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Aquatic Botany 75 (2003) 217–227

Aquatic botany

www.elsevier.com/locate/aquabot

The effect of alkalinity on photosynthesis–light curves and inorganic carbon extraction capacity of freshwater macrophytes

Sharon N. Kahara[a,b], Jan E. Vermaat[a,*]

[a] *International Institute for Infrastructural, Hydraulic and Environmental Engineering, P.O. Box 3015, 2601 DA Delft, The Netherlands*

[b] *P.O. Box 50445, Nairobi, Kenya*

Received 27 July 2001; received in revised form 30 September 2002; accepted 29 October 2002

Abstract

The influence of variable bicarbonate (HCO_3^-) alkalinity was assessed on the photosynthetic performance of four submerged macrophyte species of contrasting growth form and distribution but all reportedly capable of HCO_3^- use: *Hydrilla verticillata*, *Egeria densa*, *Potamogeton schweinfurthii* and *Potamogeton lucens*. Whole-shoot photosynthesis–irradiance curves and pH-drift capacity were measured at two alkalinities, a low and a high concentration (0.5 and 2.0 meq l^{-1} HCO_3^-). *E. densa*, *H. verticillata* and *P. lucens* had higher gross maximum photosynthesis (P_{max}) and respiration rates at the higher HCO_3^- availability. The species also differed significantly in P_{max} and respiration for both alkalinities, with P_{max} estimates ranging between 3 and 22 mg O_2 g AFDW^{-1} h^{-1}. Half-saturation constant estimates ($K_{1/2}$) did not differ between species or treatments, but were comparatively low (<10 μmol PAR m^{-2} s^{-1}). Incorporation of a light inhibition term did not improve the explained variation of the fitted curves significantly. Overall, respiration rates were high (2–13 mg O_2 g AFDW^{-1} h^{-1}), largely due to the high incubation temperature employed (25 °C). In the pH-drift experiment *H. verticillata* reached a pH above 9.5 in both alkalinities after 48 h, which is higher than all the other species tested. In the higher alkalinity pH-drift experiment, only *E. densa* failed to raise the pH. All species raised the pH more rapidly in the low alkalinity medium probably due to its weaker buffering capacity. Final (96 h) maximum pHs were also higher in low alkalinity. Across species, however, no correlations existed between any of the *P–I* curve parameters including net photosynthesis at 200 μmol PAR m^{-2} s^{-1} and carbon extraction capacity expressed as drifted pH after 48 h. In parallel growth experiments, *H. verticillata* maintained growth even at 3 meq l^{-1} (RGR = 0.06 per day), whereas

* Corresponding author. Present address: Institute for Environmental Studies, Vrije Universiteit Amsterdam, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands. Tel.: +31-20-4449596; fax: +31-20-4449553.
E-mail addresses: sharonkahara@hotmail.com (S.N. Kahara), jan.vermaat@ivm.vu.nl (J.E. Vermaat).

0304-3770/02/$ – see front matter
PII: S0304-3770(02)00179-1

in *E. densa* RGR declined rapidly beyond 1 meq l^{-1}, which is consistent with the pH-drift experiments.

Keywords: Dissolved inorganic carbon: *P–I* curves; pH-drift: Aquatic weeds

1. Introduction

Alkalinity is an important factor governing the distribution of submerged freshwater macrophytes (Wetzel, 1983; Vestergaard and Sand-Jensen, 2000). The ability to use HCO_3^-, the prevailing form of inorganic carbon in alkaline waters, probably influences species composition of macrophyte communities strongly (Maberly and Spence, 1983; Sand-Jensen and Gordon, 1986; Sand-Jensen et al., 1992). Controlled uptake or pH-drift experiments (Maberly and Spence, 1983; Sand-Jensen et al., 1992) are the usual methods used to assess this ability and compare carbon extraction capacity. However, they have rarely been combined with full photosynthesis–light (*P–I*) curves (but see Maberly, 1983, 1985), which are generally done under presumed saturating dissolved inorganic carbon (=DIC) availability (e.g. Madsen and Maberly, 1991; Hootsmans and Vermaat, 1994). Here, we combined both and contrasted four species of elodeid growth form (cf. Pieterse and Murphy, 1990): two fast growing Hydrocharitaceae and two slower growing Potamogetonaceae, i.e.: *Hydrilla verticillata* (L.f.) Royle, *Egeria densa* (Planchon), *Potamogeton schweinfurthii* (Benn), *Potamogeton lucens* L. All are known to use HCO_3^- (Denny and Weeks, 1970; Black et al., 1981; Maberly and Spence, 1983; Sand-Jensen and Gordon, 1986; van Ginkel and Prins, 1998) but the Hydrocharitaceae can be serious weed species (e.g. Pieterse, 1981; Pieterse and Murphy, 1990).

The a priori, an effective HCO_3^- use mechanism should reduce carbon limitation and thus affect the form of the photosynthesis–light curve, primarily in the section where light is no longer limiting. At higher HCO_3^--availability, maximum photosynthetic rates (P_{max}) therefore can be expected to be raised. However, Madsen et al. (1996) found the opposite for *Elodea canadensis* Michx. at 1.5 mM compared to 0.2 mM bicarbonate. For *Fontinalis antipyretica* Hedw., Maberly (1985) did find increasing P_{max} and $K_{1/2}$ with increasing DIC, but this was largely available as carbon dioxide. White et al. (1996) also observed increasing P_{max} and $K_{1/2}$ with increasing DIC for *H. verticillata*. Furthermore, White et al. (1996) observed in *H. verticillata* that significant photoinhibition only occurred at low DIC-availability (<1.3 mM DIC), whilst Spencer et al. (1994) demonstrated adverse interactive effects of CO_2-depletion and O_2-supersaturation on photosynthesis and growth within dense beds of *H. verticillata*. In such dense stands pH often increases to high values (>9, e.g. Van et al., 1976; Sand-Jensen, 1983; Spencer et al., 1994; Jones et al., 1996) on a daily basis, limiting the inorganic carbon source to HCO_3^- only. Seasonality in acclimation to such conditions has been demonstrated (Maberly and Spence, 1983; Sand-Jensen and Gordon, 1986), suggesting that some species are better equipped to maintain positive photosynthesis and hence continue growth. We postulate that such a capacity is an important trait explaining excessive biomass build-up of macrophyte species that are considered as weeds, such as *H. verticillata* (Pieterse and Murphy, 1990).

Our objectives were (1) to relate HCO_3^- use capability to photosynthesis–light curves, and (2) to test whether a more effective photosynthetic light and carbon use can explain faster growth in dense stands encountering high pH and low CO_2 availability.

2. Materials and methods

The study was carried out in the IHE laboratory facilities (Santamaria et al., 1994; Vermaat et al., 2000). Live material of all four species was obtained from the existing cultures at IHE. The *E. densa* material was originally obtained from a commercial aquarium shop, for *H. verticillata* we used the fast growing monoecious 'Rawalpindi' strain originating from an earlier collection of Dr. A. Pieterse and Prof. Dr. W. van Vierssen, *P. schweinfurthii* was originally collected by Prof. P. Denny in Lake Victoria, Uganda, and *P. lucens* was collected by Dr. M.J.M. Hootsmans from a floodplain pond near the river Meuse in Arcen, The Netherlands. Separate aquaria ($H \times W \times L = 40\,\text{cm} \times 40\,\text{cm} \times 60\,\text{cm}$) were cleaned for the initial plant cultures of each species. A 3–4 cm layer of standard IHE sediment (clay and sand in the ratio 1:3; Santamaria et al., 1994) was used with a top layer of 1–2 cm of clean sand. Each aquarium was then filled with tap water (~2.0 mM HCO_3^-, initial pH = 7.8, but this varied over the day when cultures became denser) up to about three-quarter level, and the remaining quarter filled with water from the existing IHE plant cultures. These were left to stabilise for 2 days before planting. Light availability was 200 μmol PAR $m^{-2}\,s^{-1}$, day lengths were 15 h, with half an hour dimmed light at the beginning and end of each dark period to simulate dusk and dawn. Water temperature was maintained at 25 ± 2 °C. Between 25 and 30 plant shoots from the existing IHE cultures were planted as starting material, and allowed to establish for 2 weeks before harvesting was initiated. The 0.5 g slow release pellets were added in the sediment next to each shoot as a nutrient supply (Osmocote containing 15% N, 10% P, 12% K, and 2% Mg w/w).

Whole-shoot photosynthesis–irradiance curves and pH-drift capacity were measured at two alkalinities, a low and a high concentration (0.5 and 2.0 mM HCO_3^-), created by mixing demineralised and (more alkaline) tap water, checked by titration after 12 h of equilibration at thermostatted temperature of 25 °C and brought to the desired concentration with sodium-bicarbonate when needed. Initial pH was always 7.8, with initial CO_2 amounting to 4% and bicarbonate to 96% of DIC in both alkalinities. Photosynthesis–light incubations proceeded routinely as described in Hootsmans and Vermaat (1994) and Vermaat and Verhagen (1996), using continuous O_2 recording (Campbell 21X data logger) with WTW Clark-type O_2 probes in three replicate re-circulating through flow tubes containing a number of intact shoots. These tubes were submerged in a large buffering volume (150 l) of the experimental medium in a thermostatted aquarium. Irradiance was 0, 25, 50, 100, 200, 300, 350, 400 and 500 μmol PAR $m^{-2}\,s^{-1}$. Light was measured at tube depth using a Licor 192SB sensor. After each 15 min incubation, the tubes were disconnected and fresh water allowed to enter from the surrounding aquarium. Initial O_2 content was lowered to 4 mg l^{-1} by N_2 bubbling. This was to reduce any photoinhibition effects and also must have lowered free CO_2. Since we were interested in plant performance as a function of HCO_3^- availability without interference by oxygen (e.g. Spencer

et al., 1994), this was thought the best compromise. We did not measure free CO_2 separately but measured pH before and after each 15 min incubation. The largest increase observed was from pH 7.8 to 8.1, in high alkalinity water. Oxygen increase or decrease was read from the highly significant linear slopes obtained from the 15 min lasting O_2 readings. These were then expressed per unit biomass as ash free dry weight (AFDW). For each replicate tube, plant biomass was measured separately by drying at 105 °C for 24 h, weighing, ashing at 520 °C for 3 h and final weighing. Chlorophyll was extracted from subsamples of leaves in ethanol and quantified spectrophotometrically according to Wintermans and De Mots (1965).

Per replicate, photosynthesis–light curves were fitted iteratively with a non-linear Marquardt–RSS minimalisation curve fitting procedure available in SPSS. The Michaelis–Menten rectangular hyperbola was used as P–I curve based on arguments brought forward by Ledermann and Tett (1981), Madsen et al. (1991) and Hootsmans and Vermaat (1994). To screen for possible light inhibition, an exponent $(1+C)$ was incorporated in the denominator with higher values of C leading to more pronounced inhibition. When C is set to zero the saturating hyperbola is modelled:

$$\bar{P} = \frac{P_{\max} \times I}{(K_m + I)^{1+C}} - \bar{R}$$

where P is the net rate of photosynthesis (mg O_2 g AFDW^{-1} h^{-1}), I is light intensity, $P_{\max}$ is the maximum rate of gross photosynthesis, $K_{1/2}$ is the half-saturation constant (μmol PAR m^{-2} s^{-1}) or light intensity at which gross photosynthesis is half of the maximum photosynthesis, C is a dimensionless inhibition term and R is respiration.

Three replicates were used except for *P. lucens* in low alkalinity where one widely aberrant replicate was discarded as an outlier. For each replicate curves of an alkalinity-species combination, the curve parameters were estimated individually, and then used in a two way ANOVA to assess alkalinity and species effects. Furthermore, the significance of light inhibition was assessed comparing the residual sums of squares of the fitted curve with and without inhibition term in an F-ratio with appropriate degrees of freedom (cf. Ledermann and Tett, 1981; Hootsmans and Vermaat, 1994).

For the pH-drift experiments, we followed the approach of Sand-Jensen et al. (1992). Plant shoots of the different species were incubated in similar quantity (~0.5 g FW) in closed 300 ml Winkler bottles for 96 h at constant light irradiance (200 μmol PAR m^{-2} s^{-1}). Media for the pH-drift experiments were prepared as above in open containers and allowed to stabilise for 24 h. Replication was five per alkalinity-species combination. The water was bubbled with nitrogen gas to lower the initial dissolved oxygen to ~4 mg l^{-1} O_2. Dissolved O_2, temperature and pH were measured at the beginning, after 30 min and after 16, 24, 48 and 96 h of incubation.

Data on elodeid growth as a function of bicarbonate concentration were compiled from the literature (Spencer et al., 1994; Madsen and Brix, 1997) and additional fully-factorial, randomized-block growth experiments (at least four replicates per treatment-species combination) by Okungu (2000) and Kahara (2001) carried out at IHE. We limited ourselves to experiments or treatments with a temperature range from 24 to 26 °C.

Table 1
Michaelis–Menten parameter estimates (mean ± S.E. of iteratively derived replicate curve estimates) for the four tested macrophyte species at two experimental alkalinities (low: 0.5 mM, high: 2.0 mM HCO_3^-)

Species	P_{max} (mg O_2 g AFDW^{-1} h^{-1})		Respiration (mg O_2 g AFDW^{-1} h^{-1})		$K_{1/2}$ (μmol PAR m^{-2} s^{-1})		Chlorophyll (mg chl (a+ b) g AFDW^{-1})
	Low alkalinity	High alkalinity	Low alkalinity	High alkalinity	Low alkalinity	High alkalinity	
Egeria densa	3.3 ± 0.3 a	13.1 ± 1.2 b	2.9 ± 0.1 a	9.0 ± 0.7 b	–	8.3 ± 0.9	17.3 ± 2.1
Hydrilla verticillata	7.1 ± 0.6 b	11.5 ± 0.8 b	5.3 ± 0.5 a	6.8 ± 0.5 bc	3.1 ± 0.9	8.1 ± 1.9	16.6 ± 0.1
Potamogeton lucens	15.2 ± 0.7 c	22.0 ± 1.6 c	11.9 ± 0.3 b	13.2 ± 1.0 c	–	9.7 ± 4.9	11.8 ± 1.1
Potamogeton schweinfurthii	3.2 ± 0.3 a	2.8 ± 0.2 a	3.0 ± 0.5 a	2.0 ± 0.1 a	1.2 ± 0.6	4.2 ± 1.1	18.3 ± 0.7

Also presented is the chlorophyll ($a + b$) content, in $n = 2$–3 samples of healthy shoot tips from the cultures. Chlorophyll content was not significantly different among species (one way ANOVA, $P > 0.05$). Tukey among-species multiple comparisons are presented for P_{max} and respiration within each alkalinity column.

Table 2
Two way ANOVA of effects of species and alkalinity on P–I curve parameters P_{max}, respiration and $K_{1/2}$

Factor	P_{max}			Respiration		$K_{1/2}$	
	d.f.	TSS (%)	P	TSS (%)	P	TSS (%)[a]	P
Species	3	74	0.001	80	0.001	25	0.171
Alkalinity	1	15	0.001	6	0.001	6	0.251
Species × alkalinity	3	8	0.001	10	0.001	4	0.642
Error	14	3		4		50	

Presented are the degrees of freedom (d.f.), the percentage of the variance explained (100 × factor sums of squares/total sums of squares, % TSS), and the level of significance (P).

[a] Due to empty cells, the factor sums of squares did not add to 100%.

3. Results

All fitted P–I curves had a steep initial rising part with quite low K_m values (<10 μmol PAR m^{-2} s^{-1}; Table 1), as well as light compensation points. For all *P. lucens* replicates and two of *E. densa* at low alkalinity, the iterative fitting procedure led to a negative estimate of $K_{1/2}$, i.e. a vertical asymptotic instead of a saturating curve. Of the 22 fitted curves in total, only one was not significant and had to be discarded, all others were significant and explained a substantial part of the variance ($P < 0.01$, $r^2 > 0.77$). Among species differences in P–I-parameters were larger than those due to alkalinity (Table 2), but both factors as well as their interaction were significant for P_{max} and respiration. This significant interaction is caused by the lack of a response in *P. schweinfurthii*: neither respiration nor P_{max} were affected by alkalinity, contrary to the pattern in the other three species (Table 1). Thus, in three of the four species tested, both saturated photosynthesis and respiration were significantly higher at the higher alkalinity. The difference (net P_{max} = P_{max} − respiration) remained higher at high alkalinity. Differences in $K_{1/2}$ were never significant (Table 2). Compared across species, respiration was positively correlated to P_{max} (Fig. 1).

When normalized to chlorophyll content, P_{max} ranged from 0.1 to 2 mg O_2 mg chlorophyll $(a+b)^{-1}$ h^{-1}, chlorophyll content of the experimental shoots ranged between 12 and 18 mg

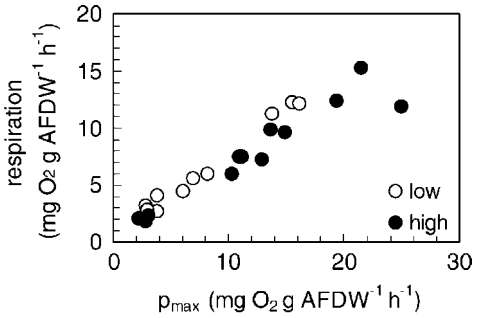

Fig. 1. Respiration estimates as a function of P_{max}. Presented are replicate estimates for each species, the filled symbols are for high and the open for low alkalinity. The regression equation for all data: $y = 0.59x + 1.03$, $r^2 = 0.91$, $P < 0.01$.

chlorophyll $(a + b)$ g AFDW^{-1}, whereas the fraction of chlorophyll b accounted for 27% of total chlorophyll for all species.

Incorporation of an inhibition term in the Michaelis–Menten curve did not increase the explained variance significantly compared to the simpler model without it for all eight combinations of species and alkalinity. However, for *H. verticillata* and *P. lucens* at low alkalinity, the F-ratios of the residual sums of squares were almost significant ($0.05 < P < 0.10$).

The pH-drift experiments showed clear contrasts between alkalinities and species (Fig. 2). As expected, in low alkalinity the pH changed more rapidly. More strikingly, in high alkalinity *E. densa* did not follow the other species as it did in low alkalinity. On the contrary, the pH declined. *H. verticillata* was able to raise the pH to the highest level in both alkalinities, and also reached such high values much earlier than the other species. If we ignore *E. densa*, the species behaved similarly in both alkalinities. A two way ANOVA was carried out on the data of 48 h (Table 3): both alkalinity and species were equally important in explaining the observed variation.

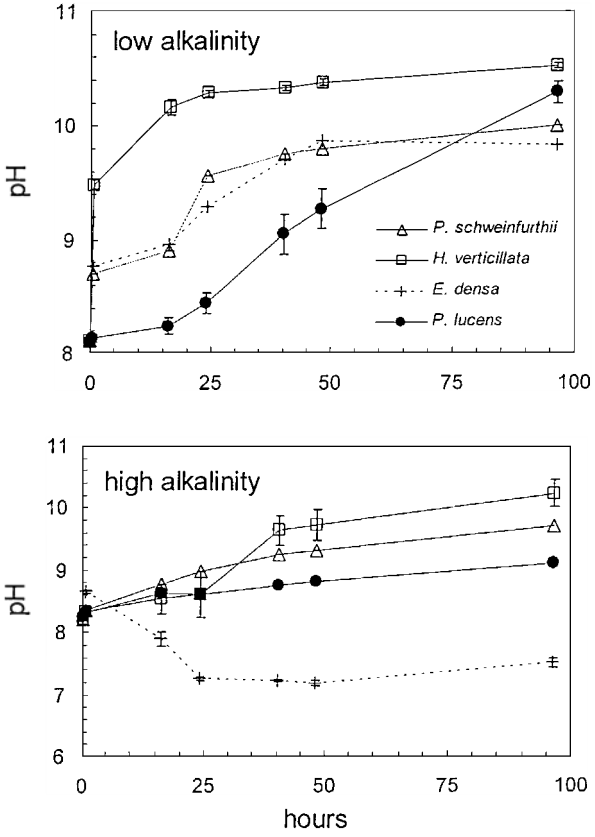

Fig. 2. Drifted pH in low and high alkalinity vs. time for the four tested macrophyte species.

Table 3
Two way ANOVA assessing the effects of species and alkalinity on pH after 48 h incubation in the pH-drift experiment, as in Table 2

Factor	d.f.	TSS (%)	P
Species	3	36	<0.001
Alkalinity	1	32	<0.001
Species × alkalinity	3	24	<0.001
Error	32	7	

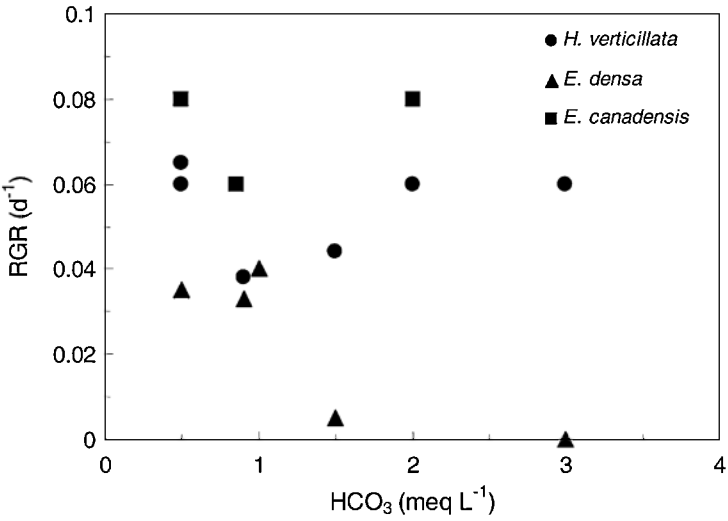

Fig. 3. Relative growth rate as a function of bicarbonate concentration for three species of Hydrcharitaceae: *Hydrilla verticillata*, *Egeria densa* and *Elodea canadensis*. Data are from growth experiments by Okungu (2000) and Kahara (2001), as well as from published literature (Barko and Smart, 1981; Haramoto and Ikusima, 1988; Madsen and Brix, 2000).

For our compilation of growth data from the literature unfortunately no data could be found on the two *Potamogeton* species. Hence, only Hydrocharitacean species are presented (Fig. 3), and clearly, *H. verticillata* as well as *E. canadensis* sustain growth at considerably higher alkalinities than *E. densa*, which is in line with our results in the pH-drift experiment.

4. Discussion

Our experiments demonstrated that increased bicarbonate availability enhanced photosynthetic performance in three out of the four species tested: both P_{max} and respiration were higher. We found no effect on the half-saturation constant $K_{1/2}$, suggesting that overall metabolism was enhanced by increased carbon availability at 2 meq l^{-1} HCO_3^-. Or reduced by a decrease. Since the plants had been cultured in alkaline local tap water, the

latter is probably the most correct phrasing. Overall, the difference in photosynthetic parameters between species was more pronounced than that between the two experimental alkalinities (Tables 1 and 2). Respiration and saturated photosynthetic rates were positively correlated (similar to, e.g. Madsen et al., 1991). Observed gross P_{max} values were high compared to the range published for freshwater plants (P_{max}: 1–15 mg O_2 g AFDW^{-1} h^{-1}; e.g. Van et al., 1976; Madsen et al., 1991; Hootsmans and Vermaat, 1994) but more comparable to those of freshwater plants incubated at elevated CO_2 (8–47 mg O_2 g DW^{-1} h^{-1}, Nielsen and Sand-Jensen, 1989) and seagrasses (3–13 mg O_2 g AFDW^{-1} h^{-1}; Vermaat et al., 1997), the latter possibly since seawater has a higher and more constant DIC concentration (Sand-Jensen and Gordon, 1984). Half-saturation constants, in contrast, were fairly low and respiration rates were quite high. The latter can partly be explained by the higher incubation temperature (25 °C versus 15–20 °C in most literature). For *Potamogeton pectinatus* L., Pilon and Santamaria (2002) demonstrate a clear increase of respiration with increasing temperature from 10 to 30 °C whilst gross P_{max} remained constant: at 25 °C respiration was about 45% of gross P_{max}. Such high percentages were also encountered in the literature: around 60% in Barko and Smart (1981) as well as Jones et al. (2000). Particularly the latter study is illustrative since it showed for *E. canadensis* and *Elodea nuttalli* (Planch.) St. John that saturated photosynthesis declined precipitously beyond pH = 8, whilst respiration remained fairly constant. Further, the high chlorophyll content of the leaves and the high proportion of (antenna-) chlorophyll *b* (Table 1; cf. Hootsmans and Vermaat, 1994) as well as the low half-saturation constants suggests that our cultures were shade-acclimated. Although the irradiances we employed are not particularly low, the dense, well-developed cultures may have been subject to self-shading. We suspect that our material may have been rich in stem material. Unfortunately, we did not separate stems and leaves. In short, we think we can explain our high respiration rates and low half-saturation constants by the high experimental temperature and the high proportion of respiring stem material as a response to self-shading. We chose this temperature because we felt it was more representative of the conditions encountered in situ. Also the high shoot density in our cultures probably resembles natural stands at the height of the growing season.

The maximal pH reached by *H. verticillata* in our pH-drift experiments was similar to the highest published elsewhere for macrophytes (pH ~10.5, Maberly and Spence, 1983; Sand-Jensen et al., 1992; Madsen et al., 1996). The other three species reached significantly lower final pH-values and/or altered pH at a slower rate. The decrease in pH in all the replicate material of *E. densa* is in line with results from growth experiments of several weeks done in our laboratory (Fig. 3): apparently the material of this species in our culture was unable to extract sufficient inorganic carbon at high alkalinity. *H. verticillata* maintained growth over a wide range of alkalinities (Fig. 3), which is also in line with the pH-drift results. A similar pattern is observed for *E. canadensis*, a species that is reportedly equally able to raise pH considerably (e.g. Maberly and Spence, 1983; Sand-Jensen and Gordon, 1986). Further, we have shown that an extension of the duration of the pH-drift experiment beyond the regular 24 h will affect the final pH: at low alkalinity, our four tested species had converged substantially compared to the situation after 24 h. Hence, the duration of a pH-drift experiment appears to affect its outcome.

From the pH-drift experiments, we conclude that the larger, slow-growing *P. lucens* and *P. schweinfurthii* were almost as efficient in extracting inorganic carbon at high pH and

high temperature as *H. verticillata*, a fast-growing, notorious weed species. We speculate that the advantage of the latter species over others gained from a more efficient carbon use is insufficient to explain its widespread occurrence as a weed. Other advantageous traits must be the high survival and dispersal capacity of vegetative fragments as well as the small absolute size of the vegetative modules and associated low construction cost that are shared with other Hydrocharitaceans.

Acknowledgements

We thank Frank Wiegman of the IHE lab for his help with the *P–I*-equipment and Patrick Denny for stimulating discussions. John Okungu is acknowledged for sharing his experimental results. George Bowes and two anonymous referees are thanked for pointing out weaknesses in an earlier version of this paper.

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