MANOMETRIC MEASUREMENTS OF PHOTOSYNTHESIS IN THE MARINE ALGA GIGARTINA

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I

We undertook the following study of photosynthesis in *Gigartina* partly in the hope of generalizing some of our conclusions drawn from work with *Chlorella*, but the results seem interesting in several other respects as well. The rather primitive methods used heretofore in measuring photosynthesis in marine algae do not permit much control of external conditions. From the existing knowledge of photosynthesis in fresh water aquatics and terrestrial plants, it is clear that results cannot be interpreted unless the degree of saturation with light and carbon dioxide is known. These essentials have never, as far as we are aware, been established in the experiments with marine algae, yet the results have been made the basis of important generalizations about the photosynthesis and ecology of these organisms. Several investigators have concluded that the Florideae are enabled, by the activity of their characteristic red pigment, to grow successfully at great depths, and in general where the color and intensity of the light are unfavorable to the development of green and brown algae.

In this paper, we cannot undertake to discuss adequately the question of the photosynthetic activity of phycoerythrin, or the distribution of the Florideae, nor do we think that a study of a single species, such as we have carried out, would provide proper basis for such a discussion. We mention these problems only because our experiments indicate the kind of information which will have to be collected before such questions can be discussed profitably.

The shortcomings in the technique of experimenters on photosyn-

thesis in marine algae are illustrated by the work of Ehrke (1931). He, like Kniep (1914), Harder (1915), and Montfort (1929), kept his material under so called "natural" conditions, because he was attacking an ecological problem. To measure photosynthesis, he enclosed samples of algae in glass vessels of special design, and of about 1 liter

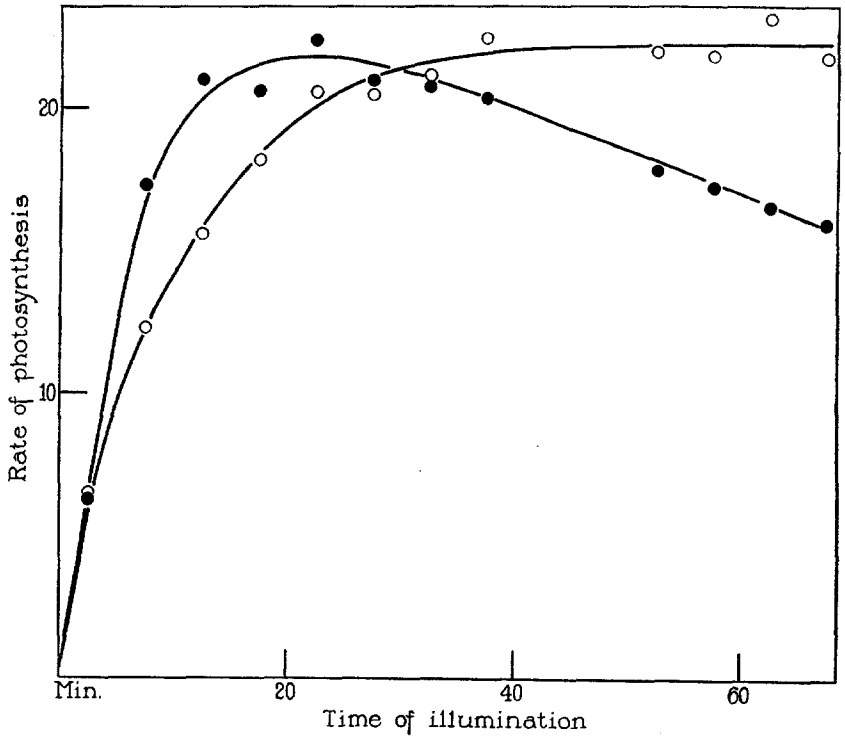

FIG. 1. *Gigartina* photosynthesis in ordinary sea water (solid circles), and in ordinary sea water saturated with 5 per cent CO_2 in air (open circles). Temperature = 15°C. Rate of photosynthesis is in mm. of Brodie per 5 minutes for 2.1 cm.^{2} of material.

capacity. They were filled to the neck with sea water, stoppered tightly, and exposed to light for several hours, either in the laboratory, or at various depths in the sea. Photosynthesis was determined by titrating the oxygen content of samples of sea water taken before and after exposure. Ehrke gives the dry weight of the material for each

experiment. Using Harder's (1915) table of dry weights, we have estimated that Ehrke used about 50 gm. of fresh material in about a liter of sea water, for a typical experiment.

In Fig. 1 we have plotted relative rates of photosynthesis against time of illumination. The curve with solid circles represents measurements with a piece of *Gigartina* weighing less than 150 mg., suspended in 8 cc. of ordinary sea water. Illumination began at zero on the time scale. Photosynthesis rises to a maximum in about 15 minutes, and soon afterwards enters an almost linear decline. This shows that when *Gigartina* and sea water are used in these proportions, only the measurements of the second 15 minutes of illumination are approximately constant. In Ehrke's experiments more material was used in proportion to the amount of sea water, and he determined only the average rate for 3 hours or more. The curve with solid circles in Fig. 1 shows that this may be something quite different from the rate of photosynthesis under truly "natural" conditions, when the algae are bathed continuously in fresh sea water.

The decline in rate is not due to irreversible injury from the experimental conditions, but to a steady fall in the carbon dioxide concentration, because of its removal in photosynthesis. This is well shown by the curve with open circles in Fig. 1. Here the same amount of material was suspended in the same quantity of ordinary sea water, this time saturated with 5 per cent carbon dioxide in air. Under these conditions the rate shows no sign of dropping for 2 hours or longer.

II

Methods

Photosynthesis was measured with Barcroft-Warburg manometers and rectangular vessels, of the type illustrated by Emerson (1929, p. 614). We shall discuss in detail the control of carbon dioxide concentration in sea water, and mention briefly the provisions made in this investigation for the other external factors.

Carbon Dioxide Concentration.—Ordinary sea water contains a small amount of dissolved carbon dioxide, a much larger amount of bicarbonate, and some carbonate. If the dissolved carbon dioxide is used for photosynthesis, some of the bicarbonate will decompose to form carbonate, and more carbon dioxide. But the concentration of the carbonate-bicarbonate system is too low to maintain a constant concentration of carbon dioxide in our experiments, and we have already

explained that the rate of photosynthesis falls unless some additional supply of carbon dioxide is provided. The curve with solid circles in Fig. 1 shows that rate measurements may be made in the second 15 minute period after the beginning of illumination, while the rate stays approximately constant. Because of the ready availability and constant composition of ordinary sea water, this may often be a desirable practice. Whenever we have used this technique, we have calculated the oxygen production, or photosynthesis, on the assumption that the pressure changes are entirely due to evolved oxygen, and that the partial pressure of carbon dioxide remains constant for this short period of time. This is a rather crude assumption, because of the poor buffering effect of the bicarbonate in the sea water. It tends to make the calculated oxygen production, and hence the rate of photosynthesis, lower than the true value.

A constant rate of pressure change can be maintained for several hours by suspending material in ordinary sea water saturated with 5 per cent carbon dioxide in air. The curve with open circles in Fig. 1 shows measurements of photosynthesis in this medium. It shows no tendency to decline, even at the end of 70 minutes, and the same is true of much longer periods. To calculate gas exchange from pressure changes in such a system, we may regard it as analogous to Warburg's Ringer solution saturated with 5 per cent carbon dioxide (*cf.* Warburg, 1924). Two vessels containing unequal volumes of fluid are required for each determination. Carbon dioxide consumption and oxygen production, and hence the photosynthetic quotient CO_2/O_2, are calculated from the pressure changes in the two vessels. Pressure changes in a single vessel can be used as a measure of relative rate of photosynthesis when the quotient is unknown. The curve with open circles in Fig. 1 was made in this way. Material in ordinary sea water saturated with 5 per cent carbon dioxide attains a steady rate of photosynthesis more slowly than material in other media, as is illustrated by Fig. 1. For this reason we have preferred to use artificial sea water without carbonate or bicarbonate, saturated with 5 per cent carbon dioxide, for measuring the photosynthetic quotient. The experiments made in ordinary sea water saturated with 5 per cent carbon dioxide serve as a useful check on those made under more artificial conditions. Either ordinary or artificial sea water saturated with 5 per cent carbon dioxide has at 15° a carbon dioxide concentration of very nearly 0.002 molar. But artificial sea water saturated with the gas mixture may be objectionable because it is considerably more acid than other media we have used. The ordinary sea water at Pacific Grove had a pH of 7.6. When saturated with 5 per cent carbon dioxide, the pH fell to 6.6, while the value for artificial sea water without carbonates, and saturated with 5 per cent carbon dioxide, was about 5.3.[1] In spite of the acidity, photosynthesis in this medium continues at a constant rate for some time.

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[1] We are indebted to Dr. K. V. Thimann for making the pH determinations for us, with a glass electrode.

In the majority of our experiments, carbon dioxide was provided by adding to artificial sea water larger amounts of carbonate and bicarbonate than are present in ordinary sea water. The advantages of carbonate mixtures for studying photosynthesis have been brought out by Warburg's work with *Chlorella* (1919). They exert a buffering effect on the carbon dioxide concentration, and maintain it nearly constant so that photosynthesis can be measured by oxygen production. Warburg has been widely criticized for his use of carbonate mixtures, which constitute a rather unphysiological medium for *Chlorella*. Whether or not these criticisms are justified,[2] they have little or no bearing on the use of carbonate mixtures for marine algae, because ordinary sea water is already a dilute carbonate-bicarbonate mixture. The normal pH of ocean water is said by Harvey (1928) to be about 8.1. In strengthening the concentration and varying the composition of the carbonate mixture, we have confined ourselves to a pH range of 7.6 to 8.6, a slight variation from the normal.

Warburg's solutions were of 0.1 molar concentration, and were well buffered. Such concentrated mixtures cannot be prepared in sea water without precipitating calcium and magnesium carbonates. Artificial sea water could be prepared with less calcium and magnesium, and the salinity made up by using more sodium and potassium chloride. Such a medium would probably be uninjurious to marine algae for short periods, and would permit the use of more concentrated carbonate mixtures, resulting in a a valuable improvement in their buffering capacities. We have made no experiments in this direction.

Our artificial sea water was prepared according to the specifications of E. J. Allen (1914, p. 421), except that the chlorides were not titrated, and ordinary distilled water from a tin-lined still was used. The following stock solutions were mixed as indicated:

Stock Solution	*Amount of stock per 100 cc. sea water*
	cc.
28.3 gm. NaCl in 500 cc. H_2O	50.0
KCl, molar solution	1.0
$CaCl_2$, " "	1.1
$MgCl_2$, " "	2.6
$MgSO_4$, " "	2.9

After mixing, distilled water was added to make 84 cc., and then 16 cc. of carbonate-bicarbonate mixture were added. The stock solutions must be mixed and diluted before adding the carbonate mixture, to avoid precipitation of carbonates.

Buch and several collaborators (1932) made an exhaustive study of carbonate equilibria in sea water, and compiled tables which facilitate the calculation of the

[2] Since preparing this paper, we have made experiments which show that at least at high concentrations of carbon dioxide, the rate of photosynthesis in *Chlorella* is quite independent of pH between 4.6 and 8.6. This objection to Warburg's procedure appears to be invalid.

carbon dioxide concentration and acidity of our various carbonate mixtures in artificial sea water. We shall indicate the form in which their data are given, and the essential steps in calculating carbon dioxide concentration.

When carbon dioxide physically dissolved in water is in equilibrium with the carbon dioxide in the air, the concentration $[CO_2]$ in the water, expressed in mols per liter, is related to the partial pressure p_{CO_2} of carbon dioxide in the air, expressed in atmospheres, by the equation

$$[CO_2] = c \cdot p_{CO_2}.$$

Here c is a constant called the molar solubility of carbon dioxide, whose value depends on the temperature and the quantity of dissolved solids. Buch's report includes a table of molar solubilities of carbon dioxide in sodium chloride solutions for temperatures from $-2°$ to 30°C., and for salt contents or salinities (S) from zero to 40 °/₀₀. The symbol °/₀₀ denotes parts per thousand by weight. The solubilities in sea water do not differ appreciably from those in sodium chloride solutions of corresponding temperature and salinity. (The "salinity" of sea water refers to the total salt content.) Therefore we can use these values in calculating the concentration of carbon dioxide in sea water if we know its partial pressure in the air. The concentrations in ordinary and artificial sea water saturated with 5 per cent carbon dioxide were calculated in this way.

The dissociation of the dissolved carbon dioxide may take place through either of two intermediate steps:

$$(a)\quad CO_2 + H_2O = H_2CO_3 = H^+ + HCO_3^-,$$

$$(b)\quad H_2O = H^+ + OH^-,\ CO_2 + OH^- = HCO_3^-.$$

Either of these pairs of reactions leads, by elimination of the intermediate step, to the following mass action expression for the equilibrium concentrations:

$$\frac{[H^+]\,[HCO_3^-]}{[CO_2]\,[H_2O]} = K.$$

Since the concentration of water molecules is constant for a water sample of given salinity and temperature, its value may be incorporated in the constant. The bicarbonate ion dissociates further:

$$HCO_3^- = H^+ + CO_3^{=}.$$

We have, then, using Buch's notation for the constants:

$$\frac{[H^+]\,[HCO_3^-]}{[CO_2]} = K_{c_1},$$

$$\frac{[H^+]\,[CO_3^{=}]}{[HCO_3^-]} = K'_2.$$

These may be solved for the carbon dioxide concentration or the hydrogen ion concentration:

$$[CO_2] = \frac{K'_2}{K_{c_1}} \cdot \frac{[HCO_3^-]^2}{[CO_3^=]}, \tag{1}$$

$$[H^+] = K'_2 \cdot \frac{[HCO_3^-]}{[CO_3^=]}. \tag{2}$$

The values of K_{c_1} and K'_2 vary with both the temperature and the salinity of the water. According to the theory of Debye and Hückel (Clark, Chapter 25), as well as to experiment, these constants are functions of the ionic strength μ of the solution, which is one-half the sum of the products obtained by multiplying the concentration of each ion in the solution by its valence. All dissolved salts are considered to be completely ionized.

Natural sea water, though varying widely in salinity, maintains a very constant relative composition, so that the concentration of a single constituent completely determines the composition. Thus, sea water is commonly characterized by its chlorine content. The ionic strength, salinity, and chlorine content of natural sea water are directly proportional to one another, as follows:

$$\mu = 0.020 \times \text{S } ‰ = 0.036 \times \text{Cl } ‰.$$

Buch's tables give values of pK_{c_1} and pK'_2, defined respectively as $-\log_{10} K_{c_1}$ and $-\log_{10} K'_2$, tabulated as functions of chlorine content, for temperatures from 0° to 30°C. and for chlorine contents up to 22 ‰. The ionic strength is the true determining factor, and only in the case of natural sea water can this factor be stated in terms of chlorine content. The importance of using these values for the constants, determined in sea water, in place of the usual values for the constants as determined in extremely dilute solutions is realized when we note that, for example, at 20°C. in extremely dilute solutions K'_2 has the limiting value 3.5×10^{-11}, while in ordinary sea water (Cl = 19 ‰) its value is 1.7×10^{-9}, about fifty times as great.

Dr. MacInnes has derived a value of K'_2 for sea water of the same salinity by extrapolation of a curve published by MacInnes and Belcher (1933), which differs greatly from the value obtained from Buch's figures. But these authors made their determinations in more dilute solutions than Buch, whose determinations were made in sea water, and a considerable extrapolation was required. Buch and others have pointed out that such constants are a different function of μ in dilute and in concentrated solutions, so we have preferred to use Buch's values, believing them to be the best obtainable at present. While future work on carbonate equilibria may lead to different values for the constants, and hence modify our absolute carbon dioxide concentrations, the relative concentrations in the various mixtures will probably remain unchanged, as Warburg pointed out in connection with his own mixtures (1919, p. 317). Warburg used constants different from ours

to calculate carbon dioxide concentrations in his mixtures, so his figures cannot be compared directly with ours, but in each case the shape of the curves and the conclusions to be drawn from them are not likely to be modified by changes in the constants.[3]

To solve equations (1) and (2), the concentrations of carbonate and bicarbonate ions must be known. The potassium carbonate and bicarbonate in our artificial sea water may be regarded as completely ionized, so we may set the initial ion concentrations equal to the concentrations of their respective salts. Here, as elsewhere, we refer to concentration per liter instead of per kilo. This involves only a negligible error.

Table I shows the initial carbon dioxide concentrations and pH values of our mixtures of carbonate and bicarbonate in artificial sea water at 15°, calculated

TABLE I

Initial Conditions in Artificial Sea Water with Various Carbonate Mixtures

$[HCO_3^-] = 0.016\beta$

$[CO_3^=] = 0.016\ (1 - \beta)$

Mixture	β	15°			4°
		pH	p_{CO_2} in atmospheres × 10^4	$[CO_2]$	$[CO_2]$
				mols × 10^6/*l.*	*mols* × 10^6/*l.*
A	5/8	8.6	7	2.7	2.4
B	3/4	8.3	15	5.8	
C	13/16	8.2	23	9.1	
D	7/8	7.9	41	16.0	
E	15/16	7.6	93	36.0	33.0

from equations (1) and (2). We also give the concentrations of carbon dioxide in mixtures A and E for 4°, because these mixtures were used in experiments at different temperatures. The pH changes only slightly with temperature.

Two factors operate to alter the initial carbon dioxide concentrations of our mixtures. The algal material withdraws carbon dioxide for photosynthesis, and carbon dioxide is exchanged between the mixture and the gas space until the two are in equilibrium. The latter is a negligible factor in our experiments, because the gas space is always small, and the partial pressures of carbon dioxide in equilibrium with our various mixtures are never much above its partial pressure in the

[3] Later work of Buch has resulted in a revision of his values of pK'_2. The revised value for K'_2 at 20°C. and Cl 19 °/₀₀ is 9.8×10^{-10}. This would reduce our calculated values for carbon dioxide and hydrogen ion concentrations about 40 per cent. *Cf.* Buch, K., *J. conseil internat. l'exploration de la mer*, 1933, **8**, 309.

atmosphere. The amount of carbon dioxide used in photosynthesis is large compared with that which may leave the mixture as gas, and it is important to know how much may be used without greatly changing the concentration. Mixture E, with the highest concentration, has the lowest buffering capacity. The

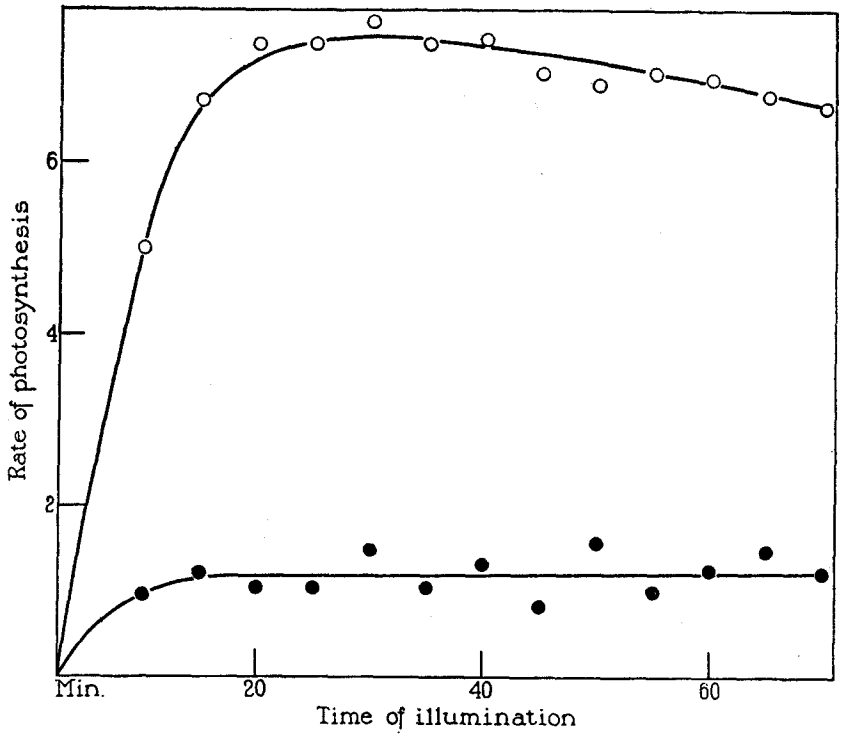

FIG. 2. *Gigartina* photosynthesis in artificial sea water to which has been added a mixture of potassium carbonate and bicarbonate. The solid circles are for a mixture giving a low carbon dioxide concentration, the open circles for a mixture giving a high concentration. Temperature = 15°C. Rate of photosynthesis is in mm.^{3} O_2 per 5 minutes for 2.1 cm.^{2} of material.

removal of 50 c.mm. of carbon dioxide from 8 cc. of mixture causes a fall of about 20 per cent in the concentration. The removal of an equal amount from mixture A causes a fall of only about 5 per cent in concentration.

The course of photosynthesis in *Gigartina* in two mixtures corresponding approximately to A and E is shown in Fig. 2. In the lower concentration (solid circles), the rate remains at the same level throughout the experiment. In the higher concentration, after the initial rise, the rate remains fairly constant for

about 20 minutes, and then begins to show a decline, because of falling carbon dioxide concentration. In making experiments in these carbonate mixtures, we have confined our measurements to the time interval when the rate is nearly constant, the second 20 minute period after the beginning of illumination.

It is evident that better buffer mixtures could have been prepared by keeping the carbonate constant, instead of decreasing it, as the bicarbonate was increased. At the time we did our experimental work, information on carbonate equilibria in sea water was not available, and we had expected to use a method of calculating carbon dioxide concentration similar to Warburg's. This requires that the concentration of the cation, potassium in our case, be kept constant, while the carbonate and bicarbonate are varied. The method of calculation outlined above, using Buch's tables, does not depend on the constancy of the cation concentration, so there is more latitude than we allowed ourselves in preparing our mixtures.

Lighting.—Illumination was provided by a row of internally frosted 60 watt lamps, placed as close to one another as possible, and about 8 cm. from the bottoms of the vessels containing the algal material. The number of lamps was always two greater than the number of vessels, not counting the control, which contained no material.

To vary the intensity, Wratten neutral filters were attached to the bottoms of the vessels with rubber bands. The manufacturer's transmission figures for this series of filters have been found to be correct within 3 per cent for incandescent light. To insure that no light entered the vessels except through the filters, the sides and tops were covered with jackets of copper foil.

Temperature.—Because of the known sensitivity of marine algae to higher temperatures, we conducted our measurements at temperatures of 16° or lower. The surface water at Pacific Grove is seldom if ever warmer than 16°C. The vessels were shaken in a large water thermostat, the temperature of which was maintained constant to less than 0.05°C.

III

Material

We made trial experiments with *Iridaea*, *Nitophyllum*, and *Gigartina*. A species of *Gigartina*, identified for us by Professor N. L. Gardner, of the University of California, as *G. harveyana*, was selected as most suitable for our purposes. Juvenile fronds are to be found in moderate quantity throughout the summer at Pacific Grove. They grow in a narrow zone about a foot below mean lower low water. The young fronds are smooth, thin, free from epiphytic algae, and of a fairly uniform red color. They were collected at low tide, and kept in a tank of running sea water in the laboratory. We found they could

be kept in good condition for 2 weeks or longer, but after this time they showed a somewhat reduced rate of photosynthesis. The experiments described in this paper were made with material kept less than 7 days in the laboratory.

Pieces of thallus to fit in the manometer vessels were punched out with a 16.5 mm. cork borer, or with an oval stainless steel punch which cut an area almost exactly twice that of the cork borer. We have used 2.1 cm^2. and 4.2 cm^2. for their respective areas.

The manometer vessels were filled with measured volumes of fluid, and then the pieces of thallus were introduced, one piece to each vessel, immediately after being cut. The change in fluid volume made by introducing the piece of thallus with its adhering water was estimated by weighing a series of pieces cut with the oval punch from fronds differing as much as possible in size and thickness. The weights varied from 270 to 330 mg. In any one experiment the variation was surely much smaller, because care was taken to cut all pieces for a given experiment from one frond, and as close together as possible. The pieces were assumed to have a density of 1, and a uniform addition to the fluid volume was made in all cases, 300 c.mm. for the oval pieces, and 150 c.mm. for the circular pieces.

The respiration of marine algae is generally supposed to be small. In *Gigartina* it is about 1/30 of the maximum photosynthesis at 15°, so it is an insignificant correction under optimum conditions. But at low intensities or carbon dioxide concentrations it becomes an appreciable proportion of the photosynthesis. In general we have dispensed with measuring it, and have made a small arbitrary correction for it. Nothing is changed in our conclusions if the correction is omitted, but the lower points in some of the figures fall more nearly on the curves when it is included.

Photosynthesis is expressed in cubic millimeters of evolved oxygen, calculated from change in pressure. In making the calculations, the absorption coefficient α given in Landolt-Börnstein for oxygen in 0.5 molar sodium chloride has been used. This value may not be quite correct for sea water, but since α_{O_2} changes only slightly with salt concentration, and constitutes only a small factor in the calculations, it may safely be used for our experiments.

IV

Description and Discussion of Experiments

One of the most characteristic features of the photosynthetic process in *Chlorella*, and probably in green plants in general, is the behavior of the temperature coefficient, Q_{10}. At high light intensities, the coefficient runs from 2 to 6, depending on the range studied. Reduction in the rate of photosynthesis by lowering the light intensity causes the coefficient to fall to nearly unity, whereas the same reduction in rate brought about by lowering the carbon dioxide concentration, keeping the light intensity high, does not greatly alter the coefficient. If photosynthesis in the Florideae involves the same reaction mechanism as in the green plants, then the Florideae may be expected to react in the same way to temperature changes under these conditions.

The choice of light intensities and carbon dioxide concentrations for the experiments at different temperatures must be determined by measurement of photosynthesis as a function of these two factors.

Fig. 3 shows a plot of rate of photosynthesis at 15°C., against light intensity in arbitrary units. The highest intensity is the direct light from a row of closely spaced 60 watt lamps, about 8 cm. from the algal material. Table II gives complete data for the experiment. The coordinates of the points in Fig. 3 are in Columns 1 and 7. The curve shows that the material is nearly saturated by the full light intensity.

Although the shape of this curve is somewhat different from Warburg's intensity curve for *Chlorella* (1925, p. 388), this does not necessarily indicate any difference in the two photosynthetic processes. Warburg used thin cell suspensions, which reduced the intensity not more than 10 per cent as the light passed through them. Our sections of *Gigartina* may have reduced the intensity of the incident light as much as 90 per cent as it passed through them. This means that for Warburg's curve, all cells will reach saturation at about the same incident intensity, because they are all nearly equally illuminated, whereas for our curve, the cells farthest from the light will reach saturation only after the incident light has far exceeded the intensity necessary to saturate the cells first receiving the light. Consequently we should expect our curve to show a much more gradual approach to saturation, and this is actually the case.

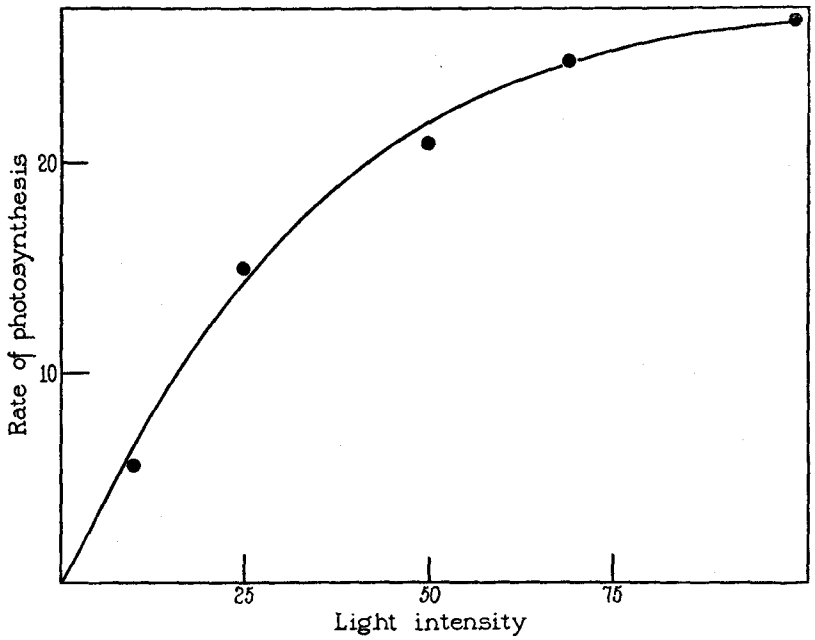

FIG. 3. Rate of *Gigartina* photosynthesis as a function of light intensity. The unit of intensity is arbitrary. Temperature = 15°C. Rate of photosynthesis is in mm.^{3} O_2 per 15 minutes per cm.^{2} of material.

TABLE II

Photosynthesis at Different Light Intensities. Detailed Data for Fig. 3

Area of algal material in each vessel = 2.1 cm.^{2} Material suspended in ordinary sea water in equilibrium with air. Light from six 60 watt lamps close together, 8 cm. below vessels.

Temperature = 15.0°C.

Relative intensity (per cent transmission of filter)	Volume of gas space	Volume of fluid, including material	Vessel constants K_{O_2}	Δh change of pressure in 15 min.	x_{O_2} volume of oxygen evolved in 15 min. = $\Delta h \cdot K_{O_2}$	x_{O_2} with uniform correction for respiration, 1 mm.^{3} in 15 min.
	cc.	*cc.*		*mm.*	*mm.*^{3}	*mm.*^{3}
10	4.16	7.15	0.41	11.1	4.6	5.6
25	4.13	7.15	0.41	33.6	14	15
50	5.26	8.15	0.52	37.6	20	21
75	5.08	8.15	0.51	47.2	24	25
100	5.33	8.15	0.53	48.9	26	27

We should mention here that the pieces of thallus never turn over while they are being shaken in the manometer vessels. During an entire experiment they remain with the same side facing the full intensity of the light, and the other side in deep shadow.

Montfort (1930) quotes Wurmser as stating that *Rhodymenia* shows a diminution in photosynthesis when exposed to intense light. He

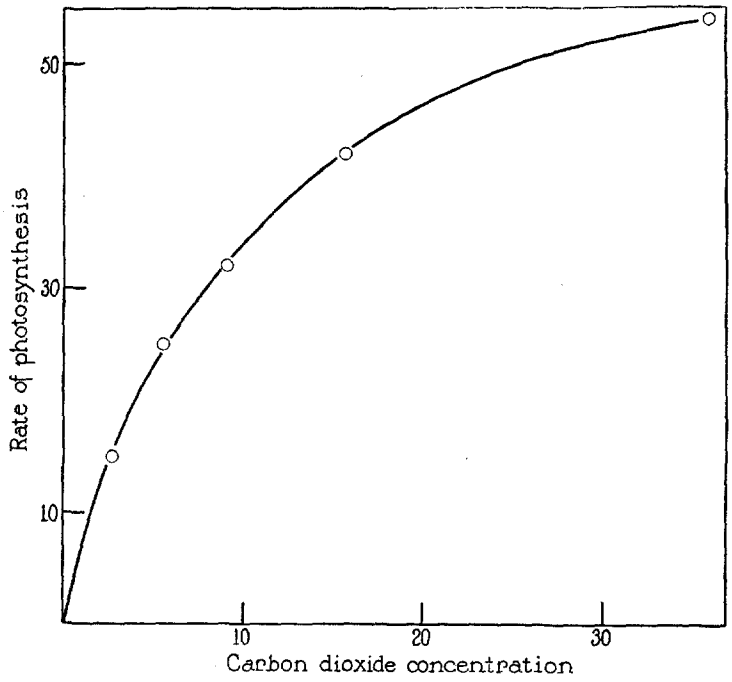

FIG. 4. Rate of *Gigartina* photosynthesis as a function of carbon dioxide concentration. Photosynthesis is in mm.^{3} O_2 per hour per cm.^{2} of material, and CO_2 concentration in mols $\times 10^5$. High light intensity; temperature = 15°C.

attributes this to the presence of phycoerythrin, because *Ulva* does not show a similar diminution. *Gigartina* may be exposed to intense light for several hours, and still show an undiminished rate of photosynthesis, provided the supply of carbon dioxide remains adequate, so we think the phenomenon observed by Wurmser can hardly be due to the presence of phycoerythrin.

Fig. 4 shows the rate of photosynthesis at 15°C., plotted against

carbon dioxide concentration. Table III gives complete data, and the coordinates of the points are in Columns 2 and 10. The highest point on the curve may be a little low, because of the poor buffering capacity of mixture E, which we discussed in the section on methods. The curve is similar to those for *Chlorella* photosynthesis obtained by Warburg (1919, p. 254) and by Emerson and Arnold (1932, p. 409). The rate is a linear function of the log of the carbon dioxide concentration.

TABLE III

Photosynthesis at Different CO_2 Concentrations. Detailed Data for Fig. 4

Area of algal material in each vessel = 2.1 cm.^{2}

Material suspended in artificial sea water with carbonate mixtures described in Table I.

Q_{O_2} is the rate of photosynthesis, expressed as volume of O_2 evolved per hour per cm.^{2} of material, corrected for respiration.

Temperature = 15.0°C.

Carbonate mixture	Mols. CO_2 per liter $\times 10^5$	Volume of gas space	Volume of fluid including material	Vessel constant K_{O_2}	$\Delta h'$, change of pressure in 20 min. in dark	x'_{O_2}, respiration per hr. $= -3\Delta h' K_{O_2}$	Δh, change of pressure in 30 min. in light	x_{O_2}, oxygen produced in light per hr. $= 2\Delta h K_{O_2}$	$Q_{O_2}\left(\frac{x_{O_2} + x'_{O_2}}{2.1}\right)$
		cc.	*cc.*		*mm.*	*mm.*^{3}	*mm.*	*mm.*^{3}	*mm.*^{3}
A	2.7	4.13	7.15	0.41	−2.3	2.8	33.3	27.3	15
B	5.8	4.16	7.15	0.41	−3.2	3.9	61.0	50.0	25
C	9.1	5.34	7.15	0.52	−2.3	3.6	61.0	63.5	32
D	15.8	5.08	8.15	0.51	−2.7	4.1	82.1	83.7	42
E	36.0	5.33	8.15	0.53	−1.7	2.7	103.5	110	54

We have investigated the effect of temperature on rate of photosynthesis in mixture E at full light intensity, and with the 10 per cent filter; and in mixture A at full light intensity. The results are shown in Fig. 5, rate being plotted directly against temperature in degrees Centigrade. The curve with solid circles is for high intensity and high carbon dioxide concentration, the open circles for low carbon dioxide concentration and high intensity; and the crosses for low intensity and high carbon dioxide concentration. Table IV gives complete data, and the coordinates of the points are in Columns 1 and 8.

We have found that exposure of our material to temperatures between 4° and 16° caused no injury, and that within this range all temperature effects were reversible.

Fig. 5 indicates that the mechanism of photosynthesis in *Gigartina* must be fundamentally like that in *Chlorella*. A Blackman reaction, with a high temperature coefficient, governs the rate at high intensities,

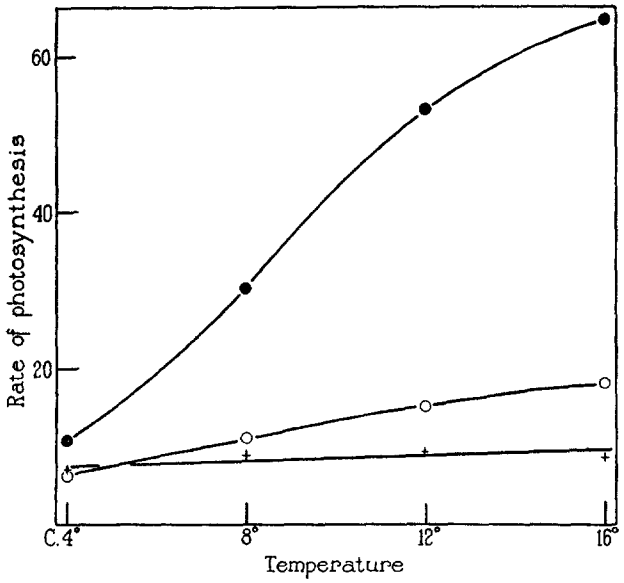

FIG. 5. Rate of *Gigartina* photosynthesis plotted against temperature in degrees Centigrade. Solid circles are for high intensity and high CO_2 concentration, open circles for high intensity and low CO_2 concentration, and crosses for low intensity and high CO_2 concentration. Photosynthesis is in mm.^{3} O_2 per hour per cm.^{2} of material.

and a photochemical reaction, with a coefficient of about 1 governs the rate at low intensities. It seems evident that at low carbon dioxide concentrations the rate is determined by a chemical reaction, rather than by the diffusion of carbon dioxide, because the value of 4.4 for Q_{10}, calculated for the increase in rate from 4° to 8°, is too high to represent a diffusion process. Van den Honert (1930), using *Hormidium*, failed to find a coefficient much higher than 1 at low concentrations of carbon dioxide, and interpreted this as showing that

the rate of photosynthesis was governed by the diffusion of carbon dioxide. From his curves, this appears likely; but we believe that they represent limiting conditions outside the cell due to his peculiar experimental method. In the case of *Gigartina*, owing to the thickness of the material it seems probable that the lower coefficient found

TABLE IV

Temperature Coefficients under Various Conditions. Detailed Data for Fig. 5

Light from four 60 watt lamps close together, 8 cm. below vessels. For low intensity experiment, vessels were covered with 10 per cent transmission filters. Q_{O_2} is the rate of photosynthesis, expressed as volume of O_2 evolved per hour per cm.^{2} of material, corrected for respiration.

Conditions of experiment	Temperature	Volume of gas space	Volume of fluid including material	Vessel constant K_{O_2}	Δk change of pressure in 20 min.	Volume of O_2 per hr. per cm.^{2} material	Respiration in dark per hr. per cm.^{2} material	Q_{O_2}
	°C.	cc.	cc.		mm.	mm.^{3}	mm.^{3}	mm.^{3}
I. High I, high [CO_2]. 2.1 cm.^{2} material suspended in artificial sea water with carbonate mixture E	4	5.33	8.15	0.56	12.4	9.9	0.7	10.6
	8	4.13	7.15	0.43	47.2	29	1.2	30
	12	5.26	8.15	0.53	66.7	51	1.6	53
	16	5.33	8.15	0.53	82.6	63	2.1	65
II. High I, low [CO_2]. 4.2 cm.^{2} material suspended in artificial sea water with carbonate mixture A	4	4.93	8.3	0.52	14.5	5.4	0.7	6.1
	8	4.01	7.3	0.42	32.7	9.8	1.2	11.0
	12	5.19	7.3	0.52	35.8	13.3	1.6	14.9
	16	4.93	8.3	0.49	45.0	15.8	2.1	17.9
III. Low I, high [CO_2]. 4.2 cm.^{2} material suspended in ordinary sea water	4	3.98	7.3	0.42	21.0	6.3	0.7	7.0
	8	4.01	7.3	0.42	24.9	7.5	1.2	8.7
	12	3.98	7.3	0.40	26.4	7.5	1.6	9.1
	16	6.18	7.3	0.60	14.8	6.3	2.1	8.4

at low carbon dioxide concentration, compared to the coefficient for high carbon dioxide concentration and high light intensity, is due in part to diffusion.

The effect of temperature on photosynthesis by marine algae has been investigated by Kniep (1914), Harder (1915), and Ehrke (1931). Kniep suggested that the ability of marine forms to develop successfully in the polar seas might depend on a greater fall in respiration than in photosynthesis as the temperature drops. Harder is also of

this opinion, but his experiments give it meager support. His figures show that the value of Q_{10}, for *Fucus* respiration, is about 1.2 between 4° and 16°. Over this range, photosynthesis usually shows a Q_{10} of 3 to 4, with a marked increase at the lower temperatures. This is certainly the case with *Gigartina*. Q_{10} for photosynthesis is 1.7 from 12° to 16°, and 13.5 from 4° to 8°.

Ehrke claims that his curves substantiate Kniep's view. They show various photosynthetic maxima at low temperatures. Sometimes there is a declining rate with rising temperature, and another maximum at a higher temperature. Because of the extreme unlikelihood of such temperature functions, we must regard Ehrke's curves as the result of a combination of poor experimental technique and injury at higher temperatures, unless they can be substantiated by reliable methods.

We realize that external conditions may be such that somewhat more oxygen is produced at low than at high temperatures, because at sufficiently low light intensity photosynthesis practically does not change with temperature. This is a perfectly general characteristic, and should not be represented as a special adaptation to enable marine algae to inhabit the polar seas. It is also far from adequate to explain Harder's statement that the oxygen production of *Fucus* increases more than twenty times as the temperature falls from 17° to 0°, especially in view of Harder's own respiration measurements on *Fucus*, which show, as mentioned above, a Q_{10} of only 1.2 between 4° and 16°.

To compare the effect of temperature on *Gigartina* photosynthesis quantitatively with similar measurements for other forms, we have plotted our results according to the Arrhenius equation. We do this only to bring out similarities and differences which are otherwise not obvious, and we do not wish to imply anything concerning the mechanism of the temperature effect.

For our purposes the Arrhenius equation may be written:

$$\frac{R_2}{R_1} = e^{\frac{\mu}{R}\left(\frac{1}{T_1}-\frac{1}{T_2}\right)},$$

where R_1 and R_2 are rates of reaction at absolute temperatures T_1 and T_2, R is the gas constant, e the base of natural logarithms, and μ

a constant whose value expresses the effect of temperature on the reaction. The work of Crozier and his collaborators has shown that μ is rarely constant for biological processes over their entire temperature range, but may be nearly constant for short intervals (see Cro-

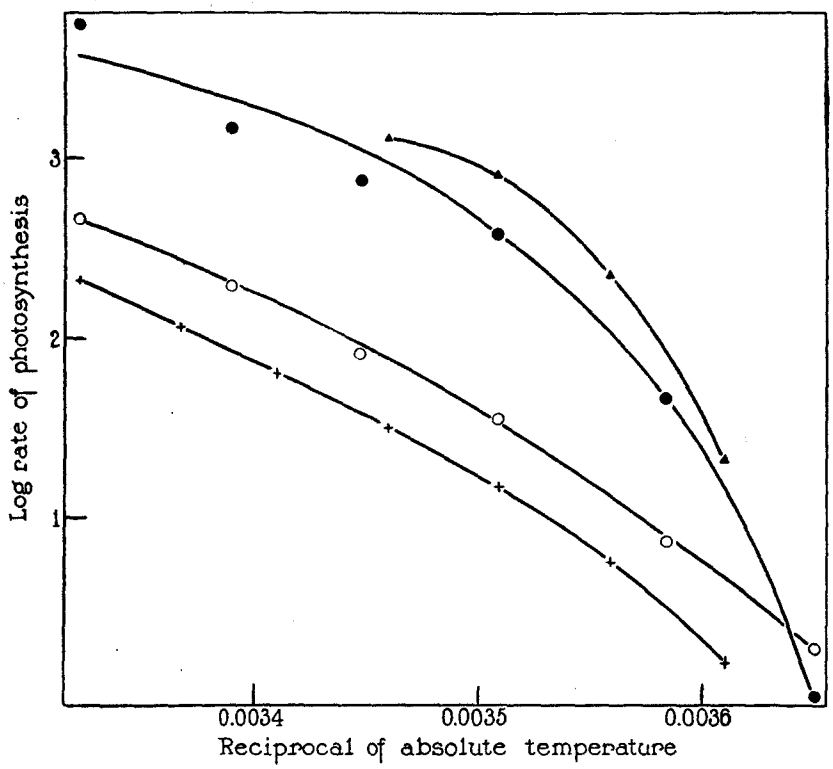

FIG. 6. Natural log of the rate of photosynthesis plotted against the reciprocal of the absolute temperature. Solid circles are for *C. vulgaris*, open for *C. pyrenoidosa*, triangles for *Gigartina*, and crosses for *Hormidium*. All measurements represent high intensity and carbon dioxide concentration.

zier, 1926). In the equation, μ usually represents heat of activation of reacting molecules, and is therefore expressed in calories. For biological processes this may be of little significance because we know so little about the reactants involved, but since it may some day be possible to attach theoretical significance to μ we have quoted it in the units used by Crozier and others in biological work. In Fig. 6,

where the natural log of the rate of photosynthesis is plotted against the reciprocal of the absolute temperature, μ represents the slope of the curves.

The solid and open circles are for *Chlorella vulgaris* and *C. pyrenoidosa* respectively. The triangles are for our measurements with *Gigartina*, and the crosses are calculated from van der Paauw's curve for *Hormidium* (1932, p. 558). The relative positions of the curves

TABLE V

Temperature Characteristics of Photosynthesis for Various Algae. Data for Fig. 6

R is the rate of photosynthesis in arbitrary units. μ is the temperature characteristic or thermal increment defined by the equation

$$\log_e R_2 - \log_e R_1 = \frac{\mu}{2}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

where T is the absolute temperature. Only maximum and minimum values of μ are recorded.

Temperature	$\frac{1}{T_{abs.}} \times 10^6$	*Gigartina harveyana*		*Chlorella vulgaris*		*Chlorella pyrenoidosa*		*Hormidium flaccidum*	
		$\log_e R$	μ	$\log_e R$	μ	$\log_e R$	μ	$\log_e R$	μ
°C.									
1	3650			0.00	54000	0.27	18000		
4	3610	1.31	45000					0.19	23000
6	3584			1.66		0.86			
8	3559	2.35						0.75	
12	3509	2.92		2.58		1.55		1.17	
16	3460	3.12	6000					1.49	
17	3448			2.88		1.91			
20	3413							1.80	
22	3390			3.17		2.29			
24	3367							2.05	
28	3322			3.75	11000	2.66	12000	2.31	12000

are arbitrary, and do not indicate relative rates of photosynthesis for the several organisms. Table V shows the rates of photosynthesis at the various temperatures, and the logarithms of the rates, adjusted to bring the curves to convenient relative positions in the figure. The maximum and minimum values of μ for each curve are also tabulated.

The minimum values of μ are roughly the same for all four species of

algae, but the *Gigartina* and *C. vulgaris* show a great increase in μ at the lower temperature, which is not shown by *Hormidium* and *C. pyrenoidosa*. If the data for *Gigartina* at low carbon dioxide concentration (open circles, Fig. 5) are plotted in this way, a curve nearly like those for *C. pyrenoidosa* and *Hormidium* is obtained. At present we can give no theoretical explanation for this, though it may be partly due to diffusion, as mentioned above. Crozier and Stier (1926) discuss other cases where the value of μ can be altered experimentally.

TABLE VI

Inhibition of Photosynthesis in Gigartina by Cyanide and by Phenyl Urethane

Cyanide experiment: 2.1 cm.^{2} algal material suspended in artificial sea water with carbonate mixture E. At the pH of this mixture the cyanide is practically all in the form of HCN at equilibrium.

Phenyl urethane experiment: 4.2 cm.^{2} algal material suspended in ordinary sea water saturated with 5 per cent CO_2 in air.

Temperature = 15°C.

Inhibitor	Concentration of inhibitor	Rate of photosynthesis	Inhibition	Rate after washing	Recovery
			per cent		*per cent*
KCN	0	33		28	
	$\frac{1}{20000}$ molar	17	49	26	86
Phenyl urethane	0	37			
	0.0075 per cent	14	62	41	100

We have found photosynthesis in *Gigartina* to be highly sensitive to traces of prussic acid and phenyl urethane. Removal of the urethane by washing results in complete reversal of the inhibition. In the case of cyanide, reversibility was not quite complete, even after short exposures to extremely low concentrations, and prolonged washing. Possibly the cyanide causes secondary injuries to the material. The inhibited photosynthesis in cyanide does not stay constant, but falls steadily, so our figures for percentage inhibition are arbitrary, being taken as soon as possible after introducing the cyanide. The results of our experiments on inhibition by cyanide and urethane are summarized in Table VI. Extreme sensitivity to cyanide and nar-

cotics is a common characteristic of photosynthesis in green plants (*cf.* Spoehr, 1926, p. 171).

Two determinations of the photosynthetic quotient were made in artificial sea water saturated with 5 per cent carbon dioxide in air. Figures for these experiments are given in Table VII. The quotient obtained is close to unity, as might have been expected. Kniep's values for red algae also run close to unity, about −0.98. In neither

TABLE VII

Photosynthetic Quotient of Gigartina in Incandescent and in Mercury Light

The photosynthetic quotient, γ, is calculated by the formula

$$\gamma = \frac{K_{CO_2} \cdot k_{CO_2}}{K_{O_2} \cdot k_{O_2}} \cdot \frac{H \cdot K_{O_2} - h \cdot k_{O_2}}{h \cdot k_{CO_2} - H \cdot K_{CO_2}},$$

where the capital letters refer to the vessel with the larger fluid volume, the small letters to the other vessel. In each vessel, 4.2 cm.^{2} material suspended in artificial sea water without carbonates, saturated with 5 per cent CO_2 in air.

Incandescent light from a row of 60 watt lamps 8 cm. below vessels. Mercury light from an intense hot cathode mercury glow discharge tube close to vessels.

Temperature = 15°C.

Light	Volume of gas space	Volume of fluid including material	Vessel constants		Change of pressure	γ
	cc.	*cc.*			*mm.*	
Incandescent	$v_G = 9.93$ $V_G = 4.01$	$v_F = 3.3$ $V_F = 7.3$	$k_{O_2} = 0.95$ $K_{O_2} = 0.40$	$k_{CO_2} = 1.25$ $K_{CO_2} = 1.06$	$h = 12.4$ $H = 94.2$	−1.07
Mercury	$v_G = 10.18$ $V_G = 4.01$	$v_F = 3.3$ $V_F = 7.3$	$k_{O_2} = 0.97$ $K_{O_2} = 0.40$	$k_{CO_2} = 1.27$ $K_{CO_2} = 1.06$	$h = 9.5$ $H = 49.9$	−0.91
Average γ						−0.99

case is the difference from unity significant, considering the accuracy of the methods used. Other determinations of the photosynthetic quotient of *Gigartina* were made in ordinary sea water saturated with 5 per cent carbon dioxide. These also gave values close to unity.

A few measurements of photosynthesis were made in different colors of light, in the hope of demonstrating whether or not phycoerythrin can act photosynthetically. The work was not completed because of lack of time, and we plan to make a more thorough investigation of

the question later. For this reason we do not wish to give a detailed discussion at present. It may be worth while, however, to indicate what bearing our results have on the widely held opinion that red algae are enabled by their phycoerythrin content to utilize the shorter wave-lengths of light, and consequently to inhabit greater depths than other forms.

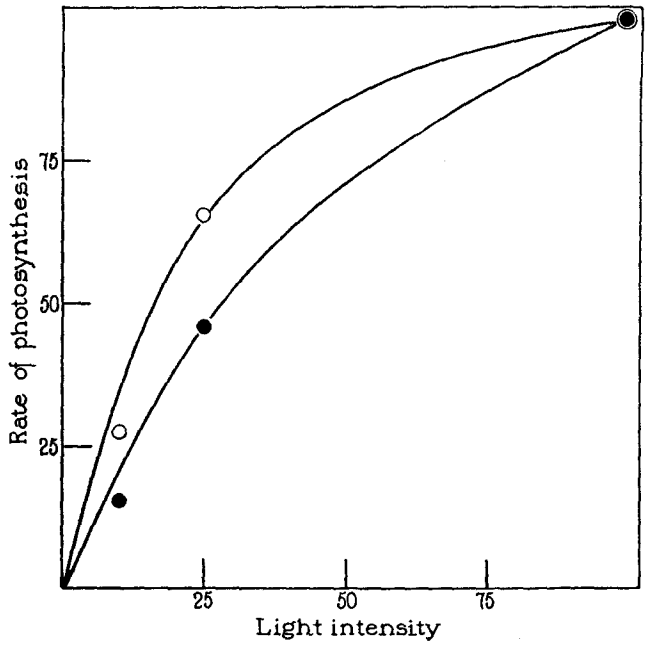

FIG. 7. Rough comparison of intensity curves for *Gigartina* and *Ulva*. Intensities and rates of photosynthesis are in arbitrary units. Open circles are for *Gigartina*, solid circles for *Ulva*. The highest rate of photosynthesis has been set at 100 in each case. Temperature = 15°C.

A number of investigators have compared the rates of photosynthesis by red and green algae in red and blue or green light, and found that the red algae do relatively better in the shorter wave-lengths than the green algae. We have been able to confirm this for *Gigartina* and *Ulva*. But von Richter (1912) has already pointed out that in certain cases at least, the results on changing from red to blue or green light

can be duplicated by using a single color of light and changing from a higher to a lower intensity. Oltmanns (1892) reported that intensity was of greater importance than color, in obtaining satisfactory laboratory cultures of marine algae.

Von Richter's results suggest that photosynthesis in the red algae may generally be light-saturated at a lower intensity than in green algae. Fig. 7 shows that for *Gigartina* and *Ulva* this is certainly the case. Photosynthesis was measured at three intensities of white light for the two forms. The maximum photosynthesis in each case was set at 100. The *Ulva* responded to a fall of 75 per cent in light intensity by a fall of 55 per cent in photosynthesis, while the *Gigartina*, for the same fall in intensity, showed only a 33 per cent drop in photosynthesis.

TABLE VIII

Comparison of Rates of Photosynthesis in Gigartina and Other Organisms

Rates are expressed as mg. of CO_2 consumed in 5 hours per hundred square centimeters of material. The figure for *Gigartina* is from our data, and all other figures are from Kniep (1914).

Material	Temperature	Rate of photosynthesis
	°C.	
Gigartina Harveyana	16.0	68.0
Padina pavonia	22.7	13.74
Ulva lactuca	23.0	6.56
Porphyra laciniata	22.7	8.98
Helianthus annuus	20.7	109.0

Differences in the light intensity curves may therefore account for the results of Engelmann (1883), Harder (1923), Montfort (1930), and Ehrke (1932), which appear to have been erroneously interpreted as showing that the red algae could use green and blue light better than the green algae. This interpretation may be correct, but at present there seems to be no conclusive evidence for it.

We also submit a comparison of the absolute value of photosynthesis in *Gigartina* with figures for other marine forms, and for leaves of *Helianthus*, in Table VIII. Kniep (1914) has collected and tabulated a number of measurements, expressed in milligrams of carbon dioxide

reduced per 100 cm². of material in 5 hours. Expressed in the same units, the rate of photosynthesis of a typical piece of our *Gigartina* compares favorably with the figure for *Helianthus*, especially considering the temperatures at which the two measurements were made. *Helianthus* is the most active of the land plants listed by Kniep. He represents the marine algae in general as having a much lower rate of photosynthesis than terrestrial plants. His finding supports our contention that the metabolism of marine algae has usually been studied under rather unfavorable conditions.

SUMMARY

A manometric method for measuring photosynthesis in marine algae is described.

Photosynthesis in the red alga *Gigartina harveyana* is shown to be similar in all important respects to photosynthesis in *Chlorella* and other Chlorophyceae.

We take this opportunity to express our thanks to members of the staff of the Hopkins Marine Station, especially Dr. Walter K. Fisher and Dr. C. B. van Niel, whose hospitality and cooperation made this work possible.

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