CV. THE RESPIRATORY EXCHANGE OF THE MUSSEL (*MYTILUS EDULIS*, L.)

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ALTHOUGH much work has been done, notably by Vernon and Montuori, on the gaseous metabolism of marine and fresh-water invertebrates [*vide* summary given by Krogh, 1916], resulting in certain generalisations as to the influence of temperature, oxygen pressure and other physical factors upon the respiratory exchange, little or no work has been recorded bearing upon fluctuations of metabolic activity associated with the sexual and seasonal cycles. That such long-period fluctuations do exist has not, of course, been doubted, and the disparities between the results of different observers, or indeed of the same observer at different times, upon a given animal, have been attributed to physiological conditions, such as age, sex, or reproductive maturity, but no attempt has been made to give quantitative expression to these factors. The reason for this neglect is doubtless the fact that the difficulties inherent in the measurement of all biological responses are heightened in this case by the necessity of maintaining the organisms for long periods under controlled cultural conditions and by the uncertainty attached to the determination of age and maturity.

For the present investigation, an organism has been selected which combines with economic importance certain qualities which render it suitable for experimental work, such as hardiness under artificial conditions, ease of measurement and manipulation, and marked regularity of physiological rhythm. Individual variations have been reduced to a minimum by the use of large numbers of mussels in each experiment, and by statistical treatment of the results. The determinations, upon a carefully selected stock of mussels, were made at intervals throughout a complete reproductive cycle of one year, under conditions of temperature and oxygen pressure similar to those obtaining at the time. In this way, the mean respiratory values obtained during the course of the experiments, without any correction or "weighting," represent a close approximation to the sequence of metabolic changes as they occur in a natural state on the mussel-beds—a sequence of which every stage is the integrated response of the organism, as a living entity, to the ever-changing factor-complex of its environment. At the same time, by a statistical sorting-out process, the same experimental values have afforded data from which the

relative effects of the separate factors, both physical and physiological, have been calculated.

Certain aspects of the results here put forward stand in a most interesting relation to a study of the seasonal changes in the chemical composition of the mussel, carried out by Daniel [1921, 1922, 1923, 1925], and are discussed in detail on pp. 845 and 846.

EXPERIMENTAL.

The stock of mussels.

In order to ensure a reasonable uniformity of size and condition in the experimental material, a special stock was set aside at the outset of the work. This consisted of about 500 mussels, selected from a hundredweight taken from the beds at Ramsey, Isle of Man. By means of a simple "hit-and-miss" gauge, mussels between 5.0 and 6.0 cm. in length were alone selected, giving a stock of mean length 5.34 cm., and these were carefully scraped clean from any barnacles or other growths, and kept in a large shallow concrete tank of 120 litres capacity in one layer for the entire period of the work. Unfiltered water, pumped daily from the sea, was run through the tank continuously, at the rate of 240 litres per hour. No food of any kind was added to the water, but the sufficiency of the plankton which it contained was evident from the apparent well-being of the mussels and the great abundance of faecal matter, which was removed at intervals by completely emptying and brushing down the tank. The monthly mean temperature of the water varied between 7.5° in March and 15.5° in July; this range is very close to that of the coastal waters of the Irish Sea, which, for the 10-year period 1914–1923 was found to be 6.9° (March) to 14.7° (August.) The conditions under which the mussels were kept did not, therefore, differ widely from those obtaining on the mussel-beds, and even when mussels were removed from the tank for a respiratory determination, the experimental temperature was arranged to lie between the maximum and minimum, as then recorded, of the immediately preceding period. There was a slight mortality among the mussels amounting, at the end of the year, to about 11 % of the initial number, the deaths being spread very evenly throughout the period.

Variations in total wet weight, tissue weight, and shell weight, are recorded in the experimental series (p. 837); comparisons were made, from time to time, with mussels of the same length-range taken directly from the Ramsey beds, and in all cases the total weight, tissue weight and shell weight of the tank-material slightly exceeded those of the "wild" specimens. The length of the mussels (maximum length, with valves closed, as measured by a calliper gauge) increased during the year by 0.04 cm., or only 0.75 % of the initial mean length; there was, however, a considerable increase of shell weight. The entire weight of the individual mussels varied between 20 and 21 g., and each contained about 6 g. (wet weight) of living tissue.

Apparatus and method.

The apparatus devised for the respiratory determinations embodies several new features, and as it is suitable for a variety of aquatic organisms and is easy of manipulation, it will be described in detail. The respiratory chamber, *A* (Fig. 1), consists of a heavy glass vessel of about 6.5 litres capacity. The lid and lower portion, which are ground into smooth contact, are provided with flat flanges to facilitate clamping together when in use. Through a rubber stopper in the tubulure of the dome-shaped lid is fitted a telescopically arranged series of glass tubes, the innermost of which, *B*, reaching to the bottom of the vessel, and bent twice at right angles, forms a syphon outflow; the tube *C*,

Fig. 1.

carrying a side branch and funnel at its upper end and reaching to the level of the lid-flange below, serves for filling the vessel or replenishing from the 10-litre reservoir, *F*, water withdrawn by the syphon, *B*; the outermost of the three tubes, *D*, carries a four-bladed ebonite stirrer, *G*, wholly contained within the dome of the lid, and a V-grooved pulley, *H*, whereby the stirrer is connected to a water-turbine or other motive arrangement. Other short lengths of tubing, *E*, associated with the central "telescope," act as bearings for the stirrer-shaft. Only one other tube, *J*, passes through the stopper. This ends flush with the underside of the stopper, and externally is fitted with a short length of pressure tubing and a spring clip; it serves as an air-escape, permitting the vessel to be completely filled with water; it is also temporarily unclipped when a sample is to be withdrawn by the syphon.

For convenience of handling, the mussels are not placed directly into the glass vessel, but into a special cage, *K*, made of perforated sheet-celluloid, which closely fits the respiratory chamber.

Such a large and heavy vessel cannot readily be placed in a thermostat of the ordinary type, but a very fair constancy of temperature is secured by having it supported, on radially projecting lugs, in a cylindrical galvanised iron tank, *L*, filled with water to the level of the broken line, and heated by a micro-burner, *P*, or cooled by ice, as the case may be. This tank has a loose drop-on lid, *M*, slotted for the tubulure of the respiratory vessel, perforated for the passage of a thermometer, and provided with two inspection holes 5 cm. in diameter. There is also a constant-level device, not shown in the figure, and a strong wire stirrup or handle, by means of which the respiratory vessel may be lifted from the tank. The tank itself is lagged with felt, to minimise heat-exchange, and the entire apparatus is mounted in a skeleton framework, *N*, of wood, and rests directly upon a "uralite" shelf, *O*, with a central perforation, to permit of heating, when required, by the burner in the space beneath. The two hinged strips of wood, forming the "lid" of the supporting framework, close tightly upon the cylindrical tank; they also carry upright rods, to which the funnel and central column are clamped when the apparatus is in use.

At the time of a respiratory determination, forty mussels are "selected at random" from the stock tank, and after a light scrubbing to remove diatoms or other surface growths and drying on a duster, their length, weight and volume are determined. The mussels are then placed in the perforated celluloid basket, which is lowered into the respiratory chamber, previously filled with seawater. The apparatus is then assembled, the lid being luted on with rubber tap-grease and afterwards clamped, and the stirrer connected up to its motor. Water is also filled into the galvanised tank. Normally, the experiment is carried out at the temperature of the water in the stock-tank, and this is readily maintained by the water-jacket for several hours, since it is not likely to differ by more than one or two degrees from the air-temperature.

The mussels remain undisturbed for about half an hour, and then all the

water is run off, to be replaced immediately from the reservoir, the respiratory chamber being completely filled.

After five minutes, the stirrer is put into gentle rotation and the initial samples run from the syphon. The samples are collected in two stoppered bottles; one, of about 230 cc. capacity, for oxygen determination (p. 834), and the other, of 270 cc. and paraffin-coated, for determination of p_H and excess base (p. 835). These two samples, together with a small amount of water for rinsing the bottles, involve the removal of about 535 cc. from the respiratory vessel, or about 1/12th of the total volume, after deducting that occupied by the mussels and the celluloid basket. A corresponding volume of water is run in from the reservoir, the clips are closed, and respiration allowed to proceed for a predetermined period, usually 30 or 60 minutes, with occasional gentle stirring. At the end of the period, another pair of samples is withdrawn, and the volume again made up, each time noting the temperature in the vessel and in the water-jacket. The entire operation of removing samples and refilling occupies $2\frac{1}{2}$ minutes, and for this no allowance is thought to be necessary when recording consecutive results taken at, say, 30-minute intervals. Several determinations are made, as a rule, with progressively diminishing oxygen content; finally, samples are taken from the reservoir itself, in order that due allowance may be made, in each determination, for the added unused water.

At the close of an experiment the mussels are removed from the vessel and 20 of them taken for determination of tissue and shell weight. For this purpose, each mussel is prised open, care being taken not to injure the tissues, and the included or "mantle-cavity" water shaken out; after this, the valves of the shell are kept apart by means of a small wedge, and the mussels, placed on edge in a rack, are allowed to drain for an hour. The moist tissues are then scraped out into a tared beaker and weighed; the empty shells are weighed also, and both results expressed as mean weight per mussel. The remaining 20 mussels are replaced in the stock-tank. Since the weight of wet tissue per mussel varies during the course of the reproductive cycle, it has been necessary, in certain of the experimental series, to express the respiratory values in terms of a constant weight (10 g.) of wet tissue.

The condition of the tissues was always observed during the scraping-out process, and in view of recent work by Yonge [1925], special note was made of the appearance of the crystalline style. In no case was any difference noted between the styles of experimental and "wild" mussels, nor was there any correlation between the size and condition of the style and the reproductive phase of the animal. Although, as Yonge observes, the style may persist, in *Mytilus*, during starvation, it rapidly disappears when respiratory conditions are unfavourable. Other workers have, indeed, alleged that the style disappears during starvation, but it is probable that other factors, including oxygen-want, have contributed to this result. In any case, the present finding of the persistence of the style points to a fair normality of the experimental conditions.

Oxygen determination.

Winkler's original method, as applied to sea-water by Bjerrum [1904], was used throughout, with very slight modification. The amount of organic matter present was not sufficiently great to warrant the use of Winkler's later [1916] modification.

The sample of sea-water was drawn into a bottle of accurately known capacity (about 230 cc.) when full to the stopper. Immediately there were added, at the bottom, by means of a long-stemmed pipette, without mixing, 2 cc. of a saturated solution of nitrite-free KOH in 10 % KI solution and then 1 cc. of a 40 % solution of manganese chloride, containing 2 cc. of conc. HCl in each 100 cc. The stopper being inserted¹, the contents were mixed by rotation, when the whole of the dissolved oxygen combined with the precipitated manganese hydroxide.

After an interval, never less than an hour, when the precipitate had settled somewhat, an excess (3 cc.) of chlorine-free conc. HCl was run in at the bottom of the bottle and the contents thoroughly mixed.

The iodine set free was titrated (using 2 % soluble starch solution as indicator) with a standard solution of sodium thiosulphate, containing about 4 g. of the crystallised salt per litre. This solution was conveniently standardised against potassium bi-iodate in the presence of excess of KI and HCl.

It was found desirable to make up the thiosulphate solution a few weeks before it was actually required for use, and to check the standardisation occasionally or before each set of readings. The strength of the thiosulphate being known, the oxygen-equivalent is given by the relation:

All experimental data have been expressed, in the first instance, in terms of cc. of oxygen, measured as at N.T.P., used per mussel, or per given weight of mussel tissue, per hour. In this, as in all physiological work, however, it is the oxygen pressure, and not the absolute amount of the gas present, which determines its availability for respiratory purposes. The partial pressure exerted by the gas in solution equilibrium depends upon several factors, notably the solubility coefficient of the gas itself, the temperature of the solution, the prevailing atmospheric pressure and, in the case of sea-water, the concentration of dissolved salts. In the present investigation, since only freshly drawn sea-water has been used, of very constant and fairly high salinity (Cl = 18 ‰), the salinity factor may be regarded as a constant, and it has further been assumed that the differences in barometric pressure were never

¹ It will be obvious that 3 cc. of the sample spill over the neck, when this is done, but since the added reagents also contain oxygen in solution, no significant error is introduced.

sufficiently great to warrant any special correction being made on that account. Equilibrium, too, is slow of attainment, and the oxygen content of a water sample is not necessarily in equilibrium with the atmospheric or oxygen pressure prevailing at the time of its collection.

Fox's data [1907] were used in estimating the partial pressure exerted by the oxygen present, as determined by the Winkler method, at the observed experimental temperature.

Carbon dioxide determination.

The determination of CO_2 is unfortunately much less satisfactory than that of oxygen, and for this reason the discussion of the respiratory activity of the mussel, in relation to biological and physical factors, has been limited in this paper to data based upon oxygen determinations. At the same time, the carbon dioxide values have a certain relative significance and, when regarded in conjunction with the oxygen data, furnish an indication of the respiratory quotient and its variations. These latter values stand in such evident relation with the chemical changes of the mussel tissue as to afford a partial if indirect confirmation of their validity.

Direct gasometric estimation being impracticable, a method depending upon the determination of p_{H} by indicators, previously outlined by the author [Bruce, 1924], was adopted. As there stated, the method is not one of a high order of absolute accuracy, but under standard conditions is capable of giving useful results of relative significance, especially in serial experiments. The indicator employed was *m*-cresol purple, specially supplied by the British Drug Houses, Ltd., in "0.04 % solution in 20 % alcohol, half-neutralised with NaOH." This was found to give a satisfactory virage, easily read to 0.02 of a p_{H} unit, and presumed to have a "salt-error," at normal salinity, of 0.2 p_{H} , to be deducted from the apparent reading, when compared with the usual Clark and Lubs buffer solutions. The method of determination of excess base and full working details of the p_{H} method will be found in the paper referred to. No significant change in excess base was observed in any of the regular determinations, even after a specially prolonged experiment (18 hours), in which the mussels were confined in a small volume of water from which practically the whole of the dissolved oxygen was extracted, and which became so acid as p_{H} 7.4. This is of importance as indicating that the rise of CO_2 pressure, consequent upon continued respiration, was not so great as to bring into play the buffer mechanism whereby, in *Mya* and other forms, the calcium carbonate of the shell is dissolved in the restoration of the acid-base equilibrium.

The carbon dioxide results have been expressed in cc. of the gas evolved by a given weight of tissue per hour.

RESULTS AND DISCUSSION.

As already indicated, determinations of respiratory activity were made at intervals, approximately monthly, throughout a year. On each occasion, the determination was carried out at several (up to eight) different oxygen pressures or temperatures, as the case might be, though a determination under normal (*i.e.* then obtaining) conditions of temperature and pressure invariably formed one of the series. In this way, a total of 43 readings was obtained. These were made on twelve different occasions, each reading representing the combined gaseous exchange of 40 mussels, for a period in no case less than 30 minutes. Despite the number and range of data thus made available, it was difficult, on account of the wide divergence between individual results, to discern on first inspection any general relationship between respiratory activity and physical or seasonal conditions. The inequality of the intervals between successive experiments, and the initial presumption that the smoothed results would not in any case lie upon a straight line, rendered it impracticable to apply the Method of Moments, and it was decided, therefore, to take averages of groups of results, plotting against a mean date the mean of the appropriate observations. In practice, the results obtained in any one month usually formed such a group, though occasionally any three successive observations have been averaged. This process was found to minimise random fluctuations, and gave results which, in graphic form, disclosed certain unmistakeable relations. It was felt that this method involved no great violence to the experimental values, especially since there was no evidence of sharp breaks in the plotted curves, and it was certainly justified in its result. It may be mentioned here that only one single experimental value was rejected in arriving at the means—a value so far anomalous as almost certainly to be the result of some untraced error.

The annual metabolic curve.

Observations were begun in March, 1925, and continued until April, 1926, spawning taking place at the end of May. As previously noted, 40 mussels were used for the respiratory determination, and 20 of these were subsequently taken for tissue and shell weight estimation. The former of these two values fluctuated rather widely, doubtless on account of differences in the rate of draining and drying which could not be eliminated. The successive determinations were for this reason averaged in groups of three, and are set out in Table I. Remarks upon the apparent condition of the mussels at the various dates, although made at the same time, are postponed to Table VI. The length measurements included in Table I are the means of several hundred individuals and are not subject to the group-averaging.

Table I. *Variations in total weight, tissue weight, shell weight and length, in Mytilus.*

Duration of Exp. (days)	Date	Mean length (cm.)	Mean entire weight (alive) wiped but not drained (g.)	Mean wet weight of tissues (g.)	Mean weight of shells (g.)	Mean weight of included water (by difference) (g.)
0	Mar. 5	5.34	—	—	—	—
25	Mar. 30	—	20.36	(7.20)	(10.16)	(3.01)
69	May 13	—	20.29	6.83	10.16	3.30
83	May 27	—	20.30	6.32	10.15	3.83
92	June 5	—	20.30	5.94	10.14	4.22
110	June 23	—	20.32	5.79	10.04	4.49
139	July 21	—	20.43	5.79	10.09	4.55
180	Aug. 31	—	20.57	5.83	10.60	4.14
196	Sept. 16	5.37	20.80	—	—	—
219	Oct. 8	—	21.04	5.78	10.72	4.54
267	Nov. 26	—	21.28	6.08	11.06	4.14
323	Jan. 22	—	21.02	6.04	10.87	4.11
370	Mar. 9	—	20.94	6.13	11.09	3.72
398	Apr. 7	5.38	21.02	—	—	—
408	Apr. 17	—	21.49	6.05	10.98	4.46

(Bracketed values are fair estimates.)

The above data show that the rapid loss of tissue weight from the 70th to the 90th day, consequent upon spawning, is made up for, in the total weight of the living mussel, by a corresponding increase in the included or so-called "mantle-cavity" water. There is a slow and only partial recovery of tissue weight, throughout the months following the spawning period, but the most striking feature is the rise in shell weight which, commencing in July, about the 140th day, continues until the end of November, when a constant weight is maintained. This gain in shell weight is not accompanied by any significant increase in length (0.2 mm.), and while it can hardly be supposed to have much, if any, influence upon the gaseous metabolism, the fact is of interest in the light of recent work by Orton [1925], on the conditions for calcareous metabolism in oysters and other marine animals. In this work, Orton adduces facts which point, in the case of the native oyster, to "a controlling factor represented by a minimum temperature, below which shell is not or cannot be laid down, and above which shell-material may be produced automatically." Other factors affecting shell growth are salinity and the reproductive activity of the animal itself. In the present instance, it is worthy of note that shell growth is at a standstill throughout, and for some time after, the actual spawning period, and that the high temperature of July and August, coupled with the cessation of the demand for gonad-material, has led to a renewal of shell deposition. The mere "relaying" of the mussels seems hardly adequate to account for the increase of shell substance, especially since it was not evident until five months after the mussels were subjected to the change of habitat.

The experimental temperature was never different by more than one or two degrees from that obtaining in the stock-tank in which the mussels were kept, and the variations of temperature in the tank itself followed closely

upon those in the coastal waters of the Irish Sea. Both stock-tank and experimental temperatures are given in Table II, as well as the "normals" for the Irish Sea, for the 10-year period 1914-23, which latter data are derived from the monthly weather reports of the Meteorological Office for those years.

Table II. *Monthly mean temperatures during course of mussel respiration experiment, compared with normal values.*

Month	Irish Sea (° C.)	Stock-tank (° C.)	Experimental vessel (° C.)
January	7.3	—	—
February	7.4	—	—
March	6.9	7.0	7.5
April	7.8	7.5	—
May	10.0	11.0	11.5
June	12.2	14.5	13.3
July	13.9	17.0	15.5
August	14.7	15.8	14.8
September	14.0	13.0	—
October	12.3	12.0	11.5
November	10.0	8.0	10.2
December	8.4	7.0	—
(January)	(7.3)	7.0	8.0
(February)	(7.4)	7.5	—

The oxygen content of the tank water was determined from time to time, consistently high values being observed. The health of the mussels, so far as could be judged from muscular tone and general appearance, remained normal, and there was no nett loss of tissue; on the contrary, there was a definite gain in shell weight, and spawning took place at the usual time. It may therefore be concluded that, as regards all the factors recognised as bearing upon the mussel's metabolism, the direct experimental values, smoothed by the monthly or ternary averaging, will afford a close parallel to the sequence of metabolic changes as they occur upon the mussel beds, in our coastal waters. This direct practical significance of the results must be emphasised; the animals were not narcotised or otherwise restrained, and while for this reason the experimental metabolic values may not strictly be regarded as "basal," it is certain that they approximate very closely to that standard. Muscular movements and slight locomotion usually occurred when the mussels were first placed in the respiration chamber, but all gross movement had ceased and the mussels were attached by their byssus-threads, before the initial period of accommodation had elapsed. During the actual experiment, the maintenance of the respiration current by ciliary action appeared to be the sole energy-requirement, apart from that involved in tissue-metabolism.

Table III embodies the complete series of monthly mean values. The respiratory exchange is expressed in cc. of oxygen (measured as at N.T.P.) absorbed *per mussel* per hour, and is not corrected in any way for temperature or other differences, as between successive members of the series. All the mussels were between 5.0 and 6.0 cm. in length. In arriving at these mean values, all experiments were disregarded in which the partial pressure of the dissolved oxygen, at the mid-period of the experiment, varied from the annual mean by more than 10 %. The actual oxygen uptake per 10 g. of wet tissue, and the

experimental temperature are also included, in order that the influence of varying body-weight and temperature upon the gross result may be judged.

Table III. *Seasonal variations in the oxygen uptake of the mussel*

(length range, 5.0-6.0 cm.), monthly means.

Date	Temperature (° C.)	Oxygen uptake per mussel per hour (cc. at N.T.P.)	Oxygen uptake per 10 g. tissue per hour (cc. at N.T.P.)	Number of experiments upon which mean is based
March 14	7.5	0.112	0.168	3
May 20	11.5	0.166	0.252	2
June 14	13.3	0.188	0.320	4
July 17	15.5	0.217	0.374	6
Aug. 31	14.8	0.161 (0.187)	0.276 (0.323)	1
Oct. 8	11.5	0.184	0.318	2
Nov. 26	10.2	0.116	0.191	4
Jan. 22	8.0	0.113 (0.114)	0.186 (0.182)	1
Annual means	11.5	0.157	0.261	—

(Figures in brackets are smoothed values, as used in the curves, to replace those based upon one observation only.)

The two sets of respiratory values, together with the corresponding temperatures, are plotted in Fig. 2, from which it will be seen that the temperature factor is the dominant one in determining the course of the metabolic rhythm. The relative importance of the several factors is dealt with more fully on p. 844. The loss of weight, consequent upon spawning, is obviously responsible for the increased disparity between the two respiratory curves, after the spawning period in May.

Fig. 2.

Effect of temperature on the gaseous metabolism.

In order to obtain a definite idea of the magnitude of the temperature effect, and a quantitative relation which would make it possible to reduce all

results to a common temperature, values were selected from the entire series of experimental data, which appeared to be least affected by conditions other than temperature. In making this selection, two criteria were observed.

(a) The values must lie within a narrow and normal range of oxygen pressure (say 20 mm.), so as to eliminate, as far as possible, variations due to that factor. The range actually tolerated was 133.3–154.0 mm. (The normal atmospheric pressure due to oxygen is 160.0 mm.)

(b) The values must not refer to physiological conditions of the mussels near the critical (spawning and fattening) periods. The period selected, being a flat region on the wet weight curve, was from June 23rd, 1925, to March 9th, 1926.

Data complying with these criteria are set out in Table IV, where the respiratory values refer, of course, to a constant weight (10 g.) of wet mussel tissue. The curve expressing these results is shown in Fig. 3, and it will at once be noted that the respiratory activity rises at a very regular rate with increasing temperature, the relation being practically one of direct proportionality over the range 3°–16°. At higher temperatures there is a falling-off, until, after passing through a maximum at about 22.5°, there is a rapid drop towards the thermal death-point, which was not, however, reached in this work, as the mussels kept for an hour at 25° and at 30°, though rendered flaccid, soon recovered, and in fact gave a normal respiratory value when tested at 15° some hours later.

Table IV. *Effect of temperature on the oxygen uptake of the mussel.*

Experimental values		Mean values	
Temperature (° C.)	Oxygen uptake per 10 g. wet tissue per hour (cc. at N.T.P.)	Temperature (° C.)	Oxygen uptake per 10 g. wet tissue per hour (cc. at N.T.P.)
3.5	0.022	3.5	0.022
5.5	0.095	5.8	0.098
6.0	0.100		
7.0	0.173	7.7	0.186
8.0	0.186		
8.0	0.198		
10.5	0.349	11.5	0.282
11.3	0.191		
11.5	0.320		
12.8	0.266		
15.0	0.472	16.2	0.430
16.5	0.448		
17.0	0.371		
22.3	0.549	22.3	0.549
25.0	0.480	25.0	0.480
30.0	0.166	30.0	0.166

From the respiration-temperature curve several interesting points may be deduced. The numerical value of Vernon's "temperature increment," defined by Krogh [1916, p. 89] as the ratio of the respiratory exchange at 24° to that at 10°, is seen to be 2.06, which is about normal for organisms of this type, hyaline forms giving a higher value. Since direct calorimetric measurement

of the respiratory energy is difficult, or impossible, in the case of slowly metabolising aquatic organisms, the calorific equivalent of the oxygen absorption could only be arrived at indirectly. On the usually accepted basis of 1 litre of oxygen being equivalent to 4.8 Cal., the oxygen uptake of the mussel-tissue, at 14°, should give rise to 0.178 Cal. per kilogram per hour. Respiratory determinations carried out by Jolyet and Regnard [1877] upon a large number of aquatic animals gave, in the case of *Mytilus*, at 14°, the value 0.06 Cal. per kilogram per hour, when the entire weight of the living animal, including the shell and presumably the included water, was used in the computation. The results obtained in the present investigation, when recalculated on this somewhat irrational basis, give a value of 0.051 Cal.—a close agreement, when it is remembered that the earlier work was based on eudiometric measurement of the extracted gases, and a totally different technique.

Fig. 3.

Finally, it is possible, by considering only that portion of the curve which most nearly approximates to a straight line, to obtain a temperature coefficient of the rate of oxygen uptake. The relation

$$t = 31r + 2.6$$

(where t = temperature in ° C.,

r = respiration-rate (cc. of O_2 per hour per 10 g. wet tissue)

determines a straight line, and from this equation it is easy to reduce the respiratory values lying within its range (3°–16°) to any desired common temperature. As a matter of fact, 10° was chosen as the standard, since in addition to its general convenience, it happens to be the mean annual temperature of the Irish Sea coastal waters (Table II).

Effect of oxygen pressure on the gaseous metabolism.

Previous observers are in general agreement that marine invertebrates are remarkably insensitive to oxygen pressure, and that the respiratory rate remains undiminished until quite low oxygen pressures, say 30 % of the normal, are reached. The results given in this paper, while bearing out the general view that oxygen pressure is a factor of minor importance, point to the conclusion that the respiratory rate is slightly and evenly affected over a range of oxygen pressures extending far below and far above those normally experienced.

Table V. *Effect of oxygen pressure upon the oxygen uptake of the mussel at 10°.*

Range of partial pressure exerted by dissolved O ₂ (mm. of Hg)	Mean oxygen uptake (corrected to 10°) per 10 g. wet tissue per hour • (cc. at N.T.P.)	Number of experiments upon which mean value is based
80-99	0.189	3
100-119	0.184	5
120-139	0.220	7
140-159	0.226	9
—	—	—
390-415	0.450	4

In order to secure a sufficiently wide range of data it was necessary to accept the validity of the temperature correction arrived at in the preceding section, and the experimental values, corrected thus to 10°, were grouped in ranges of 20 mm. oxygen pressure, the mean results being listed in Table V.

These values, though slightly irregular, may be regarded as determining a straight line, of which the equation is:

$$p = 1165r - 120$$

(where p = partial pressure of O₂, in mm., of Hg,

r = respiratory rate (cc. of oxygen per hour, per 10 g. wet tissue),
corrected to 10°).

As this line cuts the respiration ordinate at a positive value at zero oxygen pressure, it follows that it does not hold good down to the lowest pressures. It is valid, however, over the range experimentally realised (90-400 mm.), and provides a means, as in the case of the temperature curve, for reducing the readings to yet another common basis. In this case, 160 mm. is selected as the standard oxygen pressure, since that is the partial pressure exerted by the 21 % of oxygen in a normal atmosphere of 760 mm. At the same time, it is to be observed that complete saturation of the sea-water with oxygen is only occasionally realised, and that a mean annual oxygen pressure of 140 mm., or even lower in areas contaminated by sewage is likely to occur over the mussel beds.

A certain reservation must be made when interpreting the foregoing results, for although the supernormal oxygen pressures were arrived at by saturating sea-water with the gas under the pressure of a column of mercury, the

diminished or subnormal pressures were brought about, in each case, by the metabolic activities of the animals themselves. The low oxygen pressures were therefore inevitably accompanied by an increased concentration of dissolved carbon dioxide, and of hydrogen ions, and possibly also of excretory organic matter. That these factors may exert an effect upon the respiration rate, independent of that due to low oxygen pressure, is not improbable, though a careful examination of the results, corrected for both temperature and oxygen pressure, failed to reveal any significant dependence upon p_H over the range 8.15–8.55, and it may be supposed that any effect attributed to carbon dioxide is mainly brought about by the collateral change in hydrogen ion concentration. The effect, if any, of the presence of organic matter in solution was not determined.

The reproductive cycle.

It will be convenient to group under the above heading those factors of a physiological character which remain to influence the curve of respiratory exchange, after the effects of temperature and oxygen pressure have been neutralised by the appropriate correction formulae. For this purpose the combined results of each experiment are available, since it is desired to follow the effect, if any, of the reproductive factor throughout the year. The corrected results are still erratic, and in Table VI they are given, as averaged in groups of three.

Table VI. *Effect of reproductive activity on the oxygen uptake of the mussel at 10° and 160 mm. oxygen pressure.*

Date	Mean oxygen uptake (corrected to 10° and 160 mm. O ₂ pressure), per 10 g. wet tissue per hour (cc. at N.T.P.)	Remarks on condition, etc.
Mar. 30	0.276	Acclimatised 30 days in stock-tank; good condition
Apr. 17	0.288	All tissues full of spawn patches
May 13	0.241	
May 27	0.247	Spawn running freely
June 5	0.233	Spawning apparently finished
June 23	0.251	A few patches of spawn tissue remaining in some mussels
July 13	0.217	
July 21	0.207	Tissues thin and "watery" " "
Aug. 31	0.215	
Oct. 8	0.234	Small patches of spawn in the mantle
Nov. 26	0.262	Considerable development of small patches of spawn in the mantle
Jan. 22	0.241	Considerable development of small patches of spawn in the mantle
Mar. 9	0.289	Mantle full of spawn patches

These results are shown in graphic form in Fig. 4. It is evident that the period from July to March, when the reproductive reserves are presumably being built up, is marked by a 30 % increase in the oxygen uptake per unit weight of wet tissue, though this is only apparent when the readings, made on a falling temperature gradient, are reduced, as here, to a common basis of 10°. Since the unit of wet weight (10 g.) must contain, at the successive

dates, an ever-growing proportion of gonad material, it would appear that those tissues of the mussel which are impregnated with the sexual products make a higher oxygen demand, weight for weight, than when they are in a resting or non-fertile state. Daniel [1925] adduces evidence to show that even when deprived of all particulate food for a lengthy period, the mussel does not draw upon the extensive reserves of glycogen and fats destined for the up-building of the reproductive products. In the starving mammal, as is well known, the carbohydrate reserves of the liver are mobilised at an early stage, and even in the mussel it can no longer be doubted that the glycogen, with which the tissues are replete at certain seasons, or at any rate its soluble derivatives, enter into the oxidative reactions which find expression in the respiratory exchange. It is the more surprising, therefore, to find that these stores of potentially valuable reserve materials should not be available for any but the sexual activities. The point is discussed in the ensuing section.

Fig. 4.
Month (April—March)

It will be convenient at this juncture to compare the relative effects upon the gaseous metabolism of the several factors—temperature, oxygen pressure and reproductive activity—so far considered. The range of monthly mean temperature experienced in the course of the year by mussels in their natural habitat may be taken as about 8° , with a minimum temperature of 6.9° in March, and a maximum of 14.7° in August (Table II). Other things being equal, this should lead to a range of oxygen uptake (per 10 g. wet tissue per hour), of 0.24 cc., the extreme values being 0.145 cc. in March and 0.385 cc. in August, as read from the curve in Fig. 3. Variations in sexual maturity throughout the year give rise to a range of only 0.08 cc., the lowest value, 0.21 cc., occurring in July, and the highest, 0.29 cc., in March (Fig. 4). It will be seen, therefore, that the fluctuations of temperature during a normal year may give rise to a

metabolic reaction three times as great as that due to innate physiological causes in the same period. The maxima and minima of these two factors, respectively, are almost coincident in time, and this tends, of course, to a less marked oscillation of the curve of nett metabolism (Fig. 2) than would be the case if they varied in true "phase" with each other. This curve only holds good for the Manx mussel under consideration, and will be modified in the case of mussel races such as those of the Irish and Lancashire coasts which spawn at other times of the year. Variations due to oxygen pressure, in so far as that factor is dependent upon the total barometric pressure, are still smaller, the range being of the order of 0.03 cc. of oxygen used for the mean annual barometric range. The pressure factor, moreover, is naturally irregular, and not seasonal, in its incidence.

The respiratory quotient.

The respiratory quotient not infrequently affords a valuable guide to the nature of the substances primarily involved in the respiratory oxidations, and for this reason determinations of the carbon dioxide output, as well as of the oxygen intake, were made in the manner already described. The method of CO₂ determination is admittedly not of a high order of accuracy, but the results obtained, and the corresponding respiratory quotients, are relatively valid, though the numerical scale must be regarded as arbitrary. The values of the R.Q. set out in Table VII are in each case the means of several determinations made in each separate experiment, but they are not subject to any further group-averaging.

Table VII. *Variations in the respiratory quotient of the mussel.*

Date	Mean respiratory quotient (arbitrary units)	Date	Mean respiratory quotient (arbitrary units)
Mar. 30	0.95	July 13	0.59
Apr. 17	0.62	July 21	0.25
May 13	0.61	Aug. 31	0.45
May 27	0.65	Oct. 8	0.44
June 5	0.45	Nov. 26	1.22
June 23	0.53	Mar. 9	1.31

The trend of the values is unquestionably to be associated with the changing chemical composition of the tissues [Daniel, 1921, 1922]. The rising quotient, from July to March, coincides with the period when carbohydrate (glycogen) reserves are being laid down, preparatory to the rapid metabolic changes leading to fat formation and a correspondingly low respiratory quotient, from March onwards to the actual spawning period in May.

It is generally recognised that, in the mammalian economy, "different proteins, fats and sugars are not physiologically equivalent, but that certain definite chemical groups subserve special functions in the animal organism" [Dakin, 1922]. Here, in the mussel, is evidence of the same metabolic specialisation. The wide disparity between the spring and autumn values of the respiratory

quotient, and the variation of absolute oxygen uptake, which follows a very similar curve (Fig. 4), point to a profound difference of metabolism at these periods. With this difference the non-availability of the reproductive reserves for the ordinary activities of the body is almost certainly to be associated, though a full understanding of the metabolic relations can only be obtained when to the mere knowledge of the gaseous intake and output of the organism there is added an appreciation of the numerous reactions involved in the intermediary metabolism. This is a highly complex problem which awaits investigation.

SUMMARY.

1. The gaseous metabolism of the common mussel (*Mytilus edulis*, L.) has been studied throughout an entire reproductive cycle.

2. The variations in the oxygen requirement of a full-grown mussel, under the factors, both physical and physiological, normally incident upon the mussel-beds in the course of a year, have been determined.

3. Quantitative expressions have been obtained for the effect of temperature and oxygen pressure upon the rate of oxygen uptake, which is also profoundly affected by the state of reproductive maturity. The influence of the annual temperature wave is approximately three times that attributable to the sexual cycle.

4. The seasonal changes in absolute oxygen requirement, and the respiratory quotient, are intimately associated with concurrent changes in the chemical composition of the tissues.

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