ERDC/TN APCRP-EA-14
April 2007

Experimental Effects of Lime Application on Aquatic Macrophytes: 4. Growth Response of Three Species

by William F. James

PURPOSE: This investigation examined the growth response of three macrophyte species (*Elodea canadensis*, *Stuckenia pectinata*, and *Vallisnaria americana)* to lime application in experimental mesocosms.

BACKGROUND: Lime application may be an effective manipulation for temporarily stressing macrophyte growth by inducing dissolved inorganic carbon (DIC) limitation of photosynthesis. Although typically used to control internal phosphorus loading from sediments in eutrophic pelagic systems (Prepas et al. 1990), lime ($CaCO_3$ and $Ca(OH)_2$) applications have been shown to be effective in both suppressing submersed macrophyte growth and changing species composition in a variety of ponds, small lakes, canals, and dugouts (Babin et al. 1992; Chambers et al. 2001; Prepas et al. 2001a, 2001b). Submersed macrophytes favor free carbon dioxide (CO_2) but in moderately alkaline hardwater (pH range of 8 to 10) systems, bicarbonate (HCO_3^-) is the dominant form of inorganic carbon and mechanisms have evolved to use this source for photosynthesis (Prins et al. 1982, Bowes and Salvucci 1989, Madsen and Sand-Jensen 1991, McConnaughey and Whelan 1997). Addition of hydrated lime ($Ca(OH)_2$) to aquatic systems can increase pH and drive alkaline hardwater systems toward calcite formation and depletion of Ca^{+2}, CO_2, and HCO_3^- as follows:

$$Ca(OH)_2 = Ca^{+2} + 2OH^- \quad (1)$$

$$Ca^{+2} + 2HCO_3^- = Ca(HCO_3)_2 \quad (2)$$

$$Ca(OH)_2 + CO_2 = CaCO_{3(solid)} + H_2O \quad (3)$$

$$Ca(OH)_2 + Ca(HCO_3)_2 = 2CaCO_{3(solid)} + 2H_2O \quad (4)$$

In particular, lime-induced precipitation of Ca^{+2} may play as important a role in photosynthetic limitation as HCO_3^- precipitation for macrophytes that extract CO_2 from HCO_3^- via calcification (Lucas and Dainty 1977, McConnaughey 1998). Increased pH also shifts equilibrium toward bicarbonate and carbonate dominance, which can affect species that have a greater affinity for CO_2 (Maberly and Madsen 1998). As pH increases above 10.3, the dominant form of DIC is carbonate (CO_3^{-2}), which is generally unavailable for photosynthetic uptake (Lucas 1983). Thus, lime addition

can act to both remove DIC and cause shifts to unavailable forms at higher pH. Evidence also indicates that HCO_3^- uptake and carbon (i.e., CO_2, DIC, and HCO_3^-) compensation points vary as a function of macrophyte species (Allen and Spence 1981, Maberly and Spence 1983, Bowes and Salvucci 1989), suggesting the possibility that lime application rates may be adjusted for species-selective control of photosynthesis. The objectives of this study were to examine the growth response of three macrophyte species to various lime application rates that resulted in increased pH, decreased DIC, and a change in DIC species (i.e., free CO_2, HCO_3^-, CO_3^{-2}) concentrations. The author hypothesized that macrophyte growth could be temporarily or completely suppressed as a function of lime-induced DIC limitation and that growth response might vary for different macrophyte species. These hypotheses were tested using experimental mesocosms.

METHODS: *Elodea canadensis*, *Stuckenia pectinata*, and *Vallisnaria americana* were chosen as experimental plants for examination of the effects of lime treatment on growth at an outdoor mesocosm facility located in west-central Wisconsin (Eau Galle Aquatic Ecology Laboratory, Spring Valley, Wisconsin). Commercially obtained *S. pectinata* and *V. americana* tubers and apical tips (~ 10 cm length) of *E. canadensis*, collected in nearby lakes, were rooted in a sand medium in the laboratory in early May of 2005. One sprouted plant of each species was transplanted into a polyethylene container (10 cm wide x 10 cm deep x 15 cm height) filled with homogenized sediment (obtained from Eau Galle Reservoir, Wisconsin; moisture content = 71 percent; bulk density = 0.29 g mL^{-1}; total sediment N = 4.702 mg g^{-1}; porewater ammonium-N = 5.750 mg L^{-1}; total sediment P = 0.971 mg g^{-1}; porewater P = 0.359 mg L^{-1}) to a depth of 10 cm (~ 1 L of sediment) for growth in outdoor mesocosms. Eight replicate containers of each species were planted for each of three lime treatments and a control (i.e., no lime addition; see below for a description of the study design). An additional eight replicate containers were planted for the determination of biomass levels at the time of lime treatment. Thus, each mesocosm contained 16 containers of each species (total = 32 containers per mesocosm). Plants were allowed to grow in the mesocosms for 37 days before lime application.

Four clear fiberglass mesocosms (1.2 m dia x 1.2 m height; 1400 L capacity), filled with locally obtained tap water prior to the start of the experiment (Total alkalinity = 130 - 150 mg·L^{-1}; total Ca = 57 mg·L^{-1}; Conductivity = 422 µS; Mg = 28 mg·L^{-1}; NO_2NO_3-N = 0.2 mg·L^{-1}; Na = 1.6 mg· L^{-1}; K = 0.8 mg· L^{-1}; SO_4 = 21 mg·L^{-1}; initial pH = 7.8), were set up in late spring to house the plant species under different treatment conditions. Natural lighting was controlled with a 30-percent shade cloth deployed 2 m above mesocosm surfaces. Circulation pumps (Beckett Versa Gold G90AG; 0.34 m^3·min^{-1}) provided gentle water circulation in each tank during the entire study; thus, equilibration between atmospheric and aqueous phases of CO_2 occurred via diffusional processes.

Experimental lime treatment was designed to maintain pH in outdoor mesocosms within elevated ranges for approximately one week in order to manipulate HCO_3^- alkalinity and DIC concentrations in the water column during plant growth. Target pH ranges were 9.8-10.0 (referred to as the *low* application rate), 10.3-10.5 (referred to as the *moderate* application rate; at the bicarbonate-carbonate equivalence point), and 10.8-11.0 (referred to as the *high* application rate). A jar test was conducted on mesocosm water prior to lime application in order to estimate the concentration (as mg $Ca(OH)_2$·L^{-1}) required to adjust the pH to the different experimental levels (Figure 1) and lower HCO_3^- alkalinity and DIC (Figure 2 and Table 1). For initial application, commercially obtained lime (as $Ca(OH)_2$) was applied as a slurry to experimental mesocosms by mixing the appropriate dry

powder mass for each intended concentration with 8 L of tap water, then dispersing it evenly over the surface of each mesocosm. pH was monitored daily during the first week of treatment and small amounts of lime were added on day 6 of treatment to maintain pH at target levels. No further lime additions were made after 7 days and the mesocosms were allowed to re-equilibrate for the remainder of the study. Plants were allowed to grow for 42 days after treatment.

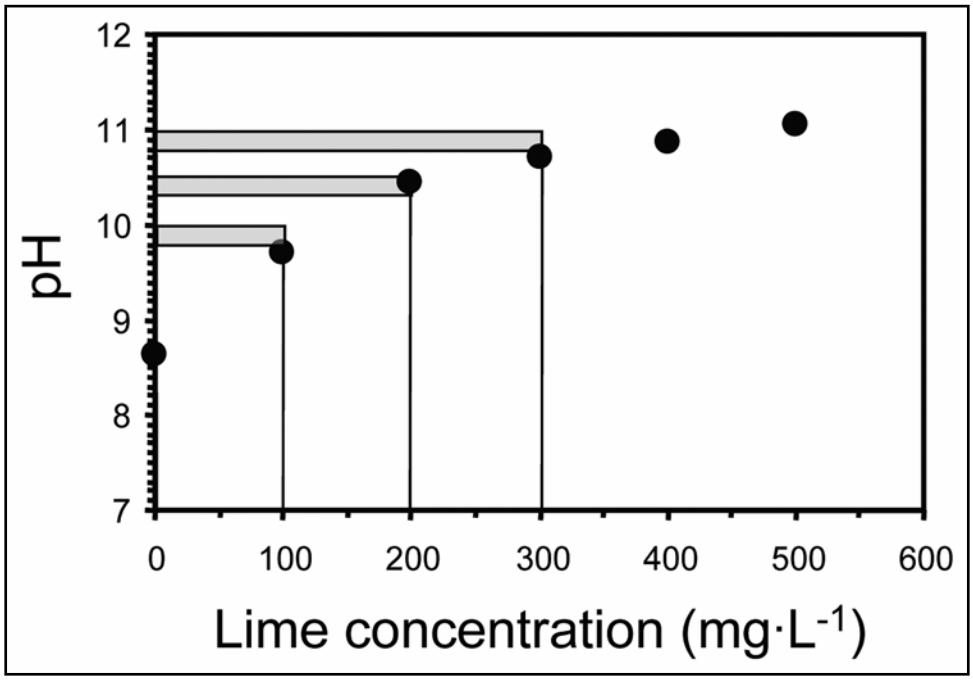

Figure 1. The effects of various concentrations of lime (as $Ca(OH)_2$) on the pH of mesocosm water. Grey horizontal bars represent the desired pH target ranges and the vertical black lines denote the required concentration of lime.

Shoot and root biomass were determined both at the time of lime application and at the end of the study. Shoot biomass was briefly soaked in a 1 N hydrochloric acid solution to remove any calcium carbonate deposits on the plant, gently rinsed several times in tap water, and dried at 65 ^{o}C for dry mass determination. Roots sieved from the sediment were dried for below-ground biomass determination (root material was not pretreated with 1 N HCl). Relative growth rates for shoots and roots (RGR; d^{-1}) were calculated as:

$$RGR = \frac{\left(\ln M_2 - \ln M_1\right)}{t} \tag{5}$$

where M_1 and M_2 were the biomass at the time of lime treatment and at the end of the study period, respectively, and t was the incubation time period (days) after lime application. Relative growth rate ratios (RGRR, dimensionless) were calculated as:

$$RGRR = \frac{RGR_{control}}{RGR_{treated}} \tag{6}$$

where $RGR_{control}$ and $RGR_{treated}$ were the relative growth rates of shoots or roots grown under control and lime treatment conditions, respectively.

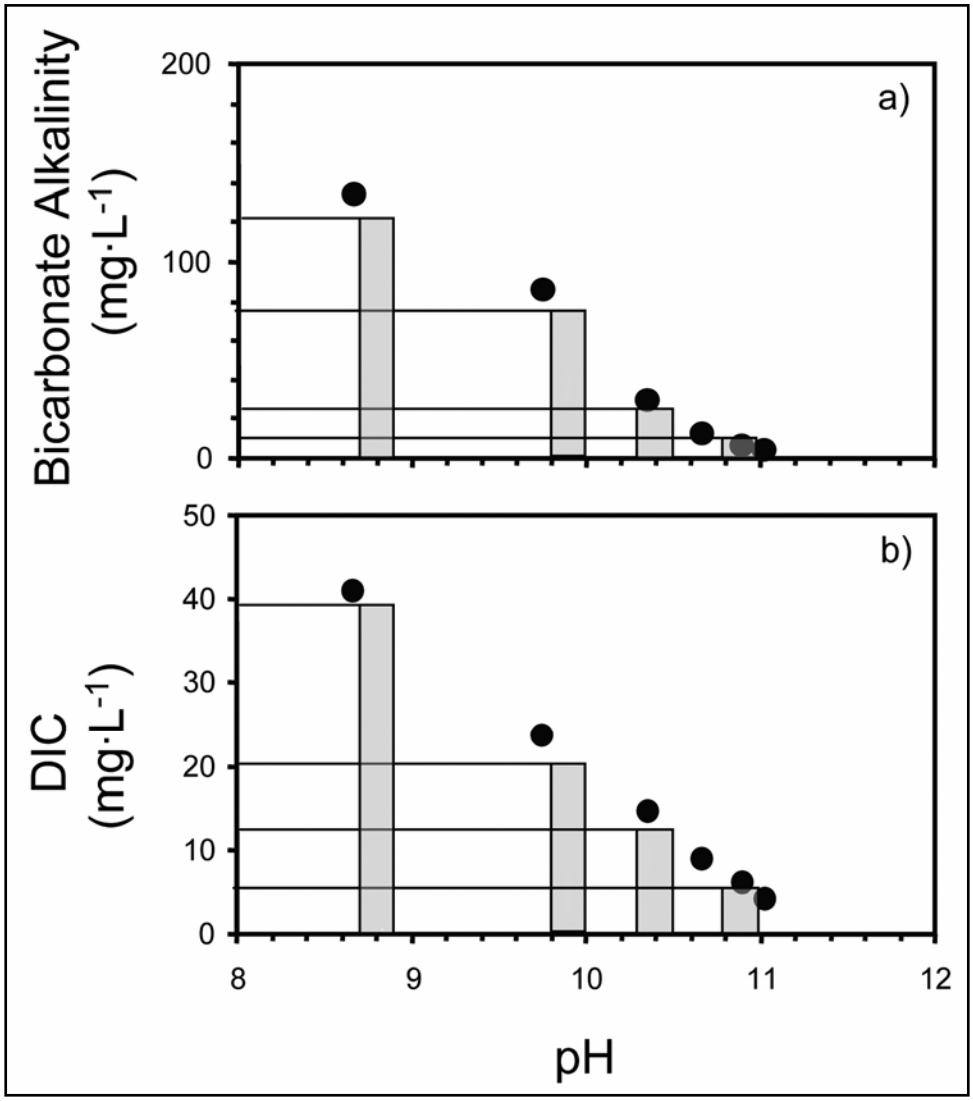

Figure 2. Variations in bicarbonate alkalinity (a) and dissolved inorganic carbon (b) as a function of lime-adjusted increases in pH.

Table 1
Initial Lime Application Rates Plus Additions Used to Maintain pH Within Three Treatment Ranges for a One-Week Period*

Initial pH	Initial total alkalinity ($mg \cdot L^{-1}$)	Initial DIC ($mg \cdot L^{-1}$)	Lime application rate ($mg \cdot L^{-1}$)		
			(*Low*) pH range 9.8 - 10.0	(*Moderate*) pH range 10.3 - 10.5	(*High*) pH range 10.8 - 11.0
8.94 (0.07)	132.0 (1.4)	29.0 (0.6)	100 + 22	200+22	300+22

* Initial means (± 1 S.E.; n = 4) for pH, total alkalinity, and dissolved inorganic carbon (DIC) represent conditions before lime treatment.

Throughout the study, in situ temperature, pH, dissolved oxygen, and conductivity were monitored in each mesocosm at 1- to 2-day intervals using a data sonde (Hydrolab Quanta System; Hach Company, Loveland, CO) that was calibrated against known buffers and Winkler titrations. Integrated water column samples were collected to determine alkalinity species, DIC, and dissolved calcium (DCa). Total alkalinity (expressed as mg $CaCO_3 \cdot L^{-1}$) was determined via titration with 0.02 N sulfuric acid to an end-point of pH 4.5 (American Public Health Association (APHA) 1998). Free CO_2 and HCO_3^-, CO_3^{-2}, and OH^- alkalinities at 25 °C were estimated by calculation based on ionization constants (APHA 1998). Mean mesocosm temperature over the study period at 24.91 °C (± 0.04 S.E.) was close to the standard temperature ionization constants. Alkalinity titrations were conducted within 1 to 2 hr of sampling. Samples for DIC were immediately filtered through a 0.45-µm syringe filter, carefully preserved in glass scintillation vials (no air headspace) in a refrigerator at 4 °C, and analyzed within 48 hr of collection by infrared spectroscopy (Shimadzu model TOC-5050; Shimadzu Scientific Instruments, Columbia, MD). DCa was determined using flame atomic absorption spectroscopy (Perkin-Elmer AA Analyst 100; Perkin Elmer Life and Analytical Sciences, Inc., Wellesley, MA) after filtration through a 0.45-µm syringe filter (APHA 1998).

RESULTS: Initial lime application to experimental mesocosms on 21 June was followed by a supplemental small lime addition on day 6 (final concentration additions = 122 $mg \cdot L^{-1}$, 222 $mg \cdot L^{-1}$, and 322 $mg \cdot L^{-1}$) in order to main pH within target ranges for one week (Figure 3). After cessation of lime application, pH recovered to near control levels for all lime-treated mesocosms and pH was less than 10.0 by 12 July. Free CO_2 declined to 0.011 $mg \cdot L^{-1}$ (91-percent reduction relative to the control) in conjunction with the low lime application rate and it was near zero (i.e., > 99-percent reduction relative to the control) in mesocosms treated at the moderate and high lime application rate. HCO_3^- alkalinity declined by 58 and 92 percent, while DIC declined by 53 and 86 percent, over control levels shortly after lime application for mesocosms treated at the low and moderate lime application rates, respectively. The highest lime application rate resulted in little additional initial decrease in HCO_3^- alkalinity and DIC; these variables exhibited a similar time series pattern as observed for the mesocosm treated at the moderate lime application rate. Although there was a trend of increasing concentration as a function of time in all treated mesocosms between June and late July, suggesting some re-equilibration with atmospheric CO_2, HCO_3^- alkalinity, DIC, and free CO_2 concentrations in treated mesocosms were well below control levels throughout the remainder of the study period.

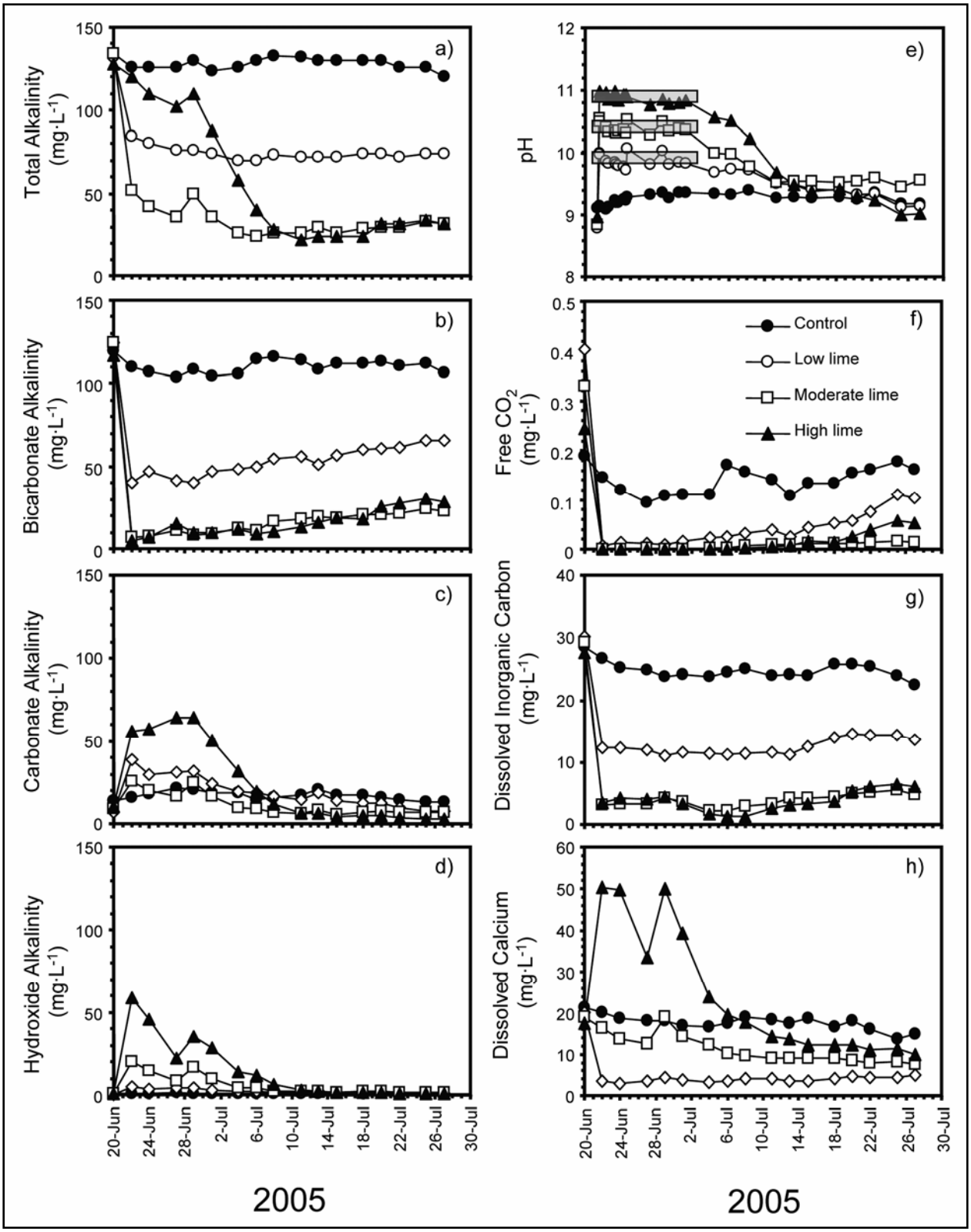

Figure 3. Variations in total alkalinity (a), bicarbonate alkalinity (b), carbonate alkalinity (c), hydroxide alkalinity (d), pH (e), free carbon dioxide (CO2, f), dissolved inorganic carbon (g), and dissolved calcium (h) in control and experimental mesocosms treated with low, moderate, and high concentrations of lime (as Ca(OH)3).

In conjunction with decreases in HCO_3^- alkalinity and DIC, DCa declined relative to control concentrations at the low and moderate lime application rates, indicating calcite precipitation. However, DCa substantially exceeded control levels at the high lime application rate between 21 June and 6 July, due to complete precipitation of HCO_3^- as calcite. As mesocosm pH rebounded from initial treatment in mid-July, DCa concentrations were greatest in the control mesocosm followed by DCa in mesocosms subjected to the high > moderate > low lime application rate (Figure 3).

Calcite precipitation after lime application resulted in declines in total alkalinity relative to controls at the low and moderate application rates (Figure 3). Declines in total alkalinity at the high application rate were initially offset by increases in CO_3^{-2} and OH^- alkalinity due to high pH. In particular, CO_3^{-2} and OH^- alkalinity increased in this mesocosm by 300 percent and 46 percent, respectively, relative to the control mesocosm. However, after cessation of lime application, total alkalinity declined rapidly in the mesocosm subjected to the highest lime application rate to levels observed in the moderate lime application rate, with accompanying decreases in CO_3^{-2} and OH^- alkalinity. Between early July and the end of the study, total alkalinity was greatest in the control mesocosm while it was lower by 38, 73, and 73 percent in mesocosms treated at the low, moderate, and high application rate, respectively.

Differential macrophyte growth response was observed as a function of the various lime application rates. For instance, net shoot growth was completely suppressed for both *S. pectinata* and *E. canadensis*, and both species exhibited some net shoot biomass loss and a negative shoot RGRR at the highest application rate (Figure 4). Net shoot growth and a positive shoot RGRR occurred for both species at the moderate and lower lime application rates; although means were significantly lower than the control. In contrast, *V. americana* exhibited negative shoot RGRR at both the moderate and highest lime application rate. Net shoot growth and positive shoot RGRR occurred at the lowest lime application rate but it was suppressed relative to shoot growth in the control mesocosm. Net root growth and root RGRR generally exhibited a similar interspecies pattern (Figure 5).

DISCUSSION: Little is known about macrophyte growth at very high pH (i.e., > 10). Titus and Stone (1982) demonstrated that inorganic carbon uptake by *Myriophyllum spicatum* and *V. americana* declined substantially when pH was increased in increments from 7.0 to 9.0 while DIC levels were held constant. Higher pH resulted in decreased CO_2 concentration and a shift in DIC equilibrium to HCO_3^- dominance, which affected uptake. However, other factors in addition to lower CO_2 such as differences in bicarbonate saturation between the two species appeared to affect uptake response. Van et al. (1976) found negative relationships between pH increase, decreased CO_2 availability, and decreased photosynthetic rate for *Hydrilla verticillata*, *Myriophyllum spicatum*, and *Ceratophyllum demersum*, indicating that pH increases indirectly impacted photosynthesis by causing shifts in equilibrium. In the present study, both DIC and HCO_3^{-2} alkalinity declined in concentration at high pH, but concentration differences were not detected between the moderate and high lime application rates. Yet, shoot and root growth and RGRR were significantly impacted at the highest lime application rate, versus the moderate lime application rate, particularly for *S. pectinata* and *E. canadensis*. CO_3^{-2} alkalinity increased substantially at the highest lime application rate while it was much lower and nearer to control levels for the other lime treatments, suggesting that perhaps CO_3^{-2} dominance was completely inhibiting growth and photosynthesis (Raven 1970, Lucas 1983,

Sand-Jensen 1983) as pH increased above ~10.5. In addition to increased CO_3^{-2}, high pH (OH^-) may have also somehow impacted plant physiology and cell structure at the highest treatment rate, causing negative RGRR at the highest lime application rate. Bowes and Salvucci (1989) suggested that OH^- may compete with HCO_3^- for transported H^+ for macrophytes that convert HCO_3^- to CO_2 by active H^+ extrusion (polar leaf mechanism, Prins et al. 1982).

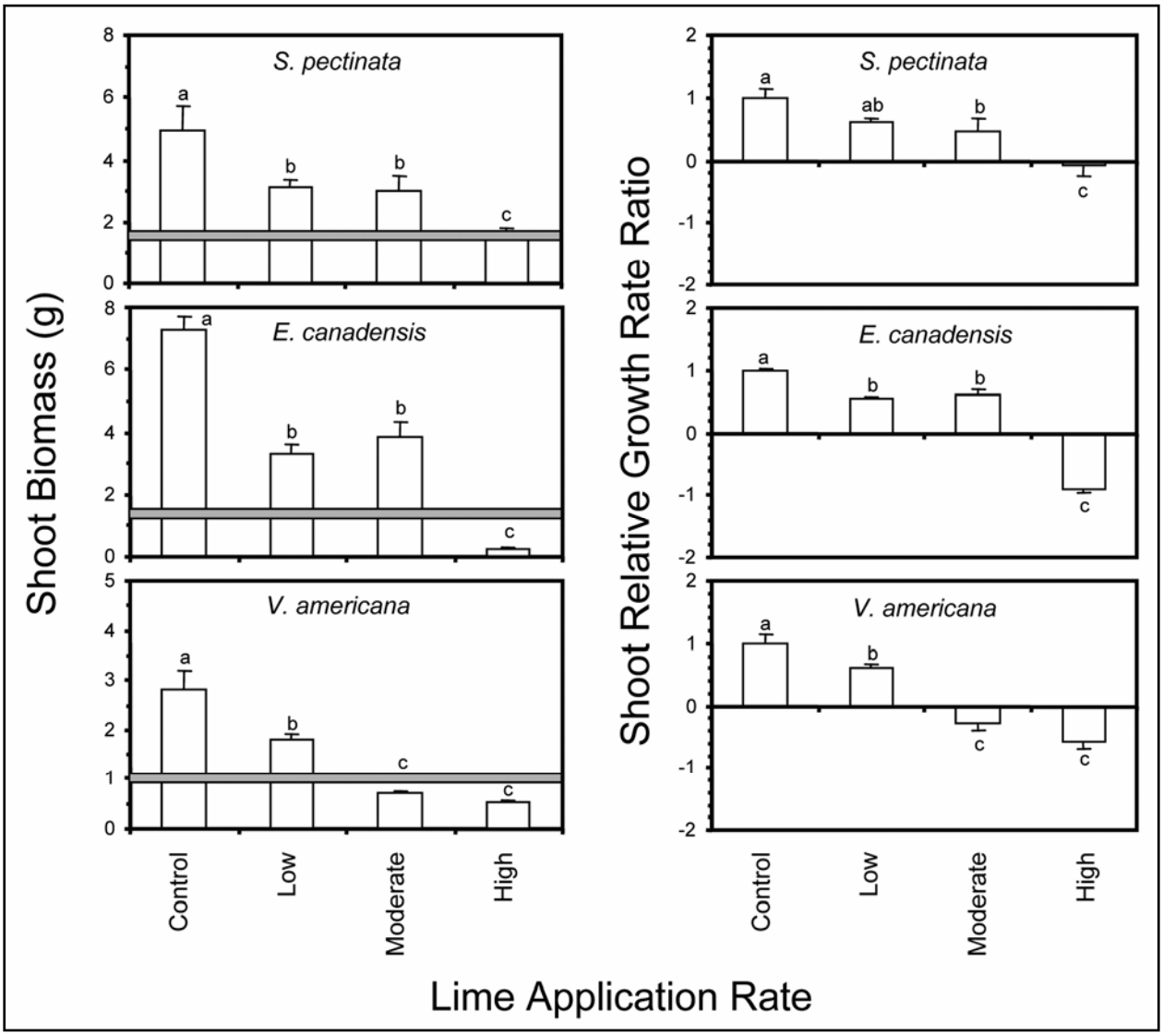

Figure 4. Variations in mean shoot biomass and relative growth rate ratio as a function of control, low, moderate, and high lime application rates. Grey horizontal bar represents mean shoot biomass at the time of treatment; its width is equal to 2 standard errors. Vertical lines represent 1 standard error of the mean. Different letters represent significant differences ($p<0.05$) based on ANOVA (Statistical Analysis System (SAS) 1994).

Related to macrophyte growth at high pH, James et al. (2005) found that *S. pectinata* lost chlorophyll pigmentation as a result of lime application. Pigment loss was temporary at the lower application rates and macrophyte chlorophyll recovered due to new growth as pH declined to nominal values. But for lime treatments that resulted in maintenance of pH above ~ 10.5 for several

weeks, they found that macrophyte chlorophyll loss was permanent, which coincided with the occurrence of net biomass loss. Chambers et al. (2001) reported similar observations of pigment loss for macrophytes subjected to lime-induced high pH. Reasons for this response are not known and more research is needed to increase the understanding of the effects of very high pH on chlorophyll and photosynthesis.

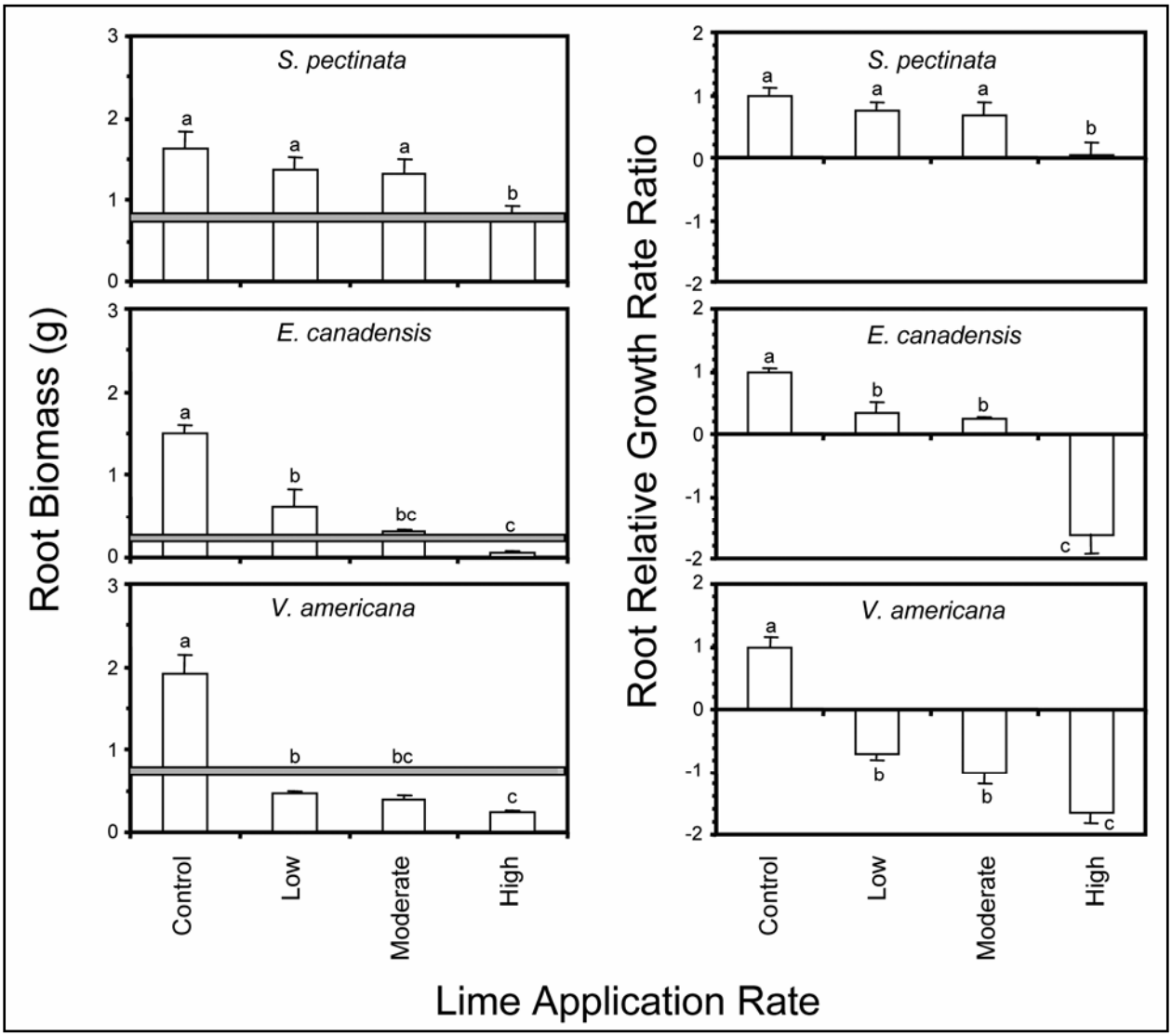

Figure 5. Variations in mean root biomass and relative growth rate ratio as a function of control, low, moderate, and high lime application rates. Grey horizontal bar represents mean root biomass at the time of treatment; its width is equal to 2 standard errors. Vertical lines represent 1 standard error of the mean. Different letters represent significant differences ($p<0.05$) based on ANOVA (SAS 1994).

The low to moderate application rates appeared to only temporarily disrupt growth of the three species examined. Some net growth occurred by the end of the study for all species under these treatments, suggesting that lime application acted as a growth inhibitor by temporarily limiting DIC availability versus acting as an herbicide. The author hypothesizes from these results that lime application at low to moderate rates stresses or temporarily stops net growth by lowering DIC

concentrations below the DIC compensation point. Some net growth then occurs as DIC levels recover to levels greater than the compensation point. James et al. (2005) found that new growth occurred in the form of buds for *S. pectinata* as HCO_3^- alkalinity increased above ~ 20 $mg \cdot L^{-1}$ after lime application. An unknown factor is the effect of exposure time to growth-limiting DIC concentrations on growth resiliency. Longer exposure times to DIC concentrations less than the compensation point would likely cause some net biomass loss if respiration is not in balance (i.e., exceeds) with net productivity, resulting in a lower probability of recovery after the stress has been removed.

Differential growth response was observed at the low and moderate lime application rates, suggesting species-specific tolerances to both DIC concentration and the form of DIC that was available for uptake. *E. canadensis* and *S. pectinata* shoot and root growth appeared to be more tolerant of very low DIC that was in the form of HCO_3^- than *V. americana* as both former species exhibited positive shoot and root RGRR at the moderate lime application rate. In contrast, *V. americana* and shoot and root growth appeared to be more susceptible to DIC limitation and HCO_3^- dominance as shoot and root RGRR was negative at the low to moderate lime application rates. All species selected for this study were efficient bicarbonate users (Maberly and Spence 1983, Madsen and Sand-Jensen 1991). However, the combination of low DIC and predominance of HCO_3^- at higher pH may have affected photosynthesis and RGRR differentially due, perhaps, to species-specific differences in the carbon (HCO_3^-) compensation point. Titus and Stone (1982) found that carbon compensation points for *M. spicatum* and *V. americana* increased as a function of increasing pH due to increased dominance of HCO_3^-. These results, in combination with observed decreases in DIC uptake, suggested that net photosynthesis was limited by HCO_3^- concentration at higher pH. Application of lime at the low to moderate rates to both lower DIC concentration and shift equilibrium to HCO_3^- dominance may have a similar impact on growth and photosynthesis as observed by Titus and Stone. Differential exploitation strategies for sequestering inorganic carbon when DIC is limiting may have explained the differing growth responses observed in the present study. Strategies include root uptake of DIC from sediment porewater, aerial leaf development, C_4 and CAM metabolism, and bicarbonate uptake (see review by Madsen and Sand-Jensen (1991)). However, the species used in this study fell predominantly within the category of HCO_3^- uptake exploitation and they are usually found in lakes with moderate to high alkalinity (Vestergaard and Sand-Jensen 2000), supporting the hypothesis that HCO_3^- compensation point played an important role in differential growth response to lime treatment.

Macrophyte growth responses to lime appeared to be consistent with results obtained from a series of field lime treatments conducted by Chambers et al. (2001); Prepas et al. (1990, 2001a, 2001b); and Reedyk et al. (2001). Those studies (summarized in Chambers et al. (2001)) suggested that lower lime dosages on the order of 100 $mg \cdot L^{-1}$ (and lower pH) resulted in suppression of growth while higher dosages (200-300 $mg \cdot L^{-1}$) usually resulted in complete elimination of macrophyte biomass. In addition, they found that treatment with lower concentrations of lime, and resultant modest pH increases (9-10), were accompanied by changes in species assemblage. For instance, lime treatment of Lower Helig Pond resulted in eradication of *M. excalbescens* but not *S. pectinata*. pH did not exceed 9.0 during post-treatment, suggesting that threshold-limiting HCO_3^- concentrations had not been achieved for the latter species. Results from the present study suggested that both selective control of species and overall biomass growth suppression may be possible by adjusting lime application rates to lower DIC in relation to the HCO_3^- compensation point of target species.

One week of exposure to lime-induced elevated pH in mesocosms appeared to be sufficient to impact net shoot and root growth for the species examined in this study. It was important to note that DIC concentrations remained well below control levels in the lime-treated mesocosms even though pH recovered in these systems within 20 days of initial lime application. Thus, exposure to low DIC was sustained in the mesocosms for about 37 growing days. In an open-water field treatment scenario, recovery from lime-induced DIC limitation may be more rapid due to reaeration and DIC inputs from benthic respiration. Thus, initial field lime treatments may need to be supplemented with additional applications to maintain desired DIC levels for a longer period of time. Lime blocks or porous containers filled with lime and deployed in the treatment area for a short period of time may aid in sustaining pH and DIC levels for a longer period of time after an initial lime application. Experimental results presented here also suggested that lime treatments probably need to be considered as a whole-lake or large embayment manipulation versus a spot treatment for controlling macrophyte biomass and species assemblage, since it appears to be acting as a growth inhibitor by modifying DIC concentration and form in the water column.

ACKNOWLEDGMENTS: Harry L. Eakin, Eau Galle Aquatic Ecology Laboratory, ERDC, and Laura J. Pommier, ASI Corporation, are gratefully acknowledged for their participation in the experimental setup and chemical analyses. The author also thanks John W. Barko for invaluable discussion on macrophyte ecology.

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This technical note should be cited as follows:

> James, W. F. 2007. *Experimental effects of lime application on aquatic macrophytes: 4. Growth response of three species.* APCRP Technical Notes Collection (ERDC/TN APCRP-EA-14). Vicksburg, MS: U.S. Army Engineer Research and Development Center. *www.wes.army.mil/el/aqua.*

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