

# Physiological Plasticity in *Elodea nuttallii* (Planch.) St. John

J. IWAN JONES, J.W. EATON AND K. HARDWICK<sup>1</sup>

## ABSTRACT

*Elodea nuttallii*, a problem weed in Britain, has been found to adapt rapidly to high pH and low CO<sub>2</sub>, conditions which are normally associated with low growth rates. This adaptation has consequences with respect to modelling plant growth and predicting weed problems in the field. Investigations indicate that *E. nuttallii* is able to utilize bicarbonate by active transport, pumping H<sup>+</sup> to the lower leaf surface and OH<sup>-</sup> to the upper surface, as proposed by Prins *et al.* (1982). For much of the time this mechanism does not operate in the field, but laboratory experiments have shown that it is switched on within a few days, over a very small pH range, as carbon dioxide supply becomes limiting. Evidence is presented that bicarbonate uptake does occur in the field and its significance to the plant's growth is discussed. These

results are compared with physiological plasticity in the closely related *Elodea canadensis*.

*Key words:* marl, bicarbonate use, photosynthesis, water plant.

## INTRODUCTION

Dissolved inorganic carbon (DIC) is present in natural waters in different ionic forms, which are freely interconvertible. These consist of free CO<sub>2</sub> (dissolved CO<sub>2</sub> and H<sub>2</sub>CO<sub>3</sub>, hereafter referred to as CO<sub>2</sub>\*), HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup>, in proportions largely determined by pH, with the equilibrium shifting toward CO<sub>3</sub><sup>2-</sup> with increasing pH. Many productive waters have pH values in the range 7 to 8, where concentrations of CO<sub>2</sub>\* are low and can be limiting as a photosynthetic carbon source, especially in still conditions where replenishment is retarded by the slow diffusion of dissolved gases in the aquatic environment (Smith and Walker 1980, Black *et al.* 1981). Plants growing in such waters often experience adverse conditions as photosynthesis raises oxygen concentrations and

<sup>1</sup>Research student and two lecturers, respectively. Department of Environmental and Evolutionary Biology, School of Life Sciences, University of Liverpool, P. O. Box 147, Liverpool L69 3BX, England.

reduces  $\text{CO}_2^*$  to very low concentrations, severely restricting further photosynthetic assimilation and inducing conditions for photorespiration (Simpson *et al.* 1980). This can occur both as rapidly changing diurnal cycles (Goulder 1970, Van *et al.* 1976) and as much longer term seasonal changes (Bindloss 1976, Talling 1976, Frodge *et al.* 1990). Any mechanism which enables a plant to overcome such limiting conditions will confer a competitive advantage, reducing photorespiration and maintaining net photosynthesis and growth when other species are under stress. One such mechanism is the ability to use bicarbonate as a carbon source. This ability has been described for many species common in eutrophic and hard waters (Maberly and Spence 1983, Madsen and Sand-Jensen 1991) and has been used to explain the field distribution of some species (Kadono 1982, Adams 1985). Other species typical of such waters, which lack the ability to use bicarbonate, avoid the problem of low  $\text{CO}_2^*$  supply in the water by using other sources, *e.g.* by having floating or emergent leaves and thereby using atmospheric  $\text{CO}_2$  (Salvucci and Bowes 1982, Maberly and Spence 1989, Madsen and Sand-Jensen 1991) or  $\text{CO}_2^*$  produced by the sediment (Wium-Andersen 1971, Sondergaard and Sand-Jensen 1979, Maberly 1985a, b, Boston *et al.* 1989) or by growing in floating waters (Sand-Jensen 1983).

To take full advantage of changing conditions, a plant must be able to adapt its physiology accordingly, using  $\text{HCO}_3^-$  by active uptake only when necessary, since it involves energy usage in order to gain carbon and the uptake of  $\text{CO}_2^*$  by simple diffusion does not. Such a capability has previously been reported for Canadian pondweed (*Elodea canadensis* Michx.) by Sand-Jensen and Gordon (1986) and suggested for common water-crowfoot (*Ranunculus peltatus* Schrank) by Madsen (unpublished, referred to in Madsen and Maberly 1991), with the former reporting that an increase of  $\text{HCO}_3^-$  use took 56 days to develop (though loss of  $\text{HCO}_3^-$  use occurred over a shorter period, see Figure 2). This extended time scale is only sufficient to allow the plant to adapt to seasonal changes or to movements between water bodies as a result of vegetative spread. It will not allow adaptation to short-term changes, such as those which might arise during periods of hot, calm weather or algal blooms. The present work was undertaken to investigate the ability of Nuttall's pondweed (*Elodea nuttallii* (Planch.) St. John), a fast-growing, dominant species, typical of eutrophic waters, to utilize  $\text{HCO}_3^-$  as a carbon source and the rate at which it can adapt its carbon uptake characteristics to changing conditions. These abilities could be crucial to the success of this submerged plant in the changeable conditions of lentic eutrophic waters.

In charophytes the deposition of marl (crystalline  $\text{CaCO}_3$  precipitated at high pH) is generally considered to be a result of the  $\text{HCO}_3^-$  uptake mechanism, being associated with the

alkaline bands involved in the process (Raven, Smith and Walker 1986), the formation of which may be more an integral part of the process than a byproduct (McConnaughey 1991, McConnaughey and Falk 1991). For submerged angiosperms it has been suggested variously that i) as photosynthesis raises the pH of the water column, marl forms and precipitates onto the leaf surfaces, ii) that marl is a byproduct of epiphyton photosynthesis, or iii) that marl is a consequence of  $\text{HCO}_3^-$  uptake by a polar leaf mechanism, through which the pH at the adaxial surface (facing toward the growing tip) is raised as a result of net  $\text{OH}^-$  efflux, while that at the abaxial surface is lowered by efflux of  $\text{H}^+$  (Prins *et al.* 1982). An investigation was made to determine whether marl is deposited on the leaves in a polar fashion, which would indicate that  $\text{HCO}_3^-$  is taken up by the polar leaf mechanism in Nuttall's pondweed.

The second part of this work was an investigation into the rate at which bicarbonate utilization is induced, as an assessment of the plant's ability to adapt to short-term changes in environmental conditions.

## MATERIALS AND METHODS

**Mechanism of marl deposition.** Twelve 10-cm-long healthy shoots of Nuttall's pondweed, collected in June 1991 from the calcium-rich Leeds and Liverpool Canal, Merseyside, UK (53°28'N, 2°57'W), were gently brushed clean of filamentous algae and loosely tied to glass rods using cotton thread. The plants were then arranged randomly, six in normal, vertical orientation and six inverted through 180° (upside down), in a glass tank with blackened sides, containing approximately 30 l of canal water filtered twice through fine plankton netting (25 TI 35, 40 by 40  $\mu\text{m}$  mesh), 2.4 mM DIC, pH 8.1,  $[\text{Ca}^{2+}] = 1.5$  mM. The rods were supported by acid-washed sand. These plants were then grown for 10 days at  $15 \pm 2^\circ\text{C}$ ,  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR, by which time a substantial layer of marl had developed on the leaves. The water was neither aerated nor mixed during this period. The plants were subsequently examined using light microscopy. Some were also prepared for examination with a Phillips 501B scanning electron microscope (SEM), by dehydration in cold ( $-10^\circ\text{C}$ ) alcohol and critical point drying, before being attached to aluminium stubs with glue and sputter-coated with gold.

**Induction of bicarbonate utilization.** Brown plastic beakers, height 7.5 cm, diameter 7 cm, were filled with canal sediment and covered with disks of black plastic. Into each beaker five 10 cm long shoots, collected in July 1991 from the canal, were inserted through small slits in the plastic, thus providing the plants with access to natural substrate, but largely isolating the  $\text{CO}_2$ -producing mud from the water body. Five plants were used to ensure an adequate supply of material for the physiological determinations. Each planted

pot was carefully placed in a glass jar containing 2 l of twice-filtered canal water and 24 such jars were incubated at each of the two temperatures, 25 and 15C, illuminated with  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR in a 16 hr light 8 hr dark cycle. The water was aerated by bubbling with air adjusted in one of the following three ways, eight jars per treatment;

- i) Low  $\text{CO}_2^*$  - air previously passed through soda lime at a rate which reduced the  $\text{CO}_2^*$  in the water to about half ambient,
- ii) Ambient  $\text{CO}_2^*$  - untreated air,
- iii) High  $\text{CO}_2^*$  - untreated air, with small quantities of dilute hydrochloric acid added each evening, as required, to the water to reduce the pH to about 7.5. The amount of acid added had a negligible effect on the conductivity of the water.

Jars were randomly removed at different times and the ability of four of the five plants in each to use bicarbonate as a carbon source was determined as below. Conductivity and pH were measured daily each morning, and a water sample was taken from each jar to determine total alkalinity by titration to pH 4.5 with 0.01 M HCl on the day it was sampled. Total DIC and the proportions of its constituent species in the water samples were calculated according to Mackereth, Heron and Talling (1978). Bicarbonate utilization by eight plants freshly collected from the field was also determined at the start of the experiment to establish a baseline.

In order to assess bicarbonate utilization, the photosynthetic rate of leaves was measured successively in media adjusted to pH 6.5 and pH 9 and bicarbonate utilization was expressed as the ratio of photosynthesis in these two media. At pH 6.5,  $\text{CO}_2^*$  is plentiful, being about 40% total DIC ( $=0.98 \text{ mM}$ ) and any limited change in pH produces little change in photosynthetic rate. At pH 9,  $\text{CO}_2^*$  is only 0.3% total DIC ( $=5 \mu\text{M}$ ) and the plant can therefore only carry out significant photosynthesis if it can utilize  $\text{HCO}_3^-$ . Use of the ratio removes effects due to variations in absolute rates between plants.

Photosynthetic rates were measured as oxygen evolution at 20C using a Clarke-type oxygen electrode (Hansatech, King's Lynn, UK), with a tungsten slide projector bulb providing saturating incident light of  $290 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR. For determinations, three leaves from a whorl 3 cm from the tip of the plant were carefully excised with a scalpel, brushed clean of epiphytes and marl with a soft paintbrush and placed in the electrode reaction chamber containing 1.5 ml of Forsberg (1965) medium, modified by omission of  $\text{Na}_2\text{SiO}_3$  and carbon sources and adjusted to the appropriate pH. In each case photosynthesis was initiated by injection of 0.1 ml of  $\text{NaHCO}_3$  solution to give a final concentration of 2.4 mM DIC. Preliminary studies showed that no significant pH change occurred on addition of  $\text{NaHCO}_3$ .

To reduce the effects of photorespiration on photosynthesis (Simpson *et al.* 1980), all photosynthetic rates were determined within  $1 \text{ mg O}_2 \text{ l}^{-1}$  amplitude change, in solutions containing  $9 \text{ mg O}_2 \text{ l}^{-1}$  (approximately 100% saturation), being sparged when necessary with either  $\text{O}_2$  or  $\text{N}_2$  to achieve this concentration before measurements began. Photosynthetic rates were determined from the measured rate of change in oxygen concentration in the reaction chamber and calculated as rate of oxygen change per unit chlorophyll. The chlorophyll content of leaves was determined by the method of Arnon (1949).

To minimize diurnal influences on photosynthetic rates, the determinations were all made within the middle 8 hr of the photoperiod.

## RESULTS AND DISCUSSION

The results of the first experiment (Table 1) showed that inversion of plants had no effect on the pattern of marl deposition. The consistent development of marl on adaxial

TABLE 1. EFFECT OF ORIENTATION ON THE DISTRIBUTION OF MARL DEPOSITS ON THE LEAVES OF *Elodea nuttallii*. The Mann-Whitney U test showed there was no difference (0.05 sig.) between the pattern of marl deposition on the abaxial surface of normal and inverted plants. All leaves looked at had marl deposits on the adaxial surface. Results:  $U'=25.5$ ,  $U_{0.05,6,6}=31$ .

Orientation of shoots	No. leaves counted	Leaves marled on abaxial surface	
		No.	(%)
Normal	32	3	(9.4)
	45	5	(11.1)
	14	3	(21.4)
	31	2	(6.5)
	20	2	(10.0)
	47	0	(0.0)
Inverted	68	3	(4.4)
	69	2	(2.9)
	60	8	(13.3)
	38	1	(2.6)
	22	0	(0.0)
	56	5	(8.9)

rather than abaxial leaf surfaces of the inverted plants is evidence that its production is not simply a general precipitation from the water above the leaves. Neither is marl a product of epiphyton photosynthesis, since SEM examination of leaves from the inverted plants showed heavy epiphytic development on abaxial surfaces, with no marl, whereas marl on the adaxial surfaces had no epiphytes amongst it (Figure 1a). Instead it confirms that marl is produced by the leaves in a

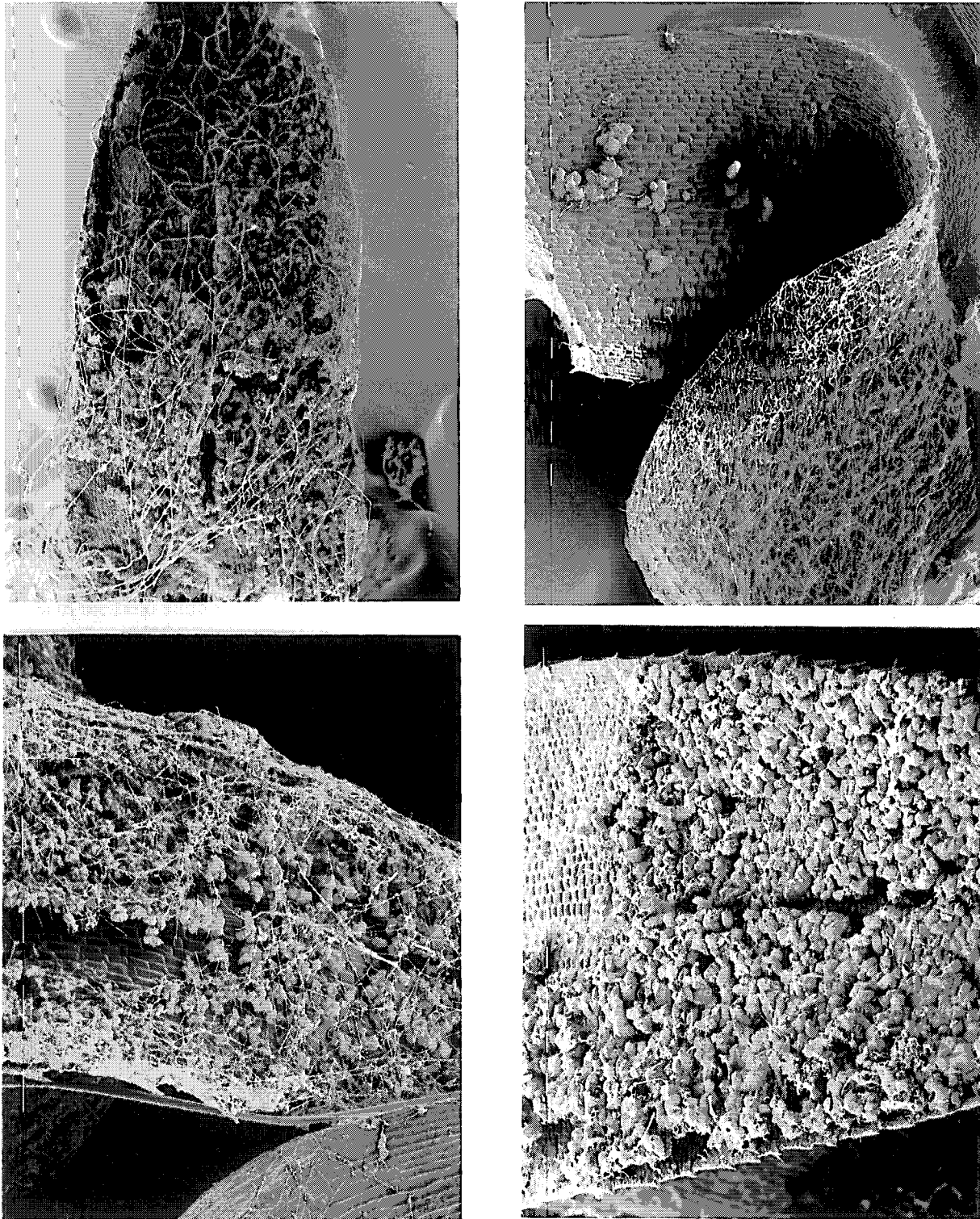


Figure 1. a) (top right) A leaf from an inverted *Elodea nuttalli* shoot showing growth of epiphyton on the abaxial surface (away from the growing point) and marl on the adaxial surface. b) (bottom right) *Elodea canadensis* leaf showing marl deposits; note absence of deposits from the midrib. c) (top left) Another leaf from an inverted *E. nuttalli* leaf, again midrib marl-free. d) (bottom left) *E. nuttalli* leaf from the field, showing similar patterning. Scale bar = 100  $\mu$ m.

polar fashion and that this polarity is retained after inversion. The detailed arrangement of the marl crystals on the leaf surface, closely associated with the photosynthesizing cells of the leaf blade, but absent from the transport cells of the midrib (Figure 1b and c), is further circumstantial evidence that leaf photosynthesis is the cause of marl accumulation. In the few cases where marl was found on the abaxial surface, in both normally orientated and inverted plants, its presence could be explained easily by dislodgement from the upper surface of the next leaf during handling, or by interaction between the leaves where they were positioned very close together.

The second part of the work concerned the rate of initiation of  $\text{HCO}_3^-$  utilization, measured as the ratio of photosynthesis at pH 9 relative to that at pH 6.5. This ratio slowly decreased in plants grown under both ambient and increased  $\text{CO}_2^*$  conditions, but increased in those grown under reduced  $\text{CO}_2$  conditions (Figure 2a and b), with a statistically significant increase in  $\text{HCO}_3^-$  use detectable after only 5 days at 25C,  $[\text{CO}_2^*]$  16  $\mu\text{M}$ , and 8 days at 15C,  $[\text{CO}_2^*]$  23  $\mu\text{M}$ . The least significant difference used is the SE of the mean from an analysis of variance of the data (for 25C SE = 0.05 and for 15C SE = 0.085). The pH range over which this switch occurred was very small, the difference between the low and ambient treatments being about half a pH unit (Table 2). When compared with the 56 days reported by Sand-Jensen and Gordon (1986) for an increase in bicarbonate use (Figure 2c), the rates reported here are rapid, and are clearly sufficient to allow plants to adapt to short-term changes in the water body. A further, similar experiment using both Nuttall's and Canadian pondweeds collected from another site, the Lancaster Canal (54°15'N, 2°44'W), where the species grow together (results not presented here), showed that bicarbonate utilization increased very rapidly in both species, demonstrating that the faster induction rate found here compared to that found by Sand-Jensen and Gordon (1986) is not due to a specific or a clonal difference. This faster induction rate could be due to differences in the physiological states of the plants at the start of the experiments. At the time of collection, the plants used here had very little affinity for  $\text{HCO}_3^-$ , whereas the Danish ones were already utilizing  $\text{HCO}_3^-$  at a substantial rate and any further increase above this could take a considerable time to develop. The  $[\text{CO}_2^*]$  of the low treatment used here (16  $\mu\text{M}$  at 25C, 23  $\mu\text{M}$  at 15C) is similar to that used by Sand-Jensen and Gordon (14.8  $\mu\text{M}$ ) to produce an increase in  $\text{HCO}_3^-$  use, and it is likely to be this low  $\text{CO}_2$  concentration, not the pH of the water, that triggers the switch to  $\text{HCO}_3^-$  utilization in both cases.

The results presented here indicate that marl is produced in a polar fashion by Nuttall's pondweed, as a result of the physiology of the leaf, a finding consistent with the polar leaf

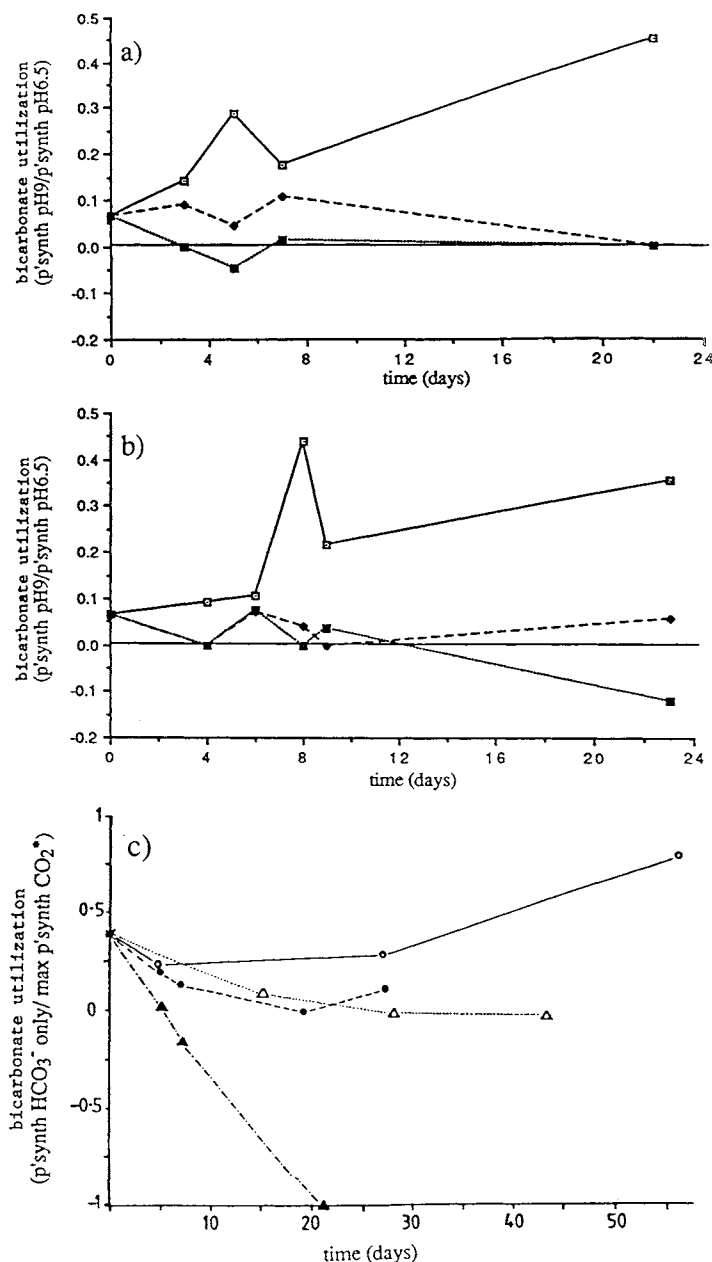


Figure 2. Changes in bicarbonate utilization of *Elodea nuttallii* when grown under different conditions, SE from ANOVA a) 25C SE = 0.05, b) 15C SE = 0.085, low  $\text{CO}_2^*$  —■—, ambient  $\text{CO}_2^*$  —●—, increased  $\text{CO}_2^*$  —□—, (see Table 2 for concentrations). Absolute values of photosynthesis were calculated as rate of oxygen evolution per unit chlorophyll. c) *Elodea canadensis* grown at 15C, 14.8  $\mu\text{M}$   $\text{CO}_2^*$  ○—○, 37  $\mu\text{M}$   $\text{CO}_2^*$  ●—●, 1,000  $\mu\text{M}$   $\text{CO}_2^*$  △·····△, 2,200  $\mu\text{M}$   $\text{CO}_2^*$  ▲---▲ (redrawn from Sand-Jensen and Gordon 1986).

mechanism of  $\text{HCO}_3^-$  utilization proposed by Prins *et al.* (1982). These findings do not support the acid-alkali banding theory of  $\text{HCO}_3^-$  uptake suggested by Eighmy *et al.* (1987), though no investigation was made into whether or not  $\text{HCO}_3^-$  uptake is an energy-requiring process utilizing a proton pump mechanism, as they concluded.

TABLE 2. MEAN CONCENTRATIONS OF DISSOLVED INORGANIC CARBON AND CO<sub>2</sub>\* (means of six measurements taken on the day the vessel was sampled), AND pH (means from daily measurements) DURING THE EXPERIMENT. ALSO SHOWN ARE MEAN SUMMER VALUES FOR THE LEEDS AND LIVERPOOL CANAL (Saednia 1980).

Temp (C)	Treatment	CO <sub>2</sub> *(μM)	DIC(mM)	pH
25	Low	16	2.08	8.42
	Ambient	39	2.27	7.90
	Increased	147	2.06	7.42
15	Low	23	2.35	8.38
	Ambient	49	2.29	7.87
	Increased	111	1.69	7.40
	Canal water	44.6	2.40	8.10

The Leeds and Liverpool Canal varies from pH 7 to pH 9, with CO<sub>2</sub>\* fluctuating accordingly (Saednia 1980), a situation typical of productive waters, so it would be expected that the plants would at times use HCO<sub>3</sub><sup>-</sup> as a carbon source. SEM investigation of fresh material from the canal showed the pattern of marl deposition typical of plants using HCO<sub>3</sub><sup>-</sup> (Figure 1d), indicating that the plants do indeed utilize HCO<sub>3</sub><sup>-</sup> at times in the field situation.

Although uptake of HCO<sub>3</sub><sup>-</sup> by active processes involves some energy costs and hence it is preferable for the plant to take up CO<sub>2</sub> by passive diffusion when available in sufficient amounts, the ability to switch rapidly to HCO<sub>3</sub><sup>-</sup> utilization confers a large advantage on plants growing in changeable aquatic environments, by allowing growth to continue when conditions of high pH and low CO<sub>2</sub> develop. The rates of induction reported here are sufficiently rapid to allow adaptation to short-term changes in the water column, such as those which might arise during periods of hot, calm weather or algal blooms. If this ability to adapt rapidly to changing environmental conditions is widespread among aquatic plants, then it will have to be taken into consideration in the construction of models predicting weed growth in relation to resource availability.

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