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Influence of seasonal variables associated with climate change on photochemical diurnal cycles of marine phytoplankton from Patagonia (Argentina)

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Abstract

The effects of temperature and ultraviolet radiation (UVR; 280–400 nm) on seasonal succession in phytoplankton assemblages of Patagonia (Argentina) were studied in the context of global change. Samples collected during pre-bloom, bloom onset, bloom, and spring were exposed to in situ and increased (+4°C) temperatures and solar radiation with and without UVR. Daily cycles of effective photochemical quantum yield exhibited a pattern of high values in the morning, decreasing towards noon, and increasing in the afternoon. The decrease in yields towards noon as the season progressed increased from 30% in the pre-bloom to 80% in the spring; in the latter there were significant differences between radiation treatments under both temperature conditions. The highest inhibition rates were during the bloom, whereas the highest recovery rates were during the spring. Inhibition rates were generally higher in treatments exposed to UVR in comparison to photosynthetically active radiation–only treatments and some stages of the succession exhibited an additional temperature effect. Increasing temperatures had little effect on pre-bloom communities but helped to counteract the magnitude of the yield decrease during the bloom onset. However, during the bloom and in the spring, temperature and UVR acted synergistically, increasing the overall photochemical inhibition. Feedback mechanisms of increased temperatures causing a shallower mixing depth will expose phytoplankton to higher radiation, which will have a negative effect on the bloom and on spring assemblages. Due to the differential effects of solar UVR and increased temperature on phytoplankton, future studies should consider the repercussions on higher trophic levels.

Global climate change is a complex process associated with the increase in atmospheric CO_2 levels since the Industrial Revolution, resulting in variations in pH, temperature, and directly or indirectly exposure to solar radiation, with profound effects on aquatic organisms and ecosystems (Häder et al. 2011). The most obvious consequences for aquatic environments are the acidification of the water and increased sea-surface temperatures (SST) (Häder et al. 2011). An increase in SST, in turn, may result in higher exposure of cells to solar radiation due to the formation of shallower and more stable thermoclines, a phenomenon that could be especially important at midlatitudes (Behrenfeld et al. 2006).

Considered individually, solar radiation, and especially the ultraviolet portion of the spectrum (UVR; 280–400 nm) is largely known to be a stressor for phytoplankton (*see* reviews by Buma et al. 2003; Villafañe et al. 2003). At the organismal level, effects such as decreased growth and photosynthetic rates (Villafañe et al. 2003; Litchman and Neale 2005), changes in morphology (Fiorda Giordanino et al. 2011), as well as damage to vital cell components such as the deoxyribonucleic acid (DNA) molecule, membrane lipids, and proteins (Buma et al. 2003), have been reported due to UVR exposure. At the community level, solar UVR can alter taxonomic composition and size distribution (Lionard et al. 2012), potentially affecting trophodynamics, biogeochemical cycles, and the general functioning of the ecosystem.

In contrast, increased temperature has been shown to be beneficial in terms of phytoplankton cell physiology, e.g., by increasing growth and photosynthesis rates (Eppley 1972). In addition, it has been related to enhanced enzymatic conversion of xanthophyll cycle pigments (Demming-Adams and Adams 1992) and the potential enhancement of ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) activity (Helbling et al. 2011), among other effects. On the other hand, temperature may also change the timing of the spring bloom as well as the community composition, further affecting trophic interactions, as seen in mesocosm experiments carried out on the coast in west Norway (Lassen et al. 2009).

Under natural conditions, however, multiple variables (including stressors) act in a complex way to produce the observed effects on individuals and communities (Folt et al. 1999). Moreover, variables may act in a synergistic or antagonistic manner (Dunne 2010) such that the response does not necessarily represent the sum of the individual effects of each variable. Some studies have evaluated the combined effects of UVR and temperature on phytoplankton photosynthesis and, in general, it has been found that they act antagonistically. For example, higher temperature either reduced the negative effects produced by UVR or promoted damage repair (Sobrino and Neale 2007; Halac et al. 2010; Helbling et al. 2011). In other studies carried

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out with natural communities, however, a spectrum of responses has been documented, ranging from a lack of interaction to different degrees of interaction between these two variables (Lionard et al. 2012). To summarize, it is obvious that the effects are not universal and mostly depend on species-specific responses that include cell size, acclimation capacity of each species, as well as the timing of the experiment, in terms of the growth phase and nutrient status of the cells.

In the Southern Atlantic Ocean, and in particular in the Northern Patagonia area, the effects of solar radiation on phytoplankton photosynthesis of natural communities have been evaluated (Villafañe et al. 2004*a*; Marcoval et al. 2008). These studies have also shown that there is a marked variability in responses that depend on the timing of the annual succession such that winter communities (i.e., blooms) are more sensitive to UVR exposure (i.e., assessed using biological weighting functions) than are pre- and post-bloom communities; however, maximum inhibition due to UVR were observed during high-irradiance periods of spring–summer (Villafañe et al. 2004*a*). The objective of this study was to determine how these different communities would respond to the combination of the different UVR levels that occur naturally throughout the year, and enhanced temperature levels, as may develop under a scenario of global climate change (IPCC 2007). The experimental approach was to expose phytoplankton communities characteristic of different stages of the annual succession (pre-bloom, bloom onset, bloom, and spring) to different combinations of radiation (by filtering out portions of the solar spectrum) and temperature treatments (in situ vs. increased by 4°C) and evaluating their photochemical efficiency during daylight exposures.

Methods

Study area—This study was conducted at Bahía Engaño, Chubut, Argentina (43°18.8′S, 65°02′W), where surface-water samples were collected in 2011 during pre-bloom conditions (six experiments conducted from 28 February to 10 March); bloom onset (seven experiments conducted from 20 May to 04 June); bloom (two experiments conducted from 08 June to 13 June); and during the spring (one experiment conducted on 07 November). The various stages of the annual succession were corroborated not only by historical records, which established that in the area blooms normally start during the months of May–June and can extend for about 2 months until early spring (end of September; Villafañe et al. 2004*a*; Helbling et al. 2005), but also supported by continuous chlorophyll *a* (Chl *a*) measurements and phytoplankton observations. In our study, we refer to the November sampling as the spring assemblage (because the experiment was conducted 5 months after the bloom sampling). However, the species composition and characteristics of this assemblage were similar to that of previously determined post-bloom conditions. The study site is located in close proximity to the Chubut River estuary where the geomorphological, biological, and chemical characteristics have been documented (Villafañe et al. 2004*a*; Helbling et al. 2010). Surface-water samples were collected during high tide from a coastal station (Sta. Egi; Villafañe et al. 2004*a*) using acid-cleaned (1 mol L^{-1} HCl) opaque containers and immediately transported to the Estación de Fotobiología Playa Unión (EFPU; 5 min from the sampling site), where short-term experiments to evaluate the combined effects of solar radiation and temperature on the photochemical efficiency of natural phytoplankton communities were carried out.

Exposures—Samples were placed into 200 mL quartz tubes under three radiation conditions: (1) full solar radiation, with samples receiving photosynthetically active radiation (PAR), ultraviolet A radiation (UV-A), and ultraviolet B radiation (UV-B), PAB treatment, 280–700 nm; (2) solar radiation without UV-B (PAR + UV-A, PA treatment, 320–700 nm) in which the samples were covered with cut-off filter foil (Montagefolie, 10155099, Folex); and (3) solar radiation without UVR (PAR treatment, P treatment, 400–700 nm), where the samples were covered with Ultraphan film (UV Opak, Digefra). For the pre-bloom, bloom onset, and bloom experiments duplicate samples were used for each radiation treatment. For the spring experiment, 5 L UV-transparent bags were used instead of quartz tubes, and triplicate samples were exposed to the PAB and P treatments.

Two temperature treatments were implemented (in situ vs. increased by 4°C ± 1°C). The in situ temperatures for the pre-bloom, bloom onset–bloom, and spring conditions were 17°C, 11°C, and 15°C, respectively. The samples were placed into a thermostatic water bath with two independent circuits to maintain the required experimental temperatures. Exposure to solar radiation lasted the entire daylight period, i.e., from 08:00 to 19:00 h, 10:30 to 15:30 h, and 08:00 to 18:00 h for the pre-bloom, bloom onset–bloom, and spring samples, respectively.

Solar radiation and temperature measurements—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. This instrument, permanently installed on the roof of the EFPU, is calibrated every year using a solar calibration procedure. In the course of the experiments carried out during the bloom period, a hand-held radiometer (ELDONET, Real Time Computers), with broadband channels for UV-B, UV-A, and PAR was used; this instrument was calibrated against the terrestrial unit described above.

Water temperature inside the thermostatic baths was controlled with sensors attached to each temperature channel and adjusted automatically throughout the experimental period. Phytoplankton communities in the study area are normally exposed to a wide range of temperatures, with mean daily values varying from 18°C in mid- to late summer to 6°C in late winter (Helbling et al. 2010). Therefore, the in situ (“control”) temperatures used during the experiments fall within the range expected for each

season. Furthermore, the increased temperature values that were used are within the predicted values for the Patagonia region, where an increase of about 3°C to 3.5°C is expected by the end of the century (IPCC 2007).

Fluorescence measurements—Subsamples (3 mL) were taken hourly (every 2 h in the spring samples) with a syringe to measure the in vivo Chl *a* fluorescence emission using a portable pulse-amplitude modulated fluorometer (Water-ED PAM, Walz). The effective photochemical quantum yield (yield) was calculated using the equations of Genty et al. (1989) as:

$$\text{yield} = \Delta F : F'_m = F'_m - F_t : F'_m$$

where F'_m is the instantaneous maximum intensity of Chl *a* fluorescence in an irradiated cell induced by a saturating white light pulse (~ 5300 μmol photons m^{-2} s^{-1} in 0.8 s) in the presence of a weak actinic light, and F_t the steady-state fluorescence induced by a weak actinic light in light-adapted cells. Each sample was measured six times.

Taxonomic analyses, Chl a, and UV-absorbing compound concentrations—Samples for the identification and enumeration of phytoplankton were placed in 125 mL brown glass bottles and fixed with buffered formalin (final concentration 0.4% of formaldehyde in the sample). Subsamples of 25 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). The biovolumes of the phytoplankton species were estimated by comparing and adjusting their shape to known geometric forms according to Hillebrand et al. (1999) and by measuring the main cell dimensions of at least 10 cells per species. From these biovolumes, biomass (as cellular autotrophic carbon concentration) was estimated using the equations of Strathmann (1967).

Chl *a* concentration was measured by filtering 100 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 Hewlett Packard spectrophotometer (model HP 8453E) 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). Once scanned, the same sample was used to determine the Chl *a* concentration fluorometrically (Holm-Hansen et al. 1965). The fluorometer (Turner Designs model TD 700) is routinely calibrated against spectrophotometric measurements. There were no significant differences between Chl *a* concentration calculated from spectrophotometric and fluorometric techniques; therefore, all the data reported in this article are those obtained with the fluorometer.

Statistical and data analysis—Exposure treatments during the pre-bloom and bloom onset–bloom experiments were carried out in duplicate, while triplicates were used when working with the spring assemblage; thus, the data

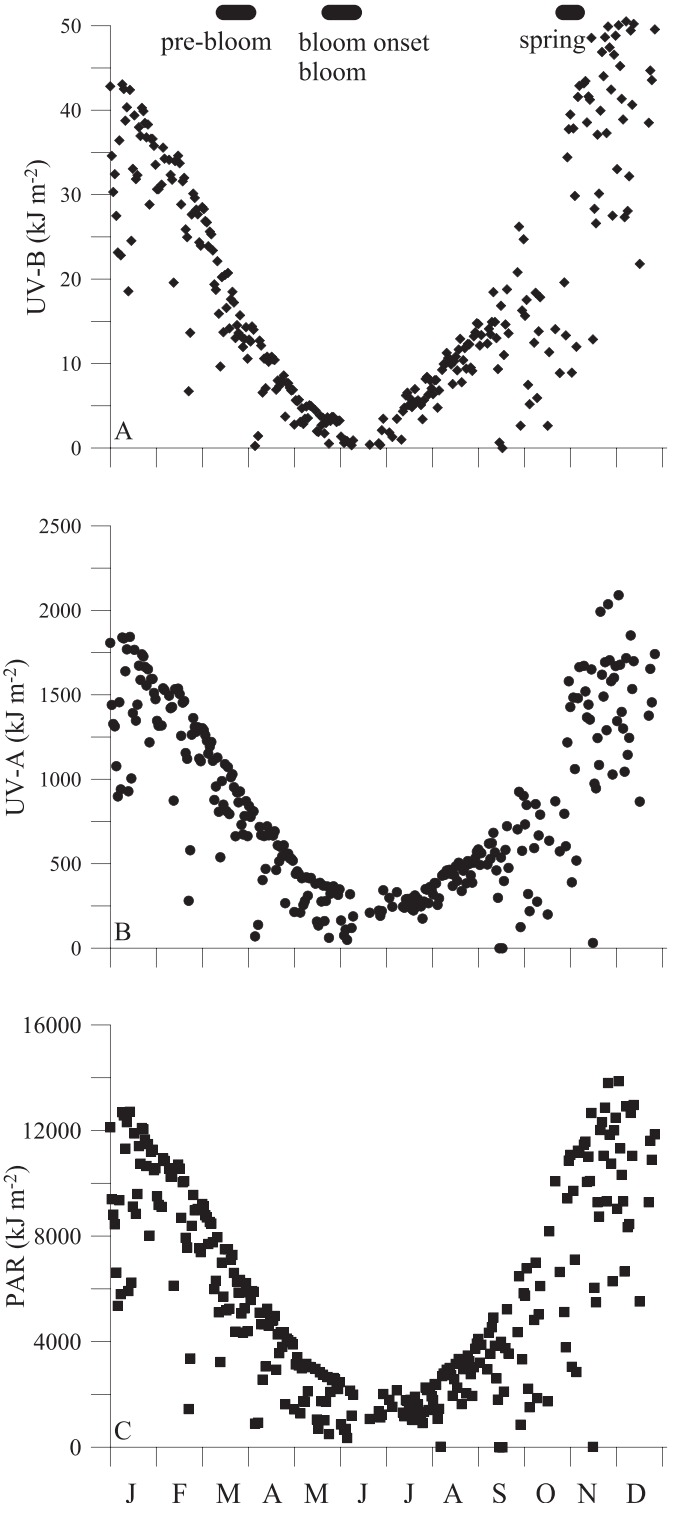
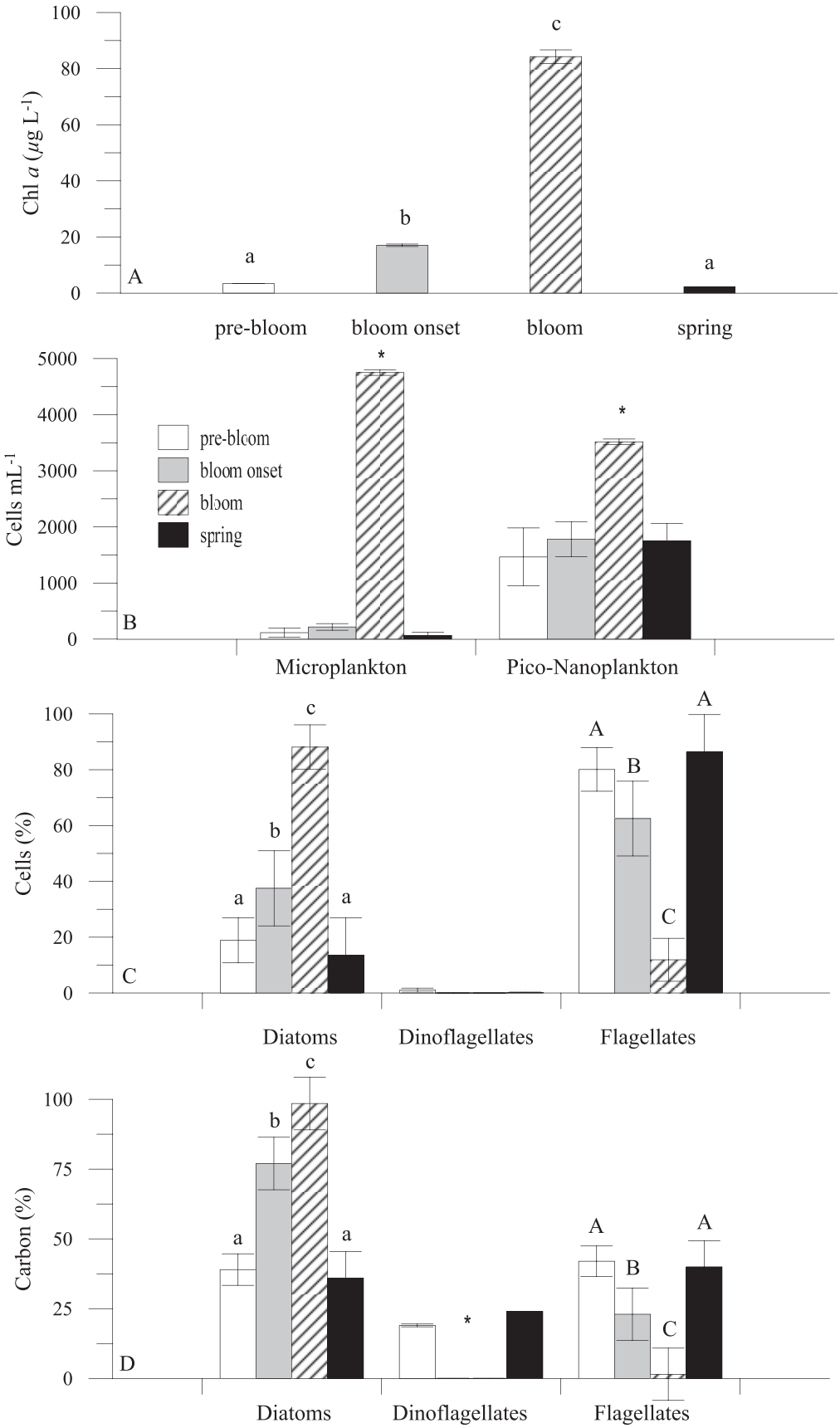


Fig. 1. Daily doses of solar radiation (kJ m^{-2}) for the year 2011 in the study area. (A) UV-B, 280–315 nm; (B) UV-A, 315–400 nm; (C) PAR, 400–700 nm. The pre-bloom experiments were carried out six times between 28 February and 10 March 2011, whereas the bloom onset experiments were conducted seven times between 20 May and 04 June 2011; bloom experiments were done twice between 08 June and 13 June 2011; the spring experiment was done on 07 November 2011.


A
Chl a (μg L-1)
100
80
60
40
20
0
a
b
c
a
pre-bloom
bloom onset
bloom
spring
B
Cells mL-1
5000
4000
3000
2000
1000
0
pre-bloom
bloom onset
bloom
spring
*
*
Microplankton
Pico-Nanoplankton
C
Cells (%)
100
80
60
40
20
0
a
b
c
a
A
B
C
A
Diatoms
Dinoflagellates
Flagellates
D
Carbon (%)
100
75
50
25
0
a
b
c
a
*
A
B
C
A
Diatoms
Dinoflagellates
Flagellates

are reported as mean and either half-mean range or standard deviation, respectively. A two-way analysis of variance (ANOVA) test was used to determine interactions between irradiance and temperature (Zar 1999) on maximum variations of yield (Δyield), or on inhibition and recovery rates. A one-way ANOVA was used to determine if there were significant differences in the effective photochemical quantum yield among radiation treatments within the same temperature treatment. In both cases a 95% confidence limit and 1 degree of freedom were used.

The maximal variations of yield (Δyield) within each radiation treatment and temperature conditions were calculated as the difference between the minimum yield value observed for each treatment and the initial value. Inhibition rates (yield h^{-1}) were calculated during the morning by applying a linear regression fit to the data between the initial time and local noon, while recovery rates (yield h^{-1}) were calculated (also with a regression fit) using the data between the local noon and the last data point measured in the evening.

Results

Solar radiation conditions—Daily doses of PAR, UV-A, and UV-B during the year 2011 are shown in Fig. 1. There was high day-to-day variability due to differences in cloud cover but, nevertheless, there was a clear trend for high values during the summer, decreasing towards the winter, and increasing again in spring–summer. Comparison of the radiation conditions among the experiments reveals that during the pre-bloom phytoplankton received relatively high daily doses, with maximum values of 29 kJ m^{-2}, and 1.3 and 9.2 MJ m^{-2}, for UV-B, UV-A, and PAR, respectively (Fig. 1A–C). The lowest daily doses were found during the bloom experiments, with maximum values of 3.8, 390, and 2800 kJ m^{-2}, for UV-B, UV-A, and PAR, respectively (Fig. 1A–C). The spring samples received the highest daily doses, with maximum values of 48 kJ m^{-2}, and 1.7 and 12.7 MJ m^{-2}, for UV-B, UV-A, and PAR, respectively (Fig. 1A–C). Differences in the ratios of UV-B to PAR were determined with maximum values of 0.0038, 0.0020, and 0.0049 during the pre-bloom, bloom onset-bloom, and spring experiments, respectively. This variability in UV-B : PAR (and also to UV-A) shows that phytoplankton in the Patagonia region receive different proportions of these wavelengths over the course of the year.

Biological characteristics—Phytoplankton abundance was different among the seasons, with the highest Chl *a* concentration, $\sim$ 84 μg Chl *a* L^{-1}, measured during the bloom, and lowest values during the pre-bloom and spring periods (3.4 and 2.3 μg Chl *a* L^{-1}, respectively), whereas during the bloom onset intermediate values of Chl *a* were determined (Fig. 2A). Cell abundances were similar during the pre-bloom, bloom onset, and spring periods with total cell values < 2500 cells mL^{-1}; however, significantly higher values were found during the bloom, i.e., $\sim$ 8300 cells mL^{-1} (Fig. 2B). The pico–nanoplankton fraction (cells $<$ 20 μm in diameter) contributed $> 80\%$ of the total cell abundance during the pre-bloom, bloom onset, and spring. In the pre-bloom and spring samples, this fraction was mostly characterized by unidentified flagellates and small centric and pennate diatoms (Fig. 2C). During the bloom onset, the abundance of small centric diatoms of the genus *Thalassiosira* increased significantly, whereas the relative proportion of flagellates decreased (Fig. 2C). Diatoms dominated in terms of carbon content during this period (Fig. 2D), with large species (e.g., *Odontella aurita*) contributing an important share to the carbon biomass even though their abundance was low. During the bloom, there was a clear dominance of microplankton cells (Fig. 2B), with the community mostly characterized by the diatom *Odontella aurita* (Fig. 2C) that contributed to almost the entire carbon allocation (Fig. 2D). The contribution of dinoflagellates (e.g., small naked species, *Prorocentrum micans*, *Alexandrium tamarense*) was very low at all sampling time points, i.e., $< 1\%$ of total cell abundance.

Photochemical responses—The variations of the effective photochemical quantum yield (yield) of natural phytoplankton communities during the daily exposures are shown in Fig. 3. Similar responses were observed in the incubations conducted within each experimental period to support the division into the four stages of the seasonal succession of phytoplankton. The data exhibited a typical pattern of high yield values during the morning, significantly decreasing towards noon, and increasing again in the afternoon. During the pre-bloom incubations, the midday yields were significantly lower ($p < 0.05$), $\sim$ 30%, than initial values (Fig. 3A,B), with significant differences among radiation treatments at some periods during the sampling. At the bloom onset (Fig. 3C,D), there were no significant differences among radiation treatments, with the exception of one sampling point. The yields of samples

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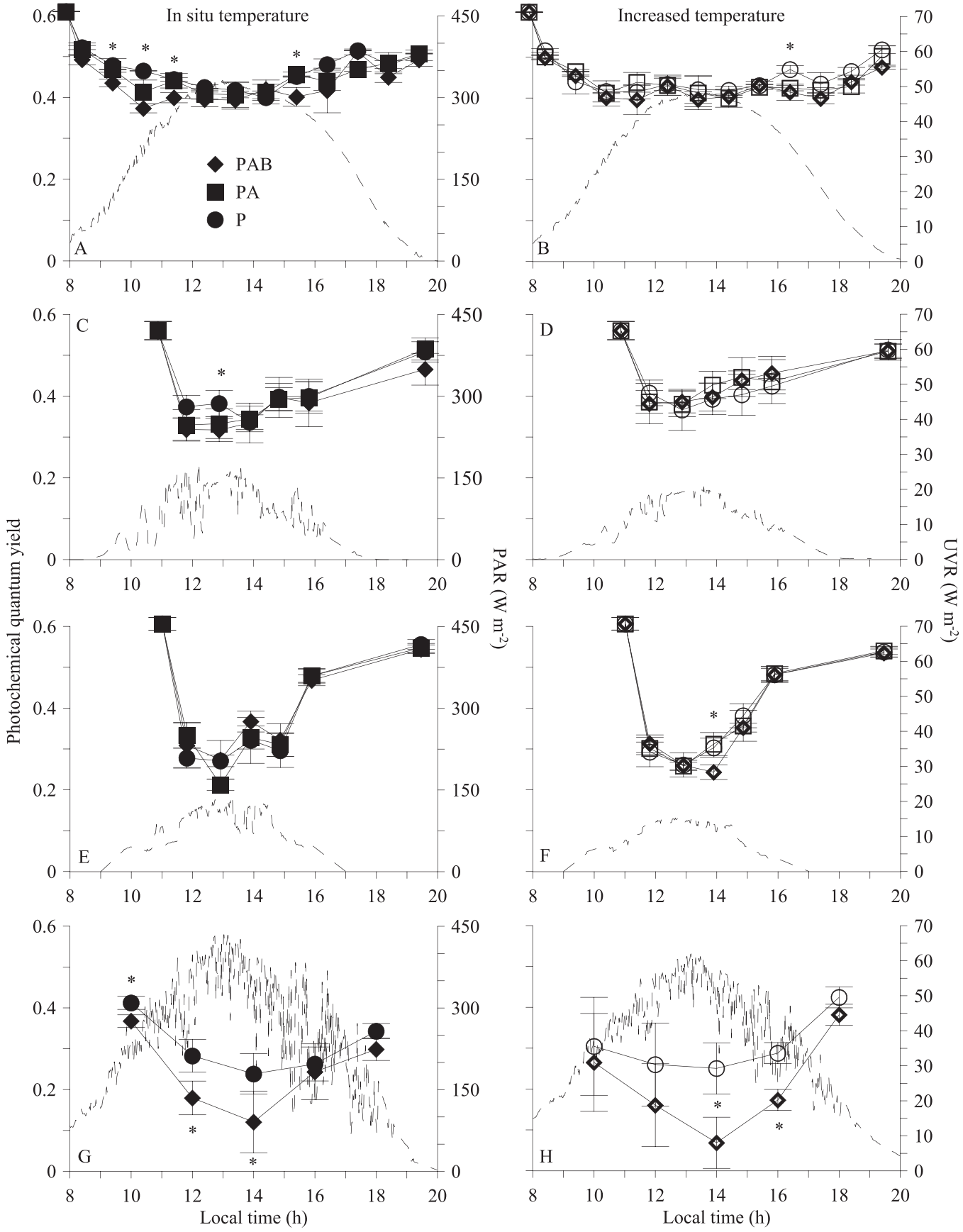
Fig. 2. Representative biological characteristics during the pre-bloom (09 March 2011), bloom onset (04 June 2011), bloom (08 June 2011), and spring (07 November 2011): (A) Chl *a* concentration (μg L^{-1}); (B) cell abundance (cells mL^{-1}) of microplankton (> 20 μm in diameter) and pico–nanoplankton cells (< 20 μm in diameter); (C) percentage (%) of cells from the different taxonomic groups—diatoms, dinoflagellates, and flagellates; and (D) percentage (%) of autotrophic carbon for diatoms, dinoflagellates, and flagellates. The different patterns of the bars indicate the stage of the seasonal succession: white = pre-bloom; gray = bloom onset; slashed = bloom; black = spring. The letters on top of the bars indicate significant differences ($p < 0.05$) among groups (lowercase for diatoms, uppercase for flagellates), whereas the asterisks in (B) and (D) over the bar indicate significant differences from the rest of the group.


In situ temperature
Increased temperature
PAB
PA
P
A
B
C
D
E
F
G
H
Photochemical quantum yield
PAR (W m-2)
UVR (W m-2)
Local time (h)
Local time (h)

incubated at increased temperatures (Fig. 3D) were, in general, significantly higher ($p < 0.05$) than those incubated at the in situ temperature (Fig. 3C). Overall, yields during this sampling period varied significantly ($p < 0.05$) over a daily cycle, with a noon decrease of $< 50\%$ of the initial value. During the bloom, there were no differences among radiation treatments, with the exception of one point at increased temperature. The decrease of yield towards midday was significantly higher ($p < 0.05$) than in the bloom onset conditions (Fig. 3E,F), with values 60–70% lower than the initial yield. During spring, however, significant differences among radiation treatments were observed: A significant decrease in yield ($p < 0.05$) was determined in samples exposed to PAR + UVR at both in situ (Fig. 3G) and increased temperatures (Fig. 3H) as compared to samples exposed to PAR. The yields determined close to local noon in this spring assemblage were $\sim$ 70–80% lower than initial values in the UVR-exposed samples. In addition, the initial yields of this spring assemblage were significantly lower ($p < 0.05$) than in the pre-bloom, bloom onset, and bloom samples.

The maximal variations of yield (Δyield), within each radiation treatment and temperature condition, were calculated as the difference between the minimum yield observed for each treatment with respect to the initial value. These Δyields were calculated for all experimental periods and are shown in Fig. 4. During the pre-bloom (Fig. 4A), there were significant differences ($p < 0.05$) among radiation treatments at both temperatures, but temperature did not cause any effects on these values. A different response was observed during the bloom onset (Fig. 4B) and the bloom (Fig. 4C): During the bloom onset, significantly lower Δyield values were determined at the increased temperature ($p < 0.05$), whereas the opposite occurred during the bloom, with higher Δyield at increased temperature ($p < 0.05$). In addition, during the bloom onset (Fig. 4B) the Δyield was significantly lower ($p < 0.05$) in samples exposed to UVR at increased temperature (i.e., PAB_{inc}), causing differences between radiation treatments. During the bloom onset and bloom, there were significant interactions between UVR and temperature ($p < 0.05$), although with opposite effects as mentioned above. The spring assemblage (Fig. 4D) had significantly higher Δyield values as compared with those of the pre-bloom and the bloom onset ($p < 0.05$). Additionally, there were significant differences among radiation and temperature treatments, so that samples receiving only PAR had lower Δyield values than those that received PAR + UVR. A significant interaction ($p < 0.05$) between UVR and temperature effects was also observed, with higher inhibition due to UVR at the increased temperature condition. It is important to consider that during the spring experiment we used 5 L bags instead of 200 mL tubes. This change in geometry of the exposure would result in less inhibition in the bags, as compared with the tubes, so in comparison with the other stages of the succession, we are underestimating the UVR effect on this community.

The Δyield values presented in Fig. 4, for the four stages of the seasonal succession, represent the maximum change of yield. These Δyields, however, do not take into account the period of time needed to attain the observed change. So, in order to relate the dynamics of the phytoplankton response to our imposed treatment conditions, we calculated the variations of yield as a function of time by determining the inhibition and recovery rates (Fig. 5): During the pre-bloom condition (Fig. 5A) under in situ temperature, inhibition rates were ~ 0.086 yield h^{-1} in the PAB treatment, whereas in those incubated in the P treatment the rates were significantly lower at ~ 0.052 yield h^{-1} ($p < 0.05$). Intermediate values were determined for samples incubated at the increased temperature (0.074 yield h^{-1}), with no significant differences among radiation treatments. Recovery rates values (Fig. 5B) were significantly lower at ~ 0.02 yield h^{-1} ($p < 0.05$) in all treatments as compared with inhibition rates. During the bloom onset (Fig. 5C), samples that received full radiation (PAB) under in situ temperature had an inhibition rate of 0.26 yield h^{-1}, but it was significantly lower (0.16 yield h^{-1}, $p < 0.05$) in samples that received PAR only and also in the samples at the increased temperature condition. Recovery rates during the bloom onset were significantly lower ($p < 0.05$) than the inhibition rates with values < 0.03 yield h^{-1}. The highest inhibition rates were determined during the bloom ($p < 0.05$), especially under the P treatment at in situ temperature (Fig. 5E); although the recovery rates (Fig. 5F) varied, they were also higher ($p < 0.05$) than during the pre-bloom and the bloom onset, ranging from 0.04 to 0.05 yield h^{-1}. Finally, in spring (Fig. 5G) there was a clear effect of radiation treatments, where samples that received UVR exhibited significantly higher ($p < 0.05$) inhibition rates than those incubated under PAR only, regardless of temperature. The recovery rates in the spring (Fig. 5H) were significantly higher as compared to the previous stages of the seasonal succession, especially in samples under the PAB treatment at both in situ and increased temperatures with the lowest recovery rates attained in samples exposed to PAR only under the in situ temperature (i.e., 0.025 yield h^{-1}). There were no significant interactions between UVR

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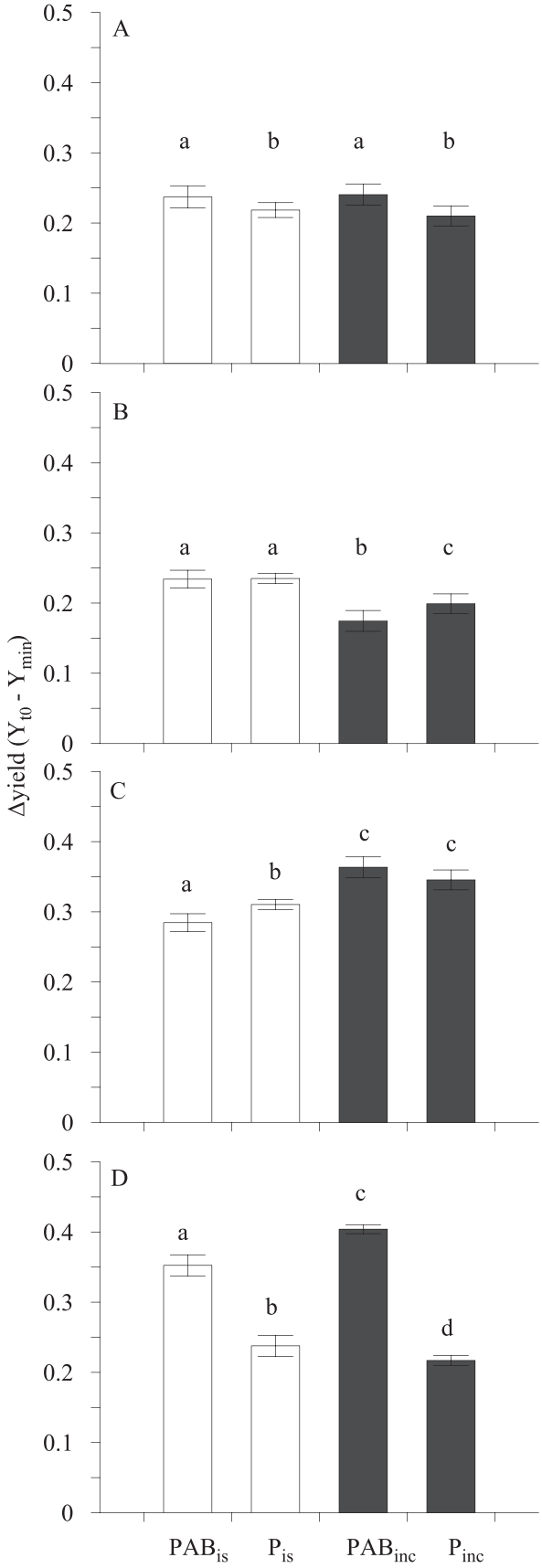
Fig. 3. Mean effective photochemical quantum yield (yield) of phytoplankton communities during daylight exposures under the three radiation treatments (PAB—diamonds, PA—squares, and P—circles) and at two temperatures (in situ vs. increased): (A) pre-bloom, 17°C; (B) pre-bloom, 21°C; (C) bloom onset, 11°C; (D) bloom onset, 15°C; (E) bloom, 15°C; (F) bloom, 19°C; (G) spring assemblage, 15°C; and (H) spring assemblage, 19°C. The lines on the symbols represent the standard deviation. Solid symbols indicate samples exposed to in situ temperatures, whereas open symbols indicate samples exposed to increased temperatures. Broken lines indicate the irradiances for (A, C, E, G) PAR and (B, D, F, H) UVR (W m^{-2}) during the daily cycles. The asterisks indicate significant differences between the PAB and P treatments.



and temperature when considering these rates on inhibition and recovery.

Even though the inhibition rates during the pre-bloom, bloom onset, and bloom (Fig. 5A,C,E) were higher than those in the spring assemblage (Fig. 5G), the actual yield values were much lower under this latter condition (Fig. 3). The reason for this apparent discrepancy is that even though the inhibition rates were high during the bloom onset and bloom, due to the shorter daylight cycle in winter, the inhibition lasted a short period (2–3 h since the beginning of the experiment), after which time the cells started to recover. In contrast, in the spring, the samples were exposed to a longer daylight cycle, the inhibition lasted from 08:00 h (the start of the experiment) until 14:00 h, after which the cells started to recover (Fig. 3G,H).

Discussion

It is clear from our results that the phytoplankton communities characteristic of the different stages of the seasonal succession in Patagonian coastal waters exhibit distinct photochemical responses throughout the year when exposed to solar UVR at in situ and increased temperatures, both variables associated with global climate change (Häder et al. 2011). There was a range of responses as the season progressed, with each stage being more or less sensitive to one or both variables. In particular, there were significant interactions between temperature and UVR during the bloom onset, the bloom, and in the spring when evaluating the magnitude of inhibition (i.e., Δyield).

Inter-seasonal variability in photochemical responses can be largely explained by differences in the taxonomic composition of the communities as the responses of phytoplankton to UVR and/or temperature have an important component of species specificity (Häder et al. 2011). The annual succession is mainly driven by differences in abiotic factors throughout the year favoring the growth of species better acclimated to the prevailing conditions. The most obvious factors that will influence the seasonal succession of phytoplankton are the variable conditions in physical factors such as solar radiation, temperature, nutrient input, and wind, among others. The daily doses of solar radiation during our experimental periods are within the values previously determined for the area (Helbling et al. 2005, 2010), but two particular features result in contrasting conditions that affect the amount of solar radiation received by the different stages of the

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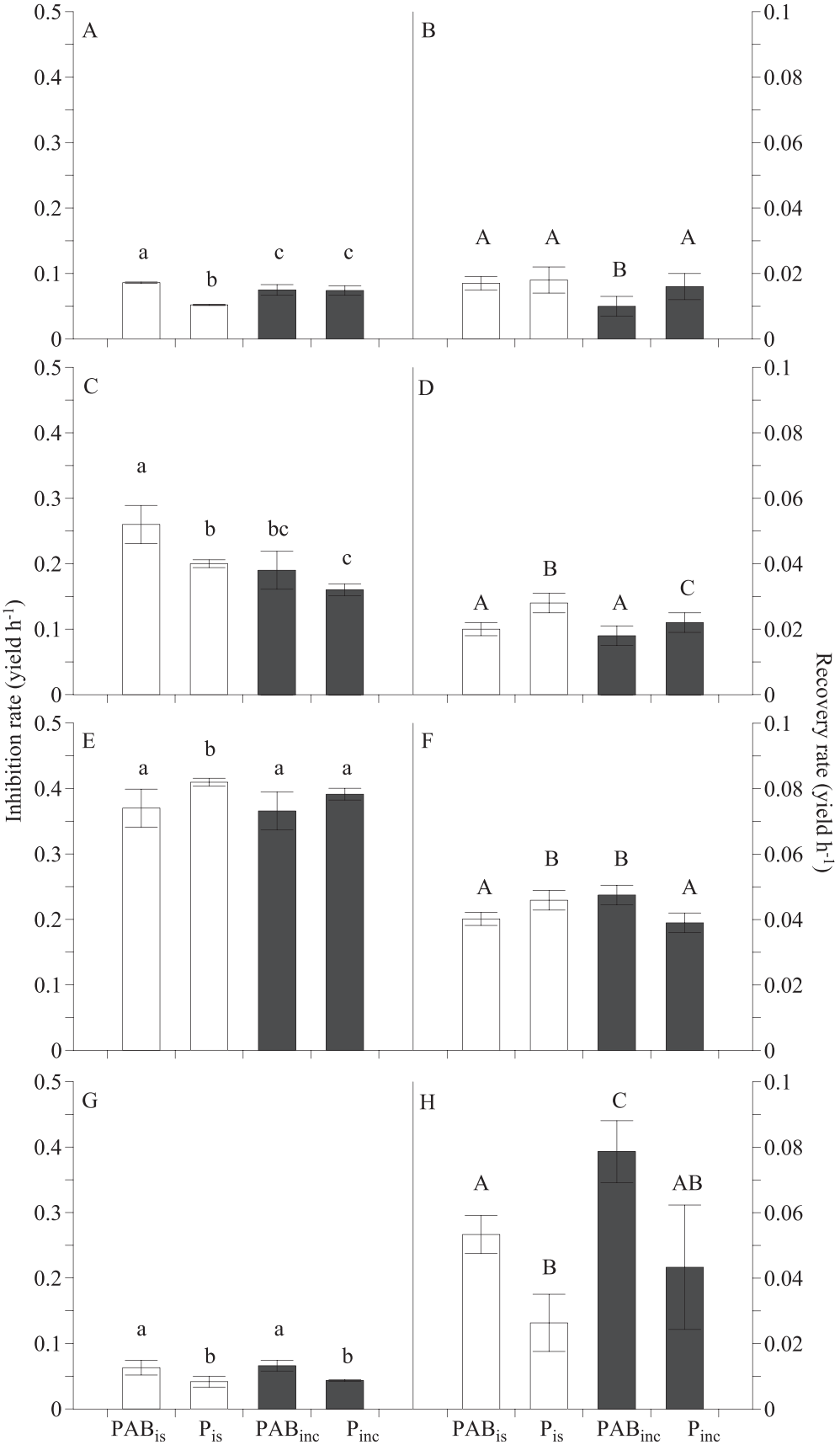
Fig. 4. Maximal variations of yield (Δyield) with respect of the initial value during the experimental periods: (A) pre-bloom; (B) bloom onset; (C) bloom; and (D) spring assemblage. White and black bars indicate samples incubated at in situ (is) and increased (inc) temperatures, respectively. PAB are samples exposed to PAR + UV-A + UV-B; P are samples exposed only to PAR. The lines on top of bars indicate the standard deviation; the different letters indicate significant differences among treatments.

seasonal succession of the phytoplankton communities. On the one hand, in the spring, the combination of long daylight hours together with high radiation levels resulted in doses comparable to those received in tropical areas in midsummer (Li et al. 2009). In addition, low values (< 310 Dobson units [DU]) of total ozone column concentration were observed over the area (http://ozoneaq.gsfc.nasa.gov/ozone_overhead_current_v8.md) during the spring with values as low as 238 DU measured on 17 November. Therefore, samples during this period might have been exposed to comparatively higher radiation levels than the usual, especially in terms of UV-B, as also noted in the ratio of UV-B to PAR. On the other hand, during the bloom onset and bloom periods, radiation levels were lower than historical records (Helbling et al. 2005), mainly due to the presence of ash in the atmosphere carried eastward after the eruption of the Puyehue volcano in the Chilean Andes, which started on 04 June and continued until 16 June. In parallel with these contrasting radiation conditions, the surface-water temperature is characterized by a rather large range throughout the year from ~ 18°C during the summer to ~ 7°C during the winter (Helbling et al. 2010). Both radiation and temperature conditions (in combination with other biotic and abiotic factors) have resulted in the characteristic phytoplankton succession, with the presence of a winter bloom, dominated by large centric diatoms. This phenomenon has been previously described for the study area (Villafañe et al. 2004*a*). Other studies (Gayoso 1999; Guinder et al. 2009) have also indicated the occurrence of a winter bloom over other areas of the Argentinean continental shelf, suggesting that this type of phytoplankton dynamic is extensive. The presence of winter blooms has been explained by the high stability of the water column favored by the low wind speeds characteristic of this time of the year. It is important to note that during the rest of the year, high speed and frequency of winds cause constant mixing of the water column, thus precluding cell growth (Villafañe et al. 2004*a*; Helbling et al. 2005).

Within the particular stages of the seasonal succession differences in responses (i.e., inter-seasonal) of the phytoplankton communities seem to be more related to the physiological conditions. One of these conditions refers to changes in acclimation capacity due to natural solar radiation exposure under variable mixing conditions. During the pre-bloom, cells are well acclimated to the high light levels experienced during the late summer as indicated by the small differences in the diurnal yield cycle; conversely, and during the bloom onset, cells are acclimated to low irradiances characteristic of the winter (Fig. 1). During the bloom, visual observation indicated the presence of a layer of ash floating on the surface waters which could have considerably affected the underwater radiation field to which the cells were exposed. Thus, and within the timeframe of a week, phytoplankton cells might have changed their exposure to solar radiation towards a more opaque environment, clearly affecting their acclimation capacity as has been observed in other turbid environments (Villafañe et al. 2004*b*). In addition, during the bloom onset, flagellates and small *Thalassiosira* characterized the assemblage, but this changed to an almost complete dominance of large *Odontella aurita* during the bloom (Fig. 2). In the spring, even though the surface irradiance is high (Fig. 1), cells seems to be acclimated to relatively low irradiances due to the deep mixing that occurs at this time of the year as a result of the high-frequency winds in the area (Villafañe et al. 2004*a*; Helbling et al. 2005). Under the incubation conditions used in our experiments, the phytoplankton were exposed to higher radiation levels as they were not mixed but just lying at the surface of the water, and thus the cells do not experience the fluctuating radiation regimes as found under natural conditions. A similar situation occurred with bloom onset and bloom samples, in which samples were acclimated to low-radiation conditions and suddenly exposed to higher radiation levels due to the experimental setup. This (experimental) enhancement in solar radiation might explain part of the observed decrease in yield, as acclimation processes would take longer than our daily exposure to be significant in providing "protection" to the cells (Van De Poll and Buma 2009).

Another factor that might influence the observed responses is the nutrient status of the cells: The nutrient concentration should be high during the pre-bloom period and at the bloom onset as has already been determined for these types of communities (Villafañe et al. 1991; Helbling et al. 1992). In contrast, during spring, nutrients are generally exhausted (Pérez pers. comm.), and the decrease in yield suggests a higher sensitivity of this nutrient-limited community towards solar UVR. Other studies have also shown a higher sensitivity of nutrient-limited phytoplankton (Beardall et al. 2009); and Litchman et al. (2002) speculated that this might be due to the limited amounts of photoprotective compounds (i.e., mycosporine-like amino acids [MAAs]) under nitrogen-limited conditions. In our case, the lack of MAAs might have accounted for a small part of the observed inhibition, as none of the communities sampled contained significant amounts of MAAs (data not shown). Another protective mechanism against UVR stress is the xanthophyll cycle as observed in diatoms and chlorophytes (Van De Poll and Buma 2009); however, in a previous study conducted in the area with summer post-bloom communities (Villafañe et al. 2008), it was established that, indeed, these cells were highly sensitive to solar UVR (when evaluating photoinhibition) and xanthophyll pigments provided only limited protection.

It has been shown (Halac et al. 2010) that increasing temperatures have positive effects in the diatom *Chaetoceros gracilis* by enhancing the dissipation of excess energy via non-photochemical quenching (NPQ). However, other diatom species (e.g., *Thalassiosira weissflogii*) do not seem to be specifically benefited by the mechanism of increasing NPQ (Halac et al. 2010), but rather increase Rubisco activity at increasing temperatures (Helbling et al. 2011). In our case, the dissipation of excess energy via NPQ was only slightly important in the increased temperature treatment during the onset of the bloom (data not shown). Temperature increase, however, had a significant role in stimulating recovery during the spring sampling, with rates of recovery being the highest of all stages of the succession (Fig. 5H).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A
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b
a
b
A
B
C
AB
Inhibition rate (yield h-1)
Recovery rate (yield h-1)
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0.1
0.2
0.3
0.4
0.5
0.02
0.04
0.06
0.08
PABis
Pis
PABinc
Pinc

In the context of global change, our results suggest that increasing temperatures would have little effect on the pre-bloom communities but will help to counteract the overall magnitude of yield decrease during the bloom onset. However, an increase in temperature acts synergistically with UVR, thus increasing the overall photochemical inhibition of phytoplankton from the bloom and the spring assemblage. As mentioned above, increasing temperatures would also have a feedback mechanism by decreasing the upper mixed layer (UML) depth, and thus would tend to expose the cells to higher radiation conditions. So, it is expected that this increase in exposure to solar radiation (due to a shallower UML) will harm the bloom and spring communities. Due to the differential effect of solar UVR and increased temperature on phytoplankton assemblages, future studies should consider the repercussions on higher trophic levels, especially considering that Patagonian coastal areas (especially during the bloom period) sustain high secondary production (Skewgar et al. 2007).

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Fig. 5. (A, C, E, G) Inhibition and (B, D, F, H) recovery rates (in yield h^{-1}) of phytoplankton communities during daylight exposures under two radiation treatments (PAB: PAR + UV-A + UV-B; and P: PAR only) and at two temperatures (in situ [is] vs. increased [inc]): (A, B) pre-bloom, (C, D) bloom onset, (E, F) bloom, and (G, H) spring assemblage. The bars are the averages with standard deviations; different letters indicate significant differences among treatments (lowercase for inhibition, uppercase for recovery). White and black bars indicate inhibition and recovery of samples incubated at in situ and increased temperatures, respectively. Note the different scales for inhibition and recovery rates.

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