Effects of dissolved inorganic carbon availability on growth, nutrient uptake and chlorophyll fluorescence of two species of marine microalgae

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Abstract

Growth of two species of marine microalgae, namely Nannochloropsis gaditana Lubiaño (Eustigmatophyceae) and Nannochloris maculata Butcher (Chlorophyceae), was investigated in cultures submitted to three different concentrations of dissolved inorganic carbon (DIC). Cultures of N. gaditana grown in the absence of DIC in the medium and aerated with less than 0.0001% (v/v) CO₂ in air (low DIC conditions) showed a reduction in final cell biomass of approximately 56% as compared with the biomass obtained in cultures grown under control conditions (2 mM DIC in the medium and aerated with air-equilibrated levels of CO₂, i.e. 0.03% (v/v) CO₂). Growth was not observed in N. maculata cultured under low DIC conditions. A concentration of 1% (v/v) CO₂ in air (high DIC conditions) did not modify growth of N. gaditana in relation to that in the control-culture but enhanced growth of N. maculata. Nutrient (NO₃⁻ and PO₄³⁻) uptake was also analyzed under the different growth conditions. The uptake of NO₃⁻ and PO₄³⁻ by N. maculata was dependent on the inorganic carbon level; thus, whereas no nutrient absorption was observed in the low DIC-culture, growth at the highest inorganic carbon concentration caused an acceleration of the uptake. Capacity to use nitrate was restricted in N. gaditana cells under low DIC conditions, but nutrient uptake was similar in cultures adapted to air levels of CO₂ and to CO₂-enriched air. Chlorophyll fluorescence measurements were used to determine the photochemical efficiency of photosystem II and the non-photochemical quenching. A similar pattern of evolution of the actual quantum yield of photosystem II (Φₚₛₛₛ) was observed in all cultures of N. gaditana.
over the growth period, without development of non-photochemical quenching. In contrast, changes in \( \Phi_{PSII} \) of \( N. \) maculata differed between treatments and were concurrent with carbon and nutrient availabilities. Non-photochemical quenching rose in this alga when carbon or phosphate limitation constrained proton dissipation from the lumen. Results are discussed in relation to the particular carbon uptake mechanism of each alga. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Nannochloropsis; Nannochloris; Growth; Inorganic carbon; Fluorescence measurements

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1. **Introduction**

Bicarbonate (HCO\(_3\)) is the predominant form of inorganic carbon (DIC) in seawater. At the normal pH of seawater in equilibrium with atmospheric air, i.e. 8.2, only 10 \( \mu \text{M} \) CO\(_2\) is available and the diffusion of CO\(_2\) is quite low (Round, 1981). This limitation in the CO\(_2\) availability may restrict its supply to marine microalgae for photosynthesis. However, it has been demonstrated that most of the microalgae examined so far have an efficient dissolved inorganic carbon concentrating mechanism (CCM), which permits them to use either CO\(_2\) or HCO\(_3\) as external sources of DIC (see Falkowski and Raven, 1997, and references therein). The functioning of such a mechanism makes it possible for cells to enhance the delivery of CO\(_2\) to the ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) and, thereby, to inhibit the oxygenase activity of the enzyme and reduce photorespiration (Sülttemeyer et al., 1993; Raven, 1997). So, even though the current concentration of dissolved inorganic carbon in seawater is high enough not to limit photosynthesis in natural environments and, consequently, productivity of marine microalgae due to the CCM (Coleman, 1991; Nimer and Merrett, 1996), a supplement of CO\(_2\), either directly or mixed with the atmospheric air used for aeration, has been shown to enhance greatly the productivity of some microalgae in mass cultures (Becker, 1984; Chrismadha and Borowitzka, 1994). However, since utilization of either CO\(_2\) or HCO\(_3\) as the preferred source for photosynthesis has been found to be species dependent, the effectiveness of CO\(_2\) supplementation is likely to be species dependent as well.

*Nannochloropsis gaditana* Lubiañ (Eustigmatophyceae) and *Nannochloris maculata* Butcher (Chlorophyceae) are two marine microalgae widely used in aquaculture for feeding rotifer cultures (Witt et al., 1981; Yufera et al., 1983; Yufera and Lubian, 1990) and, in particular, *N. gaditana* is cultured in large scale in hatcheries of marine fish located in the salt marshes of The Bay of Cadiz, where it has also been identified as a component of natural blooms (Lubian et al., 1985). Previous studies on the physiology of inorganic carbon uptake in these microalgae have shown that *N. gaditana* has the capability to take up bicarbonate from the medium, while *N. maculata* has a high affinity for CO\(_2\) and use carbon dioxide as the preferred source of inorganic carbon for photosynthesis (Huertas and Lubian, 1998). In addition, recent investigations have pointed out the presence of a light dependent bicarbonate transport system in *Nannochloropsis* species and that these cells are able to release CO\(_2\) during photosynthesis, thus increasing the CO\(_2\).
concentration in the surrounding medium (Sukenik et al., 1998; Huertas et al., in press a). These results could make questionable the use of CO₂ bubbling for a higher production of alga biomass in *Nannochloropsis* cultures. On the other hand, the occurrence of an active CO₂ uptake in *N. maculata* has also been established (Huertas et al., in press b). Both reported mechanisms have been found to be present during growth under natural conditions, but the influence of the DIC availability in relation to the specific CCM of each alga and nutrient uptake on growth in mass cultures of both species has not been considered to date. The aim of this study was therefore to assess the convenience of using CO₂ bubbling for a better performance of biomass productions in cultures of *N. gaditana* and *N. maculata* with regard to their particular CCM.

Chlorophyll fluorescence is a non-invasive technique which allows the monitoring of the status of the photosynthetic apparatus easily. An inverse relationship between chlorophyll fluorescence and photosynthetic carbon assimilation has been established (Horton, 1985; Genty et al., 1989; Seaton and Walker, 1990). Therefore, the effect of different levels of DIC on photosynthesis during growth in the two aforementioned species of marine microalgae was determined in this study by using a fluorescence modulated system, and the actual quantum yield of photosystem II (φPSII, Genty et al., 1989) was measured. Changes in nitrate and phosphate availability, and in pigment contents during growth were also determined.

2. Materials and methods

2.1. Cell growth

Cultures of *N. gaditana* Lubíán (strain B3, Eustigmatophyceae) and *N. maculata* Butcher (strain MACU, Chlorophyceae) were obtained from the Marine Microalgae Culture Collection of the Instituto de Ciencias Marinas de Andalucia (CSIC, Spain). Algae were grown on unbuffered artificial seawater (ASW) with a total DIC concentration of 2 mM (Harrison et al., 1980), which was supplemented with f/2 medium (Guillard and Ryther, 1962) modified with double nitrate and phosphate concentrations (2500 μM NO₃⁻ and 50 μM PO₄³⁻ in the initial inoculum). Experiments were carried out with cultures at three different DIC levels, which will be further referred to as low-DIC, control, and high-DIC. In the low-DIC treatment, cells were incubated in ASW lacking inorganic carbon and aerated with atmospheric air that had been passed through a 10 N KOH solution, which gave a final CO₂ concentration in air of less than 0.0001% (v/v). In the control culture, ASW was bubbled with atmospheric air (0.03% v/v CO₂); and in the high-DIC treatment, ASW was aerated with CO₂-enriched atmospheric air (1% v/v CO₂). All cultures were bubbled at a rate of 50 ml min⁻¹, which was controlled by using flowmeters connected to the output of the tubing aeration system. This experimental setup made it possible to keep a constant bubbling rate but a different CO₂ proportion in air in each culture. Cultures (1 l, in duplicate) under all treatments were grown at 20°C and at a photon flux density of 75 μmol photons m⁻² s⁻¹ at the surface.
provided by daylight fluorescent tubes. Irradiance was measured with a quantum sensor (LICOR LI-1000). Cells were counted daily with a hematocytometer and specific growth rates, $\mu$ (d$^{-1}$), were determined from regressions of the linear portion of the growth curves expressed as the natural logarithm (ln) of the cell densities versus time. Samples of cultures (10 ml) were removed every 24 h for the measurements of nutrient concentrations and fluorescence.

2.2. Nutrient measurement

Nitrate and phosphate (nutrients) concentrations in the culture medium were determined in the supernatant following centrifugation of 5 ml samples, whose pellets were used for pigment extraction, by means of a Technicon Traacs 800 Autoanalyser, using the protocols n° 818-87T and n° 812-86T for nitrate and phosphate, respectively, of Technicon Instrument Corporation. Depletion of nutrient contents in the culture medium was considered as an estimation of nutrient uptake.

2.3. Pigment analysis

Aliquots (5 ml) of the cultures were spun down, cells resuspended in 2.5 ml of absolute methanol and sonicated to extract pigments. Pigment concentrations in the supernatant were determined spectrophotometrically by measuring in a Perkin-Elmer Lambda 5 spectrophotometer at the appropriate wavelengths as follows: chlorophyll $a$ and total carotenoids were calculated according to the equations of Talling and Driver (1974) and Strickland and Parsons (1968), respectively, for $N. gaditana$, and according to equations of Porra et al. (1989) for $N. maculata$.

2.4. Chlorophyll fluorescence

Induced chlorophyll fluorescence was measured with a portable pulse amplitude modulated fluorometer FMS1 (Hansatech, Kings Lynn, UK) connected to a saturation pulse lamp, as described by Schreiber et al. (1986). The actual quantum yield of PSII (photosystem II), $\phi_{PSII}$, was measured in cell suspensions of 5 ml final volume just sampled from the cultures and under a background illumination of about 60 $\mu$mol photons m$^{-2}$ s$^{-1}$, and was calculated as $F'_m - F/F'_m$ (Genty et al., 1989), where $F'_m$ is the maximum fluorescence of illuminated cultures, and $F$ is the normal fluorescence in the steady state. Then, $F_o$, the minimum fluorescence when all reaction centers are open, and $F_m$, the maximum fluorescence when all reaction centers are closed, were measured after the cell suspension was dark-adapted for 20 min. All measurements were performed in two different samples from each duplicate culture ($n = 4$). $F_m$ and $F'_m$ were measured with a saturating pulse of 8000–12 000 $\mu$mol photons m$^{-2}$ s$^{-1}$, 0.8–1.0 s amplitude, of white light. The dependence of the PAM-fluorescence parameters on changes in chlorophyll concentration in the samples was daily checked by measurements at different saturating pulse light intensities. Most accurate measurement was then considered to be that in which the
maximum value of $F_m$ was obtained. $\phi_{PSII}$ along with the non-photochemical quenching ($q_N$), which was computed as $1 - (F_m - F_0)/(F_m - F_0)$ (Schreiber et al., 1986), with $F_0$ being the minimum fluorescence under illumination conditions, were used to assess the photosynthetic performance of cultures under the different treatments.

2.5. Statistics

Cell densities were expressed as the mean value ± standard deviation ($n = 4$, values obtained from two samples of each duplicate culture). Comparisons between treatments were tested with a model I two-way analysis of variance (ANOVA) followed by a multiple range test using Student–Newman–Keuls.

3. Results

3.1. Growth and nutrient uptake

Fig. 1 shows the results of growth and nutrient uptake in N. gaditana under the different treatments. The specific growth rates (calculated by using the part of the growth curve from days 3 to 7) were $0.54 ± 0.01 \text{ d}^{-1}$ and $0.4 ± 0.01 \text{ d}^{-1}$ for cells growing under control and high DIC conditions, respectively. From days 3 to 6, the control-culture grew significantly faster ($P < 0.001$) than that exposed to high DIC conditions, but growth of both cultures was not significantly ($P > 0.05$) different afterwards, both cultures rendering indeed a similar final cell biomass. In these cultures, nitrate and phosphate were depleted by day 5 (Fig. 1b,c). In contrast, the growth rate under low DIC conditions was significantly lower ($P < 0.001$) than under control or high DIC conditions and was reduced to $0.34 ± 0.02 \text{ divisions d}^{-1}$. Moreover, under this treatment, growth became stabilized at the 7th day, producing a final cell biomass of 44% of that obtained under air levels of CO$_2$. A high residual nitrate concentration, i.e. 900 $\mu$M (36% of the initial value), was still present in this culture medium at the stationary phase (Fig. 1a). Phosphate uptake was substantially higher than nitrate uptake, but a concentration of about 2 $\mu$M (4% of the initial value) still remained in the culture medium at the onset of the stationary phase.

Growth curves for the cultures of N. maculata are illustrated in Fig. 2. The high DIC-culture exhibited the greatest growth rate ($0.6 ± 0.01 \text{ d}^{-1}$), while growth was not observed ($P < 0.001$) under low DIC conditions, the final cell biomass decreasing in this latter case in approximately 90% in relation to the one observed in the control-culture. The culture grown on air levels of CO$_2$ (control-culture) showed a growth rate of $0.5 ± 0.01$ and the same final cell density as that obtained in the high DIC-culture. However, growth was significantly higher ($P < 0.001$) in high DIC during the entire growth period, and only by day 10 did the cell density show no significant ($P < 0.001$) difference between the two cultures. Low DIC conditions strongly affected nutrient uptake by this species, as shown by the low decrease in
the nutrient content over the entire growth period (Fig. 2a). In contrast, aeration with 1% CO₂ (v/v) caused an enhancement in the capacity to absorb NO₃⁻ as this was depleted by day 5 (Fig. 2c), whereas NO₃⁻ was depleted by day 7 in the control-culture (Fig. 2b). The uptake of phosphate was also enhanced under high DIC conditions (Fig. 2c).

Fig. 1. Growth of *N. gaditana* cells in relation to DIC concentration. (a) low DIC cultures. (b) control cultures. (c) high DIC cultures. ● cell density; □ nitrate concentration; △ phosphate concentration. Points represent the average for two separate cultures with samples from each culture in duplicate.
Fig. 2. Growth of *N. maculata* cells in relation to DIC concentration. (a) low DIC cultures. (b) control cultures. (c) high DIC cultures. ● cell density; □ nitrate concentration; △ phosphate concentration. Points represent the average for two separate cultures with samples from each culture in duplicate.

Changes in pH over the growth period are shown in Fig. 3 for both microalgae. Differences in pH between both microalgae were already observed after the first day of growth. Range of pH differed notably between both algae, in particular these of the control- and low DIC-cultures, which were from 8.7 to 10.2 for *N. gaditana*,

and from 7.8 to 8.5 for \textit{N. maculata} (values after the first day are only considered). pH of the control and low DIC-cultures of \textit{N. gaditana} rose during the first 6 days of growth but declined in the control-culture afterwards. In the high DIC-culture of \textit{N. gaditana} pH rose from 7.0 to 7.8 yet a lower value was measured on the day 10 (Fig. 3a). A slightly increasing trend was observed in the pH of the control- and low DIC-cultures of \textit{N. maculata}, but it was only after the day 4 in the low DIC-culture, and pH of these cultures never rose above 8.4 (Fig. 3b). Decreasing values of pH were observed in the high DIC-culture of \textit{N. maculata} during the first 4 days of growth, but pH rose afterwards, with values ranging between 6.9 and 7.3.

### 3.2. Pigments

Opposite patterns of pigment evolution over the growth period were observed in cultures of \textit{N. gaditana} grown under low DIC and high DIC (Fig. 4). The chl \textit{a} content initially dropped in the high DIC-culture and rose in the low DIC-culture, but these trends inversed during the exponential growth phase. After the 5th day of growth, chl \textit{a} fell in the high DIC and control cultures, which exhibited similar contents over the entire growth period. In these cultures, values ranged between 0.10 μg Chl \textit{a}/10⁶ cells and 0.03 μg Chl \textit{a}/10⁶ cells. In the low DIC-culture, chl \textit{a}
kept to a value of about 0.08 µg Chl a/10^6 cells after the 5th day of growth. Similar results were observed for total carotenoids, their contents dropping in the high DIC- and control-cultures after day 7 of growth and rising in the low DIC-culture after the 5th day of growth.

The responses of pigment content of *N. maculata* to the different DIC concentrations are illustrated in Fig. 5. Again, both chlorophylls *a* and *b*, as well as total carotenoids, were found to be similar in cultures grown under high DIC and control conditions. Pigment concentration increased until the onset of the stationary growth phase, after which pigments kept in the control-culture and slightly decreased in the high DIC-culture. Pigment concentrations in cells cultured under low DIC continuously increased over the growth period and were always higher than in cells adapted to the other growth conditions.

### 3.3. Chlorophyll fluorescence measurements

In *N. gaditana* cultures, both fluorescence parameters, the actual quantum yield (Φ<sub>PSII</sub>) and the non-photochemical quenching (q<sub>N</sub>), appeared to be independent of the DIC availability, thus remaining more or less constant during growth (Fig. 6). Φ<sub>PSII</sub> peaked on the 3rd day of growth in the high DIC- and control-cultures, but
a slight decrease with time led to a loss in $\phi_{PSII}$ of about 11% of the initial value in all cultures (Fig. 6a). Even though $q_N$ rose slightly in the high DIC-culture at the beginning of the measuring period, it subsequently dropped and equilibrated close to 0 on the 5th day of growth (Fig. 6b). In the low DIC and control cultures, $q_N$ always kept close to 0.

In contrast, fluorescence parameters of *N. maculata* were strongly influenced by the DIC level over the growth period (Fig. 7). In the low DIC-culture, $\phi_{PSII}$ declined during the first 4 days of growth from values of 0.6 to 0.4 and leveled to this value over the remaining growth period. In the control-culture, $\phi_{PSII}$ started to fall

![Fig. 5. Changes of photosynthetic pigments in *N. maculata* cells during growth at the three DIC levels; △ low DIC cultures; □ control cultures; ● high DIC cultures. The values are the means ± SD of duplicate samples from two separate cultures.](image-url)
Fig. 6. Variations of the actual quantum yield ($\phi_{PSII}$, a) and non-photochemical quenching ($q_N$, b) during growth in *N. gaditana* cultures adapted to different DIC levels. (Symbols as in Fig. 3). Errors bars are SE of the mean of duplicate samples from two separate cultures.

after the 5th day of growth, with a loss in the actual quantum yield of about 40% in relation to the initial value. The decline in $\phi_{PSII}$ with growth was notable in the high DIC-culture after rising during the first 3 days to a maximum value of about 0.65, leading to a value of about 10% of the initial value at the end of the growth period (Fig. 7a). $\phi_{PSII}$ and $q_N$ showed a complementary pattern of evolution under all growth conditions. Under the low DIC treatment, $q_N$ exhibited a continuous increase, but this increase was observed after day 6 in the control-culture and after the day 3 in the high DIC-culture (Fig. 7b).

When $\phi_{PSII}$ is expressed in a per chl a basis ($\phi_{PSII}/[\mu g\ chl\ a/10^6\ cells]$) changes with time were more evident. Thus, a continuous increase can be observed following the late exponential growth phase in the high DIC- and control-cultures of *N. gaditana*, but not in the low DIC-culture (Fig. 8a). In this latter culture, $\phi_{PSII}$ per unit chl a rose during the first 5 days of growth to decline afterwards. In the *N. maculata* cultures, a steep decrease in $\phi_{PSII}/[\mu g\ chl\ a/10^6\ cells]$ took place over the exponential growth phase in the control- and high DIC-cultures, while it was not so pronounced in the low DIC-culture (Fig. 8b).
4. Discussion

Growth limitation in marine diatoms and green algae at low CO₂ concentrations has previously been shown (Riebesell et al., 1993; Chen and Durbin, 1994) under natural conditions. However, information on the influence of carbon availability under continuous illumination and conditions of biomass production is scarce. Data presented here reveal that the low DIC-culture of *N. maculata* was clearly CO₂ limited, but CO₂ restriction did not seem to affect the growth of *N. gaditana* in the low DIC-culture, at least during the first half of the log phase, as much as the growth of *N. maculata* was affected. These findings can be explained on the basis of the capability of each algal cell to effectively use the most abundant inorganic carbon form present in the medium, especially if pH values of the culture medium are taken into account. The pH of the *N. gaditana* cultures under low DIC conditions rapidly increased up to 10 (Fig. 3a), while the *N. maculata* cultures grown under the same conditions exhibited pH values close to 8.2 (Fig. 3b). Since HCO₃⁻ is the predominant form of inorganic carbon at alkaline pH (more than 99% of the total DIC in the medium), *N. gaditana* might have taken advantage from its potential for direct HCO₃⁻ uptake (Merrett et al., 1996; Huertas and Lubián, 1998; Sukenik et al., 1998), whose removal is accompanied by strong alkalinisation of the
Fig. 8. Changes in the ratio between the actual quantum yield and the chlorophyll a content ($\phi_{psii}$/Chl a) in (a) *Nannochloropsis gaditana* and (b) *N. maculata*, over the growth period and for the three treatments (triangle low DIC cultures; square control cultures; circle high DIC cultures).

external medium (Lucas, 1983; Nimer et al., 1996). Hence, DIC limitation may have been less important for this alga than for *N. maculata*, which primarily takes up CO$_2$ and has thereby an extremely high $K_{0.5}$(DIC) at alkaline pH (1266.33 ± 66.31 μM at pH = 8.2, Huertas and Lubiana, 1998). Accordingly, the reduction of the CO$_2$ supply resulted in inhibition of growth in *N. maculata*. Nonetheless, the slight increase in pH that took place after the day 4 suggests a minimal photosynthetic activity, which would be in agreement with the observed rise in the cell density (Fig. 2a). The high pH values measured in the control-culture of *N. gaditana* indicate that the bicarbonate transport system was operative in this culture as well, while the slight increase observed in the high DIC-culture of this alga, as well as in the control- and high DIC-cultures of *N. maculata*, was likely due to the current photosynthetic activity.

The difference in the behaviour of both algae under low DIC conditions was also reflected in the evolution of the actual quantum yield ($\phi_{psii}$). So, while $\phi_{psii}$ only decreased slightly in *N. gaditana*, it dropped markedly in *N. maculata* during the first 4 days of growth until it stabilized to a value of around 0.4. In the former alga,
the gradual reduction in the phosphate content in the medium is indicative of ATPase activity over the entire growth period, which could have dissipated H⁺ from the lumen and so avoided qN development (Walker and Sivak, 1985). Photosynthesis may have been downregulated as the inorganic carbon availability decreased due to increasing cell density, with concurrent lesser rates of carbon fixation and ATP consumption (Fig. 6a). Nevertheless, differences in photosynthetic activity between the low DIC-culture and the other two cultures of N. gaditana were evident when the actual quantum yield was expressed in a per Chl a basis (Fig. 8a). Continuous decline in \( \Phi_{\text{PSII}}/\text{Chl } a \) in the low DIC-culture after the 5th day of growth may have been due to DIC limitation. In \( N. \) maculata cells, little consumption of phosphate suggests ATP formation, and maybe supply of reducing power, to proceed at expenses of pseudocyclic electron flow and/or dark respiration during the first 2–3 days of growth. It is also indicated by the relatively low values of \( q_N \) (Genty et al., 1989; Horton et al., 1990; Rees and Horton, 1990). Sültémeyer (1997) showed that the lower CO₂/O₂ concentration ratio the faster adaptation. Hence, adaptation during this period would have resulted in a slow down of the photosynthetic electron flow as well as CCM development until CO₂ was available enough for a compromise to exist between the carbon fixation rate by Rubisco and the photosystem II activity (Horton, 1985). Nevertheless, the incapability to synthesize new molecules was evident from the lack of nitrate uptake. Indeed, most of the N-sufficient green algae and cyanobacteria studied so far neither take up nor reduce NO₃ in the absence of CO₂, due to the lack of stored carbohydrates (Turpin, 1991; Huppe and Turpin, 1994). So, a minimum increase in cell density could only be observed during the last days. An increase in the photosynthetic electron flow following the adaptation period was also evident by the rise in \( q_N \).

According to our results, CO₂ limitation could also have affected the control-culture of \( N. \) maculata to some extent, since cell density of the high DIC-culture rose somewhat faster than cell density of the control-culture over the exponential growth phase. In contrast, the opposite effect occurred in \( N. \) gaditana, likely due to that CO₂-induced acidification of the medium in the high DIC-culture caused a reduction in the efficiency of bicarbonate uptake as compared with the control-culture. Therefore, even though increased growth rates as a result of CO₂ addition have been reported in microalgae (Goldman et al., 1981; Becker, 1984; Chrismadha and Borowitzka, 1994), our results point out that the effectiveness of additional CO₂ supply is rather dependent on the particular CCM operating in each species, as already explained above. Evolution of \( \Phi_{\text{PSII}} \) (both in absolute and per unit Chl a terms) did not differ substantially between the control- and the high-DIC-culture of \( N. \) gaditana, and indeed similar final cell biomass was obtained in both cultures over the same growth period. The rise in \( \Phi_{\text{PSII}}/\text{Chl } a \) which was observed after the day 4–5 of growth in both cultures (Fig. 8a) does not imply necessarily a higher electron flow, since it could have been reduced by a decrease in both the cross section and irradiance, due to the drop in pigment content and to a self-shading effect, respectively (Hofstraat et al., 1994). Additional CO₂ supply to air raised the growth rate of \( N. \) maculata cells, which was bound to a rise in the actual quantum yield until the 3rd day of growth. However the steep decrease in \( \Phi_{\text{PSII}} \) of the high
DIC-culture after that maximum was attained, along with the rise in $q_{N}$, as compared with evolution of these parameters for the control-culture suggests that lack of phosphate (and perhaps also of nitrate) was the limiting factor thereafter (Walker and Sivak, 1985; Walker, 1990).

As a general trend, pigment content and its evolution over the growth period were found to be similar between the cultures grown under control and high DIC conditions for each alga, but differed in both algae with regard to those observed in the culture grown under low DIC. In particular, under low DIC conditions $N. \textit{maculata}$ cells accumulated a higher amount of pigments than cells cultured on air levels of CO$_2$ and high DIC. Nevertheless, the continuous increase in the pigment content during the early log phase in cells of both high DIC- and control-cultures led to a pronounced decline in $\Phi_{\text{PSII}}/\text{Chl } a$, which suggests changes in the photosynthetic apparatus of these cells in order to readily acclimate to the abundance of CO$_2$ and nutrients. However, this explanation can not be valid for the $N. \textit{maculata}$ cells growing under low DIC conditions. From our knowledge, there are only few reports concerning the regulation of photosynthetic pigments during acclimation to different DIC levels in microalgae. Eley (1971) reported an increase in pigment concentrations in the cyanobacterium \textit{Anacystis nidulans} when grown at 1% CO$_2$ (v/v). In contrast, Yokota and Canvin (1986) observed that the green alga \textit{Chlorella} exhibited half of the amount of chlorophyll $a$ in cells cultured at high DIC levels compared to those grown at DIC concentrations normally found in seawater. On the other hand, no variations in pigment concentration caused by the level of DIC during growth have been detected in the green alga \textit{Scenedesmus obtusiusculus} (Larsson et al., 1985). According to the different responses reported so far and those found in this study, it seems that if the DIC level plays any role on the regulation of the pigment concentration, it could be species dependent as well.

Taking into account the high cost of CO$_2$ for commercial algal cultures, the conclusion drawn from our results is that little benefit can be achieved in adding CO$_2$ when attempting to optimise the growth of both $N. \textit{gaditana}$ and $N. \textit{maculata}$. Nonetheless, since CO$_2$ addition seems to speed up growth of $N. \textit{maculata}$ in parallel with nutrient consumption, further experimentation should intend to check whether additional nutrient supply during the log phase could enhance the final biomass that can be obtained over a shorter time period.

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