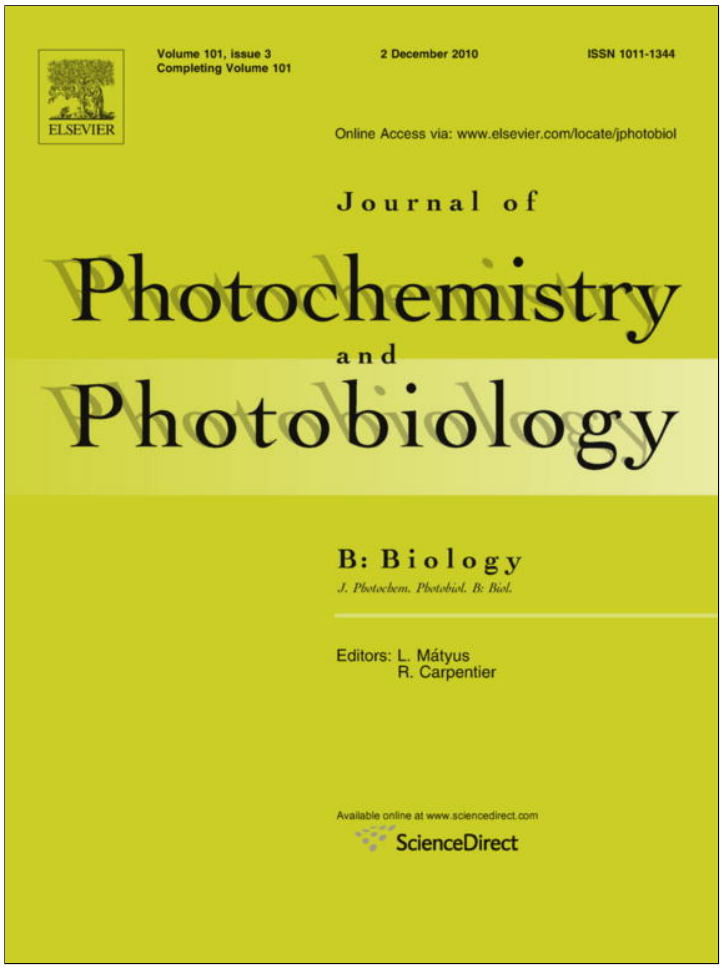

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Temperature benefits the photosynthetic performance of the diatoms *Chaetoceros gracilis* and *Thalassiosira weissflogii* when exposed to UVR

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ABSTRACT

The aim of this study was to assess the combined effects of temperature and UVR on the photosynthesis performance of two diatoms – *Chaetoceros gracilis* and *Thalassiosira weissflogii*. In particular, we evaluated the role of UVR in inducing photoinhibition and the potential mitigation of this negative effect by an increase in temperature. Cultures were pre-acclimated at two temperatures – 18 °C and 23 °C – and exposed to different radiation treatments – UVR + PAR (280–700 nm); UV-A + PAR (315–700 nm) and PAR only (400–700 nm) under two temperatures: 18 °C (local surface summer water temperature) and 23 °C (simulating a potential increase estimated by the year 2100). Exposure to natural solar radiation resulted in UVR-induced photoinhibition that was significantly higher in *T. weissflogii* than in *C. gracilis*. Both species benefited from the higher temperature (23 °C) resulting in a lower photoinhibition as compared to samples exposed at 18 °C. Inter-specific differences were determined in regard to the heat dissipation processes (NPQ) which were higher at high temperatures, and much more evident in *C. gracilis* than in *T. weissflogii*. The analyses of inhibition and recovery rates under different irradiances indicate that the balance between negative (inhibition) and positive (repair-dissipation) effects shifted towards a more positive balance with increasing temperature. Our results highlight for a beneficial effect of temperature on photosynthesis performance during exposure to UVR, although important inter-specific differences are found, probably due to differences in cell size as well as in their distribution within the oceanic realm (i.e., coastal versus oceanic species).

1. Introduction

Climate change is a very complex process that has two main outcomes of anthropogenic origin, i.e., the increase of greenhouse gases with the concomitant increase of temperature, and the enhancement of ultraviolet radiation-B (UV-B, 280–315 nm) levels due to ozone depletion events [1]. There is, therefore, an increasing interest in evaluating the magnitude and extent of climate change on organisms and ecosystems. On the one side, enhanced UV-B radiation causes stress on aquatic organisms, although now is recognized that even normal levels of ultraviolet radiation (UVR, 280–400 nm) can cause significant damage to diverse cellular components and processes [2]. For phytoplankton, effects such as reduced growth and productivity rates and damage to the DNA molecule and proteins have been reported ([3,4] and references therein). In regard to increased temperature levels, studies have shown changes in phenology and biodiversity [5] but a rather positive feedback [6] in processes such as photosynthesis, as seen in a comparative study determining Photosynthesis versus Irradiance (P versus E) relationships in different phytoplankton taxa [7].

While there is vast literature on the effects of these two stressors acting separately on phytoplankton, the impact of both of them at the same time has resulted rather difficult to assess as they can act synergistically or antagonistically; moreover, responses seem to have a high degree of species-specificity. The temperature dependence of UVR effects on pigment composition, growth rates, and photosynthetic characteristics were assessed in Antarctic cyanobacteria [8]. These authors found that UVR-induced inhibition of growth increased linearly with decreasing temperature, whereas there was no apparent effect of temperature on the magnitude of UVR-induced inhibition of photosynthesis. Other studies carried out in alpine lakes [9] determined that UVR depressed growth rates in all phytoplankton species tested at 6 °C regardless nutrients conditions, whereas at 14 °C, the negative effect of UVR was not observed for any species in the absence of nutrient; only with the addition of nutrients did UVR exposure depressed the growth of a diatom and a dinoflagellate. Finally, research carried out in mountain lakes of USA found that the cold temperatures,

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zooplankton, and UVR had negative effects on the rates of increase in phytoplankton biomass [10]. These results further demonstrated the importance of indirect as well as direct effects of climate forcing by UVR and temperature on phytoplankton community composition in mountain lakes.

One of the main targets of UVR in phytoplankton organisms is the photosynthetic apparatus [4]. UVR is known to cause photoinhibition, i.e., the reduction of photosynthetic rates [11] by damaging the photosystem components [12], particularly the D1 protein of the photosystem II (PSII). Recovery from the UVR-induced damage implies enzymatic activity to degrade the damaged PS complex proteins, as well as for re-synthesizing PS components [13]. Since temperature is a key factor that enhances enzymatic activity, an increase in its levels would, in principle, benefit species that have suffered UVR-induced damage in their photosynthetic apparatus. In fact, in a study carried out with the diatom *Thalassiosira pseudonana*, it was found that UVR-induced sensitivity (i.e., as assessed through biological weighting functions for photoinhibition) was highly affected by temperature, so that its extent increased with decreasing temperature; however, and over long periods of time, these photoinhibitory effects were slightly modified (i.e., reduced) due to acclimation processes [14].

Because of the outmost importance of Patagonia within a photobiology context, i.e., the region normally receives high levels of UVR and it is periodically under the influence of ozone depletion events [15] is that we designed experiments to assess the combined effects of temperature and UVR on the photosynthesis performance of two characteristic diatoms of the area: *Chaetoceros gracilis* and *Thalassiosira weissflogii*. In particular, we evaluated the role of UVR in inducing photoinhibition in these species, and we asked whether a potential increase in temperature would mitigate these effects and, if so, to what extent. The increase in temperature that we used in these experiments (+5 °C) represents a crude but realistic value in a scenario of climate change by the year 2100 [16].

2. Materials and methods

2.1. Culture collection/study site

T. weissflogii (Grunow) G. Fryxell & Hasle and *C. gracilis* Schütt (Bacillariophyceae) from the Microalgae Culture Collection at Estación de Fotobiología Playa Unión (EFPU, Argentina) were grown in 1-L Erlenmeyer flasks in f/2 medium [17] with a photoperiod 12L:12D in a growth chamber (Sanyo model ML 350). Cells were pre-acclimated either at 18 °C or at 23 °C for 2 weeks prior to experimentation under Photosynthetic Active Radiation (PAR, 400–700 nm) (235 μmol photons m^{-2} s^{-1}). Light was provided by cool white fluorescent lamps (Philips daylight) and photon flux densities were measured with a spherical micro quantum sensor (Walz GmbH, model US-SQS/WB). Cells were harvested during the exponential growth phase and used in the experiments as described below. Experiments to determine the short-term effects of both solar and artificial radiation on *T. weissflogii* and *C. gracilis* were carried out during the late austral winter (27 August–7 September, 2009) at the Estación de Fotobiología Playa Unión (43°18.7′S; 65°02.5′W) located in the Patagonian coast of Argentina.

2.2. Experimentation/sampling protocol

The experiments to evaluate the short-term combined effects of UVR and temperature on the photosynthesis performance of *T. weissflogii* and *C. gracilis* were conducted under solar and artificial radiation conditions as follows:

(i) *Experiments under solar radiation:* The samples were put in 50 ml quartz tubes under three radiation treatments (duplicates for each treatment): (a) Full solar radiation – UVR + - PAR (280–700 nm) – uncovered tubes – PAB treatment; (b) UV-A + PAR (320–700 nm) – tubes covered with UV cut-off filter foil (Montagefolie, No. 10155099, Folex, Dreieich, Germany) – PA treatment; and (c) Only PAR (400–700 nm) – tubes covered with Ultraphan film (UV Opak, Digefra, Munich, Germany) – P treatment. The spectra of these filters/materials are published in Figueroa et al. [18]. Samples were put in thermostatic water baths (Frío 21, Argentina) with two independent circuits that kept temperature at both 18 °C and 23 °C. Different combinations of pre-acclimation/exposure temperatures were tested: (1) Cultures pre-acclimated at 18 °C and exposed at 18 °C (18_18); (2) Cultures pre-acclimated at 18 °C and exposed at 23 °C (18_23); (3) Cultures pre-acclimated at 23 °C and exposed at 23 °C (23_23). In all experiments, samples were exposed to solar radiation during 8 h (i.e., from 10 am to 6 pm, local time) and measurements of various fluorescence parameters were obtained continuously throughout the duration of the experiments by taking alternately samples from the different radiation treatments (thus, a particular sample was measured every ∼30–35 min). Additionally, sub-samples were collected for analysis of chlorophyll-*a* (chl-*a*), carotenoids, UV-absorbing compounds and cell concentrations at the beginning of each experiment. All experiments done with *C. gracilis* (18_18; 18_23 and 23_23) were carried out on August 31, 2009, whereas those with *T. weissflogii* were done on September 3 (18_18 and 23_23) and September 4, 2009 (18_23).

(ii) *Experiments under artificial radiation:* Samples (duplicates) were put into 50 ml quartz tubes under three radiation treatments (as above) and exposed to three different UVR–PAR irradiances by adjusting the distance from the samples to a solar simulator (Hönle System Sol 1200, Hönle, Germany): (1) UV-B: 0.73 W m^{-2}, UV-A: 28.5 W m^{-2}, PAR: 375 μmol photons m^{-2} s^{-1}; (2) UV-B: 1.45 W m^{-2}, UV-A: 58.9 W m^{-2}, PAR: 698 μmol photons m^{-2} s^{-1}; (3) UV-B: 2.19 W m^{-2}; UV-A: 89.4 W m^{-2}, PAR: 1041 μmol photons m^{-2} s^{-1}. The samples were exposed submerged in a water bath just below the solar simulator. The whole set-up was placed inside an environmental chamber (Minicella, Argentina) for temperature control, but when needed, ice was added so that the temperature never rose more than 1 °C from 18 or 23 °C (depending on the experiment). The different combinations of pre-acclimation/exposure temperatures were as described above. Each experiment consisted of 1 h of exposure under the solar simulator and 1 h of recovery under dim light (30 μmol photons m^{-2} s^{-1}) with measurements of fluorescence parameters done continuously during these periods. An extra measurement of recovery was performed 1 h after the last recovery measurement.

2.3. Analyses and measurements

The following measurements and analyses were done:

2.3.1. Radiation and temperature measurements

Solar radiation reaching the cultures was continuously monitored using a broad-band filter radiometer (ELDONET, Real Time Computer, Möhrendorf, Germany) that measures UV-B (280–315 nm), UV-A (315–400 nm) and PAR (400–700 nm) every second, averages data over 1-min interval and stores them in a computer. This instrument is calibrated every year by a solar calibration procedure. The radiometer is permanently installed on the

roof of the Estación de Fotobiología Playa Unión. Water temperature inside the thermostatic baths was controlled with sensors attached to each temperature channel and adjusted automatically throughout the experimental period.

2.3.2. Fluorescence measurements

The photosystem II efficiency of charge separation is related to the proportion of photochemically active (PSII) reaction centers. This was determined by measuring the *in vivo* chl-*a* fluorescence emission using a portable pulse amplitude modulated fluorometer (Water-ED PAM, Walz, Germany). The PSII effective photochemical efficiency of charge separation ($\Delta F/F'_m$ or Y) was calculated using the equations of Genty et al. [19] as:

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

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 current steady-state fluorescence induced by weak actinic light in light-adapted cells. The non-photochemical quenching (NPQ) of chl-*a* fluorescence is a proxy of the non-radiative dissipation of light energy absorbed in excess, and it is the most important short-term photoprotective mechanism activated by saturating PAR intensities. NPQ was determined measuring F_m at the beginning of the experimentation in samples maintained in darkness during 5 min and F'_m during the exposure time, and it was calculated as:

$$\text{NPQ} = (F_m - F'_m)/F'_m \quad (2)$$

There were no significant differences between NPQ values calculated in this way and those obtained directly using the PAM fluorometer therefore, we used the data obtained with this instrument. In all cases, the chl-*a* fluorescence measurements were made six times for each sample.

2.3.3. Cell concentration and pigments quantification

Aliquots of cell cultures (10 ml) were fixed with buffered formalin (final concentration of formaldehyde in the sample = 0.4%) and counted with a Neubauer chamber under a compound microscope (Zeiss model D-7082, Germany). Chl-*a* concentration was measured by filtering 20–50 ml of sample onto a Whatman GF/F filter (25 mm) and extracting the photosynthetic pigments and UV-absorbing compounds in absolute methanol [20]. A scan between 250 and 750 nm was done using a Hewlett Packard spectrophotometer (model HP 8453E) and chl-*a* and carotenoids concentration were calculated using the equations of Porra [21]. UV-absorbing compounds were estimated by the peak at 337 nm [22]. We are aware that other studies [23] suggested the use of 20% methanol as the best extraction solvent for these compounds. Nevertheless, and since we were limited by the volume of samples, we considered that this procedure was appropriate for the purposes and aim of our investigation, even it might slightly underestimated the amount of UV-absorbing compounds. Once scanned, the same sample was used to determine the chl-*a* concentration fluorometrically [24].

2.3.4. Statistical and data analysis

Radiation treatments were done in duplicate. All data is reported as the mean and half mean range. One-way repeated measurements ANOVA test was used to determine differences among irradiances, temperatures and species while a two-ways ANOVA test was used to determine interactions between irradiance and temperature, using a 95% confidence limit and one degree of freedom [25]. The normality and homoscedasticity of data were confirmed by using a Kolmogorov–Smirnov test and plotting the residuals respectively. Thus, data transformation was not necessary. A *post hoc* Newman–Keuls test was applied to differentiate significant treatments effects. In the case of a significant interaction between the two factors (radiation and temperature), a plot of the mean Y inhibition versus the radiation levels were made for each temperature treatment to determine if the combined effect was positive (synergic) or negative (antagonist) [26].

The decrease of Y at each wavelength interval (i.e., Y in the PAB and PA treatments relative to that in the P control) over the incubation period was calculated as:

$$\text{Decrease due to UV-B} = [(Y_P - Y_{PAB}) - (Y_P - Y_{PA})]/(Y_P) * 100 \quad (3)$$

$$\text{Decrease due to UV-A} = (Y_P - Y_{PA})/(Y_P) * 100 \quad (4)$$

where Y_P, Y_{PA}, and Y_{PAB} are the PSII effective photochemical efficiency of charge separation in the P, PA, and PAB treatments, respectively. To compare the rates of photoinhibition and recovery under the different radiation/temperature treatments, the slope $\Delta Y/\Delta t$ for each interval (exposure/recovery) was determined using a linear fit.

3. Results

3.1. Solar radiation conditions

Solar radiation conditions during the study period are shown in Fig. 1. In general, the conditions during the experimentation days were of clear skies, but some cloud cover was evident during the

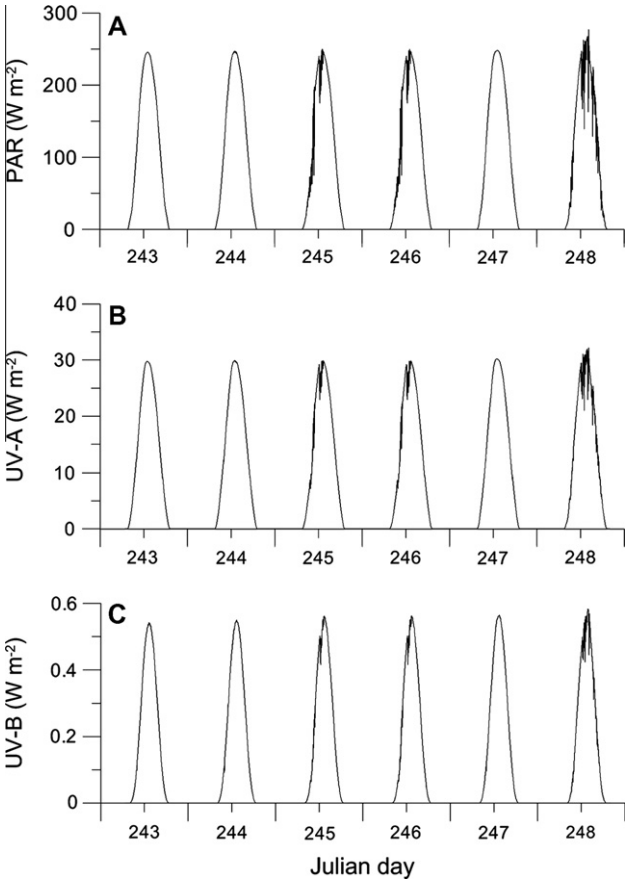

Fig. 1. Solar radiation conditions during the period August 31–September 5, 2009. Experiments were carried out on August 31 (Julian day 243), September 3 (Julian day 246) and September 4, 2009 (Julian day 247). Daily irradiance of: (A) PAR, 400–700 nm (in W m^{-2}); (B) UV-A, 315–400 nm (in W m^{-2}) and; (C) UV-B, 280–315 nm (in W m^{-2}).

morning of Julian days 245 and 246, and throughout the day on Julian day 248. Maximum PAR irradiance levels of ~250 W m^{-2} (Fig. 1A) were similar during the experiments carried out with *C. gracilis* (Julian day 243) and *T. weissflogii* – Julian days 246–247. UV-A (Fig. 1B) and UV-B irradiances (Fig. 1C) followed the same trends as PAR, with maximum values of ~30 and ~0.55 W m^{-2} for UV-A and UV-B, respectively.

3.2. Experiments under solar radiation

For simplicity, we are presenting only the data obtained in the PAB and P treatments and the data obtained under the PA treatment is presented in Table 1. Both *C. gracilis* and *T. weissflogii* had a clear pattern of inhibition of *Y* as soon as the samples were exposed to solar radiation in all radiation and temperature treatments (Fig. 2). Nevertheless, recovery was observed during the afternoon but in different degree for each species/temperature combination (Fig. 2A, B, D and E). *C. gracilis* attained higher recovery (Fig. 2A and B) than *T. weissflogii* (Fig. 2D and E) at the end of the experiment. There was a net effect of solar radiation, as well as of temperature on these daily cycles. Comparing temperature treatments, the *Y* values under both radiation treatments were significantly higher ($p \leqslant 0.001$) in samples exposed at 23 °C than at 18 °C – i.e., either pre-acclimated at 18 °C and then exposed to 23 °C, or pre-acclimated and then exposed to 23 °C (Fig. 2A–E and Table 1). Conversely, and for both species, the lower photosynthetic performance was obtained in samples exposed at 18 °C. In relation to radiation treatments, it was determined that in general, the bulk of the *Y* inhibition was due to PAR (Fig. 2B and E); UVR however, contributed to the observed inhibition by as much as 37% and 60% in *C. gracilis* and *T. weissflogii*, respectively, at 18 °C, being this percentage lower at 23 °C (Fig. 2C and F, and Table 1). Table 1 resumes the mean UVR-induced inhibition in *C. gracilis* and *T. weissflogii*. In both species, samples exposed at 18 °C were those that had higher photosynthetic inhibition during the daily exposure (Fig. 2C and F, and Table 1). At 18 °C however, the inhibition in *T. weissflogii* was significantly higher ($p \leqslant 0.001$) than that in *C. gracilis*. Both species showed significantly lower inhibition when exposed to the 23_23 treatment, i.e., 13% ($p \leqslant 0.001$) and 15% ($p \leqslant 0.05$) for *C. gracilis* and *T. weissflogii*, respectively as compared to the 18_18 treatment. There were some interesting features when comparing the responses of both species, e.g., the percentage UVR-induced inhibition in the 18_23 treatment was similar to that in the 18_18 in *C. gracilis* (Fig. 2C), but similar to the 23_23 in *T. weissflogii* (Fig. 2F). The combined effects of temperature and radiation factors were significant for all the treatments in *T. weissflogii*, but only in some of *C. gracilis* (Table 3).

Table 1
Mean maximum (±SD) UV-A and UV-B inhibition (as percentage relative to P treatment) of samples exposed to solar radiation under different temperature treatments (i.e., 18_18 °C; 18_23 °C and 23_23 °C). Mean inhibition was calculated from the period 12–15 h. Full explanation in the text.

Temperature treatments	*Chaetoceros gracilis*		*Thalassiosira weissflogii*	
	UV-A inhibition	UV-B inhibition	UV-A inhibition	UV-B inhibition
18_18 °C	29.8 ± 3.7	7.4 ± 4.0	46.1 ± 5.4	13.6 ± 3.2
18_23 °C	25.7 ± 5.8	7.8 ± 3.9	34.4 ± 4.8	5.3 ± 4.5
23_23 °C	15.9 ± 3.9	7.7 ± 3.8	36.7 ± 6.6	7.5 ± 3.9

To evaluate the potential photoprotection mechanisms against solar radiation stress in *C. gracilis* and *T. weissflogii*, we investigated the non-photochemical (NPQ) responses occurring throughout the daily cycles (Fig. 3). The most evident feature, when comparing the response of both species was of much higher NPQ values in *C. gracilis* (Fig. 3A and B) than that in *T. weissflogii* (Fig. 3C and D). The highest NPQ values in *C. gracilis* were generally determined in

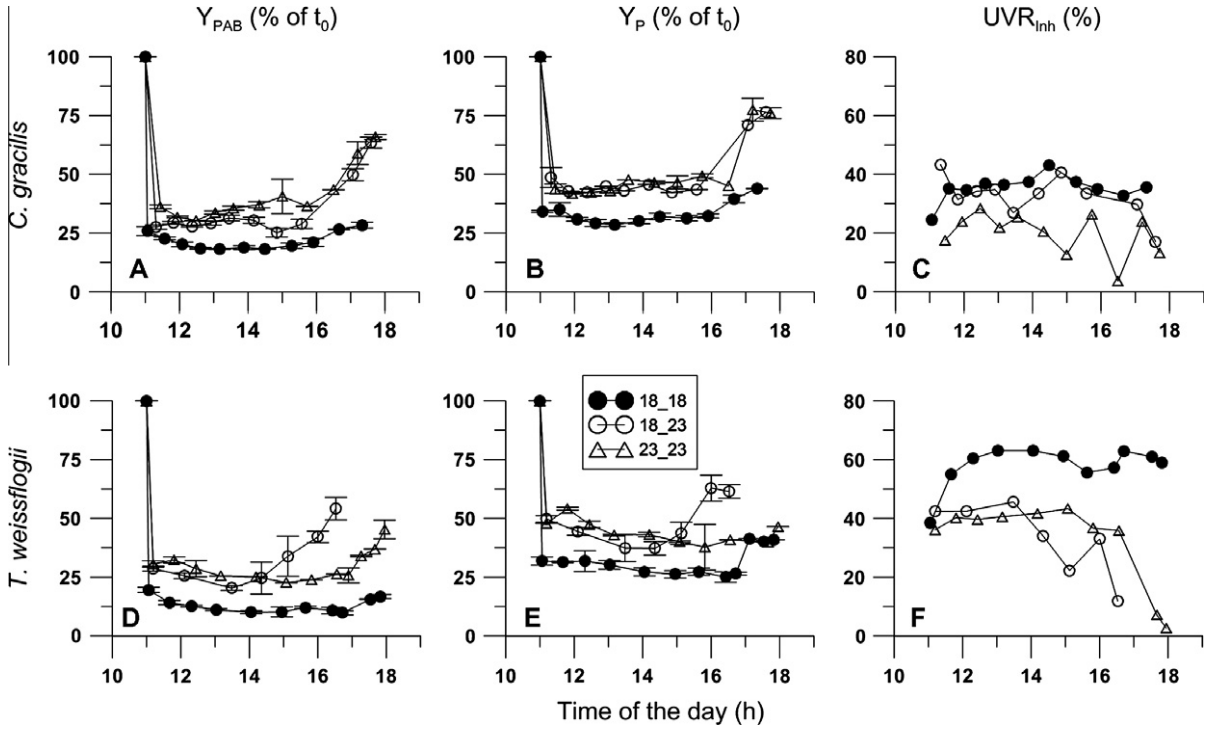

Fig. 2. Photosynthetic responses of *Chaetoceros gracilis* (A–C) and *Thalassiosira weissflogii* (D–F) in experiments carried out under solar radiation conditions. Percentage of change of yield (*Y*) relative to time zero (t_0) in *C. gracilis* samples exposed to: (A) PAR + UV-A + UV-B (PAB treatment) and; (B) PAR only (P treatment). (C) Percentage UVR-induced inhibition (relative to samples under the P treatment) during these daily cycles. Percentage of change of yield (*Y*) relative to time zero (t_0) in *T. weissflogii* exposed to: (D) PAR + UV-A + UV-B (PAB treatment) and; (E) PAR only (P treatment). (F) Percentage UVR-induced inhibition (relative to samples under the P treatment) during these daily cycles. Symbols indicate the different temperature treatments: 18_18 (black circles), 18_23 (white circles) and 23_23 (white triangles). The vertical lines on top of symbols are the half mean range.

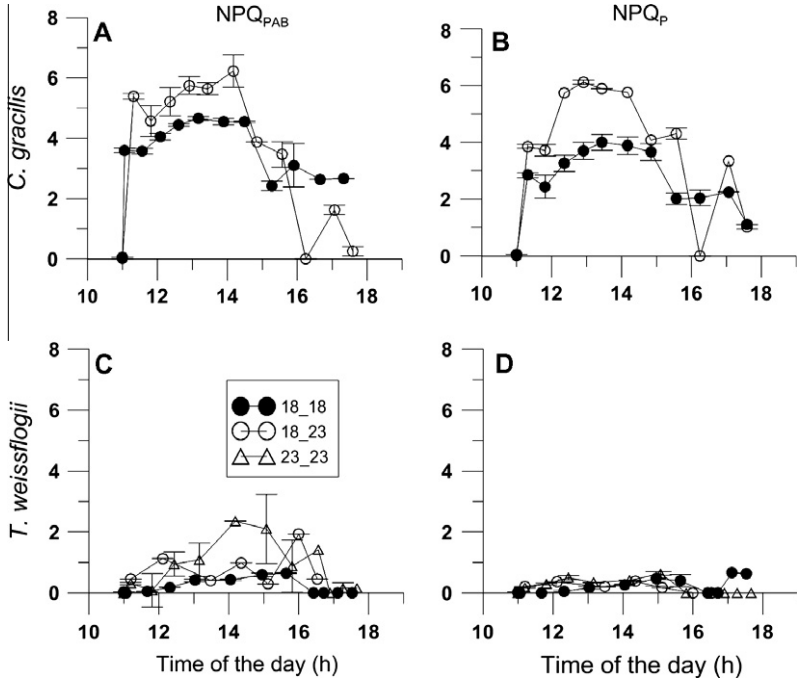

Fig. 3. Non photochemical quenching of PSII (NPQ) throughout exposure under solar radiation conditions in: (A) *Chaetoceros gracilis* samples exposed to full solar radiation (treatment PAB); (B) *C. gracilis* samples exposed to PAR only (treatment P). No data are available for the 23_23 treatment; (C) *Thalassiosira weissflogii* samples exposed to full solar radiation (treatment PAB) and; (D) *T. weissflogii* samples exposed to PAR only (treatment P). Symbols indicate the different temperature treatments: 18_18 (black circles) and 18_23 (white circles). The lines on top of symbols are the half mean range.

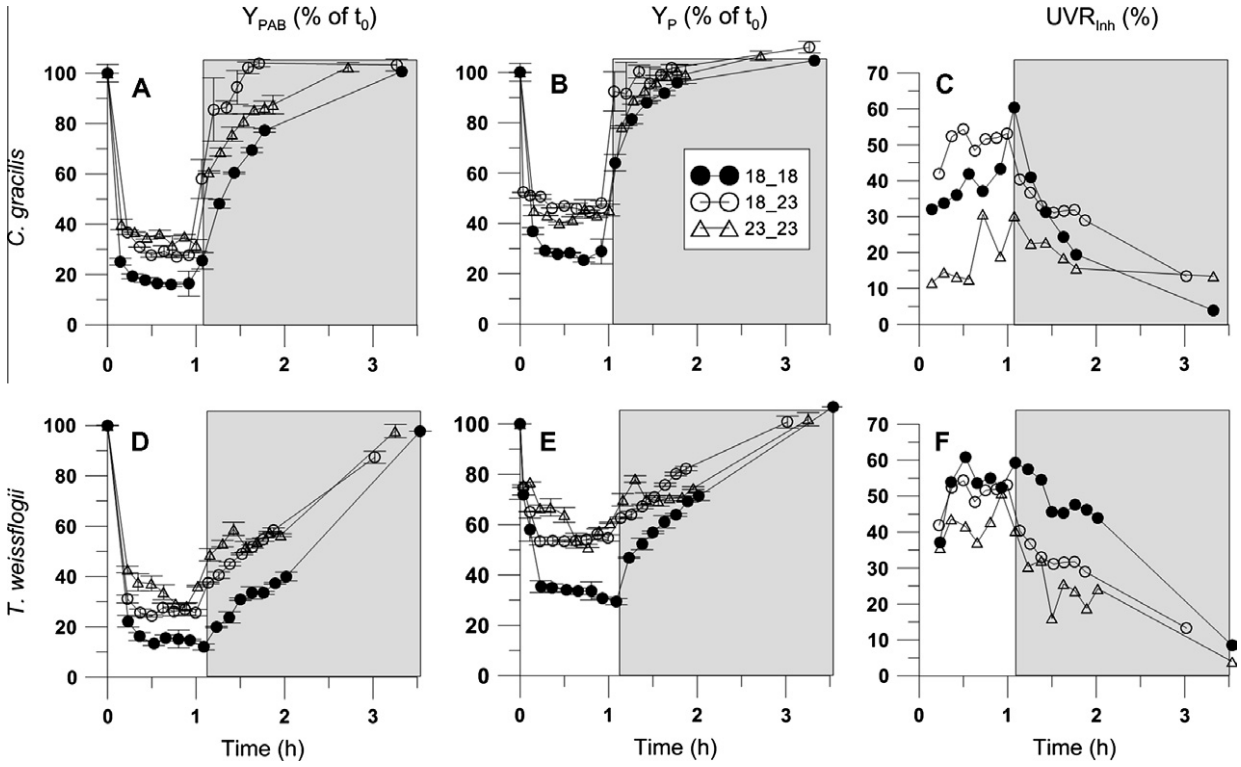

Fig. 4. Photosynthetic responses of *Chaetoceros gracilis* (A–C) and *Thalassiosira weissflogii* (D–F) in experiments carried out under artificial radiation conditions (UV-B: 1.45 W m^{-2}, UV-A: 58.9 W m^{-2}, PAR: 698 μmol photons m^{-2} s^{-1}). Percentage of change of yield (*Y*) relative to time zero (t_0) in *C. gracilis* samples exposed to: (A) PAR + UV-A + UV-B (PAB treatment) and; (B) PAR only (P treatment). (C) Percentage UVR-induced inhibition (relative to samples under the P treatment) during these daily cycles. Percentage of change of yield (*Y*) relative to time zero (t_0) in *T* exposed to: (D) PAR + UV-A + UV-B (PAB treatment) and; (E) PAR only (P treatment). (F) Percentage UVR-induced inhibition (relative to samples under the P treatment) during these daily cycles. Symbols indicate the different temperature treatments: 18_18 (black circles), 18_23 (white circles) and 23_23 (white triangles). The lines on top of symbols are the half mean range. White and gray areas indicate the period of exposure and recovery, respectively.

the 18_23 treatment for both radiation treatments whereas the lowest were found generally at 18_18 (Fig. 3A and B). Although significantly lower NPQ values were determined in *T. weissflogii* as compared to that in *C. gracilis* (Fig. 3C and D) throughout the experiment, some differences were observed among temperature treatments, with a relatively higher NPQ values in samples under the 23_23 treatment (Fig. 3C).

3.3. Experiments under artificial radiation

In order to get detailed information on the inhibition and recovery of *Y* in *C. gracilis* and *T. weissflogii* exposed under increased radiation conditions (i.e., as compared to that under solar radiation) we designed several experiments under a solar simulator. As representative of the data obtained in these experiments, in Fig. 4 we present the results obtained at intermediate irradiance conditions – i.e., UV-B: 1.45 W m^{-2}, UV-A: 58.9 W m^{-2}, PAR: 152 W m^{-2} (698 μmol photons m^{-2} s^{-1}). Similarly as with the experiments carried out under solar radiation conditions, we determined a clear pattern of inhibition and then recovery, once the stress was removed (Fig. 4A–E). There were however, differences in the responses between *C. gracilis* and *T. weissflogii*. During exposure, the highest *Y* values under PAB of *C. gracilis* (Fig. 4A) were determined in the 23_23 treatment, whereas the lowest ones were found in the 18_18 treatment. During the recovery however, *Y* values in the 18_23 treatment were significantly higher than in the rest of them. Under PAR alone (Fig. 4B) the values in the 18_23 treatment were significantly higher throughout the experiment (i.e., inhibition and recovery). After 1 h of recovery, *C. gracilis* samples reached ~90% of the initial *Y* value in the PAB treatment, whereas ca. 100% under PAR alone (Fig. 4A and B). For *T. weissflogii* however, much lower *Y* values were obtained in the PAB (Fig. 4D) as compared to those in the P treatment (Fig. 4E). Within any radiation treatment, the highest *Y* values were determined under the 23_23 treatment, and the lowest ones were obtained at 18 °C. *T. weissflogii* had a much lower recovery during the first hour than *C. gracilis*, and reached ~60% and ~80% in the PAB (Fig. 4D) and P (Fig. 4E) treatments, but attained the initial value after 2 h. Fig. 4C and F shows the contribution of UVR in inducing inhibition during these experiments: For *C. gracilis* (Fig. 4C) the lowest inhibition values during exposure were found in the 23_23, whereas the highest inhibition values were determined in 18_23 treatment. For *T. weissflogii* (Fig. 4F) the highest inhibition (up to 65%) was determined in the 18_18 whereas the lowest were found in the 23_23 treatment. Table 2 shows the mean contribution of UV-A and UV-B in inducing inhibition in *C. gracilis* and *T. weissflogii* during these experiments under artificial radiation, being UV-A the waveband that contributed to the bulk in both species. In particular, and for *C. gracilis*, the highest UV-A inhibition was generally registered in the 18_18 treatment, with a trend for increasing inhibition with irradiance. Overall, the highest inhibition values (i.e., 61.35% and 28.45% for UV-A and UV-B, respectively) were determined in the 18_18 treatment when samples were exposed to a UVR + PAR irradiance of 315 W m^{-2}. Similarly, and for *T. weissflogii* the highest UV-A-induced inhibition value (i.e., ~80%) was determined in the 18_18 treatment under the highest irradiances used in the experiments,

Table 2
Mean maximum (±SD) inhibition (as percentage relative to P treatment) of samples exposed to three levels of artificial radiation under different temperatures (i.e., 18_18 °C; 18_23 °C and 23_23 °C). Mean inhibition was calculated from the exposure period (1 h). No data are available for the highest irradiance under the 18_23 °C treatment. Full explanation in the text.

Temperature treatments	Radiation treatments	*Chaetoceros gracilis*		*Thalassiosira weissflogii*	
		UV-A inhibition	UV-B inhibition	UV-A inhibition	UV-B inhibition
18_18 °C	110 W m^{-2}	24.7 ± 5.7	13.4 ± 2.9	49.3 ± 3.8	10.0 ± 2.2
	210 W m^{-2}	36.5 ± 3.0	6.9 ± 3.9	53.2 ± 7.7	7.2 ± 4.1
	315 W m^{-2}	34.5 ± 5.7	6.5 ± 2.6	73.8 ± 4.0	15.1 ± 4.7
18_23 °C	110 W m^{-2}	30.4 ± 4.9	3.4 ± 2.6	40.6 ± 3.7	10.0 ± 2.9
	210 W m^{-2}	31.9 ± 4.3	7.6 ± 2.9	50.5 ± 4.2	7.1 ± 3.5
23_23 °C	110 W m^{-2}	21.0 ± 1.8	5.8 ± 1.8	32.2 ± 6.4	7.0 ± 4.9
	210 W m^{-2}	14.4 ± 2.1	0	41.7 ± 4.9	8.0 ± 5.5
	315 W m^{-2}	21.6 ± 3.9	0	55.4 ± 18.0	0

Table 3
Statistical results of one- and two-ways ANOVA to establish differences in *Y* inhibition between radiation treatments, between temperature treatments, and combined radiation * temperature during the solar radiation exposures. Radiation treatments are: PAB = PAR + UV-A + UV-B; PA = PAR + UV-A; P = PAR. Temperature treatments: *a* = 18_18 °C; *b* = 18_23 °C; *c* = 23_23 °C.

	Radiation treatments		Temperature treatments		Radiation * temperature
C. gracilis	18_18 °C (*a*)	$p \leqslant 0.001$		$p \leqslant 0.05$	PAB*a* = PAB*b* = PAB*c*; $p > 0.05$
		PAB = PA ≠ P	PAB	*c* = *b* ≠ *a*	PAB*a* ≠ PAB*c*; $p < 0.05$
					P*a* ≠ (P*b*, P*c*); $p < 0.05$
	18_23 °C (*b*)	$p \leqslant 0.001$		$p \leqslant 0.001$	P*b* = P*c*; $p > 0.05$
		PAB = PA ≠ P	PA	*c* = *b* ≠ *a*	PAB*a* ≠ (P*a*, P*b*, P*c*); $p < 0.05$
					PAB*b* ≠ (P*c*, P*b*); $p < 0.05$
	23_23 °C (*c*)	$p > 0.05$		$p \leqslant 0.001$	PAB*b* = P*a*; $p > 0.05$
			P	*c* ≠ *b* ≠ *a*	PAB*c* = (P*a*, P*b*, P*c*); $p > 0.05$
T. weissflogii	18_18 °C (*a*)	$p \leqslant 0.001$		$p \leqslant 0.05$	PAB*a* ≠ (PAB*b*; PAB*c*); $p < 0.05$
		PAB = PA ≠ P	PAB	*c* = *b* ≠ *a*	PAB*b* = PAB*c*; $p > 0.05$
					P*a* ≠ (P*b*; P*c*); $p < 0.05$
					P*b* = P*c*; $p > 0.05$
	18_23 °C (*b*)	$p \leqslant 0.001$		$p \leqslant 0.001$	PAB*a* ≠ (P*a*; P*b*; P*c*); $p < 0.05$
		PAB = PA ≠ P	PA	*c* ≠ *b* ≠ *a*	PAB*b* ≠ (P*b*; P*c*); $p < 0.05$
					PAB*b* = P*a*; $p > 0.05$
	23_23 °C (c)	$p > 0.05$		$p \leqslant 0.001$	PAB*c* ≠ (P*b*; P*c*); $p < 0.05$
			P	*c* ≠ *b* ≠ *a*	PAB*c* = P*a*; $p > 0.05$

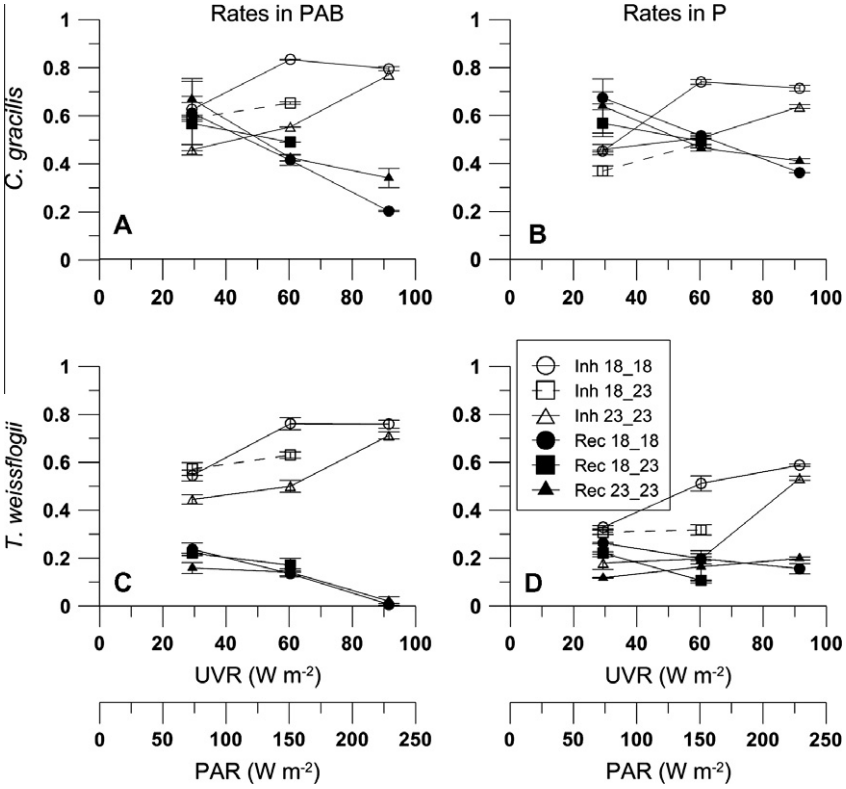

Fig. 5. Inhibition and recovery rates in *Chaetoceros gracilis* (A and B) and *Thalassiosira weissflogii* (D and E) calculated from data obtained in experiments done under artificial conditions and under increasing irradiances. Rates of *C. gracilis* under: (A) PAR + UV-A + UV-B (PAB treatment) and; (B) PAR only (P treatment). Rates of *T. weissflogii* under: (C) PAR + UV-A + UV-B (PAB treatment) and; (D) PAR only (P treatment). White and black symbols indicate inhibition and recovery rates, respectively. Circles, squares and triangles are samples exposed to the 18_18, 18_23 and 23_23 treatments. The lines on top of symbols are the half mean range.

although a high value (~74%) was also registered in the 23_23 treatment. Finally, UV-B induced inhibition reached the highest value in the 18_18 treatment, i.e., ~20% under the highest irradiances used for these experiments.

Fig. 5 resumes the photochemical dynamics of *C. gracilis* and *T. weissflogii* exposed to the different irradiance and temperature levels. For simplicity we are presenting the rates of inhibition and recovery in the PAB and P treatments as a function of irradiance. When analyzing the rates of inhibition and recovery (Fig. 5) two outcomes are clearly observed: One is that with increasing irradiance the inhibition rates increased, while the recovery rates decreased in both species and radiation/temperature conditions. In addition, temperature increase had a significant effect in decreasing the inhibition rates, but rather little effect on the recovery rates. When comparing the particular responses of the two species tested, it is seen that inhibition rates in *C. gracilis* are somehow lower under the P treatment (Fig. 5A and B); in *T. weissfloggii* these differences were much more evident (Fig. 5C and D). In regard to the recovery rates, the rates of change with increasing irradiance were steeper in *C. gracilis* under the PAB treatment as compared to that under P (Fig. 5A and B) as also determined for *T. weissflogii* (Fig. 5C and D).

4. Discussion

Our research focused on the combined effects of PAR/UVR and temperature on photosynthesis (as assessed through measurements of chl-*a* fluorescence of the PSII) of two diatom species – *C. gracilis* and *T. weissflogii*, and the main outcome of our work was of a beneficial effect of temperature by partially counteracting the UVR-induced inhibition of photosynthesis. Similarly, previous studies carried out with the diatom *Thalassiosira pseudonana* [14] determined that during short-term exposures this organism had higher sensitivity towards UVR when exposed to cold temperatures. However, this beneficial response is not universal, as other studies carried out with symbiotic dinoflagellates [27] have shown stronger UVR effects under high temperatures, due to the production of high amounts of superoxide radicals. In another study carried out with planktonic cyanobacterial surface bloom [28] elevated temperatures were more damaging to photosynthesis when cells were simultaneously exposed to high irradiance. On the other hand, Roos and Vincent [8] did not find any effect of temperature on UVR-induced photosynthesis in the cyanobacterium *Phormidium murrayi* pre-acclimated to 20 °C and exposed to UVR and PAR at 5, 10, 15 and 20 °C. So it is obvious, on the one hand, the species-specificity in responses and, on the other hand, the complexity of process taking place when exposing organisms to multifactor variables. For example, sub-optimal temperature effects on photosynthesis include rate limitation by enzymatic reactions due to thermodynamic constraints, limitation for inorganic phosphate, and feedback inhibition resulting from accumulation of photosynthetic end products [29].

Our results presented here demonstrate that photosynthesis of both *C. gracilis* and *T. weissflogii*, acclimated to 18 °C, similarly as if they were in surface waters in our study area during summer [30] is inhibited by both solar as well as by artificial radiation, as seen in the consistently reduction of *Y* during exposure (Figs. 2 and 4). As seen in other studies assessing spectral solar radiation effects on PSII photochemical efficiency of phytoplankton, most of the inhibition was due to PAR [4]; however, additional inhibition was observed due to the UVR. The observed UVR inhibition was

significantly higher in *T. weissflogii* as compared to that in *C. gracilis* (Figs. 2C and F and 4C and F) thus highlighting for a higher sensitivity of *T. weissflogii* during short-term exposures. Part of this differential sensitivity might be related to the cell size of the two species tested – *T. weissflogii* (~20 μm) and *C. gracilis* (~5 μm). In fact, previous studies have shown that large cells were more sensitive than small cells when considering photosynthetic inhibition [31] probably due to the fast acclimation kinetics of small cells [32] related in turn to the high surface/volume ratio. The size dependence of UVR effects are also related to the capacity of cells of synthesizing protective UV-absorbing compounds which is more important in large than in small cells, probably due to the fact that in small ones it would be energetically too costly [33]. During the time frame of our study, we did not register significant amounts of these protective compounds in any of the species tested (data not shown) but during long-term experiments (7 days of duration) it was determined that they were able to synthesize them in small amounts [34]. Thus although we did find obvious inter-specific differences in the photosynthetic responses, we do not know if they would vary over longer periods of time, i.e., if UV-absorbing compounds can reduce or even counteract the negative effects caused by UVR.

Other compounds however, are also important in helping cells to cope with excess radiation as it is the case of the enzymatic conversion of xanthophylls that protect cells from high PAR [35] as well as from UVR stress [36,37]. The de-epoxidation of diadinoxanthin, violaxanthin and antheraxanthin helps the energy dissipation of excess light and thus reduce any potential damage to PSII [38]. This de-epoxidation is triggered by the acidification of the chloroplast lumen and occurs within minutes from transfer of cells to excess irradiance [39] and can be adjusted on a longer time-scale (days) via by *de novo* synthesis of pigments [40]. Differences in the contribution of the xanthophyll cycle have been observed not only between green algae and diatoms [36,41] but also differences in down regulation capacity of excess energy were observed among diatom species [42]. One way to estimate this down regulation capacity is by analyzing the non-photochemical quenching process (NPQ) which occur in almost all photosynthetic eukaryotes, and help to regulate and protect photosynthesis in environments in which light energy absorption exceeds the capacity for light utilization [39]. In our study, this mechanism seemed to be significant in *C. gracilis* but in much less extent (or almost null) in *T. weissflogii* (Fig. 3). Recent studies [38] however, suggested that *T. weissfloggii* had the capacity for down regulation of excess energy via xanthophyll cycling but in those experiments the cells were exposed to higher solar irradiances (i.e., during summer time – January–February) than in those used by us. Also, Buma et al. [37] found increased down regulation capacity of *T. weissflogii* when acclimated to summer (high) irradiances but in our study it seems that the previous light history of this organism was not high enough to enhance it, as also seen in other studies [43,44]. Moreover, in a study carried out with diatoms with different spatial distribution, it was observed that the NPQ of oceanic diatoms was induced at low light intensities; on the other hand, the NPQ of estuarine diatoms was triggered only when the light intensities were 3–5 times higher than the light intensity at which the diatoms were grown [42]. The adaptation of estuarine diatoms to a variable light regime is such that NPQ can be standing-by and be readily triggered only if the PSII redox-state is very close to saturation [45]. Therefore, the different responses observed in the two studied species seems to be adaptive (i.e., genetic). Another explanation for the very low NPQ values in *T. weissflogii* could be the fact that NPQ in diatoms presents different phases: (i) A transient component generated immediately upon illumination, (ii) A steady-state component during later stages of the high-light illumination, and (iii) A fast relaxing component upon a transition of high-light to darkness [46]. In our study, the NPQ measurements were made relative to F_m measured after short dark incubation, so one possibility is that this time was not enough for relaxing some components of NPQ, and consequently, NPQ values might have been slightly underestimated (i.e., *T. weissflogii*).

In the previous paragraphs we considered the responses of cells when they were acclimated to the *in situ* summer temperature (18 °C). However, in our study we also considered a potential temperature increase of 5 °C as it would occur due to climate change by the year 2100 [16]. We simulated this temperature change in two ways, i.e., one as a sudden increase (within minutes) by incubating cells previously acclimated to 18 °C and exposing them to 23 °C, and the other by acclimating cells for a longer period of time to 23 °C. Although a sudden increase in temperature might seem unreal at first sight, previous studies deploying floats suggested that phytoplankton cells were trapped in a very shallow transient upper mixed layer (UML) in which they were exposed not only to high irradiances but also to a sudden increase in temperature [47]. Our data supported the initial view that cells of *C. gracilis* and *T. weissflogii* benefited from the increase in temperature, having a better performance by counteracting (at least partially) the UVR stress. In fact, the antagonic effects between temperature and UVR was clearly observed in all the treatments for *T. weissflogii* (but only some of *C. gracilis*, Table 3) that showed a negative interaction with UVR on yield inhibition (figure not shown; see also [26]). The differences in the PSII photochemical efficiency responses not only showed that species benefited from the pre-acclimation to 23 °C (i.e., under the 23_23 treatment) but also that they were able to respond very fast to the sudden temperature increase (i.e., from 18 °C to 23 °C). This resulted in an improved response not only by reducing the inhibition of *Y* (Figs. 2 and 4) but also increasing the down regulation of excess energy via NPQ, especially in *C. gracilis* (Fig. 3). In agreement with our findings, a better response in the photochemical activity in cells that had a fast acclimation to a high temperature was also observed in *T. pseudonana* acclimated to 20 °C and exposed to 15, 20 and 25 °C [14].

One main target for photoinhibition is the D1 protein of the PSII, which controls the electron transport after the primary photon absorption; this protein is subsequently removed by proteases resulting in a reduced photochemical efficiency [48]. During recovery, however, a new protein is restored and this process is facilitated by a rapid turn-over of the protein [49]. In our case, we expected that an increase in temperature would have resulted in a faster metabolism activity and thus a fast turn-over of the D1 protein as seen in other studies [50–52], or in a fast recovery as seen in *T. pseudonana* [14]. However, this was not clearly evident from our data, as there were slight differences during recovery among the various temperatures and irradiance conditions (Fig. 5). During exposure however, it was clear that a temperature increase diminished the rates of inhibition (Fig. 5). This indicates that during exposure the balance between negative (inhibition)/positive (repair-dissipation) effects shifted towards a more positive balance with increasing temperature, suggesting that although maximum inhibition was reached during our experiments, repair-dissipation was enhanced by rising the temperature. Of course this balance also showed some limitations, as the temperature difference was observed at the two lowest irradiances, but it was not so evident at the highest irradiance used. This fact could be related to a boundary of temperature dependence at saturating irradiances. In fact, Miller et al. [53] found that the degree of temperature stress (as assessed through measurements of carbon assimilation) was dependent upon light intensity, and this light-dependent temperature effect may have involved both reduced photochemical efficiency at sub-saturating irradiances and low saturating irradiances at both supra-and sub-optimal temperatures.

The question on why the species respond differently to the same variables can also be related to the use of different niches. It is known that a light gradient exists in the marine environment that ranges from dynamic and turbid estuarine waters, to calm and clear oceanic waters [54]. In fact, the species used in our work are characteristic from different niches, being *T. weissflogii* an estuarine species whereas *C. gracilis* is often coastal. Therefore, the better performance under UVR stress displayed by *C. gracilis* could be a consequence of adaptation to higher irradiances, including UVR, experienced during ocean water stratification events, whereas *T. weissflogii*, adapted to turbid estuarine waters with continuous mixing, has limited capability to tolerate a prolonged exposure at simulating surface water irradiances.

In summary, our data highlight the differential responses of species, due to their species-specific sensitivity as well as to their variable acclimation capacity as a consequence of different underwater light climate. These observed differences in responses among phytoplankton species are critical at the time to asses any impact of climate change. In this manuscript we demonstrated, that temperature increase might counteract the UVR negative impact. However, we are aware that extrapolation of simulated *in situ* short-term experiments needs to be considered carefully, as this type of data might explain only part of the responses due to climatic change of organisms living in natural ecosystems. In fact, studies comparing short- and long-term combined effects of radiation and temperature have shown that organisms response is quite different depending on the time-scale [14]. Moreover, and as discussed above other species/system respond differently, thus leaving a variability that would affect in various ways different regions. Therefore, more investigations considering the combined temperature-radiation effects on natural phytoplankton populations over longer periods of time need to be done in order to obtain a better understanding of possible consequences of climate change in Patagonia.

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