

Natural fluorescence and quantum yields in vertically stationary phytoplankton from perennially ice-covered lakes

Michael P. Lizotte¹ and John C. Priscu

Department of Biological Sciences, Montana State University, Bozeman 59717

Abstract

Phytoplankton in ice-covered lakes near McMurdo Sound, Antarctica, are stratified vertically in distributions similar to deep chlorophyll maxima commonly observed in lakes and seas. We measured natural fluorescence flux rates, chlorophyll concentration (Chl *a*), phytoplankton absorption spectra (a_{ph}), photosynthetic efficiency, and spectral irradiance to derive the quantum yields for photosynthesis (Φ_c) and fluorescence (Φ_f). Chlorophyll concentrations predicted from natural fluorescence based on mean a_{ph} [$0.015 \text{ m}^2 (\text{mg Chl } a)^{-1}$] and mean Φ_f [$0.044 \text{ mol photons fluoresced } (\text{mol photons absorbed})^{-1}$] correlated significantly with measured Chl *a* ($n = 122$, $r = 0.88$). Predictions of primary productivity from natural fluorescence based on mean values for Φ_f and Φ_c were poor. Relationships between $\Phi_c : \Phi_f$ and temperature and between $\Phi_c : \Phi_f$ and irradiance implied that these environmental variables would not provide good bases for correcting predictions of primary production. $\Phi_c : \Phi_f$ varied most coherently with distance from the nutricline, due primarily to a large increase in maximum Φ_c [$0.0015\text{--}0.051 \text{ mol C } (\text{mol photons})^{-1}$] with proximity to the nutricline. Our results indicate that nutrient supply may be a critical variable to consider when using natural fluorescence methods to estimate primary productivity in vertically stable phytoplankton.

Natural fluorescence of chlorophyll *a* has been proposed as a rapid method of estimating phytoplankton biomass and primary production on finer spatial and temporal scales than possible with standard water-sampling techniques (e.g. Kiefer et al. 1989; Chamberlin et al. 1990; Stegmann et al. 1992). The efficacy of using natural fluorescence to estimate chlorophyll concentrations has been demonstrated for a wide range of oceanic environments, from oligotrophic to nearshore (Chamberlin et al. 1990) waters and from deep chlorophyll maxima (Kiefer et al. 1989) to surface waters (Stegmann et al. 1992). However, the models proposed for deriving primary production rates from the natural fluorescence signal are highly sensitive to the values assumed for the quan-

tum yields of fluorescence and photosynthesis (Chamberlin et al. 1990). Because quantum yields cannot be measured on the same time and space scales as natural fluorescence, recent work has focused on predicting quantum yield relationships based on measured irradiance and temperature (Chamberlin et al. 1990; Chamberlin and Marra 1992).

Although these corrective algorithms reduced the overall variability in predictions of primary production rates, Chamberlin et al. (1990) noted that data from deep chlorophyll maxima remained as outliers. Phytoplankton in deep chlorophyll maxima may have unusual quantum yields resulting from shade acclimation (Dubinsky and Berman 1976; Kishino et al. 1986) or in response to richer nutrient conditions near a nutricline (e.g. Cleveland et al. 1989; Kolber et al. 1990). Algorithms to correct for these factors may be necessary for application of natural fluorescence techniques in studies of vertically stable, deep phytoplankton populations, such as those inhabiting deep chlorophyll maxima in oceans and lakes worldwide.

We proposed previously (Lizotte and Priscu 1992a) that shade-adapted phytoplankton populations from perennially ice-covered lakes in the dry valleys of Antarctica present a system analogous to multiple deep chlorophyll maxima. Water-column stability in these lakes

¹ Current address: Department of Biology and Microbiology, University of Wisconsin-Oshkosh, Oshkosh 54901-8640.

Acknowledgments

We thank P. J. Neale, T. R. Sharp, R. H. Spigel, I. Forne, and E. Norton for their assistance in the field. We also thank P. J. Neale, K. Arrigo, and two anonymous reviewers for comments on this manuscript. The profiling natural fluorometer used in this study was a prototype loaned from NASA/JPL thanks to arrangements made by D. J. Collins, R. C. Booth, and J. H. Morrow.

This work was supported by NSF grants DPP 88-20591, DPP 91-17907, and DPP 92-11773 to J.C.P.

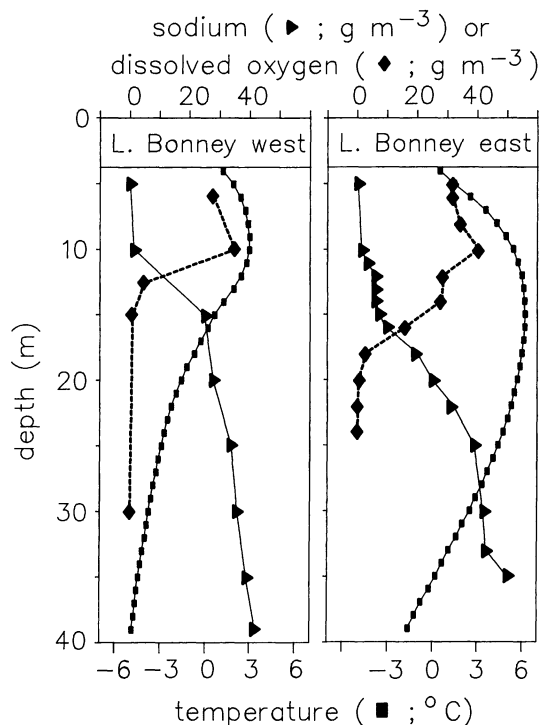


Fig. 1. Profiles of temperature, dissolved oxygen, and sodium in Lake Bonney, east and west lobes. Oxygen profile for the west lobe adapted from Parker et al. (1982).

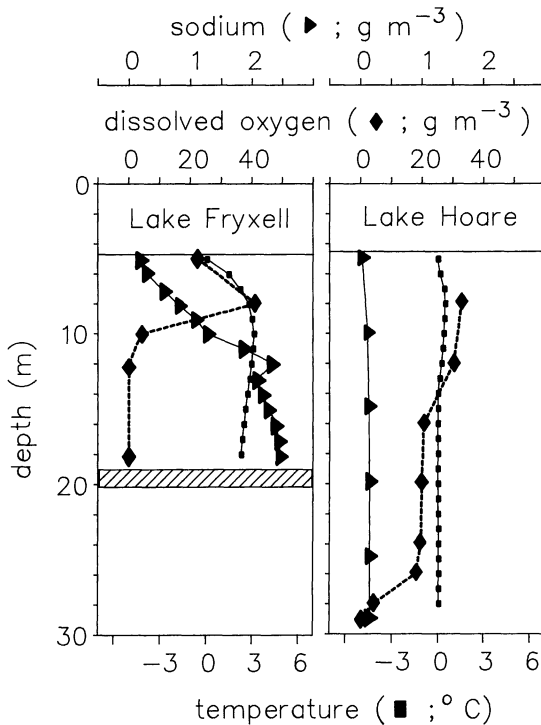


Fig. 2. Profiles of temperature, dissolved oxygen, and sodium in Lakes Fryxell and Hoare. Sodium profiles adapted from McKnight et al. (1991) and oxygen profiles from Parker et al. (1982) for Lake Fryxell and Wharton et al. (1986) for Lake Hoare. Note the scale for sodium concentration is different from Fig. 1.

is due primarily to thick ice cover (3–5 m), which eliminates wind-driven turbulence, low advective stream inflow, and strong vertical gradients in salinity (e.g. Spigel et al. 1991). These lakes are among the least turbulent aquatic systems in the world and support stratified phytoplankton populations growing under a broad range of temperature, salinity, and nutrient conditions. Here, we report on the quantum yields of photosynthesis and fluorescence in relation to the ability to predict chlorophyll concentration and primary production rates from natural fluorescence. Our results imply that, in stable water columns, natural fluorescence measurements are acceptable for estimating chlorophyll concentrations, but the prediction of primary production in deep chlorophyll maxima requires some knowledge of the nutrient regime (e.g. distance from the nutricline).

Methods

Study area—Lake Bonney, Lake Hoare, and Lake Fryxell are in the Taylor Valley in the

ice-free desert region adjacent to McMurdo Sound, Antarctica. All are covered year-round with 3.5–4.5 m of ice and have liquid water-column depths ranging from 15 to 38 m, recorded relative to the piezometric level in ice holes (~0.3 m below the ice surface). These lakes have been classified as meromictic (e.g. Angino et al. 1964; Spigel et al. 1991). Lake Bonney has two basins, an east lobe and a west lobe (max depths, ~38 m), separated by a narrow sill ~40 m wide and 12 m deep. A freshwater epilimnion can be easily distinguished from a hypersaline hypolimnion in both lobes of Lake Bonney (Fig. 1) and in Lake Fryxell (Fig. 2). Lake Hoare is a freshwater reservoir dammed by the Canada Glacier, with relatively uniform temperature and sodium profiles (Fig. 2). Oxygen-depleted deep water exists in all four basins (Figs. 1, 2). Phytoplankton populations are typically dominated by flagellates (e.g. Seaburg et al. 1983). During our

studies, HPLC pigment analysis revealed that cryptophytes dominated all samples from Lakes Hoare and Fryxell and samples from immediately beneath the ice in both lobes of Lake Bonney, chrysophytes dominated at intermediate depths (10–15 m) throughout Lake Bonney, and chlorophytes dominated in the deepest samples from the east lobe of Lake Bonney (Lizotte and Priscu 1992c).

Sample collection and water chemistry—Water samples were retrieved and instruments were lowered through holes (0.25–1-m diam) in the ice cover. Water was collected with a Niskin bottle (2 or 10 liters), transferred to HDPE bottles, and stored (<6 h) in the dark until processed at the Lake Bonney field camp.

Nutrient concentrations were determined by standard wet chemistry methods as outlined by Parsons et al. (1984). High salinity samples were diluted with distilled-deionized water to a salinity range typical of seawater (Sharp 1993). Briefly, ammonium was measured by the phenol hypochlorite method, nitrate plus nitrite was determined by cadmium reduction followed by diazotization, nitrite was determined by diazotization, and soluble reactive P (SRP) was measured by the mixed molybdate method. Nitrate concentrations were estimated by subtracting nitrite concentrations (which were relatively low) from nitrate plus nitrite.

Phytoplankton pigments and absorption spectra—Phytoplankton populations were studied from beneath the ice down to the oxycline depths in each basin. Chl *a* concentration was quantified by collecting particulate material onto Whatman GF/C filters, extracting overnight in 90% acetone at 4°C, and measuring fluorescence of extracts with a Turner Designs model 10 fluorometer as outlined by Holm-Hansen et al. (1965). Fluorescence after acidification was used to derive pheopigment-corrected chlorophyll concentrations relative to standards of purified Chl *a*.

Absorption spectra of phytoplankton (400–700 nm) were determined by spectrophotometry of material concentrated on Whatman GF/C filters (Mitchell and Kiefer 1988) based on the difference between spectra measured before and after extraction with methanol (Kishino et al. 1985). Scans were made from 380 to 750 nm, relative to a wet blank filter, with a Perkin-Elmer model Lambda-6 spec-

trophotometer. Volumes of 0.25–1.0 liter were filtered to produce maximum optical densities <0.2 absorbance units. Spectra were normalized to the average optical density in the range 730–750 nm for background drift correction. To estimate spectral variation of the path-length amplification factor (β) and derive absorption coefficients from optical density, we used the equation described by Bricaud and Stramski (1990) which is based on data from Mitchell and Kiefer (1988). We were unable to measure β specifically for this combination of equipment. Because optical density is divided by β to derive absorption coefficients, errors in estimation of β would generally be inversely proportional to estimated particulate absorption coefficients and directly proportional to estimated quantum yields.

P–I response—Photosynthesis–irradiance (*P–I*) experiments were conducted as described by Lizotte and Priscu (1992a). Incubator irradiance ranged from 2 to 240 $\mu\text{Einst m}^{-2} \text{ s}^{-1}$; it was produced by tungsten-halogen lamps and passed through a blue filter (Roscolene No. 62). Most samples were inoculated to a final activity of 5 $\mu\text{Ci ml}^{-1}$ with [^{14}C] bicarbonate and dispensed in 10-ml aliquots into 27 glass 20-ml vials. Three of these aliquots were acidified with 0.5 ml of 3 N HCl to determine background radioactivity to be subtracted from subsequent uptake values. The remaining 24 vials were incubated for 3–5 h at simulated in situ temperatures (0–6°C, Table 1).

Incubations were terminated by turning off the lamps, followed by acidification and drying at ~70°C. Each sample was rehydrated and the radioactivity determined by liquid scintillation spectroscopy. Because the dried samples had to be rehydrated in <2 ml of water (the water capacity for 20 ml of the scintillation cocktail), high salinity samples from east L. Bonney 17 m and west L. Bonney 13 m, which produced a considerable amount of residue upon drying, were limited to 2 ml of lake water per vial and were inoculated to a final activity of 25 $\mu\text{Ci ml}^{-1}$. Dissolved inorganic C concentration was determined by infrared gas analysis of acidified water samples. Carbon fixation rates were determined following Parsons et al. (1984). The *P–I* response was modeled with the hyperbolic tangent function with a *Y*-intercept term. The derived parameters in-

Table 1. Photosynthetic characteristics of phytoplankton in the dry valley lakes of Antarctica in 1990 and 1991. Temperatures are for laboratory incubations. Abbreviations and units: Chl *a* (mg m⁻³); in situ mean Chl *a*-specific absorption coefficient [a_{ph} , m² (mg Chl *a*)⁻¹]; in situ photosynthetic efficiency [α , $\mu\text{g C} (\mu\text{g Chl } a)^{-1} \text{ h}^{-1}$ ($\mu\text{Einst m}^{-2} \text{ s}^{-1}$)⁻¹]; intercept of in situ α and photosynthetic capacity (I_k , $\mu\text{Einst m}^{-2} \text{ s}^{-1}$); quantum yield of photosynthesis [Φ_i , mol C (mol photons)⁻¹]; and quantum yield of fluorescence [Φ_f , mol photons fluoresced (mol photons absorbed)⁻¹].

Lake	Depth (m)	Temp. (°C)	Chl <i>a</i>	a_{ph}	α	I_k	Φ_i	Φ_f	$\Phi_i : \Phi_f$
Bonney east 25 Nov	4.5	0.0	0.91	0.013	0.0017	23	0.0031	0.068	0.046
	6	3.0	0.69	0.015	0.0015	34	0.0024	0.071	0.034
	10	6.0	0.61	0.017	0.0054	31	0.0072	0.046	0.16
Bonney east 10 Dec	4.5	0.0	1.01	0.020	0.0015	33	0.0018	0.020	0.090
	6	3.0	0.68	0.016	0.0015	37	0.0022	0.039	0.056
	10	6.0	0.78	0.020	0.0035	52	0.0041	0.037	0.11
	17	6.0	0.71	0.015	0.0024	14	0.036	0.023	1.6
Bonney east 4 Jan	4.5	0.0	1.39	0.013	0.0047	24	0.0084		
	6	3.0	1.81	0.013	0.0040	33	0.0069		
	10	6.0	1.68	0.017	0.0073	40	0.010		
	12	6.0	1.97	0.018	0.0077	29	0.0098		
	17	6.0	0.79	0.012	0.012	23	0.023		
Bonney west 1 Dec	4	1.0	1.21	0.019	0.0012	23	0.0015	0.050	0.030
	6	3.0	1.49	0.019	0.0026	40	0.0032	0.022	0.15
	8	3.0	1.39	0.019	0.0028	33	0.0034	0.020	0.17
	13	1.0	1.37	0.019	0.0056	17	0.0068	0.033	0.21
Hoare 8 Dec	5	0.5	0.57	0.017	0.0036	33	0.0049	0.096	0.051
	10	0.5	3.15	0.010	0.0089	18	0.020	0.056	0.36
	12.5	0.5	4.62	0.010	0.018	11	0.043	0.030	1.4
Fryxell 7 Dec	5	1.0	3.33	0.017	0.0060	19	0.0082	0.047	0.17
	7	3.0	6.10	0.008	0.0059	15	0.018	0.048	0.37
	8.5	3.0	6.65	0.009	0.020	10	0.051	0.042	1.2
Mean				0.015	0.0058	27	0.012	0.044	0.37
SD				0.004	0.0051	11	0.014	0.020	0.50

clude the maximum biomass-specific photosynthetic rate (P_m^B) and the initial linear slope of the curve (α). I_k , an index of photoacclimation, was derived by dividing P_m^B by α . There was no indication of photoinhibition within the range of irradiances tested (which included irradiances up to 4-fold higher than found in situ).

Optical measurements—Profiles of natural fluorescence (upwelling nadir radiance centered at 683 nm, 1-nm bandwidth) and scalar irradiance (400–700 nm) were measured with a Biospherical Instruments PNF-300 profiling natural fluorometer (Kiefer et al. 1989). Spectral irradiance was measured with a Biospherical Instruments MER-1000 spectroradiometer (12 bands centered at 410, 441, 488, 507, 520, 540, 570, 589, 625, 656, 671, and 694 nm, with half-bandwidths of 5 nm) in the east lobe of Lake Bonney, in Lake Fryxell (Lizotte and Priscu 1992b), and in the incubator. The spectral composition of irradiance in the west lobe of Lake Bonney was assumed to be the

same as that of the east lobe. This assumption was confirmed the following year, 1991, when similar spectral irradiance profiles were recorded in both lobes. For Lake Hoare, we estimated the spectral composition of irradiance at our sampling depths from measurements made in 1982 with the same instrument (Palmisano and Simmons 1987). Depth sensor readings were corrected for large changes in water density by comparison with a calibrated line.

The maximal quantum yield of photosynthesis was determined as described by SooHoo et al. (1987) from measurements of α , phytoplankton absorption spectra, and the irradiance spectrum in the incubator. The ratio of the mean phytoplankton absorption coefficient weighted for the in situ irradiance spectrum to the coefficient weighted for the incubator spectrum was used to determine in situ α . Instantaneous primary production rates were calculated from in situ α , particulate chlorophyll concentrations, and PAR measured by the

PNF-300 at the time of the natural fluorescence profile. It is possible to estimate primary production directly from α and PAR because these phytoplankton are light limited in situ (Lizotte and Priscu 1992a; Sharp 1993). For the same reason, in situ quantum yields for photosynthesis equal the maximal quantum yields determined in the incubator. In situ quantum yields for fluorescence were estimated from ratios of natural fluorescence to PAR measured by the PNF-300 and corrected for phytoplankton absorption of PAR at each depth. Chl *a* concentration and primary production rate predicted from measurements of natural fluorescence were based on the equations presented by Kiefer et al. (1989).

Results

Profiles of natural fluorescence normalized to available irradiance [$L_u(683) : \text{PAR}$] showed maxima immediately beneath the ice in both lobes of Lake Bonney and deeper maxima in all three lakes (Fig. 3). $L_u(683) : \text{PAR}$ closely tracked Chl *a* concentrations with a vertical offset of ~ 1 m and gradually decreased to negligible values near the oxyclines. The offset is due to upwelling fluorescence, which originates up to several meters below the sensor (Kiefer et al. 1989). Therefore, phytoplankton samples from discrete depths were always compared with the natural fluorescence signal from 1 m shallower.

Photosynthetic and optical characteristics of phytoplankton varied with depth and between lakes (Table 1). In general, the deepest phytoplankton populations in each lake exhibited the lowest values for mean Chl *a*-specific absorption coefficient (a_{ph}), quantum yield for fluorescence (Φ_f), and I_k and the highest values for α and the maximal quantum yield for photosynthesis (Φ_c). The same trends distinguished lakes with higher chlorophyll concentrations (Lakes Hoare and Fryxell) from those with lower phytoplankton biomass (Lake Bonney, east and west lobes). Phytoplankton immediately beneath the ice in the east lobe of Lake Bonney showed late season increases in α and Φ_c following large inputs of glacial streamwater (see Spigel et al. 1991; Lizotte and Priscu 1992b).

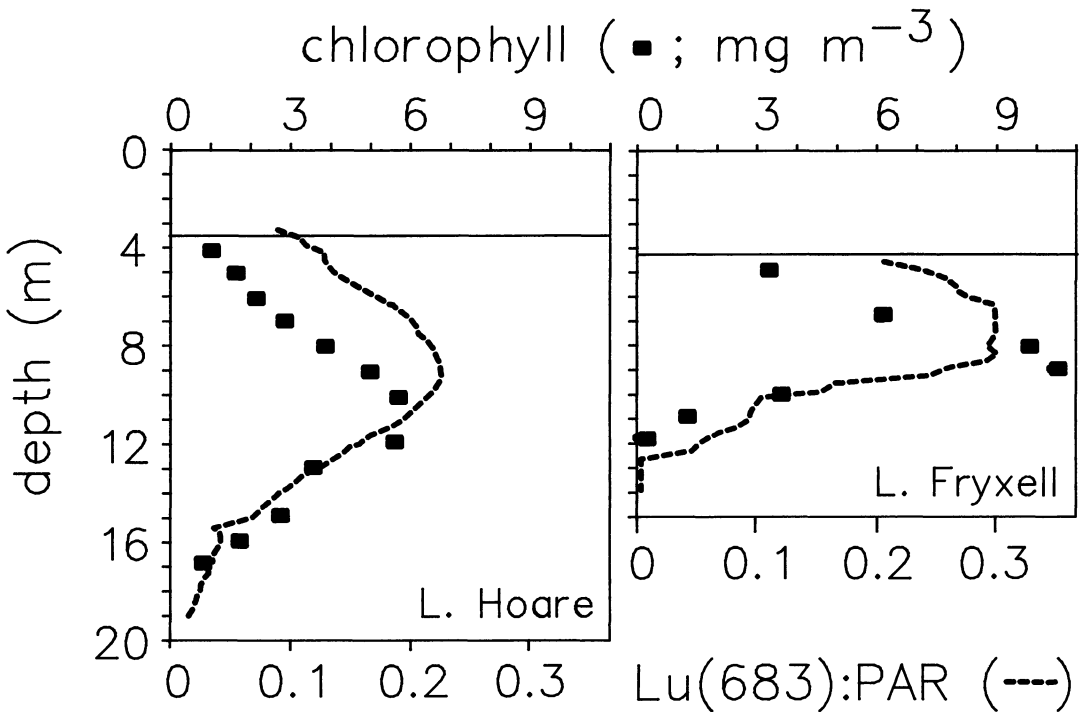
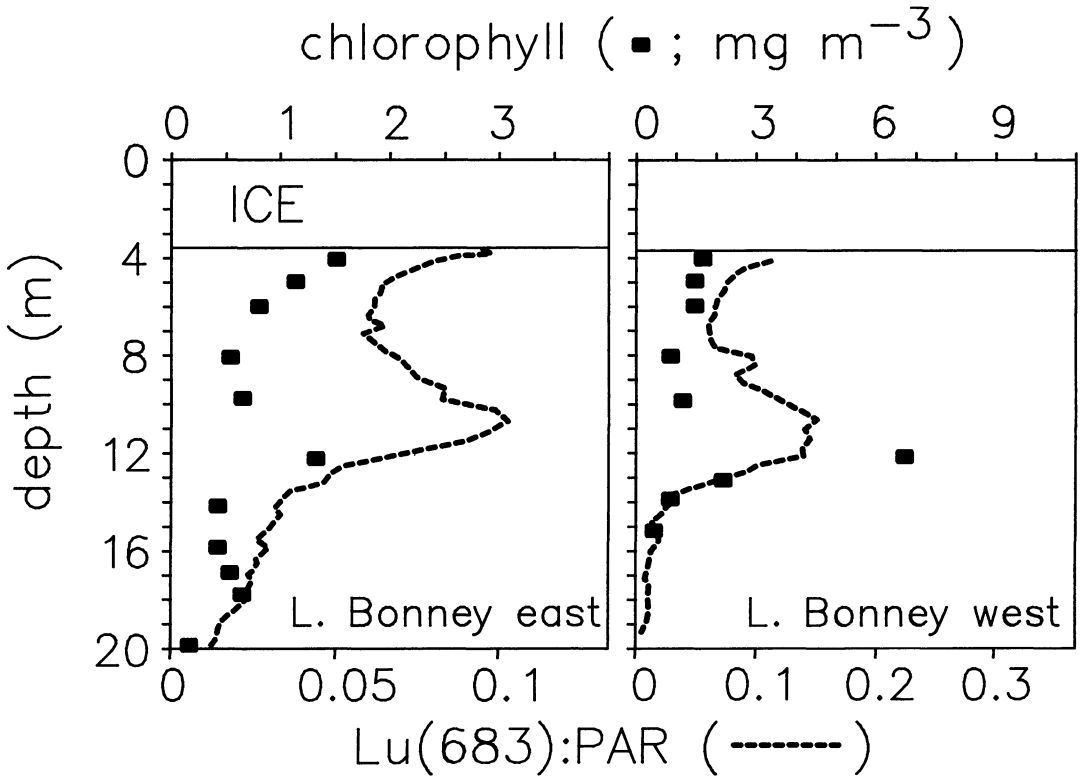
To predict chlorophyll concentration and primary production from natural fluorescence rates, one needs to estimate a_{ph} , Φ_f , and Φ_c

(Kiefer et al. 1989). The least variable of these factors was a_{ph} , which ranged from 0.008 to 0.020 $\text{m}^2 (\text{mg Chl } a)^{-1}$, with an average of 0.015. For the chlorophyll prediction, the most variable term was Φ_f , which ranged from 0.020 to 0.096 and averaged 0.044 mol photons fluoresced (mol photons absorbed) $^{-1}$. For predicting primary production, the most variable term was Φ_c , which varied over 30-fold (from 0.0015 to 0.051) and averaged 0.012 mol C (mol photons) $^{-1}$.

Chl *a* concentrations from water samples were compared with Chl *a* predicted from natural fluorescence using the mean values for Φ_f and a_{ph} (from Table 1). This larger data set ($n = 122$) showed significant correlation between measured and predicted values ($r = 0.88$), with a slope not significantly different from one (Fig. 4). Although the majority of these samples came from profiles in a transect across both lobes of Lake Bonney, there were no significant differences in this slope among the four basins studied. At high chlorophyll concentrations ($> 3 \text{ mg m}^{-3}$), the predictions were usually lower than measured chlorophyll values, which is consistent with an expected self-absorption of the fluorescence signal (Kiefer et al. 1989).

The ability to predict instantaneous primary production rates from natural fluorescence rates depends largely on the values used for Φ_c and Φ_f (or a ratio of these quantum yields). These terms can vary with irradiance (Chamberlin et al. 1990; Chamberlin and Marra 1992) and with temperature (Chamberlin and Marra 1992), and there is evidence that one or both of these quantum yields could vary as a function of distance from the nutricline (e.g. Cleveland et al. 1989; Kolber et al. 1990). Values for $\Phi_c : \Phi_f$ were plotted against temperature (measured by the PNF-300), light (% incident irradiance), and nutrients (distance from the nutricline) (Fig. 5). In all cases, the relationships are largely dictated by the high $\Phi_c : \Phi_f$ values observed in the deepest samples from Lakes Bonney, Fryxell, and Hoare (see Table 1).

For temperature, there was no clear trend in quantum yield ratios (Fig. 5A). The highest and lowest $\Phi_c : \Phi_f$ values cover the full range of temperatures in these lakes. All these points fall near or below the linear relationship between $\Phi_c : \Phi_f$ and temperature reported by Chamberlin and Marra (1992).



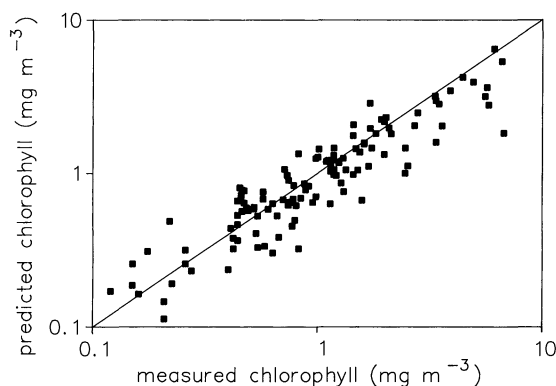


Fig. 4. Chlorophyll *a* concentration (mg m^{-3}) predicted from natural fluorescence as a function of measured values (log scales). Data from all lakes are included. Line represents a slope of 1.

Previous studies of natural fluorescence used instantaneous PAR, such as measured in situ by the PNF-300, to search for a relationship between light regime and quantum yields. It was suggested to us that photosynthetically usable radiation (PUR) may be a better measure because it could account for variation caused by spectral quality of the light field. However, vertically stable phytoplankton populations inhabit a light environment that varies predictably to produce a light history that will not be well represented by measurements of instantaneous PAR (or PUR). Thus, we normalized in situ PAR to incident irradiance ($\%I_0$). Ratios of $\Phi_c : \Phi_f$ were plotted against PAR, PUR, and $\%I_0$, then fit to the formulation used by Chamberlin et al. (1990):

$$\Phi_c : \Phi_f = \frac{k_{cf} \chi (\Phi_c : \Phi_f)_{\max}}{k_{cf} + I} \quad (1)$$

I is irradiance (PAR, PUR, or $\%I_0$), and maximum $\Phi_c : \Phi_f$ and the irradiance at which the ratio is half the maximum (k_{cf}) are derived from a best fit to the data. The best curve-fit was for $\%I_0$ (Fig. 5B), with PUR nearly as good and PAR far worse. Best-fit coefficients for this curve were 1.5 for $(\Phi_c : \Phi_f)_{\max}$ and 0.15% for k_{cf} , which produced a significant least-squares regression ($n = 17$, $r = 0.51$, $P < 0.01$).

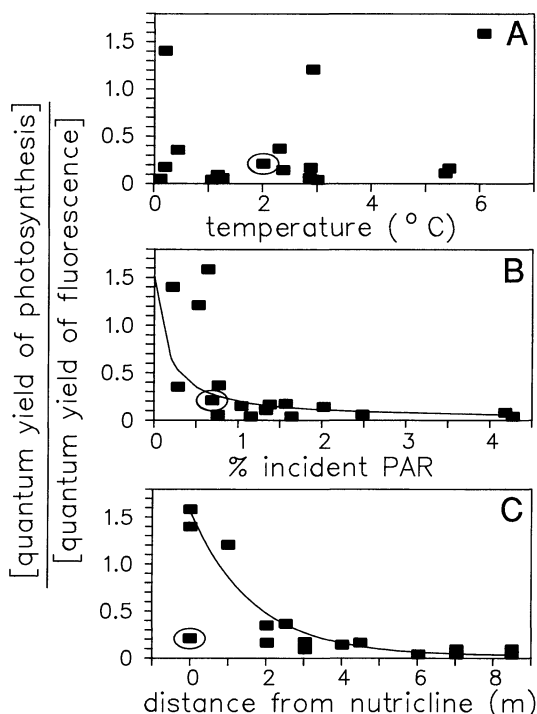


Fig. 5. Quantum yield ratios ($\Phi_c : \Phi_f$) as a function of (A) temperature, (B) percent irradiance incident at the surface, and (C) distance from the nutricline. Samples from below the nutricline are plotted at 0 m. Circled points are questionable values from 13 m in the west lobe of Lake Bonney (see text). Line in panel B is a best-fit using Eq. 1. Line in panel C is a first-order decay curve fit to the data excluding the circled point.

Distance above the nutricline was used as a qualitative estimate of nutrient supply rates that could affect quantum yields. Our study lakes had strong vertical gradients for nutrients (Table 2) corresponding to salinity (Figs. 1, 2) and maintained by a stable density structure (e.g. Spigel et al. 1991). Nutricline depths were defined as 13 m for L. Bonney-east, 10 m for L. Bonney-west, 9.5 m for L. Fryxell, and 12 m for L. Hoare. For deeper samples, distance above the nutricline was assigned a value of 0 m. The ratio $\Phi_c : \Phi_f$ was clearly highest near the nutricline (Fig. 5C).

This relationship was driven primarily by higher Φ_c values near or within the nutricline

←
Fig. 3 Profiles of natural fluorescence per unit irradiance [$L_u(683) : \text{PAR}$] and Chl *a* concentration measured in December 1990.

Table 2. Concentrations (μM) of nitrate (NO_3^-), ammonium (NH_4^+), and soluble reactive P (SRP) in the dry valley lakes of Antarctica in 1990 (ND—not detectable).

Depth (m)	NO_3^-	NH_4^+	SRP
Lake Bonney, east lobe (4 Dec)			
5	0.5	4.7	0.13
6	0.4	7.7	0.13
8	0.4	13	0.10
10	0.4	14	0.10
11		16	
12		23	
13	8.1	36	0.05
15	3.9	44	0.04
16	4.2	49	0.04
17	26	68	0.13
18	49	81	0.04
Lake Bonney, west lobe (3 Dec)			
4	0.12	5.8	0.19
6	0.24	5.9	0.16
8	0.75	1.7	0.16
10	1.1	9.8	0.14
12	0.47	15	0.14
13	41	21	0.16
14	53	21	0.21
15	140	23	0.19
Lake Fryxell (7 Dec)			
5	0.27	ND	0.14
7	0.36	0.18	0.14
8	0.39	0.23	0.29
9	0.50		0.31
10	11	0.17	1.2
11	39		5.2
Lake Hoare (8 Dec)			
5	0.24	0.11	0.20
7	0.76	0.23	0.20
9	0.44	0.08	0.35
10	0.44	0.08	0.22
12	2.5	ND	1.2
13	3.3	0.02	2.4
15	3.8	2.7	1.2
17	4.3	4.1	0.74

and to a lesser degree by increasing Φ_f values with distance above the nutricline (Table 1). The value of $\Phi_c : \Phi_f$ from 13 m in the west lobe of Lake Bonney (circled points in Fig. 5) appears to be an outlier relative to values from other deep phytoplankton. Later studies revealed that the oxycline depth was at 12–14 m in the west lobe, suggesting that the 13-m sample was compromised by the inclusion of anoxic water. This argument is supported by the observation that Φ_f (based on in situ measurements) was similar to other deep samples, while Φ_c (dependent on laboratory incubations) was much lower than other samples from

or below the nutricline (Table 1). After omitting this point from the curve-fitting analysis, we found that the relationship between $\Phi_c : \Phi_f$ and distance above the nutricline (z) could be described simply with a first-order decay function:

$$\Phi_c : \Phi_f = 1.53 \exp(-0.66z) + 0.022$$

for which predicted values showed a significant least-squares regression with the observations ($n = 16$, $r^2 = 0.92$, $P < 0.01$).

Instantaneous primary production rates predicted from natural fluorescence rates (PPR_{nf}) were compared with primary production (PPR) estimated from field data (Chl a and spectral irradiance), laboratory measurements (α and a_{ph}), and irradiance measured by the PNF-300. This comparison allowed us to test the efficacy of various corrections for PPR_{nf} based on the nonlinear relationships of $\Phi_c : \Phi_f$ to light and distance from the nutricline (Fig. 6). Using a constant value for $\Phi_c : \Phi_f$ based on mean Φ_c and Φ_f (Table 1) produced an extremely poor correlation between PPR_{nf} and PPR (Fig. 6A). Using the $\%I_0$ -based curve (Fig. 5B) to estimate $\Phi_c : \Phi_f$ produced some improvement in predicting primary production (Fig. 6B). The correction using the $\Phi_c : \Phi_f$ based on distance from the nutricline (Fig. 5C) gave the best correlation between PPR_{nf} and PPR (Fig. 6C). Obviously, Fig. 6B and C do not represent independent tests of corrections for $\%I_0$ or distance from nutricline, but they are shown to indicate the potential for improvement of PPR_{nf} , whereas temperature and incident irradiance corrections offered little improvement.

Discussion

Previous studies of natural fluorescence were done in the open ocean, with most samples collected from the surface mixed layer (e.g. Kiefer et al. 1989; Chamberlin et al. 1990; Stegmann et al. 1992). However, stratified phytoplankton populations, such as the deep chlorophyll maxima observed in many seas and lakes (e.g. Cullen 1982), may exhibit exceptional fluorescence relationships because they can acclimate more precisely to the relatively stable physical and chemical conditions. Highly stratified phytoplankton populations in perennially ice-covered lakes near McMurdo Sound show extreme physiological

acclimation to the low overall irradiance (e.g. Lizotte and Priscu 1992a) and to the strong vertical nutrient gradients. For example, the increase in Φ_c with depth that was observed is consistent with reports for marine systems in which phytoplankton show reduced nutrient stress near or within a nutricline (e.g. Cleveland et al. 1989; Kolber et al. 1990).

The key parameter for using natural fluorescence to predict phytoplankton production is $\Phi_c : \Phi_f$. Chamberlin et al. (1990) suggested that a relationship between in situ irradiance and the ratio $\Phi_c : \Phi_f$ could be used to correct for differences in phytoplankton photophysiology. Later, a correction of $\Phi_c : \Phi_f$ for temperature effects was shown to further improve the predictions of primary production for open ocean phytoplankton (Chamberlin and Marra 1992). The data presented in Figs. 5 and 6 indicate that nutrients, represented by distance from the nutricline, were more critical than temperature or light for determining $\Phi_c : \Phi_f$ in phytoplankton from stable, low light environments. One should note that our study lakes are unique in many respects, and our generalizations should be verified in other systems.

Determining the relative effects of light and nutrients on algal physiology based on data from vertical profiles can be difficult because as depth increases, both light intensity and distance to the nutricline decrease. A good example of these confounding influences can be seen in Fig. 5B and C, where both curves are determined primarily by high $\Phi_c : \Phi_f$ values from the deepest samples in three different lakes (Table 1). Species composition does not appear to be a factor, as $\Phi_c : \Phi_f$ of cryptophytes varied greatly in vertical profiles of Lakes Hoare and Fryxell, while different taxonomic groups (cryptophytes or chlorophytes, *see site description in methods*) dominated in deep samples with the highest $\Phi_c : \Phi_f$ values.

We believe that distance from the nutricline (i.e. nutrient supply) is the dominant factor determining $\Phi_c : \Phi_f$ values. First, $\Phi_c : \Phi_f$ showed a more coherent relationship with distance from nutricline than with % I_0 (Fig. 5), and this translated into better predictions of primary production (Fig. 6).

Second, the relationship between % I_0 and $\Phi_c : \Phi_f$ is extreme relative to other systems and to other measures of photoacclimation in these particular populations. For example, although

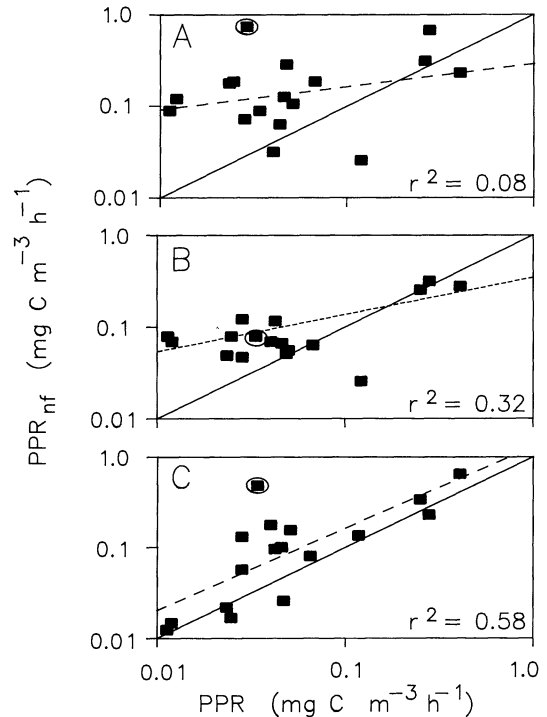


Fig. 6. Primary production rates predicted from natural fluorescence (PPR_{nf}) as a function of values modeled directly from photosynthesis-irradiance measurements (PPR). Each graph shows a different method of calculating PPR_{nf} : A—using the average quantum yields of photosynthesis (Φ_c) and of fluorescence (Φ_f); B—with $\Phi_c : \Phi_f$ corrected for percent irradiance incident at the surface; C—with $\Phi_c : \Phi_f$ corrected for distance from the nutricline. Circled points are for 13 m in the west lobe of Lake Bonney. Solid lines represent a 1–1 correlation and dashed lines a linear regression of all points with r^2 indicated.

$(\Phi_c : \Phi_f)_{max}$ in our study was similar to that reported for marine phytoplankton (Chamberlin et al. 1990; Chamberlin and Marra 1992), the k_{cf} value was far lower in our study. Estimates of k_{cf} in the ocean include 133 (Chamberlin et al. 1990) and 146–215 $\mu\text{Einst m}^{-2} \text{s}^{-1}$ (Chamberlin and Marra 1992), which are similar to I_k values for phytoplankton in these ocean systems. The k_{cf} value of 0.15% for the antarctic lake phytoplankton would equal [assuming average incident irradiance was $\sim 500 \mu\text{Einst m}^{-2} \text{s}^{-1}$ (Priscu 1991)] $0.75 \mu\text{Einst m}^{-2} \text{s}^{-1}$, compared to an average I_k value of $27 \mu\text{Einst m}^{-2} \text{s}^{-1}$ (Table 1). Thus, the half-saturation irradiance for $\Phi_c : \Phi_f$ (k_{cf}) was more than an order of magnitude lower than similar estimates for photosynthesis ($I_k/2 = \sim 13 \mu\text{Einst m}^{-2} \text{s}^{-1}$)

and fluorescence yield [$\sim 10 \mu\text{Einst m}^{-2} \text{s}^{-1}$ (Neale and Priscu 1991)].

Low k_{cf} produced the extremely sharp bend in the curve depicted in Fig. 5B, which is an attempt to fit equal numbers of low (< 0.4) and high $\Phi_c : \Phi_f$ values (> 1.2) at the lowest light levels ($\%I_0 < 1$). Therefore, our extremely low k_{cf} (and by extension the severe curve drawn in Fig. 5B) does not appear to be a reasonable description of acclimation to the relatively small gradients in light intensity that were observed. The most likely scenario is that the physiological differences between vertically stable deep and shallow populations in these lakes are determined by the much steeper chemical (i.e. nutrient) gradients.

The derived relationship between $\Phi_c : \Phi_f$ and distance from the nutricline may be unique to these antarctic lakes. In systems with less dramatic nutrient gradients, the relationship between $\Phi_c : \Phi_f$ and distance from the nutricline may be less pronounced. Also, we found that a first-order decay curve provided a reasonable fit to our data, but it is likely that high $\Phi_c : \Phi_f$ values could exist for some distance above the nutricline (e.g. Cleveland et al. 1989), which would produce a sigmoid curve. In any case, this relationship will be highly specific to each system and time, as it depends on nutrient flux rates relative to phytoplankton utilization rates, which vary depending on the physical structure of the water column, nutrient gradients, phytoplankton biomass, and physiological state. Nutrient-based correction of $\Phi_c : \Phi_f$ may only be possible for aquatic systems exhibiting extreme stability (e.g. the lakes described herein) and for highly consistent phytoplankton layers (e.g. persistent deep chlorophyll maxima).

The most variable of the terms needed to predict primary production from natural fluorescence in our study was Φ_c . If a deep chlorophyll maximum forms by in situ growth at a nutricline and the algae are light limited but not nutrient limited, then Φ_c might remain near the theoretical maximum ($\sim 0.08 \text{ mol C mol photons}^{-1}$). However, other mechanisms have been proposed for the formation of deep chlorophyll maxima, including decreased sinking rates with depth due to increasing density of water and behavioral aggregation of phytoplankton (e.g. Cullen 1982). Because both light acclimation and recovery from nutrient stress

are time-dependent processes, phytoplankton populations formed through sinking or behavior can have Φ_c values covering a wide range below the theoretical maximum. The wide range in Φ_c observed in the antarctic lake phytoplankton may be due to biomass accumulation regulated by factors other than nutrients or light, such as species-specific salinity limits or settlement to a depth of neutral buoyancy.

Other explanations for variations in phytoplankton quantum yields include high rates of respiration or nitrate reduction relative to carbon fixation. Despite constant daylight during the season we sampled, low irradiances for part or all the day in these lakes (Priscu 1991) could be near or below the compensation irradiance for photosynthesis. The relatively long incubations (4 h) used to measure photosynthesis could also lead to an underestimate of gross photosynthesis if significant amounts of [^{14}C]photosynthate were respired. Phytoplankton using nitrate as their primary source of nitrogen could have Φ_c values reduced as much as a third due to competition between nitrate reduction and carbon fixation for photochemical energy (Syrett 1981).

Finally, quantum yields could be underestimated if significant amounts of chlorophyll were associated with nonactive or dead cells at certain depths in these lakes. The water columns of these lakes are relatively cold, dim, salty, and lacking in grazers; such conditions may allow particulate chlorophyll to accumulate at depths of neutral buoyancy after phytoplankton cells have ceased to contribute to carbon fixation.

Seaburg et al. (1983) proposed that phytoplankton in these same antarctic lakes have either high light-harvesting efficiencies or high quantum efficiencies for photosynthesis. The ambivalence stemmed from difficulty in estimating in situ a_{ph} . Seaburg et al. estimated a mean extinction coefficient for suspended Chl a [$k_c = 0.033 \text{ m}^2 (\text{mg Chl } a)^{-1}$] by linear regressions of PAR and Chl a concentration; recent measurements confirmed that k_c is high [$0.027 \text{ m}^2 (\text{mg Chl } a)^{-1}$, Lizotte and Priscu 1992b]. If k_c was a reasonable estimate of in situ a_{ph} , then these phytoplankton would be exceptionally efficient at harvesting available light. However, our estimates of in situ a_{ph} ranged from 0.008 to $0.020 \text{ m}^2 (\text{mg Chl } a)^{-1}$, which is similar to values reported for other

higher irradiance environments (e.g. Mitchell and Kiefer 1988; Chamberlin et al. 1990). High k_c values were due to absorption and attenuation by constituents that covary with pigment concentrations; phytoplankton accounted for 25–83% of PAR absorption by constituents other than water, with the remainder of the absorption by detritus and gelbstoff (Lizotte and Priscu 1992b).

Furthermore, we found that quantum efficiencies for photosynthesis were not particularly high. Seaburg et al. (1983) found that using a more typical value for a_{ph} [$0.016 \text{ m}^2 (\text{mg Chl } a)^{-1}$, about half of measured k_c] produced higher estimates of Φ_c , nearing or exceeding the theoretical maximum for the deeper populations. Our estimates of maximum Φ_c values ranged from 0.0015 to 0.051 mol C (mol photons) $^{-1}$, less than half the theoretical maximum. Except for populations near or below the nutricline, maximum Φ_c was very low relative to other lakes (e.g. Dubinsky and Berman 1976; Priscu 1984) and seas (e.g. Kishino et al. 1986; SooHoo et al. 1987; Cleveland et al. 1989), where maximum Φ_c typically ranges from 0.03 to 0.08 mol C (mol photons) $^{-1}$. The only data set with a range comparable to ours was reported by Tilzer et al. (1985) for antarctic marine phytoplankton [max Φ_c from 0.0024 to 0.035 mol C (mol photons) $^{-1}$], with evidence that low Φ_c may be related to low temperature.

Nutrient stress in shallow phytoplankton populations may be alleviated by seasonal addition of glacial stream water. In late December 1990, stream flow raised the height of Lake Bonney by more than 1 m and carried in significant amounts of inorganic particles that were noticeable in filtered samples to a depth of 12 m (Lizotte and Priscu 1992b). By early January, Chl *a* had increased significantly (Sharp 1993), while ratios between taxon-specific pigments (e.g. alloxanthin, fucoxanthin, and Chl *b*) and Chl *a* remained consistent (Lizotte and Priscu 1992c), implying growth by the existing phytoplankton populations. Maximum Φ_c for shallow populations in Lake Bonney was 2–3 times higher in January than in November or December (Table 1). It is not clear whether this response indicates nutrient starvation like a batch culture (unbalanced growth) or nutrient limitation at steady state like a chemostat (balanced growth), but Cullen

et al. (1992) suggested that the former scenario is more likely to produce variations in Φ_c related to nutrient supply.

It is important to note that while incident irradiance increased slightly between late November and early January, changes in ice optics (Priscu 1991) and in the suspended particles from streams (Lizotte and Priscu 1992b) produced lower water-column irradiances in January. Thus, it is possible that decreasing irradiance was linked to the late-season increase in maximum Φ_c .

The thick ice cover of these lakes, which strongly attenuates red light (Lizotte and Priscu 1992b), effectively eliminates contamination of the upwelling fluorescence signal by backscattered sunlight. In open waters, red light contamination is a serious problem because there is no sure method for determining the depth below which natural fluorescence measurements are reliable. Kiefer et al. (1989) estimated that natural fluorescence measurements in the upper 6 m of a water column are questionable. Stegmann et al. (1992) attempted to correct the natural fluorescence signal in shallow waters by measuring downwelling radiance at 683 nm and estimating backscatter by pure water. This approach does not account for backscatter by particulate or dissolved materials, but it may be valid for many oligotrophic systems with chlorophyll concentrations that can be measured by the natural fluorescence technique ($<5\text{--}10 \text{ mg Chl } a \text{ m}^{-3}$). For ice-covered waters such as polar lakes and seas, mechanically simple instruments such as natural fluorometers are very promising for collecting information on irradiance conditions, phytoplankton biomass, and possibly (given site-specific algorithms) primary production for the entire euphotic zone of remote aquatic systems.

References

- ANGINO, E. E., K. B. ARMITAGE, AND J. C. TASH. 1964. Physicochemical limnology of Lake Bonney, Antarctica. *Limnol. Oceanogr.* **9**: 207–217.
- BRICAUD, A., AND D. STRAMSKI. 1990. Spectral absorption coefficients of living phytoplankton and nonalgal biogenous matter: A comparison between the Peru upwelling area and the Sargasso Sea. *Limnol. Oceanogr.* **35**: 562–582.
- CHAMBERLIN, W. S., C. R. BOOTH, D. A. KIEFER, J. H. MORROW, AND R. C. MURPHY. 1990. Evidence for a simple relationship between natural fluorescence,

- photosynthesis, and chlorophyll in the sea. *Deep-Sea Res.* **37**: 951-973.
- , AND J. MARRA. 1992. Estimation of photosynthetic rate from measurements of natural fluorescence: Analysis of the effects of light and temperature. *Deep-Sea Res.* **39**: 1695-1706.
- CLEVELAND, J. S., M. J. PERRY, D. A. KIEFER, AND M. C. TALBOT. 1989. Maximum quantum yield of photosynthesis in the northwestern Sargasso Sea. *J. Mar. Res.* **47**: 869-886.
- CULLEN, J. J. 1982. The deep chlorophyll maximum: Comparing vertical profiles of chlorophyll *a*. *Can. J. Fish. Aquat. Sci.* **39**: 791-803.
- , X. YANG, AND H. I. MACINTYRE. 1992. Nutrient limitation of marine photosynthesis, p. 69-88. *In* P. G. Falkowski and A. W. Woodhead [eds.], *Primary productivity and biogeochemical cycles in the sea*. Plenum.
- DUBINSKY, Z., AND T. BERMAN. 1976. Light utilization efficiencies of phytoplankton in Lake Kinneret (Sea of Galilee). *Limnol. Oceanogr.* **21**: 226-230.
- HOLM-HANSEN, O., C. J. LORENZEN, R. W. HOLMES, AND J. D. H. STRICKLAND. 1965. Fluorometric determination of chlorophyll. *J. Cons. Int. Explor. Mer* **30**: 3-15.
- KIEFER, D. A., W. S. CHAMBERLIN, AND C. R. BOOTH. 1989. Natural fluorescence of chlorophyll *a*: Relationship to photosynthesis and chlorophyll concentration in the western South Pacific gyre. *Limnol. Oceanogr.* **34**: 868-881.
- KISHINO, M., N. OKAMI, M. TAKAHASHI, AND S. ICHIMURA. 1986. Light utilization and quantum yield of phytoplankton in a thermally stratified sea. *Limnol. Oceanogr.* **31**: 557-566.
- , M. TAKAHASHI, N. OKAMI, AND S. ICHIMURA. 1985. Estimation of the spectral absorption coefficients of phytoplankton in the sea. *Bull. Mar. Sci.* **37**: 634-642.
- KOLBER, Z., K. D. WYMAN, AND P. G. FALKOWSKI. 1990. Natural variability in photosynthetic energy conversion efficiency: A field study in the Gulf of Maine. *Limnol. Oceanogr.* **35**: 72-79.
- LIZOTTE, M. P., AND J. C. PRISCU. 1992a. Photosynthesis-irradiance relationships in phytoplankton from the physically stable water column of a perennially ice-covered lake (Lake Bonney, Antarctica). *J. Phycol.* **28**: 179-185.
- , AND ———. 1992b. Spectral irradiance and bio-optical properties in perennially ice-covered lakes of the dry valleys (McMurdo Sound, Antarctica). *Antarct. Res. Ser.* **57**: 1-14.
- , AND ———. 1992c. Algal pigments as markers for stratified phytoplankton populations in Lake Bonney (dry valleys). *Antarct. J. U.S.* **27**: 259-260.
- McKNIGHT, D. M., G. R. AIKEN, AND R. L. SMITH. 1991. Aquatic fulvic acids in microbially based ecosystems: Results from two desert lakes in Antarctica. *Limnol. Oceanogr.* **36**: 998-1006.
- MITCHELL, B. G., AND D. A. KIEFER. 1988. Chlorophyll *a* specific absorption and fluorescence excitation spectra for light-limited phytoplankton. *Deep-Sea Res.* **35**: 639-663.
- NEALE, P. J., AND J. C. PRISCU. 1991. Variation of the fluorescence quantum yield in relation to photosynthesis by phytoplankton from perennially ice-covered Lake Bonney. *Antarct. J. U.S.* **26**: 228-230.
- PALMISANO, A. C., AND G. M. SIMMONS, JR. 1987. Spectral downwelling irradiance in an antarctic lake. *Polar Biol.* **7**: 145-151.
- PARKER, B. C., G. M. SIMMONS, JR., K. G. SEABURG, D. D. CATHAY, AND F. C. T. ALLNUT. 1982. Comparative ecology of plankton communities in seven antarctic oasis lakes. *J. Plankton Res.* **4**: 271-286.
- PARSONS, T. R., Y. MAITA, AND C. M. LALLI. 1984. *A manual of chemical and biological methods for seawater analysis*. Pergamon.
- PRISCU, J. C. 1984. In situ quantum yield of phytoplankton in a subalpine lake. *J. Plankton Res.* **6**: 531-542.
- . 1991. Variation in light attenuation by the permanent ice cap of Lake Bonney during spring and summer. *Antarct. J. U.S.* **26**: 223-224.
- SEABURG, K. G., M. KASPAR, AND B. C. PARKER. 1983. Photosynthetic quantum efficiencies of phytoplankton from perennially ice-covered antarctic lakes. *J. Phycol.* **19**: 446-452.
- SHARP, T. R. 1993. Temporal and spatial variation of light, nutrients, and phytoplankton in Lake Bonney, Antarctica. M.S. thesis, Montana State Univ. 166 p.
- SOOHOO, J. B., AND OTHERS. 1987. Spectral light absorption and quantum yield of photosynthesis in sea ice microalgae and a bloom of *Phaeocystis pouchetii* from McMurdo Sound, Antarctica. *Mar. Ecol. Prog. Ser.* **116**: 1-13.
- SPIGEL, R. H., I. FORNE, I. SHEPPARD, AND J. C. PRISCU. 1991. Differences in temperature and conductivity between the east and west lobes of Lake Bonney: Evidence for circulation within and between lobes. *Antarct. J. U.S.* **26**: 221-222.
- STEGMANN, P. M., M. R. LEWIS, C. O. DAVIS, AND J. J. CULLEN. 1992. Primary production estimates from recordings of solar-stimulated fluorescence in the equatorial Pacific at 150°W. *J. Geophys. Res.* **97**: 627-638.
- SYRETT, P. J. 1981. Nitrogen metabolism of microalgae, p. 182-210. *In* *Physiological bases of phytoplankton ecology*. *Can. Bull. Fish. Aquat. Sci.* **210**.
- TILZER, M. M., B. VON BODUNGEN, AND V. SMETACEK. 1985. Light-dependence of phytoplankton photosynthesis in the Antarctic Ocean: Implications for limiting productivity, p. 60-69. *In* W. R. Siegfried et al. [eds.], *Antarctic nutrient cycles and food webs*. Springer.
- WHARTON, R. A., JR., C. P. MCKAY, G. M. SIMMONS, JR., AND B. C. PARKER. 1986. Oxygen budget of a perennially ice-covered antarctic lake. *Limnol. Oceanogr.* **31**: 437-443.

Submitted: 7 May 1993

Accepted: 16 December 1993

Amended: 23 March 1994