CHAPTER IX

TRANSPORT OF WATER BY THE GILLS AND RESPIRATION

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All bivalves maintain a steady flow of water through their gills for feeding, respiration, and the removal of products of metabolism. In the literature on oyster physiology this process is described under such names as pumping, filtration, ventilation, respiratory current, and feeding. When "feeding" is used in reference to the transport of water by the gills, the term is misleading since feeding implies the acceptance of food by the organism and involves sorting and rejection of particles removed from the water by filtration. The terms ventilation and transport of water appear to be suitable expressions for denoting the processes by which the oyster maintains a flow of water in a complex system of water tubes and through the epibranchial chambers. Both terms are used in this text interchangeably, depending on whether the emphasis is on collection of food or on respiration.

Maintenance of a steady stream of water and respiration are the two principal functions of the gill. Of lesser importance are the excretion (through diapedesis) of certain products of metabolism, the absorption of substances dissolved in water, and the ingestion of particles settled on the gills by the leucocytes present on their surface.

Water transport and oxygen uptake are interdependent functions that may be considered as two phases of a single process. It is convenient, however, to discuss them separately, keeping in mind that both activities occur simultaneously.

TRANSPORT OF WATER

The preceding chapter described how a steady rate of water current inside the demibranches is produced by synchronized beats along the thousands of tracts of lateral cilia. Temporary cessation of ciliary motion along some of the tracts or a disturbance in their rhythm of beating will result in a drop of hydrostatic pressure inside the water tubes and cause a leak of water through the ostia. This disturbance may slow down or stop the cloacal current.

The adductor muscle, the edge of the mantle, the gill muscles, and the ostia all play a part in the regulation of the flow of water produced by ciliary activity. It is self-evident that, within the limits determined by the capacity of the gills' chambers, the volume of water transported depends on shell movements and the width at which the two valves are kept apart. When the shell closes the current stops. A similar effect may be produced by the edges of the mantle independently of the movements of the valves. Sometimes the pallium assumes a vertical position and the tentacles along the edges of the opposing mantles interlock while the valves remain wide open. Under this condition no water penetrates through the curtain which seals the entire pallial cavity. This behavior occurs in the presence of low concentrations of toxic substances and during the spawning of the female oyster (ch. XIV, p. 304). It is, therefore, obvious that shell movements should not be considered as indications of the feeding of the oyster, a mistake which frequently is found in papers describing the "feeding" of oysters.

The rate of water transport may also be reduced

by contractions of gill muscles bringing together the filaments of the plicae, followed by constriction of the ostia. The contraction of the ostia sometimes takes place independently of the contraction of gill muscles.

DETERMINATION OF THE RATE OF WATER TRANSPORT

The rate at which water is transported through the gills can be studied by both direct and indirect methods. In direct methods the volume of water discharged through the gills in a given time is collected and measured, or is calculated from observations of the velocity of the cloacal current. The indirect methods are based on determinations of the rate of removal of particles suspended in water. The powders used for the latter purpose include kaolin, natural silt, calcium carbonate, colloidal carbon, isolated chloroplasts, various plankton microorganisms such as *Euglena*, *Chlorella*, and *Nitzschia*, and cultures of unicellular algae that have been made radioactive.

Direct methods

There are two groups of direct methods. First, the water discharged by the gills may be intercepted, its volume measured, and the sample retained for analysis. In the second method the velocity of the current is determined by measuring the angle of deflection of a light paper cone or glass plate placed in front of the current. The rate of transport of water masses may be computed from the cross-sectional area of the column of moving water and the angular deflection of the plate. Both methods have certain advantages and disadvantages.

The methods which make it possible to collect and analyze water after it has passed through the gills are particularly suitable for studies of feeding and metabolism. The slight disadvantage is that the mollusk must be partially enclosed in rubber or plastic. In the second group of methods the mollusk is not in direct contact with any foreign material and is kept under normal conditions. The drawback is, however, that the discharged water can only be collected in very small quantities directly from the stem of the current inside the cloaca. Choice of method must be governed, of course, by the purpose of the observations.

Constant level tanks.—A very simple device consisting of two connecting vessels in which the level of sea water is kept constant was designed by Galtsoff (1926, 1928) and has been adapted and modified by many investigators making long-term observations on the feeding of the oyster. The apparatus, shown in figure 168, consists of two rectangular vessels, one large and one small, connected by a semicircular trough or by glass tubing of wide diameter inserted in the partition between the two vessels. The large vessel should be just big enough to accommodate an oyster mounted on a stand, the inlet tube for sea water, and a thermometer. The volume of the tank is made as small as possible in order to permit rapid exchange of water. Convenient dimensions for observations on adult *Crassostrea virginica* and *C. gigas*, are 10 by 6 by 4 inches for the large vessel, and 2 inches by 1½ by 2 inches for the small one.

The tanks are made of transparent plastocel, lucite, or similar nontoxic material about ¼-inch thick. The edges are sandpapered and cemented together by a solution of plastic in acetone, cellosolve, or by undiluted methylene chloride, and the small vessel is cemented to the side of the large one. After the cement is dry the tank should be washed carefully and rinsed in sea water. A semicircular trough made of thin plastic bent in hot water to the proper shape is mounted on the wall between the two vessels. The drain pipe is glass tubing, 6 to 8 mm. in diameter, inserted through the bottom of the small vessel; its upper end is slightly widened and adjusted to the exact level of water in the large vessel, which is controlled by a wide cut in the opposite wall of the vessel (left side in figure 168). The level of water is adjusted by moving the vertical overflow tube up and down (right side of fig. 168) until excess water running through the large vessel spills through the overflow and nothing enters the small vessel. Levels must be set very accu-

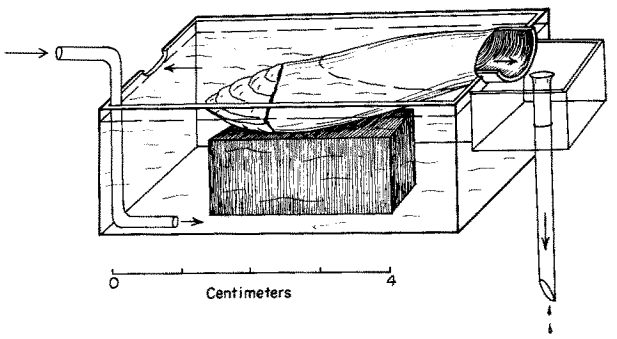

FIGURE 168.—Constant level tanks designed by Galtsoff to measure the volume of water transported by the gills of the oyster. Dimensions of the large vessel are 10 by 6 by 4 inches.

rately. When the correct height of the drain pipe is reached a few drops of water added to the contents of the small vessel will be discharged immediately. Setting the level of water in the small vessel too low may lead to serious error as the water will be forced through the gills by gravity. Since readings may be grossly influenced by poorly adjusted levels, repeated checking of the overflow must be performed at regular and frequent intervals.

The rate of supply of sea water into the large vessel should exceed the expected maximum rate of water transport by the oyster, which rarely exceeds 30 l. per hour. After equilibrium in the water levels in the two communicating vessels has been established, the tank is ready for use. Before the oyster is placed in the tank the mollusk is wrapped in thin rubber dam or in plastic sheeting cut in the shape of an apron. This technique, suggested first by Moore (1910), was adapted for oyster research by Nelson (1936, 1938). A triangular piece of sheeting cut to fit the shape and size of the oyster is spread on a table. After the valves are thoroughly scrubbed, washed, and dried, the oyster is placed on its left valve on the sheeting. The anterior half of the oyster is left free; only the posterior half is wrapped in the apron (fig. 168). The sheeting is attached to the shell with melted colophonium cement, starting with the lower (left) valve. Enough slack should be left at the edges of the shell and at the hinge side to allow free movement of the valve; small cotton balls are inserted in both places to prevent leakage of water. The two sides of the apron are then brought together to make a sleeve of the required diameter to fit the interconnecting trough of the tank and are joined using hot colophonium cement. A wide test tube is inserted in the apron and the apron sleeve is sealed by pressing the two sides of the apron against the glass while applying the cement. Before the oyster is put in the tank the apron should be tested against leakage by gently blowing air into the sleeve.

A small metal loop cut from the end of a paper clip and cemented to the flat valve is used to attach a string leading to the kymograph lever, which records the shell movements. The water levels in the tanks are checked again; the oyster is placed on a stand inside the tank and if necessary immobilized by plastic clay; the sleeve of the apron is drawn over the trough (or tubing) and is secured by a piece of string; the writing lever of the kymograph is connected to the valve. The last check for a possible leakage of water is made after the oyster opens its valves and begins to transport water. The escape of water through the seams of the apron can be detected by adding carmine suspension and watching the currents. In a correctly adjusted set all water transported by the gills enters the small vessel through the sleeve and can be collected, measured, and analyzed.

The transport of water can be recorded continuously by using various automatic devices, such as electric drop counters and dumping vessels. The latter turn over after the water has reached a certain predetermined level. Any of these devices can be connected to the kymograph lever which makes a mark on a rotating drum. The shell movements and the temperature of water are recorded simultaneously on the same sheet. The arrangement of various parts of an apparatus for recording the oyster activity and for collecting samples of water before and after its passage through the gills is shown diagrammatically in figure 169. The oyster in this diagram has been placed in a constant level tank A, which is provided with two connecting vessels B and C. A valve between A and B may be lowered to disconnect tank A, and cap L is used to close the connection between vessels B and C. Vessel B is slightly larger than C and is used for taking samples of water for plankton study or for gas analysis. The water in this vessel is protected from direct contact with air by the paraffined float H. The sample is taken through the drain tube X. The small vessel C has an overflow Y, which controls the levels of water in all three vessels. From the overflow Y water is delivered through tube Z to a dumping vessel E, which is mounted on a horizontal axis. A diagonal wall divides the vessel into two parts, of which only one (facing the reader) is being filled with water. Float F activates the system of levers and releases the catch L which holds the vessel in an upright position. The vessel overturns but snaps back into the position shown in the diagram. Proper adjustment of the vessel is obtained by attaching small weights (not shown in the diagram) to its bottom. The vessel with its levers is mounted on a solid frame D set on a heavy concrete platform. The water discharged at each dump activates the springboard G, which is connected to a writing lever N. Shell movements are recorded by the lever M; the electric time signal S connected to a timer T records time

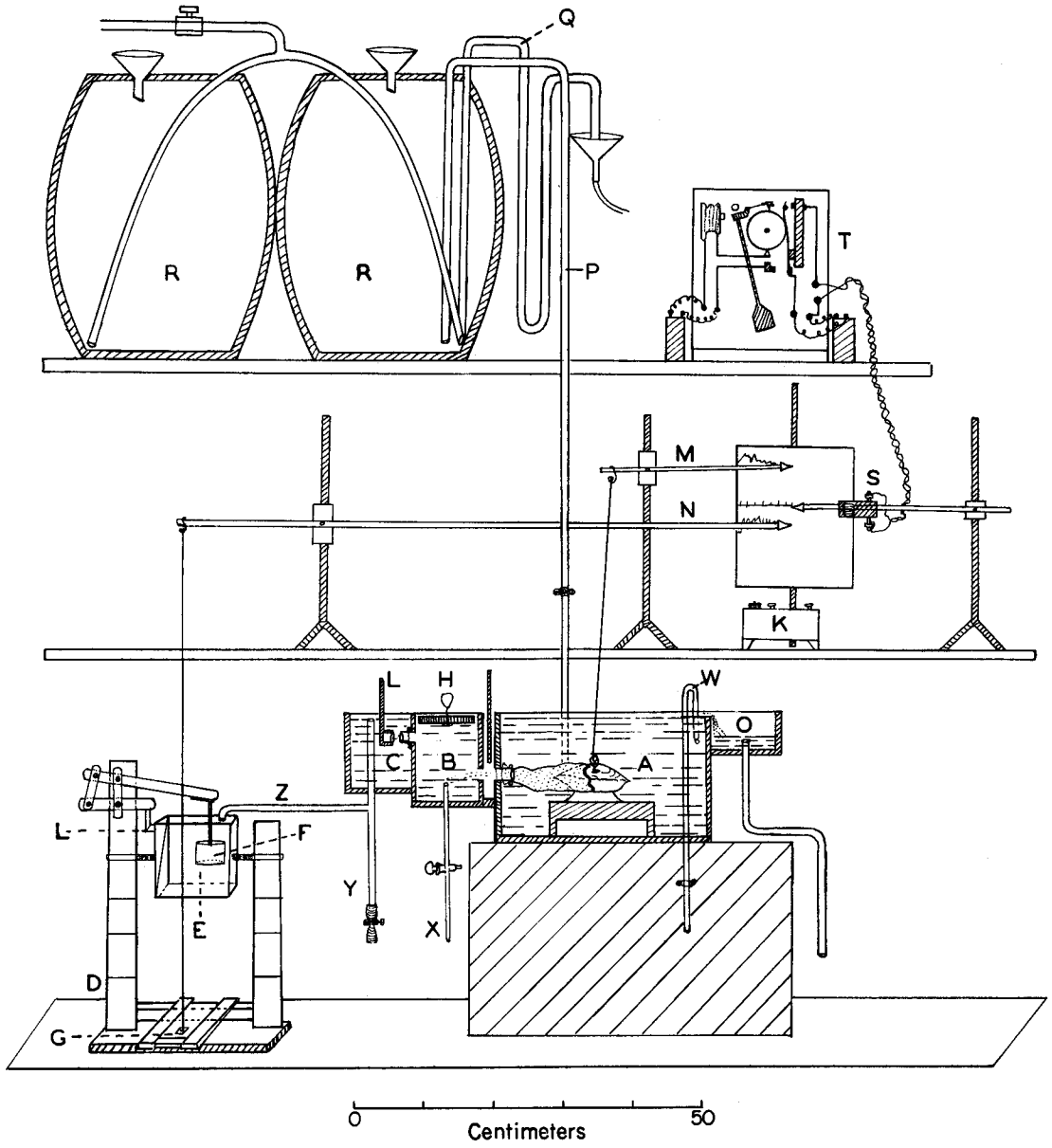

FIGURE 169.—Diagram of a setup for simultaneous recording of the rate of water transport and shell movements of the oyster. A—vessel with oyster; B and C—small connecting vessels; D—frame of the dumping vessel; E—dumping vessel; F—float; G—springboard to record each overturn of the dumping vessel; H—paraffined float; K—slow motion kymograph; L—cap to close the connection between the two vessel; M—lever recording shell movement; N—lever recording the dumping of water; O—overflow; P—sea-water supply; Q—constant level siphon; R—barrels; S—signal magnet; T—electric time recorder; X, Y, W—tubes for taking samples of water; Z—overflow tube leading to dumping vessel. Temperature recorder is not shown.

intervals. The supply of sea water is delivered to the barrels R set on top of the stand, and the overflow siphon Q keeps the water in the containers at a constant level and insures a uniform rate of delivery of water to the experimental tank *A*.

The size and shape of the dumping vessel can be modified to suit the purpose of the experiment and to facilitate its operation. The capacity of the dumping vessels made in my laboratory at Woods Hole varied from 55 ml. to 226 ml. The methods described above were successfully used in a number of investigations (Galtsoff, Prytherch, Smith, and Koehring, 1935; Chipman and Galtsoff, 1949a, 1949b).

All dumping vessels require frequent adjustments and become unreliable if used continuously

for several days. For long-term observations it is more practical to use a water wheel made with two plastic disks mounted on a horizontal glass rod about ½-inch apart. The space between the disks is divided by radial partitions into a series of triangular compartments. The wheel is placed under the overflow tubing of the small vessel which receives water discharged by the oyster. The compartments are arranged in such a way that when they are full of water the wheel turns slightly and the next empty compartment moves into position under the pipe. The wheel is kept half submerged in sea water to prevent spinning. Under this condition the rotation proceeds in smooth steps; when the wheel makes one complete turn the little bar attached to its side touches a string which moves the writing lever and makes a vertical stroke on a slow moving drum of the kymograph. The construction of the wheel (*F*) and the arrangement of different parts of the set in which it was used are shown in figure 170. The wheel is calibrated by measuring the volume of sea water needed to make it turn one complete revolution. The test is repeated at least 10 times, and the average value is taken as the true capacity of the wheel. Wheels of different dimensions may be used. In my experiments I used wheels of about 50- and 100-ml. capacities; the readings were accurate within ± 2.5 percent.

The setup shown in figure 170 is specifically designed for studying the effects of various contaminants that may be added at a known rate to the water supplied to tank E. Sea water from the laboratory supply pipe C is delivered to three 5-gal. carboys from which it runs into two tempering jars with electric heaters (group B) and vessel C. The water then passes into mixing chamber D to which the solution to be tested may be added from the two flasks O and N, which contain known concentrations of chemicals or a desired dilution of a culture of microorganisms. Test solutions in flasks O and N may be added directly to the gills (as shown in the diagram, flask N) or may be delivered to the mixing chamber D. If the solution is to go into the mixing chamber the siphon from flasks N or O is turned around 180° so that the tip of the delivery pipe is at the right end of the mixing chamber D. This

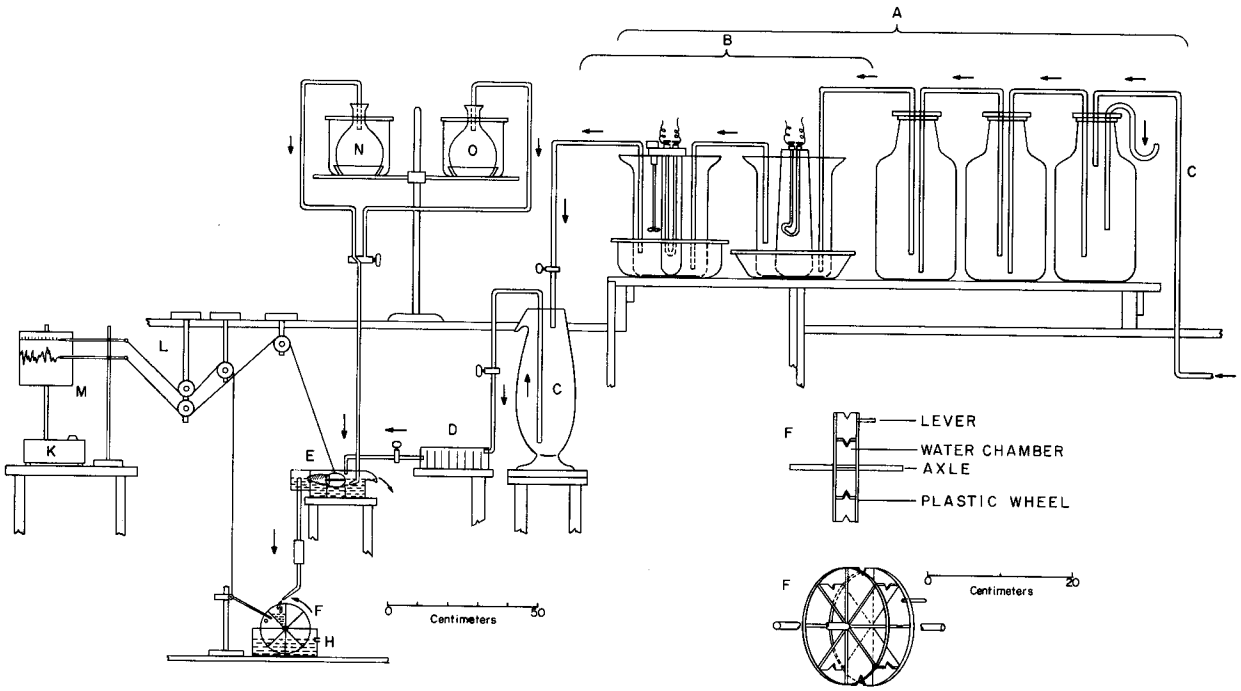

FIGURE 170.—Setup for automatic recording of the amount of water transported by the gills of the oyster. A—series of 5-gal. containers from which sea water is delivered to the tank with the oyster; B—two containers with electric heaters and thermostatic control (not shown in the diagram); C—constant level jar from which water is delivered to mixing chamber D; E—tank with oyster wrapped in apron; F—water wheel; H—container in which the water wheel is partially submerged; K—kymograph; L—writing lever activated by the turning of the water wheel; M—writing lever recording shell movement; N and O—flasks containing solutions or suspensions which may be added either to the mixing chamber or directly to the gills of the oyster.

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arrangement was used by Galtsoff and Arcisz (1954) in their work on the effects of known concentrations of *Escherischia coli* on oysters.

For observations intended to last several days or weeks it is convenient to use a slow-motion kymograph, rotating at the speed of about 1 inch per hour. A time marker of 1-hour or ½-hour intervals can be made by mounting a lucite disk on a Telechron type motor making one revolution per hour. A piece of copper wire attached to the periphery of the disk acts as a contact which slides over the two poles of an electric circuit and activates a small signal magnet. Using these methods it was possible to record the ventilation of the gills for 26 consecutive days with only occasional brief interruptions for cleaning the tank and for the removal of feces accumulated inside the apron. An excerpt of such a record is reproduced in figure 171.

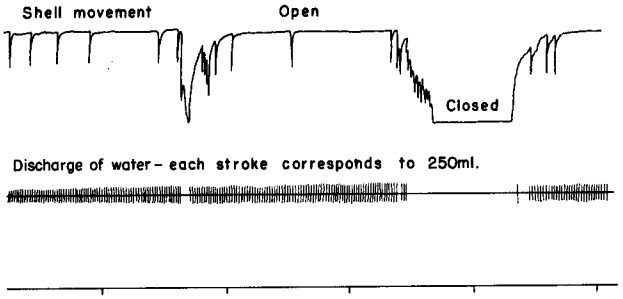

FIGURE 171.—Typical record of the shell movements (upper line) and rate of transport of water by the gills (second line) of an adult *C. virginica* kept in running sea water at the fishery laboratory in Woods Hole at the temperature of 21° to 22° C. Each vertical stroke corresponds to the discharge of a dumping vessel of 250-ml. capacity. Time interval, 1 hour.

An electric drop counter (fig. 172) may be used instead of a dumping vessel or water wheel if the volume of water passed through the gills is small, as for instance in juvenile American oysters or in *Ostrea lurida*. The drop counter is made of a short section of glass tubing with two platinum wires sealed opposite each other. Each time a drop of water falls between the wires the contact is completed and electric current from a transformer or a battery activates the signal magnet and makes a mark on the kymograph drum. The counter works satisfactorily in water of high salinity but is not suitable for brackish water. The number of drops per unit of time is counted from the kymograph record shown in figure 173.

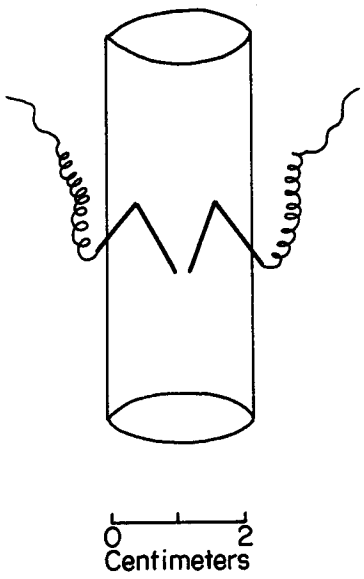

FIGURE 172.—Electric drop counter.

Current indicators.—The relative velocity of gill current can be studied by recording on a kymograph drum the deflections of a shallow cone placed in front of the cloacal opening (Hopkins, 1933). The cone C (fig. 174) about 5 cm. in diameter at its open end is made of lightweight paper waterproofed by dipping in a dilute solution of gum damar in xylene. The cone is mounted on the lower end of vertical rod F^1, which rotates on horizontal axis A and moves the horizontal lever F which indicates the current. The entire system is very light, since lever and rod are made of straw, and it is accurately balanced by connecting the vertical rod F^1 by a hair to a simple

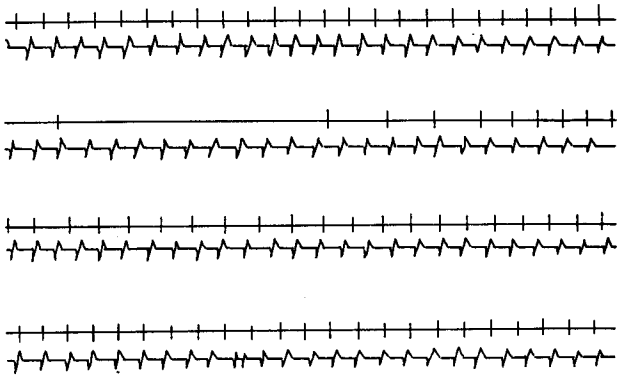

FIGURE 173.—Four parts of a record of the rate of water transport by the gills of a 2-year-old *C. virginica* obtained with an electric drop counter. Upper lines—each stroke corresponds to one drop of water discharged by the oyster; lower lines—time intervals of 1 sec. Temperature of water 22.2° C.

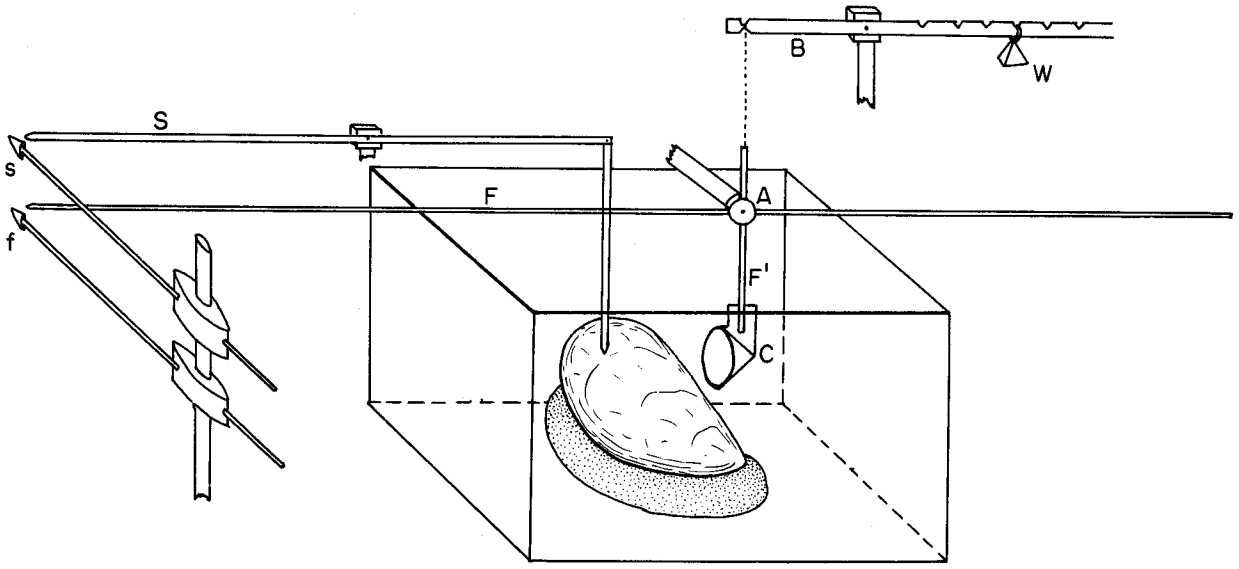

FIGURE 174.—Diagram of apparatus designed by Hopkins (1933, p. 474) to record relative rate of cloacal current and shell movements of oyster. S—lever for recording shell movements; s—fixed bar indicating on kymograph paper closed position of valves; F—lever recording cloacal current; f—fixed bar indicating zero position of F when no current is present; F'—vertical rod bearing cone C against which the current strikes and turns it on axis A; balance B; adjustable weight—W.

lever B with an adjustable weight W. The cone and current recording lever are fixed to a single stand and so placed that the cone is directly in the path of the cloacal current. The lever S records the movements of the upper valve of an oyster immobilized in cement. The two fixed bars s and f shown at left are set in such a way that they record continuously on the kymograph paper the closed position of the shell (s) and the zero position of current flow (f). The actual amount of water transported by the gill cannot be measured by this method, but the relative values corresponding to the rate of discharge are computed by measuring with a planimeter the area of the record enclosed between the lines made by the excursions of the lever during a known time. A sample of the record obtained by this method is shown in figure 175.

Records of changes in the velocity of the cloacal current obtained in this manner can not be accurately calibrated. In my experience a cone placed in front of the cloacal current frequently fails to come back to the zero position and the entire delicate system easily gets out of adjustment. Another weakness of the method is the uncertainty of the correct position of the cone in relation to the diameter of the stream; it is impossible to know whether the entire width of the column of moving water strikes the cone surface.

A method based on a similar principle was developed by Mironov (1948) in the course of studies of water filtration by Black Sea mussels. The mollusk (M, figure 176) is placed on a horizontal platform (P) mounted on the side of an aquarium about 5 to 6 cm. below the surface of the water. A cover slip (R) freely suspended by two

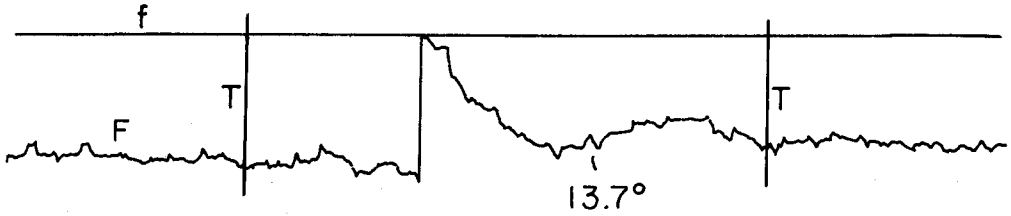

FIGURE 175.—Relative rate of water transport by the gill of *C. gigas* obtained with Hopkins' method at 13.7° C. Reproduced in part from Hopkins' paper, 1933. Portion of kymograph record shows: relative strength of cloacal current F; zero position of lever corresponding to absence of current f; and 5-minute interval on record, two vertical lines T.

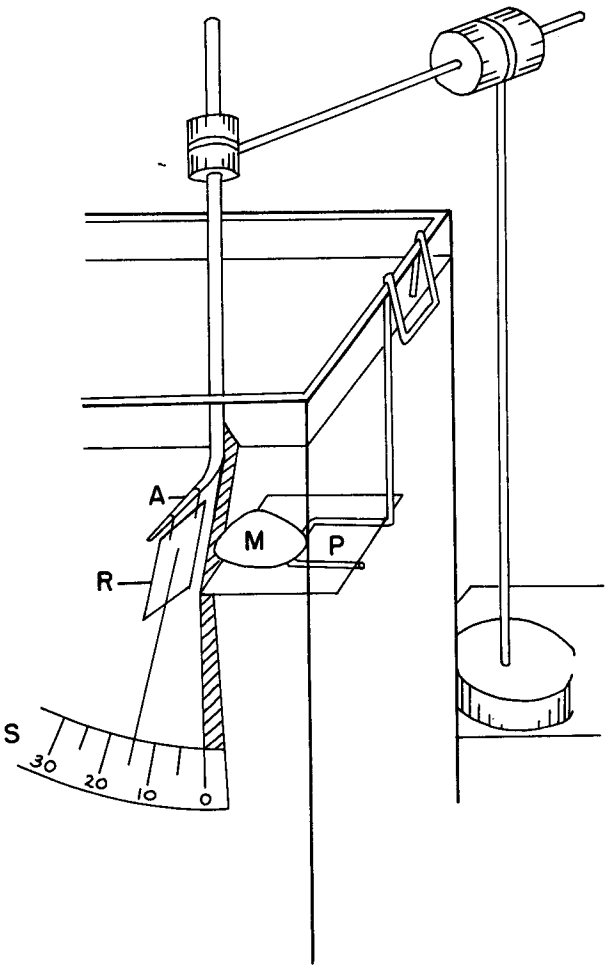

FIGURE 176.—Mironov's method of recording the rate of water filtration by sea mussel. A—horizontal arm for the suspension of cover slip R; M—mussel; P—platform; S—scale divided in angles. From Mironov, 1948.

glass strings from a horizontal arm (A) of a rod is lowered to such a position that the center of the cover slip is exactly in front of the exhalant siphon of the mussel and is perpendicular to the axis of the cloacal current. A thin glass indicator is cemented by canada balsam to the cover slip; its distal end moves along the scale (S) which is divided in angles. The deflection of the indicator is read every 5 minutes. The apparatus is calibrated by recording the deflection of the glass plate caused by a current of known velocity. For this purpose Mironov used the difference in water levels in the two aquaria A and B shown in figure 177. Water from A runs through an inverted U-tube into tank B. A small inverted T-tube (T) with one end sealed is inserted into the left arm of the siphon (U). The recording assembly of the type shown in figure 177 is placed in front of the cover slip, and the angles of deflection corresponding to the various level differences are read: millimeters on vertical scales L and L_1 placed in each tank. The velocity of the current is computed by using the formula $V=\sqrt{2gH}$ where H is the difference of the heights of water columns in the two connecting tanks and g is acceleration due to gravity. The volume is calculated by multiplying the current velocity V by the cross-sectional area of the opening of the siphon (2.4 $mm.^2$ in Mironov's experiments). The average rate of water transport was found to be 15 ml./min., or 0.9 l./hr. Mironov's observations, made within 24 hours, showed great variations in the rate of water transport which undoubtedly indicated that the experimental animals were disturbed and had not reached the steady state.

Lack of automatic registration of the rate of discharge is the obvious deficiency of the method, which could be improved by incorporating a me-

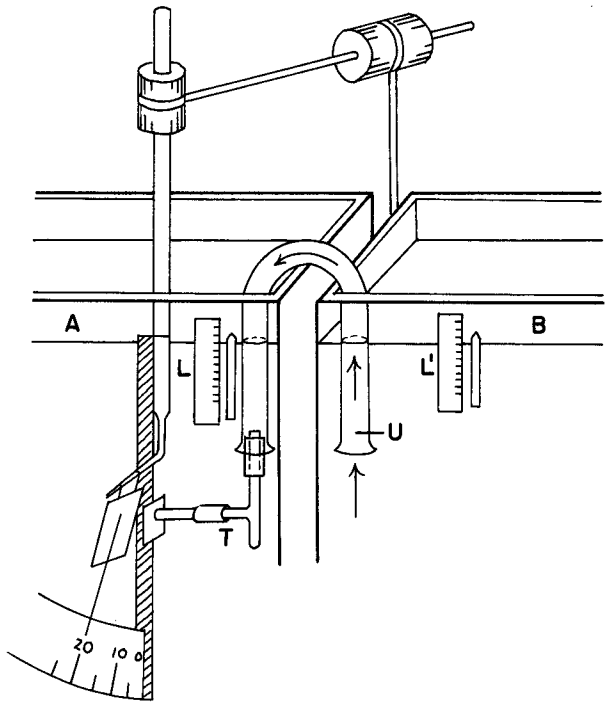

FIGURE 177.—Mironov's method of calibrating the angle of deflection of a glass plate by the currents of different velocities. A, B—two aquaria tanks; U—inverted tubing acting as a siphon; T—tubing connecting the siphon and the tank A; the horizontal arm of the tubing is placed in front of the swinging glass indicator; L, L_1—scales mounted on side walls of tanks to read the differences in water level.

chanical or optical device for continuous recording of changes in the position of the indicator.

Both Hopkins' and Mironov's methods are of limited value because the mollusks can not be kept in running sea water which would disturb the recording devices. This greatly restricts the application of their technique.

Indirect methods

All indirect methods are based on measurements of the rate of removal of particles suspended in water. Since the volume of water is kept constant, the observations must be completed in a relatively short time in order to avoid the effect of metabolites. This condition seriously limits the usefulness of the methods.

Use of turbid water.—Viallanes (1892) was the first to determine the relative rate of removal of suspended particles by bivalves. He selected a number of small *O. edulis* (18 months old), *C. angulata* of the same age, and *M. edulis* of "an average size" and placed them in separate crystallizing dishes in a tank with running sea water. Dishes of the same dimensions, but without mollusks, served as controls. After several days the sediment that accumulated on the bottom of the dishes was collected, dried, and weighed. He subtracted the quantity of material precipitated mechanically in the controls from the total quantity found in the dishes with mollusks, and assumed that the remainder was proportional to the volumes of water filtered by them. For each liter of water filtered by *O. edulis*, the *C. angulata* filtered 5 l. and the mussel 3 l. Dry clay was added to the experimental tanks in the proportion of 0.0546 g./l. In 24 hours the mussel precipitated 1.768 g. of clay, *C. angulata* 1.075 g. and *O. edulis* 0.199 g. Essentially the same crude method was employed 34 years later by Ranson (1926). He did not record the temperature during the obvervations and made no attempt to observe the shell movements of the mollusks or to note whether the valves remained open all the time during the experiment. In later years the ability of water-filtering bivalves to clear turbid water has been studied by more elaborate methods. The amount of material remaining in suspension has been computed from turbidity observations made by means of a nephelometer (Mironov, 1948) or with an electrophotometer used as a turbidimeter (Lund, 1957). A great variety of suspensions are used in this type of experiment—india ink, colloidal graphite, carmine powder, powdered eggs, ground diatoms, fuller's clay, milk, calcium carbonate, dried mud and others. Mironov (1948) reports that the best material for this purpose is nephelinic grey clay; after washing it gives a very stable suspension in which the precipitation of particles is so slow that it has virtually no effect on experimental results.

In experimental studies of the rate of water propulsion by the California mussel, *Mytilus californianus*, Fox, Sverdrup, and Cunningham (1937) used a suspension of calcium carbonate ($CaCO_3$). The water was stirred continuously to keep in suspension the calcium not removed by the mussels. At frequent intervals analyses were made of the calcium carbonate remaining in suspension and from these data the rate of water propulsion was computed. The rate of precipitation of calcium in the control tanks suggested that in the absence of mussels the amount of calcium suspended in water can be expressed as an exponential function of time and that the amount precipitated in a unit of time is proportional to the total amount which remains in suspension. In the mathematical treatment of the data Fox and his collaborators applied the following exponential equation:

$$p=P_0e-\left(\frac{nm}{M}+a\right)t=P_0e^{-bt}$$

where p is millgrams of suspended calcium per liter; P_0 is the amount of suspended calcium in the closed system at the beginning of the experiment; n the number of mussels in the vessel; m the volume of water (in liters) transported by one mussel in a unit of time; M the total volume of water in the vessel; t the time; e the Napierian base equal to 2.71828; and "A" and "B" the logarithmic decremental constants determined experimentally.

Under the specified conditions of the experiment in a closed system and using the above assumptions, the volume of water transported by one mussel was calculated by the following equation:

$$m=M\frac{b-a}{n}$$

The values obtained for medium sized mussels from 95 to 130 mm. long varied between 2.2 and 2.9 l./hr.

Several drawbacks are common to all methods based on turbidity determinations. The mollusks are kept in a closed system and are subject to abnormal conditions caused by high content of sus-

pended material and accumulation of metabolites. The methods are not suitable for continuous observations since they should be completed in the relatively short time before turbidity of the water is changed because of the aggregation and flocculation of suspended particles. Finally, computation by turbidity observations of the volume of water filtered by mollusks is based on the assumption that mechanical precipitation, due to gravity, remains constant. This, however, is not the case. Jørgensen and Goldberg (1953) found that *C. virginica* removes graphite particles from 5 to 10 times faster from a 4-hour old suspension than from a fresh one. This effect is explained by the difference in the size of the particles which in the aged suspension are about 2 to 3 μ in diameter; in the fresh one they are less than 2 μ. By adding small amounts of carmine suspension to the gill of the oyster it is easy to notice that the filtering efficiency of the gills of *C. virginica* and *C. gigas* is not high and that many small particles appear in the cloacal current. Jørgensen (1943) assumed that all particles are removed as the water is filtered through the gills, and computed the rate of water transport m by using the following formula:

$$m = \frac{(\log \text{conc}_0 - \log \text{conc}_t)\, M}{\log e \cdot t}$$

where m is the volume of water (in liters) transported in 1 hour; M is the volume of suspension in liters; conc_o and conc_t are the concentrations of cells or particles at the beginning of the observations and after t hours; and e is the Napierian base (2.71828). The formula can not be expected to give accurate results because it is based on two incorrect assumptions: first, that all suspended particles are removed from the water as it is being transported through the gills; second, that the dispersion of particles in the suspension does not change during the duration of the test. Jørgensen's observations showed considerable differences in the properties of new and old suspensions of graphite, and Chipman and Hopkins (1954) demonstrated that the efficiency of the removal of cells changes with time and is not related to cell concentrations. In their experiments the rapid rate of removal of *Nitzschia* or *Chlamydomonas* cells was followed by a decrease in the filtering efficiency of the gills and in the increased return to the suspension of the phytoplankton cells which had passed through the gills.

These difficulties introduce great uncertainty in the studies of the rate of water transport by the gills based on turbidity determinations. Some of the problems may be solved by the use of radioactive plankton.

Use of radioactive plankton.—The advance of radioisotope techniques has made it possible to employ labeled plankton algae for determining their rate of removal by water-filtering mollusks. Chipman and Hopkins (1954) and Chipman (1959) applied this method in a study of the rate of water transport in bay scallops, and Smith (1958) extended their observations to the clam, *Mercenaria* (*Venus*) *mercenaria*. Single species cultures of the diatom *Nitzschia closterium* (56 μ in length) and a species of *Chlamydomonas* (7μ in size) were made radioactive by the incorporation of phosphorus, P^{32}. The cells grown in a culture medium (modified Miquel solution) that contained virtually no phosphorus except the P^{32}, were highly radioactive. This isotope, emitting only beta particles of rather high energy, was found to be useful for this purpose. The details of the method developed in the Biological Laboratory of the Bureau of Commercial Fisheries at Beaufort, N.C., are described in a paper by Rice (1953). The method is extraordinarily sensitive and allows detection of very slight changes in cell numbers which otherwise would have remained unnoticed. The use of radioactive plankton presents several advantages in studies of the functions of the gills; it allows observations without undue increases in the concentration of suspended material and it makes possible recordings of changes in the rate of water transport which could not be detected with other methods.

CONTROL OF RATE OF WATER TRANSPORT THROUGH THE GILLS

Estimates of the rate of water transport by an adult oyster, made by investigators who have studied the problem carefully, vary from several liters to a maximum of 34 l./hr. (Loosanoff and Nomejko, 1946). Naturally the rate of water transport depends on the size of the oyster, its physiological state, and environment. The absolute figures are, therefore, of little significance unless they are accompanied by data on temperature, conditions under which the tests were made, and size of the oysters used.

It is self-evident that the quantity of water propelled by the gills must depend on the size of the mollusk. No comparative data of this

nature are available for the oyster, but determinations by Chipman (1955) and Chipman and Hopkins (1954) of the rate of propulsion of water by the bay scallop (*Pecten (Aequipecten) irradians* Lamark) clearly indicate this relationship. The data plotted in figure 178 were taken from the table of observations by these authors, who used a suspension of radioactive *Nitzschia closterium* and *Chlamydomonas*. A similar relationship was reported to exist in the California mussel in which the absolute rate of water transport through the gill was found to be a function of the weight of the soft parts of the mollusk (Rao, 1953). The relationship was well defined at temperatures of 20° and 16°, but was indefinite at 9° C. Rao also referred to the activity of mussels from various geographical regions. He stated that, regardless of temperature, mussels from higher latitudes transport water at a greater rate than mussels taken from the lower latitudes. The observations were of brief duration, lasting from 1 to 3 hours and therefore can not be considered as representative of typical behavior of bivalves over longer periods of time.

In the Bureau's shellfish laboratory at Woods Hole records taken continuously for several weeks show considerable variability among oysters of

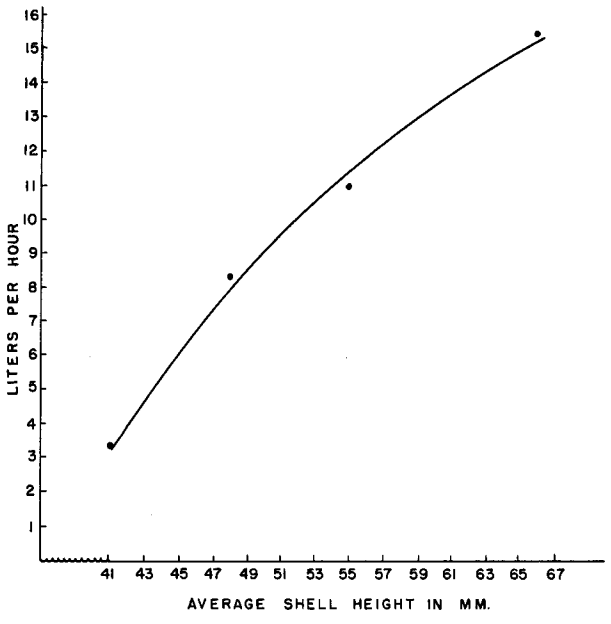

FIGURE 178.—Mean rate of water transport of scallops (*Aequipecten irradians*) of different shell length kept in sea water at room temperature ranging from 21.9° to 25.8° C. The plotted values represent the averages of 6 to 11 specimens. (From the data of Chipman and Hopkins, 1954).

equal size and origin that are kept under identical conditions. These changes in the rate of water transport can not be correlated with changes in the environment. In the tests only large oysters (10 to 12 cm. in height and 6 to 8 cm. in length) were used. They were healthy, free of boring sponge, *Polydora*, and other commensals. Daily fluctuations of temperature did not exceed 1° to 2° C. and salinity changes were less than 0.1 °/oo. The range of daily fluctuations in the rate of ventilation by a single specimen varied from 9.9 to 24.3 l./hr. in 1 day, and from 1.1 to 24.3 l./hr. 2 days later. The total quantity of water transported daily by this oyster in the 2 consecutive days of recording was 77.5 and 457 l. In the other oyster tested within the same month of July the range of daily fluctuations in the rate of water transport varied from 0.28 to 3.31 l./hr. in one day to 5.0 to 13.0 l./hr. the following day. The total quantity of water transported in these 2 days was 8.6 and 239 l./day respectively.

The more than 2,000 records of daily activities of oysters accumulated in the course of many years of my studies confirm this great variation. Some of the records were made continuously for 33 days, others were interrupted after 2 to 3 days of observations. All the records were obtained using the technique shown in figures 169 and 170.

STEADY STATE

Ventilation of the gills may continue for hours without interruption or significant changes in the rate of water transport. This condition, which may be called a steady state, occurs when temperature, salinity, and food content of the water remain constant and the oysters are not disturbed by sudden changes in illumination, vibrations, or other mechanical stimuli. The heart rhythm during the steady state remains constant. Judging by the rate of formation of fecal ribbons, the ingestion of food during these periods continues without interruption, provided the water does not contain excessive amounts of detritus, clay, or plankton which may stimulate the formation of pseudofeces and cause frequent snapping of the valves. The temperature at which the steady state was observed ranged from 15° to 25° C. It is conceivable, however, that it takes place at other temperatures.

An example of the steady state in oysters is shown in figure 179. In this experiment two oysters of approximately the same size were observed simultaneously. Their activities were slightly

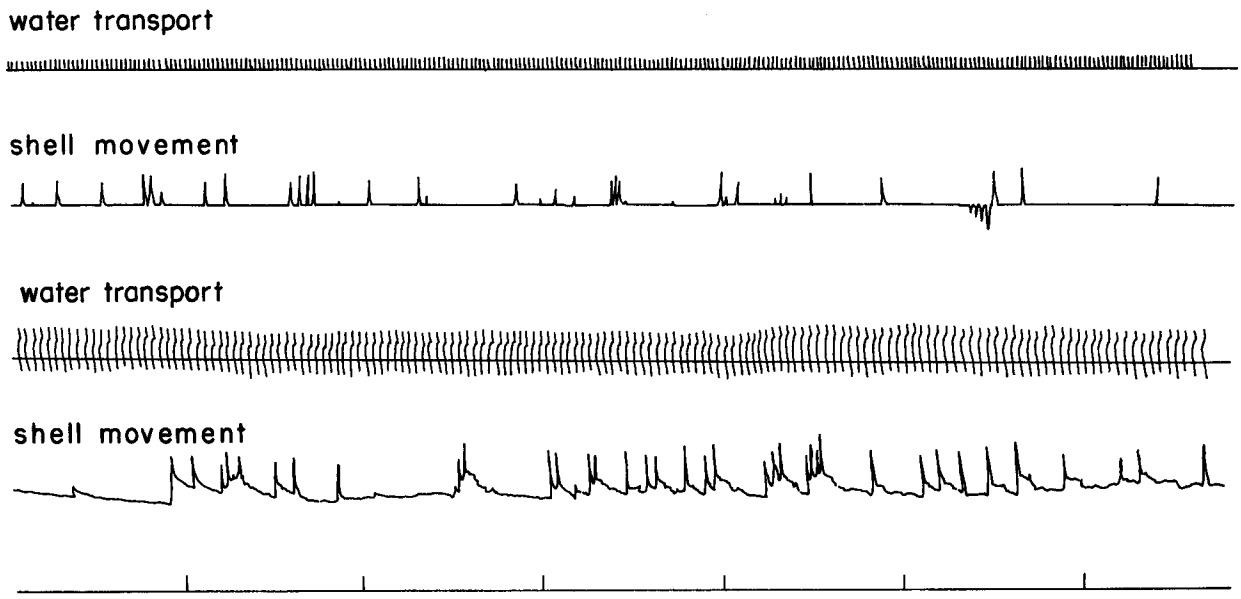

FIGURE 179.—Kymograph tracings of water transport and shell movements of two adult oysters. Each vertical stroke of the first and third line represents the discharge of 57 ml. of water (the capacity of the dumping vessel). Temperature 19.5° C. Salinity 31.2 °/oo. Oyster dimensions: 10.6 x 6.5 cm. (upper); 10.5 x 6.6 cm. (lower). Time interval, 1 hour.

different, although both were kept under identical conditions; water was delivered at a uniform rate from a common supply tank, and the temperature was kept constant at 19.5°±0.1° C. The upper record shows that during the 8 hours of observations the rate of water transport of one oyster was fairly uniform, varying between 19 and 21 l./hr. Occasional contractions of the adductor muscle were followed by an immediate return to the former tonus level. In the second oyster (lower part of figure 179) the rate of water transport decreased slightly from 14 l. per hour at the start to 11 l./hr. at the end of the record; its shell movements were more frequent and less regular than in the first oyster. It is apparent from these and other observations of the same type that under identical conditions of environment the rate of water transport may be different, depending on the intrinsic state of the organism.

Water transport by the gills is not carried on with a machine-like performance controlled entirely by such environmental factors as temperature, salinity, chemical composition of water, etc.; it is adjusted to or governed by the needs of the mollusk. It may be assumed that the variable daily requirements for food and oxygen and the necessity of eliminating the metabolites determine both the duration of the activity and the rate of water transport. In addition, the rate of water transport is affected by irregularity of gill activity shortly after spawning during the summer. Oysters often maintain a nearly constant rate of water propulsion for 2 or 3 days. Invariably these periods are followed by periods of partial or total inactivity (closed valves) manifested in greatly reduced rates of water transport or its complete cessation. It appears reasonable to deduce that the needs for food, elimination of products of metabolism, and requirements for oxygen determine both the number of hours per day the oysters stay open and the rate at which water is being transported through the gills.

REDUCTION OF WATER TRANSPORT

Increased and irregular shell movements are usually associated with a decreased rate of water transport. This relationship is apparent in tracings which were made shortly before the closing of the shells or immediately after their opening. The records of three oysters kept at a temperature of about 20° to 22° C. (fig. 180) show that in all of them the rate of water transport slowed down before the valves began to close. Complete closure of the valves took place in 72, 15, and 18 minutes after cessation of the current. In the majority of cases examined the time intervals between the resumption of the cloacal current to

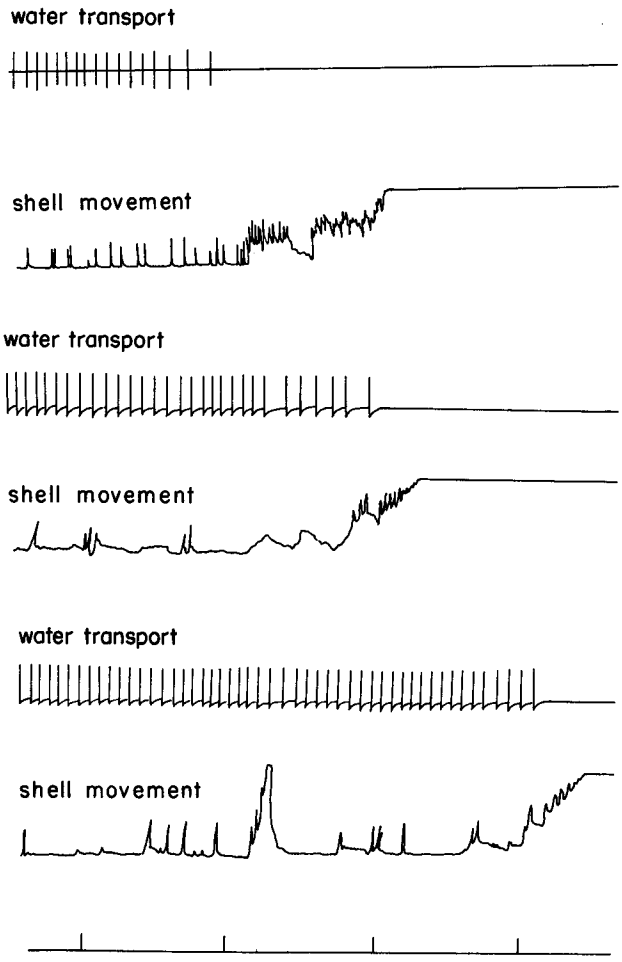

FIGURE 180.—Tracings of the rate of water transport and shell movements of three adult oysters, *C. virginica* at Woods Hole. Temperature 20° to 22° C. July. Vertical strokes of water transport line correspond to the discharge of 247 ml. of water by the dumping vessel. Time interval, 1 hour.

full velocity and the opening of the valves were about equal (fig. 181).

A reduction in the rate of water transport may also be caused by the slowing down of the lateral cilia of the gill filaments. To study the activity of these cilia it is necessary to eliminate the effects caused by the movements of the shell and mantle. A record of the activity of the lateral cilia can be obtained with an electric drop counter placed under the small tank (fig. 168) to receive water discharged through the cloaca. Parts of such a record made in the summer at Woods Hole are reproduced in figure 173. Interruption of a steady state shown on the second pair of lines in figure 173 was caused by slight tapping against the side of the experimental tank. The lateral cilia are very sensitive to minor mechanical stimuli and slow down at the slightest disturbance. These interruptions were of brief duration, and the preceding rate was restored within a few seconds (line three).

By using the drop counting technique it is possible to observe minor fluctuations in the activity of the lateral cilia and to demonstrate their responses to changes in temperature, salinity, different concentrations of drugs, food particles, etc. In a graphic summary of one such record (fig. 182) the average number of drops per 5 sec. was plotted against time shown at 5-sec. intervals. The rate of current remained fairly constant with the exception of the period between 110th and 145th sec. when a suspension of eggs was added to the gills. The reduction was temporary, and the normal rate was soon resumed.

The dilation and constriction of the ostia and the expansion and folding of the gill lamellae also affect the rate of water transport. The effect of these minor adjustments can not be measured separately from the activity of the lateral cilia. I have noticed, however, that changes in the position of the gill lamellae and the expansion or constriction of the ostia are usually associated with the muscular activities of the adductor muscle and the mantle.

DISSOLVED ORGANIC SUBSTANCES AND RATE OF WATER TRANSPORT

Collier and his associates (1950, 1953) reported that sea water of the Gulf of Mexico contains certain organic substances which have the general chemical chracteristics of carbohydrates. These substances respond to analytical tests with N-ethyl-carbazole (or with anthrone, Lewis and Rakestraw, 1955) and were reported to occur in concentrations up to 50 mg./l. The figure greatly exceeds the concentrations found in sea water by others. According to the reliable studies of Krough (1934) the content of dissolved organic matter in sea water remains fairly constant at the level of about 5 mg./l. It was further claimed by Collier that the carbohydrates occur in variable concentrations in coastal water near Pensacola, Fla., and that their presence greatly influences the shell movements and the rate of water transport by *C. virginica*. Since in their experiments the response of the oysters was not consistent with the concentrations of carbohydrates determined by carbazole reagent, the authors tried to overcome

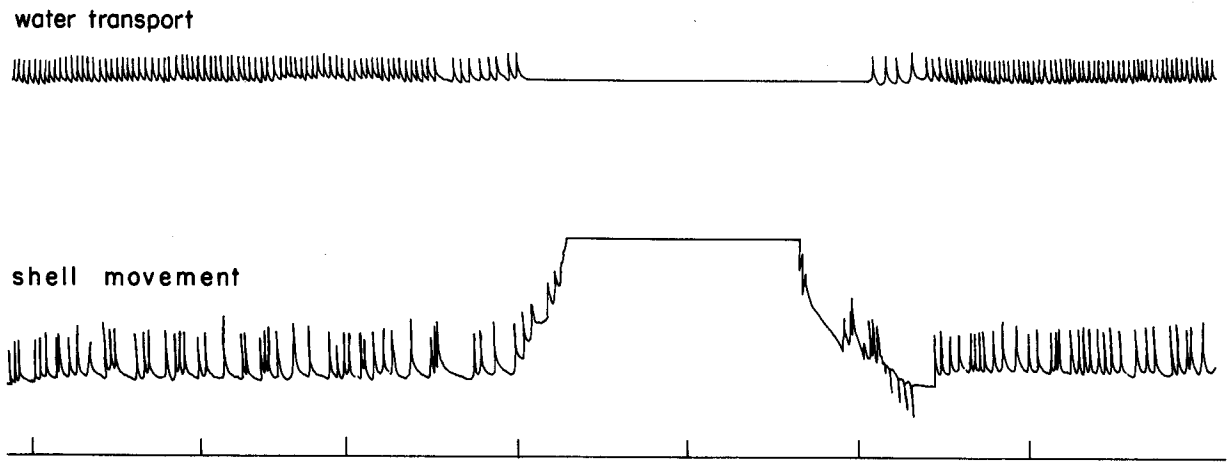

FIGURE 181.—The shell movements and rate of water transport of an adult *C. virginica* preceding the closing (left side) and following the opening (right side) of the valves. Temperature of water 22.5° C. Time interval 1 hour.

the difficulty by assuming that "each oyster appears to have a threshold limit to the carbohydrate below which it will not pump."

The existence of a general chemical factor which controls the principal activity of the oyster would be of great importance to the study of feeding and nutrition of marine invertebrates. It seems that if such a factor actually exists then Putter's theory of the significance of dissolved organic substances in the feeding of marine invertebrates should be reinvestigated, especially in view of the assertion made by Collier and his colleagues that "the oysters remove variable quantities (up to 50 mg./l.hr.) of the carbohydrates from sea water."

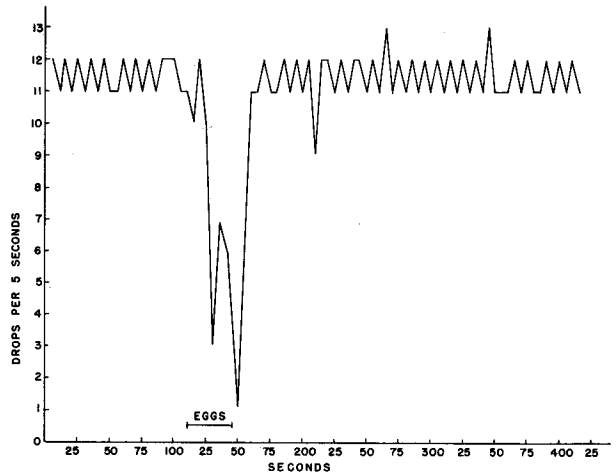

FIGURE 182.—Effects of egg suspension on the water transport by the lateral cilia of the gill of a ripe male oyster, *C. virginica*. Data from the drop counting record are plotted as averages of 5 sec. intervals. Temperature of water 22.5° C.

An attempt to identify the substances concerned was made by Wangersky (1952), who reported the isolation of a compound having the absorption spectrum of dehydroascorbic acid and the presence of a substance which "gives some indication of rhamnoside" and is found in the inshore waters of the Gulf of Mexico in concentrations up to 0.1 g./l.

A description of the reagents and methods for carbohydrate determination can be found in the paper of Lewis and Rakestraw (1955) who remark that in general, the N-ethyl-carbazole method (used by Collier, Ray, Magnitzky, and Bell) is "considerably less satisfactory" than the anthrone method.

The N-ethyl-carbazole method is as follows: 1 g. of the reagent recrystallized from ethanol and water is dissolved in one liter of 90 percent sulfuric acid cooled in ice. The acid should be of highest purity, stored in glass-stoppered bottles. The solution is stored in a dark bottle and kept refrigerated. Exposure to air and sunlight must be avoided as much as possible. Under these conditions the reagent is stable for at least 2 days.

A 2.5-ml. sample of filtered (or centrifuged) sea water is transferred to a 60-ml. bottle, 22.5 ml. of N-ethyl-carbazole reagent is added, and the sample thoroughly mixed. The sample is immediately placed in a water bath at 70° C. (± 0.2° C.) and left for exactly 30 minutes. After 15 to 20 minutes in a refrigerator the sample is allowed to come to room temperature. The optical density at 562 mμ is determined between 30 to 60 minutes after the removal of the sample from the water bath.

Standard curves and reagent blanks are determined daily with double distilled water. The reddish-violet reaction product is unstable. It is easily destroyed by sunlight and oxidizing agents with a resultant dark green coloration. In filtering the sample it is necessary to establish that passage through the filter does not introduce extraneous carbohydrates.

Collier, Ray, Magnitzky, and Bell (1953), expressed the results of their determinations in terms of concentrations of arabinose (in mg./l.) although the substance or substances present in the water were not definitely identified. They state that they "may not be true carbohydrates."

A series of analyses using the N-ethyl-carbazole method were made at Woods Hole of samples of sea water pumped from the harbor to the laboratory. In July 1953, the carbohydrate content was low, varying from 0.3 to 1.8 mg./l. Many determinations giving values of about 0.1 mg./l. were almost below the threshold of sensitivity of the method and many others had to be discarded because of contamination of the samples or deterioration of the reagent.

A study of the records of shell movements and water transport of oysters in relation to the natural fluctuation of the carbohydrate content of sea water in which they were kept gave negative results. No evidence was found to support the view that the opening of the valves and the resumption of water transport were associated with the increase of carbohydrate concentration from 0.3 to 1.8 mg./l. The oysters opened and closed spontaneously regardless of the slight fluctuations in the content of the substances which react with carbazole. Several excerpts from the laboratory records presented in table 20 illustrate this point. Within the range of concentrations found in the water, the high and low degrees of activity were not correlated with the changes in the carbohydrate content (compare the behavior of oysters B, B^1 and C, D^1). In each case the recording was continued for a period varying from 6 to 8 hours. After periods of inactivity the oysters B^1, D, and D^1 opened and resumed water transport, while there was no change in the concentration of carbohydrate. I deduce from these observations, which were repeated several times with similar results, that the presence of carbohydrates within the range found naturally in Woods Hole sea water had no effect on the oysters and is not a factor which controls the function of their gills.

TABLE 20.—*Carbohydrate concentration in sea water (mg./l.), shell movements and water transport in* C. virginica *at Woods Hole*

[Active shell movement means frequent opening and closing of the valves]

Oyster No.	Temperature	"Carbohydrate"	Shell movement	Water transport
	° C.	*mg./l.*		*l./hr.*
A	16.5	1.3	Active	0
A^1	16.5	1.1	Open	5.0
B	15.6	1.8	Active	12.0
B^1	16.0	1.8	Partly closed	0
C	21.5	0.8	Active	15.0
D	21.5	1.0	Closed	0
D^1	21.5	0.6	Closed	0

The determinations of the concentration of carbohydrates in Woods Hole water are in agreement with the findings of Lewis and Rakestraw (1955) for the Pacific Coast, where they encountered carbohydrates in quantities varying from 0.1 to 0.4 mg./l. and as high as 8 mg./l. in coastal lagoons.

If carbohydrates did in fact influence the rate of water transport it would be reasonable to expect that the addition of these substances in quantities exceeding their concentration in natural water would produce a measurable effect. Quantities of various carbohydrates were added at a known rate to the mixing chamber from which the water was supplied to the constant level tanks with oysters (Galtsoff and Arcisz, 1954). The following substances were used in concentrations ranging from 10 to 100 mg./l.: arabinose, fructose, dextrose, maltose, and ascorbic acid. The tests were continued for several hours. In all cases there was no evidence that the increase in carbohydrate concentration in any way affected the rate of water transport or shell movements of the oysters.

In some instances (first column, table 21) there was a gradual increase in the activity of the gills which cannot be attributed to the presence of arabinose since the rate of water transport continued to increase for several hours after the return of the oyster to natural sea water.

Similar results were obtained in another set of experiments using arabinose and fructose. The following rates of transport of water were recorded:

Before adding arabinose---------- 9.5 to 11.4 l./hr. during 2 hours
In arabinose solution (65 mg./l.)---------- 7.6 to 13.3 l./hr. during 4 hours
Return to natural sea water---------- 19.0 to 26.6 l./hr. during 3 hours

TABLE 21.—*Rate of water transport, in liters per hour, of three adult* C. virginica *in natural sea water and in water containing 1-arabinose in the concentrations of 65 mg., 80 mg., and 100 mg./l. Salinity 31.2 °/oo*

[In all three experiments the shift from natural sea water to arabinose solution in sea water and again to sea water was made at the end of the hour shown in the first column without disturbing the oyster. Fifteen minutes were needed to flush the experimental tank with natural sea water.]

Time in hours	Water transport in sea water and in arabinose solution in sea water								
	Water transport		Temp.	Water transport		Temp.	Water transport		Temp.
	Sea water	Arab. 65 mg./l.		Sea water	Arab. 80 mg./l.		Sea water	Arab. 100 mg./l.	
	l./hr.	*l./hr.*	*° C.*	*l./hr.*	*l./hr.*	*° C.*	*l./hr.*	*l./hr.*	*° C.*
1	5.7	-------	19.8	3.8	-------	18.2	6.6	-------	17.5
2	4.7	-------	19.8	2.4	-------	18.2	6.6	-------	17.5
3	-------	5.7	19.9	-------	1.6	18.3	0.3	-------	18.0
4	-------	3.8	20.1	-------	1.6	18.3	3.8	-------	18.5
5	-------	6.7	20.2	2.7	-------	18.0	3.8	-------	20.0
6	-------	5.7	20.3	4.1	-------	17.9	-------	5.7	19.8
7	-------	5.7	20.4	4.1	-------	17.9	-------	6.7	20.0
8	9.0	-------	20.8	-------	-------	-------	6.7	-------	20.1
9	11.4	-------	20.9	-------	-------	-------	-------	-------	-------
10	13.3	-------	21.2	-------	-------	-------	-------	-------	-------
11	10.4	-------	21.2	-------	-------	-------	-------	-------	-------

In another test using an oyster with a lower rate of water transport the results were as follows:

Before adding arabinose----3.9 l./hr. during 2 hours
After addition of arabinose (85 mg./l.)----
4.3 to 4.6 l./hr. during 2 hours
Return to natural sea water----
5.6 to 6.0 l./hr. during 2 hours

During both tests the salinity of the water was 31.2 °/oo and the temperature varied between 18.0° and 19.5° C. In the concentration of 102 mg./l. of fructose the following rates were observed:

Before adding fructose----
3.9 to 4.1 l./hr. during 2 hours
In fructose solution---2.8 to 4.7 l./hr. during 3 hours
Return to natural sea water----
4.3 to 4.5 l./hr. during 2 hours

No effects were observed even when the concentrations of carbohydrates were increased to 0.5 percent. The rate of water transport in natural sea water varied between 8.4 and 10.8 l./hr. and from 7.2 to 10.8 in the arabinose solutions. Similar negative results were obtained in 1 percent maltose and 1 percent fructose in sea water and in various concentrations of ascorbic acid.

Additional tests with ascorbic acid were made with the carmine cone method for measuring the velocity of the cloacal current. The results (table 22) show lack of any effect on the efficiency of the lateral cilia in concentrations varying from 10 to 50 mg./l. Velocity of the cloacal current is given in table 22 as an average of 10 consecutive readings made at 2-minute intervals. Fifteen minutes elapsed between each group of 10 readings. The concentration of 400 mg./l. (0.04 percent) completely inhibited the current.

My observations are in full agreement with the results obtained by Butler and Wilson (1959), who presented conclusive evidence that the increases and decreases in the rate of water transport by oysters (*C. virginica*) at the Bureau's Biological Laboratory at Gulf Breeze, Fla., (the place where Collier's experiments were conducted), are not correlated with the changes in the concentrations of carbohydrates in the water and that there is no "minimal threshold level of carbohydrate concentration below which oysters fail to pump."

The experimental studies show that the organic substances which give an N-ethyl-carbazole reaction have no effect on the water transport of oysters in the concentrations in which these substances are encountered in the tidal waters of Cape Cod.

RESPIRATION

Exchange of gases takes place primarily in the gills, but the mantle also has a role of lesser importance in the respiration of bivalves. Observations on the comparative rate of oxygen consumption by various tissues of the oyster have not been made, but the data on oxygen consumption by the gills and mantle of the hard clam *Mercenaria* (*Venus*) *mercenaria* are available. Hopkins (1946) compared the oxygen consumption of the mantle of that species with that of the gills and found that the oxygen uptake by the gills varied from 815 to 912 and that of the mantle only from 11.73 to 15.52 cu. mm./hr./g. of dry

TABLE 22.—*Effect of ascorbic acid on the velocity of cloacal current of* C. virginica

[Carmine cone method. Temperature 19.6° to 20.6° C. Each figure of velocity is an average of a group of 10 consecutive readings. Time interval between each group—15 minutes.]

Ascorbic acid	Velocity of cloacal current		pH
	In normal sea water	In water with acid	
Mg./l.	*cm./sec*	*cm./sec*	
10	2.1	2.0	8.1
10	2.6	2.9	8.1
10	3.3	3.7	8.1
15	4.3	4.0	8.2
15	2.5	2.6	8.0
15	2.6	2.8	8.0
50	2.1	2.0	8.0
50	3.3	3.7	8.0
400	4.7	no current	8.0

tissue. Similar differences in ratio were observed during all seasons although the absolute figures of oxygen consumption varied. It is probable that a similar ratio may be found in oysters and other bivalves.

METHODS OF STUDY

In the old method of determining the rate of oxygen consumption, oysters were put in a small container filled with sea water which was analyzed for oxygen content at the beginning and at the end of the test. The method was crude since no attention was paid to the increase in the concentration of metabolites in the water or to the opening and closing of the shells during the test period. The results obtained under such conditions were erratic. Some of the defects of this method were eliminated by using the open-chamber technique (Galtsoff and Whipple, 1931). The oyster was mounted on a support and placed in an open jar in sea water under a layer of paraffin oil. Shell movements were recorded on a kymograph. The water in the jar was gently stirred, and the volume drawn off for oxygen determination was replaced by fresh sea water of known oxygen content. The temperature was kept constant by placing the respiratory chamber in a water bath equipped with proper temperature control. The sea water used for the test was filtered to eliminate the effects of photosynthesis and respiration of plankton.

More reliable results were obtained by using the modified respiration chamber of Keys (1930a, 1930b) which was designed originally for studies of oxygen consumption by fishes. The method was used in my laboratory to determine the fluctuations in the rate of oxygen consumption by oysters that were kept for several hours in slowly running water of constant oxygen content (Galtsoff, 1947). During the period of testing the shell movements were recorded and the position of the borders of the mantle was observed. The apparatus (fig. 183) consists of a respiratory chamber A submerged in a large water bath in which the temperature is kept constant within ± 0.2°C. Filtered sea water of known oxygen content is supplied by gravity from a battery of carboys, and a uniform rate of delivery is controlled by head pressure kept constant in a small delivery vessel H. The water from the carboys is fed to this vessel at a constant rate; and excess water is voided by suction (on the right side of vessel H) in order to maintain the constant level in H. The tubing that leads from vessel H is divided at I into two branches of equal diameter, one leading to the collecting vessel L, the other to the respiratory chamber A. The constant rate of flow is maintained by means of two capillary glass tubings of equal diameter O inserted in the delivery tube. Stainless steel valves, pinch and glass stopcocks were found unsuitable for this purpose because of the slight shifting of their moving parts. Glass tubings of appropriate diameter were selected for each test from a set of several calibrated capillaries kept on hand, and the rate of flow of water was carefully checked at the beginning and end of each test. Before entering the respiratory chamber A, the water passes through a glass coil B (shown in figure 183 in a vertical position but actually lying flat on the bottom) which is completely submerged in water bath C. The bath is equipped with a constant temperature controller (not shown in figure 183). The water leaves the respiratory chamber through an outlet on the top and runs to one of the collecting cylinders K; by using a three-way stopcock J the flow of water may be shifted from one collecting cylinder to the other. The cylinders are suspended from pulleys and are counterbalanced by weights N. When empty the cylinders are raised above the water tank M; as they are filled with water they descend until they are partially submerged. Heavily paraffined wooden floats prevent direct contact of water collected in the cylinders with the air. For taking samples the cylinder is disconnected from the respiration chamber by turning off the stopcock J so that water is diverted to the second cylinder. Then the closed cylinder may be lifted out and the water taken through the drain cock at the bottom. Glass stoppered Erlenmeyer flasks of 100-ml. capacity were used for sampling. The sample for analysis is taken at the middle level of the cylinder between the 300- and 500-ml. marks. Water which runs directly from the supply carboy is sampled in the same manner. The difference in the oxygen content of the water running in and out of the respiratory chamber multiplied by the rate of flow through the chamber gives the quantity of oxygen consumed by the oyster in a unit of time.

Each oyster was prepared carefully for the tests. Shells were scrubbed with a wire brush, rinsed in fresh water, dried, and covered with melted paraffin applied with a small brush. During this treatment the oyster was kept vertical with its hinge at the bottom, and paraffin was applied in

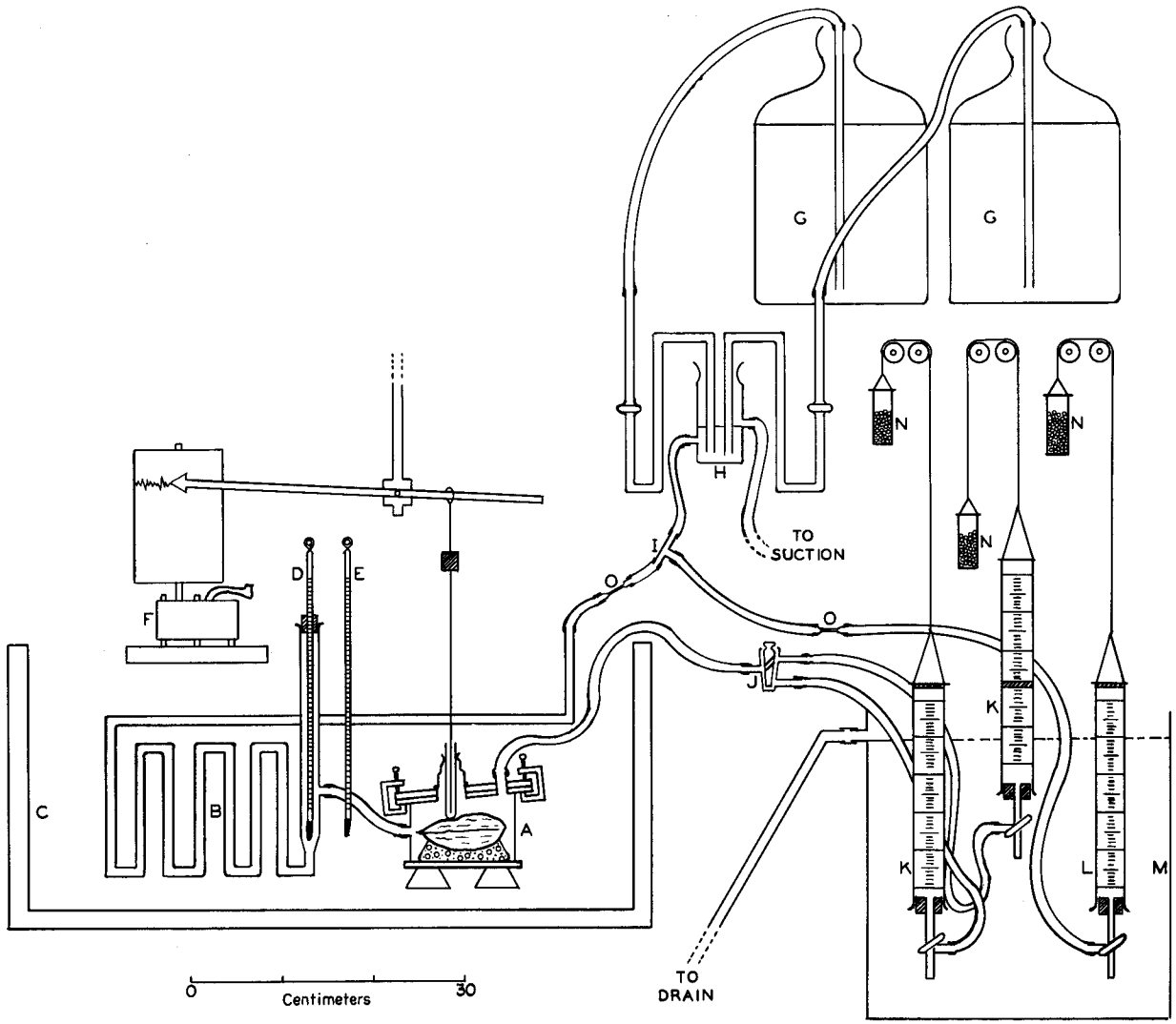

FIGURE 183.—Metabolism chamber for determining the oxygen uptake of an oyster kept in continuously renewed filtered sea water. A—respiratory chamber (for details see figure 184); B—glass coil (placed horizontally on bottom) to bring the sea water to a desired temperature; C—constant temperature water bath; D—thermometer for recording the temperature of water before it enters the respiratory chamber; E—thermometer for recording the temperature of water in the bath; F—slow-motion kymograph; G—supply of filtered sea water of known oxygen content; H—constant level tank which regulates the flow of sea water through the chamber; I—T-tube connection leading to the sampling cylinder; J—two-way stopcock; K—two sampling cylinders which receive water from the respiratory chamber; L—sampling cylinder which receives sea water directly from supply G; M—water bath in which the sampling cylinders are suspended; N—counter weights to balance the sampling cylinders; O—capillary tubing for regulating the rate of flow of sea water. Constant temperature regulator, heater or cooler in bath C, are not shown.

short strokes directed away from the edge of the shell to avoid accidental sealing of the valves. After careful examination and removal of superfluous paraffin the oyster was placed in the oval respiratory chamber (figure 184), which was built of heavy plastic with a removable slanted top (E) kept in place by two metal clamps (F). The capacity of the chamber, which rests on two heavy lead bars, is about 800 ml. When it is in operation, filtered sea water of known oxygen content is delivered through inlet B and is discharged through outlet C on the top.

The chamber is filled with filtered sea water, the oyster is placed inside, and the cover clamped down. All air bubbles are carefully evacuated. To record the shell movements a glass test tube (H) is lowered through the wide neck of the cover E until it rests on the oyster valve; the

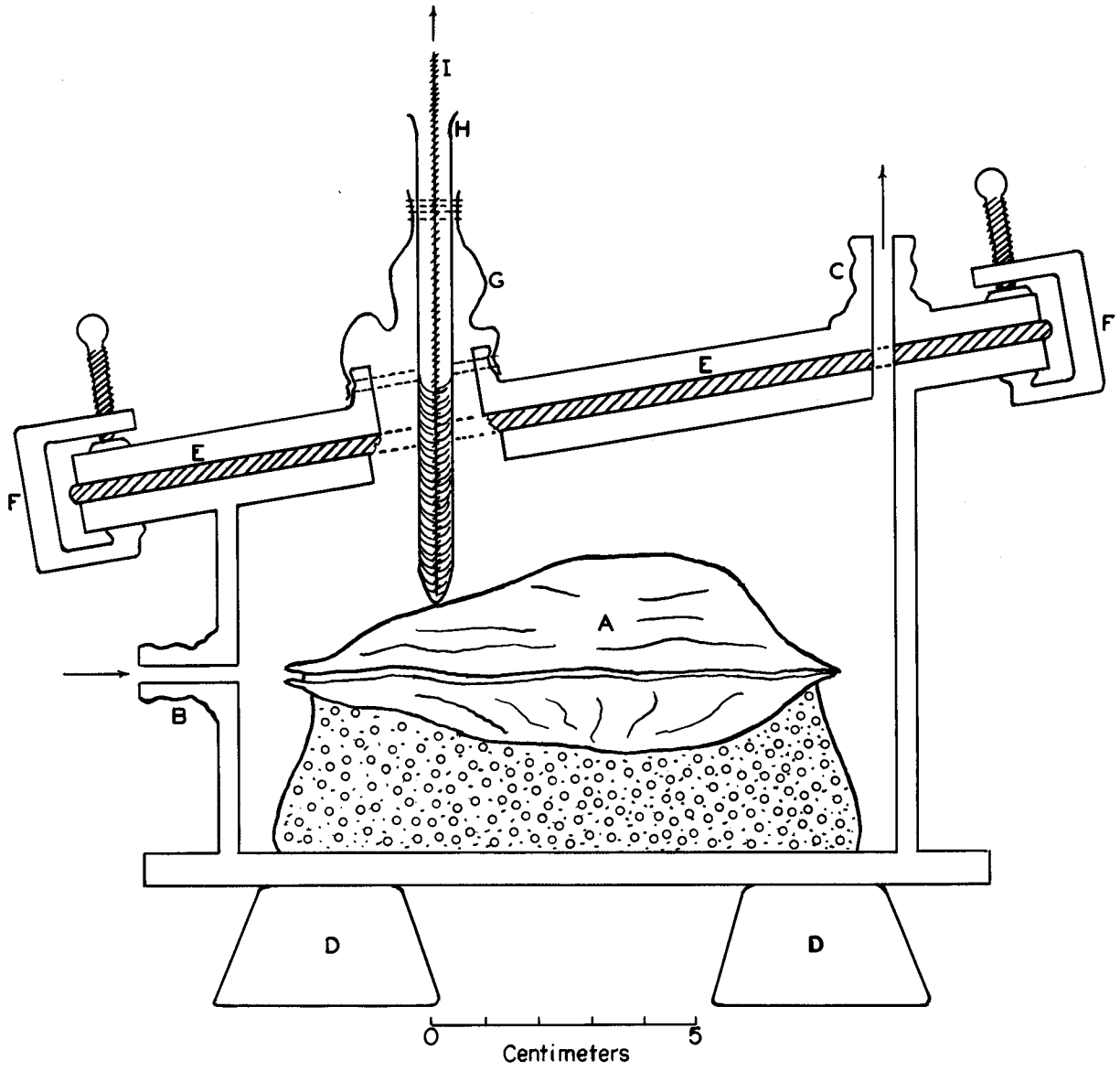

FIGURE 184.—Metabolism chamber, details of construction. A—oyster resting on heavy concrete base ready for metabolism test; B—inlet for sea water of known oxygen content; C—outlet; D—lead bars; E—slanted removable top underlined with a rubber gasket; F—clamps; G—rubber balloon which serves as a flexible gasket for test tube H which rests on the valve of the oyster; I—laboratory brush inserted into the test tube and attached to the writing lever of a kymograph.

small rubber balloon G tied with a silk thread acts as a flexible, watertight gasket with enough slack to permit slight vertical movements of the tube as the oyster opens and closes its valves. The connection with the writing lever of a kymograph is made by inserting a small laboratory brush in the glass tube and attaching the metal handle of the brush to the arm of the lever (fig. 183). Outlet C is connected to the tubing which leads to collecting cylinders K (fig. 183). The chamber without oyster must be checked first by taking simultaneous samples of water from the control and from one of the collecting cylinders. When the samples give identical values of oxygen content a second test is made with the closed oyster in place in the respiratory chamber. If the difference in the two samples exceeds the probable titration error, a search for trouble should be made. Usually it can be traced to a defective paraffin coating of the shell or to the growth of

fungi and bacteria inside the rubber connections. To avoid this growth all rubber tubings and joints should be periodically cleaned, dried, and sterilized. With these precautions duplicate determinations in the Bureau's shellfish laboratory gave consistent results, the error not exceeding ± 0.01 mg. of oxygen in the 100-ml. sample.

Oysters were kept in the respiratory chamber from 4 to 9 hours, and samples of water were taken at half-hour intervals. The duration of the test was limited by the available quantity of filtered and aerated sea water. For oxygen determination the Winkler titration was used; in some tests the Van Slyke volumetric method was employed for determining the carbon dioxide and the oxygen content of the water.

To obtain data corresponding to the level of basic metabolism, the oysters were starved for 24 hours by placing them in filtered sea water. This period was found to be long enough to cleanse the intestinal tracts and to discard the feces and pseudofeces. Throughout all the tests the temperature of water was maintained at 25° C. ± 0.1° C.

MICRODETERMINATION OF OXYGEN

Oxygen content in very small volumes of water can be found by using one of the microdetermination methods developed by Lund, 1921; Thompson and Miller, 1928; Kawaguti, 1933; Krogh, 1935b; and Van Dam, 1935a. The volume of water used for analysis in these methods varies from a few ml. to a fraction of 1 ml. Samples can be taken simultaneously or nearly simultaneously from the inhalant and exhalant currents of a bivalve. It is obvious that such a procedure requires great precision of sampling. This is made possible by Krogh's syringe pipette (Krogh and Keys, 1931) or its modification made by Van Dam (1935a). The syringe pipette designed by Krogh and suitable for delivery of small quantities of fluid with a high degree of accuracy is shown in figure 185. It is a glass cylinder with a carefully ground plunger and a heavy-walled glass capillary welded to the tip instead of the conventional metal injection needle of a hypodermic syringe. Tuberculin syringes with blue plungers are suitable for this purpose. The glass capillary is about 5-cm. long with a small bore of inner diameter of about 0.15 to 0.20 mm. The syringe is mounted on a frame of two steel rods set in a bakelite or ebonite base. The notched bar, N, determines the highest position of the plunger. The volume delivered by the syringe is adjusted to any desired fraction of its capacity by a metal collar which may be pushed into one of the notches and set in a fixed position by set screw S. The pipette with the special tip answering Van Dam's specification is not available from stock at any scientific supply store in this country and has to be made to order by an experienced glass blower.

For taking samples two syringes are mounted on an adjustable screw stand that allows fine and independent movement in the vertical and horizontal planes. The syringes are attached to the arms of the stand by ball bearing holders H, so that they can be set at any angle to the horizontal plane. The stand must be heavy and must have adjustments fine enough so that the tips of the collecting syringes can be introduced into the cloacal region of the oyster or the branchial and anal siphons of clams without touching or disturbing the sensitive tissues. The type of stand suitable for this purpose is shown in figure 1 of Van Dam's paper (1935a).

Samples of water for microanalysis also can be taken by means of a siphon; the tip is introduced deep into the cloacal chamber of a bivalve mollusk, as shown diagrammatically in figure 186. This device was used by Van Dam (1954) in his

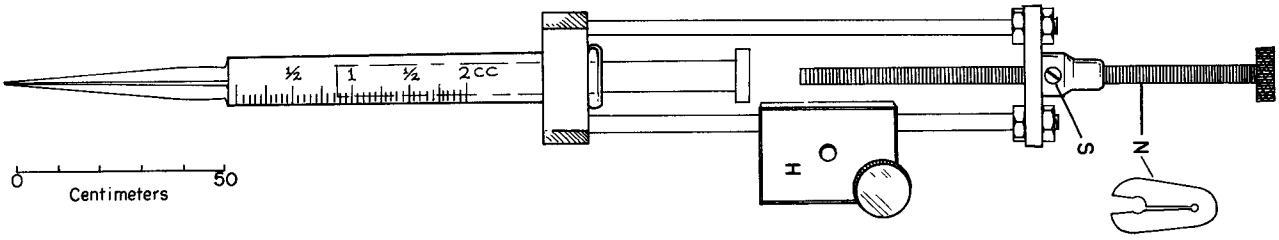

FIGURE 185.—Van Dam's modification of the syringe pipette for taking small samples of fluid with a high degree of accuracy. H—clamp holder; N—notched bar; S—set screw.

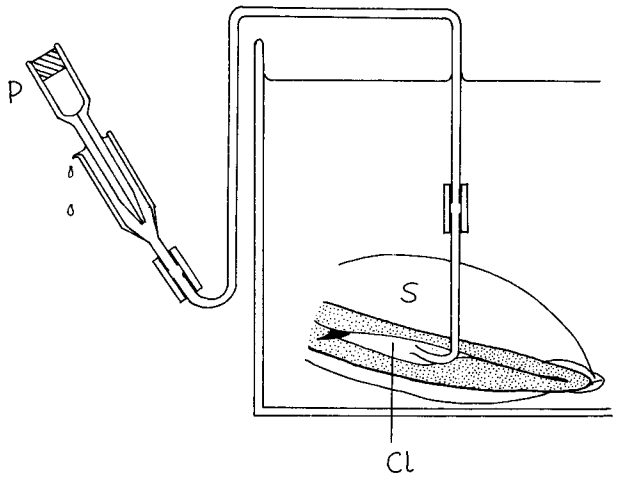

FIGURE 186.—Van Dam's method of sampling sea water from the exhalant current of sea scallop. Samples are taken by syringe pipette P (shown in part, left side) from the end of siphon with the lower part introduced into the cloaca, Cl. The horizontal arm of the siphon is about 5 mm. inside the cloaca. S—scallop. From Van Dam, 1954.

study of respiration of the scallop. The presence of the tip of the siphon in the cloaca does not interfere with the normal propulsion of water, provided the needle does not touch the scallop. Before the first samples were taken Van Dam allowed the water from the cloaca to pass through the siphon for about 1 hour at the rate of 1.5 ml. per minute. Such a slow rate of collecting was considered a guarantee that the sample was not contaminated with outside water. The difference between tne oxygen content of the water that entered the gill and of the water of the exhalant current showed the percentage of utilization of oxygen.

In bivalves with long and narrow siphons, as for instance *Mya* or *Mercenaria*, water leaving the exhalant aperture probably has a uniform content of oxygen. In species lacking siphons the cloaca opens as a wide cone-shaped slot and the stream of water escaping from the cloaca contains considerable and variable amounts of outside water depending on the distance from the epibranchial chamber. This introduces uncertainty in interpreting the results of the test. Van Dam (1954) found that in bay scallops the oxygen content of the samples taken at different positions within the cloacal current varied from 28.5 to 66.6 percent of the oxygen content of the inhaled water. Because of this uncertainty the method does not appear to be suitable for measuring tne true oxygen utilization of bivalves unless the tip of the collecting syringe is introduced deep inside the epibranchial chamber of the gills. Since the rate of propulsion of water and the volume of water transported are not known, the total quantity of oxygen used cannot be determined. This greatly limits the usefulness of the method. The main advantage of microdetermination methods is that the mollusks may be kept in running sea water under conditions which closely approach their normal environment.

The selection of a method for respiratory studies must be governed by the purpose and conditions of the experiments. All closed system methods are suitable for tests that should be completed before the depletion of oxygen begins to affect the respiratory rate. The microanalytical methods are suitable for determining the utilization of oxygen by bivalves (i.e., the percent of oxygen removed by tissues during the transport of water) provided the samples of the exhalant current are not contaminated with outside water. This is nearly impossible to avoid in species like scallops and oysters, which have no siphons. The use of the respiratory chamber with a constant rate of flow of water seems to be the most satisfactory technique for long-term observations on oysters. The method gives reproducible values of the oxygen uptake over a period of many hours. The following discussion of the respiration of the oyster is based primarily on results obtained in the Bureau's shellfish laboratory with this method.

OXYGEN UPTAKE

The rate of oxygen uptake is influenced by several extrinsic and intrinsic factors. The first group includes seasonal and diurnal changes in the temperature and salinity of water, and the occasional presence of contaminants or other environmental changes, such as an abundance of unicellular algae which may depress the rate of respiration. The existence of the intrinsic factors becomes apparent in observations of differing metabolic rates in oysters of known origin and uniform size and age kept under constant conditions (see: p. 207). Some of the intrinsic factors are associated with differences in the contents of water and glycogen in the tissues; with the loss of solids due to the discharge of sex cells during spawning; and with generally poor condition of the oysters. In a comparative study of respiratory rates several precautions are necessary to minimize the extent of individual variations.

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Oysters should be taken from a healthy population; they should be devoid of parasites and commensals; they must be of uniform size and age. The metabolism tests should be made at constant temperature and salinity.

Observations described below were made in accordance with these requirements. Oysters obtained from grounds near the laboratory varied from 9.6 to 10.3 cm. in height and from 6.5 to 7.0 cm. in length. They were fully adjusted to the salinity of the laboratory water. The temperature of the water in the respiratory chamber was kept between 24° and 25° C. but changes during each test did not exceed ±0.1° C. The salinity of water was kept constant at the concentration corresponding to the salinity of their natural environment. Tests were performed at Woods Hole and in Milford, Conn.[8]

Shell movements were recorded continuously during tests which lasted from 3.5 to 8.5 hours, depending on the behavior of the oyster. If the oysters remained closed for more than 30 minutes the test was discontinued, since it was reasonable to expect on the basis of previous experience that the period of closure would continue for several hours. Samples of water for oxygen determination were taken at half-hour intervals. For the study of seasonal changes of respiratory rates the oysters were marked by engraving a serial number on their left valves. Between tests they were kept in the harbor or in a large outdoor tank with circulating sea water. The data of oxygen uptake are expressed either as cu. ml. of oxygen (at 0° C. and 710 mm. barometric pressure) or as mg. of oxygen consumed per oyster per hour. (To convert the number of ml. into mg. of oxygen the first value should be multiplied by 1.4292.)

Oxygen uptake of animals is usually expressed per unit of their body weight. In the case of the oyster the use of the total weight may be misleading because of the great variations in the weight of metabolically inert shell material. It is, therefore, more sensible to refer to the oxygen uptake per unit of either wet or dry tissues. The use of dry weight gives more consistant results because in this way the variability caused by changes in the water content of the tissues is eliminated. The rate of oxygen uptake by a single oyster of known size is, however, of interest to ecologists who are concerned with the oxygen requirements of an entire oyster community. Furthermore, in a study of seasonal metabolic changes the experimental oysters could not be sacrificed at the end of each test. Their weight at the end of the entire series of observations would be meaningless because of the changes in solids. The data on seasonal variations in respiration are, therefore, given in the amounts of oxygen consumed by a single adult oyster in 1 hour.

The range of individual variations in the rate of oxygen uptake by adult oysters of approximately equal size is fairly large. Wide fluctuations frequently occur during a single test until the oyster reaches a steady state and remains open with a minimum of shell movement. Therefore, estimates of oxygen demand based on one or two readings made shortly after placing the mollusk in the respiratory chamber are meaningless. As the observations described below indicate, the study of the respiratory rates should be based on a series of readings continued for several hours and made at regular intervals.

Table 23 presents a summary of observations made on 11 adult Long Island Sound oysters, which prior to the tests were kept for at least 4 weeks in Woods Hole harbor and were adjusted to the salinity of the laboratory water. With a few exceptions the oxygen uptake of each oyster remained fairly constant during the test. The mean oxygen consumption per oyster per hour varied from 3.0 to 5.8 mg. The mean value for the entire group was 4.08 mg. of oxygen per hour per oyster. The group apparently divided into two classes of oysters, those with the low metabolic rate of 2.5 and 3.6 mg. of oxygen per

TABLE 23.—*Oxygen uptake in mg. per hour per oyster of 11 adult Massachusetts oysters during 3.5 hours at half hour intervals at the temperature of 24° to 25° C.*

[The tests were made at the Woods Hole laboratory in June before the beginning of spawning. Nos. 4, 8, and 11 are females, the others are male. Dimensions of oysters: height 9.6 to 10.3 cm.; length 6.5 to 7.0 cm.]

Hours after start	Oyster Number										
	1	2	3	4	5	6	7	8	9	10	11
0.5	4.5	1.8	2.9	5.1	3.9	1.9	6.3	4.8	3.6	3.7	4.8
1.0	5.4	2.8	2.9	5.0	4.2	2.5	5.7	4.7	3.3	3.8	4.8
1.5	4.3	3.0	2.9	5.2	-----	2.0	5.7	5.0	3.8	3.7	5.1
2.0	4.2	3.2	2.9	5.1	4.0	2.7	5.9	4.8	3.1	3.4	5.3
2.5	4.0	3.7	2.9	5.2	4.1	2.9	5.6	5.1	2.9	3.5	5.7
3.0	3.9	3.8	3.4	5.1	3.9	2.9	5.8	5.2	3.0	3.7	5.4
3.5	3.7	3.8	-----	5.0	3.9	2.8	-----	4.6	3.1	-----	4.9
Mean	4.29	3.16	3.0	5.13	4.01	2.54	5.84	4.89	3.26	3.64	5.17
Std. dev.	±0.56	0.72	0.25	0.07	0.11	0.42	0.26	0.24	0.33	0.16	35.0

Mean of means 4.08.
Standard deviation ± 0.337.

[8] I gratefully acknowledge the valuable cooperation of Walter A. Chipman in conducting for me a series of tests at Milford; and to two medical students, now doctors, John F. Reppun and George Mishtowt, who assisted me at Woods Hole.

hour, and those in which the mean oxygen consumption was higher varying from 4.0 to 5.8 mg. of oxygen per hour per oyster. These differences could not be associated with sex or sexual maturity. The three females of the group (Nos. 4, 8, and 11) had a high metabolic rate, but an even higher value was recorded for one of the males (No. 7). Subsequent tests showed that all 11 oysters were sexually mature and upon stimulation spawned copiously.

For comparison the metabolic rates of six oysters from Onset, Mass., were determined. The same technique was used but the duration of each observation was extended to 7.5 to 8.5 hours (table 24). The tests were completed during the last week of July and the first week of August. The mean oxygen uptake in these oysters varied from 2.99 to 4.24 mg. per hour per oyster, and the mean value for the entire group was 3.47. After the test was finished the oysters were examined and found to be partially spawned, which suggested that their lower metabolic rate possibly was associated with the loss of sex cells.

To clarify this point three tests were made with 11 adult New England oysters of approximately the same sizes as those of the Onset animals. After the first test, made early in July, the oysters were induced to spawn several times in the laboratory and were tested again 2 weeks and then 1 month after spawning. The data shown in table 25 are the mean values of six consecutive readings.

A significant decrease in the uptake of oxygen 1 month after spawning was found in all these oysters. The difference was less pronounced 2 weeks after spawning, possibly because of the incomplete discharge of sex cells. The rapid decrease in the metabolic rate is shown by plotting the rates of oxygen consumption as percentages of the initial rate observed before spawning (fig. 187). This inference that basal metabolic rate decreases after spawning is substantiated by data on seasonal changes in the composition of oyster meat (ch. XVII) which shows that the lowest

TABLE 24.—*Oxygen uptake in mg. of oxygen per hour per oyster of six adult oysters from Onset, Mass.*

[Tests made between July 12 and August 7 after the completion of spawning. Temperature 25.1° C.]

Hours after start	Oyster Number					
	1[1]	2	3	4[2]	5	6
0.5	4.2	5.4	3.5	4.6	4.2	3.6
1.0	2.2	3.4	3.9	4.3	3.6	2.0
1.5	2.5	3.8	5.7	4.3	3.6	4.8
2.0	1.3	3.6	3.8	4.2	3.4	1.0
2.5	3.2	3.6	6.2	4.2	3.5	2.0
3.0	3.5	3.7	4.5	3.8	3.2	3.1
3.5	3.5	3.9	3.8	4.1	3.9	3.1
4.0	3.8	3.3	3.8	4.0	3.4	3.0
4.5	4.6	3.0	3.9	4.0	3.4	3.4
5.0	3.4	3.5	3.6	2.6	2.1	4.4
5.5	3.4	3.7	4.2	1.5	1.4	3.4
6.0	3.6	3.7	3.8	1.8	1.7	3.4
6.5	3.3	4.0	3.8	1.4	1.3	3.4
7.0	3.8	3.4	4.3	-------	-------	3.0
7.5	3.3	-------	4.2	-------	-------	3.4
8.0	-------	-------	4.0	-------	-------	3.1
8.5	-------	-------	-------	-------	-------	3.1
Mean	3.31	3.71	4.24	3.45	2.99	3.12
Standard deviation±	0.80	0.67	0.76	1.33	0.98	0.75

NOTE: Mean of all observations 3.47±0.48 mg. oxygen per oyster per hour.

[1] Partially closed from 1.5 to 2.0 hours; fully open at 2.5 hours.
[2] Oyster partially closed from 5.0 to 6.5 hours.

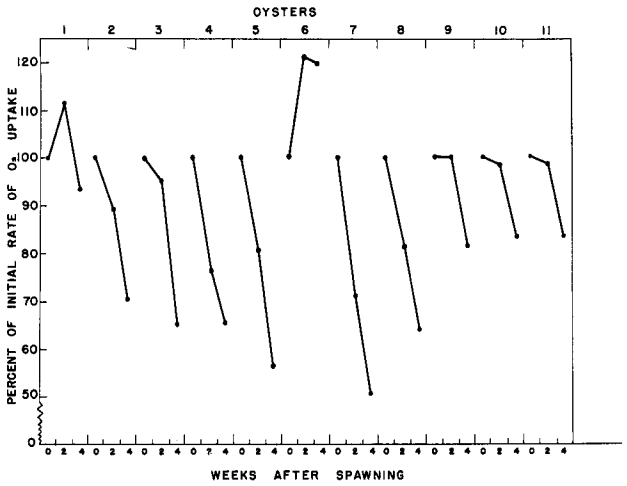

FIGURE 187.—Decrease in the rates of oxygen consumption in 11 adult *C. virginica* from Long Island Sound after spawning. The changes are plotted as percentages of the initial oxygen uptake before spawning.

TABLE 25.—*Mean oxygen uptake, in mg. per hour, per oyster, determined early in July before spawning, at 2 weeks and then 1 month after spawning*

The figures are the mean values of each test and were computed from six consecutive readings made at half hour intervals at temperature 24.0 to 25.0° C.

Time of Observation	Oyster Number												Standard deviation
	1	2	3	4	5	6	7	8	9	10	11	Mean	
Before spawning (July)	6.1	4.8	4.3	7.3	5.7	3.6	8.3	7.0	4.4	5.2	7.4	5.8	±1.51
Two weeks after spawning (August)	6.8	4.3	4.1	5.6	4.6	4.4	5.9	5.7	4.4	4.0	7.3	5.2	±1.14
One month after spawning (August)	5.7	3.4	2.8	4.8	3.2	4.3	4.2	4.5	3.6	3.2	6.2	4.2	±1.08

solid content of oysters occurs shortly after the spawning season. It will be shown later (ch. XIV) that the gonads of sexually mature oysters may constitute as much as 40 percent of the body weight and volume, exclusive of the shell. The loss of a considerable portion of gonad tissues may account for the lower oxygen uptake.

The amount of oxygen consumed by an organism during a unit of time depends on its weight. In the oyster this relationship is obscured by wide fluctuations in the proportion of solids to water. In a series of tests made during the second half of August at Woods Hole oysters of different weights were selected from an oyster bottom at Onset at the head of Buzzards Bay, Mass. The total weight of individual oysters varied from 80 to 203 g. and the wet weight of their tissues ranged from 11.35 to 23.25 g. The oysters had already spawned but still retained a substantial amount of sex cells, with the exception of one oyster in which the gonad was empty and its sex was not recognizable. The data given in table 26 are the mean values of oxygen uptake computed for each oyster from 10 consecutive readings made at half-hour intervals. The rate of oxygen consumption per oyster per hour varied from 3.97 to 7.29 mg. of oxygen. The oxygen consumption of the three heaviest oysters (Nos. 1, 2, and 3) was higher than for the others, but there are no significant differences in oxygen uptake per unit of dry weight according to oyster size (fig. 188). The oxygen demand expressed in this way varied between 2 and 3 mg. per hour.

In the majority of tests the initial oxygen consumption measured within the first hour after placing the animal in the respiration chamber was noticeably higher than in the successive samples. This phenomenon recorded for five

TABLE 26.—*Oxygen uptake per hour of adult* C. virginica *from Onset, Mass.*

[End of August. Salinity of water 31.2 to 31.3°/oo. Temperature 24.0° to 25.0° C.]

Oyster	Sex	Wet weight	Dry weight	Solids	Oxygen uptake	
					Per oyster	Per 1 g. dry tissue
		G.	*G.*	*Percent*	*Mg./hr.*	*Mg./hr.*
1	?	23.25	2.65	11.4	5.88	2.22
2	M	21.81	3.19	14.6	6.76	2.12
3	F	21.07	2.63	12.5	7.29	2.77
4	M	18.34	2.57	14.0	4.59	1.79
5	M	16.04	2.36	14.7	4.06	1.72
6	M	14.52	1.86	12.8	4.40	2.37
7	M	14.38	2.01	14.0	5.56	2.77
8	M	12.06	2.00	16.5	4.32	2.16
9	M	11.48	1.73	15.1	3.97	2.29
10	M	11.35	1.68	14.8	4.43	2.63

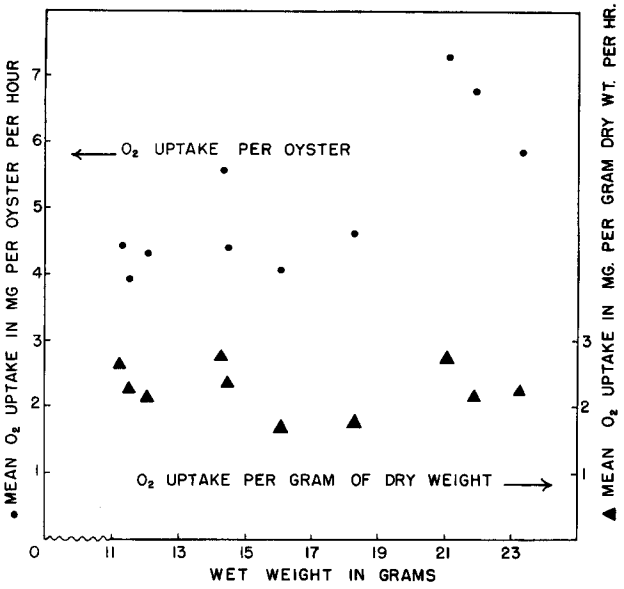

FIGURE 188.—Mean uptake of oxygen expressed in mg. per oyster (circles) and mg. per 1 g. of dry weight per hour (triangles) in relation to the wet weight of tissues. Each item is a mean value of 6 to 10 consecutive determinations of oxygen consumption of a single oyster. Temperature 24.5° C.

out of the total of six oysters (table 24), represents the effect of an oxygen debt incurred during the time the oysters were closed while being prepared for the test. The rate of oxygen uptake usually reaches a more or less stable level after a variable period of adjustment to the new situation. In figure 189 the rate of metabolism recorded continuously for 8.5 hours is plotted against time. Partial closure of the valves was accompanied by a decrease in oxygen consumption (line B). Both oysters A and B reached a steady level of oxygen uptake after the initial periods of adjustment, which in the case of oyster A required 4 hours.

The rate of oxygen uptake decreases with a partial closing of the valves, presumably because of the decrease in the rate of water transport. In the test summarized in figure 190 the two oysters A and B remained in a steady state for nearly 4½ hours. One of them (oyster A, solid line) then began to reduce the opening between the valves and completely closed them at 7½ hours. The decline in oxygen consumption corresponded to the shell movement and registered zero at the moment the shell was closed. No measurable changes in oxygen content in the water were noted after the valves remained tightly closed for some time.

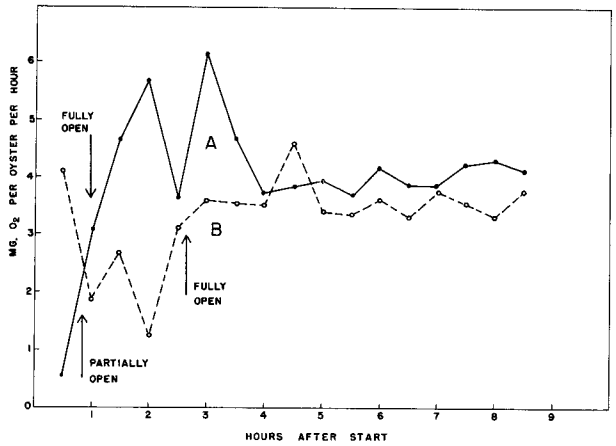

FIGURE 189.—Oxygen uptake, in mg. of oxygen per oyster, per hour, measured at half-hour intervals. Temperature 24.5° C.; salinity of water 31.9°/oo. Oyster A (solid line): wet weight of tissues 16.1 g.; dry weight 2.5 g. Oyster B (broken line): wet weight of tissues 12.5 g.; dry weight 1.4 g. Before the tests were made these 6-year-old oysters from Long Island Sound were kept for 35 days in Woods Hole Harbor. August.

Oxygen Uptake Under Continuous Pull of the Adductor Muscle

Increased shell movements of oysters are associated with an increased oxygen uptake. This has been reported by Galtsoff and Whipple (1931), who found that oxygen consumption by the oysters which made only five or less shell closures per hour was about 20 percent lower than that of oysters which closed and opened their valves 30 times or more per hour. The effect on the metabolic rate of the oyster of the resistance of the adductor muscle to a continuous pull and of the maintenance of a forced muscle tonus has been studied for large New England oysters using the equipment designed to determine the power of the adductor muscle (see: p. 176, ch. VIII). The entire platform upon which the oyster was mounted was lowered into a tank of 3 l. capacity filled with filtered sea water and covered by a layer of light mineral oil about 0.5 inch thick. The water was stirred gently, and the temperature was kept constant at 24°±0.3° C. Samples of water, of 100-ml. capacity, withdrawn from the tank were immediately replaced by an equivalent volume of filtered sea water of known oxygen tension. An accurate record was kept of the total volume of water in the tank, and its oxygen content was recomputed after every addition. During the tests, which lasted from 3 to 6 hours, sampling was made every 30 minutes. The initial oxygen tension varied in different experiments from 4 to 5 ml. per l. As the muscle was stretched by the pulling force of 4 kg. (fig. 191), the oxygen uptake, which ordinarily was 3.7 to 5.0 mg. per hour per oyster, decreased to 2.2 mg. Similar results were obtained in several tests in which the pulling force varied from 2 to 4 kg. per oyster. In all cases the adductor muscle was stretched but continued to maintain tonus at a new level. In many instances the up and down shell movements persisted but on a noticeably reduced scale. In the majority of cases the oxygen uptake decreased markedly to almost zero as the shell movements were reduced. During the test shown in figure 192 the shell movements were limited and their character did not change after the pulling force of 2 kg. was applied. The new tonus level was accompanied by an immediate decrease in the uptake of oxygen to about one-half its preceding rate. In 2 hours the consumption of oxygen almost stopped.

In several instances a sudden increase in oxygen consumption was observed when the oyster was in the respiratory chamber despite a pulling force of 2 kg. (fig. 193). Examination of the water revealed the presence of eggs or sperm released from the gonad. The metabolic rate of the sex cells contained in the gonads was suddenly increased as soon as the cells were free in water. Within the gonad tubules the sex cells are tightly packed and

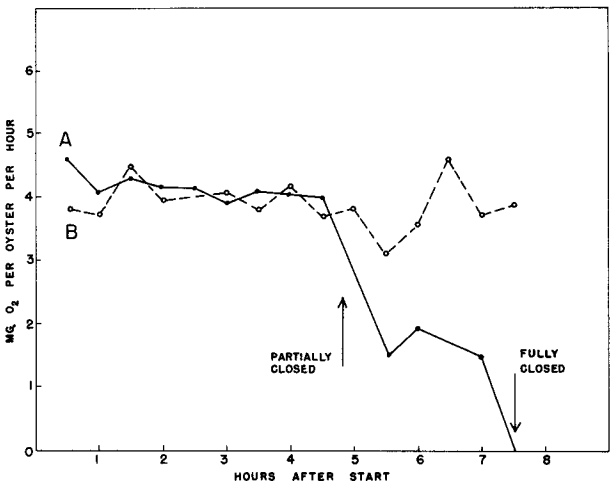

FIGURE 190.—Oxygen uptake, in mg. of oxygen per oyster per hour, measured at half hour intervals. Temperature 24.5° C.; salinity of water 31.8°/oo. Oyster A (solid line): wet weight of tissues 15.2 g.; dry weight 2.1 g. Oyster B (broken line): wet weight of tissues 12.8 g.; dry weight 1.5 g. Before the test these 6-year-old Long Island Sound oysters were kept for 30 days in Woods Hole Harbor. August.

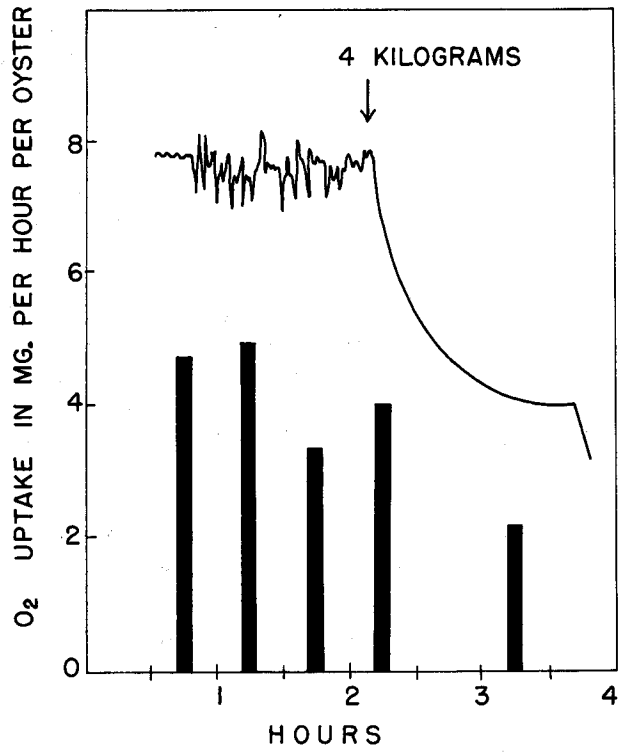

FIGURE 191.—Shell movements (upper line) and oxygen uptake (vertical bars) of an adult *C. virginica* before and during the application of a pulling force of 4 kg. Sharp inflection of the curve at about 3¾ hours corresponds to the point of tearing off the muscle. July experiment. Temperature 23.7° C.

their surface is not in direct contact with sea water. The high oxygen uptake after they are shed into the water is probably due to the greatly increased free surface areas of these cells.

In these observations the forcible stretching of the adductor muscle was always associated with a decrease and eventual cessation of ciliary current. Under the conditions of the tests the velocity of the cloacal current could not be measured, but decrease in current velocity was observed by the movements of particles suspended in the water or by the slanted position of the fecal ribbons, which in actively feeding oysters are horizontal to the axis of the cloacal current.

Two inferences can be deduced from these observations: First, the uptake of oxygen is dependent primarily upon the rate of water transport by the gills; and second, in maintaining a tonus level the locking mechanism of the adductor muscle is not dependent on the uptake of oxygen from surrounding water.

These observed rates of oxygen uptake are considerably greater than those found by Jørgensen

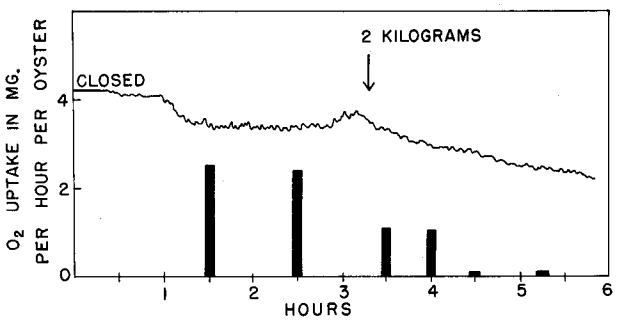

FIGURE 192.—Shell movements and oxygen uptake of an adult *C. virginica* before and after the application of a pulling force of 2 kg. Oxygen uptake in mg. per oyster per hour is shown by vertical columns. July. Temperature 22.4° C.

(1952) for adult *C. virginica* of Woods Hole. The oysters used in his experiments were supplied from the Bureau's shellfish laboratory and were approximately of the same size as those which I tested in the preceding summers. The rate of oxygen uptake determined by Jørgensen was less than 1 ml. per hour (1.5 mg. of oxygen per oyster).

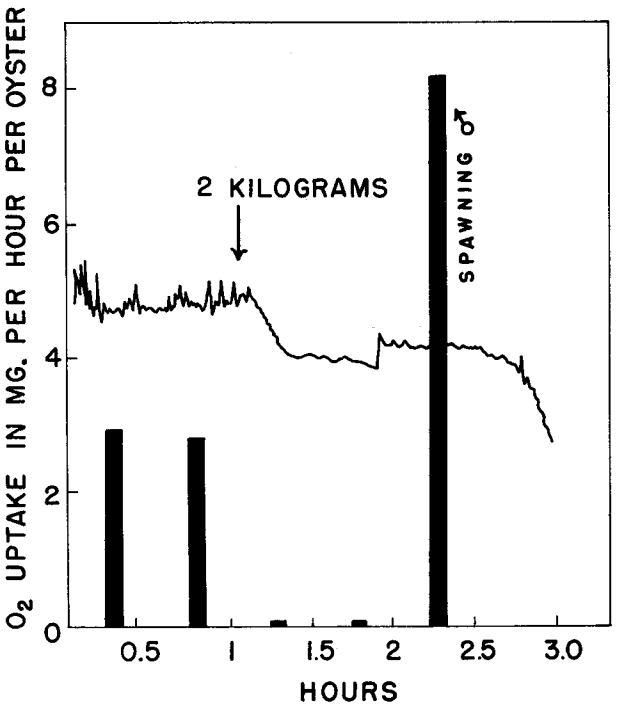

FIGURE 193.—Oxygen uptake of an adult oyster under normal conditions and after the application of a pulling force of 2 kg. Sudden increase of oxygen consumption at 2¼ hours is due to the discharged sperm. Upper line indicates shell movement. July. Temperature 24.8° C.

Unfortunately, Jørgensen does not mention the dimensions, weight, or conditions of his oysters, and does not describe the details of his technique. He states, however, (1952, p. 362) that "the oyster, *Ostrea virginica*, and the ascidians *Ciona intestinalis* and *Molgula manhattensis* filter about 10 to 20 l. of water for each ml. of oxygen consumed."

The low oxygen uptake reported by Jørgensen may have been due to the experimental conditions and particularly to the presence in the water of graphite particles used by him in determining the rate of water filtration.

Environmental effects

Seasonal changes in the rate of oxygen uptake.—After spawning the New England oysters pass through a period of lowered activity and tend to keep their valves closed, sometimes as long as 2 to 3 days; when they open again the rate of water transport is lower than it had been before the start of the reproductive period. Through the cold season of the year, from October to April, the oxygen uptake remains at a low level. In order to obtain comparable results and to eliminate the effect of temperature, tests of metabolic rates were made at 25° C., using oysters that were kept outdoors and brought into the laboratory for 3 to 4 days before testing to adjust gradually to the higher temperature. Examination of the data summarized in table 27 shows that the period of lowered metabolic activities occurred primarily during the winter.

Effect of change in salinity and pH.—No significant change in the respiratory rate was noticed in water of lowered salinity to which the oyster had become adjusted. In these tests the metabolic rate was first measured in water of 31.6 °/₀₀ salinity. After the first test, which lasted 6 hours, the oyster was transferred for 3 days into running sea water diluted with fresh water to the salinity of 24.1 °/₀₀ (approximately 76 percent of the previous concentration of salts).

TABLE 27.—*Seasonal changes in the oxygen uptake in mg. of oxygen per oyster per hour of adult Long Island oysters about 10 cm. long and 7 cm. wide*

Oyster No.	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July
50	5.0	4.2	----	----	----	2.6	----	2.6	2.3	----	----	2.0	2.7
51	3.8	----	----	----	----	----	----	2.9	----	----	----	2.1	----
52	3.5	1.5	----	1.8	----	----	1.9	2.4	1.6	----	----	1.7	----
53	4.4	----	----	----	----	----	----	----	3.6	----	----	----	----
55	5.2	----	----	3.0	----	----	3.6	----	4.0	----	2.7	4.7	4.3
56	----	4.1	----	----	2.4	3.1	----	----	2.9	----	----	4.4	2.9
62	4.7	----	----	2.6	----	----	2.0	----	3.1	----	----	5.4	4.3

The rate of respiration was measured on the 4th day under standard conditions. The results of the test (figure 194) show that the rate of oxygen uptake in the water of lower salinity was not significantly different from that observed in 31.18 °/₀₀ salinity. The tests were repeated several times with different oysters with identical results. In all experiments the oysters were left in water of lower salinity for at least 3 days to adjust to the new conditions. The effects of greater dilution of sea water have not been studied because of the technical difficulty in providing sufficient food to the oysters during the prolonged periods of adjustment.

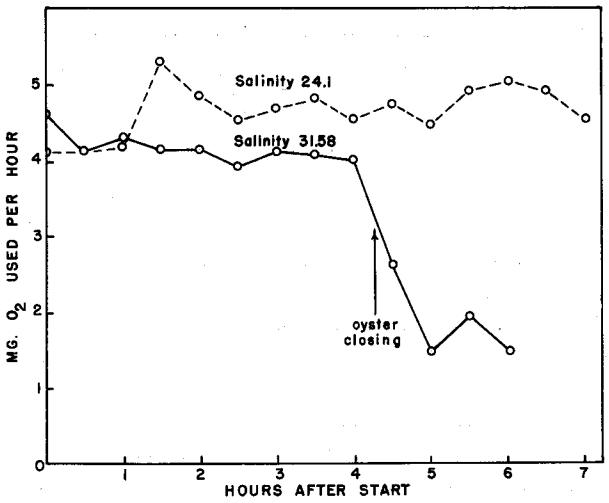

FIGURE 194.—Oxygen uptake of an adult Long Island oyster recorded at normal salinity (solid line) of Woods Hole (31.58 °/₀₀) and 4 days later at lowered salinity (broken line) of 24.1 °/₀₀. All tests were made in August under standard experimental conditions at the temperature of 25.0° C.

The pH has a very pronounced effect on the rate of oxygen uptake. The water used in metabolism tests was acidified by adding a quantity of 0.1 N hydrochloric acid. After six or eight readings with normal sea water, the acidified water was turned on and the testing continued for another 3 to 4 hours. The curve in figure 195 summarizes the results of all 10 tests performed in July to September using Long Island and Massachusetts oysters. At pH 6.5 the oxygen uptake drops to about 50 percent of the normal rate and rapidly decreases to less than 10 percent at pH 5.5. At pH 5.8 the oxygen uptake may continue for several hours at a greatly reduced rate (fig. 196).

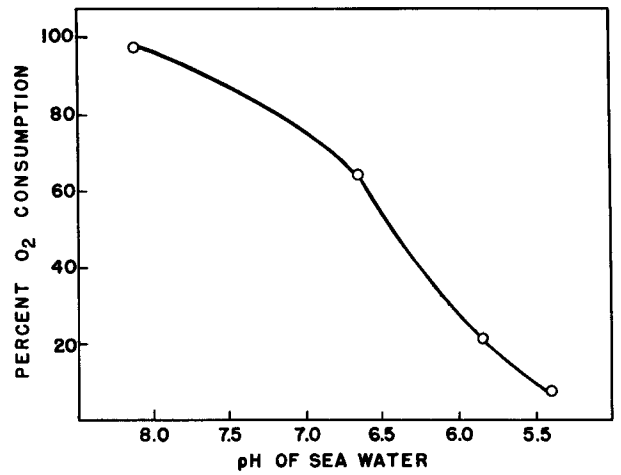

FIGURE 195.—Effect of lowered pH on the rate of oxygen uptake of adult oysters. Summary of summer experiments at Woods Hole at 25° C.

RESPIRATORY QUOTIENT, R.Q.

The respiratory quotient (R.Q.) is the ratio of the volume of carbon dioxide produced to the volume of oxygen consumed at the same time. Definite values of R.Q. have been recognized for the principal types of food (Vernon, 1895; Richardson, 1929; Krogh, 1941). The R.Q. for carbohydrate is 1.00; for protein about 0.79; and for fat 0.71. On an average mixed diet the R.Q. of man is about 0.80 to 0.85. The herbivorous animals tend to have a higher R.Q., while the carnivorous have a lower one.

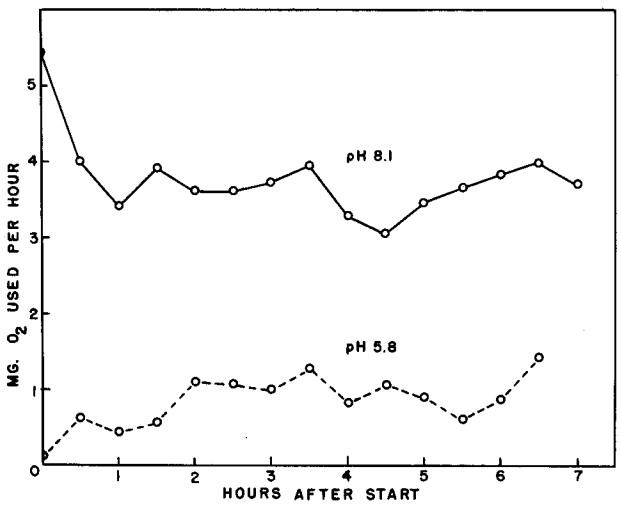

FIGURE 196.—Oxygen uptake of two oysters of approximately equal size (about 6 cm. in height) at pH 8.1 and 5.8. Salinity 31.6 °/₀₀. Measured for 6½ consecutive hours. Temperature 24.5° C.

It is of interest to find out whether the R.Q. of the oyster, which is an herbivorous mollusk, changes after the breeding season when it begins to accumulate and store glycogen. Orton (1927a, 1927b) suggested, without presenting supporting evidence, that during the reproductive cycle of *O. edulis* there is a shift from predominantly protein to carbohydrate metabolism and that this shift is correlated with the completion of the male sexual phase.

In conducting observations on carbon dioxide production it is necessary to keep in mind that deposits of calcium carbonate in the bodies of some marine invertebrates may suddenly release large quantities of this gas which would give a false R.Q. (Bosworth, O'Brien, and Amberson, 1936). For instance, the reported R.Q. 1.39 of lobster was found to be false since in lobsters coated with collodion the R.Q. was only 0.92. The shell of the bivalves is the principal storage place of carbonates which act as buffers when the valves are closed. In metabolism studies this possible source of error should be eliminated by coating the shells with paraffin.

The values of respiratory quotients vary during the conversion of food substances within the organism. Fattening of livestock and birds by forced carbohydrate feeding is usually accompanied by high R.Q., while the utilization of fats and proteins and their possible conversion to carbohydrates lowers the R.Q. values. It is, therefore, possible to expect that seasonal variations in the R.Q. of the oyster would give a clue to changes in the utilization of its food.

A Van Slyke constant volume apparatus for gas analysis was used in a series of observations of a group of marked oysters kept in live boxes in the harbor. No definite trend in the changes of R.Q. could be detected. It varied throughout the year from 0.51 to 1.44 as may be seen in the summary of observations (table 28) made under standard conditions in water containing no plankton. Addition of water soluble food in the form of dextrose resulted in an increase in R.Q. The latter determinations (table 29) were made in filtered water to which dextrose was added. There was no increase in R.Q. in water containing 0.0025 percent dextrose, but in 0.005 and 0.01 percent the R.Q. values were significantly higher.

RESPIRATION IN OTHER SPECIES OF OYSTERS

Comparison between the results described for *C. virginica* and the data published by others for

TABLE 28.—*R.Q. of* C. virginica *through the year*

[Long Island Sound oysters]

Oyster No.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
M-1	-----	0.97	1.18	-----	-----	-----	-----	-----	-----	-----	-----	0.81
M-1	-----	0.84	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
M-1	-----	0.97	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
M-2	-----	-----	-----	-----	-----	-----	1.00	-----	-----	-----	-----	-----
M-2	-----	-----	-----	-----	-----	-----	0.83	-----	-----	-----	-----	-----
M-3	-----	-----	-----	-----	-----	-----	0.89	-----	-----	-----	-----	-----
M-4	0.92	-----	0.97	-----	1.06	-----	-----	-----	-----	-----	-----	-----
M-6	0.79	-----	-----	-----	0.79	-----	0.84	-----	-----	-----	-----	-----
M-6	-----	-----	-----	-----	-----	-----	0.69	-----	-----	-----	-----	-----
M-6	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	1.19	-----
M-7	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.57
M-9	-----	0.51	-----	0.57	0.82	-----	-----	-----	-----	-----	-----	0.50
M-9	-----	0.71	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
M-10	0.51	-----	-----	0.71	-----	-----	-----	-----	-----	-----	-----	-----
R-50	-----	-----	0.72	-----	-----	-----	-----	-----	-----	-----	-----	-----
R-50	-----	-----	0.72	-----	-----	-----	-----	-----	-----	-----	-----	-----
R-51	-----	-----	-----	-----	-----	0.55	-----	-----	-----	-----	-----	-----
R-52	0.61	-----	0.80	-----	-----	0.75	-----	-----	-----	1.13	-----	-----
R-52	-----	-----	-----	-----	-----	-----	-----	-----	-----	0.97	-----	-----
R-55	0.66	-----	0.86	-----	0.92	0.74	-----	-----	-----	-----	0.89	-----
R-56	-----	-----	1.35	-----	-----	0.84	-----	-----	-----	-----	0.64	-----
R-59	-----	-----	0.54	-----	-----	-----	-----	-----	-----	-----	-----	-----
R-59	-----	-----	0.44	-----	-----	-----	-----	-----	-----	-----	-----	-----
R-62	1.44	-----	1.41	-----	-----	0.82	-----	-----	-----	-----	0.86	-----

other Ostreidae is difficult because of the different conditions under which the metabolism tests were made. In a discussion of the relation between the metabolism and temperature and its zoogeographical significance Spärck (1936) makes the statement that, "*O. edulis* consumes more oxygen than *Gryphaea* (*Crassostrea*) *angulata* of which it is shown that it is able to supplant *O. edulis* in several localities." This conclusion based on a few single determinations is not well substantiated.

Pedersen (1947) studied the respiration of *O. edulis* living in the small salt-water ponds along the Skagerrak coast in Norway. The summer temperature in these ponds rises to 25° C. and higher, while in winter ice covers the ponds for about 5 months. Prior to making the test Pedersen kept the oysters for a few days in filtered sea water, brushed them, washed the shells with 40 percent alcohol, and wrapped them in pieces of gauze to prevent small bits of shell from being broken off. For measuring the oxygen uptake the oysters were placed in hermetically closed glass containers filled with 2 l. of unfiltered sea water. The containers had to be turned over in order to mix the water. Closing and opening of shells were not recorded. Undoubtedly the turning of vessels caused the oysters to close their shells and discontinue the ventilation of the gills. For control Pedersen used blanks that contained no oysters. The difference between the blanks and the samples taken from the experimental containers was considered equal to the quantities of oxygen consumed and carbon dioxide produced by the oysters. The consumption of oxygen was expressed in mg. of oxygen per 100 g. of total weight or per 10 g. of net weight (presumably the wet weight of the meat) per 24 hours. Under these conditions and at temperatures around 24° to 25° C. the oxygen consumption of the oysters varied from 15.0 to 48.9 mg. of oxygen per 10 g. of weight per 24 hours or from 0.62 to 2.0 mg. per 1 hour. Pedersen's technique had serious drawbacks since the time the oysters were open is not known; the mollusks were disturbed by violent motions (turning over of the containers); and the animals probably were affected by accumulation of metabolites. The reported R.Q. of the Norwegian oysters varied from 0.8 to 1.0, but in some instances it was as low as 0.6 or as high as 2.6 and 3.0. The abnormally high and low values are probably fictitious because of some deficiency in technique.

TABLE 29.—*Effect of dextrose in sea water on R.Q. of* C. virginica

Date	R.Q. values in—		Percentages of dextrose
	Filtered sea water	Filtered sea water + dextrose	
Nov. 14	0.87	0.85	0.0025
Dec. 2	.57	1.56	.005
Dec. 10	.82	1.56	.01
Jan. 15	.92	1.38	.01

In a study of the energy-metabolism of *O. edulis* Gaarder and Eliassen (1955) used a closed chamber system (desiccators) of 750-ml. capacity. The shells of the oysters were kept open by glass rods inserted between the valves. The salinity of water was 32 ‰, and the temperature was kept constant within ±0.05° C. The results were expressed in ml. of oxygen consumed per 1 g. of wet weight per hour. To facilitate the comparison I have recomputed the data of the Norwegian investigators to mg. of oxygen per 10 g. of wet weight of oyster tissues. At 25° C. the oxygen consumption by *O. edulis* computed on this basis was about 2.0 to 2.5 mg. of oxygen per hour per oyster.

As one may expect from observations on the effect of temperature on ciliary motion of the gill epithelium of the oyster, the oxygen consumption increases about 1.5 times for every 10° rise (Q_{10}) of temperature between 10° and 25° C. The maximum is reached at about 25° C. Below 5° C. the oxygen uptake decreases rapidly but

measurable values were recorded by Gaarder and Eliassen even at temperatures approaching 0° C. It may be assumed that under normal conditions the valves would be closed at this low temperature and ventilation of the gill stopped.

Another experiment by Gaarder dealt with the effect of oxygen tension on oxygen consumption. The "critical oxygen tension" at which a decrease in the oyster oxygen consumption becomes apparent was found to be 4 ml. of oxygen per 1. (at 22° C.). If the figure is correct, it would indicate that *O. edulis* has a higher "critical point" than the one reported for *C. virginica* in which the rate of oxygen consumption begins to diminish when the oxygen tension is reduced to 2.5 $cm.^3$ per 1. or lower (Galtsoff and Whipple, 1931). Gaarder and Eliassen disagree with Pedersen's (1947) conclusion that *O. edulis* can live for quite a while in water poor in oxygen. They think this species shows a rather high "critical point" of oxygen tension. In both *O. edulis* and *C. virginica* the uptake of oxygen is independent of oxygen tension above the respective critical points.

O. circumpicta, observed in a closed chamber system of about 8-l. capacity containing a thick layer of liquid paraffin, was found by Nozawa (1929) to consume oxygen at the rate of about 3.2 ml. of oxygen per hour per 10 g. of wet tissues. This rate is computed from Nozawa's published data with an assumption that his figures of oxygen uptake represent the $cm.^3$ of oxygen. The R.Q. values of this species gradually increased during the 22 hours of observations from 0.85 to 2.8. The validity of the latter figure is questionable and is probably due to accumulation of metabolites. Nozawa claims that oxygen consumption of *O. circumpicta* is independent of oxygen tension until the latter is reduced to 0.1 percent of its normal content in water. The figure appears to be too low to be accepted without further verification.

UTILIZATION OF OXYGEN

Bivalves use only a small portion of the oxygen dissolved in the water which they transport through the gills. The percentage of oxygen consumed is the measure of the intensity of utilization of oxygen. In most cases less than 10 percent of the oxygen available is removed from the water (Hazelhoff, 1938). In comparison to gastropods and cephalopods, which utilize up to 80 percent of the available oxygen, the oxygen demand by bivalves is very low. The actual figures of utilizaton vary depending on the conditions of the mollusks. In *Mya arenaria* and in fresh-water *Anodonta* the normal utilization ranges from 2 to 10 percent (Van Dam, 1938). The low rate of utilization is due to the rapid transport of water which both mollusks have to maintain in order to obtain a sufficient supply of food. Van Dam reports (1937, 1938) that in many cases when the respiratory current was slowed down or when before making the test the mollusks were left in the air for 20 hours, as much as 97 percent of the oxygen was utilized.

The rate of oxygen consumption is usually higher after a period of interruption of respiratory current or after exposure of mollusks to air. This compensation by oysters for an oxygen debt has also been observed in *Mya arenaria*, and in *Anodonta cygnea* (Koch and Hers, 1943). The authors maintain that the rate of ventilation of the gills of *Anodonta* is regulated both by the need of the animal for oxygen and by the availability of oxygen in water. Oxygen determination in their experiments was made by means of a polarograph. By this method is was possible to record photographically the continuous changes in the oxygen content of water of the exhalant current. The inference the authors draw from their observations is that the regulation of the branchial current in *Anodonta* by contraction of the exhalant siphon has relation to the intensity of metabolic processes. They found that the periods of closure of siphons are longer in water rich in oxygen and become shorter when the oxygen content is low. The technique of dropping mercury electrodes (Petering and Daniels, 1938; Føyn, 1955; Brezina and Zuman, 1958) appears to be promising and should be applied in further study of respiration in mollusks.

The coefficient of oxygen utilization in the oyster (percent of oxygen removed from the water as it passes through the gills) has not been determined. The data of the metabolism tests cannot be used for this purpose because the actual rate of water transport cannot be measured in an oyster kept in the respiratory chamber. The flow of water through the chamber was maintained at a rate lower than the expected rate of water transport through the gills and, consequently, it is reasonable to expect that the water in the chamber passed through the gills several times before it reached the outlet.

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