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Impact of natural ultraviolet radiation on the free-floating liverwort *Ricciocarpus natans* – a field study in Uruguay 34°S

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ABSTRACT

Effects of natural radiation were studied on the free-floating liverwort *Ricciocarpus natans* in a field experiment conducted during 49 days in January and February 1998 in Uruguay (34°S). The plants were exposed to three different qualities of UV radiation using filters cutting different wavelengths: (1) all UV radiation, (2) UV-B radiation and (3) no UV radiation. The hypothesis was that UV-B radiation should have the most detrimental effect on *Ricciocarpus natans*. The effect parameters were production, growth measured as increase in number of thalli and photosynthetic capacity as indicated by four different fluorescence parameters.

The production of *Ricciocarpus natans* was highest in the treatment receiving the whole light spectrum, indicating that UV radiation was not harmful for the plants considering the total test period. The increase in number of thalli and the fluorescence parameters gave more detailed information about the status of the plants during the experiment. During the first part of the experiment the number of thalli was highest in treatments that did not receive any UV-B radiation, but during the final two weeks the number of thalli was highest in the treatment receiving all UV radiation. This indicates that the UV-B radiation damaged the plants in the beginning of the experiment. The UV-B/UV-A ratio seems to be important to the plants as a strong negative correlation was found between this ratio and the growth rate in the treatment receiving all UV radiation. The results from the fluorescence measurements are partly inconsistent with the growth data. Some observations indicate that, at least at the measuring occasion, there was no damage on photosystem II (PS II) caused by UV-B radiation.

Keywords

Ricciocarpus natans, coastal lagoons, UV-B, production, growth, photosynthetic efficiency

Introduction

Ultraviolet (UV) radiation can be highly destructive to living organisms, but with a sufficient amount of ozone in the stratosphere the UV radiation is filtered from the sunlight reaching the Earth. However, chlorofluorocarbons (CFCs), along with other chlorine- and bromine-containing compounds, have been involved in the accelerated depletion of ozone in the Earth's stratosphere. In the early seventies chlorine was found to be a catalytic agent in ozone destruction. Catalytic destruction of ozone removes the odd oxygen species [atomic oxygen (O) and ozone (O3)] while leaving chlorine unaffected. This process was known to be potentially damaging to the ozone layer, but conclusive evidence of stratospheric ozone loss was not found until 1984. Despite rapid phaseout of CFCs, ozone levels are expected to be lower than pre-depletion levels for several decades due to the long tropospheric lifetimes of CFCs (CIESIN 2000).

Models predict a global ozone reduction especially in the southern mid latitudes (Sze *et al.* 1989). Contrary to the Antarctic biota, which probably has an evolutionary adaptation to high UV levels, the mid latitude plant and animal species are more inclined to stress when UV radiation increases. It is therefore of great importance to obtain baseline data on effects on organisms living in areas that today receive quite low UV radiation. This knowledge is important for evaluation of the effects on these ecosystems in the future.

The ultraviolet spectrum can be divided into three different classes: UV-A (320-400 nm), UV-B (280-320 nm) and UV-C (<280 nm). UV-A is not attenuated by ozone and therefore unaffected by ozone reduction whereas UV-B is partly attenuated by ozone. UV-C is highly energetic and completely attenuated in the stratosphere as long as there is any ozone left (Stapleton 1992). UV-B is most sensitive to changes in the stratospheric ozone and small changes of the ozone concentration produce a disproportional increase of UV-B reaching the Earth's surface. As an example, a decrease of 1 % of ozone will increase the UV-B radiation by 2 %, but a 50 % depletion of ozone will cause an increment of 350 % (Booth & Madronich 1994).

The UV-B radiation that reaches the Earth is not only dependent on the stratospheric ozone. The inclination of the sun is an important factor causing variation in UV-B radiation related to latitude, season and time during the day. Cloudiness, surface reflection, altitude and local pollution can also cause the variation in UV-B radiation. For example, aerosols and tropospheric gases like NO_2, SO_2 and ozone can attenuate UV-B in polluted areas (Madronich *et al.* 1994).

In aquatic systems negative effects caused by UV-B radiation have been observed on phytoplankton photosynthesis (Smith 1989), bacterial activity (Herndl *et al.* 1993), survival of zooplankton (Williamson *et al.* 1994) and growth of phytoflagellates (Ekelund 1991, Nielsen *et al.* 1995).

Damage to plants caused by UV-B radiation can be classified in two categories:
[a)]damage to DNA and [b)]damage to physiological processes, for example photomorphogenesis, photosynthetic damage, auxin inactivation, ATPase destruction and induction of UV-absorbing pigments (flavonoids) (Stapleton 1992). Considering the effects on the photosynthesis possible targets are PS II and the CO_2-fixing enzymes (Tevini 1994).

The shallow lagoons at the Atlantic coast of Uruguay and southern Brazil are potentially vulnerable aquatic systems in case of an increase of UV-B radiation reaching the Earth. These ecosystems are among the most productive ecosystems in the world (UNESCO 1981). Coastal lagoons are considered to be ecologically complex with a high environmental diversity, many different physiological and behavioural adaptations and a complex food web (UNESCO 1981). The main characteristic is the high variability in physical and chemical properties related to the mixing of fresh and marine waters. Because of the shallow water level (mean depth between 0.5-5 m) a great proportion of the total area of these systems forms the littoral zone dominated by macrophytic vegetation, which is often continuously exposed to the UV radiation.

There is evidence that the Antarctic ozone hole can have secondary effects at mid and lower latitudes when ozone-poor air moves from Antarctic latitudes. The ozone-poor air is normally linked to the Antarctic, but by the end of October (with the disappearance of the polar vortex) it is not so strictly bound to the Antarctic and the ozone-poor air can move away. Observations in South America at 30°S in 1993 showed a sudden ozone decrease for a few days during the second half of October when the ozone is supposed to have a seasonal maximum. The region with lower ozone reached from the Antarctic in a narrow belt covering parts of northern Argentina, Uruguay and the south of Brazil (Kirchhoff *et al.* 1996). This phenomenon can cause sudden increases in the UV-B radiation at latitudes where the ecosystems are not adapted to high levels of UV radiation and this can have negative effects on these ecosystems.

Information about effects of UV-B on aquatic plant is scarce although many studies have been carried out on terrestrial plants, mainly different crops, for example sunflower, radish, mustard (Tevini *et al.* 1991) and soybean (Caldwell 1994). In all, studies of more than 300 plant species have been performed (Tevini 1994). About 50 % of the studied species were considered to be UV-sensitive and negatively affected resulting in morphological, physiological or biochemical changes. The most common effects observed in UV sensitive plants are reduced growth (plant height, dry weight, leaf area etc.), damage to the photosynthesis, accumulation of UV absorbing pigments and reduced flowering (Tevini & Teramura 1989). The majority of these studies were conducted in laboratories using artificial light. Thus the knowledge about effects on natural ecosystems is still very limited.

As free-floating plants are directly exposed to UV radiation throughout the year they are very interesting to study considering UV effects. The aim of the present study was to investigate effects of natural UV radiation on the free-floating liverwort *Ricciocarpus natans.* Production, growth and photosynthetic efficiency at different light treatments were studied under field conditions. By using filters cutting different wavelengths the plants were exposed to different qualities of UV radiation. The hypothesis was that UV-B radiation should have the most detrimental effect on *R. natans.*

Material and methods

Study area

The study was performed during the summer season (6th January to 23rd February 1998) at a field station near Laguna de Rocha, which is situated 200 km east of Montevideo, Uruguay (34°35'S, 54°17'W) (Fig. 1). The experiments were set up in a small channel (2-3 meters), which was connected with the Rocha River that entered into Laguna de Rocha. In the channel the set up was partly protected from strong winds, but at bad weather the boxes were brought into the field station to be protected. Moreover, it was necessary to check the experiment each day to take away insects (mainly crickets) feeding on the plants.

The most common aquatic plants in the channel and the surroundings were: *Schoenoplectus californicus, Polygonum* sp., *Hydrocotyle* sp., *Eichhornia azurea, Eichinodorus grandiflorus, Sagittaria montevidensis* and *Ludwigia peploides. Azolla filiculoides* and *Limnobium laevigatum* were the most common free-floating macrophyte, whereas *Ricciocarpus natans* was not observed at all in the channel this year. In 1997, on the other hand, it was quite abundant in the channel. The reason for the changes could be variation in the salinity. Conductivity and pH in Laguna de las Nutrias were measured once during the experiment.

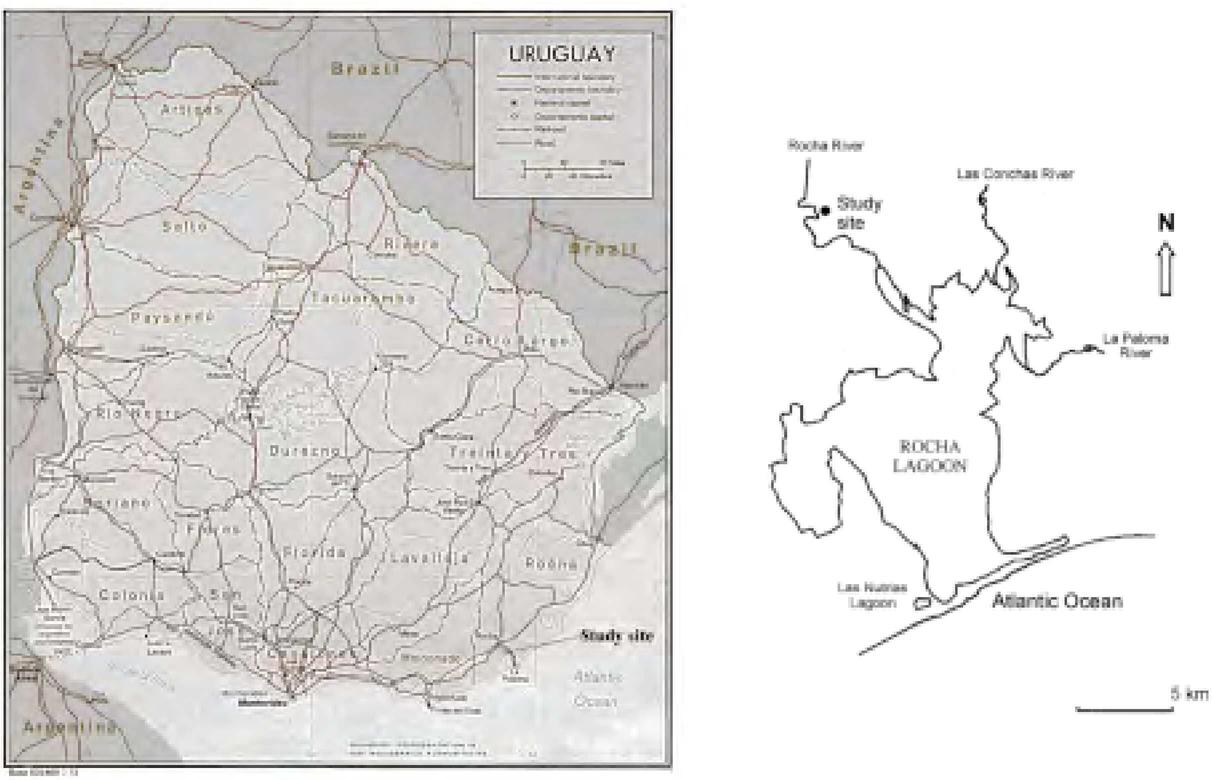

Figure 1. Map showing the study area.

Experimental set up

Ricciocarpus natans is a liverwort consisting of a thallus up to 8 mm long and 4 mm wide with scales on the lower surface. This plant is a cosmopolitan and can be very abundant (especially in tropical, eutrophic waters) covering the whole water area alone or together with other species (Alonso 1997). The plants used in this experiment were taken from Laguna de las Nutrias - a small lagoon close to Laguna de Rocha (Fig. 1).

The experimental set up consisted of three floating plastic boxes (about 50 cm x 50 cm) supplied with lids made of different materials that were cutting distinct wavelengths. Thus three different treatments were obtained:

1. PAR (photosynthetically active radiation)+ UV-A + UV-B (control) using plastic film (polyethylene) transparent to the whole spectrum.

2. PAR + UV-A using Mylar D foil (Dupont laboratories; 50% transmittance at 320 nm) to exclude the UV-B.

3. PAR using a vinyl chloride foil (CI Kasei Co., Tokyo, Japan; 50% transmittance at 405 nm) to exclude all UV radiation.

Each plastic box had four compartments and in this way four replicates for each treatment were obtained. The area of each replicate was 145 cm^2. To prevent nutrient depletion during the experiment water was regularly poured into the compartments up to a marked level. The water used in the experiment was brought from Laguna de las Nutrias. The plastic boxes were fixed in the middle of the channel by thin ropes. In this way all plastic boxes received the same quantity of light. The vegetation around the experiment did not shade the boxes during the day. The transmittance of the filters (Fig. 2) was tested initially and twice during the experiment to control that the properties of the filters did not change with time.

Radiation measurements

The radiation measurements were conducted each hour during the day (about 8.30 a.m. - 7.30 p.m.) except for 11 a.m. - 1 p.m. when the measurements were done every 30 minutes in order to get a more precise registration of the peak values around noon.

PAR was measured in the spectral range 400-700 nm with a LI-193SA (2π) radiometer (LI-COR, Inc.). The UV radiation measurements were performed with an IL1400A radiometer (International Light Inc.). The UV-A radiation was measured with a peak wavelength at 360 nm and a total bandwidth of 20 nm (350-370 nm). The UV-B radiation was measured with a peak wavelength at 295 nm and at total bandwidth of 20 nm (285-305 nm).
Given dose for one day is equal to the integrated value from all measurements that day.

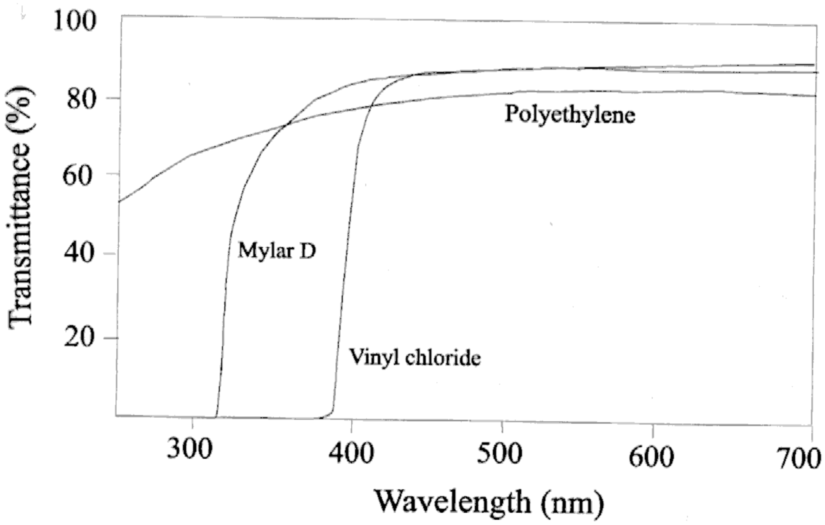

Figure 2. Transmittance of the different filters used in the experiment.

Production, growth and growth rate

The experiment was initiated with 40 individuals (=thalli) in each replicate. To estimate the initial dry weight (W_i) the dry weight of six replicates with 40 thalli of the same size as those used in the experiment was measured. The dry weight was determined after at least 48 hours in a drying oven at 85^0 C. After 49 days, when the experiment was finished, the dry weight of all thalli in each replicate (W_f) was measured. Production during the experiment was calculated using the estimated initial and final dry weight. As growth was found to be exponential, the following formula was used to calculate production:

$$(\ln W_f - \ln W_i)/(\text{time*area of each replicate})$$

Number of thalli was counted 1-3 times/week. The difference between the number of thalli at the counting occasion and the number of thalli at the start of the experiment was used to measure growth. A special criterion was used to decide when one thallus had become two; i.e. when one part of the main axis (indicated in Fig. 3 by an arrow) was longer than one third of the total thallus.

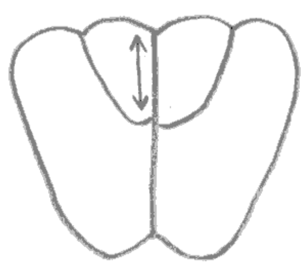

Figure 3. Illustration of how to decide when one thallus of *Ricciocarpus natans* had become two. When the part of the main axis (indicated by the arrow) was longer than one third of the total length of the axis the plant was considered to consist of two thalli.

The number of thalli at each counting occasion was plotted in a diagram. A curve was adjusted to the plotted values and growth rates were calculated from the adjusted curve.

Fluorescence measurements

All the light reaching a plant is not used for photosynthesis. Some radiation is reflected, some transmitted and some absorbed by pigments (chlorophyll a, chlorophyll b and carotenoids). There are four different ways to use the absorbed light energy and return to the ground state:

1. Energy transfer to a neighbouring pigment molecule
2. Non-radioactive de-excitation (heat)
3. Photochemical reaction
4. Light emission (fluorescence) (Krause & Weise 1984)

The fluorescence from plants has proved to be a useful instrument in the study of photosynthetic reactions (Salisbury & Ross 1992). At room temperature most of the fluorescence has its origin in photosystem II (PS II) (Krause & Weise 1984). As UV-B is considered harmful to PS II, fluorescence is a suitable tool for studies on the effects from UV-B.

The fluorescence measurements on dark-adapted plants were conducted with a Fluorescence Modulated System from the Hansatech Company (FMS II) during the dark time of the day. A lamp with a red light bulb was the only light source used in the laboratory to prevent any light exposure to the plants. The plants were adapted to darkness in plastic tubes with lids that did not allow any entrance of light, for at least 20 minutes before measuring. When the lid was removed from the tube the sensor was immediately put over the tube and the measuring started.

At the beginning of the measurement Fo (fluorescence origin) was recorded. At this moment only the modulating beam (weak 1.8 μsec pulses with long off periods) was operating. A saturated light of 10 200 $\mu mol^{-2}s^{-1}$ was applied for 0.7 s and Fm (fluorescence maximum) was recorded. A light of 32 $\mu mol^{-2}s^{-1}$ was applied for 18 s, long enough to make the sample reach a level of steady state (Fs). A second saturated light pulse (10 200 $\mu mol^{-2}s^{-1}$) was applied for 0.7 s and Fm' was determined. Finally a far red light with a peak wavelength at 735 nm was applied for 5 s. The result was a dip in the curve equivalent to the parameter Fo'. An example of a fluorescence curve is presented in Fig. 4.

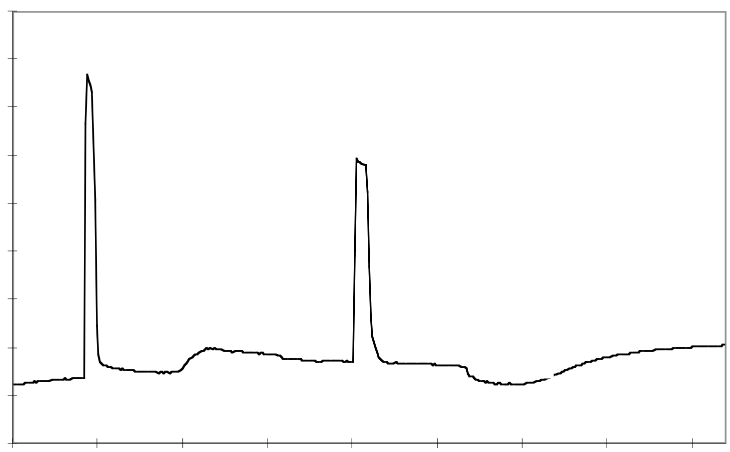

Figure 4. Fluorescence measured on an intact dark-adapted *Ricciocarpus natans*.

A number of fluorescence parameters may be calculated from these measurements and this study concentrates on four of them:

1. MQE (the maximum quantum efficiency)
This parameter expresses the efficiency of exciton energy capture (photon yield) by "open" reaction centre of PS II. It is normally very constant (0.83) for intact, unstressed leaves of many species, but environmental factors such as drought and herbicides can cause a decrease (Lawlor 1993). MQE is obtained by adapting the leaf to darkness (or after an illumination by far-red light, 700-720 nm). The difference Fm-Fo (the basal fluorescence of the system) is the variable fluorescence, Fv, and MQE= Fv/Fm.

2. QE (the quantum efficiency)
This fluorescence parameter represents the fluorescence emitted by a plant adapted to a particular light regime and working at steady state. It can be difficult to measure QE in light-adapted conditions because of variable weather conditions. An alternative, used in this study, is therefore to measure Fs and Fm' using a constant light which is applied by the fluorimeter. The quantum efficiency of PS II is calculated as (Fm'-Fs)/Fm'.

3. qP (the photochemical quenching coefficient)
The size of the parameter qP is related to processes involved in the synthesis of ATP. It normally increases after a cloudy period. It is calculated by using the parameters described earlier: qP = (Fm'-Fs)/(Fm'-Fo').

4. qNP (the non-photochemical quenching coefficient)
The size of this parameter is related to processes, which are not involved in the synthesis of ATP. Thus qNP is always opposite of qP. When qNP is high, qP is low. It is calculated in

the following way: qNP = (Fm-Fm')/(Fm-Fo').

Statistical methods
Differences between the treatments were tested by the Kruskal-Wallis one-way analysis and the Fligner-Policello test (Fligner & Policello 1981). The latter test is a modified version of the Mann-Whitney-Wilcoxon test that allows unequal variance.

Correlations between the radiation received and growth rates for the different treatments were tested with The Spearman rank correlation test.

Results

Radiation
Calculated natural daily doses for PAR, UV-A and UV-B during the experiment are shown in Fig. 5. A small downward trend can be seen for all types of radiation, but it is most apparent for UV-B. Thus, the UV-B/UV-A ratio is getting smaller during the experiment (Fig. 6). During 2nd -6th February it was very cloudy and the daily UV-B maximum was never higher than 8 $\mu W/cm^2$. After 6th February no daily maximum were ever higher than 23 $\mu W/cm^2$ and normally it was around 20 $\mu W/cm^2$. This should be compared with the highest daily maximum for the first period, which was 34 $\mu W/cm^2$. Normally the daily maximum during 6th January - 2nd February was about 25-27 $\mu W/cm^2$.

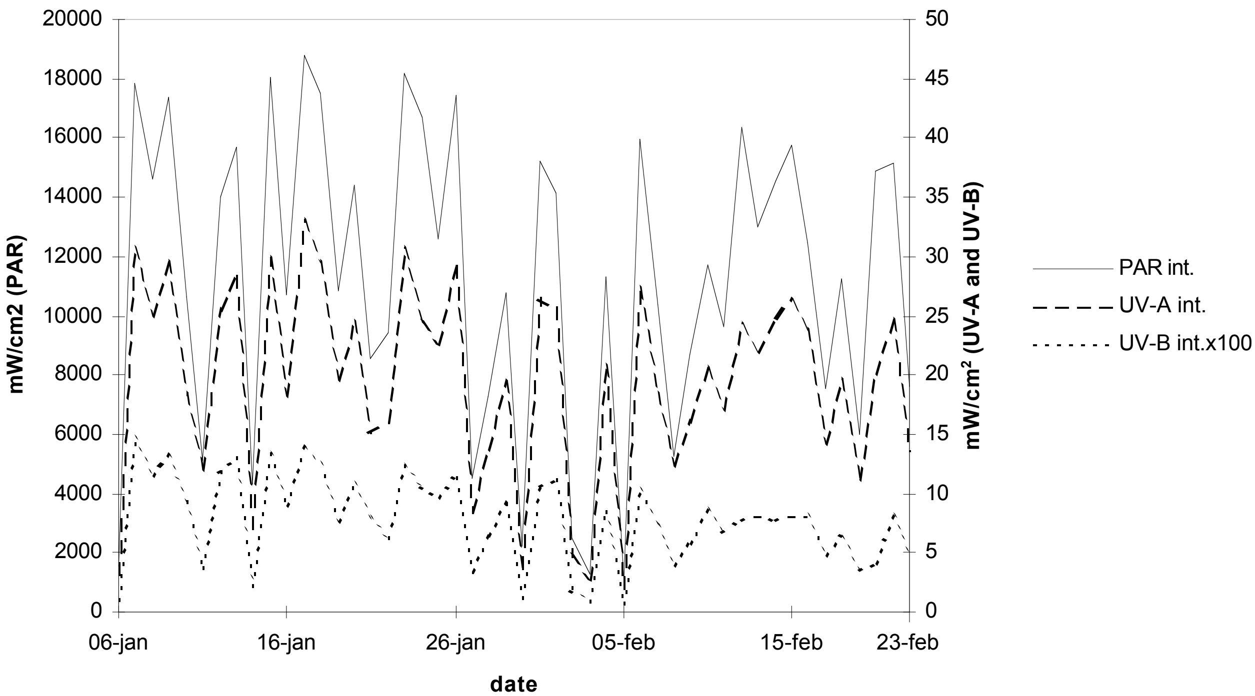

Figure 5. Calculated daily doses (integrals) of PAR, UV-A and UV-B during 49 days (6th January to 23rd February 1998) in Uruguay 34°S.

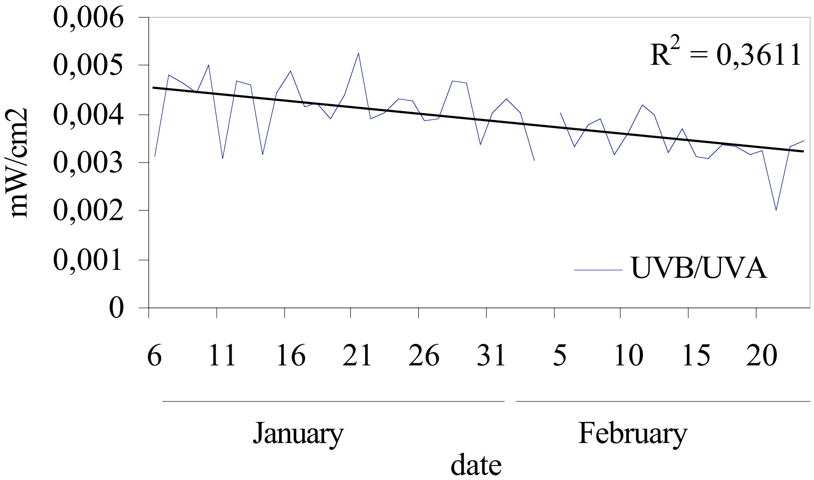

Figure 6. The UV-B / UV-A ratio during 49 days (6th January to 23rd February 1998) in Uruguay 34°S.

Production, growth and growth rate

Production in "full" light (0,204 ± 0,029 mg dw/day*cm^2) was significantly higher than if PAR and UV-A reached the plant (0,153 ± 0,026 mg dw/day*cm^2) ($p<0.05$, Fligner-Policello) or if only PAR reached the plant (0,152 ± 0,020 mg dw/day*cm^2) ($p<0.025$, Fligner-Policello).

The growth was exponential in all the treatments, which can be seen in Fig. 7 and 8. The growth at different treatments changed during the experiment, but almost all the time the plants receiving only PAR had the lowest number of thalli. The first ten days of the experiment the number of thalli was somewhat higher in the PAR+UVA treatment. The situation changed during the first week in February and after 33 days until the experiment was finished the number of thalli was highest in the PAR+UVA+UVB treatment. Significant differences between the treatments indicated by the Kruskal-Wallis test are given in Fig. 8. The results from the Fligner-Policello test are presented in Tab. 1.

There was a significant negative correlation (Spearman R= -0.650, p= 0.006) between growth rate in the control and the ratio of UVB:UVA. No other significant correlations between radiation recieved and growth rates were observed.

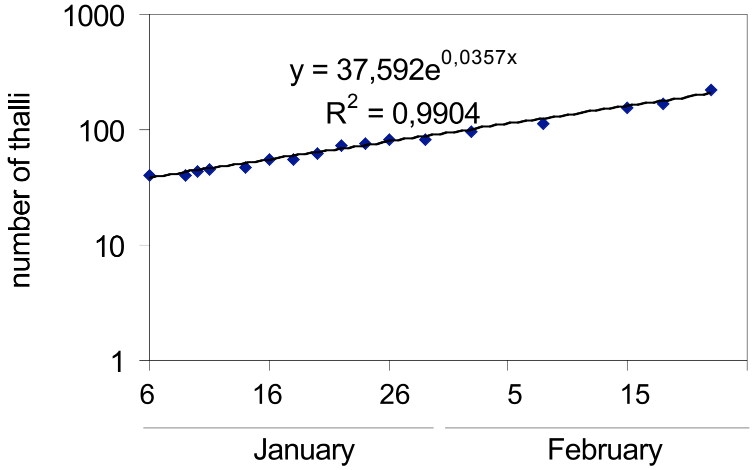

Figure 7. Number of thalli of *Ricciocarpus natans* receiving the whole spectrum of radiation (control) during 49 days (6th January to 23rd February 1998).

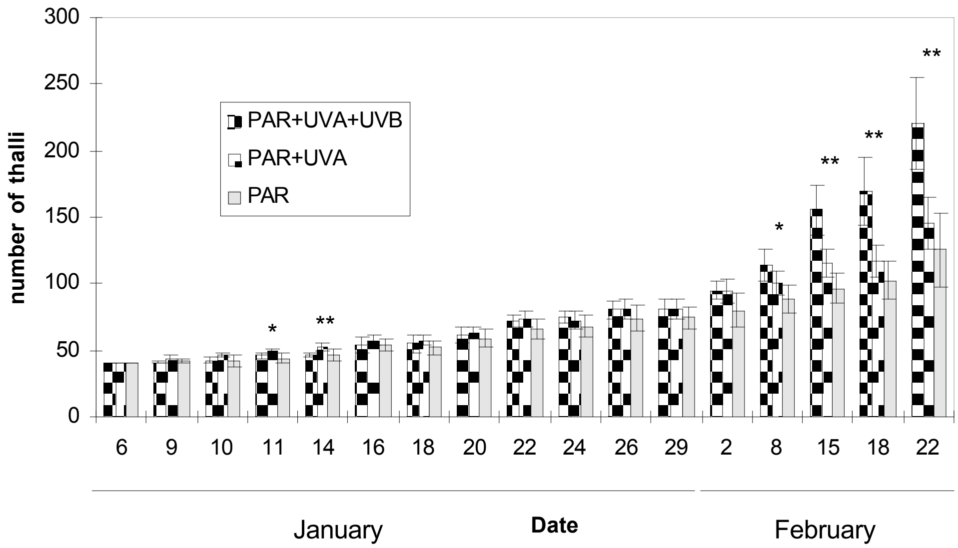

Figure 8. Number of thalli ±SD of *Ricciocarpus natans* in three different light treatments with four replicates for each treatment. Significant differences between treatments indicated by the Kruskal-Wallis test are marked with ** $p<0.025$ and * $p<0.05$.

Table 1. Significant differences in the number of thalli of *Ricciocarpus natans* receiving different light treatments (four replicates for each treatment) Fligner-Policello test. *** $p<0.01$, ** $p<0.025$, * $p<0.05$, - = non-significant

	DATE															
Treatments	9/1	10/1	11/1	14/1	16/1	18/1	20/1	22/1	24/1	26/1	29/1	2/2	8/2	15/2	18/2	22/2
PAR+UVA+UVB and PAR+UVA	-	-	*	**	-	-	-	-	-	-	-	-	-	**	***	***
PAR+UVA+UVB and PAR	-	-	-	-	-	-	-	-	-	-	-	*	*	**	***	***
PAR+UVA and PAR	-	-	**	**	-	-	-	-	-	-	-	*	-	-	-	-

Fluorescence

The results of the fluorescence measurements are presented in Fig. 9. The maximum quantum efficiency (MQE) was quite stable in all treatments during the whole experiment indicating that the capacity to capture energy in the PS II reaction centres did not change much. At every measurement MQE seemed to be somewhat higher in the treatment receiving the whole radiation spectrum. Significant difference between all the treatments was found with the Kruskal-Wallis test (Fig. 9). When tested in pairs (Fligner-Policello) many significant differences were discovered. For example, at every measuring occasion, except on the 14th of February, MQE in the PAR+UVA+UVB treatment was significantly higher compared with both or at least one of the other treatments (Tab. 2).

The quantum efficiency (QE), the photochemical quenching coefficient (qP) and the non-photochemical quenching coefficient (qNP) varied much more during the experiment compared with MQE. The highest QE and qP values were recorded on the 4th and 11th of February in all treatments.

The variation among the replicates for each treatment is often much higher for QE, qP and qNP compared with MQE resulting in high standard deviations for these parameters. Consequently significant differences between the treatments are more seldom recorded for QE, qP and qNP, though they do exist (Fig. 9 and Tab. 2).

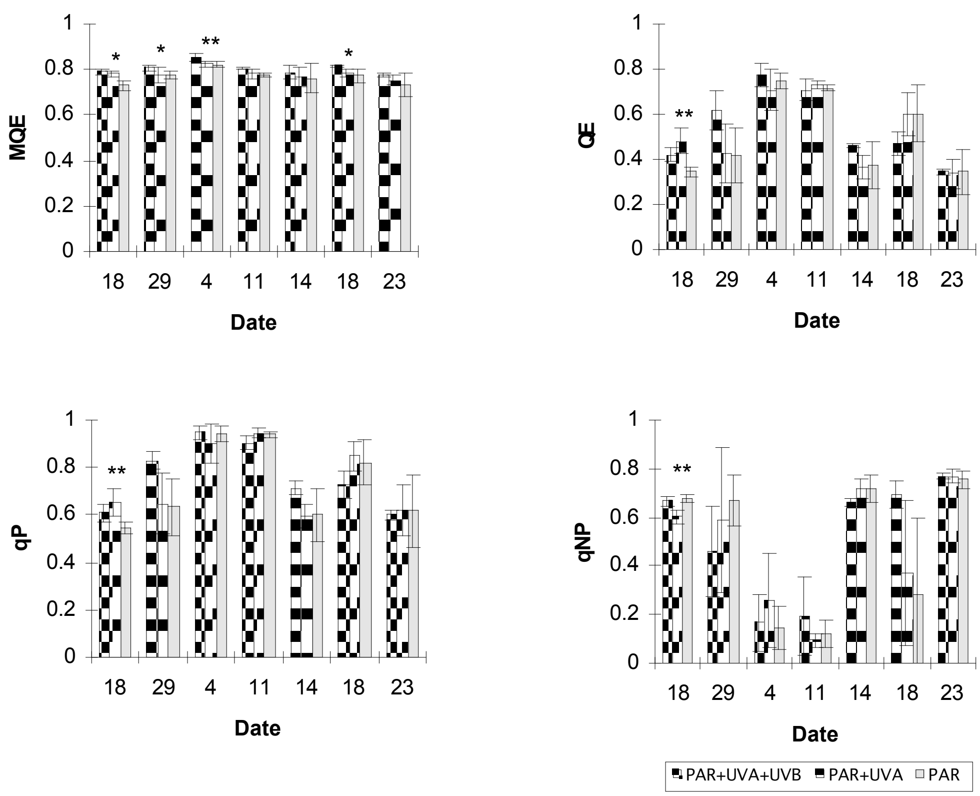

Figure 9. Four different fluorescence parameters (MQE, QE, qP and qNP) ±SD measured on dark-adapted *Ricciocarpus natans* seven times during the experiment. Three different light treatments were used and four

replicates for each treatment. Significant differences between treatments with the Kruskal-Wallis test are indicated with ** $p<0.025$ and * $p<0.05$.

Table 2. Fluorescence data for *Ricciocarpus natans* receiving different light treatments with four replicates for each treatment. Analysis by Fligner-Policello test. Fluorescence was measured on dark-adapted plants and four different parameters (MQE, QE, qP and qNP) were recorded seven times during the experiment.
*** $p<0.01$, ** $p<0.025$, * $p<0.05$, - = non-significant

Treatment	DATE 18/1	29/1	4/2	11/2	14/2	18/2	23/2
$MQE_{PAR+UVA+UVB}-MQE_{PAR+UVA}$	-	**	***	-	-	***	*
$MQE_{PAR+UVA+UVB}-MQE_{PAR}$	***	**	**	***	-	**	-
$MQE_{PAR+UVA}-MQE_{PAR}$	**	-	-	-	-	-	-
$QE_{PAR+UVA+UVB}-QE_{PAR+UVA}$	-	**	-	-	***	**	-
$QE_{PAR+UVA+UVB}-QE_{PAR}$	***	**	-	-	-	-	-
$QE_{PAR+UVA}-QE_{PAR}$	***	-	-	-	-	-	-
$qP_{PAR+UVA+UVB}-qP_{PAR+UVA}$	-	**	-	*	***	**	-
$qP_{PAR+UVA+UVB}-qP_{PAR}$	**	**	-	-	*	-	-
$qP_{PAR+UVA}-qP_{PAR}$	**	-	-	-	-	-	-
$qNP_{PAR+UVA+UVB}-qNP_{PAR+UVA}$	***	-	-	-	***	*	-
$qNP_{PAR+UVA+UVB}-qNP_{PAR}$	-	*	-	-	-	-	-
$qNP_{PAR+UVA}-qNP_{PAR}$	***	-	-	-	-	-	-

Discussion

The production of *Ricciocarpus natans* was overall higher when the plants received full radiation compared with light conditions characterised by PAR+UVA as well as only PAR. This indicates that the UV-A and UV-B radiation were not harmful for *Ricciocarpus natans* considering the whole experimental period. On the contrary, these results even indicate that radiation by UV-A and UV-B could favour the production. This holds for the whole period, but a more detailed information on UV-B effects on *Ricciocarpus natans* is given by the study of growth, growth rate and fluorescence parameters.

Considering the number of thalli (Fig. 8) it is quite obvious that the results from the experiment can be divided into two periods: 6th January - 2nd February and 8th - 22nd February. The increase in number of thalli seems to have had different rates in the different treatments during these two periods. In the first period there were significant differences between the treatments on 11th January and on 14th January. It is interesting to note that the plants in the PAR+UVA treatment at these two occasions had a higher number of thalli than in the other two treatments. This might indicate that the UV-B radiation was so strong in the beginning of the experiment that the growth in the PAR+UVA+UVB treatment was negatively affected. However, this was inconsistent with the PAR treatment, which neither received any UV-B radiation and where the growth still was relatively small. It is possible that the small growth in the PAR treatment, which is in fact smallest of the three treatments during the whole experiment, could be explained by the lack of UV-A radiation. It has been discovered that UV-A radiation can have beneficial effects on plants. For example, UV-A radiation takes part in a process called photo reactivation where damage on DNA caused by UV-B radiation is repaired by UV-A/blue radiation (Caldwell *et al.* 1994). UVA/blue radiation is

also involved in the synthesis of photosynthetic pigments, which also is beneficial for the plant (Middleton & Teramura 1994).
In the second period (8th - 22nd February) the number of thalli in the PAR+UVA+UVB treatment suddenly increased very rapidly and significant differences between the treatments could be shown at every measuring occasion during this period. The same pattern was found for the species *Spirodela intermedia* in a contemporary experiment, where the growth in the PAR+UVA+UVB treatment also tended to be higher in the end of the experiment compared with the growth in the other treatments (Braf, in prep.). It is possible that the UV-B radiation at this time of the experiment got below a threshold value where it was not harmful anymore. This can be true both considering the daily doses and the daily maximum values of UV-B. One possibility is that the UV-B radiation could be non-destructive or even beneficial at the lower doses recorded for the second period. Another possibility is that when the UV-B/UV-A ratio became smaller during this period the possible negative effects of the UV-B radiation at some point were overshadowed by positive effects of the UV-A radiation. A strong negative correlation was found between the UV-B/UV-A ratio and the growth rate in the PAR+UVA+UVB treatment. This implies the great importance of the relation between the UV-A and the UV-B radiation considering effects on the growth of *Ricciocarpus natans*. The variation among the replicates for each treatment increased during the experiment. The explanation could be due to methods, as it became more difficult to count the plants in the end of the experiment as so many individuals were sticking together. Despite this problem significant differences between the treatments were indicated also in the second period.

The results from the fluorescence measurements are quite difficult to interpret and they are not completely in accordance with the results from the growth study. Significant differences in the fluorescence parameters between the treatments were found at several occasions, although the pattern was not so clear. At every measuring occasion, except on the 14th of February, MQE in the PAR+UVA+UVB treatment was significantly higher compared with both or at least one of the other treatments. This indicates that the plants receiving the whole light spectrum at these occasions were in better condition than the plants in the other treatments. It also implies that there was no damage on PS II caused by UV-B radiation these dates or that UV-A might have had a protective effect on the plants. UV-B even seems to have had a beneficial effect on the MQE.

The parameters QE, qP and qNP are more sensitive to the dominant weather situation (i.e. light regime) before the measuring occasion. This explains why these parameters change a lot more during the experiment compared with MQE . The highest QE and qP values were recorded after periods with cloudy weather, which indicate that the major part of the energy at these occasions were used in photosynthesis. The significant differences among the treatments that were obtained for QE, qP and qNP are difficult to interpret as the relation between the treatments change in an irregular manner. There is no easy explanation to the irregular occurrence of the significant differences observed. As a comparison it can be noted that the same fluorescence parameters studied in *Spirodela intermedia* never differed significantly between the treatments (Braf, in prep.).

The in some ways unclear results of the fluorescence measurements could partly be explained by that the time for and extent of photo repair (reparation of damages caused on the photosynthesising apparatus) is not known. It is known that the photo repair in phytoplankton is induced within hours (Sommaruga, pers. comm.). If this is also the case for *Ricciocarpus natans*, provided that the plants really are damaged by UV-B radiation, it would be difficult to find effects on fluorescence parameters measured in dark conditions at night. To be able to assess the possible effect of photo reparation it is important that midday fluorescence measurements are conducted in the future.

The summer when the experiment was carried out was unusually cloudy with a very unstable weather situation changing rapidly from sunny weather to cloudy, rainy and stormy weather. This made it very difficult to interpret results in terms of light adapted fluorescence at midday. It is also likely that the very variable weather conditions led to a variable exposition to UV radiation all the time during the experiment. The radiation is normally higher in December, so similar studies should be initiated earlier than what was done in this study. Hopefully a summer with a more stable (normal!) weather situation will give more clear results.

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References

Alonso E. 1997. Plantas acuaticas de los Humedales del Este, a publication from PROBIDES, Los talleres graficos de editorial agropecuaria hemisferio sur, Montevideo, Uruguay.

Booth C.R. & Madronich S. 1994. Radiation amplification factors: improved formulation accounts for large increases in ultraviolet radiation associated with antarctic ozone depletion. In: Ultraviolet radiation in Antarctica: measurements and biological effects. Antarctic Reasearch Series 62: 39-42.

Caldwell M. M., Flint S.D. & Searles P.S. 1994. Spectral balance and UV-B sensitivity of a soybean: a field experiment. Plant, Cell and Environment 17: 267-276.

CIESIN, 2000. Center for International Earth Science Information Network at Colombia University in the city of New York. www.ciesin.org.

Ekelund N.G.A. 1991. The effects of UV-B radiation on dinoflagellates. J.Plant Physiol. 138: 274-278.

Fligner M.A & Policello G.E. 1981. Robust rank procedures for the Behrens-Fisher problem. Journal of the American Statistical Association. March 1981, Vol.76, No 373.

Herndl G.J., Müller-Niclas G. & Frick J. 1993. Major role of ultraviolet-B in controlling bacterioplankton growth in the surface layer of the ocean. Nature 361: 717-719.

Kirchhoff V.W.J.H., Schuch N.J., Pinheiro D.K. & Harris J.M. 1996. Evidence for an ozone hole perturbation at 30° south. Atmospheric Environmental Vol.30, No.9, 1481-1488. Elsevier Science Ltd. Great Britain.

Krause G.H. & Weis E. 1984. Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. Photosynthesis Research 5: 139-157 (1984).

Lawlor D.W. 1993. Photosynthesis: Molecular, physiological and environmental processes. Second edition. Lunguan (?) scientific & technical, England. 1993.

Madronich S., McKenzie R.L., Caldwell M.M. & Björn L.O. 1994. Changes in ultraviolet radiation reaching the earth's surface. Environmental effects of stratospheric ozone depletion. 1994 Update (J. van der Leun, M. Tevini and X. Tang, eds.) United Nations Environmental Programme, Nairobi, Kenya, 1994.

Middleton E.M. & Teramura A.H. 1994. Understanding photosynthesis, pigment and growth responses induced by UV-B and UV-A irradiances. Photochemistry and photobiology Vol.60, No.1, 38-45, 1994.

Nielsen T., Björn L.O& Ekelund G.A. 1995. Impact of natural and artificial UV-B radiation on mortality and growth rate of marine dinoflagellates. J. Photochem. Photobiol. 27: 73-79.

Salisbury F.B. & Ross C.W. 1992. Plant physiology. 4th edition. Wadsworth Publishing Company, Belmont, California.

Smith R.C. 1989. Ozone, middle ultraviolet radiation and the aquatic environment. J. Photochem. Photobiol. 50: 459-468.

Sze N.D., Ko K.W., Weisenstein D.K., Rodriguez J.M., Stolarski R.S. & Schoeberl M.R. 1989 Antarctic ozone hole: Possible implications for ozone trends in the southern hemisphere. J. Geophys. Res. 94: 11521-11528.

Tevini M. 1994. Physiological changes in plants related to UV-B radiation: an overview. NATO ASI Series, Vol.I 18 Stratospheric Ozone Depletion/UV-B radiation in the biosphere. Ed. by R.H. Biggs and M.E.B. Joyner. Springer-Verlag Berlin Heidelberg1994.

Tevini M., Mark U., Fieser G. & Saile M. 1991. Effects of enhanced solar UV-B radiation on growth and function of selected crop plant seedlings. Photobiology. Ed. by E. Riklis. Plenum Press, New York, 1991.

Tevini M. & Teramura 1989. Uveffects on terrestrial plants. Photochemistry and Photobiology Vol.50. No 4. pp 479-487.

UNESCO 1981. Coastal lagoon research, present and future. Report and guidelines of a seminar. Duke University Marine Laboratory, Beaufort, NC, USA. Aug. 1978. Unesco technical papers in marine science 32: 53pp.

Williamson C., Schulze P., Hargreaves B. & Seva J. 1994. The impact of short-term exposure to UV-B radiation on zooplankton communities in north temperate lakes. J. Plankton Res. 16: 205-218.