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Quantum yield, relative specific absorption and fluorescence in nitrogen-limited *Chaetoceros gracilis*

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Abstract

Decreases in cell-nitrogen quota resulted in changes in the carbon-based quantum yield of photosynthesis, the chlorophyll *a*-specific absorption coefficient, and *in vivo* fluorescence in the marine diatom *Chaetoceros gracilis* in laboratory experiments performed in 1983 and 1984. The three parameters were independently determined for the two spectral regions dominated by either chlorophyll *a* or fucoxanthin absorption. As cell-nitrogen quota decreased, the quantum yield for both pigments decreased; the specific absorption coefficient for chlorophyll *a* and the *in vivo* chlorophyll *a* fluorescence excited by each pigment increased. The observed increase in the *in vivo* fluorescence per chlorophyll *a* could be partially attributed to the increased specific absorption coefficient for chlorophyll *a*; the remainder of the fluorescence increase was related to a decline in photosystem activity. Energy transfer efficiency between light-harvesting pigments appeared to be maintained as cell-nitrogen quota decreased. The decrease in a fluorescence index $[(F_{DCMU}\text{-}F_O)/F_{DCMU}]$ with nitrogen starvation suggested a decrease in Photosystem II activity. These results imply that decreases in reaction center and/or electron-transport system activity were responsible for the decline in rates of photosynthesis under conditions of nitrogen deficiency.

Introduction

Numerous models have been developed to estimate primary production directly from chlorophyll *a* concentrations, irradiance, and other environmental parameters. These models generally assume that the quantum yield of photosynthesis and the specific absorption coefficient for chlorophyll *a* do not vary with changes in cell-nitrogen quota (Bannister, 1974; Platt and Jassby, 1976; Laws and Bannister, 1980; Kiefer and Mitchell, 1983). However, nitrogen availability is an important factor in the growth and photosynthesis of phytoplankton in many areas of the ocean (Ryther and Dunstan, 1971; Eppley and Renger, 1974). The present paper examines the effect of varied cell-nitrogen quota on quantum yield, specific absorption, and fluorescence in the marine diatom *Chaetoceros gracilis*.

Nitrogen stress has been shown to cause decreases in both light-limited and light-saturated photosynthetic rates and in quantum yield (Thomas and Dodson, 1972; Welschmeyer and Lorenzen, 1981; Prézelin, 1982; Prézelin and Matlick, 1983; Chalup and Laws, 1986; Perry *et al.*, in preparation, a, b). The observed decreases in light-limited rates of photosynthesis may be due to decreases in one or more of the following: efficiency of absorption of radiant energy per mole of pigment, energy transfer from the light-harvesting pigments to reaction centers, and turnover time or activity of reaction centers and electron-transport systems. The roles of these potential mechanisms were assessed directly or indirectly for nitrogen-limited *Chaetoceros gracilis*.

As a *Chaetoceros gracilis* culture became nitrogen-limited, chlorophyll *a* concentration per cell decreased and absorption per chlorophyll increased. Despite the increased efficiency of absorption per pigment, the overall photosynthetic rate decreased as a result of the large decline in quantum yield. Energy transfer efficiency between light-harvesting pigments was conserved, as determined from changes in quantum yield and fluorescence for light absorbed by chlorophyll *a* and fucoxanthin. *In vivo* chlorophyll *a* fluorescence excited by fucoxanthin was proportional to that excited directly by chlorophyll *a* absorption, and quantum yield based on fucoxanthin absorption was equal to quantum yield for chlorophyll *a*. Together, these results indicated that energy transfer from fucoxanthin to chlorophyll *a* was maintained during nitrogen starvation and that an uncoupling of exciton transfer was not responsible for the observed decrease in quantum yield. The fluorescence index $[(F_{DCMU}\text{-}F_O)/F_{DCMU}$, where F_O is *in vivo* chlorophyll *a* fluorescence and F_{DCMU} is fluo-

rescence in the presence of the photosynthetic electron transport inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)] has been used as an indicator of relative Photosystem II (PSII) activity by Prézelin (1981), Droop (1985), and others. The fluorescence index also declined with decreasing cell-nitrogen quota, suggesting a reduction in PSII activity as a cause of the observed decrease in quantum yield.

Materials and methods

Chaetoceros gracilis [isolated by W. Thomas (1958) from the Gulf of Tehuantepec and maintained in J. Lewin's collection, University of Washington] was grown in seawater enriched with modified Institute of Marine Resources (IMR) media (Perry *et al.*, 1981) at 20 °C, in continuous light provided by an equal mixture of cool and warm-white fluorescent lights at 365 μE m^{-2} s^{-1}. The experiments were performed in 1983 and 1984. A semi-batch stock culture was maintained at its maximal specific growth rate ($\mu = 0.083$ h^{-1}) without nutrient limitation or self-shading for two weeks prior to initiation of the experiment. Four replicate nitrogen-depletion batch cultures were grown, identified as Cultures 1 through 4. Aliquots of stock culture were added to low-nitrate IMR medium and grown with constant sterile aeration under conditions identical to those of the stock culture. For all replicate batch cultures, daily samples were taken for cell number and volume (two replicates), pigments (three replicates), and dissolved nutrients. Quantum yield was measured in Cultures 1, 2, and 3; *in vivo* chlorophyll *a* fluorescence (five replicates) in Cultures 1 and 4; and specific absorption coefficients (three replicates) in Culture 4.

Cell density and volume were measured on a Particle Data, Inc., Electrozone/Celloscope. Concentrations of nitrate and ammonium were measured using a Technicon AutoAnalyzer (Friederich and Whitledge, 1972). Cell-nitrogen quota (N_q), the total mass of nitrogen contained in each cell (Droop, 1968; Goldman and McCarthy, 1978), was calculated from the increase in cell number and the disappearance of total dissolved nitrogen from the medium:

$$N_q = (N_i - N_t)/(C_t - C_i),$$

where N_i and N_t are the dissolved nitrogen concentrations initially and at time t, and C_i and C_t are the cell numbers initially and at time t.

Concentrations of chlorophyll *a* and *c* were measured spectrophotometrically in acetone extracts (Strickland and Parsons, 1972) using the equations of Jeffrey and Humphrey (1975). Fucoxanthin was extracted in methanol and the extract shaken with hexane. The absorption at 450 nm was assumed to be due to fucoxanthin; fucoxanthin concentration was calculated from the 450 nm absorption of the methanol hypophase (Ramus *et al.*, 1977; Ramus, 1983).

Relative specific absorption coefficients for chlorophyll *a* (a^*_c) and fucoxanthin (a^*_f) were determined by normalizing absorbances to pigment concentrations. Relative absorbances were determined from the optical densities of cell suspensions measured in the integrating sphere between 400 and 450 nm for chlorophyll *a* (a_c) and between 514 and 584 nm for fucoxanthin (a_f). The absorbances were relative measurements because determination of absolute absorbance requires accurate measurement of the optical pathlength:

$$\text{absorbance} = (1/\text{pathlength}) \log (E_1/E_2),$$

where E_1 is the irradiance transmitted through distilled water and E_2 is the irradiance transmitted through a cell suspension (Beer's law: Glasstone, 1946). We were not able to correctly determine the pathlength in the integrating sphere due to multiple scattering and absorption effects and, therefore, report only relative values for absorbance and specific absorption coefficients:

$$a_c = \log (E_1/E_2)_{400-450\text{ nm}}$$

$$a^*_c = a_c\,[\text{chl}]^{-1},$$

and

$$a_f = \log (E_1/E_2)_{514-584\text{ nm}}$$

$$a^*_f = a_f\,[\text{fuc}]^{-1},$$

where [chl] and [fuc] are chlorophyll and fucoxanthin concentrations, respectively. However, the relative specific absorption coefficients measured on different days can be compared because the pathlength was maintained at a constant value by adjusting the cell density to 2×10^8 cells l^{-1}. The cell size, hence the scattering properties of the cells, remained unchanged for the duration of the experiment.

The quantum yield of photosynthesis was measured as mol C E^{-1} (moles carbon fixed per moles of photons absorbed) at irradiances limiting to photosynthesis. Carbon uptake and light absorption were measured simultanously in an integrating sphere (Welschmeyer and Lorenzen, 1981) using a General Electric 150 W Quartzline lamp as the light source. A blue, short band-pass filter (Melles Griot 03 SWP011) transmitted light of 400 to 450 nm for absorption by chlorophyll *a*. A green dichroic filter (Optical Coating Laboratory, Inc.) transmitted light of 514 to 584 nm for absorption by fucoxanthin.

The culture was concentrated for fluorescence measurements to approximately 100 μg chl *a* l^{-1} by gently siphoning the seawater supernatant through 10 μm Nitex netting. The concentrated samples were dark-adapted at room temperature for 30 to 45 min to stabilize and maximize fluorescence (Kiefer, 1973a; Harris, 1978; Heaney, 1978). Fluorescence excitation spectra were measured with a Farrand Mark I spectrofluorometer with a red-sensitive photomultiplier and a corrected excitation module to compensate for variations in lamp energy output and energy with wavelength. The excitation wavelengths scanned were 400 to 670 nm at 100 nm min^{-1}, with a 5 nm bandwidth; the emission wavelength was held constant at 680 nm, with a 10 nm bandwidth. Baseline spectra on filtered culture media exhibited no fluorescence. Replicate spectra were

performed on fresh samples. Chlorophyll *a* fluorescence was excited by light absorbed by chlorophyll *a* or by light absorbed by fucoxanthin and transferred to chlorophyll *a*. Light energy at 430 nm is primarily absorbed by chlorophyll *a*, while at 530 nm energy is primarily absorbed by fucoxanthin (Mann and Myers, 1968; Barrett and Anderson, 1980). The intensity of fluorescence excited at 430 nm ($F430$) and 530 nm ($F530$) was obtained from replicate spectra. Fluorescence was normalized to the concentration of the respective pigment ($F430$/chl or $F530$/fuc). Additionally, a relative fluorescence yield was calculated by normalizing the relative fluorescence emission to the absorbance for chlorophyll or fucoxanthin ($F430/a_c$ or $F530/a_f$).

For F_{DCMU} measurements, DCMU was added to a final concentration of 4 μM (Blasco and Dexter, 1972; Kiefer and Hodson, 1974; Cullen and Renger, 1979). Four-micromolar DCMU produced a maximal fluorescence reading. The samples were kept in the dark for 30 to 40 min prior to measurement to ensure complete uncoupling of photosynthetic electron transport; preliminary tests showed that F_{DCMU} reached a stable maximum by 20 to 30 min. Other studies have found both immediate and delayed F_{DCMU} maxima (Cullen and Renger, 1979; Prézelin and Ley, 1980), suggesting a species-specific diffusion rate through cell walls or a cell density-dependent effect. The fluorescence index $[(F_{DCMU}-F_O)/F_{DCMU}]$ was calculated for fluorescence excited at 430 nm.

Statistical significance of changes in the various parameters was examined by Student's *t*-tests of the slopes of the relationships. All trends discussed were significant at probabilities < 0.05 or better, unless otherwise noted.

Results

Nitrogen was depleted from the media between Days 1 and 2 in all replicate cultures (Fig. 1a). Initially, specific growth rate for *Chaetoceros gracilis* was maximal ($\mu = 0.083\ h^{-1}$), but with the onset of nitrogen depletion the growth rate declined to $0.020\ h^{-1}$ between Days 2 and 3. As nitrogen deprivation continued, growth ceased after Day 4 (Fig. 1b). The cell-nitrogen quota decreased from 38×10^{-13} g N cell^{-1} on the first day to 25×10^{-13} on Day 2 and to 16×10^{-13} on Day 3 (Fig. 1c). This reduction in cell-nitrogen allowed cell number to increase even after nitrogen had been completely removed from the media. Protein nitrogen was mobilized from the cells, primarily from the photosynthetic carboxylating enzyme ribulose-1,5-bisphosphate carboxylase and the light-harvesting pigment proteins (Perry *et al.*, in preparation, a, b). Mobilized nitrogen was reallocated to daughter cells and was responsible for continued cell division during the early stages of nitrogen depletion. When the cell-nitrogen quota reached a minimal value of 12×10^{-13} g N cell^{-1}, cell division ceased. However, despite the reduction in cell-nitrogen quota, cell volume showed no statistically significant variation with time or among replicate cultures (mean $= 113.4 \times 10^{-15}$ l cell^{-1}, 95% confidence interval $= 110$ to 117×10^{-15} l cell^{-1}).

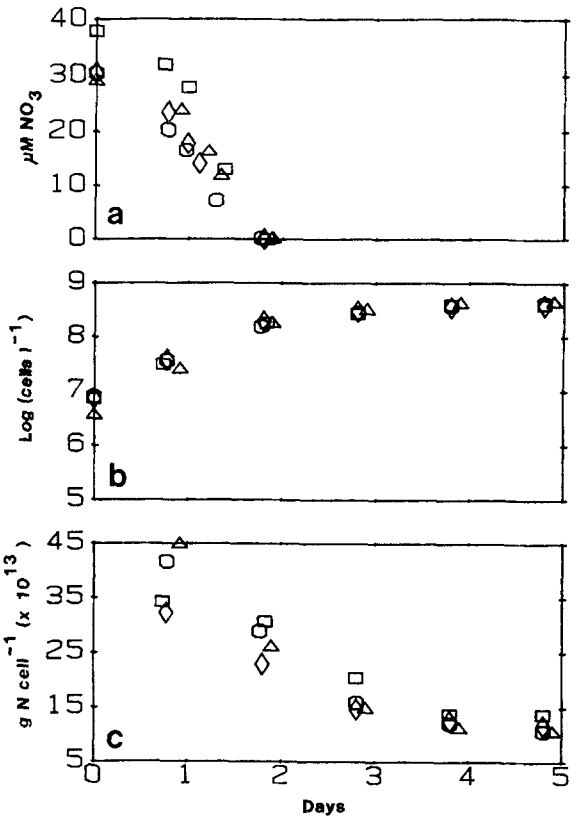

Fig. 1. *Chaetoceros gracilis.* Time course of changes in (a) media-nitrate concentration, (b) cell density, and (c) cell-nitrogen quota for four replicate cultures; △: Culture 1; □ : Culture 2; ◇ : Culture 3; ○: Culture 4

The cellular concentrations of chlorophyll *a*, *c*, and fucoxanthin declined with similar patterns; the concentrations are plotted as functions of cell-nitrogen quota in Fig. 2a, b, and c. The average coefficient of variation for pigment measurements was 8.9%. The ratio of fucoxanthin to chlorophyll *a* increased with decreasing cell-nitrogen quota (Fig. 2d), indicating that chlorophyll *a* was preferentially degraded (as in Manny, 1969). The ratios of chlorophyll *c* to chlorophyll *a* and of fucoxanthin to chlorophyll *c* did not exhibit any statistically significant trends as a function of cell-nitrogen quota.

The relative specific absorption coefficient for chlorophyll *a* (a^*_c) increased as the cellular concentration of chlorophyll *a* decreased (Fig. 3). For the lowest chlorophyll *a* concentration per cell, a^*_c increased to 155% of its original value. The specific absorption coefficient for fucoxanthin contrasted with that for chlorophyll *a* in that a^*_f did not change throughout the experiment; the small variability in a^*_f (Fig. 3) was not statistically significant. The average coefficient of variation for absorbance measurements was 2.0%.

The quantum yield of photosynthesis decreased as cell-nitrogen quota decreased (Fig. 4). Maximum quantum yield was 0.0490 mol C E^{-1} for chlorophyll *a* and 0.0483 mol C E^{-1} for fucoxanthin. The magnitude of quantum yield and the pattern of decline was statistically similar for light absorbed by either chlorophyll *a* or fu-

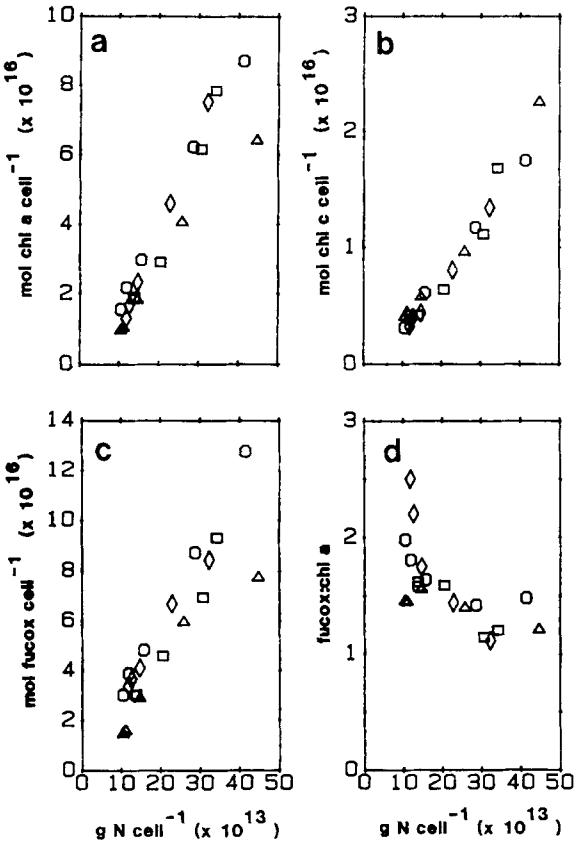

Fig. 2. *Chaetoceros gracilis.* Changes in concentrations of (a) chlorophyll *a*, (b) chlorophyll *c*, (c) fucoxanthin, and in (d) ratios of fucoxanthin to chlorophyll *a* in relation to decreasing cell-nitrogen quota during growth. Culture symbols as in Fig. 1

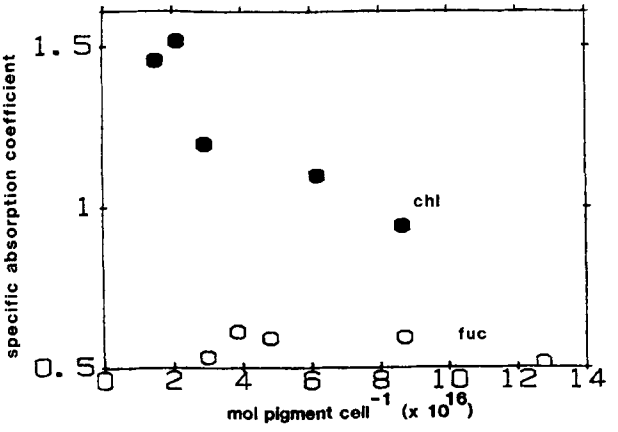

Fig. 3. *Chaetoceros gracilis.* Variations in specific absorption coefficients for chlorophyll, a_c^* (●) and fucoxanthin, a_f^* (○) as a function of cellular concentration of the respective pigment. Data are from Culture 4

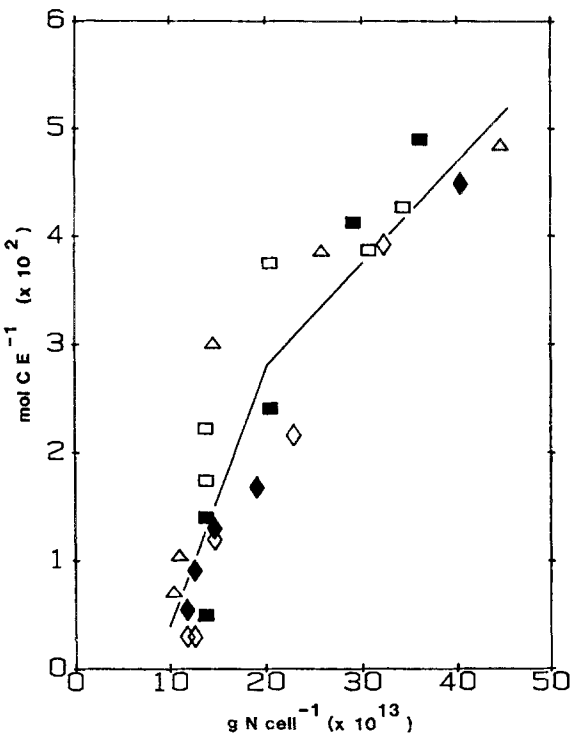

Fig. 4. *Chaetoceros gracilis.* Quantum yield of photosynthesis as a function of cell-nitrogen quota in Cultures 1 (△), 2 (■ , □) and 3 (◆, ◇). Filled symbols are chlorophyll, open symbols fucoxanthin. mol C E^{-1} = moles carbon fixed per Einstein absorbed

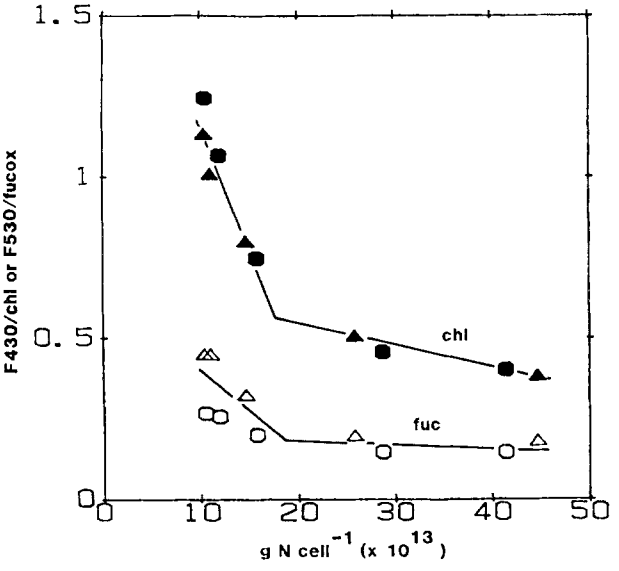

Fig. 5. *Chaetoceros gracilis.* Variations in fluorescence excited by chlorophyll and normalized to chlorophyll concentration (*F*430/chl) or excited by fucoxanthin and normalized to fucoxanthin concentration (*F*530/fuc) as a function of cell-nitrogen quota in Cultures 1 (▲, △) and 4 (●, ○). Filled symbols are *F*430/chl, open symbols *F* 530/fuc. Fluorescence is in relative units

coxanthin (Fig. 4; $p > 0.05$, analysis of covariance for logarithmic regression lines). It should be noted that changes in a^*_c are automatically incorporated into measurements of quantum yield, but such changes are not incorporated into the standard calculations of the initial slope of photosynthesis vs irradiance curves (i.e., "alpha"). Variations in a^*_c may be responsible for much of the reported variability in "alpha".

Chlorophyll *a* fluorescence, normalized to pigment concentration, increased for both chlorophyll *a* (*F*430/chl) and fucoxanthin (*F*530/fuc) as cell-nitrogen quota decreased (Fig. 5). The average coefficient of variation for

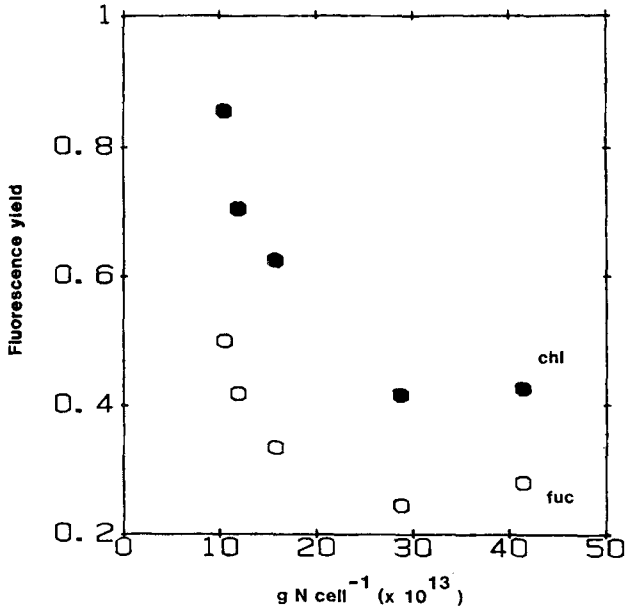

Fig. 6. *Chaetoceros gracilis.* Variations in relative fluorescence yield with cell-nitrogen quota in Culture 4. ●: $F430/a_c$; ○: $F530/a_f$. Fluorescence units are relative

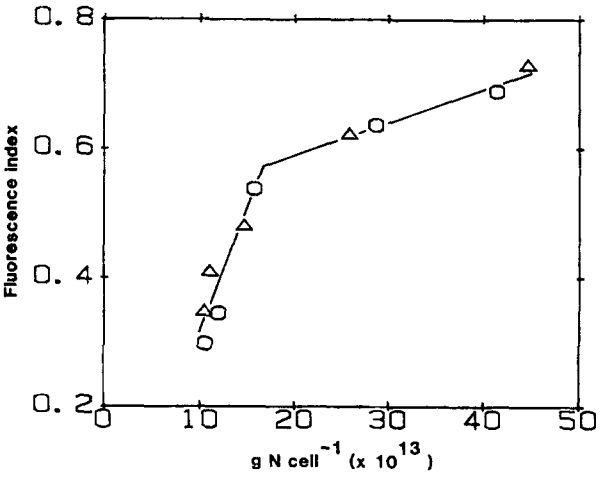

Fig. 7. *Chaetoceros gracilis.* Changes in fluorescence index $[(F_{DCMU} - F_O)/F_{DCMU}]$ as a function of cell-nitrogen quota in Cultures 1 (△) and 4 (○). Fluorescence index is dimensionless

fluorescence values was 2.4%. By the termination of the experiment $F430$/chl increased to 312% of the initial value; in contrast, $F530$/fuc increased to only 185% of its original value (Table 1). A relative fluorescence yield was also calculated as the intensity of fluorescence emission per photon absorbed; the fluorescence yield is similar in principle to the quantum yield of photosynthesis in that fluorescence is normalized to light absorbed by either chlorophyll *a* ($F430/a_c$) or fucoxanthin ($F530/a_f$). By the termination of the experiment, $F430/a_c$ had increased to 202% and $F530/a_f$ to 179% (Fig. 6). The changes in $F530/a_f$ and $F530$/fuc were identical (i.e., 179 and 185%, respectively), while the changes in $F430/a_c$ and $F430$/chl were not (i.e., 202 vs 312%). These changes were consistent with the observation that absorption per chlorophyll *a* (a^*_c) increased

Table 1. *Chaetoceros gracilis.* Percentage change in fluorescence normalized to pigment concentrations ($F430$/chl and $F530$/fuc; Column 1), relative specific absorption coefficients (a^*_c and a^*_f; Column 2) and relative fluorescence yields ($F430/a_c$ and $F530/a_f$; Column 3) for nitrogen-limited cultures at termination of a five-day experiment. F/pigment = ($a^*_{pigment}$) (F/absorbance)

Pigment	Percentage change from Day 1 to Day 5		
	(1) F/pigment	(2) $a^*_{pigment}$	(3) F/absorbance
Chlorophyll	312	155	202
Fucoxanthin	185	103	179

to 155%, but that absorption per fucoxanthin (a^*_f) did not change. The DCMU fluorescence index decreased to 46% of the initial value with decreasing nitrogen quota (Fig. 7).

The pattern of changes in quantum yield for chlorophyll and fucoxanthin, fluorescence per mole of pigment, fluorescence yield, and the fluorescence index plotted against cell-nitrogen quota all exhibited two linear trends. These parameters had steeper slopes associated with the lower cell-nitrogen quota (Figs. 4–7). The break between the two linear regions occurred at approximately 20×10^{-13} g N cell^{-1}.

Discussion

Variability in quantum yield, absorption and fluorescence are important to the success of models of primary production based on the optical and photosynthetic properties of phytoplankton (Marra and Hartwig, 1984). The changes observed in these properties in the present study on *Chaetoceros gracilis* suggested that the decrease in photosynthetic rate under nitrogen stress was due to decreased reaction center or electron-transport activity. The changes that occurred as cell-nitrogen quota decreased were: (1) cellular concentrations of chlorophyll *a*, *c*, and fucoxanthin decreased, with fucoxanthin decreasing to a lesser extent than chlorophyll *a;* (2) relative specific absorption a^*_c increased, but a^*_f not did change; (3) quantum yield decreased, with no significant difference between chlorophyll *a* and fucoxanthin quantum yields at any cell-nitrogen quota; (4) *in vivo* chlorophyll *a* fluorescence increased for both $F430$/chl and $F530$/fuc; (5) relative fluorescence yield $F430/a_c$ and $F530/a_f$ increased for chlorophyll *a* and fucoxanthin; (6) the fluorescence index $[(F_{DCMU}-F_O)/F_{DCMU}]$ decreased. The general trends of changes in pigment ratios, specific absorption coefficient for chlorophyll *a*, and fluorescence per pigment as cell-nitrogen quota decreased were consistent with earlier studies (Manny, 1969; Kiefer, 1973b; Morel and Bricaud, 1981). The specific pattern of decrease in photosynthetic parameters as a function of decreasing cell-nitrogen quota suggested a bimodal response to nitrogen limitation that could be best described by two lines (Figs. 4–7). Quantum yield and fluorescence changed less quickly in the early stages of nitrogen limi-

tation than in later stages when nitrogen limitation intensified. In contrast, the pigment content per cell exhibited a pattern that could be described by a single line. The difference between the patterns for pigment concentration and photosynthetic activity suggested an accelerated loss of photosynthetic function during the later phase of nitrogen limitation, as suggested by Perry *et al.* (in preparation, b).

Pigment changes

The increase in the ratio of fucoxanthin to chlorophyll *a* was consistent with the results of previous studies which showed an increased ratio with nitrogen starvation (Shimura and Fujita, 1975) and a decreased ratio with nitrogen enrichment (Yentsch and Vaccaro, 1958; Manny, 1969; Vince and Valiela, 1973). Assuming that the molecular ratio of pigments in the pigment-protein complexes in *Chaetoceros gracilis* were the same as have been found in the diatom *Skeletonema costatum* (Alberte *et al.*, 1981), the observed changes in pigment ratios were the result of net losses of pigment-protein complexes in the proportions of 2 chlorophyll *a* to 3 chlorophyll *a*/*c* to 1 chlorophyll *a*/fucoxanthin.

Specific absorption

The increase in a^*_c as cellular concentrations of chlorophyll *a* decreased (Fig. 3) may be a reflection of decreased self-shading of pigment molecules, photosynthetic units or chloroplasts. Other investigators have also suggested that absorption coefficients change with the cellular concentration of chlorophyll *a*. Taguchi (1976), in explaining why light-limited photosynthetic rate varies inversely with cell size, suggests that light-limited photosynthetic rate is controlled by self-shading of chloroplasts and that cells with fewer or smaller chloroplasts use incident radiation more efficiently because pigments are exposed more effectively to the incident light. Perry *et al.* (1981) observes that photosynthetic efficiency per photosynthetic unit (PSU) increases with increasing PSU size. However, for PSU sizes greater than 1 000, photosynthetic efficiency does not increase; these results suggest that self-shading occurs within large PSUs. The models of Platt and Jassby (1976) for light-limited photosynthetic rate and of Morel and Bricaud (1981) for specific absorption imply that, for constant cell size, cells with more pigment absorb less energy per molecule of pigment. These models are consistent with the results shown in Fig. 3. Privoznik *et al.* (1978) reported absorption, extinction, and scattering cross-sections for *Chlorella pyrenoidosa* in different growth stages. When we normalized their absorption cross-section at 440 nm to chlorophyll *a* per cell for *C. pyrenoidosa*, a similar trend was evident: exponentially growing cells with more chlorophyll *a* per cell had a lower absorption cross-section per chlorophyll *a* [0.0053 m^2 (mg chl *a*)$^{-1}$] than stationary phase cells with lower chlorophyll *a* per cell [0.0132 m^2 (mg chl *a*)$^{-1}$]. The influence of chloroplast density on absorption has also been demonstrated by Kiefer (1973 b), who observed decreases in absorption and fluorescence after chloroplasts migrated and aggregated in response to bright light. Although concentrations of fucoxanthin per cell decreased in the present study, a^*_f did not change (Fig. 3). Fucoxanthin is believed to be in an "exterior" position in the photosynthetic unit (Alberte *et al.*, 1981); as a result, fucoxanthin may be less subject to shading by other pigment molecules even when fucoxanthin concentration per cell is high.

Quantum yield

Quantum yield was lower when cell-nitrogen quota was low, as found in experiments with *Thalassiosira pseudonana* in white light (Welschmeyer and Lorenzen, 1981). In nitrogen-limited chemostat cultures, quantum yield for *Pavlova lutheri* decreased as growth rate decreased (Chalup and Laws, 1986). The ability of the photosynthetic unit to convert visible radiant energy to chemical energy was impaired during nitrogen deficiency; energy was absorbed, but was not efficiently utilized. Quantum yield began to decrease on Day 2 when the nitrogen in the medium became depleted (Figs. 1 a and 4). Cell division continued, causing the cell-nitrogen quota to drop. The observed decrease in quantum yield was not an artifact caused by absorption by dead cells since the cells were still dividing and growing, albeit at slower rates. Quantum yield at low light is often assumed to be constant (Bannister, 1974; Platt and Jassby, 1976; Taguchi, 1979). However, the results of the present experiments demonstrated that nutrient supply was important in determining quantum yield: quantum yield decreased to 17% of the initial value.

Energy transfer

High efficiencies of energy transfer from carotenoids to chlorophyll *a* have been established for nitrogen-sufficient algae, for example, in isolated chlorophyll *a*/fucoxanthin-protein complexes (Alberte *et al.*, 1981) and in isolated peridinin-chlorophyll *a*-protein complexes (Prézelin and Haxo, 1976; Song *et al.*, 1976; Siegelman, 1977). Our data on quantum yield and relative fluorescence yield suggested that the efficiency of energy transfer from fucoxanthin to chlorophyll *a* was unaffected by nitrogen stress. Quanta absorbed by chlorophyll *a* and fucoxanthin were equally efficient in performing photosynthesis at all cell-nitrogen quotas; quantum yield for both pigments decreased at statistically equal rates as cell-nitrogen quota decreased (Fig. 4). These results indicated that the energy absorbed by fucoxanthin and transferred to chlorophyll *a* was affected by nitrogen deficiency at a rate-limiting step that was common to both chlorophyll *a* and fucoxanthin, i.e., a step following absorption and energy transfer. [It should be noted that quantum yield and photosynthetic action spectra

are not comparable measurements. Photosynthetic action spectra exhibit large variations with wavelength (cf. Haxo and Blinks, 1950; Haxo, 1960) because action spectra are not normalized to absorption and because absorption coefficients do vary with wavelength.] The comparable increases in fluorescence yield for both chlorophyll and fucoxanthin ($F430/a_c = 202\%$; $F530/a_f = 179\%$; Table 1) indicated that efficient energy transfer from fucoxanthin to chlorophyll *a* continued during nitrogen starvation. The changes in quantum yield, absorption and fluorescence imply that decreased reaction center or electron-transport activity was responsible for the decrease in quantum yield.

Fluorescence

Fluorescence increased as cell-nitrogen quota decreased (Figs. 5 and 6; Table 1). The inverse relationship between quantum yield and fluorescence was consistent with the interpretation of variable fluorescence as a de-excitation mechanism when reaction centers are closed and photochemistry cannot be performed (Kiefer, 1973a, b; Samuelsson and Öquist, 1977; Butler, 1978; Falkowski and Kiefer, 1985). We have normalized fluorescence to both pigment concentration ($F430$/chl and $F530$/fuc) and absorbance ($F430/a_c$ and $F530/a_f$) to determine the effect of changes in absorption on fluorescence. For light absorbed by fucoxanthin, the specific absorption coefficient (a^*_f) did not change and increases in $F530$/fuc and $F530/a_f$ were identical ($\sim 180\%$; Table 1). The entire observed increase in $F530$/fuc was related to a decrease in photochemical efficiency. However, the specific absorption coefficient for chlorophyll a (a^*_c) did change (Fig. 3), and hence, the pattern of changes in chlorophyll-excited fluorescence differed from that for fucoxanthin-excited fluorescence. The chlorophyll *a*-excited fluorescence ($F430$/chl) increased to 312%, while the fluorescence yield ($F430/ac$) increased by a smaller amount, to only 202%. These results suggested that the increase in $F430$/chl was partly a result of the increase in a^*_c (Fig. 3) and partly a result of photochemical uncoupling. These data indicated the importance of changes in specific absorption to fluorescence emission per chlorophyll. Alpine and Cloern (1985) observed higher fluorescence per chlorophyll for smaller size-classes of phytoplankton from San Francisco Bay. This may be due to higher specific absorption coefficients for smaller cells (Kirk, 1975; Taguchi, 1976). In studies where variability in fluorescence per chlorophyll or variability in the relationship between fluorescence and photosynthetic rate has been observed (cf. Prézelin, 1981; Falkowski and Kiefer, 1985), changes in specific absorption may have contributed to the variability in fluorescence. Normalizing fluorescence to absorbance instead of chlorophyll concentration may provide more information about photosynthetic rates.

The fluorescence index $[(F_{DCMU}\text{-}F_O)/F_{DCMU}]$ has been used as a relative indication of PSII activity (Prézelin, 1981; Öquist *et al.*, 1982; Droop, 1985). DCMU inhibits photosynthesis by blocking electron transport from Q, the primary electron acceptor in PSII (Lavergne, 1982). When electron transport is blocked, reduced Q is unable to transfer electrons to the plastoquinone pool and cannot be reoxidized. This blockage increases *in vivo* fluorescence; the enhanced fluorescence is believed to be related to the amount of energy that would have been used for PSII photochemistry if DCMU were not present (Falkowski and Kiefer, 1985). In the present study, both the quantum yield and the fluorescence index decreased with decreasing nitrogen quota (Figs. 4 and 7). Similar results were obtained by Perry *et al.* (in preparation, a). Together, these data suggested that decreases in nitrogen-limited photosynthesis resulted from decreases in photosystem activity.

Variability in quantum yield

In a number of previous studies, quantum yield and specific absorption have been assumed to be constant (Bannister, 1974; Platt and Jassby, 1976; Laws and Bannister, 1980). Kiefer and Mitchell (1983) used quantum yield and absorption cross-section to develop a carbon-based model for phytoplankton growth: $\mu + r = \Phi\ (^{\circ}a^*_p)$ (chl/C) E_o. They modelled specific gross production rate ($\mu + r$), where μ is the specific growth rate and r is the respiration rate, as a function of quantum yield (Φ), mean specific diffuse-absorption-coefficient normalized to chlorophyll *a* concentration ($^{\circ}a^*_p$), chlorophyll to carbon ratio (chl/C), and scalar irradiance (E_o). Using data from Laws and Bannister (1980), Kiefer and Mitchell (1983) showed that the slope of $(\mu + r)$ versus [(chl/C) E_o] for nutrient-limited *Thalassiosira weissflogii* is constant, indicating that the product $[\Phi\ (^{\circ}a^*_p)]$ must be constant as well. Kiefer and Mitchell assumed both terms are constant and concluded that quantum yield does not vary with nutrient supply. However, with data from the present study, these two terms can be evaluated separately [Our coefficient a^*_c is analogous to Kiefer and Mitchell's $^{\circ}a^*_p$; a^*_c is an optical density measured over the spectral band 400 to 450 nm and $^{\circ}a^*_p$ is a diffuse absorption coefficient, measured on a glass-fiber filter and averaged over 400 to 700 nm.]. We found that Φ decreased to 17% of the initial value, while a^*_c increased to 155% of the initial value as nitrogen quota decreased. Because the two terms changed in opposite directions, the product $[\Phi\ (a^*_c)]$ changed more slowly than either individual term, and decreased to 26% of the initial value. The changes in Φ and $^{\circ}a^*_p$ in Kiefer and Mitchell (1983) may have been proportional over the range they investigated, thus generating a constant product and leading them to infer constancy of the individual terms.

Field measurements of quantum yield

The measurements of quantum yield in this study and in the work of Welschmeyer and Lorenzen (1981) provide maximal values for quantum yield; i.e., at irradiances limiting to photosynthesis and where the greatest proportion of

available irradiance is absorbed and used in photochemistry. Previous field estimates of quantum yield were based on *in situ* productivity incubations and, hence, provided both maximal and submaximal quantum yields (Tyler, 1975; Morel, 1978; Taguchi, 1979; Dubinsky and Berman, 1981; Bannister and Weidemann, 1984; Dubinsky *et al.*, 1984). Submaximal yields are obtained when irradiances are not limiting to photosynthesis (i.e., beyond the initial slope of photosynthesis vs irradiance curves). The observed increases in quantum yield with depth in the above studies were a direct function of decreasing *in situ* irradiance. As irradiance approaches values limiting to photosynthesis, quantum yield approaches a maximal value. Measurement of maximal quantum yields may be useful in diagnosing nitrogen deficiency in nutrient-impoverished areas of the ocean, whereas measurement of *in situ* quantum yields could be useful in determining the vertical transition between light-saturated and light-limited photosynthesis.

Conclusions

The quantum yield of photosynthesis, specific absorption coefficient, fluorescence per pigment, and fluorescence yield all varied as a function of cell-nitrogen quota. Quantum yield decreased, apparently due to a decrease in reaction center or electron-transport activity. Specific absorption increased, as a result of reduced self-shading as chlorophyll per cell decreased. Fluorescence per pigment increased, due in part to an increased efficiency of absorption per chlorophyll and in part to photosynthetic uncoupling (manifested in the reduced quantum yields). The trends of variability in these parameters have implications for modelling primary production from the optical properties of phytoplankton. Models such as Kiefer and Mitchell's (1983) will have to incorporate terms for variable Φ and variable a^*_c in order to estimate growth and production rates. The observation that fluorescence per photon absorbed varied less than fluorescence per unit chlorophyll has implications for correlating fluorescence with photosynthetic parameters, whether for *in situ* measurements or remote-sensing studies. Because nitrogen availability affected both quantum yield and specific absorption, direct measurement of maximal quantum yield in nutrient- deplete areas of the ocean may be a useful tool in determining nutritional status.

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