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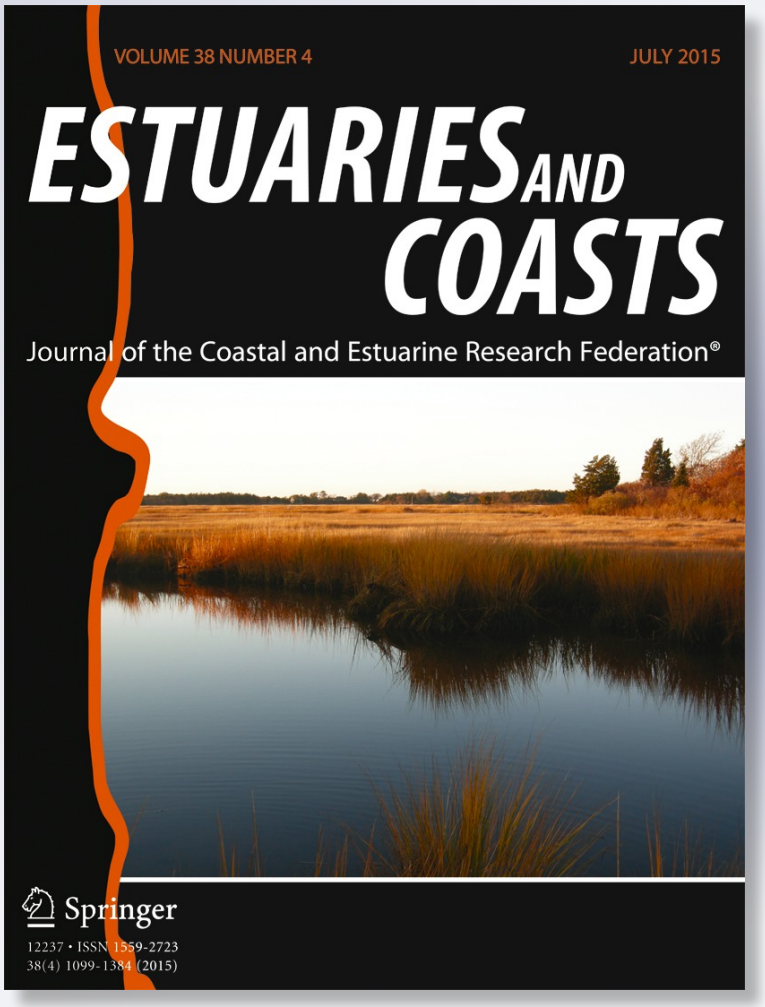

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Differential Responses of Two Phytoplankton Communities from the Chubut River Estuary (Patagonia, Argentina) to the Combination of UVR and Elevated Temperature

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Abstract The combined impact of solar ultraviolet radiation (UVR) and elevated temperature on the photosynthesis of phytoplankton communities from the two end members of the Chubut River estuary was studied using short- and long-term incubations. Samples from the river and seawater sections of the estuary were collected during the austral Spring (November 2011) and exposed to solar radiation (with and without UVR) under two temperatures: in situ and increased by 5 °C. The long-term incubations were designed to assess the potential acclimation of each phytoplankton community to the experimental conditions. Short-term incubations, at day 1 and day 5 of the acclimation period, included the determination of Photosynthesis vs. irradiance curves, Biological Weighting Functions and daily cycles of Chl-a fluorescence measurements, and were used as an estimation of the ability to cope with either increased temperature or UVR. There was a synergistic effect between UVR and temperature in the seawater community, with samples demonstrating a better photosynthetic response in the absence of UVR and at the in situ temperature. In contrast, temperature had an antagonistic effect when combined with UVR in the river community, with increased temperature counteracting the negative impact of UVR. Despite this, both communities showed an acclimation capacity toward UVR and increasing temperature. Our results point to a differential impact of global change variables on phytoplankton communities within the estuary, which affect photosynthesis and might influence the species composition with potential repercussions on the food web.

Keywords Global change · Patagonia · Photosynthesis · Phytoplankton · Temperature · Ultraviolet radiation

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Introduction

Ultraviolet radiation (UVR, 280–400 nm) is known to produce negative effects on phytoplankton, such as a decrease in growth and photosynthetic rates (Villafañe et al. 2003; Neale et al. 1998; Banaszak and Neale 2001; Litchman and Neale 2005), changes in morphology (Fiorda Giordanino et al. 2011), and damage to the DNA molecule, membrane lipids, and proteins (Buma et al. 2003). In a scenario of global change, with predictions of increased temperatures of up to 3–5 °C by the end of the century (IPCC 2007), it is highly plausible that the impact of UVR on metabolic processes and cell components will differ in comparison to present day conditions. These effects may be exacerbated because UVR exposure is expected to increase as a result of greater stratification of the water column under global change conditions (Häder et al. 2011).

The combined effects of UVR and increased temperature on phytoplankton are variable. In general, increased temperatures counteract the negative effects produced by UVR wavelengths by reducing UVR-induced photoinhibition, as shown in some species of marine diatoms (Sobrino and Neale 2007; Halac et al. 2010). A detailed study (Helbling et al. 2011), in which four different cellular targets involved in the photosynthetic process were evaluated under climate change conditions, found that increased temperature partially counteracted the negative effects of UVR on the diatom *Thalassiosira*

weissflogii by increasing the response of metabolic pathways, such as those that involve the enzyme Rubisco. However, in experiments carried out with cultures of the symbiotic dinoflagellate *Symbiodinium bermudense*, Lesser (1996) found synergistic effects of UVR and increased temperature due to increased oxidative stress that resulted in higher UVR-induced photoinhibition. Moreover, in investigations carried out with tropical marine assemblages of the Caribbean Sea, Halac et al. (2013) also found that increased temperature negatively affected the growth of some diatoms.

Such variable responses are also found in studies focusing on phytoplankton from estuaries. For example, Bergmann et al. (2002), working in the Neuse River estuary (North Carolina) evaluated the interaction between UVR and nutrient limitation on photosynthesis, finding important effects of nutrient addition during the stratification period, but none under mixing conditions. Ferreyra et al. (2006), working in the St. Lawrence estuary, did not find significant differences among radiation treatments (normal vs. enhanced levels of UV-B) in phytoplankton abundance and biomass. Likewise, Forster and Schubert (2001), working in the Darss-Zingst estuary in the Baltic Sea, did not find significant effects of UVR on chlorophyll, photosynthetic performance, or taxonomic composition. On a short-term basis, Zhou et al. (2009) compared the responses of phytoplankton to UV-B (280–315 nm) exposure between temperate and subtropical estuaries of China and found that the differences in responses (with the temperate ecosystem responding more strongly to variations in solar UV-B than the subtropical) were related not only to nutrients but also to stratification conditions. A recent study carried out with marine samples of the Chubut River estuary in Patagonia, collected at different times of the year (Villafañe et al. 2013a), determined that the combination of UVR and increased temperature had variable effects on the annual succession of phytoplankton. Increasing temperatures had little effect on prebloom communities but helped to counteract the magnitude of the decrease of the photochemical quantum yield during the bloom onset. However, during the bloom and in the spring, temperature and UVR acted synergistically, increasing the overall photochemical inhibition. These variable responses occurred because of the differences in the taxonomic composition of the assemblages and due to their differential capacity to acclimate to solar radiation. Finally, Thyssen et al. (2011) found both antagonistic and synergistic effects of UV-B (280–315 nm) combined with increased temperature on the growth of different groups (organized into clusters based on flow cytometry analysis) from natural communities of the St. Lawrence estuary. Thus, due to the antagonistic or synergistic nature of the interactions among stressors (Folt et al. 1999) together with species-specific responses, it is not possible to generalize how organisms or ecosystems respond based on studies carried out in other sites or communities.

Estuaries are transition zones between rivers and seas, with three clearly distinguishable zones: the outer (seawater), the central (mixed), and the inner (freshwater) regimes (Hansen and Rattray 1965). The central regime, characterized by a salinity gradient, is the result of mixing in various proportions of the two end members of the estuary (seawater and freshwater). The physical changes that occur along the length of estuaries will affect not only the chemistry via mixing but also the composition and distribution of the phytoplankton communities. In addition, the optical characteristics of the water column are different between the regimes, with less saline waters having, in general, higher attenuation of solar radiation than seawater, as observed in several estuarine systems of New England (Branco and Kremer 2005), thus conditioning the acclimation capacity of phytoplankton in the most turbid waters (Villafañe et al. 2004b; Helbling et al. 2013). Thus, one could expect that the phytoplankton communities, each characteristic of the two end members of the estuary, would display different responses to diverse environmental factors and in particular to stressors such as UVR. We used the Chubut River estuary, in the Atlantic coast of Argentina, as a model ecosystem to evaluate the impacts of UVR and increased temperature on the photosynthesis of phytoplankton communities from the two end members of the estuary. Specifically, our working hypothesis was that photosynthetic inhibition would be higher in phytoplankton from the inner regime (river, turbid waters) than from the outer regime (seawater, clear water) and therefore river communities would be more sensitive to UVR than the seawater communities. Furthermore, an increase in temperature should benefit phytoplankton from the inner regime as they are more acclimated to extreme changes in temperature throughout the year. Our experimental approach was to expose spring samples of phytoplankton each collected from a marine and a river site to solar radiation (with and without UVR) under two temperatures: in situ and increased by 5 °C, and analyze the responses of two mechanisms associated with the photosynthetic process: carbon incorporation and photosystem II photochemistry. The results obtained here should be of key importance for the region, as any effect on the lower trophic levels would affect the whole Patagonian coastal ecosystem, which is characterized by high productivity (Skewgar et al. 2007).

Materials and Methods

Study Area and Sampling

Water samples were collected at the two end members (Hansen and Rattray 1965) of the Chubut River estuary: the seawater-influenced ocean-side zone (outer regime—Egi station 43°18.8′S, 65°02.0′W) and the river-side zone (inner regime—43°17.5′S, 65°09.7′W). The inner regime is

considered the part of the estuary with salinities<1 (i.e., river), while the outer regime has salinities>30 (Helbling et al. 1992). The Chubut River is the most important of the Chubut province running from the Andean Mountains and flowing into the Atlantic Ocean, forming a coastal plain mesotidal estuary (Piccolo and Perillo 1999). The river discharge is regulated by the Florentino Ameghino Dam that is located ca. 120 km upstream from the mouth, with a mean historical river discharge of ca. 60 m^3 s^{-1} (Helbling et al. 1992). The Chubut River estuary is characterized by a wide range of physical, chemical, and biological variables due to the interaction between the river and the sea, having important consequences not only for the diversity of phytoplankton (Sastre et al. 1998; Villafañe et al. 2004a; Villafañe et al. 2008) but also for the succession and development of the phytoplankton bloom (Barbieri et al. 2002; Villafañe et al. 2004a; Helbling et al. 2005) and the input of nutrients of anthropogenic origin carried by the river (Helbling et al. 1992; Helbling et al. 2010). The river carries particulate and dissolved materials that condition the penetration of solar radiation, with attenuation coefficients of PAR (k_{PAR}) that vary from 4 to 6 m^{-1} along the year; in contrast, to the much clearer outer regime, with k_{PAR} values varying between 1 and 2 m^{-1} (Helbling et al. 2010). The river also transports an important load of nutrients (Helbling et al. 1992) as, in its lower part (downstream from the Ameghino Dam), it passes through agricultural areas. A large portion of these nutrients is discharged into the outer regime thus maintaining a high production in the study area. Nevertheless, variations in nutrients are observed along the year with range values of 0.20–21, 0.19–6.4, and 1.7–236 μmol l^{-1} for nitrate, phosphate, and silicate, respectively (Helbling et al. 2010). Since the river discharge is minimal during the austral summer, this is also the period when fewer nutrients are transported into the sea by the river.

Surface samples were collected on the 6th and 13th of November 2011 (seawater and river, respectively) with an acid-washed (1 N HCl) bucket, prescreened (250 μm Nitex mesh) into 50 l opaque acid-washed containers and transported to the Estación de Fotobiología Playa Unión (EFPU, 10 min away from either sampling site) to be used in experiments. We considered that surface water samples were representative of the water column as both, the seawater side and the river side, are completely mixed during austral spring due to the strong winds in the area (Villafañe et al. 2004a). The wind speed during our sampling had mean daily values of 24 and 28 km h^{-1} for the 6th and 13th of November 2011, respectively, and values>30 km h^{-1} in the previous days.

The samples (5 l) were dispensed into approx. 10 l capacity (microcosms) UVR-transparent bags (Alpax Trade Lab, São Paulo, Brazil, 72 % transmission at 280 nm) and exposed to solar radiation; seawater samples were incubated from the 7th to the 11th of November 2011, while the river samples were incubated from the 14th to the 18th of November 2011. Two radiation conditions were used: (1) PAB, samples exposed to full solar radiation, uncovered bags, and (2) *P*, samples exposed only to PAR (400–700 nm) using an Ultraphan 395 nm filter. The bags (triplicates for each radiation treatment) were placed outdoors in a water bath incubator that had two independent circuits for temperature control. One set of bags (3 PAB and 3 P) was incubated at the in situ water temperature (15 °C), while another set (3 PAB and 3 P) was incubated at the increased temperature (20 °C). The bags were manually shaken every 1 h so phytoplankton cells could not settle to the bottom of the bags and would receive homogeneous irradiances.

On day 1, initial samples for chlorophyll a (Chl-*a*), cell identification, *P* vs. *E* curves and BWFs (see below) were taken from the 50-L container, while samples for the fluorescence measurements were taken directly from the microcosms. On the last day of exposure (day 5), all samples were taken from each of the twelve microcosms. The following experiments and determinations were performed:

Daily Cycles

To study the variations of the effective photochemical quantum yield (Y) throughout the day in the initial samples (day 1) and after acclimation to the four radiation and temperature treatments (day 5), daily cycles were performed with samples from seawater or river. Every two hours between 08:00 and 18:00 h, sub-samples (3 mL) were taken from each microcosm (therefore we had triplicates for each condition) with a syringe to measure the in vivo Chl-*a* fluorescence emission as described below. The content of the bags were manually mixed just before each sampling and one syringe was dedicated to each bag to prevent cross-contamination between samples.

The rates of UV-induced damage to the photosynthetic apparatus estimated via the decrease in the photochemical quantum yield (k, min^{-1}), and repair (r, min^{-1}) were calculated using the equation of Heraud and Beardall (2000).

Photosynthesis vs. Irradiance Curves (P vs. E)

The samples were placed in 20 mL quartz tubes, inoculated with 5 μCi (0.185 MBq) of labeled sodium bicarbonate (Steemann Nielsen 1952), and exposed under a solar simulator (Hönle system, SOL 1200) for 1 hr at the corresponding incubation temperature (i.e., 15 and 20 °C). Seven irradiance levels were used (i.e., 100 %, 50 %, 25 %, 12 %, 6 %, 3 %, and 1.5 % of total irradiance output by the solar simulator); in six of them, the tubes were covered with an increasing number of neutral density screens reaching up to 6 layers, while in the 7th, the tubes were not covered with a screen. All tubes (total of 42 for each temperature) were distributed in two sets of triplicate samples (i.e., one sub-sample coming from each

replicate microcosm) at each irradiance level. One set of samples (21 tubes with samples coming from the *P* microcosms) was covered with an Ultraphan 395 nm filter to screen out all UVR, while the other set (21 tubes with samples coming from the PAB microcosms) was covered with an Ultraphan 295 nm filter to screen out any UVC output from the solar simulator. The whole setup was placed at 85 cm from the Hönle lamp, thus receiving maximum irradiances of 150, 49, and 1.22 W m^{-2}, for PAR, UV-A, and UV-B, respectively. *P* vs. *E* curves were obtained on the original community (day 1) and again on day 5 with samples coming from each of the PAB- or *P*-treated microcosms and incubated at the appropriate incubation temperature and radiation condition (e.g., samples coming from the PAB were incubated under PAB, etc.). After the 1-hr exposure period, the samples were immediately filtered onto Whatman GF/F (25 mm) filters, which were placed into 7 mL scintillation vials and exposed to fumes of concentrated HCl overnight. The filters were then dried and 2 mL of scintillation cocktail (HiSafe 3, Perkin Elmer) was added to the vials. The samples were counted using a liquid scintillation counter and the carbon incorporated into each sample was calculated from the DPM counts (Holm-Hansen and Helbling 1995).

The parameters of the *P* vs. *E* curves were obtained using the model of Eilers and Peeters (1988) with the data fitted by iteration:

$$P = E/\left(aE^2 + bE + c\right)$$

where *P* is the production [μg C (μg Chl-*a*)$^{-1}$h^{-1}], *E* is the irradiance (μmol photons m^{-2} s^{-1}), and *a*, *b*, and *c* are the adjustment parameters. The initial slope (i.e., α), the maximum production rate (P_{max}) and the light saturation parameters (E_k) were expressed as a function of the parameters *a*, *b*, and *c* as follows:

$$E_k = (c/a)^{1/2}$$
$$\alpha = 1 / c$$
$$P_{max} = 1/\left[b + 2(ac)^{1/2}\right]$$

Biological Weighting Functions (BWFs)

BWFs were determined on quadruplicate samples for the initial community measurements (day 1), while triplicates (as before, with one subsample coming from each microcosm) were used on the last day (day 5) of experimentation with samples obtained from each microcosm. The samples were placed in 50 ml quartz tubes, inoculated with 5 μCi (0.185 MBq) of labeled sodium bicarbonate (Steemann Nielsen 1952), and exposed to natural solar radiation for 1 h, around local noon, to measure photosynthesis under six different spectral treatments achieved by using sharp cut-off Schott filters (WG280, WG295, WG305, WG320, WG360, and GG400). The incubations were exposed to the same temperatures as the incubations in the microcosms (i.e., 15 and 20 °C). The samples to determine radiocarbon incorporation were treated as mentioned above for the *P* vs. *E* curves. The BWFs for carbon fixation were calculated using an exposure–response curve based on irradiance (Neale 2000). The biological responses for each wavelength interval were expressed as a function of the average irradiance (over incubation time) during the exposure interval. The irradiance in each of the UVR intervals was obtained with a USB diode array spectro-radiometer (HR 2000CG-UV-NIR, Ocean Optics, USA) attached to a 10 m fiber optics cable and a cosine-corrected sensor. The spectral dependence of the BWF to the wavelength intervals used in our experiments was extracted using the method of Rundel (1983). A third-degree polynomial function was used to fit the data from each experimental series: the best fit was then obtained by iteration (r^2>0.95) (Buma et al. 2009).

Fluorescence Measurements

In vivo Chl-*a* fluorescence parameters of photosystem II were obtained using a portable pulse amplitude modulated fluorometer (Water-ED PAM, Walz, Germany). The effective photochemical quantum yield (*Y*) was calculated using the equations of Genty et al. (1989) as:

$$\text{Yield}\ (Y) = \Delta F : F'_m = F'_m - F_t : F'_m$$

where F'_m is the instantaneous maximum intensity of Chl-*a* fluorescence in an irradiated cell induced by a saturating white light pulse (~5,300 μmol photons m^{-2} s^{-1} in 0.8 s) in the presence of actinic light (130 μmol photons m^{-2} s^{-1}), and F_t is the steady-state fluorescence induced by a weak actinic light in light-adapted cells. Each sample was measured six times.

Pigment Concentration

The samples (100 ml) were filtered onto a Whatman GF/F filter (25 mm) and the photosynthetic pigments and UV-absorbing compounds extracted 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 the manuscript are those obtained with the fluorometer.

Taxonomic Analyses

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) and species were identified and enumerated using an inverted microscope (Leica model DM IL) following the technique described by Villafañe and Reid (1995).

Statistics

Exposure to solar radiation and temperature was undertaken using triplicate microcosms for each condition or treatment. Samples for BWFs, fluorescence parameters and P vs. E curves were also incubated using triplicates from each microcosm. Pigment concentrations and taxonomic analyses were done for each microcosm so triplicate samples were obtained after each determination.

A one-way repeated measurements analysis of variance (ANOVA) was used to determine if there were significant differences in Y between conditions or treatments on day 1 and day 5. A three-way ANOVA was used to determine interactions between radiation and temperature on inhibition (k) and recovery (r) rates on day 1 and day 5, with radiation, temperature, and date (i.e., day 1 and day 5) as factors. A three-way ANOVA was also used to determine differences in specific growth rates (μ) due to radiation, temperature, and sites (i.e., river and seawater). A four-way ANOVA was done to detect differences in P_{max} due to radiation, temperature, sites, and day (i.e., day 1 and day 5). When significant differences were determined, a post hoc Fisher least significant difference (LSD) test was performed. Significant differences between the samples exposed to different conditions or treatments were established using a 95 % confidence limit (Zar 1999).

Results

Growth and Changes in Community Structure in the Microcosms

The initial concentration of Chl-*a* were 7 and 16 μg Chl-*a* l^{-1} for seawater and river water, respectively. Both river and seawater phytoplankton actively grew in the microcosms as evidenced by their specific growth rates (μ), based on Chl-*a* determinations (Fig. 1). Two important features are evident. First, samples that received UVR had significantly lower μ than those exposed only to PAR, for the corresponding temperature or site. Second, the μ were significantly higher at 20 °C than at 15 °C for the corresponding radiation and site. We did not register significant concentrations of UV-absorbing compounds (based on absorption spectra) throughout the course of the experiments neither in seawater nor in river samples.

The initial seawater phytoplankton sample (total abundance of ~1,800 cells ml^{-1}) was dominated (ca. 70 %) by small unidentified flagellates (<10 μm), while the rest were diatoms of which small (10–20 μm) species of *Thalassiosira* Cleve were the most abundant. At the end of the experiment flagellates increased their dominance slightly in the treatments at 15 °C. The diatom community was co-dominated by small *Thalassiosira* and pennate diatoms e.g., *Nitzschia* Hassall and *Pseudonitzschia* H. Peragallo (20–50 μm). At 20 °C, the community was almost completely (>97 %) dominated by small unidentified flagellates in both radiation treatments. Dinoflagellates were virtually absent throughout the experiment.

The initial phytoplankton sample collected at the river (total abundance of ~2,500 cells ml^{-1}) was dominated by diatoms (almost 50 % of the total cell concentration), with *Aulacoseira granulata* (Ehrenberg) Simonsen being the most abundant, but other discoid (e.g., *Cyclotella* Kützing Brébisson) (10–20 μm) and pennate (20–40 μm) diatoms (e.g., *Navicula* Bory de Saint-Vincent) were also abundant. The rest of the community was characterized by chlorophytes (e.g., species of *Scenedesmus* Meyen, *Closterium* Nitzsch ex Ralfs) as well as small unidentified flagellates (<10 μm). At the end of the experiment, there were few changes in the river community with diatoms still dominating but with a slight relative increase in *A. granulata* and small discoid diatoms. Dinoflagellates were not observed in this experiment.

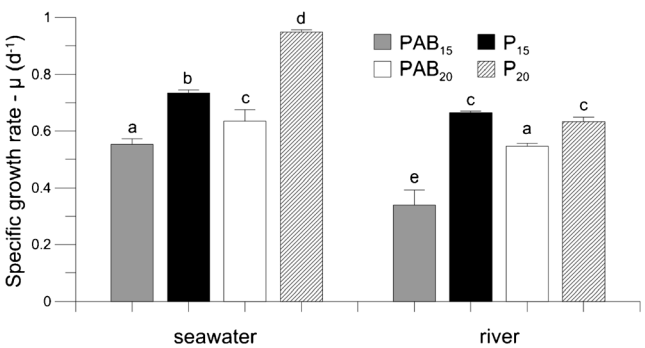

Fig. 1 Mean specific growth rates (μ), based on Chl-*a* concentration, of seawater and river communities under the different radiation and temperature conditions. The *vertical lines on top of the bars* indicate the standard deviation, while the *letters* indicate the result of the post hoc analyses

In samples from both communities, zooplankton was not observed, indicating a thorough removal of grazers by the pre-screening of the samples prior to the beginning of the experiment.

Daily cycles of Effective Photochemical Quantum Yield

Daily doses of solar radiation were similar during the 2 weeks in which the experiments were done, with values of 11.4 (SD=0.2) MJ m^{-2}, 1584 (SD=98) kJ m^{-2} and 42.8 (SD=0.8) kJ m^{-2} for seawater incubations, and 10.9 (SD=0.5) MJ m^{-2}, 1454 (SD=48) kJ m^{-2}, and 42.5 (SD=1.7) kJ m^{-2} for river water incubations. Seawater samples (Fig. 2) received similar radiation levels during the daily cycles on days 1 and 5, but with more clouds during the morning of day 1. On day 1 (Fig. 2a), there was a significant decrease ($P<0.05$) in the Y toward local noon as the samples received higher irradiances.

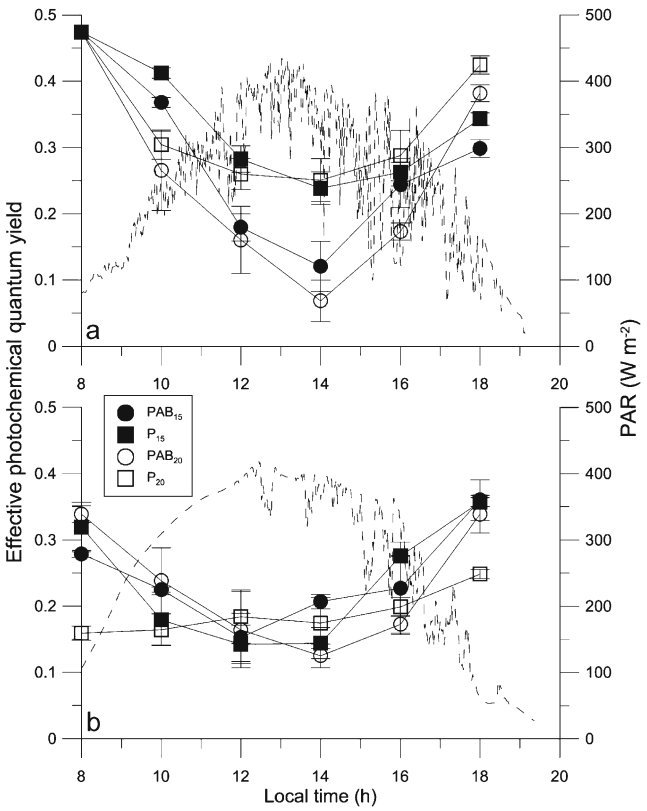

Fig. 2 Daily cycles of effective photochemical quantum yield (Y) of seawater samples exposed to solar radiation under full solar radiation (280–700 nm, *PAB*, *circles*) and PAR only (400–700 nm, *P*, *squares*) treatments and two temperatures (15 °C, *black symbols* and 20 °C, *white symbols*) during daylight exposures of: **a** Initial sample, day 1; **b** day 5. The *symbols* are the means of triplicate samples and the *vertical lines* indicate one standard deviation around the mean. The *broken lines* show the PAR irradiance during the day. The mean values of solar radiation during the incubations were 256, 38, and 1.03 W m^{-2} for PAR, UV-A, and UV-B, respectively, for day 1 (*panel a*) and 295, 43, and 1.17 W m^{-2} for PAR, UV-A, and UV-B, respectively for, day 5 (*panel b*).

The observed decay was significantly higher ($P<0.05$) in samples under the PAB treatment (75 % and 86 %, for samples incubated at 15 and 20 °C, respectively) as compared to those under the P treatment (50 % and 47 %, for samples incubated at 15 and 20 °C, respectively). Samples incubated at 20 °C had a higher decay in the Y from the start towards local noon, as compared to those incubated at the in situ temperature (15 °C). The former samples, however, had a faster recovery during the afternoon, that resulted in a higher Y at the end of the daily cycle. On day 5 (Fig. 2b), the samples still had a significant decrease in the Y toward local noon, but the UVR effect was not so evident. In addition, samples incubated at 20 °C had lower Y values than those incubated at 15 °C, in the afternoon, resulting in less recovery.

The inhibition (k) and repair (r) values of seawater samples on day 1 were higher ($P<0.05$) in samples under the PAB treatment than those exposed under the P treatment, for both temperature conditions (Fig. 3). However, by day 5, samples that received UVR+PAR (i.e., PAB treatment) had a slightly lower rate of inhibition and recovery than those receiving PAR only. In the case of the k value (P treatment) at 20 °C, there was no change in the Y from the early morning as compared to that at noon. The combined effects of UVR and temperature were significant (Table 1) and had a synergistic response with k values of samples exposed under the PAB treatment higher at 20 than at 15 °C. In the case of recovery, and as mentioned above, the samples under the PAB treatment at 20 °C had the highest values at the beginning of the experiment.

Radiation conditions during the incubation of the river samples (Fig. 4) were different, with clear skies on day 1 (Fig. 4a) but with high cloud frequency on day 5 (Fig. 4b). On day 1 (Fig. 4a), UVR had a significant effect on Y, with samples in the PAB treatments having lower ($P<0.05$) values

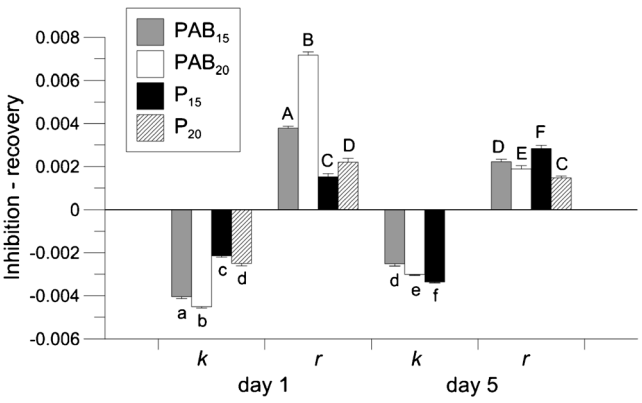

Fig. 3 Inhibition (k) and recovery (r) rates (min^{-1}), calculated from the Y data, of seawater phytoplankton communities exposed to full solar radiation (*PAB*) and PAR only (*P*) treatments and two temperatures (15 and 20 °C) during daylight exposures on day 1 and day 5. The *lines on top of the bars* indicate one standard deviation, while the *letters* indicate the result of the post hoc analyses. *Capital letters* are used for comparison of recovery rates, and *lower case letters* are used for inhibition rates.

Table 1 Results from the ANOVA analyses for inhibition (k), recovery (r), specific growth rate (μ) and P_{max}

	Seawater		River		μ	P_{max}
	K	r	K	r		
Rad	0.000	0.000	0.000	0.000	0.000	0.000
Temp	0.000	0.000	0.000	0.000	0.000	0.000
Date	0.000	0.000	0.000	0.000	n.a.	0.471
Site	n.a.	n.a.	n.a.	n.a.	0.000	0.000
Rad×temp	0.000	0.000	0.001	0.000	0.077	0.365
Rad×date	0.000	0.000	0.000	0.000	n.a.	0.000
Rad×site	n.a.	n.a.	n.a.	n.a.	0.162	0.104
Temp×date	0.000	0.000	0.000	0.000	n.a.	0.000
Temp×site	n.a.	n.a.	n.a.	n.a.	0.049	0.000
Date×site	n.a.	n.a.	n.a.	n.a.	n.a.	0.000
Rad×temp×date	0.000	0.000	0.000	0.000	n.a.	0.017
Rad×temp×site	n.a.	n.a.	n.a.	n.a.	0.000	0.000
Rad×date×site	n.a.	n.a.	n.a.	n.a.	n.a.	0.004
Temp×date×site	n.a.	n.a.	n.a.	n.a.	n.a.	0.000
Rad×temp×date×site	n.a.	n.a.	n.a.	n.a.	n.a.	0.103

Rad PAB and *P*, *Temp* 15 and 20ºC, *Date* day 1 and day 5, *Site* river and seawater, *n.a.* not applicable

than under the P treatment. In general, samples incubated at 20 °C performed better photochemically than at 15 °C, with a significant ($P<0.05$) antagonistic interaction between UVR and temperature (Table 1). On day 5, there were no differences between radiation or temperature treatments (Fig. 4b).

On day 1, river samples under the PAB treatment had higher ($P<0.05$) absolute k and r values than those under P (Fig. 5), but this difference was not that evident for k on day 5. In general, river samples incubated at 20 °C had a better photochemical response with lower inhibition rates (k values) than those incubated at 15 °C.

Photosynthesis–Irradiance Curves

The photosynthetic response of seawater samples to increasing irradiance (Fig. 6) was such that, on day 1 (Fig. 6a), cells had higher P_{max} when incubated at 15 °C than at 20 °C (Table 2). The UVR inhibition of P_{max}, however, was the same at both temperatures with a value of 13 %. A slight photoinhibition was observed toward higher irradiances with decreasing P_{max} values with the exception of samples under the P treatment at 15 °C. On day 5 (Fig. 6b), the situation reversed and cells had higher P_{max} at 20 °C than at 15 °C (Table 1). The UVR inhibition at 20 °C was about the same as on day 1 (15 %); the UVR inhibition of samples incubated at 15 °C, however, increased significantly ($P<0.05$) to a value of 46 %. Photoinhibition under higher irradiances was only significant in samples under the PAB treatment at 20 °C.

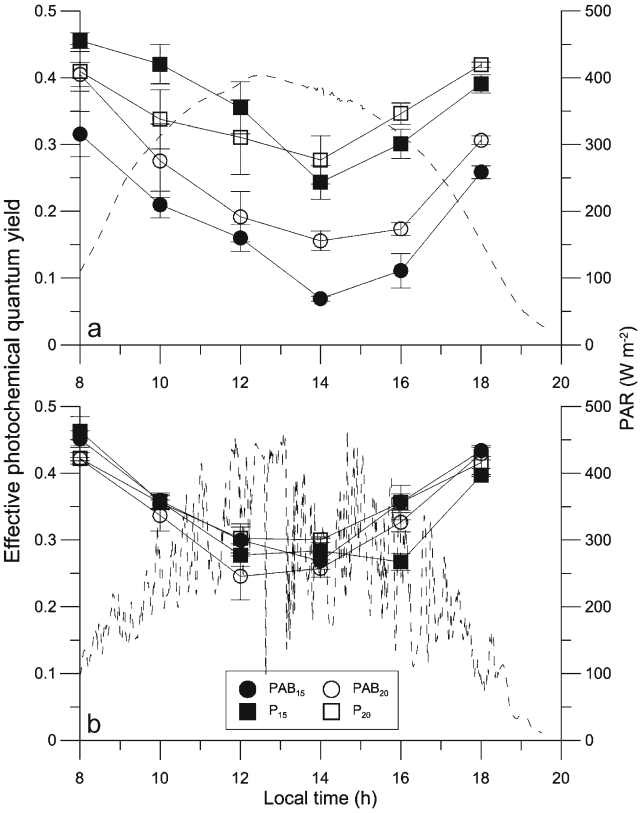

Fig. 4 Daily cycles of effective photochemical quantum yield (Y) of river water samples exposed to solar radiation under full solar radiation (280–700 nm, *PAB*, *circles*) and PAR only (400–700 nm, *P*, *squares*) treatments and two temperatures (15 °C, *black symbols* and 20 °C, *white symbols*) during daylight exposures of: **a** Initial sample, day 1; **b** day 5. The *symbols* are the mean of triplicate samples and the *vertical lines* indicate one standard deviation around the mean. The *broken lines* show the PAR irradiance during the day. The mean solar radiation during the incubations was 320, 45, and 1.25 W m^{-2} for PAR, UV-A, and UV-B, respectively, for day 1 (*panel a*) and 261, 35, and 1.11 W m^{-2} for PAR, UV-A, and UV-B, respectively, for day 5 (panel b)

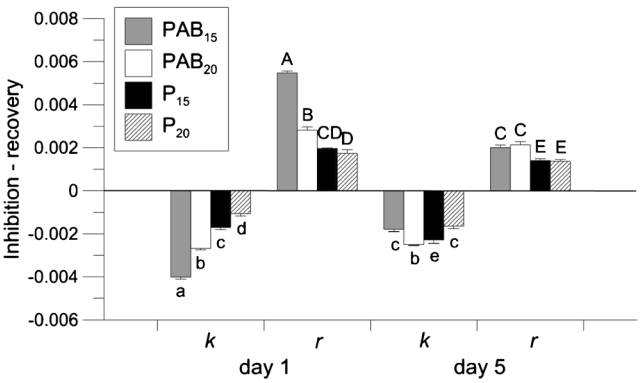

Fig. 5 Inhibition (k) and recovery (r) rates (min^{-1}), calculated from the Y data, of river phytoplankton communities exposed to full solar radiation (*PAB*) and PAR only (*P*) treatments and two temperatures (15 and 20 °C) during daylight exposures on day 1 and day 5. The *lines on top of the bars* indicate one standard deviation, while the *letters* indicate the result of the post hoc analyses. *Capital letters* are used for comparison of recovery rates, and *lower case letters* are used for inhibition rates.

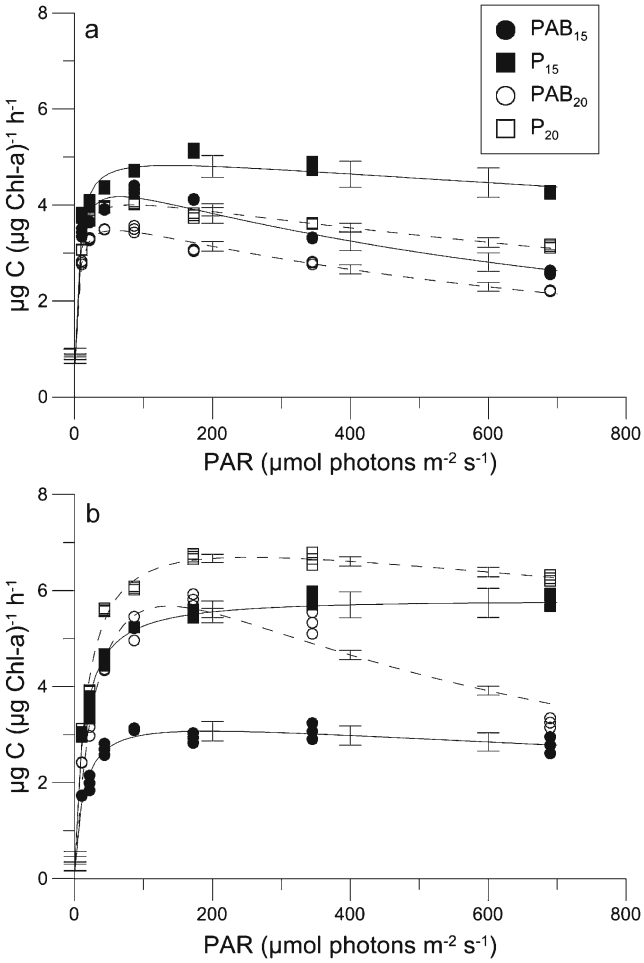

Fig. 6 Photosynthesis vs. irradiance (*P* vs. *E*) curves of seawater samples exposed to PAR+UVR (280–700 nm, *PAB*, *circles*) and PAR only (400–700 nm, *P*, *squares*) at two temperatures (15 °C, *black symbols* and 20 °C, *white symbols*). **a** Initial sample, day 1; **b** day 5. The *vertical lines* indicate the 95 % confidence limits of the model, while the *symbols* indicate the measured values

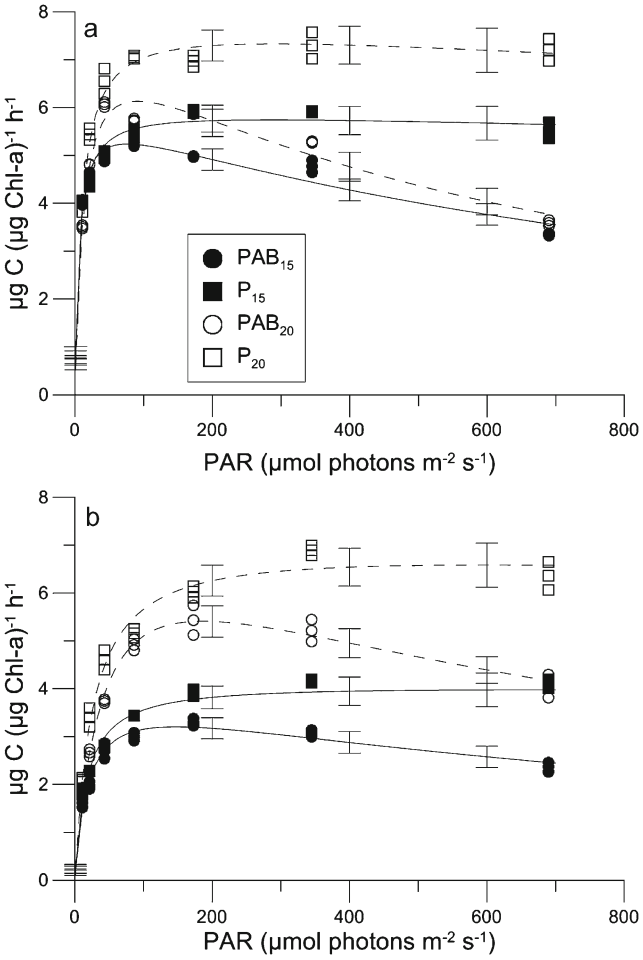

Fig. 7 Photosynthesis vs. irradiance (*P* vs. *E*) curves of river samples exposed to full solar radiation (280–700 nm, *PAB*, *circles*) and PAR only (400–700 nm, *P*, *squares*) at two temperatures (15 °C, *black symbols* and 20 °C, *white symbols*). **a** Initial sample, day 1; **b** day 5. The *vertical lines* indicate the 95 % confidence limits of the model, while the *symbols* indicate the measured values

In the case of river samples (Fig. 7), the photosynthetic response to increasing irradiance was such that, at all times (i.e., on day 1 and on day 5), samples incubated at 20 °C had higher P_{max} than at 15 °C (Table 2). The values of UVR inhibition of P_{max} at 15 and 20 °C were 8.7 % and 16 %, respectively, at the beginning, and 20 % and 18 %, respectively, at the end of the experimental period.

Table 2 Mean P_{max} values, and standard deviations between brackets, of seawater and river samples from the Chubut River estuary on day 1 and day 5 for the different radiation and temperature treatments—full radiation (*PAB*), and PAR only (*P*), at 15 and 20 °C

		Day 1		Day 5	
Sample	Radiation treatment	15 °C	20 °C	15 °C	20 °C
Seawater	PAB	4.18 (0.21)^{a}	3.47 (0.1)^{cd}	3.07 (0.22)^{c}	5.67 (0.61)^{bf}
Seawater	*P*	4.82 (0.21)^{b}	4 (0.08)^{adi}	5.76 (0.35)^{befgh}	6.7 (0.27)^{k}
River	PAB	5.24 (0.22)^{b}	6.13 (0.33)^{e}	3.2 (0.22)^{c}	5.41 (0.53)^{bfg}
River	*P*	5.74 (0.25)^{befgh}	7.33 (0.35)^{j}	3.98 (0.28)^{adi}	6.58 (0.42)^{ek}

The letters indicate the results of the post hoc analyses

Biological Weighting Functions (BWFs)

Seawater samples (Fig. 8a) had biological weights in the UV-B region that were not significantly different among treatments, whereas in the UV-A region, a differential sensitivity was observed, with the lower weights determined in samples under the *P* treatment and on day 1 at 15 °C. All other treatments had significantly higher weights (i.e., higher inhibition), but were similar among them. In river samples (Fig. 8b), significant differences were determined among the weights in the UV-B region, being higher in samples under *P* at 15 °C (day 5) and on day 1 at both temperatures than under PAB (both temperatures) and *P* at 20 °C (day 5). For the UV-A region, the highest weights were determined on day 1 (both temperatures) and the lowest in samples receiving the full spectrum of solar radiation.

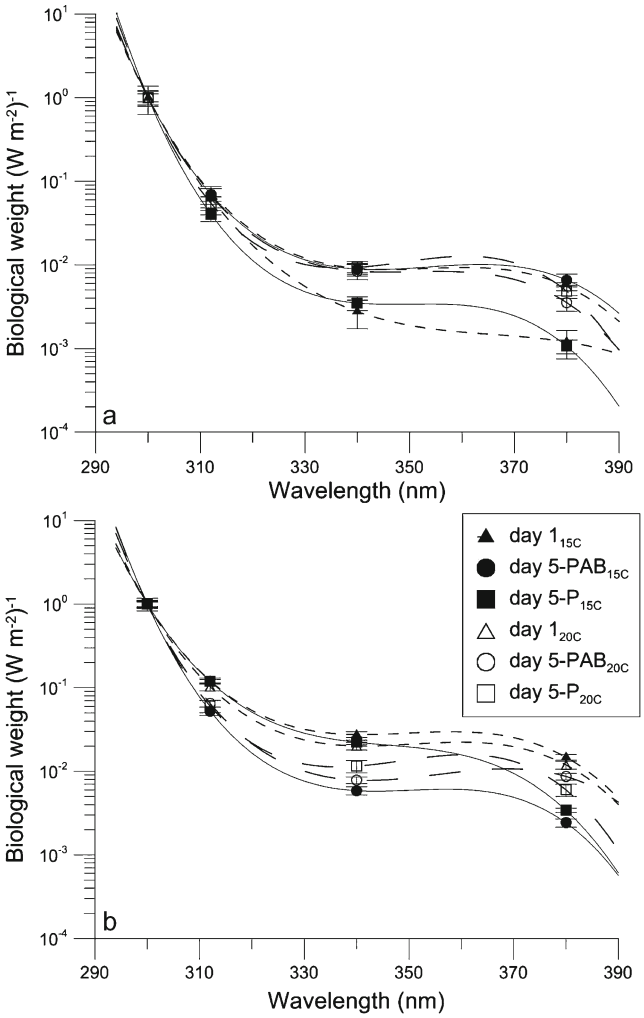

Fig. 8 Mean biological weighting functions (BWFs) of phytoplankton communities exposed to natural solar radiation under six spectral treatments and two temperatures (15 and 20 °C) on day 1 and on day 5. **a** Seawater samples; **b** River water. The *lines* indicate the results of the model output, while the *vertical lines* indicate the 95 % confidence limits of the model. The symbols indicate the biological weights determined for each Schott filter interval above 295 nm

Discussion

Estuaries, because of their key ecological importance, as nursery and breeding sites for many species, as "cleaners" due to their buffering capacity to filter out pollutants, and also as important sites for recycling of nutrients, have been the focus of several investigations devoted to evaluate the impacts of UVR on phytoplankton communities over mid- and long-term periods. However, we are not aware of any study that has evaluated simultaneously, the responses of both, seawater and river ends of an estuary, to solar UVR and increased temperature in the context of global change. The timing of our experiment (November, Austral Spring) is such that an enhancement of solar UV-B, related to the Antarctic ozone depletion, is observed over the study area (Helbling et al. 2005). Moreover, our experimental setup (i.e., with samples exposed to solar radiation under a relatively thin layer of water) represented a simulated case of "increased" radiation exposure as may happen in a scenario of global change. In regard to our experimental temperatures, we used in situ (as registered at the time of collection of samples) and an increase of 5 °C, as predicted by the year 2100 (Houghton et al. 2001). The results of our study point at two facts that are of key importance to evaluate the overall impact of global change variables on phytoplankton communities from the Patagonian coast. One refers to the ability of communities to acclimate to new (experimental) conditions and the other highlights the importance of considering synergistic and antagonistic effects among variables. Therefore, our discussion will focus on these two main aspects.

In the water column, cells receive a range of irradiances that depend not only on the attenuation of solar radiation but also on the mixing conditions and depth of the upper mixed layer (UML, Helbling et al. 1994; Neale et al. 2003). In our case, there were significant differences in these conditions between the two end members of the Chubut river estuary sampled for our experiments. The seawater side of the estuary is more transparent than the river side, with mean PAR attenuation coefficients of 0.37, and 4.8 m^{-1} for the Spring, for seawater and river, respectively; whereas the difference in the penetration of UVR wavelengths between the two water bodies is even more dramatic (Helbling et al. 2010). These differential light environments would suggest that seawater phytoplankton are high-light-acclimated and thus have, in principle, the means to cope with UVR, while river phytoplankton are low-light-acclimated, and thus would be highly sensitive to these wavelengths. This, however, was only partially observed in our experiments: for example, inhibition rates (based on PSII photochemistry) for seawater communities (Fig. 3) were higher than in river communities (Fig. 5) on day 1, which would suggest, at first look, a higher sensitivity of seawater phytoplankton. The inhibition due to UVR, however, calculated for these two communities was 88 % and 80 % for the

seawater community at 15 and 20 °C, respectively, and 136 % and 155 % for river phytoplankton at 15 and 20 °C, respectively (Figs. 3 and 5); so part of this variability was due to PAR inhibition that was higher in seawater phytoplankton. On the other hand, when looking at a different process (i.e., light and dark phase of photosynthesis) within the photosynthetic apparatus, it is seen that river phytoplankton had a better utilization of solar radiation with higher P_{max} values (Figs. 6a and 7a, Table 2). But on day 5, at the in situ temperature, phytoplankton from the river were more sensitive and with higher biological weights (Fig. 8) than seawater phytoplankton. Part of the variability for this differential response between the two communities lies in the mixing conditions and the depth of the UML: Spring is the windy season in Patagonia (Villafañe et al. 2004a), and this causes the cells to move up and down in a deeper UML (as compared to the rest of the year) and thus receive lower irradiances and doses than if they were continuously close to the surface. Even though this would be valid for both the sea and the river sections of the estuary, the bottom topography has a significant effect on the depth of the UML. On the ocean side of the estuary, phytoplankton can be mixed down to ca. 20 m, whereas in the river, the depth is shallower overall and, in the section that is close to the estuary, the depth is, on average, ca. 1 m. Taking, for example, a mean incident surface PAR irradiance of 300 W m^{-2}, the attenuation coefficients for seawater and river, and the maximum depth they can be mixed, phytoplankton moving within the UML at the seawater and river sides of the estuary, would receive a mean water column PAR irradiance of 40 and 49 W m^{-2}, respectively. Therefore, although the attenuation coefficients on the river side of the estuary are higher, the shallower depth would result in phytoplankton being exposed to similar mean irradiances as their ocean-side counterparts, although with different vertical gradients and residence time in each layer. This residence time or speed of mixing is a key factor that determines the impact of solar UVR on tropical phytoplankton (Helbling et al. 2003). Thus, these two variables, transparency and the depth of the UML might have counteracted each other in the acclimation of phytoplankton from the estuary at this time of the year.

In addition, the inhibition rates, calculated from the decrease in Y, were obtained at noon after exposure during the morning, while the P vs. E curves and BWFs are the result of a 1 h incubation at noon, thus in the former measurement (i.e., Y), acclimation processes might have already begun at day 1. Over these relatively short periods of time, acclimation of phytoplankton can be attained by processes such as reversible epoxidation/de-epoxidation associated with the dissipation of excess energy via non-photochemical quenching (NPQ; Finazzi et al. 2006; Van de Poll and Buma 2009). This de-epoxidation is triggered by the acidification of the chloroplast lumen and occurs within minutes of the transfer of cells to excess irradiance (Müller et al. 2001) and can be adjusted on a longer timescale (days) via by de novo synthesis of pigments (Kana et al. 1997). In this regard, NPQ was higher, for both phytoplankton communities, on day 1 than day 5 (data not shown), reflecting higher dissipation of excess energy on day 1. Similarly, in studies carried out in a tropical estuary of southern Brazil (Babitonga Bay), higher NPQ values were found at the beginning of the experiment (i.e., non-acclimated cells) or under less favorable conditions (i.e., ambient concentration of nutrients vs. addition of nutrients) after a few days of acclimation (Villafañe et al. 2013b).

Over longer periods of time, however, other photoacclimation mechanisms might allow cells to cope with excess irradiance as occurred in our experimental setup. One such proposed photoacclimation mechanism is the synthesis of UV-absorbing compounds (Banaszak 2003); however, the concentrations of these compounds in both the seawater and river samples were negligible. This seems to be a rather common feature of natural samples from the Patagonian coast as seen in previous studies carried out in the Chubut River estuary (Villafañe et al. 2004a, 2008, 2013a) as well as in nearby sites (Villafañe et al. 2004c; Marcoval et al. 2008). This may be associated with the fact that, in those experiments, the community was dominated by small cells within which the synthesis of photoprotective compounds would be costly in energetic terms (Garcia-Pichel and Castenholz 1993). In the present study, the communities were also dominated by flagellates and/or small diatoms. Another mechanism that allows communities to acclimate to solar radiation is by changing their composition and structure such that the fittest prevail under the changed conditions. In our experiments, the changes detected in the community structure were toward flagellates in seawater and toward diatoms in river samples, but they were more closely associated with changes in temperature than with solar radiation, as has also reported in studies carried out in the Mexican Caribbean (Halac et al. 2013). Previous studies showed that diatoms are resistant to UVR exposure by dissipating excess energy via NPQ (Halac et al. 2010) or by increasing Rubisco activity and gene expression (Helbling et al. 2011). However, Halac et al. (2009) found that the photoprotective capacity of the diatom *Phaeodactylum tricornutum* was diminished due to the negative effect of UVR on the xanthophyll cycle.

As there were variable photosynthetic responses to solar radiation not only among sites but also as a consequence of the different temperature treatments, our results also point to the importance of considering the combined effects of those variables (i.e., synergy/antagonism; Dunne 2010) rather than their individual effects. While UVR and/or excess irradiance have been found to be generally negative for different metabolic processes in phytoplankton (Villafañe et al. 2003), increased temperature is generally regarded as beneficial (Halac et al. 2010; Helbling et al. 2011; Sobrino and Neale 2007). However, some studies have determined a negative effect of increased temperature (Sobrino and Neale 2007; Halac et al.

2013), which acted synergistically with UVR. Indeed, and within a particular study site, different types or degrees of interactions have been determined between UVR and temperature, depending on the time frame of the study or the fraction of the community being analyzed (Thyssen et al. 2011; Villafañe et al. 2013a). It has been suggested that temperature interacts with UVR mainly on a short-term (h) timeframe (Sobrino and Neale 2007), but our experiments, in addition, suggest that long-term (days) incubations at increased temperature and UVR change the phytoplankton composition by favoring one species or group over another. The dynamics of change should benefit species that are normally exposed to changes in temperature and thus can acclimate faster. In fact, the temperature in the river has a higher mean range than that found downstream in the seawater (Helbling et al. 2010). In addition, due to the shallowness of the river, environmental changes should impact more quickly in the river than in the seawater, increasing the temperature faster and to higher values in comparison to the former. Thus, species in the river should be better able to cope with sudden temperature changes than seawater phytoplankton.

Our results indicate that there was a synergistic effect of UVR and temperature (i.e., higher UVR inhibition of photosynthesis at increased temperature) in phytoplankton samples collected at the seawater side of the estuary. This was observed in the *Y*, as well as in the carbon incorporation determined via *P* vs. *E* curves, and BWFs. However, there was an antagonistic effect of UVR and temperature (i.e., lower UVR inhibition of photosynthesis at increased temperature) in phytoplankton samples from the river side of the estuary. In both cases, however, the communities acclimated to the new conditions, both UVR and temperature, and had a better photosynthetic performance than when they were freshly collected. From our data reported here it is evident that climate change-related variables will differentially impact estuarine communities, with temperature playing a key role in increasing or decreasing the UVR impact. Part of this could be related to the magnitude of the disturbance in the environment as river species benefited from the increased temperature while seawater species were negatively affected. Any change in community composition might also result in a modification of the nutritional value of the different species thus affecting the food chain.

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