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Photochemical activity of PSII of tropical phytoplankton communities of Southern Brazil exposed to solar radiation and nutrient addition

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ABSTRACT

We carried out short- and mid-term incubations to assess the combined impact of solar ultraviolet radiation (UVR, 280–400 nm) and nutrient addition on tropical phytoplankton communities from Babitonga Bay, Southern Brazil. Samples were collected at a station ~10 km inward from the mouth i.e., Inlet, and incubated in microcosms under solar radiation at the surface of the water, with or without UVR, and under two nutrient conditions (ambient and with addition of macronutrients). Nutrient addition had the largest impact on specific growth rates, almost doubling the growth of algae as compared to ambient conditions. UVR-induced inhibition of the effective photochemical quantum yield (Y) at the initial conditions was higher at ambient nutrient conditions than in samples with nutrient addition, demonstrating the antagonistic effect among the two factors. Inhibition (k) and recovery (r) rates of Y at the beginning of the experiment suggest a high sensitivity of this community, but they were reduced after 4 days of exposure in the microcosms, hinting for the presence of acclimation mechanisms. Also, differences in taxonomic composition, with increasing dominance of diatoms in nutrient-enriched cultures, as compared to the initial time and in ambient nutrient conditions (with higher proportion of flagellates) may account for the observed variability in responses along the time as well as within treatments.

1. Introduction

Solar ultraviolet radiation (UVR, 280–400 nm) is a natural stress factor that affects negatively metabolic processes (e.g., growth, photosynthetic and calcification rates) as well as cellular targets (e.g., DNA molecule, fatty acids, proteins) of phytoplankton organisms (Häder et al., 2011). Because of the potentially larger impacts of enhanced UV-B (280–315 nm) due to reduced ozone concentrations (i.e., the so called ozone "hole"), a vast amount of literature has been produced about the effects of UVR on phytoplankton from polar waters. Comparatively, less studies assessing UVR effects have been performed in tropical waters, partially because it is generally assumed that these species are already adapted to high radiation levels and therefore they are less sensitive to damaging wavelengths (Helbling et al., 1992). However, a range of responses were observed when evaluating a particular process such as photosynthesis in phytoplankton from the tropical Southern China Sea (Gao et al., 2007; Helbling et al., 2003; Li and Gao, 2013; Li et al., 2011). Studies about UVR effects on natural phytoplankton communities of the tropical Atlantic Ocean are particularly scarce: Although low UV-B-induced damage on the DNA molecule was determined in picophytoplankton communities from the Caribbean Sea (Boelen et al., 2000), UVR caused significant death in this size fraction of phytoplankton (Llabrés and Agustí, 2006). Furthermore, photoinhibition and inactivation of the D1 protein (which has a key role in the photosystem II (PSII) repair cycle) due to ambient levels of UV-B were determined in phytoplankton communities off Southern Brazil (Bouchard et al., 2005; Longhi et al., 2006). It is clear thus that a low sensitivity of tropical species towards stress factors such as UVR cannot be assumed as a general rule as this seems to depend on various factors.

The overall impact of UVR on cellular targets/organisms is rather difficult to predict, due to the interactions of UVR with other biotic and abiotic factors. Several studies have demonstrated the synergistic or antagonistic nature of such interactions (Dunne, 2010; Folt et al., 1999) which tend to amplify or counteract the single effects of UVR on phytoplankton organisms. For example, increased temperature, as expected in a scenario of global change, has been found, in general, to act antagonistically with UVR, reducing its negative effects on photosynthesis (Halac et al., 2010; Helbling et al., 2011; Sobrino and Neale, 2007). However, the combination of UVR and increased temperature affected the community structure, especially by reducing the growth and biomass of some diatoms in samples collected from the Caribbean Sea (Halac et al., 2013). The addition of nutrients generally benefited photosynthetic performance, as seen in studies carried out with different phytoplankton species of Patagonian waters (Marcoval et al., 2007) as well as

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with communities from eastern Canada (Bouchard et al., 2008). However, it did not counteract UV-B-induced photoinhibition in tropical communities of Southern Brazil (Longhi et al., 2006). The interactions of UVR with mixing conditions of the water column were found to be rather complex: For example, in studies carried out in high mountain lakes of Southern Europe, it was determined that under ambient nutrient conditions there was a synergistic effect between vertical mixing and UVR, increasing phytoplankton photosynthetic inhibition and excretion of organic carbon from opaque lakes as compared to algae that received constant mean irradiance within the epilimnion. However, in clear lakes antagonistic effects were determined, with mixing partially counteracting the negative effects of UVR (Helbling et al., 2013).

Considering the relatively few photobiological studies performed in the tropics, it is thus obvious the need to gain more knowledge about the responses of natural phytoplankton communities, especially in a context of global change, where variables such as solar radiation and input of nutrients are expected to increase (Häder et al., 2011). Thus we carried out experiments to assess the combined effects of solar UVR and nutrient addition on photochemical performance of phytoplankton communities collected in the estuarine area of Babitonga Bay, Southern Brazil, at the beginning and after four days of acclimation to the experimental conditions. Based on previous studies carried out in Southern Brazil (Longhi et al., 2006) in which no effects of nutrient addition on UVR-induced photoinhibition were determined, and considering the high concentration of nutrients in the area of Babitonga Bay (Cremer et al., 2006) and the rather low penetration of solar radiation in the water column (Helbling et al., unpub. data), we hypothesized that nutrient addition will not counteract the potential negative effects of solar UVR on the photochemical activity of phytoplankton. To test this hypothesis, we conducted short- and long-term experiments using microcosms, exposing natural phytoplankton communities from Babitonga Bay to solar radiation under two quality radiation conditions and two nutrient concentrations. It is important to consider the connotations of this type of work: Babitonga Bay sustains important secondary production i.e., fishes of commercial value such as *Stellifer rastrifer* (Jordan), *Cathorops spixii* (Agassiz), *Stellifer stellifer* (Bloch), *Pellona harroweri* (Fowler) and *Centropomus parallelus* Poey, among others (Corrêa et al., 2006) thus any effect on phytoplankton might have repercussions on the local fishery economy.

2. Material and methods

2.1. Study site

The study was carried out during the period March 15–20 (Julian days 74–79), 2011, with phytoplankton communities collected from Babitonga Bay, at a station in front of San Francisco do Sul (Inlet, 26° 12.5′ S; 48° 36.2′ W — Fig. 1). Babitonga Bay (area of 1567 km^2) is the main estuary of the Santa Catarina State and it contains the largest and most austral mangrove area in South America which constitutes a huge reservoir not only of biodiversity of plants and animals, but also of organic matter that affect the chemistry and optics of water bodies. The bay is generally divided in three parts: The Canal do Linguado, the Palmital River region, and the main body of the bay itself (Cremer et al., 2006). Several studies have been carried out in the area, but those related to phytoplankton have been mainly oriented to the distribution and taxonomy of species (Brandini et al., 2006; Fernandes and Pereira Brandini, 2010; Parizzi, 2013).

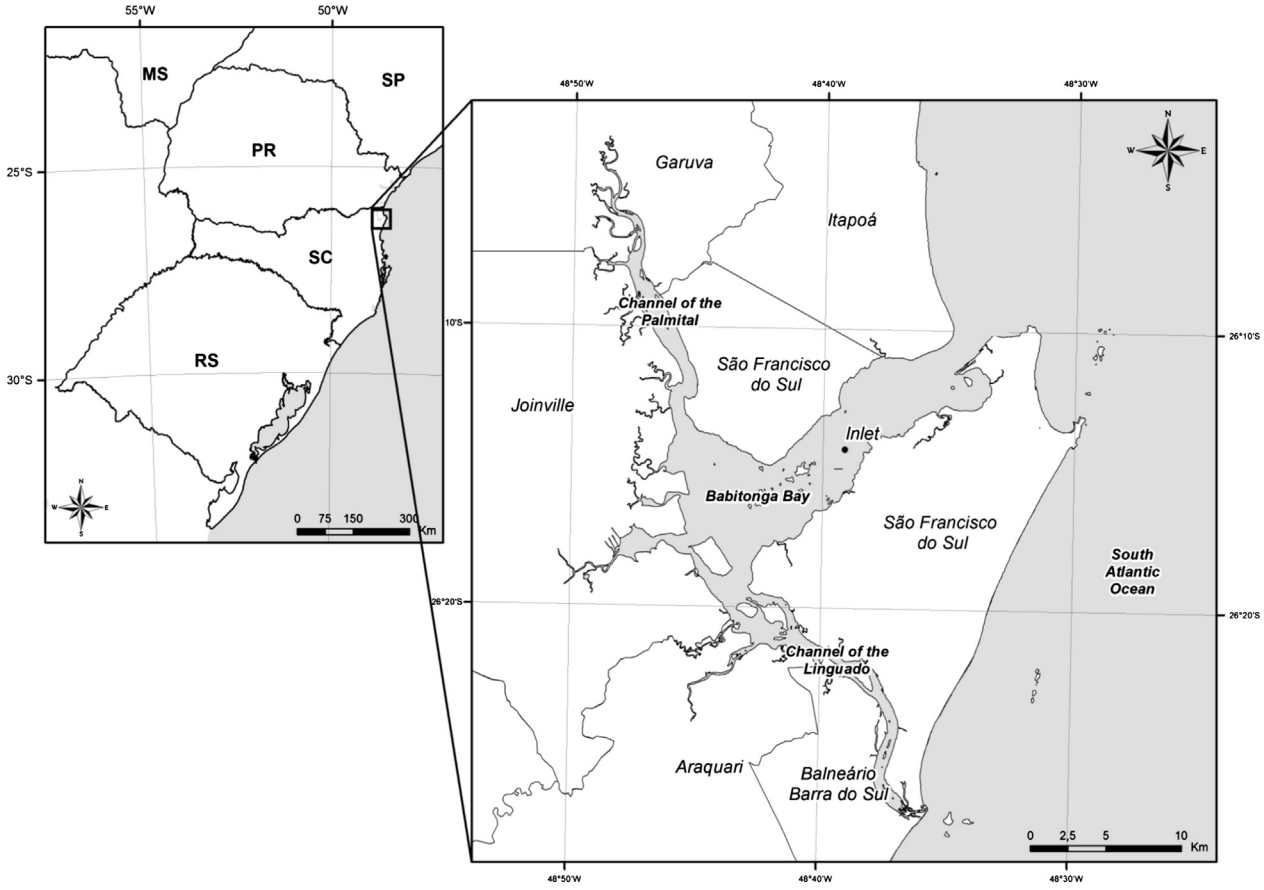

Fig. 1. Map of the study area indicating the sampling site at the Inlet of Babitonga Bay, Santa Catarina (SC), Southern Brazil. The letters are the names of the bordering states: Rio Grande do Sul (RS), Paraná (PR), São Paulo (SP) and Mato Grosso do Sul (MS).

2.2. Experimental set up

The experimental set up consisted of two phases: 1) The acclimation of samples in microcosms for 4 days and, 2) short-term exposure of the initial, as well as of the acclimated samples, to solar radiation, to determine the combined effects of addition of nutrients and solar UVR on photochemical activity.

2.2.1. Phase 1 — acclimation

Surface water samples were collected late in the afternoon from the Inlet of Babitonga Bay using acid-cleaned (1 N HCl) dark containers and immediately taken to the Marina (10 min away from the sampling site) where twelve 300-L microcosm tanks (1 m diameter × 0.4 m depth, with dark walls) were set up. The water samples were pre-screened (500 μm) while filling the microcosms up to about 3/4 of their depth. The microcosms containing the samples were placed floating just above the water to keep them at in situ temperature and to acclimate to solar radiation for 4 days under two radiation treatments (triplicates per radiation treatments). (1) PAB treatment: Samples receiving solar radiation >280 nm, tanks covered with Ultraphan 295 nm filter; and (2) P treatment: Samples receiving solar radiation >400 nm, tanks covered with Ultraphan UV Opak Digefra film. The transmission characteristics of filters and all materials used in this study were previously reported in Villafañe et al. (2003). Two nutrient treatments were implemented (triplicates per treatments): Ambient concentration versus addition of macronutrients (NO_3^-, PO_4^{3-} and SiO_3^{2-}) as in the f/2 medium (Guillard and Ryther, 1962). The water inside the tanks was manually agitated 3 times a day with a paddle. Also, and since the tanks were floating they were gently mixed by the movement of the water in the bay. Samples for experimentation and determinations were taken at the surface of the tanks after the water was thoroughly mixed.

2.2.2. Phase 2 — short term exposures

To determine the potential acclimation and short-term photosynthetic responses, two independent experiments were conducted with samples taken at the initial time, representing the in situ acclimation (day 1) and that after four days (day 4) of exposure to the different radiation and nutrient conditions as explained above. It was impossible, due to the logistics involved in collecting the water and filling the microcosms, to conduct the short-term exposure on the day of collection. However, and as the microcosms were filled during late afternoon, the samples received little or no radiation until day 1.

Experiments to determine the photochemical activity (via chl *a* fluorescence measurements) of phytoplankton to UVR and nutrient addition were conducted as follows: All the samples coming out from each microcosm replica were placed in 50 mL quartz tubes using three radiation conditions: 1) PAB (>280 nm) (as above), 2) PA (>320 nm) (tubes covered with foil Montagefolie, 10155099, Folex), and 3) P (>400 nm) (as above), and exposed for 90 min (short-term exposure) to solar radiation, followed by 90 min of recovery in dim light (ambient laboratory conditions). For each experiment we had a total of 36 tubes with triplicates for 3 radiation treatments (i.e., PAB, PA, and P) × 2 acclimated radiation conditions (i.e., PAB, and P) × 2 nutrient conditions (ambient and addition). The whole set up for each experiment was placed in a water bath for temperature control, with samples maintained at 25 °C i.e., similar as the mean at the surface waters of Babitonga Bay, and exposed to solar radiation. In order to have various data points within the 180 min of experimentation, the time involved for the measurement of Y in all tubes was kept at a minimum.

2.3. Analyses and measurements

2.3.1. Fluorescence parameters

Sub-samples (3 mL) were taken during the short-term exposures (day 1 and 4) to measure every 15–20 min in vivo chlorophyll *a* (chl *a*) fluorescence parameters of the photosystem II, using a portable pulse amplitude modulated (PAM) fluorometer (Walz, model Water-ED PAM). Samples were measured six times immediately after sampling, without any dark-adaptation time. The effective photochemical quantum yield (Y) was calculated using the equations of Genty et al. (1989) and Weis and Berry (1987) as:

$$Y = \Delta F/F'm = (F'm - Ft)/F'm$$

where F′m is the maximum fluorescence induced by a saturating light pulse (ca. 5300 μmol photons m^{-2} s^{-1} in 0.8 s) and Ft is the current steady state fluorescence induced by an actinic light in light-adapted cells.

The rates of UVR-induced damage to the photosynthetic apparatus, estimated via the decrease in the effective photochemical quantum yield during the exposure to solar radiation (k, min^{-1}), and repair in dim light conditions (r, min^{-1}) were calculated by applying an exponential function fit to the data:

$$Y = A * e^{bx}$$

where Y is the yield, A is a constant, b represents either k or r, and x is time.

The non-photochemical quenching (NPQ) of chl *a* fluorescence, used as a proxy of the dissipation of the excess energy, is normally determined by measuring Fm after an acclimation period in darkness and F′m during exposure to radiation, and is calculated as:

$$NPQ = (Fm - F'm)/F'm.$$

Previous studies carried out by our group (Halac et al., 2010) showed that there were no significant differences between NPQ values calculated when Fm was determined for each individual sample and those obtained directly using the PAM fluorometer software. The software stores a Fm value that is then used with every sample, to calculate the NPQ. In this study, we determined a new Fm value and stored it, every time just before any exposure to solar radiation using a few dark-acclimated samples, and thus we routinely used the NPQ data obtained with the PAM software based on these Fm values.

2.3.2. Chlorophyll a and UV-absorbing compounds

Chl *a* concentration was measured at the sampling time as well as on daily basis by filtering 300–1000 mL of sample onto a Whatman GF/F filter (25 mm) and extracting the photosynthetic pigments and UV-absorbing compounds in absolute methanol (Holm-Hansen and Riemann, 1978). A scan between 250 and 750 nm was done using a spectrophotometer (Shimadzu UV-1601PC), and chl *a* concentration was calculated using the equations of Porra (2002). UV-absorbing compounds were estimated by the peak at 337 nm (Helbling et al., 1996).

2.3.3. Enumeration and identification of cells

Samples for the identification and enumeration of phytoplankton were collected at day 1 and at day 4; samples were placed in 50 mL bottles and fixed with buffered formalin (final concentration 0.4% of formaldehyde in the sample). Sub-samples of 10 mL were allowed to settle for 24 h in a Utermöhl chamber (Hydro-Bios GmbH) and species were identified and enumerated using an inverted microscope (Leica model DM IL) following the technique described by Villafañe and Reid (1995).

2.3.4. Specific growth rates

The specific growth rate (μ) was calculated for the experimental period, day 1 to day 4, as:

$$\mu = \ln(N_2/N_1)/(t_2 - t_1)$$

where (N_2 / N_1) is either chl *a* or cell concentration increase during the time period ($t_2 - t_1$).

2.3.5. Solar radiation

Solar radiation was continuously monitored using an European Light Dosimeter Network (ELDONET, Real Time Computers) broadband filter radiometer that measures UV-B (280–315 nm), UV-A (315–400 nm), and PAR (400–700 nm) every second, averages the data over a 1 min interval, and stores them in a computer (Häder et al., 2007). The penetration of solar radiation in the water column was determined using an Ocean Optics radiometer (HR2000CG UV-NIR) with a 10 m fiber optics and a cosine collector.

2.3.6. Physical and chemical characteristics of the water column

Vertical profiles of temperature and conductivity (and from this, salinity) were determined using a multiparameter probe (HANNA HI 9828, with a HI 769828 sensor). Chemical characteristics of seawater were also measured at the sampling time i.e., phosphate (molybdovanadate method), silicate (silicomolybdate method), nitrate (cadmium reduction method) and nitrite (diazotization method) using kit reagents and a spectrophotometer (Hach Odyssey DR 2500) following the general techniques described in APHA (1992).

2.3.7. Data treatment and statistics

The microcosms for acclimation were done in triplicate for each radiation and nutrient condition. Also, each short-term exposure experiment was done in triplicate for each radiation/nutrient condition; thus mean values and standard deviations are reported.

The normality (by Shapiro–Wilks' W test or Kolgomorov–Smirnov) and homoscedasticity (using Cochran, Hartley & Bartlett or Levene's tests) were checked for each data group before ANOVA application. To determine differences of inhibition (k) or recovery (r) rates due to radiation or nutrient conditions at day 1, a one-way analysis of variance (ANOVA) was used. The same test was used to compare k and r values at day 1 and day 4 within each radiation or nutrient condition. To determine interactions between radiation and nutrient conditions a two-way ANOVA was used, with radiation and nutrients as factors. A comparison of the photochemical responses between dates during the short-term exposure was done using a three way-ANOVA with radiation, nutrients and dates as factors. When significant differences were determined, a post hoc Fisher LSD (Least Significant Difference) test was performed.

A two way-ANOVA was used to determine differences in specific growth rates (μ) due to radiation and nutrient conditions. In the case of Y and NPQ, a repeated measurement ANOVA test was used to determine differences between radiation or nutrient conditions for the different days. Significant differences between the samples exposed to different treatments were established using a 95% confidence limit (Zar, 1999). Due to the multiple potential comparisons between treatments, during the exposure and recovery periods, it was impractical to add symbols to the figures to indicate all possible significances; thus, we mentioned in the text the essential comparisons adding the significance when needed.

3. Results

Solar radiation in the water column as well as reaching the microcosms is shown in Fig. 2. There was a strong attenuation of UVR in the water, with wavelengths <400 nm disappearing in the upper first meters of the water column (Fig. 2A); PAR penetrated deeper, and had an attenuation coefficient (k_{PAR}) > 2.5 m^{-1}. UVR and PAR irradiances in the microcosms (Fig. 2B and C) were variable among days, reaching maximal values of ca. 70 and 500 W m^{-2} for UVR and PAR, respectively. The mean daily irradiances during the experimental period (i.e., total duration of exposure of microcosms) for UV-B, UV-A, and PAR were 0.7, 25.3 and 184 W m^{-2}, respectively. The general conditions during the experiment period were cloudy, although the third day of experimentation was cloudless for most of the time.

Depth profiles of the initial conditions of temperature, salinity and chl a in the water column are presented in Fig. 3. Temperature

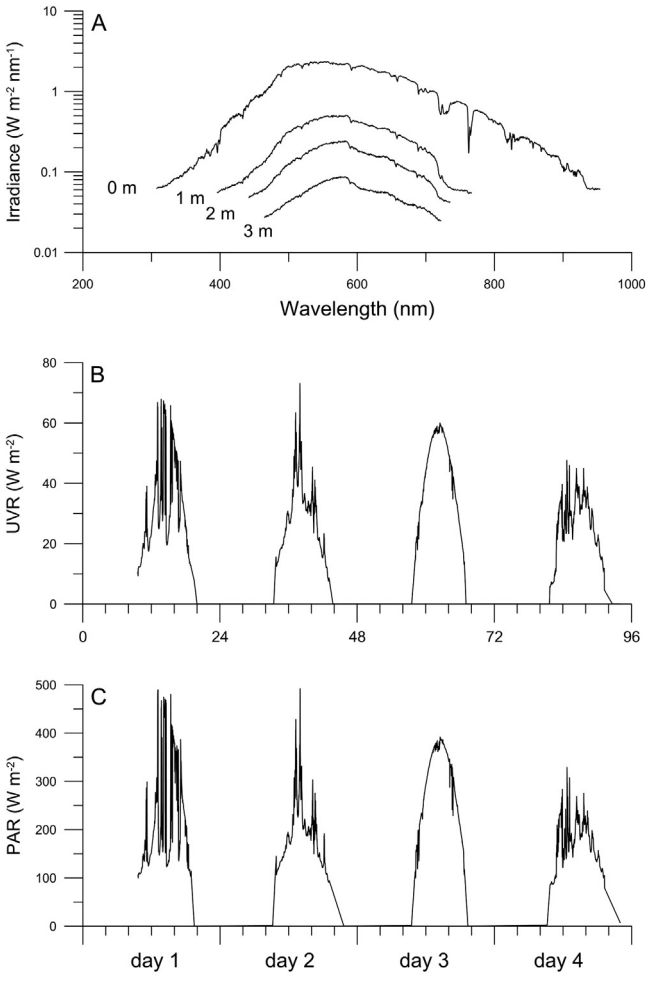

Fig. 2. Underwater spectra of downwelling irradiance (in W m^{-2} nm^{-1}) as a function of depth at the Inlet (A). Surface solar radiation (in W m^{-2}) for UVR, 280–400 nm (B), and for PAR, 400–700 nm (C) over Babitonga Bay during the exposure period (March 16–19, 2011).

presented small changes with depth, with a surface value of 25.3 °C and 25.1 °C at 10 m depth (Fig. 3A). There was an increase in salinity from its surface value of 26.5 to 29 at 10 m depth, evidencing the surface riverine influence within the estuary. Chl a concentration in the water column showed a constant increase with depth (Fig. 3B) from its surface value of 1.9 μg chl a L^{-1} to 11 μg chl a L^{-1} at 14 m. Initial surface nutrient concentrations were high, with values of 17.6, 1.12, and 15 μM for nitrate, phosphate, and silicate, respectively.

The growth rate (μ) values agreed when based on chl a or cell numbers, with significantly higher values ($p < 0.05$) in samples that received additional nutrients as compared to those under ambient nutrient concentrations. The μ values for samples at ambient nutrient concentration were 0.60 d^{-1} (SD = 0.14) and 0.76 d^{-1} (SD = 0.18) for microcosms under the PAB and P treatments, respectively; when nutrients were added, μ was the same in both radiation treatments i.e., 1.25 d^{-1} (SD = 0.01).

Abundance and taxonomic composition of the phytoplankton community are shown in Fig. 4. Total initial phytoplankton abundance was ~5000 cells mL^{-1} but it increased during exposure in the microcosms, reaching higher values (ca. 100 and 700 × 10^3 cell mL^{-1}, for ambient and addition of nutrients, respectively) at the end of the experiment (Fig. 4A), with significant differences ($p < 0.05$) among nutrient, but not among radiation conditions. This pattern was also observed among the principal taxonomic groups i.e., no significant differences were determined at the end of the experiment in both diatoms and unidentified flagellates among radiation treatments, but abundance of

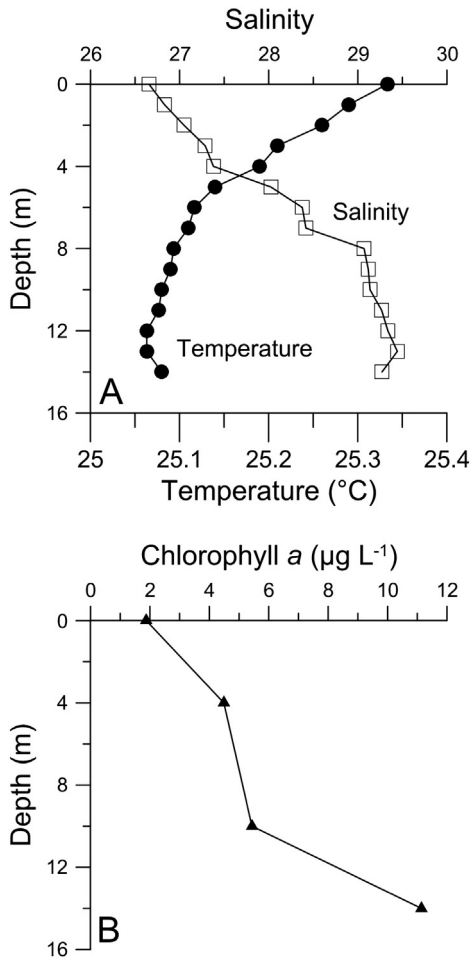

Fig. 3. Water column structure and chlorophyll *a* distribution as a function of depth. (A) Salinity and temperature (in °C) and, (B) chlorophyll *a* (in µg L^{-1}).

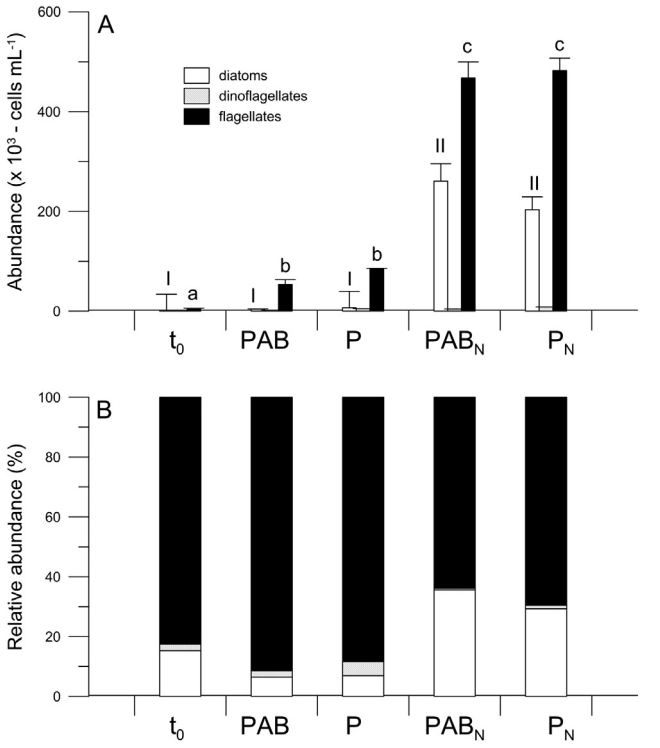

Fig. 4. Abundances, in cells × 10^3 mL^{-1} (A) and percentage of cells (B) from the different taxonomic groups – diatoms, dinoflagellates, and flagellates – at the sampling time (t_0) and at day 4, when samples were acclimated to different radiation (PAB — (PAR + UV-A + UV-B) and P — (PAR)) and nutrient conditions (ambient and addition — N). The letters and roman number on top of the bars indicate significant differences ($p < 0.05$) as a function of the treatments within the same taxonomic group. The vertical lines on top of the bars indicate the standard deviation.

these two groups was significantly higher under nutrient enriched than under ambient conditions. Overall, and in regard to the relative contribution of each of the main taxonomic group present in the community, it was determined that unidentified flagellates (<10 µm) dominated at day 1 (~81%, Fig. 4B). At day 4 unidentified flagellates still dominated in all treatments; however, their contribution was lower in treatments with additional nutrients, in which small *Thalassiosira* spp. dominated the diatom community in the P treatment (ca. 80%) whereas in the PAB they co-dominated with *Skeletonema* sp. Other diatoms species were present in the samples, but their contribution was much lower e.g., *Asterionellopsis glacialis*, *Chaetoceros* spp. and *Guinardia* spp. The contribution of dinoflagellates (mostly naked) was very low throughout the duration of the experiment.

The effective photochemical quantum yield of samples during the short-term exposures is shown in Fig. 5. The general response was of a characteristic "U" shape, with the highest values determined at the beginning of exposure, and decreasing under solar radiation; then a partial or total recovery (once the radiation stress had been removed) was observed. On day 1 (Fig. 5A) there was an initial sharp decrease in Y that was more evident in samples at ambient nutrient concentration. With a few exceptions, Y values in samples that received nutrients were significantly higher ($p < 0.05$) than at the corresponding radiation treatment that did not receive them. In addition, and during the exposure period, there were significant differences among radiation treatments in samples at ambient nutrient concentration (Fig. 5A), having higher values those that received only PAR. At day 4 (Fig. 5B and C) the initial decrease in Y was not so sharp as at day 1, and differences among radiation/nutrient treatments were in general not significant; in particular, samples acclimated only to PAR (Fig. 5C) did not present significant differences among treatments throughout the exposure and recovery periods.

The absolute rates of inhibition (k) and recovery (r) of Y (Fig. 6), calculated from the data presented in Fig. 5, were significantly higher at day 1 (Fig. 6A) than at day 4 (Fig. 6B, C and Table 1). At day 1, the absolute k values in samples under ambient nutrient conditions were significantly higher than those with nutrient addition at the corresponding radiation treatment. In addition, there were significant differences among radiation treatments at ambient nutrients, with higher absolute k values in samples under the PAB treatment and lower under the P treatment (Fig. 6A). No significant differences among radiation treatments were determined in the r values in samples with nutrient addition. However, there were differences at ambient nutrient concentration with lower recovery in samples under the P treatment as compared to the PAB. At day 4, absolute k values were still significantly higher in the PAB treatment as compared to the P treatment, especially in the samples acclimated to PAR (Fig. 6C). The r values at day 4 were either significantly higher in samples under the P treatment or there were no significant differences, as in the case of samples at ambient nutrient concentration and acclimated to PAR + UVR (Fig. 6B). The results of the statistical analyses showing probabilities of single and interactive effects of the different factors in k and r are shown in Table 1. Overall, it is seen that factors, considered either separately or in combination, presented significant differences. Moreover, it is concluded that within the time frame of the experiment, radiation and nutrients interacted significantly in an antagonistic manner, so that nutrient addition reduced the inhibition caused by UVR on PSII activity.

To evaluate the potential photoprotective mechanisms against solar radiation, we also investigated the non-photochemical quenching (NPQ) responses occurring throughout the short-term exposures. At

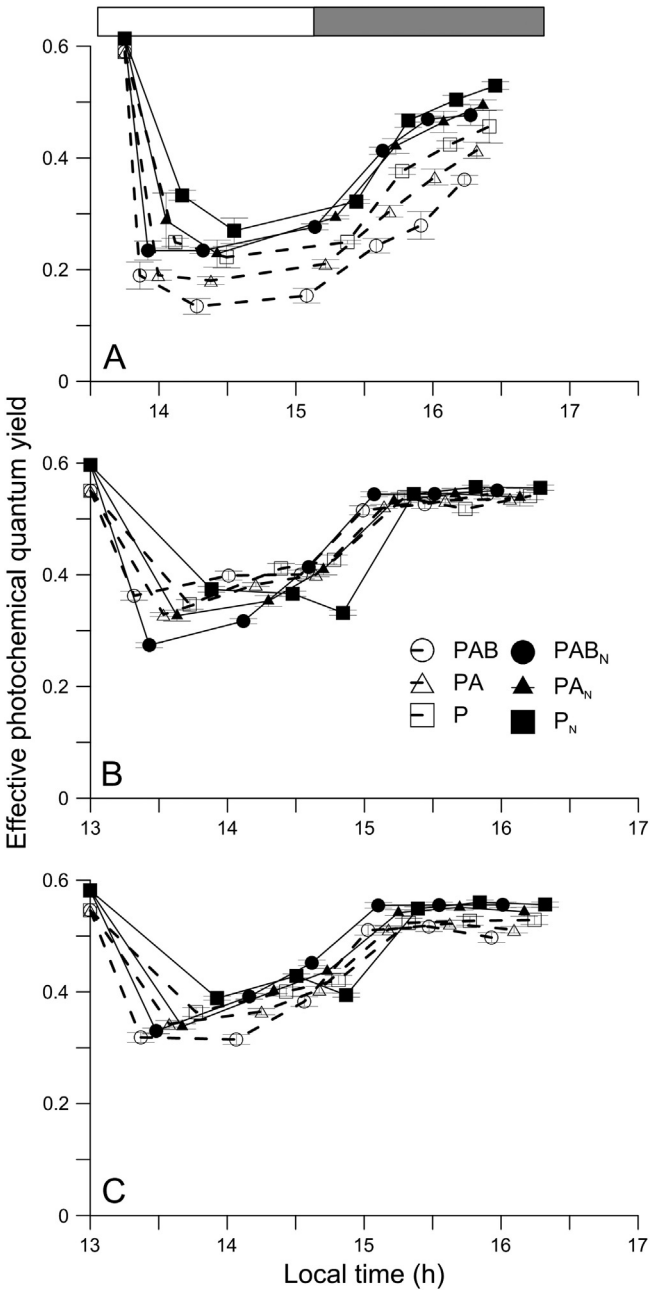

Fig. 5. Mean effective photochemical quantum yield (Y) of phytoplankton communities from Babitonga Bay during 90 minute exposure under solar radiation (horizontal white bar on top) and 90 minute recovery under dim light (horizontal gray bar on top) at day 1 (A) and at day 4 (B and C). Samples were acclimated for 4 days to two radiation treatments — PAB (B) and P (C) and two nutrient conditions: ambient (white symbols) and addition of macronutrients as in the f/2 medium (black symbols), and exposed to three radiation treatments — PAB, PA and P. The vertical lines indicate the standard deviation.

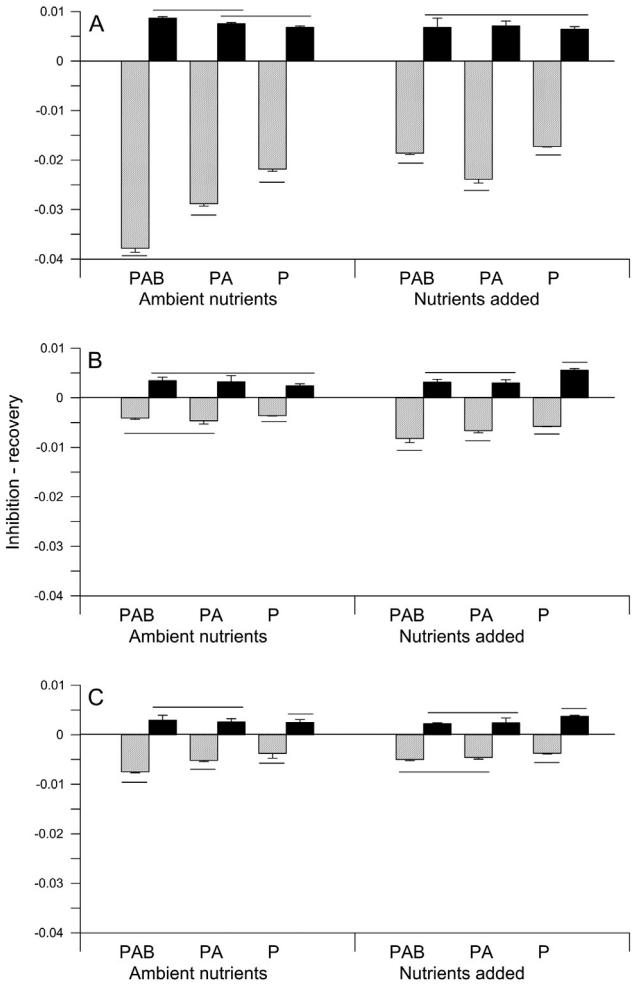

Fig. 6. Inhibition (k) (dashed bars) and recovery (r) (black bars) rates (in min^{-1}) of phytoplankton communities from Babitonga Bay at day 1 (A) and day 4 (B and C). Samples were acclimated for 4 days to two radiation treatments— PAB (B) and P (C) and two nutrient conditions: ambient and addition of macronutrients as in the f/2 medium, and exposed to three radiation treatments— PAB, PA and P. The vertical lines indicate the standard deviation. The horizontal lines on top of the bars connect treatments with no significant differences.

day 1 (Fig. 7), NPQ values were significantly higher ($p < 0.05$) in samples under ambient nutrient concentration as compared to those that received additional nutrients. At day 4, the NPQ values were significantly lower than at day 1 (data not shown), with no dissipation at all in samples acclimated to PAR + UVR, or with little (NPQ < 0.5) but significant dissipation in samples under ambient nutrients and acclimated to PAR only.

4. Discussion

Our studies combined both short- (hours) and mid-term exposures (days) to assess the impact of UVR and nutrient addition on the photochemical responses of the PSII of phytoplankton communities from Babitonga Bay. The rationale behind this experimental set up considered the fact that by performing only short-term exposures phytoplankton communities are not allowed to acclimate to new conditions, and thus, the effects on the first day of experimentation frequently over-estimate the impact of the different variables/stressors under study. Over longer periods of time, however, it is possible to evaluate the responses of cells to the changing environment. These potential

Table 1
Results from statistical analyses showing probabilities of single and interactive effects of the different factors on the rates of inhibition (k) and recovery (r).

Variable / Treatment	Inhibition (k)	Recovery (r)
rad	<0.05	<0.05
nut	<0.05	<0.05
date	<0.05	<0.05
rad * nut	<0.05	<0.05
rad * nut * date	<0.05	<0.05

The labels indicate: rad = radiation (PAB, PA and P treatments); nut = nutrients (ambient vs addition) date refers to the comparison among day 1 and day 4.

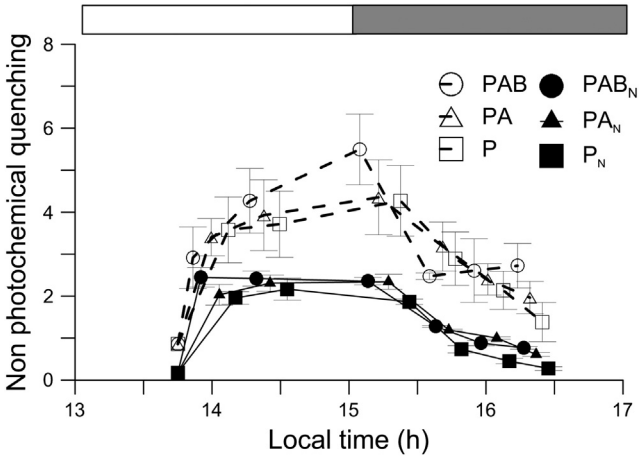

Fig. 7. Mean non-photochemical quenching (NPQ) of phytoplankton communities from the Inlet of Babitonga Bay during 90 minute exposure under solar radiation (horizontal white bar on top) and 90 minute recovery under dim light (horizontal gray bar on top) at day 1. Samples with ambient nutrient conditions (white symbols) and addition of macronutrients as in the f/2 medium (black symbols), and exposed to three radiation treatments— PAB, PA and P. The vertical lines indicate the standard deviation.

changes (which can occur within a time frame of hours) include not only transient thermoclines that might trap phytoplankton cells close to the surface of the water (Neale et al., 2003) and high frequency of clouds, but also wind speed and direction that can affect the circulation within the upper water column as well as the amount of nutrients carried into the water (Bakun, 1990). Furthermore, on a long-term basis, cells might acclimate to the experimental conditions and thus the negative effects of the tested variables are reduced in some degree. In the following paragraphs we will discuss our findings, focusing on the responses of the PSII under the different treatments, as well as on the acclimation processes, which in our study were assessed not only by comparing the differential photochemical responses of the PSII over time, but also through changes in taxonomic composition.

The photochemical activity of samples from communities from Babitonga Bay was severely impaired by exposure to solar radiation: In our study, an important reduction in Y was observed i.e., from an initial value of 0.6 to ca. 0.15 in samples exposed to full solar radiation without nutrients (Fig. 5A). Not only solar radiation levels, which were high (even under cloudy conditions, Fig. 2B and C), but also other factors such as the water transparency (and therefore the previous light history) might have accounted for part of the observed responses. In our case, the rather turbid waters of Babitonga Bay ($k_{PAR} > 2.5\ m^{-1}$) (Fig. 2A) might have resulted in cells with a shade-acclimated light history. Therefore, when these cells were exposed to the high radiation conditions as experienced in our experimental set up under a thin layer of water, they were comparatively more stressed than those that would have a previous light history of higher radiation levels in a clear environment. These responses of phytoplankton communities were also observed in Andean lakes (Villafañe et al., 2004) or in high altitude lakes of Spain (Helbling et al., 2013). However, in our study we determined clear antagonistic effects between UVR and nutrients (Table 1), with higher Y and lower inhibition due to UVR in samples in which nutrients were added. This is in agreement with previous studies that showed that nutrient conditions altered the response of phytoplankton towards UVR stress, with nutrient-depleted samples being more sensitive than those in which nutrients were not limiting (Beardall et al., 2009), as also found in several studies (Bouchard et al., 2008; Marcoval et al., 2007, 2008).

Over the time frame of experimentation, samples acclimated to the new conditions, reflected both as an overall reduction of the inhibition rates (Fig. 6B and C), as well as by changes in the taxonomic composition over time (Fig. 4). The first aspect of acclimation i.e., as seen as the reduction of inhibition rates even under high radiation levels, can be explained by a number of mechanisms that are commonly found in phytoplankton cells subjected to solar radiation stress. One of the ways to acclimate to high light conditions is via the xanthophyll cycle, with a higher xanthophyll/pigment ratio (Van de Poll and Buma, 2009), by the reversible epoxidation/de-epoxidation associated with the dissipation of excess energy which occur in a time lapse of minutes (Finazzi et al., 2006; Horton et al., 1999; Ting and Owens, 1993). In fact, dissipation of excess energy at day 1, evaluated via NPQ was in general higher, for samples at ambient nutrient concentration, a fact that can be associated with the state of the xanthophyll cycle, as was shown for other marine algae (Olaizola et al., 1994; Ruban et al., 2004; Schofield et al., 1998). In contrast, samples that received additional nutrients had lower NPQ, thus dissipated less excess energy. This might reflect a better utilization of solar radiation as it has been shown for the marine diatom *Thalassiosira weissflogii* (Halac et al., 2010; Helbling et al., 2011; van de Poll et al., 2010). In addition to NPQ, we also evaluated the presence of UV-absorbing compounds, which is considered an acclimation mechanism towards solar UVR stress that might help to mitigate the negative effects produced by these wavelengths (Banaszak, 2003). However, we did not find significant amounts of these compounds under the different experimental conditions (data not shown), in contrast to what has been observed in other environments (Ayoub et al., 2012), or even after relatively short periods of exposure to solar radiation (Marcoval et al., 2007).

The second aspect of acclimation refers to the changes in taxonomic composition towards communities better adapted to the new conditions. In fact, we noticed that within the experimental time frame, the taxonomic composition varied with respect to the initial time: While at the beginning of the experiment there was a large proportion of flagellates (Fig. 4B), after 4 days, diatoms increased their abundance only in nutrient enriched samples, taking full advantage of these experimental conditions (Fig. 5B); obviously, these types of changes in species composition are more associated to the nutrient conditions than to the exposure to solar radiation as found in studies carried out in the Patagonian coast (Marcoval et al., 2008). However, these variations in the relative proportions of taxa among the different nutrient/radiation treatments might account for the differential photochemical responses i.e., with better photosynthetic performance in the "new" (acclimated) communities, as seen in other studies carried out in temperate ecosystems (Bouchard et al., 2008).

While similar results as the ones we present here were obtained in other studies (Bouchard et al., 2008; Marcoval et al., 2008), the novelty of our work lies in two aspects: Firstly, we determined that samples from Babitonga Bay, and in contrast to the general idea of the high resistance to UVR of tropical species (Buma et al., 2003; Helbling et al., 1992), cells were indeed severely affected by these wavelengths. Secondly, our results clearly contrasts with a mesocosm study carried out in a nearby site in Southern Brazil (Longhi et al., 2006) in which the authors, working with natural communities exposed to solar UVR, did not find any particular effect of nutrient addition on photochemical performance. Specifically, the results obtained in our study led us to reject our initial hypothesis in which we proposed a general lack of effect of nutrient addition on UVR-induced effects on photosynthesis. This hypothesis was partially based on the fact that the Channel of the Linguado was closed in 1935 and the only connection of the water body with the sea is through the Mouth of the bay, with little renovation of water and high input of waste waters from various sources (Cremer et al., 2006). This resulted in a higher input not only of sediments but also of organic matter from all the nearby cities and industrial complexes around the estuary (Cremer et al., 2006). Thus, and in contrast to what we expected, phytoplankton in the Babitonga Bay does not seem to be exposed to very high levels of nutrients and instead, they might remain in the sediments. Our data, however, indicate that this community of the estuary

is sensitive to UVR and while mixing processes might help bringing up nutrients into the water column, it will also expose shade-acclimated cells to higher irradiances thus further increasing the negative effects of UVR on this phytoplankton community. Further studies dealing with climate change impact on the Babitonga Bay should consider mixing processes as well as the input of nutrients from various sources, and how this might be affecting the aquatic and benthic system in this productive estuary.

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