

Photosynthetic characteristics of a tropical population of *Nitella cernua* (Characeae, Chlorophyta)

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Photosynthetic characteristics (assessed by chlorophyll fluorescence and O₂ evolution) were analysed monthly during one year in a tropical population of *Nitella cernua* from southeastern Brazil (20°50'32" S, 49°26'15" W). Parameters derived from photosynthesis-irradiance (PI) curves by fluorescence (high values of the photosynthetic parameter, I_k, and lack or low values of photoinhibition, β) suggested adaptation to high irradiance, whereas those by O₂ evolution showed a different pattern (low values of I_k and compensation irradiance, I_c, high values of photosynthetic efficiency, α, and photoinhibition). Parameters from PI curves by O₂ evolution suggested light acclimation: I_k and maximum photosynthetic rate, P_{max} (as rETR, relative electron transfer rate) increased, whereas α became significantly lower under higher irradiance (winter). This pattern is an adjustment of both number and size of photosynthetic units. Photosynthetic performance assessed by fluorescence revealed two seasonal periods: higher values of P_{max} (rETR), I_k and non-photochemical quenching from October to March (rainy season), and lower values from April to October (dry season). Temperature responses were observed only in summer, but temperature optima were different between methods: peaks of net photosynthesis occurred at 20°C, whereas rETR increased towards higher temperatures (up to 30°C). Dark respiration increased with higher temperatures. Current velocity had a stimulatory effect on photosynthetic rates, as suggested by positive correlations with P_{max} (rETR) and α. pH experiments revealed highest net photosynthetic rates under pH 4.0, suggesting higher affinity for CO₂ than HCO₃⁻. This broad range of responses of photosynthetic characteristics of this *N. cernua* population to irradiance, temperature, and pH/inorganic carbon reflects a wide tolerance to variations in these environmental variables, which probably contribute to the wide distribution of this species.

Key words: charophytes, irradiance, *Nitella*, pH, photosynthesis, seasonal dynamics, temperature

Características fotossintéticas de uma população tropical de *Nitella cernua* (Characeae, Chlorophyta): Foram analisadas mensalmente, durante um ano, as características fotossintéticas (por meio de fluorescência da clorofila e evolução de O₂) de uma população tropical de *Nitella cernua* do sudeste do Brasil (20°50'32" S, 49°26'15" W). Parâmetros derivados das curvas fotossíntese-irradiância (FI) por fluorescência (alto valor do parâmetro de saturação de luz, I_k, e ausência ou baixa fotoinibição, β) sugeriram adaptação a alta irradiância, enquanto aqueles por evolução de oxigênio mostraram adaptações à sombra (baixos valores de I_k e de irradiância de compensação, I_c, altos valores de eficiência fotossintética, α, e β). Parâmetros das curvas FI por oxigênio sugeriram aclimação à luz: I_k e taxa fotossintética máxima, F_{max} (analisada como rETR, taxa relativa de transporte de elétrons) aumentaram e α foi significativamente menor sob irradiância mais alta (inverno). Este padrão é um ajuste pelo número e tamanho das unidades fotossintéticas. O desempenho fotossintético avaliado por fluorescência revelou dois períodos sazonais: maiores valores de F_{max}, I_k e extinção não-fotoquímica, de outubro a março, e menores, de abril a outubro. Respostas de temperatura foram observadas apenas no verão, mas ótimos de temperatura foram diferentes: picos de fotossíntese líquida ocorreram a 20°C, enquanto rETR aumentou com temperaturas mais altas (até 30°C). A respiração no escuro aumentou com a elevação da temperatura. Velocidade da correnteza teve presumivelmente papel estimulatório sobre as taxas fotossintéticas, como sugerido pelas correlações positivas com F_{max} e α. Experimentos de pH revelaram taxas fotossintéticas mais altas em pH 4,0, sugerindo maior afinidade por CO₂. Essas amplas respostas das características fotossintéticas da população de *N. cernua* à irradiância, à temperatura e ao pH/carbono inorgânico refletem grande tolerância a variações dessas variáveis ambientais, que, provavelmente, contribuem para a extensa distribuição de *N. cernua*.

Palavras-chave: carófitas, dinâmica sazonal, fotossíntese, irradiância, *Nitella*, pH, temperatura

Abbreviations: chl – chlorophyll; I_c – compensation irradiance; I_k – light saturation parameter; NPQ – non-photochemical quenching coefficient; PI – photosynthesis-irradiance; P_{max} – maximum photosynthetic rate; rETR – relative electron transport rate; α – photosynthetic efficiency; β – photoinhibition parameter

INTRODUCTION

Ecophysiological investigations on photosynthetic characteristics of charophytes mostly focus on populations from temperate regions (Libbert and Walter, 1985; Howard-Williams and Schwarz, 1995; Schwarz et al., 1996; Küster et al., 2000). In contrast, similar approaches involving charophyte populations from tropical regions are relatively scarce (Vieira and Necchi, 2003). Investigations dealing with photosynthesis-irradiance (PI) curves have received great attention (Schwarz et al., 1996; Steinman et al., 1997; Menendez and Sanchez, 1998; Küster et al., 2000), involving light adaptability based on variations of photosynthetic parameters (light saturation parameter, I_k , compensation irradiance, I_c , maximum photosynthetic rate, P_{max} , and photosynthetic efficiency, α).

Parameters derived from the PI curves (relatively low I_c and I_k , and occurrence of photoinhibition) suggested adaptation to low irradiance for some charophyte species (Andrews et al., 1984; Menendez and Sanchez, 1998; Vieira and Necchi, 2003), whereas some investigations (Steinman et al., 1997; Küster et al., 2000) clearly indicated preferences for high irradiances in other species. Photoinhibition has been rarely reported in Characeae (Menendez and Sanchez, 1998; Vieira and Necchi, 2003). However, irradiances applied in some PI curves (e.g., 60-110 $\mu\text{mol m}^{-2} \text{s}^{-1}$, Andrews et al., 1984; Schwarz et al., 1996) were not high enough to detect photoinhibition. On the other hand, some charophyte species do not grow under shade conditions (Moore, 1986) and photoinhibition has not been observed under irradiances as high as 1,600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in species adapted to high-light conditions (Küster et al., 2000). Photoacclimation has been observed in some charophytes (Küster et al., 2000; Vieira and Necchi, 2003) with species-specific acclimation to low and high irradiances. The pattern to explain photoacclimation mechanisms seems to be a combination of changes in size and number of the photosynthetic units, i.e. a reaction center and its associated light-harvesting pigment complex.

Effects of temperature on photosynthesis and respiration rates in charophytes have been shown to be somewhat variable. Libbert and Walter (1985) reported no influence of temperature on net photosynthesis but observed a positive correlation with dark respiration, whereas Menendez and Sanchez (1998) found an apparent preference for lower temperatures (highest P_{max}

at 10°C). A trend for increasing dark respiration rates with higher temperatures has been observed in some studies (Libbert and Walter, 1985; Vieira and Necchi, 2003), which implies a decreased net primary production at higher temperatures. Data on best photosynthetic performances for charophyte species have been observed at a wide temperature range, suggesting that temperature optima can be species-specific.

Charophytes can grow in waters with high pH and most species tolerate a pH range between 6 and 9, although some species of *Nitella* have been reported from waters as low as pH 5 (Moore, 1986). Several species use bicarbonate, abundant in hard waters, as carbon source for photosynthesis (King and Nash, 1994; Van den Berg et al., 1998). Results from pH experiments showed highest photosynthetic rates at pH 4.0, suggesting higher affinity for inorganic carbon in the form of carbon dioxide for most species tested (Vieira and Necchi, 2003). However, pH responses and the preference for a particular form of inorganic carbon in photosynthesis are still poorly investigated in charophytes.

Despite the investigations cited above, information is still scarce on responses of photosynthesis to environmental variables and is restricted to few species of charophytes. In addition, species or populations from tropical regions have been poorly investigated. This study involves a seasonal analysis of the photosynthetic performance in response to environmental variables (with emphasis on temperature, irradiance and pH) of a tropical population of *Nitella cernua* A. Braun under field and laboratory conditions using the techniques of chlorophyll (chl) fluorescence and oxygen (O_2) evolution. It is part of a wider study, which also included phenology and coexistence with another macrophyte (*Egeria najas* Planchon) in a shallow habitat. The hypotheses tested in this study were: 1) this population of *N. cernua* is expected to have a well-defined seasonal pattern in photosynthetic characteristics, as a response to predictable changes in environmental variables (temperature and irradiance); 2) the alga will exhibit some characteristics of shade-adapted plants, based on previous results for other charophyte species (Andrews et al., 1984; Vieira and Necchi, 2003), as well as preference for free CO_2 as inorganic carbon form for photosynthesis (Raven, 1997; Vieira and Necchi, 2003).

MATERIAL AND METHODS

Field study: The field work was carried out monthly during one year (October 2001 to October 2002) in a third-order stream segment, which was a transition between an artificial pond and its outlet channel. The site is located within the “Noroeste Paulista” Ecological Station in northwestern São Paulo State, southeastern Brazil (20°50'32" S, 49°26'15" W). The population of *N. cernua* occurs all the year round and the algal bed is mixed with a macrophyte (*E. najas*) covering an area of 12 m in length and 3.0 to 5.5 m in width and at a depth from 20 to 70 cm.

Field measurements of photosynthesis were performed by *in vivo* chl fluorescence using a Diving-PAM underwater fluorometer (Walz, Effeltrich, Germany). Measurements were taken directly in the water for five replicates from different plants at the same place late in the morning (1030 to 1130 h in winter). Apices of algal thalli were placed directly on the tip of the fluorometer fiberoptic using the supplied magnet sample holder. Rapid light curves (White and Critchley, 1999) were generated and consisted of the fluorescence responses to eight increasing actinic irradiance steps within the range of 0 to 690 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, using the “light curve” option of the Diving-PAM. The exposure time at each irradiance was 15 s, each separated by a 0.8 s saturating flash ($\sim 6,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). The illumination periods of rapid light curves are too short to achieve true steady-states but provide sound information on the overall photosynthetic performance of a plant (White and Critchley, 1999). The Diving-PAM records all relevant fluorescence parameters, as well as actinic irradiance, and data were processed using the supplied WinControl software. Three parameters were determined from each sample at each irradiance level: 1) effective quantum yield of PSII, $\Delta F/F_m'$, in which $\Delta F = F_m' - F_t$ (F_m' is the maximal fluorescence of an illuminated sample, and F_t is the transient fluorescence); 2) relative electron transport rate, $rETR = \Delta F/F_m' \times \text{actinic irradiance} \times 0.5 \times 0.95$ [the first multiplication factor (0.5) was introduced since the transport of a single electron requires the absorption of two quanta, and the second factor (0.95) represents an estimation of irradiance absorption based on measurements made with and without the alga within a circle of 0.5 cm in diameter – similar to the area of the fluorometer fiberoptic tip]; and 3) non-photochemical quenching coefficient (NPQ), as a measure of heat dissipation of absorbed light

energy, was calculated as $NPQ = (F_m - F_m')/F_m'$, in which F_m is the maximal fluorescence of a dark-adapted sample. Such a coefficient was estimated from the highest irradiance ($690 \mu\text{mol m}^{-2} \text{s}^{-1}$) applied when obtaining the light response curves. The calculations followed Schreiber et al. (1994) and Kromkamp et al. (1998), and the terminology for chl fluorescence described by van Kooten and Snel (1990) was adopted. Curves of PI were generated on the basis of rETR and the respective parameters were calculated by the equation of Platt et al. (1980): αETR , P_{max} ($rETR_{\text{max}}$), I_k , I_c and photoinhibition parameter (βETR). The values of αETR were determined by linear fitting using the first three points of the rETR versus irradiance curve (Conde-Álvarez et al., 2002).

Environmental variables were measured simultaneously with the photosynthetic performance. Temperature and pH were measured immediately below the surface with a Horiba U-10 water quality checker (Horiba, Kyoto, Japan). Current velocity and irradiance were measured close to the algal thalli (Vieira and Necchi, 2002, 2003) using, respectively, a Swoffer current velocity meter (Swoffer Instruments, Seattle, USA) and a quantum meter (Li-189, Li-Cor, Lincoln, USA) coupled to a Li-193 SA spherical quantum sensor.

Laboratory study: Measurements by O_2 evolution were made in the laboratory and followed the general procedures described in previous studies (Vieira and Necchi, 2003; Necchi 2004a,b). Photosynthesis and dark respiration rates were determined by changes in O_2 concentration using the light and dark bottle technique (Littler and Arnold, 1985; Thomas, 1988). Initial and final O_2 concentrations of incubated samples were measured with a YSI model 5000 O_2 meter (Yellow Springs Instruments, Yellow Springs, USA), equipped with a self-stirring probe, and calculations were made according to Littler and Arnold (1985). Incubations were made with orbital agitation (100 ± 5 rpm) and digital temperature control. Frontal illumination was supplied by one to three cool-white fluorescent lamps (Osram 15 W). Plants were incubated in 100 mL borosilicate glass bottles (98.5% transparency). Dark bottles were covered with thick black plastic. In order to prevent inorganic carbon depletion during incubations, stream water samples were supplied three times with 2 mM NaHCO_3 (1mL solution for each 100 mL of water sampled).

Curves of PI were made under constant temperature: $20 \pm 0.5^\circ\text{C}$ in winter (June to September) and $25 \pm 0.5^\circ\text{C}$ in summer (December to March) using eight increasing actinic irradiance levels within the range of 0 to $425 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. These encompass the saturation ranges previously reported for freshwater macroalgae (Leukart and Hanelt, 1995; Hill, 1996) and charophytes (Vieira and Necchi, 2003). Incubation lengths were of 30 min for each irradiance level. Parameters from the PI curves were calculated by the equation proposed by Platt et al. (1980) that includes a photoinhibition parameter because some charophyte species exhibited photoinhibition in previous studies (Vieira and Necchi, 2003; Necchi, 2004b).

Responses of photosynthetic performance to temperature were tested in the laboratory by the two techniques during the most contrasting seasons: August (winter) and February (summer). Five temperatures were tested (10, 15, 20, 25 and 30°C) encompassing the ranges reported for charophytes in tropical and sub-tropical regions (Vieira and Necchi, 2002, 2003; Vieira et al., 2003). The same set of specimens (fresh weights of $75 \pm 15 \text{ mg}$ in each replicate) was used first for measurements by O_2 evolution and then by the fluorescence technique. Incubations were made for 45 min at each temperature in increasing order under constant irradiance ($170 \mu\text{mol m}^{-2} \text{s}^{-1}$). This irradiance was chosen because it is an intermediary value in relation to the saturation parameter (I_k) found in PI curves for charophytes in a previous study (Vieira and Necchi, 2003) and preliminary tests.

pH experiments were conducted under constant temperature (20°C) and irradiance ($170 \mu\text{mol m}^{-2} \text{s}^{-1}$) with incubation lengths of 45 min. The pH values tested were based on the typical relationship of pH and inorganic carbon form in freshwater (Wetzel and Likens, 2000): pH 4.0 ca. 100% of $\text{CO}_2/\text{H}_2\text{CO}_3$; pH 6.5 ca. 50% of both CO_2 and HCO_3^- ; pH 8.5 ca. 100% of HCO_3^- . pH was adjusted by addition of 0.5 N HCl or KOH to the stream water samples.

Data were analyzed by Student's *t* test or one-way analysis of variance (ANOVA; Zar, 1999) and Newman-Keuls multiple comparison test to detect significant differences in photosynthetic parameters, months or environmental variables as factors. Relationships among photosynthetic parameters and environmental variables were evaluated by the Pearson moment-product correlation coefficient (Zar, 1999). Statistical tests were performed by Statsoft Statistica 6.0 software, whereas graphs were made by Microcal Origin 5.0.

RESULTS

Environmental variables: Only the environmental variables most closely related to the photosynthetic parameters are presented (Figure 1): temperature, pH, irradiance and current velocity. All these variables varied significantly along the year ($P < 0.05$) with the following annual averages (Figure 1): temperature ($25.5 \pm 0.8^\circ\text{C}$), pH (8.1 ± 0.2), irradiance ($995 \pm 110 \mu\text{mol m}^{-2} \text{s}^{-1}$) and current velocity ($6.0 \pm 4.8 \text{ cm s}^{-1}$). Temperature values were higher (above annual average) from October through April, whereas pH was lower from February through June compared to the other months (Figure 1). Irradiance was high ($> 1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$) throughout the year, except on some cloudy days (November, February, April and May), whereas current velocity was slow ($\leq 5 \text{ cm s}^{-1}$) throughout the year, except from January through March (Figure 1).

Curves of PI: Monthly variation of photosynthetic parameters derived from PI curves for chl fluorescence (Figure 2) was characterized by lack or low values of photoinhibition ($\beta = 0.05 \pm 0.11$), moderate to high values of P_{max} ($39.1 \pm 14.0 \text{ rETR}$) and α (0.20 ± 0.03), and relatively high values of I_k ($195 \pm 50 \mu\text{mol m}^{-2} \text{s}^{-1}$). Quantum yield was widely variable (0.56 ± 0.10) throughout the period, as well as NPQ (1.2 ± 0.7). Highly significant differences for all parameters were found by ANOVA ($P < 0.001$), as well as by the Newman-Keuls test (indicated by distinct letters on Figure 2), throughout the year.

From October to March P_{max} (rETR) tended to increase (except for low values in February), but showed lower values from April to October, with the lowest values in August (Figure 2). Similar trends were also observed for I_k and NPQ, which had a highly significant positive correlation ($r = 0.89-0.91$, $P < 0.001$) with P_{max} (rETR). Values of quantum yield fluctuated along the year with no clear seasonal pattern, except for consistently low values from October to December and high values from January to March (Figure 2). A similar trend was observed for α with a highly significant positive correlation ($r = 0.92$, $P < 0.001$) with quantum yield. Photoinhibition was observed only in February, May and June (Figure 2). Among the environmental variables, we only found positive correlations of P_{max} (rETR), I_k , α and NPQ with current velocity ($r = 0.60-0.80$, $P < 0.05$) and no significant correlation with temperature or irradiance.

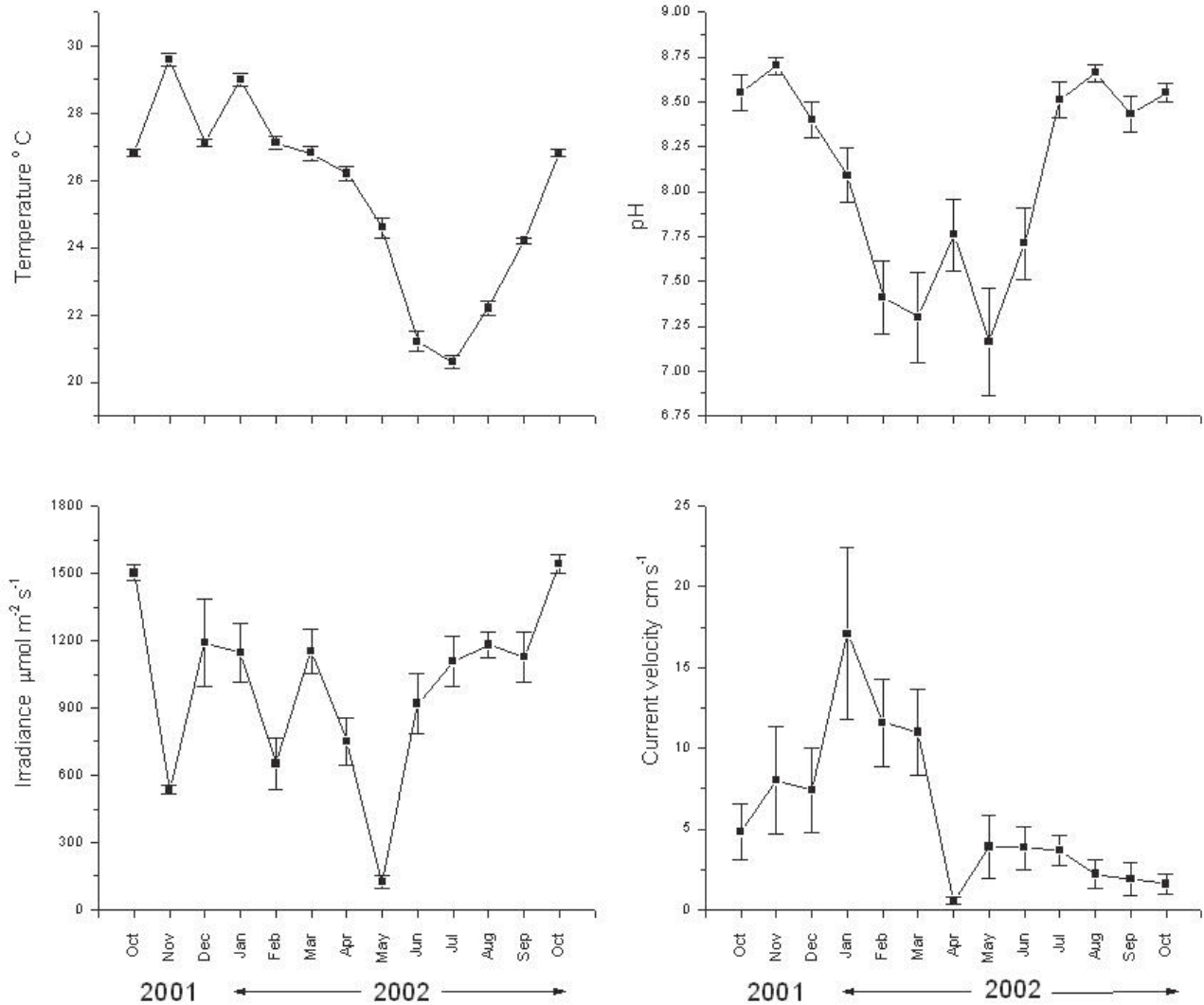


Figure 1. Values of temperature, pH, irradiance and current velocity for the population of *Nitella cernua* from October 2001 to October 2002. Each point is the mean of three (for temperature and pH) or five (for irradiance and current velocity) replicates. Bars represent SD.

Photosynthetic parameters derived from PI curves for O_2 evolution (Table 1) were all significantly different between winter and summer, except dark respiration. Higher values of P_{max} (rETR), I_k and net photosynthesis/dark respiration ratio were observed in winter, whereas I_c , α and β were higher in summer.

Responses to temperature: Rates of photosynthesis responded differently to the variations in the experimentally manipulated range of temperature (Figures 3 and 4). Fluorescence data showed that temperature had a significant effect ($P < 0.05$) on photosynthetic rates

(rETR) only in summer (Figure 4). In summer rETR was positively correlated with temperature ($r = 0.96$, $P < 0.01$). Temperature had an opposite effect on NPQ in comparison to rETR, with significant variation ($P < 0.05$) only in winter (Figure 4), with significantly higher values at 15 and 20°C. No significant correlation between rETR and NPQ in any of the seasons was found.

Temperature had a significant effect on net photosynthesis only in summer ($P < 0.01$), with the highest values at 20°C and the lowest at 30°C (Figure 3). Rates of dark respiration were significantly different ($P < 0.001$) in summer and winter and positively correlated to temperature

Table 1. Parameters of the photosynthesis-irradiance curves (P_{max} , maximum photosynthetic rate; α , photosynthetic efficiency; β , photoinhibition parameter; I_k , light saturation parameter; I_c , compensation irradiance; and P/R, P_{max} to dark respiration ratio) for the population of *Nitella cernua* measured by O_2 evolution in two periods of the year. Data are expressed as means \pm SD ($n = 5$). Distinct letters indicate significant differences ($P < 0.05$) by Student's t test

Photosynthetic parameters	Sampling periods	
	February	January
P_{max} (mg O_2 g ⁻¹ DW h ⁻¹)	0.9 \pm 0.4 a	2.3 \pm 0.5 b
α (mg O_2 g ⁻¹ DW h ⁻¹ (μ mol m ⁻² s ⁻¹) ⁻¹)	0.37 \pm 0.1 a	0.21 \pm 0.0 b
β (mg O_2 g ⁻¹ DW h ⁻¹ (μ mol ² s ⁻¹) ⁻¹)	-0.67 \pm 0.0 a	-0.08 \pm 0.03 b
I_k (μ mol m ⁻² s ⁻¹)	3.2 \pm 1.2 a	15.5 \pm 6.1 b
I_c (μ mol m ⁻² s ⁻¹)	2.7 \pm 0.3 a	2.1 \pm 0.7 b
Dark respiration (mg O_2 g ⁻¹ DW h ⁻¹)	1.3 \pm 0.2 a	1.1 \pm 0.1 a
P/R	0.7 \pm 0.2 a	2.1 \pm 0.4 b
Irradiance (μ mol m ⁻² s ⁻¹)	650 \pm 55 a	1180 \pm 115 b

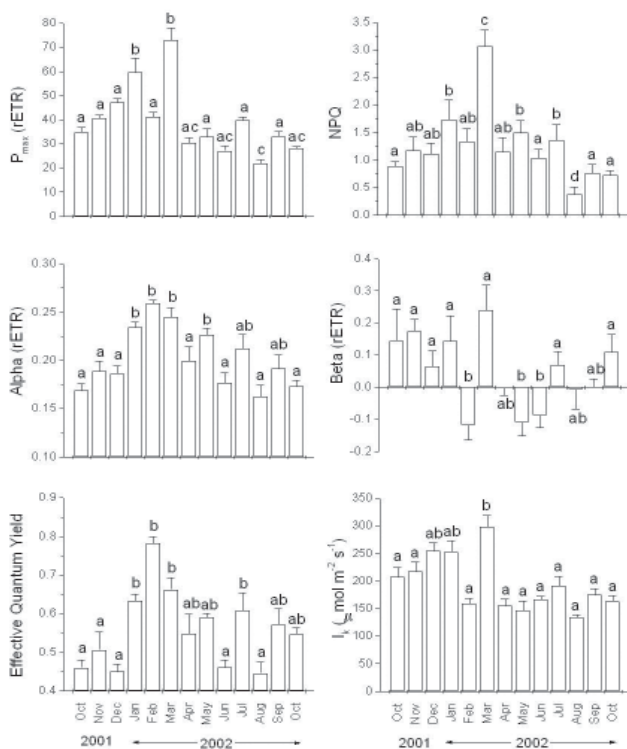


Figure 2. Parameters of the photosynthesis-irradiance curves (P_{max} , maximum photosynthetic rate; rETR, relative electron transport rate; α , photosynthetic efficiency; effective quantum yield; NPQ, non-photochemical quenching coefficient; β , photoinhibition parameter; and I_k , light saturation parameter) for the population of *Nitella cernua* measured by chlorophyll fluorescence from October 2001 to October 2002. Each column represents the mean of five replicates, and bars indicate SD. Means followed by different letters are significantly different from each other ($P < 0.05$; Newman-Keuls test).

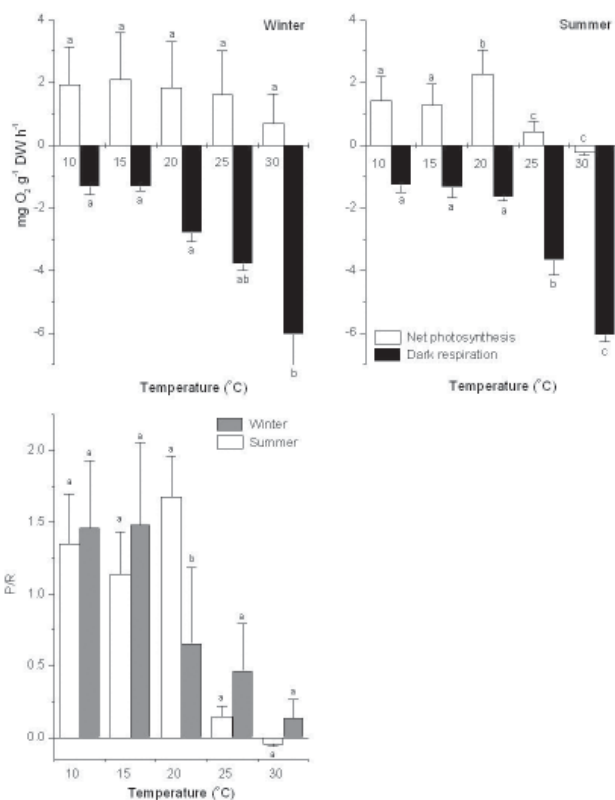


Figure 3. Rates of net photosynthesis and dark respiration under five temperatures for the population of *Nitella cernua* measured by O_2 evolution in two periods of the year at 170 μ mol photons m⁻² s⁻¹. P/R represents the ratio of P_{max} to dark respiration. Statistics as in Figure 2.

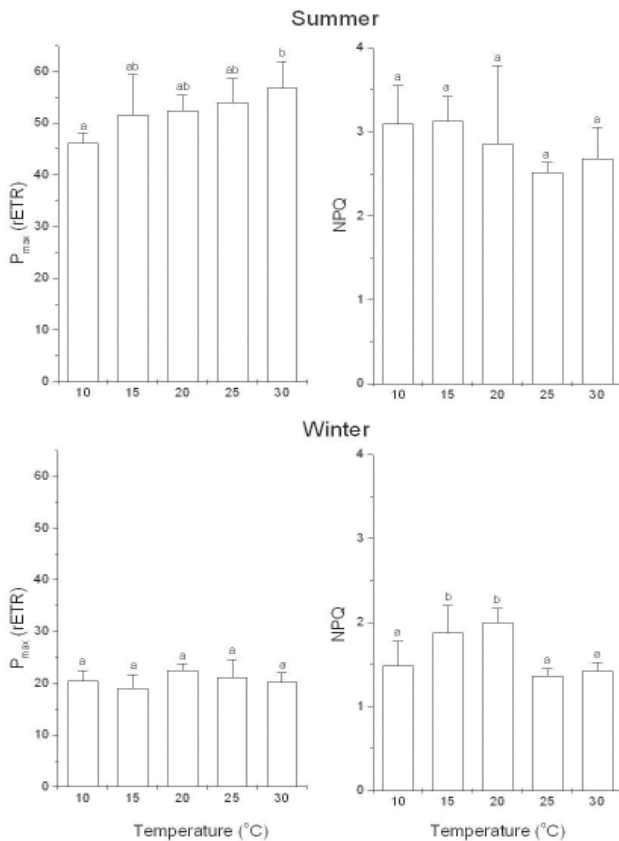


Figure 4. Photosynthetic parameters (P_{max} , maximum photosynthetic rate, and NPQ, non-photochemical quenching coefficient) for the population of *Nitella cernua* measured by chlorophyll fluorescence under five temperatures in two periods of the year. Statistics as in Figure 2.

($r = 0.91$ to 0.96). The ratio of net photosynthesis/dark respiration had a decreasing trend towards higher temperatures for both seasons, with peaks at 20°C in summer and at 15°C in winter (Figure 3). That ratio was negatively correlated to temperature ($r = -0.96$, $P < 0.01$) in winter. The alga kept best performances (higher values both of net photosynthetic rates and net photosynthesis/dark respiration ratio) at temperatures considerably lower (10 – 20°C) than the mean annual temperature ($25.6 \pm 2.8^{\circ}\text{C}$). In addition, optimum experimental temperatures (Figure 3) were far below the ambient temperatures in both seasons: winter (15 and 22.2°C ; optimum and ambient temperatures, respectively) and summer (20 and 27.1°C).

Responses to pH: Net photosynthetic rates were significantly higher ($P < 0.01$) at pH 4.0 than at the other

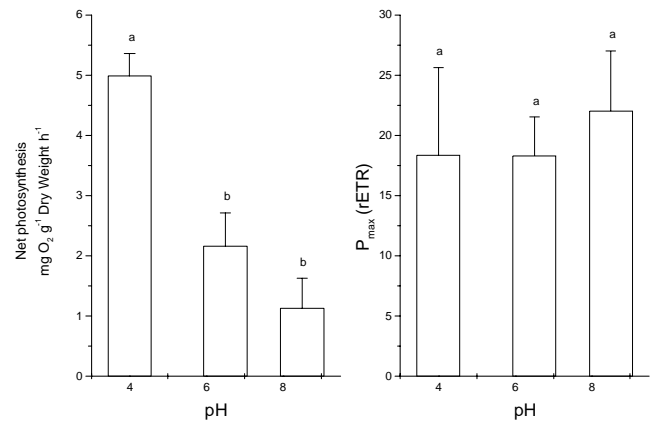


Figure 5. Photosynthetic rates for the population of *Nitella cernua* measured by O_2 evolution (left) and chlorophyll fluorescence (right) under three values of pH at 20°C and $170 \text{ mmol photons m}^{-2} \text{ s}^{-1}$. Statistics as in Figure 2.

Table 2. Initial and final pH values measured during the incubations of the population of *Nitella cernua* for photosynthetic rate determinations by O_2 evolution. Data are expressed as means \pm SD ($n = 3$). Distinct letters indicate significant differences ($P < 0.05$) by Student's t test

pH treatments	pH values	
	Initial	Final
pH 4.0	4.00 ± 0.05 a	4.96 ± 0.11 b
pH 6.5	6.50 ± 0.05 a	6.99 ± 0.03 b
pH 8.5	8.50 ± 0.05 a	7.57 ± 0.04 b

two values tested, with an evident decreasing trend towards higher pH values (Figure 5). Photosynthetic rates also changed pH values of the water, as observed by comparison before and after the incubations (Table 2). Significantly different values between initial and final pH values were observed for the three pH values tested. Greater differences were generally coincident with the pH level with higher photosynthetic rates, except at pH 8.5. In contrast, pH had no significant effect on photosynthetic rates (rETR) measured by chl fluorescence (Figure 5).

DISCUSSION

Some parameters derived from the PI curves by chl fluorescence (relatively high I_k and lack or low levels of photoinhibition) suggested adaptation to high irradiance

for this species, which is consistent with its occurrence in shallow habitats. These results agree with previous studies in shallow waters (e.g. Steinman et al., 1997; Küster et al., 2000) but contrast studies of other habitats where charophytes revealed adaptations to low irradiances (Andrews et al., 1984; Menendez and Sanchez, 1998; Vieira and Necchi, 2003). On the other hand, results by O_2 evolution showed a trend toward adaptations to shade conditions as indicated by the relatively low values of I_k and I_c , high values of α and occurrence of photoinhibition. This pattern was most evident in summer. Our values were near or below the lower limits of the ranges previously reported for I_c (4-100 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and I_k (29-554 $\mu\text{mol m}^{-2} \text{s}^{-1}$) (Howard-Williams et al., 1995; Steinman et al., 1997; Menendez and Sanchez, 1998; Vieira and Necchi, 2003), reinforcing the adaptation to shade conditions. The ratio of net photosynthesis/dark respiration revealed relatively high net carbon gain in winter and low in summer, which was essentially due to variations in net photosynthetic rates, since respiration was relatively similar. This physiological parameter, associated with the fact that the alga is relatively abundant (Vieira and Necchi, unpublished), suggest that it may contribute importantly to primary production at least during part of the year.

Parameters derived from PI curves by O_2 evolution in summer and winter suggested light acclimation: I_k and P_{max} (rETR) increased, whereas α became significantly lower under higher irradiance (winter). In this case, more light was required to saturate photosynthesis (indicated by higher I_k). This light acclimation pattern was similar to that seen for other charophytes: *Lamprothamnium papulosum* (Küster et al., 2000) and two populations of *N. subglomerata* (Vieira and Necchi, 2003).

The seasonal performance of photosynthetic parameters by chl fluorescence revealed two periods: the first from October to March characterized by higher photosynthetic activity as indicated by increasing values of P_{max} (rETR), I_k and NPQ, and the second with lower values of these parameters extending from April to October. Interestingly, these periods corresponded to some key phenological characteristics (Vieira and Necchi, unpublished): the former with specimens lacking or with few reproductive structures and large vegetative structures, whereas the latter had the opposite trend. The photosynthetic trends may indicate resource allocation for reproduction, as has been reported for charophyte species (Casanova, 1994).

Effects of temperature on photosynthesis and respiration rates have been demonstrated in charophytes (Libbert and Walter, 1985; Menendez and Sanchez, 1998; Vieira and Necchi, 2003). Libbert and Walter (1985) reported no influence of temperature on net photosynthesis, whereas Menendez and Sanchez (1998) and Vieira and Necchi (2003) showed evident responses, with preferences to definite ranges of temperatures by some species. Menendez and Sanchez (1998) reported that P_{max} (rETR) was highest at 10°C in *Chara hispida* and Vieira and Necchi (2003) found highest rates for *Chara* species at 10-15°C and for *Nitella* at 20-25°C, whereas the peak for *N. furcata* var. *sieberi* was observed at 15°C by Necchi (2004a). Highest ETR values were observed at 15°C for *N. furcata* var. *sieberi* by Necchi (2004a) but with no significant differences in the range of 10 to 30°C. In addition, temperature optima can be species-specific and differences even between populations of a same species have been reported (Vieira and Necchi, 2003). Such observations illustrate that for relatively high light (shallow) charophyte populations, where irradiance is seldom limiting for photosynthesis, some other factors (e.g. reproductive allocation, temperature and carbon availability) are likely to have a strong influence on growth. Our initial hypothesis that *N. cernua* would present some characteristics of shade-adapted plants was partially confirmed. However, this alga is not clearly shade or high light adapted.

The different performances evidenced by each measurement technique in response to irradiance and temperature can be attributed to dependence on distinct factors. Increases in temperature have been shown to generally induce higher ETR values in some species, due to increased enzymatic activity, whereas O_2 evolution generally increases at lower temperatures and decreases at when higher temperatures (Ensminger et al., 2001; Necchi, 2004a). Our results are consistent with this pattern, with higher performances of net photosynthesis during winter and increasing trend of ETR towards higher temperatures in summer. The increments of enzymatic activity are compensated by increased respiration and other mechanisms, e.g. increases in pseudocyclic electron transport (Mehler reaction) and enhanced photorespiration, leading to decreases in net photosynthesis (Davison, 1991; Ensminger et al., 2001). In addition, atypical values under extreme combinations

of irradiance and temperature (e.g. high temperature and irradiance for *N. cernua*) are also responsible for the non-linear relationships of O₂ evolution and ETR in freshwater macroalgae (Ensminger et al., 2001; Necchi, 2004a).

The increasing trend of dark respiration with higher temperatures observed in this study is consistent with data reported for freshwater macroalgae (Libbert and Walter, 1985; Necchi and Zucchi, 2001; Necchi and Vieira, 2003; Necchi, 2004a) and higher plants (Atkin and Tjoelker, 2003). This trend suggests a decreasing balance between net primary production and temperature, representing more critical conditions towards higher temperatures for most species. Our results agree with this pattern. In addition, the species had best photosynthetic performances at temperatures considerably lower than the ambient temperature. Thus, tropical species face additional constraints to adapt their physiological characteristics to the high temperatures that typically prevail during most part of the year and day period in tropical regions (Necchi, 2004a).

Current velocity seemed to have a stimulatory effect on photosynthetic rates measured by chl fluorescence, as suggested by positive correlations with P_{max} (rETR) and α . Raven (1992) hypothesized that the simple tensile force exerted by moderate flowing water has a stimulatory effect on the growth of stream macroalgae under resource-saturated conditions. Data from this study support this idea, although it has not yet been tested experimentally.

Results from pH experiments revealed highest photosynthetic rates under pH 4.0, suggesting higher affinity for inorganic carbon in the form of carbon dioxide. However, the alga has also demonstrated capacity to use bicarbonate as well, considering photosynthetic performances and changes in water pH after incubations under pH 6.5 and 8.5. These data are quite divergent of the species occurrence in the field under higher pH values (pH 8.1 ± 0.6), with high predominance of bicarbonate (>90%; Wetzel and Likens, 2000). According to Raven (1992), two main mechanisms for inorganic carbon acquisition occur in ecorticate charophytes (like *Nitella*), both involving extracellular chemical transformation (on the plant surface): 1) "acid zones" consisting of a catalytic agent (H⁺) acting to convert HCO₃⁻ (present in the water) into CO₂ (taken up by the cells); and 2) enzymatic conversion by carbonic anhydrase. Ecorticate charophytes are prime algal examples of the first mechanism. Under

experimental lower pH, direct absorption of CO₂ was presumably a more efficient and economic mode for carbon acquisition, since no need of extracellular conversion or concentrating mechanisms would be required. The findings are consistent with our initial hypothesis that *N. cernua* would be expected to present preference for CO₂.

Photosynthetic characteristics of the *N. cernua* population exhibited a relatively broad range of responses to irradiance, temperature and pH/inorganic carbon, reflecting a wide tolerance to variations in these environmental variables. Thus, the initial hypothesis that this alga would have a relatively well-defined seasonal pattern in response to environmental variables (particularly temperature and irradiance) was confirmed. This probably contributes to its wide spatial distribution in several scales, from drainage basin to regions and biomes (Vieira et al., 2003), since it is one of the most widespread charophyte species in Brazil (Bicudo and Yamaoka, 1978).

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