Effects of enhanced ultraviolet radiation on six aquatic bryophytes

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Abstract – Six aquatic bryophytes, three liverworts (*Jungermannia exsertifolia* subsp. *cordifolia*, *Marsupella sphacelata* and *Scapania undulata*) and three mosses (*Brachythecium rivulare*, *Bryum pseudotriquetrum* and *Racomitrium aciculare*) were cultured in the laboratory under artificially enhanced ultraviolet-B (UV-B) radiation to evaluate their physiological responses to a potential ozone depletion. The daily-integrated biologically-effective UV-B irradiance applied was 10.3 kJ m^{-2} (as estimated using the generalized plant damage action spectrum), mimicking a 20% ozone depletion at the latitude of the sampling site. Photosynthetic pigment composition, some variables of chlorophyll fluorescence, sclerophylly, the amount of methanol-extractable UV-absorbing compounds, and growth, were measured after 20 days of culture. The physiology of bryophytes was overall more influenced by the culture conditions than by the UV treatment, and the responses to both factors depended on the species and the variable considered. The culture conditions affected negatively some basic physiological variables (*e.g.* photosynthetic pigments and photosynthetic performance), especially in some species, but these adverse effects did not impede growth (except in *Marsupella sphacelata*). Enhanced UV-B affected negatively only some pigment variables, but not the photosynthetic performance (as derived from chlorophyll fluorescence variables) nor growth (except in *Jungermannia exsertifolia* subsp. *cordifolia*). The increase in UV-protective compounds under enhanced UV-B was rare. It may be concluded that the species studied were UV-B tolerant under the conditions considered in the present work, probably due to the fact that the samples were naturally well acclimated to high UV-B levels because they were collected at high altitudes (1850-2000 m) and near the summer solstice. Thus, they would not need additional protection against the UV-B levels used in the laboratory culture. The responses to enhanced UV-B may not depend only on the species and the environmental conditions, as it had been pointed out before, but also on the collection site and the collection date of the samples.

UV radiation / Ozone depletion / Aquatic bryophytes / Mosses / Liverworts

INTRODUCTION

Due to the anthropogenic ozone depletion, the amount of solar ultraviolet-B (UV-B: 280-315 nm) radiation penetrating through the Earth's atmosphere has increased. This has occurred not only over the Antarctic continent, but also in Arctic and mid-latitudes. At northern mid-latitudes, the last

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studies have reported ozone losses around 6% in 1997-2000 relative to 1980 levels, which might result in a UV-B increase of up to 12% (McKenzie *et al.*, 2003). Although the future pathway for UV radiation is notably uncertain, surface UV irradiance at mid-latitudes is predicted by models to peak between about 2000 and 2010 and is expected to return to the pre-1980 levels between 2040 and 2070 (McKenzie *et al.*, 2007). In addition, ozone mini-holes may cause up to 33% decreases in ozone and consequent UV increases between 43 and 75% (Antón *et al.*, 2007). Thus, the study of the ecological effects of UV radiation will be an important issue in the coming several decades.

In photosynthetic organisms, enhanced UV-B radiation may cause alterations in DNA, photosynthesis, growth and development, together with an increase in UV-screening compounds (Jansen *et al.*, 1998). The research regarding these effects has focused on terrestrial crop plants and marine phytoplankton and macroalgae (Day & Neale, 2002; Kakani *et al.*, 2003), while bryophytes have received less attention (Boelen *et al.*, 2006; Martínez-Abaigar *et al.*, 2006; Häder *et al.*, 2007). The most studied bryophytes have been terrestrial and semiaquatic species from Antarctic habitats and circumpolar heathlands and peatlands, but other ecosystems have received little attention. Bryophytes are frequently the most important primary producers in mountain streams (Stream Bryophyte Group, 1999) and should be considered when estimating the impact of UV radiation on these ecosystems. In addition, bryophytes in mountain streams are particularly exposed to the risks derived from high UV levels, because UV increases with altitude, they live at low depths where UV can easily reach them, and cold temperatures typical of these ecosystems may limit the development of protecting mechanisms, such as DNA repairing systems, antioxidants, and UV-absorbing compounds. On the other hand, structural protections against UV (*e.g.* thick cuticles, epidermis and hairs) are lacking in bryophytes.

The effects of UV radiation on bryophytes have been studied using reduced, enhanced, and ambient UV levels, respectively with the help of filters, lamps, or none of these devices (Martínez-Abaigar *et al.*, 2006). The studies using enhanced UV levels are very important in the context of UV research because they are the most appropriate tools for addressing the ozone depletion issue (Rousseaux *et al.*, 2004). A considerable number of studies on UV and bryophytes (more than 30) have used this approach (Table 1). The bryophyte responses have been assessed measuring mainly growth (both in length and dry mass), photosynthesis rates, chlorophyll fluorescence variables, photosynthetic pigment composition, DNA damage, and the accumulation of UV-protective compounds. The results obtained are still conflicting, since enhanced UV radiation has been found to either stimulate, depress or have no effect on the bryophyte performance, and thus further study is required to better characterize bryophyte responses. In this respect, currently there exist data on 30 species (25 mosses and only 5 liverworts: Table 1), but most of the results have been obtained in relatively few species. They are mainly mosses from Arctic peatlands and heathlands, such as *Hylocomium splendens* and *Sphagnum* and *Polytrichum* species, together with a liverwort and a moss from mountain streams (*Jungermannia exsertifolia* subsp. *cordifolia* and *Fontinalis antipyretica*). Testing the responses of a higher number of species would be useful to better understand the effects of enhanced UV radiation on bryophytes.

The aim of this study was to obtain, under laboratory conditions, new data on the tolerance to enhanced UV radiation of aquatic bryophytes from mountain streams. To achieve this objective, we used 6 species (3 liverworts and 3 mosses), among which we included *Jungermannia exsertifolia* subsp. *cordifolia*

Table 1. Bryophyte species used in experiments on the effects of enhanced UV radiation, together with the respective references. The first 5 species are liverworts and the remaining ones are mosses.

Species	References
Conocephalum conicum	Ihle & Laasch (1996), Ihle (1997)
Jungermannia exsertifolia subsp. *cordifolia*	Martínez-Abaigar *et al.* (2003, 2004, 2008), Núñez-Olivera *et al.* (2004, 2005), Otero *et al.* (2006), Arróniz *et al.* (2008a, 2008b)
Marchantia polymorpha	Markham *et al.* (1998)
Pellia epiphylla	Takács *et al.* (1999), Csintalan *et al.* (2001)
Riccia sp.	Prasad *et al.* (2004)
Aulacomnium turgidum	Björn *et al.* (1998)
Chorisodontium aciphyllum	Boelen *et al.* (2006)
Dicranum elongatum	Björn *et al.* (1998), Sonesson *et al.* (2002)
Dicranum scoparium	Takács *et al.* (1999), Csintalan *et al.* (2001)
Fontinalis antipyretica	Martínez-Abaigar *et al.* (2003, 2004, 2008), Núñez-Olivera *et al.* (2004, 2005)
Fontinalis neomexicana	Rader & Belish (1997)
Hylocomium splendens	Johanson *et al.* (1995), Gehrke *et al.* (1996), Schipperges & Gehrke (1996), Sonesson *et al.* (1996), Björn *et al.* (1998), Huttunen *et al.* (1998), Gehrke (1999), Phoenix *et al.* (2001), Taipale & Huttunen (2002)
Leucobryum glaucum	Takács *et al.* (1999), Csintalan *et al.* (2001)
Mnium hornum	Takács *et al.* (1999), Csintalan *et al.* (2001)
Plagiomnium undulatum	Csintalan *et al.* (2001)
Plagiothecium undulatum	Takács *et al.* (1999), Csintalan *et al.* (2001)
Pleurozium schreberi	Taipale & Huttunen (2002), Lappalainen *et al.* (2008)
Polytrichum commune	Björn *et al.* (1998), Barsig *et al.* (1998), Huttunen *et al.* (1998), Gehrke (1999)
Polytrichum formosum	Takács *et al.* (1999), Csintalan *et al.* (2001)
Polytrichum hyperboreum	Björn *et al.* (1998), Rozema *et al.* (2006)
Polytrichum strictum	Boelen *et al.* (2006)
Sanionia uncinata	Montiel *et al.* (1999), Lud *et al.* (2002, 2003), Boelen *et al.* (2006), Rozema *et al.* (2006)
Sphagnum angustifolium	Niemi *et al.* (2002a)
Sphagnum balticum	Niemi *et al.* (2002b)
Sphagnum capillifolium	Csintalan *et al.* (2001)
Sphagnum fuscum	Gehrke *et al.* (1996), Schipperges & Gehrke (1996), Björn *et al.* (1998), Gehrke (1998), Sonesson *et al.* (2002)
Sphagnum magellanicum	Niemi *et al.* (2002a)
Sphagnum papillosum	Niemi *et al.* (2002a, 2002b)
Tortula ruralis	Takács *et al.* (1999), Csintalan *et al.* (2001), Rozema *et al.* (2002)
Warnstorfia sarmentosa	Boelen *et al.* (2006)

as "control species" because of the considerable previous knowledge available on its responses to UV (Table 1). The remaining species were unknown with respect to this issue. This kind of short-term (20 days) laboratory experiments may help elucidate mechanisms of both UV action and UV protection in bryophytes.

MATERIALS AND METHODS

Plant material and environmental conditions

Bryophyte samples were collected at the oligotrophic first-order stream Lumbreras, which is located in the upper basin of the River Iregua (La Rioja, northern Spain) within the limits of the Natural Park of Sierra Cebollera. The stream flows over sandstones and quartzites (Purbeck-Weald facies, Jurassic-Cretaceous), although sporadic lime layers appear interspersed with the prevailing siliceous materials. The vegetation of the sampling zone is a subalpine shrubland dominated by *Vaccinium myrtillus* L., *Juniperus communis* L. subsp. *alpina* (Suter) Celak. and *Calluna vulgaris* (L.) Hull, and intermingled with scattered *Pinus sylvestris* L.

The six bryophyte species used for this experiment, and the collection altitudes of the respective samples, were as follows (the first three species were liverworts and the remaining three were mosses): *Jungermannia exsertifolia* Steph. subsp. *cordifolia* (Dumort.) Váňa (1850 m), *Marsupella sphacelata* (Gieseke ex Lindenb.) Dumort. (2000 m), *Scapania undulata* (L.) Dumort. (1940 m), *Brachythecium rivulare* Schimp. (1910 m), *Bryum pseudotriquetrum* (Hedw.) P. Gaertn., B. Mey. & Scherb. (1890 m) and *Racomitrium aciculare* (Hedw.) Brid. (1960 m). The nomenclature of species follows Grolle & Long (2000) for liverworts, and Magill (2008) for mosses. Voucher specimens are located in the personal herbarium of J. Martínez-Abaigar.

Samples were collected on 16 June 2003, and the coordinates of the middle of the stream stretch sampled were 42º00'30'' N, 02º38'40'' W. All the samples grew submerged under similar irradiance conditions (unshaded). Given that all the samples were collected in the same stream in an altitude range of only 150 m, the temperature measured *in situ* on the collection day showed a negligible gradient (7.1-7.5 ºC). The weather on the collection day and the two previous days was sunny, and the altitudinal variation of radiation was estimated for clear sky and aerosol level zero applying the model by Engelsen (2003). Radiation was calculated as the daily dose of biologically effective UV-B irradiance (UV-B_{BE}) using the generalized plant damage action spectrum (Caldwell, 1971). UV-B_{BE} changed from 7.01 to 7.21 kJ m^{-2} d^{-1} in the collection day and the two previous days along the altitudinal gradient considered.

Samples were rinsed with stream water, and transported to the laboratory in a portable icebox. The material was then rinsed again with stream water and green healthy shoots of each species were selected and pre-cultured separately in plastic containers filled with air-bubbled stream water. Plants were maintained at 10ºC (using an immersion chiller) with a 10:14 photoperiod (light:dark) for 5 days. The photosynthetic photon flux (PF), which was provided by True-Lite full spectrum fluorescent tubes (True Sun, USA), was 98 μmol m^{-2} s^{-1} at the water surface (LI-190SA quantum sensor, LI-COR, USA).

Experimental design

After the pretreatment, apices of the six species used were placed into separate plastic tubes with a basal net which prevented material losses. Two different tubes were used for each species. The tubes were placed in a circulating bath system within a growth chamber. The bath system was filled with stream water at a constant temperature of 10ºC. Two additional identical baths were disposed to establish replicates. The radiation was provided by a combination of three types of lamps: photosynthetically active radiation (PAR) lamps (True-Lite), UV-A lamps (QP-340, Q-Panel, USA) and UV-B lamps (Philips TL 40W/12, Philips Lighting, The Netherlands). The bryophytes were submerged at 1-2 cm depth, which attenuated less than 0.01% the photosynthetic and UV wavelengths. Half of the tubes were covered with each of the two following specific UV cut-off foils, and thus two radiation regimes were set: (1) control (PAR alone), using Ultraphan 395 (Digefra GmbH, Germany), which cut off all UV radiation; (2) UV treatment (PAR + UV-A + UV-B), using Ultraphan 295 (Digefra GmbH, Germany), which cut off UV-C radiation. To assure stability in their properties during use, the filters were pre-irradiated and replaced after every 24 h of irradiation. The tubes in each bath system were moved on a daily basis to prevent possible place-dependent differences in the irradiance received by the plants. The lamps were preburned for 100 h until they had reached a stable output. PAR lamps gave a PF of 98 µmol m^{-2} s^{-1} to the bryophytes with a 10:14 photoperiod (light:dark). UV-A and UV-B lamps were switched on around noon for 4 h 15 min per day (square-wave). UV-A lamps gave an irradiance of 10.9 Wm^{-2}. The biologically effective UV-B irradiance (UV-B_{BE}) applied was 0.67 Wm^{-2} equivalent to a daily integrated irradiance of 10.3 kJ m^{-2}, as estimated using the generalized plant damage action spectrum of Cadwell (1971) normalized to 300 nm. This UV-B_{BE} daily integrated irradiance was required to mimic a 20% ozone depletion at the latitude of the sampling site as calculated with a computer model for clear sky conditions and aerosol level zero (Björn & Teramura, 1993). The spectral irradiances were measured, and the transmission characteristics of the filters were regularly checked, with a spectroradiometer (Macam SR9910, Macam Photometrics Ltd., Scotland). The bryophytes were cultured for 20 days, a period enough to obtain different responses between control and UV-treated samples in previous experiments (Martínez-Abaigar *et al*., 2003).

Physiological variables

For the measurement of physiological variables, two bryophyte samples were taken from each tube, and thus 6 repetitions were made for each variable in each species and treatment. Before the analysis, it was microscopically confirmed that the specimens had few algal epiphytes.

The sclerophylly index (SI) was calculated as the quotient between the dry mass (DM: 80ºC for 24 h) and the surface area of the prostrate apex onto the horizontal plane (LI-3000 area meter, LI-COR, USA). Chlorophylls were extracted on fresh samples with cold 80% acetone and mortar and pestle, and quantified spectrophotometrically (Perkin-Elmer λ35 UV/Vis, Perkin-Elmer, USA) as in Martínez-Abaigar *et al*. (2003). Total chlorophyll concentrations were expressed per unit of surface area (measured with a LI-3000 area meter). Chlorophyll *a/b* ratio and the indices OD_{430}/OD_{410} and OD_{430}/OD_{665} (OD is optical density) were also obtained. These variables are often used as vitality indicators, since the two first ones decrease and the third one increases under stress situations (Martínez-Abaigar

& Núñez-Olivera, 1998). The decrease in chlorophyll *a*/*b* ratio is due to the preferential degradation of chlorophyll *a* with respect to chlorophyll *b* under the influence of adverse factors. OD_{430}/OD_{410} decreases because the proportion of phaeophytins (degradation pigments of chlorophylls) increases with respect to chlorophylls, given that the absorption maximum of chlorophylls at 430-435 nm shifts to 410-415 nm in phaeophytins. OD_{430}/OD_{665} represents the ratio of the combined chlorophylls and carotenoids (both types of pigments absorb light at 430 nm) to the chlorophylls, the only pigments which absorb light at 665 nm; a higher proportion of carotenoids with respect to chlorophylls may imply a higher photoprotection capacity to tolerate adverse situations. *In vivo* chlorophyll fluorescence of PSII was measured with a portable pulse amplitude modulation fluorometer (MINI-PAM, Walz, Germany) following Schreiber *et al.* (1995) and Núñez-Olivera *et al.* (2004). Minimal and maximal fluorescence (F_0 and F_m) were measured in samples dark-adapted for 20 min. The maximum quantum yield of PSII was given by the ratio F_v/F_m, where $F_v = F_m-F_0$, and the effective quantum yield of photosynthetic energy conversion of PSII (Φ_{PSII}) by the ratio $(F_m'-F_t)/F_m'$. Quenching due to non-photochemical dissipation of absorbed light energy (NPQ) was determined as $(F_m-F_m')/F_m'$. Also, NPQ was recorded in relation to the increment of PF from 0 to 1600 µmol m^{-2} s^{-1}, and the curve NPQ *vs.* PF was fitted to the hyperbolic tangent model equation of Jassby & Platt to calculate maximal NPQ (NPQ_{max}). The global level of methanol-extractable UV-absorbing compounds (MEUVAC) was measured in arbitrary units as the area under the absorbance curve in the interval 280-315 nm ($AUC_{280-315}$), using a Perkin-Elmer λ35 spectrophotometer, after extraction in 5 ml of methanol : water : 7M HCl (70:29:1 v/v/v) (Martínez-Abaigar *et al.*, 2003). $AUC_{280-315}$ was calculated per unit of surface area. The length of the segments of new growth was measured to the nearest 0.1 mm under a dissection microscope. Measurements were taken at the end of the experiment in 30 shoots for each species and treatment.

Statistical analysis

The effect of the species on the physiological responses was tested using a Kruskal-Wallis test because the full data set did not meet the assumption of homoscedasticity (Levene test), although data were distributed normally (Shapiro-Wilks test). Given that the species influenced significantly all the physiological variables, and that the data of each species individually met the assumptions of normality and homoscedasticity, a 2-way analysis of variance (ANOVA) was conducted for each species to test the effects of culture time and radiation regime on the different variables. The results of growth in control and UV-treated samples at the end of the experiment were compared by a Student's *t* test for each species. All the statistical procedures were performed with SPSS 13.0 for Windows (SPSS Inc., USA).

RESULTS

All the physiological variables were strongly and significantly influenced by the species ($P < 0.001$ in all species but one: Kruskal-Wallis test). The influence of culture time and radiation regime on the physiological variables of each species (2-way ANOVA) is shown in Figs 1-6. Depending on the species, the culture time
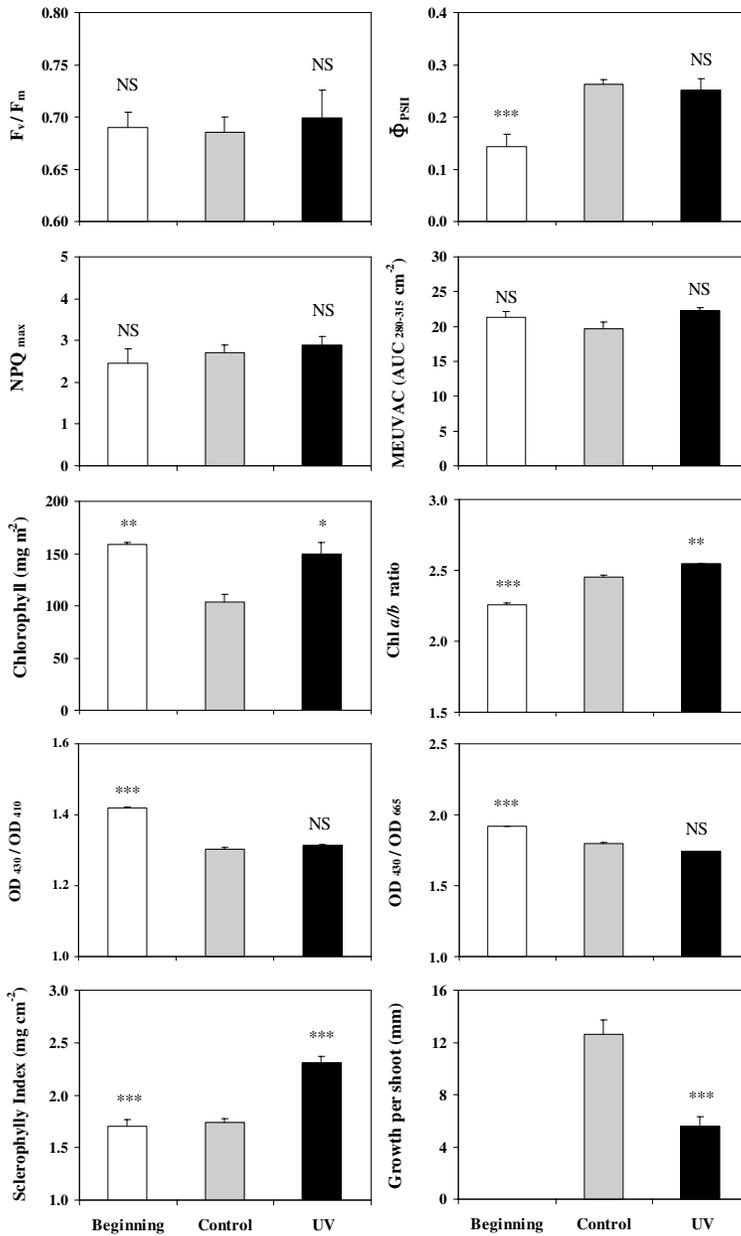
Fig. 1. Beginning values (clear bars) of the physiological variables measured in *Jungermannia exsertifolia* subsp. *cordifolia*, together with final values of the same variables in the two radiation regimes: control (PAR alone, grey bar) and UV treatment (PAR + UV-A + UV-B, dark bar). The significances of the ANOVA for the effects of the culture time and radiation regime on each variable are shown, respectively, over the bars of the beginning values and the UV-treatment final values. For growth results, the significance of the differences between control and UV values at the end of the experiment (Student's *t* test) is shown. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, NS non-significant. Means + SE are shown.
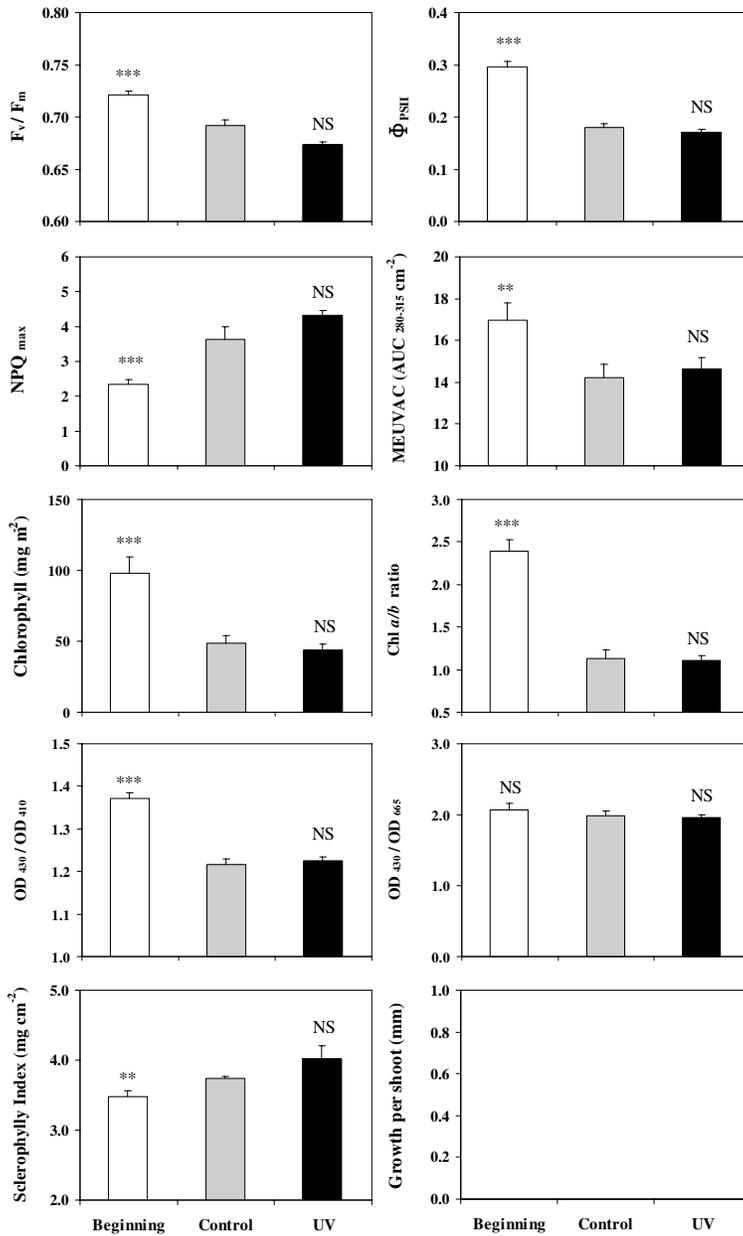
Fig. 2. Beginning values (clear bars) of the physiological variables measured in *Marsupella sphacelata*, together with final values of the same variables in the two radiation regimes: control (PAR alone, grey bar) and UV treatment (PAR + UV-A + UV-B, dark bar). The significances of the ANOVA for the effects of the culture time and radiation regime on each variable are shown, respectively, over the bars of the beginning values and the UV-treatment final values. For growth results, the significance of the differences between control and UV values at the end of the experiment (Student's *t* test) is shown. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, NS non-significant. Means + SE are shown.
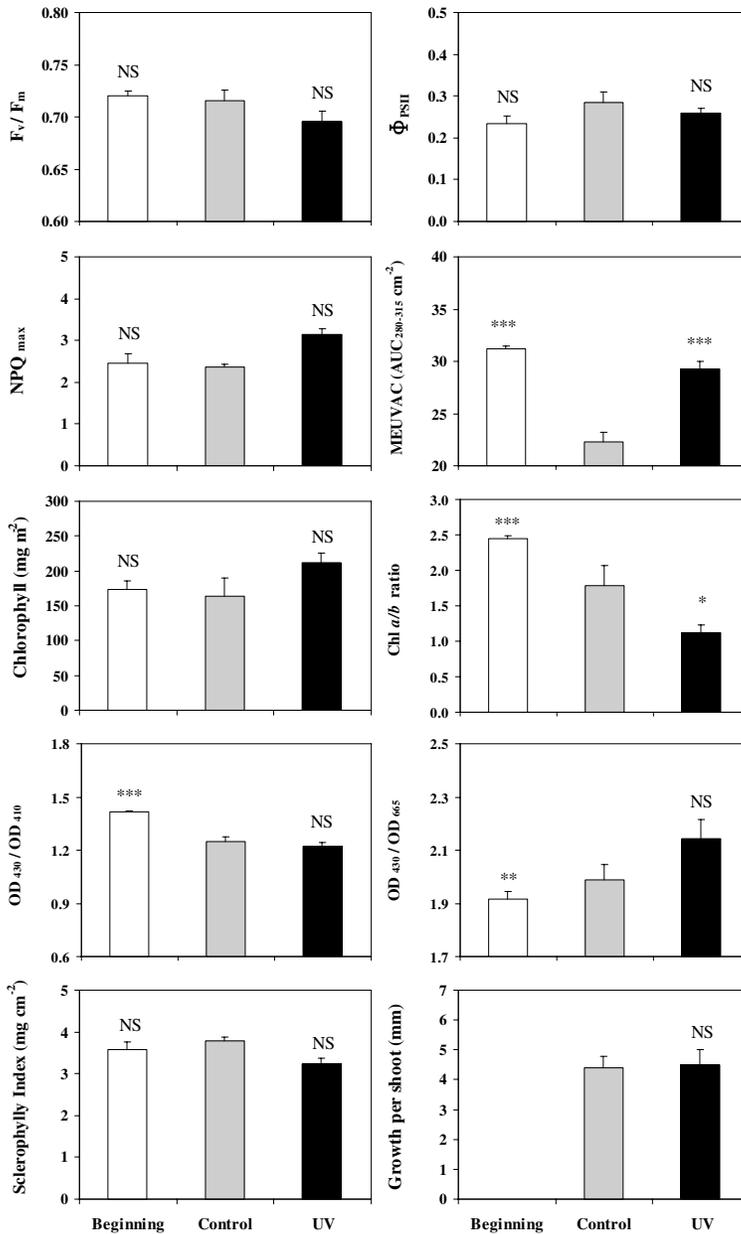
Fig. 3. Beginning values (clear bars) of the physiological variables measured in *Scapania undulata*, together with final values of the same variables in the two radiation regimes: control (PAR alone, grey bar) and UV treatment (PAR + UV-A + UV-B, dark bar). The significances of the ANOVA for the effects of the culture time and radiation regime on each variable are shown, respectively, over the bars of the beginning values and the UV-treatment final values. For growth results, the significance of the differences between control and UV values at the end of the experiment (Student's *t* test) is shown. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, NS non-significant. Means + SE are shown.
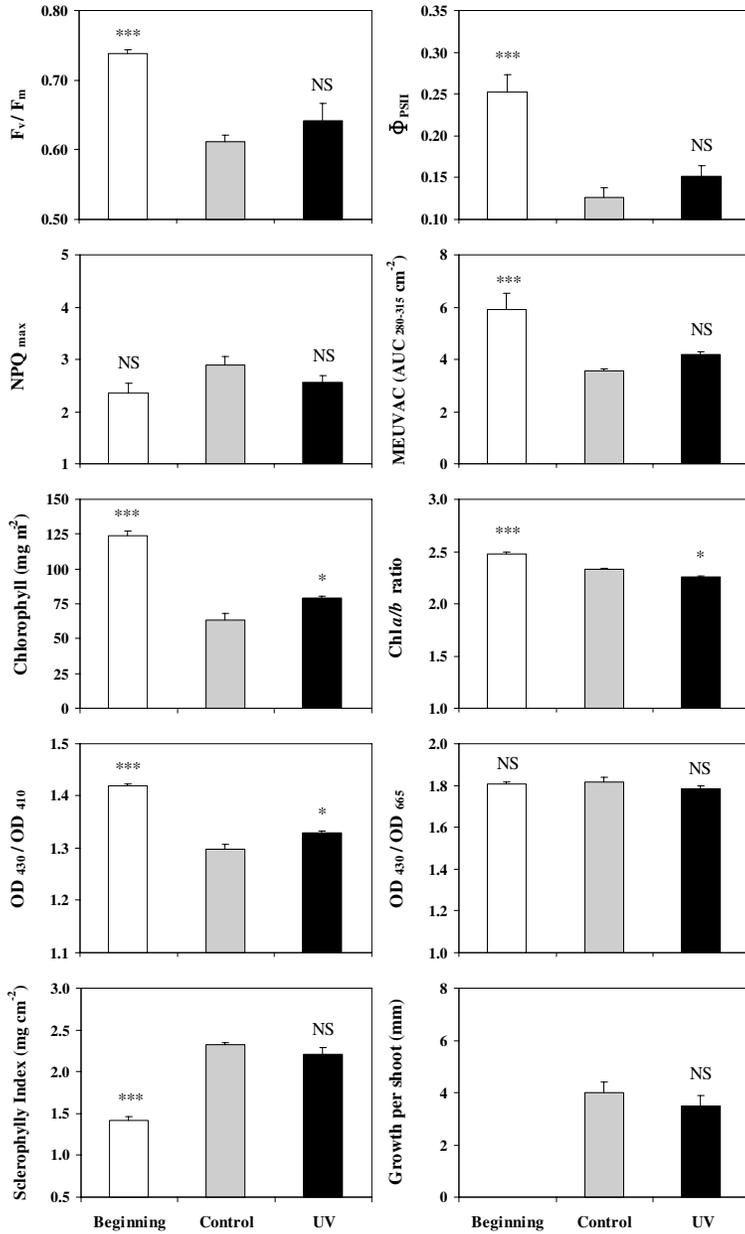
Fig. 4. Beginning values (clear bars) of the physiological variables measured in *Brachythecium rivulare*, together with final values of the same variables in the two radiation regimes: control (PAR alone, grey bar) and UV treatment (PAR + UV-A + UV-B, dark bar). The significances of the ANOVA for the effects of the culture time and radiation regime on each variable are shown, respectively, over the bars of the beginning values and the UV-treatment final values. For growth results, the significance of the differences between control and UV values at the end of the experiment (Student's *t* test) is shown. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, NS non-significant. Means + SE are shown.
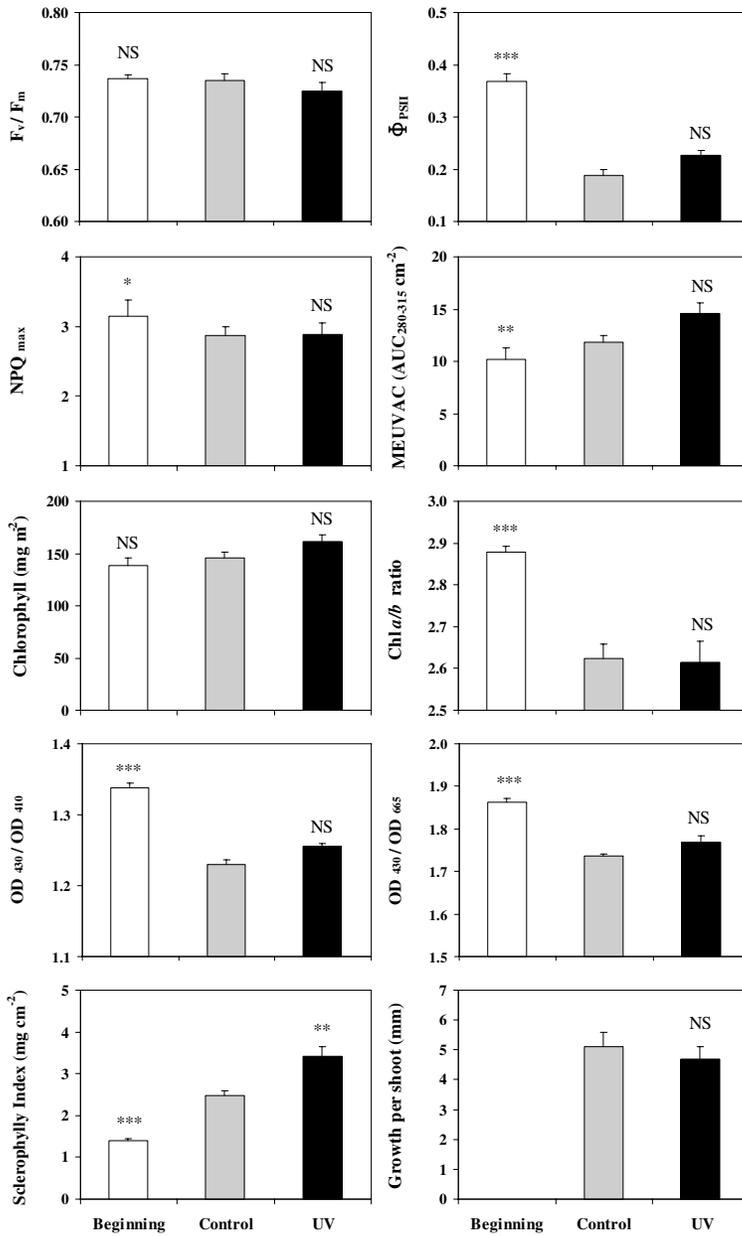
Fig. 5. Beginning values (clear bars) of the physiological variables measured in *Bryum pseudotriquetrum*, together with final values of the same variables in the two radiation regimes: control (PAR alone, grey bar) and UV treatment (PAR + UV-A + UV-B, dark bar). The significances of the ANOVA for the effects of the culture time and radiation regime on each variable are shown, respectively, over the bars of the beginning values and the UV-treatment final values. For growth results, the significance of the differences between control and UV values at the end of the experiment (Student's *t* test) is shown. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, NS non-significant. Means + SE are shown.
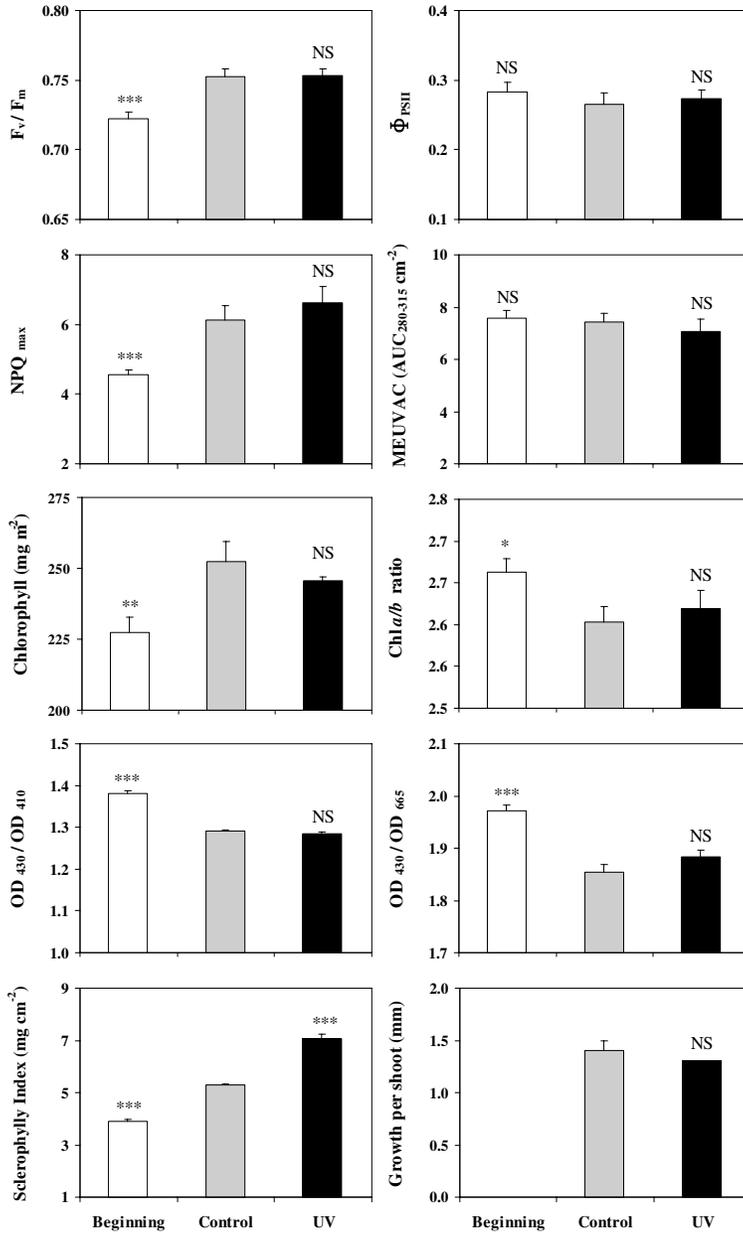
Fig. 6. Beginning values (clear bars) of the physiological variables measured in *Racomitrium aciculare*, together with final values of the same variables in the two radiation regimes: control (PAR alone, grey bar) and UV treatment (PAR + UV-A + UV-B, dark bar). The significances of the ANOVA for the effects of the culture time and radiation regime on each variable are shown, respectively, over the bars of the beginning values and the UV-treatment final values. For growth results, the significance of the differences between control and UV values at the end of the experiment (Student's *t* test) is shown. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, NS non-significant. Means + SE are shown.

significantly affected between 4 and 8 variables out of 9: 4 in *Scapania undulata*, 6 in *Jungermannia exsertifolia* subsp. *cordifolia*, 7 in the three mosses, and 8 in *Marsupella sphacelata*. The variables related to photosynthetic pigments were particularly affected by culture time. At the end of the culture period, significant decreases were recorded in OD_{430}/OD_{410} in all species and in chlorophyll *a/b* ratio in all species but one (*Jungermannia exsertifolia* subsp. *cordifolia*), whereas chlorophyll concentration and OD_{430}/OD_{665} showed contrasted responses (significant decreases in 3 species and increases in one). SI was usually affected by culture time and increased significantly in all species but one (*Scapania undulata*) at the end of the experiment. The variables of chlorophyll fluorescence were less frequently influenced by culture time: F_v/F_m decreased significantly at the end of the culture period in 2 species (*Marsupella sphacelata* and *Brachythecium rivulare*) and increased in one (*Racomitrium aciculare*); Φ_{PSII} decreased in 3 species (*Marsupella sphacelata*, *Brachythecium rivulare* and *Bryum pseudotriquetrum*) and increased in one (*Jungermannia exsertifolia* subsp. *cordifolia*); and NPQ_{max} increased in 2 species (*Marsupella sphacelata* and *Racomitrium aciculare*) and decreased in one (*Bryum pseudotriquetrum*). The amount of MEUVAC decreased at the end of the experiment in 3 species (*Marsupella sphacelata*, *Scapania undulata* and *Brachythecium rivulare*) and increased in one (*Bryum pseudotriquetrum*).

The ANOVA conducted on a variable-by-variable basis, together with the Student's *t* test applied for the analysis of differences in growth, revealed that the UV treatment affected significantly a fewer number of variables than the culture period: 0 in *Marsupella sphacelata*, 1 in *Bryum pseudotriquetrum* and *Racomitrium aciculare*, 2 in *Scapania undulata*, 3 in *Brachythecium rivulare* and 4 in *Jungermannia exsertifolia* subsp. *cordifolia* (Figs 1-6). In the samples exposed to the UV treatment, chlorophyll concentration increased in *Brachythecium rivulare* and *Jungermannia exsertifolia* subsp. *cordifolia*, chlorophyll *a/b* ratio increased in this last species and decreased in *Brachythecium rivulare* and *Scapania undulata*, OD_{430}/OD_{410} increased in *Brachythecium rivulare*, MEUVAC increased in *Scapania undulata*, SI increased in *Bryum pseudotriquetrum, Racomitrium aciculare* and *Jungermannia exsertifolia* subsp. *cordifolia*, and growth decreased in this last species. *Marsupella sphacelata* did not grow during the culture period. The interaction between the culture time and the radiation regime was significant only in the variables and species in which the influence of radiation regime was significant.

DISCUSSION

Our results show that the physiology of bryophytes was overall more influenced by the culture conditions than by the UV treatment. The responses to culture conditions depended on both the species and the variable considered. *Marsupella sphacelata* and *Brachythecium rivulare* were negatively affected by the culture and showed decreases in several physiological variables indicative of vitality, such as F_v/F_m, Φ_{PSII}, chlorophyll concentration, chlorophyll *a/b* ratio and OD_{430}/OD_{410} (Martínez-Abaigar & Núñez-Olivera, 1998; Maxwell & Johnson, 2000). In these species, the changes in photosynthetic pigments (in particular, chlorophyll loss) affected considerably their photosynthetic performance as

revealed by the kinetics of chlorophyll fluorescence. In *Bryum pseudotriquetrum*, the culture caused less damage, which was also detected by both pigment and chlorophyll fluorescence variables, although only by decreases in chlorophyll *a/b* ratio, OD_{430}/OD_{410} and Φ_{PSII}. *Scapania undulata* and *Racomitrium aciculare* showed little damage caused by the culture, and only the two pigment indices (chlorophyll *a/b* ratio and OD_{430}/OD_{410}) decreased, whereas the photosynthetic performance was not affected (even F_v/F_m increased in *R. aciculare*). *Jungermannia exsertifolia* subsp. *cordifolia* showed the least damage, because only OD_{430}/OD_{410} and chlorophyll concentration decreased, but in contrast chlorophyll *a/b* ratio and Φ_{PSII} increased. Both OD_{430}/OD_{665} and NPQ_{max} are variables related to photoprotection (Martínez-Abaigar & Núñez-Olivera, 1998; Maxwell & Johnson, 2000), but their changes were interrelated only in *Bryum pseudotriquetrum*, that showed a decrease in both variables, indicating a reduction in photoprotection. This could be due to the low PAR level supplied in the culture in comparison with natural levels, but this hypothesis needs further research because other species showed different responses in the photoprotection variables. The amount of MEUVAC also showed different changes depending on the species, and thus it is difficult to offer a clear explanation for the results obtained. SI increased significantly in all species but one (*Scapania undulata*) at the end of the experiment. A decrease in SI is usually indicative of elongation of new shoots, because young tissues are more tender than mature ones (Martínez-Abaigar *et al.*, 2003; Arróniz-Crespo *et al.*, 2008b). In contrast, the increase in dry mass per unit length (an alternative sclerophylly index to that used in our work) has been attributed to stunted growth in *Sphagnum fuscum* exposed to enhanced UV-B radiation (Gehrke, 1998). Given that all the species in our study (except *Marsupella sphacelata*) showed shoot elongation along the culture period, the SI increase observed would mean that the whole of new growth and preexistent shoots would be more sclerophyllous than only the preexistent shoots. This could have been caused by the stunted growth pointed out by Gehrke (1998), but in our case it was observed under both enhanced UV and PAR alone. *Marsupella sphacelata* was the species that least tolerated the culture conditions and the only one that did not show shoot elongation. The SI increase in this species could be due to a certain dry mass accumulation without shoot elongation along the culture period. The shoot elongation shown by the remaining species revealed that, although the culture conditions affected negatively some basic physiological variables (*e.g.* photosynthetic pigments and photosynthetic performance), especially in some species, these adverse effects did not impede growth, and thus culture conditions were only relatively damaging.

The UV treatment caused changes in a minority of variables, without a common pattern of variation among the different species. In addition, variables indicative of vitality, such as chlorophyll *a/b* ratio and OD_{430}/OD_{410}, showed contrasted tendencies even within the same species. Photosynthetic performance, as derived from chlorophyll fluorescence variables, did not change under enhanced UV-B. It may be concluded that enhanced UV-B had little damaging effect on the species studied in the short term. This was expected for *Jungermannia exsertifolia* subsp. *cordifolia*, that had shown a similar tolerance to enhanced UV-B in previous studies using diverse culture conditions (Martínez-Abaigar *et al.*, 2003; Núñez-Olivera *et al.*, 2004, 2005; Otero *et al.*, 2006; Arróniz-Crespo *et al.*, 2008a). The tolerance of this species to enhanced UV-B was attributed in previous studies to the accumulation, in the UV-B exposed samples, of MEUVAC in general (Martínez-Abaigar *et al.*, 2003; Núñez-Olivera *et al.*, 2004) or of certain hydroxycinnamic acid derivatives in particular (Otero *et al.*,

2006; Arróniz-Crespo *et al.*, 2008a). However, in the present study the global amount of MEUVAC did not increase under enhanced UV-B with respect to control samples. Other discrepancies in the UV-B responses of this liverwort with respect to previous results are that: (1) in this study, the chlorophyll concentration and chlorophyll *a/b* ratio showed a positive influence of the UV treatment and increased in the UV-exposed samples with respect to control samples, whereas in previous research these variables hardly changed; and (2) in this study, chlorophyll fluorescence variables were not affected, whereas in previous work F_v/F_m usually decreased. These slight discrepancies regarding MEUVAC, photosynthetic pigments and chlorophyll fluorescence variables could be due to the different characteristics of the samples used in each study. Probably the samples used here were naturally acclimated to high UV-B levels because they were collected: (1) at higher altitudes (1850-2000 m) than in the other studies (1350 m); and (2) in a date near the summer solstice, when UV-B irradiances are the highest in the year, whereas in other studies the collection took place in winter or spring. The high natural protection capacity against UV-B of the samples used in this study might have prevented a negative response of photosynthetic pigments and chlorophyll fluorescence variables in the laboratory culture, without the need of increasing MEUVAC levels. Thus, UV-B responses depend not only on the species and the environmental conditions (such as temperature or the presence of heavy metals), as it has been pointed out before (Martínez-Abaigar *et al.*, 2003, 2008; Núñez-Olivera *et al.*, 2004, 2005; Otero *et al.*, 2006), but also on the collection place and collection date of the samples.

It is curious that *Jungermannia exsertifolia* subsp. *cordifolia*, that has repeatedly shown to be tolerant to UV-B, grew less in UV-B exposed samples than in control ones. This result was already found in a previous study (Núñez-Olivera *et al.*, 2004). This could have occurred because in both cases length growth (and not dry mass growth) was measured, and shoot elongation could have been affected through hormonal interference in UV-B exposed samples, given that UV-B can degrade auxins (Jansen *et al.*, 1998). Thus, UV-B might cause stunted growth in this liverwort, as it has been found before in *Sphagnum fuscum* (Gehrke, 1998).

In the present study, only in *Scapania undulata* the amount of MEUVAC was higher in the UV-exposed samples than in the control ones, but in all the species except *Bryum pseudotriquetrum* the amounts at the end of the experiment were similar to or lower than the initial amounts. Thus, MEUVAC did not seem to be clearly induced by enhanced UV-B. As it has been pointed out for *Jungermannia exsertifolia* subsp. *cordifolia*, the rest of species studied could also have MEUVAC levels high enough in nature to cope with enhanced UV-B, because all of them were collected in the same place and date. It should be also taken into account that: (1) MEUVAC are rarely induced by enhanced UV-B in bryophytes, especially in mosses (Boelen *et al.*, 2006; Martínez-Abaigar *et al.*, 2006); and (2) the measurement of the bulk UV absorbance of methanolic extracts may be a method insufficient to demonstrate accumulation of UV-absorbing compounds and it should be better to analyze the individual compounds by HPLC (Arróniz-Crespo *et al.*, 2008a). The tolerance of all the species studied to enhanced UV-B could be also based on other protection mechanisms than the accumulation of MEUVAC, such as efficient antioxidant systems or DNA repairing processes. In addition, four of the species studied have a certain montane influence in their biogeographical ranges: *Jungermannia exsertifolia* subsp. *cordifolia*, *Marsupella sphacelata*, *Scapania undulata* and *Racomitrium aciculare* (Duell, 1983, 1984, 1985). It could be speculated that this montane

character could represent a sign of genetic tolerance to high UV-B levels, although not a *sine qua non* condition for that tolerance because the biogeographical ranges of the other two species studied are considerably wider and they may survive under high UV-B levels in nature and showed a similar tolerance to enhanced UV-B in the laboratory.

The results exposed here are directly related to the experimental conditions applied, in particular the relatively low PAR supplied. However, experiments conducted under high PAR level (500 μmol m^{-2} s^{-1}) showed that enhanced UV-B has little damaging effect on three of the bryopytes used in the present study: *Jungermannia exsertifolia* subsp. *cordifolia*, *Scapania undulata* and *Bryum pseudotriquetrum* (Otero *et al.*, 2006; data not shown). Thus, our results have a certain ecological relevance and may help elucidate mechanisms of both UV action and UV protection in bryophytes.

In conclusion, the species studied here were UV-B tolerant under the conditions considered, probably because the samples were collected at high altitudes and near the summer solstice. This tolerance capacity would overcome both the general structural limitations of bryophytes regarding UV protection (given that bryophytes lack thick cuticles, epidermis and hairs) and the specific ecophysiological constraints exerted by UV radiation on bryophytes from mountain streams, that are exposed to high UV levels and whose biochemical protection mechanisms may be limited by cold temperatures. Thus, the responses to enhanced UV-B may not depend only on the species and the environmental conditions, as it had been pointed out before, but also on the collection site and the collection date of the samples.

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