

## DISTRIBUTION OF THREE NYMPHAEID MACROPHYTES (*NYMPHAEA ALBA* L., *NUPHAR LUTEA* (L.) SM. AND *NYMPHOIDES PELTATA* (GMEL.) O. KUNTZE) IN RELATION TO ALKALINITY AND UPTAKE OF INORGANIC CARBON

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### ABSTRACT

Smits, A.J.M., de Lyon, M.J.H., van der Velde, G., Steentjes, P.L.M. and Roelofs, J.G.M., 1988. Distribution of three nymphaeid macrophytes (*Nymphaea alba* L., *Nuphar lutea* (L.) Sm. and *Nymphoides peltata* (Gmel.) O. Kuntze) in relation to alkalinity and uptake of inorganic carbon. *Aquat. Bot.*, 32: 45-62.

The presence/absence data of *Nymphaea alba* L., *Nuphar lutea* (L.) Sm. and *Nymphoides peltata* (Gmel.) O. Kuntze in 588 water bodies throughout The Netherlands were converted into logistic response curves with respect to alkalinity. For *Nuphar lutea* and *Nymphoides peltata* maximum probability of occurrence was found at 2.86 and 3.76 meq l<sup>-1</sup>, respectively. *Nymphaea alba* has a broad ecological amplitude with respect to alkalinity in comparison with *Nuphar lutea* and *Nymphoides peltata*. Within the 95% confidence interval no optimum for *Nymphaea* could be determined.

Uptake capacity for dissolved inorganic carbon (DIC) was examined using the pH-drift technique. In a 1-mM NaHCO<sub>3</sub> solution the DIC-extracting capacities of *Nymphaea alba*, *Nuphar lutea* and *Nymphoides peltata* were compared with those of the HCO<sub>3</sub><sup>-</sup>-using macrophyte *Potamogeton gramineus* L. Unlike the situation for *Potamogeton gramineus*, no uptake of HCO<sub>3</sub><sup>-</sup> could be registered by the floating and submerged laminae of the 3 nymphaeid species studied.

A very limited uptake of HCO<sub>3</sub><sup>-</sup> was measured when *Nymphaea alba*, *Nuphar lutea* and *Nymphoides peltata* seedlings were cultivated in solutions with a high bicarbonate concentration. Under natural conditions uptake of HCO<sub>3</sub><sup>-</sup> can be neglected.

### INTRODUCTION

Several biotic and abiotic factors determine the occurrence and abundance of aquatic macrophytes (Sculthorpe, 1967; Hutchinson, 1975). Spence (1967)

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has even suggested that macrophyte establishment and development are mainly based on water chemistry, provided that space is available and propagules of the species are present at a particular locality. It has been recognized however, that the sediment characteristics also influence the species composition of aquatic macrophyte communities (Barko and Smart, 1980, 1983).

A causal relation between the available carbon source and the distribution of aquatic macrophytes has already been reported by Steemann-Nielsen (1944). All submerged aquatic macrophytes can use dissolved  $\text{CO}_2$  for photosynthesis, but additional  $\text{HCO}_3^-$  assimilation is confined to certain algae and a limited number of aquatic angiosperms (Raven, 1970; Maberly and Spence, 1983; Spence and Maberly, 1985). Among the aquatic bryophytes examined so far (Bain and Proctor, 1980) a limited utilization of bicarbonate was ascertained only for *Fontinalis antipyretica* Hedw. (Peñuelas, 1985).

In view of this difference in C-extractive capacity, it is not surprising that a number of investigations have revealed a significant correlation between the alkalinity of a water body and the occurrence of certain species (Spence, 1967; Adams et al., 1978; Wiegleb, 1978; Pip, 1979; Hellquist, 1980; Kadono, 1982a,b).

Nymphaeid water plants do not depend entirely on the water phase for their carbon source. The upper surfaces of the floating leaves contain functional stomata, allowing  $\text{CO}_2$  from the atmosphere to be fixed by photosynthesis (Hutchinson, 1975). Little is known about the relative contributions of air and water to the photosynthesis of heterophyllous plants (cf. Spence and Maberly, 1985). However, Filbin (1980) found that the aqueous phase can be a considerable source of inorganic carbon for *Lemna minor* L. and for the floating leaves of two nymphaeacean species, although its contribution varies with the age of the leaves. Carbon assimilation of nymphaeid macrophytes is restricted to the green submerged parts of the plant during some phases of the life cycle. Seedlings and young plants of *Nymphaea alba* L. and *Nuphar lutea* (L.) Sm. produce only submerged leaves. Consequently, the biomass production of these plants during the establishing phase is completely dependent on the availability of dissolved inorganic carbon (DIC). After germination under water *Nymphoides peltata* (Gmel.) O. Kuntze develops two lanceolate cotyledons which are succeeded by some thin, circular leaves which resemble the submerged leaves of *Nymphaea alba* and *Nuphar lutea*. In contrast to the underwater leaves of *Nymphaea alba* and *Nuphar lutea*, the inundated leaves of the *Nymphoides peltata* seedlings can develop into floating leaves after they have reached the water surface.

During winter and early spring, only submerged leaves are present on the rhizome of the *Nuphar lutea* plant. In streams, *Nuphar lutea* develops only submerged leaves. *Nymphaea alba* produces less underwater leaves compared with *Nuphar lutea* and the production of these leaves ceases when the floating leaves are developing.

In view of the substantial parts of the life cycle in which the carbon source

for photosynthesis is restricted to DIC, it is of importance to study the distribution of these macrophytes in relation to alkalinity and their uptake capacity of DIC. In the present study, the occurrence of the three nymphaeids *Nymphaea alba*, *Nuphar lutea* and *Nymphoides peltata* in a large number of water bodies is related to water alkalinity, and the DIC-extractive capacity of these macrophytes is studied using the pH-drift technique (Allen and Spence, 1981).

## MATERIAL AND METHODS

### *Field studies*

During the period 1978–1984, 588 aquatic habitats were surveyed. Among them were ditches, ponds, moorland pools, oxbow lakes and rivers throughout The Netherlands. Each water body was visited 2–4 times a year, during winter and summer. The presence or absence of nymphaeids was registered and pH and alkalinity of the surface water measured. Alkalinity was determined immediately after collection of the water sample by titration with 0.1-M HCl down to pH 4.2. The pH was measured with a type EA-152 (Metrohm) combined electrode, connected to a Metrohm Herisau E-488 pH/mV meter. The data of occurrence of *Nymphaea alba*, *Nuphar lutea* and *Nymphoides peltata* in relation to water alkalinity were analysed with the Gaussian logistic regression method (GLR) (Austin, 1980; Austin et al., 1984), according to the regression analysis available in the standard statistical package (PROC LOGIST; S.A.S., 1986).

### *Experiments*

#### *Determination of inorganic carbon uptake; pH-drift experiments*

Carbon uptake by plant material was measured using a procedure which was slightly modified from the one described by Allen and Spence (1981). The measurement involved continuous registration of the pH in a DIC solution with photosynthesising plants. All experiments were conducted at 16°C ( $\pm 0.5^\circ\text{C}$ ) in a temperature-controlled chamber. During the experiments, light was provided by a 150-W halogen lamp at an intensity of  $550 \mu\text{Einstein m}^{-2} \text{s}^{-1}$ . Freshly-collected plant material or cultivated seedlings were used. The pH measurements were discontinued when no increase in pH had occurred for at least 1 h. Carbon uptake was calculated from the change in pH with time resulting from carbon assimilation. The pH was measured by means of two combined electrodes (Metrohm EA 152) and recorded simultaneously on a chart recorder (TOA EPR 200A). The electrodes were calibrated against buffers of pH 7.0 and 8.5 at 16°C. Experiments in which the two electrodes gave non-identical pH values during the measurements were rejected. The relative proportions of  $\text{CO}_2$ ,  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  were calculated from pH, temperature and

ionic strength according to Stumm and Morgan (1970). On occasions the oxygen produced at the end of the experiment was determined by Winkler titration (Drew and Robertson, 1974).

*Uptake of inorganic carbon in a 1-mM NaHCO<sub>3</sub> solution*

The carbon-uptake capacity of floating leaves of *Nymphaea alba*, *Nuphar lutea* and *Nymphoides peltata* seedlings in a 1-mM NaHCO<sub>3</sub> solution was determined. The submerged leaves of *Nymphaea alba* and *Nuphar lutea* were also tested. The carbon-extracting capacity of *Potamogeton gramineus* L., which was expected to use HCO<sub>3</sub><sup>-</sup> for photosynthesis, was measured in order to compare the obtained results.

Plant material was obtained from a cultivated stock. The plants were grown on clay in large glass aquaria or polyethylene tanks filled with tap water of moderate alkalinity (c. 1.5 meq l<sup>-1</sup>). Light was provided by a metal halide lamp (Philips HP; 400 W) at 200 μEinstein m<sup>-2</sup> s<sup>-1</sup> with a daily photoperiod of 18 h.

*Uptake of inorganic carbon by seedlings cultivated at varying alkalinity*

As HCO<sub>3</sub><sup>-</sup> uptake is dependent on the available concentration (Allen and Spence, 1981), seedlings of *Nymphaea alba*, *Nuphar lutea* and *Nymphoides peltata* were cultivated in media at varying alkalinity (i.e. varying HCO<sub>3</sub><sup>-</sup> concentration). The seedlings were grown in a mixture of sand and clay in large glass containers (40 l). The aquaria were filled with a culture medium consisting of salts added to twice-demineralized water (see Table 1). The concentration of each nutrient was within the range of the nutrient concentrations found in water bodies dominated by nymphaeid water plants (van der Velde et al., 1986). By adding different amounts of NaHCO<sub>3</sub> the alkalinity of the medium

TABLE 1

Chemical composition of the cultivation medium to which different amounts of NaHCO<sub>3</sub> were added

Major components	(μM)	Minor components	(μM)
Na <sub>2</sub> SO <sub>4</sub>	570	FeCl <sub>3</sub>	2.0
K <sub>2</sub> HPO <sub>4</sub>	1.5	CuSO <sub>4</sub>	0.0005
CaCl <sub>2</sub>	820	MnCl <sub>2</sub>	0.4
KCl	130	ZnSO <sub>4</sub>	0.04
NH <sub>4</sub> NO <sub>3</sub>	30	Co(NO <sub>3</sub> ) <sub>2</sub>	0.01
MgCl <sub>2</sub>	200	H <sub>3</sub> BO <sub>3</sub>	0.04
		EDTA	0.2
		(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub>	0.001
		NH <sub>4</sub> VO <sub>3</sub>	0.02

was varied. The culture medium was regularly replaced and filamentous algae were removed by hand, in order to prevent large fluctuations in pH and DIC concentrations. The seedlings were cultivated at alkalinities of approximately 0.6, 1.0, 2.5 and 5.0 meq l<sup>-1</sup> during 4–16 weeks. Subsequently, the C-extractive capacity of these seedlings was determined. Prior to the experiments the alkalinity of the cultivation medium was accurately determined and the carbon uptake of the seedlings was measured in a NaHCO<sub>3</sub> solution with a corresponding alkalinity.

### *Photosynthetic activity in an oxygen-depleted solution*

On occasions, when a pH-drift experiment was terminated, dissolved O<sub>2</sub> was determined. After incubations in media of high alkalinity (> 1.0 meq l<sup>-1</sup>) in particular, an oversaturation of dissolved O<sub>2</sub> was measured (155–240% air saturation; data not shown). It is not inconceivable that a high O<sub>2</sub> concentration could have a negative effect on the uptake of HCO<sub>3</sub><sup>-</sup>. Therefore, in an oxygen-depleted solution the photosynthetic activity of the nymphaeid seedlings and *Potamogeton gramineus* was measured. The plants were allowed to photosynthesize in a solution containing only HCO<sub>3</sub><sup>-</sup> as DIC (a), and in a solution containing both CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> (b).

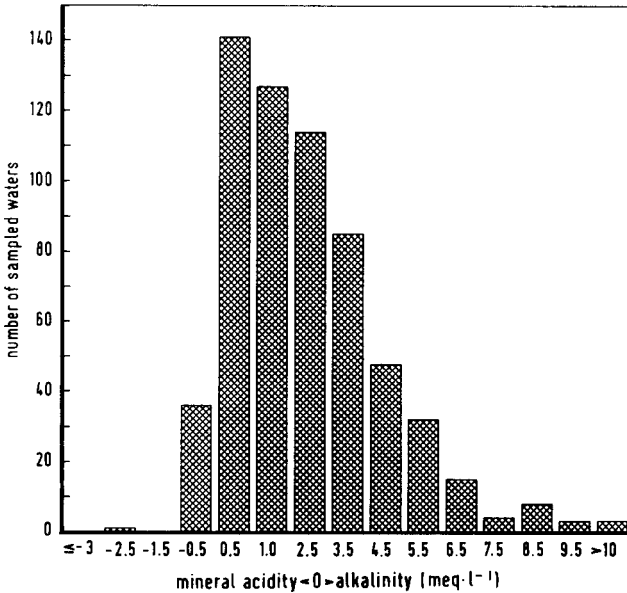


Fig. 1. Frequency distributions of the sampled waters ( $n=588$ ) with respect to alkalinity. The bars show the number of water bodies in each alkalinity class. Each class (except the extremes) is defined by  $(x-0.5 \text{ meq l}^{-1}) < x \leq (x+0.5 \text{ meq l}^{-1})$ . Mineral acidity is equivalent to negative alkalinity.

(a) The pH of a 2-mM  $\text{NaHCO}_3$  solution was adjusted to pH 8.3 with 0.1-N NaOH solution so that  $\text{HCO}_3^-$  was the only DIC source available. The bicarbonate solution was bubbled through with nitrogen gas in order to reduce the concentration of dissolved oxygen to approximately 30  $\mu\text{M}$ . Some leaves of each species (about 3-g fresh weight) were rinsed in distilled water and subsequently incubated in closed serum bottles (130 ml) containing the hypoxic 2-mM  $\text{NaHCO}_3$  solution.

(b) The laminae of *Nymphaea alba*, *Nuphar lutea*, *Nymphoides peltata* and *P. gramineus* were also incubated in the hypoxic 2-mM  $\text{HCO}_3^-$  solution. Prior to the incubation, however, acid (0.1-N HCl) was injected through the rubber stopper of the serum bottles in order to obtain a solution containing 0.5-mM  $\text{CO}_2$  and 1.5-mM  $\text{HCO}_3^-$ . Photosynthetic activity was determined by measuring the produced oxygen. Dissolved oxygen was determined according to Drew and Robertson (1974). The seedlings of the nymphaeids and *P. gramineus* used in this experiment were cultivated in a culture medium (see Table 1) containing 3-mM  $\text{NaHCO}_3$ . Prior to the experiments, plant material was carefully cleaned of debris and rinsed in distilled water. Only submerged leaves were used. The measurements were conducted at 20°C and at a light intensity of 250  $\mu\text{Einstein m}^{-2} \text{s}^{-1}$ . In all treatments the incubations were terminated before the  $\text{O}_2$  content in the serum bottles, equalled the air-saturated  $\text{O}_2$  concentration.

## RESULTS

### Field studies

Figure 1 shows the frequency distribution of the sampled water bodies with respect to alkalinity classes. It can be noted that most of the sampled water bodies had a low to moderate alkalinity (0.5–15 meq  $\text{l}^{-1}$ ), which resulted in a positively skewed sample distribution of alkalinity. As a consequence, estimation of the indicator values and ecological amplitudes by weighted averaging and calculating medians, respectively, would be unreliable. Therefore, the

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Fig. 2. The occurrence of *Nymphaea alba*, *Nuphar lutea* and *Nymphoides peltata* along the alkalinity gradient. Mineral acidity is equivalent to negative alkalinity. Water bodies in which the plant species concerned were found are marked with an open dot on the  $P=1$  level (ordinate).  $p_{\text{max}}$ , maximal probability of occurrence;  $u$ , alkalinity value of a water body with the highest probability of occurrence;  $t$ , ecological amplitude with regard to alkalinity. The optimum of the curve ( $u$ ) is the alkalinity value of a water body with the highest probability of occurrence ( $p_{\text{max}}$ ) of a species. The tolerance ( $t$ ) is a measure of peak width and provides information about the preferred range of water alkalinity (ecological amplitude). The standard errors of the estimated parameters are presented in Table 2. For a mathematical definition of the individual parameters the reader is referred to ter Braak and Looman (1986).

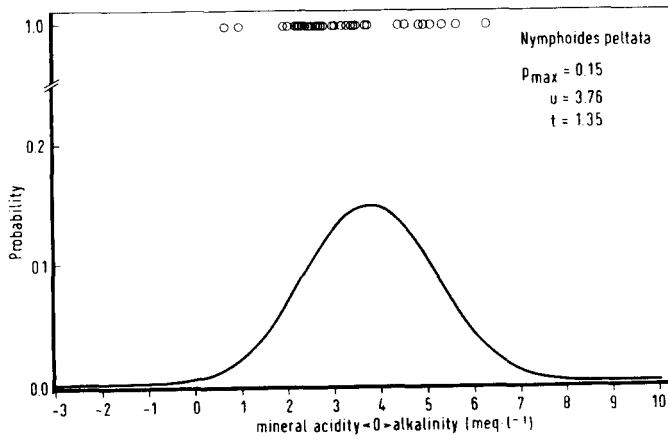
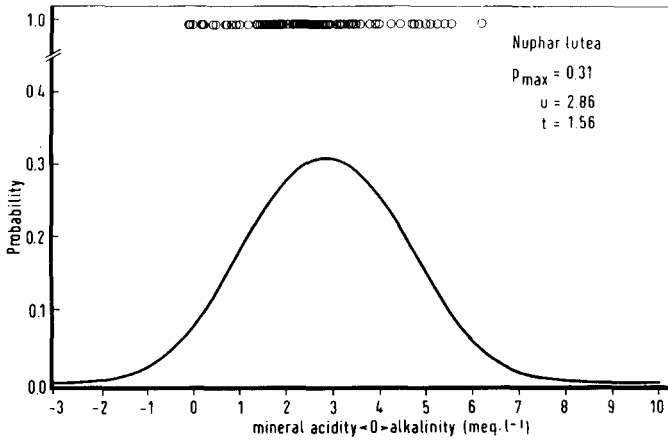
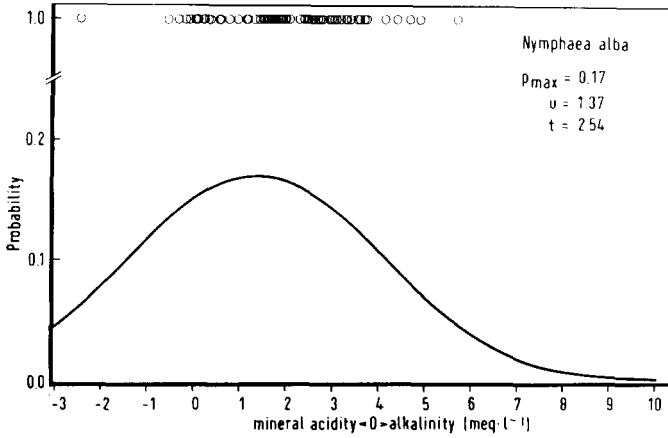


TABLE 2

The estimates for maximum probability ( $p_{\max}$ ), optimum ( $u$ ) and tolerance ( $t$ ) of the logistic response curves shown in Fig 2

	$p_{\max}$	s.e.	$t$	s.e.	$u$	s.e.	conf. intv.	lower	upper
<i>Nuphar lutea</i>	0.31	0.03	1.56	0.17	2.86	0.20	95%	2.45	3.43
<i>Nymphaea alba</i>	0.17	0.02	2.54	0.69	1.37	0.74	90%	-8.00	2.25
<i>Nymphoides peltata</i>	0.15	0.03	1.35	0.20	3.76	0.30	95%	3.21	5.03

s.e.: standard error of the estimate.

The upper and lower limits of the 95% or 90% confidence interval (conf. intv.) of  $u$  are given. Standard errors of the estimates were computed according to ter Braak and Looman (1986) and the Appendix.

Gaussian logistic regression (GLR) procedure was used to estimate species optima and ecological amplitudes from presence/absence data. These presence/absence data, modelled with GLR, result in an unimodal occurrence-probability curve. The Gaussian logit response curves for *Nymphaea alba*, *Nuphar lutea* and *Nymphoides peltata* are depicted in Fig. 2.

The relatively high values of " $p_{\max}$ " and " $t$ " of the response curve of *Nuphar lutea* indicate that this species is the most common in the waters sampled ( $p_{\max}=0.31$ ;  $t=1.56$ ), followed by *Nymphaea alba* ( $p_{\max}=0.17$ ;  $t=2.54$ ) and *Nymphoides peltata* ( $p_{\max}=0.15$ ;  $t=1.35$ ).

*Nymphoides peltata* is restricted to waters with a relatively high alkalinity ( $u=3.76 \text{ meq l}^{-1}$ ) while *Nuphar lutea* shows highest frequency in waters of a slightly lower alkalinity ( $u=2.86 \text{ meq l}^{-1}$ ). The alkalinity range of waters which are likely to harbour *Nuphar lutea* is greater than that of waters with *Nymphoides peltata* ( $t=2.54$ ,  $1.56$ , respectively). *Nymphaea alba* has a broad ecological amplitude. The optimum ( $u$ ) could not be estimated reliably. No 95% confidence interval could be calculated. The 90% confidence interval given in Table 2 shows that the species favours low alkalinities. Moreover, it may be noted that unlike *Nymphoides peltata*, *Nymphaea alba* and *Nuphar lutea* occur in acid waters in which dissolved inorganic carbon is available only as  $\text{CO}_2$ .

## Experiments

### Uptake of inorganic carbon in a 1-mM $\text{NaHCO}_3$ solution

Carbon-uptake rates calculated from pH-drift experiments in a solution of 1-mM  $\text{NaHCO}_3$  are shown in Fig. 3. The DIC uptake of *P. gramineus* can be described by a two-phased curve, which is obtained when both  $\text{CO}_2$  and  $\text{HCO}_3^-$  are removed from the solution (Allen and Spence, 1981). It has been suggested that this C-uptake curve is composed of two components. The linear



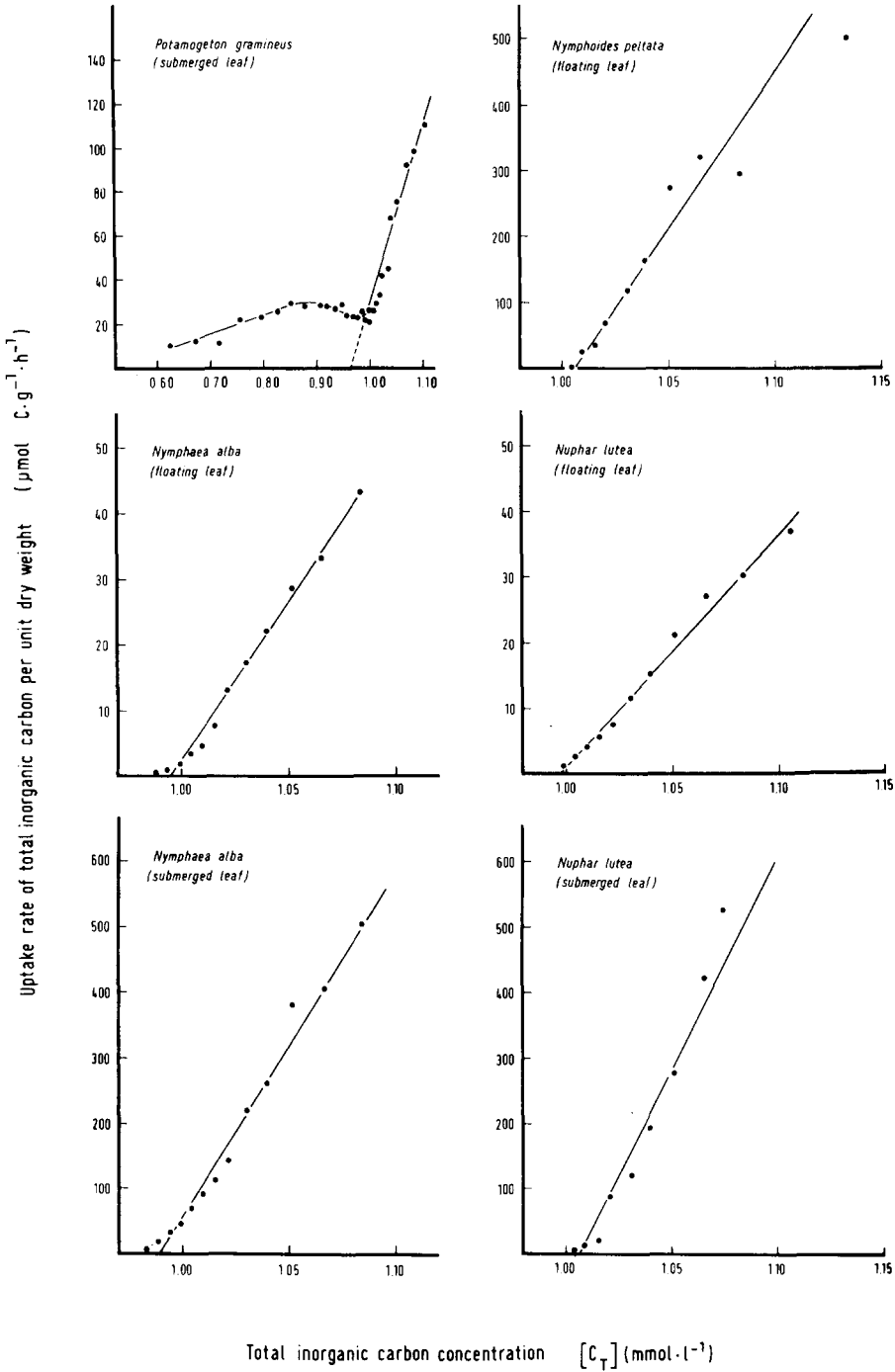


Fig. 3. Rate of carbon uptake plotted against the total inorganic carbon concentration ( $C_T$ ) in a closed constant alkalinity system. A solution of 1-mM  $\text{NaHCO}_3$  was used in all experiments.

TABLE 3

Data obtained from pH-drift experiments using an 1-mM NaHCO<sub>3</sub> solution

	Final pH	C <sub>T</sub> /Alk	CO <sub>2</sub> compensation point <sup>1</sup> (μM)
<i>Potamogeton gramineus</i>	10.30	0.51	4.1
<i>Nymphoides peltata</i> (floating leaf)	8.25	1.00	13.5
<i>Nymphaea alba</i> (floating leaf)	8.45	0.99	8.4
<i>Nuphar lutea</i> (floating leaf)	8.45	0.99	8.4
<i>Nymphaea alba</i> (submerged leaf)	8.55	0.99	6.6
<i>Nuphar lutea</i> (submerged leaf)	8.45	0.99	8.4

<sup>1</sup>CO<sub>2</sub> compensation points calculated according to Allen and Spence (1981).

TABLE 4

Data derived from pH-drift experiments with *Nymphoides*, *Nymphaea* and *Nuphar* seedlings

Species	(HCO <sub>3</sub> <sup>-</sup> ) <sup>1</sup> (mM)	(HCO <sub>3</sub> <sup>-</sup> ) <sup>2</sup> (mM)	Final pH	C <sub>T</sub> /Alk	CO <sub>2</sub> compensation point <sup>3</sup> (μM)
<i>Nymphoides peltata</i>	0.6	0.62	8.40	0.98	6.5
	2.5	2.44	9.00	0.96	18
	5.0	4.84	9.00	0.96	18
<i>Nymphaea alba</i>	0.6	0.58	8.50	1.04	5
	2.5	2.42	8.90	0.98	13
	5.0	4.80	8.80	0.98	29
<i>Nuphar lutea</i>	0.6	0.59	8.50	1.02	5
	2.5	2.49	9.10	0.93	14
	5.0	4.88	8.90	0.96	28

<sup>1</sup>The approximate bicarbonate concentration in which the seedlings were cultivated. During the culture the HCO<sub>3</sub><sup>-</sup> concentration fluctuated about 0.5 meq l<sup>-1</sup>.<sup>2</sup>The accurate bicarbonate concentration at the start of an experiment.<sup>3</sup>CO<sub>2</sub> compensation points calculated according to Allen and Spence (1981).

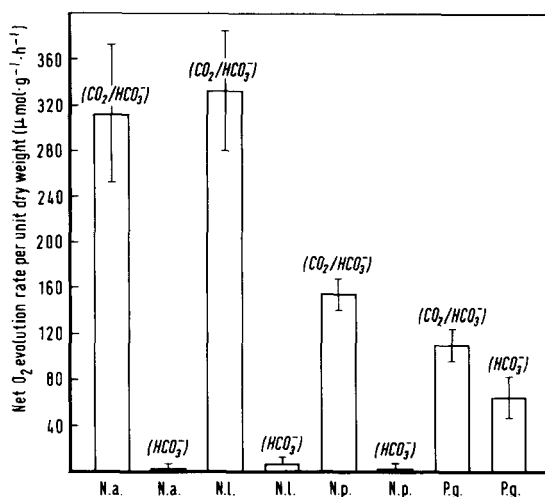


Fig. 4. Oxygen production of *Nymphaea alba* (N.a.), *Nuphar lutea* (N.l.), *Nymphoides peltata* (N.p.) and *Potamogeton gramineus* (P.g.) in a hypoxic solution under various DIC conditions. Prior to the experiments oxygen was stripped from the incubation medium using nitrogen gas, after which NaHCO<sub>3</sub> was added. In all experiments C<sub>T</sub> was 2 mM, but in half of the incubations C<sub>T</sub> consisted of 0.5 mM CO<sub>2</sub> + 1.5 mM HCO<sub>3</sub><sup>-</sup> (CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup>), while in the other half DIC was present exclusively as HCO<sub>3</sub><sup>-</sup> (HCO<sub>3</sub><sup>-</sup>). DIC composition was achieved by adding appropriate amounts of 0.1-N HCl. Each treatment was conducted in triplicate.

part of the curve represents CO<sub>2</sub> uptake, while the remaining section is the result of HCO<sub>3</sub><sup>-</sup> uptake. Extrapolation of the linear part of the curve would yield the total DIC (C<sub>T</sub>) concentration at which net uptake of CO<sub>2</sub> no longer occurs. The CO<sub>2</sub> concentration at this point can be calculated and is assumed to be the CO<sub>2</sub> compensation point. Extrapolation of the HCO<sub>3</sub><sup>-</sup> uptake curve yields the HCO<sub>3</sub><sup>-</sup> compensation point under the experimental conditions.

The ratio of DIC to alkalinity (C<sub>T</sub>/Alk) found in the solution after the plant has failed to continue the uptake of inorganic carbon is a measure of its C-extracting capacity (Maberly and Spence, 1983). It can be deduced that at a C<sub>T</sub>/Alk ratio of approximately 1.0, CO<sub>2</sub> is the main form of DIC removed from the solution. At lower C<sub>T</sub>/Alk values HCO<sub>3</sub><sup>-</sup> is used for photosynthesis as well.

In Tables 3 and 4 the results of the conducted pH-drift experiments are shown. In a 1-mM NaHCO<sub>3</sub> solution *P. gramineus* achieved a C<sub>T</sub>/Alk of 0.51 indicating that HCO<sub>3</sub><sup>-</sup> can be an important source of inorganic carbon. In view of the uptake rates for CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup>, respectively, it is evident that affinity towards CO<sub>2</sub> is considerably higher. Only linear uptake curves are obtained in pH-drift experiments with leaves of *Nymphoides peltata* seedlings and with both floating and submerged leaves of *Nuphar lutea* and *Nymphaea alba*, which reflects the very poor HCO<sub>3</sub><sup>-</sup>-extractive capacity of these macrophytes in a 1-mM HCO<sub>3</sub><sup>-</sup> solution. Compared with the floating leaves of *Nymphaea alba* and *Nuphar lutea* the C-uptake rates of the submerged leaves of *Nuphar lutea*,

*Nymphaea alba* and *Nymphoides peltata* seedlings are markedly higher. This difference is most probably due to a high diffusion resistance as a result of the thick cuticle of the floating leaves, which under natural conditions prevents desiccation. Moreover, a fast exchange between dissolved carbon and the submerged laminae is easier than it is for the relatively thick floating leaves.

pH-drift experiments conducted in solutions of high alkalinity gave lower  $C_T/\text{Alk}$  ratios than measurements in solutions with low alkalinity (see Tables 3 and 4). The  $C_T/\text{Alk}$  ratios are, however, not much lower than 1.0, indicating a very limited  $\text{HCO}_3^-$  uptake. The increased  $\text{CO}_2$  compensation points determined at high alkalinities were probably caused by the increased photorespiration.

#### *Uptake of inorganic carbon by seedlings cultivated at various alkalinities*

Photosynthesis of *Nymphaea alba*, *Nuphar lutea*, *Nymphoides peltata* seedlings and *P. gramineus* in an oxygen-depleted medium under different DIC conditions is shown in Fig. 4. Only *P. gramineus* was able to produce  $\text{O}_2$  in a solution in which DIC consisted solely of  $\text{HCO}_3^-$ . From the negligible photosynthetic activity of *Nymphaea alba*, *Nuphar lutea* and *Nymphoides peltata* in the ' $\text{HCO}_3^-$ -incubation experiment', as opposed to the considerable  $\text{O}_2$  production in the ' $\text{CO}_2/\text{HCO}_3^-$ -incubation experiment', it can be concluded that the accumulated  $\text{O}_2$  in the pH-drift experiments did not mask a limited  $\text{HCO}_3^-$  uptake by these macrophytes.

## DISCUSSION

According to Stumm and Morgan (1981) the most relevant non-carbonate bases that occur in fresh waters are the silicates ( $10^{-4}$ – $10^{-3}$  M), implicating that, generally, measurements of water alkalinity provide a reasonable estimation of dissolved  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ .

In view of the alkalinity value with the highest frequency of occurrence and the small ecological amplitude of *Nymphoides peltata* with respect to alkalinity, it can be concluded that the habitat of this nymphaeid macrophyte is restricted to well-buffered waters.

After a survey of 17 selected surface waters, representing most of the aquatic macrophyte communities in Eastern Central Europe, Pietsch (1972) also classified *Nymphoides peltata* as a species restricted to water bodies with a high calcium-bicarbonate content.

Wiegleb (1978), who investigated the hydrochemical factors and the macrophyte vegetation of 79 lakes and ponds in Germany (F.R.G.), regarded *Nymphoides peltata* as a characteristic species of waters with an alkalinity higher than  $2 \text{ meq l}^{-1}$ , which is in agreement with our results.

Like *Nymphoides peltata*, *Nuphar lutea* occurs mainly in alkaline waters, but its optimum alkalinity of occurrence is lower and its ecological amplitude, with regard to alkalinity, is larger. Hence, *Nuphar lutea* can be found in aquatic ecosystems with a low water alkalinity or even in acid waters.

Iversen (1929) presented some records of the occurrence of *Nuphar lutea* in "acidotrophic" waters in Great Britain. The pH of these waters, however, was never lower than 6. According to Heslop-Harrison (1955a,b), *Nymphaea alba* occurs more frequently in weakly acid and nutrient-poor waters than *Nuphar lutea*. In contrast to the above mentioned authors, the occurrence of *Nymphaea alba* and *Nuphar lutea* in water bodies with a mineral acidity was recorded. In none of the sampled acid waters was *Nymphoides peltata* found.

The increase in acid precipitation over the last decades has led to the acidification of a great number of poorly-buffered waters in The Netherlands in which *Nymphaea alba*, and to a lesser extent, *Nuphar lutea* were usually found (Arts, 1987). *Nymphaea alba* and *Nuphar lutea* are still present in these acidified waters, in contrast to some characteristic species of soft waters. This development may have caused a shift of the occurrence range towards the acid water bodies.

In acid waters, submerged macrophytes are completely dependent on CO<sub>2</sub> in the water column or in the sediment. The floating leaves of *Nymphaea alba* and *Nuphar lutea*, however, have access to atmospheric CO<sub>2</sub>. An additional source of inorganic carbon may be supplied by respiratory CO<sub>2</sub>. Dacey (1980, 1981) and Dacey and Klug (1982) have described an internal ventilation system which enhances oxygen transport to the rhizomes and roots of *Nuphar lutea*. Simultaneously, a more rapid movement of respiratory CO<sub>2</sub> to the leaves is achieved, where it can be assimilated. This pressurized gas transport has also been found in *Nelumbo nucifera* Gaertn. (Merget, 1874; Ohno, 1910) and *Nymphoides peltata* (Grosse and Mevi-Schütz, 1987). It appears to be a general phenomenon in aquatic plants with floating or emergent leaves.

The production of floating leaves and the successful vegetative propagation of nymphaeids may, at least partly, explain the survival of *Nymphaea alba* and *Nuphar lutea* in acid water bodies. However, little is known about the germination and seedling development of these nymphaeids. Their generative reproduction in acid moorland pools is currently under investigation.

The DIC-uptake capacity of the floating leaves is not essential for demonstrating a possible relation between the distribution of *Nymphaea alba*, *Nuphar lutea* and *Nymphoides peltata* and the quantity and composition of DIC. As pointed out, the photosynthesis of the full-grown nymphaeid has become largely independent of DIC. Juvenile nymphaeids do not possess floating leaves, and thus have no access to atmospheric CO<sub>2</sub>. In addition, there is no enhanced ventilation of the internal atmosphere which would allow an efficient refixation of respiratory CO<sub>2</sub> originating in the roots. Therefore, unlike the full-grown macrophytes, photosynthesis of *Nymphaea alba* and *Nuphar lutea* seedlings is completely dependent on DIC. This also applies to *Nymphoides peltata* seedlings, but for a significantly shorter period. *Nymphoides peltata* has no morphologically-distinct leaves similar to the underwater leaves of *Nuphar lutea* and *Nymphaea alba*. The leaves of *Nymphoides peltata* produced beneath

the water surface, however, are very thin and fragile (cf. Glück, 1924). The poor aeration of the aerenchyma of amphibious and aquatic plants during the submerged phase results in ethylene accumulation (Ridge, 1987). The petioles react to ethylene with a strong cell-wall extension (in young petioles and laminae also by an increased cell division; Funke and Bartels, 1937; Malone and Ridge, 1983; Ridge and Amarasinghe, 1984). This elongation leads to a rapid growth of the laminae to the water surface. Once the floating leaves are established, as is the case very soon after germination, the photosynthesis of the young *Nymphoides peltata* plant becomes independent of DIC.

There are several methods for measuring inorganic C uptake by aquatic macro- and microphytes. In this study the pH-drift technique introduced by Allen and Spence (1981) was used, involving continuous registration of pH in a closed system at constant alkalinity. There is good agreement between the results obtained with this potentiometric technique (indirect determination of C uptake) and those obtained with the Infra-Red Gas Analysator (IRGA) and  $^{14}\text{C}$ -method (direct determination of C uptake; Hofslagare et al., 1985). The main drawbacks of the pH-drift technique are: (1) the long incubation time which is often needed to determine the final pH (cf. also Sand-Jensen, 1987); (2) the accumulation of oxygen, which causes an increase in the  $\text{CO}_2$  compensation points. However, Maberly and Spence (1983) argued that high  $\text{O}_2$  concentrations are likely to occur in productive aquatic ecosystems, and thus C-extracting capacities estimated at elevated  $\text{O}_2$  concentrations are ecologically significant.

The floating leaves of *Nuphar lutea*, *Nymphaea alba* and *Nymphoides peltata* show comparable  $\text{HCO}_3^-$ -uptake capacities in an 1-mM  $\text{NaHCO}_3$  solution. Among other aquatic macrophytes, Maberly and Spence (1983) also determined the C-extracting ability of the floating leaves of *Nuphar lutea*, albeit under slightly different experimental conditions. However, there is good agreement with the results presented here. In a 1-mM  $\text{KHCO}_3$  solution they measured a  $\text{C}_\text{T}/\text{Alk}$  ratio for *Nuphar lutea* of 0.98 (cf. the  $\text{C}_\text{T}/\text{Alk}$  ratio of 0.99 found in this study).

Francko (1986) studied the C-assimilation of *Nelumbo lutea* (Willd.) Pers. at alkaline and acidic conditions. At pH 8.2 the assimilation rate of  $^{14}\text{C}$  exceeded the regeneration rate of  $\text{CO}_2$  from the aqueous carbonate equilibria. Hence, the observed incorporation of  $^{14}\text{C}$  could not account for  $\text{CO}_2$  uptake alone. He suggested that this relatively high assimilation rate at high pH was the result of a limited  $\text{HCO}_3^-$  uptake.

Maberly and Spence (1983) demonstrated that *Elodea canadensis* Michx. was unable to use  $\text{HCO}_3^-$  at 0.5-mM bicarbonate. When this species was tested in a 1-mM  $\text{KHCO}_3$  solution, however, a  $\text{C}_\text{T}/\text{Alk}$  ratio of 0.7 was measured. Such a concentration-dependent uptake of  $\text{HCO}_3^-$  was not found for *Nymphaea alba*, *Nuphar lutea* and *Nymphoides peltata* seedlings which had been cultivated for at least 3 weeks at varying alkalinities (Table 4). Indeed, the  $\text{C}_\text{T}/\text{Alk}$  ratios

obtained for these macrophytes were lower at higher bicarbonate concentrations, although they did not show values below 0.96 (see Table 4).

There is no sharp dividing line between  $\text{CO}_2$ - and  $\text{HCO}_3^-$ -using macrophytes. It is more appropriate to rank the species in a progressive series for bicarbonate-uptake capacity (Maberly and Spence, 1983). Comparing the  $\text{HCO}_3^-$ -uptake capacities of *Nymphaea alba*, *Nuphar lutea* and *Nymphoides peltata* seedlings with those of other macrophytes (Spence and Maberly, 1985), it is evident that these juvenile nymphaeid macrophytes belong to the group of aquatic plants which primarily use dissolved (or atmospheric)  $\text{CO}_2$  for photosynthesis.

The seedlings of *Nymphoides peltata*, but especially those of *Nymphaea alba* and *Nuphar lutea*, frequently occur in slowly moving or stagnant waters on organic sediments enriched with  $\text{CO}_2$  (A.J.M. Smits and P. van Avesaath, unpublished data, 1988). It is not known whether these seedlings are able to transport  $\text{CO}_2$  from the sediment via the roots to the laminae, where it can be used for photosynthesis. In these water bodies, however, the  $\text{CO}_2$  concentration in the water layer just above the sediment exceeds equilibrium values, so that either way ambient DIC is characterized by a relatively low  $\text{HCO}_3^-/\text{CO}_2$  ratio. Assuming that regulation of bicarbonate uptake by the  $\text{HCO}_3^-/\text{CO}_2$  ratio (Sand-Jensen and Gordon, 1986) is uniform for all aquatic macrophytes,  $\text{HCO}_3^-$  as an alternative inorganic carbon source for *Nymphaea alba*, *Nuphar lutea* and *Nymphoides peltata* seedlings under natural conditions should be of little importance. The high frequency of occurrence of *Nuphar lutea* and *Nymphoides peltata* in alkaline waters is probably based on factors which are related to high alkalinity ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  or other nutrients such as N or P; van der Velde et al., 1986; de Lyon and Roelofs, 1986). To what extent these parameters influence the occurrence of these nymphaeid macrophytes needs to be investigated.

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#### APPENDIX

BY C.J.F. TER BRAAK

Variance and confidence interval for maximum probability in the Gaussian-logit model.

Using the notation of ter Braak and Looman (1986), the estimated optimum and maximum probability are, respectively,

$$u = -b_1 / (2b_2) \text{ and } p_{\max} = 1 / \{1 + \exp(-c_{\max})\}$$

where

$$c_{\max} = b_0 + b_1 u + b_2 u^2 = b_0 - b_1^2 / (4b_2)$$

using Taylor expansion, we obtain

$$\text{var}(c_{\max}) \approx v_{00} + 2uv_{01} + 2u^2v_{02} + u^2v_{11} + 2u^3v_{12} + u^4v_{22}$$

where

$v_{kl}$  is the estimated (co)variance between  $b_k$  and  $b_l$

$$(k=0,1,2; l=0,1,2)$$

and

$$\text{var}(p_{\max}) \approx p_{\max}^4 \exp(-2c_{\max}) \text{var}(c_{\max})$$

An approximate  $100(1-\alpha)\%$  confidence interval for  $p_{\max}$  can be derived from the corresponding interval for  $c_{\max}$  by inversion of the logit transformation. The upper and lower limits of the interval for  $p_{\max}$  are, respectively,

$$p_u = 1 / \{1 + \exp(-c_u)\} \text{ and } p_l = 1 / \{1 - \exp(-c_l)\}$$

where

$c_u, c_l = c_{\max} \pm t\alpha \sqrt{\text{var}(c_{\max})}$  with  $t\alpha$  the critical value of the two-sided  $t$ -test at the chosen probability level  $\alpha$ .

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