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PHOTOINHIBITION: ALGAL RESPONSES TO BRIGHT LIGHT DURING DIEL STRATIFICATION AND MIXING IN A TROPICAL ALPINE LAKE[1]

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ABSTRACT

Near surface thermoclines form each day in the limnetic waters of Lake Titicaca (Peru-Bolivia) and thereby retain phytoplankton under extreme irradiances. This bright light exposure results in strongly depressed chlorophyll fluorescence and photosynthesis which both decay (bright light) and recover (dim light) by first order rate kinetics. During each afternoon the phytoplankton are redistributed by wind-induced mixing, and full recovery is accomplished soon after nightfall. In vivo fluorescence was measured over this diel cycle both with (F_b) and without (F_o) 3-(3,4-dichlorophenyl)-1,1-dimethyl urea. Strongest bright light effects were on the parameter ($F_b - F_o$), a crude measure of operational photosystem II reaction centers (RC IIs). On dates of strong thermocline development, surface ($F_b - F_o$) was reduced to 5% or less of that for the mixed layer maximum. Fluorescence depression was greater in the lake than in Pyrex bottles incubated at fixed depths for 4 h. Ultraviolet light intensified the photoinhibitory response, but strong ($F_b - F_o$) depression could be induced by photosynthetically available radiation alone. In Lake Titicaca, photoinhibition apparently operates by reversible inactivation of RC IIs. It occurs in the natural water column and is not simply an artifact of fixed bottle incubations.

Key index words: chlorophyll fluorescence, DCMU; diel mixing; lakes, alpine, tropical; photoinhibition; photosynthesis; photosystem II; Titicaca

Photoinhibition, the depression of photosynthetic production rates at supra-optimal light intensities, has been reported for planktonic algae at all latitudes (e.g. temperate oceans, Platt and Gallegos 1980; tropical lakes, Melack 1979; antarctic ponds, Goldman et al. 1963). This bright light effect is commonly recorded in the near-surface bottles of in situ experiments and explanations of its physiological cause have included photorespiration (reviewed in Harris 1978), enzyme destruction (Steemann-Nielsen, 1974) and depression of photosynthetic electron transport (Harris and Piccinin 1977). Recent models of the photosynthesis—light relationship (Platt et al. 1980) incorporate photoinhibition as a continuous but non-linear function of photon flux density (PFD) that acts in opposition to photo-enhanced photosynthesis over a broad range of PFDs, including P_{max}, the measured photosynthetic maximum.

Although photoinhibited production rates can be unequivocally demonstrated in fixed depth incubations, many investigators have questioned whether such effects operate in nature. Photoinhibition is strongly time-dependent; photosynthesis becomes increasingly depressed with increasing time of exposure to bright light (e.g. Takahashi et al. 1971) and then slowly recovers in dim light or darkness (e.g. Steemann-Nielsen 1974). In short term incubations (Harris and Piccinin 1977) and experiments in which bottles are continuously circulated through a PFD gradient (Jewson and Wood 1975, Marra 1978) photoinhibition is greatly reduced or eliminated. Vertical mixing processes in natural water columns might therefore move phytoplankton in and out of the near-surface zone too rapidly to permit the inhibitory effects of bright light. By this argument, photoinhibition is simply an artifact of the usual fixed depth incubation method.

Photoinhibition will occur in situ if physical conditions prolong the residence time of algae in the

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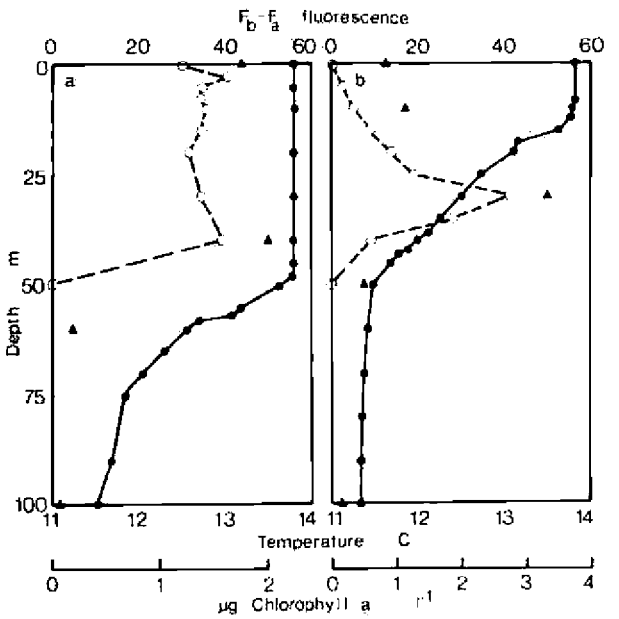


FIG. 1a, b. Vertical profiles of chlorophyll *a* fluorescence, and temperature in Lago Grande during stratification. a) 29 April 06:30 h, b) 11 November 09:00 h; ▲, chlorophyll *a*; ○, fluorescence; ●, temperature.

upper few meters of the euphotic zone or if the depression effect occurs rapidly relative to mixing rates. Such conditions occur frequently in alpine lakes and in the tropics where elevated solar energy inputs and high sun angles, respectively, promote diurnal surface thermoclines—a stable density stratification formed each day by the heating of near-surface waters (e.g. Hutchinson 1975, Dillon and Powell 1979, Bruce and Beatty 1982). Strong near-surface stratification has also been reported from various low-altitude temperate lakes (e.g. Zimmerman et al. 1981).

We report here extreme photochemical responses to bright light by the phytoplankton of Lake Titicaca, a large high altitude lake in the tropical Andes. We first document the occurrence and range of fluorescence responses during the diel stratification cycle. We then examine the relationship between fluorescence depression and photosynthetic CO_2 fixation, and attempt to relate these observations to current models of photosynthetic photochemistry.

MATERIALS AND METHODS

Study sites. Lake Titicaca is a large (8100 km²) deep (maximum depth 275 m) alpine (3803 m ASL) lake which lies on the Peru-Bolivian border at 16° S latitude (further information in Richerson et al. 1977, Lazzaro 1981). Two mid-basin sites were routinely visited for fluorescence profiling and photosynthetic assays, one in Bahia de Puno, a shallow bay (525 km², Z_{max} = 37 m, $\bar{z}$ = 14 m in the open water region) on the western side of Titicaca, the second in Lago Grande, the main basin (6315 km², Z_{max} = 275 m, $\bar{z}$ = 147 m).

Sampling and physical measurements. All samples were taken with an opaque Van Dorn water sampler. Photon flux density (PFD) was measured with a Lambda quantum irradiance probe with a 4 π sensor (Lambda Instruments, Lincoln, NE). Water temperatures were determined with a Whitney underwater thermistor, either model CTU 3B or TC-5C (Whitney Instrument Corp., San Luis Obispo, CA).

Photosynthetic CO_2 fixation. Water samples were dispensed into 125 mL transparent or opaque Pyrex bottles (filled completely), injected with ^{14}C-HCO_3 (final activity of ca. 0.05 μCi mL⁻¹) and incubated in situ for 4 h, ca. 09:30 to 13:30 h local time (Goldman 1963). At the end of the incubation the plankton were filtered onto 0.45 μm Millipore membranes which were air-dried and later counted by liquid scintillation spectrometry. Dissolved inorganic carbon was determined by pH and potentiometric titration (Anonymous 1971).

Fluorescence measurements. In vivo fluorescence of chlorophyll *a* was measured in a Turner 111 fluorometer fitted with a blue excitation filter (CS5-60) and red emission filter (CS2-64). Plankton samples were dark-adapted for 30 min. The water was then transferred to 1 cm cuvettes in dim light and the fluorescence was measured over the first 5 s exposure to the excitation beam within the fluorometer (F_a). The excitation beam was shut off and an aqueous solution of 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) injected at a final concentration of 10^{-5} M. In the samples tested, fluorescence was maximized after 30 s, and this plateau value was recorded as F_b. After dark adaptation all RCII traps are receptive (open) and fluorescence is at a minimum (F_a). DCMU blocks the reoxidation of PSII intermediates and fluorescence yield rises to a maximum (F_b). This variable yield ($F_b - F_a$), thereby provides a relative, albeit crude, measure of open RCII traps (Kok 1976). All fluorescence values reported here are expressed on a common scale of relative units, and are not normalised against extracted chlorophyll *a*.

Chlorophyll a estimates. Lake water samples were filtered through Whatman glass fiber (GF/C) filters which were then stored frozen until analysis. The filtered seston was extracted over 24 h in cold 100% methanol (Holm-Hansen and Riemann 1978). No further extraction occurred beyond this time and extraction efficiency was as high as in boiling methanol. The extracts were cleared by centrifugation and assayed with the Turner 111 fluorometer calibrated against chlorophyll *a* standards (Holm-Hansen and Riemann 1978).

RESULTS

Vertical distribution of algae. Photosynthetic and fluorescence assays were conducted throughout 1982 and spanned a wide range of vertical mixing regimes at each site. From February to May 1982 the phytoplankton of Lago Grande were distributed down a deep mixed layer to ca. 40 m (Fig. 1a), well below the 1% light level (ca. 20 m). The algae were mixed to greater depths during winter circulation (May–August), but at the onset of stratification in September a deep chl *a* maximum formed at the bottom of the euphotic zone (Fig. 1b). Surface chl *a* levels remained low and the deep maximum persisted through the final months of the year.

In polymictic Bahia de Puno algal distribution patterns ranged from vertical homogeneity during periods of complete mixing to large shifts in chl *a* concentration with depth during stratification. A one to two week period of stratification early in the year was accompanied by a marked decline in photochemical capacity as measured by DCMU-induced fluorescence and chl *a* below 20 m, suggesting that daily net photosynthesis occurred only above this depth (Fig. 2a). Later in the year the water column

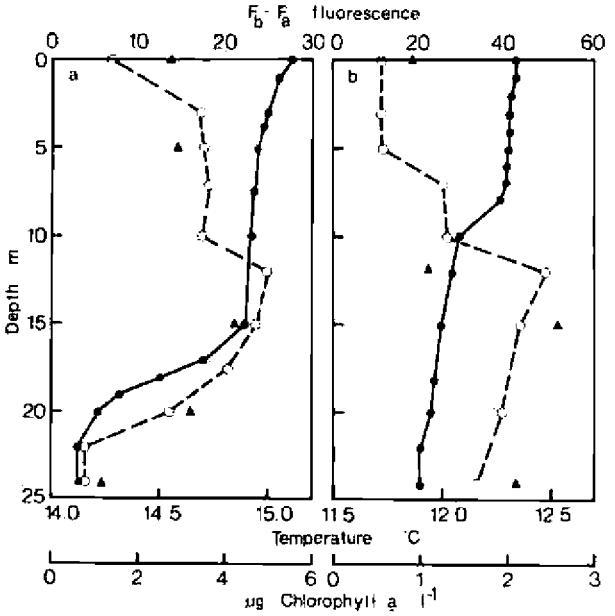


FIG. 2a, b. Vertical profiles of chlorophyll *a*, fluorescence, and temperature in Bahia de Puno during stratification. a) 31 March 09:00 h, b) 15 September 08:30 h; ▲, chlorophyll *a*; O, fluorescence; ●, temperature.

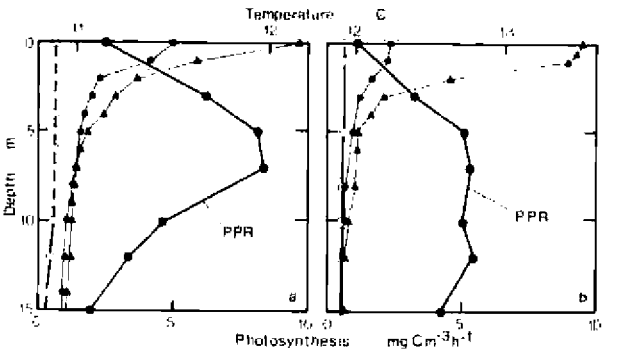


FIG. 3a, b. Near-surface photosynthesis and formation of the diel thermocline. a) Bahia de Puno, 21 July. Temperature at 07:30 h (O), 11:15 h (●), 13:00 h (▲). b) Lago Grande, 30 September. Temperature at 06:15 h (O), 09:30 h (●), 12:45 (▲). Photosynthesis (PPR) measured by 4 h midday incubation.

became more transparent—the extinction coefficient for photosynthetically available radiation (PAR) dropped from ca. 0.3 m^{-1} over January–May to ca. 0.2 m^{-1} during the rest of 1982. Over this latter period high photosynthetic capacities extended to the basin floor (26 m at the routine sampling site) and maximum chl *a* concentrations were often found below the main thermocline (Fig. 2b).

In fixed depth incubations in both Lago Grande and Bahia de Puno, the maximum photosynthetic rates for the water column (A_{max}) were typically in the region 5–10 m. Rates declined both above and below this depth to ca. 5% of A_{max} at 20–25 m and 15–20% of A_{max} in near-surface (ca. 25 cm) bottles (Fig. 3a, b). During the period of greater transparency in Lago Grande (October–December) the A_{max} region was broadened or even bimodal and the euphotic zone extended to 35–40 m.

Diel mixing cycle. Diurnal thermoclines are a characteristic feature of the upper euphotic zone in both locations. During the mornings of typical cloudless days, surface temperatures rise by 1–2° C (Fig. 3a, b). This epilimnetic temperature gradient generally begins to break down in the late afternoon or early evening, and during the night isothermy usually extends to the top of the main thermocline in Lago Grande, and often to the lake floor in Bahia de Puno. This results in a diel cycle of midday stratification in which near surface phytoplankton stay above or within the diurnal thermocline and are exposed to several hours of intense radiation, followed by afternoon and evening mixing and redistribution of cells over the mixed layer. Thus an algal cell over a typical generation time may experience extreme irradiances for several hours at the surface, yet must also, and more commonly, adjust to prolonged suboptimal light intensities lower in the water column.

Midday fluorescence profiles. On all dates of late morning or early afternoon sampling, the in vivo fluorescence parameters (F_a, F_b, $F_b - F_a$) were low at the surface and increased with increasing depth. Extracted chlorophyll *a* varied little over the same region of the water column and therefore these effects appear to operate through changes in fluorescence yield rather than in the cellular concentration of chlorophyll *a*. The magnitude of this surface depression varied with fluorescence parameter and the shape of the diurnal thermocline. Least effect was seen on F_a, the in vivo fluorescence of dark-adapted cells in the absence of DCMU. Prior to the preincubation in darkness, the fluorescence yield in surface water, or in deeper water experimentally incubated in full sunlight, was strongly depressed.

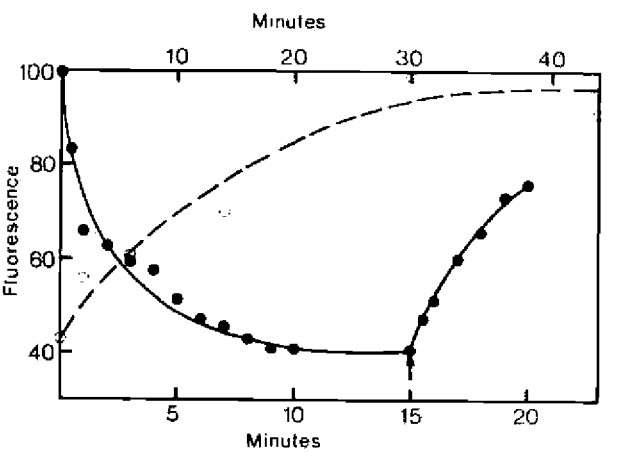
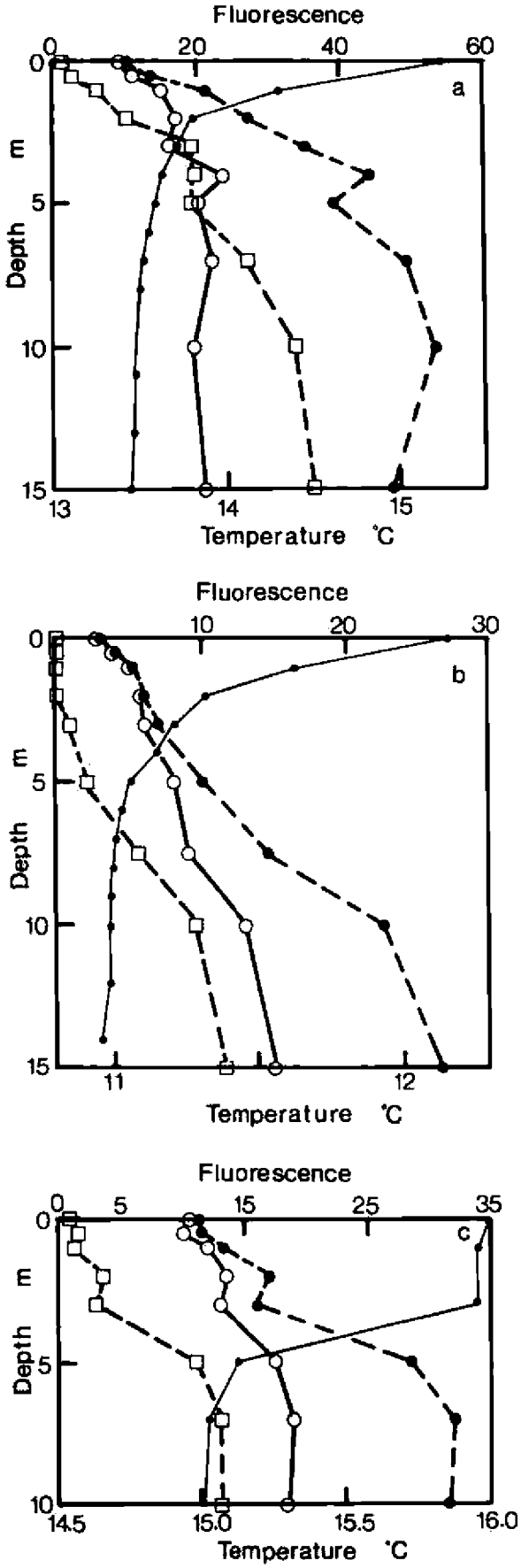


FIG. 4. Fluorescence (F_a) response to sunlight and recovery during dark incubation. Samples from Bahia de Puno; ●, 17 March 5 m sample exposed to full sunlight for 15 min, then placed in darkness (arrow, lower axis), O, 1 September 0 m midday sample dark-incubated (upper time axis). Curves fitted by eye.



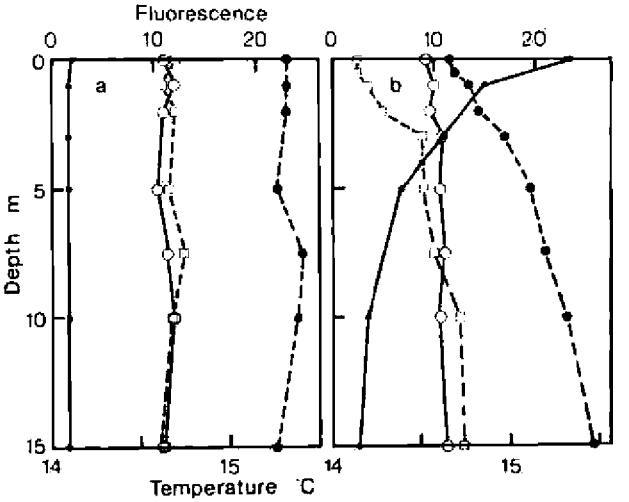


FIG. 6. Early morning and midday fluorescence and temperature profiles, 16 April, Lago Grande. a) 06:30 h; b) 12:00 h. ○, F_a; ●, F_b; □, $F_b - F_a$; ●, temperature.

However, when these samples were placed in the dark there was considerable recovery to a final plateau value within 30–40 min (Fig. 4). The 30 min dark preincubation adopted here for F_a measurements therefore removes this type of bright-light effect, which is probably attributable to chloroplast conformational changes and/or light state transitions (see Vincent 1979 for further discussion).

Near-surface F_b values at midday were always depressed to a much greater extent than F_a. The most striking change was in the RC II parameter ($F_b - F_a$) fluorescence, which on dates of strong thermocline development was reduced to 5% or less of the water column maximum. For all dates of midday profiling (total of 19) $F_b - F_a$ at 0 m averaged 21% (SD = 13%) of values at 10 m.

At midday all three fluorescence parameters (F_a, F_b, $F_b - F_a$) often followed a saturating exponential rise with depth (e.g. Fig. 5a). Deviations from this pattern were apparent under two types of conditions. When near surface responses were extreme and ($F_b - F_a$) fluorescence was zero or very low for several meters down the water column the fluorescence-depth profile was sigmoidal in shape (Fig. 5b). A similarly shaped curve was also apparent when wind induced mixing began to disrupt the diel thermocline and redistribute phytoplankton of presumably different light histories (Fig. 5c).

Morning pattern of fluorescence change. Closer examination of fluorescence changes over the course of the morning confirmed that the shift in all parameters followed the development of the near-sur-

FIG. 5. Fluorescence depression and the diel thermocline. a) 12 May, 13:30 h, Bahia de Puno; b) 21 July, 13:00 h, Bahia de Puno; c) 1 April, 11:00 h, Lago Grande. ○, F_a; ●, F_b; □, $F_b - F_a$; ●, temperature.

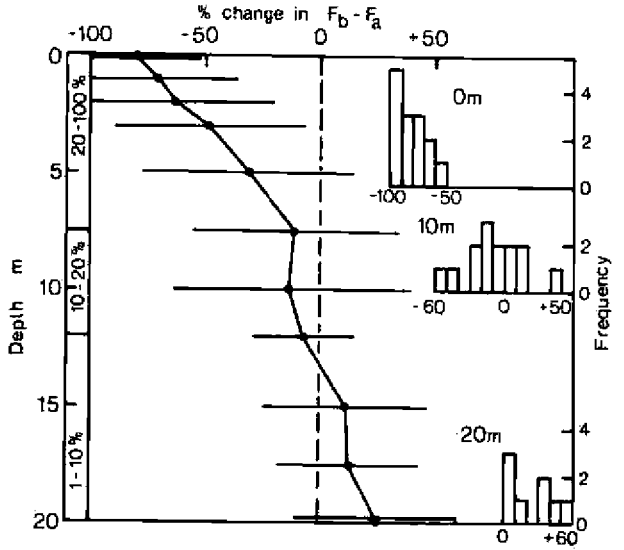


FIG. 7. Change in ($F_b - F_a$) fluorescence between early morning and midday for all dates of sampling. Each value is the mean % change ±2 SD. On right: frequency distribution of percent change at three depths. Approximate range of PFDs in three strata are indicated at left next to the depth axis.

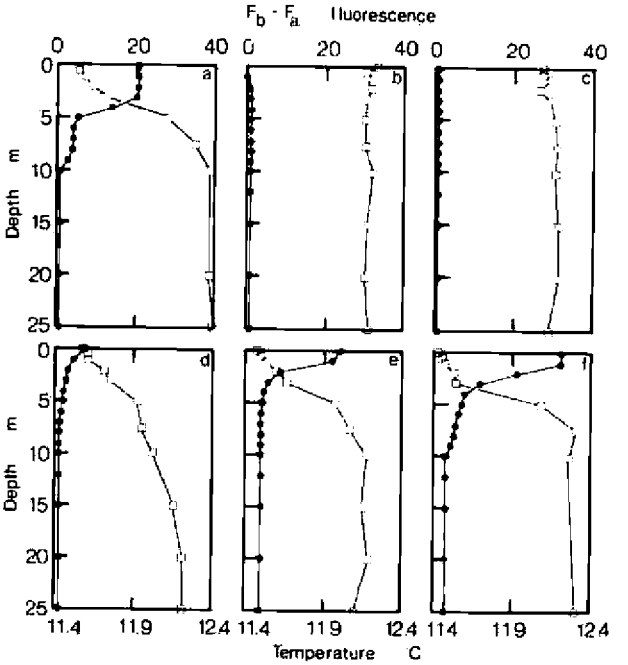


FIG. 8a–f. Diel cycle of temperature and fluorescence—thermocline development and fluorescence depression, 22 July, Lago Grande. a) 16:30 h previous day; b) 05:30 h; c) 07:30 h; d) 09:30 h; e) 11:30 h; f) 13:30 h. □, $F_b - F_a$; ●, temperature.

face thermocline. Figure 6a presents one early morning set of profiles in Lago Grande and illustrates the typically homogeneous distribution of F_a, F_b and ($F_b - F_a$) at sunrise. Later that morning, and concomitant with diurnal stratification, all fluorescence parameters were depressed near the surface (Fig. 6b). The greatest percentage reduction was in ($F_b - F_a$) fluorescence (79% decrease at 0 m) while F_a values were least reduced (18% decrease at 0 m). On this date the percentage change in fluorescence between early morning and midday followed a saturating exponential curve with depth. However this simple depth-time relationship was often distorted by wind-induced mixing, or depression to the same asymptotic minimum at several near-surface depths.

A compilation of all dates for which we have both early morning and midday profiles emphasizes the strong morning depression of fluorescence in the near surface waters, and the consistent decrease of this effect with increasing depth (Fig. 7). At depths greater than 12 m ($F_b - F_a$) values were often higher at noon then they were earlier in the day. The magnitude of fluorescence depression was highly variable from date-to-date, and for any one depth was not normally distributed (see frequency histograms in Fig. 7). These observations suggest that surface bright-light levels consistently exert an overall control on fluorescence but that several other biophysical and environmental factors may modulate this effect.

Diel fluorescence cycle. Early morning profiles (see above) clearly demonstrated that by the end of each night the strong midday fluorescence depression had been completely eliminated. To examine the processes of depression and recovery in more detail, a series of profiles were taken over a two day period in the middle of Lago Grande. Samples taken the first evening upon arrival at the mid-lake site indicated a strong surface reduction in chlorophyll fluorescence, particularly ($F_b - F_a$) levels (Fig. 8a). When sampling resumed just before sunrise the next morning (05:30 h, Fig. 8b), ($F_b - F_a$) was uniform down the water column. Near-surface temperatures were slightly lower than at greater depths because of nocturnal cooling. At 07:30 h (Fig. 8c) a 10–15% reduction in ($F_b - F_a$) was apparent. Two hours later (Fig. 8d) diel thermocline formation was well underway and fluorescence was depressed throughout the top 10 m. Over the next few hours the near-surface temperature gradient became stronger and ($F_b - F_a$) depression intensified (Fig. 8e, f).

Throughout the morning wind velocities were low (<0.5 m·s^{-1}, wave height <20 cm) but in the early afternoon wind stress increased and turbulent mixing began to erode the diel thermocline. This developing surface mixed layer was apparent in the upper 3 m by 15:30 h (Fig. 9a), and by sunset had deepened to 5 m (17:30 h, Fig. 9b). Over this two hour period (15:30–17:30 h) there was a 300% increase in surface ($F_b - F_a$), but at both 5 m and 7.5 m the fluorescence values were greatly reduced. Integral ($F_b - F_a$) fluorescence from 0–7.5 m increased over this period by only 26%, suggesting some physiological recovery of cells, but that this effect was small relative to mixing and the redistribution of phytoplankton with different light histories.

At sunset (ca. 18:00 h) wind velocities increased,

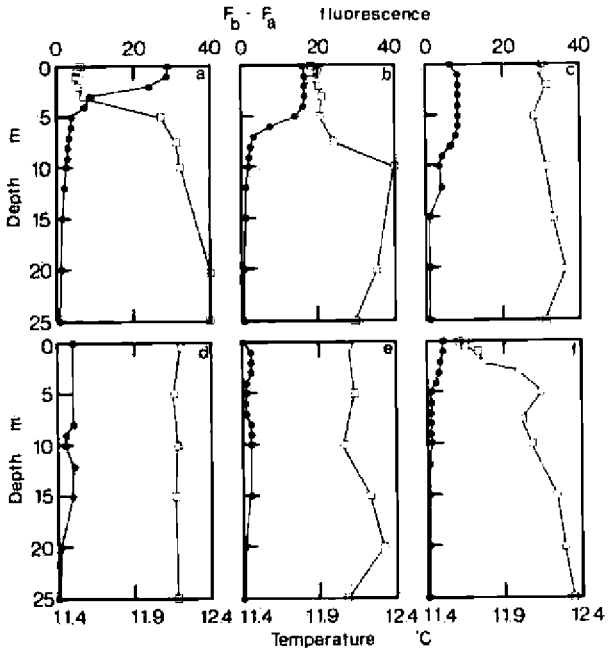


FIG. 9a–f. Diel cycle of temperature and fluorescence-thermocline erosion and fluorescence recovery, 22 July, Lago Grande. a) 15:30 h; b) 17:30 h; c) 19:30 h; d) 21:30 h; e) 09:30 h next day; f) 09:30 h next day. □, $F_b - F_a$; ●, temperature.

to a maximum of ca. 6 m·s^{-1} which persisted through the rest of the night. It was not until just before dawn that this continuous wind stress and convective cooling finally restored isothermy (Fig. 9c–e). However, by 19:30 h ($F_b - F_a$) fluorescence had returned to relatively high values at all depths suggesting considerable physiological recovery rather than just continued mixing. This homogeneity down the water column was maintained throughout the night (Fig. 9c–e) and the diel thermocline and surface fluorescence depression were reinitiated after sunrise the next morning (Fig. 9f).

The diel pattern is further summarized in Figure 10 which compares changes in ($F_b - F_a$) fluorescence at 0.5 m with variations in the turbulence parameter N^2 (square of the Brunt-Vaisala frequency) which is inversely proportional to mixing intensity (see Denman and Gargett 1983). These curves underscore the close coupling between photochemical capacity and the stratification and mixing cycle. There is a negative correlation between water column stability (as measured by N^2) and ($F_b - F_a$) fluorescence: for the period 07:30–21:30 h, $r = -0.833$, $P < 0.05$). Maximum N^2 and minimum ($F_b - F_a$) fluorescence were recorded over midday-early afternoon (Fig. 10). The mixed layer then deepened (maximum N^2 recorded at greater depths) and fluorescence recovery began. Restoration of photochemical capacity in the surface waters was first dominated by mixing which redistributed cells of varying light histories, but physiological recovery processes may have been increasingly important towards and immediately after nightfall.

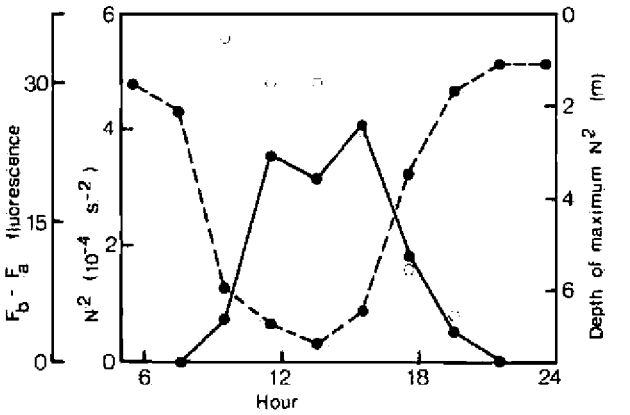


FIG. 10. Turbulent mixing and fluorescence in the surface waters of Lago Grande, 22 July. Dashed lines, $F_b - F_a$ at 0.5 m; solid line, maximum N^2 (square of Brunt-Vaisala frequency); O, depth of maximum N^2.

Additional water samples were taken from 5 m at each 2 h interval for chl *a* extraction and analysis. These values averaged 1.49 mg chl *a*·m^{-3} with no large or consistent variations over the course of the day or night. For the full cycle the coefficient of variation (SD/$\bar{x}$) was 17% for extracted chlorophyll *a*, but 60% for ($F_b - F_a$) at the same depth. Thus the diel shifts in in vivo fluorescence cannot be attributed to changes in the cellular concentration of chlorophyll *a*.

Fluorescence depression in the lake compared with bottles. Photosynthetic rates were measured May 13 in Lago Grande with the usual fixed depth ^{14}C-HCO_3^-

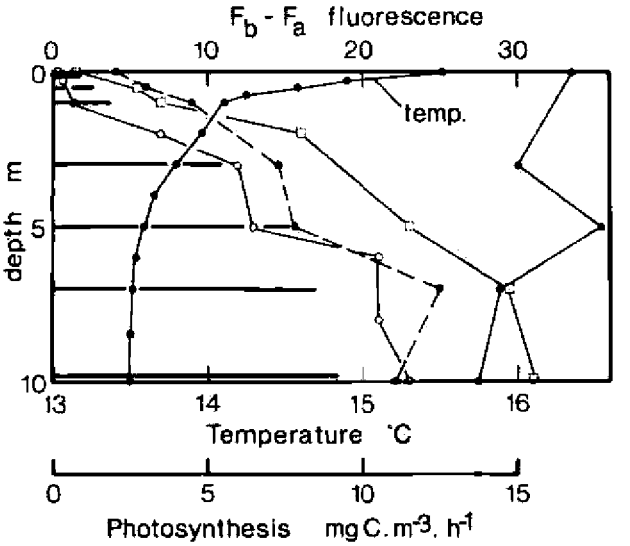


FIG. 11. Comparison between bottle incubations (photosynthesis, fluorescence), and in situ water column fluorescence, 13 May, Lago Grande. ●—●, $F_b - F_a$ in situ, 06:00 h; □, $F_b - F_a$ in situ, 09:30 h; O, $F_b - F_a$ in situ, 13:30 h; ●- - -●, $F_b - F_a$ after 4 h bottle incubation; temperature, solid line as marked; horizontal bars, photosynthesis from 4 h bottle incubation.

TABLE 1. *Parameters of variation in chlorophyll fluorescence for representative time-series experiments. Six such measurements were made over the 0.5–2 h depression and nine over the 2–6 h recovery period. K, specific rate of fluorescence change, and asymptote estimated using Gauss-Newton non-linear regression to fit the equation* $F(t) = F_a \exp(-Kt) + F_f$ *where* $F(t)$ *is time variable* ($F_b - F_a$), F_a = *maximum amount of adjustment,* F_f = *asymptotic value,* r^2 = *coefficient of determination. LG = Lago Grande, BP = Bahia de Puno.*

Source	Sample date	Mean PAR intensity $\mu E \cdot m^{-2} \cdot s^{-1}$	Type of experiment	K (min^{-1})	Asymptote[a]	r^2
LG	1 April	1881	Surface in situ[b]	0.084 (D)[c]	1.1	0.998
BP	31 March	1955	Surface in situ[b]	0.055 (D)	0.60	0.999
BP	9 April	2200	Full sunlight[d]	0.250 (D)	2.3	0.990
BP	9 April	2200	w/uv filter[d]	0.280 (D)	7.5	0.974
BP	15 April	2210	Pyrex flask, full sunlight	0.318 (D)	0.70	0.999
BP	15 April	15	Pyrex flask, shade	0.041 (R)	11.18	0.990
BP	5 May	2260	Pyrex flask full sunlight	0.108 (D)	2.45	0.997
BP	5 May	220	Pyrex flask, cool white fluorescent light	0.048 (R)	17.8	0.978
LG	1 June	1100	Surface in situ,[b] intermittent cloud	0.015 (D)	0.2	0.903
LG	1 June	15	Cubitainer, shade	0.002 (R)	19.8	0.967
BP	18 September	700	0 m water, sunlight with neutral density filters	NS[e] (D)	27.3	0.487
BP	18 September	700	20 m water, sunlight with neutral density filters	0.344 (D)	25.3	0.925

[a] In relative fluorescence units.
[b] Incubated in large (20 L) polyethylene flexible plastic containers (Cubitainers).
[c] D = depression experiment, K is rate of exponential drop; R = recovery, K = rate of saturating exponential rise.
[d] Pyrex beaker open to sunlight or covered with a Tiffen Haze – I ultraviolet filter: 0% transmission below 350 nm, 35% of 350–400 nm, 83% of 400–500 nm, 90% of PAR greater than 500 nm.
[e] NS indicates K not significantly different from zero.

method. Fluorescence profiles were taken in the lake at the beginning and the end of the 4 h incubation (09:30–13:30 h), and in addition in vivo fluorescence was measured in subsamples from the incubated bottles. At 09:30 h there was already substantial surface depression of fluorescence relative to sunrise values (06:00 h, Fig. 11) but further depression occurred in the lake over the subsequent 4 h. There was much less depression within the bottles over this period (Fig. 11). At depths less than 7 m the ($F_b - F_a$) fluorescence at 13:30 h was considerably higher in the bottles than in the lake. At near-surface depths (0, 0.5, 1 m) the bottle ($F_b - F_a$) values were higher than even 09:30 h lake samples suggesting that perhaps some slight recovery, but certainly no further depression, had taken place.

The photosynthetic CO_2-fixation profile followed the usual pattern for bottle incubations with strongly depressed surface rates and maximum values at ca. 10 m (Fig. 11). For the portion of the water column down to the depth of maximum photosynthesis there was a strong correlation between bottle ($F_b - F_a$) fluorescence and photosynthetic rates ($r^2 = 0.907$, $P < 0.01$).

Kinetics of fluorescence depression and recovery. In a series of in situ and laboratory experiments, variable fluorescence ($F_b - F_a$) and ^{14}C-HCO_3^- uptake rates followed first-order kinetics. Kinetic parameters of ($F_b - F_a$) were a function of light intensity, spectral composition, and sample location (Table 1). First-order kinetics were indicated by close statistical fits to equations describing an exponential drop to a minimum in bright light and a saturating exponential rise to a maximum during recovery in dim light (Table 1). These curves were fitted by Gauss-Newton non-linear regressions (Snedecor and Cochran 1967).

Bright-light depression to a higher asymptotic minimum ($F_b - F_a$) value than for full sunlight occurred in Bahia de Puno samples when ultraviolet light was filtered out (Table 1, Fig. 12). The water was incubated in Pyrex beakers either open to sunlight or covered with a Tiffen Haze-I ultraviolet filter. This filter allowed 90% of PAR (photosynthetically available radiation) with wavelengths greater than 500 nm to pass, but 83% transmission of 400–500 nm, 35% of 350–400 nm and 0% below 350 nm. Depression rates were similar in both treatments, but the asymptotic minimum was 69% lower in the presence of ultraviolet radiation (Table 1, Fig. 12).

Rates of decrease differed in samples from Lago Grande and Bahia de Puno at comparable PFDs. There were also differences in samples from various depths down the water column at each site, particularly when the euphotic zone was stratified for several weeks. Phytoplankton were sampled from the upper and lower euphotic zone in Bahia de Puno during one such period of stratification. On this date (18 September 1982) the Bahia had a well developed thermocline at 7 m and a markedly non-homogeneous vertical distribution of chl *a*. Average irradiance experienced by the plankton communities were 53% of surface PFD in the mixed layer, and 6% in the hypolimnion (7–25 m). Samples from 20 m responded strongly to bright light; ($F_b - F_a$) flu-

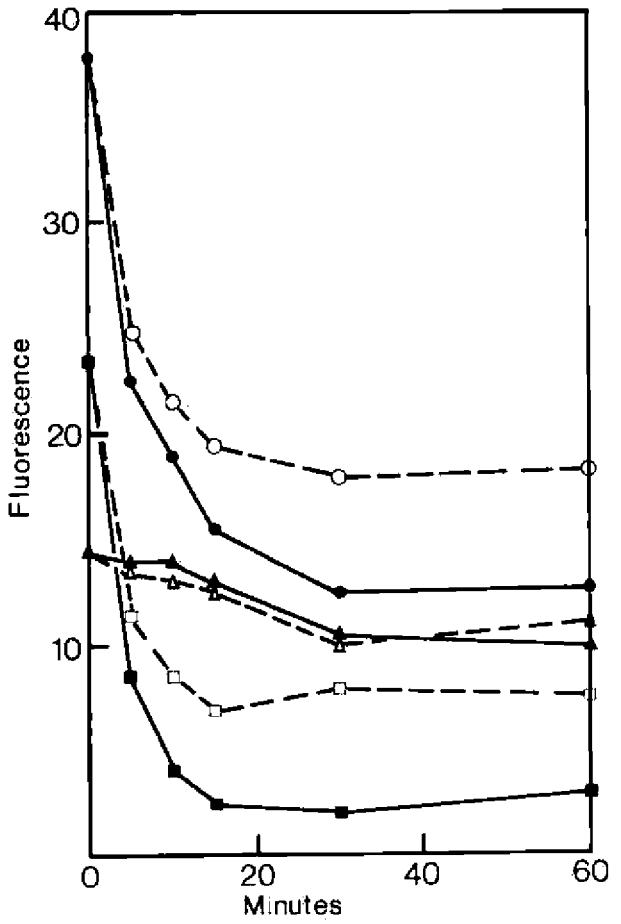


FIG. 12. Effect of ultraviolet light on fluorescence depression. Samples from Bahia de Puno, 9 April were incubated in full sunlight with (open symbols) and without (closed symbols) a Tiffen Haze-1 ultraviolet filter. F_b, circles; F_a, triangles; $F_b - F_a$, squares.

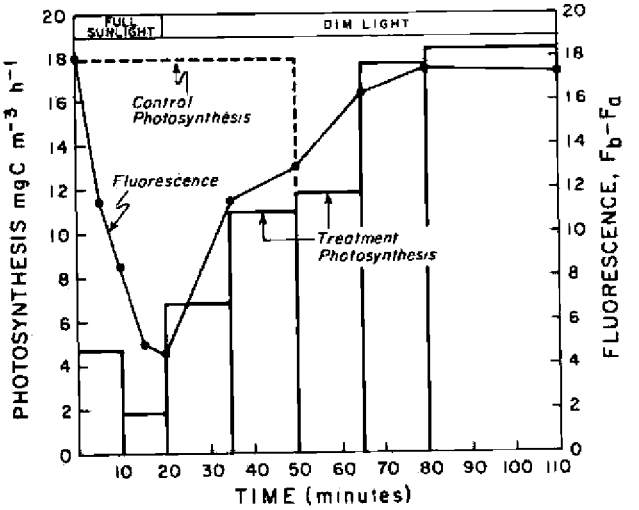


FIG. 13. Photosynthetic response to and recovery from bright light. Samples from Bahia de Puno (5 May) were incubated in full sunlight. $F_b - F_a$ fluorescence and photosynthetic rates (bars) were measured over 20 min and then the samples placed under reduced, but near saturating PFD (200 $\mu E \cdot m^{-2} \cdot s^{-1}$). Fluorescence and photosynthetic recovery were then followed over the subsequent 90 min. A control sample was incubated under 200 $\mu E \cdot m^{-2} \cdot s^{-1}$ during the first 50 min of the experiment.

orescence was rapidly depressed to a minimum, within 10 min of exposure, of 44% initial values (Table 1). The 0 m population responded much more slowly and eventually declined to a minimum 86% of the initial ($F_b - F_a$) fluorescence. The specific rate of fluorescence change was not significantly different from zero (Table 1).

In experiments where a short period (less than 60 min) of bright-light exposure was followed by transfer to dim light, all fluorescence parameters recovered to near initial levels. This recovery, however, was much slower than depression (Table 1: BP 5 May, LG 1 June). When phytoplankton samples were exposed to bright light for long periods of time (60 min or longer), ($F_b - F_a$) fluorescence was reduced to low or occasionally zero values which showed no recovery in dim light, even over several hours.

Relationship between ($F_b - F_a$) and photosynthesis. In a time-series measurement of both photosynthetic CO_2 fixation and fluorescence, changes in ($F_b - F_a$) were paralleled by changes in carbon uptake (Fig. 13). Photosynthetic rates dropped to 26% of control after 10 min, and to 10% after a further 10 min. Upon transfer to a lower light regime (approximately that at A_{max}) fluorescence and photosynthetic rates increased. ($F_b - F_a$) rose fourfold over 90 min and this increase was correlated with changes in CO_2 fixation rates ($r = 0.94$, $P < 0.01$). Changes in extractable chl *a* over the bright light incubation period were less than 10%.

To further define the photosynthetic response to bright light, carbon fixation rates were measured after a 2 h in situ full sunlight (mean PFD of 1955 $\mu E \cdot m^{-2} \cdot s^{-1}$) surface incubation and compared to replicate samples kept in dim light (10 $\mu E \cdot m^{-2} \cdot s^{-1}$) over the same period. Fluorescence again dropped exponentially in the full sunlight incubations, but did not change significantly in the control. Extractable chl *a* ($\bar{x} \pm 95\%$ confidence limits, $n = 3$) rose slightly in the dim light control from 5.3 ± 0.8 mg chl *a* to 6.1 ± 0.5 mg chl $a \cdot m^{-3}$, but did not change in the bright light treatment (5.2 ± 0.2 mg chl $a \cdot m^{-3}$). After two hours, photosynthetic rates were measured over a 30 min incubation at 5 m ($\bar{I} = 662$ $\mu E \cdot m^{-2} \cdot s^{-1}$) and 15 m ($\bar{I} = 64.4$ $\mu E \cdot m^{-2} \cdot s^{-1}$). The carbon fixation rate in the surface pre-incubated water was greatly depressed relative to dim light controls. At 5 m surface-samples had a mean (±range) fixation rate of 0.79 (±0.03) mg $C \cdot m^{-3} \cdot h^{-1}$ compared with 13.6 (±1.0) in the controls. At 15 m the rates were 0.29 (±0.07) mg $C \cdot m^{-3} \cdot h^{-1}$ vs. 5.1 (±0.4), respectively. Bright light pretreatment reduced both the maximum photosynthetic rate (P_{max}) and low light response (α) in the sample to a similar extent, to about 6% of dim light controls.

Similarly, in a related series of fixed depth experiments both α and P_{max} changed proportionately. These data suggest a shift in ability to photosynthetically utilize excitation energy at all PFDs, rather than a simple change in photochemical capacity.

DISCUSSION

In Lake Titicaca, major shifts in algal photochemistry appear to follow the diel cycle of stratification and mixing. Throughout each morning the developing diurnal thermocline retains phytoplankton near the lake surface where bright light strongly depresses, and sometimes eliminates ($F_b - F_a$) fluorescence. This effect is rapidly reversed by incubation in dim light, suggesting that it is mainly controlled by irradiance rather than by diel variations in nutrient supply or temperature. The fluorescence depression is not accompanied by major shifts in extractable chlorophyll *a* or species composition, two primary determinants of algal fluorescence in natural waters (see Vincent 1983). During the afternoon in Titicaca the surface phytoplankton populations are redistributed by wind-induced mixing, but it is not until nightfall that full photochemical recovery is reached.

Both in the laboratory and the field there was a close correlation between photosynthetic carbon fixation rates and fluorescence ($F_b - F_a$) depression. Therefore the in vivo depressions of ($F_b - F_a$) fluorescence found in the lake indicate real photoinhibition of near-surface photosynthesis of approximately the magnitude estimated by fixed-depth bottle experiments. Since fluorescence depression and carbon uptake are closely coupled (i.e. with a less than 10 min lag, Fig. 13), observed diurnal ($F_b - F_a$) depression in the near-surface layer is an nonartifactual indicator of photoinhibition.

In Lake Titicaca, Pyrex bottle incubations may even underestimate the in situ photoinhibition, particularly at the surface. Pyrex glass strongly absorbs ultraviolet radiation (Smith et al. 1980) which is a waveband that apparently intensifies ($F_b - F_a$) depression (Fig. 12). The reduced exposure to UV may therefore explain higher ($F_b - F_a$) values in the glass incubation bottles than in the lake (Fig. 11). Many studies have demonstrated the strong inhibitory effects of UV light on algal productivity (e.g. Lorenzen 1979). Lake Titicaca lies at high altitude and incoming UV radiation may therefore be intense. However, although Titicaca phytoplankton are responsive to UV, strong ($F_b - F_a$) depression could be induced by PAR alone. Ultraviolet wavelengths are rapidly attenuated in natural waters, and therefore the spectrum of photoinhibitory radiation will shift towards the visible with increasing depth of the water column (cf. Smith et al. 1980). The increasing correspondence between bottle and in situ fluorescence depression is consistent with this effect.

A current model of the photosynthesis-light relationship envisages two opposing photochemical reactions operating over a wide range of PFDs, including P_{max} (Platt et al. 1980). The fluorescence responses in Lake Titicaca lend physiological support to these models. $F_b - F_a$ depression extended well into and even below the region of A_{max}, thereby demonstrating photoinhibitory effects over a broad region of the photosynthesis-light curve. High PFDs (>10% surface, Fig. 7) provide increased energy for photosynthesis, but also reduce photochemical capacity to an extent dependent upon exposure time. As a consequence, A_{max} in stratified euphotic zones, or in fixed depth incubations, must lie below the theoretical maximum (P_s in the terminology of Platt et al. 1980) for the same phytoplankton assemblage previously incubated in dim light, or circulated continuously throughout the mixed layer. Circulating bottle experiments of the type employed by Marra (1978) modify photoinhibitory effects at all depths, not just the conspicuously depressed region from A_{max} to the surface.

The susceptibility of Lake Titicaca phytoplankton to ($F_b - F_a$) fluorescence depression varied considerably from site to site, and also with depth when the euphotic zone was stratified. These observations suggest that photoinhibition is under both short- and long-term control. At time-scales of minutes to hours the extent of depression is dependent upon recent light exposure and the sensitivity of the algae to high PFDs. This latter cellular property may be considerably modified at longer time-scales within the same light regime. Similar observations have been reported from stratified environments elsewhere. For example, deep-living phytoplankton in Baffin Bay in the eastern Arctic were highly sensitive to surface PFDs whereas surface populations were virtually uninhibited at any irradiance (Platt et al. 1982). The fluorescence data from Bahia de Puno suggest that such differences could occur within a few weeks of stratification. In the Canadian Arctic, susceptibility to bright-light could be greatly reduced over several hours exposure, but the enhanced sensitivity to high PFDs characteristic of deep populations required 2–6 weeks to develop once the algal assemblage was isolated below the pycnocline (Gallegos et al. 1983).

The dependence of photoinhibitory responses upon light conditions during growth is particularly well illustrated by sun and shade forms of terrestrial plants. *Phaseolus vulgaris*, for example, is highly sensitive to bright light when grown under low PFDs. Conversely sun-adapted plants of the same species show relatively little photoinhibition, unless they are deprived of CO_2 (Powles and Critchley 1980). Osmond (1981) concludes that photoinhibition will be observed whenever the transfer of excitation energy from antennae pigments to reaction centers is faster than it can be dissipated by non-cyclic electron transport. This imbalance occurs in shade plants when they are transferred to full sunlight because of the

disproportionate abundance of light harvesting pigments relative to electron transport intermediates (Osmond 1981). Lake Titicaca phytoplankton typically experience a low PFD environment during growth and this shade-adapted assemblage may therefore be especially susceptible to photoinhibition within the near-surface thermocline.

The observed depression in fluorescence parameters is rapid, reversible, highly correlated with decreases in CO_2 photofixation rates, and appeared to be related to changes in cellular energy utilization. The effect operated primarily through shifts in F_b, while extractable chl *a* levels remained more or less constant. A wide variety of adaptational mechanisms have been recently identified which operate both on fluorescence behavior and photosynthetic performance. However, many of these adjustment processes are rapidly reversed by a 30 min dark adaptation (light-state conversion, Vincent 1979; chloroplast conformational shifts, Kiefer 1973), affect F_a fluorescence to an equal or greater extent than F_b (changes in electron transport capacity, Fleischacker and Senger 1978; decoupling of the light-harvesting complex from RC II, Armond et al. 1980) or occur at time-scales greater than that recorded here (change in photosynthetic unit (PSU) size or number, Falkowski and Owens 1980; stoichiometry of RC I and RC II, Melis and Brown 1980; electron transport capacity, Vincent 1980). Thus these types of response do not appear to adequately explain the bright-light effects observed in Lake Titicaca.

The photoinhibition responses in Lake Titicaca must also be distinguished from effects which operate only during the period of exposure to bright light. For example, Ley and Mauzerall (1982) have reported a "total annihilation" process which occurs at RC II under very high PFDs (> 1500 $\mu E \cdot m^{-2} \cdot s^{-1}$). If two photons are received at the same reaction center trap almost simultaneously then the second can both undo the effect of the first and briefly inactivate the reaction trap. However, unlike the responses measured in Lake Titicaca, this inactivation is reversible within seconds, and does not affect the subsequent measurement of α or P_{max}.

Another light-response mechanism has been recently identified in shade-adapted terrestrial plants (Critchley and Smillie 1981, Osmond 1981, Powles and Bjorkman 1982) but has not been previously described in phytoplankton communities. When plants grown under low light (PFDs ca. 100–200 $\mu E \cdot m^{-2} \cdot s^{-1}$, similar to average light levels in the mixed layer of Lake Titicaca) are exposed to full sunlight (2000 $\mu E \cdot m^{-2} \cdot s^{-1}$) they experience a reversible inactivation of RC IIs. As a result, a higher proportion of excitation energy is lost from their chloroplasts by non-radiative processes (Powles and Bjorkman 1982). Our fluorescence and ^{14}C-HCO_3^- assays are consistent with this type of response. The measured reduction in ($F_b - F_a$) fluorescence suggests a loss of operational RC IIs. In higher plants this causes a drop in both P_{max} and α, similar to that recorded in Lake Titicaca. Furthermore, the time course of photoinhibition and recovery in such plants (Critchley and Smillie 1981, Powles and Bjorkman 1982) closely resemble the first order kinetics reported here, with rates of recovery much slower than inhibition.

Whether such extreme physiological responses, with their implications for true in vivo photosynthesis, compared to fixed-depth bottle estimates are common remains to be determined. We expect that similar responses will be found whenever high irradiances combine with weak mixing. Such conditions may frequently occur during the temperate zone summer. For example, in a eutrophic harbor of Lake Ontario, depressed DCMU-treated fluorescence but approximately constant in vivo fluorescence (not dark-adapted) were often observed at shallow depths during the day. In addition, when phytoplankton from this community were exposed to bright light (>200 $\mu E \cdot m^{-2} \cdot s^{-1}$), ($F_b - F_a$) fluorescence dropped exponentially to an asymptote 42–66% of the initial value (Harris 1980). In this Lake Ontario environment light is rapidly attenuated, self-shading from dense biomass is significant, and average light available to the mixed layer is low. There too, cells may experience prolonged exposure to high surface PFD and respond by RC II inactivation.

In the extreme irradiance setting of Lake Titicaca these responses are pronounced. Through the diel cycle of stratification and mixing cells must experience frequent changes in light conditions ranging from sustained exposure to intense PFD within the surface thermocline to severe light limitation at the bottom of the mixed layer. Cells maintain the high light-harvesting capacity in relation to electron transport and dark reaction potential needed for low light growth, but by inactivating the centers of photosynthetic photochemistry during prolonged exposure to surface PFD the damaging effects of bright light might be much reduced. In this lake, and perhaps others, an understanding of the true in situ importance of photoinhibition effects will require close attention to the photochemical response flexibility of the algae, as well as to the light field and mixing regime.

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