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Submersed macrophyte growth at low pH

I. CO$_2$ enrichment effects with fertile sediment

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Summary. *Vallisneria americana* was grown for six weeks in a greenhouse on relatively fertile sediment to test for factors other than nutrient limitation which may slow growth of this submersed macrophyte at pH 5. On the basis of dry mass accumulated, (1) low pH significantly depressed *Vallisneria* growth at constant free CO$_2$ levels; (2) free CO$_2$ enrichment, however, greatly stimulated *Vallisneria* growth at pH 5, by 2.8-fold and 10-fold at 3.2 times and 10 times air-equilibrated CO$_2$ levels, respectively; and (3) growth was greater by far at pH 5 than at higher pH with constant total dissolved inorganic carbon (DIC). Free CO$_2$ availability was thus an important controller of growth at low pH by *Vallisneria americana* on fertile sediment, and low pH was not directly deleterious. Field surveys of acidic lakes in the Adirondack Mountains of New York state revealed that DIC levels in low pH lakes were often well above equilibrium values and could potentially support vigorous macrophyte growth. Aluminum and/or iron toxicity did not appear to impair growth at low pH, and aluminum concentrations in *Vallisneria* shoots significantly decreased with increasing free CO$_2$ concentrations at pH 5.0, perhaps due to growth dilution. Rosette production (a measure of asexual reproduction), maximum leaf length, and extent of flowering within treatments were positively correlated with plant biomass, rather than with pH or free CO$_2$ levels *per se*.

Key words: Submersed macrophyte – Growth – pH – Carbon dioxide

Physiological challenges posed by acidic freshwater ecosystems to submersed macrophytes are poorly understood. Potential consequences of acidic environments, better known for terrestrial plants and likely to be imposed upon their roots, include hydrogen ion toxicity, limited nutrient availability, and toxicity of metals more soluble at low pH (Rorison 1980). In contrast, in acidi-

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fied lakes shoots of submersed macrophytes are continuously bathed in an acidic medium, while roots live in less readily acidified surroundings (Kelly et al. 1984). In addition to hydrogen ion, nutrient, or metal problems, shoots may experience reduced availability of photosynthetic carbon sources at low pH (e.g. Roelofs et al. 1984; Wetzel et al. 1985). A series of laboratory and field experiments was undertaken to evaluate effects of environmental change potentially associated with lake acidification on submersed macrophyte shoot growth. The primary objective of the work reported here was to evaluate the relative importance of pH and independently controlled CO$_2$ availability for growth of *Vallisneria americana* Michx., a submersed species rarely found at low pH.

Literature abounds on photosynthetic responses of submersed macrophytes to alterations in environmental pH or concentration of photosynthetic carbon sources (e.g. Steemann Nielsen 1947; Beer and Wetzel 1981; Titus and Stone 1982). Our experimental approach differs from most of these studies in that we focused primarily on responses to low pH where bicarbonate use is not a central issue. Further, short-term measures of photosynthetic rates may not parallel longer-term growth rates (Elmore 1980; Kramer 1981), particularly when detrimental influences such as nutrient limitation or metal toxicity at low pH may develop gradually. Our greenhouse experiment extended over several weeks to allow determination of macrophyte growth responses to different combinations of pH and free CO$_2$ availability.

Grisé et al. (1986) earlier determined that biomass accumulation by *Vallisneria americana* was sharply curtailed at pH 6 and especially pH 5 in relation to that at pH 7.5. Coupling this result with obvious leaf deterioration and high tissue concentrations of aluminum and iron in plants grown at pH 5, Grisé et al. (1986) suggested that metal toxicity may have caused growth reduction at low pH. We here test the alternative hypothesis that such a growth reduction could result from a decreased supply of photosynthetic carbon sources. Assuming a water column in equilibrium with the atmosphere, the concentration of total dissolved inorganic

carbon (DIC) should be approximately 90% less at pH 5 than at pH 7.5 (using equilibrium constants given by Stumm and Morgan 1970) by virtue of the loss of most of the bicarbonate. Another perspective on this same alternative hypothesis is that CO_2 enrichment at pH 5 should relieve growth limitation apparently imposed by low pH in the earlier work, and thus that neither the hydrogen ion concentration at pH 5 nor the regimes of soluble metals engendered at pH 5 are necessarily deleterious to *Vallisneria* growth. We tested this form of the alternative hypothesis, and also evaluated whether the DIC levels of many lakes and ponds at relatively low pH were in atmospheric equilibrium.

Materials and methods

Study species

Vallisneria americana Michx. (Hydrocharitaceae; hereafter *Vallisneria*) overwinters as a vegetative bud buried in the sediment. From the winter bud in late spring, a short stem elongates to the sediment/water interface, where a basal rosette of ribbon-like leaves is produced. The relatively shallow root system develops adventitiously near the stem apex. Additional rosettes are typically produced on elongated axillary stolons throughout the growing season. This dioecious species commonly flowers in mid-summer and sets seed by late summer. Some stolons grow down into the sediment and form winter buds prior to the senescence of the parent plant in fall.

Vallisneria occurs in a range of oligotrophic to eutrophic sites on various sediment types, but is apparently rare in low pH waters (Crow and Hellquist 1982). *Vallisneria* is hardy, grows relatively rapidly, and is easily transplanted when collected prior to the elongation of its first roots in spring.

Sediment

Sediment is the primary source of nutrients and acid-soluble metals, at least in our experimental system, and we chose a well-buffered silty sediment from alkaline Otsego Lake (Otsego County, NY). This sediment has supported robust *Vallisneria* growth in the field. We reasoned that nutrient availability in this sediment would permit vigorous growth under otherwise favorable conditions, and thus nutrient limitation would not confound our assessment of the influences of the concentrations of hydrogen ions, acid-soluble metals, and photosynthetic carbon sources.

Experimental design

Independent control of pH and free CO_2 concentration (see below) permitted the six-treatment array shown in Fig. 1a. Each is a unique combination of pH and free CO_2 concentration, and thus bicarbonate concentration, which we report as a three-part experiment: (1) pH and total DIC varied with constant free CO_2 (treatments a, b, and c; d and e); (2) free CO_2 and total DIC varied with constant pH (treatments a, d, and f; b and e); and (3) pH and free CO_2 varied with constant total DIC (treatments c, e, and f; b and d).

Planting, harvesting, and tissue analysis

Divers collected young *Vallisneria* rosettes with winter buds intact on 10 June 1986 from Otsego Lake. Rosettes were blotted to re-
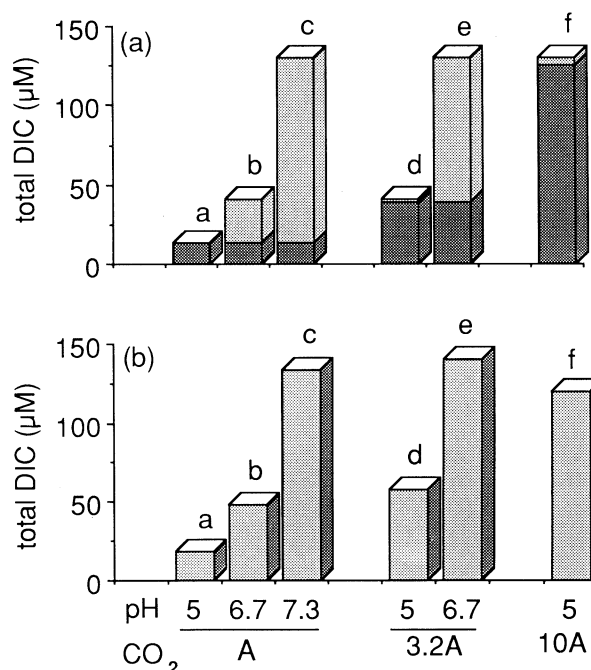

Fig. 1 a, b. Total dissolved inorganic carbon (DIC) concentrations for experimental treatments. (**a**) Experimental design, indicating pH, free CO_2 level (dark shading) and HCO_3 level (light shading) for each treatment. (**b**) Actual DIC values averaged over six weeks. Lower case letters are treatment codes

move excess water and individually weighed to determine fresh mass. Plants were grouped into one of the following size classes: (1) 1.45–2.11 g, (2) 2.12–2.48 g, (3) 2.49–2.83 g, (4) 2.83–3.34 g, or (5) 3.34–4.92 g. We randomly allocated five replicates of each size class to each treatment. On 16 June, we transplanted each rosette into 15 cm deep sediment in a 16 cm (top diameter) plastic pot, covered the sediment with ca. 2 cm of coarse quartz sand to minimize sediment/water nutrient interchange, and placed the pot in a randomly selected position within a 1200 L fiberglass tank. Each treatment was assigned to a randomly selected tank.

We immersed plants in a culture solution containing 0.63 mM $CaCl_2$, 0.15 mM $KHCO_3$, and 0.28 mM $MgSO_4$, modified from Smart and Barko (1985) by the removal of $NaHCO_3$. We increased water depth from 15 cm initially to 70 cm simultaneously in each tank as plants grew. Refrigerated circulators controlled water temperature at $23 \pm 2°$ C, Horizon 5997 pH-controllers maintained pH within 0.2 units of desired levels by automatic dropwise addition of 1 N NaOH or 1 N acid (70% H_2SO_4 and 30% HNO_3 by volume), and Tylan FC-280 mass flow controllers regulated the enrichment of compressed air lines with pure CO_2. One layer of Chicopee neutral density shadecloth (Lumite 25) placed across the top of each tank reduced incident quantum flux density by ca. one-third. The refrigerated circulators and bubbling air streams provided mixing.

Monitoring in each tank included: (1) temperature, at least twice daily, as a check on the circulators; (2) total DIC, approximately three times weekly, with infrared gas analysis (Analytical Development Co., Model 225 Mk II gas analyzer and Hewlett-Packard 3390A Integrator) of CO_2 sparged from acidified tank water samples; and (3) specific conductance and calcium concentration, both approximately once every ten days, with a Radiometer CDM 80 conductivity meter and an Orion 93-20 calcium electrode, respectively. We assumed an activity coefficient of 0.8 for calcium. Occasional algal blooms necessitated filtration with diatomaceous earth filters to maintain water clarity. Control of total DIC required frequent monitoring by analysis of tank water and readjustment of mass flow controllers. Means determined by

integrating DIC concentrations over the course of the experiment (Fig. 1b) were generally close to target values (Fig. 1a), but somewhat higher for treatments a and d and somewhat lower for treatment f. Conductivity and calcium levels both rose gradually during the experiment to average values of 440 μS·cm^{-1} and 2.0 mM.

Plants grew for six weeks, and then were harvested (except roots), and the number of rosettes, maximum leaf length, and if flowering, sex and the number of readily visible flowers (females) or inflorescences (males) for each plant noted. No winter buds had formed within the six weeks. Harvested plants were dried to constant weight at 85° C, weighed, and ground to pass a ♯ 40 screen with a stainless steel Wiley mill, except small plants, which were ground to fine powder in a mortar and pestle with liquid nitrogen. Ground samples were digested in a mixture of hydrogen peroxide and sulfuric acid (Allen et al. 1974) in a Technicon block digester, and analyzed for nitrogen content with a salicylic acid method (Havilah et al. 1977), for phosphate content with a molybdenum blue procedure (Murphy and Riley 1962), for aluminum content with a hydroxyquinoline method (James et al. 1983), and for iron content with atomic absorption spectrophotometry using a Perkin-Elmer 2380 spectrophotometer.

Statistical analysis

The experimental design allowed for four treatment comparisons to test pH effects (treatments a, b, and c; d and e; c, e, and f; b and d) and for two treatment comparisons to test CO_2 effects (treatments a, d, and f; b and e). In each case, final dry mass data were subjected to two-way analysis of variance (ANOVA; SAS Institute 1985) with pH or CO_2 as one independent variable, and initial size class as the other. Dry mass data were log-transformed (natural logarithms of 100 times dry mass values) so that significant interaction terms would indicate that treatments had a greater proportional impact on some size classes than others. Due to the use of the same means in two or three ANOVA's each, we adjusted alpha with the modified Bonferroni correction (Keppel 1982). Tissue analysis data were subjected to one-way ANOVA.

DIC survey

The marked growth responses to CO_2 enrichment at low pH (see below) motivated a survey of Adirondack Mountain (northern New York state) lakes to determine if total DIC levels were near those expected for lakes in atmospheric equilibrium. We selected 16 lakes and ponds of the southwestern and southcentral Adirondacks with relatively low pH and within 2 km of a road so that all could be sampled within two days. For each lake, water samples were collected ca. 25 cm deep in ca. 1 m of water in glass-stoppered B.O.D. bottles, and kept on ice until subjected to infrared gas analysis later in the day. We also determined pH (Orion 407A field pH meter) and temperature for each of the lakes sampled in October 1986 and monthly from May to September 1988. Below we present only growing season data (June, July, and August 1988) for one site in each of 16 lakes with the exception of two sites sampled in Big Moose Lake in August.

Results and discussion

Growth: pH effects with constant free CO_2 levels

Mean *Vallisneria* biomass at ambient free CO_2 levels increased 1.3-fold from pH 5 to pH 6.7, and 2.1-fold from pH 5 to pH 7.3 (treatments a, b, and c in Fig. 2a). These significant differences (Table 1) are in accord with biomass increases from pH 5.0 to pH 7.5 reported ear-
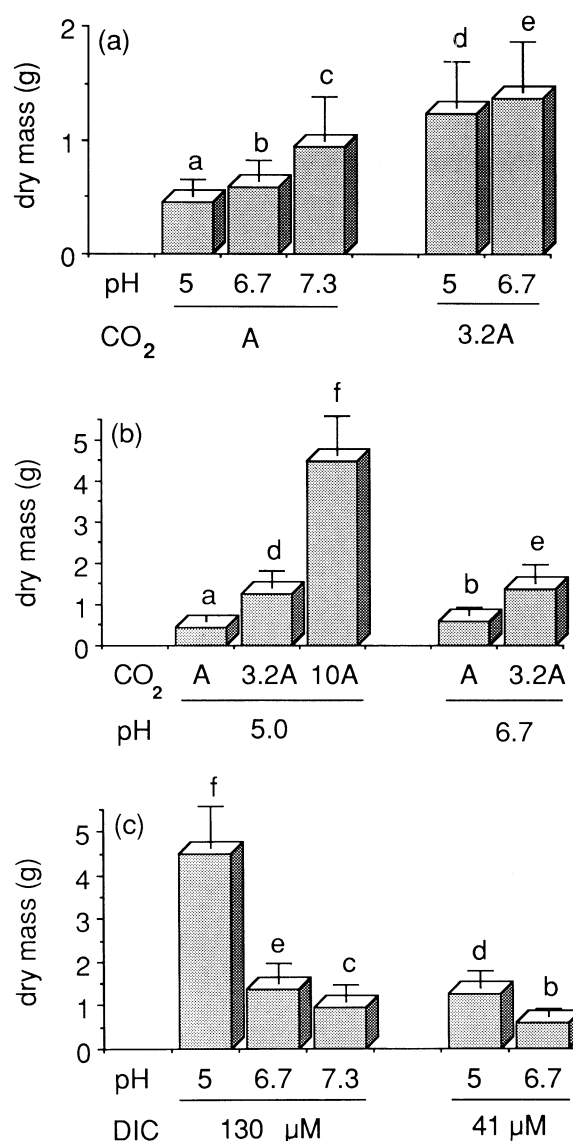

Fig. 2a–c. Dry mass for *Vallisneria* grown in different combinations of pH and free CO_2 concentration. Means shown with standard errors for treatments coded in Fig. 1. (**a**) Effects of varying pH at two controlled free CO_2 levels; (**b**) effects of varying free CO_2 concentration at two controlled pH values; and (**c**) effects of varying pH at two constant total DIC levels

lier – also for *Vallisneria* with no CO_2 enrichment – except that the earlier increases were four-fold in an eight week experiment (Grisé et al. 1986) and 17-fold in a 13 week experiment (Hoover 1984). All of these data suggest that growth nearly ceases at pH 5 and improves at higher pH values, but provide little insight into the specific effect of pH or pH-related variables on *Vallisneria* growth. The biomass differences are clearly not due to differences in free CO_2 concentrations, which were intended to be identical among these treatments but were actually slightly higher at pH 5 (Fig. 1b).

An increase in biomass occurred from pH 5 to pH 6.7 at 3.2× ambient free CO_2 levels (treatments d and e in Fig. 2a), but the difference was only 11% and not statistically significant (Table 1).

310

Table 1. Results of ANOVA's with dry mass (log scale) as the dependent variable. In all cases, when the experimental variable effect is significant, each treatment mean differs significantly from all other treatment means according to Tukey's studentized range test (SAS Institute 1985). Significance levels: n.s. = $P > 0.05$; *** = $P < 0.001$. Treatment codes as in Fig. 1

Treatment codes	Experimental variable	Experimental constant	Experimental variable effect
a + b + c	pH	free CO_2: ambient	***
d + e	pH	free CO_2: 3.2 ambient	n.s.
a + d + f	free CO_2	pH: 5.0	***
b + e	free CO_2	pH: 6.7	***
c + e + f	pH	total DIC: 130 μM	***
b + d	pH	total DIC: 41 μM	***

Growth: CO_2 effects with constant pH

Mean *Vallisneria* biomass at pH 5 increased 2.8-fold from ambient CO_2 to 3.2 × ambient CO_2, and 10-fold from ambient CO_2 to 10 × ambient CO_2 (treatments a, d, and f, Fig. 2b). Similarly, at pH 6.7 biomass increased 2.3-fold from ambient CO_2 to 3.2 × ambient CO_2 levels (treatments b and e, Fig. 2b). These highly significant (Table 1) responses support the contentions of Wetzel and Grace (1983) that carbon availability often limits macrophytes, and demonstrate convincingly that CO_2 enrichment can counter the detrimental effects of low pH reported above and previously (Grisé et al. 1986). It appears that pH *per se* does not directly influence *Vallisneria* growth, at least under the conditions of this experiment.

Grisé et al. (1986) offered the hypothesis that *Vallisneria* growth limitation at pH 5 could be attributed to the accumulation within plant tissues of aluminum or iron, each more soluble in acidic environments (Etherington 1982), to such high levels as to become toxic. Our current finding that CO_2 enrichment leads to growth enhancement at pH 5 suggests otherwise: if metal toxicity played a role in the growth limitation or tissue deterioration of *Vallisneria* at pH 5 with ambient free CO_2 levels, that toxicity was readily overcome with greater CO_2 availability. A more reasonable hypothesis to account for growth limitation at low pH (Fig. 2a; Grisé et al. 1986) is that *Vallisneria* is sensitive to decreased carbon availability due to reduced bicarbonate concentrations at lower pH. We cannot yet, however, discount the possibility of metal toxicity exacerbating carbon limitation if metals are accumulated to higher levels in tissues of slowly growing plants exposed to low pH without CO_2 enrichment (see below).

Just as the growth enhancement with CO_2 enrichment at pH 5 (Fig. 2b) weakens arguments for primary growth limitation by hydrogen ion or metal toxicity at

that pH, it also suggests that mineral nutrients were unlikely to have seriously limited growth in the ambient CO_2 treatment. Mineral nutrient supplies were clearly adequate to support the ten-fold higher biomass accumulation at 10 × ambient CO_2. We did select a sediment which has supported robust *Vallisneria* growth in the field, however, with the intention of simplifying experimental conditions to gain a clearer assessment of the relative importance of hydrogen ion, acid-soluble metal and photosynthetic carbon source concentrations. A logical extension would be to test *Vallisneria* growth at ambient and enriched CO_2 concentrations, both at low pH, on less fertile sediments from lakes susceptible to cultural acidification.

The apparently linear biomass increase with increasing CO_2 concentration at pH 5 may belie complex growth responses to CO_2. Although instantaneous net carbon fixation rate may increase approximately linearly with increasing CO_2 concentration at low to moderate CO_2 levels (Cunningham and Strain 1969; Titus and Stone 1982), biomass may accumulate disproportionately relative to the CO_2 concentration increase. This may occur if higher net carbon fixation rates are maintained and compounded by the addition of new photosynthetic surface, also fixing carbon at higher rates. On the other hand, if photosynthetic tissues acclimate rapidly to higher CO_2 levels and consequently fail to sustain higher carbon fixation rates, as has been documented for *Eriophorum vaginatum* (Tissue and Oechel 1987), biomass accumulation may be little influenced by CO_2 enrichment. Processes such as leaf turnover may further reduce the ratio of biomass accumulation to CO_2 concentration at high CO_2 levels, although we noted little leaf turnover by *Vallisneria* during this six-week experiment.

Growth: pH effects with constant total DIC

Our experimental design also assessed "pH effects" with total DIC held constant despite changing proportions of free CO_2 and bicarbonate. At a total DIC concentration of 130 μM, biomass declined 69% from pH 5 to pH 6.7, concomitant with a 60% decline in free CO_2 concentration, and 79% from pH 5 to pH 7.3, concomitant with an 85% decline in free CO_2 (treatments f, e, and c, Fig. 2c). These sharp declines occurred despite the actual DIC level in treatment f being somewhat below our target value (Fig. 1b). This highly significant (Table 1) response, similar to the 52% biomass decline from pH 5 to 6.7 at a total DIC concentration of 41 μM (treatments d and b, Fig. 2c; Table 1), appears to contradict the trend of increasing biomass with increasing pH protrayed in Fig. 2a. This response is in accord with Fig. 2b, however, in that it reenforces the notions that low pH *per se* is not detrimental, and that free CO_2 availability is a primary influence on macrophyte growth. Further, it is consistent with the idea that submersed plants use free CO_2 as a photosynthetic carbon source more readily than they use bicarbonate (e.g., Allen and Spence 1981).

Growth: effects of initial fresh mass

In each of the two-way ANOVA's with (log-transformed) dry mass as the dependent variable (Table 1), initial size class significantly (P<0.001) influenced final dry mass, reflecting the pronounced tendency in each treatment for initially larger plants to give rise to plants of greater dry mass at harvest time. There were no significant interactions. The significant effect of initial size class was removed in all cases, however, when ANOVA's were completed on relative growth rates rather than on biomass accumulation values. These ANOVA's otherwise produced the same results as those on log-transformed dry mass.

Patterns of growth and reproduction

No clear overall relationships appeared to exist between the number of rosettes, maximum leaf length, or flowering of *Vallisneria* plants and pH or DIC concentration. Each of these three parameters, however, did show a relatively well-defined, nonlinear relation to dry mass (Fig. 3). Both number of rosettes and maximum leaf length rose asymptotically with dry mass. Thus larger plants had both more rosettes and longer leaves, rather than simply one or the other (cf. Titus and Stephens 1983). Among the largest plants, rosette number was likely limited by duration of the experiment, as these plants continued to produce new rosettes throughout the experiment, and maximum leaf length may have been constrained by the 70 cm water depth above the sediment. In field situations, however, larger plants could also be expected to colonize more area by new rosette production and perhaps continue leaf expansion, although 150 cm is quite long for *Vallisneria* leaves.

Flowering also bore a close relationship to biomass at harvest (Fig. 3c). Percent flowering within a treatment rose from 12% for plants averaging 0.5 g dry mass (treatment b) to 100% for plants averaging 4.4 g dry mass (treatment f). Although flowering is but one component of successful sexual reproduction, growth limitation due to limited photosynthetic carbon supply is also likely to limit sexual reproduction in *Vallisneria*.

Tissue analysis

Grisé et al. (1986) raised the possibility of excessively high tissue levels of aluminum and/or iron leading to growth limitation for *Vallisneria* at pH 5. Tissue analysis data from the present experiment weaken this hypothesis, particularly for iron. Iron concentrations do not differ significantly among plants grown at ambient CO_2 (pH 5.0, 6.7, and 7.3: Table 2a) or among those grown at pH 5 (free CO_2 at ambient, 3.2× ambient, and 10× ambient levels: Table 2b). In both cases, biomass accu-
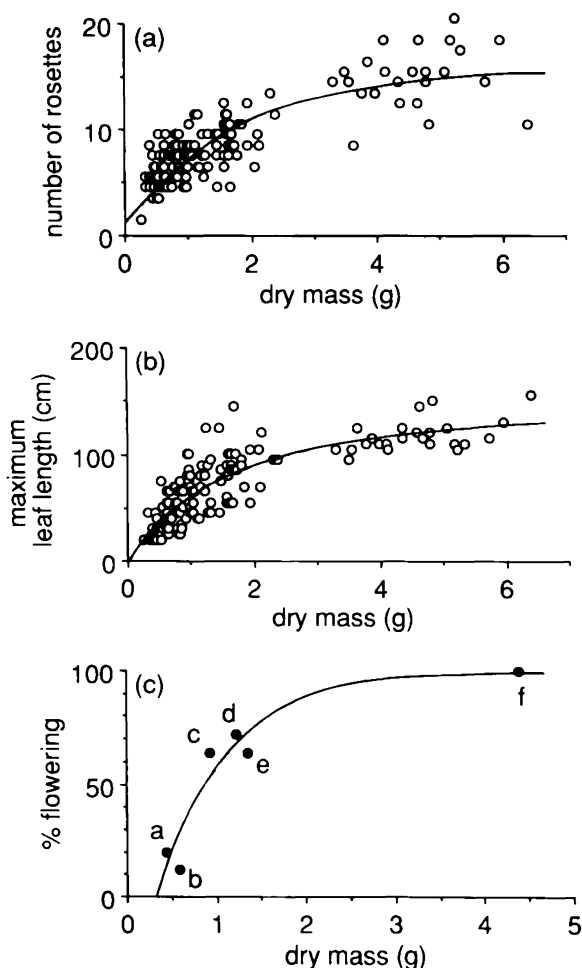

Fig. 3a–c. Parameters of growth and reproduction in relation to dry mass. (a) Number of rosettes. $R^2 = 0.70$ from regression to fit a rectangular hyperbola. (b) Maximum leaf length. $R^2 = 0.73$ from rectangular hyperbolic regression. (c) Percent flowering versus mean dry mass for each treatment as coded in Fig. 1

Table 2. Aluminum and iron concentrations ($\mu g\ g^{-1}$) in *Vallisneria* shoot tissues for plants grown (a) at ambient (A) CO_2 and varying pH's; (b) at pH 5 and three CO_2 levels: A, 3.2A, and 10A. Means shown with standard deviations. Significantly different means (P < 0.05 according to Tukey's test) indicated by different letters in each row; significance level of one-way ANOVA: n.s. = P > 0.05, * = P < 0.05, *** = P < 0.001

(a)

pH	5.0	6.7	7.3	One-way ANOVA significance level
Tissue [Fe]	468 ±140	458 ±135	456 ±118	n.s.
Tissue [Al]	693a ±209	527ab ±141	511b ±168	*

(b)

Free CO_2	A	3.2A	10A	One-way ANOVA significance level
Tissue [Fe]	468 ±140	416 ±167	326 ±80	n.s.
Tissue [Al]	693a ±209	452b ±219	311c ±105	***

Table 3. Location, sampling date and time, pH, temperature (° C), measured total dissolved inorganic carbon (DIC) concentration (μM), and atmospheric equilibrium DIC values (μM) for water samples from selected Adirondack lakes

Site (lat, long)	Date	Time	pH	Temp	Measured DIC	Equilibrium DIC
House Pond	8 JUN	11:05	5.3	17.9	56	16
(43°15′, 74°40′)	19 JUL	12:10	5.7	24.7	139	15
	16 AUG	11:40	5.5	25.0	82	14
West Pond	7 JUN	17:55	5.2	21.2	93	14
(43°49′, 74°53′)	20 JUL	13:55	5.2	26.4	120	13
	17 AUG	10:35	5.5	21.4	243	15
West Lake	8 JUN	18:15	5.4	18.7	61	16
(43°45′, 74°55′)	19 JUL	18:50	6.4	24.2	148	26
	16 AUG	17:45	6.7	26.3	134	38
Lake Rondaxe	8 JUN	18:25	5.7	19.5	74	17
(43°45′, 74°55′)	19 JUL	19:00	6.6	23.6	161	34
	16 AUG	17:55	6.8	24.4	130	43
Buck Pond	7 JUN	16:40	5.4	17.3	48	17
(43°49′, 74°56′)	20 JUL	13:05	6.4	24.5	98	25
	17 AUG	11:15	6.4	23.5	82	26
South Pond	7 JUN	15:50	4.6	17.7	26	15
(43°51′, 74°53′)	20 JUL	12:00	4.6	24.1	52	12
	17 AUG	13:20	4.8	22.2	36	13
DeBraine Lake	8 JUN	15:30	4.5	18.4	45	15
(43°22′, 74°43′)	19 JUL	16:35	5.5	24.5	75	14
	16 AUG	15:30	5.1	27.6	32	12
Silver Lake	7 JUN	15:00	4.9	18.1	10	15
(43°51′, 74°55′)	20 JUL	10:30	4.8	22.6	30	13
	17 AUG	12:10	4.9	23.3	6	13
South Pond	8 JUN	20:05	5.2	17.6	21	16
(43°56′, 74°27′)	20 JUL	16:10	6.1	24.8	39	18
	17 AUG	8:45	6.0	23.6	30	18
Twitchell Lake	7 JUN	17:05	4.7	18.0	21	15
(43°50′, 74°54′)	20 JUL	11:20	5.3	23.5	55	13
	17 AUG	13:45	5.5	23.4	36	13
Morehouse Lake	8 JUN	14:10	4.8	17.7	17	15
	19 JUL	15:30	5.7	24.5	43	15
(43°21′, 74°41′)	16 AUG	14:30	5.7	24.8	6	14
Ferris Lake	8 JUN	12:00	4.7	18.2	27	15
(43°18′, 74°38′)	19 JUL	13:15	5.7	24.1	43	15
	16 AUG	12:40	5.4	25.7	9	13
Sand Lake	8 JUN	12:55	4.6	17.8	21	15
(43°22′, 74°35′)	19 JUL	14:20	5.4	24.0	34	13
	16 AUG	13:40	5.3	25.4	5	13
Dart Lake	8 JUN	19:00	5.1	17.1	21	16
(43°48′, 74°52′)	20 JUL	14:35	5.7	23.7	34	15
	17 AUG	10:05	5.9	23.8	18	17
Big Moose Lake	7 JUN	18:20	5.3	15.9	28	17
(43°49′, 74°51′)	20 JUL	14:55	5.3	24.0	27	13
	18 AUG	11:25	5.3	22.8	16	14
inlet site	18 AUG	9:50	5.4	20.5	145	15
Spy Lake	8 JUN	9:40	5.5	16.8	53	18
(43°23′, 74°32′)	19 JUL	10:50	6.2	24.1	86	21
	16 AUG	10:25	6.4	25.2	68	24

mulation varied significantly (Fig. 2) while tissue iron levels changed little, especially in the ambient CO_2 treatments. Further, the highest mean tissue iron concentrations in this experiment (468 μg g^{-1}) fell below the lowest mean value (1034 μg g^{-1}) observed by Grisé et al. (1986). It does not appear that growth limitation can be attributed to iron toxicity.

Tissue aluminum concentrations declined 26% from pH 5.0 plants to pH 7.3 plants at ambient CO_2 levels (Table 2a). This significant trend parallels that determined by Grisé et al. (1986), except that the highest mean concentration we report here (693 μg g^{-1}) is well below the corresponding values in the earlier study (2040 μg g^{-1} in Vallisneria plants also grown at pH 5.0 with no CO_2 enrichment, and also on Otsego sediment). The values earlier considered potentially toxic were simply not attained in this study. We also noted no obvious tissue deterioration in these six-week old plants. The significant 55% decrease in tissue aluminum concentration from ambient to 10 × ambient CO_2 at pH 5 (Table 2b) suggests that biomass is accumulated more rapidly than aluminum at high CO_2 levels. Such "growth dilution" could alleviate metal toxicity. Although the possibility of metal toxicity cannot be eliminated, it is clear that CO_2 enrichment readily overcame growth limitations existing for plants grown at pH 5.0 with ambient CO_2.

Inorganic carbon regimes in Adirondack lakes

The 16 Adirondack lakes sampled proved to be quite variable in DIC content (Table 3). Of the 49 samples, only 9 (18%) were within 50% of total DIC values expected on the basis of atmospheric equilibrium (using the equilibrium constants given by Stumm and Morgan 1970) for water at specified pH and temperature; three (6%) were well below equilibrium values at the time of sampling, and 37 (76%) were well above. Fully 16% were more than five-fold higher than expected. Roelofs et al. (1984) and Grahn (1985) also reported DIC levels well in excess of atmospheric equilibrium values in acidic systems, and Wetzel et al. (1985) considered that CO_2 emanating from sediments may benefit selected macrophytes. It does appear that the free CO_2 concentrations which caused vigorous Vallisneria growth in the greenhouse at low pH are similar to values which can be observed during the growing season in nature in low pH lakes. Our results have clearly demonstrated the potential for high free CO_2 concentration to counter the detrimental effects of low pH on growth for Vallisneria americana grown on fertile sediments. These findings prompt a question to be addressed in a subsequent paper: is the mineral nutrient supply in sediment of lakes susceptible to cultural acidification sufficient to support such dramatically increased growth in response to CO_2 enrichment?

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