EXPERIMENTAL STUDY OF THE FUNCTION OF THE OYSTER GILLS AND ITS BEARING ON THE PROBLEMS OF OYSTER CULTURE AND SANITARY CONTROL OF THE OYSTER INDUSTRY

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INTRODUCTION

A study of the physiology of the oyster is of great practical importance for the oyster industry and at the same time it presents a broad and interesting field for research that hardly has been touched by scientific investigation. The present paper deals with the function of the gills of the American oyster.

The gill may be regarded as one of the most conspicuous of the organs that take part in the feeding and respiration of the oyster. The fact that great quantities of oysters are consumed raw makes the questions of what constitutes the food of an oyster and how it is taken in of great practical importance. Because the mode of feeding consists in straining great volumes of water through the gills and ingesting the microscopical material suspended therein, the purity of the oyster meat is correlated closely with the character of the water running over the oyster beds.

It has been known for many years that the general appearance of the oyster, the thickness and shape of its shell, and the quality and flavor of its meat reflect readily the conditions of the environment under which the organism grew. The constantly increasing pollution of our inshore waters caused by the discharge of domestic sewage and trade wastes has ruined many thousands of acres of profitable oyster grounds and in many instances has rendered the oysters grown in the vicinity of large cities unfit for human consumption. Long before the discovery of bacteria

as the cause of infectious diseases, a French physician, Pasquier, attributed the epidemic of intestinal illness to the eating of raw oysters taken from sewage-polluted bottoms. Since 1816, the year when his investigation was made (Pasquier, 1818), and up to the present time, oysters and other shellfish often were held responsible for the outbreaks of acute gastrointestinal disturbances and typhoid fever.

Realizing the danger to the public health brought about by the consumption of infected oysters, and conceiving the damages to the oyster industry caused by the loss of public confidence in the safeness of the oyster, the various States gradually have placed the harvesting and handling of oysters under the supervision of the municipal and State health authorities and shellfish commissions. In 1909 the Federal Government took a step in the same direction when the United States Department of Agriculture, acting under the authority of the Federal food and drugs act, issued Food Inspection Decision No. 110, which declared it to be unlawful to ship or to sell polluted shellfish in interstate commerce. In the following year, by another act (Department of Agriculture, Food Inspection Decision No. 121), it prohibited the shipment or sale of oysters floated in polluted waters. In 1927 Food Inspection Decision No. 110 was reaffirmed and it was declared unlawful to ship or to sell in interstate commerce oysters or other shellfish that have been subjected to "floating" or "drinking" in brackish water or water containing less salt than that in which they are grown. (Department of Agriculture, Food Inspection Decision No. 211). At present all the oyster-producing States have adopted a system of issuing certificates to the respective oyster growers and dealers under the supervision and with the approval of the United States Public Health Service. According to this plan every bed from which oysters are taken for the market is examined from a sanitary point of view and must conform to the established bacteriological and chemical standards of purity.

The question of standards in the sanitary control of shellfish was much discussed and is of great importance to the industry. For many years bacteriologists were using various schemes of bacteriological examination of the oysters, with the result that various Federal, State, and municipal authorities gave preference to one or another standard and enforced their regulations accordingly. In 1922 the American Public Health Association (Committee on Standard Methods for the Bacteriological Examination of Shellfish, 1922) adopted a standard method for the bacteriological examination of shellfish, which at present is widely though not universally employed in the sanitary control of the shellfish industry. Briefly speaking, it consists in determining the relative abundance of *Bacterium coli* in the shell liquor of the mollusks and expressing the results by an arbitrary numerical system known as the American Public Health Association method of scoring oysters. The technical procedure consists in making a composite sample from at least five oysters and incubating the fermentation tubes filled with lactose broth with 0.1 and 0.01 cubic centimeter of the composite liquor. The water required for dilution purposes is either sterile sea water or tap water containing 2 per cent sodium chloride. For each dilution five fermentation tubes are used; altogether 15 fermentation tubes are required for every test. Upon the formation of gas, confirmatory tests are made in accordance with the standard methods of water analysis. The presence of *Bacterium coli* in each tube, if confirmed, is to be given the value representing the reciprocal of the greatest

dilution in which the test for *B. coli* is positive. If, for instance, *B. coli* is present in 1 cubic centimeter but not in 0.1 cubic centimeter the value is 1; if present in 0.01 cubic centimeter the value is 100. The score for the whole sample is the total of these values. Sometimes, however, one or more tubes show results, for instance in 0.01 cubic centimeter, while the other tubes show negative results in 0.1 cubic centimeter. In this case the recession of values is made; the 0.01 tube is given the value 10 instead of 100, while the tube showing absence of *B. coli* is given the value 10 instead of 1. The results of the test are expressed in a tabular form.

TABLE 1.—*Method of scoring*

Tubes	1 cubic centimeter	0.1 cubic centimeter	0.01 cubic centimeter	Values
1	+	+	−	10
2	+	+	−	10
3	+	+	+	100
4	+	+	−	10
5	+	+	−	10
Score				140

The weak point in the method of scoring consists in the fact that the figures of the score do not represent exact quantitative values and are used simply as symbols to express the relative abundance or scarcity of a given microorganism. Sometimes instead of a standard method a so-called "individual" method of scoring is used. It consists in planting shell liquor from five separate oysters instead of making one composite sample. The counting is done in the same manner as prescribed by the standard method. The comparison between the two methods made by Hasseltine (1926) shows that the scores obtained by the individual method may be much lower than that obtained by the standard. For instance, the sample of five oysters tested by the individual method scored 5 while the same examined by the standard method gave a score of 5,000. Such a discrepancy is undoubtedly due to the error in the method of sampling. In order to get reliable results many more oysters should be examined, but this involves so much labor and time as to make it impracticable.

The question of the standards of purity of the oyster was much discussed during the past 17 years and different standards of purity were proposed by several laboratories. The bacteriological examinations of oysters were supplemented by an inspection of the beds from which the oysters were obtained and by the bacteriological examination of the water. Finally, the *B. coli* score not exceeding 50 was accepted by all parties interested in shellfish control as a permissible standard of purity. The committee on standard methods of the American Public Health Association (1922) failed, however, to recommend a definite standard of purity, and the members of the committee have limited their report to a description of the methods of examination without committing themselves to any definite figure. For many years various State and municipal authorities regarded oysters produced under satisfactory circumstances and having a *B. coli* score of not over 50 as safe. In 1925 the Committee on Sanitary Control of the Shellfish Industry of the United States Public Health Service recommended that pending the collection and analysis of further data the standard of a *B. coli* score not exceeding 50 be continued, with the understanding that if the facts collected warrant it this recommendation would be altered.

The question of a definite criterion of purity is of great practical importance, yet the problem has never been studied experimentally; the proposed score of 50 was accepted as standard, although no significance was established for either figure by a comprehensive investigation.

In introducing the standard of purity of shellfish the bacteriologists have followed the practice established in the sanitary control of drinking water and milk. They met with difficulties, however, which at the beginning were not fully recognized and which are caused by the very nature of the product. The oyster is a living organism and can not be treated in the same manner as milk, water, or other food products. It is capable of adjusting itself to new conditions in which it may be placed and responds quickly to changes in environment. The first difficulty encountered in the application of the *B. coli* score method to the sanitary control of the oyster consisted in sudden and wide fluctuations in score, which sometimes occurred after the oysters were taken from the water, and consequently the possibility of their further pollution was excluded. It has been found that the *B. coli* score of the shell liquor of the oyster kept in storage does not remain constant but increases and decreases rather irregularly. It happened, for instance, that oysters tested in New York and found to have a low score were shipped to Chicago, where they were condemned because of a very high score. Recently Elliot (1926) made a study of the changes in the bacterial content of market oysters and found that shucked and shell oysters kept at room temperature show a sudden and maximum rise in total count of bacteria from the second to the fourth day of storage. Unfortunately the author failed to make temperature readings but specifies that the oysters were kept in a "cool basement when the outside temperature was below freezing point." Sudden increase in the *B. coli* score indicates that the microorganism was propagating in the oyster liquor, but the increase in score after the oyster was taken from the water had no relation to the sanitary conditions under which it was grown.

It has been observed, also, by a number of investigators that there exists a definite relation between the temperature of water and the *B. coli* score in the oysters. Gorham (1912), Pease (1912), and Gage and Gorham (1925) have shown that in winter the bacterial content of oysters taken from polluted waters is abnormally low. Discussing this phenomenon, the investigators attributed it to the slowing down of biological activity of the oyster as a result of a reduction of temperature and came to the conclusion that with the decrease in temperature the oyster passes into a state of hibernation. Round (1914), working on the bacteriology of oysters, came to the conclusion that oysters close their shells for varying periods of time, depending on temperature. According to his opinion the opening and closing of the shell is controlled by the rapidity of metabolic processes, which in turn are controlled by temperature. He failed to support this statement with the experimental data, however. Cumming (1916) has shown that January and February were the months when the *B. coli* content of water taken at the mouths of rivers was highest and the lowest for oysters taken from the same locality.

The study of the effect of temperature on the *B. coli* score in the oyster was handicapped by the lack of knowledge of the relation between the abundance of *B. coli* in the water and in the oysters taken from the same locality. So little was known regarding this important question that the Committee on Sanitary Control

of the Shellfish Industry (1925) failed to recommend any precise bacterial standards for waters from which the taking of shellfish is permitted until additional data are assembled and considered. It was regarded as unnecessary, however, to apply to such waters the rigid standards that are established for drinking-water supplies in interstate commerce. It is known at present that because of the mode of feeding, the oyster is able to concentrate in the shell liquor the microorganisms present in the sea water. Undoubtedly the process is dependent on the rate of filtration of water through the gills and on the rate of ejection of the accumulated material. Both processes very likely are affected by changes in the environment. Wells (1926), working at the United States Public Health Laboratory at Fishermans Island, arrived at the conclusion that the number of *B. coli* in the shell liquor is higher as compared to that of the water, where the *B. coli* concentration was low, and that the ratio decreased as the abundance of *B. coli* in the water increased. The same relation was observed by Tarbett (1926) in the waters of lower Chesapeake Bay. He has shown that with water scoring from 0 to 0.5, the ratio of water score to oyster score was 1 to 600; in water scoring 1.4 to 5 the ratio was 1 to 44.8; and in water scoring from 14 to 50 the ratio was 1 to 7.6. Tarbett admits that the relation between the *B. coli* content in water and in the oyster is not a simple one and that temperature is an important factor. Neither Wells nor Tarbett give any explanation for the differences in the ratios they have observed. It is very likely that a number of factors, like temperature, pH, and changes in the chemical composition of water, should be held responsible for the differences in the ratios observed by these investigators. It is difficult to believe that merely the fluctuation in the abundance of *B. coli* may affect the activity of the oyster. It is very likely that the increase or decrease in *B. coli* content in shallow and polluted waters where the observations were carried out are accompanied by physical and chemical changes in the water, which in turn affect the activity of the oyster gills.

In a study of seasonal fluctuations in *B. coli* score in the oyster, and in discussing the questions of feeding and hibernation, some of the investigators (Round, 1914; Nelson, 1921, 1923) regarded the oyster as feeding whenever its shells are open. As it will be shown in the present paper, the fact that the shells are open does not necessarily mean that the oyster is feeding. The two phenomena namely, the contraction of the adductor muscle and the ciliary activity of the gill epithelium—are independent from one another and should be studied separately.

The crisis of the oyster industry in 1924 caused a revision of the methods of sanitary control of shellfish. At the same time, the question of the effect of temperature on the activity of the oyster was taken up again by the bacteriologists engaged in the sanitary inspection of shellfish and by the oyster growers interested in the safety of their product. It is the author's belief that the solution of the practical problem concerning the standard of purity of the oyster should be based on a profound knowledge of the functions of the organism and its relation to its environment. On the other hand, understanding of the activity of the organism is essential for the oyster growers who, by adopting methods that permit self-purification of the oyster, are trying to insure the cleanliness of their product.

The present investigation was made for the purpose of filling the gap in our knowledge of the physiology of the oyster and to supply information concerning the

effect of temperature on the activity of the gill. An effort was made to put the experimental work on a quantitative basis and whenever possible to give an accurate measurement of the reaction of the organism. The experiments were carried out at the United States Fisheries Biological Station at Woods Hole, Mass., in the summers of 1925 and 1926 and the winter of 1926, and in the Pease Laboratories in New York. The author wishes to acknowledge his gratitude to Dr. H. D. Pease for extending the privilege of using his laboratory and supplying technical assistance in several bacteriological experiments performed in connection with this investigation.

The oysters used in the experiments were received from Long Island Sound, Wellfleet Harbor, Mass., Wareham River, Mass., and Chesapeake Bay. No differences in the behavior of oysters from these localities were noticed.

STRUCTURE OF THE OYSTER GILL

The gill of an oyster is a complex, ciliated organ that takes part in three important functions of the organism—respiration, feeding, and excretion. One of its most noticeable activities consists in producing a strong current of water, which passes through numerous branchial chambers and insures the exchange of gases between the tissues of the organism and the surrounding medium. The material suspended in water and brought in with the current constitutes the food of the oyster. It settles on the surface of the gill and, after being entangled in the mucus excreted by numerous gland cells, is pushed by the ciliary epithelium toward the distal edges of the gill laminæ and is conveyed to the labial palps, where it is either rejected or enters into the digestive tract. When the oyster is not feeding and keeps its valves closed the gland cells of the gills continue to excrete mucus, which accumulates in a large quantity on the surface of the gills and is discharged into water at the first opportunity. The structure of the lamellibranchiate gill has been the object of numerous investigations, and for a detailed anatomical and histological description the reader is referred to the works of Peck (1877), Kellog (1892), Janssens (1893), Ridewood (1903), and Yonge (1926). It is necessary, however, for the purpose of the present paper, to give a brief account of its essential features.

The oyster has two gills, each formed by one outer and one inner demibranch. In a transverse section the gill presents the figure of the letter W. Each demibranch consists of one descending and one ascending lamella, leaving a space between them and being united at their lower ends. The upper edges of the ascending lamellæ of the inner demibranchs are united in the middle line, those of the outer are fused with the mantle. The spaces above the gill lamellæ are called epibranchial or suprabranchial chambers; they open posteriorly into one large exhalant chamber (cloaca). The spaces below the lamellæ (so-called infrabranchial chambers) are completely shut off from the epibranchial chambers, and communication between them is possible only through numerous minute pores in the gill.

The gill lamellæ are made up of numerous parallel filaments (fig. 1, *fl.*) arranged in rows, alternatingly forming crests and grooves. There are from 10 to 16 filaments to each crest. According to Herdmann (1899) there must be over 2,000 filaments for each surface of each gill, or from 8,000 to 10,000 filaments in all. Due to the peculiar arrangement of the filaments, the surface of the gill is plicate, the crests of the

plicæ being visible to the naked eye. The neighboring filaments are connected by an interfilamental junction (*int. fl. j.*) formed by a band of connective tissue running round the inner surface of the plicæ. In every groove between the two adjacent filaments there is a water pore (*p.*), through which the water enters into the interfilamental spaces. The two lamellæ of each demibranch are, in turn, united by the interlamellar junctions (*int. lm. j.*), having a form of septa and subdividing the

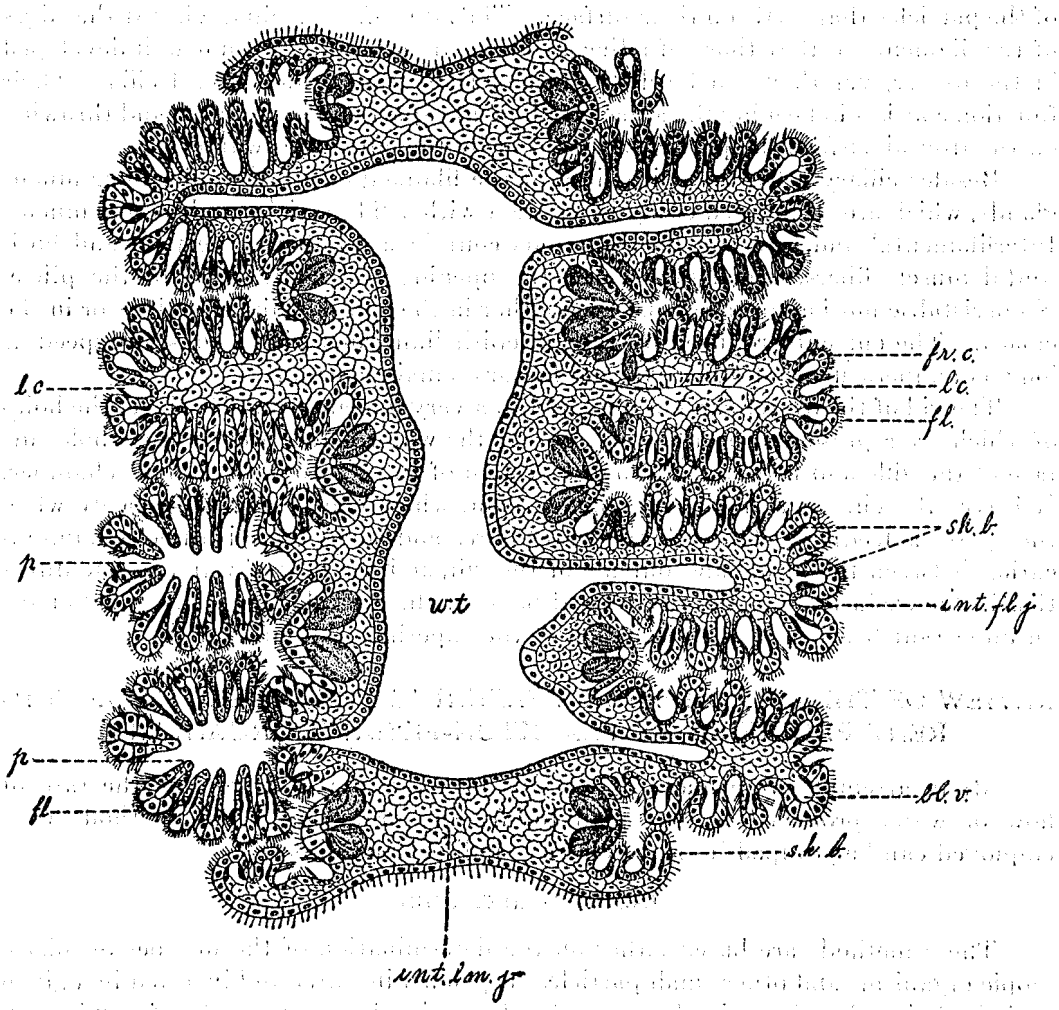

FIG. 1.—Cross section of the gill lamellæ of the oyster. Semidiagramatic. *bl. v.*—blood vessel; *fl.*—filament; *fr. c.*—frontal cilia; *l. c.*—lateral cilia; *int. lm. j.*—inter lamellar junction; *int. fl. j.*—inter filamentar junction; *sk. b.*—skeletal bar; *p.*—water pores; *wt.*—water tubes. (Slightly modified from Kellog)

epibranchial chambers into canal-like compartments or "water tubes" (*w. t.*), very narrow at their distal ends but reaching several millimeters in diameter in the proximal portion of the gill. In an adult oyster there are about 30 tubes in each demibranch, or about 120 tubes altogether. Each filament of the gill has one blood vessel (*bl. v.*), lined by a thin layer of connective tissue, and has two skeletal bars (*sk. b.*). The surface of the filament is covered with a ciliated epithelium forming

three groups of cells, frontal, (*fr. c.*) latero-frontal, and lateral (*l. c.*), the relative positions of which are shown in Figure 1. It has been fully established by the work of Orton (1912) and Gray (1922) that the ingoing current of water, passing through the water pores into epibranchial chambers and cloaca, is produced by the activity of the lateral cilia, which beat inward; i. e., across the surface of the gill. The frontal cilia beat parallel to the surface of the gill and are concerned solely with the transport of the particles that settle on their surface. The latero-frontal cilia, lying at the edges of the filaments so that those of adjacent filaments interlock, are not well developed in the oyster, yet they can be distinguished from frontal and lateral cilia. Their function consists in keeping the adjacent filaments apart and in stopping and throwing on the frontal surface the particles carried in by the current of water.

Besides ciliary cells, the epithelium of the filaments contains unicellular mucus glands, which are stimulated easily by contact with solid particles and secrete mucus. Interfilamental and interlamellar junctions contain a number of vertical and horizontal muscle fibers, which may cause the opening and contraction of the plicæ. No peristaltic motion has been observed either in the epibranchial chambers or in the cloaca. The current produced by the gill epithelium runs with a constant speed as long as temperature and other external factors remain constant.

The gill of the oyster can be compared to a very fine and complex sieve, the holes of which are represented by the water pores; the water is taken in by the whole surface of the gills and is driven through a system of tubes into one exhalant chamber. It leaves the gills as a single outgoing stream, which can be observed easily when the oyster is feeding. Through the water pores and tubes there is direct communication between the inside and outside of the gill, and the flow of water in one direction is due exclusively to the rhythmical beats of the lateral cilia. These facts have an important bearing on the discussion of the experimental data.

REVIEW OF THE METHODS FOR MEASURING THE STRENGTH OF CURRENT PRODUCED BY PLANKTON-FEEDING ORGANISMS

Many attempts were made by various investigators to determine the rate of flow of water produced by the plankton-feeding organisms. The methods they employed can be grouped into two classes—indirect and direct.

INDIRECT METHODS

These methods are based either on the determination of the number of microscopic organisms and other small particles suspended in water and ingested in a given period of time by the animal, or on the determination of the rate of respiratory exchange of a given organism. One of the first estimations of the rate of flow of water through the gills of an oyster was made by Grave (1905). He kept the oysters in filtered sea water for three days until their digestive tract became devoid of any plankton organisms. Then the oysters were placed in the natural sea water and at convenient intervals were taken up, contents of their stomachs were removed, and the number of diatoms in them counted. Knowing the average number of diatoms per liter during the three consecutive summers, and having obtained the number of diatoms collected by the oyster in a given period of time, Grave estimated the rate of

flow of water through the gills. His method is open to several objections. First, because of the seasonal and daily fluctuations in the abundance of diatoms in water the average figure may differ greatly from the diatom content at the time of observation. Second, not all of the diatoms strained by the gills are ingested; certain numbers of them may be rejected into the pallial cavity and do not reach the digestive tract.

Virtually the same method as that employed by Grave was used by Moore (1913) in studying the oysters of Mississippi. Nelson (1921), studying the feeding of oysters in Barnegat Bay, adopted the following procedure: At ebb tide oysters were placed on a platform built in the bay; when the tide began to run the investigator watched for the opening of the shells and kept them continuously under observation for one hour. Meanwhile, every two minutes a sample of the water that was passsing over the oysters was taken and the number of Tintinnopsis (a protozoan, which was taken as an index) was counted. At the close of the hour two oysters were opened, the stomachs washed out, and the contents counted. The number of Tintinnopsis collected by the two oysters compared with the number in the water gave the rate of current produced by the oyster. Nelson assumes that the stomachs of the oysters he examined were empty because, according to his observations, when an oyster opens after a period of closure of one hour or more its stomach is virtually empty of food.

An entirely different method was employed by Viallanes (1892) and Ranson (1926) in experiments on European oysters and mussels. Their method is based on the determination of the amount of clay precipitated by the mollusks during a period of 24 hours. The mollusks are kept in the crystallizing dishes placed on the bottom of a tank filled with water, to which a known quantity of clay (0.0546 gram per liter) is added. Several dishes are placed in the same tank for control without the animals. After 24 hours the sediment that has accumulated on the bottoms of the dishes is collected, dried, and weighed. The figures thus obtained are corrected by subtraction of the amount of precipitate by gravity (in the controls), and the rate of filtering of water through the gills is thus computed. These authors used their method chiefly for the determination of the relative filtering ability of European and Portuguese oysters. They fail, however, to give a record of the temperature of the water and do not state whether the shells of the mollusks were open all the time during the experiment. The possible source of error in this method lies in the fact that the mucus discharged by the gills may cause the agglutination and precipitation of the clay, and therefore the amount of precipitate on the bottom is not a safe measure of the activity of the gills.

In a series of papers Pütter (1909, 1911, 1924) has estimated the rate of flow of water by measuring the CO_2 production and O_2 consumption by various marine organisms. As a result of this study he arrived at the conclusion that in order to receive a required amount of carbon from plankton the organisms in question should filter tremendous volumes of water. Thinking that this is impossible he advanced the hypothesis, which was much criticized by other investigators (Moore, Edie, Whitley, and Dakin, 1912), that the marine organisms feed by absorption of organic matter dissolved in sea water.

DIRECT METHODS

The first measurement of the rate of flow of water through the plankton-feeding organisms was made by Parker (1914). Experimenting with the siliceous sponge Stylotella, he adopted the following method: A glass tube was inserted into the osculum and the flow of water in the tube was determined by measuring the velocity of floating particles, such as grains of carmine, that were carried up the tube by the current. By placing the tube introduced into the osculum in a vertical position he was able to observe the rise of water in the tube above the level of water in the tank where the sponge was kept and thus measured the pressure produced by the ciliary motion of the cells.

A similar method was used by Allen (1914) in a study of the feeding habits of fresh-water mussels. He introduced one end of the rubber tubing in the excurrent siphon; the other end was connected to a calibrated glass tube having a capacity of 2 cubic centimeters between given marks. A neutral coloring matter was added into the rubber tubing through a pipette thrust into it just outside the siphon and the rate of flow of colored substance in the tube was measured. Because of the contraction of the siphon, Allen had considerable difficulty in measuring the velocity of the outgoing current and made only one determination. Both Parker and Allen failed to note that the velocity of the current running in a circular pipe varies along the cross section of the pipe, the maximum velocity being at the center, with minimum velocity close to its walls. Consequently, no discharge of water can be computed from their data unless the position of the particle, the speed of which is being measured, is known. When the carmine grains flow in water they settle on the bottom gradually and are carried out at different speeds depending on the distance from the center of the tube.

METHODS EMPLOYED IN THE PRESENT INVESTIGATION

Two methods of measuring the rate of flow produced by the gills of the oyster were described by the author in 1926 (Galtsoff, 1926). It is desirable to give a more complete description of them here.

TANK METHOD

This method is designed primarily to collect the water after it had passed through the gills and to measure the pressure inside the gill cavity. The valves of the oyster are forced apart and a glass rod is placed between them to prevent their closing; a rubber tube 6 to 7 millimeters in diameter is inserted in the gill cavity and made fast by packing all the spaces around with cotton. The outgoing water passes through the tube; leakage, if any, can be noticed easily by adding a few drops of carmine suspension and watching the produced currents. The oyster is then placed in a tank (fig. 2) of about 10 liters capacity; the tank is connected through a horizontal glass tube (*b*) of 6 millimeters diameter, with a small vessel (*v*) of about 50 centimeters capacity. A vertical tube (*c*), 8 millimeters in diameter, passes through the bottom of a small vessel and serves as an overflow; its upper level is about 1 centimeter above the upper level of the horizontal tube *b*. The tank is made of celluloid, 1/8 inch thick; the walls are cemented with a solution of celluloid in acetone.

The large tank is filled with water up to the level of the vertical tube *c*. When equilibrium is established the rubber tube inserted into the oyster is connected to the horizontal tube *b* and the water from the gills begins to flow into the small vessel. The overflowing water is collected in a graduate.

The hydrostatic pressure inside the gill cavity can be measured by plugging the vertical tube *c* and watching, in the water gauge (*g*), the rise of the level in the small vessel. In a few minutes a maximum difference is reached and the flow of water through the tube *b* ceases. This indicates that there is no more difference in pressure between the inside of the gill cavity and the end of the tube *b*.

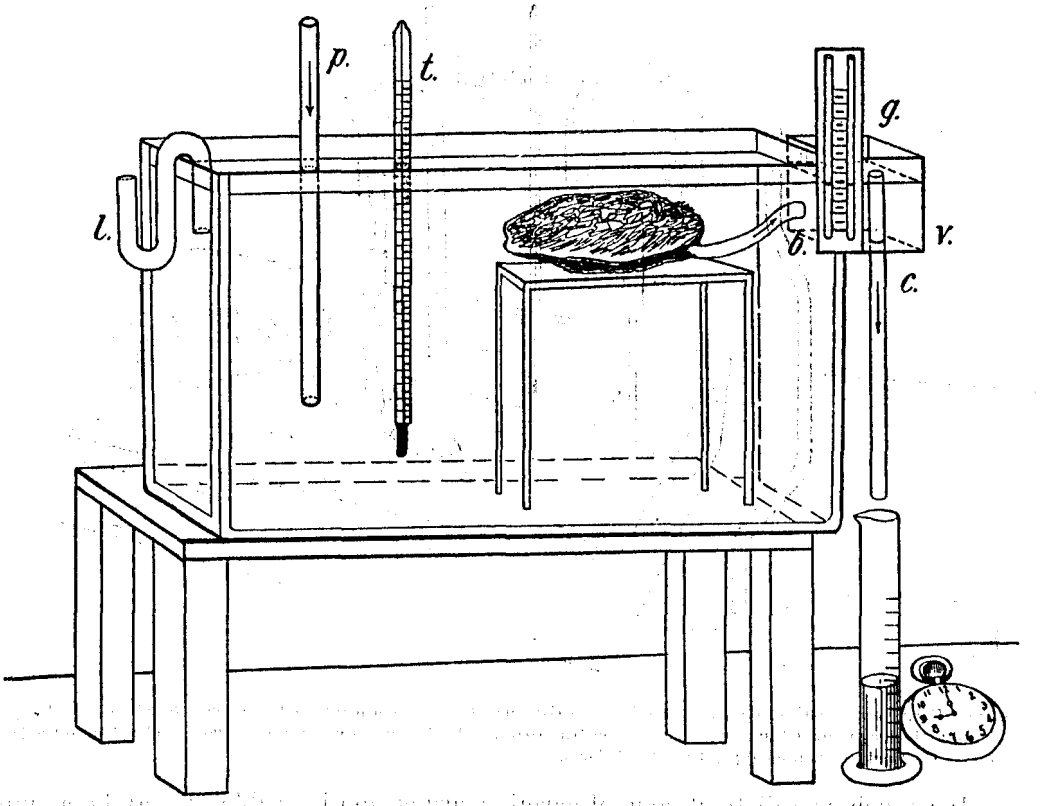

FIG. 2.—Tank for collecting the water after it passed through the gills, and measuring the hydrostatic pressure inside the gill cavity. *b*.—tube connecting the two vessels; *c*.—overflow; *g*.—water gauges; *l*.—constant level arrangement; *p*.—pipe supplying fresh sea water; *t*.—thermometer. Approximately one-third natural size

The difficulty in employing the tank method lies in the necessity of being very careful to keep the water in both vessels at a constant level. The rise of level in the large tank forces the water to flow by gravity through the gills, while a rise in the level in the small vessel retards the rate of flow because the gill epithelium is forced to work against the pressure. The method is indispensable, however, for collecting the water that had passed by the gills.

CARMINE METHOD

The rate of flow of water can be determined easily by the carmine method (Galtsoff, 1928), which is as follows: The oyster is rigged up in the same manner as in the

tank method and is placed in a glass tray of about 4 liters capacity. (Fig. 3.) The end of the rubber tube inserted in the gill cavity is connected to a ⊥ tube, the upper end of which is attached to a funnel filled with a fine suspension of carmine in sea water. The third end of the tube is connected with a graduated glass tube (*t*) 6 millimeters in diameter and 17 centimeters long. Releasing the clamp (*C*) a very small amount of carmine is allowed to enter the tube, where it forms a distinct cone moving inside the tube. The rate of movement of the apex of the cone is measured by recording, with a stop watch, the time required for it to pass from 0 to the 15-centimeter mark.

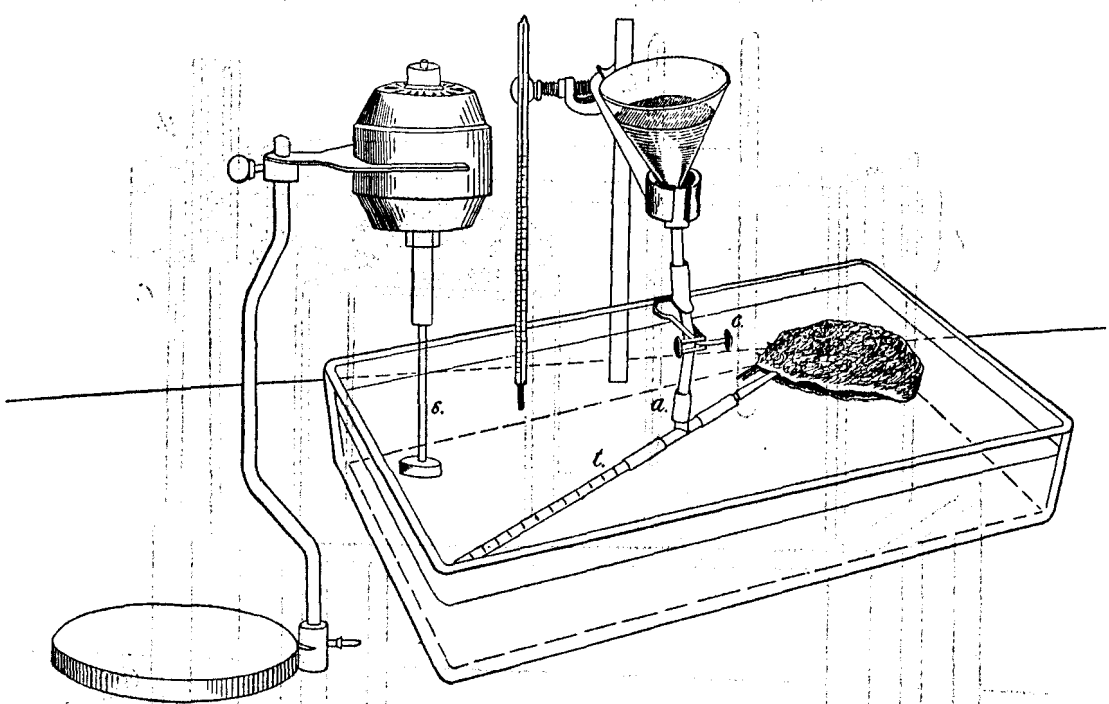

FIG. 3.—"Carmine method" to measure the velocity of the current. *a.*—vertical tube with rubber connections; *c.*—clamp; *t.*—horizontal tube graduated in centimeters; *s.*—electric stirrer. The drawing is made from a photograph taken in the laboratory. Heating and aeration apparatus are not shown

Inasmuch as a distinct cone of carmine suspension is visible, it may be assumed that in this case we have a viscuous flow or stream line, to which the Poiseuille's formula

$$S = \frac{D^2 \Delta p}{16 \mu l} \qquad (1)$$

is applicable. In this formula S = speed at the axis of the tube; D = diameter and l length of tube in centimeters; Δp = pressure drop between the two marks in dynes per cm^2; μ = viscosity in poises.

As the mean velocity S_m of the whole cross-sectional area of the tube is one-half the velocity at the axis (see Gibson, 1925, p. 63)

$$S_m = \frac{S}{2} \qquad (2)$$

the rate of discharge, V, in cm^3 per second is

$$V=\frac{\pi D^2 S}{8} \quad (3)$$

For accurate measurement of the velocity of the current in the tube (t) the quality of the carmine suspension is of great importance. The suspension must be very fine and should contain no particles that may settle inside the tube; it must have the same specific gravity as that of the sea water in which the oyster is kept, and its color must be sufficiently deep to be noticeable when a small amount of the suspension is allowed to enter the tube and forms a cone.

To make up a suspension that will conform with these requirements the following procedure should be followed: The carmine powder is ground in a glass mortar with a few drops of sea water; after a very fine paste is formed more sea water is added and the suspension is poured into a glass bottle, is well shaken, and is allowed to stand undisturbed for five minutes. Then the upper half of it is poured into another bottle, to be used in the experiments. Only fresh suspension should be used, because after 24 hours the carmine particles settle more quickly and have a tendency to form lumps.

In a study of the effect of temperature and other external factors on the function of the gills the carmine method has several advantages over the tank method. First, no error is introduced by the possible fluctuations in the levels of water in the two vessels; second, the water in the tray where the oysters are kept can be changed easily without disturbing the animals; and third, the measurement of the rate of flow can be made in a few seconds instead of several minutes, as is required by the tank method. It is interesting, however, to show how the two methods check each other. The following figures were obtained with an oyster that was placed first in a tank, where the rate of flow of water through its gills was measured; then it was transferred to a tray and after 30 minutes of rest the rate of flow of water was measured again. In both cases 10 readings were made and the arithmetic mean was computed. The results of this test are shown in the following table:

TABLE 2.—*Rate of flow of water determined by "tank" and "carmine" methods*

Method	Rate of flow (liters per hour)	Temperature, ° C.	Time
Tank	1.40	20.3	2.07–2.21 p. m.
Carmine	1.66	20.8	2.51–2.54 p. m.

It has been found that in all cases where such a comparison was made that the figures obtained by the tank method are a little lower than those obtained with the carmine method. This is due probably to the additional resistance in the tube (b) connecting the two vessels. It is obvious that the rate of flow can be measured more accurately by employing the carmine method, while the tank method makes it possible to measure the hydrostatic pressure in the gills and to collect the water after it has passed by the gills.

In the experimental study of the effect of one of the external factors on the biological reaction, particular care should be exercised in eliminating the influence

of other variables that may affect the function of the organism. Gray (1922, 1924, 1924a) has shown that not only temperature but changes in the hydrogen-ion concentration, oxygen and carbon dioxide contents, and concentration of various salts in sea water affect the ciliary activity. In the present experiments the salinity of the water, its oxygen content and the pH value were kept constant. There are two factors, however, the control of which presented certain difficulties and which may be responsible for considerable fluctuation in the experimental data. In some of the oysters, especially in those that had been exposed for a long time to a low temperature, the gills were covered with a thick layer of mucus, which blocked a free passage of water through the pores. These oysters exhibited wide and irregular fluctuations in the velocity of the current, but after the outside and the inside of the branchial chambers were washed out with sea water the current became steady.

Mechanical stimulation represents another factor that may affect the velocity of the current. Whenever the oyster attached to the apparatus was disturbed, it invariably showed a change in the rate of flow, frequently stopping the current entirely but coming back to normality in a few minutes. The following record of one of the experiments illustrates this fact very clearly.

TABLE 3.—*Effect of mechanical stimulation on the velocity of the current.—Experiment 62, August 10, 1926*

Speed at the axis of the tube (centimeters per second)	Temperature, ° C.	Time	Remarks
1.1	14.2	11.03	
1.1	14.2	11.04	
1.1	14.2	11.05	
1.1	14.2	11.06	
1.2	14.2	11.07	Oyster disturbed
.6	14.2	11.08	
.6	14.2	11.09	
1.1	14.3	11.10	
1.0	14.3	11.11	
1.0	14.3	11.12	

It is very probable that these fluctuations are due to the contraction of the branchial chambers, caused by mechanical stimulus. In the experiments described below the precaution was taken to avoid mechanical stimulation, and in case the oyster was disturbed by accident it was left for 10 minutes before the next readings were made.

The temperature of the water was changed by using either an electric hot-point immersion heater or a battery of jars filled with a freezing mixture. The water in the tray was agitated by an electric stirrer (fig. 3 *s*) and aerated. If necessary, the tray was placed in a water jacket with a mixture of salt and crushed ice packed between the walls. Readings were made after the oyster had been left for 15 minutes at a given temperature. The temperature was maintained constant within 0.5° C. At every given temperature from 10 to 20 readings were made, from which the arithmetical mean was computed. All temperature readings, unless otherwise indicated, were made in centigrade.

EFFECT OF TEMPERATURE ON THE RATE OF FLOW OF WATER THROUGH THE GILLS

SUMMER EXPERIMENTS

We begin the discussion of this problem with an analysis of the results of summer experiments The experimental material consists of the data of 64 experiments performed during the summers of 1925 and 1926, but in the following discussion the data of only 15 experiments, in which the observations were made at not less than five different temperature points, are taken into consideration.

The following technical procedure was followed in all the experiments: The first observations were made at room temperature, which in most cases was around 20° C.; then the water was cooled gradually to 5° and then warmed until the temperature of 35° and in a few cases 45° was reached, and cooled again to 20°. In 5 experiments readings were made at 2° intervals; in 10 experiments 5° intervals were used. Each determination of the rate of flow is a mean of 10 or 20 readings. Altogether 2,470 readings were made.

All the experiments were made with adult oysters varying in size from 3.5 to 5 inches. There exist considerable individual variations in the rate of flow of water that can not be correlated with the differences in size of the oysters and undoubtedly depend on the physiological conditions of the organisms. Some of the small oysters proved to be very active and produced very strong currents, while some of the largest ones were very weak. At present it is impossible to determine the cause of such differences. There was nothing in the appearance of the oysters that could be correlated with the efficiency of their gills.

The range of individual variations in the rate of flow of water at different temperatures is shown in Table 4 and Figure 4. In Table 4 the experimental data are grouped in 14 classes, each at 3° intervals, the figures in the body of the table representing the frequencies; in Figure 4 the frequency distributions for temperature, ranging from 6° to 26.9°, are presented graphically. An examination of the frequency polygons discloses that the individual variations increase with the temperature and reach their maximum between 24° and 26.9° C.

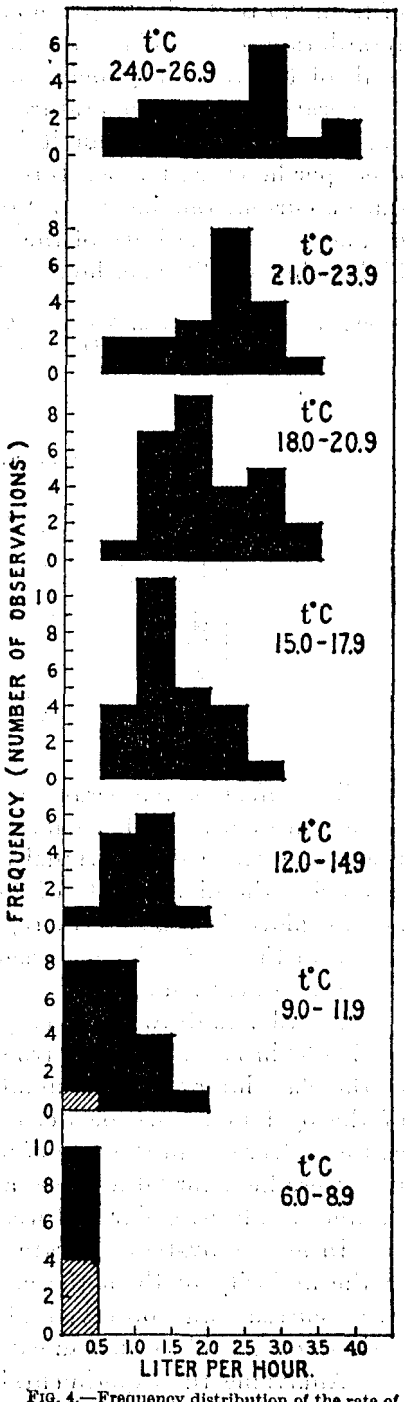

FIG. 4.—Frequency distribution of the rate of flow of water at different temperatures. Cross-hatched areas show the number of oysters that failed to produce any current

At this temperature the rate of flow of water varies in the different oysters from 0.5 to 3.9 liters per hour; the majority of the oysters, however, filter the water at a rate of from 2.5 to 2.9 liters per hour. With the cooling of the water comes a gradual retardation of the ciliary activity of the gills, and, as can be seen in Figure 4, the peak of the frequency polygons moves toward the left, following the decrease of temperature. At temperatures between 9° and 14.9° the range of individual variations is smaller than it is at higher temperatures and varies from 0 to 2.4 liters per hour; at temperatures between 9° and 11.9° the majority of oysters produce a current ranging from 0 to 0.9 liter per hour. At a low temperature between 6° and 8.9° the activity of the gills is greatly reduced and the rate of flow is never higher than 0.4 liter per hour. No current is produced below 5°.

TABLE 4.—*Frequency distribution of the rate of flow of water through the gills at a given temperature. The figures indicate the number of observations*

°C.	Rate of flow (liters per hour)							
	0–0.4	0.5–0.9	1–1.4	1.5–1.9	2–2.4	2.5–2.9	3–3.4	3.5–3.9
5–5.9	2							
6–8.9	10							
9–11.9	8	8	4	1				
12–14.9	1	5	6	5	1			
15–17.9		4	11	5	4	1		
18–20.9		1	7	9	4	5	2	
21–23.9		2	2	3	8	4	1	
24–26.9		2	3	3	3	6	1	2
27–29.9			1		2	2	1	
30–32.9			1		1	1	3	
33–35.9		1	1			2	1	
36–38.9					1			
39–41.9	2							
42–44.9	3							

The effect of temperature on the ciliary activity of the individual oysters can be seen in Figure 5. The curves represent the results of seven experiments and cover the whole range of individual variations from very slow-working oysters to those producing the highest rate of flow. The results of the remaining eight experiments are not plotted because they represent the repetition of one of the types of the curve shown in Figure 5. The average results of all 15 experiments are shown in a curve drawn in a heavy line.

In order to draw the average curve all the data were grouped in 14 classes, each having 3° intervals, and the true mean of each class was plotted against the midvalue of the class interval. Examination of the curves shows that the maximum activity of the oysters occurs between 25° and 30° C. Exposure to higher temperatures causes a decrease in the rate of flow. Below 40° the process is reversible, but oysters that were kept for 20 minutes at 40° and brought back to 20° failed to recover and produced only irregular and weak currents.

In all the oysters the rate of flow decreases with the drop in temperature, and in the majority of them the current stops at 8°. In a few cases, however, a very weak current was observed at 5.1°. Temperatures below 5° inhibit the ciliary activity of the gill to such an extent that no current is produced by the gill epithelium.

Analyzing the experimental data, it has been noticed that under the conditions of the experiments every oyster exhibited certain fluctuations in the rate of flow,

which could not be attributed directly to changes in the surrounding medium. Excluding the cases of accidental mechanical stimulation that may cause the contraction of the muscles in the gill tissue and result in a temporary decrease in the velocity or even in a complete stoppage of the current, the range of the fluctuations observed in all the experiments varied with the temperature. It has been shown in a previous paper (Galtsoff, 1928) that between 15° and 25° the fluctuations are small, ranging from 4.4 to 5.9 per cent, but that they increase considerably both below and above these temperatures. This means that the nearer we approach the temperature limits of the ciliary activity the more irregular becomes the ciliary motion of the gills. It must be borne in mind that the flow of water from the gill cavity is due to the difference in pressure between the inside and the outside of the gills, and that

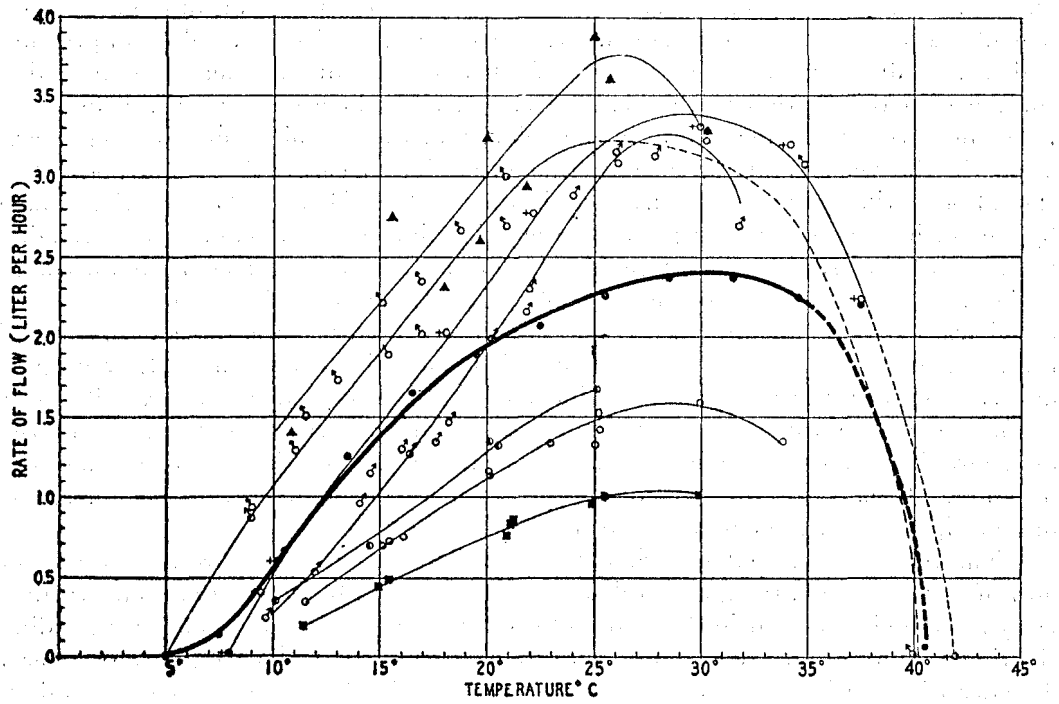

FIG. 5.—Effect of temperature on the rate of flow of water produced by the gills

the velocity of the current is a function of a pressure drop between the two points. The head pressure inside the gill cavity is maintained by the activity of the lateral cilia and is dependent upon the rhythm and coordination of the ciliary motion along the whole surface of the gill.

The beat of the ciliary cell has two distinct phases—a very rapid forward or effective stroke and a slow backward or regressive stroke. It has been shown by Weiss (1909) and Gellhorn (1925) that the work performed by the cilium during one phase is proportional to the cube of the velocity

$$W = KV^3 \qquad (4)$$

where K is a constant, W is work, and V is velocity. The ability of the ciliated cells to transport particles or produce a current depends on the difference in the velocities

of the progressive and regressive strokes. At present we have no means to measure the absolute velocity of the motion of the cilium, but Kraft (1890) has estimated that the velocity of the progressive phase is five or six times greater than that of the regressive period. This means that the work performed during the forward motion is one hundred and twenty five or two hundred and sixteen times greater than that produced during its backward movement. It is obvious that even an insignificant decrease in the ratio between the velocities of the two strokes will result in a considerable loss of efficiency of the ciliary motion.

The coordination of the ciliary activity is another factor that determines the constancy of the current produced by the gill epithelium. The maintenance of a constant pressure inside the gill cavity depends on a definite rhythm of strokes along all the filaments of the gill. As soon as the rhythmic motion in some of them becomes irregular a leakage occurs through the wall of the gill, resulting in a drop of pressure and in retarding or complete stoppage of current. In this manner even small disturbances in the rhythm of the beats of lateral cilia along one or several filaments result in considerable fluctuation in the velocity of the outgoing current. The variations in the velocity of the current that occur both below 15° C. and above 25° should be attributed to the disturbances in the rhythm of beats. Observations made by the author on the excised pieces of the epithelium kept at temperatures ranging from 5° to 15° show that the irregularity in the rhythm of the ciliary motion becomes noticeable under the microscope as soon as the temperature drops to 15°. At 10° the characteristic metachronial wave often is interrupted because the cilia in some of the filaments begin to beat simultaneously instead of in succession, as they do normally. The result is that in certain blocks of the filament all the lateral cilia beat simultaneously at the same phase, while in the other portions of the filament the metachronial rhythm is maintained. At 5° the ciliary motion becomes slow and irregular. Because of the lack of coordination at this temperature no current is produced, although the cilia are beating.

The results of present experiments on the effect of temperature on the rate of flow of water through the gills parallel the data obtained by Gray (1924) on Mytilus. Gray's method consisted in determining the relative speed of the cilia by recording the time required to move at a uniform rate a small circular plate of platinum over a distance of 1 centimeter along the surface of the gill. It should be borne in mind, however, that the transport of a particle over the ciliated surface is accomplished by the frontal cilia, while the current running through the gills is produced by the lateral cilia. As in the case of the oyster, the activity of the frontal cilia of Mytilus is a function of temperature. Gray finds that between 0° and 33° the speed of the cilia increases with the rise in temperature, although the amplitude remains normal. Between 34° and 40° a marked falling off in the amplitude of the beat occurs, followed by the reduction of the rate of beat. Experimenting with oysters, I was unable to observe the changes in the amplitude of the beats, and the attempts to apply Gray's method for measuring the mechanical activity of the frontal cilia were unsuccessful. The gills of the oyster contain numerous mucus glands that are stimulated by contact with metal and secrete mucus, which accumulates on the surface of the gill and increases the resistance to the motion of the plate. On the other hand, repeated mechanical stimulation of the cilia by contact with metal causes a complete

cessation of the ciliary motion on a given area of the gill. Readings obtained with Gray's method were so inconsistent that the method was discarded as unsuitable for the oyster gill. The temperature optimum for Mytilus gills is somewhat higher than that of the oyster. Gray's figures show that the highest activity of the Mytilus cilia takes place at a temperature between 27° and 38°, while the maximum activity of the oyster occurs between 25° and 30°.

A comparison of the results of Gray's work on Mytilus and the data obtained during the present investigation discloses the interesting fact that the curves describing the effect of temperature on the rate of flow of water and on the mechanical work produced by the oyster gills are different from the curve showing the effect of temperature on the relative speed of Mytilus cilia. (Fig. 6.) As has been shown in another

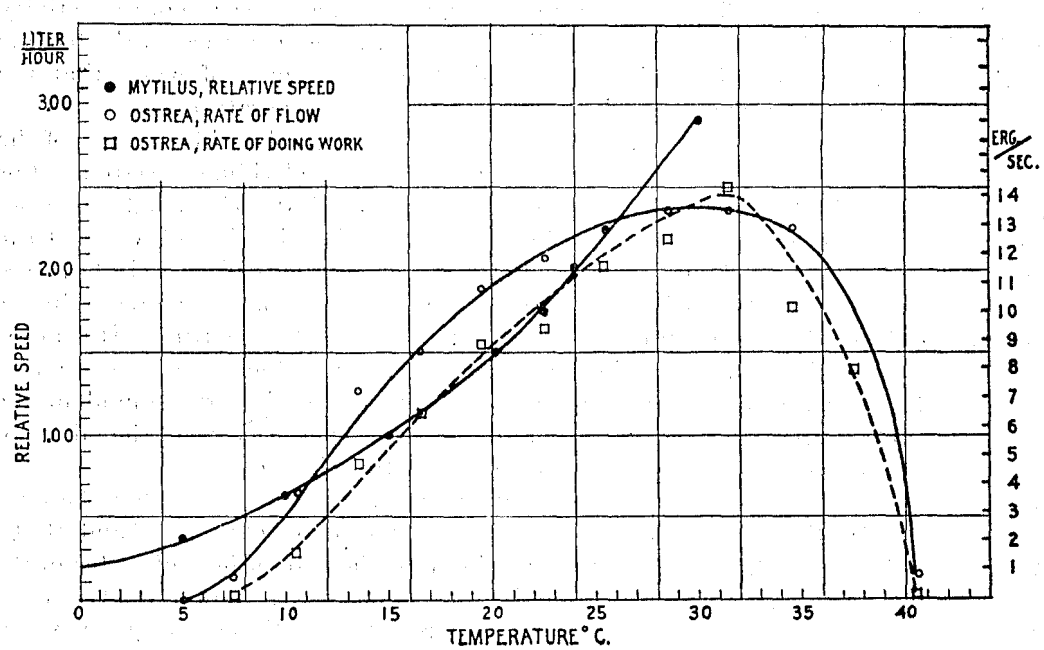

FIG. 6.—Effect of temperature on the relative speed of ciliary activity of Mytilus (Gray's data) and on the rate of flow of water and rate of doing work by the oyster. The vertical scale for the rate of doing work is shown on the right side

paper (Galtsoff, 1928), the rate of flow of water is not a true expression of the mechanical activity of the gills. With the decrease in temperature the viscosity of water increases, and therefore the resistance to fluid motion increases also; consequently more energy is required to propel cold water at a given velocity. The work expended in producing a steady current through the horizontal glass tube can be computed from the following formula (Galtsoff, 1928):

$$W = 2\pi l \mu S^2 \qquad (5)$$

where W = rate of doing work in ergs per second, l = length of the tube in centimeters, μ = viscosity of water in poises, and S = speed at the axis of tube in centimeters per second.

Analyzing the curves of the effect of temperature on the rate of doing work and on the rate of flow of water, one finds that neither curve can be described by the Arrhenius equation, which was found applicable to many instances of the effect of temperature on biological reactions. Crozier (1924) has found, however, that the effect of temperature on the relative speed of the Mytilus cilia follows this equation. The discrepancy undoubtedly is due to the fact that the rate of flow of water is controlled not only by the frequency of the ciliary beats (which may depend on a definite chemical reaction) but also is governed by several other factors, such as rhythm and coordination of the ciliary motion along the whole surface of the gill. The production of a current by the gills is a very complex phenomenon in which several reactions of the organism are involved.

Although the rate of flow of water does not give us a true measure of the activity of the gills, it supplies information regarding the effect of temperature on the feeding of the oyster; the latter is obviously dependent on the volume of water that the oyster is capable of passing through the gills at various temperatures.

It has been shown above that the rate of flow of water produced by oysters of the same age and taken from one locality is subject to wide individual variation. For instance, the highest figure of discharge of water measured at 25° was 3.9 liters per hour, while another oyster at the same temperature produced a flow of water at the rate of only 0.9 liter per hour. The results of the experiments discussed above are consistent in the respect that in all of them there was a decrease or increase in the rate of flow depending on the direction of the changes in the temperature. It is interesting to compare the data obtained in these experiments with the estimates computed by other investigators and based on the counts of planktonic forms found in the stomachs of the oysters. The comparison is difficult, however, because of the failure of the investigators to give temperature readings at the time of their experiments. Assuming that the experiments were made in summer, it is very likely that the temperature at which the observations were made was somewhere between 18° and 24° C. As is shown in Figure 4, at this temperature interval the rate of flow of water in the majority of oysters varies between 1.5 and 2.5 liters per hour. Grave (1905) states that the oyster filters 0.167 liter per hour. Moore (1913) estimates that an oyster takes in water at the rate of 40 quarts (38 liters) a day but fails to state whether the filtering was going on continuously for a 24-hour period. Wells (1916) states that "at feeding temperatures large volumes of water, from 25 to 50 gallons a day, pass through the oyster gills." In another paper (Wells, 1926) he says that "the quantity of water filtered through an oyster gill at moderate temperatures averaged greater than 2 gallons per hour." As has been shown in the present paper, "feeding temperature" covers quite a wide range—from 7° to 40° C. Unfortunately Wells does not state how he arrived at these figures. It is doubtful that there are oysters that are able to take in water at the rate of 7.5 liters (2 gallons) per hour, and Wells's figures should be regarded as guesses not supported by any experimental evidence.

Nelson's (1921) estimate of the rate of flow of water through the oyster is 6 quarts (5.7 liters) per hour. Allen (1914), for a fresh-water mussel, gives a rate of flow of 1.4 liters per hour. The filtering of water by the sea mussel has been studied in Conway Laboratory (England). According to a statement found in the Guide to

the Fisheries Exhibit (Ministry of Agriculture and Fisheries, 1922), "a mussel can pump at least 10 gallons of water through itself in 24 hours." Unfortunately I was not able to find a description of the method employed in Conway Laboratory.

The fact that the estimated figures of the rate of flow through the oyster vary from 0.167 to 7.5 liters per hour is good evidence of the unreliability of the methods employed by previous investigators. The estimation based on the count of the planktonic froms found in the stomach and intestine can not be accurate. Everyone who has had experience with quantitative plankton examination is familiar with the difficulties encountered in obtaining reliable figures. Moreover, in the estimation of the rate of flow by this method an assumption is made that all the microorganisms caught by the gills are ingested by the oyster, which obviously is incorrect, as some of them never reach the mouth of the oyster but are rejected into the pallial cavity.

EXPERIMENTS WITH HIBERNATING OYSTERS

In the experiments described above the oysters were exposed to sudden changes in temperature. Observations have shown that the period of 15 minutes during which the organism was kept at a constant temperature was sufficiently long to produce an effect on the ciliary motion. There arises a question, however, whether long-continued exposure to low temperature would produce different reaction. Nelson (1926) thinks "that with the slowly falling temperatures of autumn and early winter the oyster becomes adapted to a lower range of temperature, so that although there is a sharp decrease in ciliary movement below 5° activity does not entirely cease." For practical purposes it is very important to know whether in winter the oysters respond to the changes in temperature in the same manner as they do in summer.

In order to study this problem, several dozens of oysters were left on the bottom of Woods Hole Harbor, close to the United States Bureau of Fisheries pier, in September, 1926. The daily examination of temperature records taken at 8 a. m., noon, and 4 p. m. shows that since December 5 the temperature of the water was below 40° F. (4.4° C.), and during January and February it varied from 29.6° to 34° F. (−1.4° C. to 1.1° C.). On February 12, 1927, 36 oysters were taken from the harbor, brought into the cold laboratory room (air temperature 3.5° C.), and examined. All the oysters appeared to be healthy and showed a new growth at the edges of the shell.

The purpose of the first experiment was to determine the exact temperature at which the outgoing current begins to flow. The valves of the oysters were forced apart and glass rods were thrust between them to prevent their closing. Then the oysters were placed in cold sea water poured into a large, white enamel tray and the temperature of the water was raised gradually from 1° to 9° C. The oysters were kept from 30 minutes to one hour at a given constant temperature; observations were made at 1° intervals, and the beginning of the flow of water from every oyster was noticed by adding a few drops of carmine suspension. The temperature of the suspension was always the same as that of the water in the tray. The results of this experiment are presented in Table 5 and Figure 7. In order to facilitate a comparison with the results of summer experiments, the latter data are shown in the right column of the table.

The majority of the oysters began to produce a current at a temperature between 8.1° and 9°, though in a few of them the beginning of the flow of water took place either below or above this temperature interval. No current was observed at 5° and below. This result confirms what has been observed in the summer experiments (see right column of the table) and shows that so far as the activity of the ciliated

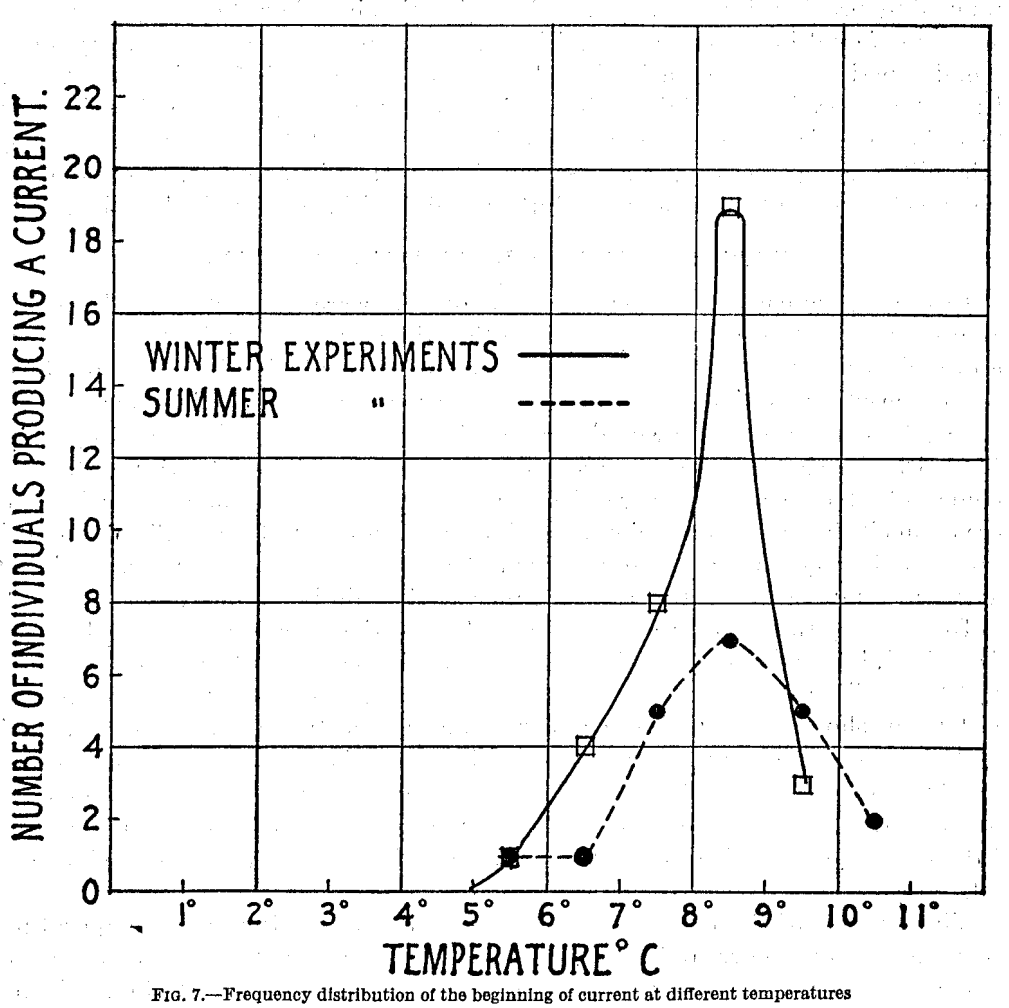

FIG. 7.—Frequency distribution of the beginning of current at different temperatures

epithelium of the gills is concerned there is no special adaptation to low temperatures; in hibernating oysters the current produced by the gills begins and ceases to flow at the same temperatures as in the summer oysters that were chilled suddenly. Examining Figure 7 one notices that in summer oysters the peak of the frequency curve is not so pronounced as it is in winter experiments, but this slight difference is insignificant and should be attributed to the greater number of winter observations.

TABLE 5.—*Temperatures at which the oysters begin to produce a current*

Temperature at which the current begins to flow (° C.)	Number of oysters		Temperature at which the current begins to flow (° C.)	Number of oysters	
	Winter	Summer		Winter	Summer
0–1	0	0	7.1–8	8	5
1.1–2	0	0	8.1–9	19	7
2.1–3	0	0	9.1–10	3	5
3.1–4	0	0	10.1–11		2
4.1–5	0	0			
5.1–6	1	1	Total number of oysters examined	35	21
6.1–7	4	1			

Another question that requires examination is whether the hibernating oysters would respond to the increase in temperature in the same manner as they do in spring or summer. In order to answer this question two experiments were performed at Woods Hole on February 14 and 15, 1927. The oysters were taken directly from the harbor and the rate of flow of water through their gills was measured with the carmine method. The results of these experiments are shown in Table 6 and Figure 8.

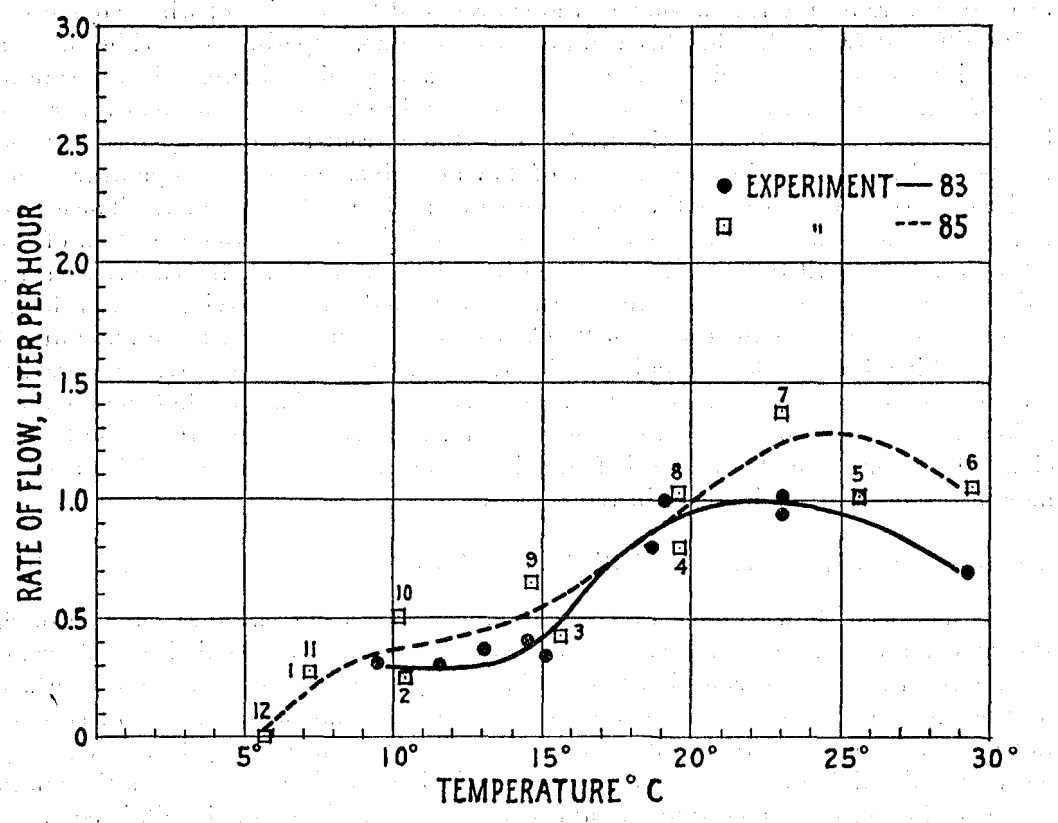

FIG. 8.—Effect of temperature on the rate of flow of water. Two experiments with hibernating oysters

TABLE 6.—*Effect of temperature on ciliary activity—Winter experiments*

Experiment 83, Feb. 14, 1927			Experiment 85, Feb. 15, 1927		
Temperature (° C.)	Speed at the axis of tube (centimeters per second)	Rate of flow (liters per hour)	Temperature (° C.)	Speed at the axis of tube (centimeters per second)	Rate of flow (liters per hour)
9.5	0.5	0.33	7.2	0.43	0.29
11.6	.45	.30	10.5	.39	.26
15.2	.51	.34	15.6	.61	.41
19.2	1.56	1.05	19.5	1.21	.81
24	1.40	.94	25.5	1.56	1.05
29.2	1.02	.68	29.4	1.81	1.22
24.2	1.56	1.05	24	2.03	1.36
18.8	1.16	.78	19.5	1.63	1.09
14.5	.61	.41	14.6	1.03	.69
13 [1]	.57	.38	10.3	.76	.51
			7.8	[2]	
			5.8	0	0

[1] Next day.

[2] Current very slow.

As can be seen from an examination of Figure 8, the curve describing the effect of temperature on the rate of flow of water produced by the gills of winter oysters is quite different from what has been obtained in summer experiments. (Fig. 5.) In both winter experiments (fig. 8) the curves show a very slight increase in the rate of flow of water between 7° and 15°, while in summer the slope of the curve at this range is quite steep. It has been noticed, also, that the current in winter oysters was less regular and the fluctuations in the rate of flow at a given constant temperature were wider than those observed at the same temperature during the summer. The explanation of such a difference in the activity of the gills is found in the condition of the gill epithelium. All the oysters examined in February had the gills covered with a thick layer of mucus accumulated during the periods of inactivity. After being kept in a tank at a temperature of 9° C., the oysters discharged large amounts of mucus, which, being of less specific gravity than water, formed long strings of gelatinous substance suspended in water. It has been mentioned above that the accumulation of the mucus clogs the water pores and interferes with the activity of the lateral cilia. It is very probable that this factor is responsible to a great extent for a slowness in response to the increase in temperature.

TEMPERATURE AT WHICH THE CILIARY MOTION CEASES COMPLETELY

It has been shown above that the production of a current that runs through the gill chambers and which is caused by the beating of the lateral cilia is also dependent on the rhythm and coordination of the ciliary motion along the whole surface of the gill. The fact that at certain low temperatures no current is produced does not necessarily mean that the cilia are at a standstill; the latter may beat irregularly without maintaining a necessary head pressure inside the gill cavity, and although in doing so they produce a certain amount of work, the efficiency of the gill is equaling zero. It is interesting to determine, however, at what temperature a complete cessation of the ciliary activity takes place. In 1926 (Galtsoff, 1926), a series of observations was made with very small pieces of gill epithelium that were kept under a

cover glass in a microaquarium. The temperature of the water in the microaquarium was regulated and kept constant within one-half of 1°. It has been found that with the decrease in temperature below 15° C. the ciliary motion becomes slow and irregular and ceases entirely at 5°. The experiments were repeated many times with the same results. Different results, however, were obtained when observations were made on large pieces of the gill. Portions of the gill lamellæ approximately 6 square centimeters in area were kept in a Stender dish of about 15 centimeters capacity which was placed on a little platform built on the bottom of a finger bowl. The space between the walls was filled with water and the temperature was kept constant. The experiments were made in September, 1926, and February, 1927. The microscopic examination made with a water immersion lens on the large pieces of gill showed that when the temperature drops to 5° C. there is a considerable slowing down of the ciliary activity and the beating continues without a definite rhythm. Due to the lack of coordination and irregularity of the ciliary motion, no current is produced. The activity of some of the cilia continues even at −2° C., when almost all the water in the dish except a narrow space just around the gill is frozen. A complete cessation of ciliary activity occurs only when the water freezes entirely. The process is reversible, and as soon as the ice melts the cilia begin to beat again. It has been noted that at low temperatures some of the cilia cease beating sooner than others; it is impossible, therefore, to speak of a definite critical temperature at which ciliary activity stops. In some of the filaments the motion stops as soon as the temperature drops to 5°; in others it goes on until all the water is frozen. There is also a distinct difference in the behavior of different kinds of cilia; frequently the lateral cilia come to rest first while the frontal cilia continue to beat.

The discrepancy observed in the experiments with small and large pieces of gills should be attributed to the different conditions of the tissues and probably to the lack of blood in the small pieces. Gray (1926) has shown that in the Mytilus gills the cells of the lateral epithelium contain a supply of available energy sufficient to maintain their activity in sea water for a limited period of time. If the gills are thoroughly washed with the sea water the lateral cilia come to rest in about 15 minutes. In a well-fed mussel the period of activity may be considerably longer. The frontal cilia, however, remain active for a very long period.

Several experiments were performed in February, 1927, with the view to determining whether the efficiency of the frontal cilia is affected by low temperature in the same manner as that of the lateral cells. The oysters were taken from the harbor when the temperature of the water was 0.8° C.; after removing the left valves and mantles the oysters, with the gills exposed, were placed in a tray filled with sea water, the temperature of which was raised gradually. A few drops of carmine suspension, having the same temperature as that of the surrounding water, were dropped on the surface of each gill, and the temperature at which the carmine particles began to move was recorded. The following is the record of one of the experiments (February 3, 1927). At 11.45 a. m. eight oysters were taken from the harbor, opened, and placed in water of 0.5° C.

TABLE 7.—*Effect of low temperature on the ciliary activity of frontal and lateral cells*

Time, p. m.	Temperature of water (°C.)	Motion of carmine particles (frontal cilia)	Outgoing current (lateral cilia)
12.37	1.0	No	No.
1.45	2.4	do	Do.
1.55	2.4	do	Do.
2.14	3.0	do	Do.
2.24	3.0	Slow in all oysters	Do.
2.32	4.0	do	Do.
2.48	4.2	do	Do.
2.54	5.2	do	Slight in 1 oyster.
3.13	5.2	do	Do.
3.18	6.4	do	Do.
3.38	6.3	do	Slight in 2 oysters.
3.42	7.0	do	Do.
4	7.2	Active	Current in 5 oysters.
4.07	8.2	do	Current in 7 oysters.
4.17	8.1	do	Do.
4.55	9.5	do	Do.
5.30	9.5	do	Do.
6.15	10.0	do	Do.
6.25	10.2	do	Current in all oysters.

The experiment shows that the frontal cilia can produce mechanical work and transport the particles along the surface of the gill at 3° while the current is produced by the lateral cilia only at temperatures above 5°. This undoubtedly is due to the fact that for the production of a current a coordinated ciliary motion along the whole surface of the gill is essential, while the transport of particles along the surface is accomplished by the coordinated beats of frontal cilia on one or several filaments only. If, for instance, the ciliary motion stops on one of the filaments (which often happens at low temperatures), it would not affect the mechanical activity of frontal ciliæ on the other filaments; as long as a sufficient ratio between the progressive and regressive strokes is maintained, the frontal cilia are pushing the particles toward the distal end of the gill, and the absence of ciliary motion in some of the filaments does not interfere with the transport of the particles by the others although the cessation of motion of lateral cilia in one of the filament interferes with the running of current through the gills.

In connection with these experiments it is interesting to mention the results of the observations that show that when the shell of the oyster is closed at low temperature the ciliary motion may be inhibited completely. The following experiments supply evidence for this statement: On February 15, 1927, six oysters were taken from the shallow water of the harbor; the temperature of the water was 0.8 ° and that of the air 1.7°. The shells were forced apart slightly and a small thermometer was thrust into the oyster meats. It registered the following temperatures: 1.4°, 1.2°, 1.3°, 1.2°, 1.4°, 1.4°. Then the oysters were opened and small pieces of gill epithelium were put in sea water immediately and examined under the microscope. The examination was made on the deck of a boat anchored in the harbor. Air temperature during the examination varied from 1.6° to 1.7°. In all the pieces of epithelium cut from the gills and placed in sea water at 1.4° there was no ciliary motion. Ten minutes later, however, the ciliary motion was active in all of them. The same experiment was repeated next day with six oysters that were taken from the harbor and left for two hours exposed to direct sunlight. The air temperature was 3.8°. When the oysters were opened the temperature of their meats was as follows: 8.6°, 8.5°, 9.6°, 10.1°, 10°, and 10°. In all of them the ciliary motion observed on the excised pieces of the gill epithelium was slow and irregular but became normal in a

few minutes. The experiments show that at low temperature in a closed shell the ciliary motion may be inhibited completely and that when the oyster is exposed to direct sunlight the temperature of its meat becomes much higher than that of the air. These facts should be taken into consideration when the hibernation of oysters is regarded from the sanitary point of view.

STRAINING OF WATER BY THE GILLS

One of the main functions of the lamellibranchiate gills consists in straining the water that passes through its body and catching planktonic organisms and other food particles suspended in it. It is interesting to study this function more carefully and to determine how completely the water is deprived of suspended material after it had passed through the gills. This can be done easily by employing the "tank" method. The oyster is placed in the tank (fig. 2) and is allowed to filter water, which is collected in a graduate. After 1 liter has been collected the water is passed through the high speed (Fœrst's) centrifuge (making about 20,000 revolutions per minute), the sediment is collected and transferred into a small volume of water, and the number of organisms in it is counted in a Sedgwick-Rafter cell. A comparison of the number of organisms present in the water before and after it had passed through the gills gives us a good idea of the efficiency of the latter as a filter. Obviously the number of organisms that can pass through the gills depends on the size and shape of the various forms. Long diatoms like Rhizosolenia or those that have long appendages, like Chætoceras, are easily retained by the gills; while minute forms, and especially bacteria, have a good chance to pass between the lateral cilia. The first experiments made in 1925 (Galtsoff, 1926) have shown that over 99 per cent of certain plankton forms may be caught by the gills. The plankton in these experiments consisted of Chætoceras, Rhizosolenia, and comparatively large dinoflagellates like *Peridinium oceanicum* and Ceratium. Different results were obtained, however, in the summer and autumn of 1926, when the plankton consisted chiefly of small organisms. Following are the results of two experiments made in August and September, 1926:

TABLE 8.—*Filtering of water by the gills*

EXPERIMENT A, AUGUST 11, 1926. TEMPERATURE OF WATER 22°; RATE OF FLOW OF WATER THROUGH THE GILLS 1.01 LITERS PER HOUR

	Number of organisms in one liter of water		
	Before it passed through the gills	After it passed through the gills	Per cent of organisms passed through the gills
Diatoms	703,000	50,000	7.1
Dinoflagellates	312,000	12,500	4.0
Total	1,015,000	62,500	6.2

EXPERIMENT B, SEPTEMBER 19, 1926. TEMPERATURE OF WATER 19.1°; RATE OF FLOW OF WATER THROUGH THE GILLS, 1.9 LITERS PER HOUR

Diatoms	194,000	24,300	12.6
Dinoflagellates	259,000	49,000	18.9
Total	453,000	73,300	16.2

In experiment B the plankton was formed mostly by minute Naviculæ and Glenodinium, while in experiment A such large forms as Coscinodiscus, Rhizosolenia, and Ceratium were present. In both experiments the water discharged by the gills contained a considerable amount of mucus.

Because of the practical importance of the bacteriological examination of the oyster, it is interesting to determine how many bacteria can be retained by the gills. Three experiments were performed with oysters kept in water to which varying amounts of fresh 24-hour-old cultures of *Bacterium coli* were added. The experiments were carried out in October, 1925, at Doctor Pease's laboratory in New York. The water in the tank to which *B. coli* was added was well stirred and the oyster was allowed to filter it for 30 minutes. During this period the small vessel of the tank (fig. 2) was twice emptied and refilled with the water passed by the gills. For counting the number of *B. coli* 1 centimeter of water was planted on Endo's plates, and for the total number of bacteria the same amount was planted on beef agar. The plates were kept for 48 hours at 37°. The results of the experiments are as follows:

TABLE 9.—*Filtering of water by the gills*

Experiment No.	Temperature of water (°C.)	Rate of flow (liters per hour	Number of bacteria in 1 centimeter of water				Per cent of bacteria passed through gills	
			Before it passed through gills		After it passed through gills			
			B. coli	Total	B. coli	Total	B. coli	Total
A	22.8	0.6	200	----------	100	----------	50	----------
B	22.8	.6	16,000	23,000	11,200	12,600	70	54.8
C	23.2	.8	14,000	16,600	12,400	14,800	88.6	89.2

As can be noticed from this table, the water, after it passed the gills, always contained less bacteria than it had before but the difference was not constant. Apparently only a small number of bacteria are retained by the gills; the microorganisms are so small that they can pass easily between the lateral cilia and escape back into the surrounding water.

OPENING AND CLOSING OF THE SHELL

Feeding and respiration in the oyster is dependent on two distinct functions—the ciliary activity of the gill epithelium and the opening and closing of the shell. The movement of the shell is controlled by the adductor muscle, the relaxation of which causes the opening of the valves, while its contraction brings the valves together and keeps them tightly closed. Because the oyster has no power of locomotion, the contraction and relaxation of the adductor muscle is the only noticeable reaction by which the organism responds to the external and internal stimuli. One should anticipate, therefore, that the opening and closing of the shell is a complex phenomenon that is controlled by a great variety of factors. No attempts were made in the present paper to study the physiology of the adductor muscle, but it seemed desirable to obtain some data regarding the duration of time the oysters keep the shell open.

The oyster was immobilized by placing it on a brick and embedding its left shell in a mixture made of 1 part of cement and 3 parts of plaster of Paris. A glass rod, attached by means of the same mixture to the right valve of the oyster, was connected to the lever of a recording apparatus (fig. 9) and the oyster was kept in a large aqua-

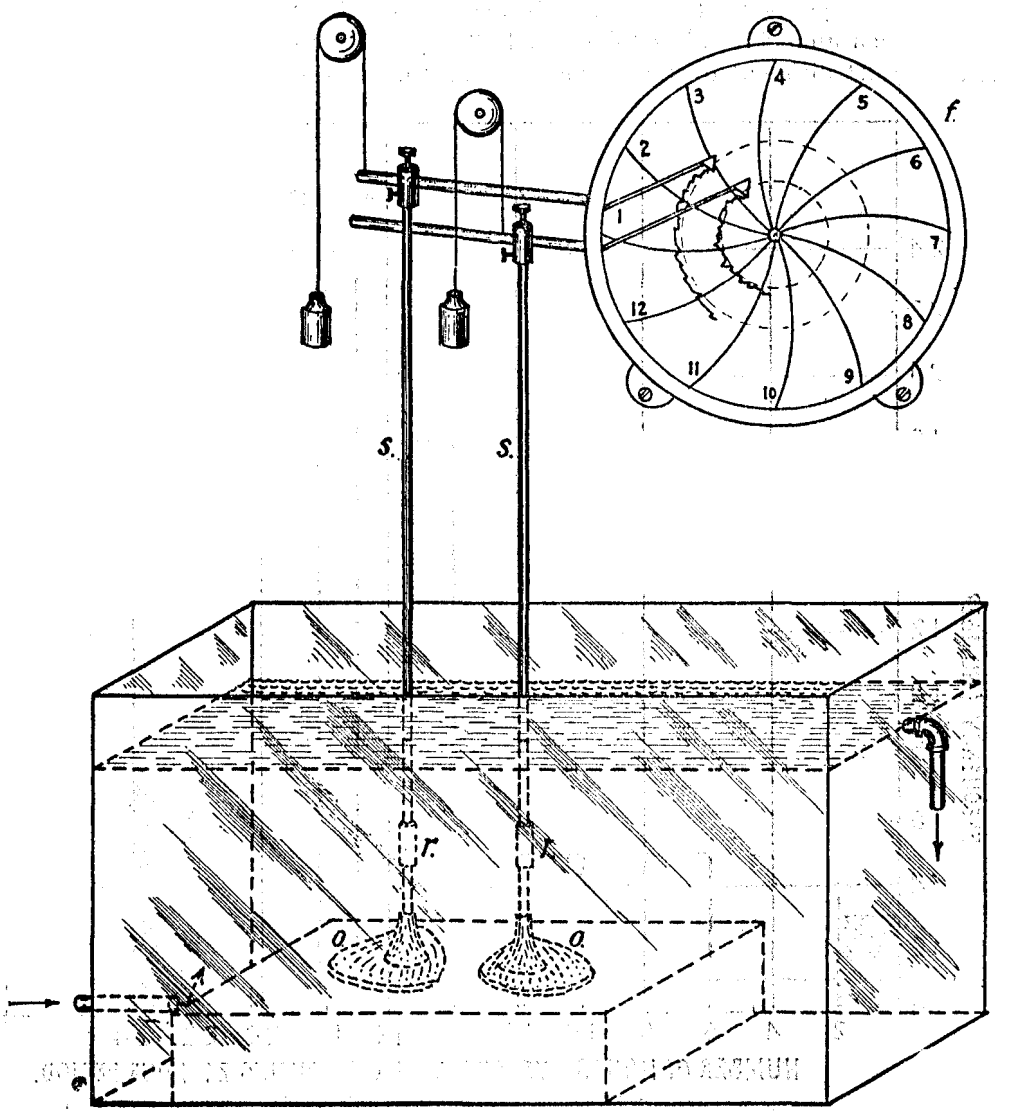

FIG. 9.—Method employed to study the shell movements of oysters. o.—oyster immobilized in plaster of Paris; r.—rubber connections; s.—glass rods attached to the levers (l) of the recording apparatus; f.—Foxboro time recorder; w.—weight

rium tank with running sea water. The records of the movement of the shells were made with a Foxboro or Bristol two-pen time recorder. The weight of the cement, glass rod, and lever was counterbalanced in such a way that the shell bore no additional pressure. (Fig. 9.) In all the experiments the records of two oysters were taken simultaneously and the temperature of the water was recorded on the thermo-

graph. For the purpose of the present experiments the use of a time recorder has several advantages over the ordinary kymograph—it eliminates the necessity of having a special time signal, and after the apparatus has been set it can be left for 24 hours without any further attention. The oysters were kept attached to the apparatus for varying periods of time ranging from one to eight days. Altogether, during the time between June 15 and October 15, 1926, there were obtained 132 daily records written by 34 different oysters. The temperature of the water varied from 13° to

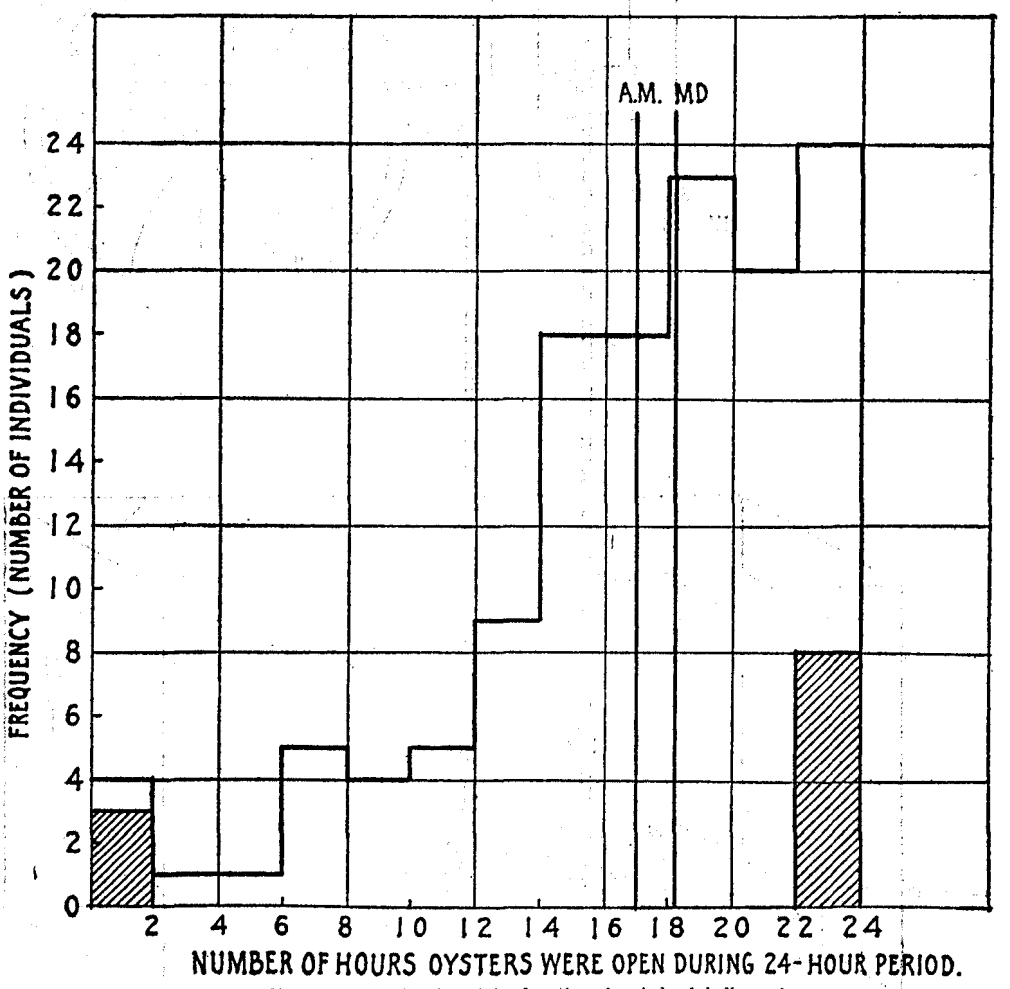

FIG. 10.—Frequency distribution of the duration of periods of shell opening

22° C. Within this range there was no definite correlation between temperature and the opening and closing of the shells. The results of all the observations are given in Figure 10. They are grouped in 12 classes, each having two-hour intervals and the frequencies are plotted as the ordinates. The number of oysters that were either closed or open for a 24-hour period are shown in cross-hatched areas. An examination of Figure 10 shows that the oyster has a tendency to keep its shell open as long as possible. The arithmetic mean of the number of hours the average oyster keeps its

shell open during a day is 17 hours 7 minutes; the median is 18 hours 5 minutes. In a preliminary paper published in 1926 (Galtsoff, 1926) it was stated that the average period of time the shells of an oyster are open during a day is 20 hours. Nelson (1921), analyzing the records of 3 oysters kept under observation for 21 days, also states that on the average the oysters were open for 20 hours. The present investigation, based on more numerous observations, shows that the average period of time when the shells are open is smaller. The decrease of the average from 20 to 17 hours and 7 minutes is due to a few instances where the oysters failed to open during the 24-hour period.

An analysis of the records shows that when the shells are open the adductor muscle contracts and relaxes periodically. The contraction is often so slight that it does not result in a complete closing of the shell (fig. 11) and is of brief duration, the muscle beginning to relax immediately after the maximum contraction is reached. It has been shown by several investigators that by periodical contractions the

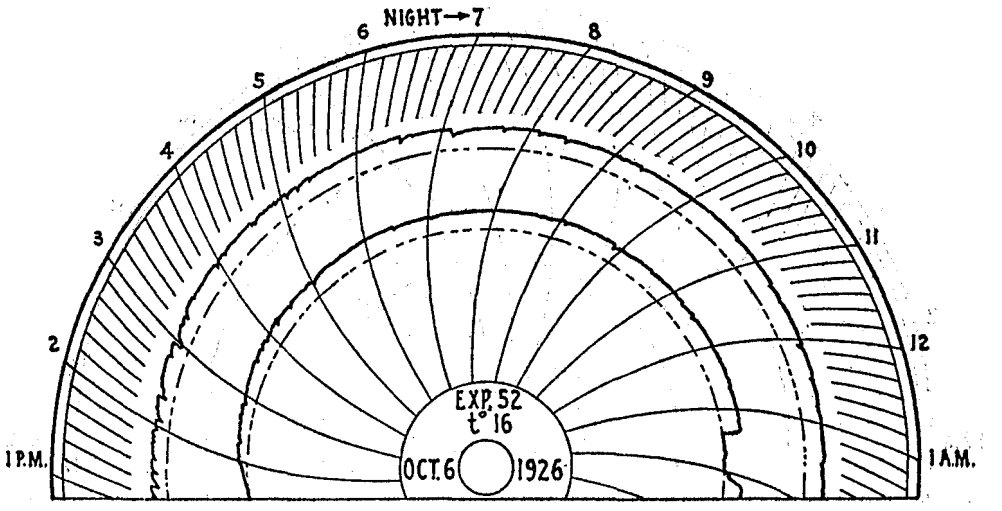

FIG. 11.—Part of the record of shell movements of two oysters made at 16° C. Dotted lines indicate the position of the pens when shells are closed

oysters cleanse themselves and discharge the material that had accumulated in the pallial cavity. Although by this reaction the organism is able to get rid of the material accumulated by the gills, the inference that every contraction of the muscle is an ejection reaction is incorrect. Nelson (1921, p. 343) states that from observation of the extent and frequency of the down strokes made by the oysters on the recording apparatus and representing the contraction of the adductor muscle, together with a knowledge of the turbidity of the water, it is possible to determine the rate of feeding. Employing this method he arrives at the erroneous conclusion (1921, p. 339) that "temperature within the limits observed during the experiment (69° to 90° F.) apparently did not operate as an independent factor in controlling the intake of food." It has been shown in this paper that the ciliary motion, which is responsible for the intake of food by the oyster, is a function of temperature. The number of contractions of the adductor muscle can not be regarded as an index of the rate of feeding, because the oyster may respond to any external or internal

stimulus by closing its shell. My observations on the oysters attached to the recording apparatus and kept in a glass tank show that ejection of the material accumulated in the pallial cavity takes place at irregular intervals and that rhythmic contractions of the adductor muscle (figs. 11 and 12) were not accompanied by a discharge of any material. Such different factors as changes in illumination, mechanical stimulation, changes in the pH or gas content in sea water, presence of certain chemicals, and so on, may cause the contraction of the muscle and temporary closing of the shell. The frequency of the contractions depends also on the physiological condition of the oyster. It has been found in the present investigation that after spawning the female oyster makes many less contractions than it does before spawning. The kymograph tracings represented in Figure 13 show this very plainly. During this experiment, in both cases the shell of the oyster was wide open and the temperature of the water and other external factors were

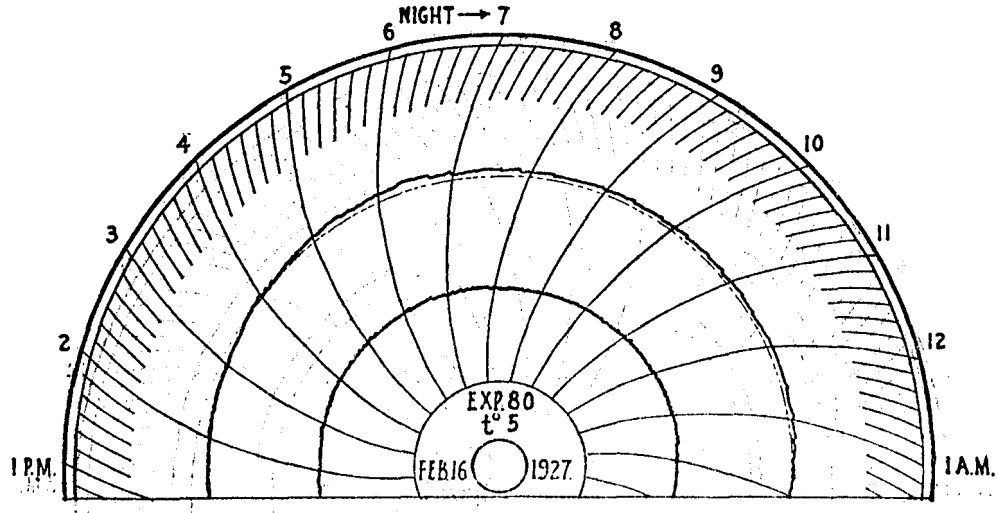

FIG. 12.—Part of the record of shell movements of two oysters made at 5° C. Dotted line indicates the position of the pen when shell is closed. In lower line the oyster was nearly closed and showed very slight motion

alike. At present we understand very little the conditions that control the periodical contractions of the adductor muscle, but it appears obvious that they can not be correlated directly with the rate of feeding, and the estimation of the latter can not be based on the frequency of the contractions.

It has been suggested by Nelson (1921) that there may be a certain amount of correlation between the times of opening and closing and the hour of the day and night. Nelson divides a day into four arbitrary periods, from 11.01 p. m. to 4.30 a. m., from 4.31 to 11 a. m., from 11.01 a. m. to 7.30 p. m., and from 7.30 to 11 p. m. Then he counts on the kymograph records the number of closures and openings that occur during each period. According to his data 50 per cent of closures take place in the 5½ hours between 11 p. m. and 4.30 a. m. (dawn). Nelson makes two somewhat contradictory conclusions—first, that "the period from 11 p. m. to dawn may almost be looked upon as a time of rest, or at least of greatly lessened activity", and second, that "the hours of inactivity on 50 per cent of all the days were confined

to periods 1 and 2" (i. e., from 11.01 p. m. to 11 a. m.). In another paper (Nelson, 1923) he states that from 60 to 70 per cent of the hours of inactivity (closure) occur during darkness. The examination of the number of closures and openings occurring during a given period of time does not convey a true idea of the activity or inactivity of the oyster. A better understanding can be gained by counting the number of hours the oyster was closed or open during a given period of a day. These observations should be made under controllable conditions, inasmuch as it has been shown by Nelson for Barnegat Bay (1921) and confirmed by Prytherch for Milford Harbor (unpublished report) that in these bays the oysters close their shells at ebb tide. We know that every change in the environment may cause the oyster to close its shell, and therefore the problem of whether oysters exhibit daily periodicity in

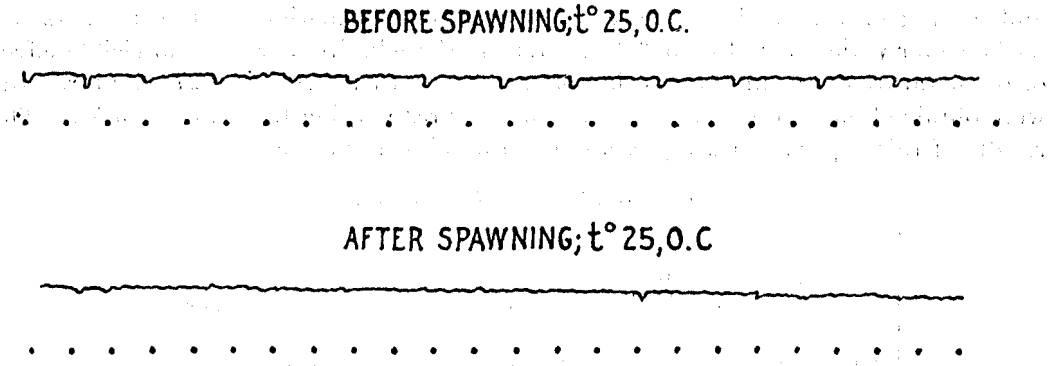

FIG. 13.—Kymograph records of the movement of the shell one hour before spawning (upper line) and one hour and a half after spawning. Oyster was open all the time. The marks under the line indicate one-minute intervals.

behavior should be studied without any possible interference of other factors that may produce the same reaction. The examination of the material obtained by the author during July–September, 1926, fails to disclose any correlation between the periods of inactivity (closure) and darkness. Analyzing the data, all incomplete records (i. e., those covering less than 24-hours periods) and all those showing that the oyster was either closed or open continuously for 24 hours were excluded, so that in each of 103 records taken into consideration the oyster was closed for a part of a day. The period of darkness was determined as beginning half an hour after sunset and ending half an hour before sunrise. The results of the examination are given in the following table:

TABLE 10.—*Opening and closing of the shell in relation to the time of day*

	Number of hours		Number of days
Total number of hours oysters were under observation	2,472	Number of days when shells were closed during day hours only	43
Number of hours oysters were closed during daytime	565	Number of days when shells were closed during night hours only	7
Number of hours oysters were closed during nighttime	266	Number of days when shells were closed during night and day hours	53

The only inference that can be drawn from an examination of this table is that there is no correlation between the closures of the shell and darkness. Out of 103 days there were only 7 during which the oysters were closed only at night. There were 43 days when the closures occurred during the daytime, and in 53 cases it took place both during day and night hours. The number of hours of night closure is 266, or 32 per cent of the total number of hours of inactivity (831). Inasmuch as the period of darkness is approximately 8 hours, or one-third of the 24-hour peri it is quite natural that one-third of the time of inactivity should fall in the night hours. Examination of these records forces us to come to the conclusion that under laboratory conditions the periods of opening and closing of the shell of the oyster are not correlated with time of day or night.

There arises the question as to whether the temperature has any effect on opening and closing of the shells. In the above-mentioned experiments the temperature of the water varied from 13° to 22° C. Within these limits there was no visible effect of temperature on opening or closing of the shell. In February, 1927, eight records were obtained on two oysters kept at temperatures varying from 4.5° to 5.5°. The results of this experiment are presented in the following table:

TABLE 11.—*Opening of shells at low temperatures*

Date	Temperature (C°)	Length of time oysters were open	
		Oyster No. 1	Oyster No. 2
Feb. 12 (half day)	5.0–5.5	12 hours	12 hours.
Feb. 13	4.5–5.5	19 hours	14 hours 50 minutes.
Feb. 14	5.0–5.5	19 hours 50 minutes	12 hours 20 minutes.
Feb. 15	2.0–5.5	13 hours 40 minutes	13 hours 50 minutes.

The shells were open very slightly, less than 1 millimeter apart (fig. 12), but the oysters exhibited typical periodical contractions. As has been shown above, no current is produced at this temperature, and several tests with carmine suspension failed to disclose any current in the oysters attached to the recording apparatus. On February 15 one oyster was attached to the kymograph and left 67 hours in cold water; during this time the temperature varied from 0.5° to 1.6° and the oyster remained tightly closed.

DISCUSSION AND CONCLUSIONS

The experimental data presented in this paper show that the functioning of the gills of the oyster is controlled by temperature. In spite of wide individual variations in the activity of the ciliary epithelium, it has been found that in all cases the ciliary motion follows strictly the changes in temperature, slowing down with its fall and increasing with its rise. Because of anatomical peculiarities, the normal functioning of the gills ceases at a temperature of 5° C., when the ciliary motion becomes irregular and is not able to maintain the head pressure necessary for the production of an outgoing current. The inhibiting effect of low temperature upon the activity of the oyster was suspected many years ago. Bashford Dean (1887) was the first to suggest that "in winter the oyster, with decreased movements of branchial cilia and reduced

heart action, may almost be said to hibernate." An indirect evidence of hibernation was supplied by Gorham (1912), Pease (1912), and Gage and Gorham (1925), who based their conclusions on the study of seasonal fluctuations in *B. coli* content in oysters taken from polluted bottoms. Physiological study of the problem was impossible, however, as no method was offered whereby the ciliary activity of the gills could be measured. The present paper, based on a quantitative study of the activity of the gills, supports fully the ideas advanced by Gorham, Pease, and Gage. However, the facts described in it contradict the conclusions reached by Nelson (1923) that "the rate of filtration of water during any given period of time, as deduced from the rapidity and extent of ejections of accumulated sediment from the mantle cavity, may vary widely, independently of the temperature and turbidity of the water." No attempts were made in the present investigation to study the effect of turbidity of the water, but the rôle of temperature in the ciliary activity of the gill epithelium was established and is shown in Figures 5, 6, 7, and 8.

Knowledge of the effect of temperature on the activity of the gill epithelium of the oyster is essential in many practical problems of the oyster industry. From the sanitary point of view, the fact that at a temperature of 5° C. and below the oyster does not take in any water and ceases feeding, supplies an additional safeguard, which can be applied in the sanitary control of oyster bottoms. In the purification of oysters by chlorination an understanding of the rôle temperature plays in the functioning of the gills is of great importance. The method of chlorination introduced in 1914 by Johnstone (1915) is based on self-purification of oysters, which are allowed to filter sterile sea water; and knowledge of the rate of filtration at a given temperature is necessary for an intelligent operation of the chlorination plant.

It is a common practice in certain areas to take oysters from slightly polluted beds and to relay them on clean, unpolluted bottoms. Sometimes this operation is carried out during the cold season when the temperature is below 5° and the oysters have no possible chance to cleanse themselves. It is obvious that the determination of the minimum period of time oysters should be left on new bottoms should be based on the rate of filtration of water at a given temperature.

For a study of the physiology of the oyster a knowledge of the rate of filtration of water is of fundamental importance. Fattening, growth, and ripening of the gonads are probably directly dependent on the amount of food consumed. At present we know almost nothing regarding these activities of the oyster, and the author is convinced that the present investigation may facilitate, to a certain extent, the attack of other problems that are of great importance for an understanding of the factors responsible for the fluctuations in the oyster crops in our waters.

RÉSUMÉ

1. Two methods are described whereby the rate of flow of water produced by the ciliary epithelium and the pressure inside the gill cavity of the oyster can be measured accurately.

2. The mechanical work performed by the oyster gills in producing a current of water can be expressed by the following formula: $W = 2\pi l \mu S^2$, where W = work in ergs per second; l = length in centimeters of the glass tube through which the

current is running; μ = viscosity of water in poises; and S = speed at the axis of the tube in centimeters per second.

3. The rate of flow of water produced by the gills is controlled by the temperature. The optimum temperature lies between 25° and 30° C. No current is produced at 5° and below, although the cilia continue to beat. Absence of current at low temperature is due to the lack of coordination of the ciliary motion along the surface of the gill.

4. Hibernating oysters do not exhibit any adaptation to low temperature; they begin to produce a current as soon as the temperature rises above the critical point. In the majority of the oysters the current begins to flow when the temperature reaches 8° C.

5. There exists considerable individual variation in the rate of flow of water produced by different oysters. The maximum rate of flow observed during the present investigations is 3.9 liters per hour at 25° C.

6. The ciliary motion may continue at temperatures below 0° but becomes very slow and irregular. There is a noticeable difference in the efficiency of the frontal and lateral cells; the first ones are able to transport the particles along the surface of the gill at 3° C., while the lateral cilia can produce a current only above 5° C.

7. In straining water through the gills the oyster catches a considerable number of plankton organisms, but a certain per cent of them (from 1 to 18.9) escapes. The number of organisms that passes through the gills depends on their shape and size; small, elongated forms, devoid of any appendages, pass easily between the lateral cilia and escape. Bacteriological examination shows that from 50 to 89.2 per cent of bacteria present in the sea water pass through the gills.

8. The analysis of 132 daily records shows that the oyster has a tendency to keep its shell open as long as possible. On the average, the shell of an oyster remains open for 17 hours and 7 minutes during every 24-hour period. There is no correlation between the opening and closing of the shell and the time of day.

9. The results of the present investigation have many bearings on various problems of oyster industry. (*a*) They confirm the theory of hibernation and show that at a temperature of 5° and below oysters cease to feed. (*b*) the knowledge of the rate of filtration of water at various temperatures is essential for a successful application of methods of self-purification, consisting either in relaying the oysters on unpolluted bottoms or in purifying them with chlorinated water. (*c*) The knowledge of the rate of filtration of water is of fundamental importance for a study of growth, fattening, and ripening of the oyster.

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