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Are there any effects of enhanced UV-B radiation on *Chara polyacantha* and *Nitella hyalina* under laboratory conditions?

Abstract

Attenuation of UV-B radiation in the water column occurs due to absorption and scatter but in shallow clear freshwater systems enhanced UV-B radiation might be of importance in affecting aquatic organisms. Two laboratory experiments were performed to determine whether two species of charophytes typical for shallow waterbodies, such as *Chara polyacantha* and *Nitella hyalina*, are negatively affected by UV-B radiation. Each experiment had two treatments: (i) the photosynthetic active radiation (PAR) treatment and (ii) the enhanced UV-B+PAR treatment. We focussed on the influence on growth rate, photosynthetic pigment concentration and the amount of methanol soluble UV absorbing compounds which can act as a screen preventing radiation damage. UV-B radiation increase in the Extremely Enhanced UV-B Radiation Experiment (> 15 $kJ \cdot m^{-2} \cdot day^{-1}$ UV-B dose) reduced average charophyte length in both species after three weeks of incubation and caused depigmentation and cracking of the charophytes. However, in the Enhanced UV-B Radiation Experiment (3.6 $kJ \cdot m^{-2} \cdot day^{-1}$ UV-B dose) we found a large variability in the length of charophyte individuals in both treatments after 22 and 49 days what caused that there were not statistically significant differences in the growth rates of both charophyte species. However, significant differences in *C. polyacantha* growth rate were found after four months of exposure. There were not either significant differences in chlorophyll *a* concentration in *C. polyacantha* charophyte tissues among the treatments. On the other hand, *N. hyalina* increased its chlorophyll concentration in the PAR+UV-B treatment. In both experiments, we did not find statistically significant increased levels of methanol soluble UV absorbing compounds under enhanced UV-B conditions. The lack of induction of this kind of compounds in *C. polyacantha* and *N. hyalina* under augmented UV-B doses during our experiments is in agreement with other Authors results for *Chara aspera*, and make us to consider charophytes as potentially sensitive organisms to UV-B radiation.

Keywords: ultraviolet radiation, charophytes, shallow water bodies, UV absorbing compounds, chlorophyll, growth rate

1 Introduction

Currently there is an increase of solar UV-B radiation reaching the earth surface resulting from stratospheric ozone depletion. This fact is known since the early 1970s –due to, among other reasons, the anthropogenic emission of halogen-containing compounds, such as chlorofluorocarbons (CFC's) – has resulted in an increase of UV-B radiation (280-320 nm) at the earth surface (Madronich 1992; Herman *et al*. 1996). The most pronounced effect of the ozone depletion is the development of the Antarctic ozone hole in the southern spring. Since the discovery of the ozone hole by Farman *et al*. (1985), it has been great public awareness of ozone depletion and its consequences for the biosphere, making a big effort to measure the levels of that kind of radiation. So, increased levels of UV-B radiation have been detected at mid and high latitudes in the Northern hemisphere during the past decades (Herman *et al*. 1996; Madronich *et al*. 1998; SORG 1999).

With a view to a global environmental change like we are facing nowadays it is essential, among other issues, to deepen the knowledge of the effects of ultraviolet radiation in water depth, particularly for shallow freshwater systems where that factor may affect the whole community (Scully & Lean 1994). Thus, several studies have emphasised the need and importance of studying the ecological implications of UV-B radiation in aquatic systems (Williamson 1995; Arts *et al*. 2000).

In aquatic environments the attenuation of the UV-B radiation through the water column (even its potential damage) is mostly due to water absorption, although it is attenuated by the dissolved organic carbon, chlorophyll (phytoplankton) and solid particles in suspension (Kirk 1994). Submerged vegetation and specially the charophytes play an important role on water transparency increases due to nutrient incorporation -in this way are not available for phytoplankton-, preventing resuspension and by producing allelopathic substances that also hinder the growth of microalgae (Scheffer *et al.* 1993; Scheffer 1998; Blindow *et al.* 2002). In the case of charophytes, which often develop a high biomass on the lake bottom, sedimentation is also stimulated (Crawford 1979; Scheffer 1998). Van den Berg (1999) stated that the restriction of resuspension and increase of sedimentation may be the primary explanation for the strong effects of *Chara* meadows on transparency. The fact that charophytes contribute to increased water transparency suggests that UV-B radiation could penetrate to a greater depth in this type of ecosystems.

Studies on the effects of ultraviolet radiation on plants show that UV-B radiation may cause damage to DNA, affect growth and induce production of protective UV-B absorbing compounds (Rozema *et al*. 1997). However, data are limited for submerged vegetation in freshwater ecosystems, especially for charophytes. From an evolutionary point of view, the charophytes constitute an important group within the green algae. Rozema and co-workers (1997) assumed that the increase in complexity of UV-B absorbing compounds during evolution may have evolutionary importance.

In relation to the UV radiation effects on charophytes, as far as we know the only available literature on this topic is De Bakker and co-workers studies (2001, 2005), in which the authors performed experiments in greenhouses and the field about the UV-B radiation influence on growth, DNA damage and reproduction on an only charophyte species, *Chara aspera*. Both studies show that UV-B radiation affects negatively charophyte growth, although the total biomass was the same under different UV-B radiation treatments in laboratory. In terms of reproductive strategies,

the radiation enhancement increases the bulbils formation (vegetative propagules). However, in response to a reduction of UV radiation the sexual reproduction structures (oogonia and antheridia) increase their number significantly. It means, in response against the increased radiation the asexual reproduction is favoured.

Within the green algae, charophytes seem to be closer to land plants (Devereux *et al.* 1990; Stafford 1991; Graham 1993). Therefore, it would be expected that their response against UV-B radiation would be similar to the higher plants (more protective compounds synthesis). However, De Bakker *et al.* (2001, 2005) suggest that charophytes do not develop protective compound against UV-B radiation, because an increase of these compounds was not recorded under the different conditions of their ultraviolet experiments. According to these authors, the results suggest that these organisms have a limited ability to produce protective compounds, therefore, they would be potentially sensitive organisms to increased UV-B radiation. The absence of protective compounds increase under UV-B radiation is remarkable and unusual, since they have been described for cyanobacteria, microalgae, macroalgae and higher plants (Büdel *et al.* 1994; Karsten *et al.* 1999; Meijkamp *et al.* 1999). However, some studies have emphasized that carotenes could have a protective role against high light or UV-radiation (Schagerl & Pichler 2000).

The aim of this study is: (i) to asses the possible changes in growth in two species of charophytes usually found in shallow coastal ecosystems under an increase of UV-B radiation, and also (ii) to determine whether or not these organisms generate UV-B absorbing compounds against UV radiation. To do that, two laboratory experiments were performed with two charophyte species, and two UV-B radiation doses. We followed variables such as the final plant length, growth rate, chlorophyll concentration and absorbance of methanol soluble UV-B absorbing compounds as a measure of protective substances against UV radiation.

2 Materials and methods

Effects of UV-B radiation on *Chara polyacantha* (A. Braun in Braun, Rabenhorst und Stizenberg 1859) and *Nitella hyalina* (De Candolle, Agardh 1824) were investigated in two laboratory experiments. We chose these species as representative of corticated charophycean algae (*Chara polyacantha*) and ecorticated (*Nitella hyalina*). *C. polyacantha* is widely distributed throughout Europe on oligosaprophytic permanent water bodies with clear water, being able to reach 14-18 meters of depth. *N. hyalina* grows on permanent and clear water bodies too, but only arriving until 4 meters deep (Krause 1997; Cirujano *et al.* 2008). The charophytes were initially collected from *Tancat de la Pipa* (Albufera de Valencia Nature Reserve, Valencia, Spain), as well as sediment used as part of growth substrate in the experimental cultures. *Tancat de la Pipa* is a restored ecosystem, where a water spring fed by subterranean waters was created. The water spring was naturally colonized by the aforementioned charophyte species. Both charophyte cultures were kept under the following conditions: room temperature of 20 ºC and a light:dark cycle of 12:12 hours. Light conditions were provided by fluorescent tubes of photosynthetically active radiation (F58W/T8 Sylvania). Illumination was from above and from the back of cultures. All the experiments were carried out at the same culture room.

Pre-experimental cultures of both charophyte species were started in 8 transparent plastic containers (Ø 14 cm, height 28 cm; 6 L of capacity) which were filled with dechlorinated tap water. After it, aquaria stood for several days before introducing the organisms. Sediment from the *Tancat de la Pipa* water spring was sterilized by autoclave in order to avoid a possible germination and growth of sexual propagules from other charophytes (unwanted) contained in the sediment. To obtain culture substrate growth, the sterilized sediment was mixed with cleaned commercial sand in a 1:2 proportion (sediment:sand). The culture substrate was put forming a uniform 2 cm thick layer in 16 plastic Petri dishes (Ø 9 cm).

Vegetative reproduction was carried out cutting the apical portions of the charophyte stocks for *Chara polyacantha*, or else pulling up very young individuals from *Nitella hyalina* cultures -checking the sexual reproduction structures absence in both cases-. Individuals obtained presented between 2 and 4 nodes (Figure 1). With the help of the forceps, 3 individuals of the same species were planted in each Petri dish -in an equidistant way- and 2 dishes (6 specimens) were placed in each aquarium. Therefore four aquaria per species were used (12 specimens (replicates) for each treatment and each species). The averaged size of the planted organisms was 1.8 ± 0.9 cm for *Chara polyacantha* and 1 ± 0.4 cm for *Nitella hyalina*.

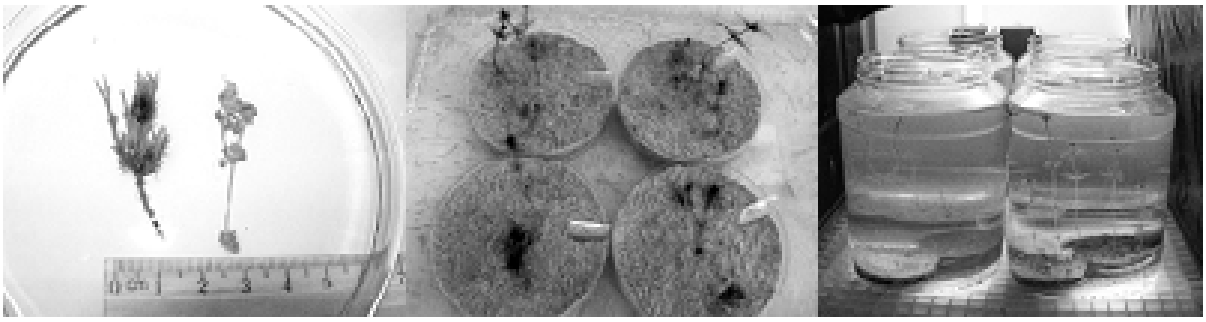

Fig. 1 a) Example of the specimens of *Chara polyacantha* (corticated) and *Nitella hyalina* (ecorticated) obtained for the vegetative reproduction in pre-experimental cultures, b) detail of the Petri dishes with the culture substrate and three charophyte specimens planted in each one (*C. polyacantha* at the picture) and, c) layout of the laboratory cultures and Petri dishes with charophytes in the culture room.

Underwater PAR radiation was measured with a spherical radiation sensor (LI-193SA), coupled to a light meter LI-COR (model LI-250), at 10 cm of depth in the aquaria. The mean irradiance was 70 µmol photons·m-2·s-1. Dechlorinated tap water was added periodically to compensate the evaporation of the cultures. During the acclimatization period, the growth of every charophyte specimen was followed by measuring the total length with the help of a ruler.

Experimental procedure

First of all the pre-experimental culture dishes were assigned at random to each treatment in every experiment. We performed two experiments: (1) the Extremely Enhanced UV-B Radiation Experiment with a simulated ten centimeters underwater UV-B dose of > 15 kJ·m-2·day-1 and (2) the Enhanced UV-B radiation experiment with a dose of 3.6 kJ·m-2·day-1. In each experiment charophyte cultures were exposed to two treatments: (i) the photosynthetic active radiation (PAR) treatment in

which charophycean algae only received this kind of radiation, and (ii) the enhanced UV-B+PAR treatment in which cultures also received radiation from an ultraviolet tube (Philips TL 40). In the middle of the room light period charophytes from PAR+UV-B treatment were exposed to UV radiation during 4 hours per day. Differences in UV-B radiation of each experiment were obtained by adjusting the height of the UV tube above the charophyte cultures. The underwater UV-B radiation was measured with a submersible BIC radiometer (Biospherical Instruments) at 10 cm of depth inside the aquaria.

Charophyte cultures position was changed once a week, following a Z-shaped path, in order to all of them could receive a similar irradiation during the experiments. Also, another day of the week, the containers were rotated 180 degrees to try to homogenize the experimental conditions. In each experiment, each container housed a single charophyte species to avoid possible effects of allelopathy or competence for nutrients that could affect the experiment results.

During the first experiment charophyte length was measured at 20 days of exposure. During the second experiment the measurements (length, chlorophyll and UV absorbing compound concentrations) were performed at 22, 49 and 120 days of exposure.

Growth rate

With the help of a ruler the maximum length of every charophyte was measured at the beginning of each experiment, at different moments and at the end. Growth rate of each specimen was calculated using the following formula:

Growth rate = (final length – initial length) / experimental time in days

Analysis of photosynthetic pigments and UV radiation absorbing compounds

Chlorophyll content from the charophytes was determined by extraction, spectrophotometric measurement and formulae of Jeffrey & Humphrey (1975). Fresh material of *Chara polyacantha* and *Nitella hyalina* (over 10 and 5 mg, respectively), after weigh it with a precision balance (Sartorius BP121S), was put in test tubes (three replicates by species). For the extractions, 3 ml of extractant (90% acetone) were added to every tube. Test tubes were sonicated in an ultrasonic water bath (ELMAsonic S30H) for 15 min to break the cell walls. After 24 hours of extraction at freezer in dark, test tubes were centrifuged (Selecta centrifuge mod 540) for 10 min at 2500 rpm. The absorbance of the solution was then measured with a spectrophotometer (Hitachi U-2001) at 630, 645 y 665 nm. Absorbance at 750 nm was also recorded as a turbidity measurement of the solution and subtracted to each other wavelength absorbance.

For the determination of methanol soluble UV absorbing compounds from charophytic algae we followed the protocol described by De Bakker *et al*. (2001), using acidified methanol (100% CH_3OH: demineralised water: 37% HCl in ratio 79:20:1) as extractant. Previously, the dry material (24 hours at 70 ºC) was weighed on a precision balance. For the extraction of the compounds, algae material from each species was added to test tubes containing 3 ml extractant (three replicas by species). After closing the test tubes to prevent evaporation, tubes were sonicated in an ultrasonic water bath for 10 min, after which they were placed in a water bath

(Clifton NE2-4DCE) at 90 ºC for 1 h. Cooled test tubes were then centrifuged for 10 min at 2500 rpm. An absorbance scanning of the supernatant was performed from 420 to 260 nm with the spectrophotometer. Absorbances at 280, 300 and 320 nm were considered as measurement of UV-B radiation absorbing compounds; and absorbances at 340, 360, 380 and 400 nm as measurement of UV-A radiation absorbing compounds. These absorbances were used to detect possible statistical differences between treatments.

Statistical methods

Firstly, starting conditions were studied in terms of initial average length of the charophyte specimens in both treatments and experiments by one-way analysis of variance (ANOVA). To detect differences in growth rates for each treatment (in each species) two separate one-way ANOVA were performed. A one-way ANOVA to analyze differences in the concentration of chlorophyll *a* and *b* for each species (depending on treatment) was performed. And another one-way ANOVA was used to detect differences in UV radiation absorption compounds in measured wavelengths (for each species). The homogeneity of variances was checked by Levene test in all cases. When no homocedasticity was achieved the non parametric Kruskal-Wallis test was applied. All statistical tests were performed using SPSS version 13.0.

3 Results

Growth rates

At the beginning of both experiments, the initial averaged length for both charophyte species did not show statistical differences between both treatments (PAR and PAR+UV-B).

Extremely Enhanced UV-B radiation experiment

There were statistically differences in growth rate of *Chara polyacantha* (ANOVA $F=99.4$; $p<0.001$) and *Nitella hyalina* (ANOVA $F=9.2$; $p=0.007$) between treatments after 20 days of exposure (the individuals of *C. polyacantha* were, on average, 57% shorter than those in the PAR treatment and 43% shorter in the case of *N. hyalina*) (Figure 2). Moreover, the charophyte *Chara polyacantha* showed visible affectation signs (whitening due to depigmentation and cracking) (Figure 3). The damage began in the whorls. In *Nitella hyalina* signs were less obvious, but also noticeable.

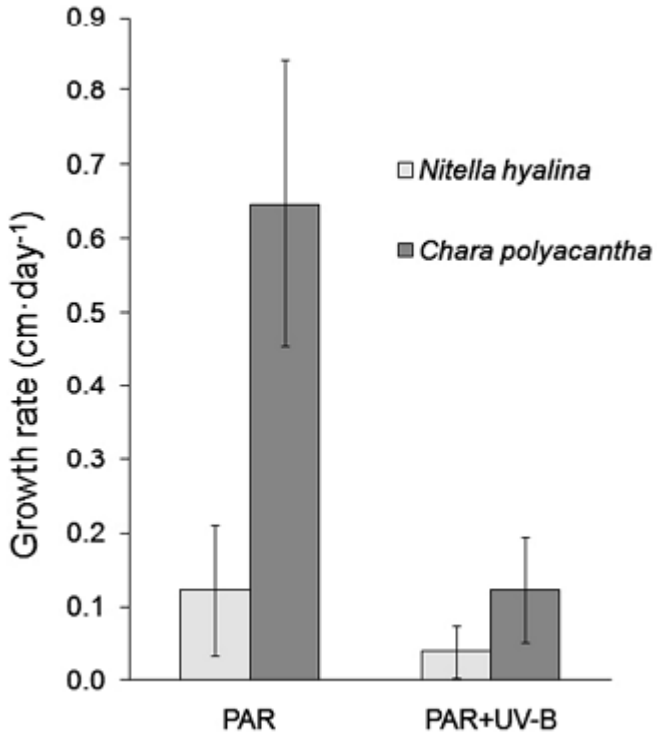

Fig. 2 Averaged growth rate (cm·day^{-1}) of *Chara polyacantha* and *Nitella hyalina* calculated at 20 days of exposure in both experimental treatments (PAR and PAR+UV-B) during the Extremely Enhanced UV-B Radiation Experiment. Vertical bars show the standard deviation of the mean (n=12).

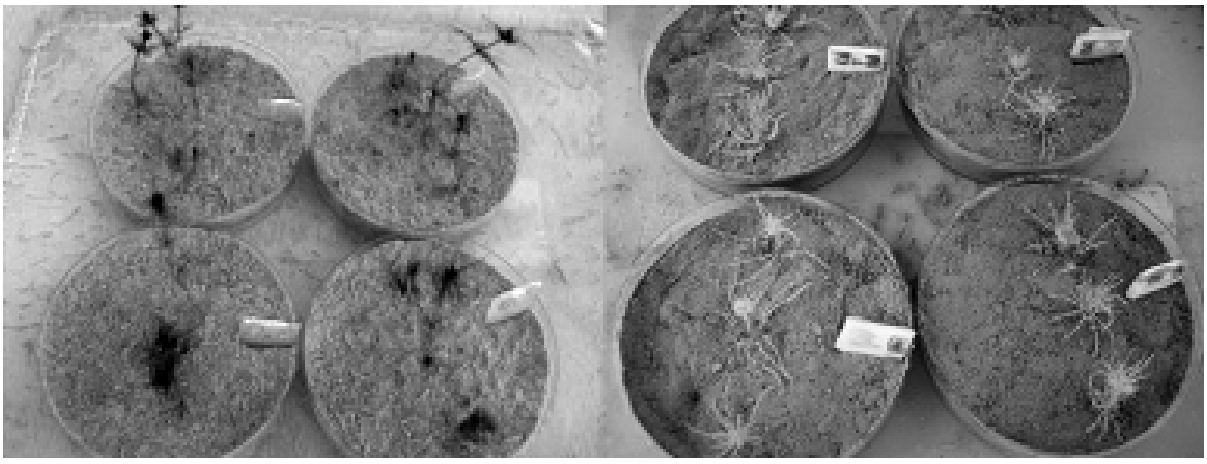

Fig. 3 Photos showing *C. polyacantha* specimens from PAR (left) and PAR+UV-B (right) treatments after 20 days of exposure during the Extremely Enhanced UV-B Radiation Experiment.

Enhanced UV-B radiation experiment

Chara polyacantha length reached 18-33 cm in average at the end of experiment, however, *Nitella hyalina* grew until 4-5 cm (Figure 4a). There were not statistically significant effects of enhanced UV-B radiation dose (3.6 kJ·m^{-2}·day^{-1}) after 22 and 49 day of exposure on the growth rate of both *Chara polyacantha* and *Nitella hyalina*. Calculated growth rates among specimens showed high variability.

However, after 120 days of exposure, a significant effect of UV-B radiation on the growth rate of *Chara polyacantha* was noted (Kruskal-Wallis 12.2; $p < 0.001$), whereas there was not a statistically significant difference in the growth rate of *Nitella hyalina* among treatments (ANOVA F=1.35; $p = 0.26$) (Figure 4b).

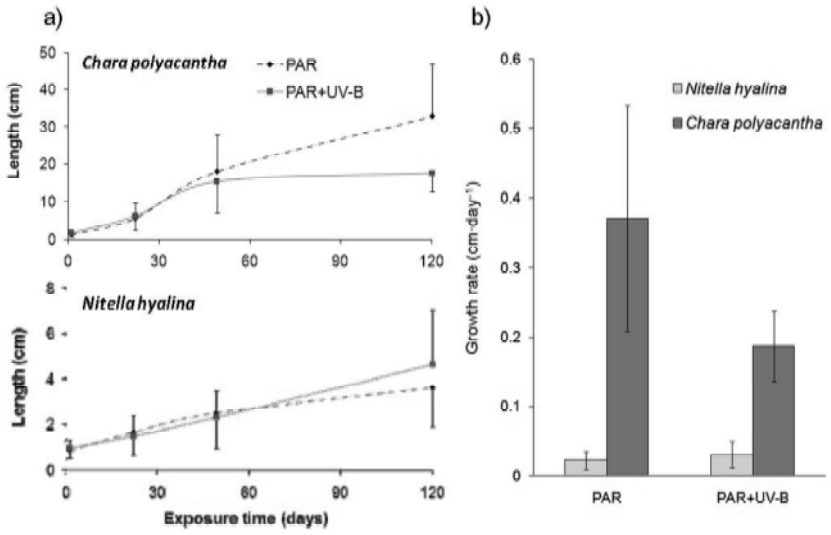

Fig. 4 a) Shoot length increases of *C. polyacantha* and *N. hyalina* throughout exposure time in both experimental treatments during the Enhanced UV-B Radiation Experiment, b) averaged growth rate (cm·day^{-1}) of *C. polyacantha* and *N. hyalina* in both experimental treatments (PAR and PAR+UV-B) after 120 days of exposure during the Enhanced UV-B Radiation Experiment. Vertical bars show the standard deviation of the mean (n=12).

Photosynthetic pigments and UV absorbing compounds

There was not statistically significant effect of UV-B radiation dose on the mean cholorophyll concentrations of *Chara polyacantha* and *Nitella hyalina* after 22 and 49 days of exposure during the Enhanced UV-B Radiation Experiment, but there was a significant increase in chlorophyll *a* and *b* concentrations in *Nitella hyalina* at PAR+UV-B treatment (ANOVA F=28.9; $p = 0.006$; ANOVA F=26.3; $p = 0.007$ respectively) (Figure 5) after 120 days of exposure. In contrast, *Chara polyacantha* did not show statistically significant differences in chlorophyll concentrations of charophyte green portions among treatments.

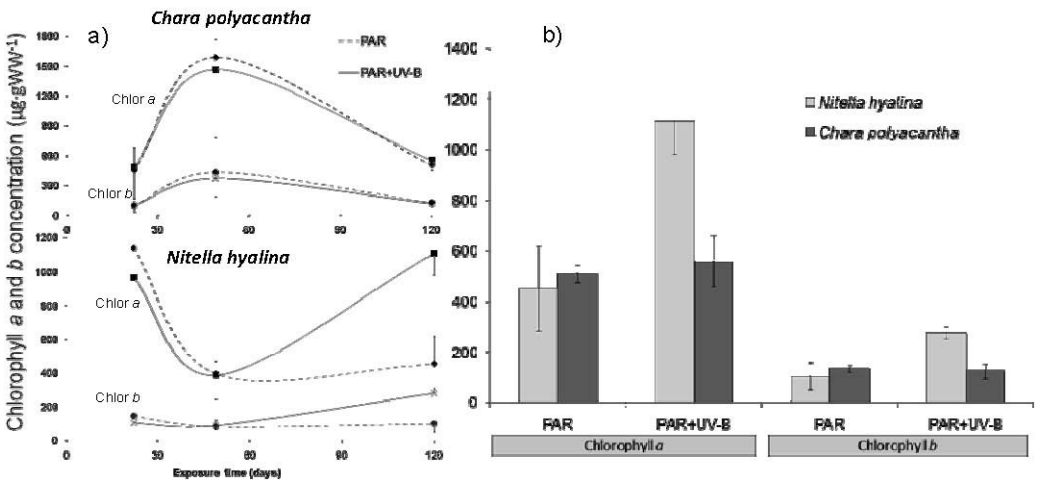

Fig. 5 a) Chlorophyll *a* and *b* concentrations at different times of UV-B exposure during the Enhanced UV-B Radiation Experiment and, b) averaged chlorophyll *a* and *b* concentrations (µg·gWW^{-1}) of *Chara polyacantha* and *Nitella hyalina* in both treatments (PAR and PAR+UV-B) at 120 days of exposure. Vertical bars show the standard deviation of replicates (n = 3).

We also recorded the absorption spectra of extracted pigments of both species after 120 days of exposure. The ratio Abs_{433}/Abs_{665} was around 2 and there were not statistical differences among treatments or species (Figure 6).

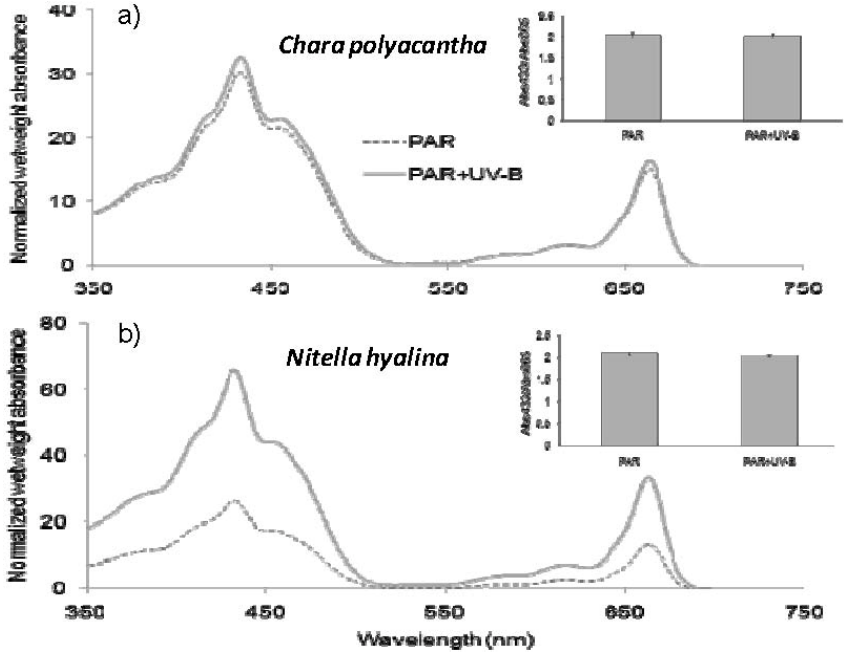

Fig. 6 Absorption spectrum of acetone extracted pigments of *Chara polyacantha* (a) and *Nitella hyalina* (b) in both experimental treatments (PAR and PAR+UV-B) after 120 days of exposure in the Enhanced UV-B Radiation Experiment (n=3). Abs_{433}/Abs_{665} ratio in the upper right corner.

There was no statistically significant effect of UV-B radiation dose on UV methanol soluble absorbing compounds of *Chara polyacantha* or *Nitella hyalina* after 22 and 49 days of exposure during the Enhanced UV-B radiation experiment. However, after 120 days of exposure the results were the same for *Chara polyacantha*. In contrast, in *Nitella hyalina*, a statistically significant reduction in absorbance of both UV-B (ANOVA: 280nm F=43, *p*=0.003; 300nm F=126; 320 nm F=389; *p* for all < 0.001) and UV-A (ANOVA: 340 nm F=653; 360 nm F=412; 280 nm F=291; 400 nm F=167, *p* for all < 0.001) absorbing compounds was detected on PAR+UV-B treatment (Figure 7).

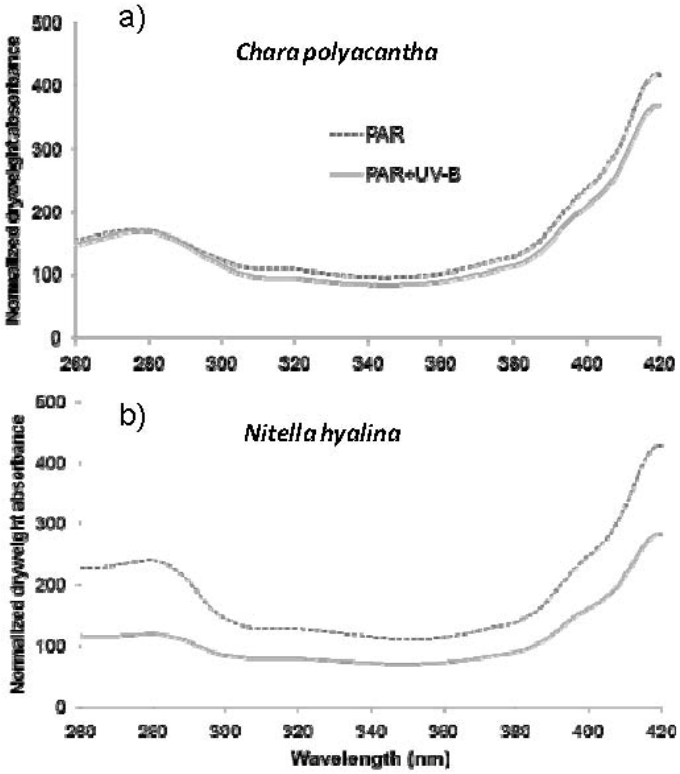

Figure 7: Methanol extracted UV absorbing compounds absorbance of *Chara polyacantha* (a) and *Nitella hyalina* (b) in both experimental treatments (PAR and PAR+UV-B) at various wavelengths after 120 days of exposure in the Enhanced UV-B Radiation Experiment.

4 Discussion

Growth rates

The high dose of UV-B radiation used during the Extremely Enhanced UV-B Radiation Experiment caused severe negative effects on both growth rates and pigments. However, when UV-B dose was smaller (3.6 $kJ \cdot m^{-2} \cdot day^{-1}$) and only after four months of daily repeated exposure to UV radiation we found significant growth reductions, but only in *Chara polyacantha*. This is consistent with growth reductions in response to UV-B exposure as found in charophyte *Chara aspera* (De Bakker *et al*. 2001; 2005), also reported for plant species from terrestrial and marine environments

(Caldwell *et al*. 1998; Searles *et al*. 2001; Van de Poll *et al*. 2001) and macroalgae (Schmidt *et al*. 2010). According to De Bakker and co-workers (2001), an explanation might be that UV-B negatively affects cell elongation of the internodes. This is in contrast with the way in which growth reduction under the influence of UV-B occurs in unicellular marine algae where cell division is inhibited by UV-B radiation so these organisms cells become larger (Karentz *et al.* 1991; Buma *et al*. 1995). Also, shorter size may affect nutrient uptake since charophytes take up nutrients via the rhizoids but also via the shoots (Krause 1997) and this can have repercussions at ecosystem level. *Nitella hyalina* growth rates seem not to be affected by the UV-B dose used during our experiment. It seems that corticated and ecorticated species react differently to UV-B radiation. We think there were other factors affecting our results. Firstly, *Nitella hyalina* has a mucilage layer surrounding the tissue (Cirujano *et al.* 2008) which is expected to have a protective important role (Ehling-Schulz *et al*. 1997; Oertel *et al*. 2004). Secondly, ecological conditions in the natural environment are different: *Nitella hyalina* is usually found in shallower waters until 4 meters of depth (so it can be better adapted to UV radiation), whereas *Chara polyacantha* can grow until 14-18 meters deep (Krause 1997; Cirujano *et al.* 2008).

Photosynthetic pigments and UV absorbing compounds

Under a really high UV-B radiation (> 15 $kJ \cdot m^{-2} \cdot day^{-1}$) mainly *Chara polyacantha* showed depigmentation and necrosis. It might be partly due to *Chara polyacantha* had a greater growth rate than *Nitella hyalina*, so it was nearer to UV-B tube and therefore receiving a higher dose than *N. hyalina*. These negative consequences could have been the result of changes in metabolism and DNA damage. Studies on higher plants, marine phytoplankton and marine macroalgae also reported that reduced growth corresponded to accumulated cyclobutane pyrimidine dimeres (CPDs) in the DNA (Mazza *et al*. 1999; Buma *et al*. 2000; Van de Poll *et al*. 2001). Depigmentation would be a consequence of cholorophyll destruction suffered after the generation of reactive oxygen species (ROS) and photoinhibition of photosynthetic pigments (Izco *et al*. 1997).

Chara polyacantha chlorophyll concentrations were not affected by Enhanced UV-B radiation dose after 120 days of exposure. In higher plants, when light intensity exceeds the absorption and transformation capacity, a photoprotection strategy is started to minimize the global damage on chloroplasts. Besides protection systems there are repair systems too: in order to keep their function despite damage, plants have biochemical machinery to solve damage constantly (Izco *et al*. 1997). Both mechanisms -protection and repair- may play an important role in these charophyte species.

On the other hand, *Nitella hyalina* increased the chlorophyll concentrations (both, *a* and *b*) in the PAR+UV-B treatment. According to Schmidt *et al*. (2010), UV-B can stimulate the synthesis of chlorophyll *a* in red macroalga *Kappaphyccus alvarezii*. However, other investigations on *Chara aspera* highlighted the lack of effect of UV-B on the chlorophyll *a* and *b* concentrations (De Bakker *et al*. 2001). In summary, the stress suffered by algae exposed to PAR+UV-B during the second experiment did not lead to degradation of chlorophylls, and some authors argue that an increase of this pigment is therefore a form of adaptation to radiation (Schmidt *et al*. 2010). The ratio between chlorophyllic and carotenoids pigments content (Abs_{433}) and chlorophyll content (Abs_{665}) was the same in both species and also in both treatments indicating that UV-B radiation dose used during the second experiment

did not stimulate a higher production of antenna pigments which are described to protect chlorophyll from photo-damage. However, Schagerl & Pichler (2000) found a clearly high carotene production on upper parts of *Chara tomentosa* in response to a high light or UV-radiation.

UV absorbing compounds are important in screening UV-B (Rozema *et al.* 2002). By lowering UV-B levels within the plant tissues damage to DNA, membranes, proteins and photosynthetic tissue can be prevented or reduced (Meijkamp *et al*. 1999). In *Chara polyacantha* no changes were observed in methanol soluble UV absorbing compounds under PAR+UV-B treatment. That is in agreement with the results of other authors (De Bakker *et al*. 2001, 2005) for *Chara aspera*. Surprisingly in our second experiment *Nitella hyalina* decreased them. Thus, it could make these algae potentially sensitive to UV-B radiation. According to De Bakker *et al*. (2001), the absence of increased UV-B absorbing compounds under enhanced UV-B is remarkable, since they are present in cyanobacteria, microalgae, macroalgae and in higher plants (Büdel *et al*. 1994; Karsten *et al*. 1999; Meijkamp *et al*. 1999), and among the green algae, charophytes appear to be most closely related to higher land plants (Devereux et al. 1990; Stafford 1991; Graham 1993).

To sum up, the present study demonstrates that high UV-B radiation doses affect negatively growth rates in both charophyte species. With UV-B lower doses the effect was not obvious at the very beginning of the experiment but at the end. The UV-B stress was able to start changes where a combination of shorter size and the remarkable no production of protective UV-B absorbing compounds would constitute a disadvantage in the charophytes competition capacity growing in shallow and clear aquatic ecosystems under an scenario of ozone depletion.

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References

Arts, M. T., R. D. Robarts, F. Kasai, M. J. Waiser, V. P. Tumber, A. J. Plante, H. Rai & H. J. De Lange, 2000. The attenuation of ultraviolet radiation in high dissolved organic carbon waters of wetlands and lakes on the northern Great Plains. Limnology and Oceanography 45: 292-299.

Blindow, I., A. Hargeby & G. Andersson, 2002. Seasonal changes of mechanisms maintaining clear water in a shallow lake with abundant Chara vegetation. Aquatic Botany 72: 315-334.

Büdel, B., U. Karsten & F. Garcia-Pichel, 1994. Ultraviolet-absorbing scytonemin and mycosporine-like amino acid derivates in exposed, rock-inhabiting cyanobacterial lichens. Oecologia 112: 165-172.

Buma, A. G. J., E. J. Van Hannen, L. Roza, M. J. W. Veldhuis & W. W. C. Gieskes, 1995. Monitoring ultraviolet-B-induced DNA damage in individual diatom cells by immunofluorescent thymine dimmer detection. Journal of Phycology 31: 314-321.

Buma, A. G. J., T.Oijen, W. H. Van de Poll, M. J. W. Veldhuis & W. W. C. Gieskes, 2000. The sensivity of *Emiliania huxleyi* (Prymnesiophyceae) to ultraviolet-B radiation. Journal of Phycology 36: 296-303.

Caldwell, M. M., L. O.Björn, J. F.Bornman, S. D. Flint, G. Kulandaivelu, A. H. Teramura & M. Tevini, 1998. Effects of increased solar ultraviolet radiation on terrestrial ecosystems. Journal of Photochemistry and Photobiology B: Biology 46: 40-52.

Cirujano, S., J. Cambra, P. M.Sánchez Castillo, A. Meco & N. Flor Arnau, 2008. Flora Ibérica. Algas continentales. Carófitos (Characeae). Real Jardín Botánico. Madrid. 132 pp.

Crawford, S. A., 1979. Farm pond restoration using *Chara vulgaris* vegetation. Hydrobiologia 62: 17-32.

De Bakker, N. V. J., A. P. Van Beem, J. W. M. Van de Staaij, J.Rozema & R. Aerts, 2001. Effects of UV-B radiation on a charophycean alga, *Chara aspera*. Plant Ecology 154: 239-246.

De Bakker, N. V. J., P. M. Van Bodegom, W. H. Van de Poll, P. Boelen, E.Nat, J. Rozema & R. Aerts, 2005. Is UV-B radiation affecting charophycean algae in shallow freshwater systems? New Phytologist 166: 957-966.

Devereux, R., A. R. Loeblich III & G. E. Fox, 1990. Higher plant origins and the phylogeny of green algae. Journal of Molecular Evolution 31: 18-24.

Ehling-Schulz, M., W. Bilger & S. Scherer, 1997. UV-B induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium Nostoc commune. Journal of Bacteriology 179: 1940-1945.

Farman, J. C., B. G. Gardiner & J. D. Shanklin, 1985. Large losses of total ozone in Antarctica reveal seasonal CLOx/NOx interaction. Nature 315: 207-210.

Graham, L. E., 1993. Origin of Land Plants. John Wiley & Sons, New York.

Herman, J. R., P. K. Bhartia, J. Ziemke, Z. Ahmad & D. Larko, 1996. UV-B increases (1979-2002) from decreases in total ozone. Geophysical Research Letters 23: 2117-2120.

Izco, J., E. Barreno, M. Brugués, M. Costa, J. Devesa, F. Fernández, T. Gallardo, T. Llimona, E. Salvo, S. Talavera & B. Valdés, 1997. Botánica. McGraw Hill, Madrid.

Jeffrey, S. W. & G. F. Humphrey, 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochemistry and Physiology Pflanzen 167: 191-194.

Karentz, D., J. E. Cleaver & D. L. Mitchell, 1991. Cell survival characteristics and molecular responses of phytoplankton to ultraviolet-B radiation. Journal of Phycology 27: 326-341.

Karsten, U., K. Bischof, D. Hanelt, H. Tüg & C. Wiencke, 1999. The effect of ultraviolet radiation on photosynthesis and ultraviolet-absorbing substances in the endemic Arctic macroalga *Devaleraea ramentacea* (Rhodophyta). Plant Physiology 105: 58-66.

Kirk, J. T. O., 1994. Light and Photosynthesis in Aquatic Ecosystems. Cambridge University Press, Cambridge.

Krause, W., 1997. Charales (Charophyceae). G. Fisher, Jena.

Madronich, S., 1992. Implications of recent total atmospheric ozone measurements for biologically active ultraviolet radiation reaching the earth's surface. Geophysical Research Letters 19: 37-40.

Madronich, S., R. L. McKenzie, L. O. Björn & M. M. Caldwell, 1998. Changes in biologically active ultraviolet radiation reaching the earth's surface. Journal of Photochemistry and Photobiology B: Biology 46: 5-19.

Mazza, C. A., D. Battista, A. M. Zima, M. Swarcberg-Bracchitta, C. V. Giordano, A. Acevedo & C. L. Scopel Al Ballare, 1999. The effects of solar ultravioleta-B radiation on the growth and yield of barley are accompanied by increased DNA damage and antioxidante responses. Plant, Cell and Environment 22: 61-70.

Meijkamp, B., R. Aerts, J. Van de Staaij, M. Tosserams, W. Ernst & J. Rozema, 1999. Effects of UV-B on secondary metabolites in plants. Pages 39-59 in J. Rozema (ed.). Stratospheric Ozone Depletion. The Effects of Enhanced UV-B Radiation on Terrestrial Ecosystems. Backhuys Publishers, Leiden, Netherlands.

Oertel, A., N. Aichinger, R. Hochreiter, J. Thalhamer & U. Lütz-Meindl, 2004. Analysis of mucilage secretion and excretion in Micrasterias (Chlorophyta) by means of immunoelectron microscopy and digital time lapse video microscopy. Journal of Phycology 40: 711-720.

Rozema, J., J. Van de Staaij, L. O. Björn & M. Caldwell, 1997. UV-B as an environmental factor in plant life: stress and regulation. Trends in Ecology and Evolution 12: 22-28.

Rozema, J., L. O. Björn, J. F. Bornman, A. Gaberscik, D. P. Häder, T. Trost, M. Germ, M. Klisch, A. Groniger, R. P. Sinha, M. Lebert, Y. Y. He, R. Buffoni-Hall, N. V. J. de Bakker, J. van de Staaij & B. B. Meijkamp, 2002. The role of UV-B radiation in aquatic and terrestrial ecosystems – an experimental and functional analysis of the evolution of UV-absorbing compounds. Journal of Photochemistry and Photobiology 66: 2-12.

Schagerl, M. & C. Pichler, 2000. Pigment composition of freshwater charophytes. Aquatic Botany 67: 117-129.

Scheffer, M., S. H. Hosper, M. L. Meijer, B. Moss & E. Jeppesen, 1993. Alternative equilibria in shallow lakes. Trends in Ecology and Evolution 8: 275-279.

Scheffer, M.,1998. Ecology in Shallow Lakes. Chapman and Hall, London.

Schmidt, E. C., M.Maraschin & Z. L. Bouzon., 2010. Effects of UV-B radiation on the carragenophyte *Kappaphycus alvarezii* (Rhodophyta, Gigartinales): changes in ultraestructure, growth and photosynthetic pigments. Hydrobiologia 649: 171-182.

Scully, N. M. & D. R. S Lean., 1994. The attenuation of ultraviolet radiation in temperate lakes.

Ergebnisse der Limnologie 43: 135-144.

Searles, P. S., S. D. Flint & M. M. Caldwell., 2001. A meta-analysis of plant field studies simulating stratospheric ozone depletion. Oecologia 127: 1-10.

SORG,1999. Stratospheric Ozone 1999. A Report from the Stratospheric Ozone Review Group (SORG) including an update by Ultraviolet-B Measurement and Impacts Review Group (UMIRG). Department of environment, transport and the regions, UK.

Stafford, H. E., 1991. Flavonoid evolution: an enzymatic approach. Plant Physiology 96: 680-685.

Van de Poll, W. H., A. Eggert, A. G. J. Buma & A. M. Breeman, 2001. Effects of UV-B induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat-related differences in UV-B tolerance. Journal of Phycology 37: 30-37.

Van den Berg, M. S., 1999. Charophyte colonization in shallow lakes. PhD Thesis, Vrije Universiteit, Amsterdam, Netherlands.

Williamson, C. E., 1995. What role does UV-B radiation play in freshwater ecosystems? Limnology and Oceanography 40: 386-392.