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Carbon acquisition mechanisms by planktonic desmids and their link to ecological distribution¹

Elly Spijkerman, Stephen C. Maberly, and Peter F.M. Coesel

Abstract: To test if different inorganic carbon (C_i) uptake mechanisms underlie the ecological distribution pattern of planktonic desmids, we performed pH-drift experiments with 12 strains, belonging to seven species, originating from lakes of different pH. *Staurastrum brachiatum* Ralfs and *Staurodesmus cuspidatus* (Ralfs) Teil. var. *curvatus* (W. West) Teil., species confined to acidic, soft water habitats, showed remarkably different behavior in the pH drift experiments: *S. brachiatum* appeared to use CO_2 only, whereas *Staurodesmus cuspidatus* appeared to use HCO_3^- as well. *Staurastrum chaetoceras* (Schr.) Smith and *Staurastrum planctonicum* Teil., species well-known for their abundant occurrence in alkaline waters, were the most effective at using HCO_3^- . Other species, to be encountered in both slightly acidic and slightly alkaline waters, took an intermediate position. Experiments using specific inhibitors suggested that *Cosmarium abbreviatum* Rac. var. *planctonicum* W. & G.S. West and *S. brachiatum* use CO_2 by an active CO_2 uptake mechanism, whereas *S. chaetoceras* and *Staurodesmus cuspidatus* showed an active HCO_3^- uptake pattern. Most likely, these active uptake mechanisms make use of H^+ -ATPase, as none of the desmids expressed significant carbonic anhydrase activity. A series of strains of *Staurastrum planctonicum* isolated from different habitats, all clustered in between the species using HCO_3^- , but no further differentiation was observed. Therefore, desmids cannot be simply characterized as exclusive CO_2 users, and the ecological distribution pattern of a desmid species does not unequivocally link to a certain C_i uptake mechanism. Nevertheless, there does appear to be a general ecological link between a species' C_i uptake mechanism and its ecological distribution.

Key words: pH drift, desmids, isolate variation, inorganic carbon acquisition.

Résumé : Afin de vérifier si différents mécanismes d'absorption du carbone inorganique (C_i) interviennent dans le patron de distribution écologique des desmidiées planctoniques, les auteurs ont effectué des expériences de dérive du pH avec 12 souches, appartenant à sept espèces, et provenant de lacs avec pH différents. Le *Staurastrum brachiatum* Ralfs et le *Staurodesmus cuspidatus* (Ralfs) Teil. var. *curvatus* (W. West) Teil., espèces qui sont confinées aux milieux acides, dans des habitats d'eau douce, montrent des comportements remarquablement différents dans les expériences de dérive du pH : le *S. brachiatum* semble n'utiliser que du CO_2 , alors que le *Staurodesmus cuspidatus* semble utiliser également le HCO_3^- . Le *Staurastrum chaetoceras* (Schr.) Smith et le *Staurastrum planctonicum* Teil., espèces bien connues pour leur grande abondance dans les eaux alcalines, utilisent le HCO_3^- de façon très efficace. D'autres espèces, qu'on rencontre à la fois dans des eaux légèrement acides ou légèrement alcalines, occupent une position intermédiaire. L'utilisation expérimentale d'inhibiteurs spécifiques suggère que le *Cosmarium abbreviatum* Rac. var. *planctonicum* W. & G.S. West et le *S. brachiatum* utilisent le CO_2 à l'aide d'un mécanisme actif d'absorption, alors que le *S. chaetoceras* et le *Staurodesmus cuspidatus* suivent un patron d'absorption active du HCO_3^- . Ces mécanismes d'absorption active utilisent vraisemblablement la H^+ -ATPase, puisqu'aucune des desmidiées ne montre une activité anhydrase carbonique significative. Un ensemble de souches du *Staurastrum planctonicum*, isolées de différents habitats, se regroupent toutes au sein des espèces utilisant le HCO_3^- , sans qu'on puisse observer d'autre différenciation. Conséquemment, les desmidiées ne peuvent pas être simplement caractérisées comme utilisatrices exclusives de CO_2 , et le patron de distribution écologique d'une espèce de desmidiée n'est pas nécessairement lié à un certain mécanisme d'absorption du C_i . Cependant, il ne semble pas exister un lien écologique général entre le mécanisme d'absorption du C_i et sa distribution écologique.

Mots clés : dérive du pH, desmidiées, variation des isolats, acquisition du carbone inorganique.

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Introduction

The concentration of C_i in a freshwater body varies with the geology of the catchment area. In soft water lakes that receive water from base-poor land, pH and the concentration of dissolved C_i are low, and CO_2 is the predominant C_i species. Conversely, in hard water lakes, receiving their water from base-rich land, pH and the concentration of dissolved inorganic carbon are high, and bicarbonate (HCO_3^-) is the predominant C_i species.

Most desmid species are known to inhabit soft water bodies, but some species are limited in their distribution to alkaline lakes (Brook 1981). The observation that the majority of desmid species are restricted to soft waters has led to the use of desmids in water quality indices and as indicator species (Habib et al. 1997; Coesel 2001). Based on growth experiments and ecological distribution, Moss (1973) hypothesized that oligotrophic desmid species are confined to free CO_2 as their C_i source for photosynthesis, and may be unable to grow below a certain carbon concentration (at least $11 \mu\text{mol}\cdot\text{L}^{-1}$). This hypothesis might be true for the species from soft water bodies, but does not explain the abundance of some desmids in alkaline lakes. So, most likely, dependence on free CO_2 is not a general desmid growth characteristic.

Inorganic carbon availability and pH are closely linked (Stumm and Morgan 1970), and therefore both variables can be correlated with the ecological distribution of an algal species or taxonomic group (Shapiro 1997), without knowing the effect of each separate parameter. As the effect of pH on the physiology of an alga is largely unknown or assumed to be rather unimportant (Talling 1976), species characteristic of soft waters (low pH) have traditionally been considered CO_2 users, and species inhabiting alkaline sites are grouped as HCO_3^- users, ignoring pH as an important factor underlying species distribution. In contrast, a recent study has shown that pH has an effect on the photosynthesis of desmids independent of CO_2 availability (Spijkerman et al. 2004). In the present study, the possible mechanisms of C_i acquisition in planktonic desmid species characteristic of different types of lakes (ranging from oligotrophic, acidic to eutrophic, alkaline) are described. In addition, physiological differences within a morphospecies, *Staurastrum planctonicum* Teil., were studied using strains isolated from a wide range of habitats.

Materials and methods

Algal species and culture conditions

Experiments were performed with seven species of desmids, *Closterium acutum* Bréb. var. *variabile* (Lemm.) W. Krieg., *Cosmarium abbreviatum* Rac. var. *planctonicum* W. & G.S. West, *Staurodesmus cuspidatus* (Ralfs) Teil. var. *curvatus* (W. West) Teil., *Staurodesmus dejectus* (Ralfs) Teil., *Staurastrum brachiatum* Ralfs, *Staurastrum chaetoceras* (Schr.) Smith, and six different strains of the species *Staurastrum planctonicum* Teil. The desmid isolates originated from a wide variety of sites, ranging from oligotrophic soft water bodies to alkaline eutrophic lakes (Table 1). For pH ranges found in other natural habitats for the various desmid species tested, see Coesel (1997). For pH-drift experiments, species were cultured in Woods Hole

medium (Nichols 1973), buffered with $2 \text{ mmol}\cdot\text{L}^{-1}$ HEPES–NaOH to a pH of 7.0, whereas for 1-d acclimation experiments at pH 6 and 8 the medium was enriched with $20 \text{ mmol}\cdot\text{L}^{-1}$ HEPES. Although HEPES is not the best possible buffer at pH 6 ($pK_a = 7.5$), only slight increases in pH over the 1-d incubation were recorded. All cultures were aerated with normal air and continuously illuminated (approx. $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, PAR) at 20°C .

pH drift experiments

Algal cells were harvested and washed with experimental medium by centrifugation ($1000g$, 10 min). After washing, the algal pellet was resuspended in the experimental medium in which the alkalinity was defined by the concentration of HCO_3^- (being confirmed by a Gran titration at the start and end of the drift, Talling 1973). This medium contained $0.25 \text{ mmol}\cdot\text{L}^{-1}$ $CaCl_2$, $0.15 \text{ mmol}\cdot\text{L}^{-1}$ $MgSO_4$, $1 \text{ mmol}\cdot\text{L}^{-1}$ $NaCl$, $50 \mu\text{mol}\cdot\text{L}^{-1}$ KCl , and equimolar concentrations of $NaHCO_3$ and $KHCO_3$ that resulted in an alkalinity of 0.1, 0.2, 0.5, 1, or 2 mequiv. $\cdot\text{L}^{-1}$. Cells were incubated in closed glass and Perspex chambers, containing a combination pH electrode and providing the cells with light that saturated photosynthesis ($90\text{--}100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, PAR). The cultures were stirred and kept in a water bath at 20°C . During the time course of the experiment, pH (starting between 7 and 8) will rise as a consequence of CO_2 or HCO_3^- used by the cells for photosynthesis. Because the alkalinity of the medium is determined by the carbonate system, C_i concentrations can be calculated from the pH of the medium solution (Maberly and Spence 1983), using the equations from Stumm and Morgan (1970). Photosynthetic C_i uptake rates were calculated from the pH change with time recorded on a data logger and the alkalinity of the solution. When photosynthetic rates were plotted against the total carbon concentration (C_T) at which the rate occurred, a two-phased response was found. The linear response at higher C_T concentrations is a result of CO_2 use, and the (extrapolated) intersection with the C_T axis represents the CO_2 compensation point. The intersection of the other linear part with the C_T axis provides the HCO_3^- compensation point (Allen and Spence 1981; Maberly and Spence 1983).

Oxygen evolution

Oxygen production measurements were carried out with a polarographic oxygen electrode in a temperature-controlled chamber (Dubinsky et al. 1987) at 20°C . The output of the electrode was recorded continuously on a chart recorder. Light was filtered through a water jacket before reaching the algae at a photon flux density of $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (PAR). The affinity for CO_2 was determined in algae adapted to pH 6 and 7. Originally, comparable measurements for HCO_3^- affinity at pH 8 were planned, but O_2 production was too low to execute these measurements. In addition, estimates of the affinity for CO_2 were calculated from pH-drift experiments. Maximum photosynthetic and C_i uptake rates, as well as affinity constants ($K_{0.5}$) were calculated using Hofstee plots (Hofstee 1952).

Inhibition studies

During the course of measurements of oxygen evolution, the effects of the addition of carbonic anhydrase (CA) and

Table 1. Desmid isolates with their clone number, the sites from which they originated, lake pH (measured values), and lake trophic status (eu-, meso- and oligo-trophic are distinguished by maximum Chl *a* concentration in summer, being >15, 7–15, and <7 µg·L⁻¹, respectively).

Species	Clone No.	Lake of origin	pH	Trophy
<i>Staurastrum planctonicum</i> var. <i>ornatum</i>	183 ^a	Lake Maarsseveen II, the Netherlands	Approx. 8	Eu
<i>Staurastrum planctonicum</i>	118 ^a	Lake Maarsseveen I, the Netherlands	7–8	Meso
<i>Staurastrum chaetoceras</i>	233 ^a	Lake IJmeer, the Netherlands	8–9	Eu
<i>Staurastrum planctonicum</i>	31 ^a	Lac Pavin, France	6–7	Meso
<i>Staurodesmus dejectus</i>	CCAP 681/1 ^b	Flass Tarn, Cumbria, UK	7–8	Meso
<i>Staurastrum planctonicum</i>	134 ^a	Glenicmurrin Lough, Ireland	6–7	Meso
<i>Staurastrum planctonicum</i>	48 ^a	Lake IJmeer, the Netherlands	8–9	Eu
<i>Staurodesmus cuspidatus</i> var. <i>curvatus</i>	137 ^a	Lough Bofin, Ireland	6–7	Oligo
<i>Cosmarium abbreviatum</i> var. <i>planctonicum</i>	229 ^a	Lake Maarsseveen I, the Netherlands	7–8	Meso
<i>Staurastrum planctonicum</i>	186 ^a	Lake Biwa, Japan	7–8	Eu
<i>Closterium acutum</i> var. <i>variabile</i>	218 ^a	Lake Maarsseveen II, the Netherlands	Approx. 8	Meso
<i>Staurastrum brachiatum</i>	232 ^a	Glennicmurrin Lough, Ireland	6–7	Oligo

Note: Ranking of species is the same as in Table 2.

^aFrom the desmid culture collection of the Department of Evolutionary Botany, University of Amsterdam, Amsterdam, the Netherlands.

^bFrom the Culture Collection of Algae & Protozoa, Dunbegg, UK.

the inhibitors acetazolamide (AZ), ethoxycarbonyl (EZ), and niflumic acid (NA) were recorded. CA was used at a concentration of 122 µmol·L⁻¹ and purchased from Sigma (St. Louis, Missouri; 6240 W-A units·(mg protein)⁻¹; 83% protein). If CO₂ limits photosynthesis, the addition of CA will increase the rate of O₂ evolution (Sültemeyer et al. 1991). AZ is an inhibitor of external CA and was used at a concentration of 35 µmol·L⁻¹. In higher concentrations (>100 µmol·L⁻¹), it is believed to inhibit intracellular CA (Williams and Turpin 1987; Sültemeyer et al. 1990). EZ is a potent inhibitor of internal CA (Badger et al. 1980) and was used at a final concentration of 30 µmol·L⁻¹. NA inhibits anion channels (Szarek and Trebacz 1999) and was used at a final concentration of 27 µmol·L⁻¹, to minimize possible side-effects.

In addition, pH drift experiments were performed in the presence of orthovanadate (VAN). VAN is the most well-known and widely used inhibitor of plasma membrane P-type H⁺-ATPase in algae (Karlsson et al. 1994). The vanadate anion only slowly penetrates the intact cell to the ATP hydrolysis site (Gilmour et al. 1985). Therefore this inhibitor was added to the culture 1.5 h in advance of the actual experiment. VAN was used at a concentration of 1 mmol·L⁻¹, following Goyal and Tolbert (1989).

Analyses

The activity of CA was measured spectrophotometrically (Pocker and Stone 1967) using the coloration of *p*-nitrophenyl acetate. Commercially acquired CA was used as a standard. Chlorophyll *a* (Chl *a*) was measured after extraction in boiling methanol (Golterman et al. 1978). Cell concentrations were determined using a Coulter Counter multisizer.

Results

pH drift

The pH in the medium of all desmid cultures rose during the pH-drift experiment to stable final pH values, which varied from 9.8 to 11.1. Results from the pH-drift experiments

are ranked according to the C_T/alkalinity (C_T/Alk) quotient at an alkalinity of 1 mequiv·L⁻¹ (Table 2). This quotient is the ratio of total carbon remaining in the medium at the end of a pH drift experiment to the alkalinity of the solution. Together with the final pH reached, the quotient indicates the C_i source used by the alga. A low C_T/Alk quotient indicates the use of HCO₃⁻, whereas a quotient higher than about 0.95 suggests CO₂ use by diffusion (Maberly and Spence 1983).

All desmid species listed in Table 2 caused the pH to drift to values above 9.8 in a 1 mequiv·L⁻¹ solution, resulting in a C_T/Alk quotient smaller than 0.95. These results suggest that all desmid clones tested, regardless of their original habitat, can use HCO₃⁻ in some way. In comparison with the pH range of the lakes of origin listed in Table 1, the data demonstrate that *S. chaetoceras*, a species abundant in alkaline waters has a lower C_T/Alk quotient than *S. brachiatum*, a species characteristic of acidic, soft water bodies. In contrast, *Staurodesmus cuspidatus*, a species characteristic of acidic, soft water bodies had a relatively low C_T/Alk quotient, and *C. abbreviatum* (isolated from a slightly alkaline lake) had a relatively high quotient. The C_T/Alk quotients of the clones of *Staurastrum planctonicum* were all relatively low, and could only partially be correlated with lake pH of origin. In general, no straightforward correlation between C_T/Alk quotient and characteristic lake pH could be detected.

CO₂ and HCO₃⁻ compensation points

From the pH-drift experiments at an alkalinity of 1 mequiv·L⁻¹, CO₂ and HCO₃⁻ compensation points were calculated (Table 2). Species with a high C_T/Alk quotient had significantly lower CO₂ compensation points than species with a low C_T/Alk quotient (ANOVA, *F* = 14.45, *df* = 1, 19, *P* < 0.01). Unfortunately, CO₂ compensation points could not be determined in all cases, as CO₂ concentrations at the start of the experiment were not always high enough to apply a linear regression.

In addition, the final concentration of CO₂ can be used as an indicator for carbon speciation usage. CO₂ compensation points of 1–3 µmol·L⁻¹ are consistent with CO₂ uptake by

Table 2. Data acquired in pH drift experiments using six desmid species and six different isolates of *Staurastrum planctonicum*.

	C_T/Alk	Max. pH	Compensation point		Min CO_2 concn. ($\text{nmol}\cdot\text{L}^{-1}$) ^a		
			CO_2 ($\mu\text{mol}\cdot\text{L}^{-1}$)	HCO_3^- ($\mu\text{mol}\cdot\text{L}^{-1}$)	0.2	1	2
<i>Staurastrum planctonicum</i> , clone 183	0.01	11.09	1.7	7	2	0.05	0.2
<i>Staurastrum planctonicum</i> , clone 118	0.02	11.09	DF	8	2	0.05	0.2
<i>Staurastrum chaetoceras</i>	0.04	11.06	0.3	4	1	0.1	0.3
<i>Staurastrum planctonicum</i> , clone 31	0.04	11.08	1.9	4	3	0.1	8
<i>Staurodesmus dejectus</i>	0.19	10.94	1.3	12	1	1	8
<i>Staurastrum planctonicum</i> , clone 134	0.25	10.85	1.5	14	2	2	4
<i>Staurastrum planctonicum</i> , clone 48	0.25	10.83	0.5	44	6	3	1
<i>Staurodesmus cuspidatus</i>	0.27	10.85	1.5	25	3	2	1
<i>Cosmarium abbreviatum</i>	0.33	10.75	0.5	100	8	4	7
<i>Staurastrum planctonicum</i> , clone 186	0.34	10.70	1.4	49	2	5	4
<i>Closterium acutum</i>	0.64	10.13	0.2	344	52	61	79
<i>Staurastrum brachiatum</i>	0.76	9.82	0.4	575	87	201	249

Note: C_T /alkalinity quotient, maximum pH reached, and CO_2 and HCO_3^- compensation point ($\mu\text{mol}\cdot\text{L}^{-1}$) are presented for a 1 mequiv. $\cdot\text{L}^{-1}$ alkalinity treatment. Species are ranked according to their C_T/Alk quotient. DF, detection failed. Data are mean of three replicates, SD typically <5% of mean.
^aMinimum concentrations of CO_2 reached ($\text{nmol}\cdot\text{L}^{-1}$) at an alkalinity of 0.2, 1, and 2 mequiv. $\cdot\text{L}^{-1}$ are presented.

passive diffusion: lower concentrations suggest some form of active uptake. If a species is restricted to CO_2 , then the final CO_2 concentration should be constant over alkalinity. In contrast, if the species is using HCO_3^- as well, then the final CO_2 concentration tends to fall with increasing bicarbonate concentration. When applying this guideline to the desmids, *S. brachiatum*, *Closterium acutum*, *C. abbreviatum*, *Staurodesmus dejectus*, and the isolates 31, 134, and 186 of *Staurastrum planctonicum* appear to be CO_2 users (Table 2); however, because the CO_2 compensation points of *S. brachiatum*, *Closterium acutum*, and *C. abbreviatum* are much lower than 1 $\mu\text{mol}\cdot\text{L}^{-1}$, they must be achieved by active CO_2 uptake.

Affinity for inorganic carbon

Photosynthetic rates were too low at pH 8 to make reliable measurements with varying C_T concentrations. Therefore maximum photosynthetic and C_i uptake rates (V_m) and affinity constants ($K_{0.5}$) were calculated from the pH drift experiments as well as from O_2 evolution rates measured in algae adapted to pH 6 and pH 7 (Table 3). For *S. brachiatum* and *Closterium acutum*, rates of photosynthesis were a function of CO_2 and independent of HCO_3^- , indicating that CO_2 was the only inorganic carbon species used (Fig. 1). Calculations of O_2 production in relation to C_T provided to the algae revealed a molar ratio of about 2.5 O_2 : C (mol:mol) for *S. brachiatum* and *Staurodesmus cuspidatus* and about 2 for *S. chaetoceras* (results not shown). These factors approximate the difference in V_m between pH drift experiments and O_2 chamber results, and therefore V_m values in both experimental methods were largely comparable. Maximum uptake rates for CO_2 were higher than those for HCO_3^- uptake (paired *t* test, $T = 5.25$, $\text{df} = 29$, $P < 0.001$), indicating the preference for CO_2 use in all desmids tested. The $K_{0.5}$ values for CO_2 uptake measured in the two different experimental methods were also comparable in *S. brachiatum*, *S. chaetoceras*, and *Staurodesmus cuspidatus*, although $K_{0.5}$ values from the O_2 production measurements were on average higher than those obtained in the pH drift experiments (Mann–Whitney *U*, $\chi^2 = -2.59$, $\text{df} = 8$, 6, $P < 0.05$). In two

Fig. 1. Rate of carbon uptake by *Staurastrum brachiatum* over different concentrations of CO_2 (A) and HCO_3^- (B) derived from pH drift experiments at 0.2, 1, and 2 mequiv. $\cdot\text{L}^{-1}$. Results are mean values of three replicates $\pm$ SD.

cases the $K_{0.5}$ values for CO_2 could not be determined in the pH-drift experiments and are marked “determination failed” (DF, Table 3). The ranking of species with increasing C_T/Alk quotients did not result in a ranking of decreasing affinity for HCO_3^- or CO_2 . Nearly all $K_{0.5}$ values were in the range between 1.0 and 10 $\mu\text{mol}\cdot\text{L}^{-1}$ CO_2 , which are among

Fig. 2. Typical O₂-production pattern over time obtained when adding acetazolamide (AZ) and ethoxzolamide (EZ) during the course of photosynthesis (150 μmol·m⁻²·s⁻¹, PAR). Addition of inhibitors is indicated by arrows. Results obtained with *Staurastrum chaetoceras* (A) and *Staurastrum brachiatum* (B), both acclimated to pH 8 for 1 d.

values recorded for other green algae (Beardall 1981; Maberly and Spence 1983).

Inhibitor results

The O₂ evolution in *S. chaetoceras* and *S. brachiatum* responded little to AZ addition, whereas it responded strongly to EZ (Fig. 2). This response was independent of pH acclimation (*t* test, *P* > 0.05 for both AZ and EZ) and was similar in all three desmids tested (Kruskal–Wallis test, *P* > 0.05; for both AZ and EZ, Table 4). Ranges of relative inhibition or stimulation by use of an inhibitor are given as a percentage of photosynthetic rates prior to addition (Table 4). The lack of decrease in O₂ evolution after addition of AZ, indicates that no periplasmic CA was synthesized by any of the three desmid species tested. The strong inhibition after the addition of EZ indicates that internal CA was important for photosynthesis. In contrast with expectations, in most cases, the addition of bovine CA did not result in increased photosynthetic rates. At pH 6, this is likely caused by the already abundant presence of CO₂ for photosynthesis. At pH 8 a slight stimulation in photosynthesis was observed in both *S. brachiatum* and *Staurodesmus cuspidatus* that was, how-

Table 3. Maximum photosynthetic rates (*V_m*, μmol C_T·(mg Chl *a*)⁻¹·h⁻¹) and affinity constants (*K_{0.5}*, μmol·L⁻¹) obtained from pH-drift experiments in planktonic desmid species in solutions differing in alkalinity (0.1, 0.2, 0.5, 1, 2 mequiv·L⁻¹).

	0.1		0.2		0.5		1		2		pH 6		pH 7	
	<i>V_m</i>	<i>K_{0.5}</i>	<i>V_m</i>	<i>K_{0.5}</i>	<i>V_m</i>	<i>K_{0.5}</i>	<i>V_m</i>	<i>K_{0.5}</i>	<i>V_m</i>	<i>K_{0.5}</i>	<i>V_m</i>	<i>K_{0.5}</i>	<i>V_m</i>	<i>K_{0.5}</i>
HCO₃⁻/CO₂ data														
<i>Staurastrum planctonicum</i> 183			56/61	40/1.4			70/86	29/3.6	85/89	57/0.5				
<i>Staurastrum planctonicum</i> 118			121/150	15/2.7			130/102	62/1.0	130/102	39/1.0				
<i>Staurastrum chaetoceras</i>			70/90	25/0.9			80/114	37/0.01	42/79	46/0.09	125	4.9	187	5.2
<i>Staurastrum planctonicum</i> 31			66/120	25/4.9			98/145	46/3.4	86/111	94/1.3				
<i>Staurodesmus dejectus</i>			48/56	40/0.9			62/58	88/1.0	58/83	101/1.4				
<i>Staurastrum planctonicum</i> 134			70/106	38/4.1			78/155	28/5.1	74/121	145/3.9				
<i>Staurastrum planctonicum</i> 48			47/100	116/2.7			62/95	233/0.8	75/100	133/1.9				
<i>Staurodesmus cuspidatus</i>			55/DF	23/DF			48/79	65/2.6	55/71	96/1.0	124	1.7	181	7.0
<i>Cosmarium abbreviatum</i>	12/75	93/9.5	17/32	101/1.3	24/26	209/0.6	43/49	241/DF	41/37	538/0.4				
<i>Staurastrum planctonicum</i> 186			81/181	60/3.5			83/176	371/5.6	81/109	563/1.0				
CO₂ data only														
<i>Closterium acutum</i>			107	1.2	94	0.7	143	0.6	147	0.6				
<i>Staurastrum brachiatum</i>	114	3.5	118	2.4			70	1.1	92	1.1	331	9.5	225	2.1

Note: For species able to use HCO₃⁻ and CO₂, *V_m* and *K_{0.5}* values for HCO₃⁻ and CO₂ are presented separated by a “/”. For O₂ evolution experiments at pH 6 and 7, data are presented as *V_m* (μmol O₂·(mg Chl *a*)⁻¹·h⁻¹) and *K_{0.5}* (μmol·L⁻¹ CO₂). Ranking of species identical to that in Table 2. DF, determination failed. Data are means of three replicates; SD was typically <10% of mean.

Table 4. Ranges of percent O₂ evolution after the addition of different inhibitors (acetazolamide, ethoxzolamide, niflumic acid, and vanadate) and commercial carbonic anhydrase in three desmid species adapted to either pH 6 or pH 8 at low CO₂ conditions to photosynthetic rates before the addition (experiments were repeated at least three times).

	<i>Staurastrum chaetoceras</i>	<i>Staurastrum brachiatum</i>	<i>Staurodesmus cuspidatus</i>
pH 6	5.0 ^a	7.2 ^a	8.4 ^a
AZ	89–103	100–108	66–102
EZ	7–36	0–38	10–76
NA	5–65	54–83	65–89
CA	63–105	90–130	74–101
pH 8	3.6 ^a	3.3 ^a	5.8 ^a
AZ	85–108	90–100	66–105
EZ	0–53	0–39	0–4
NA	81–182	90–100	64–185
VAN	30–41	36	25
CA	73–100	88–138	100–143

^aAverage rates of photosynthesis before addition of the inhibitors (mmol O₂·(10⁶ cells)⁻¹·h⁻¹).

Table 5. Mean external carbonic anhydrase activity (W-A units·(mg Chl *a*)⁻¹) in five desmid species cultured under nonaerated (low CO₂) conditions (*n* ≥ 6).

	External carbonic anhydrase activity	
	pH 6	pH 8
<i>Staurastrum chaetoceras</i>	2.8	5.9
<i>Staurodesmus cuspidatus</i>	7.2	5.0
<i>Cosmarium abbreviatum</i>	ND	0.9
<i>Closterium acutum</i>	ND	2.9
<i>Staurastrum brachiatum</i>	5.6	8.0

Note: SE varied between 5% and 20%. ND, not determined.

ever, not significant (*t* test, *P* > 0.05 for *S. brachiatum* and *Staurodesmus cuspidatus*). The addition of NA did not cause any change in O₂ evolution at pH 8, whereas it inhibited photosynthesis in the desmid species at pH 6 (*t* test, *T* = -3.24, *df* = 17, *P* < 0.01). The effect of both CA and NA did not differ among the species (ANOVA, all *P* > 0.05).

The effect of VAN was only tested in the pH drift experiments, because of its long incubation times. VAN had a strong inhibiting effect on the photosynthesis of all desmid species tested, rates typically lying between 25% and 40% of noninhibited cultures (Table 4). CO₂ and HCO₃⁻ compensation points in *S. brachiatum*, *C. abbreviatum*, *Staurodesmus cuspidatus*, and *S. chaetoceras* were 2- to 20-fold higher than noninhibited ones. These results were all gathered above pH 7 and are accordingly listed under pH 8 only.

Measurements of carbonic anhydrase

Limited CA activity was found in whole cells, suggesting that no external CA was synthesized by the desmid species (Table 5), which is in accord with the results obtained with inhibitors. Compared with activities given in the literature, activities found were about 10 times lower than those found in species that induce external CA activity at low CO₂ or high pH conditions (Williams and Colman 1995; Sültemeyer 1997). No statistical differences were detected between cells

acclimated to pH 6 and pH 8 in the desmid species tested (*t* test, *P* > 0.05 for both).

Discussion

Results of pH-drift experiments performed in this study show a wide range of C_T/Alk quotients and carbon extraction capacities for the desmids species studied. The results from this study and the literature (Table 6) show that a number of desmids have a low C_T/Alk quotient, lying in the range found previously for cyanobacteria. These species can clearly be considered HCO₃⁻ users, not depending on diffusive CO₂ use for their inorganic carbon acquisition. Regarding competition for inorganic carbon, many desmid species can likely compete for HCO₃⁻ with other green algae such as *Chlorella* and *Chlamydomonas*, as well as with cyanobacteria. Species characteristic of soft waters also had a C_T/Alk quotient below 0.95, and therefore none of the desmids used in this study can be considered exclusive CO₂ users by diffusion as has been described for two red filamentous algae, *Batrachospermum* sp., and *Lemanea mamillosa* (Raven and Beardall 1981). Nevertheless, pH-drift and C_i affinity characteristics of *S. brachiatum* and *Closterium acutum* do not appear to be consistent with bicarbonate use. A possible explanation for this is active CO₂ acquisition, since rates of C_i uptake respond to concentrations of CO₂, but not to HCO₃⁻ concentrations.

Table 6 also outlines different outcomes from pH-drift experiments performed on isolates from one single morphospecies. These differences can be a consequence of using different clones, as was shown by the use of different clones of *Staurastrum planctonicum*, originating from different lake alkalinities. Alternatively, it might be a result of the physiological status of the organism (Maberly and Spence 1983). Although all strains of *Staurastrum planctonicum* were grown under identical conditions, they were not physiologically identical as pH drift end points, maximum photosynthetic rates and affinity constants for C_i differed. This underlines physiological differences between different clones from a single morphospecies comparable with results gathered with the marine diatom *Skeletonema costatum* (Gallagher et al. 1984).

Table 6. Average values obtained in all pH drift experiments from this study (asterisks) and values of other algal species.

	C _T /Alk	Max. pH
<i>Staurastrum planctonicum</i> *	0.06/ 0.09/ 0.2/ 0.24/ 0.28	10.9/ 10.9/ 10.7/ 10.8/ 10.7
<i>Anabaena cylindrica</i> ^a	0.07/ 0.19	11.1/ 11.2
<i>Microcystis aeruginosa</i> ^a	0.08	11.1
<i>Staurastrum chaetoceras</i> *	0.10	10.9
<i>Chlorella emersonii</i> ^a	0.14/ 0.19/ 0.22	11.0/ 10.7/ 11.2
<i>Scenedesmus quadricauda</i> ^a	0.18	11.0
<i>Staurodesmus cuspidatus</i> *	0.24	10.8
<i>Staurodesmus dejectus</i> *	0.25	10.7
<i>Cosmarium botrytis</i> ^a	0.34/ 0.68	10.8/ 10.1
<i>Cosmarium abbreviatum</i> *	0.36	10.4
<i>Fragilaria crotonensis</i> ^a	0.43	10.4
<i>Chlamydomonas reinhardtii</i> ^a	0.70	10.0
<i>Closterium acutum</i> *	0.70	9.8
<i>Staurastrum brachiatum</i> *	0.75	9.8
<i>Asterionella formosa</i> ^a	0.78	9.7
<i>Melosira italica</i> ^a	0.96	8.8
<i>Lemanea mamillosa</i> ^b	ND	8.4/ 8.9
<i>Batrachospermum</i> sp. ^b	ND	7.5/ 8.2/ 8.9

Note: Different values are for different clones. ND, not determined.

^aValues from Maberly and Spence (1983).

^bValues from Raven and Beardall (1981).

Inorganic carbon can be transported into the cell via different mechanisms (Badger and Price 1992). CO₂ can be taken up passively via diffusion or actively using an H⁺-ATPase. Bicarbonate can either be transported into the cell via an anion channel or also via an ATPase coupling. Alternatively, HCO₃⁻ might be dehydrated in the periplasmic space by CA, and CO₂ might be consequently taken up. The induction of periplasmic CA in *Chlamydomonas reinhardtii* (Sültemeyer et al. 1989) is considered a major adaptation to low CO₂ conditions. The occurrence of periplasmic CA, however, varies significantly between species of microalgae (Badger and Price 1994) and, for example, in the chlorophyte alga *Eremosphaera viridis*, no periplasmic CA was detected (Rotatore et al. 1992). In general, the induction of periplasmic CA is pH sensitive, as low pH values are associated with rapid rates of uncatalyzed CO₂ supply from bicarbonate, and external CA activity is usually low in low pH media compared with high pH media (Beardall et al. 1998). The lack of effect of the inhibitor AZ on the desmid species tested strongly suggests that no significant external CA is present in *S. chaetoceras*, *S. brachiatum*, or *Staurodesmus cuspidatus*. In addition, no significant CA activity could be detected in five desmid species, activities being maximally 8 W-A units-mg Chl a⁻¹. This is largely in contrast with 40 W-A units-mg Chl a⁻¹ in *Chlorella saccharophila* (Williams and Colman 1995) and 10–350 W-A units-mg Chl a⁻¹ in *Dunaliella tertiolecta* (Sültemeyer 1997). Therefore, periplasmic CA appears not to be part of an important strategy in the C_i acquisition of desmid species, independent of the ability to use HCO₃⁻. Also addition of CA to the culture medium, found to stimulate photosynthesis in *Chlamydomonas reinhardtii* (Sültemeyer et al. 1991), had no significant effect on photosynthesis rates of our desmid isolates.

Desmids prefer CO₂ as their C_i species in accord with most other algae (Badger and Price 1992), and this was reflected in higher CO₂ uptake rates than HCO₃⁻ uptake rates (Table 3). Although Raven (1990) assumed the effect of external pH to be an insignificant factor in the physiology of algae, Williams and Colman (1996) underlined that a possible detrimental effect of high pH is not (only) a function of low CO₂. Indeed, also in some desmid species an external pH value of 8 appeared to decrease photosynthesis and growth independent of CO₂ concentration, already after exposure times of about 8 h (Spijkerman et al. 2004).

VAN had the largest effect on O₂ evolution of different inhibitors tested. Because of the long incubation time necessary to test the effect of this substance, pH-drift experiments are among the few experimental methods suitable (Choo et al. 2002; Ray et al. 2003). The major inhibition suggests that a P-type H⁺-ATPase (proton pump) was involved in carbon uptake in desmids. Because the effect was comparable in the putative CO₂ user *S. brachiatum* as in the CO₂ and HCO₃⁻ user *S. chaetoceras*, both the active acquisition of CO₂ and HCO₃⁻ are assumed to follow this strategy. The low affinity constant for CO₂ uptake and low C_T/Alk quotient in *S. brachiatum* and *Closterium acutum* also indicate active CO₂ transport. In the soft water species, *Eremosphaera viridis* (Rotatore et al. 1992), as well as in *Chlamydomonas reinhardtii* (Sültemeyer et al. 1989) and *Chlorella* (Rotatore and Colman 1991a; Rotatore and Colman 1991b), active CO₂ uptake was described. In *Chlamydomonas reinhardtii*, active uptake of HCO₃⁻ was inhibited by VAN (Karlsson et al. 1994), and HCO₃⁻ uptake can therefore also be an active process via P-type H⁺-ATPases. Hardly any inhibition of photosynthesis with NA was observed at pH 8, suggesting no HCO₃⁻ sequestration in the desmids via anion channels.

In contrast, at pH 6, NA inhibited photosynthesis, the cause of which remains to be solved.

Maberly and Spence (1983) also estimated affinity constants using data from pH drift experiments. Both their and our $K_{0.5}$ values fit in the range of affinity constants given elsewhere, suggesting that estimates from pH-drift experiments are trustworthy. Besides low affinity constants for C_T uptake, low CO_2 compensation concentrations are an indication of low rates of photorespiration and a very efficient system of C_i acquisition (Rotatore and Colman 1991c). Our values are in the same order as those of the unicellular green algae *Chlorella saccharophila* and *C. ellipsoidea* (Rotatore and Colman 1991a), and are in many cases lower than those reported for many other algae (Maberly and Spence 1983). This latter observation also holds for the HCO_3^- compensation concentrations reported here. Desmids are therefore considered efficient C_i sequesters.

The results from this paper indicate that there is a rough relation between the ecological distribution of desmids and their C_i acquisition strategy. There is not, however, a clear relation that desmids thriving in oligo- or meso-trophic lakes can be considered CO_2 users, without the ability to use HCO_3^- . The species most restricted to acidic, soft waters, *Staurostrum brachiatum*, was determined to have a relatively high C_T /Alk quotient, indicating a large dependence on free CO_2 as C_i source. As expected, *Staurostrum chaetoceras*, typically found in alkaline lakes, occurred in the lower part of the C_T /Alk range, suggesting that it is an efficient HCO_3^- user. The same holds for *Staurostrum planctonicum*, also a common inhabitant of pH-neutral to alkaline lakes. The other desmid species tested, which are encountered in both slightly acidic and slightly alkaline water bodies, appear to have an intermediate position in the C_T /Alk range presented.

Special attention should be paid to *Closterium acutum* var. *variabile*, a taxon that may cause algal blooms in eutrophic waters. Despite the fact that eutrophic waters usually are (slightly) alkaline, it has been shown that this alga does not grow well at pH values permanently above 8 (Coesel 1993). In our present study, *Closterium acutum*, with its position in the upper part of the C_T /Alk range, comes to the fore as a poor bicarbonate user, providing an explanation for the problematic growth at high pH.

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