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Short communication

Gauging submersed plant response to CO_2 enrichment: Pot size matters

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ABSTRACT

The availability of a photosynthetic carbon source may limit submersed plant growth, and experimental assessment of this limitation may depend on the volume available to belowground plant parts. To test the effect of pot size on submersed plant growth response to CO_2 enrichment, we grew the freshwater macrophyte *Vallisneria americana* in pots containing 1.01, 2.25, and 4.04 L of lake sediment in pH- and [CO_2]-controlled greenhouse tanks for nine weeks. A highly significant ($P < 0.001$) interaction between [CO_2] and pot size for final biomass, root mass fraction, dry weight gain, and relative growth rate (RGR) indicated that growth response to CO_2 enrichment depends on pot size. The ratios of growth at high [CO_2] to that at low [CO_2] increased from 3.2 in small pots to 8.1 in large pots for dry weight gain, and from 1.8 in small pots to 3.3 in large pots for RGR. Response to CO_2 enrichment was thus substantially greater for plants grown in larger pots. Further, increasing pot size from small to large did not increase dry weight gain at low [CO_2], but yielded 69% greater dry weight gain at high [CO_2]. Macrophyte experimentalists should carefully consider pot size in relation to available sediment resources, which can influence growth rate and growth response to CO_2 enrichment.

1. Introduction

Sculthorpe (1967) stated decades ago that absorption of dissolved inorganic carbon (DIC) is a major challenge facing photosynthetic organs underwater. An oft-cited contrast between water and air as media for photosynthetic organs is that the diffusion coefficient for CO_2 is about four orders of magnitude lower in water than in air. In addition, thicker boundary layers are likely to develop around submersed plants owing to the substantially greater dynamic viscosity of water (Denny, 1993). Both features can slow diffusive transport of CO_2 to sites of fixation (Raven et al., 1985). The resulting likelihood of DIC limitation has stimulated research on diverse submersed plant adaptations to acquire DIC, including the use of bicarbonate ion in the water column (*e.g.*, Maberly and Spence, 1983).

For the many species reliant on free CO_2 in the water column – including bicarbonate users in circumneutral and acid waters – the concentration of CO_2 is of central importance. It is noteworthy that CO_2 concentration in freshwater ecosystems varies spatially and temporally (Cole et al., 1994; Maberly, 1996), and is often far from equilibrium with the atmosphere (Rebsdorf et al., 1991; Weyhenmeyer et al., 2012). In turn, variations in CO_2 (or DIC) availability may influence photosynthesis (*e.g.*, Madsen and Maberly, 1991) and growth (*e.g.*, Titus et al., 1990; Vadstrup and Madsen, 1995) of submersed macrophytes, although not all species are equally affected (Madsen and Sand-Jensen, 1991; Pagano and Titus, 2007).

Determining plant growth response to CO_2 availability, germane both because CO_2 concentrations vary so widely, and because different species show varying sensitivities to CO_2 enrichment, typically involves growing plants in pots in laboratory or greenhouse environments. A specific methodological issue that has been addressed for terrestrial plants but largely overlooked for submersed plants is the influence of pot size. Meta-analysis by Poorter et al. (2012) reveals that increasing pot size generally leads to increased terrestrial plant growth. Variations in pot size, however, may or may not influence effects of CO_2 enrichment on photosynthetic capacity and growth (Arp, 1991; McConnaughay et al., 1996).

The primary objective of this study was to evaluate the importance of pot size for determining responses of a submersed vascular plant to CO_2 enrichment. Our null hypothesis was that effects of CO_2 enrichment on growth and biomass allocation are independent of pot size, in contrast to the alternative hypothesis that pot

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size changes plant response to CO_2 enrichment. Our actual expectation is that growth response to CO_2 enrichment will be greater in larger pots which provide more sediment-based resources – both in terms of volume and surface area.

Our secondary objective was to test growth response to increasing pot volume, whether at low or high [CO_2]. Poorter et al.'s meta-analysis (2012) reported a median increase in biomass production of 43% for a doubling of pot size. If water column-based CO_2/DIC limitation is relatively more important for submersed plants, as suggested by the diffusion issues cited above, pot size may be relatively less important for submersed macrophytes.

2. Materials and methods

2.1. Experimental materials

Vallisneria americana Michx. (Hydrocharitaceae; hereafter *Vallisneria*) is an important component of littoral vegetation in many mesotrophic and eutrophic lakes of eastern North America. In the northeastern United States, this plant overwinters as vegetative buds buried in the sediment. From these buds, 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 throughout the growing season on elongated axillary stolons, which we include with the roots in the belowground fraction. *Vallisneria* is easily transplanted when collected prior to the elongation of its first roots in spring, and is responsive to CO_2 enrichment (Titus et al., 1990).

Winter buds of *Vallisneria* were collected in the fall from Chenango Lake (Broome County, NY; 42°12.7′ latitude, 75°50.2′ longitude) and maintained into the winter at *ca.* 5 °C. Buds were retrieved from cold storage in early February and transplanted into sediment in the Binghamton University Research Greenhouse to stimulate their germination.

Sediment was collected from Chenango Lake, an alkaline lake (mean growing season pH 8.2 and alkalinity 2.1 meq/L – Titus and Stephens 1983). Organic content of sediment used in this experiment was 1.0% based on loss on ignition at 550 °C. We selected this sediment because Chenango Lake supports robust *Vallisneria* plants *in situ*, indicating that sediment resources, if supplied in adequate volume, would not constrain *Vallisneria* growth.

2.2. Experimental design, planting and harvesting

To test our null hypothesis of no impact of pot size on the growth response to varying [CO_2], we adopted a two-way factorial experiment with two CO_2 levels (low and high) and three pot sizes (small, medium, and large), with eight replicates for each [CO_2] x pot size combination for a total of 48 pots.

Young rosettes raised from winter buds were individually excavated from sediment before they developed root systems, gently blotted, and weighed to determine an initial fresh weight for each plant. On 17 February, we transplanted experimental plants into pots (containing 1.01, 2.25, and 4.04 L of sediment with corresponding surface areas of 143, 184, and 337 cm^2 in small, medium, and large pots, respectively). Surface area may be an important variable for a plant like *Vallisneria* with a strong horizontal component to its growth (Vermaat, pers. com.), but we cannot distinguish between volume and surface area effects because the two variables are so closely correlated in this experiment (coefficient of determination >0.95). Plants were randomly assigned to treatments and positions within 1200 L fiberglass tanks in the Binghamton University Research Greenhouse so that each of the six treatments ([CO_2] × pot size combinations) was comprised of two plants from each of four weight classes (1.8–2.0 g, 2.1–2.3 g, 2.4–2.6 g, and 2.6–3.5 g fresh weight). Tanks contained a culture solution of 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 $NaHCO_3$. The natural lake sediment provided nitrogen and phosphorus, which was not added to the tanks. Tank water was raised to a depth of 60 cm as plants grew and was maintained at 23 ± 2 °C with Remcor CFF-500 refrigerated circulators and at pH 5.0 ± 0.2 with Horizon 5997 pH controllers. The "low" CO_2 tank was bubbled with ambient air, and a Tylan FC-280 mass flow controller regulated the enrichment of a compressed air line with pure CO_2 to attain a dissolved CO_2 concentration in the "high" CO_2 tank 10-fold higher than in the low CO_2 tank – again via bubbling through tank water. Independent control of pH and [CO_2] was necessary for effective control of DIC and its components. The refrigerated circulators and bubbling air streams also provided mixing.

Plants not assigned to treatments were dried at 85 °C for determination of their mean dry weight: fresh weight ratio, thus enabling estimation of initial dry weight for all experimental plants.

As a check on our automatic control systems, we regularly monitored tank water temperature, pH, and DIC content (with infrared gas analysis of CO_2 sparged from acidified tank water samples using an Analytical Development Co., Model 225 Mk II gas analyzer and Hewlett- Packard 3390A Integrator) and adjusted if necessary to maintain desired levels. Because we carried out this experiment so early in the year, we augmented the natural light regimes in the Binghamton University Research Greenhouse with supplementary lighting from General Electric 1000 W multivapor lamps for five hours per day for three weeks at the beginning of the experiment.

Plants were harvested after nine weeks, carefully rinsed, separated into above- and belowground fractions, and dried at 85 °C. Root mass fraction (RMF) was determined as the belowground fraction of total plant biomass (*e.g.*, Poorter and Nagel 2000).

2.3. Data summary and statistical analysis

Data were summarized as final (total) dry weight, RMF, dry weight gain, and relative growth rate (RGR). Dry weight gain was calculated as the final dry weight less the estimated initial dry weight, and RGR as ln (W_f/W_i)/t, where W_f = final dry weight, W_i = initial (estimated) dry weight, and t = length of the experiment in days.

Data were subjected to two-way Analysis of Variance (ANOVA). The critical test of our hypothesis about the influence of pot size on the growth response of *Vallisneria* to CO_2 enrichment was the [CO_2] × pot size interaction term: if significant, then we reject our null hypothesis of no impact of pot size on response to CO_2 enrichment, and proceed with a Tukey means comparison test. ANOVA and Tukey tests were carried out with SAS Version 9.1.3 (SAS Institute Inc. 2003; SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA). Our data provided acceptable levels of normality and heteroscedasticity.

For presentation of plant responses to CO_2 enrichment, we determined the mean ratio of growth at high [CO_2] to that at low [CO_2] for each of eight different pairs of plants. Plants were paired on the basis of the ranks of their initial weights within each pot size, from smallest to largest, for each of three different growth measures – final dry weight, dry weight gain, and RGR.

We employed a similar approach to assess effects of pot size on dry weight gain: eight pairs of plants from the appropriate treatments (medium vs. small, large vs. medium, and large vs. small) were matched by the ranks of their initial fresh weight. Means and 95% confidence intervals were determined for the eight ratios of dry weight gain in larger to smaller pots. This approach yielded