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Blue light effect on growth, light absorption characteristics and photosynthesis of five benthic diatom strains

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Abstract

Bio-optical characteristics and photosynthetic performance of five strains of benthic diatoms belonging to three different species (*Nitzschia thermalis* Kützing var. *minor* Hilse *sensu* Podzorski, *Nitzschia laevis* Hust and *Navicula incerta* Grunow) were examined. The effects of treatments with monochromatic blue light (BL) were also determined. The values of photosynthesis rate at saturating photon fluence rate (PFR) (P_{max}) and ascending initial slope of the P-PFR curves (α) ranged from 0.22 to 3.58 μmol O_2 per mg Chl *a* min^{-1} and from 0.012 to 0.169 μmol O_2 per mg Chl *a* (μmol photons m^{-2} s^{-1})$^{-1}$ respectively. Onset of light saturation parameter (E_k) ranged from 43 to 12 μmol photons m^{-2} s^{-1}. These values were comparable to those reported from measurements performed with sub-tidal benthic communities. In vivo effective optical cross-section of Chl *a* (a^* values) was correlated to average cell surface area. Interestingly, a^* and α were also negatively correlated, probably indicating that a higher pigment packaging affects negatively to α. The acclimation of the five strains to monochromatic BL for 2 weeks produced a reduction of P_{max} and α although the effective quantum yield of photosystem II ($\Delta F/F_{m'}$) was not affected. In addition to these changes, chlorophyll *a* increased and a^* decreased in the five strains. Therefore, it was concluded

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that the reduction of a^* could contribute to the changes in the photosynthetic performance in BL.

Keywords: Bio-optic; Benthic diatoms; Chlorophyll fluorescence; Light quality; Photosynthesis

1. Introduction

Benthic diatoms are cultured usually under controlled conditions in order to be used as food for other organisms such as abalone and sea urchin (Dunstan et al., 1994) or for biotechnological applications (Stoermer and Smol, 1991). In spite of their potential uses, only a few reports describe the effects of culture condition changes in the benthic diatom strain growth (Tadros and Johansen, 1988). Recently, several species potentially usable for culturing abalone have been isolated and the effect of different treatments have begun to be studied (Correa-Reyes et al., 2001; Simental-Trinidad et al., 2001). Overall, among the factors which control the production of microalgae valuable in aquaculture, several studies have tested the effect of changes on spectral composition of light. In particular, some works have investigated the effects of the monochromatic blue light on the growth in a number of phytoplankton species (Flaak and Epifanio, 1978; Sánchez-Saavedra and Voltolina, 1994, 1995). According to these reports, this manipulation of the light field produces changes on growth rates and pigment contents which affect the nutritional value of the microalgae. However, studies with a more physiological focus demonstrate that the effects of the blue light are variable among the different algal groups. Thus, Humphrey (1983) reported that blue light stimulates the growth of Chlorophyceae while negative effects have been described in Rhodophyceae (Figueroa et al., 1995; Mercado et al., 2002). At the present, the physiological effects of the blue light on benthic diatoms remain unstudied and therefore its possible application to control the microalgal growth and biomass quality has not been determined.

In this paper, the photosynthetic characteristics of five sub-tidal benthic diatom strains are analysed after culturing in white and blue light. The five strains grow sub-tidally at their natural habitats and therefore they are subjected to a light attenuation and scattering due to the water column, sediment and self-shading within the alga mats (Rivkin and Putt, 1987; Jørgensen and DesMarais, 1988; Schwarz and Markager, 1999). A few studies have described the photosynthetic characteristics of sub-tidal benthic communities and their responses to irradiance changes (Blanchard and Montagna, 1992; Light and Beardall, 2001). The main conclusion from these experiments is that changes on irradiance modify the photosynthesis versus irradiance curves although a strong correlation between maximal photosynthesis and photosynthetic efficiency was found irrespective the irradiance regime. According to Blanchard and Montagna (1992), this correlation between maximal photosynthesis and photosynthetic efficiency indicates a strategy for photoacclimation based on an optimisation of maximal photosynthesis relative to photosynthetic efficiency. Good agreement among the photosynthetic characteristics reported for these communities and those obtained from isolated diatom species could be expected since diatoms are often dominants in sub-tidal benthic communities.

The objectives of this paper were: (1) to describe the photosynthetic characteristics of the five benthic diatom strains isolated; (2) to compare the available data regarding to sub-tidal benthic communities to the results obtained from the isolated strains and (3) to describe the effects of treatment with blue light on their photosynthetic performances. For this proposal, the bio-optical characterisation of the cells in terms of absorptance and in vivo absorption optical cross section of chlorophyll *a* (a^*) was performed. The bio-optical properties were related to the parameters of oxygen evolution versus irradiance curves. Additionally, measurements of in vivo chlorophyll fluorescence of photosystem II were performed.

2. Material and methods

2.1. Microalgae

Five diatom strains were isolated from sub-tidal sediment harvested in Baja California coast (Ejido Erendida, 31°16′33″N, 116°22′53″W, Gulf of Mexico). The five strains were identified by Correa-Reyes et al. (2001) as *Nitzschia thermalis* Kützing var. *minor* Hilse *sensu* Podzorski, three different strains of *Nitzschia laevis* Hust (called st. 1, st. 2 and st. 3 in this paper) and *Navicula incerta* Grunow. Non-axenic bath cultures of each strain were maintained in Erlenmeyer flasks of 250 ml under a constant photon fluence rate (PFR) of 150 μmol photons $m^{-2}\,s^{-1}$ and 22±1 °C. f″ Medium (Guillard and Rhyter, 1962) was used at salinity 34±1 PSU. PFR was measured inside the cultures using a QSL-100 (Biospherical Instrument) 4π quantum meter. The cultures were maintained non agitated and the medium was renewed weekly.

Triplicate cultures of *N. thermalis* and *N. incierta* were initiated starting with about 0.05×10^6 cell ml^{-1}. The initial cell concentration selected to start triplicate cultures of the three strains of *N. laevis* was 0.3×10^6 cell ml^{-1}. In order to keep the strains in exponential phase of growth, the cultures were renewed weekly starting with the initial cell density mentioned above. Two different light sources were used for the cultures: blue (BL) and white (WL) light. By using blue fluorescent lamps (Sylvania F40B) 150 μmol photons $m^{-2}\,s^{-1}$ of BL were obtained. White light at 150 μmol photons $m^{-2}\,s^{-1}$ was provided from Sylvania F40CW fluorescent lamps. The spectral photon fluence rate of the white and blue light sources were measured with a quantum radiometer Li-1800 UW (Fig. 1). PFR inside the cultures was measured with a 4π quantum meter as mentioned above. The experiments were performed at 22 °C. After 2 weeks of acclimation, the cell density was measured daily in order to calculate growth rates (GR). For this proposal, samples were sonicated and their cell concentrations estimated by direct counts using a hemacytometer. Growth rates (GR) were estimated by linear regression using the expression:

$$\mathrm{GR} = \frac{\ln(N_t/N_0)}{t}\ (\text{per day})$$

where N_t is the number of cells measured each day (from first to sixth day of culturing) and N_0 is the initial cell density.

Different samples were collected in exponential phase of growth, in order to determine the pigment cell content and the culture optical density. Oxygen evolution versus photon fluence

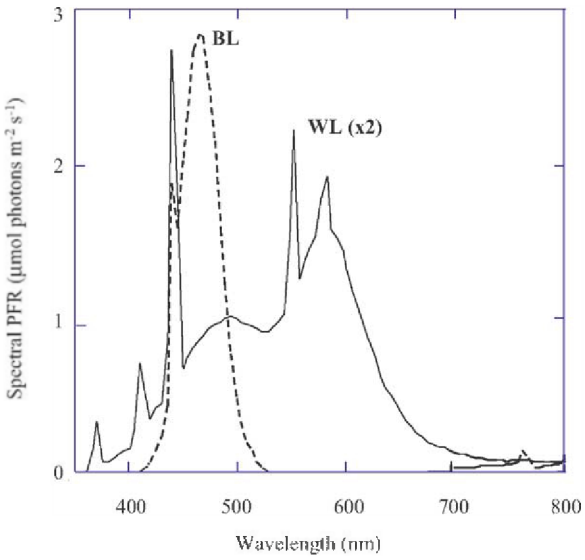

Fig. 1. Spectral photon fluence rate of light sources (WL, white light; BL, blue light) used in the cultures.

rate curves (P-PFR curves) and measurements of the in vivo chlorophyll fluorescence were also performed using cell suspensions. Additionally, the length of apical and transapical cell axis was determined for each strain using a Coulter Counter Multisizer. These values were used to calculate the average cell surface area. For this proposal, it was assumed that a ellipse matched the geometric shape of the cells.

2.2. Pigment content and light absorption characteristics

In order to determine the pigment cell content, 10 ml of culture were centrifuged at 1000 g for 10 min. The pellet was sonicated in methanol and kept at 4 °C until colourless. The concentration of Chl *a*, Chl *c*1 + *c*2 and total carotenoids in the extracts were determined by spectrophotometer (Lambda 5-Perkin–Elmer) using the trichromatic equations given by Porra et al. (1989). The different types of carotenoids contained in the extracts were determined by means of high performance liquid chromatography (HPLC) in a Waters 600E multisolvent delivery system equipped with a RP-C18 column packed with spherisorb ODS-2. Pigment extracts were filtered through a nylon membrane of 0.2 μm. Twenty microlitres of the filtrate were used to injection. Elution was developed in a two-solvent gradient system according to Minguez-Mosquera et al. (1992). A programmable photodiode array detector (Waters type 991) was used for pigment detection plotting each peak in the chromatogram at its maximum wavelength, since data were acquired three-dimensionally (absorbance-time-wavelength) over the wavelength range of 350–800 nm.

The efficiency of light capture in the cultures was estimated by calculating the ratio between optical density of the cell suspension at 678 nm (OD_{sus}) and the chlorophyll *a*

concentration (in vivo effective optical cross-section of Chl *a*, a^*). Five millilitre of culture were sonicated and afterwards filtered through glass fibber filters (1.2 μm pore size; Millipore GF/C). Optical density of the filters at 678 nm (OD_{fil}) was determined with a spectrophotometer by placing the filter between the light source and the detector of the spectrophotometer. $OD_{sus}(678)$ was calculated from $OD_{fil}(678)$ using the expression proposed by Cleveland and Weidemann (1993):

$$OD_{sus}(\lambda) = 0.378\,OD_{fil}(\lambda) + 0.523\,OD_{fil}(\lambda)^2$$

Optical density of the cultures was used in calculating $a^*(678)$ by dividing $OD_{sus}(678)$ to chlorophyll *a* concentration of the cell suspension.

2.3. Gross oxygen evolution versus photon fluence rate curves

Oxygen evolution was measured in 8 ml chambers at 22 °C with Clark-type oxygen electrodes (Yellow Spring Instruments 5221 OH, USA). Temperature was maintained constant by means of a Haake Fison DC1 equipment (Haak Mess-Technik Gmbh u. Co., Kalsruhe, Germany). The O_2 electrode signal was recorded on a strip chart recorder. The response of the oxygen evolution to changes on photon fluence rate (PFR) was determined. A halogen lamp (Xenophot HLX, Osram, Munich, Germany) was used as a source of light. PFR inside the chamber was measured with a spherical quantum sensor (20HM33CM12KG, Zemoko, Holland) connected to a quantummeter (Licor 1000). Ten different PFRs ranging from 12 to 1600 μmol photons $m^{-2}\,s^{-1}$ were obtained using glass neutral density filters. Respiration rates were measured in darkness before the first illuminated measurement. Oxygen evolution was recorded for 10–15 min after each PFR increase. The photosynthetic rate was expressed as μmol O_2 mg^{-1} Chl *a* min^{-1}. Maximal gross photosynthetic rates (P_{max}) and ascending slope at limiting PFRs (α) were obtained from the fit of the curves to the equation provided by Henley (1993):

$$P = P_{max}\left[\frac{\alpha PFR}{(P_{max} + \alpha PFR)}\right] + R_d$$

where P is the photosynthesis rate and R_d is the respiration rate in darkness. Onset of light saturation (E_k) was estimated as P_{max}/α. Three independent curves (i.e. starting from different samples) were constructed for each strain and treatment. Each curve was analysed separately. The goodness of fit to the curve model was tested by least-squares regression analysis. The average R^2 value obtained from the least-squares regression analysis was 0.99.

2.4. Measurements of PAM fluorescence

In vivo-induced chlorophyll fluorescence was determined with a portable pulse amplitude modulated fluorometer (PAM-2000, Waltz, Effeltrich, Germany). The method of quenching analysis is based on the measurement of the fluorescence parameters in response to saturating light in dark- or light-adapted specimens (Schreiber et al., 1995). In this work, the effective

quantum yield of light-adapted algae ($\Delta F/F_{m'}$) was determined by using the following expression:

$$\frac{(F_{m'} - F_t)}{F_{m'}} = \frac{\Delta F}{F_{m'}}$$

where $F_{m'}$ is the maximal fluorescence of light-exposed algae which normally decreases with increasing irradiance compared to the maximal fluorescence in dark-adapted algae (F_m), and F_t is the basal fluorescence after the saturating light pulse. The effective quantum yield was determined in cell suspensions at 150 μmol photons m^{-2} s^{-1} produced from a red light-emitting diode (LED).

In order to establish the effect of a high irradiance on the photosynthesis and its capacity of recovery, samples coming from the cultures acclimated to WL were exposed to full solar radiation (2400 μmol photons m^{-2} s^{-1}) for 1 h. The effective quantum yield at 150 μmol photons m^{-2} s^{-1} was immediately determined from at least eight replicates by immersing the optic fiber in the culture. Subsequently, the samples were transferred to darkness for 1 h. Afterwards, $\Delta F/F_{m'}$ was determined and compared to the values obtained previously.

2.5. Statistics

Oxygen determinations and pigment analysis were run in triplicate. Statistical significances of means were tested with a model 1 one-way ANOVA followed by a multirange test (Fisher's protected least significance difference). A P-level of 0.05 was used to evaluate the significance. PAM measurements for each treatment were repeated at least eight times and mean values and standard deviation were calculated.

3. Results

Growth rates (GR) ranged from 0.35 to 0.11 per day (Table 1) obtained for *N. thermalis* and *N. incerta*, respectively. In addition to these differences in GR, the three species studied showed quite different characteristics with respect to their cell size as estimated from the length of the apical and transapical cell axis. Accordingly, the average cell surface area was fourfold higher in *N. thermalis* than in the three strains of *N. laevis*. (Table 1). An intermediate figure was obtained for *N. incerta*. Blue light did not affect significantly to GR. In contrast, significant changes in the cell size were detected in *N. thermalis* and *N. laevis* st. 1 (ANOVA, $P < 0.05$). Thus, mean cell surface area of *N. thermalis* grown in BL was higher by 15% than in WL. Opposite effect was found in *N. laevis* st. 1 whose surface decreased by 60% in BL.

Chlorophyll *a* content increased in BL with respect to WL in four out of five strains analysed. It was not modified in *N. incerta* although it has been noted that this species yielded the highest Chl *a* cell content in both WL and BL. The differences on Chl *a* content and cell size among the species could account for different values of the in vivo effective optical cross-section of Chl *a* ($a^*(678)$). In fact, $a^*(678)$ varied as a function of both the strain considered and the light treatment and there was a significant negative logarithmic correlation between $a^*(678)$ and Chl *a* concentration in the cultures (Fig. 2, $R^2 = 0.90$,

Table 1
Characterization of the growth, size and bio-optical properties of the strains cultured under 150 μmol photons m^{-2} s^{-1} of white (WL) and blue (BL) PFR

	Treatment	GR (per day)	Surface area (μm²)	Chl *a* (μg 10^{-6} per cell)	a^*(678) (m^2 mg^{-1} Chl *a*)
N. thermalis	WL	0.30 ± 0.05	144	1.7 ± 0.0	0.09
	BL	0.35 ± 0.05	168	3.0 ± 0.7	0.02
N. laevis st. 1	WL	0.22 ± 0.05	36	1.3 ± 0.1	0.02
	BL	0.29 ± 0.06	14	2.7 ± 0.2	0.01
N. laevis st. 2	WL	0.24 ± 0.04	36	1.8 ± 0.1	0.02
	BL	0.28 ± 0.07	36	2.2 ± 0.8	0.01
N. laevis st. 3	WL	0.24 ± 0.04	28	1.5 ± 0.1	0.03
	BL	0.26 ± 0.08	36	2.7 ± 0.2	0.01
N. incerta	WL	0.13 ± 0.02	76	3.2 ± 0.1	0.04
	BL	0.11 ± 0.02	76	3.3 ± 0.2	0.02

Surface area: average cell surface area; GR: growth rate; a^*(678), in vivo effective optical cross-section of Chl *a* estimated at 678 nm. The values are means ± 1 S.D. Standard deviation for surface area and a^*(678) was less than 7%.

$P < 0.05$). The highest a^*(678) value (and therefore the lowest package effect) was found in *N. thermalis* cultured in WL. Lower values were obtained in the three strains of *N. laevis*. a^*(678) decreased significantly (ANOVA, $P < 0.05$) in BL with respect to WL. It is interesting to note that there was a significant linear correlation ($R^2 = 0.95$, $P < 0.05$) between cell size and a^*(678) when the cells cultured in WL were considered. However, this correlation is non significant statistically when the cells cultured in BL are considered into the calculations.

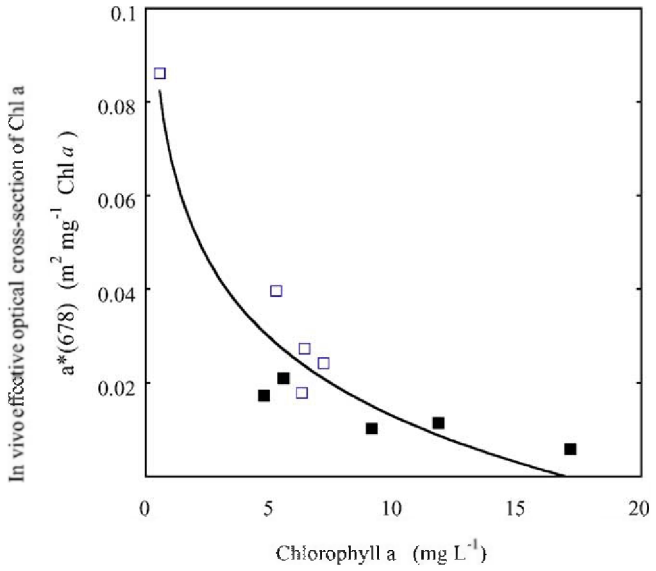

Fig. 2. Relationship between a^*(678) and the chlorophyll *a* concentration in the cultures. Open symbols: WL-treatment; closed symbols: BL-treatment.

Table 2
Concentration of carotenoids, chlorophyll $c1 + c2$ and the ratio of accessory pigment to chlorophyll *a* obtained in the five diatom strains acclimated to white (WL) or blue (BL) PFR

	Treatment	Total carotenoids (pg per cell)	Chl $c1 + c2$ (pg per cell)	Ch $c1 + c2$/ Chl *a* (%)	Carotenoids/ Chl *a* (%)	Major carotenoids(%)[a]
N. thermalis	WL	167	164	71	73	Fucox 66 Diadinox 9.5
	BL	49.8	53.4	77	72	Fucox 59 Diadinox 15
N. laevis st. 1	WL	26.7	30.5	74	65	Fucox 66 Diadinox 11
	BL	18.1	6.0	26	78	Fucox 52 Diatox 17
N. laevis st. 2	WL	42.6	42.0	54	54	Fucox 70 Fucox-type 8
	BL	65.6	51.7	51	65	Fucox 68 Fucox-type 7
N. incerta	WL	69.6	32.9	42	89	Fucox 67 Fucox-type 8
	BL	73.3	85.9	83	71	Fucox 67 Diatox 9

For all data, standard deviation was between 10 and 15%.
[a] Fucox, fucoxanthine; Diadinox, diadinoxanthine, Fucox-type, pigment fucoxanthine like; Diatox, diatoxanthine.

Accessory pigment content in four out the five strains analysed (this analysis was not performed in *N. laevis* st. 3, Table 2) showed highest total carotenoid cell content relative to Chl *a* in *N. incerta*. A lower value by 30% was found in the two strains of *N. laevis* analysed. In contrast, Chl $c1 + c2$ content was higher in *N. thermalis* than in the other strains. The major carotenoid was fucoxanthin in all the strains and it accounted for 67% of total carotenoids. In general, there was not a clear variation pattern in the accessory pigment content in BL with respect to WL. Thus, no significant changes were found in Chl $c1 + c2$ and carotenoid content in *N. thermalis* and *N. laevis* st. 2. In contrast, Chl $c1 + c2$ increased by two fold in *N. thermalis* and decreased by threefold in *N. laevis* st. 2.

The parameters of the P-PFR curves obtained for the five strains cultured are shown in Table 3. Gross photosynthesis was expressed on a Chl *a* content basis. The photosynthesis by all the strains was saturated at 800 μmol photons $m^{-2}\,s^{-1}$ and any photoinhibition was detected at 1600 μmol photons $m^{-2}\,s^{-1}$ of PFR (the highest value used in performing the curves). The highest rate of photosynthesis at saturating irradiance (P_{max}) was obtained in *N. thermalis* submitted to WL. In contrast, the P_{max} value obtained for *N. laevis* st. 1 was 16-fold lower. Initial slope of the P-PFR curves (α) varied by one order of magnitude among the strains analysed, with the highest value found in *N. thermalis* and the lowest value in *N. laevis* st. 2. The values of E_k also varied widely among the strains. Thus, E_k ranged from 43.0 to 10.2 μmol photons $m^{-2}\,s^{-1}$, figures found in WL-*N. laevis* st. 3 and BL-*N. incerta*, respectively. The effect of the BL on P_{max}, α and E_k did not show a clear pattern. Thus, although P_{max} decreased in *N. thermalis* and *N. laevis* st. 3 cultured in BL with respect to WL, the BL-treatment had a positive effect on P_{max} in *N. laevis* st. 2 and no effect was found in *N. incerta*. With the exception of *N. laevis* st. 1 and st. 2, α and E_k decreased in all the species when they were submitted to BL. It is interesting to note that P_{max} and α were correlated when the algae cultured in WL are considered (Fig. 3, $R^2 = 0.90$, $P < 0.05$). This correlation was also significant statistically when the algae cultured in BL were considered (Fig. 3, $R^2 = 0.88$, $P < 0.05$).

Table 3
Parameter of photosynthesis vs. PFR curves

Species	Treatment	P_{max} (μmol O_2 mg Chl a^{-1} min^{-1})	E_k (μmol photons m^{-2} s^{-1})	α μmol O_2 mg Chl a^{-1} min^{-1} (μmol photons m^{-2} s^{-1})$^{-1}$	$\Delta F/F_{m'}$
N. *thermalis*	WL	3.58 ± 0.06	21.2 ± 2.3	0.17 ± 0.02	0.41 ± 0.04
	BL	1.42 ± 0.01	14.4 ± 1.0	0.10 ± 0.01	0.32 ± 0.08
N. laevis st. 1	WL	0.53 ± 0.01	15.1 ± 2.5	0.03 ± 0.01	0.36 ± 0.01
	BL	0.44 ± 0.01	11.5 ± 1.4	0.04 ± 0.004	0.38 ± 0.01
N. laevis st. 2	WL	0.22 ± 0.01	29.2 ± 3.7	0.01 ± 0.001	0.39 ± 0.02
	BL	0.62 ± 0.01	25.2 ± 2.7	0.03 ± 0.002	0.34 ± 0.02
N. laevis st. 3	WL	1.55 ± 0.07	43.0 ± 9.9	0.04 ± 0.0072	0.34 ± 0.02
	BL	0.23 ± 0.01	18.7 ± 2.8	0.01 ± 0.002	0.47 ± 0.01
N. incerta	WL	1.50 ± 0.04	36.3 ± 5.3	0.04 ± 0.005	0.59 ± 0.03
	BL	1.34 ± 0.02	10.2 ± 1.1	0.13 ± 0.01	0.34 ± 0.03

$\Delta F/F_{m'}$ was estimated at 150 μmol photons m^{-2} s^{-1} of PFR. The values are means ± 1 standard deviation. (P_{max}: maximal gross photosynthetic rate; E_k, onset of saturation light; α, ascending slope) and values of effective quantum yield ($\Delta F/F_{m'}$) obtained in the five diatom strains acclimated to white (WL) and blue (BL) PFR.

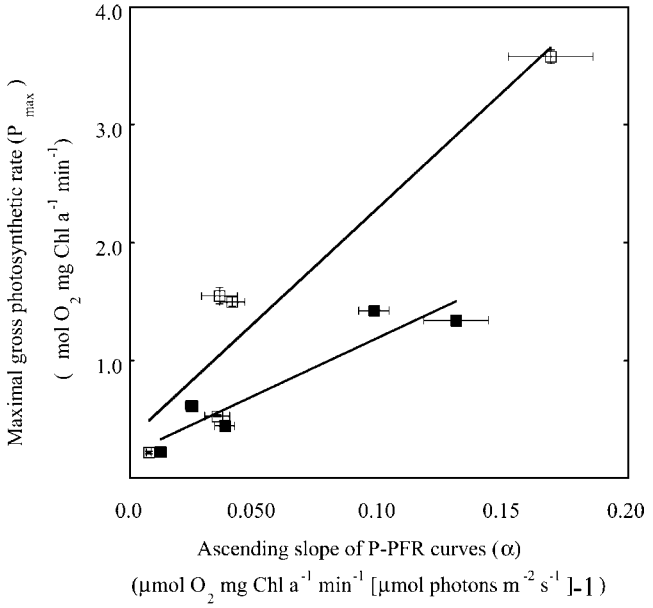

Fig. 3. Relationship between gross oxygen evolution at saturating PFR (P_{max}) and ascending initial slope of the P-PFR curves (α). Horizontal and vertical bars indicate ±1 S.D. Open symbols: WL-treatment; closed symbols: BL-treatment.

The effective quantum yield ($\Delta F/F_{m'}$) of the cultures measured at a similar PFR to during growth (i.e. 150 μmol photon m^{-2} s^{-1}) ranged from 0.59 to 0.34, with the three strains of *N. laevis* grown in WL showing the lower values (Table 3). Significant differences between the treatments were only detected in *N. laevis* st. 3 and *N. incerta* (ANOVA, $P < 0.05$). In particular, $\Delta F/F_{m'}$ decreased by 45% in *N. incerta* grown in BL with respect to WL. In contrast, $\Delta F/F_{m'}$ increased in *N. laevis* st. 3 submitted to BL. Anyway, no correlation between $\Delta F/F_{m'}$ and initial slope of the P-PFR curves was found.

In order to test the sensibility of the five strains to a photoinhibitory irradiance, they were submitted to 2400 μmol photons m^{-2} s^{-1} of full solar radiation for 1 h. This treatment was only performed with the cultures subjected to WL. The incubation under a PFR of 2400 μmol photons m^{-2} s^{-1} produced a drastic decrease of $\Delta F/F_{m'}$ measured at 150 μmol photons m^{-2} s^{-1} and declined by 90% or more. This negative effect was more pronounced in *N. thermalis* and *N. incerta*. In spite of this different sensitivity to photoinhibiton, the five strains showed the same recovery capacity provided $\Delta F/F_{m'}$ values measured 1 h after incubation in darkness were similar to those obtained initially.

4. Discussion

The photosynthetic characteristics found in the five benthic diatom strains isolated at Baja California coast are comparable to those reported from measurements performed with intact benthic biofilms (Blanchard and Montagna, 1992; Light and Beardall, 2001). Thus, the authors reported values of P_{max} ranging from 1.3–64 mg O_2 mg^{-1} Chl *a* h^{-1} and values of α ranging from 0.045 to 0.506 mg O_2 mg^{-1} Chl *a* h^{-1} (μmol photons m^{-2} $s^{-1})^{-1}$ while mean P_{max} and α obtained in this paper ranged from 0.45 to 6.88 mg O_2 mg per Chl *a* h^{-1} and from 0.023 to 0.325 mg O_2 per mg Chl *a* h^{-1} (μmol photons m^{-2} $s^{-1})^{-1}$, respectively. Large differences were found in both P_{max} and α among the strains analysed, in particular P_{max} varied one order of magnitude from *N. thermalis* to *N. laevis* st. 1 indicating a great difference in capacity for carbon assimilation (Sakshaug et al., 1997). P_{max} and α were correlated positively, i.e. the strains with a lower capacity to carbon assimilation (i.e. the three strains of *N. laevis*) had a lower light harvesting capacity. Light and Beardall (2001) also described a strong correlation between P_{max} and α in a sub-tidal benthic community through the seasonal cycle. According to these authors, this correlation indicates a strategy for photoacclimation by the benthic microalgae based on an optimisation of P_{max} relative to α. Our results suggest that modifications on the species composition could explain these changes described at the benthic community level. It is worthy to note that non correlation between E_k and P_{max} or α has been found in our experiments, as obtained by Light and Beardall (2001). According to Sakshaug et al. (1997), E_k is a more convenient indicator of photoacclimational status than P_{max} and α. The E_k values obtained demonstrate that the photosynthetic characteristics of the five strains fit well the characteristics of microalgae shade-acclimated. In fact, the values are in the lower end of the value range reported for sub-tidal benthic communities (Blanchard and Montagna, 1992; Light and Beardall, 2001).

In our experiments, the cell size appears to dictate some properties of the cells which could modulate their photosynthetic performance. Thus, the cells of the five strains exhibited

different bio-optical characteristics as demonstrated by their different values of $a^*(678)$. Kirk (1994) proposed that a^* expresses the efficiency of light capture which depends on pigment package within the cell. According to this assertion, it can be concluded that the differences in $a^*(678)$ found among the strains are related to the different degree of Chl *a* package. Changes on so-called *package effect* are a result of differences in the pigment arrangement within the chloroplasts and modifications on cell size and/or shape. In our experiments, the differences on the cell size could account for the different values of $a^*(678)$ provided there was a significant positive correlation between $a^*(678)$ and the mean cell surface. This result contrasts to the suggestion made by different authors (Duarte et al., 1995; Figueroa et al., 1997; Raven, 1998) who proposed a decrease of the package effect in small cells with respect to bigger cells. Additionally, when only the strains acclimated to WL are considered, there was a certain linear relationship between $a^*(678)$ and α ($R^2 = 0.92$, $P < 0.01$) provided both parameters were significantly higher in *N. thermalis* and *N. incerta* than in the three *N. laevis* strains. These results could indicate that a higher pigment packaging affects negatively to α (Sakshaug et al., 1997). In contrast to α, effective quantum yield ($\Delta F/F_{m'}$) appeared to be unaffected by the package effect, but the sensitivity to photoinhibition estimated as the decrease percentage of $\Delta F/F_{m'}$ after incubation under high irradiance was higher in *N. thermalis* and *N. incerta*, the species with higher $a^*(678)$ and cell size values. This result is accord with the suggestion of Raven and Kübler (2002) who pointed out that a smaller package effect increases the possibility of damage to the photosynthetic apparatus by a high irradiance.

4.1. Effect of the BL on photosynthesis

Blue light modified the photosynthetic characteristics of the five diatom strains. In general, α, P_{max} and E_k decreased when the algae were cultured in this treatment. It has to be noted that in our experiments a significant correlation between α and P_{max} was maintained in BL, indicating that both parameters varied together. According to different authors (Gantt, 1990; Sakshaug et al., 1997), α and P_{max} decrease can be due to changes in accessory pigments implied on dissipation of absorbed energy, loss of functional reaction centres and changes in electron flow. Among the five diatom strains analysed, changes in accessory pigments were produced only in *N. laevis* st. 1 and *N. incerta* whose ratio of accessory pigments to Chl *a* was modified. However, the pattern of variation was different in these two strains and no clear effect was found overall. $\Delta F/F_{m'}$ was also unaffected by BL and therefore it can be not concluded whether or not changes on electron flow associated to PSII are responsible for the reduction of α in our experiments (Gantt, 1990).

According to Sakshaug et al. (1997), additional factors such as alterations in chloroplast organization and pigment package could also contribute to a α reduction. The only factor which decreased in the five species submitted to BL with respect to WL was $a^*(678)$, i.e. the efficiency of light capture was reduced in the BL-cells. We propose that this factor could contribute to the α decrease. The reason for this reduction of $a^*(678)$ could be the higher Chl *a* content obtained in the BL-cells (e.g., in *N. thermalis* was doubled with respect to WL) although other changes on thylakoid arrangement can be not discarded. It must be noted that the cell size was not affected by BL-treatment significantly (with the exception of *N. laevis* st. 1) and then it must be not implied on the $a^*(678)$ changes.

The response to the BL by the five benthic diatoms resembles the one reported for other alga groups (Figueroa et al., 1995; Kowallik, 1987; Senge and Senger, 1991). Additionally, the five benthic diatom strains exhibited values of E_k lower in BL than in WL, indicating that the BL-response was similar to the one expected in algae acclimated to low irradiance as proposed by Senge and Senger (1991). However, the acclimation in BL did not affect growth rates, in contrast to the results published for some Chlorophyceae, Pheophyceae and Rhodophyceae (Lüning, 1992; Figueroa et al., 1995; Mercado et al., 2002). Only two reports describing the effects of BL on the growth in diatoms can be found in the literature. Sánchez-Saavedra and Voltolina (1994) described significant increase of the growth in *Chaetoceros* sp. In contrast, the growth rates were not affected in eight species of benthic diatoms, including *Nitszchia* and *Navicula* species, investigated by Correa-Reyes et al. (2001). Taking into account all these data, it can be concluded that the effect of the BL on growth of benthic diatoms could be species-dependent. The more important effect was on the photosynthetic performance. These changes affected probably the quality of the microalgal biomass. The biochemical composition of the cells cultured in BL must be determined in order to evaluate the utility of this treatment to control their nutritional value.

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