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# Long-Term UVR Effects Upon Phytoplankton Natural Communities of Patagonian Coastal Waters

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## 1. Introduction

Since the discovery of the ozone “hole” over the Antarctic continent and the concomitant increase in ultraviolet B radiation (UV-B, 280-315 nm) vast literature has been produced about the impact of these wavelengths on aquatic organisms. Nowadays it is widely accepted that ultraviolet radiation (UVR, 280-400 nm) acts as a stressor for both organisms and ecosystems, this being true not only for increased UVR but also for ‘normal’ levels (see review by Helbling and Zagarese 2003 and references therein). Many studies about the UVR impact on phytoplankton species and communities have reported negative effects on different cellular targets (e.g., photosystem II, DNA, proteins) which may affect several processes such as growth and photosynthesis (Vernet 2000; Villafañe et al. 2003). In particular, it has been found that UVR can significantly affect Photosynthesis versus Irradiance (P vs. E) parameters (Furgal and Smith 1997; Montecino et al. 2001; Villafañe et al. 2004c) thus remote sensing calculations of primary production based on them might be frequently overestimated.

On the other hand, some studies also documented positive effects of UVR, such as increased carbon uptake under UV-A (315-400 nm) exposure (Nilawati et al. 1997, Barbieri et al. 2002; Helbling et al. 2003). Exposure to UV-A can also induce the light-dependant enzymatic repair of the UVR-induced DNA damage (i.e., ‘photorepair’, Buma et al. 2003). Indirect effects of UVR such as the breakdown (photolysis / photodegradation) of dissolved organic matter (DOM) (Osburn and Morris 2003) can be either beneficial for organisms by means of increasing nutrient availability, or detrimental, by increasing water transparency so cells receive more irradiance (Cooke et al. 2006). It is also known that the interaction of UVR and other factors - e.g., nutrient availability, vertical mixing, changes in temperature, supersaturating oxygen concentrations or high pH values, may strongly condition the observed results (Forster and Schubert 2001; Neale et al. 2003; Beardall et al. 2009 and references therein) as compared to those obtained considering only UVR. This is due to the synergic or antagonistic nature of the interactions between UVR and those other factors (Dunne 2010). The evaluation of the combined effects of increased temperature and UVR is

particularly interesting because these two variables are more affected in a global change scenario (Häder et al. 2011). In this regard, some studies have demonstrated that increased temperature under UVR exposure benefits photosynthetic performance of some diatoms species by enhancing repair rates (Sobrinho and Neale 2007; Halac et al. 2010); however, in studies carried out with cyanobacteria, Fiorda Giordanino et al. (2011) found important inter-specific variability in responses (with some species benefiting from increased temperature whereas others were rather indifferent) and these variations were partially related to differences in morphology among the species.

In spite of the negative effects, there are several mechanisms that allow phytoplankton to cope with UVR over time periods of days / weeks: At the individual level, the most common strategy is the synthesis of protective compounds such as mycosporine-like amino acids – MAAs. As the maximum absorption of these compounds lies between 310 and 360 nm, they have the potential of decreasing the energy of the most damaging portions of the solar spectrum (Banaszak 2003; Korbee et al. 2006). Pigments like carotenoids can also protect organisms by dissipating excessive energy as heat i.e., via non-photochemical quenching (Müller et al. 2001). Another common protective strategy for motile organisms is to avoid UVR by performing downward migration (Richter et al. 2007). Finally, some organisms have the ability to repair damage produced to the DNA molecule (Buma et al. 2003) or photosystem components, especially the D1 protein (Andersson and Barber 1996; Halac et al. 2009). All together, the net impact of UVR is the result of a trade-off between the sensitivity of individual / community and their acclimation capacity.

## **2. Assessment of UVR effects upon phytoplankton over long-term periods**

Although extensive research has been carried out to address the short-term effects of UVR on phytoplankton (i.e., with experiments lasting less than one day, see review by Villafañe et al. 2003) the performance of communities over longer temporal scales (i.e., days / weeks) have been relatively less studied. Still, these studies are especially important because they reflect the result of a particular effect caused by UVR as well as potential photoacclimation i.e., short-term experiments frequently overestimate the impact of UVR. One way to evaluate UVR effects on aquatic autotrophic organisms on a long-term basis is by using an “enclosed ecosystem” as a model (named meso- or microcosms, depending on the volume of sample) in which a water parcel is isolated and incubated under conditions similar to those found in the natural environment (Wängberg et al. 1996). These setups make feasible to conduct controlled incubations of plankton communities while allowing the manipulation of UVR intensities (i.e., when working with artificial radiation sources) and spectral composition (by covering the containers with different filters / materials). On the other hand, natural conditions are not completely simulated in these cases, as for example water movements are restricted and larger organisms are usually excluded. In these experiments phytoplankton biomass tends to increase rapidly therefore, they often reproduce blooms conditions (Belzile et al. 2006 and references therein).

Studies devoted to evaluate the effects of UVR on phytoplankton communities over long periods of time have highlighted the high variability in responses. Some outdoor mesocosms studies showed little UVR effects on chlorophyll *a* (Chl *a*) content (Halac et al. 1997; Wängberg et al. 1999; Whitehead et al. 2000). On the other hand, Forster et al. (2001) working with phytoplankton of the Darss-Zingst estuary in the Baltic Sea found higher Chl *a* concentration in samples that did not receive UVR, as also observed in the experiments

carried out by Keller et al. (1997) with communities off Narragansett Bay. At the community level, UVR impact is frequently translated onto changes in the taxonomic composition towards more tolerant species or changes in size distribution. For example, studies carried out by Wängberg et al. (1999; 2008) found that small phytoplankton were favored by UV-B exposure, and Mousseau et al. (2000) reported a shift from diatoms to small naked flagellates that occurred more rapidly under enhanced UV-B than under its natural levels. UVR-mediated community structure shifts may result in an important impact for the whole aquatic system, either by altering the food web structure due to the differential sensitivity to UVR, or by affecting carbon allocation into different biomolecules which in turn is translated into changes of carbon and nutrient cycling in the ecosystem (Mostajir et al. 1999; Sommaruga 2003).

### **3. Why studying UVR effects upon phytoplankton communities of Patagonia?**

The ecological effects of UVR were documented more intensively in the Antarctic region at beginning of the awareness of the Antarctic ozone 'hole'. Later studies pointed out that the influence of the Antarctic ozone depletion extends to mid latitudes (Atkinson et al. 1989) and that Southern mid latitudes may be even more affected (Seckmeyer and McKenzie 1992). Still, while many studies about the effects and impact of UVR on phytoplankton have been carried out in polar areas, relatively less is known about temperate regions (see review by Gonçalves et al. 2010) such as Patagonia. The Patagonia region is located at the southern tip of South America, includes part of Argentina and Chile (Fig. 1) and has unique characteristics that would warrant UVR studies for several reasons. First, the area is occasionally under the influence of ozone-depleted air masses from the Antarctic polar vortex, thus experiencing periods of enhanced UV-B (Villafañe et al. 2001; Helbling et al. 2005). Second, its great variability in cloudiness, from high cover over the Andes and sub-Antarctic regions to the relatively clear skies on the mid-latitude Atlantic coast, creates a range of environments with variable UVR climatology. Third, it presents a high variability in the nature and bio-optical characteristics of its water bodies (e.g. the upwelling deep waters in the Pacific and the shallow and very productive Atlantic waters). Finally, high wind speed and frequency, especially during spring and summer (Villafañe et al. 2004a; Helbling et al. 2005) strongly condition the depth of the upper mixed layer (UML) and hence the underwater radiation field to which organisms are exposed. In addition the assessment of the UVR impact on phytoplankton from Argentinean Patagonia is essential since these organisms are responsible for an important share of primary productivity in the Argentinean Sea (Lutz et al. 2010) and they constitute the base of a very rich food web that includes fishes (e.g. hake, anchovy) (Skewgar et al. 2007) and invertebrate species (e.g. shrimp and mussels) of great commercial value (Caille et al. 1997).

Taking into consideration these facts, in the next section we present our study case and a review of the current knowledge about UVR effects on phytoplankton communities mainly from Patagonia, and especially focusing on effects observed in a days/weeks timeframe.

### **4. Study case: Long-term UVR effects on phytoplankton from Bahía Engaño, Patagonia, Argentina**

The study site (Bahía Engaño, Chubut, Argentina) is located at Northern coastal Patagonia (Fig. 1). Our research group had previously conducted several UVR studies with

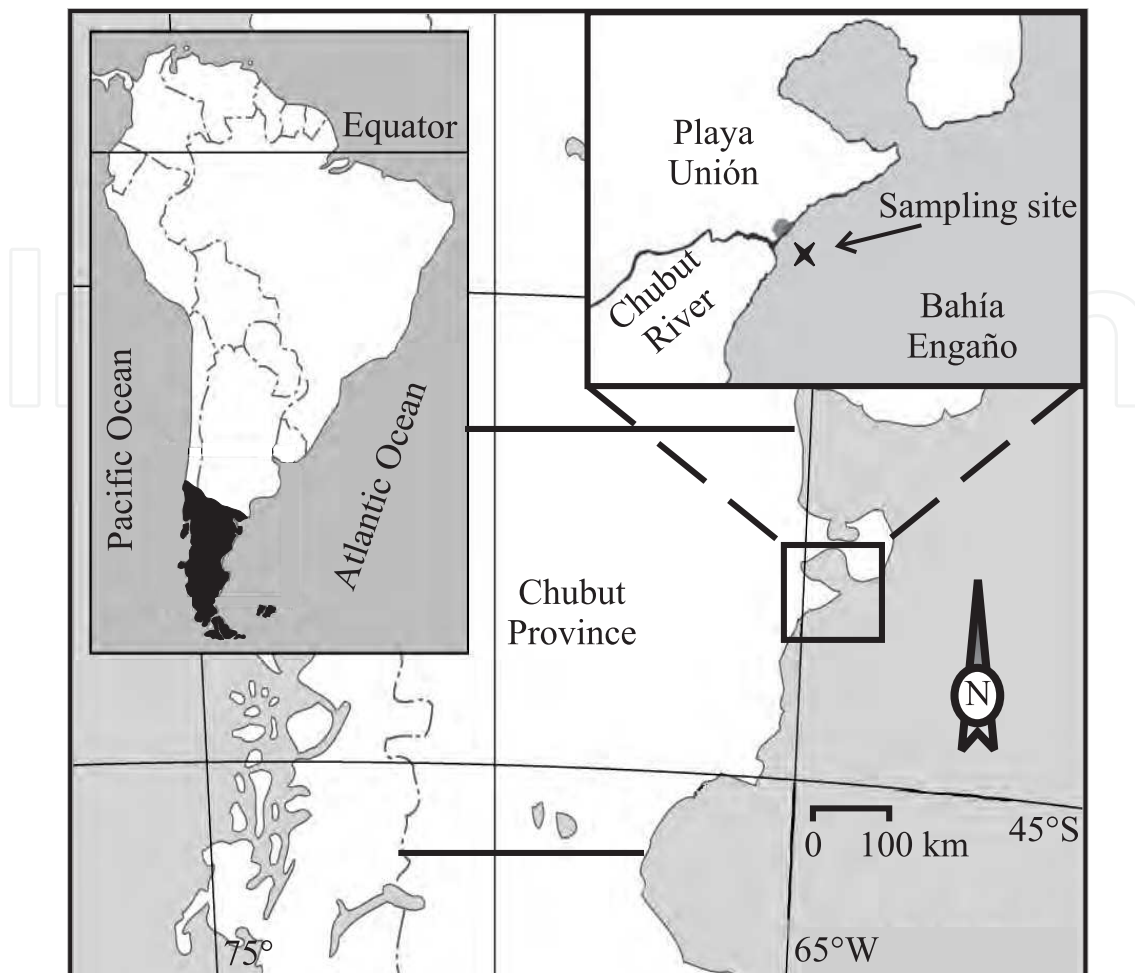


Fig. 1. Location of the study site, showing Patagonia (shaded area) and the relative position of the Chubut Province (Argentina) in South America. The inset shows the study area where microcosms experiments were done.

phytoplankton communities from this area, mostly determining short-term responses, particularly those related to inhibition of carbon fixation and photoinhibition (e.g., see Barbieri et al. 2002; Villafaña et al. 2004a; Villafaña et al. 2004b; Villafaña et al. 2008; Helbling et al. 2010), and relatively less studies to determine long-term responses to UVR in combination with nutrient addition (Helbling et al. 2005, Marcoval et al. 2008). Therefore, the results presented here aim to further elucidate aspects of UVR sensitivity and photoacclimation of phytoplankton from Patagonia occurring over longer periods of time, especially focusing on community properties such as global growth, abundance, taxonomic composition and size distribution.

An experimental approach was taken, in which natural phytoplankton samples were collected, and incubated under solar radiation during the austral summer of 2010. The experiments consisted in two microcosm incubations (hereafter MI and MII) which lasted between February 5 - 11 (MI) and February 15 - 21 (MII). The experimental setup consisted in exposing natural phytoplankton samples in 25-l, UVR-transparent bags (microcosms) under three different radiation conditions: a) **PAB**, 280-700 nm (samples receiving PAR+UV-A+UV-B); b) **PA**, 320-700 nm (samples receiving PAR+UV-A) and c) **P**, 400-700 nm (samples receiving only PAR). The microcosms (duplicates per radiation treatment) were placed in a

tank (3 m diameter, 1 m depth) with running water as temperature control and exposed to solar radiation at the surface for ca. 7 days. During the experiments, water samples from each microcosm bag was collected daily (early in the morning) for analyses of Chl *a* and UV-absorbing compounds whereas samples for taxonomic composition and size distribution were taken every other day.

During the experiments, PAR and UVR irradiance conditions (Fig. 2) presented a typical pattern of relatively high values at noon and low ones during the morning and late

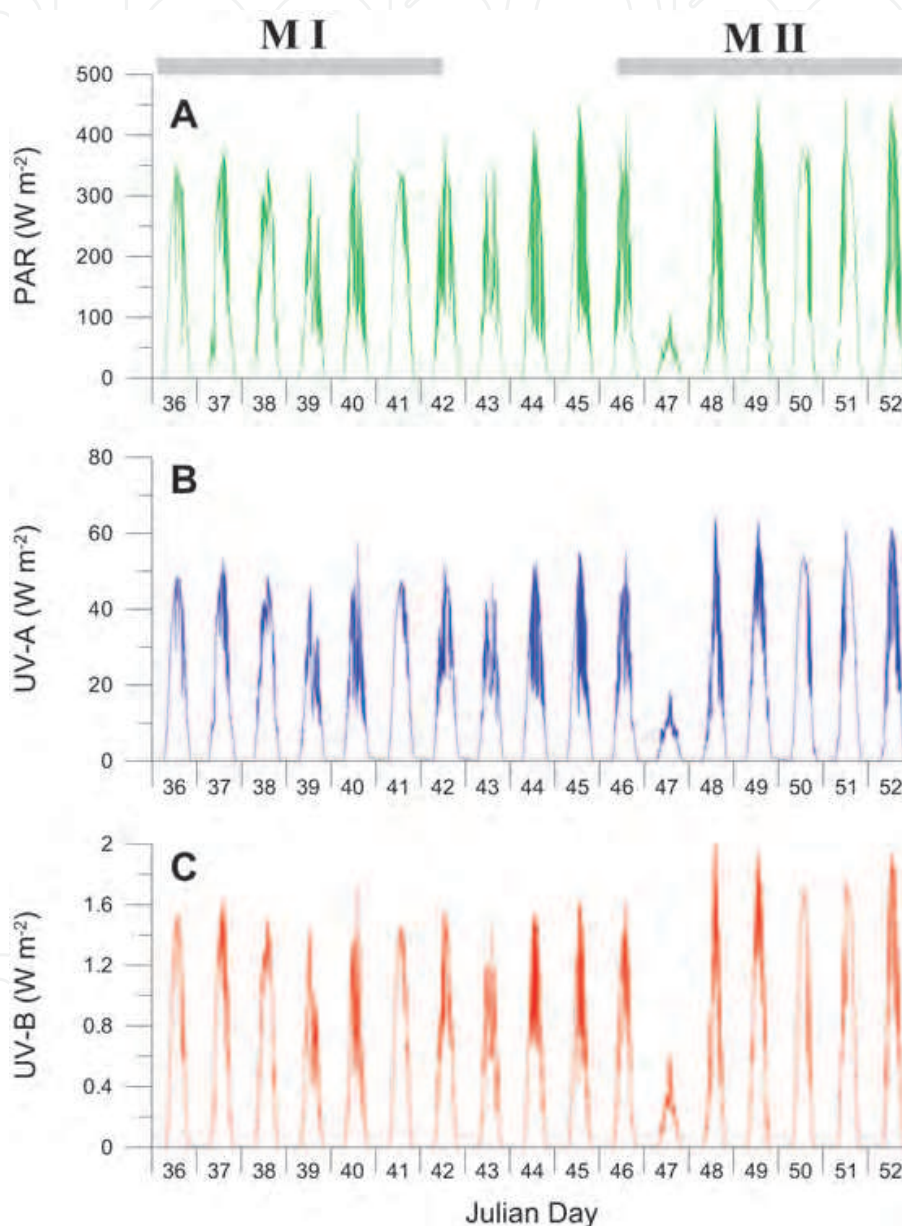


Fig. 2. Solar radiation reaching the Earth's surface at the study site during experiments carried out during February 5-11 (Julian days 36-42) (MI), and February 15-21 (Julian days 46-52), 2010 (MII). Irradiance is shown for: A) PAR, 400- 700 nm, B) UV-A, 315-400 nm and, C) UV-B, 280-315 nm. Solar radiation was continuously monitored using a broad-band filter radiometer (ELDONET, Real Time Computers, Möhrendorf, Germany, Häder et al. 2007) permanently installed on the roof of the Estación de Fotobiología Playa Unión.

afternoon; also, the presence of clouds that resulted in high daily variability in solar irradiance is characteristic for the area during summer (Helbling et al. 2005). During our experiments, maximum PAR irradiance levels were rather similar ( $\sim 440 - 460 \text{ W m}^{-2}$ ) (Fig. 2A) as also were UV-A ( $\sim 60 \text{ W m}^{-2}$ ) and UV-B ( $\sim 2 \text{ W m}^{-2}$ ) – except for the second day during MII where PAR and UVR values were very low (i.e.,  $\sim 100$ ,  $17$ , and  $0.6 \text{ W m}^{-2}$  for PAR, UV-A and UV-B, respectively; Figs. 2A-C). The high irradiance values in combination with long daylight periods result in high daily doses (Helbling et al. 2005) which are similar to those registered in tropical environments (Gao et al. 2007). Since phytoplankton in our experiments were exposed to these high irradiance conditions of solar radiation under a thin layer of water under, our results represent the ‘worst-case scenario’, i.e., as if cells were at the water surface, not allowed to move downward towards lower radiation levels.

Because the timing of our sampling (summer) that is considered a post-bloom condition for our study area (Villafañe et al. 2008), we added nutrients to each incubation bag (f/2 concentration (Guillard and Ryther 1962)) at the beginning of each experiment to avoid nutrient constraints while phytoplankton was growing. In both experiments, the phytoplankton assemblage showed an increase, as assessed by measurements of Chl *a* (Fig. 3A), cellular abundance (Fig. 3B) and autotrophic carbon (Fig. 3C). Some general features were observed: Firstly, during both experiments the variables used to calculate growth had a typical exponential increase, similar to those occurring at bloom conditions, and thus an optimum cellular response. Secondly, the observed increase was similar for both experiments, although some differences appeared for some variables; and thirdly, no general UVR effects were observed (except in a few cases) within any experiment / variable measured.

As an overview of the increase of phytoplankton assemblages, Table 1 resumes the calculated growth rates ( $\mu$ ) during both experiments. The fast growth observed during the experiments were probably due to the addition of nutrients and the low turbulence inside the incubation bags, as previously observed in long-term studies with phytoplankton communities from the area (Helbling et al. 2005; Marcoval et al. 2008). As mentioned before, a common result of long-term incubations is the lack of UVR effects on growth and biomass, as also observed in our study (i.e., no-significant differences between radiation treatments as observed in Table 1 and Fig. 3). In fact, this lack of UVR effects on growth was also observed in other studies carried out in Patagonian waters: For example, Roy et al. (2006) working with phytoplankton communities from the Beagle Channel (Tierra del Fuego) observed minor changes in biomass due to UV-B (both normal and enhanced levels), even though the UV-B enhancement imposed to the samples was important (i.e., simulating 60 % of ozone depletion). However, Hernando et al. (2006) found a significant effect of UVR on growth on these phytoplankton assemblages only when samples were exposed to solar radiation at fixed depths, in contrast to the mixed conditions imposed in the mesocosms described by Roy et al. (2006). In addition, Helbling et al. (2005) and Marcoval et al. (2008) determined variable UVR-induced inhibition of growth in natural communities off the Chubut coast under different conditions of nutrients availability, with nutrient-depleted samples being more sensitive to UVR than those in which nutrients had been added. Therefore, UVR alone is not an evident inhibitor of growth for phytoplankton off Patagonia waters, but it can have important effects when acting together with other stressors (e.g., nutrient availability, mixing conditions).

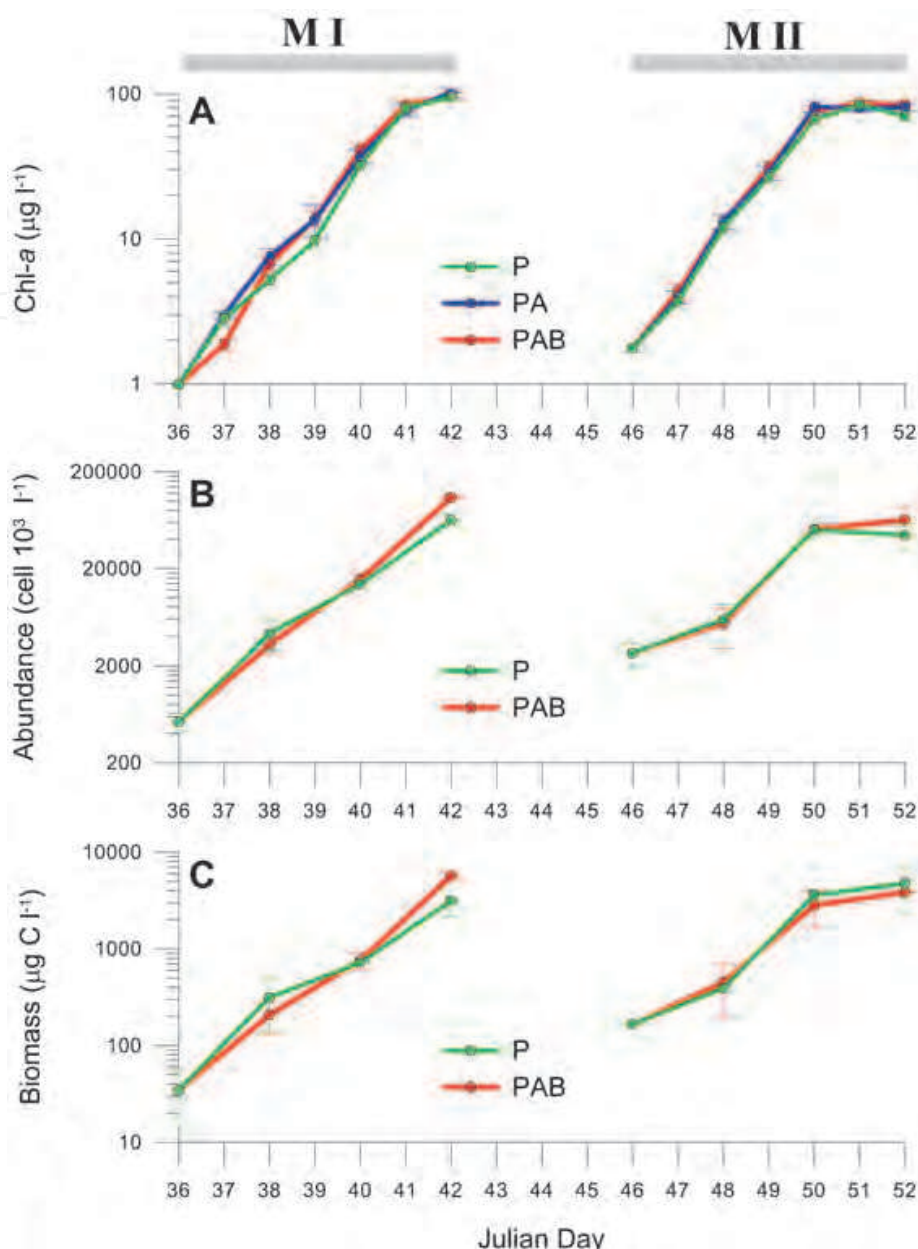


Fig. 3. Growth of the phytoplankton communities during MI and MII experiments evaluated as: A) Chlorophyll *a* (Chl *a*) content (measured by fluorometric and spectrophotometric techniques, Holm-Hansen and Riemann 1978; Porra 2002); B) Cell concentration (obtained by microscopy; Villafaña and Reid 1995); and C) Autotrophic biomass (considering biovolumes according to Hillebrand et al. 1999 and posterior transformation to carbon content following Strathmann 1967). The different radiation treatments are shown in different colors. The vertical lines on the symbols indicate the half mean range. Note the different log scales for the variables presented.

Another observed pattern in the growth response was, at first sight, a similar trend among experiments which was due not only to the similar radiation conditions (Fig. 2) but also to the initial assemblages used in both experiments (i.e., similar starting taxonomic composition). In fact, at the beginning of experiments, the communities were numerically dominated by flagellates (e.g., chlorophytes and cryptophytes) and to a less extent by



Growth rates ( $\mu$ ; d <sup>-1</sup> )						
	Chl a		Cellular abundance		Autotrophic carbon	
	PAB	P	PAB	P	PAB	P
<b>Microcosm I</b>	0.76 ± 0.01	0.76 ± 0.06	0.88 ± 0.09	0.79 ± 0.04	0.84 ± 0.14	0.75 ± 0.15
<b>Microcosm II</b>	0.94 ± 0.02	0.90 ± 0.03	0.73 ± 0.08	0.73 ± 0.11	0.70 ± 0.07	0.76 ± 0.03

Table 1. Growth rates ( $\mu$ , in d<sup>-1</sup>) during MI and MII experiments, determined from measurements of Chl *a*, cellular abundance and estimations of autotrophic carbon.

diatoms (*Thalassiosira* spp., *Nitzschia longissima*); on the other hand, the abundance of dinoflagellates (e.g., *Prorocentrum micans*, unidentified naked species) was very low. This is in agreement with previous studies carried out in the area that demonstrated the conspicuous presence of flagellates during the summer (Villafañe et al. 2004a; Villafañe et al. 2008). However, it was also evident that there were some differences in the growth rates calculated from different variables as well as when comparing experiments. For example, during MI, Chl *a*-based  $\mu$  were lower than those from cellular abundance and autotrophic carbon, while the opposite occurred in MII. The fact that Chl *a* concentration showed a slower (during MI) or faster (during MII) increase than the other two variables, suggests a differential acclimation of the assemblages as the experiments progressed. This could be due to different reasons: On the one hand, as the community grew the self-shading effect might become important and thus the Chl *a* concentration per cell would increase to keep efficiently capturing photons and maintain the exponential growth. This could be mediated by cell size, as smaller cells (i.e., higher surface-to-volume ratio) needs comparatively less Chl *a* per cell as compared to larger cells (Falkowski 1981). On the other hand, an increase in cell size, with larger cells towards the end of the exponential growth phase, means a smaller surface-to-volume ratio and thus the need of higher Chl *a* content per cell. Indeed, a combination of both factors were observed in our experiments, as the C to Chl *a* ratio - an indicator of "light acclimation" - increased in MI and decreased in MII, while the Chl *a* content per cell decreased in MI and increased in MII towards the end of the exponential phase (Table 2). In the following paragraphs we will discuss how changes in cell size, together with differential changes in species composition might have accounted for the observed patterns and variability among our experiments.

	C / Chl <i>a</i>		Chl <i>a</i> content per cell	
	PAB	P	PAB	P
MI - T <sub>0</sub>	35 ± 5	35 ± 5	1.87 ± 0.45	1.87 ± 0.45
MI - T <sub>f</sub>	58.8 ± 3.1	33.2	0.88 ± 0.33	1.48
MII - T <sub>0</sub>	94.6 ± 0.9	94.6 ± 0.9	0.65 ± 0.18	0.65 ± 0.18
MII - T <sub>f</sub>	37 ± 13	53.8 ± 15.7	1.49 ± 0.07	1.33 ± 0.02

Table 2. Mean (and half mean range) carbon to Chl *a* ratio (in  $\mu\text{g C } \mu\text{g Chl } a^{-1}$ ) and Chl *a* content per cell (in pg) at the beginning (T<sub>0</sub>) and at the end (T<sub>f</sub>) of the experiments.

To study changes in the size spectra of each treatment, we recorded digital images of each sample and analyzed them to obtain the size (area) distribution of cells at the beginning of the incubation as well as at the end of the exponential growth. The size spectra data (Fig. 4) indicates that in both experiments most of the phytoplankton assemblages (> 60 %) were dominated by small cells with an area < 100  $\mu\text{m}^2$  (Figs. 4A and C). A shift in the cumulative frequency of cell size in the range of 65-395  $\mu\text{m}^2$  was observed in all radiation treatments of MI (Fig 4B), being the P treatment the one with the higher change. On the other hand, during MII (Fig 4D) a slightly different response was observed, as the P treatment showed virtually no changes but the size distribution in the PAB treatment was slightly shifted towards larger areas in the range 85-395  $\mu\text{m}^2$ . It has been usually found in other studies that smaller cells tend to dominate the community after UVR-exposure (Mostajir et al. 1999), but in our results this might be strongly affected by the initial conditions of each microcosm. Also we can not rule out the effects of co-occurring predators (i.e., heterotrophic microplankton). Similarly to what we expressed about the lack of UVR-only effects on growth, we could speculate that UVR alone might not always show evident effects on size distribution, but depending on the starting taxonomic composition of the community, both PAR and UVR may have implications in the structure of the plankton community.

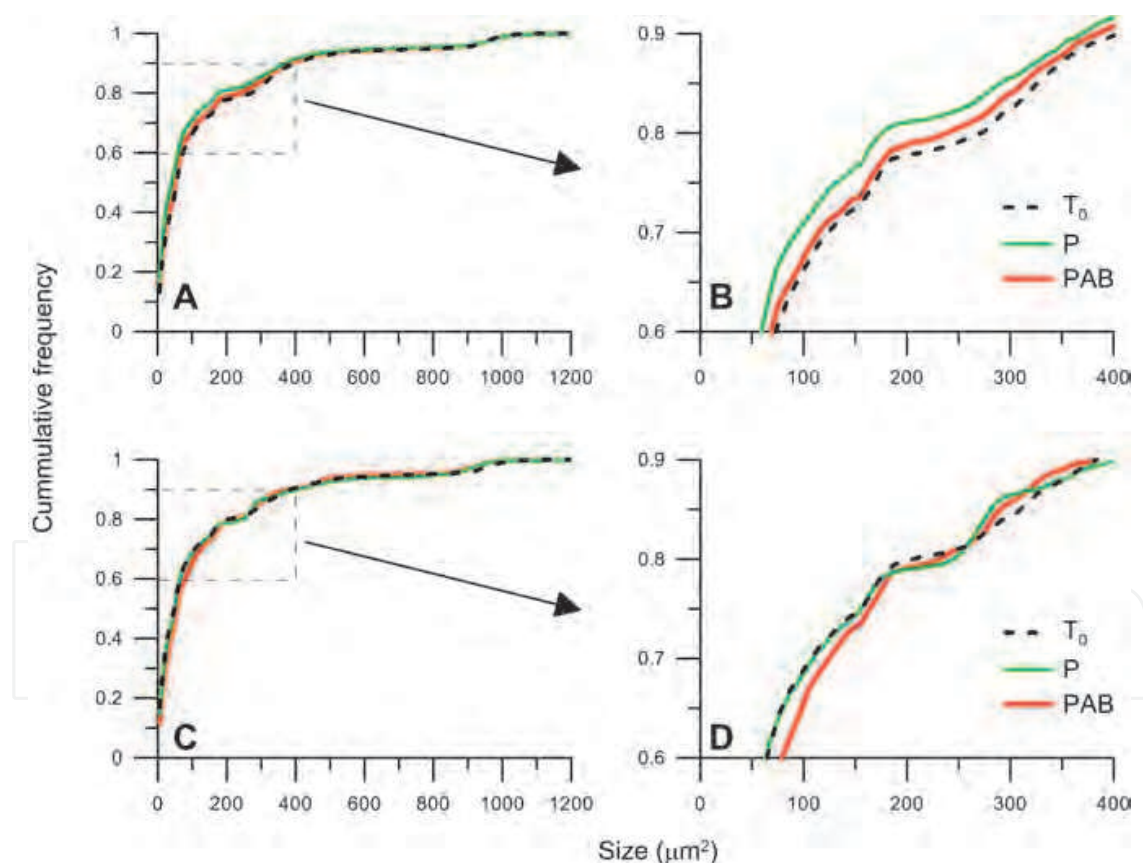


Fig. 4. Cumulative frequency of size (in  $\mu\text{m}^2$ ) at the beginning ( $T_0$ ) and at the end of the exponential growth phase for MI (A) and MII (C). In B) and C) a detailed view within the size ranges of differences is shown. The radiation treatments are shown in different colors: P (green), and PAB (red). Size distribution was evaluated in formalin-fixed samples from pictures taken under an inverted microscope; images were analyzed using Image J software (Abramoff et al. 2004).

Microscopical analyses of the communities also supported changes in cell size throughout the experiments. For example, carbon allocated in the nanoplankton fraction (cells  $< 20 \mu\text{m}$  in effective diameter) increased more rapidly than that of microplankton ( $> 20 \mu\text{m}$ ) in MI (Fig. 5A), but the opposite occurred in MII (Fig. 5 B). Also, there was a general decrease in the microplankton biomass from  $T_0$  towards the end of the experiments (Fig 5C) as was also expected from the shift towards smaller cells in MI (Fig 4B). However, the decrease was more pronounced in MI than in MII, therefore the overall result was that the relative contribution of microplankton to the total biomass in MII was higher than during MI (Fig 5C).

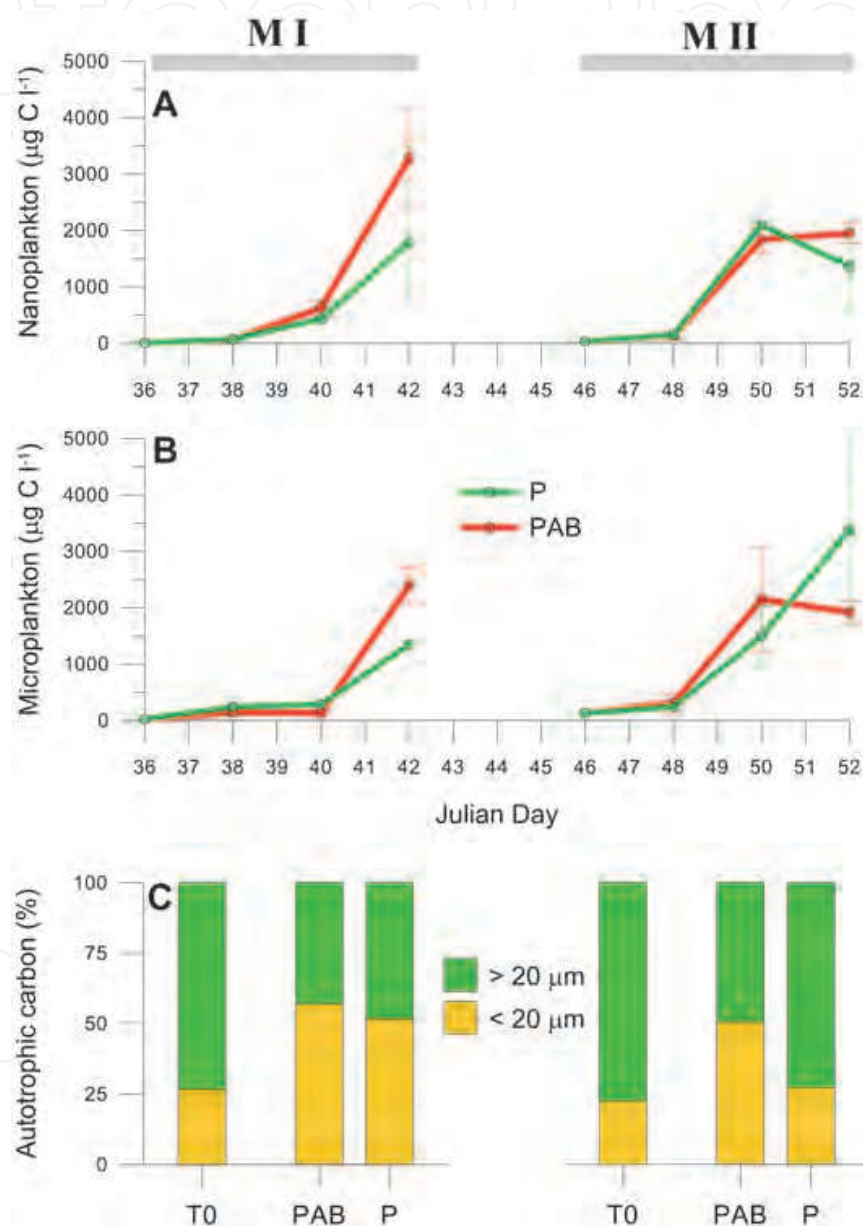


Fig. 5. Autotrophic carbon (in  $\mu\text{g C l}^{-1}$ ) of the phytoplankton size classes: A) Nanoplankton ( $< 20 \mu\text{m}$ ) and, B) Microplankton ( $\geq 20 \mu\text{m}$ ). C) Relative contribution of nanoplankton and microplankton (%) to the total autotrophic carbon at the beginning ( $T_0$ ) and at the end of the MI and MII experiments. The radiation treatments / size fractions are shown in different colors: P (green) and PAB (red) / microplankton (green) and nanoplankton (orange). The vertical lines on the symbols indicate the half mean range.

Any change in cell size and biomass allocation might occur within a particular species however they normally are associated to change in taxonomic composition towards the most resistant or acclimated groups. In fact, the most evident effect of UVR exposure (as compared to samples in which UVR was excluded) over long periods of time are the taxonomic changes produced in the community, which act as a photoacclimation mechanism. There are many studies that have reported this effect in long-term experiments (see review by Villafañe et al. 2003) but in particular, and for the Patagonia area, Hernando et al. (2006) working with the communities off the Beagle Channel observed changes from an assemblage co-dominated by phytoflagellates and diatoms at the beginning of the experiments to a progressive increase of euglenophytes, especially under static conditions of

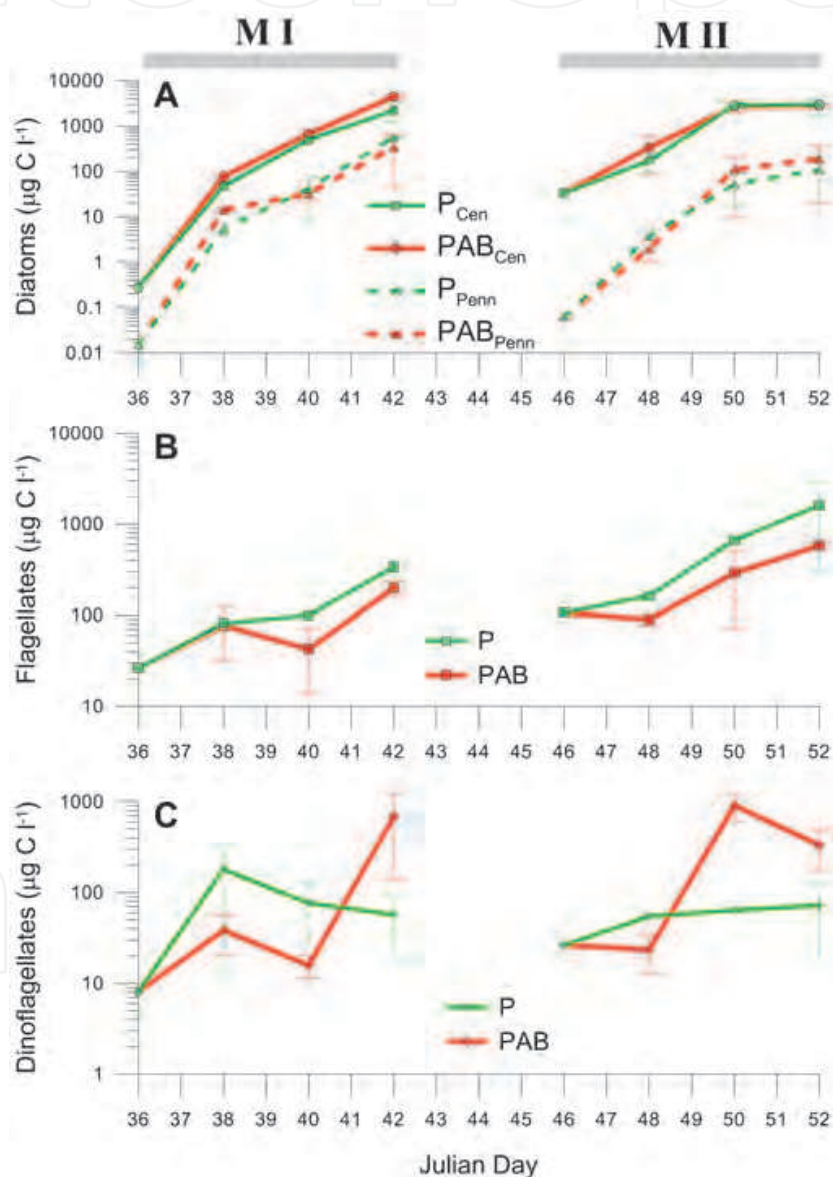


Fig. 6. Autotrophic carbon (in  $\mu\text{g C l}^{-1}$ ) of: A) Diatoms, B) Flagellates and, C) Dinoflagellates during the experiments MI and MII. The radiation treatments are shown in different colors: P (green), and PAB (red); different lines in panel A indicate the contribution to the total of centric (solid lines) and of pennate diatoms (dotted lines). The vertical lines on the symbols indicate the half mean range.

the water column. In studies carried out with communities off the Chubut coast, Helbling et al. (2005) and Marcoval et al. (2008) also found that solar radiation played a fundamental role in shaping phytoplankton communities. In order to further explore these changes in species composition in our experiments, in Fig. 6 we show the contribution of the three main taxonomic groups - diatoms (centric and pennates), flagellates and dinoflagellates.

Overall, no UVR effects were observed in the diatoms in both experiments (Fig. 6A) while significant differences among radiation treatments became evident in flagellates (Fig. 6B) and in dinoflagellates (Fig. 6C). For example, autotrophic carbon in flagellates was negatively affected by UVR, resulting in significantly lower values in samples receiving UVR (PAB treatment) as compared to those that received only PAR (P treatment). On the contrary, autotrophic carbon in dinoflagellates was higher in samples receiving UVR. Previous studies (Hernando and San Román 1999; Hernando et al. 2005) have shown similar results about the sensitivity of flagellates. In the case of dinoflagellates, their response seems to be more related to the size as shown by Helbling et al. (2008) where larger species (i.e., *Prorocentrum micans*, 50  $\mu\text{m}$  mean diameter) were less sensitive than small ones such as *Gymnodinium chlorophorum* (5  $\mu\text{m}$ ) and *Heterocapsa triquetra* (20  $\mu\text{m}$ ).

However, the overall picture in our experiments shows a significantly higher increase of autotrophic carbon in diatoms (both centric and pennates) (Fig. 6A) with centric diatoms always accounting for the higher share at the end of the experiments, as compared to flagellates (Fig. 6B) and dinoflagellates (Fig. 6C). This differential increase in autotrophic carbon caused a shift in the community dominance from a flagellate-dominated community towards a diatom-dominated one (Fig. 7).

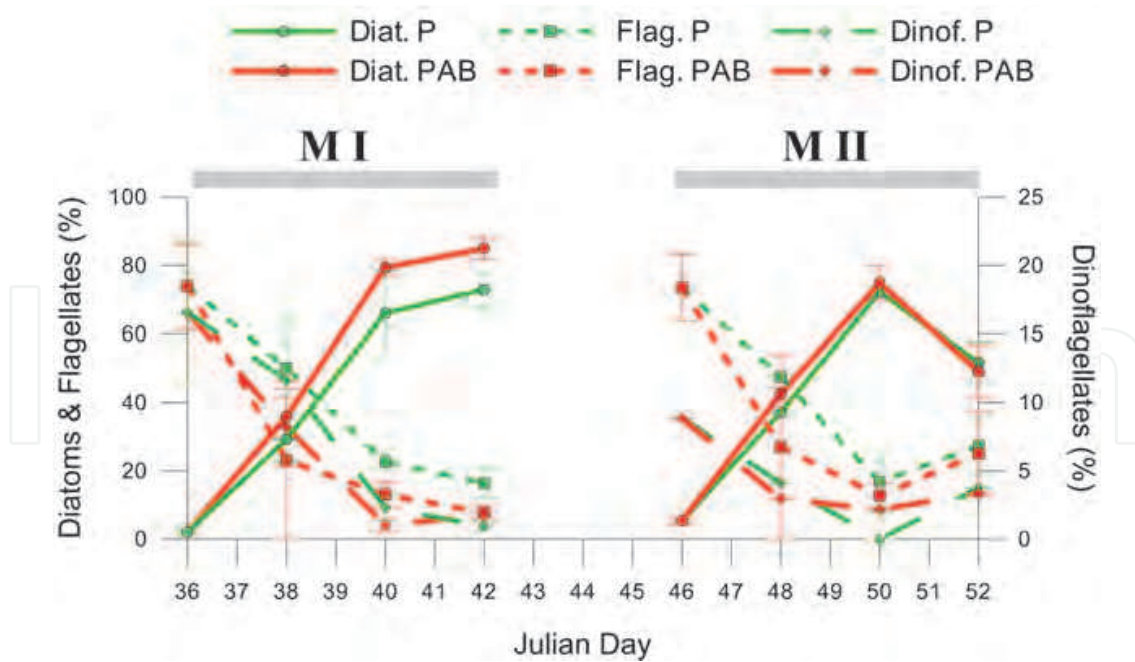


Fig. 7. Autotrophic carbon in diatoms, flagellates and dinoflagellates during the MI and MII experiments. The radiation treatments are shown in different colors: P (green), and PAB (red); filled lines: diatoms, dotted lines with squares: flagellates and dotted lines with diamonds: dinoflagellates. The vertical lines on the symbols indicate the half mean range.

It should be noted that at the beginning of the experiments, the contribution of diatoms to the total abundance and biomass was very small (Figs. 6 and 7) and they were represented by *Thalassiosira* spp., *Nitzschia longissima*, *Skeletonema costatum*, *Asterionellopsis glacialis* and unidentified pennates, among some others. However, by the end of the experiment, centric diatoms accounted for ca. 75% and 66% of the total autotrophic carbon for MI and MII, respectively. By the end of MI, and in both radiation treatments *Thalassiosira* spp. with a size range of 10-20  $\mu\text{m}$  almost completely dominated the assemblages (Fig. 8). On the other hand, in MII there was a co-dominance of *Thalassiosira* spp. of 10-20  $\mu\text{m}$  size and larger ones (> 20  $\mu\text{m}$ ).

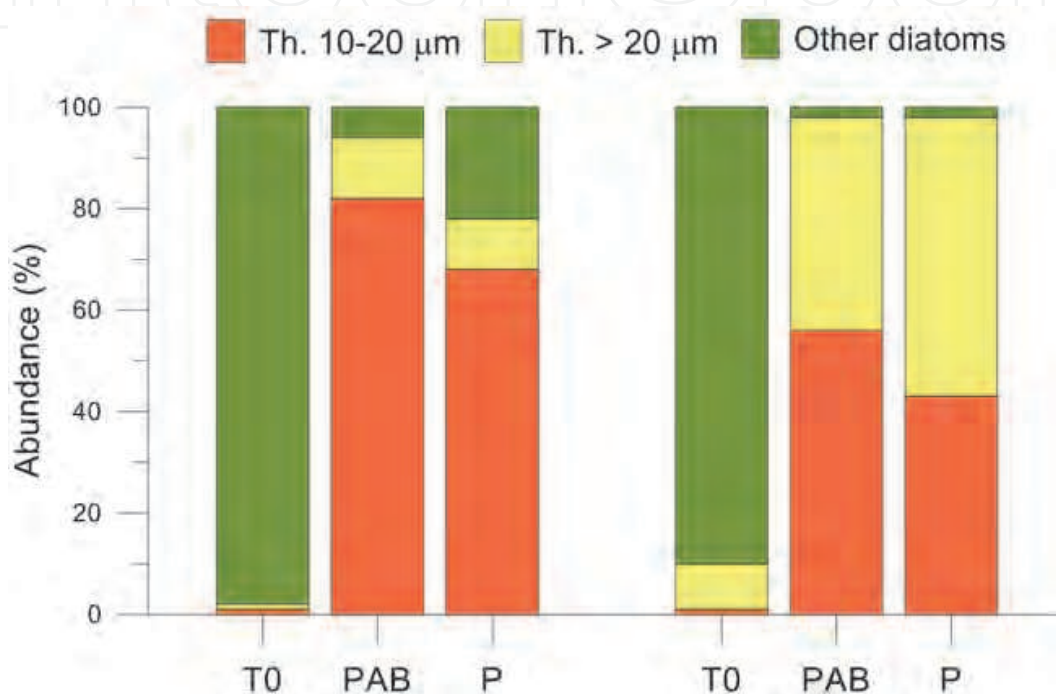


Fig. 8. Relative contribution of diatoms to the total autotrophic carbon at the beginning ( $T_0$ ) and at the end of experiments. The size classes of *Thalassiosira* (Th.) are shown in different colors: > 20  $\mu\text{m}$  (yellow), 10-20  $\mu\text{m}$  (red); other diatoms are represented in green.

So far, two main outcomes can be suggested from our data, one is related to changes in cell size (and consequently those on biomass, Chl *a* content per cell, etc.) and the other is that in species composition towards a diatom-dominated (mainly centric) assemblages. Although these are important acclimation mechanisms occurring over longer periods of time, there are also alternative ones to cope with UVR and excess of irradiance. One of such mechanism is, as previously mentioned, the synthesis of UV-absorbing compounds (mainly mycosporine like amino acids, MAAs). It has been found that even MAAs are generally very low in natural communities of Patagonia (Barbieri et al. 2002; Villafañe et al. 2004a; Villafañe et al. 2004c; Marcoval et al. 2008) but some species i.e., dinoflagellates such as *Prorocentrum micans* are able to synthesize them in high amounts after prolonged UVR exposure (Marcoval et al. 2007; Helbling et al. 2008); diatoms are also known to produce relatively high amounts (Lagunas et al., unpubl. data). It has also been shown that the synthesis of UV-absorbing compounds is more effective in large cells whereas in small species their synthesis would be too costly and osmotically disadvantageous (Garcia-Pichel 1994). In addition, previous

studies (Helbling et al. 1996) showed that centric diatoms were able to synthesize significant amounts of UV-absorbing compounds after long-term exposure to UVR, whereas pennates did not. In our study case it is interesting to note that no significant changes in the concentration of UV-absorbing compounds were observed during MI (data not shown) probably due to the dominance of small diatom species during the experiment (Fig. 8). On the contrary, during MII there was a significant increase of UV-absorbing compounds (Fig 9). This increase, relative to the Chl *a* content, was significant (Fig 9B) but there were no differences among radiation treatments, suggesting that all wavelengths of solar radiation triggered the synthesis of these compounds, as also observed for Antarctic diatoms (Helbling et al. 1996). A possible explanation is that high solar radiation in their natural environment will include PAR and UVR, therefore PAR as well as UVR may be capable of triggering the synthesis of MAAs so *Thalassiosira* spp. can obtain protection against solar UVR (Helbling et al. 1996). The presence of UV-absorbing compounds in MII probably reflects the higher proportion of large *Thalassiosira* species during this experiment (Fig 8) and also of dinoflagellates, although in lesser extent (Fig 6 C).

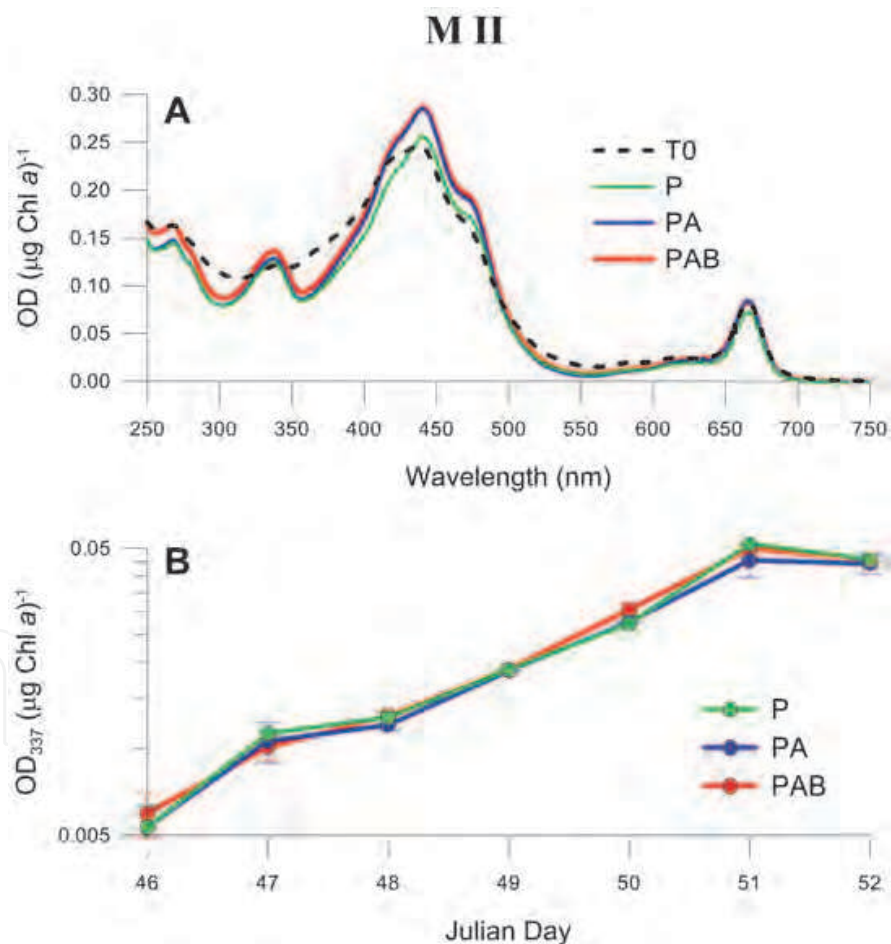


Fig. 9. A) Absorption spectra at the beginning ( $T_0$ ) and at the end of the MII experiment: B) UV-absorbing compounds relative to Chl *a* content ( $OD_{337}$  per  $\mu\text{g Chl } a^{-1}$ ) throughout the experiment. UV-absorbing compounds were estimated as described in Dunlap et al. (1995) using the peak height at 337 nm. The radiation treatments are shown in different colors: P (green), PA (blue) and PAB (red); dotted lines represent  $T_0$ .

## 5. Ecological implications

All together, this study indicates that within the experimental time frame, UVR effects are more evident in the taxonomic composition of the community than on algal growth / biomass. As stated above, the “starting point” of the studied community was very important in our incubations, therefore stressing the role of combined factors and preventing simple extrapolations. Also it suggests that it is not possible to generalize the solar radiation effects on diverse phytoplankton assemblages: Other factors such as previous light history, gradients of temperature and nutrient availability, in turn related to water turbulence and / or UML depth are very important to understand the observed responses. For example, even when both microcosms showed a sustained growth during several days, the final distribution of the autotrophic carbon was very different in each case. This may have important consequences for the available energy sources in the pelagic food web, as species and size distribution are two of the main factors affecting the chances of a phytoplankton cell being ingested by a predator. A community with a different carbon source will function and respond in a different way when exposed to UVR and other factors. It may be difficult to evaluate these scenarios with Chl *a* estimations obtained from remote sensing techniques, but additional *in-situ* research in this topic would help us to validate those estimations which proved to be useful for regional-global comparisons.

## 6. Acknowledgments

We thank V. Fiorda and E. Heimsch for their help during experiments. We are also grateful with personnel at Estación Marítima Comersonii at Puerto Rawson for sampling collection for our experiments. This work was supported by Agencia Nacional de Promoción Científica y Tecnológica (PICT2007-01651, Argentina), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina) - Deutsche Forschungsgemeinschaft (DFG, Germany) (CONICET-DFG-2009), Ministerio de Ciencia, Tecnología e Innovación Productiva (MINCyT, Argentina) - Consejo Nacional de Ciencia y Tecnología (CONACYT, Mexico) (Project N° MX/09/13) and Fundación Playa Unión. This work is in partial fulfillment of the Ph.D. thesis of SRH, supported by a scholarship from Agencia Nacional de Ciencia and Instituto Nacional del Agua (Argentina). This is Contribution N° 124 of Estación de Fotobiología Playa Unión.

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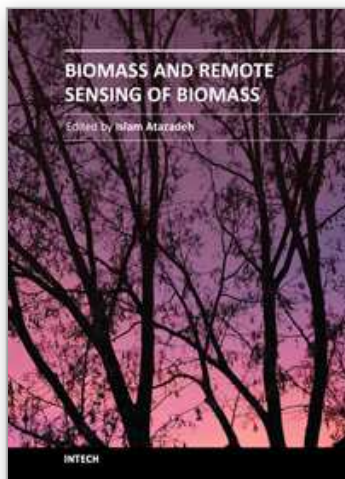
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Edited by Dr. Islam Atazadeh

ISBN 978-953-307-490-0

Hard cover, 262 pages

**Publisher** InTech

**Published online** 06, September, 2011

**Published in print edition** September, 2011

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