# OXYGEN CONSUMPTION OF NORMAL AND GREEN OYSTERS[1]

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## INTRODUCTION

The knowledge of the oxygen requirements of any bottom organism is essential in understanding the factors that control its distribution, growth, and adaptability to various local conditions. The purpose of the present investigation is to determine the rate of oxygen consumption of normal and green oysters and to evaluate some of the factors that govern the respiration of the lamellibranch mollusks.

The respiratory exchange of the oyster has been studied by Mitchell (1914), who found that at temperatures between 19° and 28° C. oysters of medium sizes used from 0.7 to 3.5 milligrams of oxygen per hour per 100 grams of entire weight (from 0.35 to 1.29 milligrams per hour per 1 gram of dried weight), and that they showed considerable resistance to lack of oxygen. He noticed also that the oxygen consumption was exceedingly variable, depending on a variety of conditions which, he thought, affected the opening and closing of the shell. No attempts were made, however, to eliminate or control these variables.

Nozawa (1929) in a study of normal and abnormal respiration of *Ostrea circumpicta* arrived at the conclusions that "the rate of the oxygen consumption is independent of the oxygen tension till its pressure is reduced to 0.1 per cent or below"; and that under abnormal conditions (pulling of the adductor muscle by a 5-kilogram weight attached to one side of a horizontally fixed shell) the gaseous metabolism of the oyster is accelerated.

There are several possible sources of error that may have an effect on the results obtained by Mitchell and Nozawa. The natural sea water used in their experiments may have contained organic matter that either consumed or liberated oxygen. In Nozawa's experiments the water in the experimental jars was not stirred and its oxygen content was probably not uniform. In Mitchell's experiments part of the oxygen was either directly absorbed by the substance of the shells or used up by

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various organisms growing on them. In neither experiment was the effect of muscular activity eliminated.

In the present investigation attempt was made to eliminate or control all these variables and to carry out experiments under standard conditions. The experiments were carried out at the United States Bureau of Fisheries laboratory at Woods Hole, Mass. Normal oysters used in the experiments were received from Onset Bay, Mass. Green oysters were obtained from the commercial beds near New Haven Harbor in Long Island Sound. All the oysters before being used in the experiments were kept for at least two weeks in floats anchored in Woods Hole Harbor.

## METHOD

The method of study of the oxygen consumption of nonambulatory organisms like the oyster is very simple. The oxygen content of the water is determined under standard conditions before the animal is put in and at given intervals thereafter; the difference, representing the amount of oxygen consumed, is calculated per unit of time and per unit of weight. In practice, however, there are a number of interfering factors (organic matter in the water, organisms grown on shells, muscular activity, etc.) that must be controlled.

Preliminary tests have shown that the sea water from the laboratory supply used in the experiment consumed appreciable quantities of oxygen. After it was passed through a ½-inch asbestos filter its oxygen content, under the conditions of the experiments, remained constant over a period of 9 hours. (Table 1.)

TABLE 1.—*Control experiment with filtered sea water. Closed chamber method*

| Time, hours | Oxygen, c. c. per liter | Time, hours | Oxygen, c. c. per liter |
|---|---|---|---|
| 0 | 4.56 | 5 | 4.59 |
| 1 | 4.62 | 6 | 4.58 |
| 2 | 4.58 | 7 | 4.62 |
| 3 | 4.60 | 8 | 4.57 |
| 4 | 4.65 | 9 | 4.60 |

The shells of the oysters were thoroughly scrubbed with a wire brush and then covered with paraffin. This treatment covered any remaining organisms and prevented the absorption of dissolved gases by the porous substance of the shell. A control experiment (Table 2) shows that during the period of 5 hours the oxygen content of the water, in which the shells treated in this way were kept, remained nearly constant.

TABLE 2.—*Control experiment with treated shells. Closed chamber method*

| Time, hours | $O_2$ content, c. c. per liter | Time, hours | $O_2$ content, c. c. per liter |
|---|---|---|---|
| 0 | 4.30 | 3 | 4.30 |
| 1 | 4.36 | 4 | 4.28 |
| 2 | 4.34 | 5 | 4.28 |

The control of the muscular activity presented a more difficult problem, which we attempted to meet in two different ways. At first the shells of the oysters were gently forced apart, great care being taken not to injure the mantle nor tear the

adductor muscle, and small glass rods were inserted to hold the shells open. It was found, however, that frequently the glass rods were expelled. This indicated that the presence of a foreign body stimulated the contractions of the adductor muscle which in turn affected the amount of oxygen consumed. To obviate this difficulty another method was devised, whereby determinations were made on single oysters, mounted as described below, so that the opening and closing of the shell was recorded on a kymograph.

Two methods were used for the determination of oxygen consumption. In the first method, which in the following discussion is referred to as a closed chamber method, six oysters, the shells of which were kept apart by glass rods, were placed in apparatus similar to that used by Bruce (1926). (Fig. 1.) It consisted of a 14-inch diameter heavy glass desiccator with ground glass flanges held together with clamps and a rubber stopper on the top of the dome-shaped lid. Three glass tubes were arranged in the stopper; one, extending to 1 inch above the bottom, formed a syphon outflow; the second one ended just below the stopper and served for replenishing the water when a sample was being taken; through the third one, filled with paraffin oil, passed the stem of the stirrer. By simultaneously regulating the outflow and the inflow of the water, a sample was withdrawn and the water in the chamber replenished from a reservoir containing filtered sea water of a known oxygen content. The chamber was kept in a constant temperature bath.

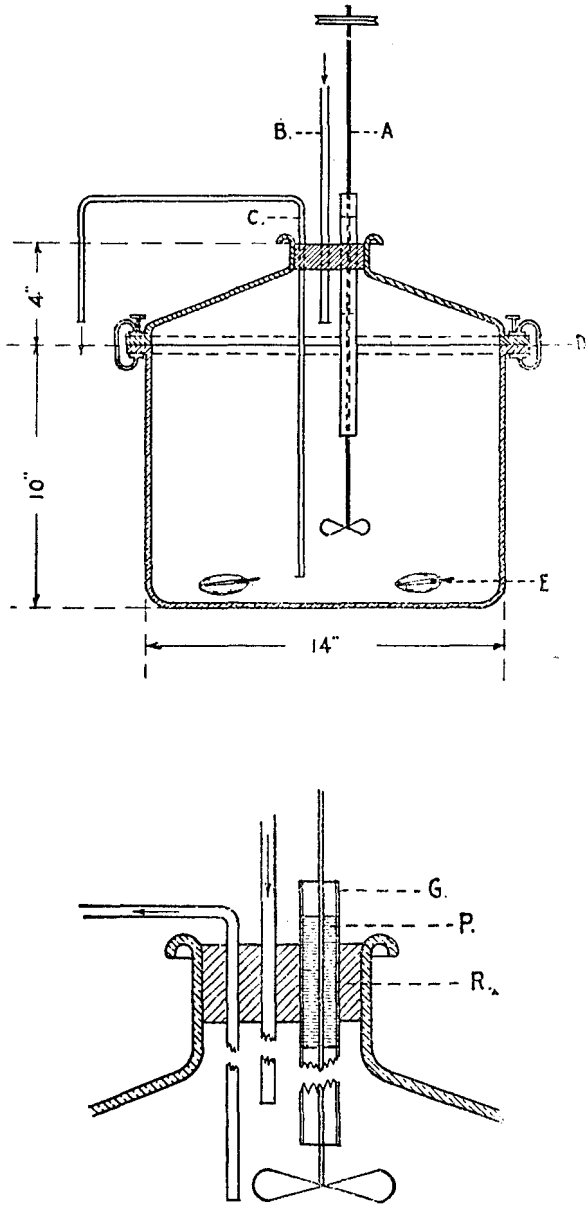


FIGURE 1.—Closed chamber for the determination of oxygen consumption. A, Glass stirrer; B, inlet; C, outlet; D, ground glass flanges on cover and base held together with clamps; E, oysters held open by means of small rods inserted between the valves; G, glass tubing in which stirrer rotates, sealed with paraffin oil; P, paraffin oil; R, rubber stopper

In the second method, which in the following discussion is referred to as open chamber method, the determinations of oxygen consumption were made on single oysters, the valves of which were connected to a recording lever of the kymograph. An oyster was placed in a 2-liter open jar filled with sea water. To prevent the exchange of gases between the air and the water the latter was covered with

a 3-centimeter layer of heavy paraffin oil. It has been demonstrated by the work of Gaarder (1918) and Nozawa (1929) that the oil layer of this thickness intercepts completely the interchange of gases between the water and the air and that, after the equilibrium between the oxygen tension in the water and in the oil has been established the oxygen tension in the water covered with oil remains practically constant. The set up of the apparatus is shown in Figure 2. The chamber *A* filled with sea water was placed in a constant temperature water bath, *B*; the water in the chamber was stirred with an electric stirrer; two glass tubes served for taking a sample of water, *O*, and replenishing the supply of it, *I*, from bottle *R*, which contained filtered sea water.

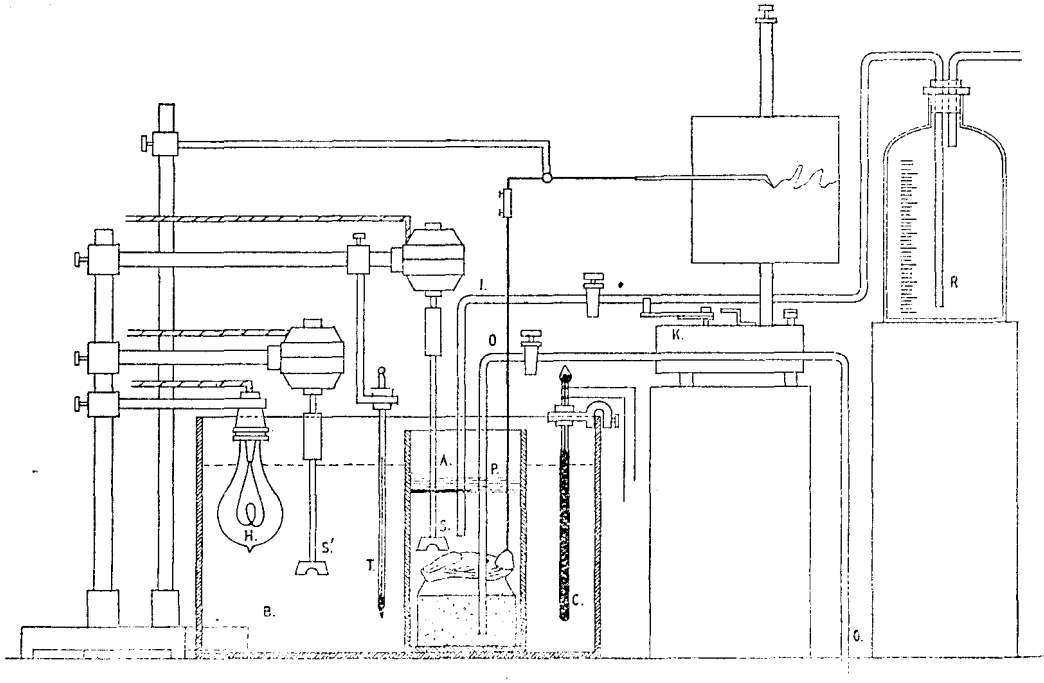


FIGURE 2.—Set up of the apparatus for the determination of oxygen consumption. Open chamber method. A, Glass jar (chamber) filled with water to the level indicated by the heavy line appearing just below oil layer (shaded ara); B, water bath; H, heater; C, metastatic temperature controller; I, glass tubing through which water is added; O, glass tubing for taking a sample; S and S′, electric stirrers; P, layer of paraffin oil; K, kymograph; R, bottle with filtered sea water of known oxygen content; T, thermometer. Relays to operate the temperature controller, dry cells, and rheostats for electric stirrers are not shown

Temperature of the bath was controlled by means of electric heater (*H*), stirrer (*S′*), and metastatic mercury temperature controller (*C*) set at 24.5° C.

At the end of the experiment the oyster was removed, the shells pried apart, and the liquor allowed to drain out for exactly 30 minutes. Then the meat was scraped out into a tarred crucible, weighed, and dried to a constant weight, first at 60° C., then at 100° C. In some instances this material was used for copper determination.

On several occasions when the oyster placed in the open chamber failed to open, opportunity presented itself to make control experiments. Determinations made at 1-hour intervals (Table 3) show that under these conditions neither the environment nor the oyster consume any oxygen. Thus, all oxygen consumed when the oyster is open may be justly attributed to the respiratory exchange of the organism.

TABLE 3.—*Control experiment showing that no oxygen is consumed when the oyster remains closed*

| Time, hours | Oxygen, c. c. per liter | Remarks |
|---|---|---|
| 0 | 4.37 | Oyster closed. |
| 1 | 4.30 | Do. |
| 2 | 4.30 | Do. |
| 3 | 4.34 | Do. |
| 4 | 4.00 | Oyster open. |

All oxygen determinations were done by the well known Winkler method, modified for the presence of organic matter. The latter modification was found necessary because considerable amounts of feces were present when the experiment had been run for more than 1 hour. All figures of oxygen content were corrected to 0° C. and 760 millimeter Hg.

All the experiments were carried on from July to September, inclusive, under the following conditions: Temperature of the water varied from 24.5° to 24.6° C.; salinity of the water from 30.0 to 31.0 per mille; pH at the beginning of the experiment from 7.9 to 8.3; oxygen tension at the beginning of the experiment varied from 3.495 to 4.66 cubic centimeters; oysters varied in size from 6.8 to 10.00 centimeters long and 4.4 to 7.8 centimeters wide; their dry weight varied from 1.019 to 2.080 grams.

The procedure of the experiments was as follows: The oyster was removed from the harbor in the late afternoon of the day preceding that on which it was to be used, was scrubbed, weighed, mounted on a brick, and both the oyster and brick were covered with a heavy coating of paraffin. The oyster was allowed to stand in the air overnight (by this treatment the oysters opened more quickly the next morning than when allowed to remain in water all night). In the morning the chamber was filled with filtered sea water to the 2-liter mark, the surface of the water covered with a heavy layer of paraffin oil, and the chamber allowed to come to constant temperature in the thermostat. Then the oyster was put in and adjusted to the kymograph and the stirrer connected. As soon as the oyster opened, a sample of water was removed for analysis and the time recorded.

In a few of the open-chamber experiments, where only a few readings were made, no fresh sea water was added to the chamber. In most of them, however, an amount of water approximately equal to that removed was added from a small reservoir, which was kept at constant temperature by the thermostat. Knowing the capacity of the chamber, the $O_2$-tension at the beginning, and at the end of the experiment, the number of cubic centimeters of oxygen used by the oysters per time interval was calculated.

## OXYGEN CONSUMPTION OF NORMAL OYSTERS

The results of the determinations of the oxygen consumption of the normal oysters are shown in Table 4. One can see from the examination of this table that under the conditions of the experiment the average oxygen consumption varied from 6.45 to 15.04 milligrams per hour per 10 grams of dry weight. There were considerable fluctuations in the rate of oxygen consumption during the period of a single experiment which are difficult to interpret. In the experiments Nos. 8–13 the determinations were made on six oysters kept together in a closed chamber. On several occasions (experiments 8 and 11) there was a sudden increase in the rate of oxygen consumption, which may be attributed to the increase in muscular activity in the attempts made by the oysters to get rid of the glass rods which were introduced between their valves.

TABLE 4.—*Oxygen consumption of normal oysters*

| Experiment | Date | Oyster | Time | pH | $O_2$ tension, c. c. per liter, 0 ° C 760 mm. | $O_2$ consumption, c. c. per hour per 10 grams dry weight | | Remarks |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Single determination | Average | |
| 8 | July 26 | 28–33 incl | 2.30 | | 4.66 | | 15.04 | Closed chamber method. |
| | | | 2.45 | | 4.60 | | | |
| | | | 3.00 | | 4.22 | 13.92 | | |
| | | | 3.15 | | 4.04 | 14.32 | | |
| | | | 3.30 | | 3.75 | 14.20 | | |
| | | | 3.45 | | 3.40 | 17.70 | | |
| 9 | July 29 | 34–39 incl | 11.10 | 8.30 | 4.33 | | 10.29 | Do. |
| | | | 11.35 | 8.20 | 3.91 | 10.65 | | |
| | | | 12.10 | 8.11 | 3.44 | 10.20 | | |
| | | | 12.40 | 8.02 | 3.31 | 10.50 | | |
| | | | 1.10 | 7.98 | 2.92 | 9.81 | | |
| 10 | Aug. 1 | 40–45 incl | 12.20 | 7.9 | 3.98 | | 6.45 | Do. |
| | | | 12.50 | | 3.08 | 6.28 | | |
| | | | 1.20 | 7.8 | 2.72 | 6.28 | | |
| | | | 1.50 | 7.7 | 2.24 | 5.93 | | |
| | | | 2.20 | 7.6 | 1.94 | 6.84 | | |
| | | | 2.50 | 7.5 | 1.69 | 5.45 | | |
| | | | 3.20 | 7.5 | 1.43 | 7.68 | | |
| 11 | Aug. 3 | 46–51 incl | 10.45 | 7.9 | 3.60 | | 8.70 | Do. |
| | | | 11.15 | 7.8 | 3.06 | 7.96 | | |
| | | | 11.45 | | 2.46 | 10.50 | | |
| | | | 12.15 | 7.6 | 2.11 | 8.66 | | |
| 12 | Aug. 6 | 52–57 incl | 10.45 | | 4.76 | | 9.16 | Do. |
| | | | 11.00 | | 4.49 | 7.86 | | |
| | | | 11.30 | | 4.02 | 9.20 | | |
| | | | 12.00 | | 3.62 | 8.95 | | |
| | | | 12.30 | | 3.05 | 9.04 | | |
| | | | 1.00 | | 2.72 | 10.01 | | |
| | | | 2.00 | | 2.07 | 9.81 | | |
| 13 | Aug. 9 | 58–63 incl | 9.50 | | 3.495 | | 10.99 | Do. |
| | | | 10.20 | | 2.865 | 14.50 | | |
| | | | 11.20 | | 2.06 | 10.52 | | |
| | | | 11.50 | | 1.713 | 9.60 | | |
| | | | 12.50 | | 1.37 | 9.35 | | |
| 74 | Aug. 17 | 74 | 11.00 | | 4.24 | | 8.97 | Open chamber method. |
| | | | 11.30 | | 3.93 | 9.80 | | |
| | | | 12.00 | | 3.53 | 11.46 | | |
| | | | 12.30 | | 3.24 | 7.65 | | |
| 75 | Sept. 4 | 75 | 9.15 | 8.0 | 4.60 | | 9.86 | Do. |
| | | | 9.45 | 8.0 | 4.26 | 10.49 | | |
| | | | 10.15 | 7.9 | 3.89 | 9.94 | | |
| | | | 10.45 | 7.7 | 3.74 | 8.15 | | |
| 76 | Sept. 12 | 76 | 1.35 | | 4.07 | | 7.73 | Do. |
| | | | 2.05 | | 3.87 | 5.71 | | |
| | | | 2.35 | | | 8.75 | | |
| | | | 3.05 | | 3.45 | 8.75 | | |
| 77 | Aug. 31 | 77 | 11.30 | 8.0 | | | 9.78 | Do. |
| | | | 12.00 | 7.9 | | 10.08 | | |
| | | | 12.30 | 7.9 | | 9.72 | | |
| | | | 1.00 | 7.9 | | 9.56 | | |
| 81 | Sept. 12 | 81 | 9.30 | | | | 10.78 | Do. |
| | | | 10.00 | | | 11.58 | | |
| | | | 10.30 | | | 10.80 | | |
| | | | 11.00 | | | 9.97 | | |

In experiments 74, 75, and 81, determinations were made on single oysters the shells of which were connected to a kymograph. An analysis of the kymograph tracings disclosed the fact that there existed a considerable difference in the muscular activity of these oysters. For instance oyster No. 74 opened almost immediately and remained wide open with scarcely any contractions of the muscle. (Fig. 3 C.) Oyster 81 remained tightly closed for a little over 1 hour, then when it did open, the muscle went through a series of vigorous contractions (fig. 3 A) which lasted as long as the experiment was continued. Oyster 75 (fig. 3 B) in respect to the muscular

activity occupied an intermediate position between the two others. As one can see from Table 4 the highest oxygen consumption was recorded in oyster 81, the muscular contractions of which were the most active.

An analysis of all the data when kymograph tracings were obtained was made. Kymograph records were grouped into three arbitrary classes (table 5) according to the number of contractions per hour. Class A comprised oysters which displayed vigorous contractions at the minimum rate of 30 per hour. Oysters that made 5 or less contractions per hour were placed in class C while those intermediate between the extremes, with more than 5 and less than 30 contractions per hour formed class B. By examining Table 5 one can see that the average consumption of oxygen was highest in class A and lowest in class C.

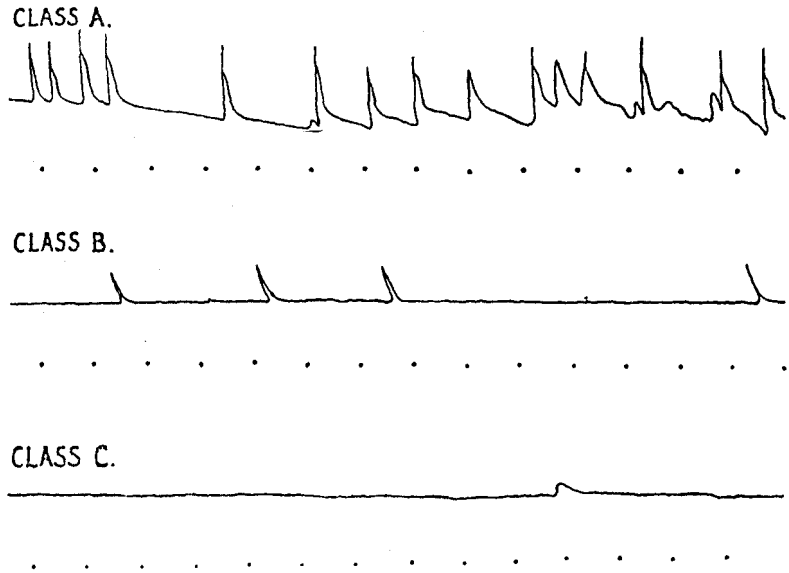


FIGURE 3.—Photograph of three kymograph tracings of shell movements used in the experiments. One-minute intervals indicated by dots. Class A, oyster No. 81; class B, oyster No. 75; class C, oyster No. 74

TABLE 5.—*Relation between the muscular activity and the oxygen consumption*

| Class: | $O_2$ consumption, c. c. per hour per 10 g. dry weight |
|---|---|
| A (more than 30 contractions per hour) | 10. 52 |
| B (more than 5, less than 30 contractions per hour) | 9. 80 |
| C (5 or less contractions per hour) | 8. 42 |

The scale is obviously only an approximation and fails to explain the fluctuations observed within each class or during one experiment. It is quite probable that besides muscular activity other factors are responsible for the differences in the rate of oxygen consumption observed during this work. One of them is related to the seasonal changes in the metabolic activity of the oyster. It has been demonstrated by Bruce (1926) that in *Mytilus edulis* the seasonal changes in absolute oxygen requirements are intimately associated with concurrent changes in the chemical composition of the tissues. It is probable that as the time of spawning approaches, the metabolic activity of the oyster increases and falls off again after the eggs or sperm have been expelled. This problem, however, was not studied during the present investigation.

Another cause of fluctuation in the rate of oxygen consumption may be found in the variation in the ciliary activity of the gill epithelium and consequently in the variation in the rate of flow of water through the gills. In a previous work one of us (Galtsoff, 1928) has shown that there exists a wide range of variation in the rate of flow of water through the gills of oysters of approximately equal size. Unfortunately, no method was suggested whereby the rate of flow of water in the oyster kept in the metabolism chamber could be determined.

## EFFECT OF OXYGEN TENSION ON OXYGEN CONSUMPTION

The results of studies made by several investigators of the effect of oxygen tension on the oxygen consumption of various invertebrates are contradictory. Apparently much more experimental material must be accumulated before better understanding of the relationship existing between these two factors is obtained.

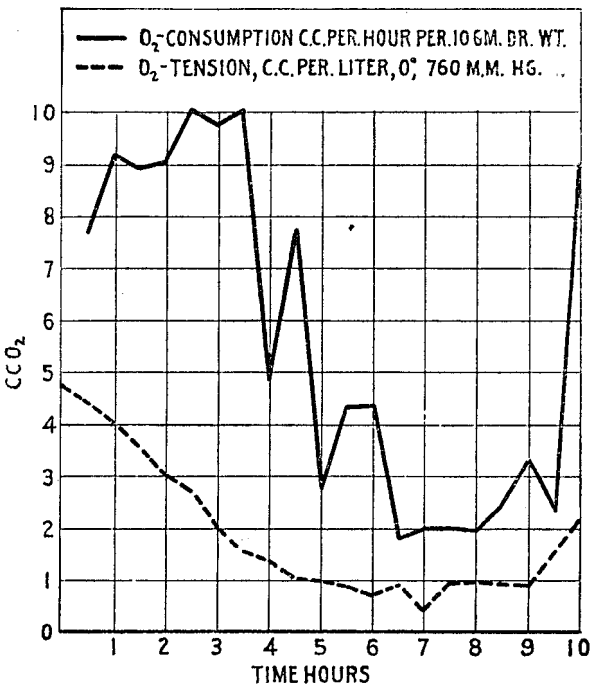


FIGURE 4.—The effect of oxygen tension on oxygen consumption

It has been recognized by Thunberg (1905) that oxygen consumption of air breathing forms like Limax, Lumbricus, and Tenebrio is dependent upon the oxygen tension of the surrounding medium. Henze (1910) extended this work on a number of marine invertebrates and has demonstrated that while in some of the lower forms (Actinia, Anemonia, Sipunculus) the oxygen consumption decreases with the decrease in its tension, in the higher forms with well developed circulatory system (Carcinus, Scyllarus, Aplysia, Eledone) the consumption of oxygen is independent of its tension over a considerable range. Lund (1921) experimenting with *Planaria agilis* found that the rate of oxygen consumption of that worm was constant up to about 36 hours, at which time the oxygen tension became equal to about one-fourth or one-sixth of the oxygen tension of air-saturated water at 20° C. Amberson, Mayerton, and Scott (1924) reported that oxygen consumption of Limulus, Callinectes, and Palaemonites is directly proportional to the oxygen tension in the sea water. The opposite conclusion was reached by Helff (1928) who found that crayfish exhibits respiratory independence from oxygen tension over a considerable range. He found also that the critical tension is different for the organisms of different sizes. In small animals averaging 4.3 grams, the critical tension occurs at 20 per cent of saturation; in those averaging 9.0 grams, the limit is 30 per cent; and for large animals averaging 17.1 grams, the limit is 40 per cent. Below these respective limits the oxygen consumption becomes erratic.

It is interesting that the respiratory exchange of unicellular forms (Paramaecium) and of the fertilized eggs (Arabacia) is virtually constant over a wide range of oxygen tenssion (Amberson, 1928).

Nozawa (1929) studied the effect of oxygen tension on respiration of the oyster and found that the rate of oxygen consumption of *Ostrea circumpicta* is independent of oxygen tension until the latter is reduced to 1 cubic centimeter per liter.

Several experiments to determine the effect of low oxygen tensions on the respiratory exchange of the American oyster were undertaken during the present investigation. The results of one of the experiments are as follows: At 10.30 a. m. six normal oysters were placed in a closed chamber and samples of water were removed at one-half hour intervals until 8.30 p. m. In spite of the fact that small amounts of fresh sea water were added each time the sample was removed, the concentration of oxygen in the water was reduced by the metabolism of the oysters themselves to a low level. The results of the experiment presented in Table 6 and Figure 4 indicate that the oxygen consumption was not affected until $O_2$ tension reached the level of approximately 1.50 cubic centimeter per liter. In order to determine whether the products of the metabolism that were allowed to accumulate in the water might conceivably influence the respiratory exchange, the oxygen in the chamber was replenished by bubbling air through the water for one-half hour (from 8.30 to 9 p. m.). In this way the oxygen was renewed and the pH rose from 7.5 to 7.9.

TABLE 6.—*Effect of oxygen tension on oxygen consumption and effect of products of metabolism on oxygen consumption*

| Date | Oyster | Time | pH | $O_2$ tension, c. c. per liter 0° C., 760 mm. | $O_2$ consumption, c. c. per hour per 10 g. dry weight | Date | Oyster | Time | pH | $O_2$ tension, c. c. per liter 0° C., 760 mm. | $O_2$ consumption, c. c. per hour per 10 g. dry weight |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Aug. 6 | 52-57, inclusive | 10.30 | 8.0 | 4.76 | ---------- | Aug. 6 | 52-57, inclusive | 7.00 | ------ | 1.005 | 1.918 |
| | | 11.00 | ------ | 4.49 | 7.86 | | | 7.30 | ------ | 0.982 | 2.452 |
| | | 11.30 | ------ | 4.02 | 9.20 | | | 8.00 | 7.5 | 0.920 | 3.362 |
| | | 12.00 | ------ | 3.62 | 8.95 | | | (1) | | | |
| | | 12.30 | ------ | 3.05 | 9.04 | | | 9.00 | 7.9 | 2.44 | ---------- |
| | | 1.00 | ------ | 2.72 | 10.008 | | | 9.30 | ------ | 2.28 | 2.315 |
| | | 2.00 | ------ | 2.07 | 9.81 | | | 10.00 | ------ | 1.64 | 8.950 |
| | | 2.30 | ------ | 1.57 | 10.012 | | | (2) | | | |
| | | 3.00 | ------ | 1.08 | 7.73 | Aug. 7 | 52-57, inclusive | 12.20 | 8.0 | 4.80 | ---------- |
| | | 4.00 | ------ | 1.03 | 2.81 | | | 1.20 | ------ | 4.26 | 6.298 |
| | | 4.30 | ------ | 0.90 | 4.36 | | | 2.20 | ------ | 3.64 | 7.580 |
| | | 5.00 | ------ | 0.78 | 4.36 | | | 3.20 | ------ | 2.775 | 6.33 |
| | | 5.30 | ------ | 0.915 | 1.895 | | | 4.20 | ------ | 2.06 | 7.04 |
| | | 6.00 | ------ | 0.468 | 2.04 | | | 5.20 | ------ | 2.281 | 5.61 |
| | | 6.30 | ------ | 0.953 | 2.04 | | | | | | |

[1] From 8.30 to 9.00 p. m., water in metabolism chamber was aerated.
[2] At 10 p. m. oysters were removed from the chamber, placed in running sea water until the next morning. Water in the chamber was then aerated for 2 hours, the same oysters put back in it, their oxygen consumption measured.

TABLE 7.—*Control on experiment in Table 6*

[Same six oysters, put in running sea water overnight. Next day oxygen consumption in fresh sea water determined]

| Date | Oyster | Time | pH | $O_2$ tension, c. c. per liter 0° C., 760 mm. | $O_2$ consumption, c. c. per hour per 10 g. dry weight | Date | Oyster | Time | pH | $O_2$ tension, c. c. per liter 0° C., 760 mm. | $O_2$ consumption, c. c. per hour per 10 g. dry weight |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Aug. 8 | 52-57, inclusive | 9.50 | 8.2 | 4.37 | ---------- | Aug. 8 | 52-57, inclusive | 12.15 | ------ | 2.05 | 7.16 |
| | | 10.20 | ------ | 3.70 | 9.85 | | | 12.45 | ------ | 1.865 | 4.38 |
| | | 10.50 | ------ | 3.28 | 6.75 | | | 1.15 | ------ | 1.345 | 9.70 |
| | | 11.10 | ------ | 2.86 | 9.70 | | | 1.45 | ------ | 1.189 | 4.55 |
| | | 11.45 | ------ | 2.51 | 7.27 | | | | | | |

At 10 p. m. the oysters were removed from the chamber and put in fresh running sea water overnight. The next morning the water which had been used the previous day and which was full of the products of metabolism, was aerated until its oxygen tension was 4.80 cubic centimeters per liter and the pH was 8.0. The same oysters were put back in this water and their oxygen consumption again measured. It remained practically the same as it had been at the beginning of the experiment. (See Table 6.)

The next day an experiment was run on the same oysters using fresh sea water of high oxygen content and normal pH (8.2) to see if the oysters had suffered any from their exposure to water full of the products of their own metabolism. No effect whatever was noticed. (See Table 7.)

In another series of experiments the sea water was boiled under vacuo until the oxygen content had been reduced to less than 1.50 cubic centimeters per liter. The temperature during the process was not allowed to rise above 30° C. An experiment was performed using this water at pH 8.0. The results given in Table 8 indicate that at low oxygen content the rate of oxygen consumption gradually declined with the drop of oxygen tension.

TABLE 8.—*Effect of reduced oxygen tension on oxygen consumption*

[(Water under vacuo 48 hours at maximum temperature 30° C.) August 27, Oyster No. 75]

| Time | pH | $O_2$ tension, c. c. per liter, 0° C., 760 mm. | $O_2$ consumption, c. c. per hour per 10 g. dry weight | Time | pH | $O_2$ tension, c. c. per liter, 0° C., 760 mm. | $O_2$ consumption, c. c. per hour per 10 g. dry weight |
|---|---|---|---|---|---|---|---|
| 10.45 | 8.0 | 1.375 | | 3.15 | 7.6 | 0.396 | 1.610 |
| 11.15 | 7.9 | .915 | 11.07 | (1) | | | |
| 12.15 | 7.8 | .825 | 7.78 | 5.30 | 7.9 | 3.70 | |
| 1.15 | 7.7 | .581 | 4.77 | 6.00 | 7.9 | 3.42 | 5.74 |
| 2.15 | 7.7 | .499 | 1.438 | | | | |

[1] Air bubbled through chamber for 2 hours.

The validity of the experiments in which the oxygen tension is decreased by the metabolism of the organism whose oxygen consumption is being measured, is open to criticism (Keys, 1930, Hall, 1929) on the grounds that the effect measured is not produced by a diminished oxygen tension alone but is due to the concurrent increase in carbon dioxide content and a corresponding decrease in the pH value of the water. In the light of the experiments of Wells (1913, 1918) and Shelford and Powers (1915), who demonstrated that fishes are very sensitive to very small changes in the pH and that their respiration is profoundly influenced by the hydrogen-ion concentration of the sea water (Powers, 1921, 1922), this criticism appears to be justified. It has been shown by the present investigation (Table 7) that the oxygen consumption of the oyster is not affected by the products of its own metabolism. The experiment fails, however, to check up the possible effect of the accumulation of the carbon dioxide which was driven out of the water when the latter was aerated. In order to distinguish completely between the effect of decreased oxygen tension and increase in $CO_2$ content, the following experiment was performed: Using the open chamber method, the rate of oxygen consumption under normal conditions ($O_2$ tension 4.0–4.5 cubic centimeters per liter; pH 7.9–8.0) was first determined. Then the $CO_2$ from a gas generator was bubbled through until the pH was reduced to 7.7. Again determinations were made and again the pH was lowered. This was continued until the pH value of 6.6 was obtained. Because the pH could not be lowered

further by bubbling in carbon dioxide, 100 cubic centimeters of N/100 acetic acid were added. This brought the pH down to 6.0. It was difficult, however, to maintain this hydrogen-ion concentration for any length of time because the calcium carbonate of the shells acts as a buffer and brings it up to 6.4 very quickly. Table 9 embodies the results of this experiment. It indicates very clearly that oysters are not susceptible to changes in hydrogen-ion concentration such as can be brought about under experimental conditions by their own metabolism. There was a noticeable decrease in the rate of oxygen consumption from pH 6.6 downward.

TABLE 9.—*Effect of decrease in pH on oxygen consumption*

[Oyster No. 75]

| Date | Time | pH | $O_2$ tension, c. c. per liter | $O_2$ consumption, c. c. per liter per 10 g. dry weight | Remarks | Date | Time | pH | $O_2$ tension, c. c. per liter | $O_2$ consumption, c. c. per liter per 10 g. dry weight | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sept. 4 | 10.30 | 8.0 | 4.520 | ---------- | | Sept. 6 | 12.45 | 6.2 | 3.62 | ---------- | $CO_2$ bubbled in. |
| | 11.00 | 8.0 | 4.448 | 11.60 | | | 1.15 | 6.2 | 3.482 | 4.96 | |
| | 11.30 | 7.9 | 4.000 | 10.07 | | | 1.45 | 6.6 | 3.250 | 2.85 | |
| Sept. 5 | 10.20 | 7.7 | 4.21 | ---------- | $CO_2$ bubbled in. | Sept. 6 | 2.15 | 6.0 | 2.658 | ---------- | 100 c. c. N/100 acetic acid added. |
| | 10.50 | 7.7 | 3.74 | 8.15 | | | 2.45 | 6.1 | 2.550 | 2.96 | |
| | 11.20 | 7.7 | 3.24 | 9.87 | | | 3.15 | 6.3 | 2.470 | 1.766 | |
| Sept. 5 | 11.25 | 7.3 | 3.60 | ---------- | Do. | Sept. 6 | 3.20 | 8.0 | 4.51 | ---------- | Fresh sea water. |
| | 11.55 | 7.3 | 3.15 | 10.16 | | | 3.50 | 8.0 | 4.270 | 6.40 | |
| | 12.25 | 7.5 | 2.85 | 10.84 | | | 4.20 | 7.9 | 4.00 | 7.95 | |
| Sept. 6 | 11.35 | 6.6 | ---------- | ---------- | Do. | | | | | | |
| | 12.05 | 6.6 | 3.655 | 8.15 | | | | | | | |
| | 12.36 | 6.6 | 3.150 | 10.84 | | | | | | | |

The experimental data presented in Table 6 and Figure 4 show that below the oxygen tension of 1.50 cubic centimeters per liter the oxygen consumption began to decrease following the further decrease in oxygen tension. Because of the small number of observations and on account of considerable individual fluctuations in the metabolic rate, it is impossible at present to determine accurately the critical oxygen tension below which the consumption of oxygen by the oyster is proportional to its pressure. It is quite possible that critical tension is also subject to certain individual fluctuation. The dependence of the oxygen consumption on oxygen tension can be demonstrated, however, by an analysis of all the data obtained with various oysters, the metabolic rate of which was measured at various oxygen tensions. Such an analysis is possible because in all the experiments the temperature was kept constant and because, as has just been demonstrated, the fluctuations in the pH values between 8.0 and 6.7 have no effect on the consumption of oxygen. An examination of Table 10 shows that in the lower half of it (oxygen tensions below 2.5 cubic centimeters per liter) there is a definite correlation between the two variables, which in the upper half of the table (range between 2.5 and 4.5 cubic centimeters per liter) appear to be independent of each other. This relationship can be expressed in mathematical terms. First a coefficient of correlation using all the data as given in Table 10 was calculated. Then similar coefficients were computed using the data of oxygen consumptions obtained at the oxygen tensions between 2.50 and 4.5 cubic centimeters and between 0.0 and 2.49 cubic centimeters per liter. The results are as follows:

Coefficient of correlation (all values)---------- $r = +.498 \pm .048$
Coefficient of correlation ($O_2$ tension above 2.5 cubic centimeters)---------- $r_1 = +.146 \pm .102$
Coefficient of correlation ($O_2$ tension below 2.5 cubic centimeters)---------- $r_2 = +.646 \pm .046$

TABLE 10.—*Correlation table showing relation between oxygen consumed and oxygen tension*

| Oxygen, c. c. per liter | Oxygen used, c. c. per hour per 10 g. dry weight | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0–0. 9 | 1–1. 9 | 2–2. 9 | 3–3. 9 | 4–4. 9 | 5–5. 9 | 6–6. 9 | 7–7. 9 | 8–8. 9 | 9–9. 9 | 10–10. 9 | 11–11. 9 | 12–12. 9 | 13–13. 9 | 14–15 | Total |
| 4.50–4.25 | | | | | 1 | | | | | | | | | 1 | | 2 |
| 4.24–4.00 | | | | 1 | | 1 | 1 | 1 | | 2 | | | | | | 6 |
| 3.99–3.75 | | | | | | 1 | | | 1 | | 1 | | | | | 3 |
| 3.74–3.50 | | | | | | 1 | | 1 | 2 | 1 | | | | | | 5 |
| 3.49–3.25 | | | | 1 | | 1 | | 2 | 1 | 1 | 1 | | | | | 7 |
| 3.24–3.00 | | | | | | 1 | 1 | | 1 | | | | | | 1 | 4 |
| 2.99–2.75 | | | | | | 6 | 1 | 1 | 2 | 1 | | | | | | 11 |
| 2.74–2.50 | | | | | 2 | | | | 1 | 1 | | | | | | 4 |
| 2.49–2.25 | | | | 1 | | | 1 | | | | | | | | 1 | 3 |
| 2.24–2.00 | | | | | | | 1 | 1 | | 1 | 2 | | 1 | | | 6 |
| 1.99–1.75 | | | | | 1 | 2 | 1 | 1 | 1 | 1 | | | | | | 7 |
| 1.74–1.50 | | | | 1 | 1 | 1 | | | 1 | 1 | 1 | 1 | | | 2 | 9 |
| 1.49–1.25 | | | | | 1 | | 2 | 1 | | 2 | | | | | | 6 |
| 1.24–1.00 | 1 | | | 3 | 1 | | 1 | | 2 | 1 | | | | | | 9 |
| 0.99–0.75 | 1 | 1 | 2 | 1 | | 3 | 1 | | | | | | | | | 9 |
| 0.74–0.50 | | 2 | 2 | 2 | 2 | 1 | 2 | | | | | | | | | 11 |
| 0.49–0.25 | 5 | | 1 | 2 | 1 | 2 | | | | | | | | | | 11 |
| 0.24–0.00 | | 1 | | | | | | | | | | | | | | 1 |
| Total | 7 | 4 | 5 | 12 | 10 | 20 | 12 | 8 | 12 | 12 | 5 | 1 | 1 | 1 | 4 | |

The above expresses in mathematical terms what can roughly be seen from the correlation table; namely, that the oxygen consumption is independent of oxygen tension when the latter is above 2.5 cubic centimeters per liter, and that below that point the rate of oxygen consumption diminishes as the $O_2$ tension decreases.

It must be borne in mind that the statistical treatment of a series of observations permits the measurement of the relationship between the two variables but can not reveal the cause or causes of the phenomena observed. For the latter purpose further experimentation is necessary. It was thought that the dependence of oxygen consumption on oxygen tension may be due to the inhibition of the ciliary activity of the gill epithelium and to the consequent decrease in the rate of flow of water through the gills. Using the method developed by the senior author and described in previous publication (Galtsoff, 1928), the rate of flow of water through the gills was measured at various oxygen tensions. The results of two experiments are presented in Table 11. An oyster was first placed in the sea water which for three days was kept under vacuo at 37° C. Oxygen determinations and pH readings were made simultaneously with the measurements of the rate of flow. Then the water was aerated for 1 hour and readings were repeated. The results of the experiments show no significant differences in the rate of flow of water at the oxygen tensions of 0.69 and 5.45 cubic centimeters per liter.

TABLE 11.—*Rate of flow of water through the gills at normal and low oxygen tensions*[1]

| Experiment and date | Time | Temperature ° C. | $O_2$, c. c. per liter | pH | Rate of flow, c. c. per hour | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Average | Maximum | Minimum | Number of readings |
| A, Sept. 1 | [2] 11. 20 | 20. 0 | 0. 69 | 7. 9 | 972 | 1, 030 | 946 | 20 |
| | 1. 27 | 19. 3 | 5. 45 | 7. 8 | 900 | 1, 011 | 810 | 20 |
| B, Sept. 4 | [2] 11. 22 | 22. 2 | 0. 975 | 7. 9 | 2, 657 | 2, 702 | 2, 430 | 20 |
| | 12. 55 | 22. 2 | 4. 52 | 7. 7 | 2, 683 | 2, 702 | 2, 430 | 29 |

[1] Size of the oysters: Experiment A, 8.4 by 7.6 cm., wet weight of meat 14.5 g.; experiment B, 9.7 by 7.8 cm., wet weight of meat 32.7 g.

[2] After these readings, air bubbled in.

Similar experiments were carried out to demonstrate the effect of the pH on the ciliary activity. The changes in the pH were obtained by bubbling the $CO_2$ through the water in which the oyster was kept. The results (Table 12) show that significant decrease in the rate of flow of water occurs when the pH is below 6.6. As has been shown before (Table 9), oxygen consumption at this pH also decreases.

These experiments demonstrate very clearly that the decrease in the rate of metabolism at low oxygen tension is not due to the failure of the gill epithelium to maintain sufficiently strong current. As the oxygen tension goes down, the amount of water passing through the oyster remains approximately the same. The rate of oxygen absorption remains at a constant level until a point is reached at which the amount of oxygen in the water is insufficient to supply the needs of the gill cells; that is, all the reduced material does not become oxidized. In normal sea water the oxygen content is far in excess of the oxygen requirements, which probably accounts for the wide range of oxygen tension to which the oyster is not sensitive.

TABLE 12.—*Effect of pH on the rate of flow of water through the gills* [1]

| Experiment and date | Time | Temperature °C. | $O_2$, c. c. per liter | pH | Rate of flow, c. c. per hour | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Average | Maximum | Minimum | Number of readings |
| C, Aug. 21, 1928 | [2] 11.38 | 22.0 | 4.59 | 8.1 | 2,527 | 2,689 | 2,112 | 20 |
| | [2] 12.20 | 21.4 | 4.65 | 7.5 | 2,961 | 3,240 | 2,696 | 20 |
| | [2] 1.16 | 21.4 | ---------- | 7.3 | 2,670 | 3,240 | 2,313 | 20 |
| | [2] 2.18 | 21.4 | ---------- | 6.4 | 2,074 | 2,203 | 1,672 | 20 |
| | 4.00 | 21.2 | 4.60 | 6.3 | 1,095 | 1,277 | 1,011 | 20 |
| D, Aug. 23, 1928 | [3] 10.35 | 21.3 | 2.62 | 8.1 | 3,078 | 3,240 | 2,864 | 20 |
| | 11.57 | 21.6 | 6.11 | 8.1 | 2,573 | 3,039 | 2,112 | 30 |
| | 1.30 | 21.0 | 5.65 | 8.1 | 2,670 | 2,864 | 2,313 | 20 |
| | [4] 2.10 | 21.0 | 5.50 | 8.1 | 2,307 | 2,430 | 2,028 | 20 |
| | [2] 2.30 | 21.1 | ---------- | 6.7 | 2,294 | 2,430 | 2,112 | 20 |
| | [2] 3.04 | 21.3 | ---------- | 6.5 | 2,093 | 2,210 | 1,944 | 20 |
| | 4.00 | 21.6 | 4.37 | 6.1 | 1,108 | 1,218 | 972 | 20 |
| E, Aug. 24, 1928 | [3] 11.10 | 27.8 | 1.58 | 8.0 | 4,802 | 5,391 | 4,413 | 20 |
| | [2] 11.27 | 27.8 | ---------- | 6.5 | 2,825 | 3,240 | 2,430 | 20 |
| | [2] 12.05 | 26.8 | ---------- | 6.3 | 2,346 | 2,702 | 1,944 | 20 |
| | 1.40 | 26.4 | ---------- | 6.5 | 2,929 | 3,264 | 2,702 | 20 |

[1] Size of Oysters: Experiment C, 9.3 by 6.4 cm., wet weight of meat 13.56 g.; Experiment D, 8.1 by 6.4 cm., wet weight of meat 9.68 g.; Experiment E, 9.2 by 7.6 cm., wet weight of meat 21.6 g.

[2] After these readings, $CO_2$ bubbled in.

[3] After these readings, air bubbled in.

[4] After these readings, water was changed and $CO_2$ bubbled in.

## INCREASED RATE OF METABOLISM

The oxygen consumption of any organism, of course is dependent on the kind of material that it is oxidizing. The normal diet of the oyster, comprising diatoms and other small plant and animal forms, is a "mixed" one containing protein, fat, and carbohydrate. It is also known (Young, 1928) that the oyster can utilize dissolved food material that is brought to it. It was thought, therefore, of interest to find out to what extent an abundance of food could be consumed. The oyster has no ability to select its food, so that anything that is in solution must necessarily pass through the animal; the question then is, what is the limit to which it can burn the material present?

To determine this the following experiment was performed: A series of control determinations was first made of the oxygen consumption of an oyster that was kept in the filtered sea water in an "open chamber" and the shell movement of which were recorded on a kymograph. After a base level of oxygen consumption had been deter-

mined, 1 cubic centimeter of a 5 per cent solution of glucose was added to the water, and the rate of oxygen consumption was measured over a period of several hours; then another cubic centimeter of 5 per cent glucose was added. Usually after the addition of 3 cubic centimeters the oyster closed up and remained so until the water had been changed.

It can be seen from Table 13 that the rate of oxygen consumption rose immediately after the introduction of the glucose, but it apparently reached its maximum very quickly, because subsequent increase in glucose was without effect until the concentration was sufficient to cause the oyster to close its shell.

TABLE 13.—*Effect of addition of glucose on oxygen consumption*

[September 6, Oyster No. 75]

| Time | $O_2$ tension, c. c. per liter 0°, 760 mm. | $O_2$ consumption, c. c. per hour per 10 g. dry weight | Time | $O_2$ tension, c. c. per liter 0°, 760 mm. | $O_2$ consumption, c. c. per hour per 10 g. dry weight | Time | $O_2$ tension, c. c. per liter 0°, 760 mm. | $O_2$ consumption, c. c. per hour per 10 g. dry weight |
|---|---|---|---|---|---|---|---|---|
| 11.00 | 4.65 | | 5.01 | | | 7.00 [2] | 2.76 | 12.38 |
| 11.30 | 4.45 | 5.62 | 5.30 | 4.11 | 13.70 | 7.01 | | |
| 12.00 | | 8.84 | 6.01 [2] | 3.96 | 14.45 | 7.30 | 2.42 | 13.87 |
| 12.30 [1] | 3.78 | 8.84 | 6.00 | | | 8.00 [3] | 1.98 | 14.12 |
| 5.00 [2] | 4.62 | | 6.30 | 3.32 | | | | |

[1] Oyster closed, and experiment was discontinued for a few hours. Oyster opened at 5 p. m.
[2] 1 c. c. of 5 per cent glucose added.
[3] Oyster closed.

## EXPERIMENTS WITH GREEN OYSTERS

The problem why oysters become green in certain localities has attracted the attention of biologists interested in a scientific study of this phenomenon and of those concerned with the oyster industry. Inasmuch as a good review of the extensive literature on the subject can be found in the papers of Herdman and Boyce (1899) and Ranson (1927), it suffices to mention here only a few essential points that have direct bearing on the problem. The present investigation refers to those green oysters the color of which is associated with the accumulation of copper in their bodies. It does not concern the so-called green-gilled oysters (from marennes) the coloration of which is due to the absorption of the pigment of a diatom, *Navicula ostearia*.

It has been demonstrated by Ryder (1882) that the green coloring matter of the American oyster is taken up by the amœboid cells which aggregate in cysts under the epithelium of the body and on the surface of the gills. Ryder suggested that the green pigment in the leucocytes may be phycocyanine. Later on it has been shown by Herdman and Boyce (1897, 1899) that comparatively large quantities of copper occur in the green leucocytes and that the intensity of the green color of the oyster is in proportion to the amount of copper present. They found that green American oysters relaid in the waters near Fleetwood, England, contained on the average 2.63 milligrams of copper per oyster, whereas normal oysters from the same source contained only 0.7 milligram of copper. Microchemical examination made by these authors proved that copper reaction coincided histologically with the presence of green leucocytes. The cause of green color was investigated by Colwell and Nelson [2] who arrived at the conclusion that copper is responsible for the bluish-green color of oysters from Narragansett Bay and certain sections of Long Island Sound. A different view was held by J. Nelson (1915) who believed that the green color is not due to copper.

[2] Unpublished manuscript in files of U. S. Bureau of Fisheries.

The problem was complicated by the fact that the early investigators did not clearly distinguish between the types of green pigmentation and confused the blue-green pigmented oysters with the green-gilled ones. It is generally agreed at the present time that the former is associated with an increase in copper content and that the latter, which may have a normal copper content, is due to the accumulation of the pigment of the diatom *Navicula ostearia*.

Hunter and Harrison (1928) have shown that oysters taken from the vicinity of manufacturing centers may contain appreciable quantities of arsenic, zinc, and lead, besides copper. In some samples taken from New York Harbor and from Connecticut waters, the metallic contamination could be easily traced to the pollution of water by trade wastes. Yet in several instances oysters from areas located far from any known source of metallic contamination were found to contain considerable quantities of zinc and copper. The question of the source of copper in the sea and of the mechanism of its accumulation by the oyster has not yet been satisfactorily solved. Hunter and Harrison (loc. cit.) believe that oysters will absorb from the water almost any substance which it contains. The problem, however, requires further investigation.

A review of the literature reveals the fact that no systematic efforts were made to determine the chemical nature of the green pigment of oysters. Such an effort was made during the summer of 1927 by the senior author and Dr. Samuel Lepkofsky. First a series of analyses for copper was made on a large number of green oysters from Long Island Sound and of normal oysters from Wellfleet Harbor, Mass. The latter locality was selected because it was known that green oysters never occurred in Wellfleet Harbor. For microchemical reactions, sections of green and normal oysters preserved in absolute ethyl alcohol and embedded in paraffin were treated with potassium ferrocyanide and with haematoxylin. The results of these analyses confirmed fully the conclusions of Herdman and Boyce that the intensity in green color was in proportion to the copper content in the oyster and that histologically the copper is located in the green leucocytes.

The next step was to isolate the green substance and to attempt to determine its chemical composition. Unfortunately, because of the separation of Doctor Lepkofsky from the bureau, this work has not been finished. It is, however, desirable to give a brief account of the results so far obtained.

The green pigment generally associated with mollusks is a copper-protein complex, hemocyanin. It was thought that the green compound in oysters might be of a similar nature. The isolation of the green substance was attempted with the view of studying its chemical properties and using this information in an effort to remove the substance from the oyster or prevent its appearance. To obtain the pigment, oysters were ground in water with pure sand, which had been previously treated with strong HCl and carefully washed.

Green extracts obtained by this method were then saturated with $(NH_4)_2SO_4$ which caused all the proteins to be precipitated, but the pigment was not thrown out of solution. This indicated it was not a hemocyanin or a copper proteinate of any kind. Treatment with $Na_2SO_4$ at 37° C. did not precipitate the green color.

Finally, dialysis showed the pigment passed through collodion sacs which held back congo red. This indicated that the green compound was of a small molecular size.

Dialysis was then used as a method of preparing the green substance and concentrating it with the hope of obtaining some crystals when sufficiently concentrated. From three to four hundred green oysters were ground very fine through a meat grinder and extracted with water by pressing through several layers of cheesecloth. The extract was first dialyzed in collodion sacs impermeable to congo red, then concentrated in vacuo. A thick sirup was finally obtained, but no crystals were formed because of the large amount of impurities.

Some of the properties of this concentrated protein-free extract are: Heating to boiling destroys the color. Addition of alkali gives the blue-purple color of the biuret test. Heating of the alkaline solution causes a precipitate of cuprous oxide, indicating the presence of sugar or other reducing substance. $H_2S$ decomposes the pigment readily with the formation of CuS. A steel spatula immersed in the green liquid soon becomes copperplated. This indicates that the compound exists in a highly dissociated state. One drop of HCl to about 5 cubic centimeters of extract causes a discharge of the green color. It apparently is not a simple copper salt as $CuCl_2$, Cu, Br, or $Cu(C_2H_3O_2)_2$, since addition of ammonia does not greatly deepen the color, as is the case with such copper salts. Boiling the extract discharges the color, thus necessitating vacuum distillation when concentrating.

An attempt was made to determine whether the compound existed as a copper ammonium complex. Aeration to remove ammonia before and after decomposition with $H_2S$ failed to reveal evidence of a copper ammonium complex.

While the evidence cited indicated that the compound was not identical with or even remotely related to hemocyanin or other copper protein complex, it was nevertheless thought possible to exist in the oyster as a copper protein complex and possibly suffer decomposition on treatment with water used in the extraction. To settle this point, the following experiment was carried out. Normal white oysters were ground up and the oyster fluids obtained by pressing through a cloth. Green oyster fluids were similarly obtained and the one dialyzed against the other. Some of the green pigment diffused through the collodion sac tested previously for tightness with congo red, leaving no doubt that the green pigment exists in the oyster as a simple, readily diffusible compound.

The green pigment is quite readily soluble in methyl alcohol, less so in ethyl alcohol, and quite insoluble in butyl or amyl alcohol. It is insoluble in such fat solvents as chloroform, ether, acetone, or benzene. It is soluble in pyridine.

On standing about three or four months, the green color disappears and the extract turns to a reddish chocolate color. It was thought that it might represent a reduced form of the green pigment. Bubbling air or oxygen through the extract does not bring back the green color. Upon shaking with methyl alcohol, ethyl alcohol, or pyridine, the color returns in these solvents.

## OXYGEN CONSUMPTION OF GREEN OYSTERS

The deposition of large quantities of copper in the oyster was regarded by Boyce and Herdman (1897) as a degenerative reaction which may be due "to a disturbed metabolism, whereby the normal copper of the hemocyanin, which is probably passing through the body in minute amounts, ceases to be removed and so becomes stored up in certain cells." It was thought that the determination of the metabolic rate of green and normal oysters would supply evidence in favor of or against this suggestion. Green oysters differing widely in depth of pigmentation were obtained from various sections of Long Island Sound. Inasmuch as previous work of Herdman and Boyce

(1899) and our observations during the summers of 1927 and 1928 have demonstrated that all intensely green oysters have high copper content, the latter was used as an index of the intensity of pigmentation.

The copper method used was that of R. Biazzo (1926), which was found to give excellent checks on very small amounts of copper, and was therefore suitable for determinations on single oysters. Because of slight variations in the method as we used it and also because the Italian journals are not always readily obtainable, the method is given below in detail:

Individual oysters were dried to constant weight in crucibles. The dried weight used was between 1 and 2 grams. The material was carefully scraped out into a large test tube and 5 cubic centimeters concentrated $H_2SO_4$ and 1 cubic centimeter of a saturated solution potassium chlorate added. After the mixture stopped foaming, it was gently heated with a microburner and boiled until all the organic matter had been oxidized. The process was usually hastened by the addition of 1 or 2 drops of hydrogen peroxide. Test tubes were covered with watch glasses to prevent any spattering during the combustion. After the solution had cleared, it was emptied into a 50-cubic-centimeter beaker and evaporated to dryness on the sand bath to render the silica insoluble. The residue was moistened with 5 cubic centimeters of 1N HCl followed by 5 cubic centimeters of water, warmed on the sand bath for one-half hour, filtered, and the precipitate washed in about 90 cubic centimeters of water. The filtrate is evaporated to a volume of 10 cubic centimeters, cooled, and enough 1N NaOH added to make the solution just alkaline to phenophthalein. Next 1 cubic centimeter of glacial acetic acid, 1 cubic centimeter of 10 per cent potassium thiocyanate solution, and 10 drops of pyridine were added. The solution was transferred to a 25-cubic-centimeter volumetric flask, 5 cubic centimeters of chloroform added, and the volume made up with water. After thorough shaking, the chloroform layer was allowed to settle, the aqueous layer removed, and the chloroform compared with a standard in the colorimeter.

Preparation of the standard: Dissolve 0.3926 gram of pure copper sulphate ($CuSO_4.5H_2O$) in water and dilute to 1 liter. One cubic centimeter of this solution contains 0.1 milligram copper. Take 1, 2, 3, and 5 cubic centimeters of this solution and treat with acetic acid, potassium thiocyanate, pyridine, and chloroform in exactly the same manner as the sample. Select the standard which has approximately the same intensity of color as the sample.

NOTE.—(1) Water distilled in glass must be used throughout, (2) a control must be run on all reagents used, (3) the copper compound formed has the formula $CuPy_2(CNS_2)$.

As a check on the method, 25 normal white oysters were dried to constant weight at 100° C., ground, and put through a 60-mesh sieve. This made a homogeneous powder, samples of which could be used for copper determinations. One-gram samples of this powder were analyzed according to the above method; 1-gram samples to which had been added 1 cubic centimeter of a standard copper solution containing 0.1 milligram of copper were also analyzed. The results are presented in Table 14.

TABLE 14.—*Control analysis of copper determinations*

| Material | Cu, mg. in 1 g. | Average | Calculated | Material | Cu, mg. in 1 g. | Average | Calculated |
|---|---|---|---|---|---|---|---|
| Mixed sample of dried oysters | 0.1138<br>0.1158<br>0.1142<br>0.1164 | 0.1151 | | Same plus 0.1 mg. Cu | 0.2136<br>0.2120<br>0.2110<br>0.2116 | 0.2121 | 0.2151 |

NOTE.—Control on the reagents gave no color.

By examining Table 15, which contains all the data of oxygen consumption of oysters of known copper content, one can notice that the respiratory rate of the green oysters was slightly higher than that of the normal ones. It must be borne in mind, however, that high oxygen consumption was observed also in the normal oysters. (See Table 4, experiment 8.) Unfortunately, no copper determination of these oysters was made, but it is very doubtful if their copper content was high, because during the course of the investigation no normal oyster was found that had high copper content. While the relationship between the oxygen consumption and copper content can not be definitely established, the results of the present investigation indicate very clearly that the metabolic rate of the green oysters was at least equal to, and probably higher than, that of the normal oysters. The conclusion can be drawn that so far as the rate of metabolism is concerned, there is no indication of any disturbance due to the accumulation of copper.

TABLE 15.—*The relation of the copper content of oysters to the oxygen consumption*

| Experiment | Date | Oyster | Dry weight | Cu. mg. per 100 g. dry weight | Cu. mg. per oyster | $O_2$ consumption, c. c. per hour per 10 g. dry weight |
|---|---|---|---|---|---|---|
| NORMAL OYSTER | | | | | | |
| 10 | Aug. 1 | 40–45 incl. | [1] 2.07 | [2] 8.21<br>8.26 | [2] 0.170<br>0.171 | 6.45 |
| 11 | Aug. 3 | 46–51 incl. | [1] 2.08 | [2] 9.90<br>9.52 | [2] 0.206<br>0.198 | 8.70 |
| 12 | Aug. 6 | 52–57 incl. | [1] 1.875 | 11.09<br>11.41 | 0.208<br>0.214 | 9.16 |
| 13 | Aug 9 | 58–63 incl. | [1] 1.275 | 13.26<br>12.55 | 0.169<br>0.160 | 10.99 |
| 75 | Aug. 21 | 75 | [3] 1.8015 | 13.77 | 0.248 | 9.86 |
| GREEN OYSTER | | | | | | |
| 72 | Aug. 16 | 72 | [3] 1.1827 | 175.02 | 2.07 | 10.28 |
| 73 | Aug. 17 | 73 | [3] 1.0188 | 121.71 | 1.24 | 11.63 |
| 76 | Aug. 29 | 76 | [3] 1.8830 | 271.91 | 5.12 | 12.09 |
| 77 | Aug. 30 | 77 | [3] 1.2494 | 186.49 | 2.33 | 10.74 |
| 78 | Aug. 30 | 78 | [3] 1.2200 | 175.74 | 2.144 | 12.25 |
| 79 | Aug. 31 | 79 | [3] 1.0190 | 241.41 | 2.460 | 13.47 |

NOTE.—Closed chamber method used in experiments 10–13, inclusive. Open chamber method used in experiments 72–79, inclusive.

[1] Average weight of 6 oysters.
[2] Determinations were repeated wherever there was sufficient material.
[3] Weights of individual oysters.

## SUMMARY

1. Two methods for the determination of oxygen consumption of the oyster were devised.

2. Oxygen consumption of normal oysters under standard conditions varies from 6.45 to 15.04 cubic centimeter per hour per 10 grams of dry weight.

3. Oxygen consumption is not influenced by oxygen tension when the amount of oxygen present is greater than 2.5 cubic centimeters per liter. Below this point oxygen consumption is affected by oxygen tension.

4. Changes in pH values such as can be brought about by the oyster under the conditions of the experiments and the accumulation of the products of its own metabolism do not alter its metabolic rate. The shell of the oyster acts as an efficient buffer, preventing the lowering of the pH below 6.0.

5. Low oxygen tension and variation in the pH values of water from 8.2 to 6.6 have no effect on the rate of flow of water through the gills.

6. Oxygen consumption of oysters can be increased by adding glucose to the water.

7. Green pigment of oysters is not a hemocyanin or copper proteinate of any kind. The compound exists in a highly dissociated state.

8. Copper content of normal oysters varies between 8.21 to 13.77 milligrams per 100 grams dry weight, or from 0.16 to 0.248 milligrams per oyster. Copper content of green oysters analyzed during the investigation varied between 121.71 and 271.91 milligrams per 100 grams dry weight, or from 1.24 to 5.12 milligrams per oyster.

9. Green oysters show a slight increase in oxygen consumption over normal oysters. The significance of this difference is, however, doubtful.

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