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The Photosynthetic Apparatus of Phytoplankton from a Perennially Ice-Covered Antarctic Lake: Acclimation to an Extreme Shade Environment

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Phytoplankton in perennially ice-covered Lake Bonney (Antarctica) are exposed to a limited range of light variation both in terms of intensity (<1–3% of incident) and spectral distribution (blue-green) during the austral spring and summer. This relative constancy is due to continuous sunlight, optical filtering through the 4.2 m ice cap and an absence of vertical mixing. The effects of this unique light environment on the structure and function of the photosynthetic apparatus were studied using measurements of P_{700} reaction center content and spectral variation in photosystem II (PSII) fluorescence kinetics. Light-induced absorbance change at both 700 nm and 810 nm was used to measure P_{700} concentration. The average ratio of total Chl/P_{700} was 743 (mol mol^{-1}), with a range of 480 to 1,039. These ratios were low in comparison to previous studies of phytoplankton growing in low-light cultures or algae growing beneath Arctic sea ice. A sample from the deep (17 m) layer dominated by *Chlamydomonas subcaudata* was grown in enriched culture media. PSII fluorescence kinetics were measured on thylakoid preparations in the presence of DCMU under blue-green (481 nm) and red (660 nm) light. *C. subcaudata* utilized blue-green light for photosynthesis more efficiently than the photobiologically well characterized *C. reinhardtii* (strain CC-124). These results, together with pigment analyses, suggest that carotenoids in Lake Bonney phytoplankton are more important in light harvesting as opposed to photoprotection.

Key words: Blue-light utilization — *Chlamydomonas reinhardtii* — *Chlamydomonas subcaudata* — Lake Bonney — Light acclimation — Photosynthetic unit size.

Modification of the structure and function of the photosynthetic apparatus is an important response to the light environment (Anderson 1986), and has advantages in increasing the quantum yield of photosynthesis (Allen et al. 1989, Chow et al. 1990, Fujita et al. 1985, Evans 1987), in optimizing allocation of plant resources (Richardson et al. 1983, Evans 1989), and in protecting against high-light stress (Demmig-Adams and Adams 1992). However, less is known about the importance of changes in the photosynthetic apparatus of suspendend microalgae (phytoplankton) in response to the planktonic light environment (reviews Richardson et al. 1983, Falkowski and LaRoche 1991), since vertical mixing introduces uncertainty about the previous light history of sampled phytoplankton (cf. Falkowski 1983).

Phytoplankton in the lakes of the Antarctic dry valleys of McMurdo Sound grow in a unique environment due to a perennial ice-cap of 3–5 m in thickness. 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 1992, Lizotte and Priscu 1992a). 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 (Lizotte and Priscu 1992a).

The ice cover also prevents stirring of lake waters by surface winds. Lake Bonney is highly stratified (Angino et al. 1964, Hawes 1985), and turbulence is very low (Spigel et al. 1991). Due to the lack of vertical mixing and the continu-

Abbreviations: PS, photosystem; P_{700}, reaction center chlorophyll of PSI; Q_{act}, intensity of actinic irradiance; Q_{abs}, absorbed irradiance.

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ous daylight characteristic of the Antarctic summer, phytoplankton in Lake Bonney grow in an unusually stable light environment (Vincent 1981, Lizotte and Priscu 1994). This offers a unique opportunity to examine the acclimation of natural phytoplankton populations to their irradiance environment.

The phytoplankton populations in Lake Bonney also show a high degree of vertical stratification. The assemblage is dominated by a cryptophyte (*Chroomonas lacustrus*) under-ice (4.5 to 8 m), a chrysophyte (*Ochromonas* sp.) in the middle layer (8 to 16 m), and a chlorophyte (*Chlamydomonas subcaudata*) in the deep layer (17 to 20 m) (Koob and Leister 1972, Parker et al. 1982, Sharp 1993). 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 (Sharp and Priscu 1992, Sharp 1993). 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 Lake Bonney phytoplankton. 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 (Larkum and Barrett 1983), but not for cryptophytes or chlorophytes. Secondly, having attained an optimal pigment composition, efficiency of utilization could be further enhanced by either increasing the number of light-harvesting pigment molecules associated with each reaction center ("antenna size") or increasing the density of reaction centers within the cell (Prézelin 1981). The majority of phytoplankton appear to adapt by increasing antenna size as indicated by the ratio of Chl to photosystem I (Perry et al. 1981), however this ratio is also affected by the variable stoichiometry between photosystem I (PSI) and photosystem II (PSII) as a function of growth irradiance spectral composition (Allen et al. 1989, Chow et al. 1990, Fujita et al. 1985) and intensity (Falkowski et al. 1981, Neale and Melis 1986, Smith and Melis 1988).

The ratio of PSI to total chlorophyll (Chl) is often used as an index of photosynthetic unit (PSU) size (Prézelin 1981). The Chl/P_{700} ratio, however, is not an antenna size per se because total chlorophyll includes pigments associated with both PSI and PSII. Measurements on eukaryotic microalgae cultured in low light ($<50\ \mu$mol photons m^{-2} s^{-1}) imply that phytoplankton can increase their Chl/P_{700} ratios to 1,000 (mol mol^{-1}) or higher (Perry et al. 1981, Smith and Melis 1988). Chlorophyte species are an exception in that Chl/P_{700} is usually less than 1,000 and there is little or no increase in Chl/P_{700} at low light (Perry et al. 1981, Falkowski et al. 1981, Guenther and Melis 1990). In the case of *Chlamydomonas reinhardtii*, photosystem functional antenna size does increase at low irradiance but the change is confounded with a shift in PSII/PSI stoichiometry (Neale and Melis 1986). Despite these differences, the Chl : PSI ratio of natural populations of chlorophytes living in low light has, to our knowledge, not been measured. When P_{700} has been measured in natural populations of sea ice microalgae (diatoms) growing at extreme low irradiance, the Chl *a*/P_{700} ratio ranged from about 800 (mol mol^{-1}) to almost 2,800 (Barlow et al. 1988). The ratio of total pigments (including the accessory pigments fucoxanthin and Chl *c*) to PSI ranged from about 1,100 to almost 4,000. On this basis, phytoplankton in ice covered lakes may also be expected to have very large Chl/PSI ratios.

The measurement of PSU size needs to be integrated with a measurement of the efficiency of light utilization, for example light-limited rates of photochemistry for PSII and PSI (Smith and Melis 1987, 1988). Measurement of PSII reaction rates can be made from the kinetics of fluorescence induction curve in the presence of DCMU (Melis and Anderson 1983). However, the requirement of a high sample concentration to obtain good kinetics limits application of the technique to cultures.

Our study is the first to examine the photosystems of phytoplankton in perennially ice-covered Antarctic lakes. This seminal study presents (i) Chl/P_{700} ratios of natural populations and cultures developed from the lake populations, (ii) the efficiency of blue-green light utilization for PSII photochemistry by a *Chlamydomonas* species (*C. subcaudata*) cultured from the lake and (iii) comparisons of blue-green light utilization in *C. subcaudata* with the photobiologically well described *Chlamydomonas, C. reinhardtii*.

Materials and Methods

Lake samples—Lake Bonney (Taylor Valley, 77°42.9′S, 162°27.6′E) was sampled during November and December 1990 through a 20 cm diameter hole in the ice cover at a mid-basin station in the east lobe. All depths are measured relative to piezometric water level in the ice hole, usually 30 cm below the ice surface. Samples were taken at the main biomass peak just below the ice cover, at a depth of ca. 4.5 m, and at secondary biomass maxima around 13 m and 17 m (further site description given in Lizotte and Priscu 1992a, b). Water was taken with a 10 liters sampler (Niskin bottle) and immediately transferred into large (20

liters) high density polyethylene carboys or pumped with a peristaltic pump directly into carboys. Samples were maintained at in situ temperature and in the dark until use, i.e. within 12 h of collection.

Samples of 40–60 liters were pre-concentrated to 1–2 liters using a tangential flow filtration device (Millipore Pellicon, 0.45 mm pore size) before final vacuum concentration on a membrane filter (nylon or polycarbonate, as indicated). Concentrates were resuspended from the filter with a minimum amount (i.e. 20–40 ml) of isolation buffer (50 mM tricine/NaOH pH 7.8, 10 mM NaCl, 5 mM $MgCl_2$, 0.2% bovine serum albumin (BSA) and 0.2% ascorbate). Suspensions were then frozen in liquid nitrogen (LN_2) and stored in LN_2 or a freezer (−80°C) until analysis. Membrane isolations were made from samples after thawing at 0°C. Additional isolation buffer was added as necessary to the sample to make a total volume of 35–40 ml, then sonicated (Virtis cell disruptor, Power Level=7) at 0°C for six 10 s intervals, with 10 s "cool down" periods in between. The sonicated suspension was sedimented by centrifugation at 90,000 × *g* for 60 min and the pellets were resuspended in a minimum volume (ca. 1 ml) of assay buffer (isolation buffer without BSA or ascorbate) and transferred to a 1.5 ml centrifuge tube. Chloroplast membranes in the broken cell preparation were solubilized by the addition of Nonidet (Sigma Co.) to a final concentration of 0.125%. The suspension was homogenized at 0°C and unsolubilized material was sedimented using a microfuge (30 s at 10,000 × *g*). The supernatant was removed and used for P_{700} and Chl determinations.

Thylakoid membranes were prepared from algal cultures using methods already described (Neale and Melis 1986). The membranes were resuspended in assay buffer with 0.125% Nonidet to the indicated Chl concentration and used for either ΔA_{810} and ΔA_{700} measurements.

Differential absorbance change—The concentration of PSI reaction centers (P_{700}) in lake samples was measured using the light-minus-dark absorbance change at 810 nm. Validation of method is discussed below in the section "Quantitation of PSI using ΔA_{810}". The time course of absorbance change at 810 nm was recorded using a Perkin-Elmer Lambda-6 spectrophotometer with computer data acquisition with PECSS software. A 1-cm pathlength, semi-micro (1 ml) cuvette was placed in a cuvette holder modified to allow mounting of a 1 cm diameter optical fiber bundle against the side of the cuvette. Actinic illumination (400 μmol photons m^{-2} s^{-1}) was provided to the fiber optic by a halogen light source filtered through a CS 4–96 Corning filter. The measuring beam bandwidth was 4 nm and instrument response time was 1 s. The spectrophotometer photomultiplier tube was shielded from actinic light and fluorescence emission by Schott RG 780 long pass filters. Absorbance measurements were made with about 0.5 ml of thylakoid preparation in the presence of 2 mM sodium ascorbate and 200 μM methyl viologen. Absorbance was recorded for 10 s in the dark to establish a baseline, the actinic light was switched on and absorbance under actinic illumination was recorded for 15 s. Each sample was illuminated for 4–5 times with 2 min dark recovery period between exposures. Individual exposures were then combined to obtain an average trace. At times an apparent transient shift in absorbance occurred when the light source was switched; these "spikes" in the time course of absorbance change were omitted from subsequent data analysis.

The concentration of PSI reaction centers in thylakoid membranes isolated from algal cultures was also determined from light-minus-dark absorbance changes at 702 nm using a laboratory-constructed spectrophotometer (Neale and Melis 1986). Buffer and detergent conditions were identical to that used for the ΔA_{810} measurements.

Chlorophyll—Chlorophyll concentration in the membrane preparations was determined in 80% acetone extracts using the equations of Arnon (1949) as modified by Melis et al. (1987).

Cultures—Chlamydomonas reinhardtii (strain CC-124, Duke University) was grown as described previously (Neale and Melis 1986) under a light intensity of 100 μmol m^{-2} s^{-1} at 20°C. Lake Bonney phytoplankton were grown in enrichment culture by directly inoculating culture media with lake water from 17 m. The growth media was similar to that used for culture of *Dunaliella salina* (Pick et al. 1986), with a NaCl concentration of 87.6 g $liter^{-1}$ (1.5 M). This salinity resulted in an ionic strength similar to that in Lake Bonney at 17 m (Spigel et al. 1991). The Lake Bonney cultures were grown at 4°C under warm-white fluorescent lamps with a light intensity of 7 μmol photons m^{-2} s^{-1} (measured with a 4-π collector immersed in a culture flask, Biospherical Instruments QSL-100). The dominant species in the culture was *C. subcaudata* (Lizotte pers. comm.). The culture was harvested at a density of 2.15×10^5 cells ml^{-1}.

Fluorescence kinetics—The rate of light absorption by PSII under light-limiting conditions was determined from the analysis of the complementary growth over the fluorescence induction curve of DCMU-treated (20 μM) thylakoid membranes isolated from cultured algae (Melis and Anderson 1983). The suspension was placed in a cuvette (0.125 cm pathlength in direction of actinic beam) at a Chl ($a+b$) concentration of 30–40 μM. The emitted fluorescence was filtered through a combination of CS 2–64 (red long pass) Corning and Balzers 690 nm interference filters. Actinic illumination in the red and blue-green regions was provided by narrow band (10 nm full bandwidth at half maximum) Oriel interference filters, with peak transmittance at 660 and 481 nm. These peak positions were chosen in order to sensitize photochemistry predominantly through light absorbed by Chl *a* (660 nm) or by light absorbed by carotenoids (481 nm). Actinic illumination was varied between 5

and 15 μmol photons m^{-2} s^{-1} (measured with a 2-π collector, LI-COR LI185).

Light utilization—Integrated light absorption by thylakoid membrane suspensions was computed for each actinic light regime using the equation

$$Q_{abs} = c2.303 \sum_{\lambda=peak-20\ nm}^{peak+20\ nm} A(\lambda)T(\lambda)\Delta\lambda \quad (1)$$

where Q_{abs} is absorbed irradiance (μmol m^{-2} s^{-1}), $A(\lambda)$ is the absorbance spectrum of the thylakoids, $T(\lambda)$ is the spectral transmission through the filter used for actinic exposure and c is a constant (units μmol photons m^{-2} s^{-1}) such that

$$c = \frac{Q_{act}}{\sum_{\lambda=peak-20\ nm}^{peak+20\ nm} T(\lambda)\Delta\lambda} \quad (2)$$

where Q_{act} is the measured intensity of the actinic irradiance. Wavelength was varied at 1 nm intervals over a 40 nm range centered on the wavelength of peak transmission of each filter.

Results

Measurement of PSI from ΔA_{810}—Spectrophotometric measurements of PSI in photosynthetic membranes are most often done in the 700 nm region, where the extinction coefficient for the light-induced bleaching corresponding to P_{700} photooxidation reaches a maximum of 64 mM^{-1} cm^{-1} (Hiyama and Ke 1972). This method has the disadvantage of requiring a narrow bandwidth (1 nm) measuring beam and a relatively large amount of material to obtain an acceptable signal to noise ratio (ca. 100 μg Chl per sample for routine measurements). Therefore the method has rarely been applied to natural phytoplankton populations (e.g. Falkowski 1983), being mostly applied to cultures (Perry et al. 1981, Prezelin 1981) or natural algae where large amounts of material can be easily obtained, e.g. ice algae (Barlow et al. 1988). Moreover, measurement of P_{700} using this method requires instrumentation that discriminates between ΔA_{700} and actinic illumination induced fluorescence emission at 700 nm. The fluorescence artifact can be eliminated by using a modulated measuring beam combined with a lock-in amplifier (Melis and Hart 1980); most commercially available spectrophotometers do not have this capability.

An alternate approach to measuring PSI that has received increasing attention is through the light-induced absorbance change in the 800–820 nm range (e.g. Klughammer and Schreiber 1991). This spectral region shows a light-induced increase of absorbance of P_{700} with a maximum extinction coefficient of 7.2 mM^{-1} cm^{-1} (Hiyama and Ke 1972). A wide measurement bandwidth compensates for the smaller extinction coefficient (relative to ΔA_{700}); and it is possible to use a lower Chl concentration per sample. In addition, the Chl fluorescence emission at 800–820 nm is insignificant. The method requires a sensitive spectrophotometer (differential resolution of at least 10^{-4} ΔA) and time-based recording of absorbance change, capabilities available on many commercially available spectrophotometers (e.g. Perkin-Elmer Lambda 6). Quantitation of PSI from ΔA_{810} and ΔA_{700} was compared using thylakoid membranes isolated from *C. reinhardtii*. Measurements of ΔA_{810} resulted in a concentration of P_{700} of 1.12 mmol mol Chl^{-1} (Chl/P_{700}=890). An example trace is shown in Fig. 1 (Trace A) with a sample concentration of 29 μM Chl $a+b$. The estimated Chl/P_{700} was the same for measurements made at 15 and 56 μM Chl $a+b$. A nearly identical P_{700} concentration (1.13 mmol mol Chl^{-1}) was determined using ΔA_{700} measurements. Thus, the ΔA_{810} method appeared to give an accurate estimate of P_{700} concentration under these measurement conditions.

Concentration of PSI in Lake Bonney phytoplankton—The biomass peak just below the ice cover was sampled several times during November and December 1990, however only the sample from December 5 was suitable for measurement of P_{700}. On this date, material was collected on a 1 μm pore size polycarbonate filter. A Chl/P_{700} ratio of 786 (mol mol^{-1}) was measured for this sample (Fig. 1,

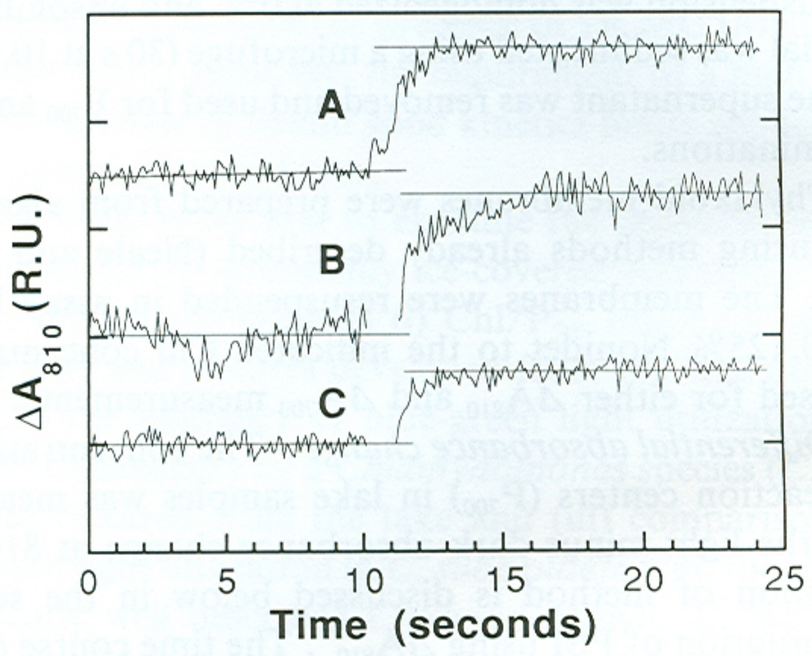


Fig. 1 Time-course of the light-induced absorption change recorded at 810 nm for membrane preparations from: (A) *Chlamydomonas reinhardtii*, Chl ($a+b$) concentration 28.48 μM; (B) sample from 17 m in Lake Bonney, November 22, 1990, Chl ($a+b$) concentration 12.4 μM; (C) sample from 4.5 m in Lake Bonney, December 5, 1990, Chl ($a+b$) concentration 13.4 μM. The reaction mixture contained 2 mM sodium ascorbate, 200 μM methyl viologen and 0.125% (v/v) Nonidet. Each trace represents the average of 4–5 exposures. A dark baseline was defined over the first ten seconds of the trace, at which time actinic illumination came on for a sufficient period to define a new steady-state absorbance level (ca. 15 s). The two mean absorbance levels are indicated by the straight lines. Further experimental details are given in the text.

Table 1 Measurements of the Chl/P_{700} ratio (mol Chl ($a+b$) [mol P_{700}]$^{-1}$) and Chl a/Chl b ratio (mol mol^{-1}) for thylakoid preparations from samples of Lake Bonney phytoplankton taken during November and December 1990

Date	Depth					
	5 m		13 m		17 m	
	Chl a/b	Chl/P_{700}	Chl a/b	Chl/P_{700}	Chl a/b	Chl/P_{700}
November 3–6	>20	nd	8.6	764		
November 9–10	>20	nd			3.6	811
November 15–16	>20	nd			5.4	830
November 22					3.6	480
December 5–6	>20	786	10.7	1,039	3.5	682

Entries are given for each time a sample was taken for the indicated depth, nd=not determined.

Trace C). The November samples were collected on 1.2 μm pore size nylon filters. Membranes prepared from this material could not be separated from scattering material which interfered with the ΔA_{810} method. However, pigment determinations on the November samples revealed that the composition was similar to December samples. Very low amounts of Chl b were found in all 4.5 m samples (Table 1) and the absorption spectra of acetone extracts were similar between samples in November and December (Fig. 2).

Better success was obtained with samples from 17 m where average cell sizes was large (>10 μm) and material could be concentrated on 3 μm pore size polycarbonate filters. The estimated Chl/P_{700} ratio decreased from around 800 (mol mol^{-1}) to 480 on the 22 November before rising again to 682 on December 5 (Table 1 also Fig. 1, trace B). This trend is inversely correlated with the variation of mean irradiance in the lake, which increased with incident irradiance through November, and then decreased in December as melting and fracturing decreased ice transparency (Sharp and Priscu 1992, Priscu 1992). This inverse relation may indicate that Chl/P_{700} decreased in response to higher irradiance (Perry et al. 1981). However the variation is relatively small, only about 20% around the mean, and could be due to assay variability. Moreover, while there was some variation in the Chl a/b ratio of the 17 m samples, being about 5.4 on November 22 and 3.6 otherwise (Table 1), higher Chl/P_{700} was not correlated with lower Chl a/b as has been observed for low-light acclimated chlorophytes in culture (Perry et al. 1981, Falkowski et al. 1981, Neale and Melis 1986). The absorbance spectra of acetone extracts of 17 m thylakoids showed little variation (Fig. 3). The pigment composition is consistent with dominance of the phytoplankton at 17 m by chlorophytes, primarily *Chlamydomonas subcaudata* (Parker et al. 1982, Lizotte and Priscu 1992a).

Samples from 13 m were taken on November 6–8

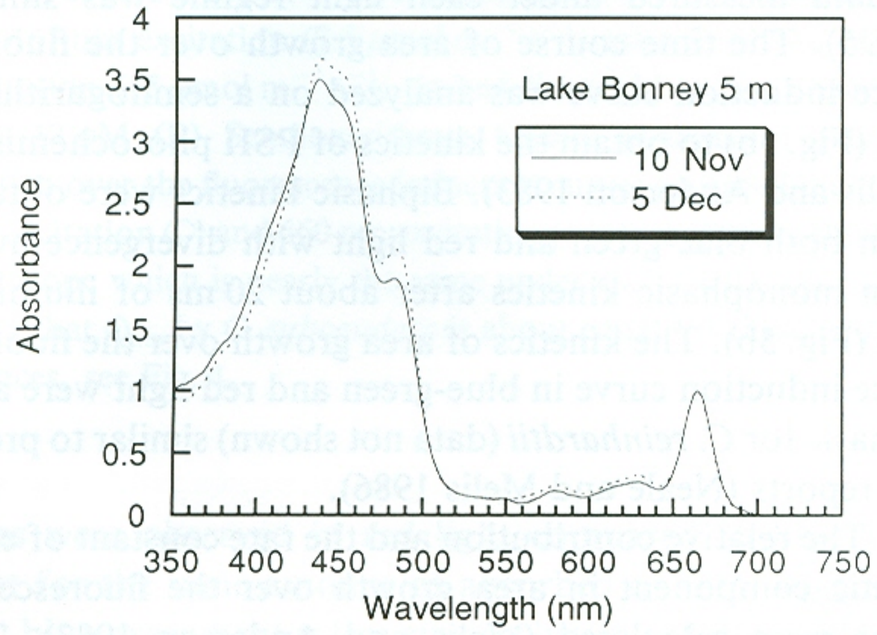


Fig. 2 Absorbance spectra for 80% acetone extracts of membrane preparations from Lake Bonney 4.5 m phytoplankton. Absorbance is relative to 1 at the 665 nm Chl a peak. Preparations from samples on November 9, 1990 (solid line) and December 5, 1990 (dashed line).

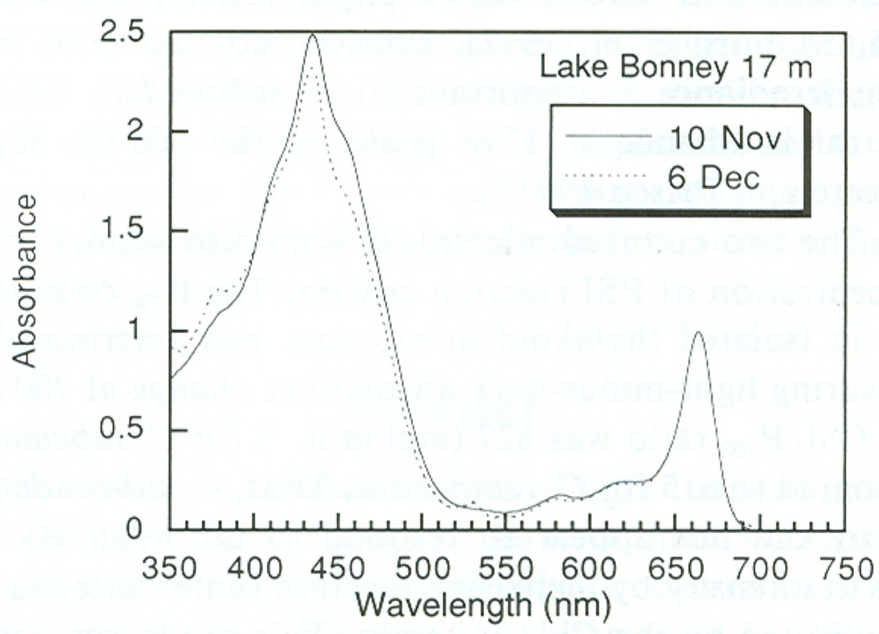


Fig. 3 Absorbance spectra for 80% acetone extracts of membrane preparations from Lake Bonney 17 m phytoplankton. Absorbance is relative to 1 at the 665 nm Chl a peak. Preparations from samples on November 10, 1990 (solid line) and December 6, 1990 (dashed line).

and December 6. Phytoplankton were collected on polycarbonate filters with a 3 μm pore size. The Chl a/b ratio (ca. 9–11) was intermediate between 4.5 m and 17 m. The Chl/P_{700} measured for the 13 m sample from November was in the same range as the Chl/P_{700} measured for 17 m samples in the same month, however the Chl/P_{700} in December was 1,039, the only time the measurement exceeded 1,000 mol Chl (mol P_{700})$^{-1}$ in any of the samples from Lake Bonney.

Comparison of fluorescence kinetics in C. reinhardtii and C. subcaudata—The efficiency of light utilization by PSII was studied by analysis of fluorescence kinetics of Lake Bonney microalgae grown in nutrient enriched culture media. The culture was inoculated with a sample taken at 17 m where the dominant species (in both the lake and the culture) was a chlorophyte alga, *Chlamydomonas subcaudata* (Parker et al. 1982). We sought to establish whether the absorption and utilization of blue-green light by this chlorophyte alga differed from the photobiologically well-described species in the same genus, *C. reinhardtii*.

The absorbance and pigmentation characteristics of these two species were similar despite the difference in growth irradiance (factor of ten lower for *C. subcaudata*). Under culture conditions the Chl a/b ratio was 2.65 for *C. reinhardtii* and 2.50 for *C. subcaudata*. The latter ratio is lower than found for freshly collected 17 m samples (Table 1), which could have been caused by the presence of other, non-chlorophyte, algae in the lake or by the different spectral irradiance regime. The absorbance spectra of thylakoid membranes were also similar (Fig. 4), with major absorption maxima near 435 nm and 680 nm, and prominent shoulders around 480 nm and 650 nm. Absorption in the red region is attributed to Chl a and Chl b, whereas in the blue and blue-green wavebands, especially around 480 nm, a significant role is played by carotenoids, such as β-carotene and several xanthophylls (Siefermann-Harms 1985, Mimuro et al. 1992). Efficient utilization of blue-green irradiance is important to *C. subcaudata* because spectral irradiance at 17 m peaks in the 500 nm region (Lizotte and Priscu 1992b).

The two cultured microalgae were also similar in the concentration of PSI reaction centers. The P_{700} concentration in isolated thylakoid membranes was determined by measuring light-minus-dark absorbance change at 700 nm. The Chl/P_{700} ratio was 627 (mol mol^{-1}) for *C. subcaudata* compared to 615 for *C. reinhardtii*. Thus, *C. subcaudata* in culture did not appear to respond to the relatively low growth intensity by increasing reaction center antenna size as indicated by the Chl/P_{700} ratio. This result was consistent with the estimated Chl/P_{700} ratios for fresh Lake Bonney samples from 17 m using ΔA_{810} (Table 1).

The relative efficiency of blue-green light utilization by *C. subcaudata* was assayed through the rate of PSII photochemistry in narrow band blue-green and red actinic illumination. The red and blue-green actinic illumination was defined, respectively, by 660 nm and 481 nm interference filters (Fig. 4). The rate of absorption was approximately two times higher in 481 nm light compared to the same quantum flux of 660 nm light for both cultures (Fig. 4). The shape of the fluorescence induction curve of *C. subcaudata* measured under each light regime was similar (Fig. 5). The time course of area growth over the fluorescence induction curve was analyzed on a semilogarithmic plot (Fig. 5b) to obtain the kinetics of PSII photochemistry (Melis and Anderson 1983). Biphasic kinetics were obtained in both blue-green and red light with divergence away from monophasic kinetics after about 20 ms of illumination (Fig. 5b). The kinetics of area growth over the fluorescence induction curve in blue-green and red light were also biphasic for *C. reinhardtii* (data not shown) similar to previous reports (Neale and Melis 1986).

The relative contribution and the rate constant of each kinetic component in area growth over the fluorescence curve were calculated (Melis and Anderson 1983). The average rate of light-limited PSII photochemistry was calculated as a weighted average of the kinetic constants (Ghirardi and Melis 1984). For thylakoids of *C. reinhardtii*, the rates of PSII turnover had a different dependence on absorbed photons in each light regime (Fig. 6b). Higher PSII

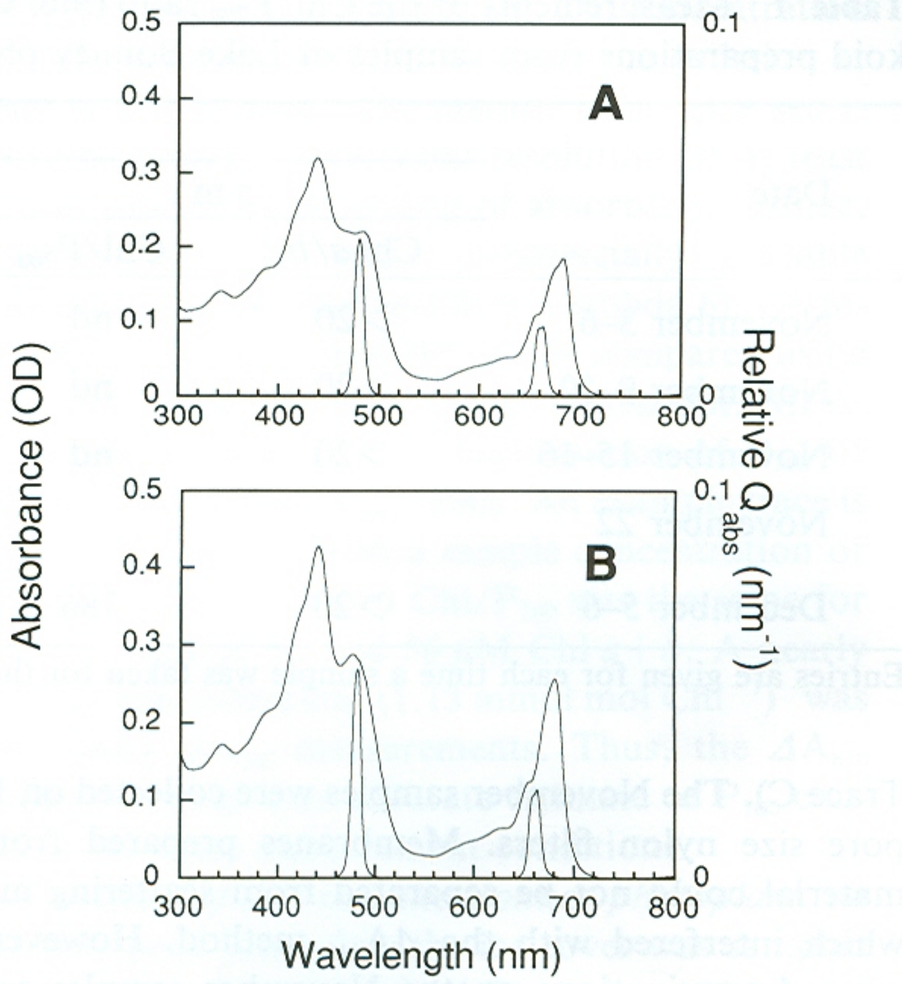
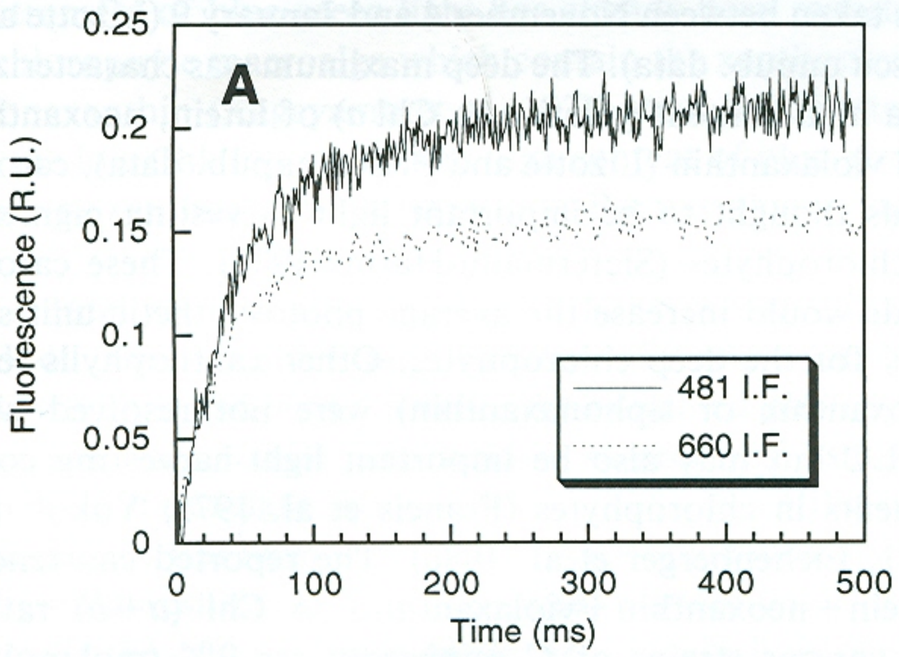


Fig. 4 Absorbance spectra for thylakoid membrane preparations from algal cultures. (A) Culture from 17 m in Lake Bonney, primarily *Chlamydomonas subcaudata*, Chl ($a+b$) concentration 33 μM. (B) Culture of *C. reinhardtii*, Chl ($a+b$) concentration 45 μM. Curves near 480 nm and 660 nm show relative Q_{abs} for equal amounts of Q_{act} calculated using Eq. 2.



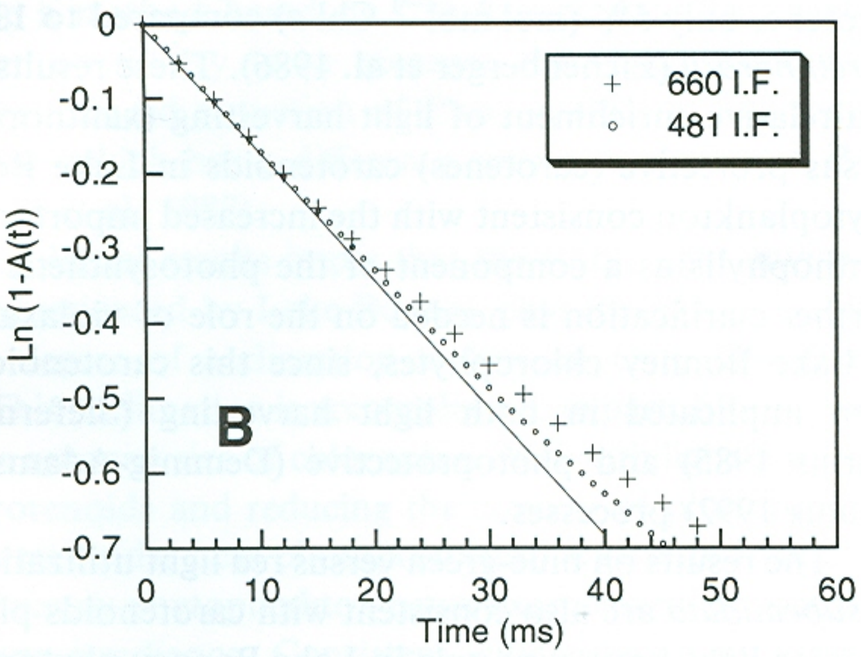


Fig. 5 Variable fluorescence induction traces for thylakoid preparations of *Chlamydomonas subcaudata* suspended in the presence of 20 μM DCMU. (A) Comparison of traces measured using 481 nm excitation (7.5 μmol m^{-2} s^{-1}, solid line) and 660 nm excitation (15 μmol m^{-2} s^{-1}, dashed line) Chl ($a+b$) concentration 33 μM (B) Semilogarithmic kinetic analysis of the area growth over the fluorescence induction curves recorded under 481 nm excitation (○) and 660 nm excitation (+). Straight line shows initial slope which is nearly the same under each excitation regime. Note that Q_{abs} by *C. subcaudata* is about equal for these two irradiances, see Fig. 4.

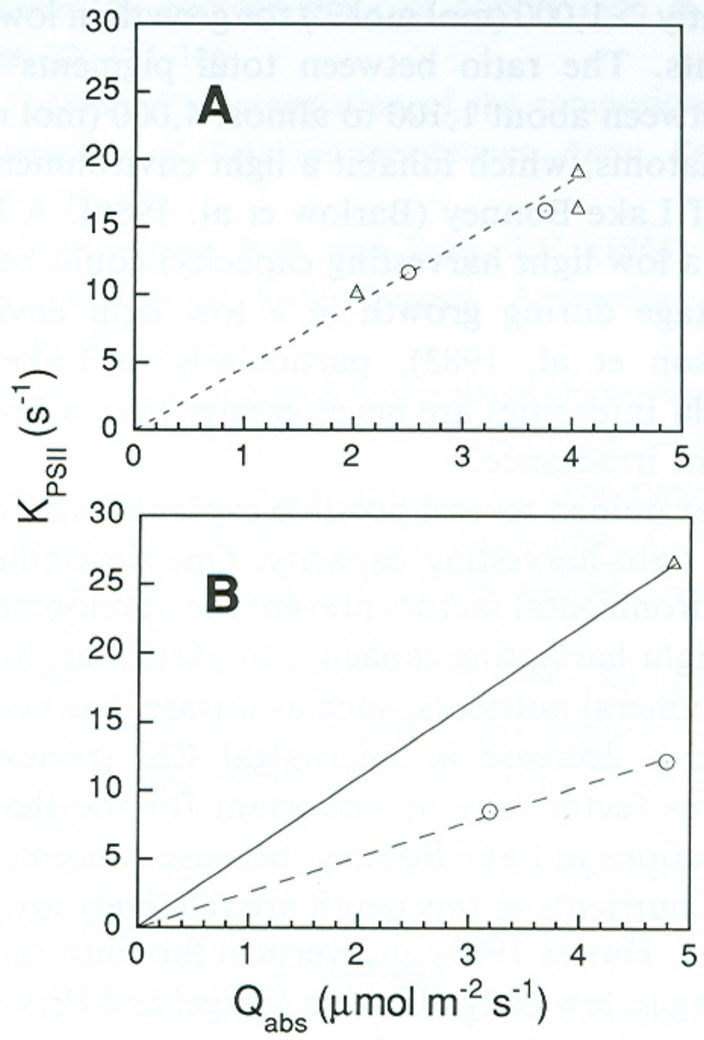


Fig. 6 Relationship between average rate of PSII photochemistry (turnovers per second) and rate of light absorption (Q_{abs}) for thylakoid membrane preparations of (A) *Chlamydomonas subcaudata* and (B) *C. reinhardtii*. Measurements were made under 660 nm excitation (triangles) and 481 nm excitation (circles). Note for *C. reinhardtii*, the slope (=utilization efficiency) was different for the different excitation spectra, but for *C. subcaudata* all measurements clustered around a single slope.

rates were observed in red light compared to blue-green light for the same amount of absorbed quanta, suggesting that blue-green light is less efficiently utilized than red light. This may have occurred because blue-green light is absorbed by pigments such as carotenoids that are not efficiently transferring excitation energy to PSII. In contrast, PSII photochemistry in *C. subcaudata* had the same dependence on absorbed quanta in blue-green and red light (Fig. 6a). Moreover, the slope of the linear relationship between PSII rate and absorbed quanta for *C. subcaudata* (dotted line in Fig. 6a) was similar (within 15%) to the slope of PSII rate per absorbed quanta of red light for *C. reinhardtii* (solid line in Fig. 6b). The observation that 481 nm photons absorbed by *C. subcaudata* are utilized with similar efficiency as 660 nm photons absorbed by *C. reinhardtii* (or *C. subcaudata*) implies that *C. subcaudata* possesses a configuration of pigments that efficiently transfers photons in the 480 nm waveband to PSII.

Discussion

We have used measurements of light-induced absorbance change at 700 nm and 810 nm to estimate the Chl/P_{700} ratio of phytoplankton in perennially ice covered Lake Bonney. Irrespective of methodology, it was found that the ratio was unexpectedly low, ranging from 480 to 1,039 Chl/P_{700} (mol mol^{-1}), with a mean of 734. This contrasts with several other studies of eukaryotic microalgae in culture (e.g. Perry et al. 1981, Falkowski et al. 1981), and sea-ice microalgae (Barlow et al. 1988) which found Chl/PSI

consistently >1,000 (mol mol^{-1}) for growth in low light environments. The ratio between total pigments and PSI varied between about 1,100 to almost 4,000 (mol mol^{-1}) in sea-ice diatoms, which inhabit a light environment similar to that of Lake Bonney (Barlow et al. 1988). A low Chl/P_{700} (i.e. a low light harvesting capacity) could be a severe disadvantage during growth in a low light environment (Richardson et al. 1983), particularly in Lake Bonney where light intensities are never greater than a few percent of incident irradiance.

We examined several possible explanations of this low apparent light-harvesting capacity. One possibility is that other environmental factors prevent the development of additional light harvesting capacity. In particular, low availability of mineral nutrients, such as nitrate, has been associated with a decrease in microalgal Chl (review Turpin 1991). This factor may be important for the shallow (4.5 m) populations in Lake Bonney, because concentrations of dissolved nutrients at this depth are relatively low (Angino et al. 1964, Hawes 1985) and vertical flux into this layer is slow owing to low eddy diffusion (Spigel and Priscu unpub. data). The 4.5 m phytoplankton populations were also observed to have a low PSII quantum yield (variable fluorescence ratio), low fluorescence quantum yield and low quantum yield for photosynthesis (Neale and Priscu, unpub. data, Lizotte and Priscu 1994). Low quantum yields have also been observed in cases of marked nutrient limitation of microalgal cultures (Cleveland and Perry 1987, Kolber et al. 1988, Geider et al. 1993).

However, efficiency of light harvesting does not always decrease under nutrient limitation (Turpin 1991) and Chl/P_{700} actually increased in sea-ice diatoms as nutrients were depleted (Barlow et al. 1988). Moreover, the population at 17 m, dominated by the large flagellate *C. subcaudata*, is associated with much higher nutrient concentrations (Sharp and Priscu 1990). It also has a much higher quantum yield of PSII, fluorescence and photosynthesis (Neale and Priscu unpub. data, Lizotte and Priscu 1994). Despite higher nutrients and quantum yield, the Chl/P_{700} was still low, even in samples grown in low irradiance and nutrient enriched media.

Another explanation for the low Chl/P_{700} ratio is that pigments other than Chl constitute a major fraction of the functional light harvesting capacity. This possibility is consistent with pigment determinations on Lake Bonney phytoplankton using high performance liquid chromatography (HPLC) (Lizotte and Priscu unpub. data). The shallow Lake Bonney populations were dominated by cryptophytes and had a high content of alloxanthin—a major light harvesting pigment in that group (Siefermann-Harms 1985). These populations also contained significant amounts of Chl *c*. Inclusion of alloxanthin and Chl *c* increases the light harvesting pigment to P_{700} ratio by 186%, based on the average alloxanthin and chlorophyll *c* content of 4.5 m samples taken between November 4 and January 9 (Lizotte and Priscu unpub. data). The deep maximum was characterized by a high content (relative to Chl *a*) of lutein, neoxanthin and violaxanthin (Lizotte and Priscu unpub. data), carotenoids thought to be important light-harvesting pigments in chlorophytes (Siefermann-Harms 1985). These carotenoids would increase the average photosynthetic unit size 27% for the deep chlorophytes. Other xanthophylls (e.g. loroxanthin or siphonaxanthin) were not resolved with HPLC but may also be important light-harvesting components in chlorophytes (Francis et al. 1975, Yokohama 1981, Eichenberger et al. 1986). The reported carotenoid (lutein+neoxanthin+violaxanthin) to Chl (*a*+*b*) ratios for various strains of *C. reinhardtii* are 9% (mol mol^{-1}, Sirevag and Levine 1973), 14% (Francis et al. 1975) and 22% (Eichenberger et al. 1986). By comparison, the 17 m population, dominated by *C. subcaudata*, not only has a high xanthophyll content but a relatively low content of β-carotene, only 5% (mol mol^{-1} Chl *a*) compared to 13% in *C. reinhardtii* (Eichenberger et al. 1986). These results imply a relative enrichment of light harvesting (xanthophylls) versus protective (carotenes) carotenoids in Lake Bonney phytoplankton consistent with the increased importance of xanthophylls as a component of the photosynthetic unit. Further clarification is needed on the role of violaxanthin in Lake Bonney chlorophytes, since this carotenoid has been implicated in both light harvesting (Siefermann-Harms 1985) and photoprotective (Demmig-Adams and Adams 1992) processes.

The results on blue-green versus red light utilization by *C. subcaudata* are also consistent with carotenoids playing a major light harvesting role in Lake Bonney phytoplankton. Various results have been previously obtained on the efficiency of algal photosynthesis in blue-green light. Action spectra of the quantum yield of photosynthesis in chlorophyte algae (Emerson and Arnold 1932) and higher plants (Evans 1987) typically have a "dip" in the blue-green (480–520) nm waveband. This has been attributed to both a lack of energy transfer from carotenoids to PSII and the preferential association of carotenoids with PSI. In the latter case, blue-green illumination would not provide balanced excitation to the photosystems. The relatively low efficiency of 481 nm light utilization by PSII in *C. reinhardtii* could be caused by either low energy transfer from PSII pigments or by the dominance of PSI pigments in this waveband. Our results showing increased efficiency of 481 nm light utilization in *C. subcaudata* also has at least two explanations. One possibility is a significantly higher PSII/PSI ratio in *C. subcaudata*, so that more of the 481 nm region pigments were associated with PSII. This alternative seems unlikely, however, in that a high PSII/PSI ratio is usually associated with a large Chl/P_{700} ratio (Neale and Melis 1986). Alternatively, there is more efficient transfer of absorbed blue-green irradiance to PSII. Efficient excita-

tion transfer of blue-green light has already been reported for chlorophyte macroalga which contain the xanthophyll, siphonaxanthin (Kageyama et al. 1977), and is consistent with the low Chl/P_{700} ratios and high content of blue-green absorbing xanthophylls found in Lake Bonney phytoplankton.

A total pigment to PSI ratio for Lake Bonney phytoplankton that includes photosynthetically-active carotenoids would significantly increase the estimated photosynthetic unit size in these populations, as previously shown for sea-ice microalgae (Barlow et al. 1988). Nevertheless, the pigment : PSI ratio remains an approximate measure of the light-harvesting capacity of an algal population. This is especially the case for chlorophyte algae in which increases in functional antenna size at low light are confounded with changes in PSII/PSI stoichiometry (Neale and Melis 1986). Thus, a method to measure PSII concentration in natural populations would clearly be useful. Kolber et al. (1988) propose a method based on Chl fluorescence flash yield. Another possibility is to measure the ΔA_{810} associated the light-induced reduction of Pheo in PSII at low redox potential, which has a difference spectrum similar to P_{700} (Demeter et al. 1987).

Our initial results imply that the stable light environment experienced by Lake Bonney phytoplankton enables a high degree of acclimation to low intensity blue-green light. This acclimation is accomplished, in part, by augmenting the antenna size of chloroplast PSII with light-harvesting carotenoids and reducing the content of photoprotective carotenoids. Less photoprotection is required because Lake Bonney phytoplankton never experience near-surface inhibiting irradiance. Consistent with minimal photoprotection, Lake Bonney phytoplankton showed a very high sensitivity to photoinhibition by PAR, UVA or UVB irradiance (Neale et al. 1994). Thus, in Lake Bonney as well in other environments, the tradeoff between lowering sensitivity to photoinhibition and increasing light harvesting and light utilization efficiency appears to be an important constraint on the structural and functional responses of the photosynthetic apparatus to the light environment.

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