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Effects of natural ultraviolet radiation on chlorophyll fluorescence, number of fronds and biomass increase of *Spirodela intermedia* in the temperate Southern Hemisphere

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Minor Field Study 98

November 2003 Uppsala

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Resumen

Evaluación del impacto de la radiacion ultravioleta en número de individuos, incremento de biomasa y emisión de flourescencia en la especie *Spirodela intermedia* de latitudes (meridionales) medias del Hemisferio Sur.

El objectivo general de la investigación es evaluar el impacto de los niveles actuales de radiación ultravioleta-B en el incremento de biomasa de las comunidades vegetales de la zona litoral de una laguna costera (Laguna de Rocha, Uruguay, 34° S) de latitudes medias del Hemisferio Ssur. Se permitirá establecer si actualmente existen o no efectos de la radiacion UV-B en esta latitud de baja radiación. El "lenteja de agua" *Spirodela intermedia* fue usado como organismo experimental en un estudio de campo realizado durante 51 días en Enero y Febrero de 1998. Al mismo tiempo se realizó un estudio similar con la especie *Ricciocarpus natans.*

Las plantas fueron expuestas a tres tratamientos diferentes con luz ultravioleta. Para ello se usaron diferentes longitudes de onda: 1) control 2) ninguna radiatión UVB 3) PAR. Cada tratamiento fue combinado con dos intensidades de luz (intensidad normal, intensidad normal / 2.). Los parámetros para probar la sensibilidad de la radiación ultravioleta fueron: numero de individuos, crecimiento de biomasa y emision de flourescencia.

Se notó cierto efecto de la UVB en el experimento con la intensidad normal. Debido a que la biomassa del control fue menos afectada que del tratamiento no-UVB. En resumen, no se encontró ningún efecto significativo en el experimento con la UVB en intensidad normal e intensidad normal / 2.

Se pudo constatar que *Ricciocarpus natans* y *Spirodela intermedia* tiene una interacción competitiva ya que *Ricciocarpus natans* fué menos affectada por la UV-B que *Spirodela intermedia* y además estas especies se encuentran en el mismo habitat.

Se considera que una redución en crecimiento e incremento de biomasa no fue resultado de cambios en los procesos fotosintéticos ya que no se obtuvó ningún efecto en la emision de flourescencia.

El resultado pudo haber sido influido por varios factores. Por ejemplo: (1) El estudio se inició al final del verano. (2) El nivel de radiación ultravioleta fue bajo. (3) Esta semana estuvo el cielo muy nublado. (4) Durante los últimos dias del estudio, *Spirodela* habia empezado su periodo de inactividad.

Abstract

This study is an attempt to evaluate the UVB effects on an aquatic plant species at a latitude of low light intensity. The outdoor experiments were carried out during the late summer season during 51 days (Jan-Feb) 1998 in Uruguay situated at a mid-latitude (32° S) where UV-radiation normally attains relatively low intensities.

A population of *Spirodela intermedia* (Lemnaceae), a species of the littoral zone of the coastal lagoon "Laguna de Rocha" was used as experimental organism. A parallel study on *Ricciocarpus natans* , another species of the littoral zone of Laguna de Rocha was done at the same time.

The plants were exposed to three different qualities of UV radiation using filters cutting different wavelengths: (1) control (2) no-UVB (3) PAR. Each treatment was combined with two intensities of light: total light and _-light. The effect parameters used for testing sensitivity to UV radiation were number of fronds, biomass increase and chlorophyll fluorescence.

The slightly lower biomass increase in the control compared to the no-UVB treatment gives a slight indication of a negative effect of UVB at the higher light intensity (tot-light).

However there was no significant negative effect of UVB on *S. intermedia* in the two light intensities. Therefore, results from this experiment do not provide evidence of a significantly negative response of UVB even at a higher light intensity. Summing up the results of fluorescence, the lower number of fronds and biomass increase in the total light intensity (tot-light) was probably not caused by damage of UV-radiation to the photosynthetic apparatus (PSII), since no damage was indicated in the dark-adapted fluorescence measurements.

Several factors might however influence the results, for example: The study was initiated too late in the summer. UV-radiation was low during the experimental period due to the unusually cloudy weather. *S. intermedia* started to be dormant in the end of the experimental period.

There seems to be a difference in adaptation to light conditions between *Ricciocarpus* and *S. intermedia*. *Ricciocarpus* seems to be more tolerant to the total light spectrum. Species composition in the plant community might be changed due to different abilities to survive at higher radiation of UVB.

Contents

Introduction

The reduction of stratospheric ozone has raised great concern about the impact of elevated solar UV-radiation flux reaching the earth's surface. The ozone reduction affects a waveband of 25 nm within the UVB (280-320 nm), which is also the waveband most damaging to life. The rest of the solar radiation affecting biological photosystems, i.e. the visible photosynthetically active radiation (PAR) (400-700 nm) and UVA (320-400 nm), are unaffected by the ozone reduction. However, the natural variation of UVB levels on earth varies markedly with altitude, latitude, season and time of day. This natural variation between min and max values of solar UVB radiation on earth exceeds the increase that results from expected stratospheric ozone depletion (Rozema et al., 1997).

From experimental findings elevated solar UVB results in environmental stress to plants. Observed physiological effects are detrimental influences on growth and competition of plants due to nucleic acid damage (DNA), damage to the photosynthetic apparatus primarily involving photosystem II, damage to membranes and inactivation of phytohormones (Rozema et al., 1997; Stapelton, 1992; Tevini et Teramura, 1989).

Responses to UVB also vary considerably among species as well as between different strains of the same species. This might result in changes in species composition when UVB radiation changes. Changes in the biodiversity of different ecosystems are therefore likely to take place (Caldwell et al., 1995).During evolution plants have adapted to enhanced UV-radiation mainly by developing protective mechanisms. One adaptive mechanism seen in terrestrial plants is to increase production of UVB absorbing metabolites like flavonoides. Flavonoides and related compounds occur in most terrestrial plants.

Another mechanism of adaptation is photoreactivation, a process of repair of DNA by visible white light. The longest wavelengths of ultraviolet radiation (UVA, 320-400 nm) are known to induce both photodamage and photoreactivation processes in living cells (Caldwell, 1986). Rather high UVA and/or blue light flux are necessary to saturate photoreactivation (Caldwell et al. 1994).

The studies of the impact of UVB on natural plant populations and communities are scarce and need to be further investigated. The negative effects on aquatic vascular plants are considered to be the same as the effects on terrestrial vascular plants.
Field experiments on UV-effects have mainly been carried out at latitudes with high light intensity (Rozema et al, 1997). Contrary to the tropical zone biota, which are evolutionary adapted to high levels of UVB, plant communities in latitudes with low light intensities might be more stressed as a response to increased UVB (Caldwell et al., 1983).

Another author reports that growth reductions were also found in wild type and stable-phytochrome-deficient mutants of cucumber indicating that growth in the latter case is regulated by an unknown photoreceptor, not by phytochrome. The molecular reasons for growth reductions can be attributed to changes in DNA and/or phytohormones (Tevini, 1983).

This study is an attempt to evaluate the UV-effects on an aquatic plant species at latitude of low light intensity. The outdoor experiments were carried out during the late summer season (Jan-Feb) 1998 in Uruguay, situated at a mid-latitude (32°) where UV-radiation normally attains relatively low intensities. A population of *Spirodela intermedia* (Lemnaceae), a species of the littoral zone of the coastal lagoon "Laguna de Rocha" was used as experimental organism. Parallel in time the same type of study was carried out with *Ricciocarpus natans*, another species of the littoral zone of Laguna de Rocha (Alm, 2003)

Hypothetis

The hypothesis tested in this study is that variations in natural levels of UVB radiation have a negative effect on number of individuals, biomass increase and fluorescence (potential photosynthetic ability) of this plant.

Material and methods

The experimental organism *Spirodela intermedia*

Spirodela intermedia is a green free-floating, freshwater plant. It belongs to the Lemnaceae family consisting of small plants, that are free or partly floating on submerged herbs (Cook, 1990). Plants of the Lemnaceae family are among the first colonisers of aquatic environments and are mainly found in calm water. Since they are floating on the water-surface and not rooted, they are easily transported away by waves and currents as well as by animals. They assimilate solar light efficiently and due to their floating way of living they are exposed to the whole range of visible radiation and UV-radiation.

The biomass of Lemnaceae per area in nature is relatively low compared with other water plants and with terrestrial plants. This is due to their special growth habit. Lemnaceae form rather thin layers of fronds and are composed of relatively high light assimilating and aerenkymatic tissue (Landolt, 1986).

In the wetland areas of Uruguay Lemnaceae often form dense covers and carpets in open areas associated with other floating hydrophytes such as *Salvinia*, *Wolffia, Azolla*, *Ricciocarpus* and *Pistia* or between rooted emergent plants called "camalotales" such as *Nymphoides indica, Hydrocleys nymphoides* and the water hyacinths *Eichhornia azurea* and *E. crassipes* (Alonso, 1997).

Spirodela intermedia occurs in warm, temperate, subtropical and tropical regions of South and Central America. The distribution area extends from 13° N (El Salvador) to 36° S (Buenos Aires). In the tropics it may reach altitudes of 2 700 metres.

Spirodela polyrhiza is closely related to *S. intermedia* and these two species are very difficult to distinguish from one another (Landolt, 1986). A common name of *S. intermedia* is ”Duckweed”(Eng.) or ”Lenteja de agua” (Spa). It has never been observed to flower in Laguna de Rocha (Alonso, 1997). It has lost floral characters and in the frond there is no difference between stem and leaves. The frond or thallus has variously been interpreted as a stem, a leaf or partly stem and leaf (Cook, 1974). The reproduction is usually vegetative through buds that are developing by splitting from 1 or 2 vegetative or reproductive pouches. Depending on species and culture conditions each frond forms up to 20 daughter fronds which become visible within different intervals (several hours up to a few days) The fronds can separate or stay united during several generations (Landolt, 1986).

In Laguna de Rocha the growing season of *S. intermedia* starts in springtime (Oct-Nov) and it is beginning to disappear in the end of the summer (February-March). In the autumn (April-May) it disappears completely (pers. communication, Mazzeo).

The study site

The experiment was performed at the Limnological station at Laguna de Rocha (34° 35'S 54° 17'W) situated 200 km east of Montevideo (Figure 1). The Faculty of Science, Univ. de la Republica, has studied the lagoon system since 1986. There are several lagoons in the southeastern and eastern coasts of Uruguay. Many of them, including Laguna de Rocha, are part of the Biosphere Reserve designated by Man and Biosphere Program (MaB) of UNESCO (ILEC, 1992) and declared a Ramsar site. This protected area denominated “Humedales del Este” is an extended net of lagoons, marshes and temporarily inundated areas. It is the place with the highest species richness of aquatic plants in Uruguay having approximately 80 % of the known aquatic species of the country (Alonso, 1997).

The depth of Laguna de Rocha varies between 0,5 and 5 metres. Due to the shallowness, a great proportion of the total area in this system forms a littoral zone dominated by macrophyte vegetation. The lagoons are ecologically complex, natural systems that are very productive but fragile due to a complex web of trophic interactions. The principal characteristic is a high temporal and spatial variability of physical and chemical factors, due to the mixing of water masses of continental and marine origin. There are five inflowing rivers of which Arroyo Rocha and the Las Conchas are the most important. Laguna de Rocha connects periodically to the Atlantic Ocean with a canal that opens up at a sand embankment at the southern side of the lagoon. The fauna of the lagoon is diverse with numerous species of fish and crustaceans and is also the habitat of many endemic and migratory birds. The high production of fish, crabs, shrimps and molluscs is of great commercial value.

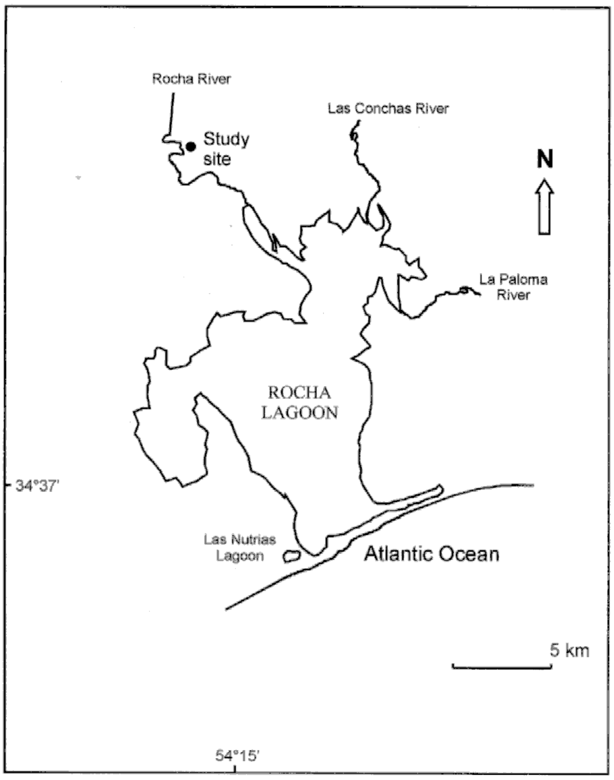

Figure 1. Map over Laguna de Rocha (34° 35'S 54° 17'W) situated at the south-eastern coast of Uruguay 200 km east of Montevideo.

Climate

The summer season in Uruguay lasts from the middle (21st) of December until the middle of March (21st). The autumn ends approximately in the middle of May. Winter ends in the end of August and the spring lasts from September until the middle of December (21^{st}). Climate data at the city of Rocha is shown in Table 1.

Table 1. Climate data at Rocha city, 1951-1980. Monthly mean temperatures (°C) and precipitation.

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mean temperature (°C)											
21.5	21.3	19.9	16.4	13.7	11.2	10.9	11.2	12.6	14.8	17.4	20.2
Annual mean temperature 15.9											
Mean precipitation (mm),											
98	102	87	81	78	97	96	102	109	92	70	61
Annual mean precipitation 1.073											
Average solar radiation, 1979-1984: 14,3 MJ $m^{-2}day^{-1}$.											

Experimental set-up, design and measurement

Spirodela intermedia *material and experimental set up*

Spirodela intermedia was sampled from ”Las Conchas” which is one of the principal tributaries to Laguna de Rocha. A total of 40 or 50 plants were placed in each of four compartments of a plastic box. In this way each treatment was kept in a plastic box divided in four replicates (145 cm^2/replicate).

Water from “Las Conchas” (conductivity=404 $\mu S\ cm^{-2}$, pH=6,11) was added to each replicate when needed and totally renewed every two or three days. In this way nutrient limitation was prevented. Controls were made daily to remove crickets and other insects that were feeding on the plants or other invertebrate animals entering by accident. The experiment was placed to float in a small channel close to the field station to receive natural ambient temperature and light. During windy weather conditions the experiment was kept on land to avoid accidents. Because of the bad weather it was sometimes necessary to bring the experiment indoors.

Different filter combinations (see below) varied the light transmittance. (se below) The filters were placed in a frame at a distance of 5-7 centimetres above the experimental boxes.

Treatments

The experimental set consisted of three treatments with different light transmittance properties (A-C), each with four replicates and combined with two intensities of light (1) total light and (2) _-light.

A. Control
The plants in the control were exposed to the full spectrum of solar radiation including the UV spectrum and the photosyntetically active radiation (=PAR+UVA+UVB), which is the natural situation for plants. To get equal gas exchange and circulation conditions as in the other treatments, the box was covered with a plastic film of polyethylene.

B. No UVB
The plants were exposed to radiation excluding UVB (= PAR+UVA) i.e. transmitting UVA and longer wavelengths, using a transparent filter of polyester of Mylar D ® (Dupont laboratories, 50 % transmittance at 320 nm).

C. PAR
The plants were exposed to sunlight excluding all UV using a filter of vinylchloride (CI Kasei Co, Tokyo, Japan, 50 % transmittance at 405 nm).

It is very likely that a population of *S. intermedia* in the stream Las Conchas grows in places that are shaded by riparian trees. In order to meet a natural situation with shading by riparian trees, the solar radiation was reduced to half the intensity of ambient radiation by means of a neutral mesh placed above the filters in one of the experimental sets. In this way the experiment was designed with two sets of different light intensities, called “**tot light**” and “**1/2 light**”. The mesh cut all wavelengths of radiation so the

relation between PAR, UVA, UVB was the same in both light intensities.
The situation of shading by a mesh is not however direct comparable to shading by foliage since there is no difference in how the mesh cut red and visible light. With the natural situation of shading by foliage light conditions are shifted towards more far-red radiation since visible radiation is almost completely reflected and absorbed by vegetation, whereas that between 700 nm and 800 nm (in the far-red range) is largely transmitted**.**

Initially spectrophotometric scanning controlled the transmittance of the different filter materials (Figure 2). The filters were checked with the radiation sensors 2 times during the experimental period to be able to register changes in the transmittance of the materials. No significant changes in the transmittance were observed.

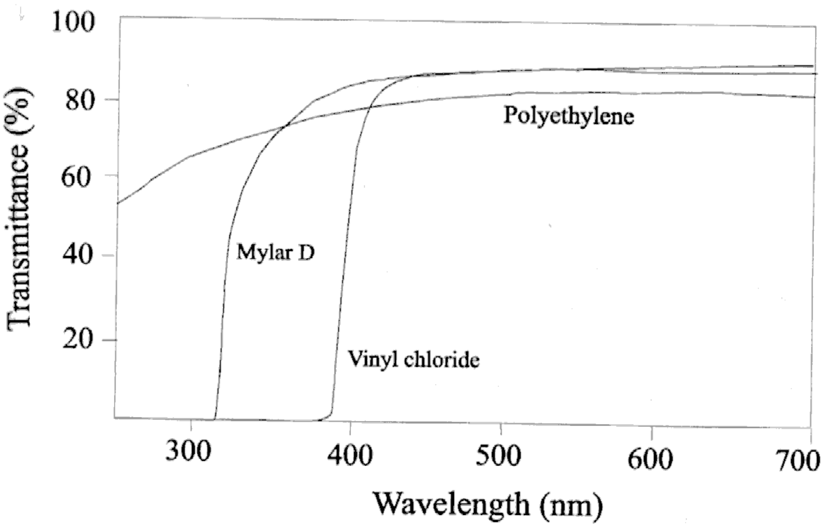

Figure 2. Transmittance of the different filter materials controlled with spectrophotometric scanning (mylar=no-UVB, vinylchloride=PAR, polyethylene=control).

Experimental period

The experiment was carried out during two periods in the summer (Jan-Feb, 1998). The duration and design of the experiments and the initial number of individuals are shown in Table 2.

Table 2. Length of the experimental period, initial number of individuals and design of the experiments.

Period	No. of days	No. of individuals	Design
2-14/1	13	40	tot-light of A, B, C
16/1-20/2	36	50	tot-light of A, B, C
16/1-22/2	38	50	1/2 - light of A, B, C

A. Control = The full solar radiation i.e. PAR+UVA+UVB
B. No-UVB = All radiation except UVB i.e. PAR+UVA
C. PAR = No UV-radiation
Light intensity: tot light and 1/2 – light.

Observations of number of fronds and biomass increase

Number of visible fronds was estimated at a 3-4 day interval during the experiments. Daughter fronds were counted as soon as they were seen from the pouch of the mother frond. Number of fronds was estimated during both the first and the second experimental period. During the first period only effects of total light intensity (tot-light) were studied. To quantify number of visible fronds, the relation between the different treatments should be estimated the 13th of February i.e. before the plants started to die.

Biomass increase was estimated only during the second experimental period. Biomass increase was actually highest before, but was impossible to quantify until, the end of the experiment. When the experiment was finished after the second experimental period (36 and 38 days respectively) the fresh (damp) and dry weight of the fronds were measured. Fresh weight was determined after blotting plants with paper towels to remove surface water. Dry weight was measured after drying the samples for 48 hours at 85 °C.

Biomass increase was determined as dry weight increment of the biomass per day. Biomass increase is expressed as the difference of the logarithms of the final dry weight (W_d) and the initial dry weight (W_0) divided by the number of days of growth and area (145 cm^2) of the replicate compartment (calculated as $k = \ln W_d - \ln W_0/(days*area)$). This equation is developed from the simplest differential equation ($dW/dt=kW$) were "W", being the number or biomass of the plant population and the change as a function of time (t), where "k" is the intrinsic rate of increase.

Solar radiation measurements

Two equipments were used to measure solar radiation. The UV sensor (Int. light inc. IL 1400 A) measures UVA (mW cm^{-2}-µW cm^{-2}) in a range of 20 nm (350-370 nm) with a centre of 360 nm. The same sensor measures UVB (µW cm^{-2}) in a range of 20 nm (285-305 nm) with a centre of 295 nm.

The PAR sensor (LI-1935 A) (2π) (LI-COR, Inc.) measures in the range of 400-700 nm (µmol $s^{-1}m^{-2}$) which is the wavelength interval most active in photosynthesis.
The daily radiation doses of PAR, UVA, UVB were determined by integration of the measurement of radiation by a Windows software. The total solar radiation was measured once every hour and once every half-hour during noontime to be able to register the peak value of radiation every day.

Chlorophyll fluorescence measurements

Fluorescence measurements were performed with a fluorescence-modulated system (FMS) supplied with a Windows software application. During measurement plastic filmtubes were used with a specially designed internal plate with a hole to be able to put the plant in the right position. Measurements were performed both indoors and outdoors. The dark-adaptated measurements were performed indoors in the night with plants that were dark-adaptated during 20 minutes before measurement. During dark adaptation the tubes were covered with a lid. Light-adaptated measurements were performed outdoors at noontime.

Description of fluorescence of chlorophyll a

UVB is known to cause damage to photochemical processes (Tevini & Teramura, 1989). Leaf chlorophyll fluorescence parameters can be used to assess damage to photosystem II (PSII; Krause & Weis, 1984). Fluorescence of chlorophyll *a* indicates variations in photosynthetic activity of measured tissue.

This study concentrated on measurement of four different fluorescence parameters. After dark-adaptation fluorescence values indicate maximal potential photosynthesis of the leaf or plant measured, i.e. *maximum quantum efficiency (MQE)* of PSII. Parameters under fully activated photosynthetic conditions (steady state) may be obtained by illuminating the leaf with an intense pulse of light to saturate the light harvesting system and close all PSII reaction centres. From this the *quantum efficiency of PSII* (QE) can be calculated.

The two main components by which the fluorescence-signal is quenched (extinguished) are *photochemical quenching (qP)* and *non-photochemical quenching, (qnP)*. The former is related to CO_2 assimilation, nitrate reduction e.t.c. The latter has several components, including the energy dependent quenching (qE) which is related to the proton concentration in the thylacoid lumen. The fluorescence parameters during dark-adaptated and steady state conditions can determine these parameters.

Statistical analyses

Statistical analyses for unplanned, non-parametrical comparison with unequal variances were applied to test the difference between the different treatments (Fligner & Policello, 1981). The test described by Flingner-Policello is a modification of the Mann-Whitney-Wilcoxon test. Correlation between the integral radiation doses and fluorescence parameters was examined with Sperman rank correlation coefficient (r_s). Significance levels are indicated in the following way: ***p=0,01, **p=0,025, *p=0,05.

Results

Observations of natural light conditions

Calculated natural daily doses (integrals) of UVA, UVB and PAR, during the days of the experiment are shown in Figures 3-5. UVA varied between 2,0 and 33 mW cm^{-2} day^{-1} and UVB between 0,007 and 0,147 mW cm^{-2} day^{-1} and PAR varied between 1050 and 18 906 µmol s^{-1}m^{-2} day^{-1} during the experimental period.

This summer the highest value of UVB radiation (34 µW cm^{-2}) was recorded at the end of December i.e. before the start of the experimental period. A small downward trend can be noticed for all types of radiation, but is most apparent for UVB. Thus the UVB/UVA ratio is decreasing during the experimental period.

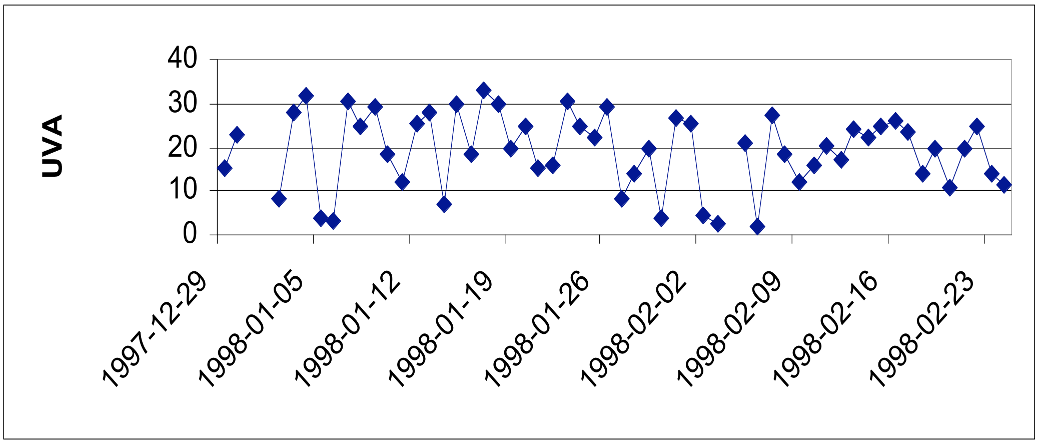

Figure 3. Calculated daily doses (integrals) of UVA-radiation (mW cm^{-2} day^{-1}) during the entire experimental period.

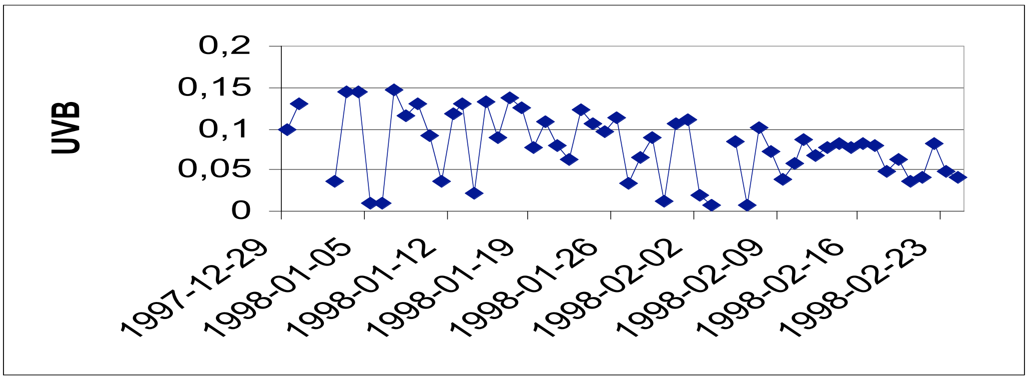

Figure 4. Calculated daily doses (integrals) of UVB-values (mW cm^{-2} day^{-1}) during the entire experimental period.

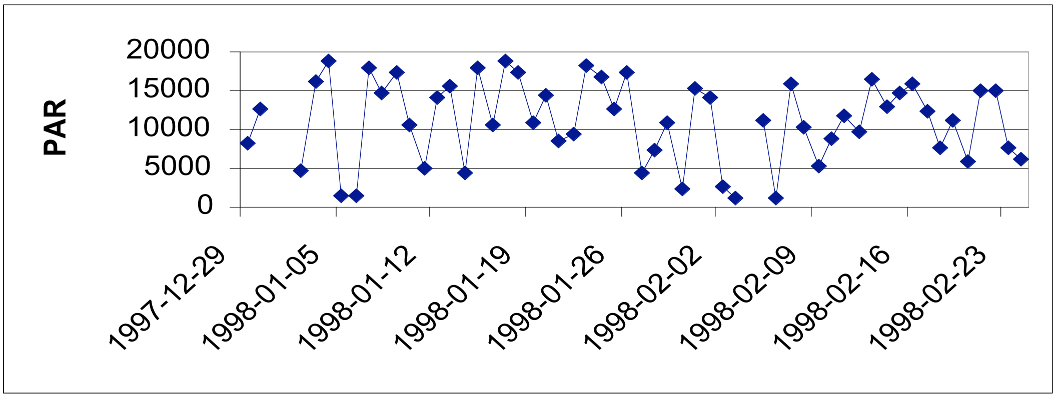

Figure 5. Calculated daily doses (integrals) of PAR (µmol s^{-1}m^{-2} day^{-1}) during the entire experimental period.

Chlorophyll fluorescence

In the total light intensity (tot-light) there were no significant differences in maximum quantum efficiency (MQE) between different treatments (Figure 6a). MQE is quite stable in all treatments during the period indicating that the capacity to capture energy in the PSII reaction centers did not change much. Three out of five measuring occasions MQE seemed to be somewhat higher (but not significantly) in the no-UVB treatment compared to control and the PAR treatment. The same pattern was found in biomass increase and number of fronds of *Spirodela intermedia*.

At half the light intensity (1/2-light) (Figure 7a) MQE was quite stable in all treatments during the period. In _-light intensity there were a few treatments that differed significantly from the others. However, there was a trend of the control being highest (except the first measuring occasion). Furthermore, at two occasions the control differed significantly from the PAR-treatment.

There was no correlation between fluorescence and integrated UVA, UVB or PAR values (Spearman rank; 0,000-0,007). QE changed in the same way as qP and the relation between the treatments at different measuring occasions was also the same (Figures 6 b-c, 7b-c).

There were very low light conditions during measurement. This resulted in relatively high photochemical quenching (qP) i.e. the major part of the energy was used in photosynthesis. This was the case on the 10th of February in the total light intensity (tot light) (Figure 6c) and the 3rd and on the 10th of February in half the light intensity (1/2-light) (Figure 7c). On the contrary, at high light intensity part of the energy disappeared in non-photochemical processes i.e. non-photochemical quenching (qnP) (Figures 6d and 7d).

The time to photorepair UVB damages in *Spirodela intermedia* is not known. As a comparison it should be mentioned that photoreparation in algae is induced within hours (R. Sommaruga, pers. comm.). In the case that midday inhibition or photosynthetic damage occurred this might be assessed by successful midday fluorescence measurements (i.e performed at noon during sunny weather conditions). However, in this study this was not successfully performed due to the high variability in daily radiation and temperature. Therefore only the dark-adapted fluorescence measurements were possible to evaluate (Figures 6a-d and 7a-d), which might not be enough to reveal photosynthetic damage.

The maximum quantum efficiency was quite stable in all treatments and at both light intensities during the period, indicating that the capacity to capture energy in the PSII reaction centre did not change much.

While measuring MQE in the half-light intensity at all measuring occasions except on 19 Jan., the control was somewhat higher (the control was twice significantly different from the PAR treatment) indicating that the total radiation spectrum, including UVA and UVB was benefical for the plant. This is consistent with the results of *Ricciocarpus natans*.

In the total light intensity, there was a tendency of a higher MQE in the no-UVB treatment compared to the control and the PAR treatment. This is the same pattern as found in biomass increase and number of fronds of *Spirodela intermedia*. However, there were no significant differences between treatments.

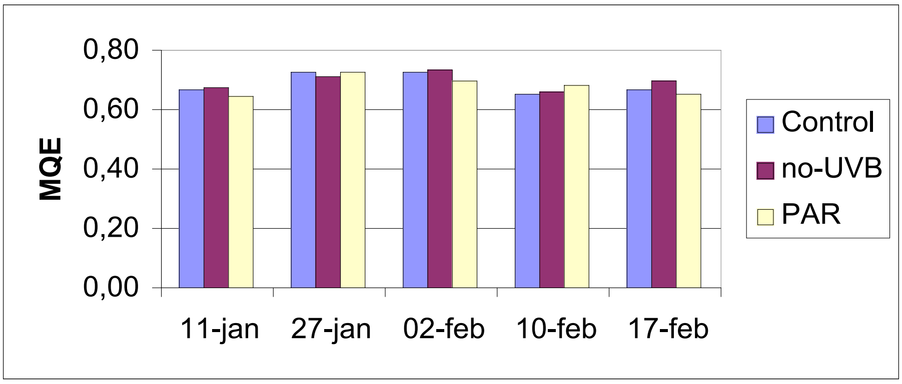

Figure 6a. Maximum quantum efficiency (MQE or Fv/Fm) of PSII (i.e. fluorescence during dark-adaptated conditions) at different filter treatments with total light intensity (tot–light*)* in *Spirodela intermedia*.

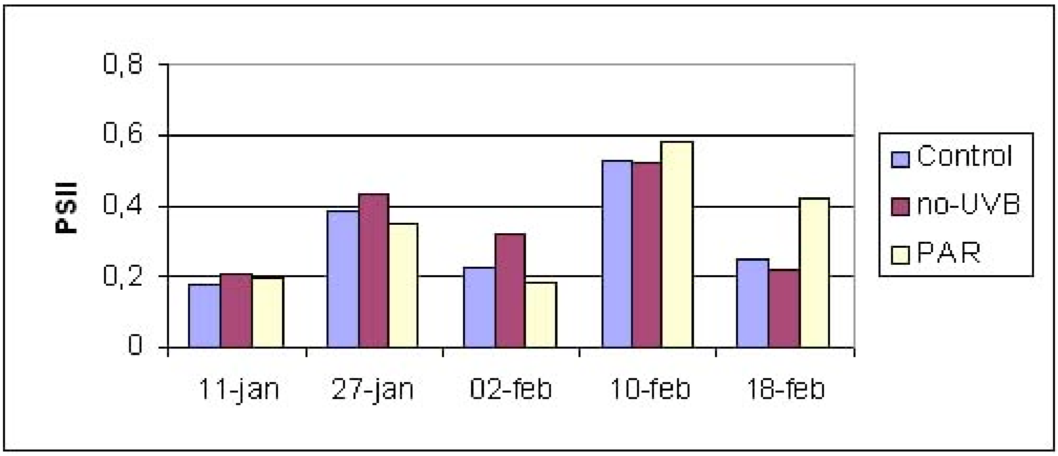

Figure 6b. Quantum efficiency of PSII (fluorescence on fully photosynthetic conditions) in the total light intensity (tot–light) in *Spirodela intermedia.*

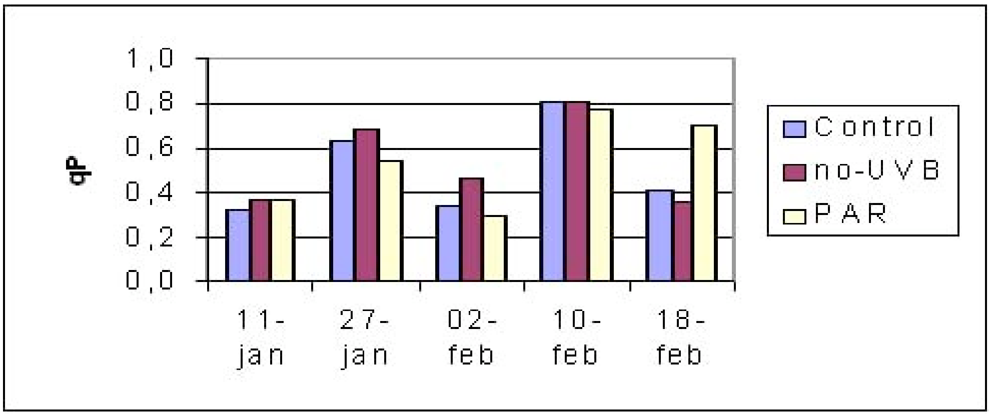

Figure 6c. Photochemical quenching (qP) (i.e. photochemical extinction of the fluorescence signal) in the total light intensity (tot–light) in *Spirodela intermedia*.

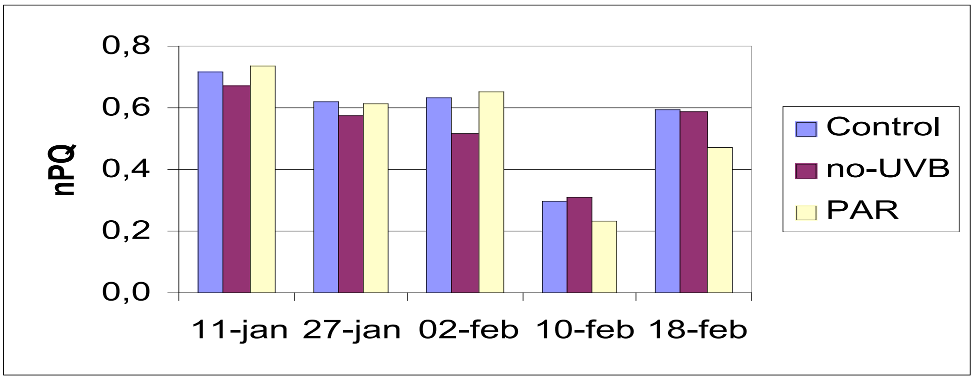

Figure 6d. Non-photochemical quenching (nPQ) (extinction of the fluorescence signal) in the total light intensity (tot–light) in *Spirodela intermedia*.

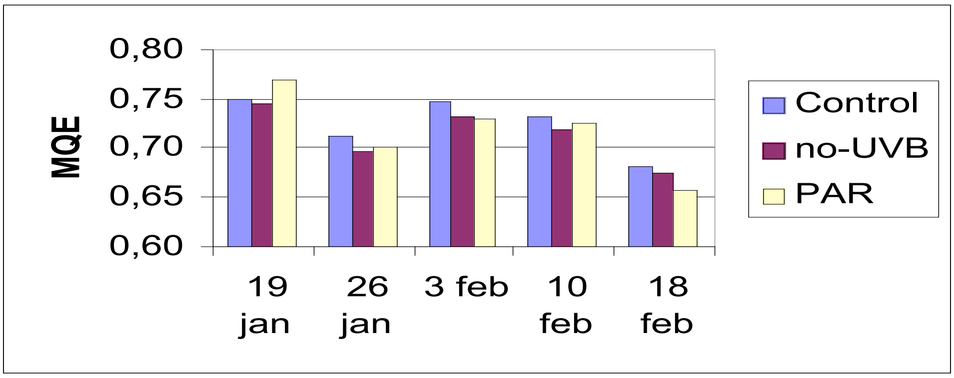

Figure 7a. Maximum quantum efficiency (MQE or Fv/Fm) of PSII (i.e. fluorescence during dark-adaptated conditions) in half the light intensity (1/2 light) in *Spirodela intermedia*.

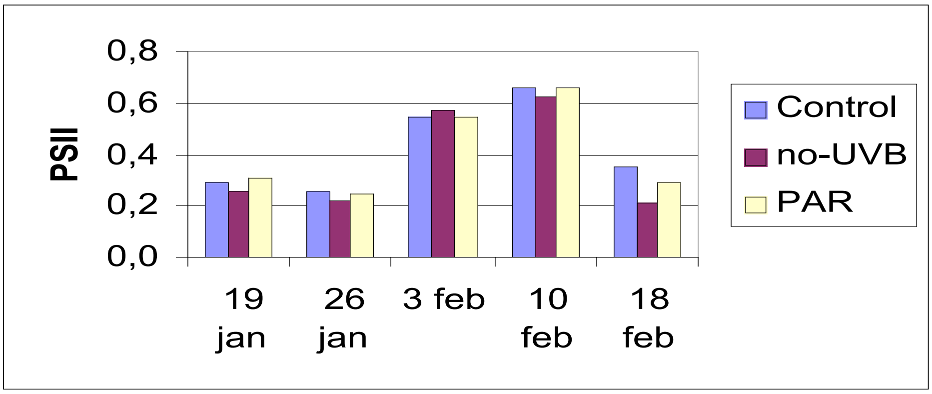

Figure 7b. Quantum efficiency of PS II (flourescense during fully photosyntetic conditions in half the light intensity (1/2 light) in *Spirodela intermedia*

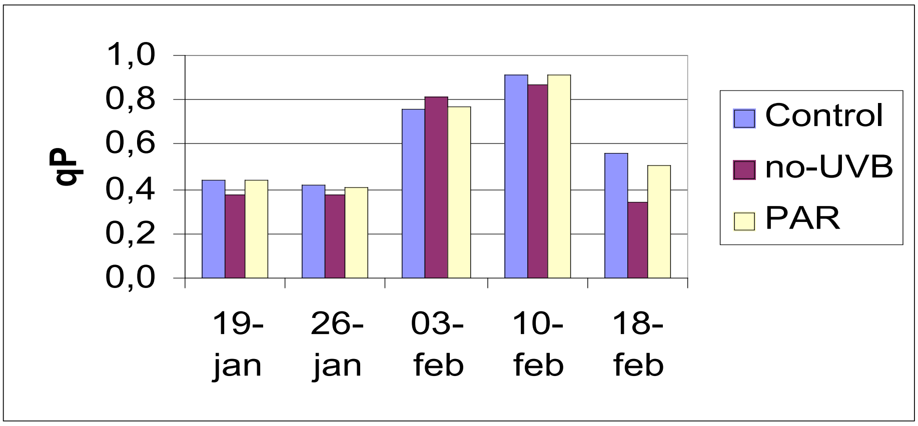

Figure 7c. Photochemical quenching (qP) (i.e. photochemical extinction of the fluorescence signal) in half the light intensity (1/2 light) in *Spirodela intermedia*.

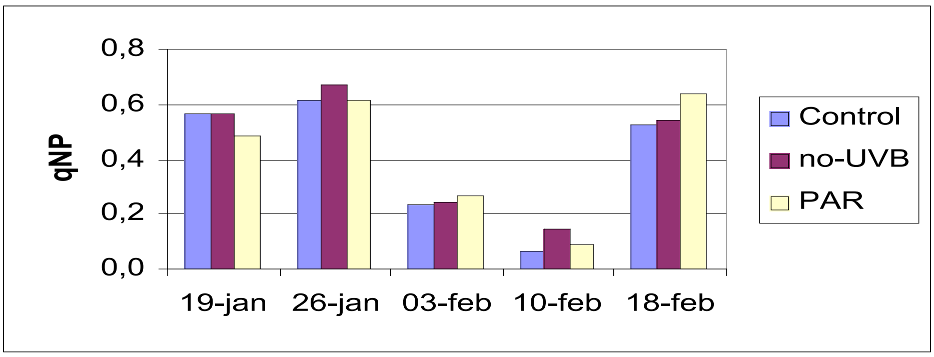

Figure 7d. Non-photochemical quenching (nPQ) (extinction of the fluorescence signal) in half the light intensity (1/2 light) in *Spirodela intermedia.*

Frond observations

The state of fronds during the experimental period

The state of fronds changed during the experimental period. At the end the number of individuals were decreasing because some plants were dying. Plants in the experiment with the total light intensity (tot light) had a very pale leaf colour, and were in a poorer condition and more affected of grazing by insects than those in the half light intensity (1/2 light). This was the reason for bringing this experiment with total light to an end 2 days before the experiment with half the light intensity.

Number of fronds

There was an increasing growth of fronds at the beginning of the experimental period. The number of visible fronds in the total light intensity was about the same during both the first and the second experimental period (Figures 8 and 9). Treatments was a few times significant from each other (Fligner-Policello, 10th, 11th, 12th, 19th, 25th, 28th of Jan and 2nd, 20th of Feb. Table 3 and 4). In the total light intensity (tot light) fronds grew best in the no-UVB treatment (maximum 80 individuals) compared with control and PAR-treatment maximum 72 respectively 75 individuals, (Figures 8 and 9). However, in the control there was a tendency of slightly higher number of fronds than in the treatment with no-UV (PAR). This pattern prevailed during both the first period (2-14 Jan) and in the beginning of the second period until the 22nd of January.

Table 3. Results from a Fligner-Policello test applied on number of fronds of different treatments of *Spirodela intermedia* receiving total light intensity (tot-light).

	Date						
Treatments	7/1	8/1	9/1	10/1	11/1	12/1	14/1
Control vs. No-UVB	-	-	-	*	-	-	-
Control vs. PAR	-	-	-	-	-	-	-
No-UVB vs. PAR	-	-	-	*	*	*	-

*** p<0,01, ** p<0,025, * p<0,05, - =non-significant.

Table 4. Results from a Fligner-Policello test applied on number of fronds of different treatments of *Spirodela intermedia* receiving total light intensity (tot-light).

	Date											
Treatments	19/1	22/1	25/1	28/1	31/1	2/2	7/2	10/2	13/2	16/2	18/2	20/2
Control vs. No-UVB	-	-	*	*	-	*	-	-	-	-	-	*
Control vs. PAR	-	-	-	-	-	-	-	-	-	-	-	*
No-UVB vs. PAR	*	-	-	-	-	-	-	-	-	-	-	-

*** p<0,01, ** p<0,025, * p<0,05, - =non-significant.

Table 5. Results from a Fligner-Policello test applied on number of fronds of different treatments of *Spirodela intermedia* receiving half-light intensity (1/2-light).

	Date											
Treatments	19/1	22/1	25/1	28/1	31/1	2/2	6/2	10/2	13/2	16/2	18/2	22/2
Control vs. No-UVB	-	-	-	-	-	-	-	-	-	-	-	-
Control vs. PAR	-	-	-	-	-	-	-	-	-	-	-	-
No-UVB vs. PAR	-	-	-	-	-	-	-	-	-	-	-	-

*** p<0,01, ** p<0,025, * p<0,05, - =non-significant.

At half light intensity (1/2-light) no treatments were significantly different during any time of the experimental period (Figure 10 and Table 5). In the latter part of the period there was a slight tendency for the control to have highest number of fronds.
A comparison between the two different radiation treatments during the second period gives the following results: plants in the control receiving half the light intensity (1/2-light) (Figure 10) grew better (at maximum 83,5 individuals) compared to plants in the control receiving total intensity (tot-light) at maximum 72 individuals (Figures 8 and 9). The same was observed in the treatment receiving light with no-UV (PAR) which also had a higher number of fronds (at maximum 80,5 individuals) in half intensity (1/2-light) compared to that in the total intensity (tot-light) at maximum 75 individuals.

On the contrary, plants in the treatment with no UVB had about the same number of fronds in half (1/2-light) (at maximum 82 individuals) and total light intensity (tot-light) (at maximum 80 individuals) at least before the 10Th of February.

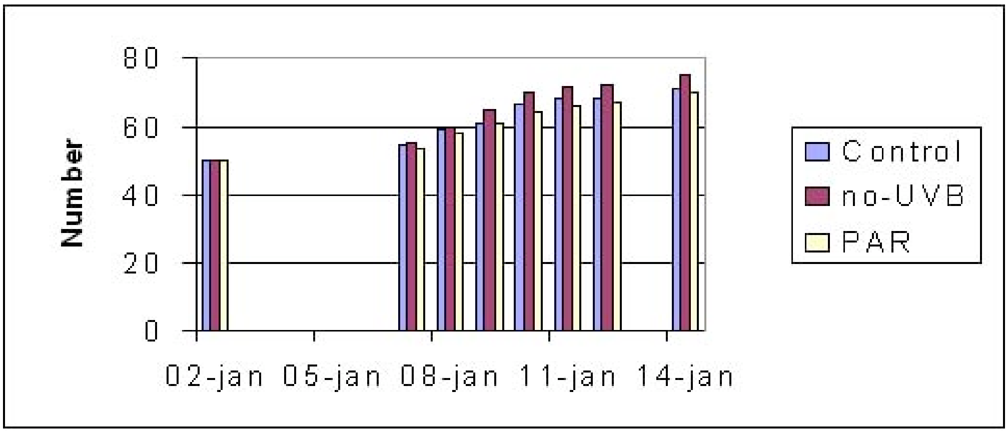

Figure 8. Number of fronds in the total light intensity (tot-light) during the first experimental period (2-14 Jan 1998).

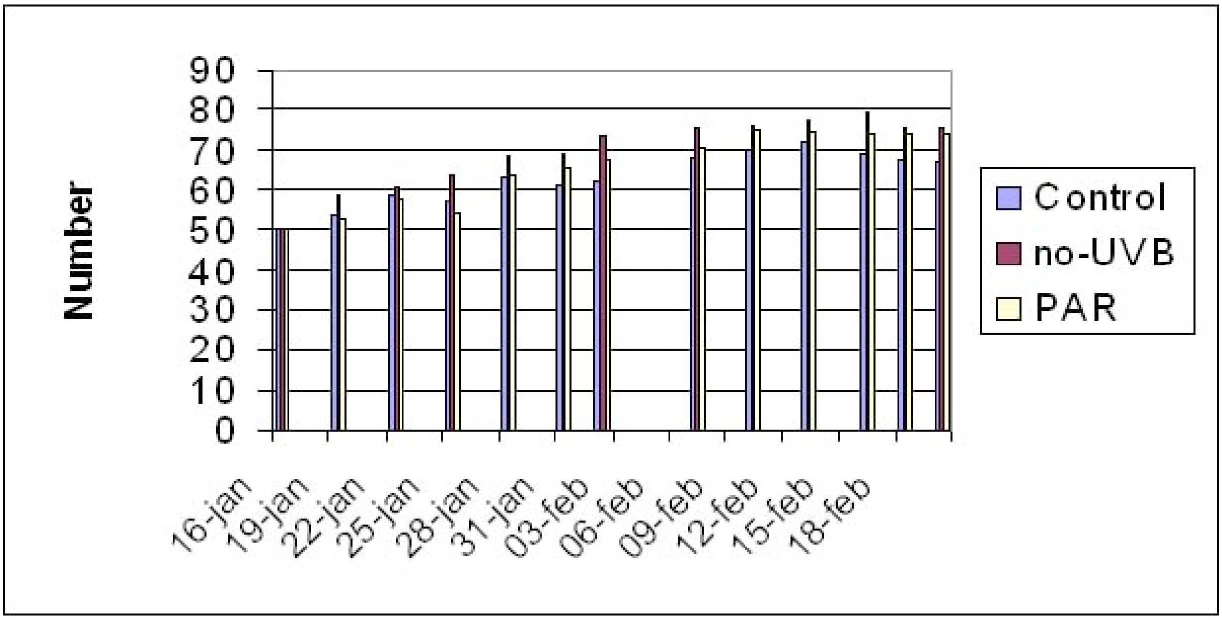

Figure 9. Number of fronds in the total light intensity (tot-light) during the second experimental period (16 Jan-20 Feb 1998).

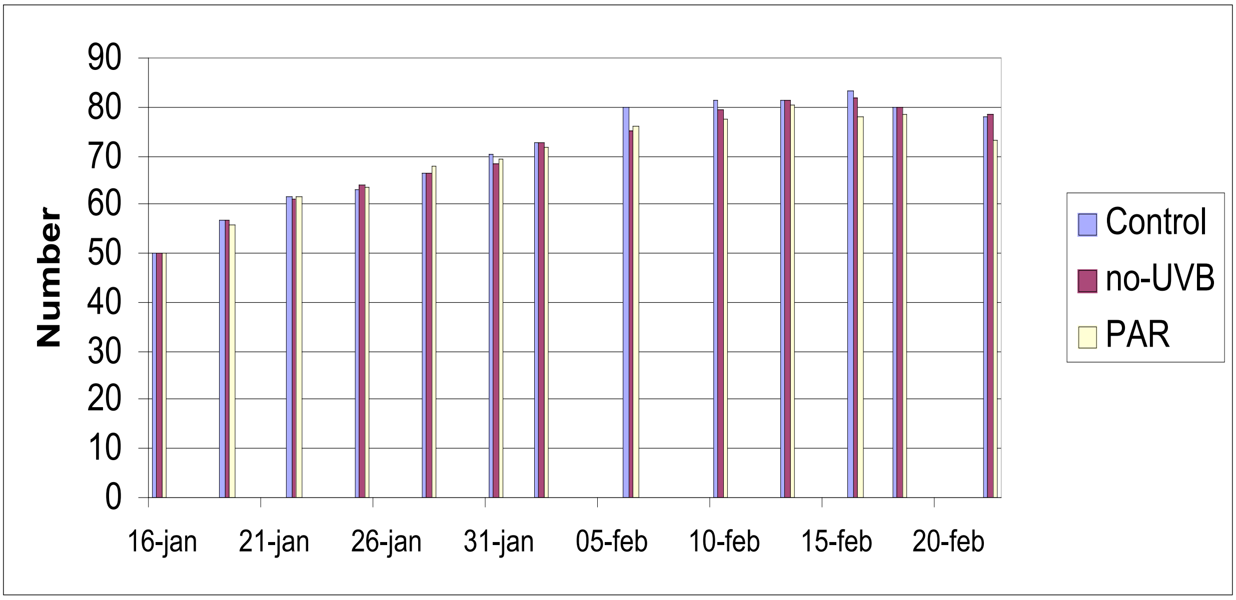

Figure 10. Number of fronds in half the light intensity (1/2-light) during the second experimental period (16 Jan-22 Feb 1998).

Biomass increase

The net biomass increase differed slightly between treatments (Figure 11).
In the total light intensity (tot-light) there was a significantly higher increase (Fligner-

Policello, test-variable 2,5) in the treatment with no-UVB compared with the PAR treatment but not compared to the control. In contrast, there were no significant differences between the treatments with half the light intensity (1/2-light).

Comparing the two light intensities, the control had a higher biomass increase (almost significant) in half the light intensity (1/2-light) compared to the total light intensity (tot-light). The treatment with no-UVB had almost the same increase in half the light intensity (1/2-light) compared to the total light intensity (tot-light). The treatment with no-UV-radiation (PAR) had a significantly higher increase (Fligner-Policello, test-variable 16) in half the light intensity (1/2-light) compared to total light intensity (tot-light).

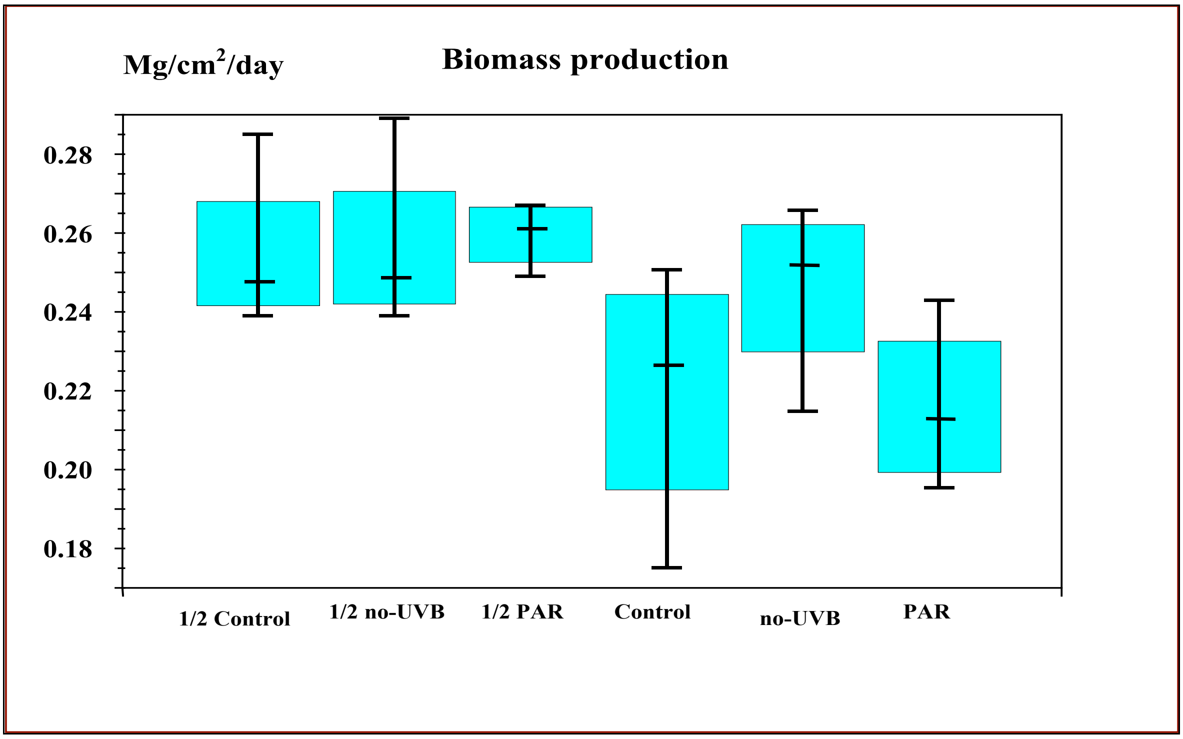

Figure 11. Biomass increase (mg cm^{-2}*day^{-1}) calculated as dryweight of *Spirodela intermedia* in the three different treatments (control, no UVB and PAR) in half the light intensity (1/2-light) and three different treatments (control, no UVB and PAR) in the total light intensity (tot-light). Box shows mean and quartiles (25% and 75%) and whiskers shows the highest and the lowest values. 16 Jan –20 Feb (tot light) resp. 16 Jan. -22 Feb. (1/2-light) 1998.

Discussion

Results from this experiment do not provide evidence of a negative response of UVB even at a higher light intensity, because there was no significant difference between the treatments of *S. intermedia.* Several factors mentioned briefly below might, however, influence the results, particularly the following:

The study was initiated too late in the summer. UV-radiation was low during the experimental period due to the unusually cloudy weather. There was no limitation of nutrients, which however might be a result in the natural situation increasing the sensitivity to UV-radiation. *S. intermedia* started to be dormant in the end of the experimental period. Plants of the higher light intensity were put under more temperature stress compared to the lower light intensity. The influence of grazing on biomass increase was different between treatments. In the natural situation plants were adaptated to a lower light environment, which might be the reason for a shorter lifespan in the higher light intensity. These factors are described more in detail in the next part.

Evaluation of experimental set-up and suggestions for future studies

Influence of weather, temperature and life cycle

UVA and visible light are wavebands that co-varies strongly whereas UVB can be somewhat uncoupled from UVA and visible light when solar angels change, and certainly, if atmospheric ozone changes (Caldwell & al., 1994). This explains the decreasing UVB/UVA ratio towards the end of the experimental period.

Since the level of UVB decreased during the experimental period it is reasonable to believe that the UVB-effect also decreased. To attain the highest values of radiation, this study should have been initiated earlier in the summer (Dec) when there were the highest levels of UVB (Figure 4).

Variations in weather and light might influence the results. During the summer of 1998 the weather was unusually cloudy and rainy because of the weather phenomenon El Niño. The unusually high variability in daily radiation of UVB (Figure 5) and temperature during this summer was due to the cloudy weather. Models predict a global ozone reduction especially in the southern mid latitudes (Sze et al., 1989). In this study unusually cloudy weather and the low radiation probably reduced potential harmful effects normally caused by UVB at these latitudes in consequence of the predictable ozone reduction. A summer with a more stable weather situation would probably have given more distinct results.

Nutrient deficiency is a factor of environmental stress and this might have had an impact on the sensitivity of the plants to UV-radiation. During the experiment nutrient limitations was prevented by regularly changing the water.

The growth of the summer generation of *S. intermedia* seemed to cease at the end of the experimental period. A way of getting a longer experimental period might be to start with plenty of time before that fronds start to die. To be able to determine the optimal experimental period the condition of the plant during different season periods needs to be further investigated. The dormancy period with declining number and dying of fronds might have influenced results in the latter part of the experimental period.

Life span of different organisms often depends on temperature. At 30° C, life span of *S. intermedia* is about half of that at 20° C. However, in a resting stage and at low temperatures, the fronds may survive for several months (Landholt, 1986). The higher light in the total light intensity (tot light) might lead to a higher temperature. This might be a factor of stress for the plants, which might be the reason for a shorter life span for this light intensity.

Fluorescence measurement

Summing up the results of fluorescence, the lower number of fronds and the biomass increase in the total light intensity (tot-light) was probably not caused by damage of UV-radiation to the photosynthetic apparatus (PSII), since no damage was indicated in the dark-adapted fluorescence measurements (Figures 6a and 7a). On the contrary there was indication of a positive effect of UVA-radiation. This is consistent with another study on soybeans where measurements of leaf fluorescence characteristics did not indicate photosynthetic damage under any radiation combination (Caldwell & al., 1994).

Other factors of stress

It was observed that there was a higher degree of grazing of crickets in the experimental boxes of total light intensity treatment. However, the effect of grazing on biomass increase was not quantified. But the extent of grazing could have been implemented as a parameter to measure to see if there was any difference between the different treatments.

The more pale/yellow leaf colour of the fronds in the total intensity treatment (tot PAR) could be explained with a lower chlorophyll need because of the high light intensity. Plants living in shade have more chlorophyll in general (pers. Mazzeo). The strain of *Spirodela intermedia* used in the experiment was probably growing in shade by the riparian trees in the Las Conchas River and thus better adapted to a lower light environment than during the experiments.

Number of individuals and biomass increase

As regards number of fronds and biomass increase of *S. intermedia*, the same tendencies and relation between treatments were observed for both parameters. Biomass increase and number of individuals were generally lower in the total light intensity (tot light), both in the control and in the treatment with no UV (PAR), compared to half the light intensity (1/2-light).

This indicates that a relatively higher intensity of the total radiation spectrum (tot light) had a more negative effect on biomass increase and number of fronds than half the correspondent radiation (1/2 light).

On the contrary, plants in the treatment with no-UVB had about the same number of fronds in half (1/2-light) and total light intensity (tot light) at least before the 10^{th} of February. This also holds for biomass increase, which was about the same in the two light intensities in the no-UVB treatment.

The positive effect on number of fronds (a few times significant, table 3 and 4) and biomass increase (significant in no-UVB compared to PAR treatment) indicates that UVA had a positive effect at the higher light intensity. The slightly lower biomass increase in the control compared to the no-UVB treatment (not significant) gives a slight indication of a negative effect of UVB at the higher light intensity (tot-light). There was also a significant effect on number of fronds at the beginning of the second experiment supporting that there was a negative effect of UVB but this result might also have been caused by an accident mentioned below.

This similar trend in the total light intensity with numbers of the control being higher than in the PAR-treatment is exactly the same during the first period and in the beginning of the second experimental period until the 22nd of January. One explanation to what happened to the growth pattern after this date might be that the number of fronds in the total light intensity was lower on the 25^{th} of January than earlier. This was due to a small incident when one of the experimental sets was pushed and some plants fell out. Some of the plants from the control treatment might have disappeared to the other treatments at this moment even if plants were immediately placed back. This might have changed the pattern between the control and the PAR-treatment making this relation different during the rest of the experimental period compared to the first period and compared to the results of the biomass increase.

Responses regulated by photoreceptors

Growth reductions might be explained by responses to different light quality by photoreceptors. To understand the mechanism behind the response to different light quality it is necessary to have knowledge of how absorption of light by photoreceptors functions.

A large number of biosynthetic processes are stimulated or regulated by UVA/blue light, including the synthesis of chlorophyll and carotenoids and photosynthetic behaviour (Tevini & Teramura, 1994). A well-known and important photoreceptor is phytochrome (P). Far-red light is in contrast to red light and wavelengths below transmitted through vegetation. Far-red light stimulation of phytochrome results in a rapid increase in the rate of internodal elongation, i.e. shade adaptation. Although phytochrome is usually thought to be red/far-red light regulated only, it has absorption peaks in the UVB waveband and in the longwave UVA region in addition to the red peak.

Model that might explain photoreceptor responses in the control treatment

A model describing responses of different photoreceptors has been suggested by Middelton and Teramura, 1994. This model is assuming that responses are additive and that plants respond independently to UVA and UVB radiation. The result in the study might be explained as the net effect of responses stimulated by the UVA/blue photoreceptor and those regulated by phytochrome. The general response pattern indicated either an antagonistic effect of UVA on UVB induced responses or a co-action between them.

In this study on *Spirodela intermedia* results like the net response in the variable number of fronds and biomass increase can be explained by using the above-mentioned model. Since the total light intensity had a lower biomass increase and number of fronds compared to half light intensity (1/2 light) this might be a result of an inhibitory response i.e. inhibiting phytochrome.

The higher biomass increase in the no-UVB treatment in the higher light intensity and equal biomass increase in both light intensities in this treatment can be explained with radiation dependant stimulation by the UVA/blue photoreceptor. The net effect of a stimulatory UVA/blue response and an inhibitory phytochrome response for the control treatment in the total light intensity (tot-light) compared to the half light intensity (1/2-light) is shown in Figure 12.

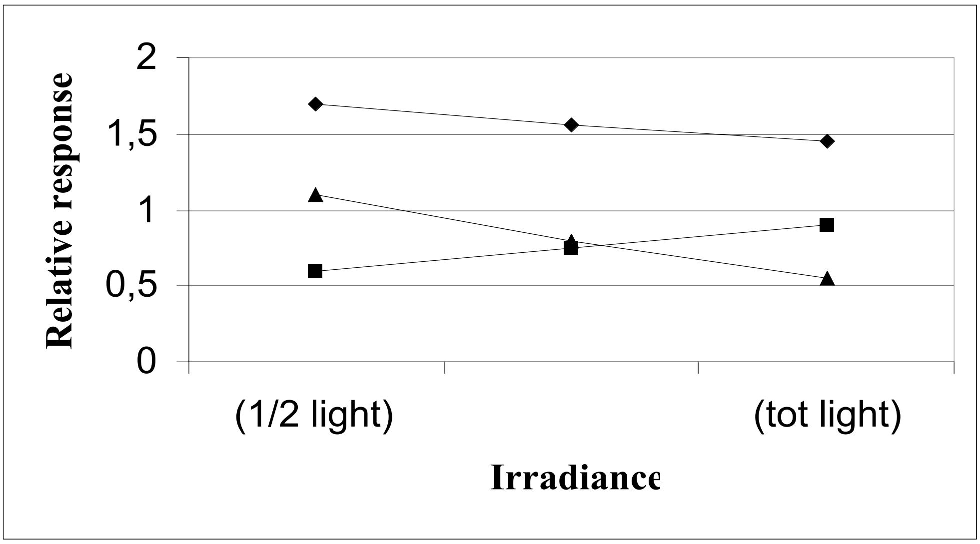

Figure 12. Model of net relative response (—◆— solid line) resulting from interaction between phytochrome (inhibitory) response (---▲--- dashed line) and responses by a UVA/blue photoreceptor (stimulatory) (····■······Dotted line) in the low light intensity (1/2 light) compared with high light intensity (tot light).

Middelton & Teramura 1994 suggested that the pigment growth pattern observed at lower UVB irradiance was a photosystem II response similar to “shade adaptation” and phytochrome was therefore involved in the UVB irradiation response.

It seemed that the characteristics of shade leaves were formed in all treatments in the lower light intensity (1/2 light). Moreover it is supposed that, in the treatment with high light intensities, the responses were not the result of UV-damage (in the usual sense) but indicated a switch from the formation of leaves with characteristics of shade conditions to those more characteristic of sunny environments. Characteristics of sun leaves were smaller, with less leaf, stem, root and therefore total biomass.

These results are consistent with this study on *Spirodela intermedia*. The PAR treatment (significant) and the control (not significant) in the total light intensity (tot light) had a lower biomass increase compared to the biomass increase in the no UVB treatment. In this study on *S. intermedia* it can have been high levels of white light that created the inhibitory response similar to characteristics of sunny conditions in the PAR treatment in the total light intensity. It can have been high levels of white light and UVB radiation that created the inhibitory response in the control.

A comparison with *Ricciocarpus natans*

The experiment with *Ricciocarpus* was carried out during the same period as *S. intermedia* (Alm, 2003). It is interesting to note that in the beginning of the experimental period of *Ricciocarpus* (6 jan- 2 feb), the no-UVB treatment twice had a significantly higher number of thalli than the other treatments. This indicates that the UVB radiation was so strong that growth of the control was negatively affected.

In the latter part of the experimental period of *S. intermedia* there was a slight tendency for number of fronds of the control to be higher. But in *Ricciocarpus* this response of a higher biomass increase in the control is much more pronounced. This indicates that the overall effect of the total radiation spectrum was beneficial with no negative impact of UVB on number of fronds.

Since this effect was detected at the total light intensity (tot-light) in *Ricciocarpus* there seems to be a difference in adaptation to light conditions between the two species. *Ricciocarpus* seems to be more tolerant to the total light spectrum compared to *S. intermedia*. Species composition in the plant community might be changed due to different abilities to survive at higher radiation of UVB.

Conclusion

The most important results of this study of *S. intermedia* are the following:

- UVB-radiation at the lower irradiance can be considered neutral since it had no impact on number of fronds and biomass increase.

- UVB-radiation of natural (total) intensity occasionally has a significant negative effect on number of fronds? This result is not reliable since it might be an effect of an artefact. Therefore results do not provide evidence of a negative response of UVB even at a higher light intensity.

- There was almost equal biomass increase in all treatments in the half-light intensity and the level was higher than in the total intensity. This indicates that there is a threshold level for an effect of UV and visible radiation.

- The higher number of fronds and biomass increase in the PAR treatment compared with other treatments in the natural (total) light intensity indicates a beneficial effect of UVA.

- The fluorescence measurements did not support photosyntetic damage by UVB. Instead it might be photoreceptors that regulated the result.

Hypothesis confirmed?
The initial hypothesis tested in this study was if natural variations in the levels of UVB radiation could have a negative effect on number of individuals, biomass increase and fluorescence of *S. intermedia*. The hypothesis can not be confirmed.

Acknowledgements

This study was financed by SIDA (Swedish International Co-operation Development Agency) as a Minor Field Study. For support and administrative help I gratefully acknowledge the Committee of Tropical Ecology at Uppsala University. I would like to express my gratitude to my supervisors Nestor Mazzeo, Universidad de la Republica, Facultad de Ciencias, Seccion Limnologia, Montevideo and Kerstin Wallström, Uppsala University, Department of Plant Ecology.

For co-operative work and hospitality during our stay I especially remember Daniel Conde, Javier Gorga, Mercedes Paradiso and many others of the staff at the Seccion Limnologia, Montevideo. I also thank my fellow student Gunilla Alm for co-operative work and company during our stay in Uruguay.

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