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Some Observations on the Respiration of the American Oyster *Crassostrea virginica* (Gmelin)

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Some Observations on the Respiration of the American Oyster *Crassostrea virginica* (Gmelin)[1]

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Abstract

The Krogh syringe and some analytical improvements by Pomeroy and Kirshman in the Winkler method for oxygen analysis were combined with the direct method of measuring the pumping rates of the oyster to obtain data on oxygen uptake at various pumping rates. When pumping at a rate of 25 liters per hour an oyster may consume as much as 50 mg of oxygen per hour. The rate of oxygen removal from the water in mg per liter per ml of shell cavity plotted against pumping rates is parabolic. When the hourly work output for various pumping rates are compared with a theoretical curve, it is found that the oyster can pump water at approximately 10 liters per hour with a maximum efficiency of 10 per cent. An oyster with a shell cavity of 40 ml can pump over 40 liters per hour but at this rate the efficiency falls to less than two per cent. Some unknown carbohydrate-like substances are removed from the water in quantities commensurate with the oxygen uptake. The role of algae in the energy budget of the oyster as reflected by oxygen uptake is discussed, and certain points relative to the production of extracellular carbohydrates by algae are considered.

Contents

Introduction

The quantity of oxygen consumed by an animal is related to such metabolic processes as growth, repair of tissue, excretion, digestion, and osmoregulation. Besides these basic processes, any act, which results in the performance of mechanical work, increases con-

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sumption. In the oyster, for instance, there are shell movements and the pumping of water through the branchial chambers; activities necessary for respiratory and feeding purposes. Associated with the latter there is often a great production of mucus which entrains silt and other dejecta, the whole being voided as pseudo-feces. Both the production of this mucus and the work done by the ciliated epithelium in transporting it entails an additional demand for oxygen.

Water pumping and the associated activities of the oyster are analogous to food hunting on the part of non-sedentary animals. But since, as a sedentary animal, the oyster cannot search for favorable feeding areas it should (1) expend energy only when the water being pumped contains sufficient available nourishment to allow a net gain of energy over that required by the work being done and (2) be able to accumulate a reserve of potential energy which can be drawn on in times of famine and to augment the development of gonadal tissue as the spawning season approaches.

These two phases of the oyster's struggle for existence impel its complete integration with its immediate environment. The first has been quantitatively assessed only in recent years (Collier *et al.*, 1953) and is, in effect, a method of conserving energy. The second, the storage of energy in the form of glycogen, has been long established and is largely responsible for the commercial value of oysters.

To understand the natural factors which cause fluctuations in the abundance and condition of oysters it would be useful to know how much work is done by the oyster in pumping water and how the resulting respiratory and nutritional requirements are related to environmental factors. The most expedient means of measuring the work performed by an organism is to estimate the amount of oxygen consumed per unit of time per unit of respiring tissue. This has been done for a number of invertebrate animals and several studies have been made on the oxygen uptake of oysters.

Mitchell (1914) and Galtsoff and Whipple (1931) published the first reports on the oxygen uptake of American oysters. The principle of the experimental technique, in both cases, was the measurement of the differences between the initial oxygen content of a mass of standing water containing the oysters and samples drawn at succeeding intervals. Galtsoff and Whipple concluded from these experiments that the oxygen varied from 6.45 to 15.04 ml (760 mm Hg, 0°C) per hour per 10 grams of dry weight. No attempt was made to measure the uptake as related to pumping rate but kymograph traces were made of shell movements. The movements illustrated in his paper were not typical of normal pumping oysters. Mitchell's results were 0.35 to 1.29 mg per hour per gram of dry weight; reduced to the same terms, Galtsoff and Whipple's results were 0.92 to 2.1 mg per hour per gram of dry weight. Jørgensen (1952) made correlative studies of respiration and pumping rate. To estimate the pumping rate he used the indirect method of Fox (1937) while oxygen levels were estimated by the Winkler method. Jørgensen concluded that his oysters pumped 10 to 20 liters of water for each milliliter of oxygen consumed. This frame of reference does not permit reduction of the data in terms of variable pumping rates and amounts of respiring tissue.

Observations on oxygen uptake made simultaneously with the measurement of pumping rates by the direct method are reported for oysters obtained at Pensacola, Florida.

I wish to acknowledge the assistance of my co-workers, Dr. Sammy Ray and Mr. A. W. Magnitzky, whose industry and expert attention to detail made this work possible.

Methods, Materials, and Terminology

The Determination of Dissolved Oxygen

For many years the Winkler method has been the standard method of estimating the quantity of oxygen dissolved in water. Krogh (1935) introduced a modification utilizing syringe pipettes and correspondingly small samples. Yoder and Dresher (1934) studied the effects of oxygen contained in reagents and tested the various types of starches as end-point indicators. After studies of the solubilities of sodium iodide in sodium hydroxide solutions (Pomeroy and Kirshman, 1944), and potassium iodide in potassium hydroxide solutions (Kirshman and Pomeroy, 1943), and potassium iodide in sodium hydroxide (Kirshman and Pomeroy, 1944), Pomeroy and Kirshman (1945) suggested a modified Winkler method which we combined with the Krogh syringe pipette method for use in these studies. The modification was chiefly in the high iodide concentration for which Pomeroy and Kirshman claimed the following advantages: less interference from other reducing agents, reduced loss of iodine vapor, and a sharper end-point. The routine procedure used in these experiments follows.

A set of 10 ml syringes were prepared and calibrated according to the method of Krogh (1935).

The solutions were as follows:

I. 90 grams of NaI and 40 grams of NaOH in 55 ml of water.

II. 40 grams $MnCl_2$, and 10 ml 6N HCl made up to 100 ml with water.

III. 6N HCl.

IV. 0.003572 N sodium thiosulfate. Standardized with standard solution of $K10_3$ or recrystallized potassium dichromate.

V. Starch in saturated NaCl.

Reagents I and II were kept in rubber capped vaccine bottles. The routine determinations were performed as follows:

1. Syringe needle (18 gauge) inserted through rubber cap into inverted vial containing Reagent I. Reagent drawn in and expelled by pushing plunger to bottom of syringe. All air bubbles expelled. This left about 0.1 ml of solution in dead space of syringes.

2. Water sample and Reagent I left in dead space; drawn in by pulling plunger back to first stop.

3. After water sample drawn, syringe held in inverted position and care taken not to collect air bubbles. Needle inserted through rubber cap of vial containing Reagent II. Plunger withdrawn to second stop. The $MnCl_2$ reacts with the NaOH originally in the dead space and subsequently mixed with the sample to produce a precipitate of manganous hydroxide which absorbs all of the oxygen dissolved in the water in about five minutes.

4. After the five minute waiting period, discharge the contents of the syringe beneath the surface of 1 ml of 6N HCl. The syringe was rinsed with the acid solution and then with two rinses of water.

5. Titrate with microburette. 1 ml of Reagent IV equals 0.02000 ml of oxygen (0°, 760 mm).

To determine the amount of oxygen removed from the water by an oyster, a sample was taken from the inhalant side and at the same time from the exhalant side. The difference in the oxygen concentration of the two samples indicated the removal rate. The syringe needles were brought as close to the apertures as possible without causing the oyster to close.

This method of collecting the oxygen samples was similar to that originally described by van Dam (1954), and was subject to certain errors pointed out by him. He showed that different oxygen values could be obtained by placing the syringe tip at different points in the exhalant stream. Such errors probably entered these data and were possibly responsible in part for some of the aberrant points obtained.

Methods of Measuring and Recording Pumping Rate

The experimental apparatus used for recording the pumping rates of oysters has been described elsewhere (Collier and Ray, 1948 and Collier *et al.*, 1953). The method was a combination of the rubber dam and the constant level tank. This is a direct method of measurement to which certain objections are commonly raised. The criticisms center around the attachment of the rubber dam and the possible interference with shell movements which may be caused. Any experimental method is subject to such criticisms, but I can offer evidence that these oysters were not overburdened by the artificial environment under which they were kept. During these experiments the oysters were maintained in the experimental apparatus for months at a time and in some cases substantial growth occurred. For instance, an oyster placed in the apparatus on January 23 increased in length 15.5 mm and in total volume 17.5 ml by June 23. Under the experimental conditions imposed, this particular oyster exhibited a maximum pumping rate of approximately 35 liters an hour. Other animals, although not retained as long, showed signifigrowth and exhibited pumping rates over 40 liters per hour.

The samples for oxygen determinations were all drawn while the oyster was pumping water. In addition to the automatically recorded pumping rates, the rates were directly determined for the precise moment that the oxygen sample was drawn by collecting the exhalant water in a graduated cylinder.

The Oysters

The oysters used in these experiments came from various localities around Pensacola, Florida. The individuals were initially selected according to the adaptability of their shape to the attachment of the rubber dam and their freedom from boring organisms. Their condition could not be determined until a sufficient record of their valve and pumping activities was made. Even then their glycogen content, freedom from disease, and internal parasites could not be ascertained. As the work progressed, the investigator recognized a normal pattern of activity which was characteristic of those animals showing significant growth and maximum pumping.

Terminology

In work of this nature, dry weight of the respiring tissue is ordinarily used as the common denominator for the comparison of animals of different sizes. In this case the observations for a given individual were made over a long period of time and determi-

nation of the dry weight of the tissues prior to the completion of the experiment was impossible. Because of the variability in the glycogen content, the state of the gonads during the spawning season, and a variety of other physiological factors, the weight of the meat at the end of the experiment would have had little meaning relative to the pumping function throughout the long experimental period. The volume of the shell cavity was used as the best available measurement providing some degree of constancy. In examining the data and making interpretations, the source of error in using cavity as a measure of the animal's average volume is to be borne in mind.

The volume of the shell cavity for calculations was determined by the difference between the total volume of the intact oyster and that of the valves after the meat was removed; both volumes were obtained by displacement measurement. The primary data are reduced to milligrams of oxygen per liter of water (at a specified pumping rate) per milliliter of shell cavity.

The transport of water through the branchial systems of sedentary filter feeders has been designated by the terms "filtration rate," "feeding rate," and as I have done here, "pumping rate." Filtration infers that the animal may be filtering water but not necessarily feeding, while "feeding rate" implies actual feeding. By definition, pumping rate implies neither. It has not been shown that all water which is transported through the body of an oyster has been filtered; it is possible that part is passed through for respiratory purposes. I have used "pumping rate" throughout this paper to connote the total water passed through the body of the animal without designating whether it is filtered for food, just filtered, or utilized as a respiratory medium.

In the analysis of the data which follows it will be necessary to refer to the types of shell movement. These are Phases I, II, and III described by Collier *et al.* (1953). Phase I is the brief period during which the valves move from the position of complete closure to the degree of gape characteristic of the testing period or Phase II. During Phase I there is often a dripping of water from the exhalant side of the animal which is difficult to sample. This is probably water which was entrapped by the valves when they closed. This fluid, when successfully collected, was always nearly depleted of oxygen. In Phase II the valves are only partly opened and the pumping rate fluctuates between about 4 and 12 liters per hour. The above authors regarded this as a "testing period" because they observed that if certain unknown substances responding to the N-ethyl-carbazole test for carbohydrates were not above threshold levels the animal would close without going into Phase III, or the phase of active pumping. Phase III was characterized as one in which the maximum gape and maximum pumping rates occurred. For example, an oyster with a shell cavity of approximately 40 ml pumped over 40 liters an hour during this phase.

Sea Water Conditions

Our experimental arrangements did not include provisions for controlling temperature or salinity. The sea water circulating system drew water directly from Santa Rosa Sound, Florida. Temperature and salinity were determined regularly, but the influence of pumping rates of the oysters on oxygen uptake proved to be so great that the effects of other factors were obscured. There were undoubtedly some variations due to salinity, temperature, and viscosity but our data are not sufficiently abundant to demonstrate their effect. The discussion of the experimental results will, therefore, be confined to oxygen uptake and pumping rate only.

Experimental Results

Oxygen Uptake From Pumping Rates

The results from the four oysters, on which we obtained the greatest amount of information and the most consistent data, are presented in A and B of Figure 1. For this group of animals the most noticeable attribute common to all four cases (curves A and B) is the tendency for the points to group roughly along the locus of a parabola. Since the lowest pumping rates generally follow periods of closure, oxygen tends to be removed from the water more rapidly at the lower pumping rates with a decreasing rate of removal as the flow rate of the water increases. The total oxygen removed from the water

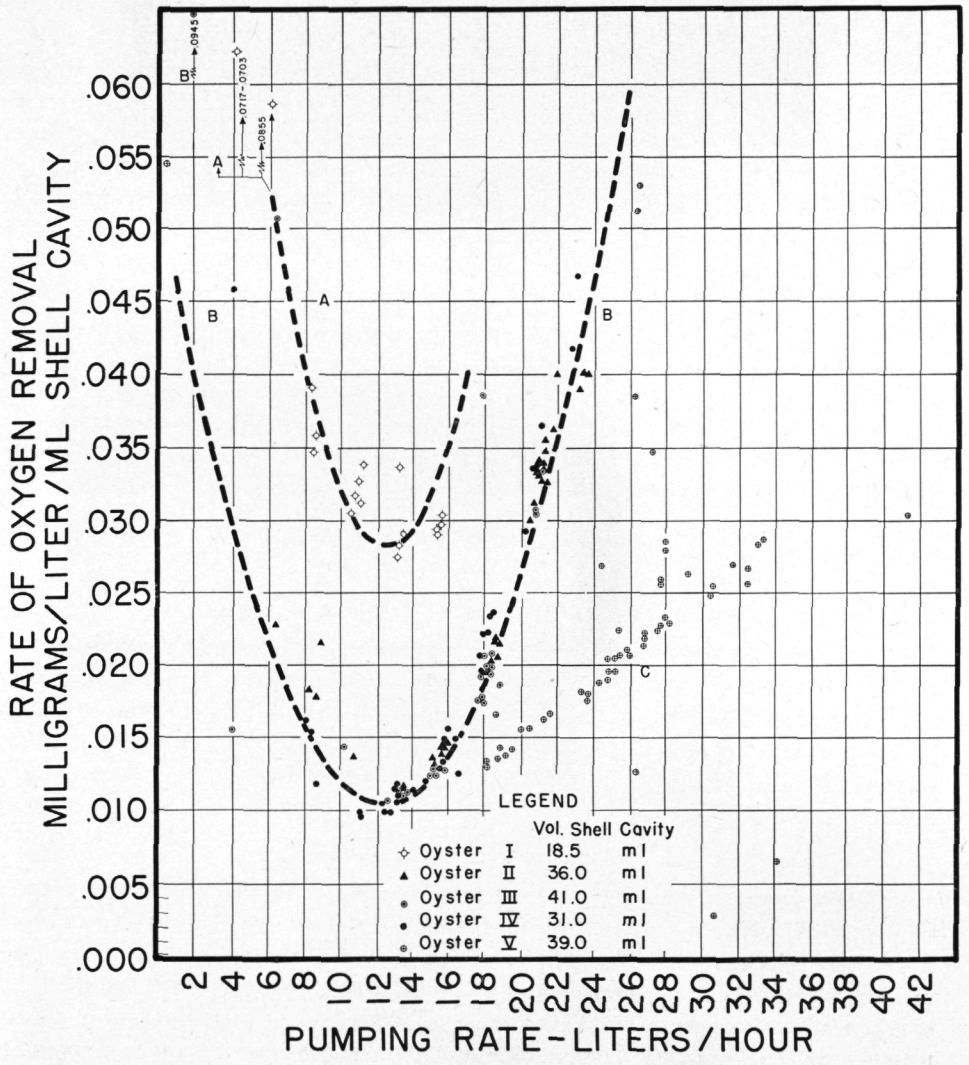

Fig. 1. Rate of oxygen removal by oysters, milligrams of oxygen removed per liter per milliliter of shell cavity plotted against pumping rate. To obtain hourly oxygen utilization, multiply values on ordinates by total volume of shell cavity times desired pumping rate.

actually increases as the curve approaches the minimum because the increased volume of water more than makes up for the decreased rate of removal of oxygen per unit of water (Figure 2). At a point between 12 and 13 liters per hour the rate of removal of oxygen per unit of water pumped begins to increase with increased pumping rates, and the right limb of the parabola is formed. This limb represents the pumping rates characterized as Phase III pumping and is characteristic of an actively pumping oyster. The 12 liter

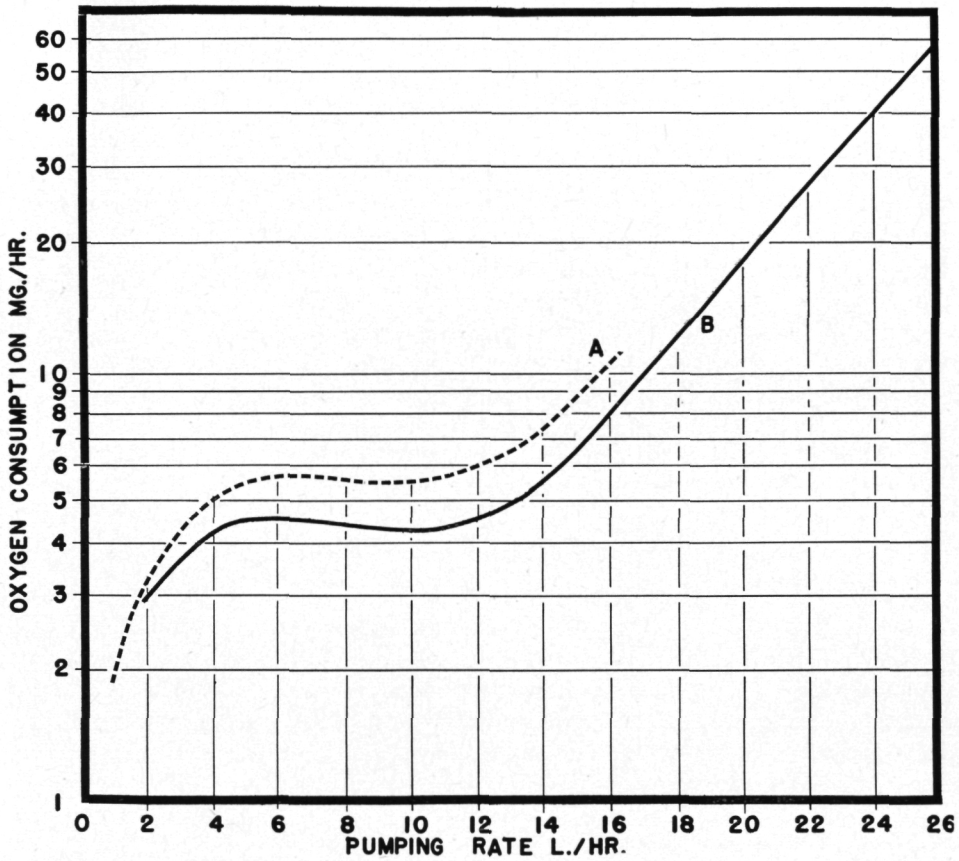

FIG. 2. Total oxygen consumption of oysters pumping at various rates. "A" is for oysters of 18 ml shell cavity and "B" is for oysters of 36 ml shell cavity. Curves derived from Figure 1.

per hour point is at the maximum pumping rate of the testing period and since the oxygen uptake is low this may represent the rate at which the oyster can circulate water through a minimum portion of the branchial system with a minimum of work. Above this, the total oxygen consumption may reach 40 mg per hour for an oyster with a shell cavity of 36 ml (about a three inch oyster) at a pumping rate of 24 liters per hour (Figure 2).

The curve for the rate of oxygen removal from the water for a small oyster is shown by curve A, Figure 1. Although there are insufficient observations to completely resolve the curve, it is clear that the smaller animal has a higher rate of intake per unit size than the larger one, an observation consistent with general knowledge concerning respiration. The parabolic relationship is suggested in this case because of the similar case for the larger oysters.

The loci of the parabolas, shown as dashed lines through the points, were approximated empirically and are expressed by the following equations:

$$\text{For curve A } y = \frac{(x - 12.4)^2 + 45.6}{1627}$$

$$\text{For curve B } y = \frac{(x - 12.4)^2 + 38.4}{3661}$$

where x = pumping rate in liters per hour and y = rate of oxygen removal from the water in mg/liter/ml shell cavity.

The results obtained from one oyster to the next were not as precise as these equations infer. Curve C, in Figure 1, illustrates this point. There is no information which will satisfactorily explain this type of respiratory behavior, although it is to be remembered that these observations were made over a period of some months and the variations could represent some metabolic change in the oyster during that time. Even so, the total uptake for this oyster during Phase III pumping rates was much more than the values of Mitchell 1914 and Galtsoff and Whipple 1931 and while pumping at 32 liters per hour it utilized approximately 37 mg of oxygen per hour. In view of the relatively low oxygen requirements reported by other workers, these large values require an examination of their significance in terms of nutritional requirements and work output.

Discussion

Work and Pumping Efficiencies

The principal difference between our results and those reported by other workers is that the oxygen uptake was measured while the oyster was doing measurable work. Jørgensen (1952) attempted this using the method of Fox (1937). This permitted measurement of pumping rates up to 16 liters per hour, slightly beyond the 12.4 liter per hour transitional point mentioned above. As a result, it is impossible to compare values simply in terms of oxygen uptake. The constants for computing the data used in the discussion of the next few paragraphs are listed in Table 1.

Table 1
Data for computations

1 ml oxygen (STP) weight 1.43 mg
1 joule = 10^7 ergs
1 calorie = 4.185 joules
1 mg carbohydrate = 4.1 calories
1 mg oxygen equivalent to 1.17 mg carbohydrates
1 mg oxygen yields 3.5 calories in combining with carbohydrate
1 gram = 980 dynes
Mean density of sea water pumped by oysters = 1.015 gm/ml
1 ml sea water, mass 1.015 gm/ml weighs 995.38 dynes.

In Figure 3 the rate of oxygen consumption (illustrated in Figure 1) has been converted to work done in joules for the various pumping rates, B-B′. For comparison, the theoretical curve for the transfer of potential energy required to move similar volumes of water is also plotted, A-A′. The latter is calculated according to Jørgensen (1955) from the relationship:

ml pumped times pressure in cm times weight of 1 cm in dynes/cm^2 = work in ergs.

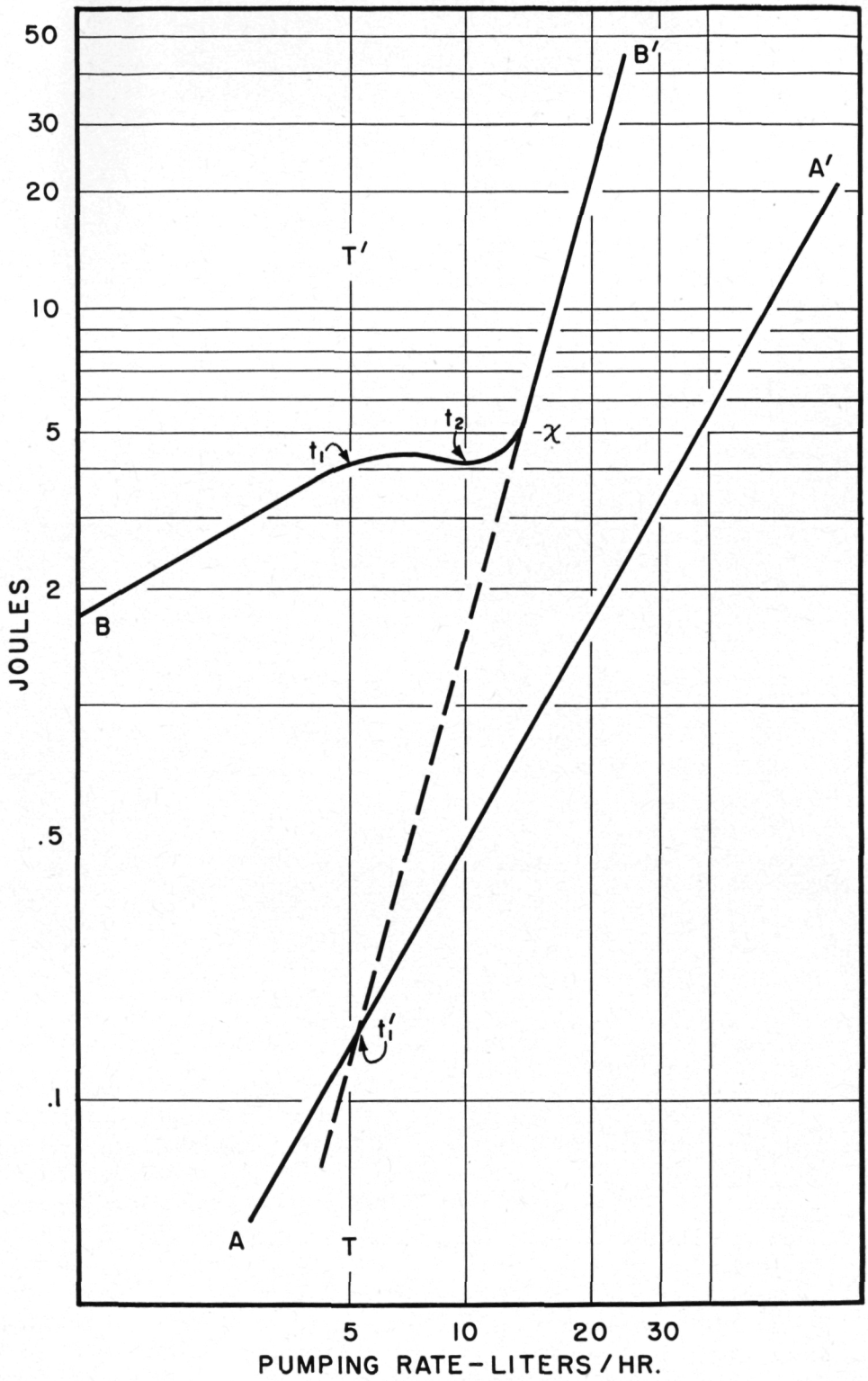

FIG. 3. Comparison of work done at various pumping rates as calculated from oxygen uptake with theoretical requirements (without internal resistance, etc.). B-B′ curve computed from oxygen uptake values derived from parabolic relationship shown in Figure 1. A-A′ theoretical work required for pumping rates indicated (Based on equation given by Jørgensen, 1955).

The form of B-B′ is determined by the parabolic nature of the primary oxygen uptake curve. That part of the curve to the left of the 12 liter/hr. intersect comes from the left limb of the parabola and that to the right from the right limb. It is clear that the two limbs concern two entirely different aspects of oyster respiratory behavior. That part of the curve B-B′ up to t_1, as mentioned earlier, represents the recovery from the oxygen debt incurred during closure. The region t_1–t_2 encompasses the range of pumping rates which has been designated as Phase II (testing period). Our interpretation here is that from 5 to 10 liters per hour can be pumped with little difference in oxygen uptake, with the differences in volume varying according to the amount of ciliated tissue involved in actual pumping. It is possible that the oxygen uptake during this interval approximates that of the basal metabolism of the animal. At any rate, the benefits accrued from the water pumped within this range are gained by a minimum expenditure of energy.

If the right extension of the curve is extended from the inflection X to where it intersects A-A′ at t'_1 we can fix the point at which pumping would theoretically be 100 per cent efficient. It may be more than coincidence that this point is nearly at the five liter per hour level and corresponds to t_1 on curve B-B′. In other words, beyond t_2 all cilia available for pumping are at work and the inefficiency of the ciliated epithelium comes into play. As in other power-work systems, efficiency decreases as the rate of work output increases beyond a certain critical point. For these oysters, the efficiency at 10 liters per hour is 10 per cent, at 25 liters per hour it is five per cent. By extrapolation we would expect the ciliated epithelium of an oyster pumping at 40 liters per hour to be about 1.5 per cent efficient. The latter is in close agreement with Ludwig's (1931) estimate of two per cent for the individual ciliated cell but my value for 10 liters per hour is not. One and five tenths (1.5) per cent is approaching zero efficiency and is probably indicative that 40 liters per hour is near the maximum pumping capacity of the animals in this size range. Larger animals could pump greater quantities of water but they would also require greater amounts of oxygen.

In computing the values for A-A′, I used a pressure range of 0.1 cm at one liter per hour to 0.9 cm at 25 liters per hour. These were interpolated from my observation that an oyster pumping at approximately 40 liters per hour produces a one cm hydrostatic head. This is consistent with Nelson's (1936) observation of 0.7 cm at 16 liters per hour.

Relationships Between Oxygen Uptake, Energy Requirements, and Available Food

Adequate data on the caloric values of suspended and dissolved organic materials are not available. However, some useful information can be derived from the literature indirectly by examining data for types of organisms which may serve as oyster food. *Nitschia closterium* is representative of the size of some of the smaller phytoplankton organisms and *Prorocentrum* is a typical member of the flagellate flora of the marshes and is somewhat larger. The contribution that these organisms can make towards meeting the caloric requirements of the oyster will be compared in the following paragraphs.

The dry weight of *N. closterium* is given by Ketchum and Redfield (1949) as 2.32×10^{-11} grams per cell. These organisms were from laboratory cultures and may not be typical. Also, the species may not be as large as many organisms available to the oyster as food. Based on other material from Ketchum and Redfield (1949) the average composition of algae can be approximated as: protein 45%; carbohydrate 30%; and

lipids 25%. Unfortunately, no data are available on the caloric values of these materials as they occur in plankton, but from Hawk, Oser, and Summerson (1947) we have the following: mixed plant and animal protein 4.1 large calories per gram, carbohydrates 4.1 large calories per gram, and fats 9.3 large calories per gram. For the present application these figures will aid us in getting the overall picture, although it is realized that some revision will be in order as more experimental results become available. The computations to be explained are summarized in Table 2.

TABLE 2

Postulated composition and energy content of *Nitschia* type cells
(Postulated dry weight of 10^6 cells 0.02 mg. Such cells as *Isochrysis galbana* are similar in size)

Food class	Percent	mg/10^6 cells	cal*/mg	cal*/10^6 cells
Protein	45	.009	4.1	0.0369
Carbohydrate	45	.006	4.1	0.0246
Lipids	25	*.005*	9.3	*0.0465*
Totals		.020		0.1080

* Note that these units are *small* calories.

Bainbridge (1957) has reviewed the literature on phytoplankton concentrations and from this we can judge that a range of 0 to 10 million cells per liter would be reasonable for inland waters although μ-flagellates are reported in much greater concentration (Droop, 1954). This then gives us an estimate of up to 1.08 calories available per liter of water if all the organisms are similar to *N. closterium* in size.

Let us assume that the basal metabolism of the oyster is indicated by the oxygen uptake during the testing period. At a pumping rate of 10 liters per hour, 4.4 mg O_2 per hours is consumed. At 10 liters per hour and a concentration of 10^6 cells per liter of *N. closterium* 1.080 calories could be acquired, assuming for the moment 100 per cent filtration and assimilation. An uptake of 4.4 mg O_2 represents 15.4 calories or 14.3 more than available in the phytoplankton present. If these oxygen consumption values are even approximately correct it is clear that the animal is either using a reserve of stored energy or is capable of utilizing dissolved organic materials directly from the water, or else the *Nitschia* type of food is not adequate except in unlikely concentrations. Even at 12×10^6 cells per liter the caloric value just barely matches the 10 liter per hour expenditure of energy by the oyster.

The dinoflagellate genus *Prorocentrum* is a common organism in the marsh and estuarine plankton. Collier (1958) published data relative to its carbohydrate content and its production of non-particulate carbohydrate in laboratory cultures. Although it is not presumed that these data are applicable to field conditions, they will be useful in discussing the problem at hand.

The cell volume of this organism is estimated at 104 cubic microns. This amounts to 10^{-2} $cm^3/10^6$ cells, and using 25 per cent as a median value from the estimates of Ketchum and Redfield (1949) for solids, we find a dry weight of approximately 2.5 mg/10^6 cells. Using the same food-class composition as applied in the case of *Nitschia* we derive the caloric value of *Prorocentrum* as shown in Table 3.

One million cells provide an estimated 13.6 calories which is about 125 times the value for *N. closterium*. Since there are no estimates on the caloric values based on actual calorimetry this difference is due solely to volume of cells. It is interesting to

TABLE 3

Postulated food-class composition and energy vaule of *Prorocentrum* type cell

Food class	Percent dry weight	mg/10^6 cells	cal*/mg	cal*/10^6 cells
Protein	45	1.13	4.1	4.663
Carbohydrates	30	0.75	4.1	3.075
Lipid	25	0.63	9.3	5.859
Totals		2.51		13.597

* Note that these units are *small* calories.

note that an estimate of 0.75 mg carbohydrate/10^6 cells based on the 25 per cent value arbitrarily selected from the data of Ketchum and Redfield differs only by 0.55 mg measured by the N-ethylcarbazole method (Collier, 1958).

If 10 liters of water containing 10^6 cells per liter of this organism were filtered, the total yield could be as much as 135 calories or almost nine times the caloric equivalent of the 4.4 mg O_2 uptake.

Beyond this minimal phase of oxygen uptake, beginning at about 12 liters per hour, the increase in oxygen consumption with increased pumping rate is very rapid. At the 24 liter per hour level the caloric equivalent would be approximately 150 calories per hour. A population of *Nitschia* of 12×10^6 cells per liter being filtered at 24 liters per hour could provide a maximum of only 35.0 calories. However, at this pumping rate, a concentration of only 10^6 cells per liter of *Prorocentrum* would supply about 350 calories. In the latter case there would be no problem of the oyster using more energy to pump than it can take from the water. The concentrations indicated, therefore, seem reasonable, particularly when it is considered that other types of cells are usually present and contribute to the total nutritional value of the water.

Another phase of the relationship between the oyster's pumping activity and the organic content of the water pumped involves a possible sensory stimulation by non-particulate carbohydrates. This was reported in some detail by Collier *et al.* (1953). After making successive hourly observations (day and night) for over 18 consecutive months, it was found that pumping responded directly and quantitatively to substances whose concentration could be estimated by the N-ethyl-carbazole reagent for carbohydrates. The evidence suggested that these materials were at least in part dissolved and were produced by microorganisms. If their concentration was not above certain thresholds when the oyster opened for testing the water the oyster closed. If the concentration was sufficiently high, pumping continued at a rate which thereafter fluctuated according to changes in the carbohydrate concentration.

Such a mechanism could have high survival value for the species. Immobile filter feeders are dependent on what the water circulation brings to them. Continual filtration would soon use up all energy reserves. (A fat oyster three inches in length with a six per cent glycogen content pumping at 10 liters per hour would burn all of its reserve in about 400 hours if there were no replenishment.) Random filtration would provide only a moderate improvement. This mechanism enables the oyster to test the water and pump when the likelihood of obtaining food is greatest.

This stimulating function of the dissolved materials, whatever they may be, is not necessarily their only significance. If the basal metabolism maintains a level at which five mg of O_2 per hours is consumed, the carbohydrate equivalent would be 4.3 mg of

carbohydrate on combustion. The combustion equivalents of carbohydrate would be on the order of 35 to 40 mg per hour at the higher levels of pumping rates. A series of determinations of the rate of removal of carbohydrate from the water pumped by a single oyster were made to learn if the quantities actually removed were significant. The results are shown in Figure 4. The actual observations are indicated as points in the scatter diagram and the theoretical amount of carbohydrate required for the various oxygen consumptions (shown in Figure 1) are represented by the curve about which the points are scattered. The agreement of the points with the curve is surprising. Since this makes it appear that the oyster actually removes carbohydrates from the water at rates commensurate with the energy requirements of the work done in pumping water, these and related questions must be answered: (1) Can the animal automatically adjust its pumping rate to the level of energy available in the water? (2) Are the ciliated cells

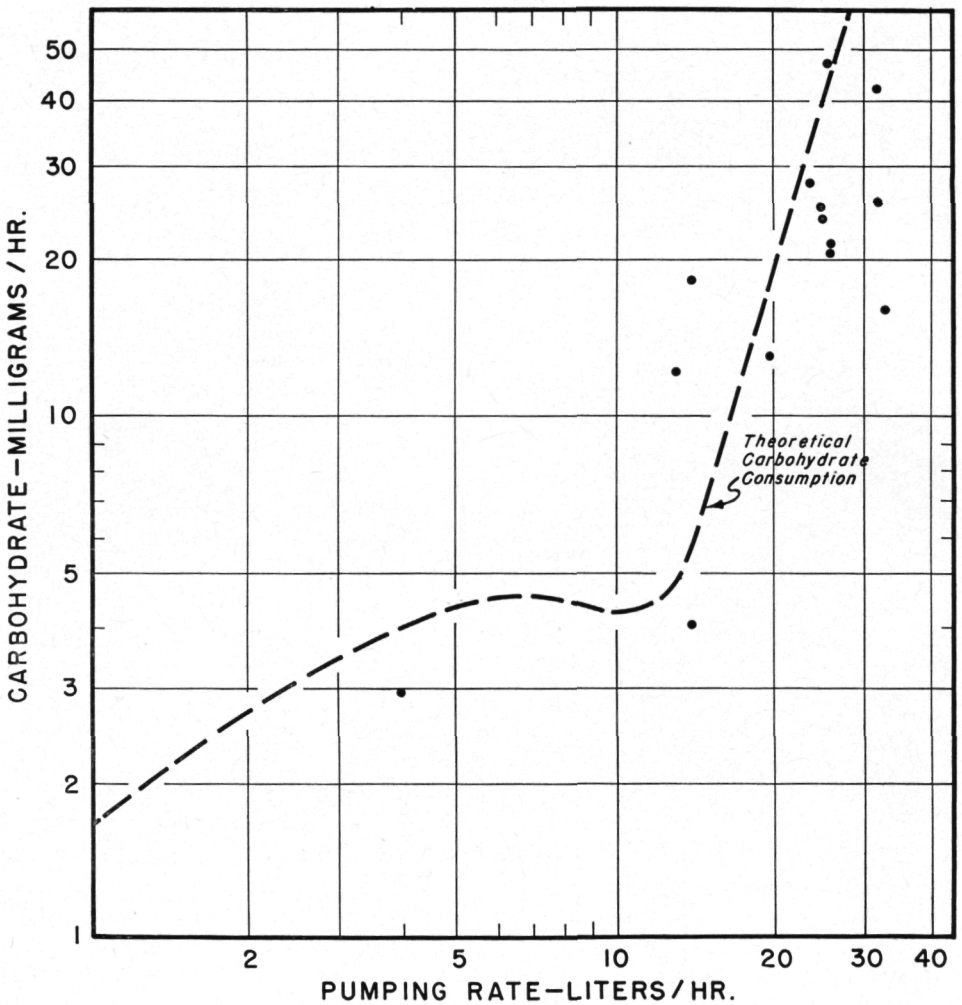

FIG. 4. Carbohydrate consumption for various pumping rates derived from oxygen uptake curve of Figure 1. Dots represent actual observations on carbohydrate removed from water by oyster at pumping rates indicated.

capable of utilizing dissolved materials directly from the water? (3) Is there another explanation? The available data are insufficient to resolve these problems but additional effort would be justified.

Jørgensen (1955, p. 423) may have generalized too broadly in indicating that Collier, Ray, and Magnitzky (1950) and Wangersky (1952) reported up to 100 mg of carbohydrate per liter for all sea water and in indicating that one mg removed for each liter of water pumped is several times the food requirement of oysters. Up to 100 mg of material per liter was found in some rich marsh waters and a concentration of dense plankton from a red tide outbreak yielded values of 50 mg per liter. However, as shown in the report of 1953 (Collier *et al.*), the Gulf of Mexico waters are generally poor in carbohydrates while the estuaries are comparatively rich. The results reported on oxygen uptake in this paper would indicate that the oyster has a high energy requirement when pumping at maximum rates and that the amount of carbohydrate removed is consistent with this.

Guillard and Wangersky (1958) published an account of some experiments designed to demonstrate the relationship between concentrations of algal cells in artificial cultures and the accumulation of extracellular carbohydrates in the culture medium. They concluded that ". . . carbohydrate production in cell-free medium did not parallel cell numbers in cultures of any of the organisms studied. Such accumulation as occurred began with the end of the logarithmic phase of growth, and in most instances, increased during the stationary phase or senescence of the cultures. The fact that killing cells liberates comparatively large amounts of carbohydrate supports the idea that its presence, particularly during the phase of actual growth, is due largely to the death of the cells."

These statements contain the premises on which the authors, in speaking of natural populations, base the additional conclusion that "assuming that these organisms behave like stationary cultures the maximum amount of carbohydrate liberated would not exceed one mg/L." They pointed out that the relative sizes of organisms were not considered.

When the cell counts of Guillard and Wangersky (1958) are converted to mg dry weight/l and plotted with extracellular carbohydrates in mg/l against time on semi-log paper, it is seen that an interpretation different from theirs is possible.

Using an estimated volume of 72 cubic microns for *Monchrysis lutheri* and applying the same constants as used for the other organisms in an earlier section of this paper, we can estimate the dry weight at about 0.018 mg/10^6 cells. Applying these factors to Guillard and Wangersky's data and plotting as described above, Figure 5 is obtained. This method of graphing reveals a definite tendency of extracellular carbohydrates to increase with the growth of the culture, even during the log phase. The tendency is even more clear in the case of *Prymnesium parvum*. The fact that my estimates of cell volume for these organisms may be in error would change the positions of the curves but not their slopes.

A point of special interest is the curve for carbohydrate production per mg of cells. Apparently the cells are producing carbohydrate at a declining rate during the logarithmic phase but, nevertheless, they are producing it. The total carbohydrate increases because cell multiplication more than compensates for the decreased rate of production per unit of cells. It is noticed that the minimum rate of carbohydrate production coincides with the termination of the logarithmic phase.

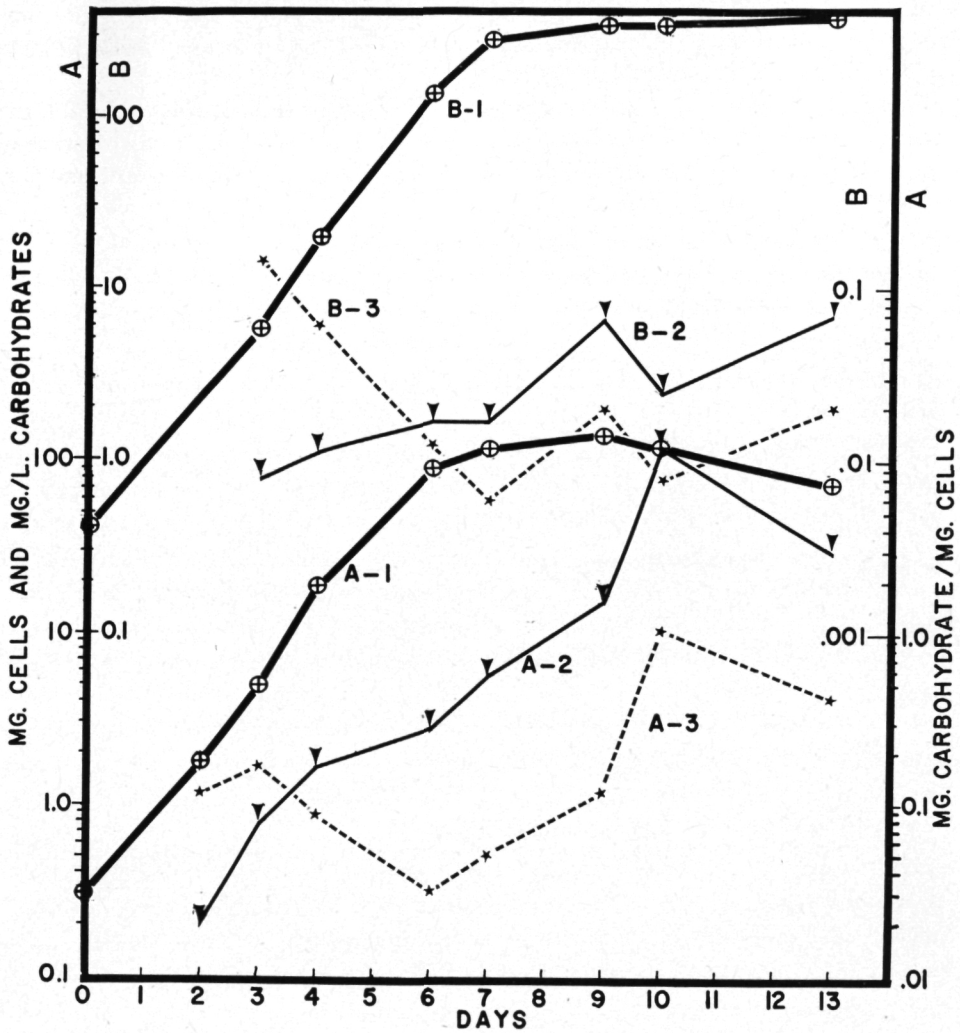

FIG. 5. Production of extracellular carbohydrates by unicellular algae. For *Prymnesium paruvim* "A-1" represents growth rate of algae in mg dry weight. "A-2" is the rate of accumulation of total extracellular carbohydrate as mg/l and "A-3" is the rate of production of carbohydrate per mg of cells (dry weight). For *Monochrysis luthuri* "B-1," "B-2," and "B-3" are respectively the same as "A-1," "A-2," and "A-3" above. Data from Guillard and Wangersky, 1958, p. 452, Table 1.

Since the oyster may be able to regulate its pumping activity according to the food content of the water, these points may become pertinent. In addition, it is conceivable that the "free" carbohydrates could be used as an energy source directly by the ciliated epithelium. Thus, they would be useful to the animal as a means of conserving energy if their abundance were related to the algal concentration.

Summary

1. Several workers have studied oyster respiration (Mitchell, 1914, Galtsoff and Whipple, 1931, and Jørgensen, 1952), but none of these have measured oxygen uptake over the full range of pumping rates of which the oyster is capable.

2. A modified Winkler method using the syringe pipette of Krogh (1935) was adopted for the determination of dissolved oxygen. Reagents were modified according to Pomeroy and Kirshman (1945).

Oxygen uptake by the oyster was determined by difference between inhalant and exhalent water. The experimental arrangement of the oysters was a combination of the rubber dam method and the constant level tank as described by Collier and Ray (1948) and Collier *et al.* (1953).

3. Shell cavity volume in ml was used as a common denominator for reduction of the data because of differences in size of oysters and possible changes in glycogen content over long periods of observation.

4. "Pumping rate" is used to designate the rate at which the oyster moves water through its branchial systems to avoid the ambiguities of "filtration rate" or "feeding rate."

5. The oyster exhibits three different phases of gape (or degrees of openness) of valves, each characterized by a magnitude of pumping rate. These phases must be included in a consideration of respiration.

6. Analysis of oxygen uptake data in terms of pumping rates yielded parabolic curves, with the minimal uptake in each case occurring at a pumping rate between 12 and 13 liters per hour. Beyond this point the uptake increased to its maximum very rapidly. A three inch oyster pumping at a rate of 24 liters/hr. may consume 40 mg of O_2 per hour.

7. A comparison of oxygen uptake and work performed in pumping water suggests a maximum efficiency during Phase II pumping (the testing phase). A theoretical efficiency of one to two per cent at maximum pumping rates is deduced.

8. By theoretical considerations, the caloric value of different types of plankton organisms are compared to the energy demand of the oyster in terms of oxygen uptake. It is found that high concentrations of cells of the *Nitschia* type could not provide sufficient energy to balance that expended in pumping, even with 100 per cent utilization, while cells of the *Prorocentrum* type could easily provide adequate energy.

9. It is suggested that extracellular carbohydrate (as reported by Collier *et al.*, 1953) could supply a directly available source of energy for the ciliated epithelium in pumping water and at the same time provide a means for the oyster to regulate its pumping according to the concentration of derivable plankton elements. It is possible that the necessary sensory mechanism for accomplishing this exists.

10. The data of Guillard and Wangersky (1958) suggests that extracellular carbohydrates are produced in a manner related to the numbers of cells in a population of algae, although these authors conclude that such is not likely.

11. Also from the data of Guillard and Wangersky (1958), it is found that the rate of carbohydrate production per unit of cells diminishes as the logarithm phase progresses, but the decrease is more than compensated for by the increasing number of cells. The net result is an increasing amount of extracellular carbohydrates during the logarithmic phase of cell growth.

12. It is believed that the problem of extracellular carbohydrates is of greatest importance in the study of oyster nutrition.

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