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Relative effects of nitrogen or phosphorus depletion and light intensity on the pigmentation, chemical composition, and volume of *Pyrenomonas salina* (Cryptophyceae)

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ABSTRACT: The nitrogen-rich phycobiliproteins are efficient light-harvesting pigments of cryptophytes, cyanobacteria, and rhodophytes. Whereas it is well established that certain cyanobacteria mobilize their phycobiliproteins in response to nitrogen deprivation, the effect of nitrogen stress on cryptophyte physiology has been studied only rarely. We compared the effects of nitrogen and phosphorus depletion on the biovolume, chemical composition, and pigment composition of the marine cryptophyte *Pyrenomonas salina*, grown in batch cultures under light-limiting and light-saturating conditions. Cellular phycoerythrin content declined during the late exponential growth phase in nitrogen-depleted cultures, and the cells were nearly devoid of phycoerythrin (< 3 % of the phycoerythrin content of nitrogen-replete cells) at the beginning of stationary growth. Phycoerythrin content also decreased during late exponential phase in phosphorus-depleted cultures, but these latter cells contained more than 10 times the phycoerythrin concentration of nitrogen-depleted cells at the same growth stage. Chlorophyll *a* content declined in nitrogen-depleted cultures, but at a slower rate than the decrease in phycoerythrin, such that the phycoerythrin to chlorophyll *a* ratio decreased by more than 95 % from the early exponential to early stationary growth phase. In nitrogen-depleted cultures grown at a low light intensity, the decline in phycoerythrin content preceded the decline in chlorophyll *a* content, and resulted in only slight changes in cellular nitrogen content and biovolume. These data indicate that phycoerythrin is preferentially lost from *P. salina* during nitrogen deficiency, and support the hypothesis that, similar to certain cyanobacteria, *P. salina* responds to nitrogen deprivation by mobilizing phycoerythrin in order to help sustain cellular nitrogen requirements.

INTRODUCTION

Phycobiliproteins are the major light-harvesting pigments in cyanobacteria, rhodophytes, and cryptophytes. Cryptophyte phycobiliproteins are immunochemically related to cyanobacteria and rhodophyte phycobiliproteins (MacColl et al. 1976, Guard-Friar et al. 1986), suggesting a common evolutionary ancestry to the 3 pigment groups. However, the phylogenetic status of cryptophyte phycobiliproteins is complicated by their distinct physical properties and molecular structures (Glazer 1981), and especially by the unique way in which they are organized within the chloroplast (Gantt 1979). Whereas the phycobiliproteins of cyanobacteria and rhodophytes are organized within discrete phycobilisomes located on the outer thylakoid membrane, cryptophytes lack phycobilisomes, and contain phycobiliproteins within the thylakoid lumen. Cryptophytes also differ from cyanobacteria and rhodophytes in containing 2 light-harvesting pigment complexes, the phycobiliprotein-associated protein complex and a chlorophyll *a/c* protein complex. The latter is integrally bound to the thylakoid membrane, and transfers energy to either photosystem (Lichtlé et al. 1980).

Cryptophytes resemble cyanobacteria and rhodophytes, however, in that Photosystem II receives excitation energy primarily through the phycobiliprotein light-harvesting protein complex (reviewed in Larkum & Barrett 1983, MacColl & Guard-Friar 1987), and this energy transfer is highly efficient (Haxo & Fork 1959, Holzwarth et al. 1983, Hanzlik et al. 1985, Bruce et al. 1986). Efficient energy transfer in cyanobacteria and rhodophytes can be partly explained by the highly

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ordered juxtapositioning of antenna pigments within the phycobilisome and the close spectral overlap between phycoerythrin, phycocyanin, allophycocyanin, and chlorophyll *a* (reviewed in Scheer 1981). Efficient energy transfer to Photosystem II is not as thoroughly understood in cryptophytes, where phycobiliprotein complexes are seemingly less efficiently organized, allophycocyanin is absent, and phycoerythrin and phycocyanin have never been demonstrated in the same organism (reviewed in Gantt 1979). However, the presence of multiple forms of phycoerythrin or phycocyanin within a species (Hiller & Martin 1987 and references therein), and the organization of phycobiliproteins into discrete units closely associated with the thylakoid membrane (Lichtlé et al. 1987, Ludwig & Gibbs 1989) may partly account for efficient energy conversion in cryptophytes. Clearly, cryptophytes have evolved a unique system for harvesting light, combining strategies of other phycobiliprotein-containing organisms and chromophytes.

One issue that has been extensively studied in cyanobacteria, but not cryptophytes, is the response of the photosynthetic system to changes in nutrient status. In general, cyanobacteria react to nitrogen depletion by decreasing the rate of phycobiliprotein production (reviewed in Grossman et al. 1986). Constant rates of growth and chlorophyll *a* production were maintained while phycobiliprotein content declined during the initial stages of nitrogen depletion in cultures of *Cyanophora paradoxa* (Schenk et al. 1987), *Spirulina platensis* (Boussiba & Richmond 1980), *Anacystis nidulans* (Bayer & Schenk 1986), and *Agmenellum quadruplicatum* (Stevens et al. 1981a). De novo synthesis of phycocyanin was shown to be selectively depressed by nitrogen-deficient *Anacystis nidulans* (Lau et al. 1977). This is not surprising given that the light-harvesting machinery associated with phycobiliproteins requires substantial amounts of nitrogen relative to the chlorophyll/protein complexes (Raven 1984), and that photosynthesis can function in the absence of phycobiliproteins (Lemasson et al. 1973, Arnon et al. 1974).

In addition to reducing the rate of phycobiliprotein production, cyanobacteria also appear to be able to degrade phycobiliproteins already present in the cell when organisms become nitrogen-stressed (Allen & Smith 1969, Foulds & Carr 1977, Lau et al. 1977, Yamanaka & Glazer 1980). Phycobiliproteins can, under certain conditions, account for a significant portion of the cellular nitrogen content (Myers & Kratz 1955, Bennett & Bogorad 1973, Kana & Glibert 1987a). Phycobiliprotein degradation is believed to be a mechanism for mobilizing nitrogen for processes required to sustain cellular growth or metabolism during times when the exogenous supply of nitrogen is depleted.

Analogous information on the effects of nitrogen availability on the pigment concentration of cryptophytes is rare, and the relative importance of light intensity and nutrient status to cryptophyte pigment production is unclear. Results from 2 studies indicate that phycobiliproteins may be selectively lost during nitrogen-deficient conditions in cryptophytes. Lichtlé (1979) compared the pigment content and chloroplast ultrastructure of nitrogen-replete *Cryptomonas rufescens* cultures grown at a relatively high light intensity, nitrogen-replete cultures grown at a relatively low light intensity, and nitrogen-deficient cultures grown at a relatively low light intensity. Cells from the nitrogen-replete cultures grown at the low light intensity and sampled during the exponential growth phase contained higher concentrations of all photosynthetic pigments and a greater proportion of phycoerythrin to total chlorophyll than cells from the nitrogen-deficient cultures grown at the low light intensity. This result is consistent with the selective degradation of phycoerythrin and/or inhibition of phycoerythrin production by *C. rufescens* in response to nitrogen stress. Pigment determinations from the nitrogen-replete cultures grown at the high light intensity were made at, or just prior to, the stationary growth phase. Because these cultures were grown in media with a dissolved nitrogen to phosphorus ratio of ca 8, it is possible that the cells were nitrogen-deficient during this growth stage. Therefore, the effects of light intensity and nitrogen availability on the pigment content of *C. rufescens* cannot be adequately distinguished based on these results.

Similarly, Rhiel et al. (1985, 1986) found that nitrogen-deficient *Cryptomonas maculata* cells, grown under a relatively high light intensity, contained a much lower concentration of phycoerythrin, a lower proportion of phycoerythrin to chlorophyll, and fewer Photosystem II particles than nitrogen-sufficient cells grown at a lower light level. Resupplying the nitrogen-deficient cells with nitrate caused a restoration of the red color characteristic of cultures growing exponentially, but the pigment content was not quantified. Therefore, the relative importance of light intensity and nitrogen availability on the cryptophyte photosynthetic apparatus is difficult to differentiate from these results.

The effects of nutrient status (nitrogen depletion, phosphorus depletion) and light intensity on photosynthetic pigment production in the marine cryptophyte *Pyrenomonas salina* were examined in this study. The physiological response of *P. salina* to nitrogen depletion resembled that of certain cyanobacteria in that, independent of light intensity, cellular phycobiliprotein content declined before cell division stopped or chlorophyll *a* concentration decreased. Based on patterns of pigment concentration, biovolume, and

chemical composition, we hypothesize that *P. salina* mobilizes phycoerythrin during nitrogen-stressed growth in order to supply the biosynthetic nitrogen demands for cellular growth.

METHODS

Pyrenomonas salina Santore (formerly *Cryptomonas salina* Wislouch; *Chroomonas salina* Butcher) clone 3C (Culture Collection of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences) was obtained from Dr Diane Stoecker (Woods Hole Oceanographic Institution). Batch cultures used for inocula in the experimental treatments were grown axenically at 21 °C in artificial seawater medium (ASPM Base; Table 9 in Guillard [1975], except that 20 mM $CaCl_2$, 0.2 mM H_3BO_3, and 0.81 mM KBr were used) with f-mix vitamins and trace metals (Guillard 1975). The nitrogen source was $NaNO_3$ and the phosphorus source was NaH_2PO_4. The pH of the medium was 7.4 and the salinity was 29.5 ‰.

Cultures were grown under 2 different nutrient regimes at each of 2 light intensities. Continuous illumination was provided by cool-white fluorescent lights at intensities of 325 ('High Light') or 15 ('Low Light') $\mu E\ m^{-2}\ s^{-1}$. Irradiance was measured with an integrating 4π quantum sensor (Biospherical Instruments, QSL-100P probe). The effects of nitrogen or phosphorus depletion were examined by growing batch cultures of *Pyrenomonas salina* in media that were nitrogen-depleted or phosphorus-depleted during the stationary growth phase. Data from previous experiments (Fig. 1) indicated that the population density of cultures in the stationary growth phase correlated directly with the initial NO_3^- concentration in media with initial molar nitrogen to phosphorus ratios between 0.2 and 4, and correlated with the initial PO_4^{-3} concentration when the initial nitrogen to phosphorus ratio ranged between 50 and 50 000. In this study, cultures were grown in medium with a molar nitrogen to phosphorus ratio of 1 (5 μM NO_3^- and 5 μM PO_4^{-3}), and were nitrogen-depleted during the stationary growth phase, or 80 (25 μM NO_3^- and 0.3 μM PO_4^{-3}), and were phosphorus-depleted during the stationary growth phase.

Cultures were acclimated to high or low light levels (see above) by repeated transfer of cells in the mid-exponential growth phase. Exponentially growing populations were inoculated into fresh media to begin the experiment (initial population densities of ca 10^3 cells ml^{-1}). Cell numbers, biovolumes, and cellular concentrations of phycoerythrin (PE), chlorophyll *a*, carbon, and nitrogen were determined periodically throughout the growth cycle of the alga. Cultures were tested for bacterial contamination throughout the experiment by inoculating tubes containing 0.5 % yeast extract with 1 ml of the culture, and examining these tubes for bacterial growth.

Biovolumes and cell numbers were measured using a Coulter Multisizer particle counter with a 70 μm aperture. Cell counts also were determined microscopically from triplicate samples with counting chambers. A Fuchs-Rosenthal Ultra Plane chamber (0.2 mm depth) was used when cell counts were below 2×10^4 cells ml^{-1}, and a Reichert Bright-Line hemacytometer (0.1 mm depth) was used for higher population densities. Counts obtained with the 2 chambers were normalized to each other by performing duplicate counts of a number of samples with both chambers, and normalizing to Reichert Bright-Line estimates.

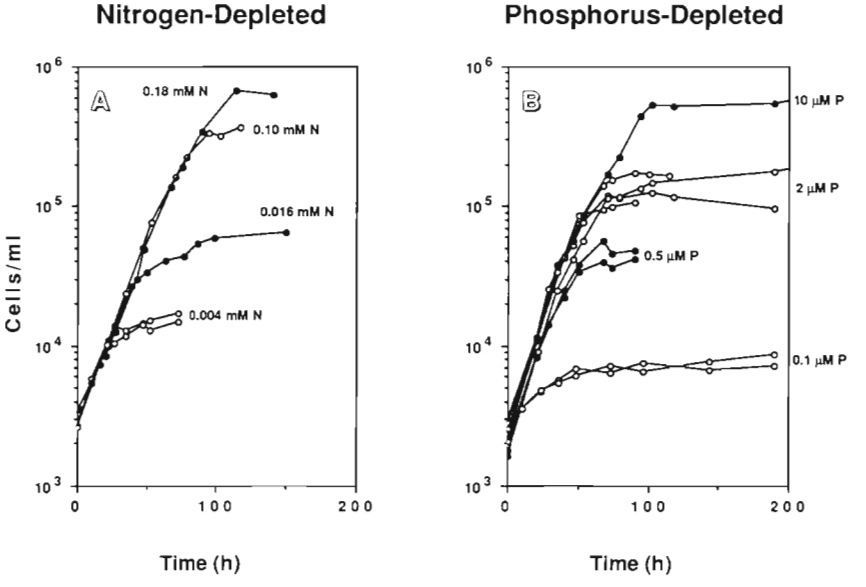

Fig. 1. *Pyrenomonas salina*. Growth curves when cultured at 325 $\mu E\ m^{-2}\ s^{-1}$ in different nutrient regimes. (A) Cultures grown in media with NO_3^- as the sole nitrogen source (molar N : P ratio ranged from 0.2 to 4). (B) Cultures grown in media with PO_4^{-3} as the sole phosphorus source (molar N · P ratio ranged from 50 to 50 000)

Specific growth rates, μ (d^{-1}), were determined from regressions of the linear portion of the growth curves expressed as the natural log of the population densities versus time (i.e. the exponential growth phase).

PE was extracted by freeze-thawing in 0.05 M phosphate buffer (pH 6.8) (Thinh 1983). Cells were harvested by centrifugation, and the pellet was resuspended in buffer. The solution was rapidly frozen in liquid nitrogen and thawed in water at room temperature. One freeze-thaw cycle was sufficient for maximal PE extraction in this species, regardless of population density. Additional cycles did not increase the amount of soluble PE recovered from cultures over a wide range of population densities. After freeze-thawing, the sample was centrifuged at 6800 × *g* (Sorvall Superspeed RC2-B; Type SS-34 rotor) for 30 min at 7 °C to remove particulate material. The concentration of extracted PE in the supernatant was determined fluorometrically, using an SLM Aminco SPF-500C Spectrofluorometer. Dilution series of extracted PE were used to standardize the peak PE fluorescence (550 nm excitation; 585 nm emission) against peak absorption (549 nm), as measured with a Hewlett Packard 8451A Diode Array Spectrophotometer. PE concentrations were determined based on an extinction coefficient of 12.6 ml mg^{-1} cm^{-1} (R. MacColl pers. comm.).

Chlorophyll was extracted with 100% acetone, following filtration onto HA Millipore membrane filters (mean pore size = 0.45 µm). Chlorophyll *a* concentrations were then determined spectrophotometrically (Jeffrey & Humphrey 1975), using a Shimadzu Spectrophotometer UV-260.

Particulate carbon and nitrogen were measured with a Perkin Elmer 240 elemental analyzer, after filtration onto pre-combusted Whatman GF/F glass-fiber filters. The amount of nitrogen contained in the PE of *Pyrenomonas salina* was estimated by assuming that 19% of the weight of PE from *P. salina* is nitrogen. This value was calculated, based on the amino acid composition of PE from *Rhodomonas lens* (MacColl et al. 1976), which has the same spectral characteristics as PE from *P. salina* (R. MacColl pers. comm.).

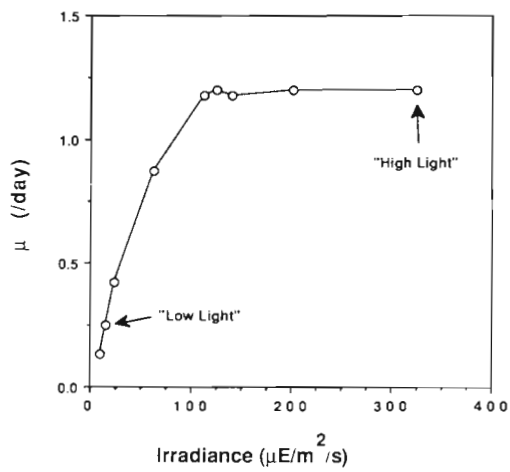

Fig. 2. *Pyrenomonas salina*. Effect of irradiance on growth rate (μ). Growth rates at the High Light intensity (325 µE m^{-2} s^{-1}) and Low Light intensity (15 µE m^{-2} s^{-1}) used in this study are indicated

In a separate experiment, growth rates of *Pyrenomonas salina* were determined in batch cultures that had been acclimated to 325, 200, 140, 125, 110, 60, 25, 15, and 10 µE m^{-2} s^{-1} (see Fig. 2). Methods for acclimation to light intensity and determination of growth rates were as previously described. The culture medium for this experiment was identical to that described above except that NH_4Cl was provided as the nitrogen source.

RESULTS

Preliminary growth experiments carried out with *Pyrenomonas salina* demonstrated that the growth rate of this cryptophyte in defined medium is light-saturated at ca 100 µE m^{-2} s^{-1} (Fig. 2). The growth rate of *P. salina* at light intensities between 115 and 325 µE m^{-2} s^{-1} was constant at 1.2 d^{-1}. The highest light intensity (325 µE m^{-2} s^{-1}) was the 'High Light' intensity employed in the present study. Below 115 µE m^{-2} s^{-1}, the growth rate of *P. salina* was proportional to light intensity. The growth rate at 15 µE m^{-2} s^{-1} (the 'Low Light' intensity of this study) was 0.25 d^{-1}.

The average biovolume of *Pyrenomonas salina* varied with the light level and the growth stage of the alga (Fig. 3; Table 1). Mean biovolume was relatively constant throughout the exponential growth phase in each culture, but was not the same for the various treatments. Differences in biovolumes for cultures grown at the different light intensities were most conspicuous during the exponential growth phase. Biovolume averages for both cultures at High Light were significantly higher (t-test, $p > 0.05$) than the biovolumes of the cultures at Low Light during exponential growth. Average biovolume decreased during the stationary growth phase at High Light. The initiation of this decline occurred at an earlier growth period in the nitrogen-depleted culture than in the phosphorus-depleted culture (Fig. 3A, C), such that phosphorus-depleted cells were, on average, approximately twice as large as nitrogen-depleted cells 100 h after the beginning of stationary growth (Table 1). Contrary to the patterns found in cultures grown at High Light, the average biovolume increased during stationary growth in cultures grown at Low Light (Fig. 3B, D). However, the increase in volume of phosphorus-depleted cells was greater than that of nitrogen-depleted cells. At the last sampling time, the average volume of nitrogen-depleted cells grown at

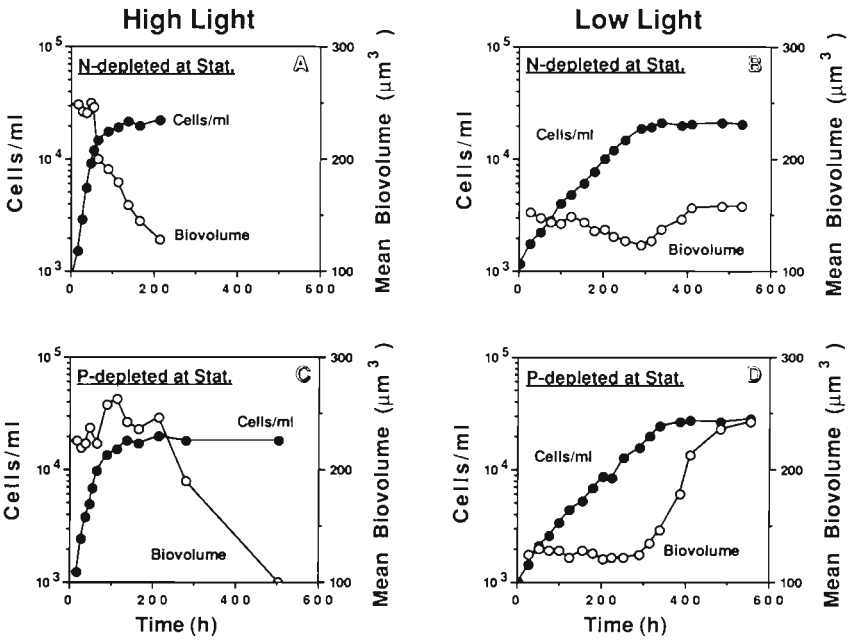

Fig. 3. *Pyrenomonas salina*. Cell numbers and mean biovolume in: (A) High Light cultures nitrogen-depleted during stationary growth, (B) Low Light cultures nitrogen-depleted during stationary growth, (C) High Light cultures phosphorus-depleted during stationary growth, (D) Low Light cultures phosphorus-depleted during stationary growth

Table 1. *Pyrenomonas salina*. Mean biovolume (Biovol), carbon, nitrogen, and C:N ratio of cells during the exponential and stationary growth phases. Exponential growth phase data are the mean values obtained from cells in early and middle exponential growth. Means of cell carbon and nitrogen from exponentially growing cultures were calculated from 2 and 3 sampling time points for High Light and Low Light cultures, respectively. Stationary phase data were obtained from cells 100 h after the beginning of stationary growth, with the exception of the Low Light culture grown at an N:P ratio of 80. Data from the latter culture were obtained from cells 50 h after the approximate beginning of stationary phase. The standard deviations for the biovolume distributions, as calculated from cell size histograms, ranged from 7 to 10 % of the mean

Treatment	Exponential phase						Stationary phase					
	Biovol. (μm^3)	Carbon (pmol $cell^{-1}$)	Carbon (fmol μm^{-3})	Nitrogen (pmol $cell^{-1}$)	Nitrogen (fmol μm^{-3})	C:N	Biovol. (μm^3)	Carbon (pmol $cell^{-1}$)	Carbon (fmol μm^{-3})	Nitrogen (pmol $cell^{-1}$)	Nitrogen (fmol μm^{-3})	C:N
High Light												
N-depleted at stat. phase	246	5.7	23	0.89	3.6	6.5	128	3.8	30	0.36	2.8	10.6
P-depleted at stat. phase	226	7.9	35	1.2	5.2	6.6	246	9.6	39	1.1	4.5	8.7
Low Light												
N-depleted at stat. phase	144	4.6	32	0.69	4.8	6.7	152	6.2	41	0.67	4.4	9.4
P-depleted at stat. phase	126	4.9	39	0.81	6.4	6.0	178	5.7	32	0.61	3.4	9.5

Low Light was ca 60 % less than that of phosphorus-depleted cells (Fig. 3B, D).

The mean molar carbon to nitrogen (C:N) ratios of exponentially growing *Pyrenomonas salina* did not differ significantly with light intensity (t-test, $p > 0.05$), despite the differences in average biovolumes (Table 1). Cells from the stationary growth phase had significantly greater C:N ratios than exponentially growing cells for all treatments.

During the exponential growth phase, PE concentration $cell^{-1}$ and PE concentration $(cellular\ carbon)^{-1}$ were significantly higher (t-test, $p > 0.05$) in cultures grown at Low Light than at High Light (Fig. 4; Table 2). PE $cell^{-1}$ decreased during late exponential growth in all cultures. However, the extent of the reduction of the PE content during late exponential and early stationary growth was affected by the nutrient regime of the culture medium. When cells were grown in medium that became nitrogen-depleted in the stationary growth phase, PE $cell^{-1}$ decreased to

near-zero values during early stationary growth (Fig. 4A, B; Table 2). During this growth phase, cells in these cultures contained less than 10% of the PE concentration of phosphorus-stressed algae. The PE content of *Pyrenomonas salina* grown in a medium that became phosphorus-depleted declined steadily during stationary growth at the High Light intensity (Fig. 4C). PE concentration also decreased in cells grown in the same medium at Low Light, but remained relatively constant at around 2 pg PE cell^{-1} for the duration of the experiment (Fig. 4D).

Unlike PE concentration cell^{-1}, there were no significant differences (t-test, $p > 0.05$) in the concentration of chlorophyll *a* cell^{-1} at the 2 light levels during exponential growth (Fig. 4; Table 2). This difference between PE and chlorophyll *a* concentration in the 2 light regimes resulted in a higher cellular ratio of PE to chlorophyll *a* in cultures grown at Low Light than those grown at High Light during this period (Fig. 5; Table 2). The chlorophyll *a* content of cells grown in the nitrogen-depleted medium began to decline during late exponential growth, but the rate of decrease was much less than the rate of decrease of PE cell^{-1} (Fig. 4A, B), leading to a sharp decline in the ratio of PE to chlorophyll *a* during this period (Fig. 5; Table 2). The chlorophyll *a* content of cells

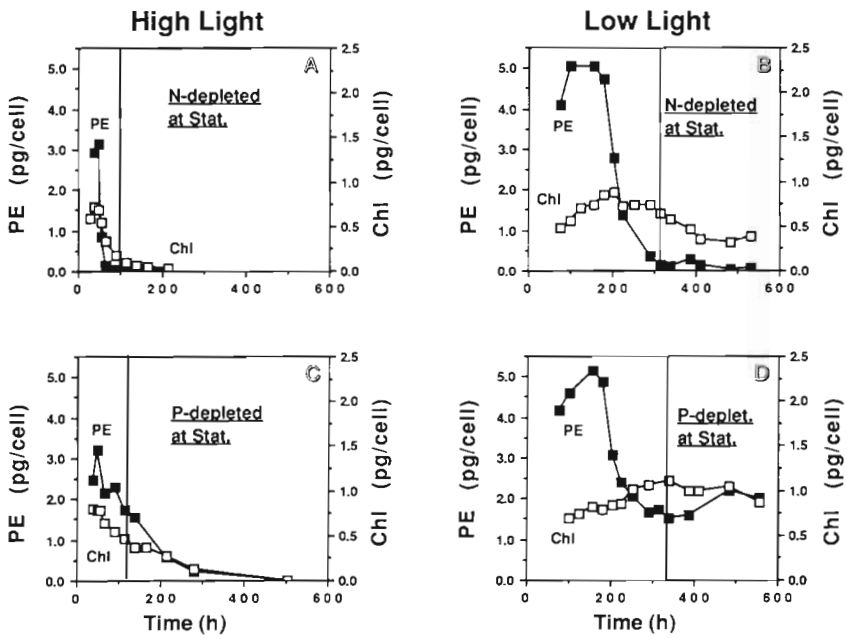

Fig. 4. *Pyrenomonas salina*. Cellular PE and chlorophyll *a* in: (A) High Light cultures nitrogen-depleted during stationary growth; (B) Low Light cultures nitrogen-depleted during stationary growth; (C) High Light cultures phosphorus-depleted during stationary growth; (D) Low Light cultures phosphorus-depleted during stationary growth. Vertical lines indicate approximate beginning of stationary growth

Table 2. *Pyrenomonas salina*. Mean PE content, chlorophyll *a* content, and PE to chlorophyll *a* ratio of cells from the early exponential and early stationary growth phases. Data from the early exponential phase are the mean values obtained from cells prior to the time when the decline in PE content began. Data from the early stationary phase were obtained from cells 24 h after the approximate beginning of stationary phase.

Treatment	Early exponential phase					Early stationary phase		
	PE (pg cell^{-1})	PE (g(mg cell C)$^{-1}$)	Chl (pg cell^{-1})	Chl (g(mg cell C)$^{-1}$)	PE · Chl	PE (pg cell^{-1})	Chl (pg cell^{-1})	PE Chl
High Light								
N-depleted at stat. phase	3.0	44	0.70	10	4.3	0.02	0.10	0.20
P-depleted at stat. phase	2.9	31	0.75	8	4.0	1.7	0.46	3.7
Low Light								
N-depleted at stat. phase	4.9	89	0.65	12	7.6	0.14	0.67	0.21
P-depleted at stat. phase	4.9	83	0.77	13	6.2	1.7	1.1	1.6

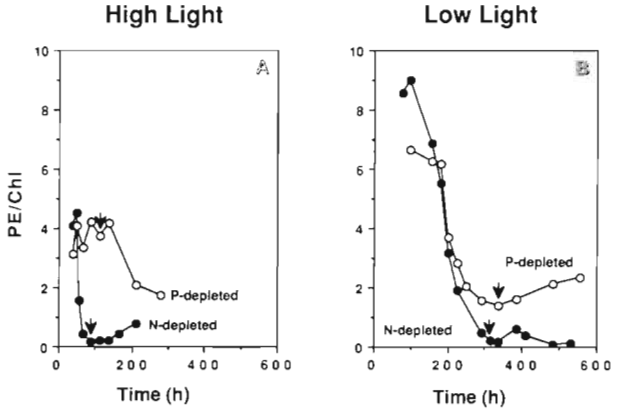

Fig. 5. *Pyrenomonas salina*. Cellular PE to chlorophyll *a* ratio in cultures grown under different nutrient regimes at: (A) High Light and (B) Low Light intensities. 'N-depleted' and 'P-depleted' refer to cultures that were nitrogen- and phosphorus-depleted, respectively, during stationary growth. Arrows indicate approximate beginning of stationary growth

grown in the phosphorus-depleted medium decreased during the late exponential growth phase in High Light and continued to decrease during the stationary growth phase at a rate similar to that of PE cell^{-1} (Fig. 4C), resulting in comparable PE to chlorophyll *a* ratios in cells from the exponential and early stationary growth phases (Fig. 5A; Table 2). The chlorophyll *a* concentration actually increased during the late exponential growth phase in cells grown at Low Light in the phosphorus-depleted medium (Fig. 4D), possibly due to culture self-shading effects. Because PE cell^{-1} decreased during this same period, the cellular PE to chlorophyll *a* ratio of this culture decreased by ca 75% during the transition period from exponential growth to early stationary growth (Fig. 5B; Table 2). These cells maintained a relatively high chlorophyll *a* content throughout the stationary growth phase (ca 1 pg cell^{-1}).

Algae from all cultures experienced a decline in nitrogen content cell^{-1} and PE content cell^{-1} in the stationary growth phase relative to the exponential growth phase (Table 3). The decrease in cellular nitrogen during this period, however, was less than the decrease in the amount of nitrogen contained in PE (calculation described in 'Methods'). That is, as the cultures approached the stationary growth phase, a greater percentage of the PE nitrogen was lost from the cells than indicated by the change in total nitrogen. The largest discrepancy was observed for algae grown at the low light intensity in the nitrogen-depleted medium. During early stationary growth, these cells contained only 3% of the PE nitrogen and 87% of the total cellular nitrogen of the same culture during the exponential growth phase (Fig. 6A; Table 3). In contrast to PE, chlorophyll *a* cell^{-1} did not change significantly (t-test, $p > 0.05$) during this same period (Fig. 6A). Nitrogen contained in PE contributed significantly (ca 9% or ca 4% in cultures grown at Low Light or High Light, respectively) to total cellular nitrogen of exponentially growing *Pyrenomonas salina* (Table 3). This contribution decreased dramatically as the cultures reached the stationary growth phase in the nitrogen-depleted medium (e.g. Fig. 6B).

Table 3. *Pyrenomonas salina*. Mean cell nitrogen and nitrogen contained in cell's PE (PE-N) of cultures from the early exponential and early stationary growth phases. See 'Methods' for procedure for calculating PE-N. Also included are the % decrease in cell nitrogen and PE-N from the early exponential to early stationary growth phase, and the quotient of these 2 parameters. Early exponential growth phase data are the mean values obtained from cells prior to the time when the decline in PE content began. Means of cell nitrogen from exponentially growing cultures were calculated from 2 and 3 sampling time points for High Light and Low Light cultures, respectively. Early stationary growth phase data were obtained from cells 24 h after the approximate beginning of stationary phase

Treatment	Early exponential phase		Early stationary phase		% Decrease in cell N	% Decrease in PE-N	% PE-N dec. / % Cell N dec.
	Cell N (pg cell^{-1})	PE-N (pg cell^{-1})	Cell N (pg cell^{-1})	PE-N (pg cell^{-1})			
High Light							
N-depleted at stat. phase	12.4	0.57	6.4	0.004	48	99	2.1
P-depleted at stat. phase	15.9	0.55	12.7	0.32	16	42	2.6
Low Light							
N-depleted at stat. phase	9.7	0.90	8.4	0.027	13	97	7.5
P-depleted at stat. phase	11.4	0.93	8.5	0.32	25	66	2.6

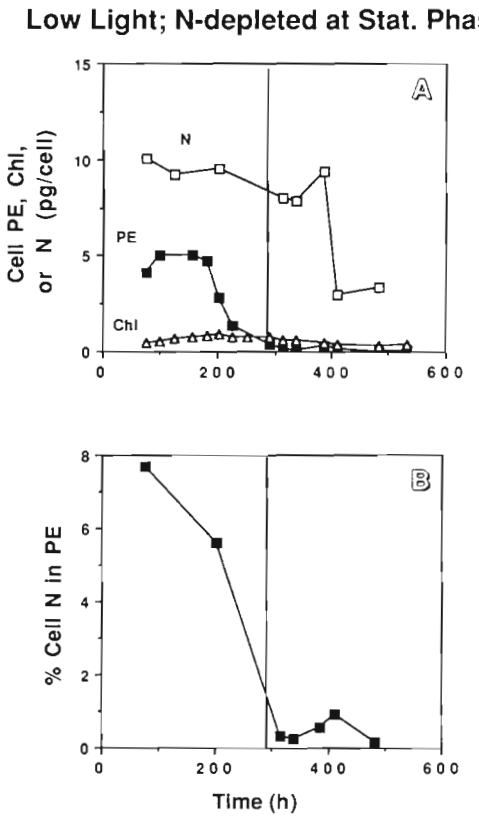

Fig. 6. *Pyrenomonas salina*. (A) Cellular PE, chlorophyll *a*, and nitrogen contents in the Low Light culture nitrogen-depleted during stationary growth. (B) Fraction of cellular nitrogen contained in PE of this same culture. Vertical lines indicate approximate beginning of stationary growth

DISCUSSION

A common response of phytoplankton to nitrogen deprivation is the selective loss of proteins associated with the photosynthetic apparatus (e.g. Hipkin & Syrett 1977, Perry et al. 1981, Coleman et al. 1988a, b). It has been suggested that the mobilization of nitrogen from the photosynthetic components allows continued synthesis of proteins essential for cell maintenance and growth during periods of nitrogen deficiency (Perry et al. 1981). In some species of cyanobacteria, phycobiliprotein content decreases in response to nitrogen stress prior to decreases in chlorophyll *a* content and growth rate (Boussiba & Richmond 1980, Stevens et al. 1981a, Bayer & Schenk 1986, Schenk et al. 1987). Thus, the mobilization of PE as an endogenous nitrogen source appears to be an adaptive response of these cyanobacteria to early stages of nitrogen deficiency. To our knowledge, this study is the first to compare the relative importance of nitrogen deficiency, phosphorus deficiency, and light intensity on the chemical composition and pigment content of a cryptophyte. The patterns in cellular volume, carbon and nitrogen content, and pigment composition observed in this study indicate that, similar to some cyanobacteria, the mobilization of PE may serve as an important endogenous source of nitrogen in *Pyrenomonas salina*, but that phosphorus stress and light intensity also have significant effects on PE content.

Analogous to the situation in cyanobacteria, the adaptive significance of mobilizing nitrogen from photosynthetic components by *Pyrenomonas salina* may lie in the ability of the cell to support growth and/or maintenance during periods of nitrogen deficiency. Because nitrate concentration of the culture medium was not measured in our study, the precise point of nitrogen exhaustion in the nitrogen-depleted cultures was not determined. However, we can assume that, in cultures grown in the medium with a nitrogen to phosphorus ratio of 1, nitrogen was depleted no later than at the onset of stationary growth, and that physiological adaptations in response to nitrogen stress were initiated at some point prior to the stationary growth phase. Based on these assumptions, we present evidence supporting the hypothesis that nitrogen mobilization from PE may sustain growth and/or cellular maintenance in nitrogen-stressed *P. salina*.

Nitrogen-depleted cultures reached the stationary growth phase only after the cellular PE content declined considerably (from 3 to 0.03 pg cell^{-1} at High Light and from 5 to 0.4 pg cell^{-1} at Low Light). Relative to PE, chlorophyll *a* content decreased only slightly during this period (i.e. the PE to chlorophyll *a* ratio decreased precipitously), indicating a preferential loss of PE relative to chlorophyll *a* prior to the cessation of cell division. It is noteworthy that the decline in PE content began during exponential growth. Physiological adaptations to nitrogen depletion in phytoplankton batch cultures are known to occur during exponential growth (Prézelin 1982). The decrease in the PE content of nitrogen-stressed *Pyrenomonas salina* in this study occurred prior to changes in the growth rate, chlorophyll *a* content, biovolume, carbon content, or nitrogen content of the alga. This was especially clear for the nitrogen-depleted culture grown at Low Light (Figs. 4B and 6). The latter values did not change significantly until the stationary growth phase was reached (and after PE content decreased to near-zero values).

The probability that PE was mobilized by *Pyrenomonas salina* for other cellular uses in nitrogen-depleted cultures is supported by the relative changes in nitrogen contained in PE and in the total cellular nitrogen of *P. salina*. These patterns are shown for the culture at Low Light in Fig. 6. The cellular nitrogen content of this nitrogen-depleted culture did not change substantially during the late exponential and early stationary growth phases. The PE concentration

cell^{-1} of this culture, however, decreased dramatically during this same period. It is possible that the mobilization of PE nitrogen allowed the cells to maintain a relatively high nitrogen content during this period, thus delaying the eventual decrease in total cellular nitrogen that occurred in the late stationary growth phase.

Nitrogen depletion had a marked effect on the PE concentration cell^{-1} in *Pyrenomonas salina* as described above. However, both light and phosphorus depletion also had significant effects on the PE concentration of this alga. The PE content and PE to chlorophyll *a* ratio of phosphorus-depleted cultures decreased during the late exponential growth phase, similar to the pattern in the nitrogen-depleted cultures. It is incongruous that PE cell^{-1} declined in a phosphorus-stressed culture if PE is mobilized for nitrogen, because the breakdown of PE would not provide a biosynthetic source of phosphorus. However, loss of phycocyanin in response to phosphorus depletion has been observed in the cyanobacterium *Agmenellum quadruplicatum* concurrent with a decrease in the nitrate uptake rate and total cellular nitrogen content (Stevens et al. 1981b). Given that deficiencies in sulfur (Lawry & Jensen 1979), carbon dioxide (Miller & Holt 1977), or iron (Öquist 1971) can also lead to phycobiliprotein loss in cyanobacteria, the decline in cellular PE content in phosphorus-depleted *P. salina* may reflect a more general response to nutrient deprivation. Interestingly, the decrease in PE concentration cell^{-1} in the nitrogen-depleted culture at Low Light was much greater than the decrease in the phosphorus-depleted culture in this light regime (Fig. 4B, D). At High Light, the decrease in the PE concentration cell^{-1} in the phosphorus-depleted culture occurred at a much slower rate than in the nitrogen-depleted culture, although PE concentration cell^{-1} eventually reached very low values in both cultures (Fig. 4A, C).

The possibility that a decline in the PE content of *Pyrenomonas salina* may be a generalized response to stress is supported by the interaction of phosphorus depletion and the light regime in this study. Although phosphorus depletion resulted in a decrease in the PE concentration cell^{-1}, the magnitude of the decrease was different at the 2 light intensities as mentioned in the previous paragraph (Fig. 4). Measurable concentrations of PE ($>$ 1 pg cell^{-1}) were always present in the phosphorus-stressed culture grown at Low Light. The PE content of the culture grown in the same medium at High Light eventually attained very low values, although the rate of decrease of PE concentration in this culture was much slower than that of the nitrogen-depleted culture. The lower concentrations of PE in the phosphorus-depleted culture at High Light (relative to Low Light) are consistent with our expectations if the loss of PE is a generalized response to stress. The additive effects of phosphorus depletion and continuous high light intensity could account for the greater loss of PE. It is perhaps noteworthy that, in this latter culture, chlorophyll *a* concentration cell^{-1} decreased concomitantly with PE concentration (but did not decrease in the culture grown at Low Light). The interaction of light intensity and PE concentration cell^{-1} was not as evident in the nitrogen-depleted cultures because of the overriding influence of nitrogen deficiency on PE concentrations (Fig. 4A, B).

One consistent effect of the light regime that may have affected PE concentration cell^{-1} was the changes in biovolume of the cryptophyte over the growth cycle (Fig. 3). Increases in cell size during the late exponential and stationary growth phase of batch cultures have been commonly observed in phytoplankton (Fogg 1959, Epstein et al. 1961, Prakash et al. 1973, Lehman 1976). Increased cell size during this period apparently can result both from a decrease in the rate of cell division relative to biomass production and a general shift in cellular metabolism from protein to storage carbohydrate or lipid synthesis (Prakash et al. 1973, Lehman 1976, Ben-Amotz et al. 1985). *Pyrenomonas salina* in the stationary growth phase was larger and contained a greater proportion of carbon to nitrogen than exponentially growing cells for both cultures grown at Low Light. This result is consistent with a shift from protein to carbohydrate or lipid production. The large decreases in average biovolume of *P. salina* during the stationary growth phase of the cultures at High Light are more problematical to explain, but, at least during early stationary growth, may be related to the expenditure of cellular reserves in response to the high metabolic demands of rapidly growing cells that suddenly encounter nutrient-depleted conditions (Prézelin 1982). The continual decline in mean biovolume through late stationary growth is perhaps indicative of cells becoming severely stressed or even dying as a consequence of the additive effects of high light intensity and nutrient deficiency.

This study was not designed to address whether or not 2 separate pools of PE exist in *Pyrenomonas salina*, one for light-harvesting and one for nitrogen storage. Such a controversy exists concerning the role of PE in the cyanobacteria *Synechococcus* spp. (Wyman et al. 1985, 1986, Yeh et al. 1986, Kana & Glibert 1987a, b). In *Synechococcus* WH7803, Kana & Glibert (1987a) argued against a non-photosynthetically coupled pool of PE based partly on the large differences in PE concentration observed in cyanobacteria grown at different light intensities. Although PE concentration in exponentially growing *P. salina* was affected by light intensity (Fig. 4), these differences in PE cell^{-1} and PE (cellular carbon)$^{-1}$ were much less than the differences observed for *Synechococcus*. Given this observation, and the

finding that much of the PE in another cryptophyte, *Rhodomonas lens*, was loosely arranged in the thylakoid lumen (Spear-Bernstein & Miller 1987, but see Ludwig & Gibbs 1989), it is at least possible that PE in *P. salina* provides a small pool of endogenous nitrogen that can be mobilized under the appropriate circumstances.

In summary, we have presented evidence that supports the hypothesis that mobilization of nitrogen from PE is an adaptive response of *Pyrenomonas salina* to nitrogen depletion. In addition, we have demonstrated that phosphorus depletion and the light regime also affected the PE concentration cell^{-1}. These results extend the observations of Lichtlé (1979) and Rhiel et al. (1985) that in cryptophytes, as in cyanobacteria and rhodophytes, phycobiliproteins may be selectively lost during nutrient stress conditions.

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