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Photoacclimation by the Antarctic flagellate *Pyramimonas* sp. (Prasinophyceae) in response to iron limitation

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In this study we tested the hypothesis that iron limitation suppresses photoacclimation in cultures of the Antarctic flagellate *Pyramimonas* sp. The cultures were exposed to two different irradiances under iron-rich and iron-poor conditions. Light-harvesting capacity was determined by assessing the pigment composition and measuring *in vivo* absorption spectra. Light utilization efficiency (α) was determined from photosynthesis versus irradiance curves. The quantum yield of photosynthesis (ϕ_m) was calculated using α and the absorption spectra. Iron limitation led to commonly observed changes in cells of *Pyramimonas*, that is, a decrease in cellular pigment content and a reduction in cellular carbon and nitrogen quota. A reduction in α^{cell} followed a decrease in ϕ_m and light-harvesting capacity. Interpretation of the effects of iron limitation was different when considered on a carbon basis. Because iron limitation resulted in a decrease in cellular carbon content, the carbon-specific absorption coefficient was not affected. Consequently, the observed decrease in α^C was mainly due to the decrease in ϕ_m, showing that iron limitation did not control light utilization via pigment synthesis but exerted control on energy transfer. This is supported by the findings that at high irradiance a shift in pigment ratios within the total pool of violaxanthin, antheraxanthin and zeaxanthin towards zeaxanthin, which is indicative of photoacclimation to high irradiance, was observed for iron-replete cells as well as for iron-depleted cells. In contrast to what is generally hypothesized, the effects of iron limitation were not enhanced at low irradiance. Low irradiance led to an increase in the cellular light-harvesting pigment content. This increase was less pronounced in iron-depleted cells than in iron-replete cells. However, looking at the light-harvesting capacity of the cells on a carbon basis, it was found that iron-depleted cells responded similarly to iron-replete cells. We therefore conclude that the light-harvesting capacity was governed by light conditions and not by iron limitation. In addition to the increase in absorption capacity at low irradiance, an increase in light utilization efficiency was measured, again under both iron-rich and iron-poor conditions. Notably, the relative increase in α^C was strongest in iron-depleted cells. Photoacclimation was clearly demonstrated by normalizing α to chl *a*. For iron-replete cells, α^{chl} was highest at high irradiance. In contrast, for iron-depleted cells α^{chl} was highest at low irradiance. We argue that iron-depleted cells can photoacclimate to low irradiance by a reduction in the 'package effect' and reducing growth rates.

Key words: Antarctic, iron, irradiance, photoacclimation, photosynthesis, phytoplankton, pigments.

Introduction

The growth of phytoplankton is determined by the capacity of the cell to make use of available light. The efficiency of light utilization (α) depends on the light absorption capacity of the cell and on the efficiency of energy conversion, that is, quantum yield of photosynthesis, ϕ_m. Two important factors that can control growth are light and iron; together they act synergistically on photosynthesis. Both factors may play a crucial role in the Southern Ocean, where the growth of phytoplankton is thought to be limited by the low concentrations of iron, coupled with the variable light conditions (de Baar *et al.*, 1990; Martin *et al.*, 1990; Chisholm & Morel, 1991; Mitchell & Holm-Hansen, 1991; Nelson & Smith, 1991; van Leeuwe *et al.*, 1997).

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When cells are exposed to low light conditions, they increase the number of light-harvesting chlorophyll–protein complexes (Falkowski & LaRoche, 1991). Subsequent changes in their pigment composition can also occur, which result in an increased ratio of accessory light-harvesting pigments to chlorophyll. The changes in pigment–protein complexes may involve alterations to the structure of the photosystems. Acclimation to low irradiance sometimes involves an increase in the 'package effect', which results in a reduction of the absorption efficiency per unit pigment (Morel & Bricaud, 1981; Berner *et al.*, 1989). Photoacclimation to low irradiance is often coupled with an increase in the quantum yield of photosynthesis (ϕ_m) (Kolber *et al.*, 1988; Hofstraat *et al.*, 1994). The magnitude of subsequent changes in light utilization efficiency (α) will vary with changes in absorption efficiency and quantum yield.

Iron is essential for phytoplankton productivity, due to its involvement in photosynthesis and in many other pathways of cell metabolism (reviewed by Geider &

LaRoche, 1994). Iron limitation results in a decrease in protein synthesis related to the light-harvesting complexes. Iron-depleted cells show a reduction in chlorophyll *a*, which is the core pigment of the photosystems (Guikema & Sherman, 1983; Greene *et al.*, 1992). The effects of iron limitation on the accessory light-harvesting pigments have been extensively studied in higher plants (Terry, 1980; Abadia *et al.*, 1989; Pascal *et al.*, 1995), and have also been demonstrated in microalgae (Geider *et al.*, 1993; van Leeuwe & Stefels, 1998). These studies showed a decline in total cellular pigment content for iron-depleted cells. Iron limitation induces synthesis of photoprotective pigments because iron-limited cells are susceptible to photoinhibition (Geider *et al.*, 1993; Morales *et al.*, 1994). Therefore, iron-depleted cells are expected to show an increase in pigments from the xanthophyll cycle as opposed to other accessory pigments.

Iron limitation not only affects pigment synthesis, it also controls the efficiency of energy conversion (Greene *et al.*, 1991; Greene *et al.*, 1991; Vassiliev *et al.*, 1995). The transformation of light energy into chemical energy involves photosynthetic electron transport. The photosynthetic electron transport chain includes many iron-containing molecules (cytochromes and FeS proteins). Studies on the photosynthetic apparatus of the diatom *Phaeodactylum tricornutum* have shown clearly how iron limitation affected the photosystems, resulting in a reduction in cellular pigment content (Greene *et al.*, 1991). This led to a subsequent decrease in the 'package effect', and therefore to an increase in the chlorophyll *a*-specific absorption capacity. However, a decline in electron transport efficiency through the photosystems led to a reduction in the quantum yield ϕ_m. The overall effect of iron limitation in *P. tricornutum* cells was a reduction in the efficiency of light utilization, α.

We propose that synergistic growth control is exerted by light and iron. Because iron controls pigment synthesis, iron-depleted cells may not be able to acclimate their photosynthetic apparatus to changes in light conditions. The influence of iron on photoacclimation may be stronger at low irradiance than at high irradiance. At low irradiances, a reduction in iron-use efficiency has been theorized (Raven, 1990). This means that per mole iron, less carbon will be fixed, and less energy will be available for metabolic processes per unit time. An increase in cellular iron demand under low light conditions has in fact been recorded for several algal species (Sunda & Huntsman, 1997).

The study described here was carried out to test the hypothesis that iron limitation does suppress photoacclimation. *Pyramimonas* sp., an Antarctic species abundant in the open ocean, was studied under a combination of light and iron limitation. The pigment composition and cellular carbon and nitrogen levels were measured to see whether iron would affect the synthesis of structural cell components, and the photosynthetic performance of the cells was further examined in the light of changes in biochemical composition. To study the overall effect of iron limitation on photosynthesis, the light utilization efficiency (α) was determined by measuring photosynthesis–irradiance curves. In addition, the light-harvesting capacity of the cells was measured by determining *in vivo* absorption spectra. The quantum yield of photosynthesis ϕ_m could be calculated using α and the absorption spectra. We will show that effects of iron on α are mainly determined by ϕ_m, at both high and low irradiances.

Materials and methods

Culture conditions

The strain of *Pyramimonas* sp. used for the experiments was isolated from the Weddell Scotia Confluence (Buma *et al.*, 1991). Cultures were grown in 2.7 l polycarbonate Fernbach culture flasks (Nalgene) at 4 °C. All material used was soaked for 48 h in 6 N HDl and rinsed five times with Milli-Q water to remove iron contaminants. Cultures were grown at two different irradiances (30–40 and 90–100 μmol photons m^{-2} s^{-1}) at a 16 h light : 8 h dark regime. Light was provided by cool white fluorescence tubes (Philips TLD 36W/54) and measured with a spherical sensor (LI-COR, SPQA III). The seawater medium was prepared as described by Morel *et al.* (1979), with slight modifications. Natural (nutrient-depleted) seawater collected in the northern North Sea was passed over a Chelex-100 ion exchange resin and sterilized by filtration. The seawater was enriched with nitrate and phosphate to 120 μM and 10 μM respectively. $FeCl_3$ and EDTA were added separately at final concentrations of 10^{-5} M EDTA for all cultures, 10^{-6} M Fe for iron-rich cultures and 10^{-9} M Fe for iron-poor cultures. Initial iron concentrations (measured by organic ligand solvent extraction followed by atomic absorption spectrophotometry; Nolting & de Jong, 1994) ranged from 2 to 10 nM Fe in iron-poor cultures.

Before starting the experiments, cultures were stressed by iron deficiency and acclimated to different levels of irradiance by repeated transfers. Experiments were started by transferring cells to a fresh medium. Cultures were shaken daily and sampled at regular intervals. To avoid contamination with iron and bacteria, samples were taken in a Class-100 laminar flow bench.

Cell counts

Cell numbers were determined by microscopy (inverted microscope) after fixation with Lugol's solution. Growth rates were calculated following the formula

$$\mu = 1/t[\ln(N_t/N_0)] \quad (1)$$

where μ (d^{-1}) is the growth rate, t is the period of time after the start $t = 0$ of the experiment and N is the number of cells.

C and N analysis

Particulate carbon (POC) and nitrogen (PON) were analysed on a Carlo Erba elemental analyser, after gentle filtration (< 15 MPa) of 25 ml aliquots over pre-combusted GF/F Whatman filters.

Pigments

Samples taken for pigment analyses were filtered gently (< 15 MPa) over GF/F Whatman filters. Pigments were extracted in 100% methanol (buffered with 2% ammonium acetate) and analysed by high-performance liquid chromatography according to Kraay *et al*. (1992), using a RoSil C18 HL column.

Absorption spectra

In vivo light absorption spectra (350–700 nm) were measured immediately after filtering a variable volume of sample on GF/F Whatman filters. Sample volumes were taken to an approximate optical density of *c*. 0·3. Spectra were recorded using a Varian-Cary spectrophotometer equipped with an integrating sphere. The pathlength amplification factor was determined empirically (on the basis of a dilution series). Data were corrected for scattering by subtracting a baseline value as measured at 725 nm. The spectrally weighted absorption coefficient $\bar{a}_{ph}(m^{-1})$ could then be calculated according to

$$\bar{a}_{ph} = \frac{\int_{400}^{700} a_{ph}(\lambda) * Q(\lambda) d\lambda}{\int_{400}^{700} Q(\lambda) d\lambda} \quad (2)$$

where $Q(\lambda)$ is spectral irradiance (μmol photons m^{-2} s^{-1} nm^{-1}).

P–I curves

Photosynthesis–irradiance relationships were measured by ^{14}C incorporation; 9·25 kBq of $NaH^{14}CO_3^-$ was added to 50 ml of sample. Samples were incubated for 1 h under light ranging from 0 to 100% irradiance (100% incident irradiance was 2000 μmol photons m^{-2} s^{-1} (Philips TL8W/33 white fluorescence tubes)). After filtration of the samples, the filters (GF/F Whatman) were exposed to fuming HCl and placed in scintillation vials. Following addition of 20 ml of Ultima Gold Luma Gell, activity was recorded using an LKB scintillation counter. The photosynthetic parameters maximal photosynthesis (P_{max}) and light utilization efficiency (i.e. initial slope α) were determined using a non-linear fit (Systat) based on the P–I equation of Platt & Gallegos (1980). The light saturation parameter I_k was determined as P_{max}/α.

Maximum quantum yield

The maximum quantum yield of photosynthesis ϕ_m(mol C × mol photons^{-1}) was derived from the initial slope α and the weighted absorption coefficient $\bar{a}_{ph}$, using the following formula:

$$\Phi_m = \frac{\alpha}{\bar{a}_{ph}} \quad (3)$$

Data handling

Data are presented from several experiments performed over the course of 15 months. Consequently, different sets of variables were measured for different experiments (P–I curves and absorption spectra were thus determined for a different set of cultures). Measurements were always made on cells harvested in the early exponential phase. Differences between treatments were tested for significance by use of a two-factor analysis of variance.

Results

Growth rates

Exponential growth of the cells was reduced by both light and iron limitation (ANOVA, $p < 0{\cdot}05$; Table 1). Maximum growth rates of 0·31 d^{-1} were measured at high irradiance and high iron conditions (HL+). Reduction of growth due to light limitation was most severe under iron limitation. At low irradiances, iron-depleted cells (LL−) experienced a growth reduction of 43%; in iron-replete cells (LL+) growth was reduced by only 23%. The combination of light and iron stress resulted in the lowest growth rates of 0·16 d^{-1}.

Elemental composition

The cellular carbon and nitrogen contents were unaffected by irradiance. Iron limitation resulted in a decrease in POC/cell and PON/cell (ANOVA, $p < 0{\cdot}05$). As POC/cell was reduced to a lesser extent than PON/cell, a shift in the C:N ratios was observed (Table 1; ANOVA, $p < 0{\cdot}05$).

Pigment composition

Pyramimonas sp. contained chlorophyll *a* and *b*, neoxanthin, lutein, violaxanthin, antheraxanthin, zeaxanthin, and five carotenes. Chl *a* and chl *b* were the main light-harvesting pigments.

Overall, at low irradiance, the cellular pigment content increased significantly for both iron-replete and iron-depleted cells, except for zeaxanthin and the carotenes (ANOVA, $p < 0{\cdot}05$; Table 2). The highest values for the latter pigments were recorded for cells cultured at high iron and high irradiances. The different irradiances also had an effect on the VAZ pool (violaxanthin, anthera-

Table 1. Growth rates and elemental composition of *Pyramimonas* sp.

	HL+	LL+	HL−	LL−
μ (d^{-1})	0·31 (0·04)	0·24 (0·02)	0·28 (0·04)	0·16 (0·01)
pg PON/cell	10·39 (2·21)	8·98 (1·07)	4·23 (0·91)	4·36 (0·76)
pg POC/cell	59·02 (5·42)	53·47 (1·39)	32·11 (5·19)	31·48 (2·47)
C/N (molar)	4·96 (0·64)	5·23 (0·53)	6·57 (0·56)	6·30 (0·79)
chl *a*/POC (g/g)	0·019 (0·002)	0·045 (0·000)	0·020 (0·004)	0·034 (0·001)

Values are the means (±SD), $n = 4$–7.
HL+ : high light, high iron; LL+ : low light, high iron; HL− : high light, low iron; LL− : low light, low iron.

Table 2. Cellular pigment content (pg/cell) and pigment:chl *a* ratios (±SD), $n = 4$

	HL+	LL+	HL−	LL−
(*a*) *Per cell*				
Neoxanthin	0·221 (0·038)	0·432 (0·131)	0·110 (0·027)	0·187 (0·040)
Lutein	0·216 (0·028)	0·280 (0·038)	0·125 (0·030)	0·174 (0·039)
Violaxanthin	0·105 (0·033)	0·324 (0·189)	0·118 (0·046)	0·188 (0·047)
Antheraxanthin	0·063 (0·022)	0·070 (0·022)	0·038 (0·012)	0·041 (0·007)
Zeaxanthin	0·291 (0·071)	0·248 (0·133)	0·145 (0·035)	0·158 (0·050)
Chlorophyll *b*	1·444 (0·289)	2·896 (0·885)	0·766 (0·169)	1·333 (0·318)
Carotenes[a]	0·734 (0·070)	0·662 (0·113)	0·371 (0·048)	0·413 (0·067)
VAZ pool	0·459 (0·086)	0·641 (0·128)	0·301 (0·069)	0·387 (0·063)
Chlorophyll *a*	1·105 (0·256)	2·401 (0·909)	0·614 (0·165)	1·046 (0·267)
(*b*) *Per chl* a				
Neoxanthin	0·203 (0·016)	0·185 (0·018)	0·180 (0·007)	0·181 (0·011)
Lutein	0·208 (0·016)	0·128 (0·036)	0·205 (0·012)	0·168 (0·008)
Violaxanthin	0·095 (0·023)	0·127 (0·041)	0·189 (0·045)	0·183 (0·029)
Antheraxanthin	0·057 (0·018)	0·030 (0·006)	0·063 (0·014)	0·040 (0·009)
Zeaxanthin	0·268 (0·063)	0·126 (0·087)	0·243 (0·064)	0·156 (0·044)
Chlorophyll *b*	1·315 (0·052)	1·234 (0·097)	1·261 (0·078)	1·281 (0·048)
Carotenes[a]	0·687 (0·129)	0·311 (0·125)	0·633 (0·148)	0·412 (0·102)
VAZ pool	0·420 (0·035)	0·283 (0·055)	0·495 (0·025)	0·379 (0·044)

HL+ : high light, high iron; LL+ : low light, high iron; HL− : high light, low iron; LL− : low light, low iron.
VAZ pool, violaxanthin + antheraxanthin + zeaxanthin.
[a] The pool of carotenes consisted of five, strongly related carotenes. The individual carotenes were hard to distinguish, and are therefore not discussed further.

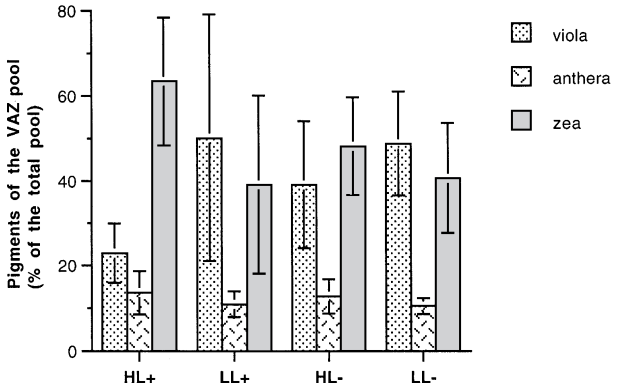

Fig. 1. Proportion of violaxanthin (viola), antheraxanthin (anthera) and zeaxanthin (zea) as a percentage of the total VAZ pool under different conditions (±SD): high irradiance, high iron (HL+); high irradiance, low iron (HL−); low irradiance, high iron (LL+) and low irradiance, low iron (LL−).

xanthin and zeaxanthin; Fig. 1). At low irradiance, violaxanthin was the main pigment of the VAZ pool. However, at high irradiance, 50% (HL− ; high irradiance low iron) to 65% (HL+) of the VAZ pool was made up of zeaxanthin (ANOVA, $p < 0{\cdot}05$).

Iron limitation resulted in a reduction in cellular pigment content, except for violaxanthin at high irradiances (ANOVA, $p < 0{\cdot}05$; Table 2). The largest decreases in chl *a* and *b* were measured at low irradiance. Chl *a* was reduced by 44% and 53% at high and low irradiances, respectively; for chl *b* the reductions were 47% and 56% respectively. A 40% reduction of the lutein content was recorded at both irradiances. Similarly, iron deficiency resulted in a reduction in the VAZ pool by about 35%.

At low irradiance, the ratios of chl *a* to POC increased (ANOVA, $p < 0{\cdot}05$). The increase was stronger for iron-replete cells (136%) than for iron-depleted cells (70%).

The ratio of chl *b* to chl *a* was not significantly affected by light or iron stress (Table 2). The ratio of lutein to chl *a* clearly increased at high irradiance, the increase being greatest for iron-replete cells (ANOVA, $p < 0{\cdot}05$). The ratio of the VAZ pool to chl *a* was also the highest at high irradiance. Iron limitation resulted in an increase in this

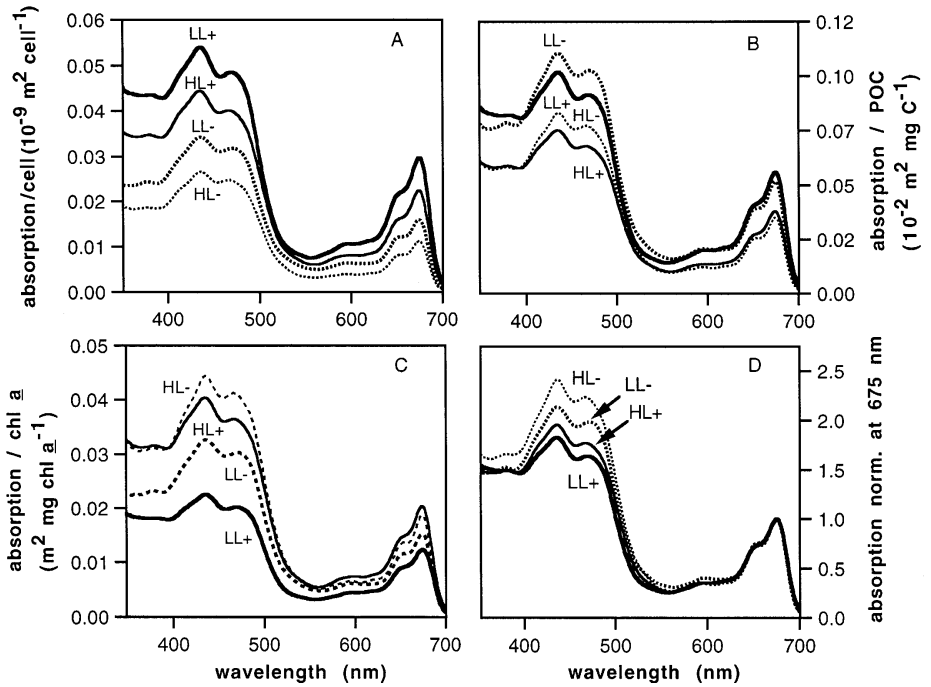

Fig. 2. *In vivo* absorption spectra for different cultures (abbreviations as in Fig. 1). (*A*) absorption per cell; (*B*) absorption normalized to POC; (*C*) chl *a*-specific absorption; (*D*) spectra normalized to the 675 nm peak of chl *a*. Note the different scales of the figures.

Table 3. Photosynthetic characteristics of *Pyramimonas* sp.

	HL+	LL+	HL−	LL−
P_{max}^{cell}	0·265 (0·065)	0·352 (0·032)	0·064 (0·008)	0·107 (0·029)
P_{max}^{C}	4·496 (1·109)	6·623 (0·661)	1·719 (0·121)	2·967 (0·888)
P_{max}^{chl}	0·241 (0·102)	0·157 (0·002)	0·107 (0·013)	0·109 (0·031)
α^{cell}	0·478 (0·194)	0·780 (0·131)	0·045 (0·009)	0·155 (0·037)
α^{C}	0·961 (0·312)	1·237 (0·212)	0·264 (0·036)	0·866 (0·074)
α^{chl}	0·482 (0·048)	0·365 (0·047)	0·088 (0·001)	0·123 (0·004)
I_k	57·4 (9·6)	46·1 (11·9)	143·7 (11·9)	69·2 (2·2)
ϕ_m	5·26 (1·81)	6·76 (1·39)	0·89 (0·37)	2·10 (0·44)

Values are the means (±SD), $n = 4$ (for ϕ_m, $n = 2$).
HL+: high light, high iron; LL+: low light, high iron; HL−: high light, low iron; LL−: low light, low iron.
P_{max}^{cell} = pg C cell^{-1} h^{-1}; P_{max}^{C} = mg C g C^{-1} h^{-1}; P_{max}^{chl} = g C g chl a^{-1} h^{-1}; $\alpha^{cell} = (10^{-2}) \times$ (pg C cell^{-1} h^{-1}) $\times$ (μmol m^{-2} s^{-1})$^{-1}$;
$\alpha^{C} = (10^{-1}) \times$ (mg C g C^{-1} h^{-1}) $\times$ (μmol m^{-2} s^{-1})$^{-1}$; $\alpha^{chl} = (10^{-2}) \times$ (g C g chl a^{-1} h^{-1}) $\times$ (μmol m^{-2} s^{-1})$^{-1}$; I_k = μmol photons m^{-2} s^{-1};
$\phi_m = (10^{-3})$ mol C mol quanta^{-1}.

ratio (Table 2) at both high and low irradiance. The increase found was significant only at high irradiance (ANOVA, $p < 0{\cdot}05$).

In vivo *absorption spectra*

Cell-specific absorption coefficients were highest for cells cultured at low irradiance (Fig. 2*A*). Iron-replete cells had a higher absorption per cell than iron-depleted cells. Distinct peaks in the red and blue could be observed at 675 nm (chl *a*), 650 nm (chl *b*), 470 nm (carotenoids) and 430 nm (chl *a*). Carbon-specific absorption coefficients were also highest for cells grown at low irradiances (Fig. 2*B*). The overall effects of iron limitation were of minor importance relative to the effects of light limitation. In contrast, light absorption per unit chl *a* was highest for cultures adapted to high irradiances (Fig. 2*C*), and iron-depleted cells had a higher absorption in the blue part of the spectrum (Soret band) than iron-replete cells. When normalized to the absorption at 675 nm, absorption in the Soret band was highest for cultures adapted to high irradiances (Fig. 2*D*). Moreover, iron limitation resulted in enhanced absorption in the Soret band.

Photosynthesis versus irradiance

The effects of the treatments on P_{max} and α were not always significant (Table 3). The trends displayed in Fig. 3, however, were consistent within each series of experiments. On the whole, rates of photosynthesis determined using ^{14}C appeared low relative to recorded growth rates. Maximum photosynthesis per cell (P_{max}^{cell}) was highest

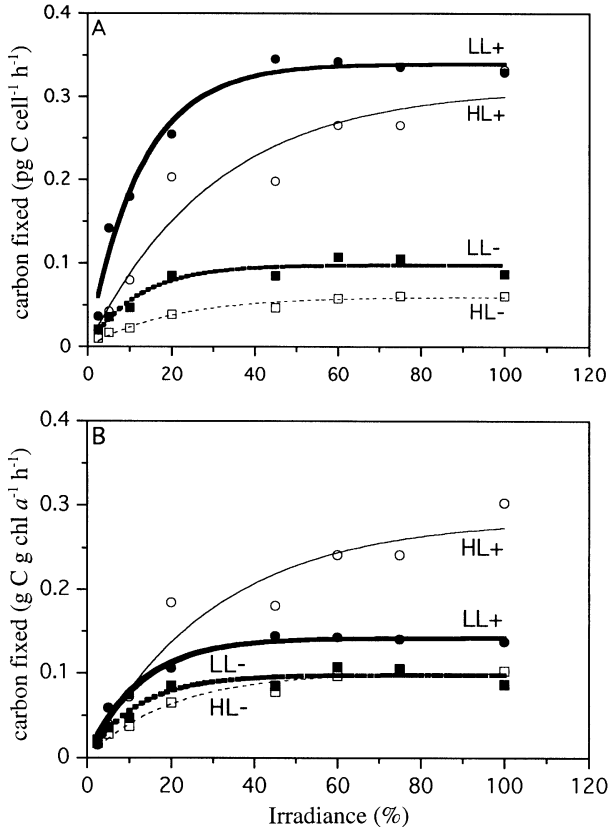

Fig. 3. Representative photosynthesis versus irradiance curves for one single experiment, for different cultures (abbreviations as in Fig. 1). (*A*) Carbon fixation per cell; (*B*) chl *a*-specific carbon fixation.

for cells grown at low irradiance and was clearly impaired following iron limitation (Fig. 3 *A*): P_{max}^{cell} was more than 3 times higher for iron-replete cells than for iron-depleted cells (ANOVA, $p < 0{\cdot}08$). A similar trend was found when P_{max} was normalized to cellular carbon (P_{max}^{C}; Table 3). When normalized to chl *a* (P_{max}^{chl}), P_{max} was highest for cells grown at high irradiance in iron-replete conditions. P_{max}^{chl} was also reduced by iron limitation, although here the trend found was not significant (Fig. 3 *B*).

The light utilization efficiency α was highest for cells grown at low irradiance and reduced by iron limitation, on both a cell (α^{cell}) and a carbon basis (α^{C}) (ANOVA, $p < 0{\cdot}08$; Table 3). When normalized to chl *a* (α^{chl}), a significant effect of the interaction of the two factors, irradiance and iron, was observed (ANOVA, $p < 0{\cdot}05$). Whereas for iron-replete cells α^{chl} decreased for cells grown at low irradiance, for iron-depleted cells α^{chl} was highest for cells grown at low irradiances (ANOVA, $p < 0{\cdot}05$).

The light saturation level I_k was highest for cells grown at high irradiance, for both iron-replete and iron-depleted cells. The highest value (143·7 μmol photons m^{-2} s^{-1}) was found for HL− cells. For iron-depleted cells, high irradiances resulted in an increase of I_k by 100%. For iron-replete cells, the increase in I_k was only 25%. The P–I curves showed no photoinhibition on the short time scale used for the ^{14}C experiments.

Quantum yield of photosynthesis

Highest values for the maximum quantum yield (ϕ_m) were found at the low irradiance (Table 3). The quantum yield was strongly reduced by iron limitation: ϕ_m was lowest for iron-depleted cells cultured at high irradiance (0·89 mmol C mol^{-1} quanta). The values for ϕ_m are fairly low (average value for ϕ_m according to Senger, 1982: 0·1 mol O_2 mol quanta^{-1}), but match, for example, ϕ_m measured for natural plankton populations by Dubinsky *et al.* (1984).

Discussion

Iron limitation led to the commonly observed changes in the biochemical composition of *Pyramimonas* cells, that is, a reduced pigment content and a reduction in carbon and nitrogen quota (Tables 1, 2). The light utilization efficiency (α) was reduced due to a decrease in the quantum yield of photosynthesis ϕ_m (Table 3). However, the light-harvesting capacity of the cells was not affected by iron limitation (Fig. 2). Iron limitation did not suppress photoacclimation of the photosynthetic apparatus; within the limits of iron limitation, *Pyramimonas* sp. was capable of adjusting the make-up of its photosynthetic apparatus to the prevailing light conditions.

Pigment content

Pyramimonas sp. adapted to low irradiance by increasing the cellular content of chlorophyll *a* and *b* (Table 2). At the same time, the ratio of chl *a* to POC increased (Table 1). Such an increase is generally observed under light limitation (Geider, 1987). Under iron limitation, the response in chl *a* and *b* content was less prominent: the increase in chl *a* to POC was nearly twice as high for iron-replete cells as for iron-depleted cells. Greene *et al.* (1991) have already described how iron limitation results in a more pronounced decrease in cellular chl *a* content than in carbon fixation. The ratio of chl *a* to POC thus decreases under iron limitation. In the experiments with *Pyramimonas* this decrease was only observed at low irradiance. These results are consistent with the findings of Kudo & Harrison (1997), who observed a less pronounced increase in chl *a*/cell for iron-depleted cells relative to iron-replete cells in the cyanobacterium *Synechococcus* cultured at low irradiance.

Iron-limited cells are known to be more susceptible to photoinhibition (Geider & LaRoche, 1994). A shift in pigment composition within the VAZ pool has been observed under iron depletion (Morales *et al.*, 1994). However, in cells of *Pyramimonas* sp. iron limitation did not control the response of the VAZ pool, which was related to changes in irradiance. At high irradiance, both iron-replete cells and iron-depleted cells showed an increase in photoprotective pigments, as reflected in an

increase in the ratio of the VAZ pool to chl *a* (Table 2). Even so, for both iron-depleted and iron-replete cells, the contribution of zeaxanthin, the de-epoxidized photoprotective product of violaxanthin (Pfündel & Bilger, 1994; Lichtlé *et al*., 1995), was greatest at high irradiances. We suggest that lutein also functioned as a photoprotective pigment. The ratio of this pigment to chl *a* was considerably higher at high relative to low irradiances (Table 2). Lutein is generally described as a light-harvesting pigment (Siefermann-Harms, 1987). However, a photoprotective function was also found in another *Pyramimonas* species (Kohata & Watanabe, 1988), and has been recorded for several other algae (Schäfer *et al*., 1994).

Light-harvesting capacity

The increase in cellular absorption in response to low irradiance was similar for iron-depleted and iron-replete cells (Fig. 2*A*), despite the apparent differences in pigment composition. Moreover, light absorption per unit chl *a* was higher for iron-depleted cells than for iron-replete cells (Fig. 2*C*). The increase in specific absorption capacity can be attributed to a reduction in the 'package effect', which resulted from the lower pigment content of iron-depleted cells (Mitchell & Kiefer, 1988; Greene *et al*., 1991; 1992; Johnson & Sakshaug, 1993). This was also reflected in changes in α (discussed below). Light absorption per unit carbon was also governed by the growth irradiance. The carbon-specific rate of light absorption was not suppressed by iron limitation (Fig. 2*B*) because the cellular carbon content was significantly lower for iron-depleted cells than for iron-replete cells. On a carbon host basis, it can thus be concluded that, even at low irradiance, the light-harvesting capacity of cells of *Pyramimonas* is not suppressed by iron limitation, despite the observed decline in cellular pigment content.

The increased weight of photoprotective pigments in iron-depleted cultures, inherent to iron limitation (Geider *et al*., 1993), related to the increased absorption capacity in the blue region relative to absorption in the red part of the spectrum (Fig. 2*D*). The increase in the Soret region was even more pronounced for HL− cells, and reflected the increases of lutein and the pigments of the VAZ pool relative to chl *a*. This again showed that iron-depleted cells were capable of modifying their pigmentation in response to high irradiances.

Light utilization efficiency

Iron limitation has been shown to result in a decrease in photosynthetic capacity, both the light utilization efficiency and the maximum rate of photosynthesis being affected (Greene *et al*., 1991, 1992). The decrease in light utilization efficiency α resulted from a pronounced decrease in the quantum yield of photosynthesis, which could not sufficiently be compensated for by an increase in absorption efficiency (Greene *et al*., 1991, 1992; Vassiliev *et al*., 1995). A decrease in α due to iron limitation was also found in cells of *Pyramimonas* sp. (Table 3), whereby both the absorption capacity and the quantum yield of photosynthesis were suppressed.

Concurrently, light limitation led to an increase in light utilization (Table 3). This increase was stronger for iron-depleted than for iron-replete cells, which can be ascribed to different changes in the 'package effect' and quantum yield. For both iron-depleted and iron-replete cells the chlorophyll *a*-specific absorption coefficient decreased (Fig. 2*C*), due to an increased 'package effect' often associated with low light acclimation as a result of higher pigment concentrations in the cells (Berner *et al*., 1989; Iriarte & Purdie, 1993). The 'package effect' was, however, not as strong for iron-depleted cells as for iron-replete cells. Moreover, under iron-replete conditions, light limitation resulted in a decline in chl *a*-specific absorption as well as in α^{chl}, which is indicative of the 'package effect' (Berner *et al*., 1989). For iron-depleted cells, the increase in cellular pigment content was not accompanied by a decrease in α^{chl}. In fact, α^{chl} was higher at low irradiance than at high irradiance. At the same time, an increase in the maximum quantum yield of photosynthesis was observed under iron-replete as well as iron-depleted conditions (Table 3). Contrary to our observations, it has so far been hypothesized that in iron-depleted cells, light limitation would lead to a decrease in ϕ_m. Findings by Sunda & Huntsman (1997), however, support our results. They showed that iron-depleted cells could maintain a relatively high cellular iron quota by a decrease in growth rates. This strategy may enable the cells to support the functioning of the photosynthetic apparatus. The relatively high efficiency of light utilization observed in our experiments, not only for iron-replete cells but also for iron-depleted cells, thus again underlies the capacity of cells to photoacclimate despite iron limitation.

For cells grown at high irradiance, iron limitation led to a pronounced increase in the parameter of light saturation I_k. This indicated that under these light conditions, iron-depleted cells had a very poor capacity to utilize low levels of irradiance. The low efficiency of light utilization (α for HL− cells was less than 10% of α in HL+ cells) actually explains the high saturation level of photosynthesis; a decrease in α is often coupled with an increase in I_k (Henley, 1993).

Growth rates

For iron-depleted cells, growth as determined by cell counts was strongly reduced by light limitation. Growth reduction was less strong for iron-replete cells. Maximum rates of photosynthesis as determined by carbon incorporation did not match this observation. Iron limitation often results in a decrease in P_{max}, caused by a decrease in the number of reaction centres and an increase in turnover time (Greene *et al*., 1991). Iron-depleted cells of *Pyramimonas* indeed showed such a decrease. The effects of iron limitation on P_{max} were not any stronger at low than at high irradiance, as discussed above. Whereas

growth rates were lowest for LL− cells, P_{max} values were lowest for HL− cells. This discrepancy between P_{max} determined over a short time scale and growth rates determined over a long time scale has been observed more often, and may relate to the different-sized photosynthetic apparatus of cells cultured at low irradiance versus cells cultured at high irradiance (Falkowski *et al*., 1985; Garcia & Purdie, 1992). Moreover, small differences in temperature and irradiance may affect P_{max}, and minor offsets in P_{max} may result in a large relative error when extrapolated to daily growth rates. The larger reduction in growth on the basis of cell counts, observed in iron-depleted cells grown at low irradiance, may also be related to differences in respiration. Respiration cannot be accounted for accurately when measuring carbon isotope incorporation, and this may bias net production rates. A decrease in light availability results in an increase in respiration rates; maintenance costs may be higher in cells with larger light-harvesting systems (Konopka & Schnur, 1981; Garcia & Purdie, 1992). Iron limitation also results in an increase in respiration relative to gross photosynthesis (Trick *et al*., 1995; Henley & Yin, 1998). Under conditions of low irradiance, the relative increase in respiration rates linked with an increased iron demand may account for the strong decrease in cell growth observed in iron-depleted cells.

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