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EFFECTS OF WATER CHEMISTRY ON AQUATIC PLANTS: GROWTH AND PHOTOSYNTHESIS OF
MYRIOPHYLLUM SPICATUM L.

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Final Report
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**Title:** Effects of Water Chemistry on Aquatic Plants: Growth and Photosynthesis of Myriophyllum Spicatum L.

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**Abstract:**

Growth of the submersed macrophyte, Myriophyllum spicatum L., was examined in nine solutions differing in major cation (Ca, Mg, Na, and K) and inorganic carbon $C_\text{in}$ levels. Growth experiments were conducted under two conditions differing in the relative magnitudes of gas exchange and solution volume. Under conditions of limited solution volume, growth was adversely affected by the combination of low cation (particularly Ca) and low $C_\text{in}$ levels. However, where solution volume was greater, there was no effect of cations. Irrespective of...
20. ABSTRACT (Continued)

volume, growth was increased by increasing \( C_T \) from 3.5 to 10.5 mg/L, but was unaffected by further increase in \( C_T \) to 21.0 mg/L.

Photosynthetic uptake of \( C_T \) during growth reduced levels of \( C_m \) and acidity and increased pH. Alkalinity and Ca levels were also reduced in some solutions due to the precipitation of \( CaCO_3 \). Diurnal analyses indicated \( C_T \) was not appreciably replenished during the night; thus, the experimental systems continually lost \( C_T \) throughout the study.

Initial photosynthetic rates, measured in the same solutions, indicated a strong relationship between photosynthesis and solution \( C_m \). Photosynthetic rates were adversely affected by the combination of low cation and low \( C_m \) levels. At favorable cation levels, photosynthetic \( C_m \) uptake rates followed Michaelis-Menten kinetics, with a half-saturation constant \( K_m \) of 25 mg C\( \cdot L^{-1} \). Photosynthetic \( C_T \) uptake was relatively unaffected by pH over the range 8.0 to 10.2.

Measurements of the exchange of CO\(_2\) between the atmosphere and the solution during the photosynthesis experiments revealed the potential importance of the atmosphere as a significant source of carbon. As photosynthetic \( C_T \) uptake reduced the concentration of solution \( C_m \), the rate of supply of atmospheric \( CO_2 \) rapidly increased to a near maximal value which was unaffected by the initial solution composition. Atmospheric \( CO_2 \) exchange thus moderates the influence of solution \( C_T \).

Comparisons of growth with measured photosynthetic rates indicated that these measures of plant response to solution composition were not closely related. While photosynthetic \( C_T \) uptake was strongly affected by solution \( C_T \), growth was less affected. In an attempt to resolve this difference, a carbon budget for growth of \( M.\ spicatum \) was constructed. The atmosphere was a major source of carbon in the growth experiments, particularly in the lower \( C_T \) solutions. While the influence of solution \( C_T \) on photosynthesis and growth diminishes over time (due to decreases in solution HCO\(_3\)), the rate of atmospheric \( CO_2 \) exchange increases to a maximal value independent of the initial level of \( C_T \). Thus, as the duration of the experiment increases, the plant response (either photosynthesis or growth) becomes independent of the initial level of \( C_T \). In the short-term photosynthesis experiments photosynthesis was strongly affected by solution \( C_T \); however, in the longer term growth experiments, plant growth was relatively less affected by solution \( C_T \).

In order to determine the potential importance of carbon as a factor limiting the growth of submerged aquatic plants, an additional experiment was conducted to examine the growth response of \( M.\ spicatum \) to the combined effects of varying solution \( C_T \) and atmospheric \( CO_2 \) supply. Augmenting the airstream \( CO_2 \) concentration resulted in increased growth in each of the solutions and also increased the responsiveness of growth to solution \( C_T \). The increased supply of \( CO_2 \) prevented solution \( C_T \) levels from being depleted in spite of the increased photosynthetic carbon uptake associated with the increased growth. By maintaining higher levels of solution \( C_T \), the enhanced \( CO_2 \) supply allowed the plants to photosynthesize at higher rates, thus resulting in an increase in growth in relation to solution \( C_T \).

Growth and distribution of \( M.\ spicatum \) are unlikely to be limited by cation concentrations. While growth of this species may be limited by carbon availability, the degree of limitation will depend on the magnitude of the various sources of carbon supply and not on the level of \( C_T \) at any one time.
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This report should be cited as follows:

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EFFECTS OF WATER CHEMISTRY ON AQUATIC PLANTS: GROWTH
AND PHOTOSYNTHESIS OF *MYRIOPHYLLUM SPICATUM* L.

PART I: INTRODUCTION

**Background**

1. It has long been realized that the chemical composition of the water is of primary importance in affecting the distribution of aquatic plants (West 1905; Iversen 1929, cited in Hutchinson 1975; Moyle 1945). Various investigators have attempted to relate the species distributions of submersed macrophytes to generalized water chemistry parameters such as hardness, alkalinity, conductivity, pH, total dissolved solids, or salinity (see reviews by Moyle 1945; Hutchinson 1975). In this regard, *Myriophyllum spicatum* L. and the closely related *Myriophyllum exalbescens* Fern. have been characterized as typically occurring in waters that are hard (particularly with respect to Ca), moderate to high in alkalinity, neutral to high in pH, high in electrical conductivity, and eutrophic (Moyle 1945; Spence 1967; Hutchinson 1970; Seddon 1972; Kadono 1982). Many exceptions, however, have been noted to these general patterns of occurrence (Hutchinson 1970; Giesy and Tessier 1979; Kadono 1982), and it remains difficult to explain observed distributions on the basis of general water chemistry conditions.

2. As Hutchinson (1970) noted, many water chemistry parameters are interrelated, with pH, Ca concentration, bicarbonate concentration (or alkalinity), and total electrolyte content generally varying in a correlative manner. Although these parameters may act independently in affecting plant distribution, it is difficult to clearly separate their effects through examinations of species distributions in relation to water chemistry alone (Hutchinson 1970; Seddon 1972; Kadono 1982). Another difficulty is that sites differing in water chemistry are likely to differ in other environmental parameters (notably sediment composition) as well (Pearsall 1920; Moyle 1945). For these reasons it is necessary to determine the effects on growth of independently varying important water chemistry parameters under otherwise uniform environmental conditions.

3. Many previous studies on the effects of water chemistry on submersed
macrophyte growth have concentrated on the macronutrients N and P (Mulligan and Baranowski 1969; Ryan, Reimer, and Toth 1972; Mulligan, Baranowski, and Johnson 1976; Nichols and Keeney 1976; Best and Mantai 1978; Patterson and Brown 1979; Barko 1983). Results of these studies indicate that rooted submersed macrophytes are generally capable of acquiring both N and P by either root uptake from sediment or shoot uptake from the water. Consequently, the effects of N and P in the water column are dependent, in part, on levels of these in the sediment. Likewise, the role of solution K as a nutrient for submersed macrophyte growth has also been addressed (Barko 1982; Huebert and Gorham 1983), and there is some evidence that individual species may differ in their requirements for K in the water column. Other studies have considered the effects of salinity on submersed macrophyte growth and distribution (Bourn 1932; Haller, Sutton, and Barlowe 1974; Kadono 1982). These studies have indicated that *M. spicatum* is moderately tolerant of brackish waters.

4. Relatively little attention has been devoted to the effects on growth of other water chemistry parameters such as alkalinity, dissolved inorganic carbon \( C_T \), or major cation concentrations. Huebert and Gorham (1983) demonstrated that *Potamogeton pectinatus* L. required Ca, Mg, and \( \text{HCO}_3^- \) in solution to achieve normal growth. Barko (1983) indicated that low levels of Ca and \( C_T \) in solution depressed growth of *M. spicatum*.

5. While little is known of the effects of \( C_T \), alkalinity, and major cations on growth of submersed macrophytes, there is a considerable amount of information on the effects of these on photosynthesis. Among the many published reports on photosynthesis in relation to water chemistry, the most notable concerned with *M. spicatum* are Steemann Nielsen (1947), Adams, Guilizzoni, and Adams (1978), and Titus and Stone (1982). These authors indicate that *M. spicatum* can use \( \text{HCO}_3^- \) as a carbon source for photosynthesis with uptake following Michaelis-Menten kinetics. Steemann Nielsen (1947) demonstrated that cations, particularly Ca, stimulated photosynthesis in *M. spicatum*. A stimulatory effect of Ca was also observed by Stanley (1970). Photosynthetic uptake of \( \text{HCO}_3^- \) in *M. spicatum* has been shown to be little affected by pH over the range of 8.0 to 10.2 (Steemann Nielsen 1947); however, more recently Titus and Stone (1982) indicated that photosynthesis in this species declines over the range of 7 to 9. In view of the diurnally and seasonally occurring high pH values commonly observed in submersed macrophyte populations (McCracken et al. 1975; Van, Haller, and Bowes 1976; Halstead and
Tash (1982), the effects of pH on photosynthesis should be clearly resolved.

Objective and Scope

6. The objective of this report is to evaluate the effects of major cation and inorganic carbon levels on the growth and photosynthesis of M. spicatum. A secondary objective is to evaluate the relationship between growth and photosynthesis. If these two measures of plant response to water chemistry are closely related, it might be possible to predict potential growth of submersed macrophytes in a system based on photosynthetic responses measured in the laboratory.
7. Growth and photosynthesis experiments were conducted using a 3 by 3 factorial design with three levels of major cations (Na, K, Ca, Mg) at each of three levels of $C_T$. Major cation concentrations and $C_T$ levels (Table I) were based on analyses of bicarbonate lake waters (Hutchinson 1957) and were selected to cover a broad range in water composition. Concentrations in the high level solution were six times those in the low level solution, with the intermediate (mid) level solution being three times the concentration of the low level solution. Solutions were formulated by additions of reagent grade chemicals to deionized (reverse osmosis) water. Gaseous $CO_2$ was administered to solution prior to adding required amounts of CaCO$_3$ to achieve solubility (Smart and Barko 1985). Concentrations of Ca and $C_T$ in the high level solution were near the upper limit for solutions of this type in equilibrium with atmospheric $pCO_2$.

8. The chemical composition of the experimental solutions was verified by chemical analysis. Initial and final solution samples were collected and analyzed for Na, K, Ca, and Mg by atomic absorption spectrophotometry. Electrical conductivity (25° C) was determined with a YSI Model 32 conductivity meter. Dissolved inorganic carbon was determined with a Beckman Model 915A total organic carbon analyzer. pH was measured with a Beckman Expandomatic IV expanded scale pH meter. Equilibrium calculations are based on equations provided in Stumm and Morgan (1981). Specific experimental details regarding the individual experiments will be presented in subsequent sections.
PART III: GROWTH EXPERIMENTS

Methods

9. Sediment used in the growth experiments was collected from Lake Washington in Washington State. This sediment was selected based on earlier investigations which indicated a high potential for supporting luxuriant submersed macrophyte growth (Barko and Smart 1981, 1983, in press). Physical and chemical characteristics of the sediment were similar to those provided earlier (Barko and Smart 1983, in press). Sediment was stored at room temperature under anaerobic conditions until needed. The sediment was thoroughly mixed with a rotary mixer and allocated to sediment containers a few days prior to experimentation.

10. Apical shoots, 15 cm in length, were taken from greenhouse cultures of *M. spicatum* which had been maintained at 25°C and at light levels similar to those to be used in the experiments. Four apices were randomly selected and allocated to each container. After planting, a 2-cm layer of washed silica sand was placed over the sediment to minimize physical exchanges with the overlying water. Plants were allowed to grow for 6 weeks. At termination of the experiments plant shoots were clipped at the sediment surface, bagged, and dried at 80°C in a forced draft oven to constant weight. Roots were washed over a 1-mm sieve to remove sediment and debris and dried as for shoots. Weights of root and shoot samples were recorded to the nearest milligram. Biomass accrual (final biomass minus initial biomass) was used as a measure of plant growth in all experiments. Root and shoot samples were ground in a Wiley mill to pass a 40 mesh sieve. Subsamples of the dried and ground material were combusted in a muffle furnace at 550°C to determine ash content. Additional subsamples were digested in $\text{H}_2\text{O}_2 - \text{H}_2\text{SO}_4$ (Allen et al. 1974) and analyzed for total Kjeldahl nitrogen (TKN) and total P on a Technicon Autoanalyzer. Major cations (Na, K, Ca, Mg) were determined by atomic absorption spectrophotometry. Data were subjected to one- and two-way analysis of variance procedures as appropriate (Statistical Analysis System, Cary, N. C.).

11. Experiments were conducted under two conditions differing in the relative magnitudes of atmospheric gas exchange (aeration) and solution volume in an attempt to evaluate the relative importance of the atmosphere and
solution as sources of inorganic carbon. Gas exchange rate coefficients $K$ for the two experiments were evaluated by measuring $O_2$ exchange (American Public Health Association (APHA) 1980) between the atmosphere and deoxygenated, deionized water under the appropriate experimental conditions. Dissolved $O_2$ and temperature were measured with a YSI Model 57 dissolved oxygen meter. Mass transfer coefficients $D/z$ and boundary layer thicknesses $z$ were calculated according to Emerson (1975) and Goldman and Dennett (1983) using the diffusion coefficient $D$ for $O_2$ of $8.28 \times 10^{-6} \text{ m}^2 \cdot \text{hr}^{-1}$ (Broecker 1974).

Table 2 provides a summary of the characteristics of the two experiments.

High $D/z$, low solution volume experiment

The high $D/z$, low solution volume experiment was conducted in 150-cm-tall by 15-cm-outside-diameter lucite columns in an environmental chamber. (See Barko and Smart 1980 for additional details.) Air temperature was maintained at 25°C providing water temperatures of 25°C ± 1°C. Light was provided over a 14-hr photoperiod by a combination of Hg vapor and Na vapor lamps. Photosynthetically active radiation (PAR) levels measured at the midpoint of the experimental columns were about 350 $\mu\text{E} \cdot \text{m}^2 \cdot \text{sec}^{-1}$. Gas exchange and mixing were facilitated by administering humidified, compressed air at the bottom of the water column. After 2 weeks, and weekly thereafter, one half the volume of solution was removed and replaced with fresh solution.

Low $D/z$, high solution volume experiment

The low $D/z$, high solution volume experiment was conducted in 150- by 90- by 90-cm-deep, white fiberglass tanks in a greenhouse. Experimental solutions were randomly assigned to tanks in the greenhouse. Solutions were maintained at 25°C ± 1°C with thermostatically controlled liquid circulators (Remcor Corp., Chicago, Ill.). Gas exchange and mixing were facilitated by administering humidified, compressed air through twin air lifts in each tank. Experimental tanks were fitted with lucite covers to prevent the entry of dust and other contaminants. Neutral density shade fabric was used to reduce solar irradiance to PAR levels of about 750 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$, measured at midday.
Growth Responses

High D/z experiment

14. Weekly height measurements obtained during the high D/z, low solution volume experiment indicated no appreciable effect of cation treatments on plant shoot height. However, shoot height was appreciably affected by C_T level (Figure 1). Plants grown in the higher C_T solutions were significantly taller than those grown in the low C_T solution, and this difference in plant height increased throughout the study period.

15. Plant growth responses were analyzed by two-way ANOVA (analysis of variance); however, the occurrence of significant interaction terms (Appendix A, Tables A1, A2) indicated that the effects of cation and C_T treatments were not additive. These interactions preclude the use of the two-way ANOVAs for determining the significance of the main effects due to cation and C_T levels. Therefore, the experiment was re-analyzed as a series of six one-way ANOVAs to determine the effects of cations at each C_T level and the effects of C_T at each cation level (Appendix A, Tables A1, A2). From these analyses it was found that shoot, root, and total biomass accrual increased with increasing cation levels only under low C_T conditions. Total biomass accrual increased with increasing C_T at each of the cation levels (Figure 2). However, consideration of ash-free biomass indicates that the increase in shoot and total biomass accrual between the mid and high C_T levels was due to an increase in ash content of plants grown under the higher C_T and cation levels (Figure 2). Consideration of ash-free biomass thus provides a clearer interpretation of the effects of solution composition on submerged macrophyte growth.

16. Under these experimental conditions, growth of M. spicatum (as ash-free biomass) was responsive to an increase in C_T from 3.5 to 10.5 mg·l^{-1}. Under similar conditions, Barko (1983) noted an increase in biomass (uncorrected for ash content) of the same species by increasing C_T from 2.5 to 5.5 mg·l^{-1} or increasing Ca from 7.5 to 20 mg·l^{-1}. In the present investigation growth increases under low C_T conditions were associated with Ca increases from 10 to 30 mg·l^{-1}; however, since major cations were increased collectively, it is not possible from these data to attribute the increased growth to Ca alone.
Specific cation experiments

17. In order to determine which cations were responsible for the increased growth under low CT conditions, two additional experiments were conducted in which the levels of specific cations were varied independently. General experimental conditions were the same as in the previous high D/z experiment. The cation addition experiment used the low cation, low CT solution as a control and independently added individual cations (Na, K, Ca, Mg) to achieve concentrations equivalent to those in the high cation, low CT solution. Also included, for reference purposes, was a treatment in which all cations were increased to the high level and a treatment in which all cations were held at the low level with CT increased to the high level. The cation reduction experiment used the high cation, low CT solution as a control and independently omitted a portion of each cation (Na, K, Cd, Mg) to achieve the low level for each particular cation.

18. Independent additions of any of the major cations except Na resulted in an increase in total ash-free biomass accrual (Figure 3, graph A). Addition of all cations in combination was no more effective in increasing growth than additions of K, Mg, or Ca alone. However, addition of CT was more effective in increasing growth than addition of cations. Plants grown in low cation, low CT solution exhibited premature senescence after only a few weeks of growth. Addition of K slightly moderated this occurrence while additions of Na or Mg had no effect (Figure 3, graph B). Addition of Ca, either alone or in combination with other cations, completely prevented senescence, as did addition of CT alone.

19. Reduction of K or Ca concentrations from the high level to the low level resulted in a slight reduction in plant growth under low CT conditions (Figure 3, graph C). Reduction in the level of all major cations produced a similar effect. Senescence was not appreciable under high cation conditions; however, reduction in the level of Ca, either alone or in combination with other cations, resulted in a high degree of senescence (Figure 3, graph D).

20. These results indicate that increasing levels of K partially moderate the deleterious effects on growth of low Ca in combination with low CT. However, K, alone or in combination with Na and Mg, cannot substitute for Ca in preventing premature senescence. The importance of Ca in the photosynthetic process of HCO$_3^-$ utilization has been documented (Steemann Nielsen 1947; Lowenhaupt 1956; Stanley 1970; Lucas 1976; Lucas and Dainty 1977), and the
premature senescence noted here may be a symptom of the inability of
*M. spicatum* to efficiently utilize bicarbonate under conditions of low $C_T$
and low Ca. However, the substantial increase in growth due to $C_T$ addition
under low Ca conditions suggests that $\text{HCO}_3^-$ utilization is not impaired under
low Ca conditions. Calcium may also be involved in maintaining membrane
integrity (Poovaiah and Leopold 1976) or in preventing the toxicity of other
ions (Huebert and Gorham 1983); however, these possible explanations for the
stimulatory effect of added Ca under low $C_T$, low Ca conditions do not
explain enhanced growth due to increased $C_T$ under low Ca conditions.

**Low D/z experiment**

21. Plant growth responses in the low D/z, high solution volume
experiment were analyzed by both two-way and one-way ANOVA procedures
(Appendix A, Tables A3, A4). As in the high D/z experiment, the $C_T$ and
cation interactions were significant. From the one-way ANOVAs it was found
that both shoot and total biomass accrual increased with increasing cation
levels only under higher $C_T$ conditions. Thus, the stimulatory effect of
increasing cation levels under low $C_T$ conditions noted in the high D/z
experiment was not observed in the low D/z experiment. Total biomass
accrual increased with increasing $C_T$ at each of the cation levels (Figure 4); however, the increase in total biomass between the mid and high $C_T$
solutions was entirely due to an increase in the ash content of plants grown
under higher levels of cations. Ash-free biomass accrual, which is a better
measure of plant response, was relatively unaffected by cation levels and
increased with increasing $C_T$ only up to the mid level.

**Experimental comparison**

22. The effect of cations on growth of *M. spicatum* at low $C_T$ in the
high D/z experiments was not observed in the low D/z experiment. Like-
wise, there was no evidence of premature senescence in the low D/z experi-
ment. These differences in results were likely due to the large differences
in solution volume between the two experiments (Table 2). In the low solution
volume experiment (high D/z), levels of both Ca and $C_T$ may have been
depleted due to the demands of a relatively high mass of plant tissue in rela-
tion to the relatively low volume of solution. This depletion may have been
particularly acute in the low Ca, low $C_T$ solution. While the weekly
replacement of one half of the solution volume in the high D/z experiment
should have ensured a steady supply of these elements, it is possible that
levels were occasionally depleted. However, analysis of the solutions at the end of the experiments (data not presented) indicated that, while \( C_T \) levels were depleted in the low \( C_T \) solutions in the high \( D/z \) experiment, Ca levels were not. The depletion of \( C_T \) in the low \( C_T \) solutions was less severe in the high solution volume (low \( D/z \)) experiment. Thus, plants growing in the low \( C_T \) solutions in the high \( D/z \) experiment may have been subject to more stressful conditions with regard to low levels of \( C_T \). The possible implications of this stress will be considered in a subsequent section.

23. As in the high \( D/z \) experiment, biomass accrual was significantly higher in the mid and high level \( C_T \) treatments than in the low level \( C_T \) treatment. In both experiments growth of \( M. \) spicatum was proportional to solution \( C_T \) only up to a level of 10.5 mg·L⁻¹.

Diurnal Metabolism

24. After 5 weeks of growth in each experiment, water samples were taken every 2 hr during the photoperiod and at the end of the following dark period. These samples were analyzed for \( C_T \) and pH as described earlier to provide estimates of diurnal uptake, precipitation, and recharge of the carbonate system. In addition, samples of the aerating gas were collected at midday to determine the efficiency of \( CO_2 \) exchange between the airstream and the experimental solutions.

High \( D/z \) experiment

25. Diurnal changes in pH and \( C_T \) in the high \( D/z \), low solution volume experiment are presented in Figure 5. Maximal pH levels were higher in the mid and high level \( C_T \) solutions than in the low \( C_T \) solution (Figure 5). The high pH levels reported here (10 to 10.8) are similar to those measured in some natural submerged macrophyte populations (Van, Haller, and Bowes 1976; Halstead and Tash 1982). The attainment of these high values is somewhat surprising considering the high gas exchange coefficient measured in this experiment (Table 2). Another significant feature of these diurnal pH curves is that pH decreases during the dark phase were slight except under low \( C_T \) conditions. This indicates that, in spite of the high gas exchange rate, equilibration of the carbonate system was not attained during the night. High cation solutions generally exhibited a greater decrease in pH during the dark phase suggesting that cations may be involved in the regeneration of the
carbonate system. Examination of diurnal $C_T$ levels (Figure 5) indicates that this is only partially correct. Regeneration of the carbonate system was greater in the higher $C_T$, higher alkalinity solutions. All solutions exhibited increased pH and decreased $C_T$ relative to initial conditions in spite of the weekly replacement of half of the solution.

26. Diurnal changes in alkalinity and acidity, calculated from pH and $C_T$, are presented in Figure 6. Alkalinity was reduced relative to initial conditions, particularly in the solutions containing higher levels of $C_T$ and cations, indicating probable precipitation of CaCO$_3$ during the experiment (Appendix B). However, alkalinity changed little over the diurnal cycle, indicating that diurnal changes in $C_T$ were largely due to photosynthetic uptake of $H_2CO_3$ or HCO$_3^-$. Photosynthetic $C_T$ uptake and regeneration of $C_T$ are reflected in the rather large diurnal changes in acidity (Figure 6).

27. Measured CO$_2$ concentrations at midday in the effluent airstreams from each of the experimental solutions varied only slightly among the different solutions. The transfer of airstream CO$_2$ in this experiment was operating at near the maximum rate, and 86 to 95 percent of the airstream CO$_2$ was removed during passage through the solutions. Thus, in spite of the relatively minor decreases in $C_T$ during the day, photosynthetic carbon uptake was operating at a considerable rate. The rate of carbon uptake was roughly equivalent to the influx of atmospheric CO$_2$.

Low D/z experiment

28. Diurnal changes in pH and $C_T$ in the low D/z, high solution volume experiment are presented in Figure 7. Maximum pH values (8.8 to 9.5) were less than those attained in the high D/z experiment, due in part to the lesser depletion of $C_T$ in the low D/z experiment. As in the high D/z experiment, equilibration of the carbonate system did not occur during the dark phase, and levels of $C_T$ increased only slightly in the low $C_T$ solutions and not at all in the higher $C_T$ solutions. All solutions lost $C_T$ over the 24-hr period.

29. Alkalinity (Figure 8) was reduced relative to initial conditions and decreased over the 24-hr period suggesting that changes in $C_T$ over the diurnal period were partly due to precipitation of CaCO$_3$. Changes in acidity (Figure 8) were similar in magnitude to those in alkalinity, indicating that photosynthetic uptake of $H_2CO_3$ or HCO$_3^-$ and precipitation of CaCO$_3$ were roughly proportional.
30. Measured CO₂ concentrations at midday in the effluent airstreams from each of the experimental solutions varied only slightly, ranging from 140 × 200 μl·l⁻¹. Thus, in contrast with the high D/z experiment where approximately 90 percent of airstream CO₂ was removed during passage through the solutions, airstream CO₂ supply in the low D/z experiment was operating at only ≈ 50-percent efficiency. The difference in the efficiency of CO₂ transfer from the atmosphere into solution was partly due to the differences in the degree of C_T depletion between the two experiments. In the high D/z experiment, C_T depletion was considerably greater than in the low D/z experiment.

31. Results of the diurnal metabolism studies indicate that, in spite of the high mass transfer coefficients and high efficiencies of airstream CO₂ exchange, atmospheric CO₂ supply was inadequate for overnight attainment of equilibrium in the carbonate system. Moreover, C_T levels were considerably reduced relative to the initial conditions, particularly in the high D/z experiment. Reductions in C_T were due to both photosynthetic C_T uptake and CaCO₃ precipitation. Precipitation of CaCO₃ was greater in the solutions containing higher levels of C_T and Ca. As a result of these depletions in C_T, photosynthetic carbon uptake was increasingly dependent on the transfer of atmospheric CO₂ into solution. These observations suggest that growth of M. spicatum in these experiments may have been limited by the availability or supply of carbon.

**Growth Limitation**

32. If biomass accrual was limited by carbon, it is difficult to explain the lack of a growth response in either experiment to increasing concentrations of C_T between the mid (10.5 mg·l⁻¹) and high (21.0 mg·l⁻¹) levels. One possible explanation is that photosynthesis (and growth) was impaired by the higher pH levels attained in the higher C_T solutions. However, pH levels were similar in the mid and high level C_T solutions, and in the low D/z experiment there was little difference in pH between the low C_T solutions and some of the higher C_T solutions. In addition these pH levels were considerably lower than those in the high D/z experiment, yet biomass accrual exhibited the same pattern in both experiments.

33. Another possible explanation for the lack of a growth response to
increasing $C_T$ from 10.5 to 21.0 mg·l$^{-1}$ is that growth was limited by some other factor. Space and light limitations can be ruled out on the basis of the lack of visible crowding relative to that observed in prior experiments and in dense cultures grown under similar conditions (Smart and Barko 1985).

34. Nutrient limitation is unlikely on the fertile Lake Washington sediment used in this investigation. Tissue levels (on an ash-free dry weight basis) of P and K in plants grown in the low D/z experiment (see Appendix A, Table A5) were above critical (limiting) concentrations established for $M$. spicatum (Gerloff 1975). Although tissue N levels of plants grown under mid and high $C_T$ conditions were within the critical concentration range, we have twice failed to demonstrate N limitation of plant growth on the sediment used in this investigation (Barko and Smart 1983, and unpublished data). Limitation of growth on this sediment by other sediment nutrients is also unlikely (Smart and Barko 1985, Barko and Smart in press).
PART IV: PHOTOSYNTHESIS EXPERIMENTS

Methods

35. Photosynthetic carbon uptake was measured in each of the experimental solutions using an apparatus that allowed for measuring changes in atmospheric CO$_2$ from an open system airstream as well as changes in solution C$_T$ (Figure 9). This configuration was chosen because it was felt that while aquatic systems are generally considered open with respect to exchange of gases, the environment of littoral submersed macrophyte populations is relatively closed with respect to water exchange over short time periods.

36. The photosynthetic apparatus consisted of a 1,000-ml volumetric hydrometer cylinder fitted with a four-hole rubber stopper. Solution was recirculated at 1 l·min$^{-1}$ with a peristaltic tubing pump by withdrawal from the bottom of the cylinder and delivery to the top of the cylinder. An in-line sample port consisted of a polypropylene "Y" connector fitted with a rubber serum bottle stopper. Samples for C$_T$ analysis were obtained with 1-cc disposable syringes. Compressed atmospheric air, from an outside source, was humidified by passing through a water column, and delivered to a 40-l carboy for mixing and damping short-term changes in atmospheric CO$_2$ concentration. Air was then pumped at 1 l·min$^{-1}$ through a diffuser positioned at the bottom of the cylinder and exited through the top of the cylinder. The effluent airstream then passed through a 500-ml mixing reservoir, fitted with a two-hole rubber stopper containing a sample port fitted with another rubber serum bottle stopper. Gas samples were taken from the influent and effluent airstreams with 3-cc disposable syringes. Photosynthesis cylinders were contained in a water bath to maintain temperature at 25$^\circ$ ± 0.5$^\circ$ C. Three cylinders were operated concurrently by using multichannel pumps and by staggering sample periods by 1 min. Samples were collected every 4 min during the initial 24 min and every 8 min thereafter until changes in C$_T$ and airstream CO$_2$ were minimal (96 to 120 min). Additional water samples were taken at the beginning and end of the experiments for analysis of Ca to determine the magnitude of CaCO$_3$ precipitation. These analyses indicated that precipitation of CaCO$_3$ had occurred in some of the solutions containing higher levels of C$_T$ and Ca, resulting in overestimates of photosynthesis in these solutions. The onset of CaCO$_3$ precipitation was determined by calculating CaCO$_3$ ion activity
products in relation to measured changes in $C_T$. Photosynthetic rates were determined over the change in $C_T$ occurring prior to the onset of CaCO$_3$ precipitation. Photosynthetic rates uncorrected for CaCO$_3$ precipitation are referred to here as "apparent" photosynthetic rates.

**Initial Photosynthesis**

37. Apparent initial photosynthetic $C_T$ uptake rates were calculated from the slope of $C_T$ over time during the initial, linear portion of the relationship. These rates were unaffected by CaCO$_3$ precipitation except in the high Ca, high $C_T$ solution (Table 3). Initial photosynthetic rates in the remaining solutions increased with increasing $C_T$ at all cation levels.

38. Photosynthetic $C_T$ uptake was also affected by cation levels. Under low $C_T$ conditions photosynthetic $C_T$ uptake was greatly diminished at lower cation levels. This result is consistent with the effect of cations on growth of *M. spicatum* in the high D/z and specific cation experiments described earlier. Steemann-Nielsen (1947) likewise demonstrated increased photosynthesis of *M. spicatum* due to cation additions to low $C_T$ solutions. Stanley (1970) also demonstrated that additions of Ca increased photosynthesis in the same species. These results, in conjunction with the results of the high D/z and specific cation experiments, suggest that Ca is involved in photosynthetic uptake at HCO$_3^−$. The nature of this involvement remains unclear, however, as the requirement for Ca decreases with an increase in $C_T$. Possible explanations are that Ca is required for the transport of HCO$_3^−$ across the plasmalemma under conditions of severe HCO$_3^−$ depletion (Lucas and Dainty 1977) or that the precipitation of CaCO$_3$ on the leaf surfaces ameliorates the stress of high pH and excessive CO$_3^{−}$ concentrations at sites of OH$^−$ efflux (Raven 1970).

39. Kinetics of photosynthetic $C_T$ uptake in the experimental solutions are provided in Figure 10. Kinetic constants were determined by linear transformation (Hanes-Woolf plot of $S/v$ vs. $S$) of initial photosynthetic rate ($v$) in relation to solution $C_T$ ($S$) according to Segel (1976). Solutions in which CaCO$_3$ precipitated or which contained unfavorably low cation concentrations were excluded from the analysis. Transformed data were linear ($r^2 = 0.98$) and provided a half-saturation constant $K_m$ of 24.5 mg $C_T$·l$^{-1}$. This estimate of $K_m$ is within the range of published estimates for *M. spicatum*.
(Adams, Guilizzoni, and Adams 1978; Titus and Stone 1982). However, our estimate of $V_{\text{max}}$ of 20.9 mg $C_\text{T} \cdot g^{-1} \cdot \text{hr}^{-1}$ is over twice those of Adams, Guilizzoni, and Adams (1978). This difference may reflect the differences in physiological state between plants obtained from laboratory cultures, which were essentially free of senescent tissue, epiphytes, adherent sediment, and carbonate encrustation (Smart and Barko 1985), and those obtained directly from the field. Direct comparison of kinetic constants is also hampered by differences in methodology (Titus and Stone 1982) and will not be further attempted here.

**Nonlinear Photosynthetic Rate Dynamics**

40. The concentration of $C_\text{T}$ decreased exponentially with time over the course of the photosynthesis experiment (Figure 11). Curves were fit to these data using an iterative, nonlinear, least-squares estimation technique (Statistical Analysis System, Cary, N. C.). The curve-fitting equation used was:

$$C_\text{T} = ae^{-bt} + c$$

where

- $a$ = an empirical constant
- $b$ = the photosynthetic rate coefficient
- $t$ = time
- $c$ = the asymptotic limit of $C_\text{T}$

Taking the first derivative with respect to $t$ gives the first-order photosynthetic rate as a function of $C_\text{T}$:

$$P = b(C_\text{T} - c)$$

where $P$ = photosynthetic $C_\text{T}$ uptake rate for values of $C_\text{T}$ between the initial concentration and the asymptotic limit of $C_\text{T}$ uptake. The advantage of this method of analysis is that it provides continuous estimates of the rate of photosynthesis over the change in $C_\text{T}$ between the initial condition (maximum rate) and the limit of $C_\text{T}$ depletion (rate = 0).
41. The influence of CaCO$_3$ precipitation on the measured C$_T$ uptake rates was evaluated as stated in the previous section on initial linear photosynthesis. Since the nonlinear analysis employed a longer measurement period, these rates were more likely to be affected by precipitation.

42. Apparent photosynthetic C$_T$ uptake rates are expressed in Table 4 as the product of a rate coefficient (corrected for system volume and plant mass) and the difference between C$_T$ and c. Calculated values of pH at the C$_T$ uptake limit are also shown except where CaCO$_3$ precipitation occurred. In these cases changes in alkalinity precluded the calculation of final pH levels. Precipitation of CaCO$_3$ significantly affected the measured nonlinear rates of C$_T$ uptake in the high C$_T$ solutions containing mid and high levels of cations. These rates will not be considered further. Although precipitation also occurred in the mid C$_T$, high cation solution, the precipitation did not occur until near the end of the measurement period and was readily discernible by an abrupt change in the slope of C$_T$ versus time. The nonlinear rate of C$_T$ uptake in this solution was calculated from the initial portion of the curve, prior to the onset of precipitation.

43. Photosynthetic rates calculated for the initial C$_T$ concentrations (Table 4) generally compare favorably with rates determined from the initial linear portion of the C$_T$ concentration curve (Table 3). Linear rates were generally somewhat less than initial rates determined by nonlinear analysis due to the decrease in photosynthetic rate over the time interval required in the former analysis. However, this slight difference did not affect estimated kinetic constants. The kinetics curve fit to the initial nonlinear photosynthetic C$_T$ uptake rates (Figure 12) is virtually identical ($K_m = 25.8$, $V_{max} = 23.4$) to that fit to the initial linear phase photosynthetic rates ($K_m = 24.5$, $V_{max} = 20.9$).

44. The increase in solution pH due to H$_2$CO$_3$ or HCO$_3^-$ uptake is proportional to the quantity of C$_T$ taken up (assuming precipitation does not occur), and this relationship has been used to measure photosynthesis (Allen and Spence 1981). For this reason final pH levels attained in the higher C$_T$, higher alkalinity solutions were higher than those in the lower C$_T$, lower alkalinity solutions (Table 4). Estimated C$_T$ uptake limits (roughly analogous to C$_T$ compensation points) increased with increasing C$_T$ and alkalinity, except where precipitation occurred (Table 4). This increase in C$_T$ compensation point undoubtedly reflects the increasing proportion of C$_T$
in the form of CO$_3^{--}$ (which cannot be used in photosynthesis) in the higher alkalinity solutions at high pH. The utility of experimentally determined C$_T$ compensation points for comparative purposes is thus severely limited, as the C$_T$ compensation point is as much dependent on the properties of the solution (alkalinity, pH, and C$_T$) as on the photosynthetic capacity of the plant.

Photosynthetic C$_T$ uptake in relation to C$_T$ and pH

45. As photosynthetic C$_T$ uptake proceeds, the level of C$_T$ decreases and pH increases. Since photosynthetic C$_T$ uptake rates are first order with respect to C$_T$, the rate of C$_T$ uptake also decreases. Examination of photosynthetic C$_T$ uptake under the changing conditions of C$_T$ and pH should provide information on the relative importance of these parameters in affecting photosynthetic rates.

46. The divergence of photosynthetic C$_T$ uptake rates from the kinetics curve (Figure 12) might be considered as an indication of a pH effect on photosynthesis. For example, C$_T$ uptake at 10 mg C$_T$·l$^{-1}$ in the mid level solution (initially 10.5 mg C$_T$·l$^{-1}$) was 6.5 mg·g$^{-1}$·hr$^{-1}$ at a pH of 8.8 compared with the rate of 2.4 mg·g$^{-1}$·hr$^{-1}$ in the high level solution (initially 21.0 mg C$_T$·l$^{-1}$) at a pH of 10.6. However, this difference in photosynthetic rate is largely due to the difference in HCO$_3^-$ concentration between the two solutions—9.7 mg HCO$_3^-$·l$^{-1}$ in the mid level solution and 3.5 mg HCO$_3^-$·l$^{-1}$ in the high level solution.

47. Replotting the photosynthetic rate data against HCO$_3^-$ (Figure 13, graph A) rather than C$_T$ indicates that photosynthetic C$_T$ uptake rates in each of the solutions are similar at equal HCO$_3^-$ concentrations, in spite of large differences in pH (Figure 13, graph B). In addition, photosynthetic C$_T$ uptake in individual solutions approximates the kinetics curve down to a HCO$_3^-$ concentration of ~4 mg·l$^{-1}$. In neither the mid nor the high level solutions is there an appreciable difference in the slopes of the photosynthesis vs. HCO$_3^-$ lines below a pH of ~10.2, indicating that photosynthetic HCO$_3^-$ uptake in T. spiracatum was unaffected over the pH range 8.0 to 10.2. This observation is in agreement with the earlier findings of Steemann Nielsen (1947) who indicated that photosynthetic HCO$_3^-$ uptake in the same species was relatively unaffected over the same pH range. In contrast, the recent work of Titus and Stone (1982) indicates that photosynthesis in T. spiracatum is greatly
reduced with increasing pH over the range 7 to 9. One possible reason for this discrepancy is that Titus and Stone used buffered solutions to obtain different pH levels. Recent work, reviewed by Lucas (1983), has repeatedly demonstrated inhibitory effects of buffers on HCO₃⁻ assimilation (Lucas 1977; Prins, Snel, and Zanstra 1982).

48. It is interesting that photosynthetic Cₜ uptake in each of the solutions approaches zero at a HCO₃⁻ concentration of ≈1.5 mg·l⁻¹ (Figure 13, graph A). This observation may be indicative of a HCO₃⁻ compensation point in M. spicatum of 1.5 mg·l⁻¹.

Effects of atmospheric CO₂ exchange

49. Plots of open system gas phase CO₂ concentration against time (not presented) were roughly similar in shape to those for Cₜ. Exponential equations were fit to these data as described earlier. Photosynthetic CO₂ uptake rates were determined by calculating the product of the flow rate and the difference in influent and effluent airstream CO₂ concentrations.

50. Concurrent with the decrease in Cₜ due to photosynthetic Cₜ uptake, the equilibrium between the solution and the atmosphere is disturbed, resulting in a gradient in pCO₂ between the atmosphere and solution. Atmospheric CO₂ exchange thus increases with time while both Cₜ and Cₜ uptake decrease (Figure 14). Atmospheric CO₂ exchange rates rapidly reached similar maximal values in all solutions, averaging 5.0 ± 0.2 mg C·g⁻¹·hr⁻¹. The exchange of CO₂ was thus not affected by the initial solution composition. Atmospheric CO₂ exchange was a significant source of carbon in these experiments, supplying 20 to 80 percent of maximal carbon demands. As Cₜ declines to the asymptotic limit, a steady-state is approached with uptake of Cₜ being balanced by CO₂ exchange from the atmosphere (Figure 14). At the asymptotic limit of Cₜ declines, the atmosphere is effectively supplying all of the carbon required for photosynthesis.

51. The exchange of CO₂ across the air/water interface is a function of the mass transfer coefficient and the gradient in pCO₂. The gradient in pCO₂ across the air/water interface rapidly approaches a maximum as solution Cₜ is depleted, regardless of the initial Cₜ level (Figure 15). This explains the relative similarities in atmospheric CO₂ exchange rates measured at steady-state in the different solutions.

52. Although photosynthetic Cₜ uptake rates were proportional to
solution $C_T$, the uptake of atmospheric carbon was unaffected by solution $C_T$. Thus, consideration of uptake from only the liquid or only the gas phase could result in very different interpretations of the effects of solution on photosynthesis. Also, since atmospheric $CO_2$ exchange was relatively unaffected by the initial solution composition, consideration of atmospheric exchange in photosynthesis measurements lessens the effects of solution composition on the total (air + water) photosynthetic rate.

Kinetics including atmospheric $CO_2$ exchange

53. Considering the importance of the atmospheric $CO_2$ exchange component on the total rate of photosynthesis, it is of interest to examine the kinetics of the overall photosynthetic process. In attempting to describe the kinetics of total photosynthetic carbon uptake in relation to solution $C_T$, it is not possible to use initial rates because the initial atmospheric $CO_2$ exchange rate is zero and total photosynthesis equals solution $C_T$ uptake. Therefore, the maximum total carbon uptake rates were used to determine kinetic constants. The resulting kinetics curve (Figure 16) provided a good fit to the maximum rate data. The $K_m$ value of 8.5 mg C·l⁻¹ determined from this analysis is quite different from the $K_m$ values based on solution $C_T$ uptake alone. This difference indicates the large effect on $K_m$ of considering atmospheric $CO_2$ exchange in addition to direct $C_T$ uptake from solution. Quite different conclusions regarding the importance of $C_T$ might be reached depending on the analysis employed.
PART V: GROWTH IN RELATION TO PHOTOSYNTHESIS

54. The nearly linear response of photosynthetic $C_T$ uptake to solution $C_T$ contrasts with the slight responses in growth. Plotting growth of *M. spicatum* (as ash-free biomass accrual) in the two experiments against initial photosynthetic $C_T$ uptake in the same solutions (Figure 17, graph A) suggests that growth was not strongly related to photosynthesis. Integration of photosynthetic rate over the change in solution $C_T$ measured in the low D/z experiment (Figure 17, graph B) does not improve the relationship between growth and photosynthesis. Inclusion of atmospheric $CO_2$ exchange in the integrated photosynthetic rate (Figure 17, graph C) likewise does not improve the relationship due to the similarity in atmospheric $CO_2$ exchange rates among the different solutions used in the photosynthesis experiment.

Carbon Sources Used in Growth

55. To determine the relative importance of the atmosphere and solution as sources of carbon, a carbon budget was constructed for growth in the low D/z experiment (Figure 18). Total carbon uptake during growth was the sum of plant organic carbon ($0.465 \times$ ash-free dry weight) and plant inorganic carbon ($CaCO_3$ encrustation). Plant inorganic carbon was determined by measuring weight loss of ashed plant samples ($550^\circ C$) after reheating to $1,100^\circ C$ for 4 hr to convert $CaCO_3$ to $CaO$. This procedure was verified by analyzing tissues for $Ca$ and also by direct measurement of total carbon in ashed plant samples (LECO Carbon Determinator, Model WR 12). The quantity of carbon taken up from solution was the difference between the molar change in solution $C_T$ and the molar change in solution $Ca$ which was used as a measure of $CaCO_3$ precipitation. Precipitation of $CaCO_3$ was further partitioned between $CaCO_3$ encrustation (measured) and precipitation in the tanks (by subtraction). The remainder of the total carbon uptake was considered to have been supplied by atmospheric $CO_2$ exchange. Measurement of heterotrophic respiration of the water column was neglected. Microbial respiration should be minor in these experimental solutions, which lack $N$, $P$, and organic carbon (Smart and Barko 1985). An estimate of sediment respiration as a source of inorganic carbon was based on measurements of $CO_2$ release from Lake Washington sediments (Kuivila and Murray 1984). This estimate was considerably less than 1 g and...
was not included in the figure. Direct uptake of CO$_2$ by roots of *M. spicatum* has likewise been shown to be insignificant (Loczy, Carignan, and Planas 1983) and was not included.

56. Much of the carbon used for growth in these experiments was apparently derived from the atmosphere. This is not surprising considering the large gradients in pCO$_2$ across the air/water interface and the high mass transfer coefficient measured here. The effect of initial solution composition, in particular C$_T$ and alkalinity, on atmospheric CO$_2$ exchange was slight as in the photosynthesis experiments. In the high C$_T$, high cation solution, atmospheric CO$_2$ supply was diminished, probably due to the lessening of the gradient in pCO$_2$ by CaCO$_3$ precipitation and the consequent decrease in alkalinity and pH of this solution.

57. The quantity of carbon derived directly from solution C$_T$ increased with increasing C$_T$ and to a lesser extent with increasing solution cations. The latter effect is due to the increase in CaCO$_3$ precipitation with increasing solution Ca and C$_T$. Although carbon derived from solution increased with increasing C$_T$ between 10.5 and 21.0 mg·l$^{-1}$, this increased uptake from solution was offset by decreased CO$_2$ supply from the atmosphere. The magnitude of atmospheric CO$_2$ exchange is dependent on the gradient in pCO$_2$ across the air/water interface. As demonstrated earlier (Figure 15), the gradient in pCO$_2$ rapidly approaches a maximum as C$_T$ uptake proceeds. In low C$_T$ solutions, CO$_2$ exchange reaches a maximum after a relatively small depletion of C$_T$. In higher C$_T$ solutions, maximal CO$_2$ exchange requires a greater depletion of C$_T$. Moreover, in situations where CaCO$_3$ precipitation occurs, the gradient in pCO$_2$ is diminished, and atmospheric CO$_2$ exchange may be reduced. For these reasons the importance of atmospheric CO$_2$ exchange in the carbon budget is not easily predicted from the initial solution composition.

58. The inability to relate growth of *M. spicatum* in these experiments to photosynthetic rates measured in the same solutions is due to an inability to assess changes in the role of atmospheric CO$_2$ exchange during the changing water chemistry conditions occurring in the growth experiments. However, photosynthetic C$_T$ uptake rates, integrated over the change in C$_T$ occurring in the low D/z experiment, were linearly related to the quantity of C$_T$ supplied by solution during growth (Figure 19). Thus, while photosynthetic measurements may be useful for assessing the uptake of solution C$_T$, they may not be good predictors of plant growth in natural systems.
59. Many of the environmental parameters known to affect the relative availability of atmospheric and solution carbon were controlled in these experiments. These parameters include temperature, mass transfer coefficients, mixing, and water exchange. In addition, other sources of carbon were minimized. These sources include water column respiration, sediment respiration, diffusion of HCO$_3^-$ from the sediment, and dissolution of sedimentary carbonates. In view of the much greater complexity of natural systems, the use of photosynthetic rates as a diagnostic tool for assessing the submersed macrophyte growth potential of natural systems may be unwarranted without a better understanding of the importance of these other sources of carbon.

Carbon Limitation

60. The growth responses of *M. spicatum* in these experiments suggest that growth was limited by the availability of carbon at the low level of C$_T$ (3.5 mg·l$^{-1}$) but not at the mid or high levels. In order to assess the possible limitation of growth by carbon, an additional experiment was conducted in the lucite columns in the growth chamber. In this experiment the same three levels of C$_T$ were examined and each solution was balanced with respect to cations. For example, the low C$_T$ solution had the low level of cations and the high C$_T$ solution had the high level of cations (see Table I). Two sets of columns were used with one set receiving ambient CO$_2$ aeration ($\approx 350$ µl·l$^{-1}$) and the other receiving about 3.5 x ambient CO$_2$ ($\approx 1,200$ µl·l$^{-1}$). The high CO$_2$ aeration was accomplished by metering compressed CO$_2$ into the metered airstream. Other experimental details were as described earlier.

61. Augmenting the airstream CO$_2$ concentration produced a dramatic increase in growth of *M. spicatum* in all solutions (Figure 20). In addition to the increased growth in each solution, a difference in growth between the mid and high level C$_T$ solutions was also noted, suggesting that addition of CO$_2$ increased the availability of C$_T$ under high C$_T$ conditions. This increased availability of C$_T$ was due to the lessened C$_T$ depletion in all solutions and decreased CaCO$_3$ precipitation in high Ca, high C$_T$ solutions (data not presented).

62. In these short-term (6 weeks) growth experiments, biomass accrual is directly proportional to growth rate and can be used to examine the
kinetics of growth in relation to \( C_T \). Figure 21 is a plot of biomass accrual under ambient and elevated CO\(_2\) conditions during the high D/z experiment along with Michaelis-Menten kinetics curves calculated for these data. The half-saturation constant for growth under ambient CO\(_2\) (\( K_m = 2.1 \text{ mg C}_T^*\cdot l^{-1} \)) is quite different than that presented earlier for photosynthesis. The half-saturation constant for growth in the low D/z experiment (\( K_m = 1.9, \text{ data not shown} \)) is essentially identical to the \( K_m \) for growth in the high D/z experiment. Augmenting the CO\(_2\) supply during growth changed both \( K_m \) and \( V_{\text{max}} \) (Figure 21). Interestingly, the 3.5-fold increase in airstream CO\(_2\) produced a 3.4-fold increase in \( V_{\text{max}} \).

63. These changes in kinetic constants indicate that the growth response of \( M. \text{ spicatum} \) to solution \( C_T \) is highly dependent on the characteristics of both solution composition and atmospheric CO\(_2\) exchange. Photosynthesis experiments involving only the direct uptake of \( C_T \) generally produce high values of \( K_m \) (Figure 10). Consideration of atmospheric exchange during photosynthesis results in a considerably lower \( K_m \) (Figure 16). Growth experiments conducted under conditions favoring depletion of solution \( C_T \) and the exchange of CO\(_2\) between the atmosphere and solution produce even lower estimates of \( K_m \) (Figure 21). Enhancing the replenishment of solution \( C_T \) by augmenting the airstream CO\(_2\) concentration produced an intermediate value of \( K_m \).

64. These results indicate that growth of \( M. \text{ spicatum} \) was limited by availability of carbon under all experimental conditions employed here. The lack of a growth response to increasing \( C_T \) above the mid level (10.5 mg \cdot l^{-1}) under ambient CO\(_2\) conditions may have been due to both the magnitude of, and the relative similarity in, the transfer of atmospheric CO\(_2\) into solution (Figure 18). The differences in the kinetics of growth and photosynthesis in these experiments arise from differences in the two measurement parameters. In the photosynthesis experiments, both \( C_T \) uptake and atmospheric CO\(_2\) exchange varied in relation to changes in solution \( C_T \). However, in the growth experiments, biomass accrual integrated the changes in photosynthesis occurring over the course of the experiment. In the growth experiments photosynthetic \( C_T \) uptake lowered the concentration of \( C_T \) in solution. The decrease in \( C_T \) resulted in a corresponding decrease in photosynthetic \( C_T \) uptake rate. Atmospheric CO\(_2\) exchange increased in response to \( C_T \) depletion (Figure 15), but was unable to maintain the initial \( C_T \) levels. In both
growth experiments, measured CO₂ concentrations in the effluent airstreams varied only slightly among the different solutions. The transfer of airstream CO₂ in these experiments was presumably operating at near the maximum possible rates for each of the experimental systems. Approximately 50 and 90 percent of the airstream CO₂ was removed during passage through the solutions in the low and high D/z experiments, respectively. Thus, as C_T depletion continued, the systems approached steady-states, with C_T uptake from solution being balanced by atmospheric CO₂ exchange. For sufficiently long experimental periods a similar steady-state will be attained for all starting conditions, and growth would be independent of the initial C_T conditions. In the growth experiments conducted under ambient atmospheric CO₂ conditions, the mid and high C_T solutions had achieved equivalence with respect to their ability to support photosynthesis through the combined processes of C_T supply and atmospheric CO₂ exchange. Under elevated atmospheric CO₂ conditions, CO₂ exchange was sufficient to maintain the solutions at higher levels of C_T. Therefore, steady-state conditions were not approached, and differences in growth among the different solutions were observed. In the experiments reported here, the influence of C_T on growth was therefore dependent on the rate of supply of atmospheric CO₂.
PART VI: GENERAL DISCUSSION

Cation Effects

65. The influence of specific cations on the growth and photosynthesis of *M. spicatum* seems to be limited primarily to the involvement of Ca in the photosynthetic process. As noted earlier, the deleterious effects of low Ca are only observed under low $C_T$, high pH conditions. Under conditions of limited solution volume (i.e. photosynthesis experiments), $C_T$ depletion rapidly becomes acute, and the deleterious effects of low Ca are pronounced. As solution volume increases (i.e. growth experiments), $C_T$ depletion is less severe, and the requirement for Ca seems to decrease. Increasing either $C_T$ or the airstream CO$_2$ concentration alleviates the effects of low Ca by reducing the severity of $C_T$ depletion. These observations suggest that Ca may be involved in alleviating stress due to the occurrence of low levels of HCO$_3^-$ in combination with relatively high levels of pH and CO$_3^-$. The CO$_3^-$ has been shown to be a competitive inhibitor of HCO$_3^-$ uptake (Lucas 1977), and Ca may moderate this inhibition by precipitation of CO$_3^-$ at sites of OH efflux.

66. Although Ca is clearly required for photosynthesis and growth, these results indicate that low levels of Ca (or other cations) are unlikely to limit the growth or distribution of *M. spicatum*. The establishment of *M. spicatum* in ponds of extremely low Ca (Giesy and Tessier 1979) substantiates this finding. Earlier evidence that Ca may affect the growth or distribution of this species (Hutchinson 1970; Barko 1983) should be reevaluated in light of these findings. Observed patterns of the distribution of *M. spicatum* in relation to Ca levels may reflect the natural correlation between Ca and $C_T$, rather than an environmental requirement for high Ca levels.

Carbon Effects

67. The influence of $C_T$ on the growth and photosynthesis of *M. spicatum* is more difficult to quantify. Photosynthetic $C_T$ uptake from solution is strongly related to solution $C_T$ concentration. However, the influence of $C_T$ on growth is more dependent on the ability of the system to maintain levels of $C_T$ than on the actual concentration of $C_T$ in solution. Thus, the effect of $C_T$ on the growth of submersed macrophytes cannot be
determined a priori, as it will more likely depend on the rate of replenishment of the \( C_T \) pool than on the actual quantity of \( C_T \). Unlike the experiments reported here, in which the atmosphere was the major source of carbon, in natural plant populations it is likely that sediment respiration, water column respiration, and atmospheric exchange are all significant sources of carbon input to the \( C_T \) pool.

68. The importance of \( C_T \) in affecting the growth and distribution of \textit{M. spicatum} in nature involves all components of the carbon budget. Systems containing low levels of \( C_T \) are likely to also exhibit low rates of carbon regeneration (respiration), and are unlikely to support excessive growth of submersed macrophytes. Systems chronically supporting luxuriant growth of submersed aquatic plants are likely to exhibit high rates of both water column and sediment respiration, resulting in a high rate of supply of carbon to the \( C_T \) pool. In systems which have not previously been exposed to submersed macrophytes, the potential influence of the \( C_T \) level on macrophyte growth potential will depend on the intrinsic capacity of carbon regeneration. If the carbon-regenerating capacity of the system is low, the development of invasive submersed macrophytes (e.g., \textit{M. spicatum}) may be short lived. It is of interest in this regard that the decline of \textit{M. spicatum} in Lake Wingra, Wis., coincided with a 31-percent reduction in \( C_T \) levels in the lake (Titus and Stone 1982).
69. Based on this research and a review of the technical literature on the effects of water chemistry on *M. spicatum*, the following conclusions and recommendations are made:

a. Under conditions of limited Ca and $C_T$ availability, growth and photosynthesis of *M. spicatum* can be adversely affected. While K can partially substitute for Ca, neither Na nor Mg are effective substitutes. Additions of carbon as either $C_T$ or $CO_2$ lessen the requirement for Ca. The requirement for Ca seems to involve the total quantity available rather than the concentration in solution. In spite of these findings, levels of the major cations in most surface waters should be sufficient to support nuisance growths of *M. spicatum*. Observed patterns of the distribution of *M. spicatum* in relation to Ca levels may reflect the natural correlation between Ca and $C_T$, rather than an environmental requirement for high Ca levels.

b. While photosynthetic $C_T$ uptake is strongly affected by solution composition, exchange of atmospheric $CO_2$ is relatively unaffected. Atmospheric $CO_2$ exchange therefore moderates the influence of solution $C_T$. Additional studies on photosynthesis of submersed aquatic plants should consider the interrelationship between uptake of solution $C_T$ and exchange of atmospheric $CO_2$ into solution.

c. Photosynthetic $C_T$ uptake is dependent on the concentration of HCO$_3^-$ and, at equivalent HCO$_3^-$ levels, is relatively unaffected by pH over the range 8.0 to 10.2. Additional studies on the effects of solution $C_T$ and pH on photosynthesis of submersed aquatic plants should be conducted in unbuffered solutions, allowing pH levels to be controlled naturally by changes in solution $C_T$. This method, in addition to being more natural, will allow study of the combined effects of changes in $C_T$ and pH on the photosynthetic process.

d. Photosynthesis and growth of *M. spicatum* can result in considerable changes in water chemistry conditions, and levels of $C_T$ in solution can be rapidly depleted through the combined processes of photosynthetic $C_T$ uptake and CaCO$_3$ precipitation. Continuous addition of gaseous $CO_2$ can be effective in moderating $C_T$ depletion and CaCO$_3$ precipitation, thus maintaining high levels of $C_T$ in solution. Elevated $CO_2$ levels can thereby enhance the growth response of *M. spicatum* to solution $C_T$. Regeneration of carbon through ecosystem respiration seems to be required to maintain $C_T$ levels adequate to support luxuriant growth of *M. spicatum*. Therefore, the carbon-regenerating capacity, rather than the level of $C_T$ in solution, will limit the submersed macrophyte production potential of the system. Additional studies of the effects of water chemistry on the growth of submersed aquatic plants should
consider the major fluxes of inorganic carbon and their relationship to the maintenance of the $C_T$ pool.

e. The relationship between photosynthesis and growth is complicated by growth-related changes in solution composition as well as by changes in the rate of exchange of atmospheric $CO_2$. The utility of photosynthesis measurements as indicators of plant growth potential cannot be realized without a better understanding of the roles of atmospheric $CO_2$ exchange and carbon regeneration in maintaining the level of $C_T$ in solution.

f. Carbon limitation of $M. spicatum$ growth is likely in lentic systems exhibiting low rates of carbon regeneration. The prevalence of carbon limitation of submersed macrophyte growth should be evaluated in a variety of natural systems.

g. The responses of other species of submersed aquatic plants to water chemistry conditions should be evaluated and compared with results obtained for $M. spicatum$. These comparative evaluations may suggest mechanisms to explain observed patterns of growth and distribution.

h. Although water chemistry conditions (specifically those affecting carbon availability) may set limits on the growth potential of submersed macrophyte populations, it is not readily apparent that increased understanding of these limits will lead to efficacious aquatic plant management techniques. Although levels of $C_T$ could be reduced in localized areas through chemical precipitation, this technique may be thwarted by redissolution of CaCO$_3$ by respiratory $CO_2$. Field investigations of the feasibility of CaCO$_3$ precipitation as a means of reducing submersed macrophyte growth might be initiated on a small scale. If successful, this technique might provide an inexpensive and environmentally acceptable alternative to the use of herbicides in certain situations.
REFERENCES


<table>
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<tr>
<th>Treatment Levels</th>
<th>Chemical Composition, mg·L⁻¹</th>
<th>Conductance (S·cm⁻¹ @ 25°C)</th>
<th>pH</th>
<th>Ionic Strength mM</th>
<th>Alkalinity meq·L⁻¹</th>
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### Table 2
Characteristics of Experimental Environments

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<th>Low D/z</th>
<th>High D/z</th>
<th>Experiment</th>
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<td>Growth chamber</td>
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<td>2</td>
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<td>Maximum O₂ transfer rate</td>
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<td>Solution volume</td>
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### Table 3
Apparent Initial (Linear Phase) Photosynthetic Cₜ Uptake Rates (mg·g⁻¹·hr⁻¹) of *M. spicatum* in the Experimental Solutions

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<th>High</th>
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</table>

* Measured rate includes CaCO₃ precipitation.
Table 4
Apparent Photosynthetic $C_T$ Uptake Rate Coefficients, and Photosynthetic $C_T$ Uptake of *M. spicatum* in the Experiment Solutions

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<th>Coefficient Limit</th>
<th>Initial Rate</th>
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<td>Mid</td>
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<td>Mid</td>
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<tr>
<td>High</td>
<td>High</td>
<td>21.0</td>
</tr>
</tbody>
</table>

Note: The pH Limit for $C_T$ uptake is calculated from the asymptotic limit of photosynthetic $C_T$ uptake (c) where CaCO₃ precipitation did not occur. Initial photosynthetic $C_T$ uptake rates were calculated from these values and Equation 2.

* Indicates precipitation of CaCO₃.
** Indicates that the calculated rate is overestimated due to CaCO₃ precipitation.
Figure 1. Shoot height of *M. spicatum* during the high D/z experiment. Data are pooled across cation levels within $C_T$ levels. High and mid level $C_T$ treatments are similarly pooled.
Figure 2. Total biomass and total ash-free biomass accrual of *M. spicatum* in relation to solution cation and $C_T$ levels in the high D/z experiment. Cation levels are indicated within bars: L = low, M = mid, H = high
Figure 3. Total biomass and total ash-free biomass accrual (graphs A and C) and percent senescence (graphs B and D) of *M. spicatum* in relation to specific cation additions and reductions in the specific cation experiment. Cation additions increased the level of cation from the low (control) level to the high level. Cation reductions reduced the cation from the high (control) level to the low level. Asterisks denote a significant difference from the control treatment at α < 0.05, as determined by Dunnett's procedure.
Figure 4. Total biomass and total ash-free biomass accrual of *M. spicatum* in the low D/z experiment. Cation levels are indicated within bars: L = low, M = mid, H = high.
Figure 5. Diurnal changes in pH and $C_T$ during the fifth week of the high D/z experiment. Horizontal bars indicate light and dark portions of the experimental period.
Figure 6. Diurnal changes in alkalinity and acidity during the fifth week of the high D/z experiment. Data were calculated from measured pH and $C_T$ values. Horizontal bars indicate light and dark portions of the experimental period.
Figure 7. Diurnal changes in pH and C<sub>T</sub> during the fifth week of the low D/z experiment. Horizontal bars indicate light and dark portions of the experimental period.
Figure 8. Diurnal changes in alkalinity and acidity during the fifth week of the low D/z experiment. Data were calculated from measured pH and CT values. Horizontal bars indicate light and dark portions of the experimental period.
Figure 9. Diagrammatic representation of the experimental apparatus used for concurrent measurement of gas phase CO$_2$ concentrations and liquid phase C$_4$ concentrations during photosynthesis by *M. spicatum*. (1) 1-l glass hydrometer cylinder fitted with neoprene stopper; (2) peristaltic tubing pump for water circulation; (3) 40-l mixing vessel for damping changes in influent compressed airstream; (4) peristaltic tubing pump for air flow; (5) 1-l mixing vessel; (6) sampling ports fitted with rubber serum bottle stoppers. Arrows show direction of liquid and gas flow in the system.
Figure 10. Relationship between initial (linear phase) photosynthetic $C_T$ uptake by *M. spicatum* and $C_T$ in the photosynthesis experiment. The kinetics curve was obtained by linear transformation of initial rate data in relation to solution $C_T$ at each of the experimental $C_T$ levels. Solutions containing unfavorable cation levels (o) were excluded from the analysis. The measured photosynthetic $C_T$ uptake rate in the high $C_T$, high cation solution (not shown) was affected by CaCO$_3$ precipitation and was likewise excluded. $K_m =$ half-saturation constant; $V_{\text{max}} =$ maximal photosynthetic rate.

$K_m = 24.5 \text{ mg} \cdot \text{l}^{-1}$

$V_{\text{max}} = 20.9 \text{ mg} \cdot \text{g}^{-1} \cdot \text{hr}^{-1}$
Figure 11. Representative curves showing temporal changes in $C_T$ during the nonlinear photosynthesis experiment. Curve fitting was accomplished with a nonlinear least-squares estimation technique.
Figure 12. Relationship between photosynthetic $C_T$ uptake by *M. spicaturn* and solution $C_m$. The kinetics curve (solid line) was obtained by linear transformation of initial rate data in relation to solution $C_m$ at each of the experimental $C_T$ levels. Rates obtained in solutions containing unfavorably low cation levels (o) were excluded from the analysis. Photosynthetic $C_T$ uptake rates which were affected by CaCO$_3$ precipitation (not shown) were also excluded. Photosynthetic $C_T$ uptake rates in each solution (dashed lines) decreased linearly as $C_T$ decreased. The dotted portion of the line was extrapolated
Figure 13. Relationship between photosynthetic $C_T$ uptake and pH versus $\text{HCO}_3^-$ concentration. (A) Relationship between photosynthetic $C_T$ uptake by *A. spicatum* and $\text{HCO}_3^-$ concentration. The kinetics curve was obtained by linear transformation of initial rate data in relation to solution $\text{HCO}_3^-$ in each of the experimental solutions. Photosynthetic $C_m$ uptake rates in each solution (dashed lines) decrease as $\text{HCO}_3^-$ decreases. (B) Relationship between solution pH and $\text{HCO}_3^-$ during photosynthesis in each of the solutions.
Figure 14. Temporal changes in rates of photosynthetic $CT$ uptake (dashed line), atmospheric CO$_2$ exchange (dotted line), and total photosynthetic carbon uptake (solid line) by $M. spicatum$ in solutions containing low, mid, and high levels of $CT$.
Figure 15. Relationship between the gradient in pCO$_2$ across the air/water interface and the depletion of C$_m$ from solutions of different C$_T$ levels. Curves are calculated from carbonate equilibria for each of the solutions. Alkalinity was held constant at the initial level.
Figure 16. Relationship between total photosynthesis by *M. spicatum* and solution $C_T$. Photosynthetic $C_T$ uptake rates and $C_T$ uptake kinetics are shown for reference. Total photosynthesis (dashed lines) is the sum of $C_T$ decline and atmospheric CO$_2$ exchange. Dotted portions of the lines are extrapolated. The horizontal portions of the total photosynthesis curves are equivalent to the steady-state maximum rate of atmospheric CO$_2$ exchange ($\Delta C_T = 0$). The kinetics curve for total photosynthesis (bold line) was calculated from linear transformation of maximal rate data in relation to solution $C_T$ at each of the experimental $C_T$ levels.
Figure 17. Relationship between growth (total ash-free biomass accrual) in the low D/z (○) and high D/z (■) experiments and several measures of photosynthesis of M. spicatum: (A) growth in relation to initial linear photosynthetic $C_r$ uptake rates, (B) growth in relation to integrated apparent photosynthetic $C_T$ uptake rates, and (C) growth in relation to integrated total apparent photosynthetic rates. Open points (○,□) indicate photosynthetic rates affected by CaCO$_3$ precipitation. Integrated photosynthetic rates were obtained by integration of photosynthetic rate equations over the change in solution $C_T$ measured during the low D/z growth experiment.
Figure 18. Sources of carbon used during growth (cross-hatched bars) of *M. spicatum* in the low D/z experiment. Solution cation levels are indicated within bars: L = low, M = mid, and H = high.
Figure 19. Relationship between the quantity of carbon supplied from solution during growth and integrated apparent photosynthetic $C_T$ uptake rates. Photosynthetic $C_T$ uptake rates were integrated over the measured changes in $C_T$ occurring during the growth experiment. Carbon supplied from solution includes the quantities of $C_T$ removed from solution and either incorporated into plant organic matter or precipitated on the plant tissues (○). Alternate values (■) also include $C_T$ removed from solution by precipitation of CaCO$_3$ from the water column.
Figure 20. Total biomass and total ash-free biomass accrual of *M. spicatum* in relation to solution composition and atmospheric CO$_2$ supply.
Figure 21. Kinetics of growth of *M. spicatum* in relation to solution $C_T$ in the high D/z experiment under ambient and elevated airstream $CO_2$ conditions. Kinetics were determined by linear transformation of ash-free biomass accrual values in relation to solution $C_T$. 

$K_m = 4.7 \text{ mg } C_T \cdot \text{l}^{-1}$  
$V_{max} = 13.9 \text{ g}$  

$K_m = 2.1 \text{ mg } C_T \cdot \text{l}^{-1}$  
$V_{max} = 4.1 \text{ g}$
APPENDIX A: STATISTICAL COMPARISON OF MEAN VALUES FOR GROWTH AND NUTRIENT CONCENTRATIONS IN RELATION TO SOLUTION CATION AND INORGANIC CARBON $C_T$ LEVELS IN THE LOW AND HIGH $D/z$ EXPERIMENTS
Table A1  
Treatment Means and Statistical Comparisons of Plant Growth Parameters  
(g Dry Weight) Among Treatments in the High D/z Experiment  

<table>
<thead>
<tr>
<th>Cation Level</th>
<th>Low</th>
<th>Mid</th>
<th>High</th>
<th>Pooled</th>
</tr>
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<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<tr>
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<td>4.30Ca</td>
<td>3.48ab</td>
</tr>
<tr>
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<td>3.66Ba</td>
<td>4.56Cb</td>
<td>3.58b</td>
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<tr>
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<td>4.25C</td>
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<td></td>
<td></td>
<td></td>
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<tr>
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<tr>
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Note: Individual treatment means (n = 4) followed by different letters (upper case for $C_T$ levels, lower case for cation levels) are significantly different ($\alpha \leq 0.05$) as determined by Duncan's Multiple Range Test applied to individual one-way analysis of variance (ANOVA). Main effects (from two-way ANOVAs) of $C_T$ and cation treatments are similarly indicated for values pooled over cation levels and $C_T$ levels, respectively.  
* Denotes a significant ($\alpha \leq 0.05$) $C_T \times$ cation interaction.
Table A2
Treatment Means and Statistical Comparisons of Plant Growth Parameters (g Ash-Free Dry Weight) Among Treatments in the High D/z Experiment

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<th>Pooled</th>
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<tr>
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<td>3.22Ba</td>
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<td>Ash-free root biomass</td>
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<td>0.37Ba</td>
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<td>0.32a</td>
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<tr>
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<td>0.38Ba</td>
<td>0.36ABAa</td>
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<tr>
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<td>0.43B</td>
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<tr>
<td>Low</td>
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<td>3.70Ba</td>
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<tr>
<td>Mid</td>
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<td>3.31Ba</td>
<td>3.76Ba</td>
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<tr>
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<td>3.45B</td>
<td>3.59B</td>
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Note: Individual treatment means (n = 4) followed by different letters (upper case for C<sub>T</sub> levels, lower case for cation levels) are significantly different (α ≤ 0.05) as determined by Duncan's Multiple Range Test applied to individual one-way ANOVAs. Main effects (from two-way ANOVAs) of C<sub>T</sub> and cation treatments are similarly indicated for values pooled over cation levels and C<sub>T</sub> levels, respectively. 
* Denotes a significant C<sub>T</sub> x cation interaction.
Table A3

Treatment Means and Statistical Comparisons of Plant Growth Parameters
(g Dry Weight) Among Treatments in the Low D/z Experiment

<table>
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<th>C/T Level</th>
<th>Cation Level</th>
<th>Low</th>
<th>Mid</th>
<th>High</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot biomass</td>
<td>Low</td>
<td>6.65Aa</td>
<td>9.00Ba</td>
<td>9.80Ba</td>
<td>8.48a</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>7.08Aa</td>
<td>9.62Ba</td>
<td>11.48Cb</td>
<td>9.39b</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>6.96Aa</td>
<td>10.56Bb</td>
<td>13.29Cc</td>
<td>10.27c</td>
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<tr>
<td>Pooled</td>
<td></td>
<td>6.90A</td>
<td>9.73B</td>
<td>11.52C</td>
<td>**</td>
</tr>
<tr>
<td>Root biomass</td>
<td>Low</td>
<td>0.95Aa</td>
<td>1.10Ba</td>
<td>1.21Ba</td>
<td>1.08a</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>1.04Aa</td>
<td>1.10Aa</td>
<td>0.98Ab</td>
<td>1.04a</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>1.03Aa</td>
<td>1.18Bb</td>
<td>1.04ABb</td>
<td>1.08a</td>
</tr>
<tr>
<td>Pooled</td>
<td></td>
<td>1.01A</td>
<td>1.13B</td>
<td>1.08AB</td>
<td>*</td>
</tr>
<tr>
<td>Total biomass</td>
<td>Low</td>
<td>7.60Aa</td>
<td>10.10Ba</td>
<td>11.00Ca</td>
<td>9.51a</td>
</tr>
<tr>
<td></td>
<td>Mid</td>
<td>8.11Aa</td>
<td>10.72Ba</td>
<td>12.46Cb</td>
<td>10.43b</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>7.99Aa</td>
<td>11.74Bb</td>
<td>14.33Cc</td>
<td>11.35c</td>
</tr>
<tr>
<td>Pooled</td>
<td></td>
<td>7.90A</td>
<td>10.85B</td>
<td>12.60C</td>
<td>**</td>
</tr>
</tbody>
</table>

Note: Individual treatment means (n = 6) followed by different letters (upper case for C/T levels, lower case for cation levels) are significantly different (α ≤ 0.05) as determined by Duncan's Multiple Range Test applied to individual one-way ANOVAs. Main effects (from two-way ANOVAs) of C/T and cation treatments are similarly indicated for values pooled over cation levels and C/T levels, respectively.

* Denotes a significant (α ≤ 0.05) C/T x cation interaction.
** Denotes a significant (α ≤ 0.01) C/T x cation interaction.
### Table A4

**Treatment Means and Statistical Comparisons of Plant Growth Parameters (g Ash-Free Dry Weight) Among Treatments in the Low D/z Experiment**

<table>
<thead>
<tr>
<th>Cation Level</th>
<th>CT Level</th>
<th>Low</th>
<th>Mid</th>
<th>High</th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash-free shoot biomass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td>6.11Aa</td>
<td>8.49Ba</td>
<td>9.23Cb</td>
<td>7.94a</td>
</tr>
<tr>
<td>Mid</td>
<td></td>
<td>6.51Aa</td>
<td>9.03Bab</td>
<td>9.71Bb</td>
<td>8.42a</td>
</tr>
<tr>
<td>High</td>
<td></td>
<td>6.37Aa</td>
<td>9.58Cb</td>
<td>8.33Ba</td>
<td>8.10a</td>
</tr>
<tr>
<td>Pooled</td>
<td></td>
<td>6.33A</td>
<td>9.03B</td>
<td>9.09B</td>
<td>**</td>
</tr>
<tr>
<td>Ash-free root biomass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td>0.76Aa</td>
<td>0.87Ba</td>
<td>0.96Eb</td>
<td>0.87a</td>
</tr>
<tr>
<td>Mid</td>
<td></td>
<td>0.83ABa</td>
<td>0.88Ba</td>
<td>0.78Aa</td>
<td>0.83a</td>
</tr>
<tr>
<td>High</td>
<td></td>
<td>0.82Aa</td>
<td>0.94Aa</td>
<td>0.83Aa</td>
<td>0.86a</td>
</tr>
<tr>
<td>Pooled</td>
<td></td>
<td>0.80A</td>
<td>0.90B</td>
<td>0.86AB</td>
<td>*</td>
</tr>
<tr>
<td>Ash-free total biomass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td>6.87Aa</td>
<td>9.36Ba</td>
<td>10.19Cb</td>
<td>8.81a</td>
</tr>
<tr>
<td>Mid</td>
<td></td>
<td>7.33Aa</td>
<td>9.91Bab</td>
<td>10.49Cb</td>
<td>9.25a</td>
</tr>
<tr>
<td>High</td>
<td></td>
<td>7.19Aa</td>
<td>10.52Cb</td>
<td>9.16Ba</td>
<td>8.96a</td>
</tr>
<tr>
<td>Pooled</td>
<td></td>
<td>7.13A</td>
<td>9.93B</td>
<td>9.95B</td>
<td>**</td>
</tr>
</tbody>
</table>

**Note:** Individual treatment means (n = 6) followed by different letters (upper case for CT levels, lower case for cation levels) are significantly different (α ≤ 0.05) as determined by Duncan's Multiple Range Test applied to individual one-way ANOVAs. Main effects (from two-way ANOVAs) of CT and cation treatments are similarly indicated for values pooled over cation levels and CT levels, respectively.

* Denotes a significant (α ≤ 0.05) CT × cation interaction.

** Denotes a significant (α ≤ 0.01) CT × cation interaction.
### Table A5
Treatment Means and Statistical Comparisons of Plant Shoot Nutrient Concentrations (mg·g⁻¹ Ash-Free Dry Weight) Among Treatments in the Low D/z Experiment

<table>
<thead>
<tr>
<th>C₄ Level</th>
<th>Shoot N</th>
<th></th>
<th></th>
<th>Pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Mid</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>11.3Aa</td>
<td>7.4Ba</td>
<td>6.1Ca</td>
<td>8.3a</td>
</tr>
<tr>
<td>Mid</td>
<td>10.2Ab</td>
<td>6.7Ba</td>
<td>5.9Ba</td>
<td>7.6a</td>
</tr>
<tr>
<td>High</td>
<td>10.3Aab</td>
<td>5.7Cb</td>
<td>6.8Bb</td>
<td>7.6a</td>
</tr>
<tr>
<td>Pooled</td>
<td>10.6A</td>
<td>6.6B</td>
<td>6.3B</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Shoot P</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Mid</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>4.95Aa</td>
<td>3.76Ba</td>
<td>3.34Cab</td>
<td>4.01a</td>
</tr>
<tr>
<td>Mid</td>
<td>4.84Aa</td>
<td>3.46Ba</td>
<td>3.25Ba</td>
<td>3.85a</td>
</tr>
<tr>
<td>High</td>
<td>4.86Aa</td>
<td>3.53Ba</td>
<td>3.59Bb</td>
<td>4.00a</td>
</tr>
<tr>
<td>Pooled</td>
<td>4.88A</td>
<td>3.58B</td>
<td>3.39B</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Shoot K</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Mid</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>20.0Aa</td>
<td>11.7Ba</td>
<td>11.4Ba</td>
<td>14.3a</td>
</tr>
<tr>
<td>Mid</td>
<td>19.2Aa</td>
<td>14.0Bb</td>
<td>13.0Bc</td>
<td>15.4a</td>
</tr>
<tr>
<td>High</td>
<td>10.5Aa</td>
<td>12.5CAbb</td>
<td>15.8Bb</td>
<td>16.3a</td>
</tr>
<tr>
<td>Pooled</td>
<td>19.9A</td>
<td>12.7B</td>
<td>13.4B</td>
<td></td>
</tr>
</tbody>
</table>

Note: Individual treatment means (n = 2) followed by different letters (upper case for C₄ levels, lower case for cation levels) are significantly different (α ≤ 0.05) as determined by Duncan's Multiple Range Test applied to individual one-way ANOVAs. Main effects due to C₄ and cation treatments are similarly indicated for values pooled over cation levels and C₄ levels, respectively.
APPENDIX B: PHOTOSYNTHETIC CaCO₃ PRECIPITATION
1. In order to obtain photosynthetic rates of plants under conditions resulting in CaCO$_3$ precipitation, the authors elected to repeat the photosynthesis experiments with modifications to include measurement of Ca and pH in addition to inorganic carbon $C_T$. At each sampling interval an additional 10-ml water sample was collected in a disposable syringe. This sample was split and pH and Ca were determined as described earlier. In order to minimize the effect of the larger sample volume requirements, an additional vessel containing the experimental solution was included in the recirculation loop of the experimental apparatus. This served the dual purpose of increasing the system volume and slowing the rate of change of water chemistry parameters. Total sample volume withdrawn during the period was <5 percent of the system volume. This slight change in volume did not require correction.

2. Results obtained in the high $C_T$, low cation solution are presented in Figure B1, graphs A, B, C, and D. The $C_T$ declined almost linearly over the measurement period, while Ca remained virtually constant at 9.5 ± 0.1 mg·l$^{-1}$ (0.24 mM). Concurrent with the reduction in $C_T$, pH increased to 10.0 by the end of the measurement period. The ion activity product $[Ca^{++}][CO_3^{--}]$ increased from a level near saturation to over ten times the solubility product. In spite of this supersaturation, precipitation of CaCO$_3$ did not occur. Alkalinity, calculated from $C_T$ and pH, remained virtually constant ($\bar{x} = 1.74 \pm 0.01$ S.E.). Acidity, also calculated from $C_T$ and pH, declined almost linearly over the measurement period in a manner similar to that for $C_T$.

3. Photosynthetic $C_T$ uptake of H$_2$CO$_3$ or HCO$_3$-, in the absence of associated CaCO$_3$ precipitation, results in a decline in acidity while alkalinity remains constant—the change in acidity equaling 2 meq/mM change in $C_T$. In the high $C_T$, low cation solution, precipitation of CaCO$_3$ did not occur, and alkalinity (calculated from $C_T$ and pH) remained constant (Figure B1, graph D). Thus, in short-term experiments such as these, changes in alkalinity due to cation uptake can be neglected.

4. Results obtained in the high cation, high $C_T$ solution are presented in graphs E, F, G, and H of Figure B1. The $C_T$ declined linearly over the first 60 min, then declined exponentially over the remainder of the measurement period. Calcium remained constant at 59.4 ± 0.2 mg·l$^{-1}$ (1.48 mM) during the first 60 min, then declined exponentially coincident with the exponential decrease in $C_T$. Both pH and the ion activity product $[Ca^{++}][CO_3^{--}]$ increased during the first 60 min, then declined as CaCO$_3$ precipitated.
Alkalinity, calculated from $C_T$ and pH, declined in a manner similar to that of Ca. Acidity declined almost linearly over the measurement period. By calculating the quantity of $C_T$ precipitated as CaCO$_3$ and adding this to the measured value of $C_T$, the decline in $C_T$ due to photosynthesis can be plotted (Figure B1, graph E). This correction for precipitation of CaCO$_3$ results in a nearly linear decrease in $C_T$ due to photosynthetic $C_T$ uptake in contrast with the exponential decrease in $C_T$ due to the combined effects of photosynthesis and CaCO$_3$ precipitation.

5. Precipitation of CaCO$_3$, in the absence of photosynthetic $C_T$ uptake, results in a decline in alkalinity while acidity remains constant—the change in alkalinity equaling 2 meq/mM change in $C_T$. In the high $C_T$, high cation solution, precipitation of CaCO$_3$ resulted in changes in alkalinity (Figure B1, graphs E and F). In this experiment alkalinity could be calculated from $C_T$ and pH as well as from changes in Ca. Linear regression of the two measures of alkalinity resulted in a slope of 1.00, an intercept of 0.011, and an $r^2$ of 0.98. The close agreement between the two indicates that the change in alkalinity was entirely attributable to precipitation of CaCO$_3$.

6. Since acidity is unaffected by CaCO$_3$ precipitation, and alkalinity is unaffected by photosynthetic $C_T$ uptake, these parameters should be useful in determining the individual rates of each process, even when both are occurring simultaneously. One obvious advantage of this approach is that it does not require analysis of solution Ca, only $C_T$ and pH.

7. Plots of the change in acidity against the change in $C_T$ due to photosynthesis should result in a slope of 2 meq acidity/mM $C_T$. Data obtained in the high $C_T$, low cation solution, where CaCO$_3$ precipitation did not occur, are plotted in Figure B2. These data are in close agreement with the theoretical slope ($m = 1.9$, $r^2 = 0.996$), indicating that acidity can be used as a measure of photosynthetic $C_T$ uptake under conditions where CaCO$_3$ does not precipitate.

8. Plots of the change in alkalinity against the change in $C_T$ or Ca due to CaCO$_3$ precipitation should likewise result in a slope of 2 meq/mM change $C_T$ or Ca. Data obtained from the high $C_T$, high cation solution, where CaCO$_3$ precipitation did occur, are plotted in Figure B3, graph A. The close agreement between the calculated slope (2.0) and the theoretical slope indicates that alkalinity (calculated from $C_T$ and pH) is an accurate measure
of CaCO$_3$ precipitation, even during photosynthetic $C_T$ uptake. Similarly, linear regression of acidity against the change in $C_T$ due to photosynthesis ($\Delta C_T - \Delta Ca$) results in a slope of 1.9, closely approximating the theoretical slope (Figure B, graph B). These results indicate that the parameters alkalinity and acidity can be used to differentiate the effects of CaCO$_3$ precipitation and photosynthetic $C_T$ uptake on measured changes in $C_T$ and pH. This method allows for the simultaneous determination of rates of photosynthesis and CaCO$_3$ precipitation under conditions where photosynthetic $C_T$ uptake results in the precipitation of CaCO$_3$, a fairly common occurrence in hard waters.

9. The similarity of the slopes of alkalinity and acidity during photosynthesis in the high cation, high $C_T$ solution (Figure B1, graph H) indicates that both the rate and quantity of CaCO$_3$ precipitation are similar to those for photosynthetic $C_T$ uptake. These data are plotted in Figure B4. Photosynthetic $C_T$ uptake initially exceeds CaCO$_3$ precipitation as can also be seen in Figure B1. However, as photosynthesis continues, the rate of precipitation increases and the two processes appear to be closely coupled. The slope calculated from linear regression ($r^2 = 0.998$) is 0.96 mM $C_T$ precipitated per mM $C_T$ assimilated in photosynthesis. This slope is in close agreement with the ratio of 0.90 calculated for this solution from carbonate equilibria and the solubility product for CaCO$_3$. It should be noted that the aberrant data points to the lower left in Figure B4 correspond to the period prior to the occurrence of steady-state conditions with respect to CaCO$_3$ precipitation in relation to photosynthetic $C_T$ uptake (Figure B1, graphs G and H). These data would thus not be expected to fit equilibrium calculations. The principles applied here might be used to predict the relationship between CaCO$_3$ precipitation and photosynthetic $C_T$ uptake in solutions of various alkalinity, $C_T$, and Ca$^{++}$ levels.
Figure B1. Temporal changes in $C_T$, Ca, pH, CaCO$_3$ ion activity product (IAP), alkalinity, and acidity during photosynthesis of *M. spicatum* in solutions containing the high level of $C_T$ and the low (graphs A, B, C, D) or high (graphs E, F, G, H) level of cations. Corrected $C_T$ values (graph E) are the sum of measured $C_T$ plus the quantity of $C_T$ precipitated as CaCO$_3$. Dual scales (mg·l$^{-1}$ and mM) are provided for $C_T$ and Ca for stoichiometric comparison.
Figure B2. Relationship between Δ acidity and ΔCₜ during photosynthesis of *M. spicatum* in the high Cₜ, low cation solution. The line represents the theoretical stoichiometric relationship
Figure B3. Alkalinity and acidity vs. $\Delta C_T$ and $\Delta C_T - \Delta Ca$. (A) Relationship between $\Delta$ alkalinity and $\Delta Ca$ during photosynthetic CaCO$_3$ precipitation in the high $C_T$, high cation solution. The line represents the theoretical stoichiometric relationship. (B) Relationship between $\Delta$ acidity and $\Delta C_T - \Delta Ca$ ($\Delta C_T$, corrected for CaCO$_3$ precipitation) during photosynthesis of *M. spicatum* in the high $C_T$, high cation solution. The line represents the theoretical stoichiometric relationship.
Figure B4. Relationship between CaCO$_3$ precipitation and photosynthetic $C_T$ uptake during photosynthesis of *M. spicatum* in the high $C_T$, high cation solution. The regression line was calculated from the data obtained during steady-state conditions.
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