FLUORESCENCE QUENCHING IN PHYTOPLANKTON OF THE MCMURDO DRY VALLEY LAKES (ANTARCTICA): IMPLICATIONS FOR THE STRUCTURE AND FUNCTION OF THE PHOTOSYNTHETIC APPARATUS

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Phytoplankton in perennially ice-covered lakes of the McMurdo Dry Valleys experience a light environment that is unusually stable due to constant shade (< 1–3% of incident) and a narrow spectral distribution (blue-green). This relative constancy is due to optical attenuation and spectral filtering through the ice cover and an absence of vertical mixing. We have studied the structure and function of the photosynthetic apparatus of phytoplankton in Lakes Bonney, Fryxell, and Hoare (Taylor Valley) using a variety of methods. Some photosynthetic characteristics of phytoplankton in the dry valley lakes indicate low-light acclimation, including a low irradiance for the onset of light saturation of photosynthesis (I_k) and high sensitivity to photoinhibition. Other characteristics seem contrary to expectations for an extreme shade environment. Antenna sizes are not large (average Chl:P_{700} = 743 mol mol^{-1}) and the maximum quantum yield of photosynthesis is low. We obtained further information on the structure and function of the photosynthetic apparatus in these phytoplankton through analysis of the slow (minutes time-scale) fluorescence transients. Our approach used a sensor for upwelling radiance at 683 nm (natural fluorescence) mounted over a transparent container and illuminated with fixed intensity blue-green irradiance. For samples from Lake Bonney and Lake Hoare, the steady-state fluorescence yield (F_s) after about five minutes of illumination was lower (quenched) for irradiances greater than 10 µmol photons m^{-2} s^{-1}. This indicated induction of protective mechanisms to dissipate excess excitation irradiance (non-photochemical quenching) at an unprecedented low irradiance. In situ, these assemblages appear to be at, or just below, the threshold for the induction of non-photochemical quenching. Conversely, the Lake Fryxell assemblage had a high maximum quantum yield of photosystem II photochemistry and did not show F_s quenching at irradiances up to 100 µmol photons m^{-2} s^{-1}. Overall the results are consistent with photosynthetic acclimation to minimize excitation pressure, i.e., an energetic imbalance between photochemical sources and metabolic sinks. Acclimation of the dry valley phytoplankton assemblages is constrained by a strikingly narrow irradiance range from low to high excitation pressure. We hypothesize that optimum light harvesting subject to maintenance of low excitation pressure is possible because of the constant shade environment.

INTRODUCTION

Phytoplankton in the lakes of the McMurdo Dry Valleys, Antarctic grow in a unique environment due to a perennial ice-covers 3–5 m thick. In Lake Bonney, irradiance beneath the ice is less than 1 to 3% of incident, with maximum irradiance less than 50 µmol photons m^{-2} s^{-1} [*Priscu*, 1991; *Lizotte and Priscu*, 1992b]. In addition, the ice cover selectively transmits blue-green irradiance, with maximum flux near 500 nm. Irradiances at wavelengths shorter than 440 nm have intensities of <50% relative to the spectral peak, and irradiances at wavelengths longer than 600 nm have intensities lower than 10% of the spectral peak

TABLE 1. Symbols and Abbreviations Used in the Text

Symbol	Description	Units
Chl	Chlorophyll-*a*	mg m^{-3}
F'_0	Minimum fluorescence yield after actinic illumination	m^{-1} (see footnote)
F'_m	Maximum fluorescence yield under actinic illumination	m^{-1}
F_0	Minimum fluorescence yield after dark adaptation	(see footnote)
F_m	Maximum fluorescence yield after dark adaptation	(see footnote)
Φ_m	Maximum quantum yield of PSII photochemistry (electrons generated per photons absorbed)	dimensionless
Φ_p	Quantum yield of PSII photochemistry (electrons generated per photons absorbed)	dimensionless
F_s	Steady state fluorescence yield under actinic illumination	m^{-1}
$F_s(max)$	Maximum F_s	m^{-1}
I	Irradiance	µmol photons m^{-2} s^{-1}
Lu_{683}	Upwelling radiance at 683 nm	nmol photons m^{-2} s^{-1} steradian^{-1}
P_{700}	Photosystem I reaction center	
PAM	Pulse Amplitude Modulation fluorometry	
PAR	Photosynthetically Available Radiation (400–700 nm)	µmol photons m^{-2} s^{-1}
P^B_m	Light saturated rate of photosynthesis normalized to biomass	mg C mg Chl^{-1} h^{-1}
PSII	Photosystem II	

The fluorescence yields measured with the PNF have not been adjusted for absorption, thus units are given as m^{-1}, this is equivalent to the term "fluorescence coefficient" used by *Chamberlin et al.* [1990]. Fluorescence measured on the Turner Designs (F_0 and F_m) are on a arbitrary output scale (volts).

[*Lizotte and Priscu*, 1992b; *Howard-Williams et al., this volume*].

The ice cover also prevents stirring of lake waters by surface winds. The McMurdo Dry Valley lakes are highly stratified [*Angino et al.*, 1964], and turbulence is very low or non-existent [*Spigel and Priscu,* this volume]. Owing to the lack of vertical mixing and the continuous daylight characteristic of the Antarctic summer, the phytoplankton in the dry valley lakes grow in an unusually stable shade environment [*Vincent*, 1981b]. This setting offers a unique opportunity to examine the acclimation of natural phytoplankton populations to their irradiance environment.

Light availability appears to be a primary factor regulating phytoplankton growth in the dry valley lakes. Photosynthesis-irradiance response curves imply that phytoplankton photosynthesis is always light limited [*Lizotte and Priscu*, 1992a]. Likewise, seasonal variation in primary production by different biomass maxima is linearly related to available irradiance [*Lizotte et al.*, 1996]. Survival of a given population depends on the efficiency with which light is absorbed and used for photosynthesis, both processes are dependent on the structure and function of the photosynthetic apparatus.

In addition to low intensity, irradiance spectral distribution affects light utilization by phytoplankton of the dry valleys. Most non-phycobilin containing eukaryotic microalgae (and in particular chlorophytes) have a lower efficiency of photosynthesis in blue-green light compared to other wavebands (review *Larkum and Barrett* [1983]). Better utilization of blue-green light could be achieved through synthesis of light-harvesting pigment-protein complexes which absorb in the predominant spectral bands and efficiently transfer energy to the reaction center, increasing the maximum quantum yield for photosynthesis. Such chromatically

selective pigmentation has been reported for several algal groups, but has been rarely reported for cryptophytes and chlorophytes [*Larkum and Barrett*, 1983], flagellate groups commonly found in the dry valley lakes [*Lizotte and Priscu,* this volume]. Nevertheless our studies provide two lines of evidence that pigmentation of Lake Bonney populations responds to spectral irradiance. Firstly, phytoplankton have an abundance of light-harvesting carotenoids such as alloxanthin (in cryptophytes) and violaxanthin (in chlorophytes) that are particularly efficient in absorption of blue-green light [*Neale and Priscu*, 1995; *Lizotte and Priscu,* this volume]. Secondly, blue-green and red light are utilized with equal efficiency for light-dependent electron transport by photosystem II (PSII) in *Chlamydomonas subcaudata* isolated from Lake Bonney [*Neale and Priscu*, 1995]. This contrasts with the common strain of *C. reinhardtii* that is much less efficient at utilization of blue-green *versus* red light in PSII photochemistry [*Neale and Priscu*, 1995].

With the low average light intensity below the ice cover, low-light acclimation of photosynthesis [*Falkowski and LaRoche*, 1991] would be expected in phytoplankton populations of the dry valley lakes. Observations on the relationship of photosynthesis to irradiance (P-I) curves are consistent with this expectation [*Lizotte and Priscu*, 1992a; *Lizotte and Priscu*, 1994]. Low-light acclimation is also indicated by high sensitivity of photosynthesis to inhibition by photosynthetically available radiation (PAR) and ultraviolet radiation (UV) [*Neale et al.*, 1994]. This sensitivity implies that phytoplankton in the dry valley lakes lack defense mechanisms required to minimize damage resulting from exposure to the full solar spectrum at near surface intensities. However not all characteristics of the phytoplankton in dry valley lakes match those expected of low-light acclimated populations. Maximum quantum yields for photosynthesis are low relative to the theoretical maximum, especially in shallow populations situated away from the nutricline [*Lizotte and Priscu*, 1992a; *Lizotte and Priscu*, 1994]. Also, it was expected that the overall antenna size of the light-harvesting chlorophyll in photosynthesis, indicated by the ratio of total chlorophyll to the photosystem I reaction center [Chl:P_{700} ratio] would be large in Lake Bonney phytoplankton. This expectation has precedent in another microalgal population living under low irradiance conditions, sea-ice algal diatoms that have very large ratios of Chl:P_{700} [*Barlow et al.*, 1988]. In contrast average Chl:P_{700} in Lake Bonney phytoplankton (743 mol mol^{-1}) is similar to many measurements with high-light grown microalgae [*Neale and Priscu*, 1995]. However the Chl:P_{700} ratio may significantly underestimate the actual photosynthetic antenna size given the high concentration of light-harvesting carotenoids.

In this report, we present data on the fluorescence characteristics of phytoplankton from Lake Bonney and other perennially ice-covered lakes in the Taylor Valley. Our objective is to better elucidate responses of the photosynthetic apparatus to the interacting effects of low light intensity, light spectral composition, nutrient supply, and temperature. In particular, we focus on the dynamic aspects of the variation in fluorescence yield in these populations. Studies of the dynamic response of fluorescence to variations in incident irradiance on time scales of seconds to minutes is a powerful tool to diagnose the regulation of light harvesting and photosynthetic electron transport [*Büchel and Wilhelm*, 1993]. In general these studies have been aided by developments of instruments to measure fluorescence yield during on-going illumination of photosynthetic tissue. One approach that has been widely used in higher plants and dense cultures of algae is Pulse Amplitude Modulation (PAM) fluorometry [*Schreiber et al.*, 1986]. Studies using this or similar techniques have shown that chlorophyll fluorescence yield can either increase or decrease as illumination increases. The direction of this change in fluorescence yield depends on the balance between the ability to absorb light and store energy in chemical form (light reactions) and the enzymatic capacity to utilize that energy in metabolic processes, in particular carbon fixation. Here we use such fluorescence trend analysis as a tool to better understand the structure and function of the photosynthetic apparatus of phytoplankton in the lakes of the McMurdo Dry Valleys.

Changes in fluorescence yield are linked to changes in the dissipation of the excitation energy absorbed by PSII, the primary source of chlorophyll fluorescence at ambient temperature [see reveiw by *Büchel and Wilhelm*, 1993]. Light absorbed by the photosynthetic apparatus has three possible fates: 1) photochemical production of reduced energy carriers, ultimately leading to photosynthetic electron transport; 2) re-emission as fluorescence; or 3) non-radiative decay (heat production). When light is low and limiting to photosynthesis, a maximum proportion of absorbed photons is used for photochemistry. Under these conditions, fluorescence emission and non-radiative dissipation are low, and fluorescence is said to be quenched photochemically. When light intensity

increases, the inherent turnover rate of PSII starts to limit the efficiency of photochemistry; some of photons find occupied or "closed" reaction centers. Fluorescence yield rises and remains elevated as long as the increased photochemical production is balanced by downstream dissipation. However continued increases in irradiance can result in a dangerous imbalance, which is little tolerated by photosynthetic organisms. In particular, electrons from reduced chromophores and electron carriers can be diverted to formation of possibly damaging radicals. There are mechanisms by which the overall activity of PSII is down regulated to better match downstream demand for the products of photosynthetic electron transport, primarily carbon fixation (for a more detailed discussion see review by *Büchel and Wilhelm,*[1993]). The mechanisms deactivate the excitation energy absorbed by PSII and increase dissipation to heat, so that neither photochemistry or fluorescence emission can occur. As a consequence of this increase in the so-called "non-photochemical quenching," fluorescence yield is lowered. Detection of non-photochemical quenching of fluorescence is therefore indicative of limitation of photosynthesis by downstream processes.

Because of feedback to PSII photochemistry, variations in the yield of in vivo fluorescence can be caused by many factors besides the absolute light intensity, for example nutrient supply and temperature. The basic principle is that photosynthetic systems appear to be highly regulated to keep photochemistry and coupled metabolic processes in balance. This principle has been described as "excitation pressure" regulation [*Maxwell et al.*, 1995a]. *Maxwell et al.* showed that *Chlorella vulgaris* acclimates to minimize excitation pressure under growth conditions. As a result, *C. vulgaris* grown under high light and temperature are comparable to those grown at lower light and low temperature in terms of the structure and function of the photosynthetic apparatus, e.g., pigment content photosynthesis-irradiance relationship, and resistance to photoinhibition. In both cases acclimation counteracted a growth environment induced imbalance between upstream generation and downstream utilization of reductant, whether because of overexcitation of photochemistry (due to high light) or thermodynamic limits on metabolism (due to low temperature).

In this report, we describe a simple method for measuring fluorescence transients over slow time scales of minutes to seconds in natural populations of phytoplankton. The method uses a sensor designed to measure upwelling irradiance at 683 nm, Lu_{683} or "natural fluorescence." Instead of using the sensor as commonly employed to measure profiles of in situ fluorescence stimulated by solar irradiance, we measured fluorescence in the laboratory with the instrument mounted over a sample in a transparent container illuminated by a blue-green light source with similar spectral composition as in situ irradiance. We then measured relative fluorescence yield as the ratio of measured Lu_{683} to measured incident PAR in the container. We have used this method to measure slow fluorescence transients as a function of irradiance for phytoplankton from perennially ice-covered lakes of the Taylor Valley (Lake Bonney, Lake Fryxell, and Lake Hoare). The transients are interpreted in terms of excitation pressure theory to infer factors controlling the structure and function of the photosynthetic apparatus of phytoplankton in the dry valley lakes.

METHODS

Study Sites

Phytoplankton populations were sampled from the depths of peak biomass in the east lobe of Lake Bonney (4.5 and 17 m), Lake Hoare (10 m), and Lake Fryxell (8.5 m). Biomass ranged from 0.7 to 6.6 mg Chl m^{-3} [*Lizotte and Priscu*, 1994]. More extensive physical, chemical and biological descriptions of these lakes are given elsewhere in this volume [*Lizotte and Priscu, Spigel and Priscu, Howard-Williams et al.*, this volume]. Phytoplankton are typically dominated by phytoflagellates in all lakes. In Lake Bonney, the assemblage is dominated by a cryptophyte (*Chroomonas lacustrus*) under-ice (4.5 to 8 m) and a chlorophyte (*Chlamydomonas subcaudata*) in the deep layer (17 to 20 m) [*Koob and Leister, 1972; Parker et al., 1982; Sharp 1993*]. Based on HPLC pigment studies, cryptophytes also dominated the depths sampled at Lake Hoare (10 m) and Lake Fryxell (8.5 m) [*Lizotte and Priscu*, this volume; see also *Vincent*, 1981b]. All samples were collected from October through December, 1990.

Fluorescence Measurements

A 10 liter sample was taken using a Niskin bottle through a drill hole in the ice cover and stored in the dark at ambient temperature. After at least 30 min of dark-adaptation, a 1 liter aliquot was placed in a clear polycarbonate container and the Lu_{683} (Chl fluorescence) sensor of a Biospherical Instruments PNF-300 profiling natural fluorometer was positioned directly

over the uncovered top of the container. The container was then illuminated by a halogen projector (500W) source filtered through two blue-green (Corning 4-97) filters and one or more nickel screen neutral density filters to vary overall light intensity. The spectral composition of the actinic irradiance was measured with a MER-1000 spectroradiometer as described by [*Lizotte and Priscu*, 1992b] and was similar to underwater irradiance in Lake Bonney [*Lizotte and Priscu*, 1992b] (Figure 1). The intensity of actinic irradiance was measured in the center of container using a scalar quantum sensor (Biospherical Instruments QSL-100) for PAR (400-700 nm). The PNF-300 output was sampled once per second; instrument response time (90% of step change) was 3.5 seconds. Chlorophyll fluorescence was corrected for a background measured on filtered lake water and then scaled by the factor of 4π divided by scalar irradiance to estimate a relative fluorescence yield (m^{-1}), uncorrected for the presence of the air-water interface.

Maximum quantum yield of PSII photochemistry (ϕ_m) was also measured on samples after 30 min dark adaptation using a Turner Designs Model 10 fluorometer. Sample (2–3 ml) was placed in a cuvette and an initial reading was taken as soon as a stable reading was attained (e.g., within 10 s). The cuvette was then removed from the fluorometer and the inhibitor dichlorophenyl-dimethyl urea (DCMU), which blocks PSII electron transport, was added from a stock solution of 325 mM in 95% ethanol. The final concentration was 10 μM as previously described [*Vincent, 1981a; 1981b*]. The sample was returned to the fluorometer and a second reading (F_m) was taken at a higher stable emission level attained within 30 s. These two measurements were used to calculate ϕ_m according to the formula $(F_m-F_0)/F_m$ ([cf., *Vincent* [1981a]). All field measurements were performed in dim light (< 1 μmol photons m^{-2} s^{-1}) and at ambient air temperature (about 0°C).

Additional laboratory measurements were made with a PAM fluorometer (Walz, Effeltrich, Germany) using the basic fiber optic setup as described [*Schreiber et al.*, 1986]. The tip of the fiber optic bundle of the PAM was placed next to a Pyrex flask containing a culture of *Thalassiosira pseudonana* (Clone 3H, CCMP) with a biomass of 200 mg Chl m^{-3}. The flask was illuminated with irradiance from a halogen light source filtered through a neutral density screen to obtain a range of intensities. Scalar quantum irradiance in the flask was measured with a Biospherical Instruments QSL-100 light meter.

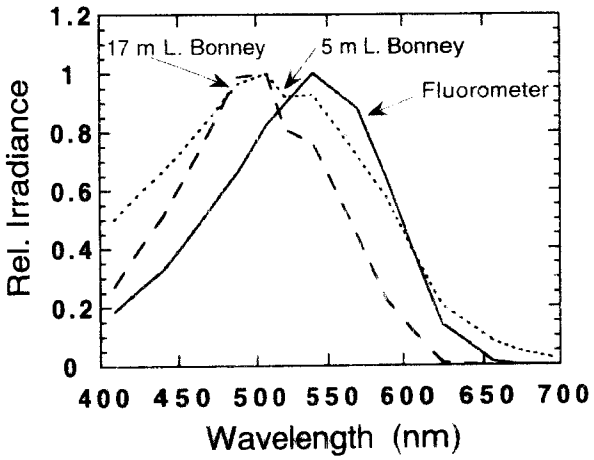

Fig. 1. Spectral downwelling irradiance normalized to maximum irradiance for two depths in Lake Bonney (December 10, 1990 measurements redrawn from *Lizotte and Priscu* [1992b]), and for the actinic illumination used for laboratory measurements of fluorescence transients using the PNF-300. The profile of spectral irradiance at 17 m is plotted with long dash, at 5 m with short dashes, and of the actinic irradiance with a solid line. The actinic illumination consists of a halogen (500W) source filtered through two blue-green (Corning 4-97) filters. In all cases peak irradiance was in the 500 to 550 nm range.

RESULTS

Maximum Quantum Yield of PSII

The vertical stratification and differentiation characteristic of the phytoplankton community in the dry valley lakes also applies to differences in the maximum quantum yield of PSII photochemistry (ϕ_m). In the east lobe of Lake Bonney during October through early December 1990, ϕ_m was significantly lower in the assemblage immediately below the ice (4.5 m) than in the deep chlorophyll (Chl) maximum (15–18 m) (Figure 2). In contrast, ϕ_m was highest near the surface in Lake Hoare and was mostly lower at depth including the biomass peak near 10 m (Figure 2). In Lake Fryxell, only the depth of the biomass peak (8.5 m) was sampled for fluorescence assay. At this depth, ϕ_m was 0.64. The Lake Fryxell assemblage was the only assemblage with a ϕ_m in the range expected for "healthy" populations or cultures (0.6 to 0.7) [*Büchel and Wilhelm*, 1993; *Geider et al.*, 1993].

Time Course of Fluorescence Yield

An example of the time-dependent variation in fluorescence yield for shallow (4.5 m) phytoplankton

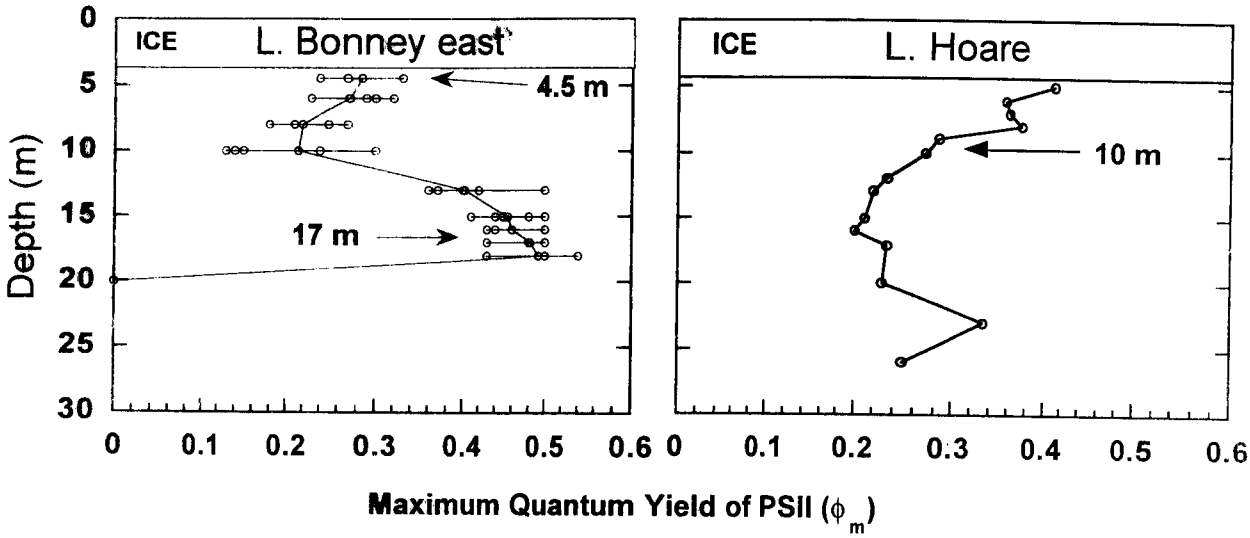

Fig. 2. Profiles of the maximum quantum yield of PSII (ϕ_m) as estimated from the ratio $(F_m - F_0)/F_m$ of fluorescence measured in a Turner Designs fluorometer in absence (F_0) and presence (F_m) of the photosynthetic inhibitor dichlorophenyl-dimethyl urea (DCMU). Composite of five profiles sampled in the east lobe of Lake Bonney during October through December 1990 (line connects means) and a profile from Lake Hoare on December 9, 1990. Arrows indicate position of chlorophyll maxima sampled for fluorescence transient analysis. All samples were dark adapted at least 30 min before measurement. Horizontal lines in the upper portion of each panel denote ice thickness.

from Lake Bonney is presented in Figure 3. At steady, low irradiance (<6 μmol photons m^{-2} s^{-1}), light limiting for photosynthesis [*Lizotte and Priscu*, 1992a], fluorescence yield was constant. However at irradiances near or above the threshold of light saturation of photosynthesis (I_k, 15–40 μmol photons m^{-2} s^{-1}, [*Lizotte and Priscu*, 1992a]), fluorescence rose to a peak and then rapidly declined to a new steady state (F_s) after about five minutes of illumination. The initial peak is called the Kautsky induction and reflects PSII dynamics on time scales of seconds [*Büchel and Wilhelm*, 1993]. Of particular interest is the steady state F_s level attained after the induction and whether this is lower (more quenched) compared to the F_s level in low irradiance. For the 4.5 m samples from Lake Bonney, F_s was progressively lower as irradiance increased above 10 μmol photons m^{-2} s^{-1} (Figure 3).

Also shown in Figure 3 is the fluorescence transient that occurs after addition of DCMU. There is an immediate fluorescence yield increase from F_s to a maximum yield under illuminated conditions (F'_m). The latter yield continues to rise as illumination continues in the presence of DCMU, indicating a steady reversal in non-photochemical quenching when photosynthetic electron transport is blocked. At increasing irradiance, there is a decline in both F_s and F'_m as well as in the difference $F'_m - F_s$, the steady state variable fluorescence.

A different type of time course was exhibited by the deep (17 m) assemblage in Lake Bonney (Figure 4). The reduction in F_s is not as rapid as at 4.5 m, and addition of DCMU leads to a rapid reversal of quenching. This progressive rise in fluorescence after addition of DCMU is so rapid that it was not clear what F'_m would have been immediately post addition, and so a steady-state variable fluorescence is difficult to estimate. The 17 m assemblage was also unusual in that, after DCMU addition, fluorescence first increased and then decreased again. This implies that some portion of the quenching was inducible even in the presence of DCMU. This decrease was not observed in the Lake Hoare and Lake Fryxell samples, even though they also had rapid increases in F' with continued exposure in the presence of DCMU (data not shown).

The fluorescence transients defined by the PNF for phytoplankton from the dry valley lakes allow for a systematic examination of the dependence of fluorescence yield on incident irradiance (F_s versus I). This

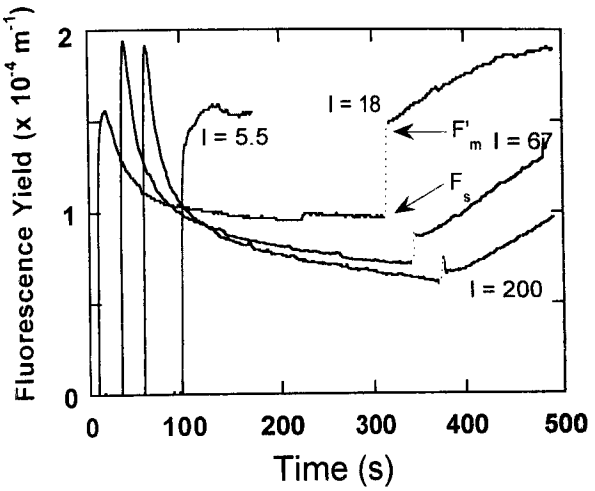

Fig. 3. Time course of relative fluorescence yield for Lake Bonney phytoplankton sampled at 4.5 m on November 20, 1990. Measurements were made with four separate 1 l aliquots exposed to scalar irradiances of 5.5, 18, 67, and 200 μmol photons m^{-2} s^{-1}; the corresponding trace is labeled with the actinic intensity. For clarity start times are offset 25 seconds. The discontinuity (dotted line) at the elapsed time of 300 seconds corresponds to the addition of DCMU at a final concentration of 10 μM. As intensity increases, the steady state yield (F_s) and DCMU enhanced yield (F'_m) is progressively lower. There is a 10 s pause in data acquisition during the addition of the DCMU which is not shown on the plot.

type of study has rarely been done for any natural population of phytoplankton, much less in the dry valleys, despite "natural fluorescence" being advocated as an indicator of phytoplankton photosynthesis [*Kiefer et al.*, 1989]. A summary of the irradiance-dependent variation of F_s at 4.5 m in Lake Bonney is shown in Figure 5. Experiments from different dates were combined by normalizing to average yield for irradiance less than 10 μmol photons m^{-2} s^{-1}. A consistent decrease in F_s relative to low-light levels (quenching) occurred for all irradiances greater than 10 μmol photons m^{-2} s^{-1}. Based on the fit to a rectangular hyperbola, F_s reached one-half of the average low light value at approximately 80 μmol photons m^{-2} s^{-1} (Figure 5). While there are few other observations to compare with this, the induction of quenching at such a low irradiance appears to be unprecedented. *Chamberlin et al.* [1990] combined data from various open ocean and coastal marine environments and, though there is a large amount of scatter in their analysis, the data indicate that quenching of F_s only occurs at "high" irradiance (e.g., > 500 μmol photons m^{-2} s^{-1}).

Other published F_s versus I curves, which are mostly for cultures, also show no F_s quenching until irradiance is 10 or 100 times higher than the threshold in Lake Bonney. An example result is shown in Figure 5, the F_s versus I curve determined by PAM fluorometry for a culture of *Thalassiosira pseudonana* grown at 170 μmol photons m^{-2} s^{-1}. In this culture, F_s increased until irradiance exceeded 165 μmol photons m^{-2} s^{-1}. Quenching below the average F_s in low light did not occur until irradiance exceeded 300 μmol photons m^{-2} s^{-1}. The overall pattern of level or increasing yield at lower irradiance, followed by decreased yield at higher irradiance, is similar to the F_s versus I curve of the Lake Bonney sample, however the pattern occurs at irradiances about 30 times higher in the *T. pseudonana* culture (Figure 5). Similar results have been reported for a related diatom species, *T. weisflogii* [*Falkowski et al.*, 1986]. *Ting and Owens* [1993] measured the steady-state fluorescence parameters of *Phaeodactylum tricornutum* grown at 100 μmol photons m^{-2} s^{-1}. They did not report F_s per se, but reported that significant increases in non-photochemical quenching only occurred at irradiances exceeding 400 μmol photons m^{-2} s^{-1}.

Such high thresholds for fluorescence quenching are not only a feature of diatom cultures. In measurements

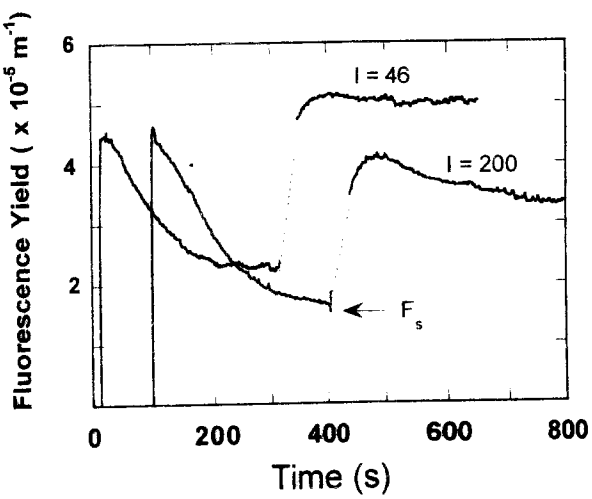

Fig. 4. Time course of relative fluorescence yield for Lake Bonney phytoplankton sampled at 17 m on November 22, 1990. Measurement conditions as in Figure 3, except only scalar irradiances of 46 and 200 μmol photons m^{-2} s^{-1} are shown. The discontinuity corresponding to DCMU addition is shown with a dotted line. Fluorescence yield increased rapidly with continued illumination after DCMU addition, therefore no attempt was made to back extrapolate to a F'_m yield.

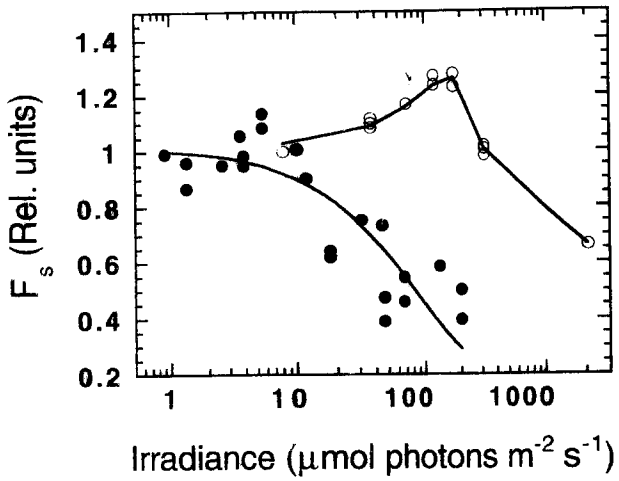

Fig. 5. Relative fluorescence yield (F_s) as a function of scalar irradiance for Lake Bonney phytoplankton (4.5 m) sampled during November and December, 1990 (closed circles), and of *Thalassiosira pseudonana* (Clone 3H) grown at 170 µmol photons m^{-2} s^{-1} (open circles) as measured with a PAM fluorometer. Note log scale for irradiance, fluorescence yield is normalized to the average low light (I < 10 µmol photons m^{-2} s^{-1}) for each sample. The data from Lake Bonney was fit to the function $F_s = 1/(1 + kI)$ using nonlinear regression, the estimate of k was 0.012 with a standard error of 0.003 (R^2 = 0.70), implying a light intensity of 80 µmol photons m^{-2} s^{-1} (with approximate standard error of ± 25) for 50% quenching of F_s.

with cultures of the chlorophyte alga *Ankistrodesmus braunii* growing at 80 µmol photons m^{-2} s^{-1}, *Schreiber et al.* [1995b] found that F_s yield increased as irradiance was increased to 600 µmol photons m^{-2} s^{-1}. Using blue-light illumination similar to that used in the present study, *Falkowski et al.* [1988] found that F_s increased up to an intensity of 60 µmol photons m^{-2} s^{-1} in cultures of *Chlorella vulgaris* that were low light acclimated (growth at 40 µmol photons m^{-2} s^{-1}). The increase in F_s was eliminated when irradiance was supplemented with far-red (photosystem I absorbed) irradiance, and in both cases quenching only occurred when irradiance exceeded 165 µmol photons m^{-2} s^{-1}.

A low threshold for fluorescence quenching was also observed in other dry valley phytoplankton assemblages. The 17 m assemblage from Lake Bonney and the 10 m assemblage from Lake Hoare exhibited similar F_s versus I curves, with significant quenching at irradiance greater than 10 µmol photons m^{-2} s^{-1} (Figure 6). At irradiances between approximately 1 and 6 µmol photons m^{-2} s^{-1}, fluorescence appeared to increase. Variability in the data precludes a definitive assignment of curve shape, however all curves from Lake Bonney and Lake Hoare have higher yields around 5 µmol photons m^{-2} s^{-1}. F_s decreases to one-half the low light level at 61 µmol photons m^{-2} s^{-1} (Lake Hoare) and 138 µmol photons m^{-2} s^{-1} (17 m Lake Bonney).

The Lake Fryxell assemblage is the one exception to the general pattern of low quenching thresholds. These phytoplankton exhibited a general increase in F_s over the range 1 to 100 µmol photons m^{-2} s^{-1} (Figure 6), a response that resembles the previously cited culture studies.

The large decreases in F_s in Lake Bonney and Lake Hoare suggest induction of non-photochemical quenching at extremely low irradiances. This conclusion can be confirmed directly for the 4.5 m assemblage in Lake Bonney through analysis of F'_m. In the

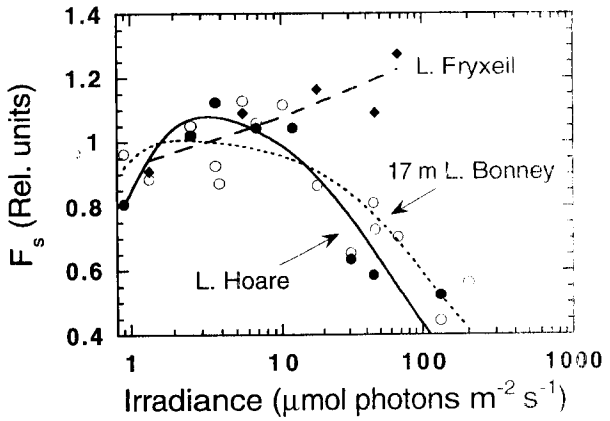

Fig. 6. Relative fluorescence yield (F_s) as a function of scalar irradiance for Lake Bonney phytoplankton (17 m) sampled during November and December, 1990 (open circles), 10 m Lake Hoare (closed circles), and 8.5 m Lake Fryxell (closed diamonds), both sampled in December, 1990. Fluorescence yield is normalized to the average F_s in low light (I < 10 µmol photons m^{-2} s^{-1}), The Lake Hoare and 17 m Lake Bonney data were fit to the function $F_s = f(I)/(1+kI)$, where f(I) is a saturating function $F_s(max)(1-\exp(-\alpha I))$ and $F_s(max)$ is peak F_s, and α is a saturation parameter. Based on the estimated k's, the corresponding estimated irradiances for 50% quenching from $F_s(max)$ are 61 µmol photons m^{-2} s^{-1} (approximate standard error ± 20) for Lake Hoare (R^2 = 0.85) and 138 µmol photons m^{-2} s^{-1} (approximate standard error ± 40) for 17 m, Lake Bonney (R^2 = 0.73).

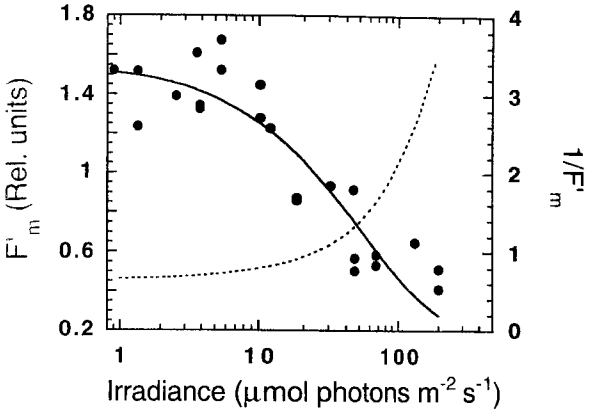

Fig. 7. Maximum fluorescence yield (F'_m) as a function of scalar irradiance for Lake Bonney phytoplankton (4.5 m) sampled during November and December, 1990 (solid line, left hand axis). Fluorescence yield is normalized to the average F_s in low light ($I < 10$ µmol photons m^{-2} s^{-1}) in each case. The data were fit to the function $F'_m = F'_m(max)/(1 + kI)$. The corresponding estimate irradiances for 50% quenching from $F'_m(max)$ are 44 µmol photons m^{-2} s^{-1} (approximate standard error ± 9, $R^2 = 0.83$). The fitted curve was then used to estimate $1/F'_m$ (dotted line, right hand axis), a measure of non-photochemical quenching.

case of an active fluorometer, such as the PAM, F'_m is measured concurrently with F_s by applying a short duration, high intensity flash [*Schreiber et al.*, 1995a]. However pulsed illumination of the large volume used for the PNF measurements was not possible; therefore F'_m was obtained by blocking the PSII acceptor side with DCMU. In samples from 4.5 m in Lake Bonney, addition of DCMU led to a rapid rise to F'_m followed by a slower secondary increase. The F'_m yield is readily distinguished since the secondary increase was comparatively slow (Figure 3). The decrease in F'_m with irradiance for the 4.5 m Lake Bonney assemblage was stronger than the decrease in F_s (Figure 7). A decrease to 50% of the low light F_m occurred at approximately 50 µmol photons m^{-2} s^{-1}. The relative increase in non-photochemical quenching is estimated as $1/F'_m$ [*Havaux et al.*, 1991], which shows a sharp increase at irradiances exceeding 10 µmol photons m^{-2} s^{-1}. Non-photochemical quenching is more conventionally estimated by the ratio $1 - (F'_m - F'_0) / (F_m - F_0)$ [*Büchel and Wilhelm*, 1993], however this could not be implemented since F'_0 (F_0 immediately after cessation of actinic illumination) cannot be measured after the addition of DCMU.

The post-illumination level of DCMU-induced fluorescence was also measured in the 17 m Lake Bonney (Figure 4), Lake Hoare, and Lake Fryxell assemblages (data not shown). In all cases the fluorescence immediately after addition was lower than the F_m attained under low light (data not shown). However the secondary increase was much more rapid in these samples. Thus F'_m could not be quantitatively determined in these cases. Qualitatively, quenching of F'_m was apparent for irradiance greater than 10 µmol photons m^{-2} s^{-1} for 17 m in Lake Bonney and Lake Hoare, and greater than 20 µmol photons m^{-2} s^{-1} in Lake Fryxell.

A primary motivation for simultaneous measurement of F'_m and F_s is the estimation of ϕ_p, where $\phi_p = (F'_m - F_s) / F'_m$. The parameter ϕ_p is interpreted as the quantum yield of PSII photochemistry, also known as the Genty yield [*Genty et al.*, 1989]. PAM measurements of ϕ_p been shown to be highly correlated with the quantum yield of photosynthesis in leaves [*Genty et al.*, 1989] and algal cultures [*Kroon et al.*, 1993]. Photosynthesis calculated from ϕ_p measured by a related type of instrument, the "pump and probe" fluorometer, was correlated with ^{14}C based estimates of primary production in the northwest Atlantic [*Kiefer and Reynolds*, 1992; *Kolber and Falkowski*, 1993]. Apart from these latter measurements, little is known about how closely ϕ_p is correlated with the quantum yield of photosynthesis in natural assemblages of phytoplankton. The ϕ_p versus irradiance relationship for phytoplankton at 4.5 m in Lake Bonney is shown in Figure 8. A single curve was fit to the composite data based on the assumption that quantum yield of PSII would be proportional to

$$(1 - e^{(-I/I_k)}) / I$$

where I_k is irradiance for the onset of light saturation of photosynthesis. This analysis suggested an overall I_k of 30 µmol photons m^{-2} s^{-1} for phytoplankton from 4.5 m in Lake Bonney. An independent estimate of I_k was made using photosynthesis (carbon assimilation) versus irradiance curves [*Lizotte and Priscu*, 1992a; *Lizotte and Priscu*, 1994]. The I_k of the phytoplankton from 4.5 m in the east lobe of Lake Bonney ranged from 23 to 33 (average 28) during November and December 1990. Thus there is good agreement between the light-saturation characteristics of PSII photochemistry and photosynthesis in this assemblage.

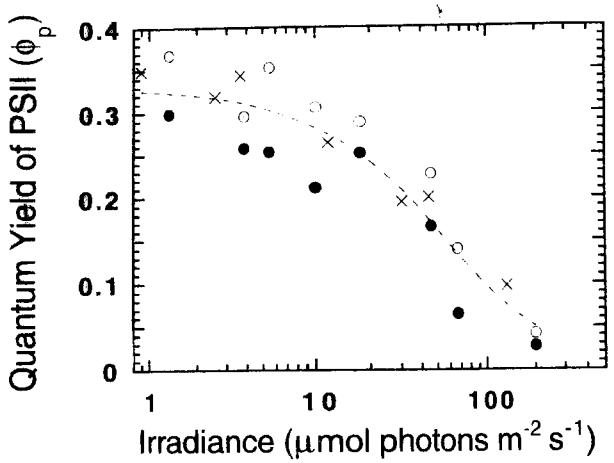

Fig. 8 Quantum yield of PSII photochemistry (ϕ_p) in relation to incident irradiance for phytoplankton at 4.5 m in Lake Bonney for three sample dates: November 20 (open circles), November 21 (solid circles), and December 9 (x), 1990. Data from all three dates were used to fit the function $M(1-\exp(-I/I_k))/I$, where M is an arbitrary scaling constant (effectively the molar ratio of incident to absorbed photons). The fitted value for M was 10, and I_k was 30.5 µmol photons m^{-2} s^{-1} (approximate standard error ± 5.1, $R^2 = 0.87$).

DISCUSSION

Quenching Analysis Using Lu_{683}

We report here a novel application of a natural fluorescence (Lu_{683}) sensor for determination of fluorescence quenching parameters in natural populations of phytoplankton. Much of the debate about the relationship between Lu_{683} and rates of photosynthesis centers on how to properly adjust for fluorescence quenching [*Chamberlin et al.*, 1990; *Cullen and Lewis*, 1995; *Kiefer and Reynolds*, 1992; *Lizotte and Priscu*, 1994]. Published results on the relationship of fluorescence yield (F_s) to ambient irradiance show much scatter, possibly due to physiological variations between the different populations included in the analysis. Improved precision might be obtained using field measurements of F_s versus I curves for specific assemblages of interest. Recently this has been facilitated by the development of active fluorometers using low measuring irradiance, i.e., the PAM with a high-sensitivity cuvette system [*Schreiber*, 1994] or the Fast Repetition Rate (FRR) fluorometer [*Falkowski and Kolber*, 1995]. However if this type of sophisticated instrumentation is lacking, useful laboratory measurements can be with a Lu_{683} sensor as described in this report. At biomass levels below 0.5 mg Chl m^{-3}, a larger volume of sample water may needed to obtain sufficient signal. Other types of active excitation field fluorometers, such the Sea-Tech fluorometer, generally use too high excitation irradiance to measure a true F_s yield [*Neale et al.*, 1989].

Implications for Acclimation of the Photosynthetic Apparatus

Viewed strictly from the standpoint of growth in a low-light environment, the phytoplankton of dry valley lakes present a paradoxical picture. This is best seen with the relatively well-studied assemblages from the east lobe of Lake Bonney. In some respects, these assemblages appear to be low-light acclimated, for example in their extreme sensitivity to UV inhibition [*Neale et al.*, 1994] and low I_k [*Lizotte and Priscu*, 1992a]. However I_k is comparable to that measured in other Antarctic habitats (see review in *Cabrera and Montecino* [1990]), and lower I_k values have been measured [*Priscu et al.* 1987; *Lizotte and Priscu*, 1992a]. Indeed there are several aspects that seem contrary to extreme low light adaptation. Antenna sizes, based on Chl:P_{700} ratios, are not particularly high [*Neale and Priscu*, 1995]. Also, α, the initial slope of P^B versus I curve, and the quantum yield of photosynthesis are well below the highest values measured for phytoplankton in low-light environments [*Lizotte and Priscu*, 1994].

This seemingly paradoxical picture is perhaps better understood if acclimation of the photosynthetic apparatus is viewed more as a response to excitation pressure [*Maxwell et al.*, 1995a] than to the light environment, per se. The results of the fluorescence quenching analysis on dry valley lakes phytoplankton show that irradiance even slightly above the normal range of in situ irradiance results in a large drop in the quantum yield of PSII photochemistry (ϕ_p) and an increase in non-photochemical quenching. Though excitation pressure ($= 1 - q_p$, sensu *Maxwell et al.*, [1995a]) was not quantitatively estimated (F'_0 could not be measured), the drop in ϕ_p implies that excitation pressure was high. *Maxwell et al.* [1995a] interpreted induction of non-photochemical quenching at low irradiance as indicative of acclimation to low excitation pressure. Characteristics of acclimation to low excitation pressure also include higher sensitivity to photoinhibition and lower accumulation of xanthophyll quenching pigments, which are also properties of the

Lake Bonney assemblage [*Neale et al.*, 1994; *Neale and Priscu*, 1995; *Lizotte and Priscu*, this volume]

These characteristics were especially evident in the 4.5 m assemblage. Midday irradiance just below the ice is sufficient to nearly saturate rates of photosynthesis and induce non-photochemical quenching. Interestingly, this assemblage is acclimated to low excitation pressure, even though it is apparently often on the verge of experiencing high excitation pressure. Apparently, the maximum sustainable rate of electron transport only slightly exceeds the rate occurring under mean in situ irradiance, a condition consistent with the extremely low maximum rates of photosynthesis (P^B_m) in this assemblage [*Lizotte and Priscu*, 1992a]. At least two factors appear to be limiting overall photosynthetic capacity: 1) chronic nutrient stress (at least during the November–December period [*Priscu*,1995]); and 2) low temperature, i.e., 0°C just below the ice cover. These factors may also explain the low maximum quantum yield of PSII observed in this assemblage, though it unknown whether this arises because of a direct effect on PSII structure [*Kolber et al.*, 1988], a limitation of the PSII repair cycle with accumulation of inactive units, [*Smith et al.*, 1990], or some other mechanism.

The threshold light intensities for fluorescence quenching in the 17 m assemblage from Lake Bonney and the 10 m assemblage in Lake Hoare are similar to the 4.5 m assemblage. However these assemblages experience generally lower light intensities than the 4.5 m assemblage. Thus there is a greater "safety margin" between in situ irradiance and "high" irradiances in these assemblages. The margin is probably made possible by a more ample nutrient supply to these phytoplankton, owing to their proximity to the lake nutricline [*Lizotte and Priscu*, 1994; *Priscu*, 1995] and higher water temperatures. Both P^B_m and maximum quantum yield of photosynthesis are higher in these assemblages compared to 4.5 m assemblage [*Lizotte and Priscu*, 1994]. Nevertheless the maximum quantum yield of PSII (ϕ_m) was still below the level observed in "healthy" phytoplankton. This may be due to low flux rates of nutrients to these populations since supply is by molecular diffusion [*Priscu*, 1995; *Spigel and Priscu*, this volume].

The one exception to this general paradigm of photosynthetic acclimation in the dry valley lakes is the 8.5 m assemblage from Lake Fryxell. In contrast to phytoplankton in Lake Bonney and Lake Hoare, this assemblage had a high ϕ_m and a relatively high maximum quantum yield of photosynthesis [*Lizotte and Priscu*, 1994]. The biomass peak in Lake Fryxell is also larger than in the other lakes, suggesting more robust growth. The F_S was not quenched at low intensities signifying a low excitation pressure even at irradiances above the reported I_k for this assemblage of 10 μmol photons m^{-2} s^{-1} [*Lizotte and Priscu*, 1994]. This assemblage appears to be responding like a nutrient sufficient culture, suggesting higher nutrient flux to this environment despite low ambient concentrations. A slight inconsistency with this interpretation is the reported low P^B_m as determined by ^{14}C incorporation over several hour incubations [*Priscu et al.*, 1989]. One possible explanation is that these phytoplankton are able to sustain high rates of electron transport over the short exposures used for the fluorescence measurements, yet rates decrease over longer exposures (cf. [*Marra*, 1978]).

The remarkable feature of the Lake Bonney and Lake Hoare phytoplankton is that they exhibit low excitation-pressure acclimation despite the fact that a strikingly small increase in growth irradiance would apparently shift them into a high excitation-pressure state. The success of this growth strategy is undoubtedly aided by the relatively stable shade regime experienced by phytoplankton in the lakes of the McMurdo Dry Valleys. Thus the photosynthetic apparatus acclimates so as to have the maximum light harvesting capacity consistent with the requirement of low excitation pressure under growth conditions. These phytoplankton may be physiologically capable of increasing light harvesting capacity, but such an increase would quickly overwhelm cellular metabolic capacity to utilize that energy as manifested by induction of non-photochemical quenching. Overall these results lead to the hypothesis that growth in an environment of low nutrient supply, low temperature, and stable shade entails an extremely fine-tuned allocation of resources between the light harvesting (e.g., PSII) and energy utilization (e.g., Calvin cycle enzymes) components of the photosynthetic apparatus. Indeed there is evidence that excitation pressure, perhaps as manifested as a change in the chloroplast ATP/NADPH ratio [*Melis et al.*, 1985], regulates chloroplast gene expression in *Dunaliella salina* [*Maxwell et al.*, 1995b]. Further experimentation would be needed to show whether such a fine-tuned allocation is actually operating within the phytoplankton assemblages in the lakes of the McMurdo Dry Valleys.

Acknowledgments. We thank Michael Lizotte, Thomas Sharp, Robert Spigel, and Ian Forne for field assistance. P.J.N. thanks Anastasios Melis for use of laboratory facilities

in the Dept. of Plant Biology, University of California at Berkeley. This work was supported by NSF grants DPP-88-20591, OPP-92-11773 and OPP-94-19423 to J.C.P.

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(Received October 1, 1996;
accepted April 24, 1997)