The effects of UVB radiation on charophycean algae and bryophytes

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Effects of UVB radiation on a charophycean alga, *Chara aspera*

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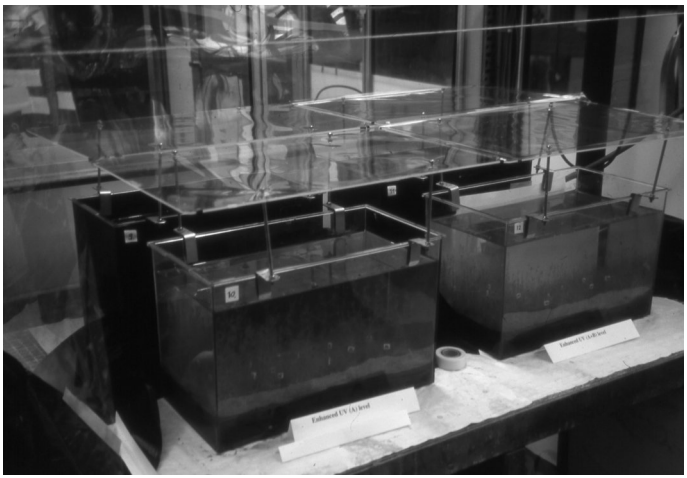

Abstract

The charophycean algal species *Chara aspera* was exposed for 73 days to three levels of UVB radiation (weighted according to Caldwell's generalized plant action spectrum): 1.9 kJ m^{-2} day^{-1} ('No UVB'), 6.4 kJ m^{-2} day^{-1} ('Ambient') and 10.5 kJ m^{-2} day^{-1} ('Enhanced UVB'), the latter level simulating 30% ozone reduction in The Netherlands.

Charophycean algae are mainly freshwater organisms and are thought to be the algae most closely related to higher land plants. Therefore we expected that responses of charophycean algae to UVB radiation might be more related to those observed in the higher land plants than those of other 'lower' algal groups.

Under elevated UVB radiation algal length was reduced. There was no induction of UV absorbing compounds under enhanced UVB. This might relate to a sensitive response to UVB radiation. The charophycean algae show similar adaptations to UVB radiation as terrestrial plants, while not having UV screens as occur in many angiosperms. Vegetative reproduction (bulbils) increased in the presence of UVB radiation, while generative reproduction (antheridia and oogonia) decreased.

Introduction

The stratospheric ozone layer has declined since the early 1970s, due to the anthropogenic emission of halogen-containing compounds, such as chlorofluorocarbons (CFCs). This decline in ozone thickness has resulted in an increase of UVB radiation (280-315 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, but also at temperate latitudes the ozone layer has declined (Herman *et al.* 1996, Madronich *et al.* 1998, SORG 1999).

Since the discovery of the ozone hole by Farman *et al.* (1985), much attention has been paid to the effects of UVB radiation on terrestrial and marine organisms and ecosystems. UVB radiation can cause damage to DNA (Karentz *et al.* 1991a, Buma *et al.* 1995, Taylor *et al.* 1996), membranes (Kramer *et al.* 1991) and damage affecting photosynthesis (see Sullivan and Rozema 1999). Some organisms are able to protect themselves or reduce the UVB damage by repairing the damage, e.g. by 'light' and 'dark' repair of DNA damage (Sancar and Sancar 1988) or by avoiding the damage. Avoidance can be realised via changes in morphology (Tevini and Teramura 1989) and by changes in chemical composition filtering out deleterious UVB, the so-called 'UVB screens' (see Meijkamp *et al.* 1999).

Most research has focussed on effects of UVB radiation on marine and land organisms and ecosystems. However, less attention has been paid to fresh water systems (Williamson 1995). The charophytes, from an evolutionary point of view, constitute an important group of the green algae. These algae occur mainly in fresh water systems. The evolution of the land plants is believed to have taken place from algae, via mosses to higher land plants (Bhattacharya and Medlin 1998). Rozema *et al.* (1997) assumed that it might be of evolutionary importance that UVB absorbing phenolic compounds, acting among other functions as UVB screens, increase in complexity during evolution. Among the green algae, charophytes appear to be most closely related to the higher land plants (Devereux *et al.* 1990, Stafford 1991, Graham 1993). Therefore we expect that effects of or adaptations to UVB radiation of charophycean algae might be in line with their evolutionary position: more close to higher land plants than to other algal groups. As far as we know, no literature is available on the effects of UVB radiation on these algae.

Here we report results of a greenhouse experiment on the effects of UVB radiation on charophycean algae. This study focuses on morphological and

chemical changes and reproductive characteristics in the charophyte, *Chara aspera*, in response to UVB radiation.

Methods

Experimental set-up

The effects of UVB radiation on *Chara aspera* Deth. Ex Wild. were investigated in a greenhouse experiment. Sediment containing spores and bulbils was collected from Lake Veluwe, The Netherlands, on 29 April 1998 and stored dark at 4°C until the start of the experiment. Natural water of this Lake Veluwe was collected on 17 June 1998 and stored dark at 4°C as well. Lake Veluwe is a de-eutrophicated shallow lake with a dense charophyte vegetation, where *C. aspera* is the dominant species. For a detailed description of the Lake Veluwe see Van den Berg (1999). The experiment was conducted from 3 July till 14 September 1998.

Aquaria (length 39 cm, width 21 cm, and height 25 cm) were filled with a 4 cm thick layer of sediment. Natural lake water was added to a height of 17 cm above the sediment. Adding demineralised water compensated for evaporation during the experiment. Air temperature in the greenhouse was around 24°C (day) and 16°C (night). The aquaria were wrapped in black plastic to prevent algal growth on the sides of the aquaria. In addition to natural light,

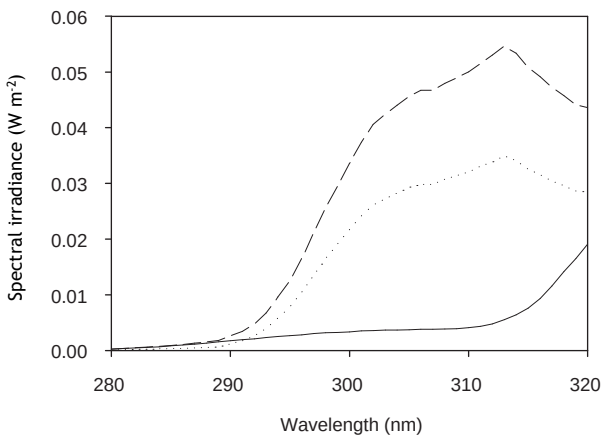

Figure 2.1: The average above water spectrum (280-320 nm) of the treatments; solid line is the 'No UVB' treatment; dotted line is the 'Ambient' treatment and dashed line is the 'Enhanced UVB' treatment.

170 mol m^{-2} s^{-1} PAR was supplied by Philips HPI/T lamps (400 W) to the middle of each aquarium. The light:dark cycle was 14:10 hours.

In the middle of the light period the charophycean algae were exposed to UV radiation for 6 hours. Philips TL 40 UV tubes provided UV radiation. Three UV treatments were applied: No UVB, Ambient UVB and Enhanced UVB (Figure 2.1). The 'No UVB' treatment had a biologically effective dose (UV_{be}) of 1.9 (±0.7) kJ m^{-2} day^{-1}, calculated using the generalized plant action spectrum (Caldwell 1971) normalized at 300 nm. In this treatment, UVB radiated by the UV tubes was filtered out with 0.13 mm thick polyester foil (mylar, Dupont Industries, USA), which absorbs radiation < 313 nm. The 'Ambient' treatment had a UV_{be} of 6.4 (±0.5) kJ m^{-2} day^{-1} reflecting ambient plus up to 4-7% ozone reduction levels around the end of June in The Netherlands (52°N), calculated using the model of Green (1980) and assuming cloudless sky conditions. The 'Enhanced UVB' treatment, which simulated 30% ozone reduction, corresponded to a UV_{be} of 10.5 (±1.2) kJ m^{-2} day^{-1}. In both the 'Ambient' and the 'Enhanced UVB' treatment the UV tubes were covered with 0.1 mm thick cellulose acetate foil (Tamboer and Co. Chemie B.V., Haarlem, The Netherlands), which absorbs radiation with wavelengths < 290 nm. Differences between the treatments were obtained by adjusting the height of the UVB tubes above the aquaria. Both the mylar and the cellulose acetate foils were changed weekly to avoid changes in the radiation regimes due to photodegradation of the foils. The UVA irradiance emitted by the Philips HPI/T lamps and the Philips TL 40 UV tubes and the natural light was on average 1.64 W m^{-2} for the 'No UVB' treatment and 1.93 and 1.91 W m^{-2} for the 'Ambient UVB' and 'Enhanced UVB' treatment, respectively. Four aquaria were used in every treatment.

The spectral irradiance of the UVB lamps was measured with a double-monochromator spectroradiometer (Optronics Model OL 752, Orlando, FL, USA). With a portable UV-X radiometer with a UV-X 31 sensor (San Gabriel, CA, USA), prepared for underwater measurements, UV measurements in the aquaria at various depths were performed after the vegetation was collected and the sediment stabilised (Figure 2.2).

Harvest and morphological measurements

After four days charophycean algae started emerging above the sediment in all aquaria. Electric conductivity (EC), a measure for total dissolved salts in the water, and pH were measured weekly. After 73 days the charophycean algae in the aquaria were harvested from 187 cm^2 of the soil surface for measurements of

morphological characteristics. The other part of the aquaria was harvested and used for chemical analysis. Two species occurred in the aquaria: *Chara aspera* and *Chara contraria* A. Braun ex Kützing. About 94% belonged to the species *C. aspera* and 6% to the species *C. contraria*. The two species were separated to measure characteristics of individuals of the species *C. aspera*. Length was only measured for charophytes longer than 25 mm. Charophytes shorter than 25 mm were counted. Furthermore, the number of branch whorls was measured. Dry weight of the harvested algal material was determined after drying for 4 days at 70°C. The following reproductive characteristics were measured: generative reproduction by the presence of branch whorls containing oospores and/or spores, and vegetative reproduction by the number of bulbils, which are vegetative starch-rich organs (Moore 1986).

Chemical analysis of photosynthetic and UV absorbing pigments

Chlorophyll content was measured according to the method of Arnon (1949). For the extractions 100 mg of fresh *C. aspera* material was put in test tubes. After adding 5 ml of 80% acetone (Fluka, Germany), test tubes were sonicated for 15 minutes to break the cell walls. After 3 hours of extraction at 4°C in dark, test tubes were centrifuged for 10 minutes at 2500 rev. min^{-1}. The absorbance of the solution was then measured with a spectrophotometer (Perkin-Elmer Lambda UV-Vis) at 645, 652 and 663 nm.

For the determination of methanol soluble UVB absorbing compounds 10 mg of lyophilised *C. aspera* was added to test tubes containing 6 ml extractant. The extractant was composed of 100% CH_3OH (J.T. Baker, Deventer, The Netherlands), demineralised water and 37% HCl (Riedel-de Haen, Germany) in the ratio 79:20:1 (v:v:v). After closing the test tube to prevent evaporation, test tubes were sonicated for 10 minutes after which they were placed in a water bath at 90°C for 1 hour. Cooled test tubes were then centrifuged for 10 minutes at 2500 rev. min^{-1}. The absorbance of the resulting solution was measured at 280, 300, 320, 340, 360, 380 and 400 nm (Perkin-Elmer Lambda UV-Vis spectrophotometer) (Meijkamp *et al.* 2001, Visser 1997).

Data analysis

For statistical analysis of treatment effects on the length of the charophycean algae, only plants longer than 25 mm were used. A Kolmogorov-Smirnov test was performed to test for normality. The data were logarithmically transformed. Although the variances of the data were not homogeneous (tested by a Bartlett Box), the most suitable statistical test was a nested ANOVA to analyse these

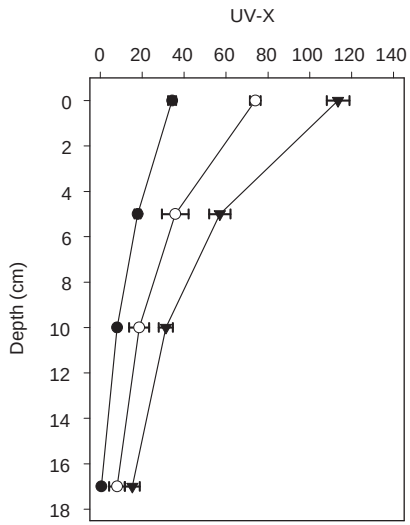

Figure 2.2: Below water UV conditions of the different treatments. Attenuation of UV radiation within the water column, measured with a broad band sensor; line with closed circles represents the 'No UVB' treatment; the line with open circles represents the 'Ambient' treatment and line with closed triangles represents the 'Enhanced UVB' treatment; errors bars are shown (n=4).

data. The different aquaria were nested within the UVB treatments. Length measurements were taken as replicates within an aquarium.

For data on dry weight, charophycean algal density, both chlorophyll a and b concentrations and number of bulbils, a one-way ANOVA was used, after testing for normality and homogeneity of variance of the data with the tests mentioned above. The data on generative reproduction were first transformed using the arcsinus square root transformation, after which a one-way ANOVA was done. For the average number of branch whorls a Kruskal-Wallis test was performed. Significance level in all statistical tests was 0.05. All statistical tests were performed using the program SPSS (SPSS Inc., Chicago).

Results

Experimental conditions

There was no significant difference in pH between the applied treatments. At the start of the experiment the average pH in the aquaria was 8.5 (± 0.1). During the course of the experiment the average pH increased to 9.4 (± 0.1) at the day of the harvest. The electric conductivity (EC) decreased from 877 ±10 to 770 ±16 μS cm^{-1}. There was also no significant difference in EC between the aquaria in all treatments. The radiation profile measured with the UV-X radiometer (Figure 2.2) shows that UV is attenuated by the water column.

Table 2.1: ANOVA table of the statistics of length of *C. aspera*

	Sum of Squares	df	Mean Square	F	Sig.
Between UVB treatment	2.140	2	1.070	7.810	=0.01
Within UVB treatment	1.237	9	0.137	4.496	< 0.001
Error	59.33	1941	0.030		

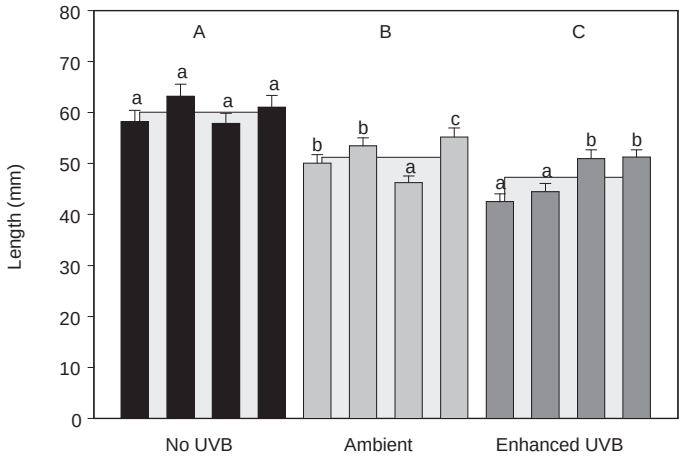

Figure 2.3: Average length of *C. aspera* per treatment (wide bars) and the average length (+ SE) of *C. aspera* per aquarium within a treatment (small bars; n varies from 91 to 232). Different capital letters indicate significant difference between treatments; different lowercase letters indicate significant difference within a treatment.

Table 2.2: Average (± SE) plant density, average number of branch whorls and average biomass per treatment (n=4).

	No UVB	Ambient	Enhanced UVB
Density (nr of plants cm^{-2})	3.8 ± 0.5	4.3 ± 0.2	3.6 ± 0.1
Average number of branch whorls	3.3 ± 0.3	3.4 ± 0.2	3.1 ± 0.2
Biomass (g DW cm^{-2})	0.80 ± 0.06	0.75 ± 0.02	0.83 ± 0.04

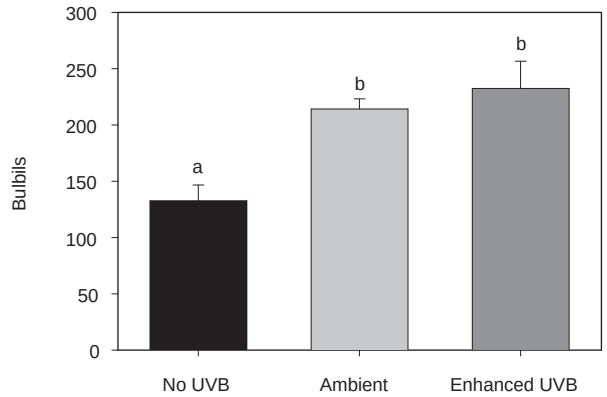

Figure 2.4: Average number of bulbils collected per aquarium per treatment (n=3). Standard errors are shown; significant differences of number of bulbils between treatments are indicated with different lower-case letters.

Morphology

There was a significant effect of UVB radiation on length of *C. aspera* (P=0.01) at the end of the experiment. The 'No UVB' treatment resulted in the largest charophycean algae, followed by the ambient treatment. Under enhanced UVB the charophycean algae were the shortest. Besides this effect between the UV treatments, there was also a significant difference between aquaria within some of the treatments (P<0.001; Figure 2.3, Table 2.1). This might be related to differences in irradiance, as the aquaria could not be rotated in order not to disturb the growth of the charophycean algae. Although there was some variation within the treatments, the effect of UVB radiation is clear.

Biomass of the charophycean algae was not affected by the different UV treatments, nor was there a significant difference in charophycean algal density. There was also no difference in average number of branches per plant (Table 2.2).

Table 2.3: The average (±SE) chlorophyll concentration (mg l^{-1}) in the charophyte samples per treatment (n=4).

	Chlorophyll a	Chlorophyll b
No UVB	6.16 ± 0.10	2.38 ± 0.08
Ambient	6.01 ± 0.77	2.41 ± 0.31
Enhanced UVB	6.38 ± 0.82	2.57 ± 0.32

Reproduction

The ambient and enhanced UVB treatment had more bulbils (vegetative reproductive structures) than the treatment without UVB ($P<0.01$; Figure 2.4). However, in the no UVB treatment the number of branches containing generative reproductive organs was significantly higher than in the ambient and enhanced UVB treatments ($P<0.01$; Figure 2.5).

Photosynthetic and UVB absorbing compounds

For the chemical analysis the charophyte samples comprised both *C. aspera* and *C. contraria*. There was no effect of UVB on the chlorophyll a and chlorophyll b concentrations in the samples (Table 2.3), neither was there a significant difference in the absorption of the methanol soluble UVB absorbing compounds between the treatments at the wavelengths measured (Figure 2.6).

Discussion

Experimental conditions

The pH and electric conductivity did not differ between aquaria during the experiment. Therefore, there were no differences in growth conditions between

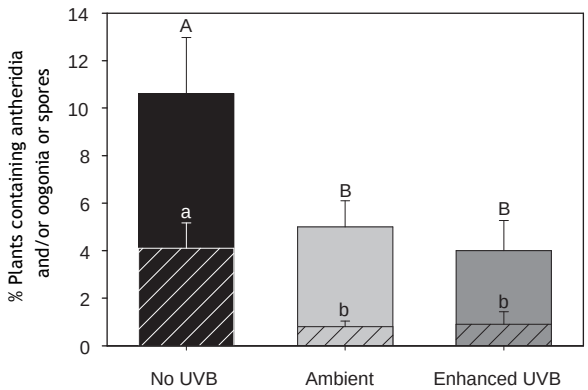

Figure 2.5: Percentage (+SE; n=4) of *C. aspera* plants that contain generative reproductive structures on the branch whorls as antheridia and/or oogonia (solid bars) or spores (dashed bars). Significant differences of percentages of plants with antheridia and oogonia between treatments are indicated with different capitals; different lower-case letters indicate significant difference in percentages of plants containing spores between treatments.

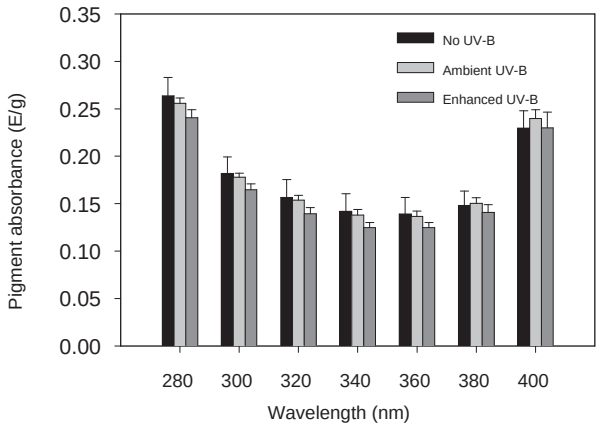

Figure 2.6: Absorbance of methanol soluble UVB absorbing pigments in *C. aspera*. Bars represent average pigment absorbance (+SE) per treatment at different wavelengths (n=4).

the UV treatments. *C. aspera* grows under natural conditions at a pH of 6-9 (Nat *et al.* 1994). In the aquaria, the pH increased from 8.5 (±0.1) to 9.4 (±0.1), which slightly exceeds the range of natural occurrence. However, under cultivated conditions a higher pH than under natural conditions may be observed (Van den Berg 1999, J. Simons personal comment).

Morphology

Charophytes, like *C. aspera*, are composed of multicellular nodes, from which branch whorls are formed, and single celled internodes. These internodes can be surrounded by modified laterals (e.g. in *C. aspera*), forming a one cell layer thick cortex (Moore 1986). There was a negative effect of UVB radiation on the length of *C. aspera* plants. As there was no effect of UVB on plant density and on total biomass or on the average number of branch whorls per algae, it seems that UVB negatively affects cell elongation of the internodes. This is in contrast with the way in which growth reduction under the influence of UVB occurs in unicellular marine algae. In these organisms cells become larger, because cell division is inhibited by UVB radiation (Karentz 1994, Buma *et al.* 1995). However, in higher land plants internode length (Meijkamp *et al.* 2001) or shoot elongation can be reduced as well, e.g. by changes in phytohormones (Ros and Tevini 1995). Thus changes in morphology can result in avoidance of UVB radiation (Tevini and Teramura 1989).

Reproduction

C. aspera propagates both by the formation of diploid oospores and by forming vegetative, starch-rich organs, the bulbils (Moore 1986, Krause 1997). There seemed to be a trade-off in reproduction strategy. Under enhanced UVB radiation *C. aspera* formed more vegetative reproduction structures than in the absence of UVB, while the opposite was found for the generative reproduction. Bulbils can serve as structures for propagation during the growing season, but also play an important role in wintering (Krause 1997). More bulbils might perhaps result in a denser vegetation in the next growing season, where competition with other algae and macrophytes might be influenced. On the other hand less generative reproductive structures, both antheridia, oogonia and spores, were formed under enhanced UVB radiation. Spores are important for dispersion and recolonisation (Krause 1997). Therefore, enhanced UVB might negatively influence dispersion. This trade-off between generative and vegetative reproduction in *C. aspera* under enhanced UVB has not been observed in higher terrestrial plants. It is generally known that in higher land plants the investment in generative reproductive structures might increase under stress conditions (Grime 1989).

UV absorbing compounds

UV absorbing compounds are important in screening UVB. By lowering UVB levels within the plant tissues damage to DNA, membranes, proteins and photosynthetic tissue can be prevented or reduced (Meijkamp *et al.* 1999). In *C. aspera* no changes were observed in methanol soluble UV absorbing compounds under enhanced UVB. This makes these algae potentially sensitive to UVB radiation. The absence of increased UVB absorbing compounds under elevated UVB is remarkable, since both in cyanobacteria, algae and in higher land plants increased absorbance has been reported under influence of UVB (Büdel *et al.* 1994, Meijkamp *et al.* 1999). In algae, both in marine and fresh water, induction of mycosporine like amino acids (MAAs) has been shown (Garcia-Pichel and Castenholz 1991, Karentz *et al.* 1991, Xiong *et al.* 1997), whereas in higher land plants, e.g., flavonoid concentrations may be enhanced under elevated UVB (Meijkamp *et al.* 1999). Preliminary measurements on the presence of flavonoids in *C. aspera* in our experiment did not show these types of secondary metabolites (data not shown). Markham and Porter (1969) reported the presence of flavonoids in charophytes. However, this finding has not been reproduced since then (Harborne 1986, Wegner-Hambloch 1983). Thus, the presence of flavonoids in charophytes is questionable (cf. Stafford 1990).

Ecological effects of changes in penetration of UV-radiation

Light is attenuated in the water column (Kirk 1994). The UVB dose received by the charophycean algae therefore was lower than applied at the water surface (Figure 2.1, Figure 2.2). Under enhanced UVB charophycean algae were shorter. As UVB radiation is absorbed much more by water than in air, in aquatic ecosystems reduced algal length will result in a lower UVB dose at plant level. Thus, the shorter charophytes received a lower UVB dose. This might be advantageous to avoid UVB radiation, but also photosynthetically active radiation (PAR) decreases with depth and the growth rate of charophytes may be reduced as well.

Penetration of UV into the water depends on local water properties and may be influenced by e.g. the amount of dissolved humic substances and the amount of particles present in the water (Kirk 1994). De Lange (1999) measured UVB radiation in Dutch lakes. Measurements in the clearest lake showed that the depth at which 1% of the UVB radiation was present was 0.5 m, while the euphotic depth reached to 5.5 m. In Lake Veluwe *C. aspera* occurs at depths of 30-80 cm (Van den Berg 1999). Above charophyte meadows in Lake Veluwe the water transparency for PAR is high (a light attenuation coefficient , K_d (m^{-1}), < 1). However in unvegetated sites the K_d is > 4, which is due to high detritus, inorganic suspended solids and chlorophyll a (Van den Berg 1999). Therefore *C. aspera* might be exposed to solar UVB under natural conditions as well, depending on the water transparency.

Besides receiving less PAR, another disadvantage for short algae is lower nutrient uptake. Charophycean algae take up nutrients both via the rhizoids and via the shoots (Krause 1997). However, as charophytes were smaller under enhanced UVB, the total surface of the charophycean algae was smaller as well and therefore nutrient uptake can be limited. This might reduce the competition capacity to algae and macrophytes that do not suffer growth depression.

In general, it can be concluded that charophycean algae show some adaptations to enhanced UVB similar to terrestrial plants. However, charophycean algae are potentially sensitive to UVB radiation, as there seems to be no induction of UVB absorbing compounds under influence of enhanced UVB levels. Avoiding UVB by reducing length may be effective (Tevini and Teramura 1989), but reduced length also affects the competitive ability of the species, by reduced light interception and perhaps lower nutrient uptake. There was a change in reproduction strategy, which might affect dispersion of the species.

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References

Arnon DI. 1949. Copper enzymes in isolated chloroplasts. Polyphenyloxidase in *Beta vulgaris*. *Plant Physiology* 24:1-15.

Bhattacharya D, Medlin L. 1998. Algal phylogeny and the origin of land plants. *Plant Physiology* 116: 9-15.

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

Buma AGJ, van Hannen EJ, Roza L, Veldhuis MJW, Gieskes WWC. 1995. Monitoring ultraviolet-B-induced DNA damage in individual diatom cells by immunofluorescent thymine dimer detection. *Journal of Phycology* 31: 314-321.

Caldwell MM. 1971. Solar UV radiation and the growth and development of higher plants. In: Giese AC (ed.) *Photophysiology*, Vol.6, pp 131-268. Academic Press, New York.

De Lange HJ. 1999. *Effects of ultraviolet-B radiation on phytoplankton-zooplankton interactions*. Doctoral thesis. Wageningen Agricultural University, Wageningen, The Netherlands.

Devereux R, Loeblich III AR, Fox GE. 1990. Higher plant origins and the phylogeny of green algae. *Journal of Molecular Evolution* 31: 18-24.

Farman JC, Gardiner BG, Shanklin JD. 1985. Large losses of total ozone in Antarctica reveal seasonal ClO_x/NO_x interaction. *Nature* 315: 207-210.

Garcia-Pichel F, Castenholz R. 1991. Characterization and biological implications of scytonemin a cyanobacterial sheath pigment. *Journal of Phycology* 27: 395-409.

Graham LE. 1993. *Origin of land plants*. John Wiley & Sons, Inc. New York.

Green AES, Cross KR, Smith LA. 1980. Improved analytic characterization of ultraviolet skylight. *Photochemistry and Photobiology* 31: 59-65

Grime JP. 1989. Whole-plant responses to stress in natural and agricultural systems. In: Jones HG, Flowers TJ & Jones MB (eds.) *Plants under stress*, pp.31-46. Cambridge University Press.

Herman JR, Bhartia PK, Ziemke J, Ahmad Z, Larko D. 1996. UV-B increases (1979-1992) from decreases in total ozone. *Geophysical Research Letters* 23: 2117-2120.

Karentz D. 1994. Prevention of ultraviolet radiation damage in Antarctica marine invertebrates. In: Biggs RH and Joyner MEB (eds.) *Stratosperic Ozone depletion/UV-B radiation in the biosphere*, vol. I 118. NATO ASI series. Springer Verlag, Berlin.

Karentz D, Cleaver JE, Mitchell DL. 1991a. Cell survival characteristics and molecular responses of phytoplankton to ultraviolet-B radiation. *Journal of Phycology* 27: 326-341.

Karentz D, Mc Euen FS, Land MC, Dunlap WC. 1991b. Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. *Marine Biology* 108: 157-166.

Kirk JTO. 1994. *Light & photosynthesis in aquatic ecosystems*. Cambridge University Press, Cambridge, U.K.

Kramer GF, Norman HA, Krizek DT, Mirecki RM. 1991. Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cumcumber. *Phytochemistry* 30: 2101-2108.

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, McKenzie RL, Björn LO, Calwell MM. 1998. Changes in biologically active ultraviolet radiation reaching the earth's surface. *Journal of Photochemistry and Photobiology B: Biology 46*: 5-19.

Markham KR, Porter LJ. 1969. Flavonoids in the green algae (chlorophyta). *Phytochemistry* 8: 1777-1781.

Markham KR. 1988. Distribution of flavonoids in the lower plants and its evolutionary significance. In: Harborne JB (ed.) *The flavonoids*. Chapman and Hall, London.

Meijkamp B, Aerts R, Van de Staaij J, Tosserams M, Ernst W, Rozema J. 1999. Effects of UV-B on secondary metabolites in plants. In: Rozema J (ed.) *Stratopheric ozone depletion, the effects of enhanced UV-B radiation on terrestrial ecosystems*, pp.71-99. Backhuys Publishers, Leiden.

Meijkamp B, Doodeman G, Rozema J. 2001. The response of *Vicia faba* to enhanced UV-B radiation under low and near ambient PAR levels. *Plant Ecology* 154: 135-146.

Moore JA. 1986. *Charophytes of Great Britain and Ireland*. Botanical Society of the British Isles, London.

Nat E, Simons J, De la Haye MAA, Coops H. 1994. *Verspreiding van kranswieren in Nederland*. RIZA report 94.148x, RIZA, Lelystad.

Ros J, Tevini M. 1995. Interaction of UV-radiation and IAA during growth of seedlings and hypocotyls segments of sunflower. *Journal of Plant Physiology* 146: 295-302.

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

Sancar A, Sancar GB. 1988. DNA repair enzymes. *Annual Review of Biochemistry* 57: 29-67.

Sokal RR, Rohlf FJ. 1998. *Biometry*, 3rd edition. W. H. Freeman and Company, New York.

SORG 1999. *Stratospheric ozone 1999*. Department of Environment, Transport and the Regions, U.K.

Stafford HE. 1991. Flavonoid evolution: an enzymatic approach. *Plant Physiology* 96: 680-685.

Sullivan J, Rozema J. 1999. UV-B effects on terrestrial plant growth and photosynthesis. In: Rozema J (ed.) *Stratopheric ozone depletion, the effects of enhanced UV-B radiation on terrestrial ecosystem*, pp.39-57. Backhuys publishers, Leiden.

Taylor RM, Nikaido O, Jordan BR, Rosamond J, Bray CM, Tobin AK. 1996. Ultraviolet-B-induced DNA lesions and their removal in wheat (*Triticum aestivum* L.) leaves. *Plant, Cell & Environment* 19: 171-181.

Tevini M, Teramura AH. 1989. UV-B effects on terrestrial plants. *Photochemistry and Photobiology* 50: 479-487.

Van den Berg MS. 1999. *Charophyte colonization in shallow lakes: processes, ecological effects and implications for lake management*. Doctoral thesis. VU University Amsterdam, The Netherlands.

Visser AJ. 1997. *Growth and physiology of Triticum aestivum and Vicia faba in response to increasing atmospheric CO_2 concentrations*. Doctoral thesis. VU University Amsterdam, The Netherlands.

Wegner-Hambloch S. 1983. *Polyphenole aus der Phaeophycee Cystoseira granulata Agardh., sowie untersuchungen uber das Flavonoidvorkommen in Chara contraria Kutz und Nitella flexilis Agardh.* Doctoral Thesis. Rheinischen Friedrich-Wilhelms-Universitat, Bonn.

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

Xiong F, Komenda J, Kopecky J, Nedbal L. 1997. Strategies of ultraviolet-B protection in microscopic algae. *Physiologia Plantarum* 100: 378-388.