

# Dynamics of potentially protective compounds in Rhodophyta species from Patagonia (Argentina) exposed to solar radiation

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## Abstract

The impact of solar radiation upon potentially protective compounds (i.e., UV-absorbing compounds and carotenoids) was assessed in four Rhodophyte species from Patagonia (i.e., *Ceramium* sp. Lyngbye, *Corallina officinalis* Linnaeus, *Callithamnion gaudichaudii* Agardh and *Porphyra columbina* Montagne) during short-term (i.e., 46 h) experiments. Algae were exposed to solar radiation under two treatments (PAR only: 400–700 nm, and PAR + UVR: 280–700 nm) and sub-samples were taken every 3 h (or longer periods at night) to determine the spectral absorption characteristics and concentration of UV-absorbing compounds, carotenoids and photosynthetic pigments. Except for *C. gaudichaudii* which displayed a decrease in chl-a concentration throughout the experiment, photosynthetic pigments had small variations in all species. UV-absorbing compounds concentration varied as a function of solar irradiance, with maximum values around local noon. In *C. officinalis* and *P. columbina* UV-absorbing compounds concentration increased as compared to that of chl-a; in *Ceramium* sp. and *C. gaudichaudii*, however, there was no relationship between UV-absorbing compounds content and chl-a concentration. Carotenoids, on the other hand, did co-vary with chl-a in all species. Our data suggest that, with the exception of *C. gaudichaudii*, the differential responses of UV-absorbing compounds concentrations are more associated to the previous light history of the algae (i.e., in turn due to their position in the intertidal zone) rather than to the radiation treatment imposed to the samples. Based on our results, the variable impact of solar radiation upon productivity (and eventually biodiversity) of macroalgae from Patagonia might consequently differentially affect higher trophic levels of the aquatic food web.

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**Keywords:** PAR; Patagonia; Rhodophyta; UV-absorbing compounds; UVR

## 1. Introduction

Solar radiation plays a crucial role upon aquatic autotrophs: On one hand, visible radiation (PAR, 400–700 nm) is responsible for most of the energy transfer for photosynthesis, but is also associated to the photoinhibition process [1–3] and bleaching of photosynthetic pigments [4]. On the other hand, short wavelengths of the electromagnetic spectrum, i.e., ultraviolet radiation

(UVR, 280–400 nm) is known to cause a wide range of negative effects upon these organisms [5]. In marine macroalgae not only photoinhibition [1–3] but also damage to photosynthetic targets [6,7], to the DNA molecule [8,9], and UVR-induced degradation of pigments have been reported [5,10,11].

The overall impact of solar radiation upon macroalgae depends on several variables, such as the irradiance levels under which organisms are exposed and the strategy used to cope with this abiotic factor [5,12]. Macrophytes living in the intertidal zone are, in general, a group especially vulnerable to solar radiation because

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they are exposed to fluctuating radiation regimes during the day as a consequence of their growth site – i.e., attached to the bottom or in tide pools. These radiation fluctuations are in turn associated to tide levels, resulting in algae completely exposed to solar radiation during low tide or partial/completely submerged during high tides. Particularly, littoral macroalgae may be exposed to relatively high radiation levels, especially when low tides coincide with the local noon [1,13]. It is obvious then that macroalgae must have developed several physiological strategies to enable them to live under their ambient PAR and UVR levels. Such strategies include the dynamic photoinhibition (that helps to dissipate excess energy as fluorescence and heat by interrupting the electron transport chain) [3], the synthesis of protective UV-absorbing compounds (e.g., mycosporine-like amino acids – MAAs) [2,14–16] or carotenoids [17] and repair mechanisms of DNA damage [8]. In particular, the presence of MAAs has been frequently cited within Rhodophyta species [15,18–20]. Their protective function have been shown for *Porphyra yezoensis* by Misonou et al. [21], who demonstrated that these UV-absorbing substances blocked thymine photodimer production; moreover, Yabe et al. [22] suggested that these compounds secreted by red algae played a role in protecting the germination of *Laminaria*. Other studies carried out with macroalgae though, did not find any evidence of the protective role and instead, MAAs were rather acting as side products of certain biochemical pathways [12].

In Patagonian waters, macroalgae constitute a considerable economic resource, especially for harvesting of commercial species of Rhodophyta [23]. A number of descriptive studies on the taxonomy and distribution of Rhodophyta species have been carried out in this region [23–26], but very few have addressed the impact of solar radiation upon this group [27–31]. Protective mechanisms in Rhodophyta from Patagonia have been addressed only in some studies carried out by Häder et al. [28] and by Korbée Peinado et al. [31]. Therefore, the aim of this study is to establish acclimation mechanisms to UVR via the presence of potentially protective compounds, and to determine the presence and variations of them as a function of the irradiance levels and radiation quality received by the organisms. The study especially focuses on the synthesis/degradation of compounds that would help these organisms to cope with relatively high solar radiation fluxes as found during late summer in the study area.

## 2. Material and methods

### 2.1. Study area and specimens

The study was carried out with Rhodophyta species collected from Playa Barrancas Blancas-Rawson, Chu-

but, Argentina (43°19'S, 65°3'W). The area has a semi-diurnal tidal regime with mean amplitude of 3.3 m [32]; the radiation regime throughout the year in this habitat has been published in Barbieri et al. [33] and in Villafañe et al. [34]. The study site is characterized by a large intertidal zone (~200 m) of flat rocky shore with small tide pools of maximum depths ~15 cm. The sub-littoral has crevices (~60 cm deep and ~50 cm wide) that run perpendicular to the shore. The whole intertidal zone is covered with different species of macroalgae and during low tide it is completely exposed to solar radiation, with some algae in the upper-littoral area suffering severe desiccation. The algae used in this study were *Ceramium* sp. Lyngbye, *Corallina officinalis* Linnaeus, *Callithamnion gaudichaudii* Agardh and *Porphyra columbina* Montagne. *Ceramium* sp. and *P. columbina* were collected in the mid-littoral, whereas *C. officinalis* and *C. gaudichaudii* were collected in the sub-littoral. Specimens were collected during low tide and immediately taken to the Estación de Fotobiología Playa Unión (EFPU) where experiments were conducted as described below. Two experiments were performed, each lasting 46 h: one during 8–10 of February and the other during 20–22 February, 2002.

### 2.2. Experimentation and measurements/determinations

During early morning whole intact specimens of macroalgae were harvested still attached to large pebbles or stones and kept in a large volume of seawater during transport to the laboratory (approximately 20 min away). Macroalgae samples were transferred to flat open plastic containers (15×15×4 cm) filled with sea water and covered with filters so that two radiation treatments were implemented: (a) Samples exposed to PAR + UVR (280–700 nm), containers covered with Ultraphan-295 film (UVT 100, Digefra, Munich, Germany) and (b) Samples exposed to PAR (400–700 nm), containers covered with Ultraphan –395 film (UV Opak, Digefra, Munich, Germany) (the spectra of these materials are published in Figueroa et al. [10]). The containers were floating on a water bath for temperature control and exposed to natural radiation for 46 h. Every 3 h during daytime (and at longer intervals at night) duplicate samples from the thalli were collected; then, samples were gently dried over absorbent paper, weighted (i.e., fresh weight) and placed in tubes with 7 ml of absolute methanol. The samples were extracted for 24 h and then centrifuged; the spectral absorption (250–750 nm) was determined with a scanning spectrophotometer Hewlett Packard (model 8453E). The peak height calculations for the various compounds present in the algae (i.e., UV-absorbing compounds, carotenoids and chl-a, with absorption at about 330, 470 and 665 nm, respectively) were done by peak analysis. A previous work carried out with phytoplankton species [35] has shown that peak

height at 330 nm was a good estimator of MAAs concentration.

Selected specimens of each type of algae were analysed at Friedrich-Alexander Universität (FAU, Germany) using HPLC techniques [36] to determine MAAs at the time of collection. Briefly, MAAs were extracted in 2 ml of 20% methanol (v/v); after centrifugation, the supernatant was lyophilized and redissolved in 0.2% acetic acid. Then the samples were filtered through 0.2  $\mu\text{m}$  pore-sized microcentrifuge filters and analysed using an HPLC system (Merck Hitachi; Interface D-7000, UV-Detector L-7400, Pump L-7100, Darmstadt, Germany) equipped with a LiCrospher RP 18 column and guard (5  $\mu\text{m}$  packing; 250  $\times$  4 mm ID). The samples were then injected into the HPLC column (detection at 330 nm, flow rate of 1 ml min<sup>-1</sup>, mobile phase of 0.2% acetic acid). MAAs were identified by co-chromatography by comparing the absorption spectra and retention times with several standards available at FAU.

Solar radiation was monitored throughout the study period with a broadband ELDONET radiometer (Real Time Computers Inc.). This instrument is permanently installed at the roof of the EFPU since 1998, and collects one data point per minute in three wavebands – PAR (400–700 nm), UV-A (315–400 nm) and UV-B (280–315 nm) – as well as surface temperature.

Non-parametric analyses (i.e., Kruskal–Wallis test) [37] were used to establish differences among treatments; a confidence level of 95% was used in all analyses.

### 3. Results

Incident solar radiation over the study area during the month of February 2002 is shown in Fig. 1. Solar radiation showed a day-to-day variability due to changes in cloud cover. Daily doses of PAR (Fig. 1(a)) varied between 12 MJ m<sup>-2</sup> and ~4 MJ m<sup>-2</sup>; UVR displayed a similar pattern, with UV-A ranging from ~1.6 to 0.46 MJ m<sup>-2</sup>, and UV-B from ~40 to 10 kJ m<sup>-2</sup> (Fig. 1(b)). Fig. 1(c) shows PAR irradiance levels during the experiment performed in the period 20–22 of February 2002 (Exp. #2). It is seen that although maximum PAR irradiances were similar in this two-day period (i.e., ~340 W m<sup>-2</sup>), the second day was characterized by some variability due to scattered clouds, whereas the first day of experimentation was very clear; the same pattern for PAR irradiance was determined during Exp. #1.

The initial absorption characteristics (i.e., optical density (OD) per fresh weight) at the time of collection of macroalgae are shown in Fig. 2. High species-specific variability in both the concentration of UV-absorbing compounds and photosynthetic pigments were determined: *P. columbina* presented the highest concentrations of UV-absorbing compounds (peak at 330 nm),

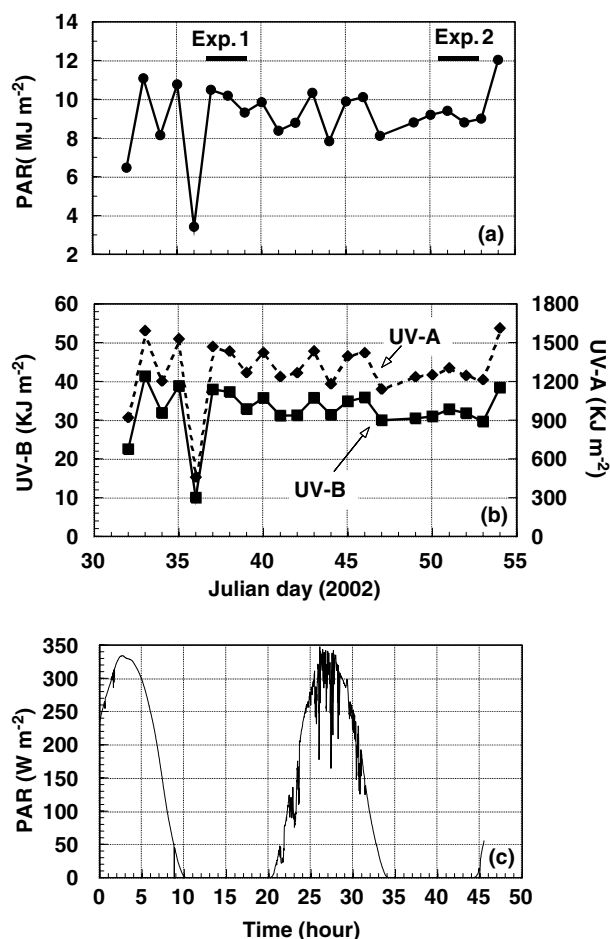


Fig. 1. Solar radiation levels at the study site during February 2002. (a) Daily doses of PAR (MJ m<sup>-2</sup>). (b) Daily doses of UV-B and UV-A (kJ m<sup>-2</sup>). (c) PAR irradiance (W m<sup>-2</sup>) during Exp. #2, February 20–22, 2002.

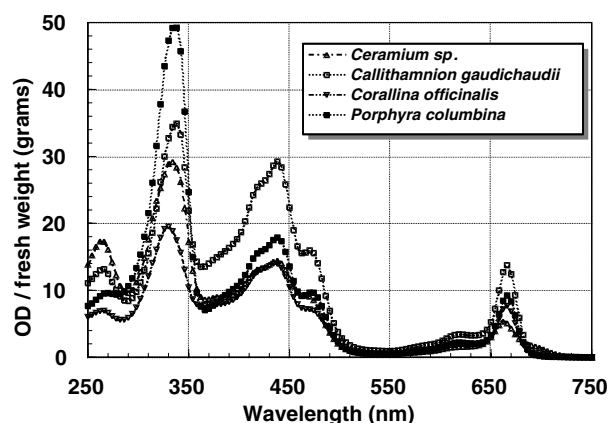


Fig. 2. Absorption characteristics (OD/fresh weight) as a function of wavelength at the time of sampling in: *Ceramium* sp., *Callithamnion gaudichaudii*, *Corallina officinalis* and *Porphyra columbina*.

whereas the lowest were determined in *C. officinalis*; *Ceramium* sp. and *C. gaudichaudii* displayed intermediate values. The absorption due to photosynthetic

Table 1

Mycosporine like amino acids present in Rhodophyte species from Patagonia

Macroalgae	MAAs
<i>Ceramium</i> sp.	S, P
<i>C. gaudichaudii</i>	S, P, P-334
<i>C. officinalis</i>	S
<i>P. columbina</i>	S, P-334

S, Shinorine; P, Palythine; P-334, Porphyra-334.

pigments – chlorophylls, phycobilins and carotenoids (i.e., within the 400–700 nm region) was highest in *C. gaudichaudii*, but rather similar in the other species. HPLC analyses of UV-absorbing compounds revealed the presence of mycosporine-like amino acids (MAAs) in the four species used in this study (Table 1). Shinorine was present in all species, whereas palythine was additionally present in *Ceramium* sp. and *C. gaudichaudii*; porphyra-334 was determined in *C. gaudichaudii* and *P. columbina*.

In the two experiments we determined similar patterns of algae responses when exposed to solar radiation, so for the sake of clarity here we present only data from the Exp. #2 on the daily course of UV-absorbing compounds and photosynthetic pigments (Fig. 3). High variability between radiation treatments (i.e., full radiation and PAR only) was especially evident in *Ceramium* sp. (Figs. 3(a) and (b)) and *C. gaudichaudii* (Figs. 3(c) and (d)). In *Ceramium* sp. exposed to full radiation (Fig. 3(a)) the concentration of UV-absorbing compounds followed approximately the daily irradiance cycle, with high concentrations during the day and then decreasing in the evening; during the day, OD/fresh weight in this radiation treatment was significantly higher ( $p < 0.05$ ) than that in samples exposed only to PAR (Fig. 3(b)). In this species, photosynthetic pigments did not display significant variations between radiation treatments, with absorption values remaining relatively low throughout the experiment. *C. gaudichaudii* also displayed high variability in the concentration of both UV-absorbing compounds and photosynthetic pigments when exposed to full solar radiation (Fig. 3(c)), with significantly high values ( $p < 0.05$ ) during early morning and then decreasing during the day. In samples exposed only to PAR (Fig. 3(d)) there was a clear steady reduction in photosynthetic pigments throughout the experiment and UV-absorbing compounds were undetectable after 5 h of exposure to visible radiation. Photosynthetic and UV-absorbing compounds in *C. officinalis* in both radiation treatments (Fig. 3(e) and (f)) were similar and much lower than in the other three species – just a slight trend of somewhat higher UV-absorbing compounds was noticeable during daytime. Finally, UV absorbing compounds in *P. columbina* generally decreased throughout the experiment in both radiation treatments

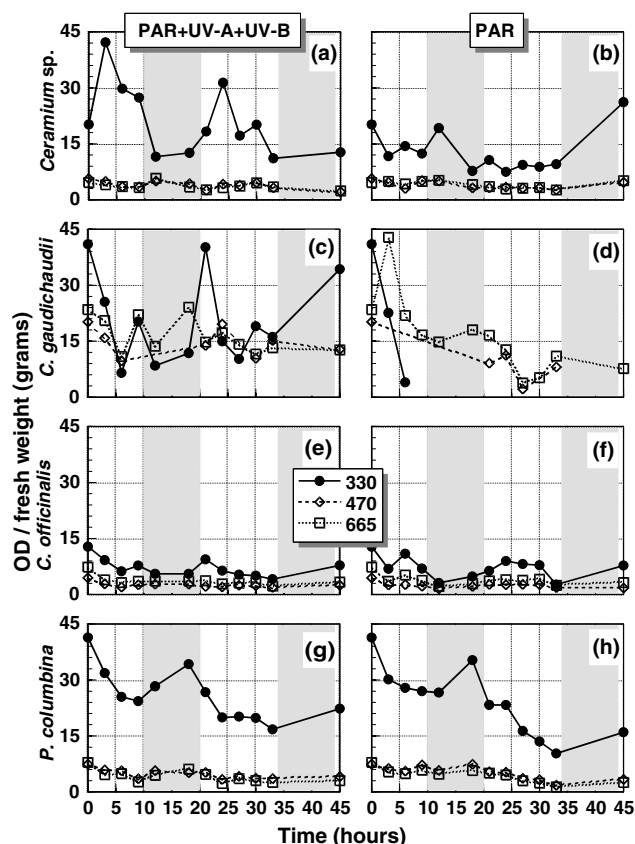


Fig. 3. Mean absorption (OD/fresh weight) at different wavelengths. Data from the peak analysis (330, 470 and 665 nm) at different times of the experiment in: *Ceramium* sp. (a and b), *Callithamnion gaudichaudii* (c and d), *Corallina officinalis* (e and f) and *Porphyra columbina* (g and h) exposed to two radiation treatments (PAR + UV-A + UV-B, and PAR-only). (a), (c), (e), and (g) are samples exposed to full solar radiation, whereas (b), (d), (f), and (h) are samples exposed to PAR only. The shaded areas indicate night time while the clear areas indicate daytime.

(Fig. 3(g) and (h)) but, contrasting to the other three species, higher values were determined at night. A very similar pattern of these compounds was determined in both radiation treatments, except for slightly lower values at the end of the experiment in those samples exposed only to PAR (Fig. 3(h)). Photosynthetic pigments concentrations, on the other hand, remained low throughout the experiment.

Since UV-absorbing compounds varied during our experimentation, we further investigated if these variations were also observed in the photosynthetic pigment or if there were variations due to higher synthesis/degradation of these potentially protective compounds as a function of the irradiance treatments. The co-variation of UV-absorbing compounds and carotenoids as a function of chlorophylls (i.e., the ratio of the peak at 330 or 400 to 665 nm) is shown in Fig. 4. For all species and for both radiation treatments, carotenoids co-varied with chlorophyll with a ratio close to one. On the other hand, UV-absorbing compounds had a wide range of

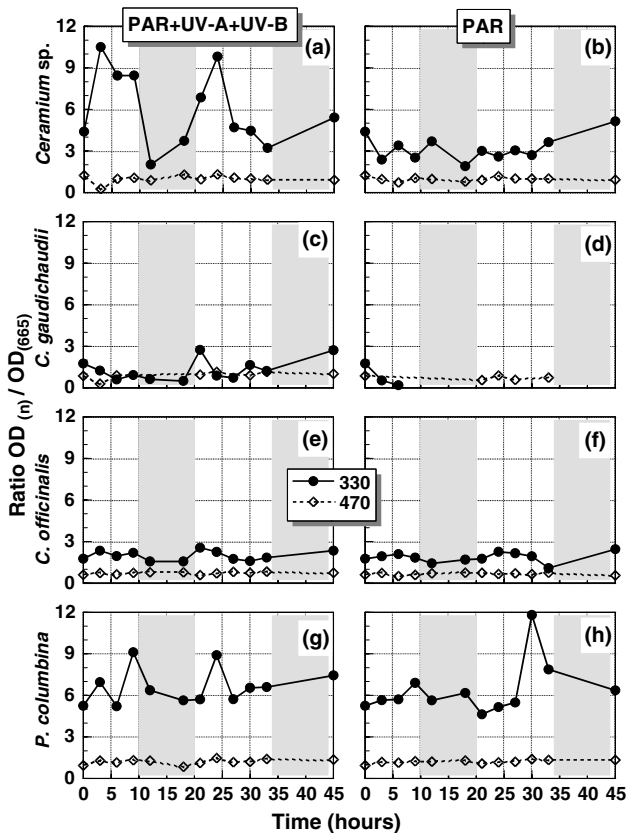


Fig. 4. Mean ratio of UV-absorbing compounds (peak at 330 nm) and carotenoids (peak at 470 nm) to chlorophyll-a (peak at 665 nm) at different times of the experiment in *Ceramium* sp., *Callithamnion gaudichaudii*, *Corallina officinalis* and *Porphyra columbina* exposed either to PAR + UV-A + UV-B, or to PAR-only. (a), (c), (e), and (g) are samples exposed to full solar radiation whereas (b), (d), (f), and (h) are samples exposed to PAR only. The shaded areas indicate night time while the clear areas indicate daytime.

responses according to the species and the radiation treatment imposed to the samples: In *Ceramium* sp., the ratio 330/665 was highly variable, especially under full radiation (Fig. 4(a)) being generally higher during daytime than at night; this high variability, however, was not observed in the PAR-only treatment (Fig. 4(b)). For both *C. gaudichaudii* (Fig. 4(c) and (d)) and *C. officinalis* (Fig. 4(e) and (f)), a relatively high degree of co-variance between UV-absorbing compounds and chlorophylls was determined in both radiation treatments. However, the ratio 330/665 in *C. gaudichaudii* was rather low (Fig. 4(c)) or inexistent due to the fact that UV-absorbing compounds were not detected (Fig. 4(d)), but in *C. officinalis* this ratio was close to 2 and displayed a slight circadian behaviour in both radiation treatments (Fig. 4(e) and (f)). In *P. columbina*, UV-absorbing compounds/chls were variable in the full radiation treatment (Fig. 4(g)), but rather constant when samples were exposed to PAR only, except for the high values observed at the end of the second day of experimentation (Fig. 4(h)).

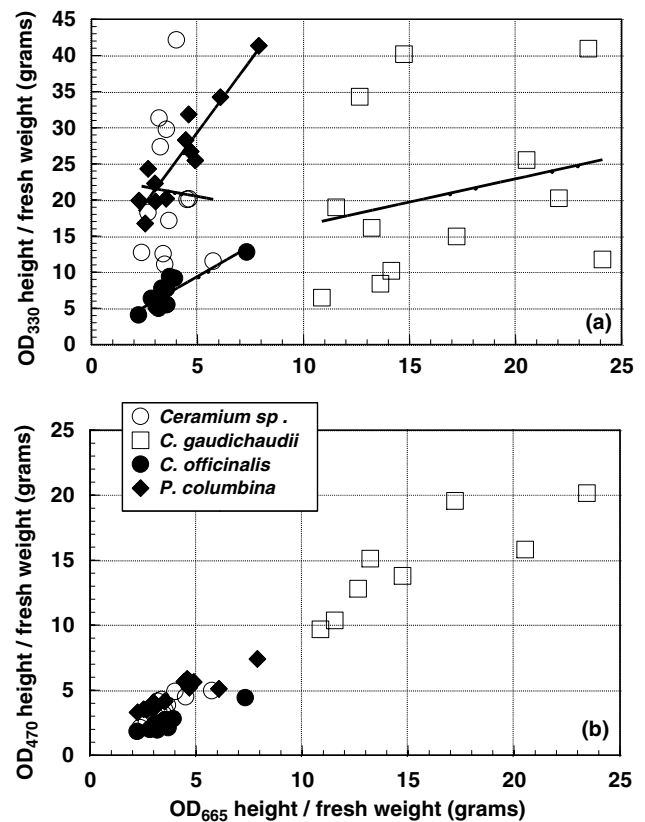


Fig. 5. Mean absorption characteristics (OD/fresh weight) of *Ceramium* sp., *Callithamnion gaudichaudii*, *Corallina officinalis* and *Porphyra columbina* exposed to UVR, as a function of chl-a concentration ( $OD_{665\text{ nm}}$ /fresh weight). (a) Variations in MAAs ( $OD$  at 330 nm). (b) Variations in carotenoids ( $OD$  at 470 nm).

Finally, for *C. officinalis* and *P. columbina* exposed to full radiation, we determined a significant positive correlation between UV-absorbing compounds and chl-a concentrations ( $R^2 = 0.74$ ,  $p < 0.005$  and  $R^2 = 0.85$ ,  $p < 0.0001$ , respectively). On the other hand, in *Ceramium* sp. and in *C. gaudichaudii* a poor correlation between these compounds was found ( $R^2 = 0.0025$ ,  $p = 0.875$  and  $R^2 = 0.0625$ ,  $p = 0.421$ , respectively) (Fig. 5(a)). Carotenoids, however, showed a significant positive correlation with chl-a in the four species studied (Fig. 5(b)), with  $R^2$  varying from 0.67 in *Ceramium* sp. to 0.88 in *C. officinalis*. All data fell within the same fit, with an overall relation of: carotenoids =  $0.9 \times \text{chl-a}$  ( $R^2 = 0.89$ ,  $p < 0.0001$ ).

#### 4. Discussion

Macroalgae living in the littoral zone can be especially affected by solar radiation because they can be exposed to high irradiances, especially when local noon coincides with low tides [1,13]. In particular, our study site in the Patagonian coast was covered with many

macroalgae species that are exposed for relatively long periods to high PAR and UVR levels, especially during summer. In fact, during February, samples were exposed to doses of PAR, UV-A and UV-B as high as  $12 \text{ MJ m}^{-2}$  and 1600 and  $40 \text{ kJ m}^{-2}$ , respectively (Fig. 1(a) and (b)); these values fell within the normal ranges for the study area during this period, as shown in previous studies [33,34]. In addition, relatively clear waters characterize the area, being the PAR attenuation coefficient ( $k_{\text{PAR}}$ )  $\sim 0.3 \text{ m}^{-1}$  [33] so that even with high tides, algae living in the intertidal zone are exposed to relatively high radiation fluxes. Under these conditions, it is expected that macroalgae undergo a series of negative effects produced by natural PAR and UVR levels but, on the other hand, adaptive and acclimation mechanisms should have been developed for such radiation regimes.

Macroalgae exposed to solar PAR and UVR show a number of responses, which can be related to intrinsic factors (i.e., species-specific sensitivity) as well as to the radiation environment (i.e., quantity and quality) in which they live. In addition, factors of morphological nature (e.g., thickness), physiological (i.e., nutritional) status of cells, as well as their capacity to acclimate to the prevailing conditions [2] account for the observed effects. One of these acclimation mechanisms is the reduction in the quantum yield of photosystem II (i.e., dynamic photoinhibition, [3]) which occurs at local noon and in the early afternoon hours in macroalgae from different regions: tropical and temperate zones [38,39] as well as from polar areas [40]. This mechanism has been also determined in several macroalgae species from the Patagonia area [41–43], including red algae [27–31]. Other common acclimation mechanism to UVR is through the presence of UV-absorbing compounds, mainly mycosporine like aminoacids (MAAs). These compounds have been determined in many macroalgae species – especially in red algae – from different environments [14,15,19,20, among others], and its protective function is thought to occur either by acting as sunscreens or by having antioxidant activity [12]. Moreover, a recent study [21] have found an important ecophysiological role of these UV-absorbing compounds in the protection of the DNA molecule, not only by filtering UVR, but also by quenching the excited thymine residue.

In our study we have found that all surveyed species had UV-absorbing compounds (Fig. 2) which were later identified as MAAs (Table 1). The concentration of these compounds (as determined by peak height) varied between species (Fig. 2), probably related not only to intrinsic (i.e., genetic) factors, but also to the relative position of the organisms within the inter-tidal zone or in the water column. Depth distribution of UV-absorbing compounds has been observed in several macroalgae species, with organisms containing relatively higher amounts when living in more exposed areas or in

shallower places [20,44,45]. For example, in our study, the species collected in more exposed and upper zones of the mid-littoral (i.e., *Ceramium* sp. and *P. columbina*) had higher MAAs content than those collected in the lower part of the sub-littoral (i.e., *C. officinalis*).

The general dynamics of UV-absorbing compounds for the four species studied was characterized by a species-specific response (Fig. 3), with their concentration varying differentially throughout the experiment. *Ceramium* sp. exposed to full radiation was the only species in which the concentration of UV-absorbing compounds (i.e., both as a function of fresh weight (Fig. 3(a)) and of chl-a (Fig. 4(a))) closely followed the daily cycle of solar UVR with maximum values close to noon (Fig. 3(a)), as also found in studies carried out by Wood [38] and Karsten and Wiencke [20]. Our data (Figs. 3(a) and 5(a)) as well as its collecting place in the mid-littoral suggest that *Ceramium* sp. is acclimated to high irradiances, with photosynthetic pigments being rather constant during exposure to solar radiation; in fact, Häder et al. [30] found that this Patagonian species, although more sensitive than others they studied, has the ability to acclimate to high radiation regimes as normally found in the area. The fact that the concentration of UV-absorbing compounds are much lower in the PAR only treatment (Fig. 3(b)), together with the daily course of these compounds clearly hints for a UVR-induced production mechanism and thus a potential protective function – i.e., associated to high solar radiation levels. *C. gaudichaudii* displayed a continuous decrease in chl-a (Fig. 3(d)), probably as a consequence of the irradiance change from its original low irradiance environment as received in the crevices where it grow, to the full solar radiation conditions in the experimental set up. A bleaching of photosynthetic pigments within 8 h of exposure to solar radiation was previously observed in this species in our area [30], even though in that study *C. gaudichaudii* was collected from exposed rocky pools rather than from the low-light crevices in the sub-littoral as in this study. In addition, *C. gaudichaudii* was the only species in which UV-absorbing compounds were not detected after 5 h of exposure (PAR – only treatment) (Figs. 3(d) and 4(d)). Together with this, UV-absorbing compounds poorly co-varied with chl-a or with solar radiation (Figs. 4 and 5(a)), suggesting a poor protective function of these compounds in this species. Instead, UV-absorbing compounds may either have an indirect function or act as passive shielding substances by dissipating the absorbed radiation energy in form of harmless heat without generating photochemical reactions [46]. Protection against high PAR and UVR levels in this species is probably related to the morphology of *C. gaudichaudii*, which form a dense thallus protecting effectively the inner part of the organism [30]. In *P. columbina*, on the other hand, UV-absorbing compounds per fresh weight displayed a trend of steady

decrease during the day in both radiation treatments, but then it increased at night (Fig. 3(g) and (h)). When their concentration were normalized by chl-a (Figs. 4(g) and (h) and 5(a)), however, there was a co-variation between these compounds. Thus, it seems that UV-absorbing compounds in this species are not closely related to a protective function against high UVR levels. However, a recent study [31] has shown that MAAs concentration is naturally high in *Porphyra columbina* from Patagonian waters, but its synthesis can be induced if the species is exposed to UVR under high nitrogen levels ( $\sim 400 \mu\text{M}$ ), as could occur in Nature by nutrients supply by river runoff at certain periods of the year [32]. A similar response to that of *P. columbina* was found in *C. officinalis*, but this latter species had much lower content of UV-absorbing compounds as well as UV-absorbing compounds/chl-a ratios (Fig. 4), reflecting its acclimation to low radiation levels as those found in its habitat in the lower part of the sub-littoral as also seen in studies carried out by Häder et al. [29]. It should be noted, however, that the cell wall of *C. officinalis* contains calcium carbonate that effectively absorbs UVR and thus minimize the exposure of vital targets in the algae. Häder et al. [29] nevertheless, working with the same species, but other strain that growth deeper in the sub-littoral, noted a strong photobleaching of photosynthetic pigments when the algae was exposed for a day to full solar radiation.

One should be aware though, that these variable responses – either synthesis or degradation of compounds – can be the result of different processes. On one hand, the differential stability of UV-absorbing compounds when exposed to solar radiation may account for part of the intra and inter-specific variability in their cellular content. For example, Conde et al. [47] demonstrated the high stability of porphyra-334 even under high radiation levels, so while we can assume the stability of this compound, the other MAAs present in our samples (Table 1) might have changed during the experimentation period. On the other hand, the time frame of experimentation may have been not enough (i.e., in *C. officinalis*), so that the poor synthesis of MAAs could be just the result of a short exposure period to solar radiation. For example, a study carried out with the red algae *Porphyra umbilicalis* [19] revealed that 72 h of exposure to solar radiation were not enough for inducing the synthesis of MAAs. The high temporal variability for synthesis/degradation of MAAs has also been determined in phytoplankton species: In the dinoflagellate *Alexandrium excavatum* (= *A. tamarense*) MAAs synthesis occurred within hours [48], whereas in *Symbiodinium microadriaticum* it occurred within weeks [49]; on the other hand, degradation of MAAs in polar diatoms occurred within a time frame of several days [50].

We conclude from this study that a number of responses can be found among native Rhodophyta species

from Patagonia hence production (and eventually diversity) could be variably affected in response to the solar radiation environment. Moreover, the highly exposed area of the Patagonia coast supports various trophic levels thus any decrease or alteration in macroalgae production/diversity might have a higher impact upon the ecosystem than previously thought. In order to fully understand the dynamics of these marine ecosystems, as well as the overall impact of solar radiation, future studies should also address long-term acclimation and repair mechanisms in these species.

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## References

- [1] D.-P. Häder, F.L. Figueroa, Photoecophysiology of marine macroalgae, *Photochem. Photobiol.* 66 (1997) 1–14.
- [2] L.A. Franklin, R. Forster, The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology, *Eur. J. Phycol.* 32 (1997) 207–232.
- [3] D. Hanelt, Photoinhibition of photosynthesis in marine macroalgae, *Sci. Mar.* 60 (Suppl. 1) (1996) 243–248.
- [4] G. Döhler, E. Hagmeier, C. David, Effects of solar and artificial UV radiation on pigments and assimilation of  $^{15}\text{N}$  ammonium and  $^{15}\text{N}$  nitrate by macroalgae, *J. Photochem. Photobiol. B: Biol.* 30 (1995) 179–187.
- [5] V.E. Villafañe, K. Sunbäck, F.L. Figueroa, E.W. Helbling, Photosynthesis in the aquatic environment as affected by ultraviolet radiation, in: UV effects in aquatic organisms and ecosystems, in: E.W. Helbling, H.E. Zagarese (Eds.), *Comprehensive Series of Photoscience and Photobiology*, vol. I, The Royal Society of Chemistry, Cambridge, 2003, pp. 357–397.
- [6] A. Flores-Moya, D. Hanelt, F.L. Figueroa, M. Altamirano, B. Viñegla, S. Salles, Involvement of solar UV-B radiation in recovery of inhibited photosynthesis in the brown alga *Dictyota dichotoma* (Hudson) Lamouroux, *J. Photochem. Photobiol. B: Biol.* 49 (1999) 129–135.
- [7] M. Altamirano, A. Flores-Moya, F.L. Figueroa, Long-term effects of natural sunlight under various ultraviolet radiation conditions on growth and photosynthesis of intertidal *Ulva rigida* (Chlorophyceae) cultivated in situ, *Bot. Mar.* 43 (2000) 119–126.
- [8] H. Pakker, C.A.C. Beekman, A.M. Breeman, Efficient photoreactivation of UVBR-induced DNA damage in the sublittoral macroalga *Rhododymenia pseudopalmeta* (Rhodophyta), *Eur. J. Phycol.* 35 (2000) 109–114.
- [9] W.H. van de Poll, A. Eggert, A.G.J. Buma, A.M. Breeman, Effects of UV-B induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat related differences in ultraviolet-B tolerance, *J. Phycol.* 37 (2001) 30–37.
- [10] F.L. Figueroa, S. Salles, J. Aguilera, C. Jiménez, J. Mercado, B. Viñegla, A. Flores-Moya, M. Altamirano, Effects of solar

- radiation on photoinhibition and pigmentation in the red alga *Porphyra leucosticta*, Mar. Ecol. Prog. Ser. 151 (1997) 81–90.
- [11] J. Aguilera, C. Jiménez, F.L. Figueroa, M. Lebert, D.-P. Häder, Effect of ultraviolet radiation on thallus absorption and photosynthetic pigments in the red alga *Porphyra umbilicalis*, J. Photochem. Photobiol. B: Biol. 48 (1999) 75–82.
  - [12] A.T. Banaszak, Photoprotective physiological and biochemical responses of aquatic organisms, in: UV effects in aquatic organisms and ecosystems, in: E.W. Helbling, H.E. Zagarese (Eds.), Comprehensive Series of Photoscience and Photobiology, vol. I, The Royal Society of Chemistry, Cambridge, 2003, pp. 329–356.
  - [13] K. Bischof, D. Hanelt, C. Wiencke, UV-radiation can affect depth-zonation of Antarctic macroalgae, Mar. Biol. 131 (1998) 597–605.
  - [14] U. Karsten, T. Sawall, D. Hanelt, K. Bischof, F.L. Figueroa, A. Flores-Moya, C. Wiencke, An inventory of UV-absorbing mycosporine like amino acids in macroalgae from polar to warm-temperate regions, Bot. Mar. 41 (1998) 443–453.
  - [15] U. Karsten, L.A. Franklin, K. Lüning, C. Wiencke, Natural ultraviolet radiation and photosynthetically active radiation induce formation of mycosporine-like amino acids in the marine macroalgae *Chondrus crispus* (Rhodophyta), Planta 205 (1998) 257–262.
  - [16] A. Gröniger, R.P. Sinha, M. Klisch, D.-P. Häder, Photoprotective compounds in cyanobacteria, phytoplankton and macroalgae – a database, J. Photochem. Photobiol. B: Biol. 58 (2000) 115–122.
  - [17] J. Aguilera, K. Bischof, U. Karsten, D. Hanelt, C. Wiencke, Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defense systems against high light stress, Mar. Biol. 140 (2002) 1087–1095.
  - [18] D.-P. Häder, A. Gröniger, C. Hallier, M. Lebert, F.L. Figueroa, C. Jiménez, Photoinhibition by visible and ultraviolet radiation in the red macroalga *Porphyra umbilicalis* grown in the laboratory, Plant Ecol. 145 (1999) 351–358.
  - [19] A. Gröniger, C. Hallier, D.-P. Häder, Influence of UV radiation and visible light on *Porphyra umbilicalis*: photoinhibition and MAA concentration, J. Appl. Phycol. 11 (1999) 437–445.
  - [20] U. Karsten, C. Wiencke, Factors controlling the formation of UV-absorbing mycosporine-like amino acids in the marine red alga *Palmaria palmata* from Spirsbergen (Norway), J. Plant Physiol. 155 (1999) 407–415.
  - [21] T. Misonou, J. Saitoh, S. Oshiba, Y. Tokitomo, M. Maegawa, Y. Inoue, H. Hori, T. Sakurai, UV-absorbing substance in the red alga *Porphyra yezoensis* (Bangiales, Rhodophyta) block thymine photodimer production, Mar. Biotechnol. 5 (2003) 194–200.
  - [22] K. Yabe, M. Makino, M. Suzuki, The influence of ultraviolet irradiation on marine organisms, 4: the secretion of UV-absorbing substances from *Palmaria palmata*, Jpn. J. Phycol. 46 (1998) 167–172.
  - [23] E.E. Romanello, G. Arnoldi, H. García, R. Taylor, M. Medina, Evaluación del área y biomasa de la pradera de *Gracilaria verrucosa* (Hudson) Papenfuss en la bahía Melo, provincia del Chubut, Argentina, Nat. Pat. Cienc. Biol. 1 (1993) 111–114.
  - [24] M.L. Piriz, Phenology of a *Gigartina skottsbergii* Setchell et Gardner population in Chubut Province (Argentina), Bot. Mar. 39 (1996) 311–316.
  - [25] A.L. Boraso de Zaixso, *Gracilaria verrucosa* in Golfo Nuevo, Chubut, Argentina. Biological parameters and environmental factors, XII Int. Seaw. Symp. Proc. Hydrobiol. 151/152 (1987) 239–244.
  - [26] G.N. Casas, E.E. Romanello, H. García, Observaciones sobre el crecimiento y brotación de *Gracilaria gracilis* (Gracilariales, Rhodophyta) en Golfo Nuevo (Chubut, Argentina), Nat. Pat. Cienc. Biol. 5 (1997) 39–54.
  - [27] D.-P. Häder, M. Lebert, R.P. Sinha, E.S. Barbieri, E.W. Helbling, Role of protective and repair mechanisms in the inhibition of photosynthesis in marine macroalgae, Photochem. Photobiol. Sci. 1 (2002) 809–814.
  - [28] D.-P. Häder, M. Lebert, E.W. Helbling, *In situ* effects of solar radiation on photosynthesis in the Patagonian Rhodophyte, *Porphyra columbina* Montagne, Recent Res. Devel. Biochem. 4 (2003) 931–944.
  - [29] D.-P. Häder, M. Lebert, E.W. Helbling, Effects of solar radiation on the Patagonian rhodophyte, *Corallina officinalis* (L.), Photosynth. Res. 78 (2003) 119–132.
  - [30] D.-P. Häder, M. Lebert, E.W. Helbling, Variable fluorescence parameters in the filamentous Patagonian rhodophytes, *Callithamnion gaudichaudii* and *Ceramium* sp. under solar radiation, J. Photochem. Photobiol. B: Biol. 73 (2004) 87–99.
  - [31] N. Korbee Peinado, R.T. Abdala Díaz, F.L. Figueroa, E.W. Helbling, Ammonium and UVR stimulate the accumulation of mycosporine-like amino acids (MAAs) in *Porphyra columbina* (Rhodophyta) from Patagonia, Argentina, J. Phycol. 40 (2004) 248–259.
  - [32] E.W. Helbling, J.M. Santamarina, V.E. Villafañe, Chubut River Estuary (Argentina): Estuarine variability under different conditions of river discharge, Rev. Biol. Mar. Valparaíso 27 (1992) 73–90.
  - [33] E.S. Barbieri, V.E. Villafañe, E.W. Helbling, Experimental assessment of UV effects upon temperate marine phytoplankton when exposed to variable radiation regimes, Limnol. Oceanogr. 47 (2002) 1648–1655.
  - [34] V.E. Villafañe, E.S. Barbieri, E.W. Helbling, Annual patterns of ultraviolet radiation effects on temperate marine phytoplankton off Patagonia, Argentina, J. Plankton Res. 26 (2004) 167–174.
  - [35] W.C. Dunlap, G.A. Rae, E.W. Helbling, V.E. Villafañe, O. Holm-Hansen, Ultraviolet-absorbing compounds in natural assemblages of Antarctic phytoplankton, Antarct. J. US 30 (1995) 323–326.
  - [36] R.P. Sinha, M. Klisch, D.-P. Häder, Induction of a mycosporine-like amino acid (MAA) in the rice-field cyanobacterium *Anabaena* sp. by UV irradiation, J. Photochem. Photobiol. B: Biol. 52 (1999) 59–64.
  - [37] J.H. Zar, Biostatistical Analysis, second ed., Prentice-Hall, Englewood Cliffs, NJ, 1984.
  - [38] W.F. Wood, Photoadaptive responses of the tropical red alga *Euchema striatum* Schmitz (Gigartinales) to ultraviolet radiation, Aquat. Bot. 33 (1989) 41–51.
  - [39] L.A. Franklin, G.G.R. Seaton, C.E. Lovelock, A.W.D. Larkum, Photoinhibition of photosynthesis on a tropical reef, Plant Cell Environm. 19 (1996) 825–836.
  - [40] I. Gómez, D.N. Thomas, C. Wiencke, Longitudinal profiles of growth, photosynthesis and light independent carbon fixation in the Antarctic brown alga *Ascoseira mirabilis*, Bot. Mar. 38 (1995) 157–164.
  - [41] D.-P. Häder, M. Lebert, E.W. Helbling, Photosynthetic performance of the chlorophyte *Ulva rigida* measured in Patagonia on site, Recent Res. Develop. Photochem. Photobiol. 4 (2000) 259–269.
  - [42] D.-P. Häder, M. Lebert, E.W. Helbling, Effects of solar radiation on the Patagonian macroalgae *Enteromorpha linza* (L.) J. Agardh – Chlorophyceae, J. Photochem. Photobiol. B: Biol. 62 (2001) 43–54.
  - [43] D.-P. Häder, M. Lebert, E.W. Helbling, Photosynthetic performance of marine macroalgae measured in Patagonia on site, Trends Photochem. Photobiol. 8 (2001) 145–152.
  - [44] K. Hoyer, U. Karsten, T. Sawall, C. Wiencke, Photoprotective substances in Antarctic macroalgae and their variation with respect to depth distribution, different tissues and developmental stages, Mar. Ecol. Prog. Ser. 211 (2001) 117–129.



- [45] M. Maegawa, M. Kunieda, W. Kida, The influence of ultraviolet radiation on the photosynthetic activity of several red algae from different depths, *Jpn. J. Phycol.* 41 (1993) 207–214.
- [46] W.M. Bandaranayake, Mycosporines: are they nature's sunscreens?, *Nat. Prod. Rep.* 15 (1998) 159–172.
- [47] F.R. Conde, M.S. Churio, C.M. Previtali, The photoprotector mechanisms of mycosporine-like amino acids. Excited-state properties and photostability of porphyra-334 in aqueous solution, *J. Photochem. Photobiol. B: Biol.* 56 (2000) 139–144.
- [48] J.I. Carreto, M.O. Carignan, G. Daleo, S. De Marco, Occurrence of mycosporine-like aminoacids in the red-tide dinoflagellate *Alexandrium excavatum*: UV-photoprotective compounds?, *J. Plankton Res.* 12 (1990) 909–921.
- [49] A.T. Banaszak, R.K. Trench, Effects of ultraviolet (UV) radiation on marine microalgal-invertebrate symbioses. II. The synthesis of mycosporine-like amino acids in response to exposure to UV in *Anthopleura elegantissima* and *Cassiopeia xamachana*, *J. Exp. Mar. Biol. Ecol.* 194 (1995) 233–250.
- [50] E.W. Helbling, B.E. Chalker, W.C. Dunlap, O. Holm-Hansen, V.E. Villafañe, Photoacclimation of Antarctic marine diatoms to solar ultraviolet radiation, *J. Exp. Mar. Biol. Ecol.* 204 (1996) 85–101.