Macronutrient uptake and carotenoid/chlorophyll *a* ratio in the dinoflagellate *Amphidinium carteri* Hulburt, cultured under different nutrient and light conditions

Consumo de macronutrientes y proporción de carotenoides/clorofila *a* en el dinoflagelado *Amphidinium carteri* Hulburt, cultivado bajo diferentes condiciones de luz y nutrientes

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ABSTRACT

Population growth, macronutrient (NO_3^-, PO_4^{3-}) uptake and carotenoids/chlorophyll *a* ratio we determined in *Amphidinium carteri* Hulburt, cultured under conditions of continuous light (50, 150, 300 and 750 µmol quanta m^{-2} s^{-1}) and three nutrient concentrations $NaNO_3/NaH_2PO_4$ at 441.5/18.1 µM (low) 883/36.3 µM (medium) and 1766/72.6 µM (high). Both nutrient and irradiance had a significant effect ($p < 0.05$) on cellular abundance during the period of culture, except for the fourth and seventh day for nutrients ($p > 0.05$). In cultures under low nutrient condition, NO_3^- and PO_4^{3-} were almost depleted by the fifth day and in cultures with medium nutrient this condition occurred in the sixth day; whereas, at high nutrient condition the nutrients were not depleted. We concluded that *A. carteri* had higher growth rates and nutrient consumption at 300 µmol quanta m^{-2} s^{-1} during the first five days and in general the effect of nutrients on the pigment ratios was not significant ($p > 0.05$). However, the average peridinin/Chl*a* ratio decreased up to 72% from the lowest and the highest irradiance. The opposite was observed for the average of diadinoxanthin/Chl*a* ratio that increased almost two-fold, and the averages dinoxanthin and diatoxanthin to Chl*a* ratios that increased from low to high irradiances. The average diatoxanthin/Chl*a* ratio at 750 µmol quanta m^{-2} s^{-1} increased up to 2.7-fold from the exponential to the stationary phase only in the low and medium nutrient concentration. Likewise, the average peridinin, dinoxanthin and diatoxanthin to Chl*a* ratios were not significantly different at 50 and 150 µmol quanta m^{-2} s^{-1}. These results indicate important changes in average carotenoids/Chl*a* ratios in *A. carteri* cultured under different irradiances.

Key words: Macronutrient uptake, carotenoid/Chl*a* ratio, irradiance, *Amphidinium carteri*.

RESUMEN

Se evaluaron el crecimiento poblacional, consumo de macronutrientes (NO_3^-, PO_4^{3-}) y proporción de carotenoides/clorofila *a* en *Amphidinium carteri* Hulburt, cultivado durante 7 días, bajo condiciones de luz continua: 50, 150, 300 y 750 µmol quanta m^{-2} s^{-1} y tres concentraciones de nutrientes $NaNO_3/NaH_2PO_4$ (441.5/18.1 µM (bajo); 883/36.3 µM (medio)

y 1766/72.6 µM (alto). La abundancia celular fue significativamente afectada ($p < 0.05$) tanto por el nivel de nutrientes como por la irradianza durante el periodo de cultivo, excepto para el nivel de nutriente en el cuarto y séptimo día. En los cultivos con bajo nutriente, el NO_3^- y PO_4^{3-} se agotaron casi totalmente al quinto día, y en cultivos con medio nutriente ocurrió al sexto día; mientras que en alta concentración de nutrientes, éstos no se agotaron. Se concluye que *A. carteri* tuvo la mayor tasa de crecimiento y consumo de nutrientes en 300 µmol quanta m^{-2} s^{-1} durante los primeros cinco días y en general el efecto de nutrientes sobre las proporciones de pigmentos no fue significativo ($p > 0.05$). Sin embargo, la proporción promedio de peridinina/Chl*a* disminuyó hasta un 72% de bajas a altas irradianzas. Lo contrario ocurrió para el promedio de las proporciones de diadinoxantina/Chl*a*, que se incrementó casi 2 veces, y los promedios de las proporciones de dinoxantina/Chl*a* y diatoxantina/Chl*a* se incrementaron de altas a bajas irradianzas. Los promedios de las proporciones de diatoxantina/Chl*a* en 750 µmol quanta m^{-2} s^{-1} aumentó casi 2.7 veces de la fase exponencial a la estacionaria sólo en la condición baja y media de nutrientes. Así mismo, no hubo diferencias significativas en los promedios de las proporciones de peridinina, dinoxantina y diatoxantina respecto a la clorofila a en las irradianzas de 50 y 150 µmol quanta m^{-2} s^{-1}. Estos resultados indican cambios importantes en los promedios de las proporciones de carotenoides/Chl*a* en *A. carteri* cultivada en diferentes irradianzas.

Palabras clave: Consumo de macronutrientes, proporción carotenoides/clorofila *a*, irradianza, *Amphidinium carteri.*

INTRODUCTION

Amphidinium carteri Hulburt, is a marine dinoflagellate that is frequently present in coastal waters under bloom conditions. This dinoflagellate possess chlorophyll *a* (Chl*a*) and the accessory photosynthetic pigment peridinin (Jeffrey & Vesk, 1997). Some authors have noted that the pigment ratios vary as a function of light, nutrient status and specific growth rate (Goericke & Montoya, 1998; Henriksen *et al*., 2002). Field studies have demonstrated changes in pigment composition in relation to the structure and nutrient content of the phytoplankton community (Stón & Kosakowska, 2000; Stón & Kosakowska, 2002). Under laboratory conditions, changes in pigment ratios are induced by irradiance variability and nutrient limitation during exponential and stationary growth phases in batch cultures of marine phytoplankton (Henriksen *et al*., 2002). However, different species have distinct growth rates and pigment composition in response to light and nitrogen stress (Sciandra *et al*., 2000).

Evaluation of the phytoplankton pigment composition using high performance liquid chromatography (HPLC), Chemical Taxonomy (CHEMTAX) and supplementary microscopy analysis, have been conducted to identify phytoplankton species and functional groups by relating specific marker pigments to total Chl*a* contents (Mackey *et al*., 1996; Fietz & Nicklisch, 2004; Llewellyn *et al*., 2005). Previous investigations utilizing *A. carteri* have focused on the light-harvesting system and energy transfer in the peridinin-chlorophyll-protein complex (Sharples *et al.,* 1996; Lohuis & Miller, 1998; Damjanović *et al*., 2000; Polívka *et al.,* 2005). However, few studies have been conducted in relation to nutrient uptake and the individual pigment composition in this species under different growth conditions in laboratory cultures. In this respect, the objective of the present work was to evaluate daily changes in macronutrient uptake and carotenoid/chlorophyll *a* ratios of *A. carteri* cultured under different nutrient and light conditions.

MATERIALS AND METHODS

Amphidinium carteri cultures. The marine dinoflagellate *A. carteri* was obtained from the monoalgal culture collection of the Instituto de Investigaciones Oceanológicas of the Universidad Autónoma de Baja California. All nutrients in the f/2 medium (Guillard, 1975) except nitrogen and phosphorus were added to one liter of filtered seawater to obtain the following concentrations: 11.7 µM $Na_2EDTA{\cdot}2H_2O$; 11.7 µM $FeCl_3{\cdot}6H_2O$; 0.04 µM $CuSO_4{\cdot}5H_2O$; 0.08 µM $ZnSO_4{\cdot}7H_2O$; 0.05 µM $CoCl_2{\cdot}6H_2O$; 0.9 µM $MnCl_2{\cdot}4H_2O$; 0.03 µM $Na_2MoO_4{\cdot}2H_2O$; 0.1 mg thiamine HCl; 0.5 µg biotin; 0.5 µg vitamin B_{12}. Silicate was omitted because it should only be used for the growth of diatoms. Cultures were started in 250 mL Erlenmeyer flasks containing 200 mL of medium. The flasks were autoclaved at 120 °C with 1.05 kg cm^{-2} of pressure for 15 minutes, and then inoculated with 10 mL of *A. carteri* culture to obtain an average initial population of 1.17×10^5 cells mL^{-1}. The cultures were maintained during seven days at 150 µmol quanta m^{-2} s^{-1} of irradiance, 33 PSU of salinity and a temperature of 25 ± 1 °C.

Experimental setting. A factorial experiment was designed using four levels of continuous light (50, 150, 300 and 750 µmol quanta m^{-2} s^{-1}) and three nutrient concentrations. Light was provided by fluorescent lamps (daylight 75 W); the irradiance was measured with a scalar photosynthetic active radiation (PAR) irradiance meter (4π sensor, Biospherical Instruments model QSL-100). $NaNO_3$ and NaH_2PO_4 were added at concentrations of 441.5/18.1 µM (low), 883/36.3 µM (medium) and 1766/72.6 µM (high), keeping a nitrogen/phosphorus ratio of ~24. All experiments were carried out in 3 L Fernbach flasks (2.9 L of media, n = 2 for each treatment). These flasks were sterilized as described above. A volume of 200 mL of culture were obtained at the end of the exponential phase from the previous level Erlenmeyer and was added to each experimental unit under controlled conditions. The initial average cell densities at the experimental units

was $1.22 \pm 0.2 \times 10^5$, $1.15 \pm 0.2 \times 10^5$, $1.13 \pm 0.2 \times 10^5$ cells mL^{-1} for the low, medium and high nutrient conditions, respectively. The culturing temperature was 25 ± 1 °C, and the salinity was 33 PSU. The pH was measured daily using a pH meter (Altex) and adjusted between 7.3 and 8.5 by the addition of CO_2 at a flow rate of 270 mL min^{-1}. Each flask was hand shaken every 12 hours. The cell density was determined daily with a Coulter (Beckman Coulter) particle counter Multisizer 3 in duplicate. The specific growth rate (μ) day^{-1} was calculated as follows:

$$\mu = [\ln(N_2) - \ln(N_1)]/(t_2 - t_1),$$

where t_1 and t_2 are the initial and final time, and N_1 and N_2 are the initial and final cell densities, respectively.

NO_3^- and PO_4^{3-} uptake. Daily samples of 10 mL were taken to quantify nitrate and phosphate removed from the culture during the experiment. These samples were filtered through 25-mm fiber glass filters (GF/F) of 0.7 µm and stored immediately at –20 °C for later analysis. Prior to analysis, calibration curves were performed for each nutrient. The filtered water was used to determine nitrate and phosphate concentrations according to Parsons *et al.* (1985). The nitrate and phosphate uptake were determined by measuring the disappearance of the nutrient from the media each day. The uptake was calculated as follows:

$$V = C_i - C_f/t$$

Where V is the daily uptake rate (µM), C_i and C_f are the initial and final nutrient concentration respectively and t is the time period of uptake (d^{-1}).

Pigment analysis. For Chl*a* and carotenoids analysis, 10 mL samples were filtered through 25 mm GF/F glass filters during the first three days of the culture, and thereafter, only 5 mL were filtered up to the seventh day. The filters were frozen, stored in liquid nitrogen and analyzed within the next three months. Prior to the analysis, the filters were placed in 4 mL of 93% acetone, sonicated for 10 seconds and stored in the freezer for 24 hours. Subsequently, the samples were centrifuged at 2,000 × *g* for 5 minutes and then filtered through 0.2 µm filters. From the acetone extract, 100 µL were injected into a HPLC system according to the method described by Bidigare *et al.* (2003). The pigments measured were Chl*a*, the photosynthetic carotenoid (PSC) peridinin, and the photoprotective carotenoids (PPC) diadinoxanthin, dinoxanthin and diatoxanthin.

Statistical analysis. Two-way analyses of variance were used to determine the individual and interactive effects of nutrients and irradiance on dependent variables (cellular density, nitrate and phosphate uptake and carotenoids/Chl*a* ratios). For variables that were significantly affected by nutrients and/or irradiance a multiple comparison procedure of Tukey was performed ($\alpha = 0.05$).

RESULTS

Microalgae cultures. The mean growth of *A. carteri* cultured under different nutrient and light conditions is shown in Fig. 1.The specific growth rate (µ) day^{-1} during the exponential phase was typically higher at high irradiance (0.49 to 0.66) than at low irradiances (0.42 to 0.63) per day. Subsequently, slow growth phase was observed with a reduction in specific growth rate during the latter part of the culture period in all treatments. These results indicated that the cultures had initiated the stationary growth phase (Fig. 1). The maximum cell densities at the low, medium and high nutrient concentrations were $19.1 \pm 0.24 \times 10^5$, $23.3 \pm 0.09 \times 10^5$ and $21.6 \pm 0.008 \times 10^5$ cells mL^{-1}, respectively and were observed at 750 µmol quanta m^{-2} s^{-1} (Fig. 1). However, during the first five days of the culture, the highest mean cell density was observed at 300 µmol quanta m^{-2} s^{-1}, with a significant interaction between nutrient and irradiance in the majority of the days (Table 1A).

NO_3^- and PO_4^{3-} uptake. Both nitrate and phosphate uptake was significantly affected by the levels of nutrients and light during the majority of the days of the culture, with a significant interaction between the factors only for nitrate uptake (Table 1B, C). The average nitrate and phosphate uptake during the first five days for low nutrient condition was 429.7 µM and 13.2 µM, which represents 98% and 93% of the total concentration of nitrogen and phosphate added to the culture respectively (Figs. 2A, 3A; Table 2). When cells were growing in the medium nutrient condition, in all light conditions, the average uptake was 744.4 µM of nitrate on the fifth day, representing 84% from the initial nutrient in the culture (Figs. 2B; 3B; Table 2); likewise the phosphate uptake was 27.6 µM representing the 76% from the initial nutrient in the culture. Cells growing at medium nutrient and 300 µmol quanta m^{-2} s^{-1} almost depleted the nutrients during the first five days, the nutrient uptake during this period of time was 817.5 µM of nitrate and 32.5 of phosphate representing the 92% and 90% of the initial content in the media, these results were higher than those observed at irradiances of 50, 150 and 750 µmol quanta m^{-2} s^{-1}, for the same period of time. Under high nutrient concentrations and irradiances of 50 and 150 µmol quanta m^{-2} s^{-1}, similar nutrient uptake were taken up from the media during the first five days (613.5-676.6 µM NO_3^- and 41.1-39.8 µM PO_4^{3-}). However, these values were lower than those observed in cultures that received 300 µmol quanta m^{-2} s^{-1} but higher than those in cultures exposed to an irradiance of 750 µmol quanta m^{-2} s^{-1}, where only 575.6 and 24.4 µM of NO_3^- and PO_4^{3-} were taken up from the media, these values representing the 32 and 33.6% of the initial content in the media (Figs. 2C; 3C; Table 2). A multiple comparison procedure showed that nitrate uptake by nutrient factor were significantly different ($p < 0.05$) from fourth to seventh day of culture, and the phosphate uptake between low and medium versus high, also were significantly different during the last two days of culture ($p < 0.001$).

After the fifth day in all light conditions, the nitrate was almost exhausted at low nutrient concentrations (Fig. 2A). However,

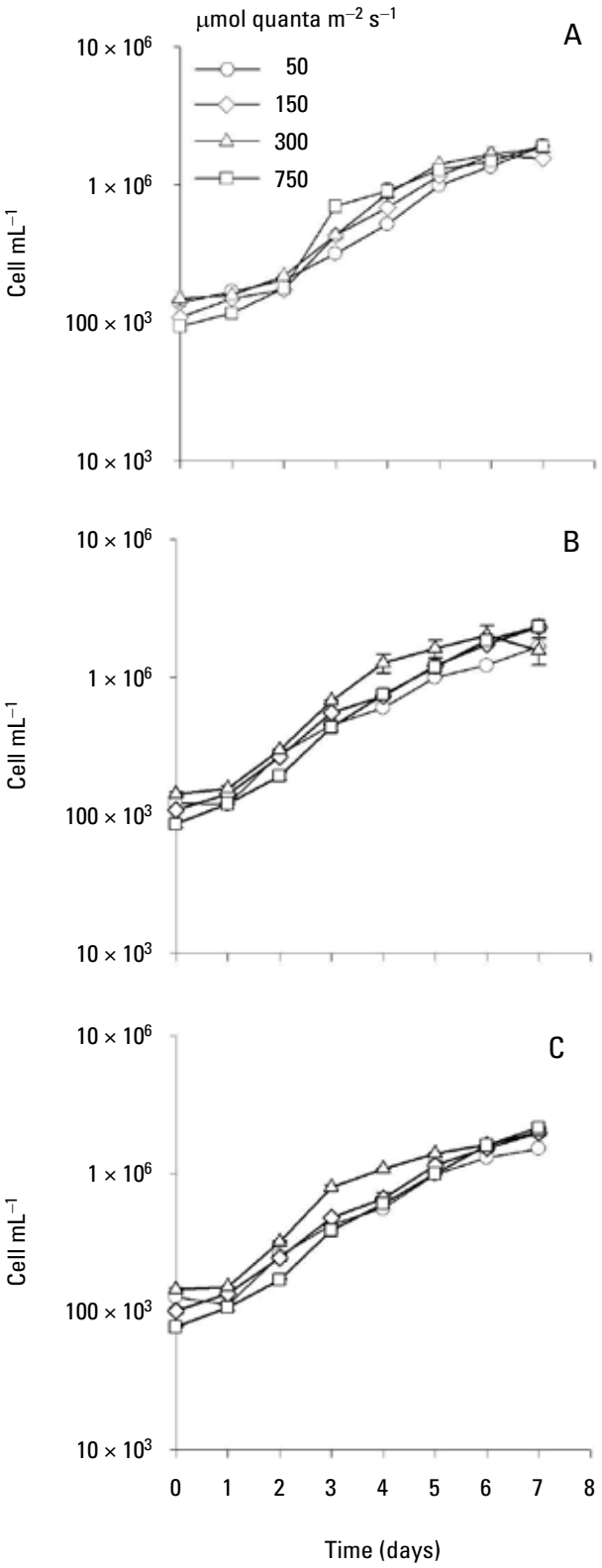

Fig. 1. A-C. Average growth of *Amphidinium carteri* in cultures with different concentrations of $NaNO_3/NaH_2PO_4$: A) 441.5/18.1 µM; B) 883/36.3 µM; C) 1766/72.6 µM and four levels of irradiances. Vertical bars indicate the standard error.

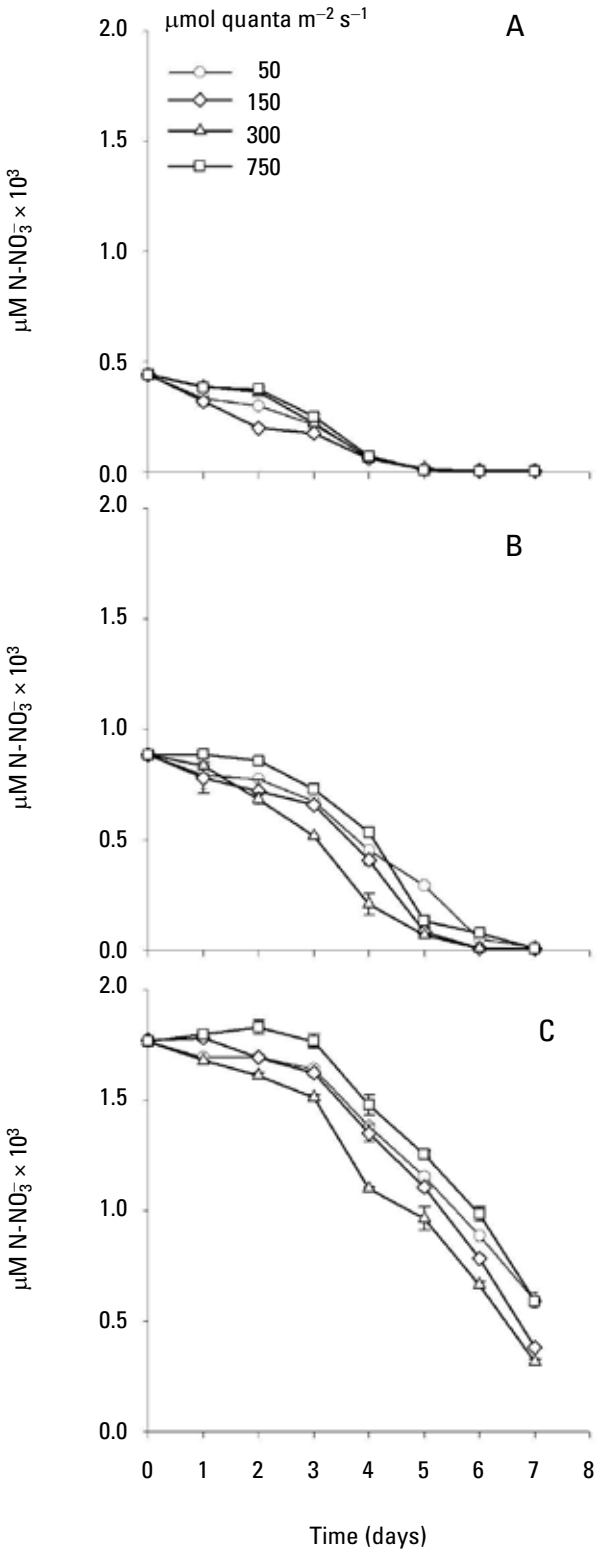

Fig. 2. A-C. Average nitrate concentration in the media of cultures of *Amphidinium carteri* grown under concentrations of $NaNO_3/NaH_2PO_4$: A) 441.5/18.1 µM; B) 883/36.3 µM; C) 1766/72.6 µM and four levels of irradiance. Vertical bars indicate the standard error.

Table 1. Two way analysis of variance: A) Cell density, B) Nitrate uptake and C) Phosphate uptake by cultures of *Amphidinium carteri* under different nutrient and light conditions. NS = No significative: S = significative ($\alpha = 0.05$).

A)

	d. f. error = 24								
Day	Nutrient		$p < 0.05$	Light		$p < 0.05$	Interaction		$p < 0.05$
	F value	P value		F value	P value		F value	P value	
1	28.101	<0.001	S	51.381	<0.001	S	10.993	<0.001	S
2	6.743	0.011	S	1.939	0.177	NS	0.865	0.547	NS
3	4.559	0.034	S	12.291	<0.001	S	7.584	0.002	S
4	2.532	0.121	NS	26.033	<0.001	S	3.220	0.040	S
5	1.883	0.194	NS	16.677	<0.001	S	1.093	0.420	NS
6	2.107	0.164	NS	6.114	0.009	S	0.904	0.523	NS
7	1.528	0.256	NS	5.615	0.012	S	4.134	0.018	S

B)

	d. f. error = 24								
Day	Nutrient		$p < 0.05$	Light		$p < 0.05$	Interaction		$p < 0.05$
	F value	P value		F value	P value		F value	P value	
1	1.495	0.263	NS	1.941	0.177	NS	1.533	0.249	NS
2	33.573	<0.001	S	49.460	<0.001	S	16.45	<0.001	S
3	2.497	0.124	NS	5.316	0.015	S	0.816	0.578	NS
4	29.210	<0.001	S	5.361	0.014	S	1.963	0.151	NS
5	65.858	<0.001	S	13.460	<0.001	S	6.881	0.002	S
6	125.49	<0.001	S	2.900	0.079	NS	5.097	0.008	S
7	1070.1	<0.001	S	8.404	0.003	S	8.098	0.001	S

C)

	d. f. error = 24								
Day	Nutrient		$p < 0.05$	Light		$p < 0.05$	Interaction		$p < 0.05$
	F value	P value		F value	P value		F value	P value	
1	39.396	<0.001	S	8.707	0.002	S	0.897	0.528	NS
2	5.624	0.019	S	3.538	0.048	S	1.495	0.260	NS
3	3.062	0.084	NS	8.019	0.003	S	2.970	0.051	NS
4	2.546	0.120	NS	2.605	0.100	NS	1.551	0.243	NS
5	23.913	<0.001	S	1.244	0.337	NS	2.888	0.056	NS
6	22.047	<0.001	S	1.787	0.203	NS	1.060	0.436	NS
7	50.250	<0.001	S	9.930	0.001	S	7.759	0.001	S

at medium nutrient concentrations, depletion occurred after the sixth day (Fig. 2B). At high nutrient concentration neither nitrate nor phosphate was depleted (Figs. 2C; 3C). Comparison for light factor showed that the fourth day of culture the nitrate uptake at 300 µmol quanta m^{-2} s^{-1} was higher and significant with respect to the other irradiances ($p < 0.05$).

Pigment analysis. Both nutrient and light intensity had an effect on carotenoids/Chl*a* ratios. Nutrient had not significant effect for diadinoxanthin/Chl*a* ratio ($p > 0.05$; Table 3B) and light intensity had a significance effect for all carotenoids/Chl*a* ratios ($p < 0.001$; Table 3, 4) at the end of the seven days experiment of *A. carteri*. For peridinin and dinoxanthin/Chl*a* ratios a significant interaction

Table 2. Average cell density* ($\times 10^5$ mL^{-1}) and nutrient uptake by day of *Amphidinium carteri* cultured under different nutrient (L, low; M, medium and H, high) and light conditions.

Day	NO_3^-/PO_4^{3-} (μM)	Irradiance (μM quanta m^{-2} s^{-1}) 50		150		300		750	
		Cells*	Uptake (μM)	Cells*	Uptake (μM)	Cells*	Uptake (μM)	Cells*	Uptake (μM)
0-1	L	1.68	106.5/1.1	1.48	120.8/—	1.58	53.8/3.0	1.17	57.1/0.1
	M	1.19	94.5/2.0	1.42	105.2/—	1.55	51.4/8.0	1.20	—/2.8
	H	1.13	73.1/11.8	1.35	—/7.7	1.52	87.4/17.2	1.08	—/9.1
1-2	L	2.04	34.0/—	1.74	120.8/2.3	2.17	25.2/2.3	1.77	7.6/2.8
	M	2.76	15.1/1.1	2.63	59.2/2.8	2.97	149.4/7.4	1.92	41.7/2.8
	H	2.56	—/4.0	2.45	88.9/8.6	3.20	68.1/8.6	1.71	—/1.1
2-3	L	3.15	85.6/5.1	4.29	24.1/7.4	4.29	141.7/3.4	6.97	125.2/3.4
	M	4.50	100.7/5.7	5.54	62.5/17.2	6.74	168/1.1	4.34	128.5/3.4
	H	4.26	56/4.6	4.80	69.2/4.6	7.94	99.9/4.0	3.90	63.7/0.5
3-4	L	5.17	149.4/3.4	6.84	114.2/1.1	8.58	158.2/5.1	9.00	180.1/8.0
	M	5.99	222.8/5.1	7.26	250.1/2.3	12.62	307.6/10.3	7.48	195.5/6.9
	H	5.62	258.1/8.6	6.62	274.6/7.4	10.81	413/5.7	6.05	288.9/8.6
4-5	L	9.87	56.4/1.1	11.63	48.4/1.7	14.08	46.7/1.7	12.87	63/—
	M	9.93	160.2/8.6	12.04	323.6/5.7	16.06	141.1/5.7	11.85	400.8/11.5
	H	9.90	226.3/12.1	11.50	243.9/11.5	14.01	134/6.9	10.03	223/5.1
5-6	L	13.53	9.3/1.7	16.41	6.8/—	16.59	7.1/—	14.80	4.7/1.7
	M	12.17	241.9/5.1	17.41	76.3/1.7	20.18	59.2/0.5	18.42	56/2.8
	H	13.15	266.9/9.2	15.41	323/12.1	16.20	301/8.6	16.05	271.3/13.2
6-7	L	18.94	—/0.5	15.38	0.5/—	18.31	4.3/—	19.18	—/0.5
	M	16.63	36/1.7	22.99	1.5/0.5	15.63	0.5/0.5	23.32	71.2/2.3
	H	15.21	292.2/4.0	19.70	402.1/4.0	20.02	348.2/13.2	21.61	394.4/18.4

was found between nutrient and light for the days 2-4 and 1-2 respectively ($p < 0.05$; Table 3A, 4A). Also a multiple comparison procedure (Tukey test) for factor nutrient showed that the average peridinin/Chl*a* ratio on the second and third day was significantly different ($p < 0.05$) in high versus low nutrient. However, comparison for factor light among the different levels of nutrient indicated significant differences for all light conditions except for 50 and 150 μmol quanta m^{-2} s^{-1} ($p > 0.05$; Table 5).

At low nutrient conditions and irradiance of 50, 150 and 300 μmol quanta m^{-2} s^{-1}, the mean peridinin/Chl*a* ratios were 0.39, 0.39, and 0.37 respectively, which were higher than at irradiance of 750 (Fig. 4A). Likewise, the average peridinin/Chl*a* ratio in the different growth phase showed little variation among the different irradiancies. On the other hand, the averages diadinoxanthin, dinoxanthin, and diatoxanthin/Chl*a* ratios were always greater in high irradiance than low irradiances (Fig. 5A, 6A, 7A). For diadinoxanthin/Chl*a* ratios, a multiple range test suggested that for majority of these ratios was not significant different between 50 versus 150 and 300 versus 750 μmol quanta m^{-2} s^{-1} ($p > 0.05$; Table 5). However, the most conspicuous increase in the average of diadinoxanthin/Chl*a* ratio was detected at an irradiance of 750 μmol quanta m^{-2} s^{-1}, increasing up to 0.46 on the fourth day of culture (Fig. 5A), while averages dinoxanthin and diatoxanthin/Chl*a* ratios were higher at 750 μmol quanta m^{-2} s^{-1} (Fig. 6, 7) and almost all values were significantly different than the treatments that received a light intensity of 50; 150 and 300 μmol quanta m^{-2} s^{-1} ($p < 0.05$; Table 6).

When *A. carteri* was cultured under medium nutrient concentrations there was not significant different ($p > 0.05$) in the mean peridinin/Chl*a* ratios (0.40 and 0.39) at 50 and 150 μmol quanta m^{-2} s^{-1} (Fig. 4B, Table 5). At an irradiance of 300, the average peridinin/Chl*a* ratio was 0.36 and showed small variations during the time culture (Fig. 4B), and the ratio obtained at an irradiance of 750 μmol quanta m^{-2} s^{-1}, was 0.26 during the first three

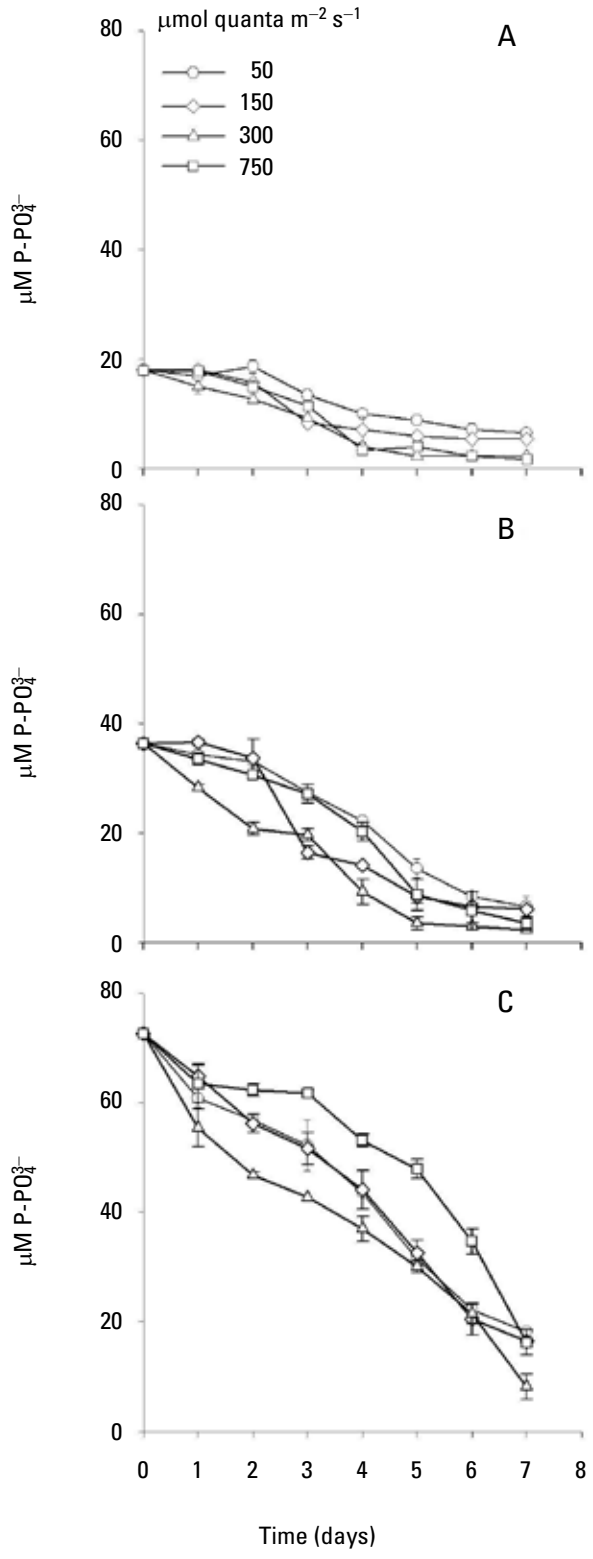

Fig. 3. A-C. Average phosphate concentration in the media of cultures of *Amphidinium carteri* grown under concentrations of $NaNO_3$/NaH_2PO_4: A) 441.5/18.1 μM; B) 883/36.3 μM; C) 1766/72.6 μM and four levels of irradiance. Vertical bars indicate the standard error.

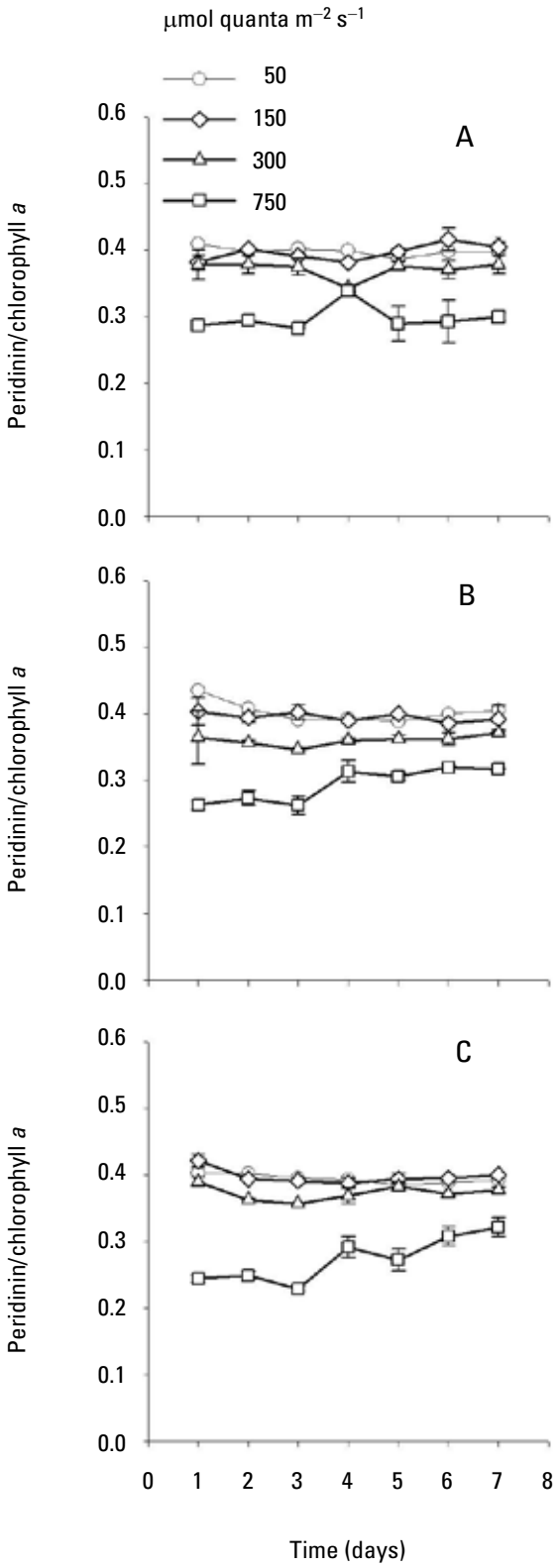

Fig. 4. A-C. Average peridinin/Chl*a* ratios of *Amphidinium carteri* in cultures grown under different nutrient concentration: A) 441.5/18.1 μM; B) 883/36.3 μM; C) 1766/72.6 μM and four levels of irradiance. Vertical bars indicate the standard error.

Table 3. Two way analysis of variance: A) peridinin/Chl*a*, B) diadinoxanthin/Chl*a* ratios for cultures of *Amphydinium carteri* under different nutrient and light conditions. NS = no significative; S = significative, (α = 0.05).

A)

	d. f. error = 24								
Day	Nutrient		$p < 0.05$	Light		$p < 0.05$	Interaction		$p < 0.05$
	F value	P value		F value	P value		F value	P value	
1	0.0384	0.962	NS	57.865	<0.001	S	1.7767	0.189	NS
2	6.590	0.012	S	292.19	<0.001	S	3.891	0.022	S
3	6.946	0.010	S	233.11	<0.001	S	3.952	0.020	S
4	0.393	0.683	NS	51.921	<0.001	S	3.204	0.041	S
5	0.299	0.747	NS	70.910	<0.001	S	1.168	0.384	NS
6	0.0726	0.930	NS	33.911	<0.001	S	0.968	0.486	NS
7	0.774	0.926	NS	41.817	<0.001	S	0.634	0.701	NS

B)

	d. f. error = 24								
Day	Nutrient		$p < 0.05$	Light		$p < 0.05$	Interaction		$p < 0.05$
	F value	P value		F value	P value		F value	P value	
1	0.121	0.887	NS	19.039	<0.001	S	0.945	0.499	NS
2	1.245	0.323	NS	89.726	<0.001	S	1.253	0.347	NS
3	0.766	0.486	NS	60.591	<0.001	S	2.226	0.112	NS
4	0.281	0.760	NS	197.31	<0.001	S	8.356	0.001	S
5	0.286	0.756	NS	17.128	<0.001	S	0.547	0.763	NS
6	3.316	0.071	NS	89.898	<0.001	S	5.239	0.007	S
7	1.640	0.235	NS	9.593	0.002	S	1.411	0.287	NS

days and then increased to 0.30 (Fig. 4B). A multiple range test indicated significant difference ($p < 0.05$; Table 5) between these treatments.

The average ratio of photoprotective pigments/Chl*a* increased with increasing irradiance (Fig. 5, 6, 7). A multiple range test indicated that at irradiances of 300 and 750 µmol quanta m^{-2} s^{-1} the diadinoxanthin/Chl*a* ratio was not significantly different except in the fourth day ($p > 0.05$; Table 5), but was higher than those obtained at low irradiances (Fig. 5B). The average values of the dinoxanthin/Chl*a* and diatoxanthin/Chl*a* ratios were smaller than the diadinoxanthin/Chl*a* ratio (Fig. 6, 7) whereas averages dinoxanthin and diatoxanthin/Chl*a* ratios at 750 µmol quanta m^{-2} s^{-1} were higher and significantly different than the treatments that received a light intensity of 50, 150 and 300 µmol quanta m^{-2} s^{-1} ($p < 0.05$; Table 6).

In high nutrient cultures (Fig. 4C), the average peridinin/Chl*a* ratios (0.39) of *A. carteri* was not significantly affected by the irradiance at 50 and 150 µmol quanta m^{-2} s^{-1} ($p > 0.05$; Table 5). However, at 300 and 750 µmol quanta m^{-2} s^{-1}, the average values of the peridinin/Chl*a* ratios were 0.37 and 0.27 respectively (Fig. 4C) and a significant difference was observed ($p < 0.05$; Table 5). In addition, at 750 µmol quanta m^{-2} s^{-1} this ratio was 0.24 during the lag phase, which showed differences with respect to the ratios obtained during the exponential and stationary phases (0.26, 0.31) respectively (Fig. 4C). The average diadinoxanthin/Chl*a* ratios at irradiances of 300 and 750 µmol quanta m^{-2} s^{-1} changed from the lag to the stationary phase from 0.34 to 0.38 and from 0.36 to 0.45 respectively, without significant differences ($p > 0.05$; Table 5) between irradiances. From the multiple range test results, we found that averages dinoxanthin and diatoxanthin/Chl*a* ratios at 50 and 150 µmol quanta m^{-2} s^{-1} there were not a significant difference ($p > 0.05$) and these ratios showed little variation during the seven days of culture (Fig. 6, 7; Table 6).

DISCUSSION

Microalgae cultures. The cellular density and the growth rate of *A. carteri* may vary in response to interactions between irradiance and nutrient concentrations. Comparing all treatments, the

Table 4. Two way analysis of variance: A) dinoxanthin/Chl*a*, B) diatoxanthin/Chl*a* ratios for cultures of *Amphydinium carteri* under different nutrient and light conditions. NS = no significative; S = significative, ($\alpha = 0.05$)

A)

	d.f. error = 24								
Day	Nutrient		$p < 0.05$	Light		$p < 0.05$	Interaction		$p < 0.05$
	F value	P value		F value	P value		F value	P value	
1	1.586	0.245	NS	125.86	<0.001	S	3.653	0.027	S
2	13.914	<0.001	S	287.03	<0.001	S	9.831	<0.001	S
3	2947	0.091	NS	70.276	<0.001	S	2.301	0.103	NS
4	5.627	0.019	S	86.155	<0.001	S	1.613	0.226	NS
5	2.517	0.122	NS	24.584	<0.001	S	1.420	0.284	NS
6	1.105	0.363	NS	25.739	<0.001	S	1.043	0.445	NS
7	0.0223	0.978	NS	18.133	<0.001	S	0.976	0.482	NS

B)

	d.f. error = 24								
Day	Nutrient		$p < 0.05$	Light		$p < 0.05$	Interaction		$p < 0.05$
	F value	P value		F value	P value		F value	P value	
1	7.840	0.007	S	307.04	<0.001	S	5.482	0.006	S
2	1.028	0.387	NS	225.59	<0.001	S	1.494	0.261	NS
3	5.075	0.025	S	53.217	<0.001	S	1.734	0.196	NS
4	3.880	0.050	NS	47.807	<0.001	S	2.120	0.126	NS
5	1.8854	0.199	NS	29.868	<0.001	S	0.794	0.592	NS
6	0.757	0.490	NS	16.531	<0.001	S	1.037	0.448	NS
7	4.282	0.039	S	40975	<0.001	S	3.011	0.049	S

ANOVA results indicated that the cellular density was affected both by the nutrient as irradiance, but mainly by the irradiance (Fig. 1; Table 1A). However, cells growing under different nutrient and light conditions showed low densities at the beginning of the culture period. This result indicates that *A. carteri* cells undergo a period of conditioning during the first two days of culture (Fogg & Thake, 1987). Likewise, the specific growth rate during the exponential phase was similar in all treatments with values from 0.40 to 0.66, these results agree with those reported by Sosik & Chisholm (1989). García and Purdie (1992) point out a maximum growth rate of 0.37 doubling day^{-1} for *Gyrodinium* cf. *aureolum* Hulburt grown under a light intensity of 235-380 µmol m^{-2} s^{-1} and at ~500 µmol m^{-2} s^{-1} the growth rate decreased. In contrast, Dae-IL *et al.* (2004) have found that the optimum irradiance for the growth of *Cochlodinium polykrikoides* Margalef is higher than 90 µmol m^{-2} s^{-1}. In this study, the best response in the growth of *A. carteri* was at 300 µmol quanta m^{-2} s^{-1}at the fifth day of culture.

NO_3^- and PO_4^{3-} uptake. The uptake of nitrate and phosphate by *A. carteri* was affected for the different nutrient conditions. In low nutrient condition, during the first five days of culture the nitrate concentration was depleted, this result probably indicate a limitation of nitrate at the end of the culture period (Fig. 2A), which can lead to a decrease in protein synthesis (Bucciarelli & Sunda, 2003). Phosphate uptake showed variation with respect to different light levels, this has been observed by other authors who suggest that altering light status will change the uptake of phosphate (Lemasson *et al.*, 1980; Nalewajko & Lee, 1983). But others, Lean & Pick (1981) point out that the phosphate uptake was not stimulated by light. However, the phytoplankton photosynthesis requires phosphate to synthesize ATP and NADPH, which are used in the the reduction and assimilation of nitrate (Clark & Flynn, 2002).

The results of the present work are also consistent with other studies (Clark & Flynn, 2002; Clark, 2001) that quantified the utilization of dissolved inorganic nitrogen and nitrate assimilation and found that the nitrate was exhausted between the fifth and sixth day. In addition, Needoba & Harrison (2004) have reported that NO_3^- uptake is modified by light and that the greatest concentrations of internal pools are detected during the dark period.

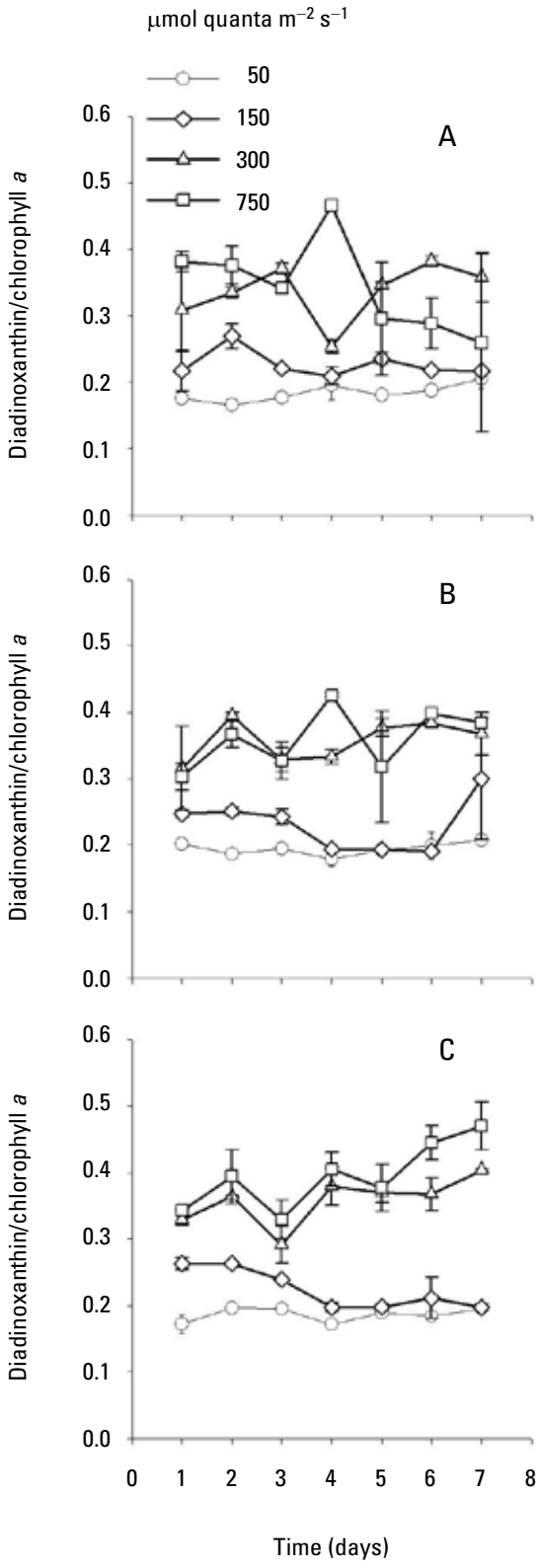

Fig. 5. A-C. Average diadinoxanthin/Chl*a* ratios of *Amphidinium carteri* in cultures grown under different nutrient concentration: A) 441.5/18.1 μM; B) 883/36.3 μM; C) 1766/72.6 μM and four levels of irradiance. Vertical bars indicate the standard error.

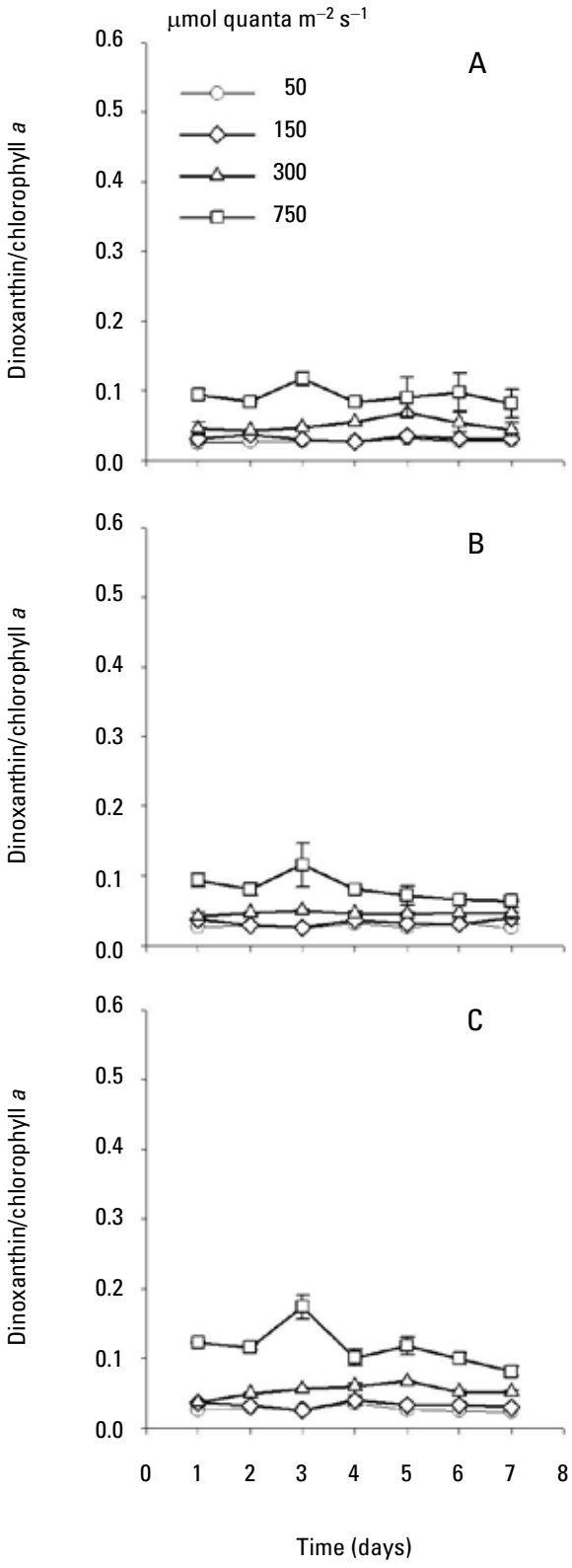

Fig. 6. A-C. Average dinoxanthin/Chl*a* ratios of *Amphidinium carteri* in cultures grown under different nutrient concentration: A) 441.5/18.1 μM; B) 883/36.3 μM; C) 1766/72.6 μM and four levels of irradiance. Vertical bars indicate the standard error.

Table 5. Multiple range test (Tukey) of peridinin and diadinoxanthin/Chl*a* ratios between nutrient condition and irradiance. No significative = >0.05; Significative = <0.05 (α = 0.05).

Ratios		Contrast by irradiance	Days 1	2	3	4	5	6	7
			d. f. error = 24						
			p values						
Peridinin/Chl*a* Nutrient condition	Low	50 vs. 750	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001
		50 vs. 300	0.505	0.188	0.101	0.003	0.888	0.447	0.612
		50 vs. 150	0.595	0.970	0.760	0.471	0.866	0.742	0.957
		150 vs. 750	0.005	<0.001	<0.001	0.022	<0.001	<0.001	<0.001
		150 vs. 300	0.999	0.093	0.429	0.041	0.473	0.101	0.343
		300 vs. 750	0.007	<0.001	<0.001	0.982	<0.001	0.005	0.001
	Medium	50 vs. 750	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001
		50 vs. 300	0.034	<0.001	0.006	0.091	0.322	0.219	0.174
		50 vs. 150	0.503	0.400	0.657	0.997	0.848	0.882	0.824
		150 vs. 750	<0.001	<0.001	<0.001	<0.001	<0.001	0.014	0.002
		150 vs. 300	0.341	0.004	<0.001	0.125	0.093	0.562	0.548
		300 vs. 750	0.003	<0.001	<0.001	0.012	0.009	0.136	0.018
	High	50 vs. 750	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	0.003
		50 vs. 300	0.935	0.003	0.013	0.260	0.997	0.781	0.764
		50 vs. 150	0.826	0.782	0.967	0.974	0.926	0.980	0.954
		150 vs. 750	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.001
		150 vs. 300	0.499	0.016	0.028	0.451	0.844	0.563	0.470
		300 vs. 750	<0.001	<0.001	<0.001	<0.001	<0.001	0.019	0.017
Diadinoxanthin/Chl*a* Nutrient condition	Low	50 vs. 750	0.002	<0.001	<0.001	<0.001	0.175	0.012	0.873
		50 vs. 300	0.030	<0.001	<0.001	0.063	0.034	<0.001	0.192
		50 vs. 150	0.739	0.005	0.258	0.903	0.725	0.682	0.998
		150 vs. 750	0.008	0.004	0.001	<0.001	0.659	0.087	0.933
		150 vs. 300	0.168	0.073	<0.001	0.191	0.195	<0.001	0.245
		300 vs. 750	0.323	0.351	0.590	<0.001	0.764	0.020	0.524
	Medium	50 vs. 750	0.113	<0.001	<0.001	<0.001	0.128	<0.001	0.111
		50 vs. 300	0.068	<0.001	<0.001	<0.001	0.018	<0.001	0.164
		50 vs. 150	0.683	0.078	0.196	0.872	1.000	0.985	0.581
		150 vs. 750	0.545	0.002	0.014	<0.001	0.130	<0.001	0.640
		150 vs. 300	0.382	<0.001	0.012	<0.001	0.019	<0.001	0.776
		300 vs. 750	0.990	0.650	1.000	0.003	0.674	0.940	0.995
	High	50 vs. 750	0.006	<0.001	<0.001	<0.001	0.016	<0.001	0.011
		50 vs. 300	0.011	<0.001	0.005	<0.001	0.021	<0.001	0.054
		50 vs. 150	0.172	0.066	0.267	0.608	0.999	0.754	1.000
		150 vs. 750	0.254	<0.001	0.008	<0.001	0.021	<0.001	0.011
		150 vs. 300	0.403	0.006	0.148	<0.001	0.027	<0.001	0.055
		300 vs. 750	0.985	0.605	0.372	0.599	0.999	0.058	0.789

Table 6. Multiple range test (Tukey) of dinoxanthin and diatoxanthin/Chl*a* ratios between nutrient condition and irradiance. No significative; = >0.05; Significative = <0.05 (α = 0.05).

Ratios		Contrast by irradiance	Days 1	2	3	4	5	6	7
						d. f. error = 24			
						p values			
Dinoxanthin/Chl*a* Nutrient condition	Low	50 vs. 750	<0.001	<0.001	<0.001	<0.001	0.008	0.001	0.004
		50 vs. 300	0.071	0.015	0.554	0.008	0.107	0.232	0.594
		50 vs. 150	0.853	0.140	0.997	0.999	0.996	0.990	0.998
		150 vs. 750	<0.001	<0.001	<0.001	<0.001	0.012	0.002	0.005
		150 vs. 300	0.254	0.587	0.668	0.007	0.153	0.352	0.702
		300 vs. 750	<0.001	<0.001	0.003	0.006	0.476	0.030	0.036
	Medium	50 vs. 750	<0.001	<0.001	<0.001	<0.001	0.071	0.085	0.212
		50 vs. 300	0.921	0.007	0.396	0.578	0.766	0.620	0.934
		50 vs. 150	1.000	1.000	1.000	1.000	1.000	1.000	1.000
		150 vs. 750	<0.001	<0.001	<0.001	<0.001	0.071	0.085	0.212
		150 vs. 300	0.921	0.007	0.396	0.578	0.766	0.620	0.934
		300 vs. 750	<0.001	<0.001	0.005	0.001	0.323	0.507	0.467
	High	50 vs. 750	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002
		50 vs. 300	0.459	0.003	0.282	0.015	0.068	0.221	0.138
		50 vs. 150	0.510	0.960	0.999	0.891	0.969	0.926	0.954
		150 vs. 750	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.005
		150 vs. 300	1.000	0.008	0.233	0.052	0.143	0.497	0.300
		300 vs. 750	<0.001	<0.001	<0.001	<0.001	0.019	0.017	0.110
Diatoxanthin/Chl*a* Nutrient condition	Low	50 vs. 750	<0.001	<0.001	0.003	0.003	<0.001	0.005	<0.001
		50 vs. 300	1.000	0.128	0.712	0.075	0.214	0.939	0.833
		50 vs. 150	0.946	0.953	1.000	0.995	0.992	0.992	0.991
		150 vs. 750	<0.001	<0.001	0.003	0.002	<0.001	0.009	<0.001
		150 vs. 300	0.961	0.282	0.685	0.050	0.321	0.994	0.944
		300 vs. 750	<0.001	<0.001	0.017	0.291	0.007	0.014	<0.001
	Medium	50 vs. 750	<0.001	<0.001	<0.001	0.001	0.004	0.004	<0.001
		50 vs. 300	0.999	0.618	0.885	0.930	0.960	0.937	0.492
		50 vs. 150	0.416	0.997	0.997	0.995	1.000	1.000	0.999
		150 vs. 750	<0.001	<0.001	<0.001	0.001	0.004	0.004	<0.001
		150 vs. 300	0.350	0.499	0.794	0.836	0.949	0.923	0.569
		300 vs. 750	<0.001	<0.001	<0.001	0.004	0.010	0.012	0.003
	High	50 vs. 750	<0.001	<0.001	<0.001	<0.001	0.016	0.263	0.073
		50 vs. 300	0.635	0.219	0.028	0.143	0.658	0.829	0.933
		50 vs. 150	0.748	<0.001	0.978	1.000	0.999	1.000	1.000
		150 vs. 750	<0.001	1.000	<0.001	<0.001	0.013	0.292	0.076
		150 vs. 300	0.997	0.192	0.014	0.130	0.583	0.863	0.939
		300 vs. 750	<0.001	<0.001	0.005	<0.001	0.117	0.703	0.191

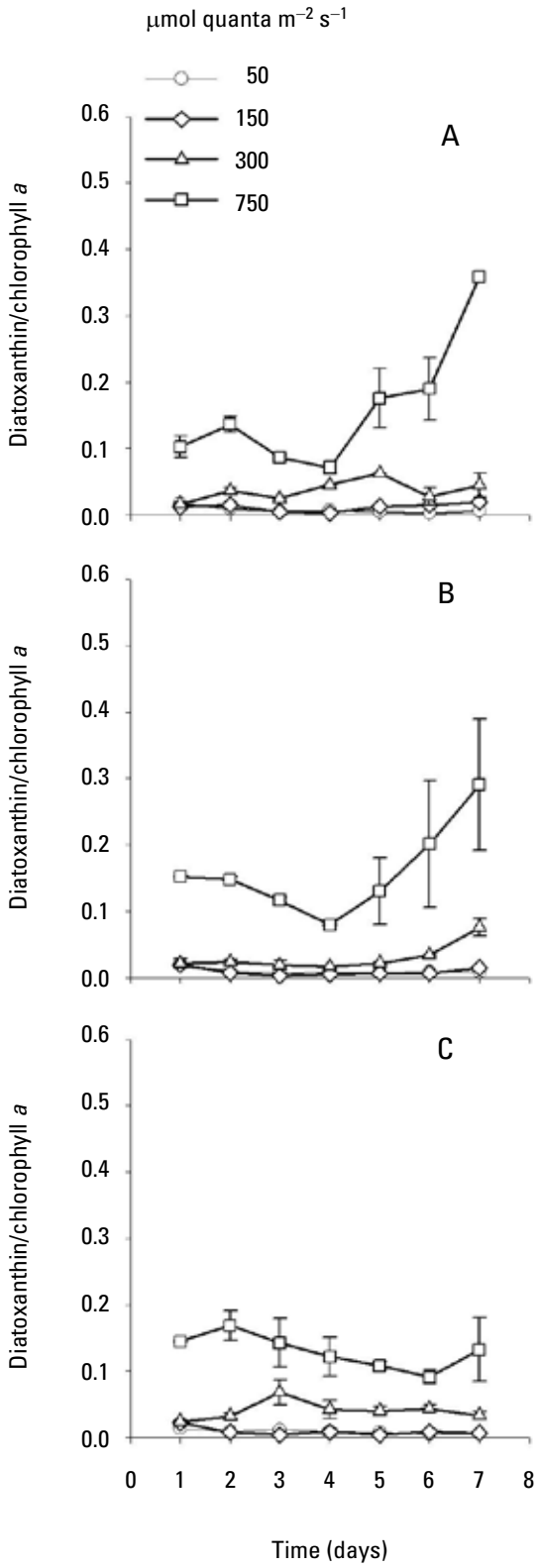

Fig. 7. A-C. Average diatoxanthin/Chl*a* ratios of *Amphidinium carteri* in cultures grown under different nutrient concentration: A) 441.5/18.1 μM; B) 883/36.3 μM; C) 1766/72.6 μM and four levels of irradiance. Vertical bars indicate the standard error.

Pigment ratios. The average peridinin/Chl*a* ratio was higher at irradiances of 50 and 150 than at 300 and 750 μmol quanta m^{-2} s^{-1}. The converse was observed for the diadinoxantin, dinoxanthin, and diatoxanthin/Chl*a* ratios, which were higher at 300 and 750 μmol quanta m^{-2} s^{-1}. Considering the photoprotective functions of these pigments, their concentrations might be expected to co-vary with the amount of chlorophyll *a* and the irradiance, as was demonstrated in this work. Other studies have noted that in dinoflagellates the pigment diadinoxanthin is transformed into dinoxanthin under high light exposure and under limited light conditions, the peridinin concentration in *A. carteri* co-varies with Chl*a* to absorb light (Jeffrey & Vesk, 1997) and transfer the absorbed energy to the reaction centers to carry out the photosynthetic process (Kirk, 1994). These observations are in agreement with the results reported by Schlüter *et al.* (2000) who cultured two species of dinoflagellates and found high concentration of peridinin in response to low light. Likewise, the peridinin/Chl*a* ratio decreased with increasing irradiance due to a reduction in the cellular content of accessory pigments and Chl*a*. Field studies have also confirmed that high concentrations of PSC are a result of chromatic adaptation to low light intensity and when phytoplankton are exposed to high irradiances, the cells produce pigments that are capable of protecting the reaction center against photo-oxidation and damage (Trees *et al.,* 2000). On the other hand, Goericke & Montoya (1998) have pointed out that the pigment concentrations change as a function of the irradiance or the growth rate. The results of the present work indicated that the irradiance modified the pigment ratios, and a major effect was observed at 750 μmol quanta m^{-2} s^{-1}, with changes detected between the exponential and stationary phases. However, changes in the growth rate at high irradiances, specifically at 300 μmol quanta m^{-2} s^{-1}, improved the cell density in the cultures. This result might explain the changes observed in the growth rate with increasing light intensity, which were not observed when the nutrient concentration varied. Even though, it was not observed a limitation of the maximum population density achieved (Fig. 1), however the nutrient concentration observed at the end of the culture period, might explain that there is limited potential to increase cell yield, but the cell division continue even when the limiting nutrient is present at or near detection limit. This occurred in our experiments at the beginning of the stationary phase at low and medium nutrients (Figs. 2A, B; 3A, B). In addition, intracellular store of nitrate or phosphate might be sufficient for several hours or days of growth, whereas intracellular pools can be depleted (Leonardos & Geider, 2004). Likewise, the average carotenoid/Chl*a* ratios were similar among the different nutrient conditions used to culture *A. carteri.* From our results we concluded that *A. carteri* had higher growth rates and nutrient consumption at 300 μmol quanta m^{-2} s^{-1} during the first five days of the experiment. The pigment ratios were affected both by nutrients and irradiance, but mainly by the irradiance. However, the average peridinin/Chl*a* ratio decreased up

to 72% from the lowest to the highest irradiance. The opposite was observed for the average of diadinoxanthin/Chl*a* ratio that increased almost two-fold. Whereas, the average dinoxanthin and diatoxanthin to Chl*a* ratios increased from low to high irradiances, and these ratios were lower than the average diadinoxanthin/Chla ratio, instead the average diatoxanthin/Chl*a* ratios at 750 µmol quanta m^{-2} s^{-1} increased almost 2.7-fold from the exponential to the stationary phase only in the low and medium nutrient concentrations. On the other hand, the average peridinin, dinoxanthin and diatoxanthin to Chl*a* ratios there were not significantly different at 50 and 150 µmol quanta m^{-2} s^{-1} and similar results were observed for diadinoxanthin/Chl*a* at 300 and 750 µmol quanta m^{-2} s^{-1}. Our results indicate important changes in average carotenoids/Chl*a* ratios in *A. carteri* cultured under different irradiances.

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