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# Effects of pH on the growth and carbon uptake of marine phytoplankton

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ABSTRACT: This study examines the growth and photosynthetic response of marine phytoplankton to a naturally occurring range of pH's. Growth rates were determined for *Thalassiosira pseudonana* and *Thalassiosira oceanica* via *in vivo* fluorescence measurements; photosynthetic rates were measured via $^{14}$carbon uptake using mesocosm tank assemblages of phytoplankton. A pH range of 7.0 to 9.4 was used for both sets of experiments and consistent declines of growth rate and photosynthesis were observed at high pH levels (> pH 8.8). The pH response of the 2 phytoplankton species and the tank assemblages appeared to correlate with calculated concentrations of free carbon dioxide indicating a possible carbon substrate limitation at high pH. Half-aturation values for calculated [$CO_2$] were determined for cell growth rates and for photosynthesis using the Monod equation ($K_\mu$ = 0.5 µM) and Michaelis-Menten equation ($K_s$ = 1.3 µM), respectively. These values were within the range of values measured for phytoplankton in previous studies. Based on this evidence, it is suggested that at high pH levels the availability of $CO_2$ may become limiting to marine phytoplankton growth and photosynthesis.

KEY WORDS: Phytoplankton · pH · Carbon uptake · Algal growth rates · $CO_2$ limitation

## INTRODUCTION

Variation in pH can affect algal growth in a number of ways. It can change the distribution of carbon dioxide species and carbon availability, alter the availability of trace metals and essential nutrients, and at extreme pH levels potentially cause direct physiological effects. Most studies of pH effects on algae have been conducted in freshwater systems where the carbonate buffering system is weaker than in seawater and pH may fluctuate dramatically. However, a number of studies have demonstrated that pH also changes significantly in marine systems despite the strong buffering capacity of the carbonate system in seawater (Marshall & Orr 1948, Park et al. 1958, Frithsen et al. 1985, Pegler & Kempe 1988). In many marine systems, pH may be an important factor regulating algal abundance and distribution.

In general, changes in pH levels in marine systems appear to correlate with changes in temperature, dissolved oxygen, and phytoplankton production. Conditions of high pH, high phytoplankton production, and low oxygen conditions are characteristic of nutrient-enriched systems and often are found in coastal waters or enclosed bodies of water (lagoons, salt ponds, embayments, etc.) which receive anthropogenic inputs such as sewage effluent or agricultural runoff (Marshall & Orr 1948, Park et al. 1958, King 1970, Oviatt et al. 1986). In these systems, pH ranges approaching pH 9 have been measured. pH levels exceeding pH 9.0 have been recorded in a number of coastal marine systems (Hires et al. 1963, Emery 1969), and in experimental mesocosms receiving nutrient enrichment (Frithsen et al. 1985, Hinga 1992).

Several studies have shown that pH variations within these ranges influence phytoplankton abundance and species distribution. Freshwater studies have suggested that species succession is determined by the ability of certain species to proliferate at high pH's presumably due to their tolerance of low $CO_2$ levels (Brock 1973, Goldman & Shapiro 1973, Shapiro 1973). In marine mesocosms, Hinga (1992) also correlated pH changes with the succession of diatoms to dinoflagellates. Goldman et al. (1982) suggested that the domi-

nance of *Phaeodactylum tricornutum* over other species in outdoor mass cultures of marine phytoplankton was due to its ability to grow at pH levels up to pH 10.3. Pruder & Bolton (1979) provided evidence for an interaction of pH and $CO_2$ causing decreased growth of *Thalassiosira pseudonana*, a coastal diatom species.

The relative concentrations of $CO_2$, $HCO_3^-$, and $CO_3^{2-}$ of the carbonate system and the pH of seawater are closely linked. As pH increases, carbonate increases and bicarbonate and molecular $CO_2$ decrease. At the average pH of seawater (pH 8.2), only about 1% of total $CO_2$ is found as molecular $CO_2$, 90% as $HCO_3^-$, and the rest as $CO_3^{2-}$ (Steeman Nielsen 1975). At any given pH, the concentrations of these species are set by any one of the following: partial pressure of $CO_2$, total alkalinity, or $\Sigma CO_2$. The removal of $CO_2$ by photosynthetic uptake leads to an increase in pH and a decrease in $CO_2$ partial pressure when $CO_2$ replacement processes occur more slowly than the utilization. These replacement processes include atmospheric $CO_2$ influx via diffusion, respiration, fermentation, and the slow hydration and dehydration reactions of dissolved $CO_2$ (Goldman et al. 1974, Owens & Essias 1976).

In this study, we examine the effects of pH variation on marine phytoplankton by measuring 2 types of phytoplankton response. First, we tested the effects of pH and alkalinity changes on growth in the laboratory of a coastal and an oceanic species. Second, we examined photosynthetic response of natural phytoplankton assemblages to changes in pH. Based upon existing pH data for natural marine systems and experimental marine mesocosms (Hires et al. 1963, Emery 1969, Frithsen et al. 1985), a pH range of 7.0 to 9.4 was chosen for both sets of experiments. Finally, carbon species concentrations were calculated in order to examine the potential relationship between carbon availability and phytoplankton growth rate and photosynthesis.

## METHODS

**Growth rate response of phytoplankton cultures.** The diatoms *Thalassiosira pseudonana* (3H), a coastal species, and *Thalassiosira oceanica* (13-1), an oceanic species, were used in the growth rate experiments. Stock cultures were obtained from T. Smayda (Graduate School of Oceanography, URI) and maintained in batch culture at 18°C. Continuous light was provided by cool white fluorescent tubes at an irradiance of 75 $\mu E\ m^{-2}\ s^{-1}$.

Experimental cultures were grown in acid-cleaned 250 ml polycarbonate bottles with 0.2 µm filtered Sargasso Seawater containing modified F/2 nutrient concentrations (Guillard 1972). Experimental treatments were incubated in a water bath at 20°C with continuous light at 100 $\mu E\ m^{-2}\ s^{-1}$. Inocula for each experiment were taken from exponentially growing cells. The volume of the inoculum was chosen such that the initial cell concentration was just above the lower detection limit of the fluorometer (Turner Designs) used to measure cell densities. This provided a period of about 40 h before a measurable change in pH (0.1 pH units) was caused by the $CO_2$ uptake of the phytoplankton. Prior to inoculation of an experiment, the fluorescence and pH of the stock culture were measured to determine the appropriate volume of inoculum.

***pH adjustment:*** The pH of the experimental culture media was adjusted using 2 different methods. The first method required bubbling with compressed gases ($CO_2$, compressed air, and a mixture of nitrogen and oxygen). The pH of a 4 l volume of culture medium was raised to pH 8.9 prior to bubbling by adding diluted NaOH. The medium was then divided into four 1 l volumes. Three of these were bubbled for 2 h by either $CO_2$, compressed air, or a mixture of $N_2$ and $O_2$, respectively producing pH levels of approximately 7, 8, and 9. The fourth 1 l volume remained unchanged and was used as a control for phytoplankton growth in the initial pH and alkalinity levels. For the second method of pH adjustment, both alkalinity and pH levels were altered. An initial acid/base titration of the medium was conducted and appropriate acid and base additions were made to 1 l volumes of culture medium to attain the desired pH levels. Generally, 6 or more pH levels were tested simultaneously in these experiments.

The pH was measured prior to and after pH adjustments and at the beginning and end of experiments with an Altex Model O71 pH meter and a Ross combination electrode. An effort was made to maintain the pH of all treatments within 0.1 pH units of the initial measurement. However, in the lower pH treatments (pH 7.0 to 7.5) pH levels sometimes increased up to 0.4 pH units during the course of the 40 h incubations.

***Calculation of carbon speciation:*** Concentrations of $CO_2$ and $HCO_3^-$ were calculated for each experiment to examine the possible effect of carbon substrate limitation. The following equations were used:

$$[CO_2] = \frac{CA\, a_H^2}{K_1 (a_H + 2K_2)}$$

$$[HCO_3^-] = \frac{CA\, a_H}{a_H + 2K_2}$$

in which CA = carbonate alkalinity; $a_H = 10^{-pH}$, activity of the hydrogen ions; $K_1$ = 1st apparent dissociation constant of carbonic acid; $K_2$ = 2nd apparent dissociation constant of carbonic acid.

Table 1. Experimental conditions for growth rate experiments on *Thalassiosira pseudonana* (3H) and *T. oceanica* (13-1)

| Date | pH range | Control pH | No. of pH treatments | pH adjustment |
|---|---|---|---|---|
| ***T. pseudonana*** | | | | |
| 29 Sep 1984 | 7.03 – 8.83 | 8.34 | 4 | Bubbling |
| 3 Oct 1984 | 7.01 – 9.18 | 8.50 | 4 | Bubbling |
| 15 Jan 1985 | 7.07 – 9.42 | 8.37 | 8 | Acid/base titration |
| 25 Jan 1985 | 8.32 – 9.33 | 8.32 | 6 | Acid/base titration |
| ***T. oceanica*** | | | | |
| 7 Nov 1984 | 7.10 – 9.36 | 8.45 | 4 | Bubbling |
| 8 Feb 1985 | 8.35 – 9.48 | 8.35 | 6 | Acid/base titration |

Carbonate alkalinity was calculated from measured total alkalinity and calculated borate alkalinity. CA values were adjusted for any additions of NaOH or HCl to the media. The dissociation constants were calculated using algorithms based on Plath et al. (1980).

***Growth rate experiments:*** Phytoplankton growth rates were determined from the rate of increase of *in vivo* fluorescence in 6 different experiments (Table 1). In all experiments, measurements were taken at approximately 6 h intervals during the 40 to 48 h incubation period. Growth rate was calculated from a linear regression of the log of the daily fluorescence readings versus time in days. The slope of the regression line, which represents growth rate as $\text{logarithm}_{10}$ units of increase per day, was multiplied by 3.322 in order to obtain the number of divisions per day (Guillard 1973).

In 4 of the experiments, growth rates of *Thalassiosira pseudonana* were measured in cultures where the pH had been adjusted using either the bubbling technique or titration of acid or base, as previously described (Table 1). Two experiments were conducted to measure growth rates of *T. oceanica* in which either one of the 2 pH adjustment procedures was used. In bubbled experiments, there were 3 replicate bottles for each pH level and 2 replicates in each pH treatment in the titrated experiments.

**Photosynthetic response of mesocosm assemblages.** The response to pH of natural phytoplankton assemblages was measured using $^{14}C$ uptake as a relative measure of the photosynthetic rate of the phytoplankton under saturating irradiance. Samples for these experiments were taken from mesocosms at the Marine Ecosystem Research Laboratory (MERL) in Narragansett, Rhode Island, USA, during a eutrophication experiment in 1985. The experimental mesocosm tanks held 13 $m^3$ of Narragansett Bay water overlying sediment taken from the Bay. Water was pumped into the tanks from the Bay at a rate sufficient to give a 27 d turnover. The tanks were mixed for 4 h out of every 6 h period (Lambert & Oviatt 1983).

Five experiments were conducted to measure $^{14}C$ uptake of phytoplankton from the mesocosms (Table 2). Four of the experiments were conducted on nutrient-enriched tanks (2 each from Tank 4 and Tank 8). Both nutrient-enriched tanks were enriched with nitrogen, phosphorus, and silica at levels 4 times the loading of sewage and runoff in Narragansett Bay (Nixon et al. 1983) and contained a phytoplankton assemblage comprised of large diatoms, flagellates, and an abundance of *Phaeocystis* sp. In the fifth experiment, phytoplankton from a control tank (Tank 6) was used. This tank received no nutrient enrichment and contained a phytoplankton community dominated by diatoms. These tanks were chosen in order to obtain a range of ambient pH levels from which phytoplankton assemblages were taken (> pH 8.8 in the nutrient-enriched tanks and pH 8.2 in the control tank).

***pH adjustment:*** A surface water sample was collected from an experimental tank 1 h prior to the beginning of an experiment. Temperature, pH and fluorescence were measured immediately. A pH titration of the sample was conducted using dilute HCl and NaOH in order to determine the appropriate volumes needed to adjust pH in the experimental treatments. A 4 l volume of tank water was then collected from the surface for the actual experiment and 250 ml

Table 2. Experimental conditions for carbon uptake experiments using MERL tank phytoplankton assemblages

| Tank (treatment) | Date | pH range | Tank pH | No. of pH treatments | Acclimation time (h) |
|---|---|---|---|---|---|
| Tank 4 (4×) | 12 Apr 1985 | 7.61 – 9.28 | 9.05 | 8 | 5 |
| | 13 Apr 1985 | 7.33 – 9.51 | 8.93 | 9 | 1 |
| Tank 8 (4×) | 17 Apr 1985 | 7.25 – 9.44 | 8.82 | 8 | 1 |
| | 18 Apr 1985 | 7.25 – 10.09 | 8.94 | 12 | 1 |
| Tank 6 (1×) | 22 Apr 1985 | 7.28 – 9.14 | 8.21 | 12 | 1 |

aliquots poured into acid-cleaned polycarbonate bottles. The predetermined volumes of dilute HCl and NaOH were added to the bottles to adjust pH to the desired treatment levels (Table 2). The bottles were then placed in an illuminated water bath which was set for continuous light (100 µE $m^{-2}$ $s^{-1}$) at the same temperature as the MERL tanks in order to acclimate the phytoplankton cells to the new pH levels. Two of the experiments conducted on phytoplankton from Tank 4, a nutrient-enriched tank, examined the effect of acclimation time (1 h or 5 h) on the pH response (Table 2). The 3 other experiments were acclimated for 1 h. During the acclimation period, each bottle was removed briefly and weighed in order to determine the gravimetric volume.

***Carbon uptake experiments:*** Carbon uptake was measured in the 5 separate experiments using $NaH^{14}CO_3$. After pH adjustment and acclimation of the natural phytoplankton assemblages, the pH treatment samples were poured into replicate acid-cleaned, 85 ml polycarbonate centrifuge tubes, and 100 µl of 10 µCi $ml^{-1}$ $NaH^{14}CO_3$ solution was inoculated into each tube. The changes in alkalinity and pH due to the labeled bicarbonate addition were 0.0067 meq. $l^{-1}$ and at most 0.05 pH units at the lowest pH levels. Experimental tubes were inserted into a rack and placed into the illuminated water bath (100 µE $m^{-2}$ $s^{-1}$) for approximately 3 h. The actual activity added to each tube was determined separately by measuring the activity of 100 µl aliquots of the stock $NaH^{14}CO_3$ solution. Initial pH and fluorescence of the experimental treatments were measured using the remaining unlabeled sample from each 250 ml bottle.

After the 3 h incubation, the $^{14}C$ labeled treatments were filtered immediately using 25 mm A/E glass fiber filters at 125 mm Hg maximum vacuum. The filters were then transferred to glass scintillation mini-vials and 3 ml of scintillation fluid were added. All samples were shaken continuously for 8 h to allow the cocktail to fully saturate the filter and to allow any degassing of residual inorganic $^{14}C$. The vials were then counted in a Beckman LS 3801 scintillation counter. Counts per minute were converted to disintegrations per minute using a third order polynomial regression (least-squares cubic fit) to relate sample quench to counting efficiency. Sample quench was determined using a cesium 137 external standard. Counting efficiency was about 92% for all experiments (Lambert & Oviatt 1983).

***Calculation of carbon uptake:*** Radiocarbon activities were converted into carbon uptake rates (mg C $m^{-3}$ $h^{-1}$) using the following equation (Strickland & Parsons 1965):

$$U = \frac{R \times W \times 1.05}{A \times T}$$

where $U$ = uptake (mg C $m^{-3}$ $h^{-1}$); $R$ = activity counted for each sample (DPM); $A$ = activity added to each sample (DPM); $W$ = weight of carbonate carbon (mg C $m^{-3}$); $T$ = incubation time (h); 1.05 = isotope discrimination factor ($^{14}CO_2$ vs $^{12}CO_2$).

The available carbon concentration ($W$) of the sample water in each pH treatment was calculated from the following equation:

$$W = \text{mg C m}^{-3} = 12000\,(\text{TA} - \text{BA})\,F_t$$

Total alkalinity (TA) of the initial tank water sample was determined using the method of Culberson et al. (1970). Any changes due to the addition of an acid or base were then calculated by adding (for a base) or subtracting (for acid) the number of milliequivalents of acid or base added to the sample. Borate alkalinity (BA) was calculated as a function of the sample chlorinity, temperature, and pH using the dissociation constant for boric acid (Lyman 1956). Carbonate alkalinity (CA) was determined from the difference of the two (TA – BA = CA). $F_t$, which is a conversion factor to derive total $CO_2$ from carbonate alkalinity, was calculated by using the $a_H$ and the first and second dissociation constants for carbonic acid (Plath et al. 1980).

## RESULTS

### Growth rate response of phytoplankton cultures

Both *Thalassiosira pseudonana* and *T. oceanica* showed consistent decreases in growth rates at higher pH levels (Fig. 1a, b) and lower [$CO_2$] and [$HCO_3^-$] (Table 3). In the 3 experiments where pH levels were adjusted by bubbling, both *T. pseudonana* and *T. oceanica* grew at maximal rates at all pH treatments up to pH 8.8 but growth rates began to decrease at levels above pH 8.8 (Table 3). Both phytoplankton species also showed similar trends in experiments in which pH was adjusted by acid and base titrations. Since the growth rates of both phytoplankton species in the bubbled experiments (in which alkalinity was unchanged) responded similarly to the titrated experiments in which alkalinity was altered, we concluded that the response to high pH was attributed to the pH level rather than alkalinity (Table 3).

In order to determine whether the pH response was related to carbonate chemistry, growth rates measured at the different pH levels for each phytoplankton species were plotted against the calculated $HCO_3^-$ concentrations (Fig. 2a, b) and $CO_2$ concentrations (Fig. 3a, b). The growth rate and external substrate concentration relationships for *Thalassiosira pseudonana* and *T. oceanica* were calculated by fitting the

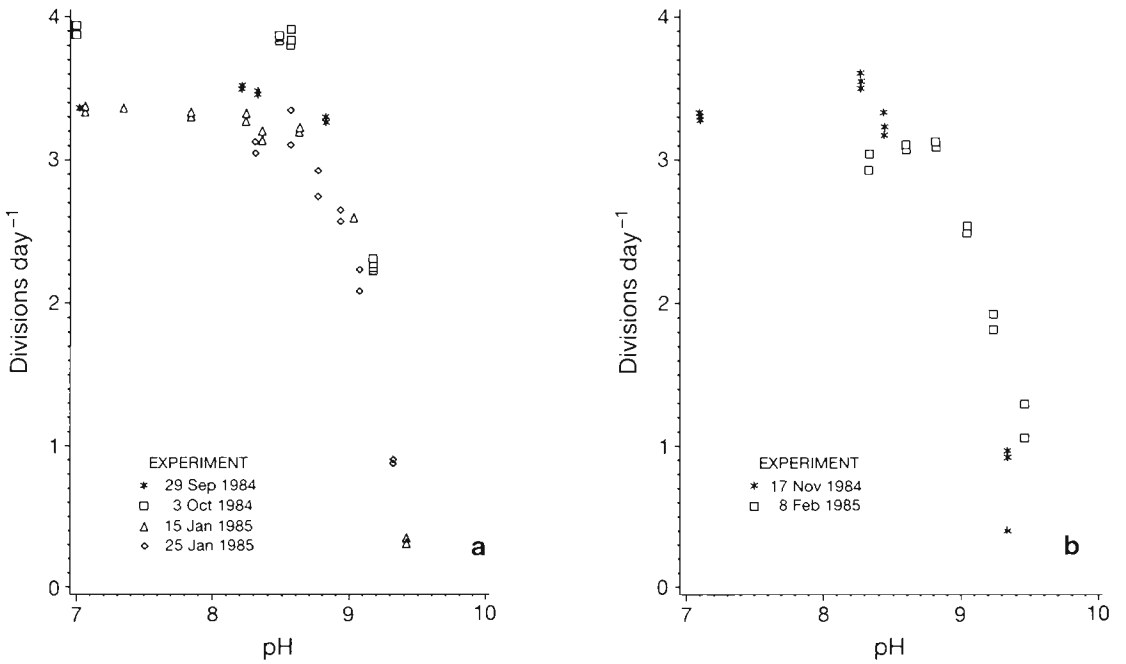


Fig. 1. (a) *Thalassiosira pseudonana*; (b) *T. oceanica*. Growth rate vs pH. Growth rates calculated from fluorescence measured over 40 h

predicted curves to a model using the Monod equation as follows:

$$\mu = \mu_{max} S/(K_\mu + S)$$

where $\mu$ = specific rate of growth in divisions $d^{-1}$; $\mu_{max}$ = maximum growth rate; $S$ = concentration of substrate ($CO_2$ or $HCO_3^-$); $K_\mu$ = half-saturation constant for growth.

From the $HCO_3^-$ relationship to growth rate, the estimated value of $\mu_{max}$ for *Thalassiosira pseudonana* was 5.6 divisions $d^{-1}$ with a $K_\mu$ of 999 µM $HCO_3^-$. For *T. oceanica*, $\mu_{max}$ was 6.8 divisions $d^{-1}$ and the $K_\mu$ was 2146 µM $HCO_3^-$. The calculated $\mu_{max}$ values determined by this $HCO_3^-$ model were approximately 2 times any growth rates observed in these experiments. The relationship of growth rate to [$CO_2$] for *T. pseudonana* gave estimated values for $\mu_{max}$ and $K_\mu$ of 3.63 divisions $d^{-1}$ and 0.45 µM $CO_2$, respectively (Fig. 3a). Values for *T. oceanica* were $K_\mu$ = 0.49 µM and $\mu_{max}$ = 3.38 divisions $d^{-1}$ (Fig. 3b).

### Photosynthetic response of mesocosm assemblages

The photosynthetic response of the natural phytoplankton assemblages to pH showed a consistent pattern in all 5 experiments. Results of each experiment were expressed as a proportion of the maximum carbon uptake and all 5 experiments were combined in a single figure (Fig. 4). At the lower range of pH treatments (pH 7.2 to 7.8) uptake rates increased from 70 to 80% of maximum to the maximum (100%) at pH 7.9 to 8.2. Above the optimum pH, the uptake response decreased to approximately 10% of maximum at pH 9.4. There was no effect of different acclimation times (5 h and 1 h, respectively) on the manner in which the relative rates of photosynthesis changed with pH. The pH values at which maximum uptake was observed were both at approximately pH 8. Only the magnitude of carbon uptake differed between the 2 acclimation experiments: $^{14}C$ uptake was higher for phytoplankton acclimated for only 1 h than for assemblages acclimated for 5 h. Similarly, there was no difference in the pattern of responses between the nutrient-enriched and control tanks. The difference in magnitude of carbon uptake (greater uptake in the nutrient-enriched tank) was most likely due to the differences in cell densities as well as species composition in these tanks.

Carbon uptake showed a linear relationship to bicarbonate concentration in all the experiments (Fig. 5). In contrast, there was a curvilinear relationship between carbon uptake and $CO_2$ concentration (Fig. 6). These data were fitted to the Michaelis-Menten equation for enzyme kinetics to determine the relationship between substrate concentration and photosynthesis rate. The Michaelis-Menten and Monod equations are mathematically identical and, under steady-state conditions, specific uptake rates can be equated with specific growth rate (Kilham & Hecky 1988). They have been

Table 3. *Thalassiosira pseudonana* and *T oceanica*. Growth rates based on fluorescence measured for each pH treatment and calculated concentrations of available inorganic carbon

| pH adjustment | pH | Slope ($log_{10}$Fluor. $d^{-1}$) | Growth rate (div. $d^{-1}$) | $[CO_2]$ (µM) | $[HCO_3^-]$ (mM) | TA (meq. $l^{-1}$) |
|---|---|---|---|---|---|---|
| ***T. pseudonana*** | | | | | | |
| Bubbling | 7.03 | 1.01 ± 0.001 | 3.4 | 170 | 1.60 | 1.62 |
| | 8.23 | 1.06 ± 0.004 | 3.5 | 8.8 | 1.32 | 1.62 |
| | 8.34[a] | 1.04 ± 0.006 | 3.5 | 6.3 | 1.22 | 1.59 |
| | 8.83 | 0.98 ± 0.006 | 3.3 | 1.4 | 0.84 | 1.62 |
| Bubbling | 7.01 | 1.18 ± 0.01 | 3.9 | 182 | 1.63 | 1.65 |
| | 8.50[a] | 1.16 ± 0.02 | 3.8 | 4.03 | 1.11 | 1.59 |
| | 8.58 | 1.16 ± 0.02 | 3.8 | 3.28 | 1.09 | 1.65 |
| | 9.18 | 0.68 ± 0.01 | 2.3 | 0.40 | 0.54 | 1.65 |
| Titration | 7.07 | 1.01 ± 0.008 | 3.4 | 174 | 1.80 | 1.83 |
| | 7.35 | 1.01 ± 0.001 | 3.4 | 95.0 | 1.89 | 1.95 |
| | 7.86 | 1.00 ± 0.005 | 3.3 | 30.1 | 1.90 | 2.10 |
| | 8.26 | 0.99 ± 0.01 | 3.3 | 11.5 | 1.82 | 2.27 |
| | 8.37[a] | 0.95 ± 0.01 | 3.2 | 8.53 | 1.77 | 2.34 |
| | 8.64 | 0.97 ± 0.006 | 3.2 | 3.89 | 1.48 | 2.36 |
| | 9.04 | 0.78 ± 0.001 | 2.6 | 1.01 | 0.97 | 2.39 |
| | 9.42 | 0.10 ± 0.009 | 0.32 | 0.24 | 0.54 | 2.44 |
| Titration | 8.32[a] | 0.93 ± 0.02 | 3.1 | 9.78 | 1.82 | 2.34 |
| | 8.58 | 0.97 ± 0.05 | 3.2 | 4.70 | 1.56 | 2.36 |
| | 8.77 | 0.85 ± 0.04 | 2.8 | 2.51 | 1.31 | 2.37 |
| | 8.94 | 0.74 ± 0.02 | 2.6 | 1.42 | 1.10 | 2.39 |
| | 9.08 | 0.65 ± 0.03 | 2.2 | 0.86 | 0.91 | 2.41 |
| | 9.33 | 0.26 ± 0.006 | 0.88 | 0.34 | 0.63 | 2.44 |
| ***T. oceanica*** | | | | | | |
| Bubbling | 7.10 | 0.97 ± 0.007 | 3.2 | 217 | 2.38 | 2.42 |
| | 8.28 | 1.04 ± 0.01 | 3.5 | 11.4 | 1.92 | 2.42 |
| | 8.45 | 0.95 ± 0.02 | 3.1 | 6.73 | 1.68 | 2.34 |
| | 9.36 | 0.22 ± 0.09 | 0.72 | 0.30 | 0.60 | 2.42 |
| Titration | 8.35[a] | 0.87 ± 0.02 | 2.9 | 9.19 | 1.79 | 2.34 |
| | 8.62 | 0.90 ± 0.007 | 3.0 | 4.11 | 1.50 | 2.36 |
| | 8.85 | 0.91 ± 0.004 | 3.0 | 1.95 | 1.21 | 2.37 |
| | 9.07 | 0.74 ± 0.01 | 2.4 | 0.89 | 0.92 | 2.39 |
| | 9.26 | 0.55 ± 0.02 | 1.8 | 0.44 | 0.70 | 2.41 |
| | 9.48 | 0.34 ± 0.05 | 1.1 | 0.18 | 0.49 | 2.44 |

[a]Control treatments

used interchangeably throughout the literature and are assumed to be the same in this study also. The $V_{max}$ and $K_s$ values were calculated for carbon uptake as a function of $CO_2$ (Table 4). $K_s$ values ranged between 0.86 and 2.41 µM with a mean of 1.33 ± 0.65 µM.

A lack of fit test was conducted to determine any lack of fit of the experimental data with either $CO_2$ or $HCO_3^-$ models (Neter et al. 1983). P-values were calculated for the linear regression relationship of photosynthetic rate to bicarbonate and for the Michaelis-Menten calculation for the photosynthetic rate/carbon dioxide relationship (Table 5). With the linear model, one of the nutrient-enriched tank experiments (17 April 1985) and the control tank experiment (22 April 1985) showed a significant lack of fit. With the Michaelis-Menten model, 3 experiments showed a significant lack of fit (Table 5). The reason for this lack of fit may have been a decrease in carbon uptake rates which occurred at lower pH values (Fig. 4). Since carbon uptake was not likely to have been $CO_2$ limited at these low pH levels, these data were omitted and the Michaelis-Menten equation fitted to the revised data sets. New $V_{max}$ and $K_s$ values were determined (Table 4) and the data were analyzed again with a lack of fit test (Table 5). With the the lowest pH treatments removed from the data analysis, there was still a sig-

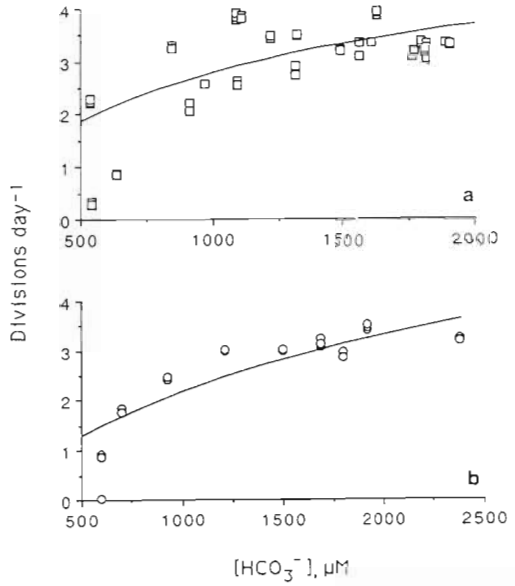


Fig. 2. (a) *Thalassiosira pseudonana*; (b) *T. oceanica*. Growth rate vs $[HCO_3^-]$. Solid line depicts the predicted Monod response curve

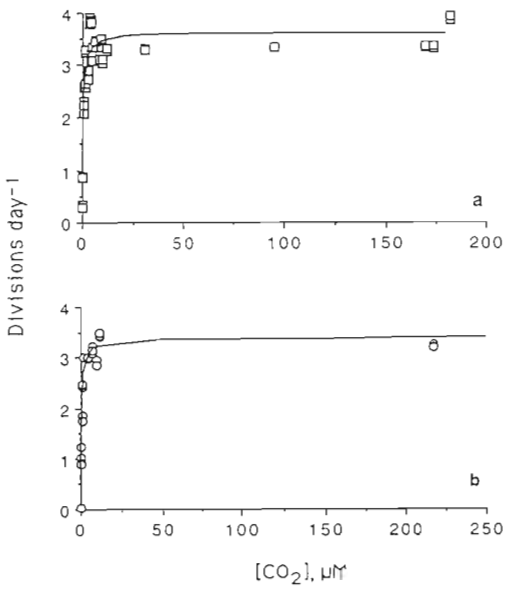


Fig. 3. (a) *Thalassiosira pseudonana*; (b) *T. oceanica*. Growth rate vs $[CO_2]$. Solid line depicts the predicted Monod response curve

nificant lack of fit for one of the nutrient-enriched tank experiments (17 April 1985) and the control tank experiment. Given the reasonably good fit of the model to the data (Fig. 6), the continued statistical lack of fit is due more to the limitations of the test than to the appropriateness of the curvilinear function. It is clear from the plots of the carbon uptake response against

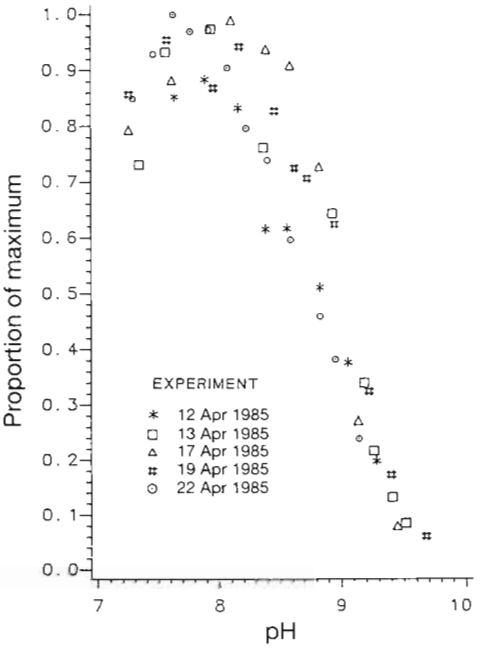


Fig. 4. Photosynthetic rate expressed as proportion of maximum carbon uptake vs pH for carbon uptake experiments using phytoplankton assemblages from marine mesocosms

$[CO_2]$ that the Michaelis-Menten model accounts for the major features of the photosynthetic response particularly at the lower levels of $CO_2$. The lack of fit is actually due to the very small variance at each $CO_2$ level and the resulting deviations from the curvilinear function because the test compares the data by taking mean values for each level rather than averaging over the entire curve.

## DISCUSSION

In the past, pH has not been considered to be an important chemical parameter influencing biotic processes in marine environments. However, a number of studies have shown that pH and, in some cases, inorganic carbon may be important in regulating the growth rate and distribution of marine algae (Yoo 1991, Hinga 1992, Riebesell et al. 1993). The results of this study indicate a limitation of phytoplankton growth and photosynthesis at elevated pH levels. Both *Thalassiosira pseudonana* and *T. oceanica* exhibited a decline in growth above pH 8.8 and minimal growth at pH 9.0. In a similar response, natural populations taken from nutrient-enriched tanks showed diminished carbon uptake at elevated pH levels. When pH was adjusted from ambient levels (8.8 to 8.9) to lower levels (pH 8.0 to 8.3), carbon uptake rates of the natural assemblages were enhanced.

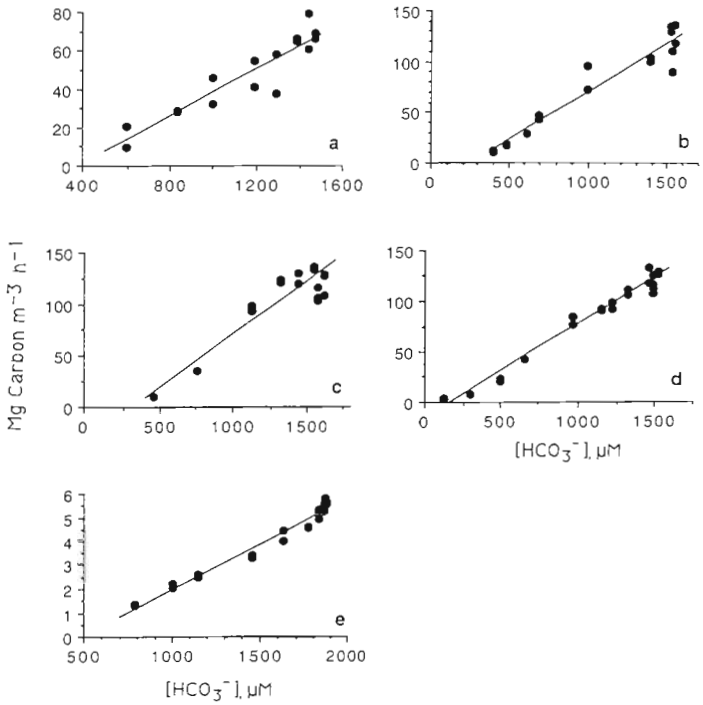


Fig. 5. Carbon uptake rate vs $[HCO_3^-]$ plotted with linear regression (solid line) for carbon uptake experiments using phytoplankton assemblages from marine mesocosms. Experiment dates: (a) 12 April 1985, (b) 13 April 1985, (c) 17 April 1985, (d) 19 April 1985, and (e) 22 April 1985

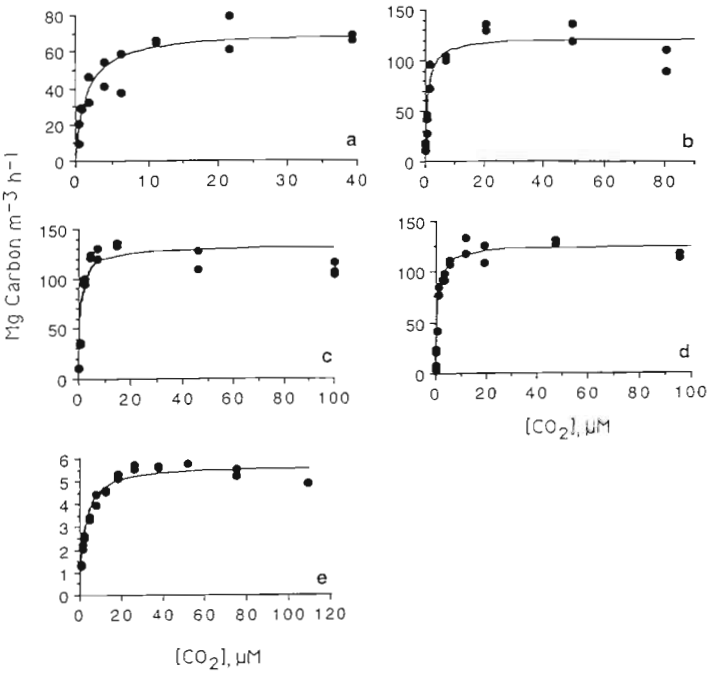


Fig. 6. Carbon uptake vs $[CO_2]$ and predicted Michaelis-Menten response curve (solid line) vs pH for carbon uptake experiments using phytoplankton assemblages from marine mesocosms. Experiment dates: (a) 12 April 1985, (b) 13 April 1985, (c) 17 April 1985, (d) 19 April 1985, and (e) 22 April 1985

Goldman's (1976) study of marine phytoplankton in wastewater documents a decline in growth of *Thalassiosira pseudonana* and *Dunaliella tertiolecta* at pH values approaching pH 9 even when excess nutrients were present. *Phaeodactylum tricornutum*, on the other hand, grew at pH values greater than pH 10. Pruder & Bolton (1979) also observed a decline of growth in *T. pseudonana* as pH approached 8.8. A decline of growth rate with increasing age of culture and pH was observed for the marine diatom *Cylindrotheca closterium* (Humphrey & Subba Rao 1967) even though nitrate and phosphate were both available. The authors hypothesized a carbon deficiency. Kain & Fogg (1958) observed a similar significant inhibition of growth above pH 8.75 for *Isochrysis galbana* and above pH 8.5 for *Asterionella japonica* Gran. These data suggest a general pattern of a limitation of growth at high pH, although there may be species-specific responses.

The decline of both growth rate and photosynthesis at high pH in the present study may have been caused by a number of different mechanisms including trace metal toxicity or limitation, reduced nutrient availability, or changes in the availability of carbon substrate. Trace metal toxicity or nutrient availability are unlikely to have been responsible for the decline in growth rate and carbon uptake at high pH for a number of reasons. First, differences have been observed in the nutrient requirements and toxicity responses of *Thalassiosira pseudonana* and *T. oceanica*. Brand et al. (1983) and Murphy et al. (1984) have observed that iron, manganese, and zinc requirements of the 2 species differ by several orders of magnitude. Similar differences have been observed in their copper tolerances (Gavis et al. 1981). Thus, *T. pseudonana* would have required higher concentrations of free iron and tolerated higher concentrations of free copper than *T. oceanica*. If trace metals were influencing growth rate, the change in metal speciation and the free ion concentrations over the pH range studied would have resulted in different responses between the 2 phytoplankton species. In the present study, both species responded to pH and presumably metal concentrations in a similar fashion.

Limitation of nitrogen, phosphorus, or silica was also unlikely to cause the observed pH response in this study because these were all in considerable excess in the experiments. Concentrations in the growth media for the 2 phytoplankton clones were high enough to sustain

Table 4. Predicted parameters for carbon uptake (mg C $m^{-3}$ $h^{-1}$) from Michaelis-Menten models based on calculated concentrations of $CO_2$ (μM)

| Experiment date | $R^2$ | $V_{max}$ (mg C $m^{-3}$ $h^{-1}$) | $K_s$ (μM) |
|---|---|---|---|
| 12 Apr 1985 | 0.98 | 70 | 1.5 |
| 13 Apr 1985 | 0.98 | 122 | 0.93 |
| 17 Apr 1985 | 0.98 | 132 | 0.95 |
| | 0.99[a] | 141[a] | 1.2[a] |
| 18 Apr 1985 | 1.00 | 125 | 0.86 |
| | 1.00[a] | 128[a] | 0.93[a] |
| 22 Apr 1985 (control) | 1.00 | 5.7 | 2.4 |
| | 1.00[b] | 5.6[b] | 2.4[b] |

[a]Recalculated values after omission of lowest pH data point
[b]Recalculated values after omission of 5 lowest pH data points

exponential growth for periods much longer than the 40 h incubation time. Assemblages used in the carbon uptake experiments were taken from mesocosm tanks which were enriched with $NH_4$, $PO_4$, and $SiO_4$ at 4 times the nutrient loading into Narragansett Bay. Meanwhile, the control tank assemblage showed a similar pH response to the nutrient-enriched tanks indicating that nutrient availability was not determining the pH response.

A number of studies have implicated inorganic carbon limitation of growth as the main mechanism in phytoplankton response to pH. In most of these studies, molecular $CO_2$ is considered the major species of inorganic carbon utilized in photosynthesis (Blackman & Smith 1911, Rabinowitch 1945, Riebesell et al. 1993). It has been shown by Cooper (1969) that $CO_2$ is the carbon compound within a cell that is critical to photosynthesis in binding with the enzyme ribulose bisphosphate carboxylase. Although relatively few studies have been conducted to investigate the specific forms of carbon substrate used by marine plants, evidence for $CO_2$ utilization by marine algae has been documented. Isotopic studies using $\delta^3C$ have identified $CO_2$ as the carbon source for diatoms (Degens et al. 1968, Deuser 1970) and have shown $HCO_3^-$ to be utilized by coccolithophores (Falkowski 1991). Pruder & Bolton (1979) also studied *Thalassiosira pseudonana* and attributed the reduction in growth rate at high pH to a combination of pH effect and low $CO_2$ levels. Algal growth rates were reduced in media bubbled with $CO_2$ free air but not changed at elevated $CO_2$ levels. More recently, Riebesell et al. (1993) also measured growth rates and DIC uptake in 3 species of diatoms and found them to be limited by the supply of $CO_2$. Some species of marine phytoplankton also appear capable of concentrating the supply of $CO_2$ within cells to levels higher than the ambient concentration allowing cells grown at low concentrations to be more efficient at utilizing inorganic carbon from the medium (Zenvirth & Kaplan 1981, Badger & Andrews 1982, Burns & Beardall 1987, Raven & Johnston 1991).

The results in this present study concur with those of Riebesell et al. (1993) and other studies using diatoms to support the hypothesis that $CO_2$ limitation was the cause of the pH effect on growth and photosynthesis. In both sets of experiments, the relationship of growth and photosynthesis, and $[CO_2]$ were similar to Monod

Table 5. *F*-test for lack of fit of linear regression function (mg C $m^{-3}$ $h^{-1}$ vs $[HCO_3^-]$) and Michaelis-Menten model (mg C $m^{-3}$ $h^{-1}$ vs $[CO_2]$)

| Date | Linear regression SSLF[a] | MSPE[b] | p-value | Michaelis-Menten SSLF[a] | MSPE[b] | p-value |
|---|---|---|---|---|---|---|
| 12 Apr 1985 | 107 (6) | 634 (8) | 0.9541 | 891 (6) | 634 (8) | 0.202 |
| 13 Apr 1985 | 956 (7) | 699 (9) | 0.2107 | 1043 (7) | 699 (9) | 0.1781 |
| 17 Apr 1985 | 1377 (6) | 400 (16) | 0.0002 | 4832 (6) | 400 (16) | 0.0000 |
| | 822 (5)[c] | 313 (14)[c] | 0.0023[c] | 800 (5)[c] | 313 (14)[c] | 0.0007[c] |
| 18 Apr 1985 | 336 (10) | 345 (12) | 0.3925 | 947 (10) | 345 (12) | 0.0276 |
| | 277 (9)[c] | 335 (11)[c] | 0.4854[c] | 715 (9)[c] | 335 (11)[c] | 0.0682[c] |
| 22 Apr 1985 (control) | 0.82 (10) | 0.19 (12) | 0.0049 (10) | 2.26 (12) | 0.19 | 0.0001 |
| | 0.16 (5)[d] | 0.13 (7)[d] | 0.2565[d] | 0.54 (5)[d] | 0.13 (7)[d] | 0.0215[d] |

[a]Lack of fit sum of squares (associated degrees of freedom)
[b]Pure error mean square (associated degrees of freedom)
[c]Recalculated values after omission of lowest pH data point
[d]Recalculated values after omission of 5 lowest pH data points

and Michaelis-Menten curves for substrate-limited growth rate and uptake. The subsequent half-saturation constants for $[CO_2]$ calculated from the 2 data sets were also within the limits of values determined in previous studies. The results of the $^{14}C$ uptake experiments using natural assemblages show $K_s$ values to be between 0.89 and 2.41 µM for nutrient-enriched and control populations. The $K_\mu$ values determined in the growth rate experiments for *Thalassiosira pseudonana* and *T. oceanica* were 0.45 and 0.49 µM, respectively. The difference in values derived from the growth rate and photosynthesis experiments was most likely due to the metabolic functions being measured.

Past studies of inorganic carbon acquisition in marine algae in the literature contain values of half-saturation constants similar to those in this study, suggesting that carbon limitation is not uncommon. Raven & Johnston (1991) compiled data from a number of studies showing that most marine species have $K_s[CO_2]$ values less than 1.7 µM. Burns & Beardall (1987) determined the $K_s[CO_2]$ values for 6 species of marine microalgae by measuring rates of photosynthetic oxygen evolution and found values to range between 0.25 and 1.35 µM $CO_2$. The $K_s[CO_2]$ values for *Stichococcus bacillaris* and *Phaeodactylum tricornutum* have been measured to be 1.5 µM (Munoz & Merrett 1988) and 0.54 µM (Patel & Merrett 1986), respectively. Badger & Andrews (1982) calculated the $K_s[CO_2]$ for *Synechococcus* sp. to be 0.40 µM and Zenvirth & Kaplan (1981) determined a half-saturation value for photosynthesis to be 2.2 µM $CO_2$ at pH 7.5 for *Dunaliella salina*.

While there was a positive relationship between $[HCO_3^-]$ and carbon uptake in this study, there are several reasons why $HCO_3^-$ was not likely to be the cause of the observed growth limitation in these experiments. First, there was no apparent saturation plateau in the $^{14}C$ uptake experiments at levels of $HCO_3^-$ used in this experiment or at levels observed for saturation in other studies. Either the saturation plateau for carbon uptake is at an extremely high level of $HCO_3^-$ as indicated by the growth rate experiments or there is no plateau which would indicate the lack of an enzyme-mediated uptake or an active transport mechanism as described by Lucas (1983) for other aquatic plants. Second, carbon uptake measured in the present study was reduced at $HCO_3^-$ concentrations below about 1 mM. This result contrasts with a number of earlier studies. *Scenedesmus quadricauda*, a freshwater alga, exhibited maximum growth rates at $HCO_3^-$ concentrations as low as 10 µM (Osterlind 1950). In *Sargassum muticum*, which was reported to assimilate $HCO_3^-$ directly, rate of carbon assimilation remained constant while pH shifted from 8.97 to 9.9 and $pCO_2$ fell from 62 to 13 ppm (Thomas & Tregunna 1968). In the present study, carbon uptake rates were consistently lower at these lower levels of $pCO_2$ and higher pH. Finally, Burns & Beardall (1987) found the half-saturation values for $HCO_3^-$ to be approximately 20 µM for 2 species of marine algae, whereas Badger & Andrews (1982) found values to be 42 µM at pH 8 for *Synechococcus* sp. The $K_\mu[HCO_3^-]$ for growth rate in this study was not at all comparable, ranging between 1200 and 2100 µM. If we assume that carbon acquisition in these alga is governed by Michaelis-Menten kinetics, then one would have to conclude that $HCO_3$ was not the limiting factor.

Some algal species have been shown to absorb exogenous $HCO_3^-$ which is dehydrated to $CO_2$ within the cell (Badger et al. 1980, Kaplan et al. 1980, Beardall & Raven 1981). Imamura et al. (1983) found that at pH 8.0, freshwater algal cells grown under ordinary air (low-$CO_2$ cells) utilized $HCO_3^-$ as their major form of inorganic carbon while high-$CO_2$ cells utilized $CO_2$. For the marine cyanobacterium *Synechococcus* sp. both $CO_2$ and $HCO_3^-$ could be used as a substrate but in steady-state photosynthesis in seawater, $HCO_3^-$ uptake into the cell was the primary source of internal inorganic carbon (Badger & Andrews 1982). In contrast, Riebesell et al. (1993) suggested that bicarbonate uptake does not compensate for the $CO_2$ limitation in diatoms and that $CO_2$ could be limiting during intense blooms. In the present study it was also concluded that bicarbonate is not the limiting substrate.

Several studies have investigated the effects of elevated $CO_2$ concentrations on phytoplankton. In freshwater algae, Sorokin (1962, 1964) reported $CO_2$ to have an inhibitory effect on cell division at high concentrations. Steeman Nielsen (1955) described a decrease in photosynthetic rate of *Chlorella pyrenoidosa* with increasing $CO_2$ concentrations and hypothesized that $CO_2$ could act as a toxin at high concentrations in saturating light levels. In marine species, high $CO_2$ concentrations induced growth lags in *Cricosphaera elongata* (Swift & Taylor 1966), a phenomenon not observed in this study for *Thalassiosira pseudonana* or *T. oceanica*. Growth rates of *Skeletonema costatum* and *Isochrysis galbana* (Small et al. 1977) at elevated $CO_2$ levels were dependent on the acclimation of the cells to the new elevated concentrations. Unacclimated cells exhibited clumping and less than optimal growth rates.

In the *in vivo* fluorescence experiments conducted in this study, growth rates for *Thalassiosira pseudonana* and *T. oceanica* showed a similar response to that observed by Pruder & Bolton (1979) where they remained relatively unchanged between pH 7.0 and 8.8. However, in the carbon uptake experiments, a consistent decline in uptake rate was shown at the lowest pH levels (< pH 7.5) where the highest $CO_2$ con-

centrations were found. The response of the natural assemblages of phytoplankton in the present study to low pH and high $CO_2$ could not be interpreted since potential effects of $CO_2$ and pH could not be separated. While the reduction in photosynthesis may have been due to a toxic effect, other possible causes include a reduction in electrochemical potential of the cell membrane or acclimation effects such as those described by Small et al. (1977). The relatively short acclimation and incubation periods in the present study may have increased the effects of these factors.

Two different approaches for characterizing phytoplankton response to pH used in this study yielded similar results over a broad pH range. Furthermore, consistent trends in response to high pH were observed despite the difference in organisms used, i.e. diatom cultures grown in media versus natural populations from the MERL tanks. Based upon the findings in this study, it is suggested that $CO_2$ was the limiting substrate at levels above pH 8.8. High pH levels which have been observed in nutrient-enriched marine systems may have an inhibitory effect on phytoplankton metabolism via the carbonate system. At high pH levels, the availability of $CO_2$ decreases and may become limiting to photosynthesis and growth of marine phytoplankton. Therefore, pH mediated through $[CO_2]$ may be an important abiotic factor affecting the ecology of marine phytoplankton.

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