Plant Physiol. (1976) 58, 761-768

Comparison of the Photosynthetic Characteristics of Three Submersed Aquatic Plants[1]

Received for publication May 10, 1976 and in revised form August 13, 1976

THAI K. VAN, WILLIAM T. HALLER, AND GEORGE BOWES[2]
Departments of Agronomy and Botany, University of Florida, Gainesville, Florida 32611

ABSTRACT

Light- and CO_2-saturated photosynthetic rates of the submersed aquatic plants *Hydrilla verticillata*, *Ceratophyllum demersum*, and *Myriophyllum spicatum* were 50 to 60 μmol O_2/mg Chl·hr at 30 C. At air levels of CO_2, the rates were less than 5% of those achieved by terrestrial C_3 plants. The low photosynthetic rates correlated with low activities of the carboxylation enzymes. In each species, ribulose 1,5-diphosphate carboxylase was the predominant carboxylation enzyme. The apparent $K_m(CO_2)$ values for photosynthesis were 150 to 170 μM at pH 4, and 75 to 95 μM at pH 8. The $K_m(CO_2)$ of *Hydrilla* ribulose 1,5-diphosphate carboxylase was 45 μM at pH 8. Optimum temperatures for the photosynthesis of *Hydrilla*, *Myriophyllum*, and *Ceratophyllum* were 36.5, 35.0, and 28.5 C, respectively. The apparent ability of each species to use HCO_3^- ions for photosynthesis was similar, but at saturating free CO_2 levels, there was no indication of HCO_3^- use. Increasing the pH from 3.1 to 9.2 affected the photosynthetic rate indirectly, by decreasing the free CO_2. With saturating free CO_2 (0.5 mM), the maximum photosynthetic rates were similar at pH 4 and 8. Carbonic anhydrase activity, although much lower than in terrestrial C_3 plants, was still in excess of that required to support HCO_3^- utilization.

***Hydrilla* and *Ceratophyllum* had CO_2 compensation points of 44 and 41 μl/l, respectively, whereas the value for *Myriophyllum* was 19. Relatively high CO_2 compensation points under 1% O_2 indicated that some "dark" respiration occurred in the light. The inhibition of photosynthesis by O_2 was less than with terrestrial C_3 plants. Glycolate oxidase activity was 12.3 to 27.5 μmol O_2/mg Chl·hr, as compared to 78.4 for spinach. Light saturation of photosynthesis occurred at 600 to 700 μeinsteins/m²·sec in each species grown under full sunlight. *Hydrilla* had the lowest light compensation point, and required the least irradiance to achieve the half-maximal photosynthetic rate.**

Field measurements in a *Hydrilla* mat indicated that in the afternoon, free CO_2 dropped to zero, and O_2 rose to over 200% air saturation. Most photosynthetic activity occurred in the morning when the free CO_2 was highest and O_2 and solar radiation lowest. The low light requirement of *Hydrilla* probably provides a competitive advantage under these field conditions.

Hydrilla verticillata is a submersed, fresh-water angiosperm in the family Hydrocharitaceae. Since its introduction into Florida in 1960, it has become widely distributed, and is now regarded as a major aquatic weed problem in the Southeastern states (19). Once established in a body of water, it readily dominates and replaces native submersed species (19), such as *Ceratophyllum demersum*. The reason for this rapid dominance is uncertain. *Myriophyllum spicatum* is a submersed macrophyte that was also introduced, although earlier, into the United States. It has become widespread in the Northeast, but for reasons unknown, its distribution in Florida is limited. A major objective of this study was to compare the photosynthetic characteristics of these aquatic species in an attempt to explain the competitive success of *Hydrilla*.

The majority of terrestrial plants can be classified as C_3 or C_4 plants, based on specific characteristics associated with their photosynthetic pathways (4). C_4 plants typically have higher photosynthetic rates and greater productivity than C_3 plants (4). The photosynthetic mechanisms of submersed macrophytes, although basic to their productivity, have received limited attention. For *Hydrilla* and *Ceratophyllum*, the photosynthetic pathways and most of the associated characteristics are unknown. *Myriophyllum* apparently exhibits characteristics of both C_3 and C_4 plants. The initial product of CO_2 fixation is 3-P-glycerate (29), as in C_3 plants; but it reportedly also has a high optimum temperature for photosynthesis and a low CO_2 compensation point (29), which are characteristics usually associated with C_4 plants. In contrast, the submersed macrophytes *Egeria densa* and *Lagarosiphon major*, which belong to the same family as *Hydrilla*, possess CO_2 compensation points similar to those of C_3 plants, and their photosynthesis is inhibited by O_2 (9). Thus, aquatic macrophytes appear to exhibit some diversity in regard to their photosynthetic mechanism.

In an aquatic environment, the inorganic carbon can exist in several forms: free CO_2, H_2CO_3, HCO_3^-, or CO_3^{2-}, depending on the pH. For both aquatic and terrestrial plants, free CO_2 is the form most readily utilized for photosynthesis (25). A number of submersed plants, including *Myriophyllum*, reportedly can use HCO_3^- ions in addition to free CO_2 for photosynthesis (30). Recent work, however, suggests that several aquatic species are unable to use HCO_3^- ions (9). It has been argued that the ability to use HCO_3^- ions would provide an aquatic plant with a competitive advantage in alkaline waters (21). A further factor that may influence the competitive success of an aquatic plant is its photosynthetic response to light. *Egeria*, for example, reportedly replaces both *Elodea* and *Lagarosiphon* because of its lower light requirement for photosynthesis (9). In this study, we have examined the ability of *Hydrilla*, *Ceratophyllum*, and *Myriophyllum* to use HCO_3^- ions for photosynthesis and also the photosynthetic responses of these plants to varying irradiance. The possible ecological implications of these factors are discussed.

MATERIALS AND METHODS

Materials. *H. verticillata* (L.F.) Royle, *C. demersum* L., and *Cabomba caroliniana* Gray were collected from Rodman Reservoir, Lake Killarney, or Orange Lake, and *M. spicatum* L. was collected from Crystal River, Fla. Photosynthetic and enzymic

[1] This study was supported in part by a Florida Department of Natural Resources Research grant, and by funds from the Center for Environmental Programs of the Institute of Food and Agricultural Sciences. Published as Journal Series No. 6189 of the Florida Agricultural Experiment Stations.

[2] Department of Botany. To whom reprint requests should be sent.

measurements were made within 2 to 3 days of collection. Plants were held at 25 C in aerated lake water under a 12-hr day. All plants were washed free of epiphytes and bacteria before use. Spinach (*Spinacia oleracea* L.) was locally purchased. Soybean (*Glycine max* [L.] Merrill) and maize (*Zea mays* L.) were greenhouse-grown.

As used in this paper, $NaHCO_3$ refers to the total inorganic carbon, HCO_3^- refers to bicarbonate ions, and free CO_2 refers to H_2CO_3 and CO_2, calculated on the basis of published equilibrium constants (16, 21).

Field Measurements. Environmental parameters were measured in Lake Killarney, in the surface 5 cm of a *Hydrilla* mat over a 24-hr period. Free CO_2 and HCO_3^- levels were determined according to standard methods (1). Temperature and O_2 were measured with a Yellow Springs O_2 meter, model 54. Irradiance was determined with a Lambda quantum meter, model LI-185.

Polarographic O_2 Determinations. Unless otherwise stated, photosynthetic rates were measured as O_2 evolution at 30 C using a Clark O_2 electrode (Yellow Springs Instrument Co., model 53). The reaction vessels were irradiated with two 150-w incandescent lamps, which gave a saturating intensity of 1000 μeinstein/m^2·sec (400–700 nm). Leaves from the apical portions of the plants were immersed in 3 ml of 25 mM buffer and photosynthesis was initiated by the injection of 0.1 ml $NaHCO_3$ at various concentrations. Citrate-phosphate buffer was used to obtain pH values between 3.1 and 5.8, phosphate buffer for 5.8 to 6.9, and tris-HCl for 6.9 to 9.2. The buffering capacity of the higher $NaHCO_3$ concentrations was compensated for by the addition of predetermined amounts of 0.2 M HCl. Preliminary experiments indicated no effect of the buffers *per se* on rates of O_2 evolution during the 10 min required for one rate determination. All treatments were run in triplicate.

Infrared Gas Analyzer Determinations. To measure CO_2 compensation points, three or four apical plant segments, 12 cm long, were excised under water and immersed in 150 ml of 10 mM MES-NaOH, pH 5.5, and 5% v/v Hoagland solution contained in a 200-ml gas washing bottle fitted with a fritted glass gas filter. The bottle was then immersed in a glass-sided water bath and connected into a circuit consisting of a peristaltic pump, a moisture trap, a silica gel drying column, a Beckman model 215A infrared CO_2 gas analyzer, and a Beckman model 1008 O_2 analyzer. The plant material was irradiated through the water bath by a 1000-w quartz-halogen lamp (Berkley Colortran, multi-10A) providing 1000 μeinstein/m^2·sec (400–700 nm). An equilibrium period of 1 hr was used to reduce interference by residual gases in the lacunal system of the plants. The system was flushed with gas mixtures containing accurately metered concentrations of O_2, CO_2, and N_2; it was then closed and the gases were circulated by the peristaltic pump at 1 liter/min. The equilibrium point attained by the CO_2 concentration was taken as the CO_2 compensation point. Determinations were usually made in duplicate, the first starting above the CO_2 compensation point, the second from below it. The CO_2 leakage rate was found to be negligible. To avoid complicating effects from HCO_3^- formation, the medium was held at pH 5.5.

This system was also used for measurements of photosynthetic rates at air levels of CO_2, by determining the time required for the plant material in the closed system to reduce the CO_2 level of the circulating gas mixture from 340 to 335 μl CO_2/l. The amount of CO_2 taken up was calculated from the volume of the total system (550 ml). Where necessary, the irradiance was varied with neutral density filters.

Enzyme Assays. Leaf samples of approximately 500 mg fresh weight were ground at 4 C in a TenBroeck homogenizer with 10 ml of 50 mM tris-HCl, pH 8, containing 10 mM $MgCl_2$, 0.1 mM EDTA, 5 mM DTT,[3] 5 mM D-isoascorbate, and 2% w/v PVP-40. Aliquots were taken for Chl determinations and the homogenates were then centrifuged for 10 min at 1000*g* and 4 C. The supernatants were assayed immediately for PEP and RuDP carboxylase activity. The assay solutions for RuDP carboxylase contained in 1 ml: 50 mM tris-HCl, pH 8; 10 mM $MgCl_2$; 0.1 mM EDTA; 5 mM DTT; 0.4 mM RuDP; 20 mM $NaH^{14}CO_3$ (0.2 μCi/μmol); and 0.1 ml leaf extract. The PEP carboxylase assays were similar, except 5 mM PEP was substituted for RuDP and only 10 mM $NaH^{14}CO_3$ was used. Before addition of buffer, $NaH^{14}CO_3$, RuDP or PEP, and the leaf extract, the assay solutions at pH 5 were flushed and shaken for 5 min with He gas to remove dissolved CO_2 and O_2. The assay flasks were sealed and the remaining components, with the exception of RuDP or PEP, were injected and preincubated for 3 min. The reactions were initiated with RuDP or PEP and halted after 3 min at 30 C with 0.1 ml of 6 M HCl saturated with 2,4-dinitrophenylhydrazine. Aliquots were placed in scintillation vials, dried at 35 C under an air stream, and the radioactivity determined by liquid scintillation spectroscopy. All assays were run in triplicate.

Glycolate oxidase activity was assayed polarographically at 30 C in the presence of 14 mM glycolate (15). Carbonic anhydrase activity was determined by a modified Veronal indicator method (13). The Chl concentration was determined by the method of Arnon (2).

RESULTS

Field Measurements. In many Florida lakes during spring through autumn, *Hydrilla* plants form mats of vegetation just below the water surface. Light penetration through the mat is small, and net photosynthesis appears confined to the apical portions of the plant at the mat surface (19). In Figure 1 are plotted various photosynthesis-related parameters, measured over a 24-hr period in the surface 5 cm of water covering a naturally occurring *Hydrilla* mat. Day 1, when measurements

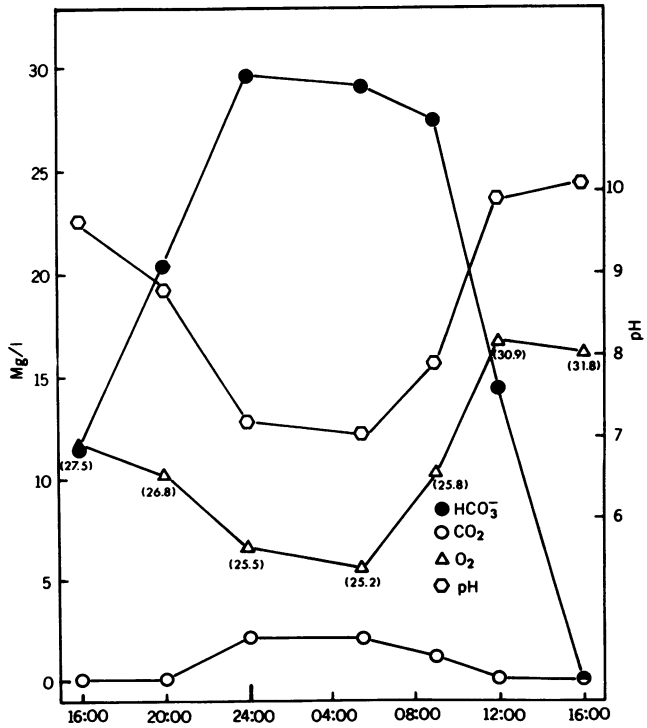

FIG. 1. Diurnal fluctuations in free CO_2, HCO_3^-, O_2, and pH measured in the surface 5 cm of water over a *Hydrilla verticillata* mat. Data were collected on October 14 and 15, 1975, at Lake Killarney, Fla. Figures in parentheses refer to the water temperature (C) at the time of sampling.

[3] Abbreviations: DTT: dithiothreitol; PEP: phosphoenolpyruvate; RuDP: ribulose 1,5-diphosphate.

commenced, was overcast, whereas day 2 was clear and warmer, which accounts for the slight variation in results between the two days. Large diurnal fluctuations in pH, O_2, HCO_3^-, and free CO_2 were found. Free CO_2 and HCO_3^- were highest at night, presumably due to respiration, but soon after sunrise (06.30 hr), they began to decline, and by the afternoon of day 2, they were both virtually zero. Conversely, due to the changes in inorganic carbon, the pH reached a low value of 7.1 at night but rose to a high of 10.2 in the afternoon of day 2. Oxygen during the night was low, being less than the air-saturated level, but by noon of day 2, it reached a maximum of 16.7 mg/l, equivalent to over 200% air saturation. Similar diurnal fluctuations have been previously reported for dense areas of aquatic vegetation (9). During this 24-hr period, temperatures varied from 25.2 to 31.8 C, however July values as high as 38 C have been measured in *Hydrilla* mats.

From the O_2 and temperature data in Figure 1, it was possible to calculate the approximate photosynthetic and respiratory activity of the mat over the 24-hr period (18). The data indicate (Table I) that the bulk of the photosynthetic activity occurred between sunrise and noon; net photosynthesis in the afternoon hours was very low. The negative values for the evening and night hours denote respiratory O_2 uptake.

In Figure 2 is plotted the irradiance intercepted by a mat as a function of time after sunrise. The day was clear and the full sun value was 2150 μeinsteins/m^2·sec. Even 3 hr after sunrise, the irradiance at 5 cm depth was still only about half of the maximum attainable. Corresponding values for the surface, and light penetration in clear water at 30 and 60 cm depth are also shown.

Table I. Photosynthetic and Respiratory Activity in a Hydrilla verticillata Mat over a 24 hr Period. Data calculated from the O_2 and temperature values in Figure 1.

Time Period	O_2 Evolution (+) or Uptake (-)
hr	g O_2/m^3
16:00 - 20:00	- 1.25
20:00 - 05:30	- 4.87
05:30 - 12:00	+11.68
12:00 - 16:00	+ 0.35
16:00 - 16:00 (24 hr)	+ 5.91

Laboratory Measurements. Unless otherwise stated, all measurements were made at 30 C, which corresponds closely to the mean day temperature for Florida lakes in the summer. Optimal temperatures for photosynthetic CO_2 fixation by *Hydrilla, Myriophyllum,* and *Ceratophyllum* were found to be 36.5, 35.0, and 28.5 C, respectively (data not shown). All three species exhibited measurable CO_2 fixation at temperatures as low as 10 C and as high as 44 C.

A number of laboratories have reported that the photosynthetic rates of submersed aquatic macrophytes decrease as the pH of the bathing medium increases (28, 30). With 0.6 mM $NaHCO_3$ as the inorganic carbon source, we observed similar results for O_2 evolution by *Hydrilla* (Fig. 3). The concentration of 0.6 mM $NaHCO_3$ (equivalent to 36.6mg HCO_3^-/l) approximated the highest inorganic carbon levels in the lakes from which the plants were collected. The highest, light-saturated, photosynthetic rates were obtained at pH values between 3.1 and 5.8. Above pH 5.8, the rate declined, falling to almost zero at 9.2 (Fig. 3). Although experimentally the highest photosynthetic rates were obtained at low pH, the measured pH values in *Hydrilla* mats from which the plants were collected ranged from pH 7.1 to 10.2 (Fig. 1). The data in Figure 3 for pH values above 7 probably reflect the range of light-saturated photosynthetic rates that occurs in the natural environment. In Figure 3, the per cent free CO_2 present at the various pH values is also shown. With an exception at about pH 5.8, the photosynthetic rate paralleled the free CO_2 concentration.

In Figure 4, photosynthetic rates of *Hydrilla, Ceratophyllum,* and *Myriophyllum,* as a function of the free CO_2 concentration, are compared at pH 4 and pH 8. At pH 4, free CO_2 comprised 99.3% of the total inorganic carbon in solution, while at pH 8 it was only 1.4%. For reference, the total concentrations of $NaHCO_3$ at pH 8 are also plotted on the abscissa. For each species, the maximum rate of O_2 evolution was similar at pH 4 and pH 8. However, at pH 4, saturation was achieved with 0.5 mM $NaHCO_3$, whereas at pH 8, 35 mM $NaHCO_3$ was required to saturate photosynthesis. The free CO_2 level at these two $NaHCO_3$ concentrations was similar, approximately 0.5 mM. It would appear that the apparent inhibition by high pH in Figure 3 was an indirect effect of high pH decreasing the free CO_2 level. There was no evidence that pH *per se* had any direct effect on the photosynthetic rate during these short term experiments. For a given subsaturating level of free CO_2, the photosynthetic rates of the three species were actually higher at pH 8 than at pH 4 (Fig.

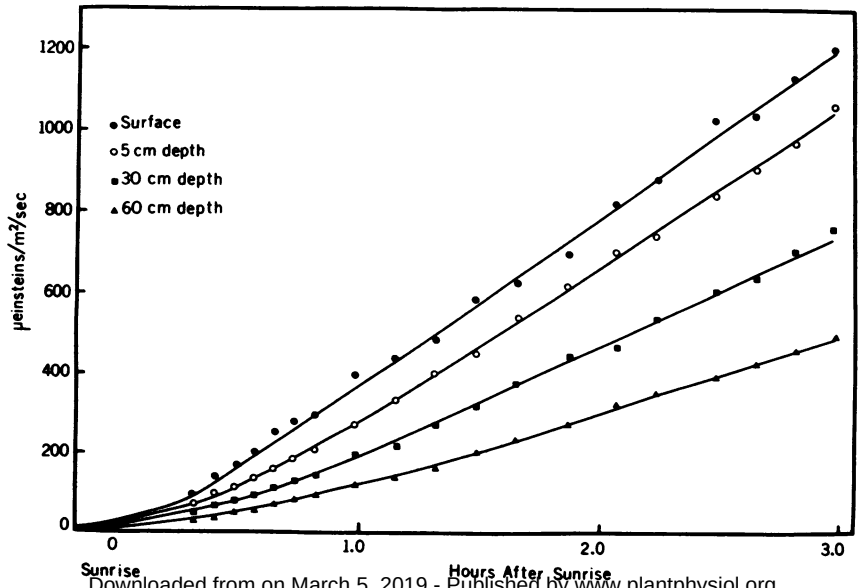

FIG. 2. Light penetration in clear lake water as function of the time after sunrise. Full sun value was 2150 μeinsteins/m^2·sec.

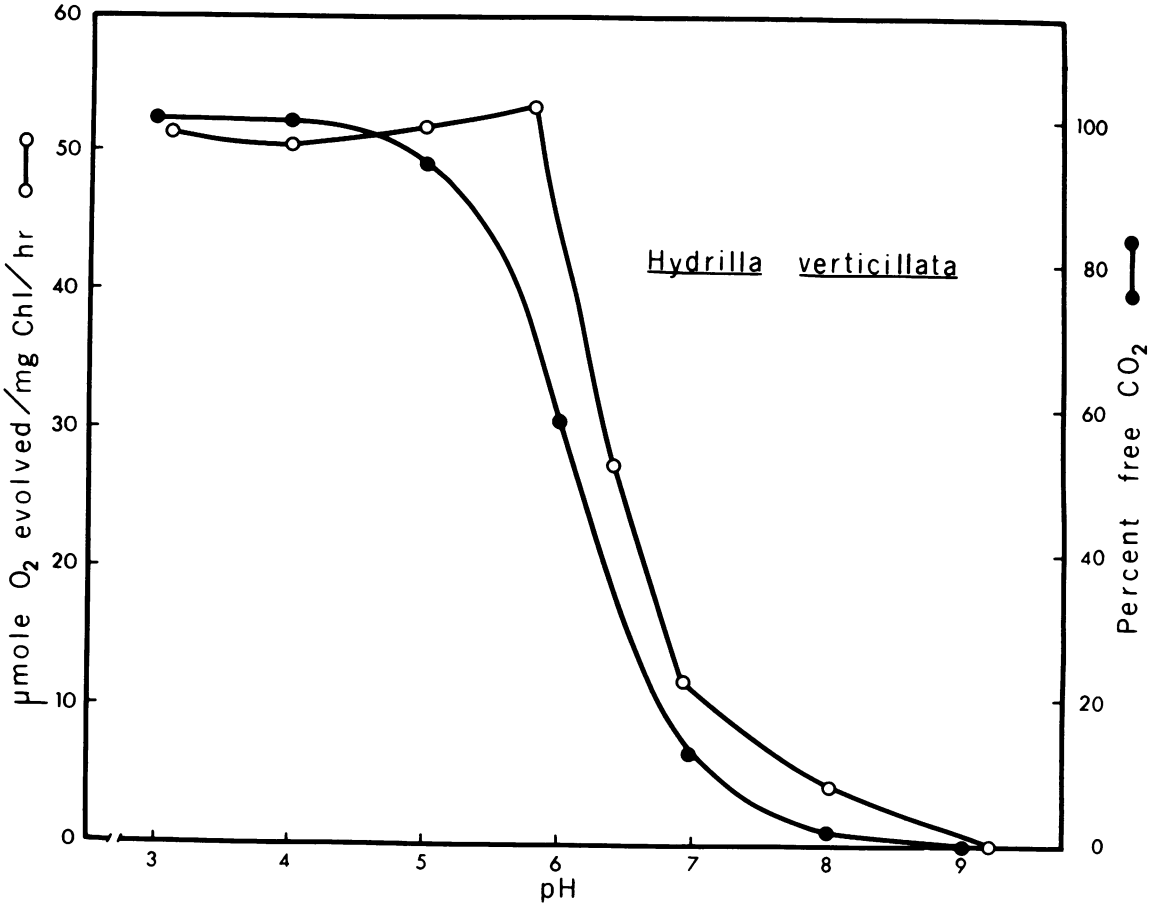

FIG. 3. Effect of pH on the photosynthetic rate of *Hydrilla verticillata* and on the calculated per cent free CO_2 in solution. Total concentration of inorganic carbon was 0.6 mM.

4.) The higher rates at pH 8 may be attributable to use of HCO_3^- ions in addition to free CO_2 (25, 30). The enhancement of photosynthetic rate at pH 8 was similar for each of the three species. On a percentage basis, the enhancement was greatest at the lowest levels of free CO_2 and declined to zero at saturating free CO_2 concentrations. It would appear that free CO_2 was the preferred form of inorganic carbon, because at saturating levels of free CO_2, increasing the HCO_3^- concentration had a negligible effect on the photosynthetic rate (Fig. 4) and compared with HCO_3^-, much lower concentrations of free CO_2 were required to achieve a given photosynthetic rate.

Carbonic anhydrase, which catalyzes the reversible hydration of CO_2, may be involved with the ability of plants to use HCO_3^- ions for photosynthesis (13, 25). The activity of this enzyme in leaf extracts of *Hydrilla*, *Ceratophyllum*, and *Myriophyllum* was found to be only 253, 292, and 207 units/mg Chl, respectively, as compared to 6283 units/mg Chl in a spinach leaf extract, spinach being representative of terrestrial C_3 plants. However, in terms of absolute rates at 0 C (31), the carbonic anhydrase activity in the aquatic species was still far in excess of that required to support HCO_3^- utilization.

In Table II are listed photosynthetic rates, for the three aquatic species at light and CO_2 saturation at pH 8. They probably represent close to the maximum rates attainable by these three species at 30 C, and as far as can be ascertained, they are the highest rates reported for submersed aquatic angiosperms. However, they are only about 10% of the maximum light and CO_2-saturated photosynthetic rates achieved by terrestrial angiosperms (26) and by the fresh-water alga, *Chlamydomonas* (5). Also listed in Table II are light-saturated photosynthetic rates at air levels of CO_2 (340 μl CO_2/l in the gas phase, or 0.42 mg CO_2/l in the aqueous phase) at pH 5.5. The rates observed for the three aquatic species were less than 5% of those obtainable with terrestrial C_3 plants under comparable CO_2 levels (24). The observation in Table II that the apparent $K_m(CO_2)$ values for photosynthesis of the three aquatic species were as high as 150 to 170 μM at pH 4 is probably related to the very low photosynthetic rates seen at air levels of CO_2. Apparent $K_m(CO_2)$ values for the photosynthesis of terrestrial plants are reportedly much lower (17), being approximately 10 μM CO_2 (0.03%). Increasing the pH from 4 to 8 decreased the apparent $K_m(CO_2)$ of photosynthesis (Table II), although still not to the level attained by terrestrial plants. This effect of pH on the apparent $K_m(CO_2)$ may be related to the decreased $K_m(CO_2)$ observed at high pH with RuDP carboxylase from spinach (8), or it may reflect the use of HCO_3^- ions at alkaline pH values.

Among terrestrial C_3 plants, RuDP carboxylase has been suggested to be an important rate-limiting step in light-saturated photosynthesis (3). The activity of this enzyme, together with the activity of PEP carboxylase, was determined in crude extracts of the three aquatic species (Table III). In each case, RuDP carboxylase was the predominant carboxylation enzyme. On a Chl basis, the RuDP carboxylase activities corresponded closely with the maximum photosynthetic rates attained by the three species (Table II). Although for the aquatic species, the activities of both carboxylases were lower than for spinach (Table III), the ratio of PEP to RuDP carboxylase was similar to spinach, being in the range generally found with C_3 plants (6, 23). *Hydrilla* and spinach were also similar with regard to the apparent $K_m(HCO_3^-$ and $CO_2)$ values of their extracted RuDP

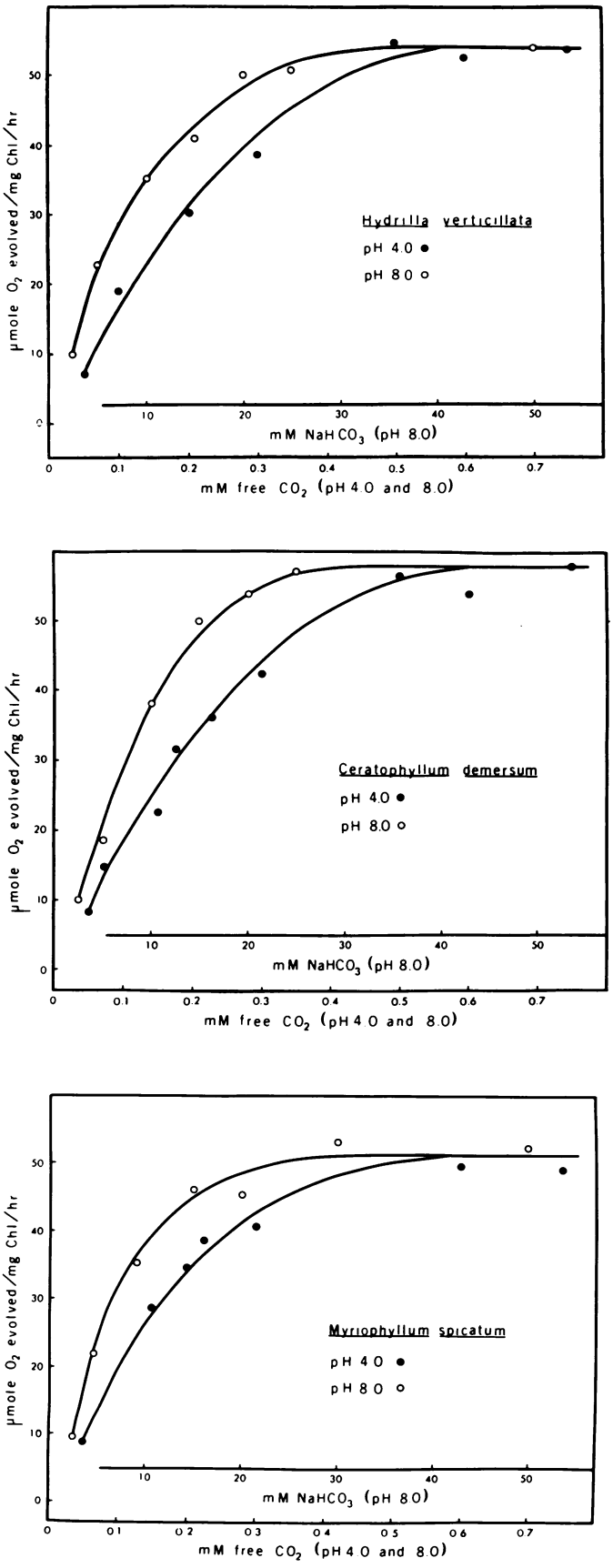

FIG. 4. Photosynthetic rates of *Hydrilla verticillata*, *Ceratophyllum demersum*, and *Myriophyllum spicatum* at pH 4 and pH 8 as a function of the free CO_2 concentration. For reference, the total inorganic carbon ($NaHCO_3$) concentration is also shown.

carboxylases (Table III), despite the high apparent $K_m(CO_2)$ for photosynthesis in the aquatic species (Table II).

Terrestrial C_3 plants, because of the O_2 sensitivity of the initial carboxylation enzyme, RuDP carboxylase (6, 7), exhibit a marked inhibition of photosynthesis at atmospheric levels of O_2. Figure 5 shows the effect of O_2 on photosynthetic CO_2 fixation by the three aquatic species at 340 μl CO_2/l in the gas phase. On the abscissa is plotted per cent O_2 in the gas phase, and for reference, the equivalent mg O_2/l dissolved in the aqueous phase. The photosynthesis of all three species was inhibited by O_2. At 21% O_2 (7.9 mg/l), *Hydrilla* and *Ceratophyllum* were inhibited 20.5% and 20.2%, respectively, whereas *Myriophyllum* was only inhibited 9.3%.

A further characteristic of C_3 plants is a high CO_2 compensation point due to photorespiratory CO_2 release in the light (12). Table IV presents the CO_2 compensation points at 25 C of the three aquatic species in 21% and 1% O_2 gas phase, and for comparison the values for soybean and maize leaves. The aquatic species were freshly collected from their aquatic habitats. The pH of the medium was held at pH 5.5 because in preliminary experiments, high pH levels reduced the CO_2 compensation point, confirming the report of Brown *et al.* (9). In our system, this was found to be an artifact. Bicarbonate formation in high pH solutions acted as a sink for free CO_2, and thus reduced the CO_2 in the gas phase measured by the analyzer. At pH 5.5 and 21% O_2, the aquatic species exhibited higher CO_2 compensation points than are found with terrestrial C_4 plants such as maize (Table IV). *Hydrilla* and *Ceratophyllum* were similar to the terrestrial C_3 plant, soybean; however *Myriophyllum* was intermediate between soybean and maize (Table IV). In terrestrial C_3 plants at 1% O_2, photorespiration is negligible, and hence the CO_2 compensation point is typically close to zero. For the three aquatic species, relatively high values were obtained at 1% O_2 (Table IV).

Glycolate oxidase activity is associated with an active photorespiratory pathway, being relatively high in C_3 plants and usually much lower in C_4 plants (22). In the three aquatic species under investigation, glycolate oxidase activity was detectable in leaf extracts, but at a level lower than in spinach (Table IV). It was appreciably higher than measured in leaf extracts of maize (Table IV).

In Figure 6 is shown the effect of increasing irradiance on the photosynthetic CO_2 fixation rate of the three aquatic species and also *Cabomba caroliniana*, a native submersed macrophyte common to Florida waters. As in the natural habitat, the CO_2 concentration was subsaturating at 340 μl/l in the gas phase. All of the plants were freshly collected from surface waters exposed to full sun. The species tested did not differ greatly in the irradiance required to saturate photosynthesis; saturation occurred at about 600 to 700 μeinsteins/m^2·sec (Fig. 6). *Hydrilla* and *Ceratophyllum* had similar photosynthetic rates at light saturation (Fig. 6), *Cabomba* had the lowest rate. The rates of dark respiration were similar for the species investigated, being 2 to 3 μmol CO_2 evolved/mg Chl·hr. Of the species examined, *Hydrilla* exhibited the lowest light compensation point (Fig. 6) and the lowest irradiance requirement to achieve half of the light-saturated rate ($^1/_2V_{max}$). Thus, at the light compensation of *Ceratophyllum* (35 μeinsteins/m^2·sec), *Hydrilla* was capable of photosynthesizing at 27% of its light-saturated rate.

DISCUSSION

The classification of submersed fresh-water plants in terms of their photosynthetic carbon fixation pathway is somewhat obscure (4). In this study, *Hydrilla*, *Ceratophyllum*, and *Myriophyllum* exhibited a number of characteristics associated with the C_3 photosynthetic pathway. The predominant carboxylation enzyme was RuDP carboxylase, which supports the finding that 3-PGA is the major first product of photosynthetic carbon fixa-

Table II. Photosynthetic Rates at Saturating and Air Levels of CO_2, and the Apparent $Km(CO_2)$ Values for Photosynthesis of Hydrilla verticillata, Myriophyllum spicatum, and Ceratophyllum demersum.

Species	Light and CO_2 Saturated Photosynthetic Rate	Light Saturated Photosynthetic Rate at 340 µl CO_2/l (gas phase)	Apparent $Km(CO_2)$[1] for Photosynthesis	
			pH 4	pH 8
	µmole O_2/mg Chl.hr	µmole CO_2/mg Chl.hr	µM	
H. verticillata	54	4.6	170	90
M. spicatum	51	3.3	150	75
C. demersum	58	4.9	165	95

[1] Derived from 1/2 Vmax.

Table III. RuDP and PEP Carboxylase Activities in Leaf Extracts of Hydrilla verticillata, Myriophyllum spicatum, Ceratophyllum demersum, and Spinacia oleracea.

Species	Carboxylase Activity		Km[1] Values for RuDP Carboxylase	
	RuDP	PEP	HCO_3^-	CO_2
	µmole CO_2 fixed/mg Chl.hr		mM	µM
H. verticillata	50.6	18.5	3.2	45
M. spicatum	69.7	7.1	-	-
C. demersum	69.9	5.5	-	-
S. oleracea	329.6	40.2	3.6	50

[1] Derived from 1/2 Vmax.

tion in *Myriophyllum* (29); however, the activity of this enzyme was much lower than in spinach. The aquatic species had low photosynthetic rates, in fact substantially lower than terrestrial C_3 plants, and were inhibited by 21% O_2 in the gas phase. At similar O_2 and CO_2 levels, terrestrial C_3 plants show 30 to 40% inhibition of net photosynthesis (12, 14); for the aquatic species, the inhibition was only 10 to 20%. Under 21% O_2, the CO_2 compensation points of *Hydrilla* and *Ceratophyllum* were similar to those of C_3 plants, but with *Myriophyllum*, it was significantly lower. It was not, however, zero, as Stanley and Naylor (29) inferred. It appeared to be intermediate between C_3 and C_4 plants, and in this respect, resembled *Panicum millioides*, a terrestrial C_3 plant with a reduced O_2 inhibition of photosynthesis and CO_2 compensation point (10, 23). With several aquatic macrophytes, we have observed large, intraspecific variations in the CO_2 compensation point that appear to be environmentally related (Bowes, G. and A. S. Holaday, unpublished

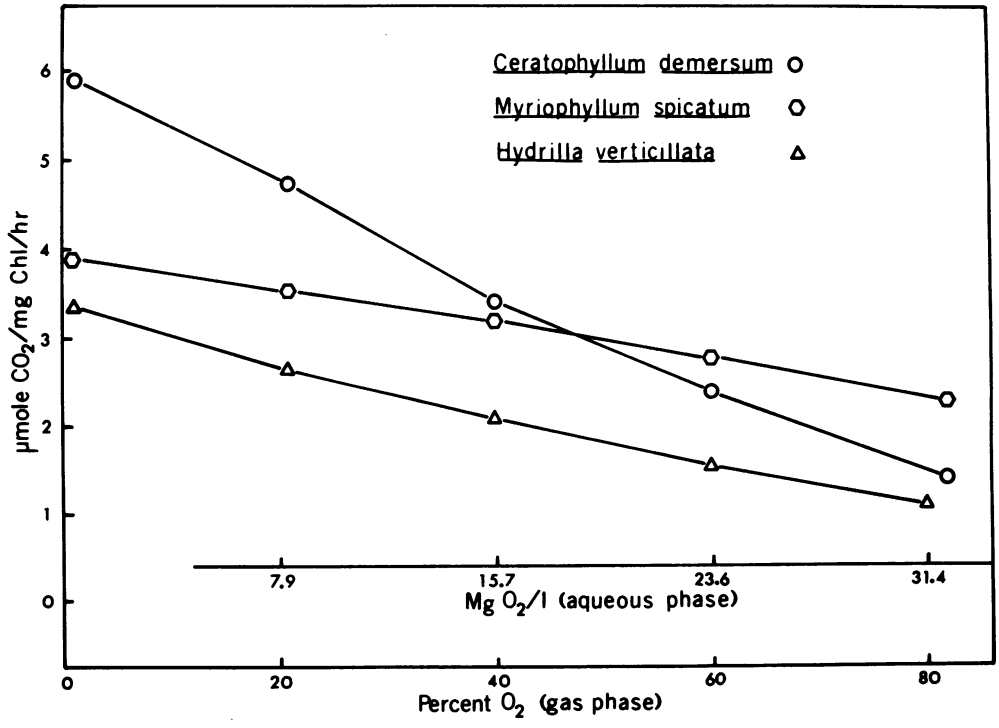

FIG. 5. Effect of O_2 on the photosynthetic CO_2 fixation rates of *Hydrilla verticillata*, *Ceratophyllum demersum*, and *Myriophyllum spicatum* at pH 5.5. Measurements were made at 340 µl CO_2/l in the gas phase (equivalent to 0.42 mg CO_2/l in the aqueous phase).

data), which may account for the conflicting reports with submersed aquatic plants (9, 29). Unlike more typical C_3 plants, the CO_2 compensation points of the aquatic species remained quite high under 1% O_2 in the gas phase. A similar result has been reported for *Egeria densa* (9). It may indicate that dark respiration in these aquatic species continues unabated in the light. At 340 μl/l CO_2 in the gas phase, dark respiration was equivalent to over 50% of the light-saturated net photosynthetic rate. For terrestrial plants, dark respiration in the photosynthetic tissues is typically only 5 to 10% of photosynthesis (33).

A high CO_2 compensation point under 21% O_2 suggests an active photorespiratory pathway, with concomitantly high glycolate oxidase activity (4, 12, 22). In the aquatic species examined, the activity of glycolate oxidase was between 15 and 35% of that found in spinach leaves. This may indicate that the photorespiratory capacity of aquatic species is less than terrestrial C_3 plants. Hough and Wetzel (20) reported that photorespiratory $^{14}CO_2$ release by the submersed macrophyte *Najas flexilis* was limited in comparison with terrestrial C_3 plants because of the limited solubility of O_2 in water. However, for aquatic and terrestrial plants, O_2 has to dissolve to become available to the cells. Our field measurements (Fig. 1) suggest that aquatic plants under certain conditions may be exposed to dissolved O_2 levels far in excess of those faced by terrestrial plants. It appears more likely that any reduced photorespiration in aquatic plants is a function of generally lower enzyme activities. When the low photosynthetic rate and reduced activities of RuDP carboxylase and glycolate oxidase are taken into account, the ratio of photosynthesis to photorespiration may be similar to that occurring in more typical C_3 plants, a conclusion supported by the CO_2 compensation point measurements (Table IV).

Table IV. CO_2 Compensation Points and Glycolate Oxidase Activity of Hydrilla verticillata, Myriophyllum spicatum, and Ceratophyllum demersum in Comparison with Terrestrial C_3 and C_4 Species.

Species	CO_2 Compensation Point		Glycolate Oxidase Activity
	21% O_2	1% O_2	
	µl/l		µmole O_2/mg Chl.hr
H. verticillata	44	17	12.3
M. spicatum	19	9	27.5
C. demersum	41	11	22.2
G. max	44	5	-
S. oleracea	-	-	78.4
Z. mays	0	0	8.6

A further characteristic associated with the C_3 pathway is a low (25 C) optimum temperature for photosynthesis (4, 12). *Ceratophyllum*, with a value of 28.5 C, is thus similar to C_3 plants. A lower optimum of 20 C has been observed with *Ceratophyllum* growing in cooler waters (11). Both *Hydrilla* and *Myriophyllum* exhibited high optimum temperatures for photosynthesis reminiscent of C_4 plants (4). The high optimum temperature in C_4 plants is at least partially related to the absence of an inhibitory effect of 21% O_2 on photosynthesis (22). This explanation is unlikely to apply to *Hydrilla* and *Myriophyllum*, as both species exhibited some O_2 inhibition.

Leaf anatomy has been widely used to differentiate between C_3 and C_4 plants (4). In C_3 plants, the chloroplasts are distributed throughout the leaf, whereas in C_4 plants, they are generally restricted to the bundle sheath cells and an abutting ring of mesophyll cells. The leaves of *Myriophyllum* (27), *Hydrilla*, and *Ceratophyllum* (Bowes, G., unpublished observations), appear

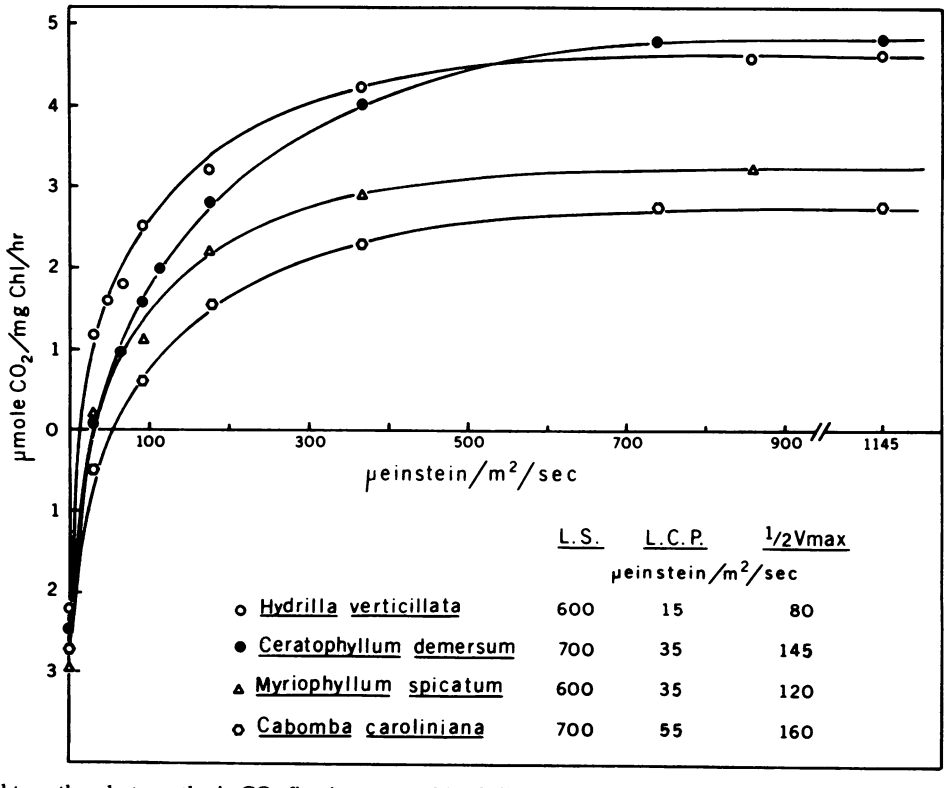

FIG. 6. Effect of light on the photosynthetic CO_2 fixation rates of *Hydrilla verticillata, Ceratophyllum demersum, Myriophyllum spicatum*, and *Cabomba caroliniana* at pH 5.5. All measurements were made at 340 μl CO_2/l in the gas phase (equivalent to 0.42 mg CO_2/l in the aqueous phase). L.S.: irradiance required for light saturation; L.C.P.: irradiance at the light compensation point; $^1/_2V_{max}$: irradiance required for half-maximal photosynthetic rate.

anatomically more similar to C_3 than to C_4 plants. From these considerations, it appears that the three aquatic species examined are basically C_3 plants, although in certain respects they are atypical of this grouping.

In the field, *Hydrilla* often appears luxuriant, and this, coupled with its ability to dominate large areas of water, belies its low photosynthetic capability. The ability to cover large areas despite a low photosynthetic rate is probably related to the low dry to fresh weight ratio of aquatic plants. The dry weight of a *Hydrilla* plant comprises only 8.8% of the fresh weight, whereas the value for a terrestrial soybean leaf is about 22%. In aquatic systems, it is likely that less photosynthate has to be used for support of the plant. The low photosynthetic rate measurements reflect productivity data, as on a dry weight basis, submersed fresh-water plants are less productive than terrestrial systems (32).

The low photosynthetic rates cannot be attributed solely to diffusion limitations in an aquatic environment, as certain aquatic multicellular and unicellular algae photosynthesize at rates comparable to terrestrial angiosperms (5, 26). The low RuDP carboxylase activity and the high apparent $K_m(CO_2)$ values are probably contributory factors to the low photosynthetic rates. In the lake, the maximum free CO_2 level was equivalent to only 48 μM, indicating that in the natural habitat these plants not only photosynthesize at CO_2 levels below saturation but also below their apparent $K_m(CO_2)$. The high apparent $K_m(CO_2)$ values for photosynthesis are not of universal occurrence in aquatic species as the fresh-water alga, *Chlamydomonas*, reportedly has a low value similar to terrestrial plants (5). It is unlikely that the high apparent $K_m(CO_2)$ values are due to RuDP carboxylase kinetics, as *Hydrilla* and spinach RuDP carboxylases exhibited similar $K_m(CO_2)$ values.

At subsaturating CO_2 levels, the enhancement in photosynthetic rate at pH 8 as compared with pH 4 has traditionally been regarded as evidence for HCO_3^- utilization (25, 30). However, an increase in the affinity of RuDP carboxylase for CO_2 at high pH and HCO_3^- concentrations (8) may provide an alternative explanation for the phenomenon. If the enhancement does reflect HCO_3^- use, it appears unlikely that it could be a factor enabling *Hydrilla* to replace native species, because for the three species examined, the degree of enhancement was similar. The importance of HCO_3^- use for the growth of aquatic plants is difficult to estimate, and has yet to be unequivocally demonstrated.

In areas of dense aquatic vegetation most of the daily photosynthetic activity occurs during the early morning hours (Table I). It is during this period that solar radiation is low (Fig. 2), and thus the photosynthetic response of the plants to light should be an important determinant of competitive success. For the aquatic species examined, the irradiance required to saturate photosynthesis was similar. Saturation occurred at 28 to 33% of the equivalent full sun intensity. Thus, in the field, photosynthesis in the surface regions should not be light-limited for large parts of the day, and *Hydrilla* and *Ceratophyllum* might be expected to have equivalent photosynthetic rates (Fig. 6). However, the aquatic species differed in the irradiance required to achieve the half-maximal photosynthetic rate and the light compensation point. To achieve a given, light-limited photosynthetic rate, *Hydrilla* had the lowest light requirement of the species examined. In the hours following sunrise, when free CO_2 is highest and most photosynthetic activity occurs, this low light requirement would appear to provide *Hydrilla* with a distinct advantage.

Acknowledgments – We wish to thank L. A. Garrard for helpful discussions and M. H. Gaskins for use of the gas analyzer.

LITERATURE CITED

1. American Public Health Association. 1971. Standard Methods for the Examination of Water and Waste Water. Government Printing Office, Washington, D. C.
2. Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24: 1-15.
3. Björkman, O. 1968. Carboxydismutase activity in shade-adapted and sun-adapted species of higher plants. Physiol. Plant. 21: 1-10.
4. Black, C. C., Jr. 1973. Photosynthetic carbon fixation in relation to net CO_2 uptake. Annu. Rev. Plant Physiol. 24: 253-286.
5. Bowes, G. and J. A. Berry. 1972. The effect of oxygen on photosynthesis and glycolate excretion in *Chlamydomonas reinhardtii*. Carnegie Instit. Year Book 71: 148-158.
6. Bowes, G. and W. L. Ogren. 1972. Oxygen inhibition and other properties of soybean ribulose 1,5-diphosphate carboxylase. J. Biol. Chem. 247: 2171-2176.
7. Bowes, G., W. L. Ogren, and R. H. Hageman. 1971. Phosphoglycolate production catalyzed by ribulose diphosphate carboxylase. Biochem. Biophys. Res. Commun. 45: 716-722.
8. Bowes, G., W. L. Ogren, and R. H. Hageman. 1975. pH Dependence of the $K_m(CO_2)$ of ribulose 1,5-diphosphate carboxylase. Plant Physiol. 56: 630-633.
9. Brown, J. M. A., F. I. Dromgoole, M. W. Towsey, and J. Browse. 1974. Photosynthesis and photorespiration in aquatic macrophytes. *In:* R. L. Bieleski, A. R. Gerguson, and M. M. Cresswell, eds., Mechanisms of Regulation of Plant Growth, Bulletin 12. The Royal Society of New Zealand, Wellington. pp. 243-249.
10. Brown, R. H. and W. V. Brown. 1975. Photosynthetic characteristics of *Panicum milioides*, a species with reduced photorespiration. Crop Sci. 15: 681-685.
11. Carr, J. L. 1969. The primary productivity and physiology of *Ceratophyllum demersum*. II. Micro primary productivity, pH, and the P/R ratio. Aust. J. Mar. Freshwat. Res. 20: 127-142.
12. Chollet, R. and W. L. Ogren. 1975. Regulation of photorespiration in C_3 and C_4 species. Bot. Rev. 41: 137-179.
13. Everson, R. G. and C. R. Slack. 1968. Distribution of carbonic anhydrase in relation to the C_4 pathway of photosynthesis. Phytochemistry 7: 581-584.
14. Forrester, M. L., G. Krotkov, and C. D. Nelson. 1966. Effect of oxygen on photosynthesis, photorespiration, and respiration in detached leaves. I. Soybean. Plant Physiol. 41: 422-427.
15. Frederick, S. E., P. J. Gruber, and N. E. Tolbert. 1973. The occurrence of glycolate dehydrogenase and glycolate oxidase in green plants. An evolutionary survey. Plant Physiol. 52: 318-323.
16. Gibbons, B. H. and J. T. Edsall. 1963. Rate of hydration of carbon dioxide and dehydration of carbonic acid at 25 C. J. Biol. Chem. 238: 3502-3507.
17. Goldsworthy, A. 1968. Comparison of the kinetics of photosynthetic carbon dioxide fixation in maize, sugarcane and its relation to photorespiration. Nature 217: 6.
18. Hall, C. A. S. and R. Moll. 1975. Methods of assessing aquatic primary productivity. *In:* H. Lieth and R. H. Whittaker, eds., Primary Productivity of the Biosphere. Springer-Verlag, New York. pp. 19-53.
19. Haller, W. T. and D. L. Sutton. 1975. Community structure and competition between *Hydrilla* and *Valisneria*. Hyacinth Contr. J. 13: 48-50.
20. Hough, R. A. and R. G. Wetzel. 1972. A ^{14}C-assay for photorespiration in aquatic plants. Plant Physiol. 49: 987-990.
21. Hutchinson, G. E. 1957. A Treatise on Limnology, Vol. I. John Wiley & Sons, New York.
22. Jackson, W. A. and R. J. Volk. 1970. Photorespiration. Annu. Rev. Plant Physiol. 21: 385-432.
23. Kestler, D. P., B. C. Mayne, T. B. Ray, L. D. Goldstein, R. H. Brown, and C. C. Black. 1975. Biochemical components of the photosynthetic CO_2 compensation point of higher plants. Biochem. Biophys. Res. Commun. 66: 1439-1446.
24. Lilley, R. McC. and D. A. Walker. 1975. Carbon dioxide assimilation by leaves, isolated chloroplasts, and ribulose bisphosphate carboxylase from spinach. Plant Physiol. 55: 1087-1092.
25. Raven, J. A. 1970. Exogenous inorganic carbon sources in plant photosynthesis. Biol. Rev. 45: 167-221.
26. Raven, J. A. and S. M. Glidwell. 1975. Photosynthesis, respiration and growth in the shade alga *Hydrodictyon africanum*. Photosynthetica 9: 361-371.
27. Sculthorpe, C. D. 1967. The Biology of Aquatic Vascular Plants. E. Arnold, London.
28. Shiyan, P. N. and A. I. Merezhko. 1972. Effect of hydrogen ion concentration on photosynthesis and radiocarbon metabolism in aquatic plants. Hydrobiol. J. 8: 23-29.
29. Stanley, R. A. and A. W. Naylor. 1972. Photosynthesis in Eurasian watermilfoil (*Myriophyllum spicatum* L.). Plant Physiol. 50: 149-151.
30. Steemann Nielsen, E. 1960. Uptake of CO_2 by the plant. *In:* W. Ruhland, ed., Encyclopedia of Plant Physiology, Vol. 5, Part 1. Springer-Verlag, Berlin, pp. 70-84.
31. Waygood, E. R. 1955. Carbonic anhydrase (plant and animal). Methods Enzymol. 2: 836-846.
32. Westlake, D. F. 1963. Comparisons of plant productivity. Biol. Rev. 38: 385-425.
33. Zelitch, I. 1975. Improving the efficiency of photosynthesis. Science 188: 626-633.