

Vertical mixing within the epilimnion modulates UVR-induced photoinhibition in tropical freshwater phytoplankton from southern China

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SUMMARY

1. The importance of vertical mixing in modulating the impact of UVR on phytoplankton photosynthesis was assessed in a tropical, shallow lake in southern China from late winter to mid-spring of 2005.
2. Daily cycles of fluorescence measurements (i.e. photosynthetic quantum yield, Y) were performed on both 'static' and *in situ* samples. Static samples were of surface water incubated at the surface of the lake under three radiation treatments – PAB (PAR + UVR, 280–700 nm), PA (PAR + UV-A, 320–700 nm) and P (PAR, 400–700 nm). *In situ* samples were collected every hour at three different depths – 0, 0.5 and 1 m.
3. The general daily pattern was of a significant decrease in Y from early morning towards noon, with partial recovery in the afternoon. Samples incubated under static conditions always had lower Y than those under *in situ* conditions at the same time of the day.
4. Under stratified conditions, no overall impact of UVR impact could be detected *in situ* when compared with the static samples. Further rapid vertical mixing not only counteracted the impact of UVR but also stimulated photosynthetic efficiency.
5. Based on these measurements of fluorescence, the mixing speed of cells moving within the epilimnion was estimated to range between 0.53 and 6.5 cm min⁻¹.
6. These data show that mixing is very important in modulating the photosynthetic response of phytoplankton exposed to natural radiation and, hence, strongly conditions the overall impact of UVR on aquatic ecosystems.

Keywords: mixing, photosynthesis, photosynthetically available radiation, phytoplankton, ultraviolet radiation

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Introduction

Vertical mixing, as produced by solar heating, wind and storms, causes phytoplankton to move within the water column and thus exposes cells to fluctuating radiation (Neale, Helbling & Zagarese, 2003). It has been found that such radiation fluctuations produce varied effects, ranging from an increase (Marra, 1978), decrease (Kroon *et al.*, 1992) or no change (Yoder & Bishop, 1985) in phytoplankton primary productivity.

Most of these studies, however, have considered the responses of phytoplankton under variable photosynthetically available radiation (PAR, 400–700 nm), but it is now recognised that fluctuating ultraviolet radiation (UVR, 280–400 nm) can also affect the performance and fitness of aquatic organisms (Helbling, Villafañe & Holm-Hansen, 1994; Neale *et al.*, 2003).

Studies addressing the combined impact of fluctuating UVR and vertical mixing on phytoplankton photosynthesis (Helbling *et al.*, 1994, 2003; Neale, Cullen & Davis, 1998a; Neale, Davis & Cullen, 1998b; Köhler *et al.*, 2001; Barbieri, Villafañe & Helbling, 2002) have found a variety of responses. For example, research in Antarctic waters has shown that a shallow upper mixed layer depth (Z_{UML}) enhanced short-term effects of UVR-induced photoinhibition as compared with conditions of deep mixing (Helbling *et al.*, 1994; Neale *et al.*, 1998b). Barbieri *et al.* (2002), working with postbloom phytoplankton populations in Patagonia, further demonstrated the importance of the combined effects of mixing intensity (i.e. the portion of the euphotic zone that was being mixed) and UVR on carbon fixation. In that study, Barbieri *et al.* (2002) showed that the integrated impact of UVR varied from an inhibition of carbon fixation under shallow mixing conditions, to an enhancement under deep mixing, thus demonstrating the use of UVR as a source of energy for photosynthesis. In fact, the use of longer UVR wavelengths (i.e. UV-A) for photosynthesis has been also demonstrated in studies carried out in the tropics, when cells were exposed to fast mixing in the water column (Helbling *et al.*, 2003). An opposite response was determined in Antarctic phytoplankton, however, in studies that demonstrated higher photosynthetic inhibition under deep and rapid mixing conditions (Helbling *et al.*, 1994; Neale *et al.*, 1998b).

It is thus evident that the prevalent mixing regime plays a key role in modulating the impact of UVR on phytoplankton photosynthesis. However, it also appears that the response to UVR of the phytoplankton, when exposed to different mixing conditions, is species-specific (Barbieri, Villafañe & Helbling, 2006) and may vary from place to place (Helbling *et al.*, 1994; Barbieri *et al.*, 2002). In this paper we report data on the importance of mixing in moderating the effect of UVR on phytoplankton photosynthesis, obtained by the non-invasive, pulse amplitude modulated (PAM) fluorescence technique. We also calculated

the circulation speed of phytoplankton cells moving within the epilimnion. We used as a case study a small lake in southern China which is exposed to naturally high radiation levels because of its tropical location. Our results are of general relevance, but also increase our knowledge of the impact of UVR on aquatic organisms in this relatively under-sampled region, where photobiological investigations have recently begun (Helbling *et al.*, 2003, 2006b; Dobretsov, Qian & Wahl, 2005; Villafañe, Gao & Helbling, 2005; Wu *et al.*, 2005).

Methods

Study area

This study was conducted at Shantou, southern China (23°26'N, 116°42'E) from late winter to mid-spring of 2005 (6 March–5 May, Julian days 65–125). The experiments were conducted *in situ* once a week with water samples collected from an eutrophic, turbid, modified natural lake (hereafter referred to as Lake Pipino: area, 2 km²; mean depth, 3 m) located on the campus of Shantou University.

Experimentation/measurements of in situ fluorescence parameters

Using the lake as a model system, we determined the interactive impact of UVR and mixing upon phytoplankton circulating within the epilimnion. In each experiment, data collection started early in the morning (around 08.00 hours) well before the sun's rays shone directly on the water surface. Measurements of the fluorescence of photosystem II (PSII) were made every hour until 17.00 hours on 'static' and *in situ* samples. We defined as 'static' water samples collected just below the lake surface at the start, dispensed into six 500 mL quartz tubes and incubated at the surface of the lake under three radiation treatments: two samples each were exposed to (i) PAB: full solar radiation (PAR + UVR, 280–700 nm), uncovered quartz tubes; (ii) PA: (PAR + UV-A, 320–700 nm) tubes covered with UV cut-off filter foil Montagefolie N°10155099 Folex (Germany, 50% transmission at 320 nm) and (iii) P: (PAR, 400–700 nm) quartz tubes covered with Ultraphan UV Opak Digepra film (Germany, 50% transmission at 395 nm) (see Figueroa *et al.*, 1997 for spectra).

In situ samples were defined as those collected every hour (08.00–17.00 hours) at three different depths – 0, 0.5 and 1 m – and so they represented the *in situ* fluorescence characteristics of cells under the natural mixing conditions. Sampling at different depths was performed with a small plastic aquarium-type pump (3-cm diameter, 5-cm length) that gently collected samples at a flow rate of 1 L per min. Because of the size of the pump and to the flow rate no disturbance was expected in the water column; in addition, there was no indication of broken cells when samples were immediately observed under the microscope. Thus, ‘static’ samples were intended to mimic stratified conditions, while *in situ* samples reflected the natural mixing regime.

On both, ‘static’ and *in situ* samples, fluorescence parameters were determined within 1 min after collection, with a portable PAM fluorometer (PAM – WATER-ED, Walz, Germany). The photosynthetic quantum yield (Y) was determined by measuring the instant maximal fluorescence (F'_m) and the steady state fluorescence (F_t) of light-adapted cells using a saturating white light pulse (approximately $5300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in 0.8 s) in the presence of a weak measuring actinic light. The yield was calculated according to Van Kooten & Snel (1990) and Genty, Briantais & Baker (1989) as:

$$Y = (F'_m - F_t)/F'_m = \Delta F/F'_m \quad (1)$$

Additional analysis and measurements

The procedure for each determination/measurement during the experiments was as follows:

1 Chlorophyll-*a* (Chl-*a*) and UV-absorbing compounds: Chl *a* and UV-absorbing compounds were measured by filtering a variable volume (50–100 mL) of sample onto a Whatman GF/F (Whatman, New Jersey, U.S.A.) glass fibre filter (25 mm), followed by extraction with absolute methanol (Holm-Hansen & Riemann, 1978) for 2 h, and subsequent determination of the optical density in a scanning (250–700 nm) spectrophotometer (Shimadzu UV 2501-PC, Kyoto, Japan). Chlorophyll-*a* concentration was calculated from the optical density using the equation of Porra (2002).

2 Taxonomic analysis: Counting and identification of cells was performed using an inverted microscope (Leica DM IL, Wetzlar, Germany) after settling overnight 10 mL of sample; cells counts were made

following the methodology described in Villafañe & Reid (1995).

3 Measurements of solar radiation: Incident solar radiation was measured continuously using a broadband filter radiometer (ELDONET, Real Time Computers, Möhrendorf, Germany) installed on the roof of the Institute of Marine Biology (Shantou University). The instrument records irradiance in the UV-B (280–315 nm), UV-A (315–400 nm) and PAR (400–700 nm) wavebands at a frequency of once per minute. The downward radiation was measured using a submersible filter radiometer (ELDONET, Real Time Computers) which has the three radiation channels (as above) and additional sensors for temperature and pressure, from which depth is calculated automatically by the software.

4 Measurements of underwater temperature and conductivity: Profiles of conductivity and temperature at the sampling site were obtained every 2 h; data were collected with a conductivity – temperature – depth sensor (YSI 600XL Sonde, Yellow Spring, OH, U.S.A.).

5 Statistics/treatment of data: The data are reported as the mean and SD for the different measurements. In each experiment, duplicate samples were obtained for the different radiation treatments (in static samples) and different depths (in *in situ* samples), and each sample was measured six times with the PAM fluorometer. A total of eight experiments were performed during the study period. The non-parametric Kruskal-Wallis test (Zar, 1984) was used to assess for significant differences ($P < 0.05$) between the samples exposed to different radiation treatments (in static samples) and at different depths (for the *in situ* measurements).

The photosynthetic inhibition for each wavelength interval (i.e. photosynthetic quantum yield in the PAB and PA treatment relative to that in the *P* control) over the incubation period was calculated as:

$$\text{UV-B inhibition} = \{[(Y_P - Y_{PAB}) - (Y_P - Y_{PA})]/Y_P\} \times 100 \quad (2)$$

$$\text{UV-A inhibition} = [(Y_P - Y_{PA})/Y_P] \times 100 \quad (3)$$

where Y_P , Y_{PA} and Y_{PAB} are the photosynthetic quantum yield in the *P*, *PA* and *PAB* treatments, respectively.

We used the data from all experiments to estimate the variation in Y as a function of irradiance and time; we used a power function ($Y = A x^B$), where A and B

are constants and x is either time or irradiance (see below). This function was the best fit for our data and B was used either as a decay rate constant (with a negative value), in the case of inhibition, or as a repair rate constant (with positive value), in the case of recovery of Y . We also determined the first derivative of the power function to calculate a mean rate of change of Y as a function of time during the morning; this rate was used to estimate mixing time and speed (see Results).

Results

The mean PAR irradiance at local noon was approximately 280 W m^{-2} and it ranged between 50 and 400 W m^{-2} (Fig. 1a). Both UV-A and UV-B (Figs 1b,c)

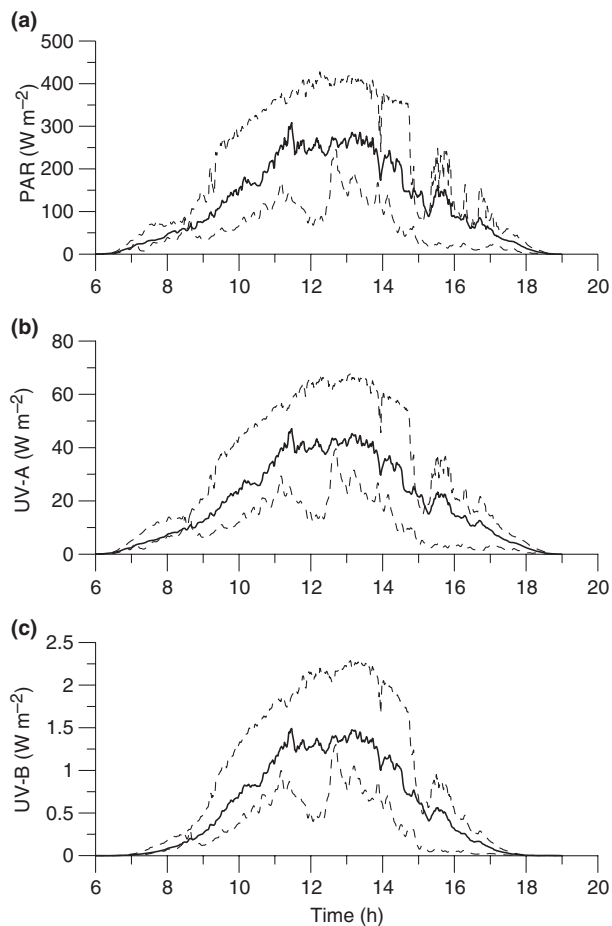


Fig. 1 Mean (solid lines) and range (maximum and minimum, broken lines) incident solar radiation (in W m^{-2}) measured between dawn and dusk over Lake Pipino throughout the study period. (a) PAR (400–700 nm); (b) UV-A (315–400 nm) and (c) UV-B (280–315 nm).

followed the same trend, with a mean UV-A irradiance at noon of approximately 40 W m^{-2} (range: $10\text{--}70 \text{ W m}^{-2}$) (Fig. 1b) and of UV-B of approximately 1.4 W m^{-2} (range: $0.5\text{--}2.3 \text{ W m}^{-2}$) (Fig. 1c). Daily variability in irradiance was mostly caused by passing clouds.

There were no significant changes in the attenuation coefficient at Lake Pipino during the study period, so a single representative underwater radiation profile could be applied (Fig. 2). The lake had a PAR attenuation coefficient ($K_{d\text{-PAR}}$) of 0.96 m^{-1} so that the mean depth (3 m) encompassed 2.9 optical depths and thus, at 4.8 m, the euphotic depth (i.e. the depth where 1% of incident radiation is determined) would exceed the lake depth. As expected, the attenuation coefficients for UV-A and UV-B were higher than that for PAR, with $K_{d\text{-UV-A}}$ and $K_{d\text{-UV-B}}$ being 2.68 and 2.96 m^{-1} , respectively. During the study period, mean Chl-*a* concentration was $10.7 \mu\text{g L}^{-1}$ (SD, $5.0 \mu\text{g L}^{-1}$) and, on average, the nanoplankton fraction ($<20 \mu\text{m}$) accounted for approximately 85% of this total. Taxonomic analyses revealed the dominance of monads and flagellates (i.e. mainly chlorophytes of the genera *Tetraedron* Kützing, *Staurastrum* Meyen and *Closterium* Nitzsch) throughout the study period. Diatoms represented a minor fraction of the community and they were represented by *Aulacoseira granulata* (Ehrenberg) Simonsen and unidentified pennate species. Dinoflagellates were numerically scarce during this period and were represented mostly by the genus *Peridinium* Ehrenberg.

Water temperature and wind speed were variable throughout the study and during the day. The range

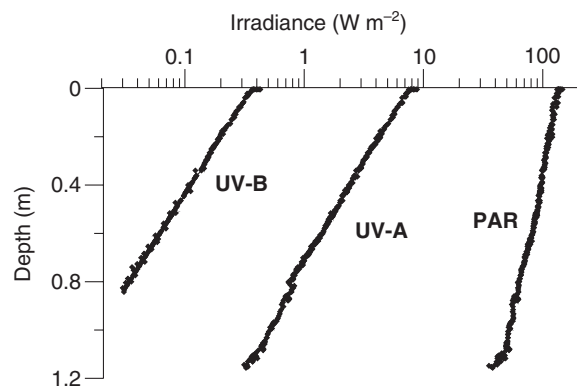


Fig. 2 Representative profile showing the underwater radiation field (PAR, UV-A and UV-B) close to our experiments; radiation is expressed in W m^{-2} . The attenuation coefficients for PAR, UV-A and UV-B were 0.96 , 2.68 and 2.96 m^{-1} , respectively. Profile measured on 8 April 2005 (14.00 hours).

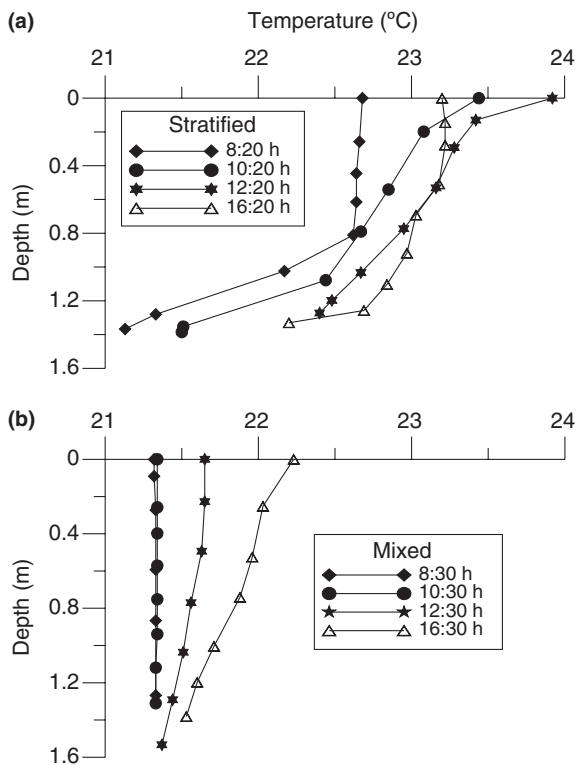


Fig. 3 Representative temperature profiles obtained at Lake Pipino. Profiles were measured every 2 h throughout the duration of the experiment. (a) Stratified condition, profiles measured on 8 April 2005; (b) Mixed condition, profiles measured on 15 April 2005.

of water temperature was 21–24 °C (Fig. 3) whereas wind speed ranged from 0.3 to 7.3 m s⁻¹ (data provided by Shantou Weather Station). Consequently, stratification changed and two main conditions (stratified and mixed) typically characterised the period from late winter to mid-spring. When the lake was stratified, the epilimnion extended down to approximately 0.8 m depth in the early morning and the temperature difference between surface and 1.4 m was approximately 1.5 °C (e.g. 8 April, Fig. 3a). As the day progressed, the epilimnion depth increased (to approximately 1.2 m) and some heating of the first 10 cm of the water column was observed at noon. By the end of the day the temperature was higher throughout the water column and the surface-to-depth difference was <1 °C. When the lake was mixed there was little variations in temperature between surface and 1.4-m depth (e.g. 15 April, Fig. 3b). However, surface temperature at the end of the day was higher (approximately 0.9 °C at the surface) than that early in the morning. Note that, under stratified

conditions (Fig. 3a), the variation in surface temperature during the day was about 1.3 °C; on the other hand, under mixed conditions (Fig. 3b) they were less, approximately 0.9 °C.

The effects of solar radiation on photosynthetic quantum yield (Y) throughout a daily cycle under stratified and mixed conditions are shown in Figs 4 & 5. Static samples on a representative day under stratified conditions had a relatively low Y value of approximately 0.3 early in the morning (Fig. 4a), and it decreased significantly towards noon in all radiation treatments; a significant though not complete recovery, was observed in the afternoon. Samples under the P treatment had a significantly higher Y than those under the PA or PAB treatments (Fig. 4a). Photosynthetic inhibition caused by UVR (Fig. 4b) was up to 70% during the morning/early in the afternoon, with UV-A accounting for most of the inhibition and UV-B for <20%. *In situ* Y at different depths in the water column (Fig. 4c) had a similar daily pattern to the static samples, with the lowest values at or close to noon; these samples had significantly higher Y than those under static conditions at the surface. The differences of Y measured at 0 and 1 m depth in *in situ* samples – $\Delta Y_{(1-0m)}$ (Fig. 4d) varied between 0.05 and 0.23, with large differences during mid-morning and early afternoon.

During the mixed condition (Fig. 5) static samples had a significant decrease in Y from a relatively high mean value of 0.52 during early morning, to approximately 0.1 under full radiation at noon; during the afternoon, however, samples partly recovered their photosynthetic efficiency (Fig. 5a). Samples under the P treatment had significantly higher Y than those under the PA or PAB treatments (Fig. 5a). There were significant effects of the different wavebands, with the bulk of inhibition due to UV-A (maximum of approximately 40% during the morning) and UV-B contributing <10% throughout the day (Fig. 5b). *In situ* samples (Fig. 5c) displayed a similar pattern of inhibition/recovery, with all samples (i.e. collected at different depths) being similarly inhibited during the morning, reaching a Y value of approximately 0.23. However, samples collected at 1-m depth recovered much faster in the afternoon than those collected at the surface. In fact, when comparing $\Delta Y_{(1-0m)}$ (Fig. 5d) it is evident that this difference was close to zero during the morning, whereas in the afternoon it was significant and with values as high as 0.2.

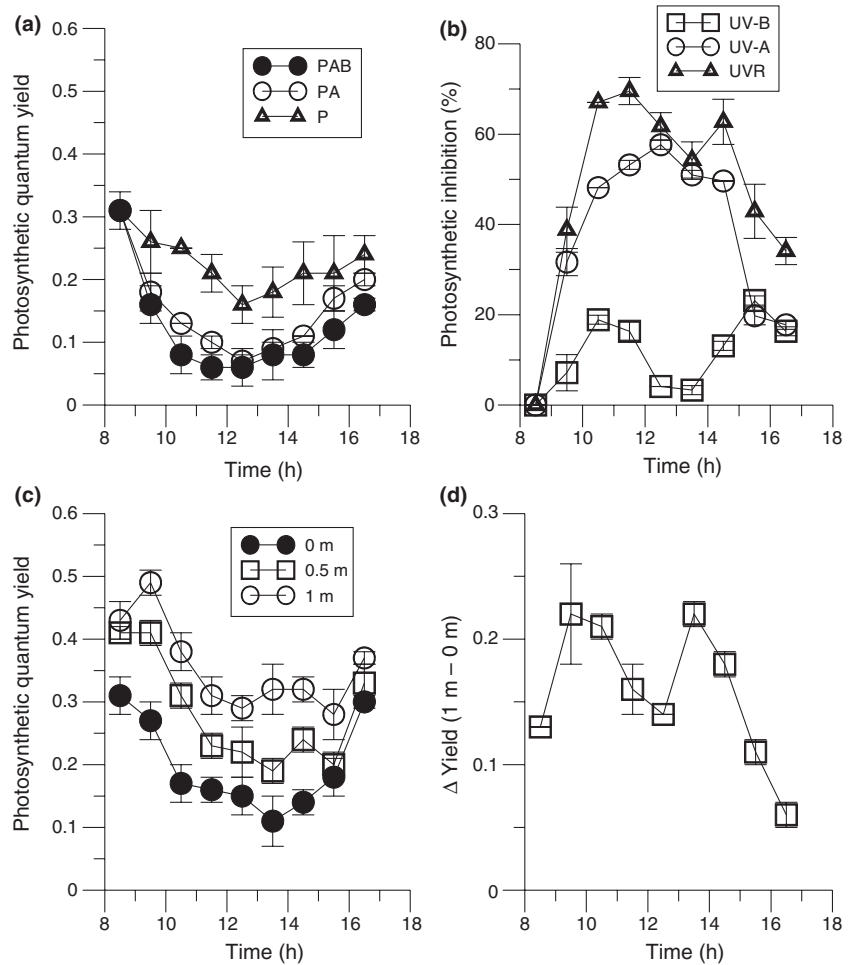


Fig. 4 Daily variations in photosynthetic parameters and inhibition during stratified condition (8 April 2005). (a) Photosynthetic quantum yield (Y) in the surface static samples exposed to three radiation treatments PAB (280–700 nm), PA (315–700 nm) and P (400–700 nm); (b) Photosynthetic inhibition (%) due to UV-B, UV-A and UVR in static samples exposed to maximum radiation at surface waters; (c) Photosynthetic quantum yield (Y) of *in situ* samples collected at 0, 0.5 and 1-m depth of the water column; (d) Difference in the photosynthetic quantum yield ($\Delta Y_{(1-0m)}$) between samples collected at 1 and 0-m depth. The data represent the mean (symbols) and SD (vertical lines) of duplicate samples collected on this representative experimental day.

In the static samples there was an overall decrease in the photosynthetic quantum yield (Y) with increased irradiance (Fig. 6a), the data being well described by a power function ($R^2 > 0.85$, $P < 0.05$). There were no significant differences in Y between samples under the PA or PAB treatments. At irradiances $> 50 \text{ W m}^{-2}$, Y in the P treatment was significantly higher than that under PA and PAB. If we consider data from the *in situ* samples taken from the surface only the mean Y values were significantly lower during stratified than during mixed conditions (Fig. 6b). These *in situ* Y data have been added to Fig. 6a for the purposes of comparison, where it is evident that Y values at 0 m, when the lake was stratified, were similar to those in the static samples under the P treatment. On the other hand, during mixed conditions, *in situ* Y values were higher than those in the static samples in any of the radiation treatments.

Temporal variations in mean Y in static samples for the P and PAB treatments showed that, as expected, values were generally low around noon, but higher early in the morning and/or late in the afternoon (Fig. 7). We used the morning data to fit a power function ($R^2 > 0.9$, $P < 0.05$) and, from that, to calculate the rate of change of Y (i.e. the first derivative $\delta Y / \delta t$). The calculated $\delta Y / \delta t$ in the PAB treatment was 0.07 Y h^{-1} . As the *in situ* samples received full solar radiation (as did the PAB treatment for the static samples) we used that value and, together with those of $\Delta Y_{(1-0m)}$ (i.e. data from Figs 4d & 5d) to calculate the mixing speed for this depth interval. Essentially, we calculated how much time was needed to keep the observed $\Delta Y_{(0-1m)}$ with the $\delta Y / \delta t$ determined in the static sample. For example, if $\Delta Y_{(1-0m)}$ was 0.018 during the mixed condition this time would be 15.4 min and so, the estimated mixing speed would be 6.5 cm min^{-1} . Similarly, if $\Delta Y_{(1-0m)}$

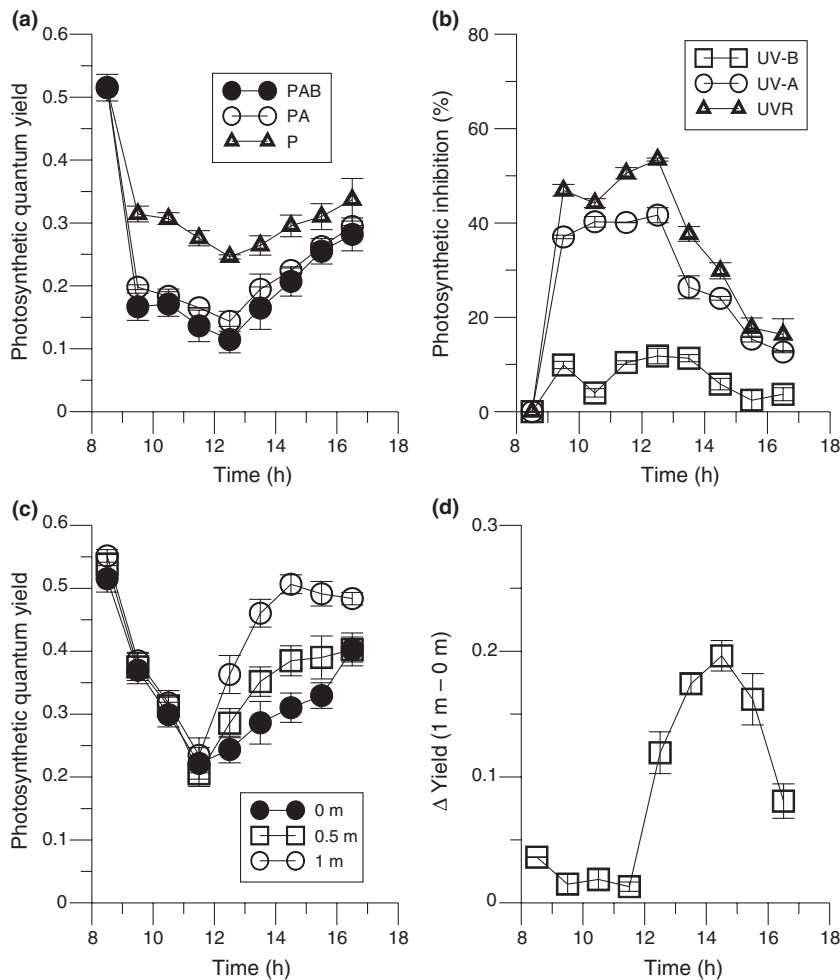


Fig. 5 Daily variations in photosynthetic parameters and inhibition during mixed condition (15 April 2005). (a) Photosynthetic quantum yield (Y) in the surface static samples exposed to three radiation treatments PAB (280–700 nm), PA (315–700 nm) and P (400–700 nm); (b) Photosynthetic inhibition (%) due to UV-B, UV-A and UVR in static samples exposed to maximum radiation at surface waters; (c) Photosynthetic quantum yield (Y) of *in situ* samples collected at 0, 0.5 and 1-m depth of the water column; (d) Difference in the photosynthetic quantum yield ($\Delta Y_{(1-0m)}$) between samples collected at 1 and 0-m depth. The data represent the mean (symbols) and SD (vertical lines) of duplicate samples collected on this representative experimental day.

was 0.22 during the stratified condition, mixing speed would be 0.53 cm min^{-1} . Similar calculations could have been performed with data from any specific day, but the calculation of $\delta Y / \delta t$ was based on the static samples as most of the variability in Y was due to solar irradiance and time.

Discussion

Previous research has demonstrated that vertical mixing is a very important ecological variable in the aquatic environment as it exposes cells to a variable radiation field (Neale *et al.*, 2003). In the phytoplankton, these fluctuations can affect their photoacclimation (Falkowski & Wirick, 1981; Cullen & Lewis, 1988) as seen in variations in P–E parameters, fluorescence yield or cellular chemical composition (Marra, 1978; Denman & Gargett, 1983; Cullen & Lewis, 1988). Although these studies have given new insights about

the importance of fluctuating PAR for phytoplankton photosynthesis, current knowledge on the role of variable UVR is relatively poor. Moreover, results indicate that it is not possible to generalise the contribution of fluctuating PAR and UVR (as produced by vertical mixing) in inhibiting/enhancing phytoplankton photosynthesis. Variables associated with mixing (e.g. depth and speed) seem to act either synergistically or antagonistically with UVR, depending on species composition and on environmental conditions, thus leading to different responses such as increasing or decreasing carbon fixation (Helbling *et al.*, 1994; Neale *et al.*, 1998b; Barbieri *et al.*, 2002; Helbling *et al.*, 2003). Our data show that mixing does play an important role by modulating UVR-induced photoinhibition, as seen in the different response of phytoplankton when exposed to solar radiation under stratified/mixed conditions within the epilimnion at Lake Pipino (Figs 4 & 5).

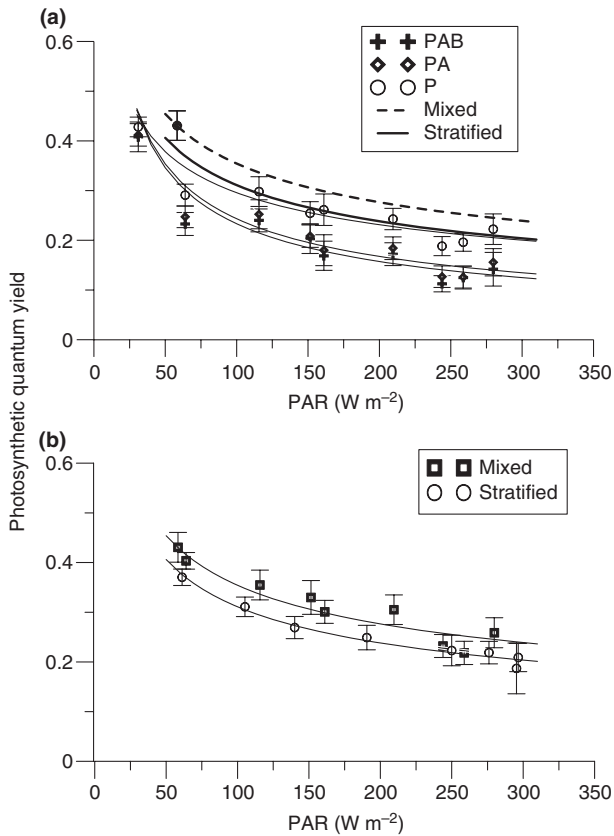


Fig. 6 Mean photosynthetic quantum yield as a function of PAR irradiance (in $W\ m^{-2}$); the fitted lines are power functions ($Y = Ax^B$, $R^2 > 0.85$, $P < 0.05$, full explanation in the text). (a) Static samples (thin lines) exposed to three radiation treatments: PAB (280–700 nm), PA (315–700 nm) and P (400–700 nm) at the surface. For comparison the data from *in situ* samples collected at 0 m in stratified and mixed conditions are presented. The B coefficients of the power functions were -0.3552 , -0.5388 and -0.5685 for stratified conditions under P, PA and PAB treatments, respectively. (b) *In situ* samples collected at 0 m in mixed and stratified conditions. B coefficients of the power functions were -0.3579 and -0.3848 for mixed and stratified conditions, respectively. The data represent the mean (symbols) and SD (vertical lines) of all the data obtained during the experiments.

As a tropical lake, Lake Pipino received relatively high radiation during the late winter to mid-spring (Fig. 1). Nevertheless, maximal irradiance was rather similar to that found in summer in temperate latitudes of Patagonia (Villafañe, Barbieri & Helbling, 2004). In tropical China, however, the ratios UV-B or UV-A to PAR (0.0058 and 0.18, respectively) were higher than those determined at mid-latitudes at comparable times of the year (0.0044 and 0.15, respectively) (Helbling *et al.*, 2005). From an ecological point of view, these energy ratios are very important as they

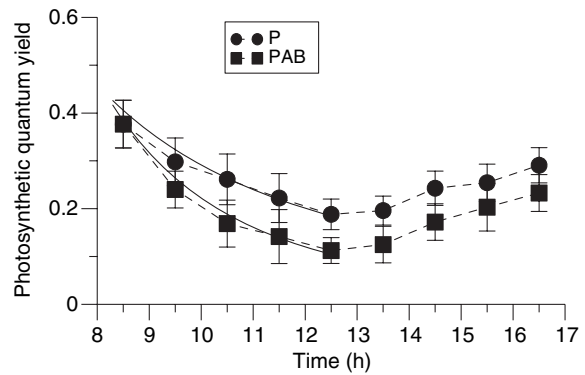


Fig. 7 Photosynthetic quantum yield as a function of time in static samples under P (400–700 nm) and PAB (280–700 nm) treatments at surface waters. The solid lines represent the best fit (power function, $R^2 > 0.9$, $P < 0.05$). The points represent the mean (symbols) and SD (vertical lines) of all the data obtained during the experiments.

determine the balance between damage/effect and repair (Buma, Boelen & Jeffrey, 2003) and thus they are clearly involved in the assessment of the overall impact of UVR on organisms. Moreover, and based on the attenuation coefficients of PAR, UV-A and UV-B (0.96, 2.68 and $2.96\ m^{-1}$, respectively, Fig. 2), Lake Pipino can be considered as relatively ‘clear’ because it provides a well illuminated environment for cells (especially as the euphotic depth exceeds the lake depth). In addition, the depth of 1% incident UV-B and UV-A would be at 1.55 and 1.72 m, respectively, suggesting that UVR might be important in the upper half or two-thirds of the water column. On a global scale, however, the transparency of Lake Pipino is only intermediate. Thus, extreme K_{d-PAR} values of 5.21 and $0.08\ m^{-1}$ have been determined, for instance, in turbid American lakes and clear lakes in the Tyrolian Alps, respectively (Morris *et al.*, 1995; Laurion *et al.*, 2000).

Our study focussed on the effects of solar radiation on phytoplankton photosynthesis and specifically on the reduction of photosynthetic rates under high (PAR and UVR) radiation levels (Osmond, 1994). Such photoinhibition is rather common in aquatic organisms and has been well documented in macroalgae and in phytoplankton inhabiting a wide variety of aquatic environments globally (see review by Villafañe *et al.*, 2003). There are, however, a wide range of responses, depending on the radiation climate, specific sensitivity and acclimation capacity. When addressing UVR-induced photoinhibition on phytoplankton,

however, it seems that tropical species are more resistant than those inhabiting higher latitudes (Helbling *et al.*, 1992; Villafañe *et al.*, 2003). In our study of Lake Pipino we demonstrate further that the overall impact of UVR on phytoplankton photosynthesis is associated with the particular characteristics of the environment under study, and in this case with the mixing conditions. This photosynthetic response has previously been determined in a freshwater reservoir in Australia (Oliver *et al.*, 2003), although in that study the authors addressed only the impact of visible radiation.

One of the most striking features of our data was that, under mixed condition, the *in situ* Y at the surface was approximately 20% higher than in any of the static samples (Figs 4–6). There were however, no significant differences between the *in situ* Y at the surface when the lake was stratified and that in the P treatment of static samples (Fig. 6a). Thus, under stratified conditions, no overall impact of UVR can be determined when compared with the static samples. However, vertical mixing not only counteracted the impact of UVR but also resulted in higher photosynthetic efficiency at all irradiances (Fig. 6). Part of this response of the *in situ* samples might be due to a replacement of part of the population: it is evident that mixing is important, not only by bringing stressed cells from the surface to deeper water where active repair can take place, but also by bringing non-stressed (or less stressed) cells to the surface and thus increasing the observed Y value. Similar 'diluting' effects have been observed for UVR-induced DNA damage in phytoplankton (Helbling *et al.*, 2001, 2006a). This replacement of part of the phytoplankton population might also have occurred during stratified conditions (Fig. 4), where a significant gradient of Y with depth was observed for the *in situ* samples. Based on our data, however, it seems that under these conditions any effect of replacement was slight, as a high $\Delta Y_{(1-0m)}$ was maintained (Fig. 4d).

Another interesting point is how fast and to what extent cells recovered during the afternoon and at night. In both, static and *in situ* samples, there was a recovery in Y during the afternoon, although in most of the cases it was not complete (Figs 4 & 5). Independently of the mixing rate, however, our data suggest that recovery was complete during the night except for some days (e.g. Fig. 4a,c). On the one hand,

under the stratified condition (Fig. 4) there were relatively low Y values (between 0.3 and 0.43) and there were significant differences between surface and depth (Fig. 4c) suggesting chronic damage that was not repaired during the previous night. This might have been due to cells that had been continuously exposed to relatively high solar radiation under stratified condition for a longer period time (i.e. several days of calm, stratified conditions) than our sampling span, and thus, because of the previous light history, were not able to cope with the damage. On the other hand, full recovery during the previous night was observed for the mixed condition (Fig. 5) as our samples collected early in the morning had high Y values and there were no significant differences in Y among depths (Fig. 5c). Again, 'dilution' effects, as discussed above, might also have accounted for part of the observed variability.

One of the critical points when assessing the combined effects of UVR and mixing is to determine how fast phytoplankton cells are moving within the epilimnion/upper mixed layer. There have been several attempts to assess mixing rates (Denman & Gargett, 1983; Scully, Vincent & Lean, 2000; Steffen & D'asaro, 2001). Some of them included the use of tracers to estimate physical variables. For example, Scully *et al.* (1998) determined vertical eddy diffusion coefficients based on hydrogen peroxide measurements. Here, we presented an alternative and simple approach to estimate mixing speed of phytoplankton circulating within the epilimnion, based on fluorescence measurements. We estimated mixing speed based on changes in Y , so physically we mean that we are obtaining a mixing rate based on a rate of change of Y (considering both static and *in situ* samples). In this way, we are not only taking into account the incident solar radiation but also the attenuation of the radiation in the water column (*in situ* samples). The circulation rates estimated with our approach ranged between 0.53 and 6.5 cm min⁻¹ for the two conditions observed. These rates are much lower than the 4 min needed for a displacement of 4 m estimated by Köhler *et al.* (2001) and between the time (minutes–hours) needed for a displacement of 10 m estimated by Denman & Gargett (1983).

Overall, the data presented in our study strongly support the fact that the prevalent mixing regime, in terms of both the extent and intensity, is an important variable that needs to be considered in assessing the

overall impact of UVR on phytoplankton. In addition, our data support earlier findings (Helbling *et al.*, 2003) in which it was demonstrated that vertical mixing in this tropical area enhances photosynthesis, in contrast to others sites where it limits photosynthesis (Helbling *et al.*, 1994; Neale *et al.*, 1998b). If our findings also apply to other tropical areas that normally receive relatively high solar radiation, this might result in greater carbon uptake than has been reported from static incubations, as is normally made from ships or in coastal areas.

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