THE EFFECTS OF MANIPULATIONS OF THE CONCENTRATIONS OF O_2, CO_2, TOTAL INORGANIC CARBON, AND GLYCIDATE ON THE WARBURG EFFECT IN TWO SPECIES OF MARINE UNICELLULAR ALGAE

by

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B.Sc., University of Victoria, 1973

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
in the Department
of
Biology

ACCEPTED
FACULTY OF GRADUATE STUDIES

[redacted]

DATE Sept 1980

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August 1979

ABSTRACT

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The photosynthetic CO_2 assimilation rate of *Isochrysis galbana* was depressed by dissolved $[O_2]$ greater than zero. The depression was linear between 80 and 945 μM O_2 and independent of $[HCO_3^-]$ or $[CO_2]$ at high $[O_2]$. The depression caused by 330 μM O_2 compared to 115 μM O_2 was relieved by 5.05 mM carbon. At least 10 μM CO_2 was required for half-maximal photosynthesis, and 300 μM O_2 was sufficient to cause half-maximal O_2 inhibition. The pH optimum of photosynthesis was narrow (7.25 to 7.50) and correlated with the $[CO_2]$. Glycidate inhibited photosynthesis and the depression was independent of $[O_2]$.

Optimal photosynthesis in *Thalassiosira fluviatilis* occurred at $[O_2]$ between 220 and 500 μM at 2.0 mM carbon and between 125 and 225 μM at 0.2 mM carbon. A slight depression in photosynthesis was sometimes observed at $[O_2] > 800$ μM compared to $[O_2] < 100$ μM. This depression was sensitive to [carbon] and relieved by 2.0 mM carbon. Only 2.5 μM CO_2 was required for half-maximal photosynthesis and at least 700 μM O_2 was required for half-maximal O_2 inhibition. The pH optimum of photosynthesis was broad (< 7.00 to 8.25) and not closely correlated with $[CO_2]$. Glycidate stimulated photosynthesis and the stimulation was inversely related to $[O_2]$.

These data are consistent with exclusive C-3 metabolism in *I. galbana* and the presence of photorespiration, although the absence of stimulation of photosynthesis by glycidate is not consistent with previous studies on C-3 terrestrial plants. In *I. galbana* the Warburg effect at high [O_2] was not relieved by elevated [CO_2] which indicated that O_2 inhibition can be caused by mechanisms other than photorespiration. The absence of a Warburg effect and the high affinity for CO_2 in *T. fluviatilis* are consistent with efficient recycling of photorespiratory CO_2 by C-4 metabolism. However, other mechanisms can explain these results; these include the dehydration of HCO_3^- in the chloroplast through the activity of carbonic anhydrase or absence of photorespiration *per se* resulting from a low intrinsic activity of RuDP oxygenase. Regardless of the causes of these differences, the high affinity for CO_2, broad pH tolerance, and absence of a Warburg effect in *T. fluviatilis* suggest that this speices would have a considerable competitive advantage over a species like *I. galbana* in near-shore areas where high cell densities and high photosynthetic rates could result in alkaline pH values and O_2 supersaturation.

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ACKNOWLEDGEMENTS

I would like to thank Dr. Louis Hobson for his financial and intellectual support and for the considerable time he devoted to editing this thesis. I would also like to thank Dr. David Ballantyne for bringing the results of Brändén (1978) to my attention.

INTRODUCTION

It is common knowledge that all life depends, ultimately, upon the flow of energy from the sun, which is rendered useful by a unique physio-chemical process known as photosynthesis. Within a photoautotrophic cell the various reactions of photosynthesis undoubtedly function as an integrated unit, but it is convenient and logical to consider photosynthesis as consisting of two distinct groups of reactions, those involving the direct capture and conversion of light energy and those responsible for the assimilation of organic carbon. In terms of the latter, "true" or "gross" photosynthesis results from the reaction of ribulose-1,5-diphosphate (RuDP) with CO_2 to form two molecules of 3-phosphoglycerate (3-PGA). This reaction is catalysed by RuDP carboxylase (EC 4.1.1.39) which has an absolute requirement for CO_2 as opposed to HCO_3^- (Cooper & Filmore, 1969). The resulting 3-PGA enters the Calvin cycle which serves to simultaneously synthesize hexose molecules and to regenerate the CO_2 acceptor, RuDP. Gross photosynthesis is an appropriate measure of the photosynthetic capacity of an organism, but in relation to ecological concepts, such as primary productivity rates or the energy flow in food webs, the relevant parameter is "apparent" or "net" photosynthesis. Gross photosynthesis is reduced to net photosynthesis by the activities of: (1) conventional respiratory pathways which have not been conclusively shown to occur in photosynthetic cells or tissues in the light and (2) photorespiration. In this study, only the effects of photorespiration will be discussed.

Photorespiration can be defined as light dependent O_2 consumption and CO_2 release by photoautotrophic organisms or tissues. Considerable evidence indicates that the CO_2 released during photorespiration is derived from the oxidation of glycolate through the activity of a complex, but apparently superfluous, pathway involving several intermediates and three cell organelles (Jackson & Volk, 1970; Tolbert, 1974; Zelitch, 1971). Although large amounts of recently fixed carbon may appear as glycolate, glycolate is not among the intermediates of the Calvin cycle; consequently the source of glycolate is not obvious and is the subject of a long-standing controversy. A currently popular hypothesis of glycolate biosynthesis derives from the discovery by Bowes *et al.* (1971) that, *in vitro,* RuDP carboxylase catalyses the oxidation of RuDP as well as the familiar carboxylation reaction that initiates the Calvin cycle. One of the products of the oxidation of RuDP is phosphoglycolate which can be readily hydrolysed to glycolate by phosphoglycolate phosphatase, an enzyme commonly found in chloroplasts (Randall, 1976). Accordingly, many authors have suggested that glycolate synthesis *in vivo*, hence photorespiration, also results from the oxygenase activity of RuDP carboxylase. The validity of the RuDP oxygenase hypothesis of photorespiration has not been fully evaluated, but the behavior of the *in vitro* reaction has been shown to have many similarities to photorespiration (Tolbert & Ryan, 1975). In particular, CO_2 and O_2 apparently act as competitive inhibitors of the oxygenase and carboxylase activities of the purified enzyme, a fact that dovetails with the observation that photorespiration is directly proportional to the O_2 concentration and inversely proportional to the concentration of

CO_2 (at least according to some methods of estimating photorespiration). The half saturation (Km) values of *in vitro* RuDP carboxylase/oxygenase for the two substrates, CO_2 and O_2, are similar to the concentrations of these gases in aqueous systems (e.g. cytoplasm) in equilibrium with the atmosphere (Jensen & Bahr, 1977). This provocative correlation has led to the conclusion that photorespiration may be an inevitable consequence of the high levels of O_2 in our atmosphere, or, restated in ecological terms, that photorespiration is merely a manifestation of habitual CO_2 limitation of photosynthesis (Lorimore & Andrews, 1973). Although numerous arguments exist in the literature concerning the causes, magnitude, function, and biochemical details of photorespiration, most authors would agree that photorespiration substantially reduces the rate of gross photosynthetic CO_2 assimilation in terrestrial plants possessing only the Calvin cycle (C-3 plants) when they are exposed to atmospheric levels of CO_2 and O_2. If photorespiration is the result of an oxygenase reaction of RuDP, then aquatic environments, in which O_2 and CO_2 are frequently at or near equilibrium with the atmosphere, should be no less intrinsically favourable to photorespiration than terrestrial environments. However, despite extensive investigation, photorespiration has not been unequivocally demonstrated in aquatic autotrophs in general and algae in particular. Although a vast body of literature indicates the presence of photorespiration-like phenomena in algae, a few methodologically important studies have failed to detect photorespiration in these organisms. These contradictions and the generally unsatisfactory understanding of algal photorespiration is largely the result of technical limitations imposed by the aquatic environment.

Photorespiration in terrestrial plants is best characterised by light dependent CO_2 exchange rates in various gas mixtures, measurements of which depend upon the use of an infrared CO_2 analyser. In terms of CO_2 exchange, photorespiration is indicated by: (1) a CO_2 compensation point of more than 40 $\mu l\ l^{-1}$, (2) a greater release of CO_2 into a stream of CO_2-free air in the light than in darkness, (3) inhibition of photosynthetic CO_2 assimilation by elevated O_2 concentrations (the Warburg effect), and (4) a greater release of $^{14}CO_2$ than $^{12}CO_2$ when $^{14}CO_2$ is initially supplied. The fourth measurement indicates that recently fixed carbon is preferentially metabolised and is supposed to allow the separation of residual dark respiration in the light from photorespiration *per se* (Ludwig & Canvin, 1971). Gas analysis systems have occasionally been adapted for use with unicellular algae (Björkman, 1966; Fock *et al.*, 1971; Lloyd *et al.*, 1977a) by layering algal cells on filter paper, 10µ Nitex mesh, or membrane filters saturated with culture medium. Applied to algae, the gas analysis technique yields quantitative estimates of photorespiration that can be directly compared to studies with terrestrial plants and has the further advantage of allowing direct and virtually instantaneous measurements of the CO_2 assimilation rate. The only drawback of this technique is that the algae must be exposed to an unnatural gaseous environment which introduces an uncontrolled variable. Nevertheless, algal cells under these conditions show typical photosynthetic responses to light intensity and CO_2 concentration (Lloyd *et al.*, 1977a) and their photosynthetic rates are in the range of values reported by other authors using aqueous systems (Burris, 1977). However, the results obtained with algae exposed to gaseous environments have been inconsistent with the view that photorespiration occurs in these organisms. For example, Lloyd *et al.* (1977a), using a variety of marine and freshwater species, reported less release of CO_2 or $^{14}CO_2$ in the light than in darkness, CO_2 compensation points of less than 10 μl l^{-1}, and no inhibition of photosynthetic CO_2 assimilation by 21%- or 50%-O_2 air compared to 2%-O_2 air. These results are in marked contrast to C-3 terrestrial plants under the same conditions or algae assayed in aqueous environments. The causes of this discrepency are not known, but, until these contradictory observations are reconciled, results with algae in gaseous environments must be considered tentative.

With the exceptions outlined above, studies with algae have relied upon qualitative indications of photorespiration which include: (1) indirect estimates of the CO_2 compensation point (Brown and Tregunna, 1967; Tolbert and Garey, 1976); (2) comparisons of the release of $^{14}CO_2$ from labelled cells into $^{14}CO_2$-free medium in the light and in darkness (Hough, 1976); (3) measurements of glycolate metabolism such as the incorporation of ^{14}carbon into glycolate-pathway intermediates, the excretion of glycolate with and without inhibitors of glycolate metabolism, and the measurement of the activity of glycolate-pathway enzymes (Beardall *et al.*, 1976; Bruin *et al.*, 1970; Burris *et al.*, 1976; Burris, 1977; Codd & Merrett, 1971; Colman *et al.*, 1974; Fogg, 1976; Han & Eley, 1974; Ingle & Colman, 1976, Kowallik, 1971; Mukerji *et al.*, 1978; Nelson & Tolbert, 1969; Paul & Volcani, 1974; Randall, 1976; Tolbert & Zill, 1956; Tolbert, 1976; and others); and (4) inhibition of photosynthetic CO_2 assimilation or O_2 evolution by algae assayed in culture medium containing elevated concentrations of O_2 (Warburg effect) (Beardall *et al.*, 1976; Belay & Fogg, 1978; Black *et al.*, 1976;

Burris, 1977; Downton *et al.*, 1976; Findenegg & Fischer, 1978; Griffiths, 1976; and many others reviewed by Tolbert, 1974). The first three methods contain implicit assumptions and internal errors that make quantitative interpretation and comparison with terrestrial plants difficult. The fourth method, O_2 inhibition of photosynthesis, is conveniently applicable to aqueous systems and constitutes the largest body of data on photorespiration in marine algae.

While the Warburg effect is probably not identical with photorespiration (Bunt & Heeb, 1971), there exists in terrestrial plants an excellent correspondence between the presence of a measurable Warburg effect and the presence of photorespiration in a given species. Therefore the method is probably a valid measure of photorespiration in algae, however, previous studies utilizing this measurement have suffered from several limitations. Most studies have used continuous aeration of the cultures during experiments. This author (unpublished data) found bubbling with N_2 at the rate of 100 ml min^{-1} through 50 ml cultures of *Isochrysis galbana* reduced the rate of CO_2 fixation by more than 50% compared to unagitated controls in a two hour experiment. If CO_2 fixation rates in cultures aerated with N_2, He, air, or O_2 are to be validly compared, then the inhibition caused by aeration alone must be independent of the gas used. No evidence exists to support this assumption. In the present study, even brief periods of vigorous bubbling with O_2 caused the pH to shift in artificial seawater (see Materials and Methods). Such a shift would influence CO_2 fixation since the concentration of free CO_2 is inversely related to the pH in seawater. Further complications of continuous aeration arise from the difficulty of ensuring that all experimental chambers are receiving the same rate of bubbling when several chambers are used simultaneously and the unknown effects of frequent exposure of algal cells to a gas/water interface. For these reasons continuous aeration introduces uncontrolled variables and should be avoided. Rarely in previous studies has the actual concentration of dissolved O_2 been reported and frequently the pH of the medium has been ignored (Beardall *et al.*, 1976; Black *et al.*, 1976; Burris, 1977; Downton *et al.*, 1976; Griffiths, 1976; Beardall & Morris, 1975) making estimates of free CO_2 concentration impossible. These oversights have made comparisons with terrestrial plants and correlations with RuDP carboxylase/oxygenase activities difficult. The effect of O_2 on algal photosynthesis has been limited to a few points, generally at O_2 concentrations near zero (N_2 or He aeration), at maximum supersaturation (O_2 aeration), and sometimes at intermediate values. Rarely has a single species of unicellular algae been exposed to the full range of O_2 concentrations possible in seawater. Therefore, it has been difficult to determine the probable impact of O_2 inhibition under natural conditions. Lastly, some studies have used unrealistically high cell densities (>10^6 cells ml^{-1}). Such high cell densities would rapidly deplete the available CO_2 resulting in overstimates of O_2 inhibition.

An analysis of the deficiencies of previous studies stimulated me to develop a new method for measuring the Warburg effect in unicellular marine algae which permits fine control over the critical variables of pH, O_2, and CO_2. Although lacking the convenience and experimental precision of gas analysis systems, the method employed here has the advantage of retaining the natural aqueous environment of algae. The method is adaptable to low cell densities, eliminates the effects of continuous aeration, and allows a full range of dissolved O_2 concentrations to be tested. Most importantly, the technique permits the effects of O_2 on photosynthesis to be examined with respect to simultaneous or independent changes in CO_2 and total inorganic carbon concentrations. In contrast to the simple bubbling experiments most frequently used, this method has several advantages in characterizing the photorespiratory responses of marine algae in comparison with the well documented behaviour of terrestrial plants. For example, it is possible to measure the concentration of CO_2 required for half-maximal saturation of photosynthesis and the O_2 concentration required for half-maximal inhibition of photosynthesis. These values can be quantitatively compared to the apparent Km values for CO_2 and O_2 of terrestrial plants subject to photorespiration and to *in vitro* RuDP. carboxylase/oxygenase. Similarly, the responses of the algae to changes in O_2 and CO_2 can be compared with the predictions of the RuDP oxygenase hypothesis of photorespiration. It is also possible to evaluate (to a first approximation) the potential involvement of HCO_3^- in the photosynthesis of marine algae from their responses to ultra low CO_2 concentrations. The effects of a specific inhibitor of photorespiration would constitute an independent assay for algal photorespiration. Accordingly, the effects of glycidate (2,3-epoxy propionic acid, the potassium salt), a reported inhibitor of photorespiration (Zelitch, 1974), are included in this report.

The choice of the species used in this study, *Isochrysis galbana* Parke (Chrysophyceae) and *Thalassiosira fluviatilis* Huestedt (Bacillariophyceae), was based on the work of Beardall *et al.* (1976) who have demonstrated considerable diversity in the photosynthetic mechanisms of various taxa of marine algae. Their work implicates the presence of C-4 pathway in diatoms which also have relatively low rates of O_2 inhibition, while the photosynthetic and photorespiratory behaviour of *I. galbana* is consistent with exclusive C-3 metabolism. The question of C-4 pathway in marine algae is highly controversial and tied to the larger question of HCO_3^- involvement in photosynthesis. Bicarbonate ion may be used in photosynthesis in either of two ways: directly through the C-4 metabolism in which HCO_3^- is initially fixed into organic substrates by the activity of phosphoenol pyruvate (PEP) carboxylase (EC 4.1.1.31) or (2) indirectly through the dehydration of HCO_3^- to CO_2 ($HCO_3^- + H^+ \rightleftharpoons CO_2 + H_2O$) by carbonic anhydrase at the site of the Calvin cycle. Seawater contains large quantities of HCO_3^- (about 2.2 mM); if marine algae can utilize HCO_3^- as a carbon source for net photosynthesis, then carbon limitation (and therefore, photorespiration, if the RuDP oxygenase hypothesis is correct) may not readily occur in the marine environment. The purpose of this study is to evaluate the roles of photorespiration and HCO_3^- in photosynthetic CO_2 assimilation in two taxonomically and morphologically dissimilar species of marine phytoplankton and relate these roles to the general question of carbon limitation in the marine environment as it applies to phytoplankton ecology.

MATERIALS AND METHODS

Cultures

I. galbana and *T. fluviatilis* were grown as 1.5-litre batch cultures in the medium described by Guillard and Ryther (1962). The cultures were maintained in an environment chamber at 20° on a 12:12 hours light:dark cycle. Irradiance, measured with an Alphametrics model dc 1020 irradiance meter, was 12.2 joules cm^{-2} $hour^{-1}$ at the vessel surface which is near the minimum required for light saturated division rates in these species (Hobson, 1974). Cell counts were measured daily and the experiments were conducted with log phase cultures at cell densities between 4×10^4 and 1×10^5 cells ml^{-1} for *I. galbana* and between 1×10^4 and 4×10^4 cells ml^{-1} for *T. fluviatilis*.

Preparation of Artifical Seawater with Known Carbon and Oxygen Concentrations and pH

Artificial seawater was prepared as 20 litre volumes using the formula of Brujewicz (Sverdrup *et al.*, 1942) omitting $NaHCO_3$. After the salts had completely dissolved the salinity was adjusted to 32 ‰, which was similar to the culture medium, and stored under N_2. 2.0-litre aliquots of the artificial seawater were dispensed under N_2 pressure into 4-litre erlenmyer flasks; glass beads were added and the solution was boiled vigorously for 60 minutes with a condensor fitted to prevent volume loss. Immediately after boiling, the artificial seawater was purged with N_2 bubbled through an air stone for 20 minutes, stoppered and allowed to cool for at least 12 hours. The residual carbon (ΣCO_2) was measured by the method of Martin (1972) and found to be less than 0.04 mM. Nutrients, trace elements, and vitamins were added as in the culture medium (Guillard & Ryther, 1962). Carbon was brought to the desired molarity with $NaHCO_3$, and 10 μci litre^{-1} of $NaH^{14}CO_3$ (New England Nuclear) was added. Glycidate (2,3-epoxy propionic acid, the potassium salt - Sigma Chemical Company) was added at this time for experiments using this inhibitor.

The pH was then adjusted by dispensing under N_2 pressure a 50.0-ml aliquot of the artificial seawater and titrating it to the desired pH with 0.010 N NaOH or HCl. The aliquot was then discarded and an appropriate amount of 0.100 or 0.010 N NaOH or HCl was added to the entire volume. After mixing another 50.0-ml aliquot was dispensed and the pH measured. If the pH deviated by more than 0.01 pH units from the desired value, the entire procedure was repeated. All pH determinations were made with a Fischer Accumet model 220 b pH meter fitted with a Fischer pH electrode and an Orion double junction reference (Calomel) electrode.

Dissolved oxygen concentration was adjusted by bubbling O_2 through an air stone at the rate of 2 litres min^{-1} for time periods from 5 to 180 seconds. The oxygen concentration was then determined by the Winkler method. After the addition of oxygen, the pH was measured and readjusted if necessary, since O_2 bubbling sometimes caused a slight shift in pH.

When the pH and O_2, carbon, and glycidate concentrations were all at the desired levels, the artificial seawater was dispensed under N_2 pressure into 60-ml Pyrex bottles which were filled to overflowing, fitted with glass stoppers and stored at 20° until used.

Harvesting the Cultures

The cells were harvested at 2000 rpm in a refrigerated (10^o) centrifuge (Sorvall model RC2-B) fitted with a Szent-Gyorgi and Blum continuous flow apparatus. The flow rate was adjusted to drain the 1.5-litre culture in 20 minutes. The cell concentrate was resuspended in 500 ml of CO_2/O_2-free artificial seawater and centrifuged at 2000 rpm for 10 minutes. Most of the supernatant was discarded and the cells resuspended in the remaining medium. A 0.50 ml aliquot of the cell concentrate was diluted to 10.0 ml with seawater and the cell density estimated using a haemocytometer. Accordingly, the volume of the remaining cell concentrate was then adjusted with CO_2/O_2-free artificial seawater such that, when 0.5 ml of the volume-adjusted cell concentrate was added to the 60.0-ml volume of the experimental vessels, the resulting final cell densities fell in the range of 1.6 to 4 x 10^4 cells ml^{-1} for *I. galbana* and 2.4 to 6.0 x 10^3 cell ml^{-1} for *T. fluviatilis*.

After centrifugation cells were examined by phase contrast microscopy for obvious signs of damage. No damage was visible in *T. fluviatilis*, but *I. galbana* showed reduced motility and about 10% of the cells were clumped in groups of 3 to 6.

Experiments

Each experiment was initiated by adding 0.50 ml aliquots of the cell concentrate to the prepared 60-ml bottles and placing the bottles in the same environment chamber used for the growth of the cultures. After two hours the experiment was terminated by filtering the cells onto Gelman glass fiber filters. The pressure differential across the filter was 6 mm of Hg created by an aspirator.

Preparation of Filters and Measurement of Photosynthetic CO_2 Assimilation

The filters were fumed with HCl for 20 minutes to remove inorganic ^{14}C, dried at 60^{o} for 30 minutes and transferred to scintillation vials containing 10 ml of Econoflour (New England Nuclear). The radioactivity was measured with a Beckman model 133 liquid scintillation spectrophotometer; the counting efficiency was determined using the internal standard technique. Carbon-14 as counts per minute was converted to photosynthetic CO_2 assimilation by the method of Strickland and Parsons (1972) and normalized on cell counts. The photosynthetic rate for each species varied considerably from one experiment to another (see Tables 1 - 8). This was probably due to differences in the physiological states of the cultures and to damage caused by harvesting the cells. To eliminate these variations within an experiment, a single population of cells was used for each experiment.

Excretion of dissolved organics was estimated by acidifying the filtrates with HCl, purging with N_2 for 2 hours, and transferring 1.0-ml aliquots of the acidified purged filtrates to scintillation vials containing 10 ml of Aquasol (New England Nuclear). Radioactivity and counting efficiency were determined as described above. Initial experiments, in which all samples were checked for excretion, generally revealed undetectable levels of dissolved organic ^{14}C. Thereafter excretion was monitored by checking random samples from each experiment. In no case did ^{14}C in the dissolved organic fraction exceed 5% of the particulate ^{14}C, and usually the level was at the limit of detection (about 1%).

RESULTS

Photosynthetic CO_2 Assimilation as a Function of Oxygen Concentration at Constant pH and Inorganic Carbon Concentration

The effect of dissolved oxygen concentration on the photosynthetic rate of *I. galbana* at pH 8.00 and 2.0 and 0.2 mM inorganic carbon is shown in Table 1 and Figure 1. Oxygen concentrations greater than 80 μM (and, by inference, greater than zero) inhibited the rate of CO_2 fixation, and within the limits of experimental error the inhibition was linear and independent of the concentration of inorganic carbon between 0.2 and 2.0 mM, 2.0 and 20 μM CO_2 respectively. (At pH 8.00 1% of the total inorganic carbon is in the form of CO_2 at 20^o and 32^o/oo salinity (Riley & Skirrow, 1965); this convenient relationship and the fact that pH 8.00 is roughly the middle of the range of pH values found in natural seawater are the reasons that pH 8.00 was chosen for these experiments.) Assuming that the maximum rates of CO_2 assimilation are represented by the y-intercept values of the lines calculated by linear regression (dashed lines on Figure 1), the rate of CO_2 assimilation was reduced by 6% and 20% at 240 μM O_2 (100% of saturation, defined as the concentration of dissolved O_2 in seawater of this temperature and salinity when in equilibrium with the atmosphere) and 710 μM O_2 (300% of saturation) respectively compared to zero O_2. However, these values must be considered the lower limits of O_2 inhibition, since the depression appears to be proportionally greater at lower O_2 concentrations and therefore the y-intercept values probably underestimate the actual rate

TABLE 1. The effect of dissolved oxygen concentration on the photosynthetic CO_2 assimilation rate of *Isochrysis galbana* at pH 8.00 and two concentrations of inorganic carbon.

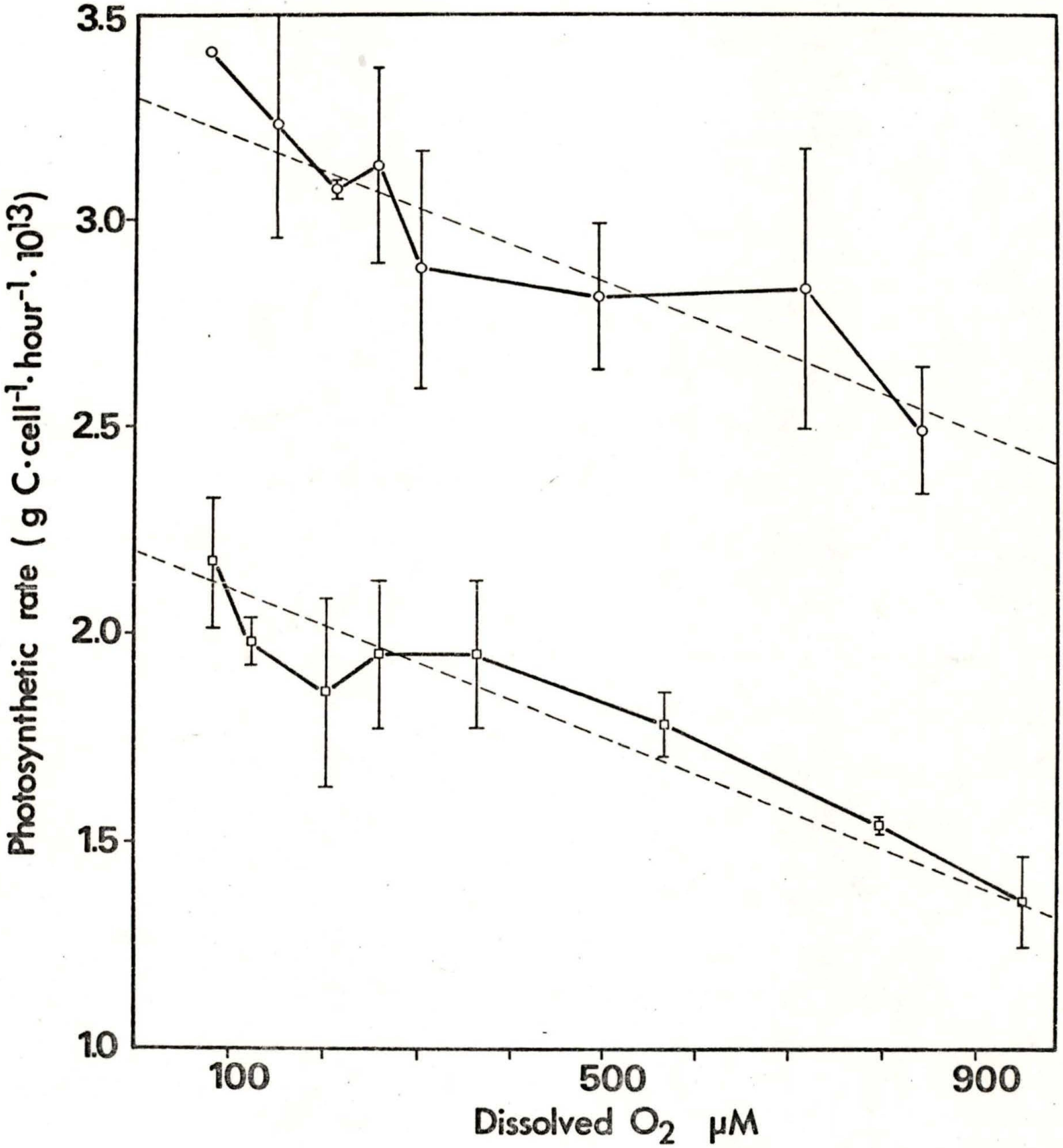
Inorganic Carbon		Dissolved Oxygen		Photosynthetic Rate	
Total (mM)	CO_2 (μM)	O_2 (μM)	% of saturation	$g\ C \cdot cell^{-1} \cdot hour^{-1} \cdot 10^{13}$ ± $1.96 \cdot S.E.$ of $\bar{X}$	% of rate at 2.0 mM C and 80 μM O_2
2.0	20	80	34	3.41 ± 0.00	100.0
"	"	150	63	3.23 ± 0.28	94.7
"	"	220	91	3.07 ± 0.02	90.0
"	"	260	110	3.13 ± 0.24	91.8
"	"	305	129	2.88 ± 0.29	84.5
"	"	495	209	2.81 ± 0.18	82.4
"	"	715	302	2.83 ± 0.34	83.0
"	"	840	355	2.49 ± 0.15	73.0
0.2	2.0	85	36	2.17 ± 0.16	63.6
"	"	125	53	1.98 ± 0.06	58.1
"	"	205	87	1.86 ± 0.24	54.5
"	"	260	110	1.95 ± 0.18	57.2
"	"	365	154	1.95 ± 0.11	57.2
"	"	565	239	1.78 ± 0.08	52.2
"	"	770	336	1.54 ± 0.02	45.2
"	"	945	399	1.36 ± 0.11	39.9

FIGURE 1. The photosynthetic CO_2 assimilation rate of *I. galbana* as a function of the dissolved O_2 concentration at pH 8.00. Circles = 2.0 mM inorganic carbon (20 μM CO_2); squares = 0.2 mM inorganic carbon (2.0 μM CO_2). Error bars represent ± 1.96 x S.E. of the mean (n = 2). Dashed lines were calculated by linear regression

(upper line, $y = 3.35 + (-9.68 \times 10^{-4})X$, $r = -0.85^*$

lower line, $y = 2.15 + (-7.94 \times 10^{-4})X$, $r = -0.92^*$)

(* Significant at the 99% confidence level)


3.5
3.0
2.5
2.0
1.5
1.0
Photosynthetic rate (g C·cell^{-1}·hour^{-1}· 10^{13})
100
500
900
Dissolved O$_2$ µM

of CO_2 assimilation at zero O_2. Notice that reducing the inorganic carbon concentration from 2.0 to 0.2 mM reduced the rate of CO_2 assimilation by 33%, but did not alter the percentage inhibition caused by O_2.

Table 2 and Figure 2 present the data for an identical experiment performed on *T. fluviatilis*. Unlike *I. galbana*, optimum CO_2 assimilation at 2.0 mM carbon occurred between 200 and 495 μM O_2 (90 to 209% of saturation). The rates of CO_2 assimilation at very low and very high O_2 concentrations (80 and 840 μM O_2 respectively) were identical within the limits of experimental error. When inorganic carbon was reduced from 2.0 to 0.2 mM, the rate of CO_2 assimilation was reduced by 25% and optimal rates occurred between 125 and 260 μM O_2 (53 to 110% of saturation). A slight inhibition occurred at 0.2 mM carbon when the O_2 concentration exceeded 565 μM. To facilitate comparison, Figure 3 expresses these data as percentages of the rates measured for each species at 2.0 mM carbon and 80 μM O_2.

Photosynthetic CO_2 Assimilation as a Function of Total Inorganic Carbon Concentration at Constant pH and Three Concentrations of Dissolved O_2

Figure 4 and Table 3 show the response of *I. galbana* to changes in carbon concentration at pH 8.00 and three concentrations of dissolved O_2. Assuming that the rate of CO_2 assimilation at 5.05 mM carbon was near the maximum, then half saturation of CO_2 assimilation occurred at 1 mM carbon (10 μM CO_2). This value is clearly a lower limit, since Figure 4 shows that the CO_2 assimilation rate is approaching, but has not reached CO_2 saturation at 5.05 mM carbon (50.5 μM CO_2). *I. galbana* is apparently incapable of utilizing HCO_3^- in photosynthesis, since

TABLE 2. The effect of dissolved oxygen concentration on the photosynthetic CO_2 assimilation rate of <u>Thalassiosira fluviatilis</u> at pH 8.00 and two concentrations of inorganic carbon.

Inorganic Carbon		Dissolved Oxygen		Photosynthetic Rate	
Total (mM)	CO_2 (μM)	O_2 (μM)	% of saturation	$g\ C \cdot cell^{-1} \cdot hour^{-1} \cdot 10^{12}$ ± 1.96·S.E. of $\bar{X}$	% of rate at 2.0 mM C and 80 μM O_2
2.0	20	80	34	5.35 ± 0.21	100.0
"	"	150	63	5.55 ± 0.05	103.7
"	"	220	91	5.92 ± 0.24	110.7
"	"	260	110	5.74 ± 0.05	107.3
"	"	305	129	5.51 ± 0.54	103.0
"	"	495	209	5.75 ± 0.17	107.5
"	"	715	302	5.50 ± 0.06	102.8
"	"	840	355	5.33 ± 0.15	99.6
0.2	2.0	85	36	3.98 ± 0.25	74.4
"	"	125	53	4.22 ± 0.56	78.9
"	"	205	87	4.17 ± 0.07	77.9
"	"	260	110	4.03 ± 0.03	75.3
"	"	365	154	3.93 ± 0.04	73.5
"	"	565	239	3.93 ± 0.36	73.5
"	"	770	336	3.87 ± 0.21	72.3
"	"	945	399	3.61 ± 0.22	67.5

FIGURE 2. The photosynthetic CO_2 assimilation rate of *T. fluviatilis* as a function of the dissolved O_2 concentration at pH 8.00. Circles = 2.0 mM inorganic carbon (20 μM CO_2); squares = 0.2 mM inorganic carbon (2.0 μM CO_2). Error bars represent ± 1.96 x S.E. of the mean (n = 2).

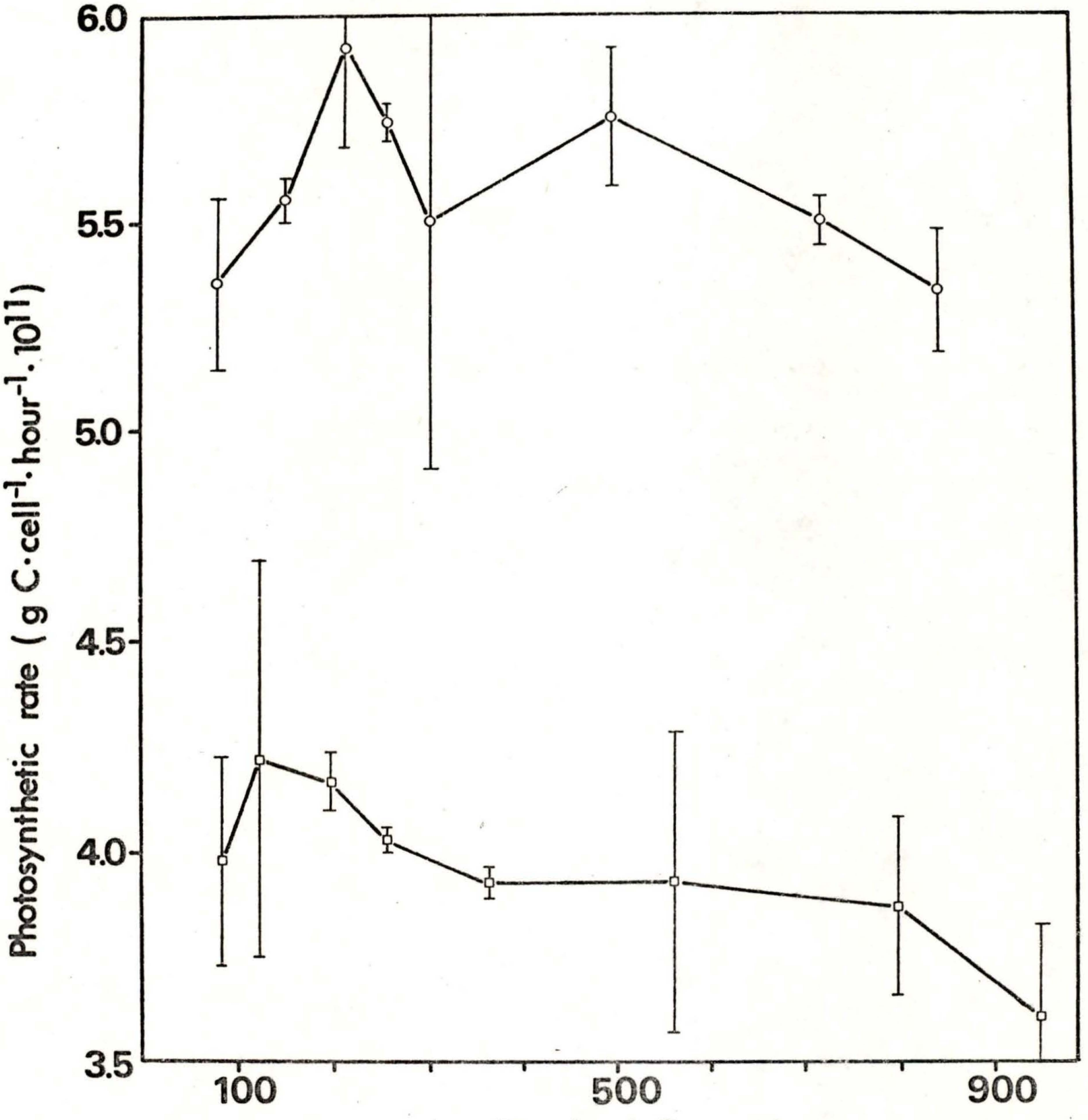

6.0
5.5
5.0
4.5
4.0
3.5
Photosynthetic rate (g C·cell^{-1}·hour^{-1}·10^{11})
100
500
900
Dissolved O_2 µM

FIGURE 3. The photosynthetic CO_2 assimilation rate of *I. galbana* and *T. fluviatilis* as a function of the concentration of dissolved O_2 at pH 8.00. Values are expressed as a percent of the value measured for each species at 80 μM O_2 and 2.0 mM carbon. Circles = *T. fluviatilis*, closed = 0.2 mM carbon, open = 2.0 mM carbon. Squares = *I. galbana*, closed = 0.2 mM carbon, open = 2.0 mM carbon.

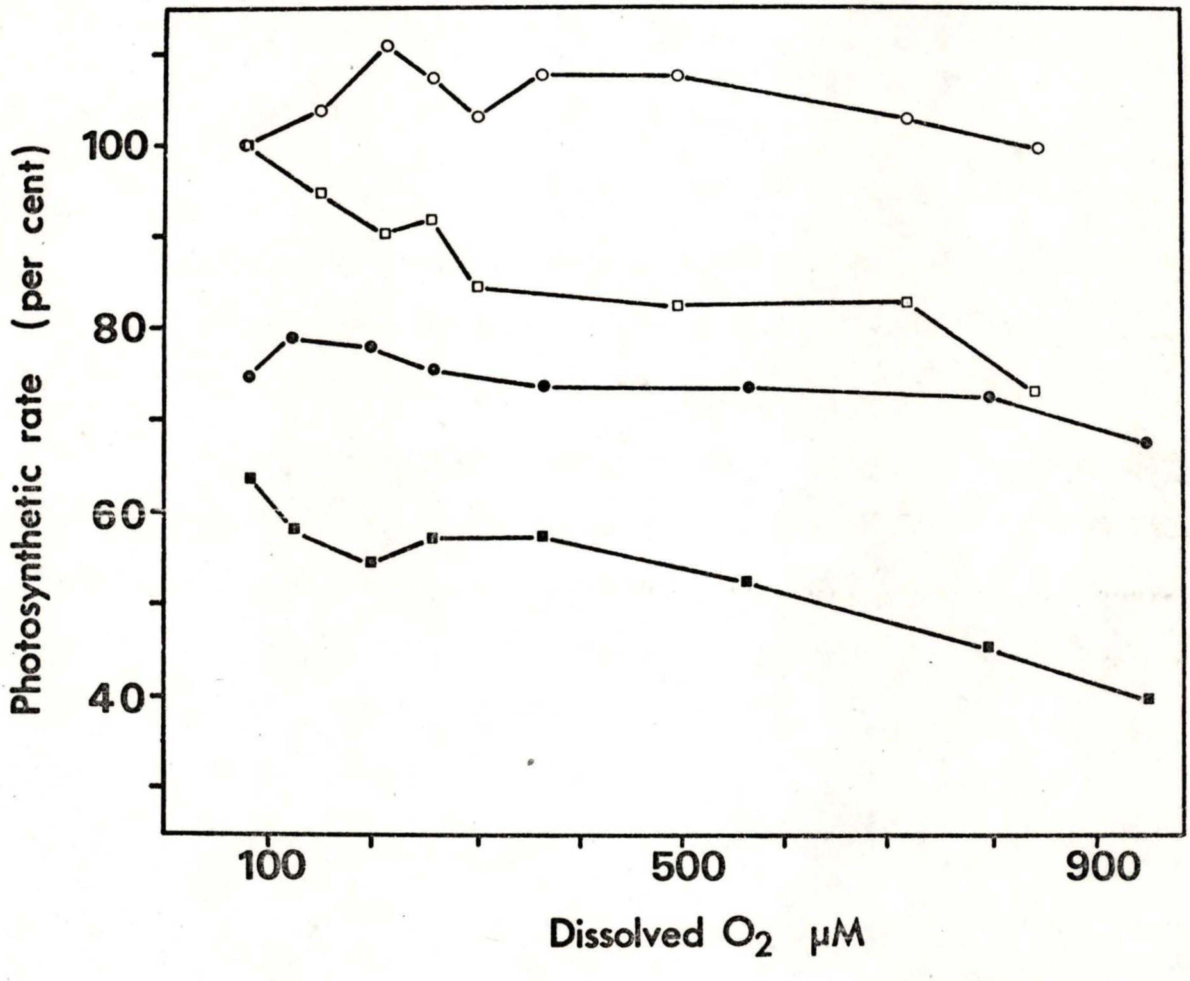
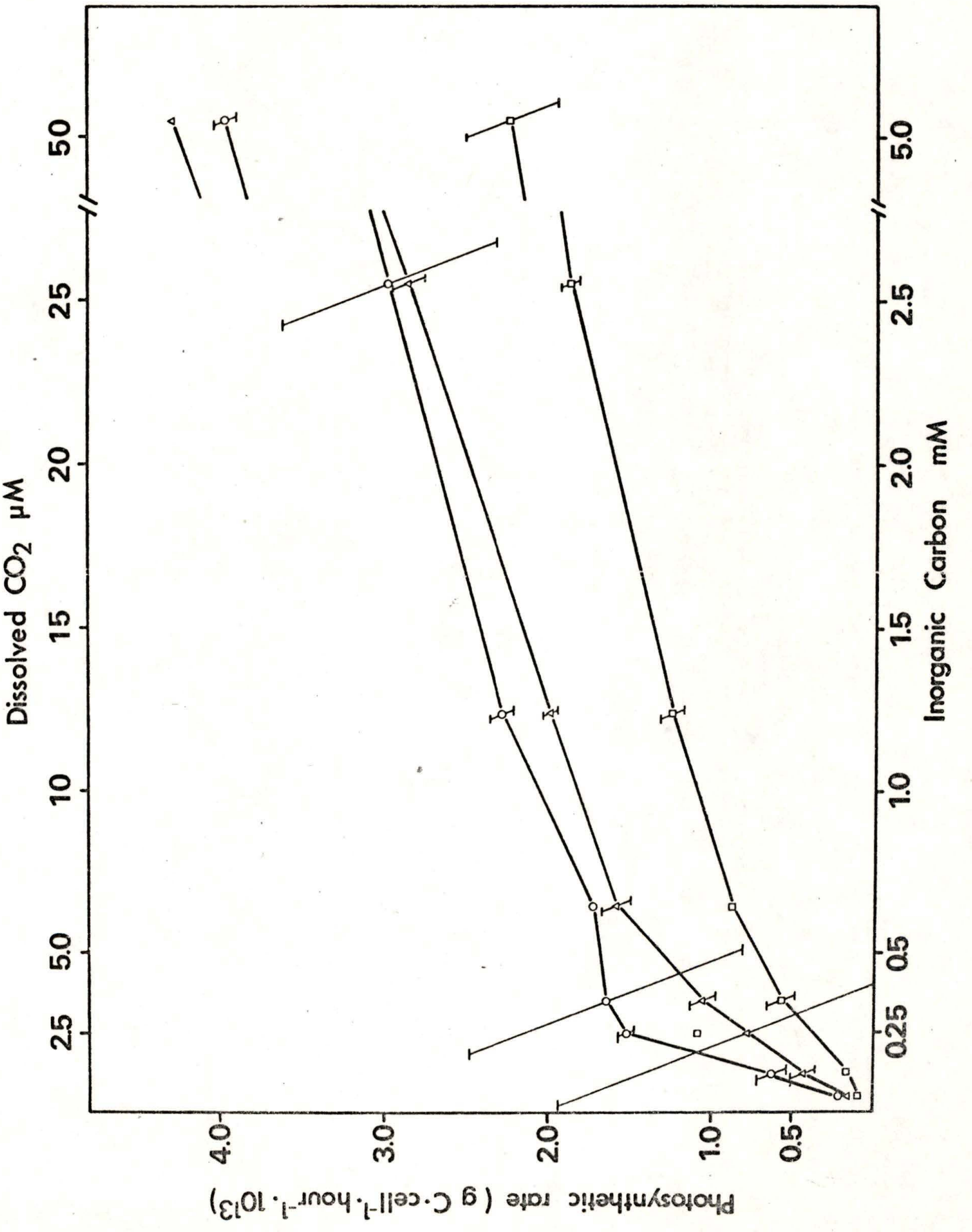

Photosynthetic rate (per cent)
100
80
60
40
100
500
900
Dissolved O_2 µM

TABLE 3. The effect of inorganic carbon concentration on the photosynthetic CO_2 assimilation rate of <u>Isochrysis galbana</u> at pH 8.00 and three concentrations of dissolved oxygen.

Inorganic Carbon		Dissolved Oxygen		Photosynthetic Rate	
Total (mM)	CO_2 (μM)	O_2 (μM)	% of saturation	g C·cell^{-1}·hour^{-1}·10^{13} ± 1.96·S.E. of $\bar{X}$	% of rate at 5.05 mM C and 115 μM O_2
5.05	50.5	115	49	3.97 ± 0.06	100.0
2.55	25.5	"	"	2.97 ± 0.73	75.5
1.24	12.4	"	"	2.29 ± 0.03	57.7
0.65	6.5	"	"	1.72 ± 0.00	43.5
0.35	3.5	"	"	1.65 ± 0.97	41.6
0.25	2.5	"	"	1.52 ± 0.05	38.4
0.12	1.2	"	"	0.63 ± 0.11	15.8
0.05	0.5	"	"	0.23 ± 0.03	5.7
5.05	50.5	330	138	4.29 (replicate lost)	108.1
2.55	25.5	"	"	2.85 ± 0.01	72.0
1.24	12.4	"	"	1.98 ± 0.03	50.0
0.65	6.5	"	"	1.59 ± 0.07	40.2
0.35	3.5	"	"	1.04 ± 0.08	26.3
0.25	2.5	"	"	0.79 ± 1.20	19.9
0.12	1.2	"	"	0.44 ± 0.09	11.0
0.05	0.5	"	"	0.15 ± 0.01	3.7
5.05	50.5	846	360	2.24 ± 0.32	56.4
2.55	25.5	"	"	1.82 ± 0.05	45.8
1.24	12.4	"	"	1.24 ± 0.04	31.2
0.65	6.5	"	"	0.88 ± 0.00	22.1
0.35	3.5	"	"	0.58 ± 0.16	14.7
0.25	2.5	"	"	1.08 ± 0.02	27.5
0.12	1.2	"	"	0.18 ± 0.02	4.5
0.05	0.5	"	"	0.099 ± 0.025	2.5

FIGURE 4. The photosynthetic CO_2 assimilation rate of *I. galbana* as a function of the concentration of inorganic carbon at pH 8.00 and three concentrations of dissolved O_2. Circles = 115 µM O_2; triangles = 330 µM O_2; squares = 846 µM O_2. Error bars represent ± 1.96 x S.E. of the mean (n = 2).


Dissolved CO_2 μM
2.5
5.0
10
15
20
25
50
Photosynthetic rate (g C · cell^{-1} · hour^{-1} · 10^{13})
4.0
3.0
2.0
1.0
0.5
Inorganic Carbon mM
0.25
0.5
1.0
1.5
2.0
2.5
5.0

the CO_2 assimilation rate was reduced to less than 6% of its maximum value when the total inorganic carbon was reduced to 0.05 mM (45 μM HCO_3^-).

Raising the dissolved O_2 from 115 μM to 330 μM reduced the rate of CO_2 assimilation between 30.4% and 48% at carbon concentrations between 0.05 and 0.35 mM. At carbon concentrations between 0.65 and 2.55 mM the depression caused by 330 μM O_2 was in the range of 4.0% to 13.5% and at 5.05 mM carbon no inhibition occurred. These results conflict somewhat with those in Figure 1, and suggest that elevated CO_2 concentrations act to alleviate O_2 inhibition. However, when the O_2 concentration was increased further to 846 μM O_2, CO_2 assimilation rates were depressed by an average of 48.7% compared to 115 μM O_2 and the depression was quite constant over the entire range of carbon concentrations tested (0.05 to 5.05 mM).

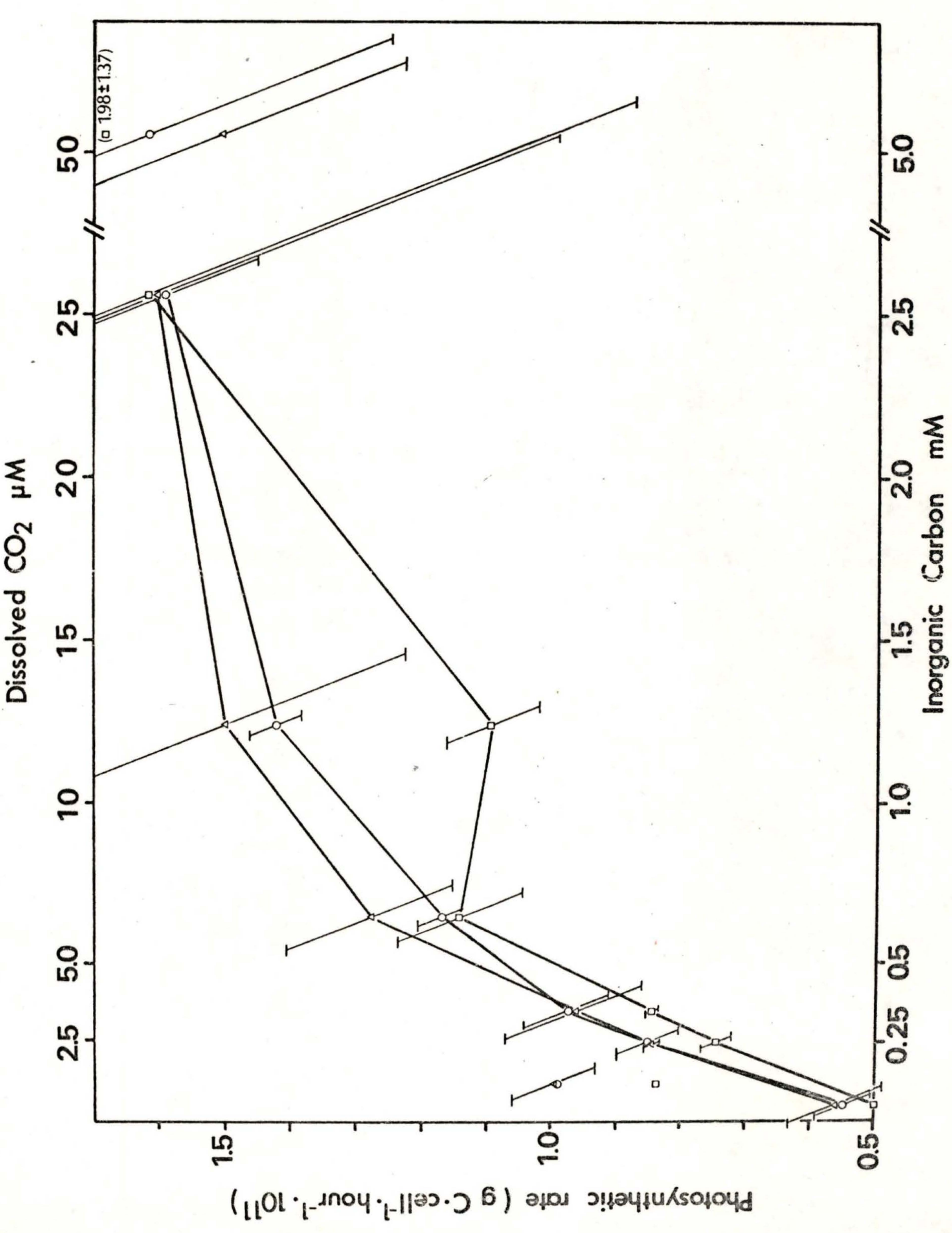
Identical experiments performed on *T. fluviatilis* yielded markedly different results as shown in Table 4 and Figure 5. The CO_2 assimilation rate was CO_2 saturated at 2.55 mM carbon (25.5 μM CO_2) and half-saturation occurred at 0.25 mM carbon (2.5 μM CO_2). *T. fluviatilis* retained 34% of its maximum CO_2 assimilation rate at 0.05 mM carbon, indicating a far greater capacity to scavenge CO_2 or to utilize HCO_3^- than *I. galbana*.

Raising the dissolved O_2 from 115 μM to 330 μM had little effect on the CO_2 assimilation rate when the concentration of inorganic carbon was between 0.05 and 0.35 mM; above 0.35 mM carbon, 330 μM O_2 stimulated the CO_2 assimilation rate compared to 115 μM O_2. When the O_2 concentration was increased further to 846 μM O_2 a slight depression ($\overline{X}$ = 12.7%) occurred at carbon concentrations between 0.05 and 1.24 mM. At 2.55 mM carbon the CO_2 assimilation rate was similar at 115 or 846 μM O_2, and

TABLE 4. The effect of inorganic carbon concentration on the photosynthetic CO_2 assimilation rate of <u>Thalassiosira fluviatilis</u> at pH 8.00 and three concentrations of dissolved oxygen.

Inorganic Carbon		Dissolved Oxygen		Photosynthetic Rate	
Total (mM)	CO_2 (μM)	O_2 (μM)	% of saturation	g $C \cdot cell^{-1} \cdot hour^{-1} \cdot 10^{11}$ ± 1.96·S.E. of $\bar{X}$	% of rate at 5.05 mM C and 115 μM O_2
5.05	50.5	115	49	1.16 ± 0.50	100.0
2.55	25.5	"	"	1.59 ± 0.14	98.3
1.24	12.4	"	"	1.42 ± 0.04	88.0
0.65	6.5	"	"	1.17 ± 0.04	72.6
0.35	3.5	"	"	0.98 ± 0.07	60.7
0.25	2.5	"	"	0.86 ± 0.05	53.1
0.12	1.2	"	"	0.99 ± 0.00	61.3
0.05	0.5	"	"	0.55 ± 0.06	34.1
5.05	50.5	330	138	1.51 ± 0.33	93.2
2.55	25.5	"	"	1.60 ± 0.75	98.9
1.24	12.4	"	"	1.50 ± 0.47	93.1
0.65	6.5	"	"	1.28 ± 0.14	79.3
0.35	3.5	"	"	0.97 ± 0.12	59.9
0.25	2.5	"	"	0.84 ± 0.01	52.2
0.12	1.2	"	"	0.99 ± 0.07	61.6
0.05	0.5	"	"	0.56 ± 0.08	34.9
5.05	50.5	846	360	1.98 ± 1.37	122.9
2.55	25.5	"	"	1.62 ± 0.87	100.2
1.24	12.4	"	"	1.09 ± 0.08	67.7
0.65	6.5	"	"	1.14 ± 0.09	70.8
0.35	3.5	"	"	0.84 ± 0.01	52.3
0.25	2.5	"	"	0.73 ± 0.03	45.2
0.12	1.2	"	"	0.87 ± 0.01	54.0
0.05	0.5	"	"	0.50 ± 0.01	30.8

FIGURE 5. The photosynthetic CO_2 assimilation rate of *T. fluviatilis* as a function of the concentration of inorganic carbon at pH 8.00 and three concentrations of dissolved O_2. Circles = 115 μM O_2; triangles = 330 μM O_2; squares = 846 μM O_2. Error bars represent ± 1.96 x S.E. of the mean (n = 2).


Dissolved CO$_2$ μM
2.5
5.0
10
15
20
25
50
Inorganic Carbon mM
0.25
0.5
1.0
1.5
2.0
2.5
5.0
Photosynthetic rate (g C·cell^{-1}·hour^{-1}·10^{11})
0.5
1.0
1.5
(□ 1.98 ± 1.37)

the latter concentration stimulated CO_2 assimilation at 5.05 mM carbon.

To permit direct comparisons between the two species, the data of Tables 3 and 4 have been expressed as percentages of the values measured at 115 μM O_2 and 5.05 mM carbon for each species in Figures 6, 7 and 8. The curves for both species at 115 μM O_2 are shown in Figure 6; Figure 7 shows both at 330 μM O_2 and 846 μM O_2 is shown in Figure 8.

Photosynthetic CO_2 Assimilation as a Function of pH at Constant Concentrations of Inorganic Carbon and Dissolved Oxygen

The concentration of free CO_2 in seawater is inversely proportional to the pH. *I. galbana* and *T. fluviatilis* were subjected to variations in pH at a limiting carbon concentration (0.2 mM) to further characterize their photosynthetic responses to low CO_2. The pH of natural seawater rarely falls outside the range of 7.8 to 8.3, although the extremes of its range extend from 7.3 to 8.6 (Riley & Skirrow, 1965). By virtue of this relatively constant pH environment (compared to freshwater), marine algae probably have fairly narrow pH optima. Therefore, changes in photosynthetic CO_2 assimilation as a function of pH may reflect both the intrinsic pH tolerance of a given species and its ability to utilize HCO_3^- or effectively remove CO_2 at low concentrations.

The effects of pH on the CO_2 assimilation rate of *I. galbana* are shown in Figure 9 and Table 5. The pH optimum is narrow and lies between 7.25 and 7.50 (11 to 6.5 μM CO_2). The CO_2 assimilation rate drops significantly at pH 7.00; the concentration of CO_2 at pH 7.00 is higher than at 7.25, so this reduction probably reflects the intrinsic pH tolerance of *I. galbana*. The rate of CO_2 assimilation dropped rapidly above pH 7.75; at pH 8.50 the rate was only 34.5% of the rate

FIGURE 6. The photosynthetic CO_2 assimilation rate of *I. galbana* and *T. fluviatilis* as a function of the concentration of inorganic carbon at pH 8.00 and 115 µM O_2. Open symbols = *I. galbana*; closed symbols = *T. fluviatilis*. Values are expressed as a percent of the value recorded for each species at 5.05 mM inorganic carbon and 115 µM O_2.

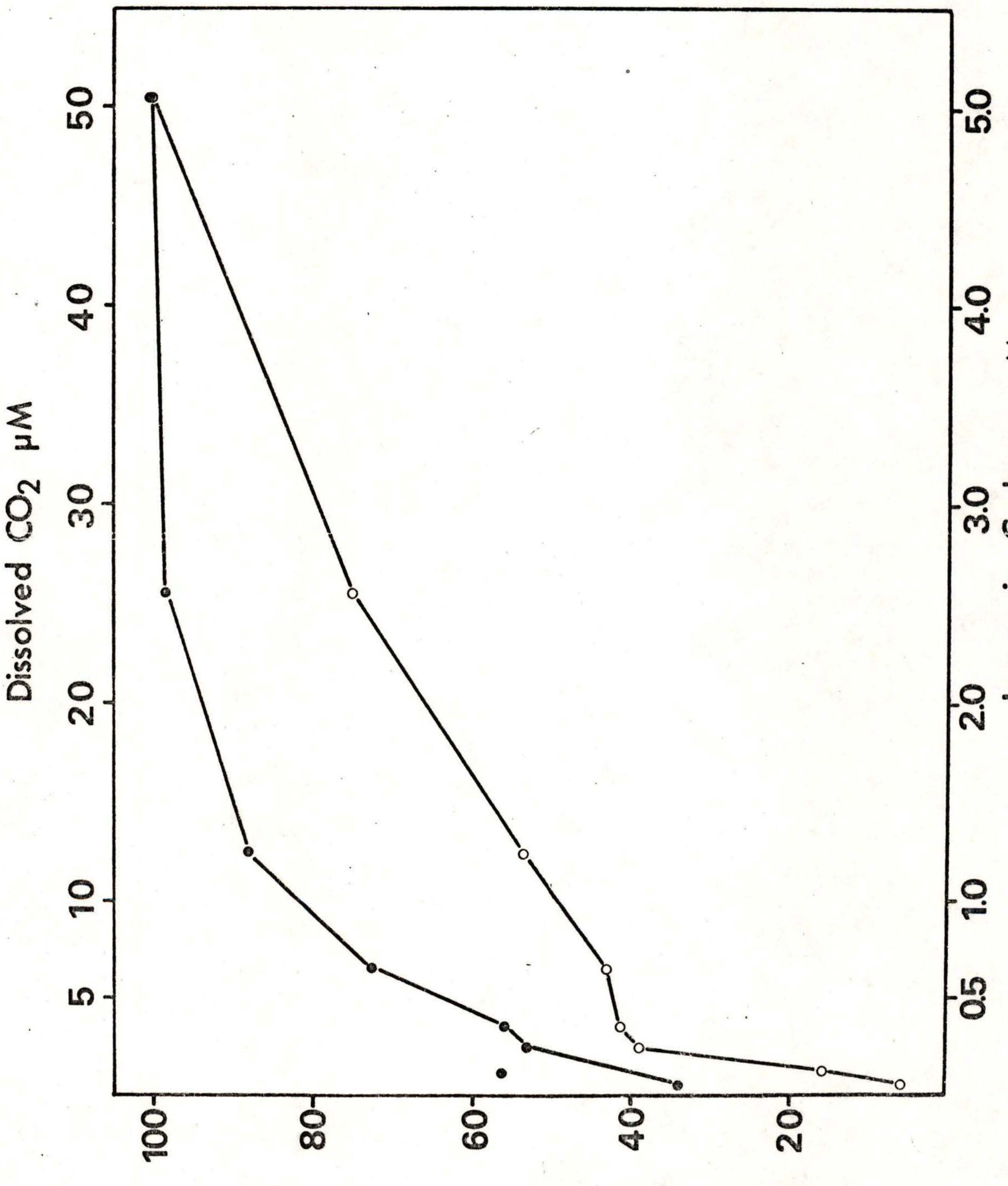

Dissolved CO_2 µM
5
10
20
30
40
50
Inorganic Carbon mM
0.5
1.0
2.0
3.0
4.0
5.0
Photosynthetic rate (per cent)
100
80
60
40
20

FIGURE 7. The photosynthetic CO_2 assimilation rate of I. galbana and T. fluviatilis as a function of the concentration of inorganic carbon at pH 8.00 and 330 μM O_2. Open symbols = I. galbana; closed symbols = T. fluviatilis. Values are expressed as a percent of the value recorded for each species at 5.05 mM inorganic carbon and 115 μM O_2.

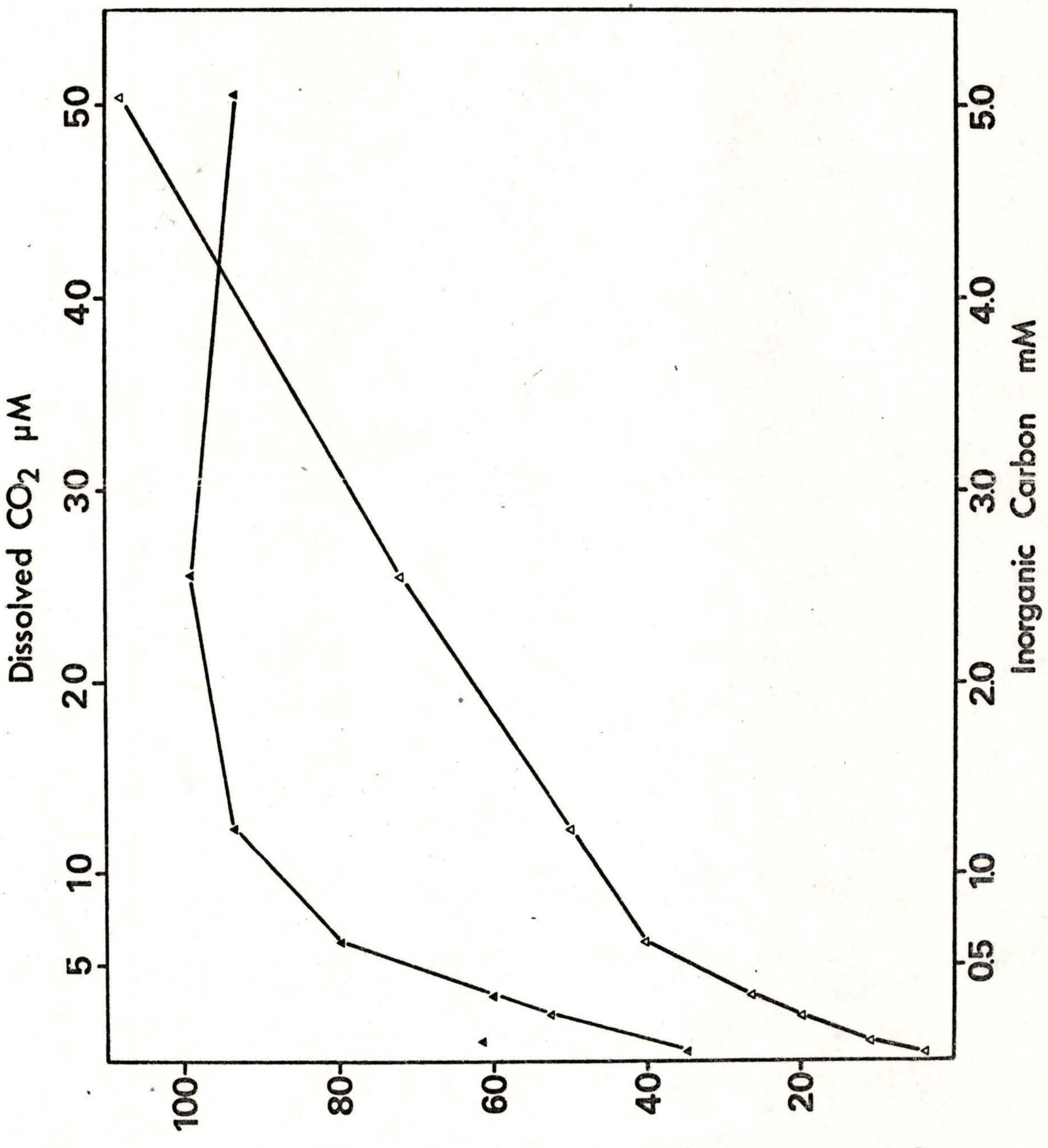

Dissolved CO_2 μM
5
10
20
30
40
50
Photosynthetic rate (per cent)
100
80
60
40
20
Inorganic Carbon mM
0.5
1.0
2.0
3.0
4.0
5.0

FIGURE 8. The photosynthetic CO_2 assimilation rate of I. galbana and T. fluviatilis as a function of the concentration of inorganic carbon at pH 8.00 and 846 μM O_2. Open symbols = I. galbana; closed symbols = T. fluviatilis. Values are expressed as a percent of the value recorded for each species at 5.05 mM inorganic carbon and 115 μM O_2.

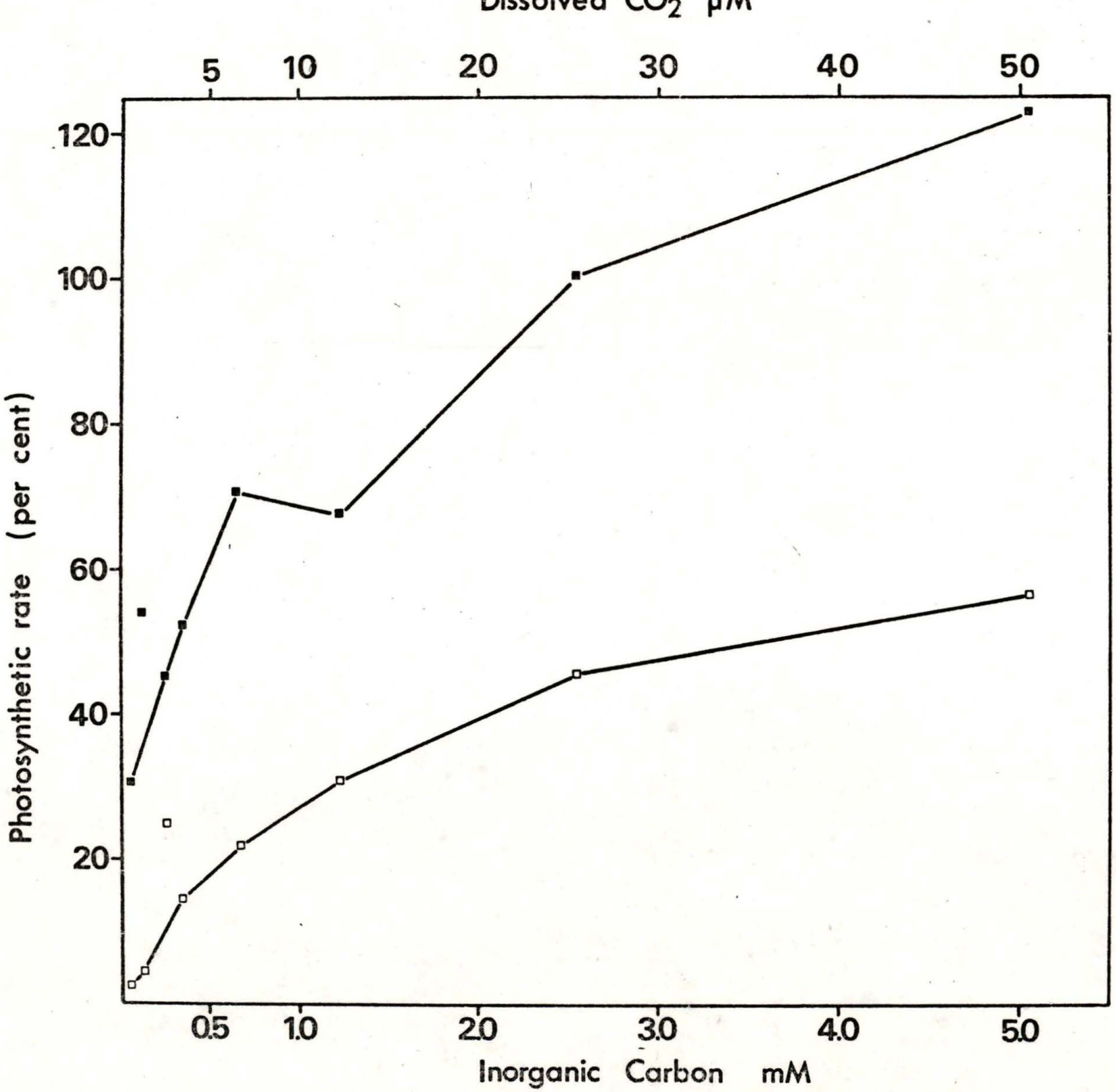
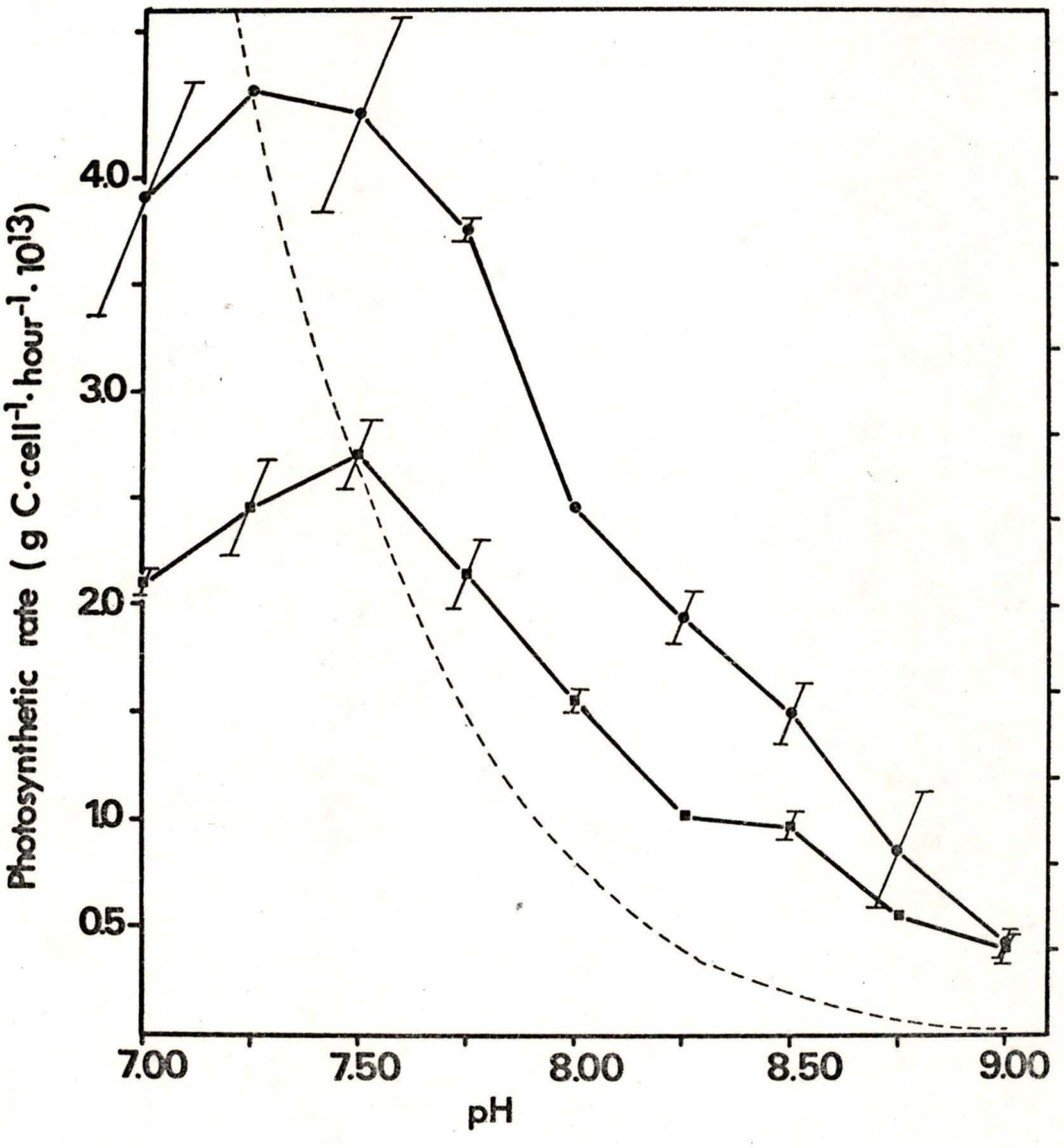

Dissolved CO2 µM
5
10
20
30
40
50
120
100
80
60
40
20
Photosynthetic rate (per cent)
0.5
1.0
2.0
3.0
4.0
5.0
Inorganic Carbon mM

TABLE 5. The effect of pH on the photosynthetic CO_2 assimilation rate of *Isochrysis galbana* at 0.2 mM inorganic carbon and two concentrations of dissolved oxygen.

pH	CO_2 (µM)	Dissolved Oxygen		Photosynthetic Rate		
		O_2 (µM)	% of saturation	g $C \cdot cell^{-1} \cdot hour^{-1} \cdot 10^{13}$ ± $1.96 \cdot S.E.$ of $\bar{X}$	% of maximum (pH 7.25 & 38 µM O_2)	% change in 835 µM O_2
7.00	18.0	38	16	3.92 ± 0.62	88.9	
"	"	835	352	2.10 ± 0.06	47.6	-46.4
7.25	11.0	38	16	4.41 ± 0.03	100.0	
"	"	835	352	2.46 ± 0.24	55.8	-44.2
7.50	6.4	38	16	4.35 ± 0.52	98.6	
"	"	835	352	2.70 ± 0.17	61.2	-37.5
7.75	3.6	38	16	3.77 ± 0.05	85.5	
"	"	835	352	2.16 ± 0.18	49.0	-42.7
8.00	2.0	38	16	2.46 ± 0.02	55.8	
"	"	835	352	1.57 ± 0.06	35.6	-36.2
8.25	1.0	38	16	1.95 ± 0.13	44.2	
"	"	835	352	1.03 ± 0.03	23.4	-47.7
8.50	0.5	38	16	1.52 ± 0.16	34.5	
"	"	835	352	0.98 ± 0.07	22.1	-36.2
8.75	0.2	38	16	0.86 ± 0.33	19.6	
"	"	835	352	0.55 ± 0.03	12.5	-36.2
9.00	< 0.1	38	16	0.44 ± 0.06	9.9	
"	"	835	352	0.42 ± 0.05	9.6	-2.8

FIGURE 9. The photosynthetic CO_2 assimilation rate of *I. galbana* as a function of pH at 0.2 mM inorganic carbon and two concentrations of dissolved O_2. Circles = 38 μM O_2; squares = 835 μM O_2. Dashed line = the concentration of CO_2 as a function of pH. Error bars represent 1.96 x S.E. of the mean (n = 2).


Photosynthetic rate (g C·cell^{-1}·hour^{-1}. 10^{13})
4.0
3.0
2.0
1.0
0.5
Dissolved CO$_2$ µM
10
5
1
7.00
7.50
8.00
8.50
9.00
pH

measured at pH 7.25. The drop in CO_2 assimilation at pH values above 7.75 roughly parallels the concentration of free CO_2 in the medium (dashed curve on Figure 9) and probably reflects CO_2 limitation of photosynthesis. The decline in the CO_2 assimilation rate is slower than the decline in CO_2 concentration indicating that the relative affinity of *I. galbana* for CO_2 increases as the concentration of CO_2 decreases. Raising the dissolved O_2 from 38 μM to 835 μM did not alter the pH optimum. Over the range of pH from 7.00 to 8.75 at 835 μM O_2, the molar ratios of O_2:CO_2 varied from 46.4 to more than 4000, but the inhibition caused by 835 μM O_2 remained relatively constant between 36.2 and 47.7% ($\overline{X}$ = 41%).

The response of *T. fluviatilis* to changes in pH is shown in Figure 10 and Table 6. The maximum CO_2 assimilation rate occurred at pH 7.50, but the rate was relatively constant between pH 7.00 and 8.25. The rate of CO_2 assimilation at pH 8.50 was 73.5% of the rate at pH 7.50. Greatly reduced rates of CO_2 assimilation resulted from pH values higher than 8.50, the minimum occurred at the highest pH tested (pH = 9.00). Unlike *I. galbana*, the rate of CO_2 assimilation did not closely parallel the CO_2 concentration in the medium (dashed curve in Figure 10). Raising the O_2 concentration to 835 μM from 38 μM stimulated CO_2 assimilation at pH values below 8.00 (except at 7.50). At pH 8.00 the rate of CO_2 assimilation was similar at either 38 or 835 μM O_2, and above pH 8.00 high O_2 inhibited CO_2 assimilation. To aid direct comparison, the data from the pH experiments for both species is combined in Figure 11 and expressed as a per cent of the maximum for each species.

TABLE 6. The effect of pH on the photosynthetic CO_2 assimilation rate of <u>Thalassiosira fluviatilis</u> at 0.2 mM inorganic carbon and two concentrations of dissolved oxygen.

pH	CO_2 (µM)	Dissolved Oxygen		Photosynthetic Rate		
		O_2 (µM)	% of saturation	g $C \cdot cell^{-1} \cdot hour^{-1} \cdot 10^{13}$ ± 1.96·S.E. of $\bar{X}$	% of maximum (pH 7.50 & 38 µM O_2)	% change in 835 µM O_2
7.00	18.0	38	16	2.10 ± 0.14	87.9	
"	"	835	352	2.26 ± 0.03	94.6	+7.1
7.25	11.0	38	16	2.19 ± 0.22	91.6	
"	"	835	352	2.27 ± 0.22	95.0	+4.5
7.50	6.4	38	16	2.39 ± 0.26	100.0	
"	"	835	352	2.31 ± 0.20	96.7	-3.3
7.75	3.6	38	16	2.06 (replicate lost)	86.2	
"	"	835	352	2.20 ± 0.08	92.0	+6.4
8.00	2.0	38	16	2.12 ± 0.92	88.7	
"	"	835	352	2.11 ± 0.16	88.3	-0.5
8.25	1.0	38	16	2.16 ± 0.02	90.4	
"	"	835	352	1.97 ± 0.15	82.4	-8.8
8.50	0.5	38	16	1.75 ± 0.10	73.2	
"	"	835	352	1.45 ± 0.14	60.7	-17.1
8.75	0.2	38	16	0.95 ± 0.04	39.6	
"	"	835	352	0.75 ± 0.05	31.5	-20.4
9.00	< 0.1	38	16	0.50 ± 0.05	21.1	
"	"	835	352	0.43 ± 0.06	18.2	-15.9

FIGURE 10. The photosynthetic CO_2 assimilation rate of *T. fluviatilis* as a function of pH at 0.2 mM inorganic carbon and two concentrations of dissolved O_2. Circles = 38 M O_2; squares = 835 M O_2. Dashed line = the concentration of CO_2 as a function of pH. Error bars represent 1.96 x S.E. of the mean (n = 2).

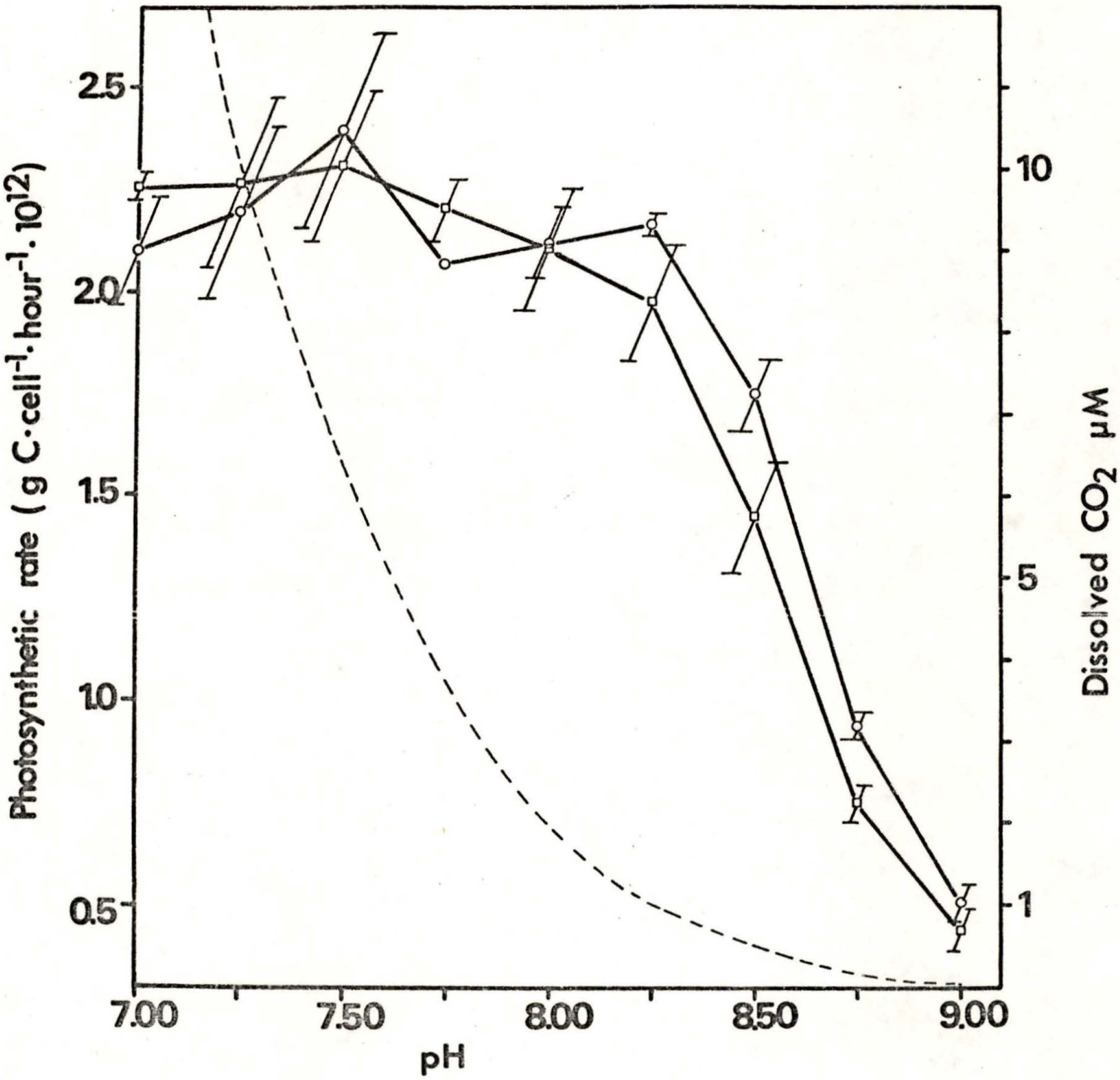
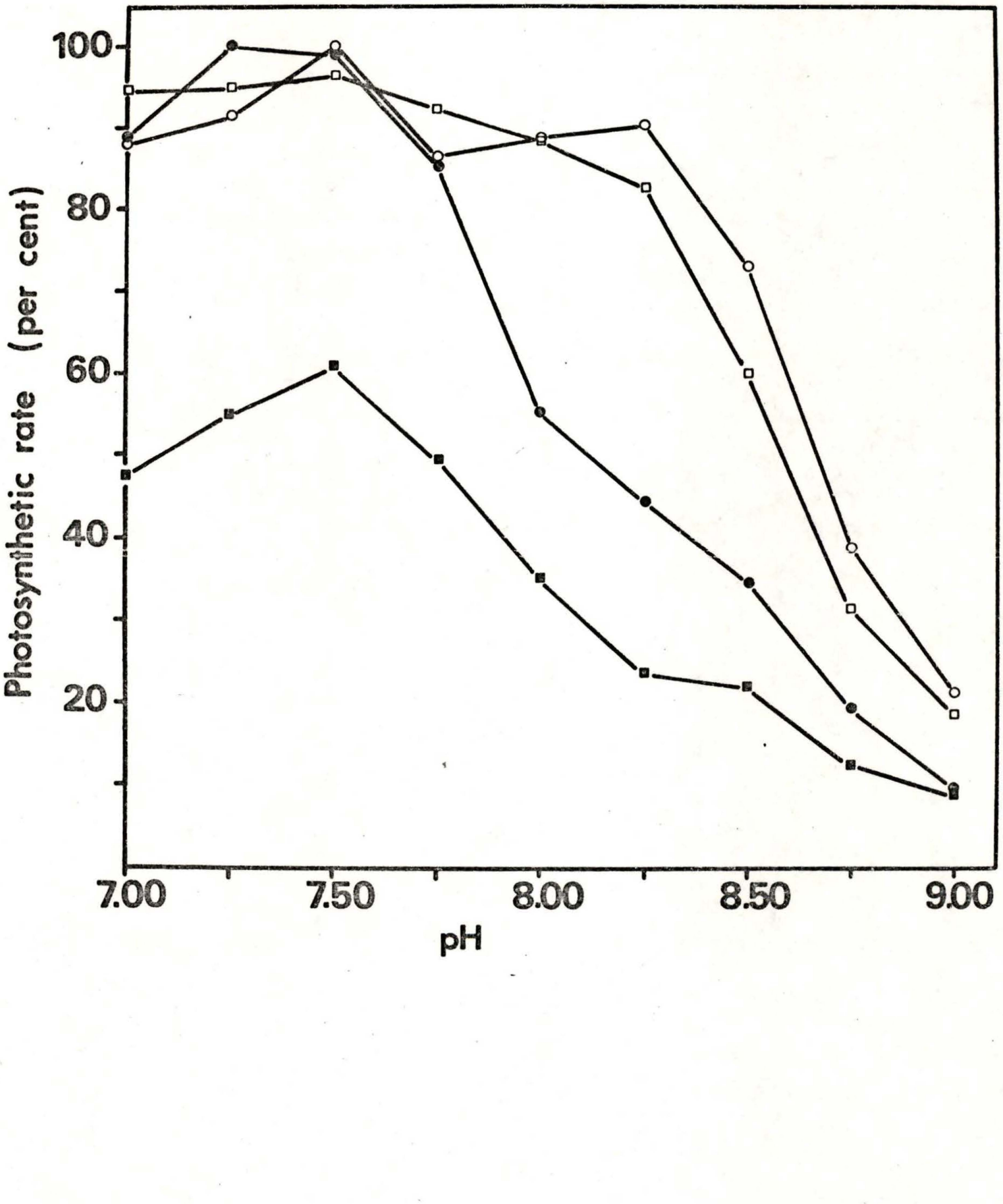

Photosynthetic rate (g C·cell^{-1}·hour^{-1}·10^{12})
2.5
2.0
1.5
1.0
0.5
Dissolved CO$_2$ µM
10
5
1
7.00
7.50
8.00
8.50
9.00
pH

FIGURE 11. The photosynthetic CO_2 assimilation rate of *I. galbana* and *T. fluviatilis* as a function of pH at 0.2 mM inorganic carbon and two concentrations of dissolved O_2. The values are expressed as a percent of the maximum rate observed for each species. Closed symbols = *I. galbana*; open symbols = *T. fluviatilis*. Circles = 38 μM O_2; squares = 835 μM O_2.


Photosynthetic rate (per cent)
100
80
60
40
20
7.00
7.50
8.00
8.50
9.00
pH

Photosynthetic CO_2 Assimilation as a Function of Glycidate concentration at Constant pH and Inorganic Carbon Concentration and Three Concentrations of Dissolved O_2

Since glycidate has been implicated in photorespiratory metabolism, *I. galbana* and *T. fluviatilis* were subjected to a limiting concentration of inorganic carbon (0.2 mM carbon = 2.0 μM CO_2 at pH 8.00) and a range of dissolved O_2 concentrations to study the effects of glycidate on these species under conditions favourable to photorespiration. Photosynthetic CO_2 assimilation in *I. galbana* was inhibited by all concentrations of glycidate tested (0.5 to 50 mM) as shown in Figure 12 and Table 7. The inhibition was proportionally greater at low concentrations of glycidate and was independent of the O_2 concentration between 85 and 890 μM.

The effects of glycidate on *T. fluviatilis* (Figure 13 and Table 8) were in marked contrast to *I. galbana*. At 85 μM O_2, glycidate between 0.5 and 10 mM stimulated CO_2 assimilation; the maximum stimulation of 67% was observed at 1 mM glycidate. At 289 or 890 μM O_2, 1 mM glycidate stimulated CO_2 assimilation by only 21%. 10 mM glycidate slightly reduced the rate of CO_2 assimilation at 289 or 890 μM O_2, and 50 mM glycidate was strongly inhibitory at all O_2 concentrations. Figure 13 also reveals that O_2 concentrations of 289 and 890 μM increased CO_2 assimilation by 33.3% and 20.6% respectively compared to 85 μM O_2. These values are higher than those reported in the previous experiments of this study and are indicative of the high degree of variability in the O_2 stimulation of CO_2 assimilation in *T. fluviatilis*. To facilitate comparison, Figure 14 presents the data on glycidate for both species as percentages of their respective rates without glycidate at 85 μM O_2.

TABLE 7. The effect of glycidate on the photosynthetic CO_2 assimilation rate of <u>Isochrysis galbana</u> at pH 8.00, 0.2 mM inorganic carbon (2.0 µM CO_2), and three concentrations of dissolved oxygen.

Glycidate (mM)	Dissolved Oxygen		Photosynthetic Rate	
	O_2 (µM)	% of saturation	g $C\cdot cell^{-1}\cdot hour^{-1}\cdot 10^{14}$ ± 1.96·S.E. of $\bar{X}$	% of the rate at 0.0 mM glycidate and 85 µM O_2
0.0	85	36	6.19 ± 0.03	100.0
0.5	"	"	6.21 ± 0.28	89.9
1.0	"	"	5.55 ± 0.07	79.6
5.0	"	"	4.34 ± 0.24	62.8
10.0	"	"	1.74 ± 0.02	25.2
50.0	"	"	0.41 ± 0.03	5.9
0.0	289	122	6.31 ± 0.02	91.3
0.5	"	"	5.76 ± 0.59	83.4
1.0	"	"	4.86 ± 0.16	70.3
5.0	"	"	3.46 ± 0.43	50.1
10.0	"	"	1.50 ± 0.05	21.7
50.0	"	"	0.39 (replicate lost)	5.6
0.0	890	375	4.45 ± 0.29	64.4
0.5	"	"	4.07 ± 0.06	58.9
1.0	"	"	3.42 ± 0.05	49.5
5.0	"	"	1.95 ± 0.17	28.2
10.0	"	"	1.04 ± 0.21	15.1
50.0	"	"	0.30 ± 0.05	4.3

FIGURE 12. The photosynthetic CO_2 assimilation rate of I. galbana as a function of the concentration of glycidate at pH 8.00, 0.2 mM inorganic carbon (2.0 μM CO_2), and three concentrations of dissolved O_2. Circles = 85 μM O_2; triangles = 289 μM O_2; squares = 890 μM O_2. Error bars represent 1.96 x S.E. of the mean (n = 2).

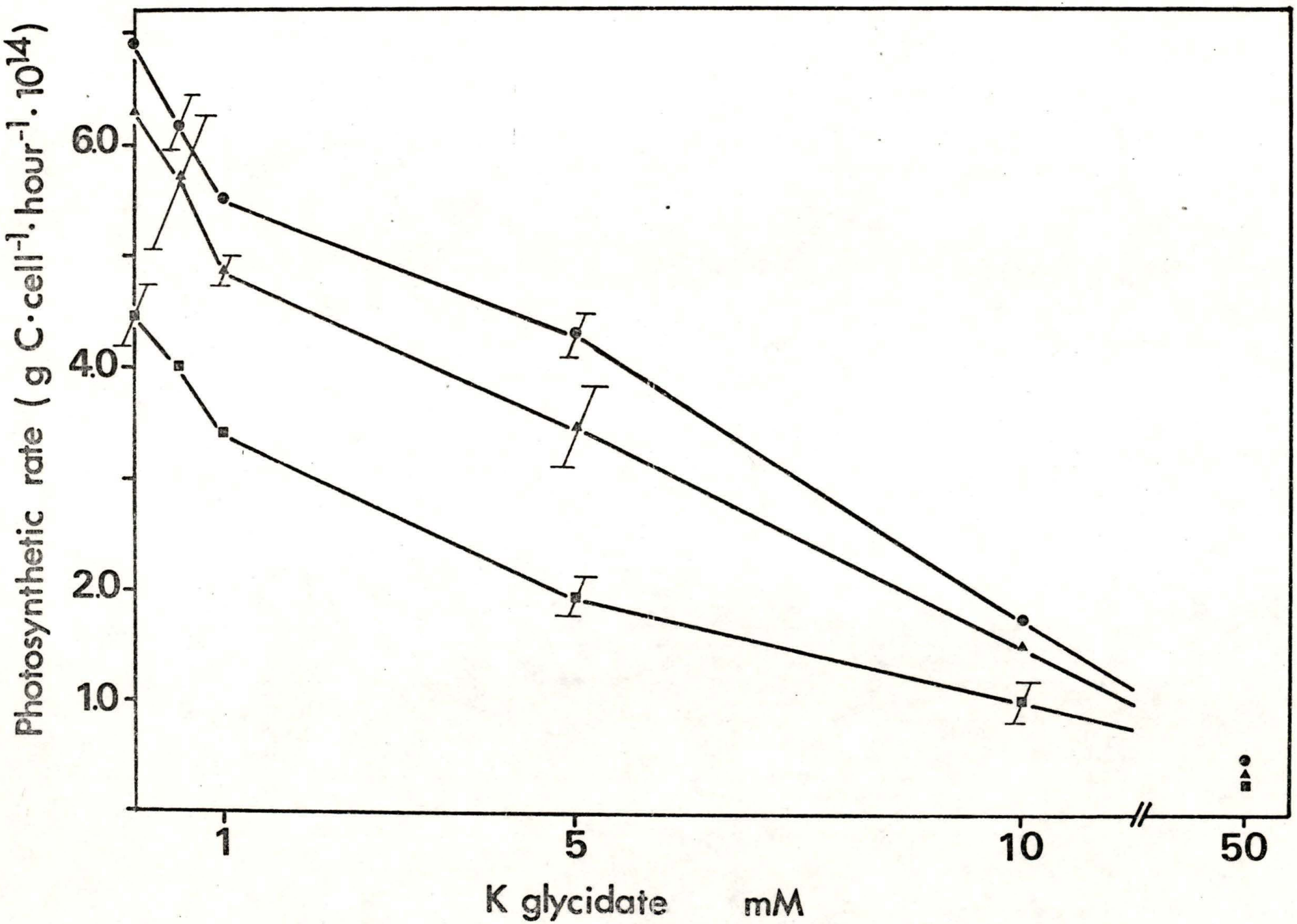

Photosynthetic rate (g C·cell^{-1}·hour^{-1}·10^{14})
6.0
4.0
2.0
1.0
1
5
10
50
K glycidate mM

TABLE 8. The effect of glycidate on the photosynthetic CO_2 assimilation rate of <u>Thalassiosira fluviatilis</u> at pH 8.00, 0.2 mM inorganic carbon (2.0 M CO_2), and three concentrations of dissolved oxygen.

Glycidate (mM)	Dissolved Oxygen		Photosynthetic Rate	
	O_2 (μM)	% of saturation	$g\ C\cdot cell^{-1}\cdot hour^{-1}\cdot 10^{12}$ ± 1.96·S.E. of $\bar{X}$	% of the rate at 0.0 mM glycidate and 85 μM O_2
0.0	85	36	1.89 ± 0.10	100.0
0.5	"	"	1.98 ± 0.09	104.8
1.0	"	"	3.15 ± 0.17	166.7
5.0	"	"	2.78 ± 0.09	147.1
10.0	"	"	1.86 ± 0.00	98.4
50.0	"	"	0.66 ± 0.04	36.6
0.0	289	122	2.52 ± 0.34	133.3
0.5	"	"	2.47 ± 0.29	130.7
1.0	"	"	3.02 ± 0.02	159.8
5.0	"	"	2.77 ± 0.02	146.6
10.0	"	"	1.66 ± 0.04	87.8
50.0	"	"	0.65 ± 0.03	34.6
0.0	890	375	2.28 ± 0.24	120.6
0.5	"	"	2.31 ± 0.19	122.2
1.0	"	"	2.73 ± 0.13	144.4
5.0	"	"	2.40 ± 0.03	127.0
10.0	"	"	1.64 ± 0.17	86.8
50.0	"	"	0.58 ± 0.02	30.8

FIGURE 13. The photosynthetic CO_2 assimilation rate of _T. fluviatilis_ as a function of the concentration of glycidate at pH 8.00, 0.2 mM inorganic carbon (2.0 μM CO_2), and three concentrations of dissolved O_2. Circles = 85 μM O_2; triangles = 289 μM O_2; squares = 890 μM O_2. Error bars represent 1.96 x S.E. of the mean (n = 2).

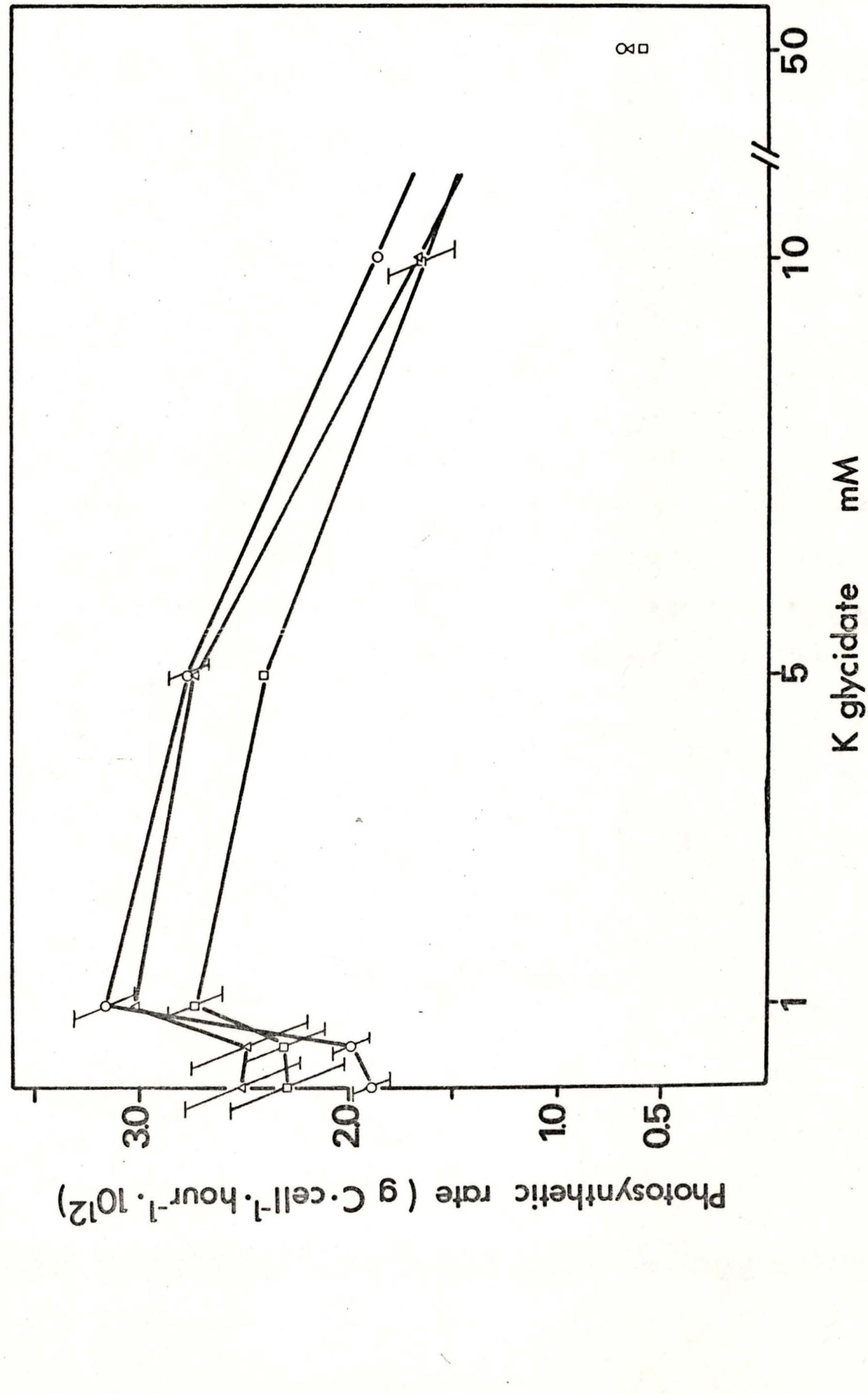
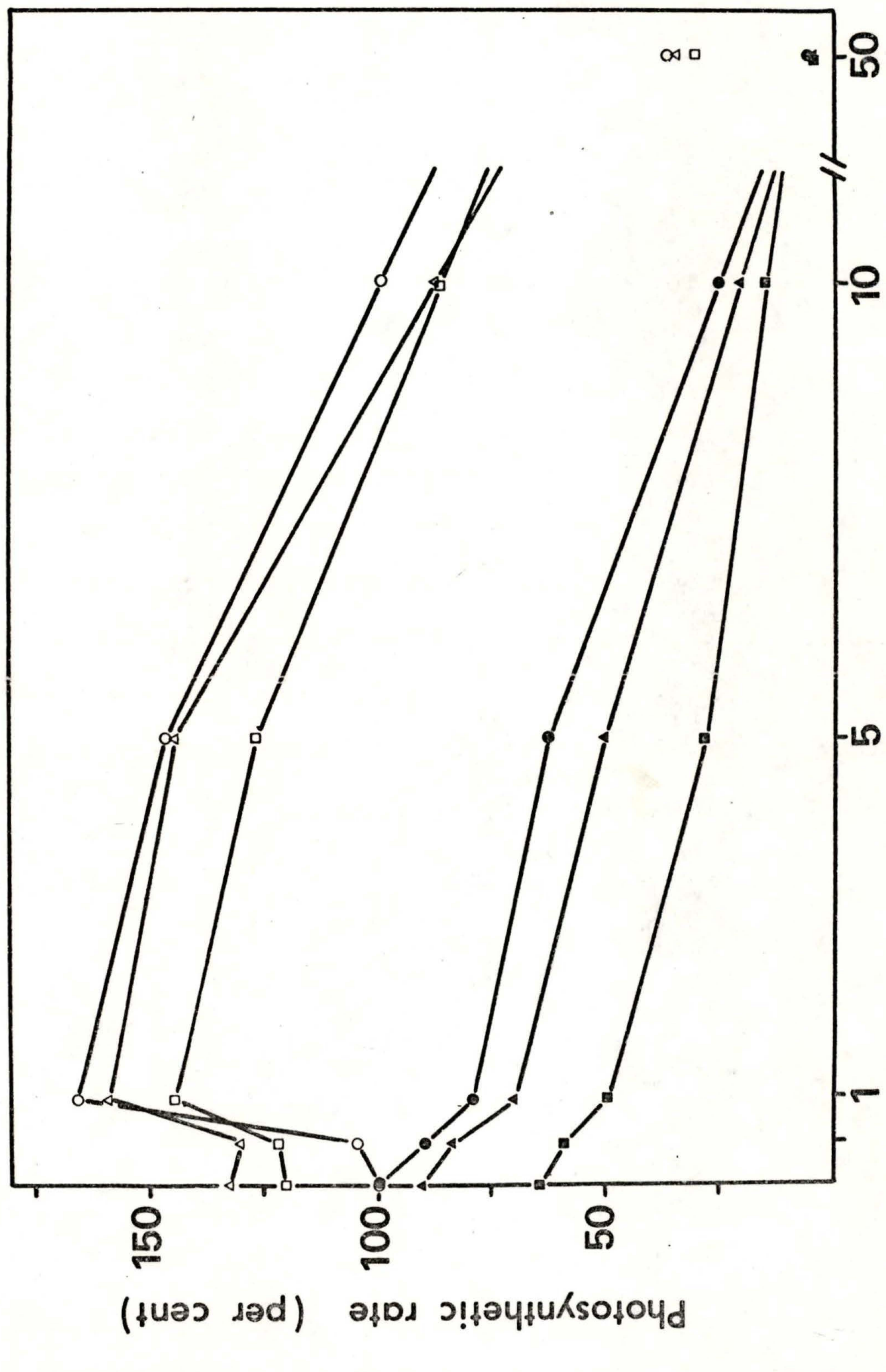

Photosynthetic rate (g C · cell-1 · hour-1 · 10 12)
3.0
2.0
1.0
0.5
1
5
10
50
K glycidate mM

FIGURE 14. The photosynthetic CO_2 assimilation rate of I. galbana and T. fluviatilis as a function of the concentration of glycidate at pH 8.00, 0.2 mM inorganic carbon (2.0 μM CO_2), and three concentrations of dissolved O_2. The values are expressed as a percent of the rate measured for each species at 85 μM O_2 without glycidate. Closed symbols = I. galbana; open symbols = T. fluviatilis. Circles = 85 μM O_2; triangles = 289 μM O_2; squares = 890 μM O_2.


Photosynthetic rate (per cent)
150
100
50
K glycidate mM
1
5
10
50

Summary of Results

A *Isochrysis galbana*

1. The concentration of CO_2 required for half maximum CO_2 assimilation was at least 10 μM.
2. The rate of CO_2 assimilation was inhibited by O_2 concentrations greater than zero.
3. The inhibition caused by O_2 was in the range of 5 to 15% at typical seawater concentrations of carbon and O_2.
4. The inhibition caused by moderate O_2 (50 to 150% of saturation) was generally greater at low concentrations of carbon, and in one experiment, elevated carbon alleviated the inhibition caused by moderate concentrations of O_2.
5. At maximum supersaturation (> 350% of saturation), O_2 depressed CO_2 assimilation by 30 to 50%.
6. The depression caused by very high O_2 was insensitive to the concentration of free CO_2 or total inorganic carbon when these were varied through a wide range.
7. The pH optimum of photosynthetic CO_2 assimilation was narrow and correlated with the concentration of free CO_2.
8. Glycidate inhibited photosynthetic CO_2 assimilation at all concentrations tested.
9. The depression caused by glycidate was independent of the concentration of dissolved O_2.

B *Thalassiosira fluviatilis*

1. The concentration of CO_2 required for half maximal photosynthetic CO_2 assimilation was 2.5 μM.
2. Optimal CO_2 assimilation occurred at O_2 concentrations between 125 and 500 μM depending on carbon concentration.
3. The magnitude of the stimulation caused by O_2 was highly variable and directly related to the concentration of CO_2.
4. Very high concentrations of O_2 (> 800 μM) were generally inhibitory when CO_2 was held at limiting concentrations. This was the case whether the CO_2 limitation was caused by reducing the total inorganic carbon concentration or raising the pH above 8.00.
5. The pH optimum for photosynthetic CO_2 assimilation was broad and not well correlated with the concentration of free CO_2.
6. Low concentrations of glycidate stimulated CO_2 assimilation; concentrations of 10 mM or more were inhibitory.
7. The stimulation caused by glycidate was much greater at low O_2 than at moderate or high O_2.

DISCUSSION

The results of this research show that *Isochrysis galbana* and *Thalassiosira fluviatilis* respond differently to manipulations of concentrations of O_2, CO_2, and total inorganic carbon, pH, and the metabolic inhibitor, glycidate. In general, the photosynthetic rate of *I. galbana* was inhibited by all concentrations of O_2 greater than zero while that of *T. fluviatilis* was stimulated by moderate concentrations. This response of photosynthesis to O_2 was similar to responses to glycidate. Also, photosynthetic rates of *I. galbana* were limited by low concentrations of CO_2 while those of *T. fluviatilis* remained relatively high. These general results suggest that *I. galbana* has a traditional C-3 metabolism using only CO_2 as a substrate for photosynthesis while *T. fluviatilis* can use HCO_3^- as a substrate through some mechanism such as either β-carboxylation of phosphoenol pyruvate (PEP) or dehydration of HCO_3^- by the enzyme, carbonic anhydrase. A detailed discussion is aided by first comparing the differences in the Warburg effect and photorespiration in these species and then considering the possible causes of these differences.

Warburg effect

Results of the present study have shown that O_2 concentrations greater than zero inhibit the rate of CO_2 assimilation in *I. galbana*, a marine chrysophyte. At typical seawater concentrations of inorganic carbon (2.5 mM) and dissolved O_2 (200-300 μM) the inhibition was in the range of 5 to 15 %, while at high O_2 (800-900 μM) the inhibition frequently exceeded 40%. These results are in agreement with previous studies using different methods to estimate the Warburg effect in algae. Downton *et al.* (1976) reported a substantial reduction in O_2 evolution ($\overline{X}$ = 48%) when various species of macroalgae were exposed to seawater in equilibrium with atmospheres containing 55 to 90 % O_2 compared to 21% O_2, but little effect when 21% O_2 was compared to 5% O_2. Black *et al.* (1976) compared CO_2 assimilation in various macroalgae, a bluegreen bacterium, and isolated zooxanthellae in seawater bubbled with N_2, He, air, and O_2 and showed a reduction of 0 to 83 % ($\overline{X}$ = 34%) when O_2 was compared to N_2 or He and 0 to 65 % ($\overline{X}$ = 19%) when air was compared to N_2 or He. Beardall and Morris (1975) and Beardall *et al.* (1976) reported a 0 to 33 % ($\overline{X}$ = 11%) reduction in CO_2 assimilation when various species of unicellular marine algae were bubbled with air compared to N_2 and a 9.3 to 66 % ($\overline{X}$ = 36%) reduction when O_2 was compared to N_2. Burris (1977) found a 5 to 38 % inhibition of CO_2 assimilation with air verses N_2 and 12 to 72 % when O_2 was compared to N_2 in various marine and freshwater algae including macrophytes, a diatom, a dinoflagellate, a chlorophyte, and isolated zooxanthellae. Other studies with unicellular algae, usually involving bubbling cultures or natural populations with O_2, air, and N_2 have shown a Warburg effect although with considerable variability (Tolbert, 1974). However, a study using an open gas analysis system (Lloyd *et al.*, 1977a) showed no inhibition of photosynthesis in 10 species of unicellular marine and freshwater algae in 21 or 50 %-O_2 air compared to 2%-O_2 air. Causes of these different results are not known but may be related to exposure of cells to air in the open gas analysis system.

In contrast to *I. galbana*, even high concentrations of dissolved O_2

(> 800 μM) did not inhibit the CO_2 assimilation rate of *T. fluviatilis* compared to the lowest concentration tested when the total inorganic carbon equalled at least 2.0 mM. When free CO_2 was reduced below 2 to 4 μM by increasing the pH to shift the equilibrium toward HCO_3^- or by reducing the total inorganic carbon, high O_2 sometimes caused a slight reduction in photosynthesis (about 10%). A most unexpected result of this research is that O_2 stimulated photosynthesis in *T. fluviatilis*. Although O_2 has been shown to be required for the normal reproductive development of some terrestrial plants (Quebedeaux and Hardy, 1975), optimal photosynthesis in C-3 terrestrial plants occurs at O_2 concentrations near zero as reported here for *I. galbana*. However, in *T. fluviatilis* maximum rates of CO_2 assimilation occurred when the dissolved O_2 concentration was between about 200 and 400 μM (83 to 175 % of saturation). Thus, optimal photosynthesis in this species is correlated with the average range of dissolved O_2 expected in its natural environment. Although O_2 stimulation of photosynthesis in eukaryotic algae has occasionally been reported (Lloyd *et al.*, 1977a; Burris, 1977), these reports have been limited to isolated observations, generally falling within the limits of experimental precision and have not received any attention by these authors. Oxygen stimulation of photosynthesis in bluegreen bacteria is a well characterized response. In studies with *Anacystis nidulans*, Miyachi and Okabe (1976) reported that the maximum stimulation (50%) occurred when cultures were aerated with 10% O_2 and the magnitude of the stimulation was inversely related to the concentration of inorganic carbon; the latter result was confirmed by Lloyd *et al.* (1977a) with *Anabaena flosaquae*. In contrast to these results with cyanophytes, the stimulation of photosynthesis in *T. fluviatilis* was generally lower in magnitude, maximal at higher O_2

concentrations, and directly related to the concentration of inorganic carbon. These results suggest that the O_2 stimulation of photosynthesis in the diatom is qualitatively different from that reported for cyanophytes.

The Warburg effect is usually considered an indication of photorespiration in C-3 terrestrial plants. If this consideration is correct, then *I. galbana* photorespires in a manner similar to C-3 terrestrial plants and *T. fluviatilis* either does not photorespire or efficiently recycles photorespiratory CO_2 to carboxylation sites. However, it seems that for Warburg effect to be equated to photorespiration requires concomittant examination of photosynthesis as a function of CO_2 concentrations. This view is supported by observations that the Warburg effect is reduced by elevated CO_2 in C-3 terrestrial plants (Ellyard & Gibbs, 1979; Jackson & Volk, 1970; Robinson *et al.*, 1977), and that *in vitro* activity of RuDP oxygenase is competitively inhibited by CO_2 (Jensen & Bahr, 1977).

Photorespiration

Half-maximal rates of photosynthetic CO_2 assimilation in *I. galbana* occurred at about 10 μM CO_2 (1.0 mM total inorganic carbon at pH 8.00), and 300 μM O_2 was sufficient for half-maximal rates of O_2 inhibition. These values are close to those reported by Serviates and Ogren (1977 and 1978) for isolated soybean leaf cells, and the Km values of fully activated RuDP carboxylase for CO_2, (6 to 25 μM) and O_2 (196 to 220 μM) (Jensen & Bahr, 1977). In *I. galbana* the depression in photosynthesis caused by 330 μM O_2 compared to 115 μM O_2 was reduced and finally eliminated when the concentration of inorganic carbon was incrementally increased from 0.05 to 5.05 mM (a change in free CO_2 from 0.5 to 50.5 μM), however, the depression in photosynthesis caused by 846 μM O_2 was not changed. Increasing the inorganic carbon from 0.2 to 2.0 mM (from 2 to 20 μM CO_2) at pH 8.00 resulted in a 33% increase in the rate of photosynthesis, but did not alter the cells' response to O_2. Altering the CO_2 concentration from 0.2 to 18 μM by adjusting the pH while holding the concentration of inorganic carbon constant at 0.2 mM did not effect the percent inhibition caused by 835 μM O_2 compared to 35 μM O_2. These results are consistent with photorespiration occurring at moderate concentrations of O_2 but not at high concentrations. At high concentrations of O_2, it appears that net photosynthesis is reduced by some mechanism other than photorespiration.

Oxygen inhibition of photosynthetic CO_2 assimilation in *T. fluviatilis* was relieved by increasing concentrations of CO_2 or total inorganic carbon. However, half-maximal CO_2 assimilation rates occurred at 2.5 μM CO_2 (0.25 mM total inorganic carbon at pH 8.00), and O_2 inhibition did not become noticeable at this concentration of carbon until the dissolved O_2 reached 800 μM. These values are not similar to the Km for either CO_2 or O_2 of *in vitro* RuDP carboxylase/oxygenase (Jensen & Bahr, 1977) or isolated soybean leaf cells (Serviates & Ogren, 1977). Apparently this species is not subject to photorespiration except at very high concentrations of O_2.

I. galbana is probably a C-3 plant subject to photorespiration. This conclusion is based on the presence of a Warburg effect at moderate O_2 concentrations which is subject to relief by elevated concentrations of inorganic carbon. At high O_2 concentrations, the

Warburg effect was not relieved by elevated carbon which suggests that O_2 inhibition in algae can also result from oxidative damage to the photosynthetic apparatus at high O_2 concentrations as concluded by Bunt and Heeb (1971). Beardall *et al.* (1976) have reported that the short-term labelling pattern of *I. galbana* is consistent with C-3 metabolism, although their results also indicated the presence of low rates of β-carboxylation. The results of this study cannot eliminate the possibility of some β-carboxylation activity in *I. galbana*, but the low affinity for CO_2 observed is consistent with exclusive utilization of CO_2 via the Calvin cycle. However, the results with glycidate weaken the conclusion that *I. galbana* is a typical C-3 plant.

Results of studies with glycidate are difficult to interpret, assuming *I. galbana* is a C-3 plant and O_2 inhibition was due to photoreapiration. Glycidate should relieve part or all of the O_2 inhibition of CO_2 assimilation if glycidate is an irreversible inhibitor of glycolate synthesis (Zelitch, 1974). However, glycidate caused a concentration-dependent inhibition of photosynthetic CO_2 assimilation. This result implies that either: (1) glycidate is not an inhibitor of photorespiration, or (2) inhibition of CO_2 uptake by O_2 in *I. galbana* was not due to photorespiration. The first implication is supported by results in the literature. Zelitch (1974) originally concluded that glycidate alleviated photorespiration by blocking glycolate synthesis on the basis of results from experiments that have been challenged (Chollet, 1978). Also, many studies have failed to show that glycidate is a specific inhibitor of photorespiration in plants, including tobacco leaf discs (Chollet, 1978), intact chloroplasts (Chollet, 1976; Wildner & Larsson, 1979), bean seedlings (Poskuta & Kochańska, 1978) and the photosynthetic bacterium, *Chromatium* sp. (Asami & Akazawa 1976).

Previous studies have shown that low concentrations of glycidate stimulate photosynthesis in C-3 terrestrial plants, while high concentrations are inhibitory. The cause of glycidate's stimulatory effect is not known, but it may involve allosteric inhibition of glycolate metabolism (Zelitch, 1974; Oliver & Zelitch, 1977a and 1977b) or stimulation of RuDP synthesis (Poskuta & Kochańska, 1978). The optimum concentration of glycidate for stimulation of photosynthesis has been reported as 40 mM for tobacco leaf discs (Zelitch, 1974), 5.0 mM for bean seedlings (Poskuta & Kochańska, 1978), and 3.0 mM for isolated spinach chloroplasts (Chollet, 1976). In *I. galbana* photosynthesis was inhibited by all concentrations tested (0.5 to 50.0 mM) which suggests underlying differences in the regulation of photosynthesis and glycolate metabolism in this species.

This research has revealed no evidence for photorespiration in the diatom, *T. fluviatilis*, except the presence of a slight Warburg effect under conditions of simultaneous high O_2 and low CO_2. The interpretation of these results is complex since the absence of apparent photorespiration may involve at least three mechanisms and these need not be mutually exclusive.

1. Photorespiration could be masked by efficient recycling of photorespiratory CO_2 via C-4 metabolism.

2. Intra-chloroplastic carbonic anhydrase could act to dehydrate HCO_3^- and thereby raise the concentration of CO_2 high enough to inhibit the activity of RuDP oxygenase.

3. The low levels of photorespiration in *T. fluviatilis* could be the result of an intrinsically low activity of RuDP oxygenase.

C-4 Metabolism

In its simplest form C-4 metabolism involves the β-carboxylation of phosphoenol pyruvate (PEP) to oxaloacetate which in turn is reduced to malate or transaminated to aspartate. The resulting malate or aspartate may then enter other pathways for the formation of essential metabolites. The existence of this form of C-4 metabolism is well established in algae where it is responsible for dark fixation of carbon via PEP carboxykinase (Kremer & Küppers, 1977) and may supply the aspartate group of animo acids and substrates for the TCA cycle (Benedict, 1978). In terms of total photosynthetic carbon flux, C-4 metabolism may be of considerable importance, since algae frequently divert large amounts of recently fixed carbon into amino acids, proteins, and TCA cycle intermediates (Patil & Joshi, 1970; Burris *et al.*, 1976; Morris *et al.*, 1974).

Alternatively, malate and aspartate may act as temporary reservoirs of CO_2 in certain tropical grasses with Kranz anatomy and plants possessing CAM physiology. In these plants, malate and aspartate are decarboxylated to supply CO_2 for the Calvin cycle and the resulting 3-carbon products are recycled to PEP. This process is referred to as C-4 pathway to distinguish it from simple β-carboxylation of PEP which is called C-4 metabolism. The absence of apparent photorespiration in C-4 pathway plants is probably the result of efficient recycling of photorespiratory CO_2, since the bundle sheath cells, the site of the Calvin cycle and therefore photorespiration, are surrounded by mesophyll cells rich in PEP carboxylase (Black, 1973). Studies of C-4 pathway in marine plants, based on the activities of C-4 pathway enzymes (Kremer & Küppers, 1977; Ting, 1976) and δ-^{13}C values (Black & Bender, 1976) have generally yielded negative results. However, Joshi *et al.* (1974) and Karekar and Joshi (1973) have implicated the presence of C-4 pathway in marine macroalgae based on labelling patterns and the presence of high levels of PEP carboxylase in *Enteromorpha* sp., although these results have been challenged (Kremer & Küppers, 1977). Also, Benedict and Scott (1976); Black and Bender (1976); and Smith and Epstein (1971) have reported δ-^{13}C values consistent with initial carbon fixation via β-carboxylation in some marine cyanophytes and angiosperms. Of direct relevance to my results is a group of recent studies which have emphasized the role of C-4 pathway in marine diatoms.

Beardall *et al.* (1976) found short-term labelling patterns in the diatoms, *S. costatum* and *P.tricornutum* indicative of high rates of β-carboxylation; these results contrasted with those obtained with *I. galbana, G. tamarensis* and *D. tertiolecta.* Experiments comparing labelling after 2-second and 60-second exposures to $NaH^{14}CO_3$ showed label in malate decreasing and a simultaneous increase in 3-PGA and sugar phosphates in *S. costatum* (Beardall *et al.,* 1976) and in stationary-phase cultures of *D. tertiolecta* (Mukerji *et al.,* 1978). These results are consistent with C-4 pathway. These authors also found PEP carboxylase activities as high or higher than RuDP carboxylase in both diatoms and in stationary-phase *D. tertiolecta.* PEP appeared to act as the primary carbon acceptor, since the photosynthetic rate of *S. costatum* was higher when HCO_3^- was initially added rather than CO_2. Stationary-phase *D. tertiolecta* cells had similar rates of photosynthesis in either CO_2 or HCO_3^- but log-phase cells had much higher rates in CO_2. To fully evaluate the evidence for C-4 pathway presented by Beardall *et al.* (1976) and Mukerji *et al.* (1978) other features of their methods and results must be examined. High cell densities were used throughout their work (2×10^5 to 1.4×10^6 cells ml^{-1}). The combined activities of PEP and RuDP carboxylase could never account for more than 10% of the $^{14}CO_2$ fixation rate in intact cells, and the extremely high Km HCO_3^- (12.7 mM at pH 8.0) they reported for RuDP carboxylase in their assays indicate that the authors greatly underestimated the activity of the enzyme (Mukerji & Morris, 1976). For comparison, this study has shown that the apparent Km HCO_3^- at pH 8.00 for intact cells of *I. galbana* was about 0.9 mM and that of *T. fluviatilis* was even lower at 0.22 mM. Beardal *et al.* (1976) and Mukerji *et al.* (1978) have also reported that high ratios of RuDP carboxylase:PEP carboxylase are correlated with high rates of photosynthesis; this ratio was lower in older cultures which had low rates of photosynthesis. One interpretation of these results is that healthy, log-phase cells use conventional Calvin cycle with an accessory β-carboxylation pathway, the magnitude of which varies among species. Depletion of CO_2 due to high cell densities or high pH values in stationary cultures (which may exceed pH 9.3) results in declining capacity of the Calvin cycle and an increase in the relative importance of β-carboxylation. Other authors have shown that growth in low CO_2 or high pH can result in a shift to β-carboxylation in algae (Graham & Whittingham, 1968).

In the present study the pH was controlled and complications resulting from high cell densities and senescent cells have been eliminated. Nevertheless, the diatom, *T. fluviatilis* is shown here to have a much higher affinity for CO_2 than *I. galbana* and an absence of apparent photorespiration. These differences can be explained by the involvement of C-4 metabolism in the diatom. The results of this research and that of Beardall *et al.* (1976) and Mukerji *et al.* (1978) support the following conclusions.

When CO_2 is not limiting, optimal photosynthesis in diatoms depends on a balance of Calvin cycle and an active β-carboxylation pathway. Assuming that PEP carboxylase is restricted to the cytoplasm, as in terrestrial plants (Kelly *et al.*,1976), β-carboxylation could account for the absence of apparent photorespiration by acting to refix photorespiratory CO_2. Under these conditions, the substrate, PEP, could be derived form Calvin cycle 3-PGA or from serine produced in the glycolate pathway. The latter possibility is of particular interest since low O_2 concentrations could act to limit glycolate synthesis which in turn could result in substrate limitation of β-carboxylation if PEP were derived from serine. Thus, low O_2 could inhibit photosynthesis, assuming that β-carboxylation is essential for optimum photosynthesis through some mechanism such as the production of amino acids and TCA cycle intermediates. This scheme is hypothetical, but it could explain the observation that optimal photosynthesis in *T. fluviatilis* occurred at 200 to 400 μM O_2. If correct, this hypothesis implies that *T. fluviatilis* is adapted to average seawater concentrations of O_2 and experiences reduced rates of photosynthesis when photorespiration is inhibited by low O_2. At very high O_2 the rate of CO_2 and serine production could exceed the capacity of the β-carboxylation pathway and result in reduced net photosynthetic CO_2 assimilation through loss of photorespiratory CO_2. The results with the effects of glycidate on *T. fluviatilis* are relevant to this hypothesis. Unlike *I. galbana*, glycidate stimulated photosynthetic CO_2 assimilation in *T. fluviatilis*, and the stimulation was greater at low O_2 (67% at 1 mM glycidate) than at moderate or high O_2 (21% at 1 mM glycidate). Glycidate is known to increase the pool size of serine (Zelitch, 1974; Poskuta & Kochańska, 1978). If the inhibition of photosynthesis in *T. fluviatilis* at low O_2 is the result of inadequate serine synthesis to supply the β-carboxylation pathway with PEP, then glycidate could act to relieve this inhibition and therefore stimulate photosynthesis proportionately more in low O_2 than at high O_2 where serine production via the glycolate pathway is higher to begin with. An alternative to this hypothesis of O_2 stimulation of photosynthesis in *T. fluviatilis* which is also consistent with the glycidate results derives from research showing O_2 stimulation of photosynthesis in *Phaseolus* and *Populus* leaves (Viil *et al.*, 1977). These authors have attributed the O_2 stimulation (10 to 20 % in 21%-O_2 air compared to 2%-O_2 air) to enhanced RuDP synthesis. As mentioned previously, Poskuta and Kochańska (1978) have shown that glycidate stimulates RuDP synthesis in bean seedlings. Either of these hypotheses could account for the observed stimulation by O_2 in *T. fluviatilis* and the fact that glycidate seemed to relieve the inhibition caused by low O_2. However, given the controversy surrounding glycidate, these results can not be considered convincing evidence for either hypothesis.

Under conditions of severe CO_2 limitation, diatoms may be able to shift to a form of C-4 pathway. This conclusion is supported by the results of this research. For example, at 0.05 mM total inorganic carbon and pH 8.00 (0.5 μM CO_2) the photosynthetic rate of *I. galbana* was reduced to 2.5 to 5.7 % (depending on the O_2 concentration) of the rate at 5.05 mM carbon (50.5 μM CO_2) at the same pH, whereas *T. fluviatilis* retained more than 30% of its maximum rate at 0.05 mM carbon. This relationship also held for the case where the concentration of inorganic carbon remained constant at 0.2 mM and the concentration of CO_2 was varied by adjusting the pH. In both species the maximum rate of photosynthesis occurred when the pH was relatively low (pH 7.25 to 7.50), but *T. fluviatilis* retained 75% of its maximum rate at pH 8.50 (0.4 μM CO_2) while *I. galbana* was reduced to only 35%. In the above experiments HCO_3^- was relatively abundant and since PEP carboxylase has an absolute requirement for HCO_3^- (Coombs *et al.*, 1975), it follows that high rates of carbon assimilation via β-carboxylation could occur under these conditions. However, C-4 metabolism alone cannot produce net photosynthate, since it has no mechanism for regenerating the HCO_3^- acceptor, PEP. Regeneration of PEP requires Calvin cycle activity via RuDP carboxylase which is substrate specific for CO_2 (Cooper & Fillmore, 1969). Therefore, these experiments imply that *T. fluviatilis* is capable of maintaining substantial Calvin cycle activity in spite of vanishingly low concentrations of CO_2. This could be accomplished by diverting the 4-carbon acids produced by β-carboxylation to the chloroplast where they are decarboxylated to supply CO_2 to the Calvin cycle and exporting the 3-carbon products to the cytoplasm where they are recycled to PEP. This is C-4 pathway at an intracellular level as proposed by Beardall *et al.* (1976). There is no evidence in this research or, in my opinion, in that of Beardall *et al.* (1976) and Mukerji *et al.* (1978) to support the conclusion that C-4 pathway occurs in diatoms except in extreme conditions of CO_2 deprivation, conditions which are unlikely to occur outside the laboratory.

Between the two extremes described, shifts in the exchange of metabolites among the Calvin cycle, β-carboxylation, and the glycolate pathway probably act to maintain the maximum photosynthesis possible at a given pH, O_2 and CO_2 concentration when other factors are not limiting. Beardall *et al.* (1976) and Mukerji *et al.* (1978) have shown that other taxa of unicellular algae are capable of such responses, however, *T. fluviatilis* and, possibly, diatoms in general seem particularly well adapted to environmental conditions that tend to limit the concentration of free CO_2. This probably explains the high rates of primary production of diatoms in field studies (Thomas *et al.*, 1978) and their dominance in nutrient-rich coastal waters where high cell densities can cause the pH, O_2 and CO_2 concentrations to reach extreme values.

The results of this research are consistent with the presence of C-4 metabolism in *T. fluviatilis*; a conclusion that is also supported by the literature. Furthermore, it is not necessary to postulate C-4 pathway except under conditions of severe CO_2 limitation. However, other mechanisms can account for the absence of apparent photorespiration and the high affinity for CO_2 of *T. fluviatilis*.

Carbonic Anhydrase

The high affinity for CO_2 shown by *T. fluviatilis* is a strong indication of the involvement of HCO_3^- in the photosynthetic metabolism of this species, while the relatively low affinity displayed by *I. galbana* suggests that this species is restricted to free CO_2. Bicarbonate ion could be utilized indirectly in photosynthesis through the activity of carbonic anhydrase, which catalyses the reversible hydration of CO_2. When comparing HCO_3^- involvement in the photosynthesis of terrestrial and marine plants, there is one over-riding consideration. Carbon dioxide is the only carbon source for photosynthesis in the terrestrial environment, and any HCO_3^- involvement is merely the result of enzyme specificity. But in the marine environment where HCO_3^- is, on average, 90 times more abundant than CO_2, the involvement of a HCO_3^--fixing mechanism would represent an effective increase in the carbon available for photosynthesis.

Carbonic anhydrase is widely distributed in photoautotrophs (Atkins *et al.*, 1972; Graham & Smillie, 1976), and has long been implicated in the photosynthesis of terrestrial plants and algae (Colman *et al.*, 1974; Findenegg, 1976; Findenegg & Fischer, 1978; Han & Eley, 1973; Ingle & Colman, 1976). Although many roles for carbonic anhydrase in photosynthesis have been proposed (Werdan & Heldt, 1972) these roles are not mutually exclusive and only one of them is directly relevant to this discussion. That proposal is that carbonic anhydrase acts to dehydrate HCO_3^- at the site of the Calvin cycle. This function is suggested by the observation that the ability of algae to excrete glycolate is dependent on the cells being grown at elevated CO_2 concentrations (0.2 to 5 % CO_2 aeration) and immediately transferred to a low CO_2 environment (Colman *et al.*, 1974; Findenegg & Fischer, 1978; Han & Eley, 1973; Ingle & Colman, 1976; Nelson & Tolbert, 1969). Adaptation to low CO_2, requiring 20 minutes to several hours, depending on the species, results in an increase in the activity of carbonic anhydrase and cessation of glycolate excretion. It is not clear whether the reduction in glycolate excretion is the result of increased glycolate metabolism (Findenegg & Fischer, 1978: Nelson & Tolbert, 1969) or reduced glycolate synthesis (Colman *et al.*, 1974; Codd & Merret, 1971), but it seems probable that both mechanisms contribute with the latter being the more significant in most cases. The most convincing results are those of Findenegg (1976) who showed that adaptation to low CO_2 by *Scenedemus* grown at high CO_2 consists of two distinct phases. Cells grown in CO_2-rich environments could not assimilate HCO_3^-, but after 1-3 hrs exposure to low CO_2 the cells could assimilate HCO_3^-. However, the CO_2 concentration required for half-maximum CO_2 assimilation at low pH was still as high as for CO_2-adapted cells (100 μM). During this period carbonic anhydrase activity increased, but the enzyme was restricted to the cytoplasm. After longer than four hours of adaptation to low CO_2, HCO_3^- assimilation at high pH was retained, but the CO_2 concentration required for half maximum CO_2 assimilation at low pH dropped to only 5 μM. Carbonic anhydrase activity was now present in the chloroplasts as well as the cytoplasm. These results clearly show that the activity of intra-chloroplastic carbonic anhydrase was able to greatly reduce the Km CO_2 of a unicellular alga. Since other studies have shown this effect to be correlated with a reduction in glycolate excretion in the presence of inhibitors of the glycolate pathway (Colman *et al.*, 1974; Findenegg & Fischer, 1978), it seems reasonable to conclude that carbonic anhydrase can increase the concentration of CO_2 in the chloroplast and by so doing inhibit the RuDP oxygenase reaction, thereby reducing the rate of glycolate synthesis. Such an effect could account for the apparent high affinity for CO_2 and the absence of a Warburg effect observed in this study with *T. fluviatilis*.

The pH of the chloroplast stroma is known to be alkaline and therefore favours the hydration of CO_2 rather than dehydration. Thus, it is not obvious how carbonic anhydrase could function in the environment of the stroma to release CO_2 from HCO_3^-. As envisioned by Werdan and Hedlt (1972) carbonic anhydrase and RuDP carboxylase may cooperate in the following way. The carboxylation of RuDP results in the release of two protons, which, together with the local concentration gradient caused by the removal of CO_2, acts to create a microenvironment favourable to the dehydration reaction. A separate, but related, scheme involves both enzymes in close association with the F_0/CF_1 complexes which are embedded in the thylakoid membranes. According to the chemiosmotic hypothesis (Racker, 1976), protons leaving the interior of the thylakoid into the stroma pass through F_0/CF_1 complexes (large enzyme complexes where the free energy of the proton gradient across the thylakoid membrane is used to synthesize ATP). This could result in temporary, local accumulations of protons in the stroma, thus forming microenvironments favouring the dehydration reaction. While experimental evidence is lacking for either proposal, it is also true that almost nothing is known about the organisation of stromal proteins.

An obvious question is, since seawater contains large quantities of HCO_3^-, why cannot a mechanism of HCO_3^- dehydration act to provide essentially unlimited CO_2 for photosynthesis in the marine environment? The answer probably lies in the fact that the dehydration of HCO_3^- results in the release of an hydroxyl ion. The continual use of HCO_3^- in photosynthesis, in the absence of some mechanism to balance the pH, would ultimately result in the intracellular and extracellular pH rising above the pH tolerance of the cells. Synthesis of carboxylic and amino acids, as in C-4 metabolism would tend to balance the rise in pH resulting from the production of hydroxyl ions, but this is only a temporary solution. The ultimate products of photosynthesis, starch, proteins, nucleic acids, etc, are either neutral or only weakly acidic or basic and could not be expected to balance the pH, given the rate of hydroxyl ion production that would result if HCO_3^- were the only or even a major source of carbon for photosynthesis. Nevertheless, this research indicates that HCO_3^- was utilised to some extent in the photosynthesis of *T. fluviatilis*. The markedly different rates of photosynthesis in *I. galbana* and *T. fluviatilis* as a function of pH may reflect not only differences in their respective abilities to utilize HCO_3^-, but also other adaptations which permit *T. fluviatilis* to survive in the high pH environment that results from the use of HCO_3^- in photosynthesis. Such adaptations could play a major role in the seasonal succession of species observed in natural marine phytoplankton.

The high rates of photosynthesis reported here for *T. fluviatilis* at CO_2 concentrations of less than 0.5 μM are strong evidence that the diatom is able to utilize HCO_3^- in photosynthesis. However, it is not possible to decide on the basis of this research whether this utilization is the result of C-4 metabolism or the activity of carbonic anhydrase. On the basis of the work of Beardall *et al.* (1976), it is probable that C-4 metabolism is involved, but nothing in their results or other literature suggests that both mechanisms cannot operate simultaneously. This research does not rule out the possibility that either or both of these mechanisms may also operate in *I. galbana*, but if they do, they are much less efficient than in *T. fluviatilis*.

Carbonic anhydrase or C-4 metabolism or both can explain the high affinity for CO_2 and the absence of apparent photorespiration in *T. fluviatilis*. However, the lack of apparent photorespiration could also result from the absence of photorespiration *per se* which implies the absence of RuDP oxygenase activity.

RuDP Oxygenase

Extensive evidence supports the hypothesis that the CO_2 released during photorespiration results from the oxidation of glycolate formed in photosynthesis (Jackson & Volk, 1970; Tolbert & Ryan, 1975; Zelitch, 1971). Although several origins of glycolate have been proposed (Gibbs, 1971; Wilson & Calvin, 1955; Zelitch, 1971), the only hypothesis that accounts for all the known features of glycolate synthesis (see Tolbert & Ryan, 1975) derives from the discovery by Bowes *et al.* (1971) that RuDP carboxylase, *in vitro*, catalyses the oxidation of RuDP in which one of the products is phosphoglycolate. This discovery has led to the hypothesis that *in vivo* fraction-one protein, the complex of stromal enzymes which is largely composed of RuDP carboxylase, catalyses the production of phosphoglycolate from RuDP in the presence of O_2. Implicit in this hypothesis is the idea that photorespiration is inevitable whenever photosynthesis depends on the activity of the Calvin cycle and whenever O_2 and CO_2 occur in appropriate relative abundances, as in the atmosphere or in aquatic environments at equilibrium with the atmosphere. If the observed differences in the photorespiration of *I. galbana* and *T. fluviatilis* are to be explained as a result of intrinsic differences in their rates of glycolate synthesis, then the validity of the RuDP oxygenase hypothesis must be examined. Although the hypothesis is widely accepted, there are indications in the literature that it can be challenged.

Many reports have shown that the carboxylase and oxygenase activities of fraction-one protein co-purify, including even crystalline preparations (Marsho & Kung, 1976). Furthermore, the factors which activate or inhibit the carboxylase reaction also activate or inhibit the oxygenase reaction (Badger & Lorimer, 1976; Chollet & Anderson, 1976). For these reasons it has been assumed that both reactions are catalysed by the same enzyme. However, Brändén (1978) has reported that, when RuDP carboxylase is purified at pH 8.3, a shoulder appears on the 280 NM absorbance peak that contains most of the oxygenase activity while the main peak retains most of the carboxylase activity. The shoulder is not resolved when the purification is carried out at pH 7.4 conforming to previous work. The author has interpreted his results to mean that RuDP carboxylase/oxygenase is really two enzymes, however, this conclusion must be considered preliminary since he has not isolated the enzymes. Obviously, Brändén's results do not alter the basic concept that glycolate is synthesized by an enzyme catalysed oxidation of RuDP, but they do imply that this oxidative reaction, and therefore photorespiration, need not be a universal feature of Calvin cycle photoautotrophic organisms. A more general challenge to the RuDP oxygenase hypothesis comes from the work of Bravdo and Canvin (1975 and 1979) and Mahon *et al.* (1974) who have shown that the rate of release of photorespiratory CO_2, as opposed to the Warburg effect, is unchanged when the concentration of CO_2 is increased from 20 to 1150 $\mu l\, l^{-1}$ (approximately equivalent to a change from 0.7 to 40 µM CO_2 in the aqueous phase). These authors base this conclusion on tracer studies with photorespiring sunflower leaves. The validity of this technique is difficult to interpret, but this is the only method of estimating the release of photorespiratory CO_2 during photosynthesis. If CO_2

is a competitive inhibitor of RuDP oxygenase, as required in the RuDP oxygenase hypothesis, then it is inconsistent for the rate of photorespiration to be independent of the concentration of CO_2 as these author's results imply. With reference to algae, other studies have been inconsistent with the existence of oxygenase activity. In extracts of fraction-one protein from various marine macroalgae, Akazawa and Osmond (1976) found the oxygenase activity did not exceed 1% of the carboxylase activity although these authors attributed this result to unspecified problems with activation of the oxygenase reaction. Lloyd *et al.* (1977a) reported no evidence for photorespiration in ten species of marine and freshwater unicellular algae, including no Warburg effect. While I have questioned the validity of their technique, it is nevertheless puzzling that these authors failed to detect photorespiration in algae while the same methods yielded positive results with aquatic angiosperms (Lloyd *et al.* 1977b). The extent to which *in vivo* oxygenase activity is responsible for the Warburg effect observed in algae remains to be determined.

A definite resolution of the increasingly tenuous relationship between *in vitro* RuDP oxygenase activity and *in vivo* photorespiration is urgently required. In addition to the hundreds of publications that support or accept the validity of the RuDP oxygenase hypothesis and the unique ability of the hypothesis to explain the various properties of glycolate formation, the one-enzyme RuDP carboxylase/oxygenase hypothesis has supplied a unifying concept for the study of photorespiration. With the exception of some photosynthetic bacteria, all known photoautotrophic organisms rely upon the Calvin cycle for the net fixation of CO_2 (Benedict 1978). The central role of the Calvin cycle is a direct result of its unique autocatalytic nature; that is, its ability to assimilate net organic carbon and to simultaneously regenerate the CO_2 acceptor molecule, RuDP. Just as photoautotrophy depends upon the Calvin cycle, the Calvin cycle depends upon RuDP carboxylase activity. It is precisely the pivotal role of RuDP carboxylase in photosynthesis that makes its oxygenase activity both profoundly puzzling and yet explicable. It seems illogical for such a critically important enzyme to be subject to competitive inhibition by O_2, a substance which is not only abundant but a by-product of photosynthesis. Yet, as pointed out by Lorimer and Andrews (1973), the Calvin cycle must have evolved when O_2 was not abundant and the oxygenase reaction would have been insignificant. Once the Calvin cycle was established, the enzyme's very importance may have assured its conservative evolution. The idea that the enzyme has changed little through time has been confirmed by Takabe and Akazawa (1975) and Akazawa and Osmond (1976) who have shown, through tryptic peptide analysis and immunodiffusion experiments, that the enzyme's catalytic subunit is quite homologous when isolated from unrelated organisms. Noteably, the catalytic subunit is coded for on chloroplast DNA while a smaller, presumably regulatory, subunit is coded for on nuclear DNA and is highly variable among species (Jensen & Bahr, 1977).

Despite many attempts, no one has ever found an essential metabolic function for the glycolate pathway and many authors have concluded that none exists. The oxygenase hypothesis provides an obvious explanation for the superfluous pathway. If glycolate formation is merely a necessary, but undesirable, by-product of the Calvin cycle, then the glycolate pathway is a method of minimizing the impact of the oxygenase reaction by conserving 75% of the carbon traversing the pathway. Likewise C-4 metabolism can be seen as a secondary adaptation to high O_2 environments which sacrifices quantum efficiency in order to either: (1) raise the intrachloroplastic CO_2 concentration high enough to inhibit the oxygenase reaction or (2) to permit the effective refixation of the CO_2 released by photorespiration.

The validity of these theoretical considerations rests primarily with the future confirmation or refutation of Brändén's (1978) results. Unless it can be shown that the two enzymes must co-exist for proper catalysis or that they actually represent two quasi-stable states of the same enzyme, one is again faced with the central dilemma of photorespiration - why does it exist?

CONCLUSIONS

Within the vast and rapidly growing literature on photorespiration, it seems possible to find a published result to contradict any given result. In part these contradictions result from the numerous assay techniques employed by various authors, but they also reflect the lack of any well established theory to explain why photorespiration occurs or what causes it. It appears certain that photorespiration is an integral part of the Calvin cycle, but as to whether it is necessary for the proper activity of the Calvin cycle or merely an in-built error in photosynthesis (or neither of these) remains to be determined. The RuDP oxygenase hypothesis of photorespiration faces critical tests of its validity, and there are no competing hypotheses, which are consistent with the known facts, ready to fill the vacuum if it should fail. At least in terrestrial plants photorespiration is known to occur, but in algae even this is uncertain. Two things have made the study of photorespiration in marine algae particularly difficult. In the first place the best characterized and least ambiguous assays for photorespiration require the use of a CO_2 gas analyser which is not directly adaptable to studies in aqueous systems. The alternative of adapting algae to a gaseous environment has yielded results which are almost impossible to interpret when compared to terrestrial plants or algae assayed in aqueous environments (Lloyd *et al.*, 1977a). This could be due to basic differences in the photosynthesis of algae compared to terrestrial plants or to artifacts imposed by the exposure of the algae to air. The resolution of this question is vital to the understanding of photorespiration in algae. The second derives from what appears to be a fundamental difference between the terrestrial and marine environments, the high concentration of HCO_3^- in the latter. In one sense photorespiration is related to carbon limitation of photosynthesis and no treatment of photorespiration in marine plants can be complete without considering the potential involvement of HCO_3^- in photosynthesis.

Rather than add to the already considerable confusion, the purpose of this research was to unequivocally demonstrate the presence or absence of photorespiration in marine algae, because previous studies have, in my opinion, failed to do so. To accomplish this a new technique was developed which eliminated most of the artifacts and limitations that have made other studies unreliable. The application of this technique did unequivocally show the presence of a Warburg effect in *I. galbana;* however, it has not been possible to conclude from this result that photorespiration occurs in *I. galbana* for two reasons. The first is a direct result of a refinement made possible by this technique which allowed changes in the concentrations of CO_2 and total inorganic carbon to be correlated with the Warburg effect. These experiments revealed that the Warburg effect in *I.galbana* at high O_2 concentrations was not relieved by increasing the CO_2 or total inorganic carbon and therefore unlike that found in C-3 terrestrial plants. This result also implies that bubbling cultures or natural populations of algae with O_2 is not a valid assay for photorespiration. The second reason involves the questionable cause and effect relationship between the Warburg effect and photorespiration (Bravdo & Canvin, 1979). Although it remains probable that at least some of the O_2

inhibition observed in *I. galbana*, specifically that which occurred at low and moderate O_2 concentrations, is due to photorespiration, it is clear that this conclusion is not fully supportable. The only effect of the metabolic inhibitor glycidate that most authors agree on is that it stimulates photosynthesis in C-3 plants, but in *I. galbana* glycidate did not stimulate photosynthesis. The lack of stimulation by glycidate, along with the different character of the Warburg effect at high O_2, implies that *I. galbana* may not be so typically C-3 as its affinity for CO_2 and short-term labelling pattern (Beardall *et al.*, 1976) have indicated. However there is no evidence of C-4 metabolism or other known varients of photosynthesis in *I. galbana*. Perhaps the photosynthesis of *I. galbana* incorporates novel features, but the results of this study are not sufficient evidence to warrant such a conclusion.

The results with the diatom *T. fluviatilis* regarding its high affinity for CO_2, lack of a Warburg effect except in extreme conditions, broad pH tolerance, and, in particular the O_2 stimulation of photosynthesis, were largely inconsistent with previous studies. The lack of apparent photorespiration is probably the result of efficient recycling of photorespiratory CO_2 via C-4 metabolism, as in the case of C-4 terrestrial plants (Black, 1973), rather than an absence of photorespiration *per se*. This conclusion is supported by the results of Beardall *et al.* (1976), however, it is not necessary for this C-4 metabolism to result in C-4 pathway as concluded by those authors except under conditions of severe CO_2 deprivation. Two hypotheses were proposed to account for the O_2 stimulation of photosynthesis in *T. fluviatilis* and both were shown to be consistent with the results on the effects of glycidate in *T. fluviatilis*. All the results with *T. fluviatilis* are unified within the interpretation that the absence of a Warburg effect and high affinity for CO_2 result from C-4 metabolism, but other interpretations are possible and two were discussed.

This research has shown that the photosynthetic metabolism of *T. fluviatilis* is qualitatively different than that of C-3 terrestrial plants and *I. galbana*. Further study is required to determine the nature of these differences and whether these results apply to all marine diatoms. Regardless of the causes of these differences, the relatively broad pH optimum for photosynthesis and the absence of a Warburg effect in *T. fluviatilis* under typical seawater conditions, should give this species a distinct competitive advantage over a species like *I. galbana* when high cell densities and high photosynthetic rates result in supersaturation of O_2 and strongly alkaline pH values. If these results are found to hold for diatoms in general, then this may help explain the dominance of diatoms in near-shore areas and their relatively high rates of primary production.

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Title of Thesis

THE EFFECTS OF MANIPULATIONS OF THE CONCENTRATIONS OF O_2, CO_2, TOTAL INORGANIC CARBON, AND GLYCIDATE ON THE WARBURG EFFECT IN TWO SPECIES OF MARINE UNICELLULAR ALGAE

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Dec. 19th, 1979