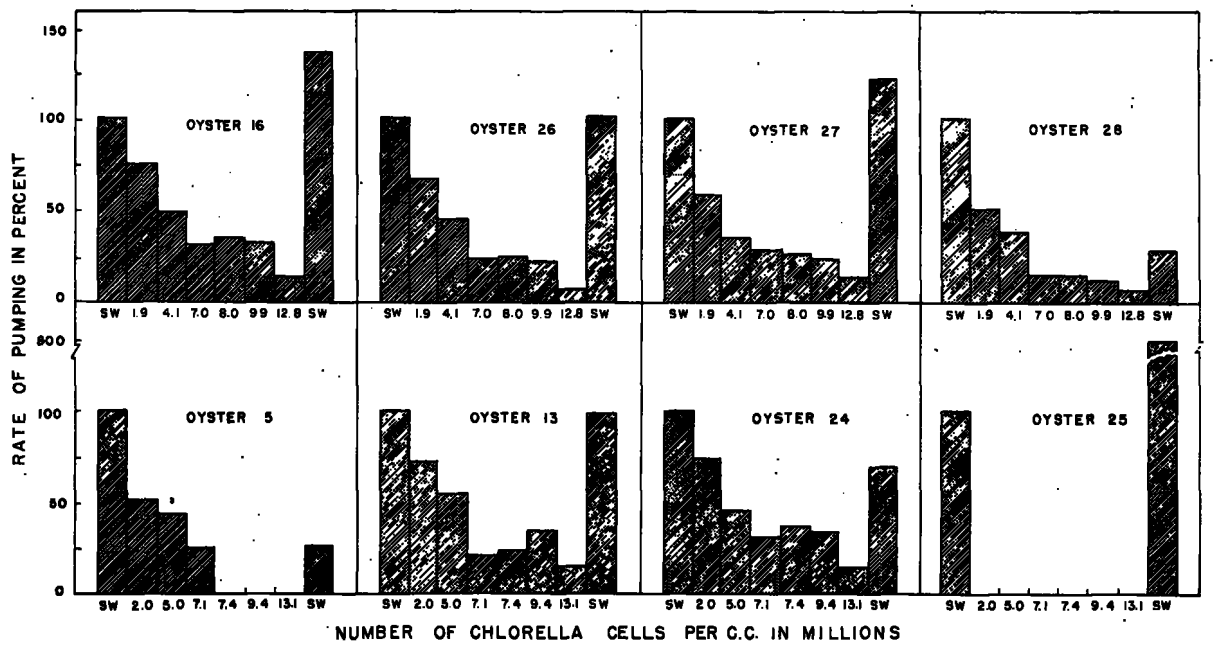

FIGURE 10.—Histogram showing rate of pumping (percentage) of eight oysters exposed to increasing concentrations of *Chlorella*. Rate of pumping in sea water (SW) at the beginning of the experiment was taken as 100 percent.

made by diluting the original heavy culture with the needed quantities of sea water.

Upon the change from ordinary sea water to that containing about 2 million cells of *Chlorella* per cc. the rate of pumping of the oysters sharply declined. Some oysters, as oyster No. 25, ceased to pump entirely, although the shells remained open (table 1) and moving (fig. 10). In other oysters the rate of pumping was reduced from 52 to 76 percent of the original rate.

Subsequent increases in the density of the *Chlorella* resulted in further decreases in the rate of pumping. In general, the decrease became more pronounced as the concentration of the cells increased. In the heaviest concentration, which was approximately 13 million cells per cc., several oysters, although open, did not pump any water. In other oysters the rate of pumping was only from 8 to 21 percent of that recorded in sea water, before the oysters were subjected to water containing *Chlorella*.

TABLE 1.—*Percentage of time oysters remained open when subjected to increasing concentrations of Chlorella*

Number of cells per cc.	Temperature ° C.	Percentage of time open			
		Oyster number			
		16	26	27	28
Sea water	22.0	100	100	100	100
1,900,000	21.4	100	100	100	100
4,125,000	21.2	100	100	100	100
7,000,000	21.2	100	100	100	100
8,000,000	21.0	100	100	100	100
9,875,000	20.0	100	100	100	100
12,750,000	20.0	100	39	75	75
Sea water	20.5	100	100	100	100
		Oyster number			
		24	5	25	13
Sea water	22.0	100	100	100	100
2,000,000	22.0	100	100	92	100
5,000,000	21.8	100	100	58	100
7,125,000	22.0	100	78	17	58
7,375,000	21.8	100	0	75	58
9,375,000	21.6	100	0	0	100
13,125,000	21.0	77	15	0	64
Sea water	21.3	100	100	100	100

When at the end of the exposure to *Chlorella* the oysters were again in sea water their rate of pumping usually showed a marked increase (figs. 10 and 11). Often they pumped more vigorously than during the period at the beginning of the experiment when their normal rate of pumping was ascertained. Such intensive pumping suggested that the oysters tried to cleanse themselves of the cells which accumulated in the gills and

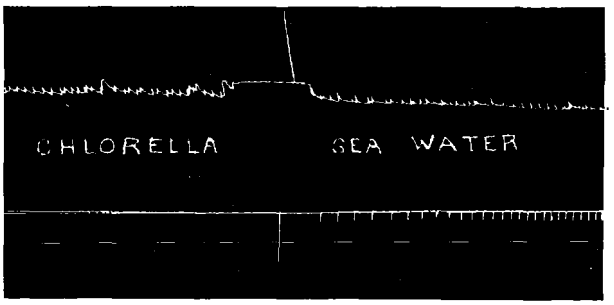

FIGURE 11.—Section of the kymograph record showing the resumption of rapid pumping by the oyster when heavy *Chlorella* culture of approximately 13 million cells per cc. of sea water was substituted. Note that while in the *Chlorella* culture the shells of the oyster were moving although no water was being pumped. Each vertical line of the lower record represents 246 cc. of water pumped by the oyster.

mantle chamber. The cleansing action, as shown by very fast pumping, became evident almost immediately after the oysters were returned to sea water, but reached its maximum intensity somewhat later.

Exposing the oysters to increasing concentrations of *Chlorella* did not immediately change the percentage of time the shells remained open (table 1). However, as the concentrations were increased, the shells of several oysters were closed for some time, and in the heaviest concentration the time the shells remained open was reduced in almost every case. Nevertheless, no definite correlation was found between the increase in the number of *Chlorella* cells and the time the oyster shells remained open.

In the next series of experiments the process of exposure of the oysters to different concentrations of *Chlorella* was reversed. The oysters, after having been kept in sea water for several hours, were subjected to decreasing concentrations of the strength indicated in figure 12 and table 2. They were kept in each concentration for a period of 1 hour.

The first concentration, used immediately after the sea water, was very heavy, containing approximately 16 million cells per cc. (fig. 12 and table 2). The contact of the oysters with such a heavy cell population always resulted in a rapid and marked decrease in the rate of pumping. Some oysters ceased pumping entirely, as if they were unable to cope with the mass of cells present in the surrounding water (fig. 13). Apparently the initial

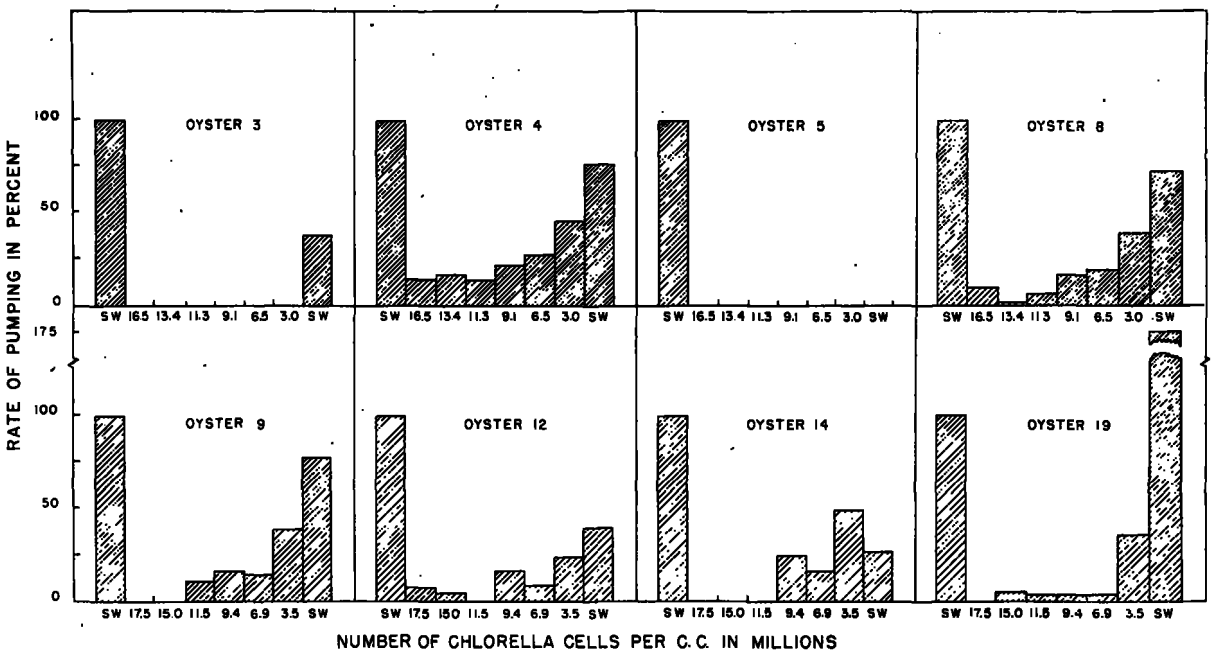

FIGURE 12.—Histogram showing rate of pumping (percentage) of eight oysters exposed to decreasing concentrations of *Chlorella*. Rate of pumping in sea water (SW) at the beginning of the experiment was taken as 100 percent.

shock received by the oysters had a lasting effect, because many of them kept their shells closed, or were open but not pumping any water for several hours, although, meanwhile, the concentrations of *Chlorella* were gradually reduced (fig. 12).

Some oysters, the pumping activities of which were stopped by the initial strong concentration, later showed an increase in the rate of pumping, which roughly corresponded to the decrease in the number of *Chlorella* cells (fig. 12, oysters Nos. 4, 8, and 9). The increase was especially noticeable in the weakest concentration tried, which was approximately 3 million cells per cc. However, the quantities of water pumped during that period were much smaller than those recorded at the beginning of the experiment when the oysters were kept in sea water.

Following the weakest concentration of *Chlorella*, sea water was again flowed into the chambers

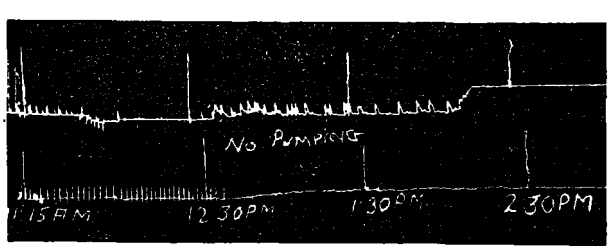

FIGURE 13.—Section of the kymograph record showing cessation of pumping and the change in the type of shell movements of the oyster when sea water was substituted at 12:30 p. m. with a flow of *Chlorella* culture containing approximately 16 million cells per cc. Each short vertical line of the lower record represents 259 cc. of water pumped by the oyster.

TABLE 2.—*Percentage of time oysters remained open when subjected to decreasing concentrations of Chlorella*

Number of cells per cc.	Temperature ° C.	Percentage of time open			
		Oyster number			
		3	4	5	8
Sea water	22.9	100	100	100	100
16,500,000	22.7	100	100	67	100
13,375,000	20.2	75	100	0	17
11,250,000	20.9	0	100	33	75
9,125,000	22.1	0	100	0	100
6,500,000		0	100	0	100
3,000,000	22.3	0	100	0	100
Sea water	22.9	100	100	100	92
		Oyster number			
		9	12	14	19
Sea water	21.2	100	92	83	75
17,500,000	18.8	0	79	0	0
15,000,000	18.7	0	8	0	13
11,500,000	19.7	9	0	0	100
9,375,000	20.4	100	42	67	100
6,875,000	20.9	100	100	60	100
3,500,000		100	100	97	100
Sea water	21.9	100	100	50	100

containing the oysters. As soon as the water replaced the culture, the oysters resumed a rapid rate of pumping. However, in the majority of oysters the rate of pumping, although rather rapid, did not equal or exceed that recorded in sea water at the beginning of the experiment (fig. 12). We ascribed this to a prolonged exposure to dense concentrations of *Chlorella* which probably temporarily affected the pumping mechanism of the oysters.

Our observations on the shell movements of oysters in these experiments, as well as in many other studies of a similar nature which we will describe later in this article, showed two facts of considerable significance. First, we noted that sometimes the shells remained open for long periods, often lasting several hours (table 2), although the oysters did not pump any water (fig. 13). These observations showed conclusively that, although the shells of the oysters may be open, this fact cannot be always interpreted that the oysters are actively feeding. Hopkins (1933) working with *Ostrea gigas* was the first to observe that sometimes, when the shells of the oysters are wide open, no flow of water is produced. He found that in most of such instances the entrance to the branchial cavity is completely closed by the borders of the mantle. Under such a condition the animals are incapable of pumping water and, therefore, feeding.

Our second observation concerned the change in character of the shell movements of the oysters after sea water was substituted with *Chlorella*. We noted that the shell movements usually became of greater amplitude and of a type different than that observed in the same individuals under normal conditions (fig. 13). Our numerous records showed that this type of shell movement was always made when the oysters were kept in heavily laden water and were ejecting large quantities of pseudo feces at frequent intervals. Incidentally, this type of shell movement closely resembles that shown by Nelson (1921) in his figure 3, which he interprets as the type indicating active feeding of oysters. Actually, as is seen on the kymograph record shown in our figure 13, the rate of feeding of oysters may be markedly reduced or even entirely stopped when the shell movements are of this type.

Experiments in which we used *Nitzschia closterium* fully substantiated and corroborated all general conclusions formed during the studies of effects of *Chlorella* upon the rate of pumping and character of the shell movements of oysters. We found that the concentrations of *Nitzschia* of less than 70 to 80 thousand cells per cc. usually did not adversely affect the activities of the oysters (fig. 14). Heavier concentrations, however, not only

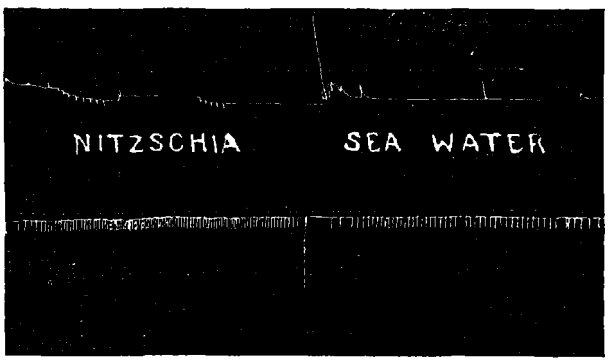

FIGURE 14.—Section of the kymograph record showing that a light concentration of *Nitzschia* of approximately 30,000 cells per cc. did not depress the pumping rate of the oyster. In this case the rate of pumping in *Nitzschia* was somewhat greater than in sea water. Each vertical line of the lower record represents 246 cc. of water pumped by the oyster.

reduced the rate of flow but also affected the character of the shell movements (fig. 15). Data of the same nature were obtained when *Prorocentrum* cultures were used (fig. 16).

EXPERIMENTS WITH *EUGLENA VIRIDIS*

We devoted the next series of experiments to studies of the behavior of oysters in different con-

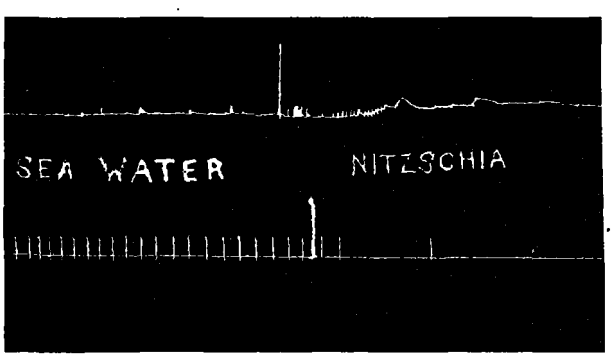

FIGURE 15.—Section of the kymograph record showing a very prominent reduction in the rate of pumping, and a change in the type of shell movements of the oyster subjected to a heavy concentration of *Nitzschia* of approximately 200,000 cells per cc. Each vertical line of the lower record represents 310 cc. of water pumped by the oyster.

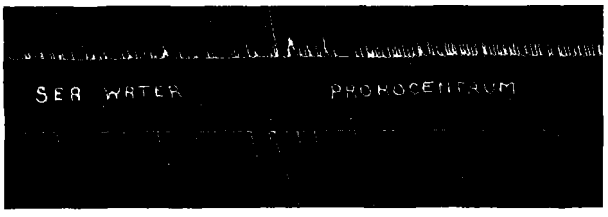

FIGURE 16.—Section of the kymograph record showing a very prominent reduction in the rate of pumping, and a change in the type of shell movement of the oyster subjected to a heavy concentration of *Prorocentrum*. Each vertical line of the lower record represents 267 cc. of water pumped by the oyster.

centrations of *Euglena viridis*, a form which is of a considerably larger size than any of the three microorganisms tried before. *Euglena* was easily cultivated but in working with it certain precautions were necessary to maintain the cells uniformly distributed in the water. Because of their relatively large size the euglenoids did not easily remain in suspension, as was the case with *Chlorella*, *Prorocentrum*, and *Nitzschia*, but quickly settled on the bottom. Also, they were negatively phototropic, showing a constant tendency to move away from the side of the aquarium receiving the most light. We easily overcame these difficulties by a strong agitation of the aquarium content and by regulation of the light.

The organism was identified for us by Dr. James B. Lackey, chief biologist of the United States Public Health Service who in his letter to the senior author said: "This I would call an absolutely typical *Euglena viridis*. However, this species has never been reported, to the best of my knowledge, from salt water and judging from other organisms in your sample it was evidently salt water which you sent me. If you have acclimated this species it is an interesting biological accomplishment." In our work this species was easily cultivated in the outdoor tanks placed under a roof and in the laboratory aquaria kept away from direct sunlight. The salinity of the water in which the cultures were raised was usually about 27 parts per thousand. However, in some aquaria *Euglena* lived well even when the salinity was 32 parts per thousand.

As in the previous experiments, our first observations consisted in determining the effects of very light concentrations of the cells upon the oysters. The first concentrations tried contained only about 1 or 2 thousand *Euglena* per cc. of water. These concentrations did not disturb the oysters. In several instances the rate of pumping even showed a considerable increase. The type of the shell movements remained the same as that recorded before the flow of sea water was substituted with a weak concentration of *Euglena*.

Somewhat stronger concentrations, ranging from 3 to 5 thousand cells per cc., began to affect the oysters (fig. 17). Usually, as soon as the oysters came in contact with these concentrations, the pumping decreased and the type of shell movements changed. The change was often accompanied by the formation of large quantities of pseudo feces. However, some cases were also recorded where the animals exposed to such concentrations remained apparently undisturbed or even pumped larger quantities of water.

In heavier concentrations, containing approximately 8,000 or more cells of *Euglena* per cc. of water, the oysters were obviously under unfavorable conditions because the rate of pumping was sharply reduced and the type of shell movements changed (fig. 18). Many oysters kept their shells open and moving, but did not pump water. We also noted that after being changed from a heavy culture of *Euglena* to sea water the oysters began to pump water very rapidly as if to cleanse their gills (fig. 19). It may be recalled that we made similar observations when forms other than *Euglena* were used.

To demonstrate the rapid changes in the rate of pumping and in the type of shell movements shown of the oysters when they are changed from sea water to *Euglena* culture, and vice versa, we made a supplementary series of experiments. It consisted in subjecting oysters intermittently for

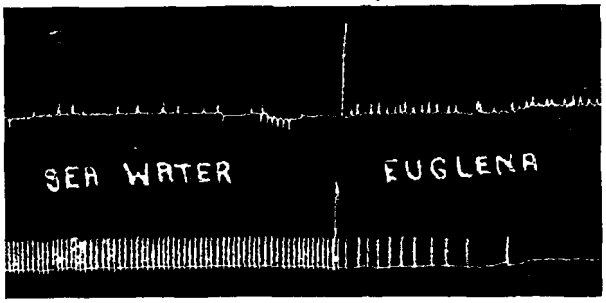

FIGURE 17.—Section of the kymograph record showing a reduction in the rate of pumping, and a change in the type of shell movements of the oyster subjected to a *Euglena* concentration of approximately 5,000 cells per cc. Each vertical line of the lower record represents 265 cc. of water pumped by the oyster.

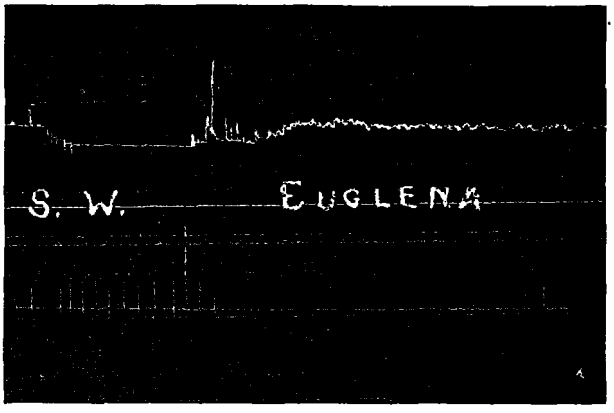

FIGURE 18.—Section of the kymograph record showing a drastic reduction in the rate of pumping, and a change in the shell movements of the oyster subjected to a culture of *Euglena* of approximately 12,000 cells per cc. Each vertical line of the lower record represents 267 cc. of water pumped by the oyster.

periods of 2 hours to a flow of sea water and to *Euglena* culture. Four experiments, each employing four oysters, were made. In the first one 3,300 cells of *Euglena* per cc. of sea water were used and in the following ones we employed concentrations of 8,000, 11,300, and 25,000 cells per cc. Care was taken to keep the temperature, salinity, and hydrogen-ion concentration of the ordinary sea water and of that containing *Euglena* alike, or nearly alike. For the entire series the temperature was about 21.0° C. and the salinity, approximately 27.0 parts per thousand. The pH of both the sea water and the culture varied between 7.8 and 8.2.

Even while the lightest concentration was tried the difference in the behavior of the oysters in sea water and in *Euglena* culture cells was noted, although it was not always sharply defined. In the stronger concentrations of 8,000 and 11,300 cells

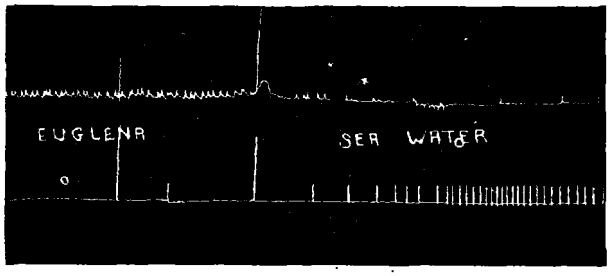

FIGURE 19.—Section of the kymograph record showing the changes in the rate of pumping and shell movements of the oyster after a flow of strong *Euglena* culture of approximately 12,000 cells per cc. was substituted with sea water. Each short vertical line of the lower record represents 265 cc. of water pumped by the oyster.

per cc. the difference became clear. Usually, the rate of pumping decreased upon the change from sea water to *Euglena*, and increased when the oysters were again exposed to sea water. Sometimes, during the periods when the oysters were exposed to *Euglena*, pumping was entirely discontinued, although the shells of the experimental animals remained open and moved, vigorously expelling a large mass of pseudo feces.

The strongest concentration of *Euglena* which we used in this series of experiments contained 25,000 cells per cc. (fig. 20). Always, when the

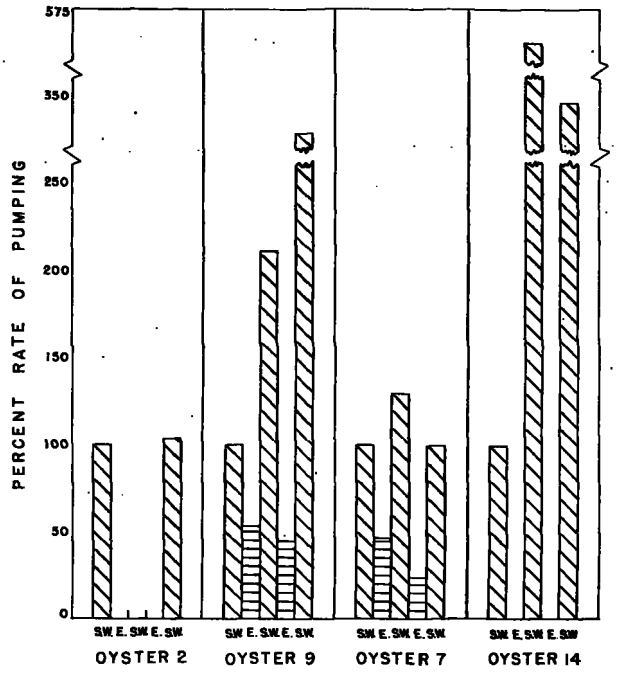

FIGURE 20.—Diagram showing percentage rate of pumping of four oysters subjected intermittently for periods of 2 hours to sea water (SW) and to *Euglena* culture (E) containing approximately 25,000 cells per cc. Rate of pumping in sea water prior to the first exposure to *Euglena* was taken as 100 percent.

oysters were open, the change from sea water to *Euglena* culture resulted in a decrease in the rate of pumping. Oyster No. 2 closed after the flow of the culture was substituted for sea water and remained closed for 6 hours. Three other oysters, however, had their shells open. While exposed to *Euglena*, oyster No. 14 was wide open and moved its shells vigorously, although pumping ceased. Usually the change from *Euglena* culture to sea water resulted in the resumption of pumping, and

the rate of flow often exceeded that of the initial period.

The final series of observations in which we used *Euglena viridis* consisted in subjecting the oysters to increasing concentrations of this form (fig. 21). Two separate experiments, each with four oysters, were run. In the first one the oysters, after their rate of pumping in sea water had been determined, were subjected for hourly periods to various dilutions of strong *Euglena* culture ranging from 8,600 to 63,000 cells per cc. In the second experiment the number of cells ranged from 12,600 to 94,600 per cc. As a rule, the change from sea water to *Euglena* culture always resulted in a considerable decrease in the rate of pumping. A further increase in the density of the *Euglena* population showed even greater interference with the pumping activites of the oysters. Some oysters, such as No. 31, had their shells open for several hours while exposed to dense concentrations of *Euglena*, but did not pump water. With the exception of oyster No. 40, which remained closed for several hours, and oyster No. 38, which closed its shells while exposed to concentrations of 54,000 and 94,600 cells per cc., the shells of all the other oysters were kept open during the entire experiment.

As the experiments progressed, we noticed that the oysters became sluggish and their responses to stimuli diminished. For example, if tapped with a glass rod, they would not close their shells as rapidly as under normal conditions. Sometimes the shell would not close at all, as if the tonus of the adductor muscle had been partially lost. This condition became more apparent as the experiment progressed perhaps indicating that the oysters exposed to strong concentrations of *Euglena* became partly paralyzed. Similar observations were made in the previous experiments where forms other than *Euglena viridis* were used. We thought that these conditions might be due partly to purely mechanical causes, such as clogging of the gills with a mass of cells which interfered with the respiration of the oysters, and partly to the toxic effects of the metabolites of the culture. We shall discuss this matter more fully in the latter part of this article.

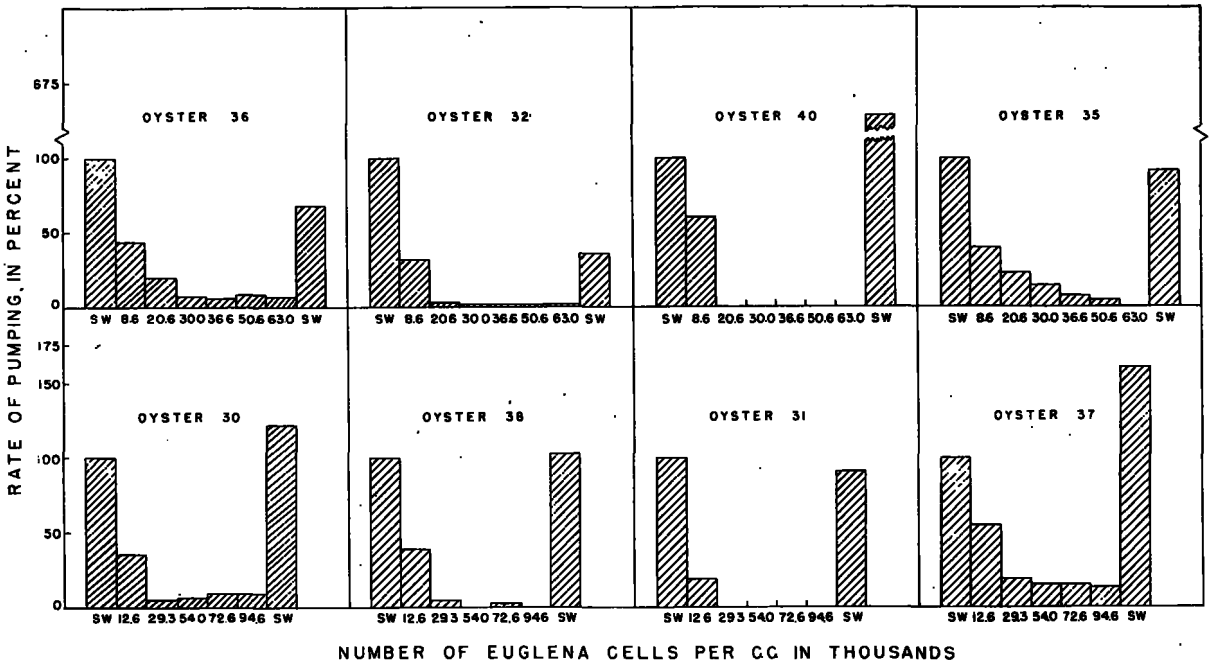

FIGURE 21.—Histogram showing rate of pumping (percentage) of oysters subjected to increasing concentrations of *Euglena*. The rate of pumping in sea water (SW) prior to exposure to *Euglena* was taken as 100 percent.

After the exposure to increasing concentrations of *Euglena* the oysters were again subjected to a flow of sea water. In all instances they soon resumed pumping, which at first was somewhat slow, but within a short period, rarely exceeding 30 minutes, became faster and reached a very rapid

rate usually during the second or third hour after the oysters had been returned to sea water. The post-exposure behavior of the oysters resembled the cleansing process observed in the earlier experiments where *Chlorella*, *Prorocentrum*, or *Nitzschia* were used.

RETAINING OF THE CELLS BY THE GILLS OF THE OYSTER

Our experiments offered us the opportunity to determine the number and percentage of cells retained by the oysters when these mollusks were feeding in different concentrations of *Chlorella*, *Nitzschia*, or *Euglena*. We accomplished this by comparing the numbers of the cells present in the water before and after it passed through the oyster gills. The method consisted in allowing the oyster to pump the water until we were certain that the overflow chamber (*G* in fig. 4) contained a true sample of the fluid passed through the gills, and then taking simultaneously two samples, one from the oyster chamber (*A* in fig. 4), where a continuous stream of a culture was running, and the second from the overflow chamber. Each sample was immediately checked with the Yoe colorimeter and the number of cells per cc. ascertained by reference to the curves used for the conversion of the microammeter readings into the number of microorganisms per cc.

In the experiments with *Chlorella* these determinations were made for a large number of oysters which were kept in concentrations ranging from 200,000 to 12,750,000 cells per cc. (fig. 22). The majority of the observations, however, were made with comparatively weak concentrations, because when strong ones were used the oysters either ceased pumping, or pumped at such a slow rate that it was often impossible to obtain reliable and large enough samples of water.

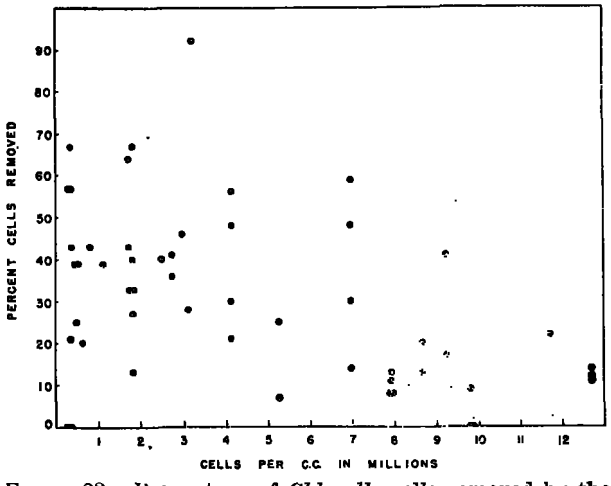

FIGURE 22.—Percentage of *Chlorella* cells removed by the oysters kept in different concentrations of this form.

Analysis of our data showed that the percentage of *Chlorella* cells retained by the gills of the oysters varied from 0 to 92. In the majority of oysters, however, less than 50 percent of the cells were retained. It appeared possible that in lighter concentrations the gills functioned more efficiently retaining a larger percentage of food organisms. However, this relationship was not very clearly defined (fig. 22).

Similar experiments were performed using *Nitzschia closterium* and *Euglena viridis*. In the experiments with *Nitzschia* we obtained data for concentrations ranging from 5 to 370 thousand cells per cc. The percentage of cells retained by the gills varied from 0 to 85. Usually more than 50 percent passed through the gills. In the experiments with *Euglena* the percentage of cells retained varied from 15 to 80. In neither case could we find a correlation between the number of cells originally present and those removed.

Incidentally, in our experiments we noticed that the pH of the water passed through the gills of the oysters was usually 0.1 or 0.2 lower than that of the water fed in the chamber containing an experimental oyster. The slight reduction of the pH was probably due to the removal of oxygen and to the addition of carbon dioxide to the water during its passage through the gills.

PRESENCE OF LIVING CELLS IN FECES OF OYSTERS

During this work we often noted that the feces of the oysters were composed almost exclusively of apparently living plankton organisms. Similar observations were made earlier on different lamellibranchs by other investigators.[7] To ascertain whether or not the plankton cells which passed through the digestive tract of the oysters underwent unfavorable physiological changes or suffered certain not easily detectable injuries we conducted the following experiments.

Oysters, the shells of which were cleaned of all attached material, were kept for 24 hours in sev-

[7] Blegvad 1914; Allen 1921; Churchill and Lewis 1924; Yonge 1926.

eral changes of sea water passed through bacteriological filters. When at the end of the period their digestive tracts were empty the oysters were placed to feed in sea water containing light concentrations of *Chlorella* or *Nitzschia*. Microscopic examination of the fecal ribbons which were soon formed by the oysters showed that they were composed of tightly packed cells of the species which was fed to them. Comparison of these cells with those constituting our standard cultures did not show any difference in color or structure. Many cells composing the feces were in the stage of active division, the condition indicating beyond all doubt that they were alive and uninjured.

Further proof of the healthy condition of the cells of *Chlorella* or *Nitzschia*, which passed through the digestive system of the oysters, was offered by the following experiment: small pieces of the fecal ribbons were carefully washed in five changes of filtered sea water and then placed in the culture media, where they were broken apart by vigorous agitation. Several days later the media flasks contained rich cultures of *Chlorella* or *Nitzschia*.

Thus, we were able to show that living plankton organisms can pass undamaged through the oyster gut. Yonge (1926) thought that this is possible because of the poor development of extracellular digestion in the oysters, and is especially due to the complete absence of extracellular protease and lipase. Petersen and Jensen (1911) and Blegvad (1914) maintained that the presence of living micro-organisms in the feces indicates that they are not important and perhaps useless as oyster food. This theory finds support in the work of Coe and Fox (1944), and Coe (1945) on the biology of the California mussel, *Mytilus californianus*. Coe (1945) thinks that the principal food supply of mussels "consists of minute particles of organic detritus derived from the disintegration of the cells of all kinds of marine organisms, both animals and plants, supplemented by living and dead unicellular organisms of minute size as well as living and dead gametes."

INHIBITING EFFECT OF THE CELLS AND OF THE LIQUID PORTION OF THE CULTURES

Our experiments gave sufficient evidence to prove that large quantities of micro-organisms present in the surrounding water adversely affect the oysters. Naturally, the question arose as to which component of a culture, i. e., the cells or the liquid, or perhaps the combination of the two, is causing the depressing effects. A review of the literature shows that numerous cases are known where an excessive growth of plankton was responsible for the unbalanced biological conditions which resulted in the mortality of aquatic animals. Prescott (1939) in discussing the relationship of phytoplankton to aquatic communities, stated that certain algae, while alive, liberate substances toxic to many forms. Decaying algae also produce large quantities of poisonous substances, such as hydroxylamine. Pratt (1942) in his extensive studies on *Chlorella vulgaris* found that these organisms manufacture and release in the surrounding medium a growth-inhibiting substance. This substance has not been identified as yet, but Pratt thinks that it is probably an organic base.

The mortality of oysters and other mollusks caused by the presence in the water of extremely large numbers of micro-organisms, usually dinoflagellates, has been observed in many parts of the world. Whitelegge (1891) reported a heavy mortality of oysters and mussels in Port Jackson, New South Wales, caused by *Glenodinium rubrum*. Nishikawa (1901) reports the destruction of pearl oysters in Japanese waters caused by *Gonyaulax polygramma*. Kofoid (1907) described the case of mortality of fish and shellfish occurring along the California coast, between San Pedro and San Diego, where *Gonyaulax polyhedra* was present in large numbers. Bückmann (1934) reported heavy injury to the oyster set of Helgoland caused by large numbers of the diatom *Skeletonema costatum*.

An interesting review of the literature on the occurrence of large numbers of dinoflagellates and their influence upon marine animals, especially mollusks, was recently offered by Nightingale (1936), who expressed the opinion that both the cells of micro-organisms and the products of their decomposition and metabolism may be fatal to marine animals. However, he did not arrive at a final conclusion regarding this matter, but suggested further studies of these problems.

To ascertain the relative importance of the cells and the fluid part of cultures in affecting the activities of oysters we carried on several experiments. We obtained *Chlorella* filtrate by filtering the culture through Berkefeld filters. To replace the

oxygen, which might have been lost during filtration, the fluid was afterwards vigorously aerated. Other methods employed to separate the cells from the medium, such as filtering through cotton, or through the finest grade of filter paper, or using the Foerst centrifuge, were unsatisfactory. The cells, owing to their small size, either penetrated through the filters or could not be separated by centrifuging.

Chlorella cells (residue) were collected also by using Berkefeld filters. After a large quantity of *Chlorella* had accumulated on the walls, we placed the filters in a vessel containing a very small quantity of water, and applied air pressure to drive the cells from the crevices of the filter. Usually the cells were used within one or two hours after having been filtered off. They were added to sea water in the quantities necessary to create the same concentration as that used at the beginning of the experiments, when unadulterated culture was used.

At first the oysters were placed in running sea water (fig. 23). Two hours after they opened and began to pump, a flow of a strong culture of *Chlorella*, containing approximately 10 million cells per cc., replaced the water. After another 2-hour period the flow of *Chlorella* was stopped and running sea water reintroduced. Thus, the effect of that particular culture of *Chorella* upon the oysters was determined. Upon elapsing of the second 2-hour period in sea water the oysters were subjected to a flow of filtrate of *Chlorella* which was obtained from the same culture, part of which was used earlier in the experiment. Then the oysters were again given running sea water for 2 hours, and finally subjected to *Chlorella* cells (residue).

The behavior of 16 oysters which we used in these experiments usually followed the pattern shown on the kymograph record reproduced in figure 23. It showed conclusively that the filtrate of the culture containing metabolic products of *Chlorella* cells, and the cells, affected the oysters. The rate of pumping was sharply reduced or even stopped when the oysters were subjected to either component.

A series of similar experiments in which we used a culture of *Nitzschia closterium* containing approximately 150,000 cells per cc. corroborated the results obtained with *Chlorella*. Each chief component of the culture, the cells or the filtrate, caused a reduction of the rate of pumping and changed the type of the shell movements of the oysters. Upon return from either one of these components to sea water the oysters soon showed a normal behavior.

EXPERIMENTS WITH YEAST

Our observations may be criticized on the assumption that the abnormal behavior of the oysters was perhaps caused by some of the ingredients of the media in which the microorganisms used in the experiments were grown. Such a criticism can be partly invalidated by pointing out that the material used for growing the culture contained no toxic substance. Furthermore, we applied the strict rule to use only those cultures which had been prepared long in advance, and where all signs of decomposition of fertilizing substances were absent. It was also our practice to aerate the cultures before using them, to be sure that no lack of oxygen existed and that the obnoxious gases which might have been present were driven off. Finally, we were able to show that even if the fluid part

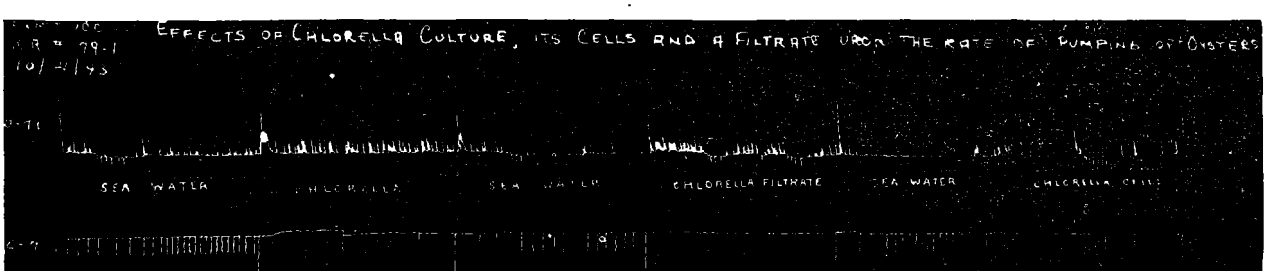

FIGURE 23.—Kymograph record showing behavior of the oyster in sea water and when subjected for periods of about 2 hours to a strong *Chlorella* culture of approximately 10 million cells per cc., to filtrate of *Chlorella*, and to cells of *Chlorella* suspended in sea water. Upper line shows the shell movement and the lower one, the rate of pumping of the oyster. Each short vertical line of the lower record represents 244 cc. of water pumped by the oyster.

of a culture were removed, the cells alone would, nevertheless, adversely affect the oysters. To substantiate further the latter conclusion we ran additional experiments in which cells of yeast instead of regular plankton micro-organisms were used. The yeast cells of the regular commercial variety were about 5 microns in diameter thus closely resembling in size those of *Chlorella*.

The experiments consisted in keeping the oysters for a period of about 3 hours in running sea water and then subjecting them for the same period to a flow of sea water to which a certain quantity of yeast had been added. The concentrations of yeast used were 0.1, 0.25, 0.5, 1.0, and 2.0 grams per liter. For each concentration records of seven or more oysters were taken. The temperature of the control sea water and of the sea water containing the yeast was near 20.0° C., the pH varied between 7.7 and 8.0, and the salinity was about 27.0 parts per thousand.

In the weakest concentration, containing 0.1 gram of yeast per liter of sea water, approximately one-half of the experimental oysters showed an increase in the rate of pumping which ranged from 51 to 240 percent over the original rate (table 3). The other half, however, showed a decrease ranging from 10 to 54 percent. The character of the shell movement was not noticeably changed by the introduction of yeast suspension.

In a concentration of 0.25 gram per liter, however, the depressing effect became quite evident (fig. 24). Of the 12 oysters used 11 showed a decrease in the rate of pumping ranging from 27 to 86 percent (table 3). In all heavier concentrations the presence of yeast cells in sea water always decreased the rate of pumping (table 3). In the concentration 0.5 gram of yeast per liter of sea water, the decrease ranged from 39 to 96 percent. In the concentration of 1.0 gram per liter the rate of pumping showed a decrease from 5 to 84 percent. In two-thirds of the oysters, however, the decrease was 63 percent or greater. In the strongest concentration, which contained 2.0 grams of yeast per liter of sea water, the reduction in the rate of pumping varied from 47 to 89 percent of the original rate. Therefore, the conclusion that the presence of a large number of micro-organisms in sea water compels the oysters to reduce the rate of pumping has been again confirmed.

In many instances after sea water was substituted for the yeast suspension the oysters displayed a very rapid rate of pumping (figs. 24 and 25). For example, two oysters pumped for a short period at the rate of 25,970 cc. per hour, while four others pumped from 20,916 to 22,550 cc. for the same period. It should be recalled that we noticed the rapid rate of pumping in our other experiments when the culture of micro-organisms, such as *Chlorella*, was substituted for sea water. Thus, we believe that sufficient data have been collected to show that this behavior, which may be considered as cleansing of the gills from the mass of cells and mucus, is of common occurrence among the oysters which were exposed to the water containing large quantities of suspended matter.

The exposing of oysters to a suspension of yeast of 0.25 gram per liter or heavier was invariably followed by a change in the type of the shell movements (figs. 24 and 25). Usually the contraction and relaxation of the adductor muscle becomes

TABLE 3.—*Effect of different concentrations of yeast cells upon the rate of pumping of oysters*

Oyster Number	Yeast concentration, gram per liter	Rate of pumping cc. per hour		Percentage of increase (+) or decrease (−) in rate of pumping in yeast suspension
		In sea water	In yeast	
6	0.1	1,027	1,602	+56
10	0.1	1,685	2,553	+51
91	0.1	7,735	6,949	−10
79	0.1	4,928	10,585	+115
93	0.1	4,335	6,600	+52
15	0.1	2,600	8,120	+212
54	0.1	2,456	8,342	+240
16	0.1	14,000	9,800	−30
2	0.1	12,611	3,713	+71
20	0.1	10,582	4,845	−54
19	0.1	10,950	8,906	−19
19	.25	12,358	6,630	−46
48	.25	2,066	1,033	−50
21	.25	8,493	1,773	−79
17	.25	10,349	6,478	−38
91	.25	14,933	3,080	−79
6	.25	1,343	1,343	0
79	.25	17,593	8,500	−51
10	.25	7,227	3,431	−53
54	.25	4,853	2,530	−48
51	.25	2,449	2,986	+22
93	.25	8,246	6,035	−27
15	.25	12,702	1,728	−86
95	0.5	10,956	396	−96
77	0.5	11,952	7,272	−39
92	0.5	9,204	4,758	−48
64	0.5	18,333	2,750	−85
96	0.5	13,197	7,844	−41
58	0.5	5,250	1,890	−64
91	0.5	11,466	3,042	−73
79	0.5	7,975	2,118	−73
47	1.0	2,158	2,063	−5
68	1.0	4,970	1,611	−68
94	1.0	10,179	3,276	−68
48	1.0	8,591	2,760	−68
80	1.0	5,291	1,412	−73
50	1.0	2,832	1,038	−63
52	1.0	4,950	797	−84
96	2.0	8,663	2,154	−75
93	2.0	5,565	2,451	−56
83	2.0	10,881	1,658	−85
80	2.0	2,213	699	−68
93	2.0	5,225	550	−89
83	2.0	21,200	2,562	−88
92	2.0	12,987	3,822	−71
80	2.0	2,148	1,141	−47

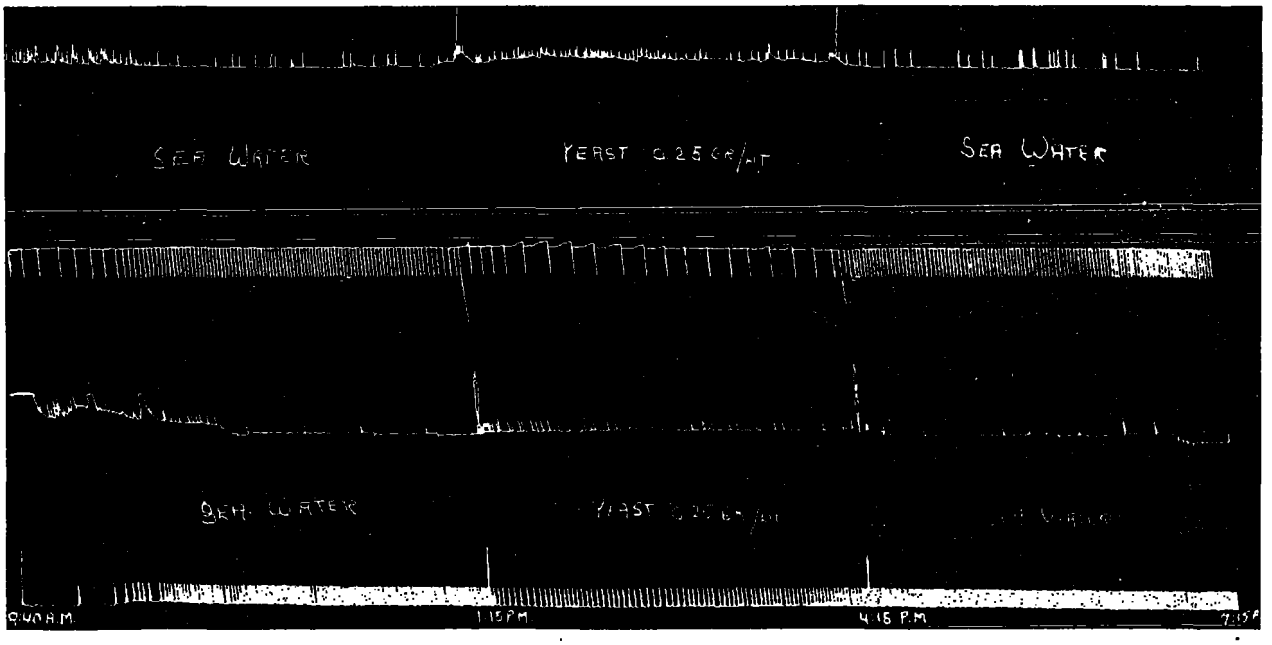

FIGURE 24.—Kymograph record showing effect of 0.25 gram of yeast per liter of sea water upon the shell movement (1st and 3d lines) and rate of pumping (2d and 4th lines) of two oysters. Each short vertical mark of the second line designates emptying of the tripping vessel of 280 cc., while each mark on the fourth line shows the dumping of 237 cc. of water.

more frequent. Nevertheless, in the majority of cases the shells remained open during the entire exposure while in the remaining instances the shells were closed only for very brief periods.

While in the suspensions of yeast the oysters formed very large quantities of pseudo feces which were composed almost entirely of yeast cells and mucus. The true feces, however, were formed in small quantities only. We noted that while the pseudo feces were white in color, the true feces were a dark brown. Microscopic examination of the feces showed that, in contrast to the pseudo feces, they consisted largely of plankton forms and detritus. These observations suggested that oysters exercise a certain degree of selectivity in their feeding. In some rare cases, however, the

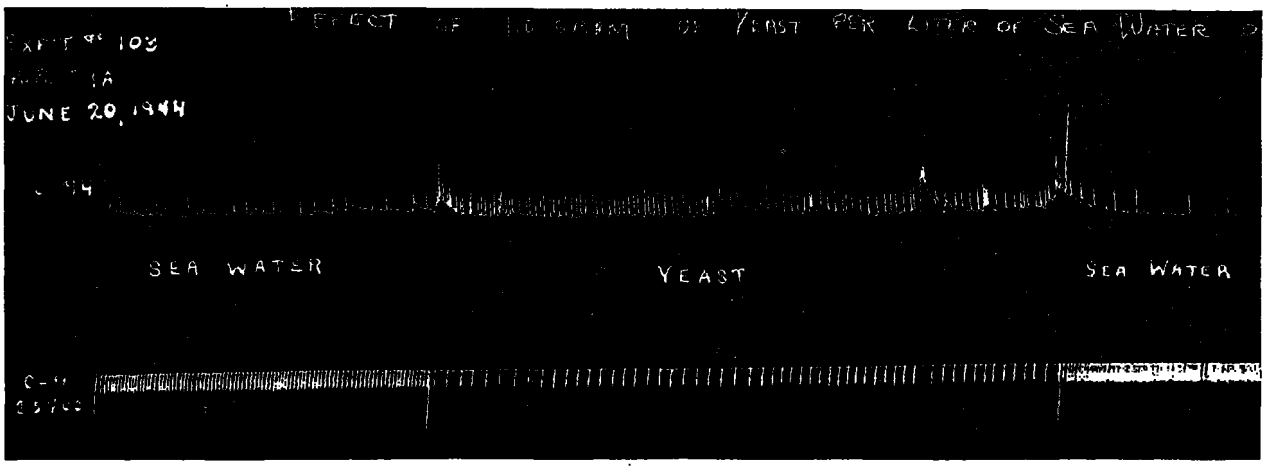

FIGURE 25.—Kymograph record showing effect of 1.0 gram of yeast per liter of sea water upon the shell movement (upper line) and rate of pumping of the oyster (lower line). Note the increase in the rate of pumping after the change from yeast suspension to sea water. Each short vertical line of the lower record represents 234 cc. of water pumped by the oyster.

feces were composed mostly of yeast cells. This indicated, perhaps, that the ability of food selectivity was not equally possessed by all oysters.

As in the experiments where *Chlorella*, *Nitzschia*, or *Euglena* were used, we made tests to find the relative quantities of yeast cells removed from suspension by the oyster gills. Although we did not count the number of yeast cells per cc. of water before and after it passed through the oyster, we formed, nevertheless, the quite reliable conclusion, by checking the samples with the Yoe colorimeter, that a relatively very small number of the yeast cells was retained by the gills, and that the largest portion passed through them and was expelled on the cloacal side of the oyster.

In general, this series of experiments showed that the behavior of the oysters subjected to a flow of sea water containing a large number of yeast cells strongly resembled that of the oysters exposed to heavy concentrations of plankton organisms, such as *Chlorella*, *Prorocentrum*, *Nitzschia*, or *Euglena*.

EFFICIENCY OF FEEDING AND FORMATION OF TRUE AND PSEUDO FECES

Our observations suggested that the relative quantities of true feces and pseudo feces formed by the oysters may indicate the efficiency of their feeding. To verify this the following experiments were run. Single oysters were placed in glass jars each containing 3 liters of *Chlorella* culture the density of which varied from 18,000,000 to 450,000 cells per cc. The different densities were made by diluting with sea water the original heavy culture, containing 18 million cells per cc. A control oyster was placed in a jar containing 3 liters of sea water. The oysters used were of almost uniform size, shape, and weight. Before being placed in the jars with *Chlorella* they were kept overnight in filtered sea water to have their digestive tracts emptied of the food.

During the experiment, which lasted three days, the temperature of the water in all the jars was the same ranging between 18.0 and 21.0° C., depending upon the room condition. The salinity in all jars was identical, being 26.55 parts per thousand. However, the pH of the different jars showed a difference. In the strongest concentration of *Chlorella* it was 8.3, whereas in the weakest one it ranged between 7.7 and 7.8, being identical with the pH of the sea water in which the control oyster was kept.

Our observations on the formation of true and pseudo feces by the oysters kept in different concentrations of *Chlorella* follow:

Concentration of 18,000,000 cells per cc.—The oyster opened its shells within a few minutes and soon began to expel large quantities of pseudo feces, although no true feces were discharged. After 35 minutes of activity the oyster shut its shells and remained closed until the end of the experiment. Apparently the concentration of the cells in the water was too great to allow the oyster to function normally. Therefore, after the initial effort of pumping the water, which resulted in the discharge of a large quantity of pseudo feces, the oyster stopped pumping and closed its shell. The density of the *Chlorella* population in the jar at the end of the experiment was practically the same as at the beginning. At the end of the third day we opened the oyster and found that its stomach was empty and the crystalline style was absent.

Concentration of 11,000,000 cells per cc.—The oyster opened and began pumping, forming pseudo feces 3 minutes after having been placed in the culture. When, after approximately 1 hour, the density of the culture had been somewhat reduced by the formation of pseudo feces, the oyster began to expel a small quantity of true feces. However, as in the previous concentration, the animal soon closed its shell, remaining inactive until the end of the experiment. The quantity of true feces formed during the active period was much smaller than that of pseudo feces. At the end of the experiment the stomach of the oyster was empty and there was no crystalline style.

Concentration of 5,400,000 cells per cc.—The oyster began pumping as soon as it was placed in the jar, ejecting a large quantity of pseudo feces. After a period of about 45 minutes the concentration of *Chlorella* was reduced to such an extent that the cells apparently ceased to interfere seriously with the feeding. As a result, a large quantity of true feces was gradually formed and finally the water was almost completely cleared of microorganisms. We noticed that when the density of the culture was reduced to approximately 1,200,000 cells per cc., the formation of pseudo feces stopped almost entirely, while that of true feces proceeded uninterruptedly. The stomach of the oyster contained food and the crystalline style was present.

Exposure to this concentration showed that by forming pseudo feces the oyster was able to reduce the number of cells in suspension to approximately 1,500,000 cells per cc. and then proceeded to feed almost normally. We should emphasize, however, that the reduction of the number of cells was possible only because the oyster was kept in a small volume of water. Undoubtedly, if kept in a much larger vessel containing *Chlorella* of the same density as that existing at the beginning of the experiment, the oyster would be unable to reduce substantially the concentration of micro-organisms and would finally become inactive. It should also be remembered that in addition to the action of the cells, the fluid part of the culture would eventually contribute to the inhibition of the pumping activities of the oyster. We think, therefore, that the concentration of approximately 5,000,000 *Chlorella* cells per cc. should be considered as still being above the threshold which would permit the oyster to function normally.

Concentration of 3,600,000 cells per cc.—The observations made on the oyster exposed to this concentration were almost identical with those of the preceding one.

Concentration of 2,000,000 cells per cc.—The results obtained with this concentration closely resembled those of the two preceding ones with the exception that, because the number of cells was smaller the oyster could reduce it below the unfavorable level within a few minutes, and then proceed to eject true feces, the quantity of which greatly exceeded that of pseudo feces. Within a few hours the water in the jar was almost transparent.

Concentration of 1,200,000 cells per cc.—The oyster opened and began to filter water almost as soon as it was placed in the jar. The amount of pseudo feces formed was very small. The true feces, which were rather abundant, were dark green in color and were composed almost exclusively of *Chlorella*.

Concentration of 900,000 cells per cc.—The results of this experiment were almost the same as those observed in the previous concentration with the exception that even less pseudo feces were formed.

Concentration of 450,000 cells per cc.—The plankton content of the water was quickly ingested by the oyster and discharged in the form of true feces. No pseudo feces were produced. This probably indicated that the animal was able to feed very efficiently.

Control—sea water.—The oyster placed in sea water utilized almost the entire content of micro-organisms within a very short time. The brown feces consisted mostly of the organisms commonly found in sea water. No pseudo feces were formed.

Experiments of the same nature were performed on two more occasions. New oysters were used but the concentrations chosen closely approximated those of the first experiment. The results of all three experiments were in agreement with the exception that in the strong concentrations of the last two experiments some of the oysters remained open but apparently not pumping because neither feces nor pseudo feces were formed.

These experiments showed once more that heavy concentrations of *Chlorella*, containing more than 5 million cells per cc., seriously interfere with the oysters which cease pumping if kept in such a concentration for long periods. In intermediate concentrations, ranging from approximately 2 to 5 million cells per cc., the oysters may continue to feed. However, the rate of feeding is low because the activity of oysters is directed primarily to the cleansing of their gills, and results in the formation of a very large quantity of pseudo feces. If these conditions persist, the oysters may eventually become inactive.

In light concentrations, containing less than 2 million *Chlorella* cells per cc., the oysters feed normally. Under these conditions the quantity of true feces ejected is relatively large, while pseudo feces are either absent or formed in very small quantities.

On the basis of these and the previously described experiments we may formulate a general rule, namely, that the quantities of pseudo feces formed by the oysters are usually roughly proportional to the quantities of plankton and other material suspended in the water, whereas a reverse relationship exists in the formation of true feces. The latter relationship, naturally, does not include the condition when the water is devoid, or almost devoid, of suspended matter.

The presence of large quantities of pseudo feces also indicates that the feeding of oysters proceeds under unfavorable conditions caused by heavy concentrations of material suspended in the water. Small quantities of pseudo feces, or their absence, in the presence of large quantities of true feces

show, on the other hand, that the oysters are feeding efficiently.

The concentrations of cells which began to interfere noticeably with the normal feeding activities of the oysters varied with the different species, depending upon the size of the micro-organisms. In general, the larger the size of the micro-organisms, the smaller the number necessary to create unfavorable conditions. Obviously, because of the great variety of plankton forms in sea water, it is impossible to determine the maximum concentration of each species that could be tolerated by the oysters. The problem becomes even more difficult when it is remembered that in nature it is not a single species but a combination of many which usually populates the water where oysters exist. We think, however, that during these studies we found a general criterion which we believe may permit us to determine the lowest concentration of a single or of a combination of different plankton forms which would depress the normal rate of pumping of the oysters. This criterion is based upon the determination by the Yoe colorimeter (Yoe and Crumpler 1935) of the relative density of the plankton population in the water. Within the scope of our experiments we found that regardless of the type of species, or of their size, the oysters began to show signs of abnormal behavior when, because of the presence of micro-organisms, the turbidity of the water, as registered on the microammeter, dropped below the 23 mark. This point corresponded to approximately 2,000,000 *Chlorella*, 70,000 *Nitzschia*, or 3,000 *Euglena* per cc. of water. Of course, as should be expected, the individual variations among the oysters were of considerable magnitude.

DISCUSSION

It is axiomatic that the physiological behavior of an animal depends upon and is controlled by changes of its environment. In the case of the oyster, *O. virginica*, which lives either in the intertidal zone or in comparatively shallow water affected by the tides, the changes are frequent and often abrupt. Because of the tidal currents flowing over the beds the quantity and quality of the micro-organisms change almost continuously. The seasonal changes in the plankton population diversify even greater the variety and concentrations of the microscopic forms. Thus, as far as the quantity of food supply is concerned, the oysters are subjected to an almost continuous change and, therefore, must be equipped anatomically and physiologically to cope, within certain limits, with the change of the condition.

As mentioned earlier, Yonge (1936) expressed the opinon that oysters evolved from forms which lived in clear water, but gradually developed a more efficient cleansing mechanism that enabled some of the species, such as *O. virginica* and *O. angulata*, to exist in less clear waters. However, in the opinion of some investigators (Kellogg 1915) this tolerance of the oysters is still very limited, the animals being able to feed only when the water contains in suspension relatively small quantities of food organisms or other materials. Grave (1916) and Nelson (1921, 1923) on the contrary, maintained that oysters would feed rapidly in water bearing large quantities of suspended matter. The results of our experiments indicate that both schools of thought are correct but only to a certain extent.

The opinion advanced by Kellogg is fully supported by our experiments in the respect that oysters will feed efficiently only if the water contains small quantities of suspended material. Contrary to Kellogg's opinion, however, we found that oysters are also capable of feeding, at least for short periods, in heavily laden water. Under such conditions, nevertheless, the rate of feeding is depressed. Consequently, the conclusions of Grave and Nelson (loc. cit.) could be considered correct only if they were qualified with additional statements emphasizing the time element and showing that the efficiency of feeding of oysters decreases in water too rich in micro-organisms. Since no such statements were made by those authors, their conclusions create the impression that it is unimportant whether or not the water is clear or heavily laden.

Nelson's opinion (1923) that the rate of water filtration by oysters is independent of the quantity of material suspended in the water cannot be corroborated by our studies, which gave ample evidence that, in general, the rate of pumping decreases with an increase in the number of micro-organisms. We think that Nelson's conclusions were formed because of the wrong interpretation of his experimental data. Nelson's (1921) experiment, as recently pointed out,[8] was designed to

[8] Loosanoff and Nomejko 1946.

study only the shell movements of oysters and not the rate at which these mollusks were pumping water. Obviously, because he had no data on the rate of pumping, which is the criterion of feeding, no definite conclusions should have been formed regarding the latter subject. Nevertheless, assuming that the frequent and rapid closing and opening of the shells signified active pumping and feeding, whereas they actually might have shown repeated ejection of pseudo feces, the phenomenon indicating that the efficiency of feeding was reduced, Nelson (1921, 1923) concluded that the rate of filtration of water is independent of the quantities of material suspended in the water.

As shown in the earlier part of this article, there are rather definite concentrations of the micro-organisms above which the feeding of oysters was inhibited. In concentrations below this threshold, the rate of pumping was not reduced, and often the rate of pumping of the oysters kept in light concentrations was even greater than when they were kept in running sea water. In concentrations above the threshold, however, a reverse correlation was noticed between the density of micro-organisms and the rate of pumping. In heavy concentrations pumping entirely ceased. Under such unfavorable conditions little or no food was ingested by the oysters and the crystalline style was usually absent. The oysters were forming large quantities of pseudo feces to cleanse their gills and palps of the excessive accumulation of food cells.

Upon coming in contact with the large number of micro-organisms the type of shell movement of the oysters changed, showing frequent attempts to expel the masses of cells and mucus accumulating on the gills and palps. Under these conditions the shells often remained open for long periods although no water was pumped.

In many of our experiments we noticed cessation of pumping when the oyster shells were open and the edges of the mantle were widely separated. Therefore, the decrease in the rate of pumping, or its complete cessation, could not be attributed to the fact that the water could not enter the gill cavity of the oysters because the latter was blocked by the edges of the mantle.

The results of our experiments at first appeared to be rather paradoxical. They showed that the rate of feeding of oysters decreased when plankton was heavy, while if given only a limited quantity of food, the oysters acted normally. Our conclusion contradicted the established opinion that a rich plankton population was necessary for a favorable existence of these mollusks. For example, Nelson (1921) stated "Any conditions which result in an increase in the number of food organisms in the water will make for fatter and better oysters." Naturally, an explanation was needed to clarify our conclusions. We offer this explanation by referring to our experiments which showed that large numbers of micro-organisms in sea water affect the oysters mechanically, by interfering with the proper functions of the gills and palps, and also chemically. The latter apparently is due to the presence of a certain inhibiting substance released by micro-organisms in the surrounding water. At present we do not know the nature of this substance, but we have determined that it is heat resistant, and that vigorous aeration for a period of 24 hours does not reduce its toxicity.

The mechanical interference apparently causes a disturbance of the synchronization of the ciliary motion and results in a decrease in the flow of water through the gills. Detailed studies of these phenomena, as well as of the chemical effects of the filtrates of the various cultures of micro-organisms upon the mantle gills, palps, and adductor muscles of the oysters, will be discussed by Loosanoff in a separate article. It is sufficient to say here that the gills of the oysters kept in water rich in plankton became quickly covered with a mass of food cells which imbedded in the gill mucus. This interferes with the normal feeding of the oysters and, probably, with their respiration. In extreme cases the mass of micro-organisms covering the gills, undoubtedly, was slowly suffocating the oysters. Miyake, whose report was not available to us but whom Nightingale (1936) mentions in his paper, came to the same conclusions while studying the mortality of the pearl oyster in 1933 in Gakasho Bays, in Toba, Japan. He found that losses among pearl oysters, estimated at 15 million yen, were caused by clogging of the respiratory organs of the mollusks with a mass of *Gymnodinium*.

In our experiments the oysters exposed to dense cultures of micro-organisms were trying to keep their gills and palps clean. This was shown by the characteristic movements of the shells directed to expel large quantities of pseudo feces. At the same time little or no true feces were formed, which indicated that little or no food was ingested.

These and the observations mentioned in the preceding paragraph provide the explanation as to why the presence of a rich population of micro-organisms creates an unfavorable condition for the existence of oysters.

Our conclusions agree with those of Kellogg (1915) who found that when relatively large quantities of suspended material were collected by the gills of the oyster and then brought to the palps all or most of this matter was rejected and expelled as pseudo feces. Yonge (1926) also observed that if the gills of an oyster were heavily covered with a mass of particles, these particles, regardless of their individual size, would be all removed before being carried to the mouth.

Our laboratory experiments were to a large extent corroborated by observations on the conditions existing in nature. For example, it has been observed that the oysters of Great South Bay in the State of New York have been poor for several years. During the same period, however, the water of the bay was extremely rich in plankton. Nevertheless, regardless of the presence of large quantities of food the oysters remained poor. Joseph B. Glancy, who studied the conditions in Great South Bay, told us that the oysters usually become poor when micro-organisms are present in large numbers. Perhaps other undiscovered factors also contributed to the poor condition of the oysters of that body of water, but the fact remains that they were in such a state regardless of the abundant food supply.

As an example of the opposite type, we may mention Gardiners Bay, Robin Island Sound, Shelter Island Sound, and other basins of the State of New York where the water is usually very clear containing but a small number of micro-organisms. Yet it is a well known fact to the members of the industry and to the biologists working in this vicinity that these basins are used to improve the condition of the oysters which in those waters accumulate large quantities of glycogen. Thus, despite comparatively small numbers of micro-organisms, the oysters of those areas become "fatter" than those living in water much richer in plankton, such as Great South Bay.

Perhaps the most interesting and significant conclusion may be made from a letter received by the senior author in April 1946 from P. O. Mercer, manager of the largest oyster company of Great South Bay, which states: "It is true that the meats of the oysters in Great South Bay have been of excellent quality during the past year. The water in the bay has been and is still unusually clear which indicates that the microscopic organisms which were in the water during the last several years have disappeared."

In connection with the discussion we would like to mention that recent plankton studies of Long Island Sound, carried on by Riley (1941), showed that plankton was least abundant during October, November, and the early part of December or, in other words, during the period when the oysters in our waters accumulate large quantities of glycogen. This observation appears to be of considerable significance perhaps indicating nature's provision to create more favorable conditions for the oysters during the season when large quantities of glycogen should be stored in their bodies.

In nature the appearance of masses of micro-organisms dense enough to kill the oysters in a short time is of rather rare occurrence but was, nevertheless, noticed in a number of cases in different parts of the world (loc. cit.). The presence of relatively heavy concentrations, not dense enought to kill the oysters within a short time, but heavy enough to interfere with their normal existence and to make them poor has also been observed. For example, during the seasons when the water of Great South Bay contained over 3,000,000 cells of small green algae per cc. the oysters were usually very poor. We realize, however, that such cases are of unusual nature, and that the numbers of micro-organisms in the water flowing over the oyster beds are considerably smaller than those found in Great South Bay or employed in some of our experiments. Nevertheless, our studies showed that the feeding of oysters proceeds efficiently only when the water is relatively clear. The appearance of micro-organisms in numbers exceeding the threshold suggested in this article unfavorably affects oysters by depressing their rate of pumping and, therefore, feeding.

Before advancing final conclusions, however, more research on the subjects discussed in our paper is necessary. Thus far, our studies have been confined to oysters of the same general locality, living under approximately the same conditions. Consequently, it is not known whether the conclusions which we offer would apply to oysters of other geographical areas, where environmental conditions are radically different

from those of the New York and New England beds. We also are well aware of the existence of "claires" at Marennes and other places along the French coast where oysters, although of different species than ours, are fattened in the presence of abundant supplies of diatoms, peridinians, algal spores, and other micro-organisms. Obviously, further extensive studies are needed before final conclusions may be safely and correctly formed.

SUMMARY

1. Experiments performed with *Chlorella* sp., *Nitzchia closterium*, *Prorocentrum triangulatum*, *Euglena viridis*, and a common variety of yeast have demonstrated that there are rather definite concentrations of these forms above which the density of the micro-organisms begins to interfere with the feeding of the oysters.

2. In heavy concentrations of micro-organisms the rate of water pumping of the oysters was reduced, and the character of the shell movement noticeably changed. In many instances a correlation was noticed between the density of the micro-organisms and the rate of pumping. Pumping entirely ceased in very heavy concentrations.

3. When the cells were too abundant little or no food was ingested by the oysters and the crystalline style was usually absent. The oysters were forming large quantities of pseudo feces to cleanse their gills and palps of an excessive accumulation of plankton cells.

4. In concentrations below the threshold the rate of pumping was not reduced and the shell movements remained normal. In many instances the rate of pumping of the oysters kept in light concentrations of micro-organisms was even greater than when kept in sea water.

5. The size of the cell played an important part in affecting the activities of the oysters. A much greater number of small-sized cells, such as *Chlorella*, was needed to produce the same effect as caused by a smaller number of larger organisms, such as *Euglena*.

6. In heavy concentrations of micro-organisms the shells of the oysters sometimes remained open for periods of several hours although no water was pumped.

7. If the oysters were kept in heavy concentrations for prolonged periods, the tonus of the adductor muscle became impaired or partially lost. The oysters became sluggish and their responses to stimuli diminished.

8. Both the filtrate of the cultures, containing metabolic products of the cells, and the cells were found to affect the oysters. The rate of pumping was reduced or entirely stopped when the animals were subjected to strong concentrations of either component.

9. When, after exposure to heavy concentrations of micro-organisms the oysters were again subjected to a flow of sea water, their rate of pumping usually showed a marked increase. Such intensive pumping on the part of the animals suggested an attempt to cleanse themselves of the micro-organisms which had accumulated in the gills and mantle chamber.

10. The percentage of cells of *Chlorella* sp. removed by the gills of the oysters during the feeding processes varied from 0 to 92. In the majority of cases, however, less than 50 percent of the cells were retained. In the case of other micro-organisms usually more than 50 percent of the cells passed through the gills. No correlation was found between the number of cells originally present in the water and those retained by the gills.

11. The oysters exercised a certain degree of selectivity in their feeding. This ability, however, was not equally demonstrated by all the oysters.

12. It has been observed that living plankton organisms, such as *Chlorella* and *Nitzschia*, can pass undamaged through the digestive system of the oyster.

13. The quantities of pseudo feces produced by the oysters were usually roughly proportional to the quantities of plankton and other materials suspended in the water, whereas a reverse relationship existed in the formation of true feces.

14. The presence of large quantities of pseudo feces usually indicated that feeding of oysters proceeded under unfavorable conditions caused by heavy concentrations of material suspended in the water. Small quantities of pseudo feces, or its absence, in the presence of large quantities of true feces showed that the oysters were feeding efficiently.

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