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Sensitivity of photosynthesis to UV radiation in several *Cosmarium* strains (Zygnematophyceae, Streptophyta) is related to their geographic distribution

Stamenković Marija*, Hanelt Dieter

*corresponding author

Fax: ++49-40-42816-565

e-mail: marie110@gmail.com

Biozentrum Klein Flottbek

Ohnhorst Str. 18

22609 Hamburg, Germany

Abstract

Photoinhibitory effects of ultraviolet radiation (UVR) on four *Cosmarium* strains were studied with respect to their geographic distribution pattern. This study dealt with two strains of a cosmopolitan taxon (*C. punctulatum* var. *subpunctulatum*) collected from high-mountain tropical and lowland polar regions, one typical tropical species (*C. beatum*) and one typical polar representative (*C. crenatum* var. *boldtianum*). Physiological characteristics of the strains during and after various UVR spectral combinations at two temperature gradients were determined by the measurement of chlorophyll fluorescence, oxygen evolution rates and using an inhibitor of chloroplast-encoded protein synthesis (streptomycin). All of the *Cosmarium* strains investigated exhibited consistent geographic distribution patterns in accordance with the UVR prevailing at their sampling sites, despite a long-term cultivation under constant laboratory conditions. It appeared that moderate ultraviolet-B radiation (UVBR) treatment did not exert large damages to photosystem II in all of the *Cosmarium* strains, compared to ultraviolet-A radiation (UVAR) treatment at 21°C. Interestingly, an ameliorating effect of UVBR at 21°C was observed in *C. beatum* as concluded from higher rates of recovery of maximum quantum yield after moderate UVBR treatment, compared to that after UVAR application. This study also reveals that the mucilage of desmids has a limited role in the protection against UVR as demonstrated by the measurements of absorption in the UVR range, in contrast to previous assumptions. Increased UVBR (i.e. high UVBR:PAR ratio) severely decreases oxygen evolution in all of the *Cosmarium* strains, pointing to possible consequences to peat bogs which are native habitats of desmids, as they are particularly poor in oxygen.

Keywords: *Cosmarium*, distribution pattern, maximum quantum yield, mucilaginous sheath, oxygen evolution, photoinhibition, streptomycin, ultraviolet radiation

Abbreviations

PAR – photosynthetically active radiation (=P), UVR – ultraviolet radiation; UVAR (UVA) – ultraviolet-A radiation, UVBR (UVB) ultraviolet-B radiation, PA – PAR+UVA, PAB –

PAR+UVA+UVB, AB – UVA+UVB, NF (no filter) – unfiltered radiation of a sun simulator, PSII – photosystem II, Fv/Fm – maximum quantum yield of PSII, SM – streptomycin, PQ – plastoquinone

Introduction

So far, extensive investigations on geographic and depth distribution patterns of seaweeds induced by ultraviolet radiation (UVR) have been performed (summarized by Bischof and co-workers[1]), revealing that majority of macroalgae occupy specific ecological niches in accordance with their resistance to UVR. Although numerous investigations of UVR effects on microalgae have been performed at ecosystemic, physiological and ultrastructural levels,[2–5] comparative studies on the impacts of UVR on possible geographic patterns of microalgae have been a topic of only a few investigations.[6–10]

Desmids are a group of exclusively freshwater microalgae, named according to the Greek word 'desmos' (bond or chain) since cells of the majority of taxa are transversally carved by a constriction (*sinus*) on two symmetrical semicells connected with *isthmus* (modified from Brook 1981).[11] Desmids are members of the algal class Zygnematophyceae (former Conjugatophyceae, Chlorophyta), characterized by conjugation mediated sexual reproduction and the total absence of flagellated life cycle stadia.[12] Except for a few cosmopolitan representatives, desmids are principally known for their preference for specific habitats and climatic regions.[13–15] Actually this attribute may render desmids as an ideal object for the study of impacts of UVR on the global distribution pattern of freshwater microalgae.

Conjugatophycean algae represent abundant and frequently predominant organisms in shallow freshwater habitats (such as peat bogs, fens, marshes, puddles, ditches and boggy margins of pools) and are exposed to intensive and varying solar radiation. Up to date, laboratory investigations on the tolerance of desmids to UVR revealed contradictory results. Strong intensities of UVBR stopped photomovement in a long-term cultivated *Cosmarium* species by the inhibition of the mucilage production, hence disabling cells to find a field of suitable irradiance.[16] On the other hand, freshly

isolated *Micrasterias denticulata* Brébisson ex Ralfs demonstrated a marked resistance against UVR wavelengths down to 284 nm.[17,18]

Although one of the main effects of UVR on algae is photoinhibition,[2,6,19,20] it is worth noting that UVR cannot be regarded as an 'excessive energy input' in a proper sense. Its maximal irradiance is much smaller than of photosynthetically active radiation (PAR) and the UV wavebands do not contribute significant energy supply for photosynthetic chemistry.[21] Interestingly, positive effects of moderate fluxes of UVBR, as demonstrated from the delayed recovery of photoinhibition if the natural UVB wavelength range is removed from solar spectrum, were noted in several macrophytes.[21–23] Therefore, it is necessary to investigate the effects of all wavebands of the solar spectrum (i.e. PAR, UVA, and UVB) on the possible geographic distribution of several *Cosmarium* strains. In addition, as UVR increases with increasing solar intensity (i.e. concurrently with the increasing of PAR) it is assumed that the *Cosmarium* strains may develop a considerable *de novo* protein synthesis under relatively low to moderate UVR stress. This incidence is supposed to occur in the so-called 'sun-plant' strategists,[24,25] as estimated by the addition of an inhibitor of chloroplast-encoded protein synthesis.[26,27]

Taking into account the previously mentioned facts, this study is aimed to unveil whether the *Cosmarium* strains are capable to occupy specific geographic areas regarding the prevailing UVR regime, as judged from their physiological behaviour under a set of UVR conditions applied *in vitro*. Considering that the *Cosmarium* strains are particularly sensitive to temperature decrease,[28] the UVR photoinhibitory treatments were done at both permissive and cold temperatures, to observe physiological characteristics of geographically different strains when both of the stressors were present. Furthermore, studies of the sensitivity of the *Cosmarium* strains to various UVR spectral combinations with regard to oxygen evolution are necessary as desmids are important primary producers in circumpolar peat bogs,[13,15] which may be exposed to elevated UVR due to the thinning of the ozone layer.[29,30]

Finally, considering that some of the *Cosmarium* strains produce a vast amount of mucilage,[31] which is hypothesized to have a role in screening cells against UVBR as it has been observed in

cyanobacteria,[32,33] the measurements of the absorption in the UVR range by isolated mucilage are performed to explore this assumption.

Material and methods

Algal strains and culture conditions

The four medium-celled *Cosmarium* clones examined in this study were isolated from various parts of the world within approximately the same time period, to exclude the influences of sampling time and, therefore, the influences of constant nutrient, light and temperature regime in laboratory conditions. This was done to enable approximately the same cultivating conditions for all the desmid strains, as it is known that in some cases long-term subcultivation may lead to the accumulation of mutations and/or selection in microalgal strains studied, which may be a source of genetic variation.[34] The investigation was based mainly on the geographic origin of the individual isolates; however, we tried to make a narrow link between known general distributions and the origins of the clones for the selected *Cosmarium* taxa. Two strains of a cosmopolitan taxon (*C. punctulatum* var. *subpunctulatum*) were collected from a high-mountain tropical site (pool on Mt. Cotopaxi at 1600 m a.s.l., Ecuador) and from a lowland polar region (pool near Skarsvåg at 80 m a.s.l, the North Cape, Norway). A typical tropical species (*C. beatum*) was isolated from a marshy area near Ol Bolossat Lake in Kenya, while a typical polar taxon (*C. crenatum* var. *boldtianum*) was isolated from peat mosses spread in Northbrook Island, Franz Joseph Land, Russia. Details of the taxonomic and ecological attributes of the investigated taxa, as well as the climatic characteristics of the sampling locations are published elsewhere.[31] For a summary of all data, including yearly mean of daily irradiation in UVR (280–400 nm) of the sampling localities (based on data from Mines ParisTech, France, http://www.soda-is.com/eng/index.html[35]) see Table 1.

All of the investigated *Cosmarium* strains were grown under standard conditions (16°C; ~30 µmol photons m^{-2} s^{-1}) in a climate chamber of the Microalgae and Zygnematophyceae Collection Hamburg (MZCH) during several years. Taking into account that 16°C is a sub-optimal temperature

for the tropical species (*C. beatum*),[31,36] while temperatures above 22 or 25°C are recognized as sub-optimal for polar microalgal representatives,[37,38] all of the experimental *Cosmarium* strains were pre-cultured at 21°C, which was considered as a roughly compromising optimal temperature for both tropical and polar strains studied (see Stamenković and Hanelt 2013a[28]). The strains were grown in a mineral medium (L-d)[31] and had been acclimated to 21°C at least 12 months before the stress experiments began (at a daily light regime of 14 h of light and 10 h of darkness, 30 µmol photons m^{-2} s^{-1}). In sterilized 1-litre Erlenmeyer flasks 500 ml of medium was inoculated with cells to a final concentration of 1500 cells ml^{-1}. Cultures were aerated with humidified air at a rate of about 10 l h^{-1} to prevent CO_2 limitation. The cultures were mixed regularly with a magnetic stirrer to prevent self-shading of cells. Another set of inoculated Erlenmeyer flasks was grown for 5 days at 21°C, and then transferred to a climate chamber at 7°C (30 µmol photons m^{-2} s^{-1}) for 7 days. For the photoinhibitory tests, cells were sampled from the middle of the logarithmic growth phase, i.e. 12 days from the beginning of the cultivation at 21°C, or at the end of the acclimation at 7°C.

Photoinhibition and recovery

Photosynthetically active radiation and ultraviolet radiation (PAR and UVR) were provided by a sun simulator (SonSi, iSiTEC GmbH, Germany), as described by Hanelt and co-workers.[23] The samples were positioned in small plastic beakers and mounted on a rotating plate within a double-walled, water-filled glass jar. The temperature of the jar was kept at 21°C or 7°C (±0.5°C) by a thermostated water jacket. The samples were irradiated with a stabilized Metallogen lamp (Philips MSR 400 HR, Germany) which emanates a solar-like continuum. Wire meshes acted as neutral filters to reduce the irradiance up to 700 µmol photons m^{-2} s^{-1} without changing the spectrum. Considering that application of 700 µmol photons m^{-2} s^{-1} at 21°C had no large damaging effect to the *Cosmarium* strains studied, as judged from the relatively small depressions of Fv/Fm during 6 h treatment,[37] this irradiance was selected as a 'background' for the study of impacts of UVR. To investigate effects of UVR spectra on the physiological behaviour of the *Cosmarium* strains UV absorbing filters (Schott, Mainz, Germany) were placed between the experimental units and the light source, achieving the different light/UV

conditions: GG400 ($\lambda > 400$ nm) was used to exclude UV radiation, WG320 ($\lambda \geq 320$ nm; PAR+UVA), WG295 ($\lambda \geq 295$ nm; PAR+UVA+UVB), and UG5 ($\lambda < 400$ nm; UVA+UVB) (Table 2). PAR and UVR experiments were done at two temperature levels (21 and 7°C).

Units for PAR (µmol photons m^{-2} s^{-1}) were converted to W m^{-2}, according to McCree.[39] An additional UVR experiment was performed without filters, while PAR intensity was adjusted up to 700 µmol photons m^{-2} s^{-1} by means of wire meshes, to observe the action of the full spectrum at 21°C (NF treatment). The spectrum within the sun simulator was measured using a SonSi spectrometer (Isitec, Germany). This spectrometer is equipped with a Zeiss monolithic miniature spectrometer module (MMS) including a diode array with sensitivity from 198 to 738 at about 2.2 nm intervals. Data were analysed by a Sonsi associated software. Data from the Sonsi-spectrometer were compared with the data from a LI-1000 (LI-COR Biosciences, USA) equipped with LI-190 quantum light sensor (400–700 nm) for PAR measurement, UVA-Sensor Type 2.5 (310–400 nm), and UVB-Sensor Type 1.5 (265–315 nm) sensors (Indium Sensor, Germany) for UVA and UVB measurements, respectively. The UVA sensor measured the impinging unweighted energy (W m^{-2}) while the UVB sensor measured the erythemally weighted energy (µW cm^{-2}). Data were converted to unweighted UVB irradiance according to McKenzie and co-workers.[40]

Desmids samples were exposed to the spectral combinations in series of time treatments (1, 4 and 6 h; n = 3 per treatment combination) at 21 or 7°C, in accordance with the pre-acclimation temperature. Afterwards, the samples were returned to climate chambers at 21 or 7°C (30 µmol photons m^{-2} s^{-1}) for recovery. Measurements on the chlorophyll fluorescence and oxygen evolution were performed after 1, 4 and 6 h of inhibition and after 15 min, and 1, 2, 4, and 24 h of recovery. All of the experiments were repeated three times.

Chlorophyll fluorescence measurements

Photosynthetic efficiency was measured as variable fluorescence of PSII using a Pulse Amplitude Modulation fluorometer (PAM 101) connected to a PC with WinControl software (Heinz Walz GmbH, Effeltrich, Germany). Prior to measurements, the number of cells was adjusted to 4000 cells

189 ml^{-1} by adding a quantity of thermally adjusted L-d medium, estimated by an electronic particle
190 counter (Beckman Coulter Electronics ZB, Munich, Germany). For the samples treated with
191 streptomycin, L-d medium was enriched with a respective concentration to obtain the desired
192 concentration (see below). Immediately after sampling, the algal suspension was subjected to 3 min of
193 dark adaptation in a water bath at the experimental temperature and filled into 5 ml quartz cuvettes
194 (Hellma, Müllheim, Germany). A pulse of weak, far-red light was applied to empty the electron pool
195 from Q_A. The maximum quantum yield (Fv/Fm) was measured at time zero as described by Hanelt.[41]
196 Initial fluorescence (Fo) was measured with red measuring light (~0.3 µmol photons m^{-2} s^{-1}, 650 nm),
197 and maximal fluorescence (Fm) was determined using 600 ms of completely saturating white light
198 pulse (~3500 µmol photon m^{-2} s^{-1}). To eliminate the possible handling effect due to repeated
199 measurements, chlorophyll fluorescence was also measured in time zero control at time-series in
200 synchrony with recovery of treated samples, and designated as a disturbed control. Another set of
201 controls in parallel to each replicate was separately prepared and cultured at 21 or 7°C and 30 µmol
202 photons m^{-2} s^{-1}, and designated as undisturbed controls. Photosynthetic efficiency of undisturbed
203 controls was measured at the end of the recovery period (24 h) of the experiment. Time-series
204 recovery in maximum quantum yield of the *Cosmarium* strains after exposure to different spectral
205 irradiance was expressed as a percent recovery of the disturbed control.
206
207 *Inhibitor studies*
208 To assay the influence of chloroplast-encoded protein synthesis on the degree of photoinhibition,
209 streptomycin (SM) (Sigma, Germany) was added to samples 1 h before the photoinhibitory
210 experiments started. The final concentration of SM in samples was 20 µg ml^{-1}; 0.5% ethanol was used
211 to enhance the absorption of the antibiotic.[43] This concentration had no effects on fluorescence
212 parameters of the *Cosmarium* strains exposed to dim white light, at 21 and 7°C (control experiments).
213 Absorptions of UV radiation by L-d medium and SM solution, measured by a UVPC-2101 UV-VIS
214 spectrophotometer (Shimadzu, Japan), were negligible (data not shown).
215

Oxygen evolution

In synchrony with measurements of maximum quantum yield, the changes in oxygen production were measured using a Presens Fibox 3 fiber-optic oxygen meter (Precision Sensing GmbH, Germany), attached to a PC running OxyView PS3 software. After different times of photoinhibition, 5 ml of the homogenized algal sample was transferred to the cuvette containing a planar oxygen-sensitive foil and bubbled with helium for 1 minute to lower the O_2 concentration and to avoid O_2 saturation during the measurements. Prior to the measurements, 100 µl of a 1M HCO_3^- solution was added to achieve saturating carboxylating conditions. The amount of oxygen in cuvettes before each measurement was approximately 10% of the saturated concentration, and the cuvettes were tightly closed by rubber stoppers during measurements. The light source was a projector fitted with a halogen lamp (Xenophot, Osram, Germany), and samples were irradiated with a light intensity of 100 µmol photons m^{-2} s^{-1}, using red light (650 ± 20 nm) as measuring light. The measurements lasted 10 min, until a steady state level of oxygen evolution was achieved. The sample was stirred continuously with a small magnetic bean during the measurements. The sample was maintained at the desired temperature (21 or 7°C) by means of a thermostat-controlled water jacket. Oxygen evolution of each non-photoinhibited control was standardized to 100% and the degree of photoinhibition after different treatments was related to these controls.

Absorption of PAR and UV radiation by mucilaginous envelopes of the desmid strains

To investigate the UVR absorption of mucilaginous layers after various spectral combinations, the *Cosmarium* strains were treated 6 h under 700 and 1200 µmol photons m^{-2} s^{-1} as well as under PAR+UVA+UVB (PAB) and UVA+UVB (AB) spectral combinations. 10 ml of a treated algal sample, containing around 35000 cell ml^{-1}, was filtrated under vacuum-pressure using a net with mesh size of 15 µm to extract mucilage layers from desmid cells, which remained on the net surface. The filtrates with mucilage were placed in test tubes and homogenized for 5 min by means of a small laboratory shaker. Afterward the filtrates were placed in 5 ml quartz cuvettes (Hellma, Müllheim,

Germany) and UV absorption was estimated by means of a UVPC-2101 UV-VIS spectrophotometer (Shimadzu, Japan).

Statistical analysis

All of the statistical analyses were conducted using the SPSS program (SPSS, Chicago, USA). Data were tested for normality (Kolmogorov-Smirnov test) and for homogeneity of variance (Levene statistics). Student's t-test was done to compare differences in Fv/Fm between disturbed and undisturbed controls.

Photosynthetic responses to varying irradiance, exposure time and interaction effect were tested using the multivariate analyses of variance (MANOVA). As previous investigations demonstrated that spectral irradiance and exposure time showed significant correlations, this fact allowed treating these variables as two dependent variables (outcomes), hence multivariate test was performed (i.e. values of Fv/Fm time-series were split for the two dependent variables). In this way MANOVA has a greater power to detect an effect, because it can detect whether groups differ along a combination of variables, whereas ANOVA can detect only if groups differ along a single variable.[43] Fv/Fm values sampled during and after different exposure times for all of the investigated spectral combinations (applied at 7 and 21°C) were used as variables for the estimation of between-subjects effects of the MANOVA test. Since the assumption of multivariate normality cannot be tested on SPSS, the test of univariate normality (i.e. Kolmogorov-Smirnov test) was done for each dependent variable in turn. Although this solution does not guarantee multivariate normality, it is practical and used in multivariate statistics.[44] The assumptions of equality of covariance matrices were compared between groups using Box's test, which is non-significant if the matrices are the same. The test statistics Pillai-Bartlett trace (V) was used to determine the significance of the main MANOVA test. The analysis of contrasts (repeated method) was used to estimate differences between irradiation treatments, for each desmid strain studied.

A set of the one-factor between subjects (one-way) ANOVAs was applied to find significant changes of the UVR absorption of desmid mucilaginous sheaths isolated from PAR, PAB and AB treated samples, compared to control samples (30 μmol photons m^{-2} s^{-1}) (including Tukey HSD test).

Correlations were performed to determine relationships between Fv/Fm and gross oxygen values (expressed as % of controls) at the end of photoinhibitory treatments (at 7 and 21°C), for all of the strains and UVR spectral combinations.

Results

Chlorophyll fluorescence

Measurements of Fv/Fm of disturbed controls exhibited no significant handling effect on the photosynthetic performance of the *Cosmarium* strains. Comparison between disturbed and undisturbed controls after 24 h showed no significant variation in all of the *Cosmarium* strains studied, at both temperatures (21 and 7°C) (t-test, $p > 0.05$; data not shown).

According to the main MANOVA test, the *Cosmarium* strains studied differed significantly regarding the application of various spectral irradiances and time exposure at 21°C, taking into account the Pilai's trace ($V = 0.36$, $F(5, 51) = 13.7$, $p < 0.05$); also the strains differed at 7°C ($V = 0.44$, $F(5, 51) = 10.2$, $p < 0.05$). In addition, tests of between-subjects effects demonstrated significant effects of irradiance and exposure time at 21°C on Fv/Fm for all of the strains studied (Table 3). Interactions of these variables were significant for *C. punctulatum* No. 571 and *C. crenatum*.

The high-mountain strain of *C. punctulatum* (No. 570, Fig. 1a) displayed the highest resistance under all of the UV spectral combinations, indicated by the smallest depression of maximum quantum yield during UVR treatments and the highest recovery degree, among all of the strains studied. This strain showed a complete recovery of Fv/Fm within 24 h even after the prolonged (6 h) stress under PAR+UVA (PA), PAR+UVA+UVB (PAB) and UVA+UVB (AB) spectral combinations, while the recovery after the NF (no filter) treatment achieved 84% – the highest recovery value among all of the *Cosmarium* strains studied. In contrast, even short-term treatments under any of UVR combinations

294 provoked an incomplete recovery of the polar strain of *C. punctulatum* (No. 571, Fig. 1b). Analysis of
295 contrasts revealed non-significant differences in Fv/Fm between PA and PAB treatments for both
296 strains of *C. punctulatum* (*C. punctulatum* No. 570 $p = 0.102$; *C. punctulatum* No. 571 $p = 0.115$),
297 while NF and AB treatments caused a significant decrease of Fv/Fm compared to the other treatments
298 in the polar strain of *C. punctulatum* ($p < 0.05$).

299 The typical tropical species, *C. beatum*, achieved a full recovery of Fv/Fm within 1 h after the
300 short-term PA and PAB applications; thus showing its insensitivity under moderate UVA and UVB
301 radiation. Interestingly, the application of a moderate UVB intensity (0.89 W m^{-2}, PAB treatment)
302 during 4 and 6 h treatments initiated a faster recovery of Fv/Fm, compared to that after the UVA (PA)
303 application. This difference was statistically significant ($p < 0.05$), as estimated by the analysis of
304 contrasts of MANOVA test. The arctic taxon, *C. crenatum* var. *boldtianum*, demonstrated rather a low
305 sensitivity under 1 h PA and PAB treatments, as concluded from a small depression of Fv/Fm during
306 these treatments (around 60%), after which a full recovery was attained within 1 h or 4 h for PA and
307 PAB treatments, respectively. The difference in Fv/Fm values for PA and PAB treatments appeared
308 statistically non-significant ($p = 0.063$) in *C. crenatum*. However, this species appeared rather
309 sensitive under 6 h AB treatment, as concluded from the strong Fv/Fm decrease (20%) which caused
310 limited recovery. The maximum quantum yield dropped down to zero during 6 h NF application in
311 both typical tropical and arctic species, after which recovery reached only up to around 26%.

312 UVR caused deeper inhibition of maximum quantum yield in all of the *Cosmarium* strains pre-
313 acclimated at 7°C (Fig. 2). The typical arctic taxon, *C. crenatum*, displayed relatively high resistance
314 to 1 h PA and PAB treatments at 7°C, achieving a complete recovery after 24 h (difference between
315 PA and PAB treatments was non-significant, $p = 0.071$) Longer treatments provoked a marked
316 inhibition of Fv/Fm (below 20%), causing an incomplete recovery in this taxon. Both strains of *C.*
317 *punctulatum* showed noticeably stronger inhibition when treated under PA or PAB at 7°C for all of
318 the treatment times, compared to that at 21°C. MANOVA demonstrated significant effects of UV
319 spectral combinations and exposure time in both strains of *C. punctulatum* and *C. crenatum*, which
320 were pre-acclimated and treated at 7°C (Table 4). Maximum quantum yield of *C. beatum* rapidly

decreased under all of the UVR treatment combinations at 7°C thereby showing severe cell damages. NF treatment applied at 7°C caused a drastic decrease of Fv/Fm in all of the *Cosmarium* strains studied, compared to the treatment at 21°C, and it was excluded from Fig. 2.

Oxygen evolution

The averaged O_2 production of the *Cosmarium* strains studied (expressed in $\mu MolO_2$ mg Chl^{-1} min^{-1}) grown at 7 and 21°C is shown in Table 5. Acclimation at 7°C decreased oxygen evolution in all of the *Cosmarium* strains; yet, *C. crenatum* exhibited the highest oxygen production at this temperature.

Concomitantly with measurements of Fv/Fm during the time-series of inhibition and recovery, oxygen evolution was measured. To observe effects of UVR on total cell metabolism of the *Cosmarium* strains, the measurement of oxygen evolution included photosynthesis as well as respiration rates (see Lütz and co-workers[18] and Samuelson and co-workers[44]). Oxygen and Fv/Fm values measured during UVR inhibitions were expressed as percents of controls (for both temperature grades) and these percentages were plotted for all of the spectral combinations (Fig. 3).

Significantly positive correlations between oxygen evolution rates and Fv/Fm were found during the PA treatment, taking into account all of the treatment times (Fig. 3a). The addition of a moderate UVB intensity (0.89 W m^{-2}) did not exert significantly larger damage to the total oxygen evolution in the *Cosmarium* strains, as concluded from approximately the same range of oxygen evolution rates as measured during PA applications (Fig. 3b). The UVR treatment with low PAR (AB) caused a stronger decrease of oxygen evolution rates than that of Fv/Fm in both desmid strains collected from the polar region, demonstrating damaging effects of UVR to PSII (Fig. 3c). The intensive UVB radiation (1.28 W m^{-2}, NF treatment) caused a severe decrease of oxygen production which dropped lower than 10% of a control after longer treatments (Fig. 3d).

Relationships between total oxygen evolution rates and Fv/Fm for the *Cosmarium* samples treated under UVR spectral combinations at 7°C were significantly positive for all of the strains studied (data not shown).

Effects of a translation inhibitor

Fv/Fm values were measured for SM-treated samples in parallel with untreated samples during photoinhibitory UVR treatments and in recovery at 7 and 21°C. The measured values were expressed as percentages of the controls, and Fv/Fm percentages of SM-treated samples were subtracted from untreated samples. These differences (for 1 and 6 h treatment times at 21°C) are shown in Fig. 4; the higher values of the difference in Fv/Fm indicate the higher photodamage.

All the SM-treated *Cosmarium* strains demonstrated a rapid decrease of Fv/Fm during the UVR treatments at 21°C, achieving considerably lower values compared to that of untreated samples. This indicated a marked inhibition of synthesis of chloroplast-encoded proteins, in accordance with previous observations showing that SM-induced inhibition of *de novo* synthesis of chloroplast-encoded proteins was in correlation with an increase of the inhibition rate in several SM-treated plant samples.[26]

The prolonged (6 h) PA application caused more pronounced inhibition of *de novo* protein synthesis during recovery in both of the strains of *C. punctulatum* (Fig. 4a, b), compared to that of the shorter treatment (1 h). Interestingly, 6 h PAB application caused a smaller difference in the *de novo* protein synthesis in the high-mountain strain of *C. punctulatum* (when compared to 6 h PA, NF and AB treatments, Fig. 4a), indicating rather low damages and a low *de novo* protein synthesis caused by the moderate UVBR applied. In contrast, 6 h PAB treatment seemed more stressful than PA for the polar strain of *C. punctulatum* (Fig. 4b), as judged from the higher depression of *de novo* protein synthesis in SM-treated samples under PAB. Yet, recovery after the 6 h NF treatment demanded an intensive *de novo* protein synthesis in both strains of this cosmopolitan species, as concluded from a high difference between Fv/Fm of SM-treated and untreated samples during the recovery period. The typical tropical species, *C. beatum*, showed markedly high Fv/Fm depressions of SM-treated samples compared to that of untreated samples under all of UVR treatments (Fig. 4c), thus pointing to the distinctly high chloroplast-encoded protein synthesis during recovery. On the contrary, the arctic species, *C. crenatum*, displayed minor Fv/Fm inhibitions in SM-treated samples compared to that of untreated samples, for all of the treatment times and UVR spectral combinations (Fig. 4d). This

pointed to a noticeably small degree of chloroplast-encoded *de novo* protein synthesis in *C. crenatum* after the photoinhibitory UVR influence.

The application of SM to samples acclimated at 7°C and treated under UVR treatments caused no significantly stronger decrease of Fv/Fm compared to that of untreated samples, in *C. beatum* and both of the strains of *C. punctulatum*. This observation indicated that limited *de novo* protein synthesis occurs at the low temperature in these strains, whereas higher *de novo* protein synthesis rates were observed in *C. crenatum* (data not shown).

UVR absorption by isolated mucilaginous sheaths of the Cosmarium strains

All of the *Cosmarium* strains grown at 21°C exhibited a relatively low UVR absorption by their mucilaginous envelopes in the range 280 – 400 nm, pointing to an insignificant role in the protection from UV radiation (shown for 280, 320 and 380 nm, Fig. 5). Absorption was larger in the UVB range (280 – 320 nm), while it decreased greatly towards longer wavelengths. The thickness of mucilaginous layers in all of the *Cosmarium* strains treated under photoinhibitory PAR intensities (700 and 1200 μmol photons m^{-2} s^{-1}) slightly increased (data not shown) consequently leading to an increase of the UVR absorption.

Main one-way ANOVAs demonstrated statistically significant absorption of UVR by isolated desmid sheaths taking into account all PAR and UVR spectral combinations, for all of the strains and wavelengths (not shown). The increase of UVR absorption was significant during 1200 μmol photons m^{-2} s^{-1} treatment for all wavelengths, in all *Cosmarium* strains studied (Tukey HSD test, $p < 0.05$). Additionally, PAB treatment caused only a slightly higher UVR absorption compared to that of control samples (significantly increased in *C. beatum* for 280 nm), while AB treatment appeared detrimental to the mucilage development in all of the *Cosmarium* strains leading to a significant decrease of the UVR absorption. The UVR absorption by mucilaginous shields of all *Cosmarium* strains acclimated at 7°C and treated under PAR, PA and PAB was somewhat larger when compared to that at 21°C (data not shown).

Discussion

Consistently with the defined geographic distribution patterns of the *Cosmarium* strains regarding temperature or PAR regimes,[28,36] the impact of experimentally applied UVR on desmid physiology furthermore revealed that the *Cosmarium* strains might prefer specific ecological niches regarding the prevailing UV-radiation conditions. In general, UVR exerted an additional stress on the photosynthetic apparatus of the *Cosmarium* strains when compared to the moderate PAR intensity (700 µmol photons m^{-2} s^{-1}). Both short- and long-term applications of UVA radiation (PA treatment) at 21°C caused a twofold larger depression of maximum quantum yield compared to that under the moderate PAR treatment, in all of the investigated *Cosmarium* strains. Although the measurable effects of both PAR and UVR in the reduction of photosynthetic efficiency are similar, UVA radiation was found to be damaging to PSII by decreasing the electron flow from reaction centers to plastoquinone affecting electron transport both at the water-oxidizing complex and the binding site of the Q_B quinone electron acceptor.[45,46] Pronounced photoinhibition, as observed in the desmid samples exposed under PA, could be of ecological relevance since the intensity of UVA spectral range in the natural sunlight is at least 10 times higher than UVB, and UVA is not attenuated by the ozone layer.[20,47,48] Studies performed with Antarctic and high-mountain phytoplankton have demonstrated that at least half of the damage caused by solar radiation between 290 and 400 nm is induced by the UVA range.[6,20] The addition of a moderate UVB radiation (0.89 W m^{-2}; PAB treatment) by a WG295 cut-off filter, imitated the UVBR:PAR ratio which is comparable to that of temperate climate zones.[21,23,27,49] Generally, this treatment did not provoke larger damage to PSII compared to that during PA in all of the *Cosmarium* strains studied. Although UVBR has stronger detrimental effects on the photosynthetic apparatus than UVAR,[50] protein repair capacity in intact cells may be enhanced when UVB is accompanied by a moderate intensity of visible light and provides protection against photodamage.[48,51–53] This UVBR-induced positive effect becomes non significant at high light intensity characteristic of strong sunlight.[54]

Interestingly, moderate UVB radiation caused ameliorating effect to photosynthesis of the tropical species, *C. beatum*, as demonstrated by the lower Fv/Fm recovery kinetics under PA- compared to PAB-treatment.[21] So far, this phenomenon was noted in tropic marine macrophytes that had been previously adapted to a high UV environment and the studies were conducted at high PAR and UV ratio.[21–23] Possibly, the UVBR ameliorating effect may have a large ecophysiological significance for algae inhabiting circumtropical areas, which receive high amounts of UVB radiation, as it seems that moderate UVBR induces or it is even involved in the repair mechanism during the high solar irradiation.[21] Máté and co-workers noted that the UVB-induced transcription of *PsbA* genes, which encode the D1 protein, appeared in microalgae and, hence, might explain the intensive recover capacities in some tropical algae.[55] In addition to our study and observations on high-light adapted macrophytes, positive effects of intermediate fluxes of UVBR on some Antarctic microalgae have been observed,[56] which pointed that this interesting phenomenon should be thoroughly investigated. However, unfiltered radiation from a sun simulator has a considerably high UVBR intensity (1.98 W m^{-2}) and a high UVBR:PAR ratio (Table 2), which reached more than a twofold value compared to that of temperate or (sub)tropic zones.[21,27,49,57] This treatment appeared as exceedingly detrimental for all of the *Cosmarium* strains studied, as judged from the drastic decrease of Fv/Fm, causing an incomplete recovery after 24 h. UVBR can cause degradation of D1/D2 heterodimer,[58] direct molecular damage by absorption by aromatic and disulfide-containing biomolecules,[59] DNA lesions[60] and induction of reactive oxygen species,[61] and so apparently this treatment may provoke multiple damages to the *Cosmarium* cells.

Streptomycin binds to the small 16S rRNA of the 30S subunit of the prokaryotic ribosome which leads to codon misreading and inhibition of protein synthesis;[62] hence, SM is commonly used as an inhibitor in the estimation of the turnover of the chloroplast-encoded protein synthesis (principally D1 protein).[27,44] Schnettger and co-workers demonstrated that blocking of the D1 protein synthesis by SM in high-light treated plants leads to a substantial increase in photoinhibition (estimated by means of chlorophyll fluorescence) and to net loss of D1 protein.[26] Interestingly, our study showed that SM exhibited relatively weak action at the beginning of recovery after UVR

treatments, while the SM action was the highest after 24 h of recovery. This indicates that SM may have an influence on the *de novo* synthesis of proteins damaged by reactive oxygen species (ROS), which may be produced during the UVR treatments, similarly as it has been observed after the application of photoinhibitory PAR.[36]

The high-mountain, tropical strain of the cosmopolitan species, *C. punctulatum*, displayed a reliance on high rates of *de novo* chloroplast-encoded protein synthesis after the prolonged PA, NF and AB treatments at 21°C. It is known that phytoplankton species of high-mountain lakes situated in circumequatorial region are well adapted to solar UVR as a result of the high radiation fluxes received at the high-altitude, low-latitude environment[6] and possess fairly developed DNA- and photosynthesis-repair mechanisms.[63,64] The NF treatment decreased *de novo* protein synthesis in the polar strain of *C. punctulatum* at a higher rate than in the high-mountain one, thereby revealing the damaging effects of the strong UVBR both to the PSII complex and gene expression.[50,65]

The typical tropical species, *C. beatum*, displayed the exceedingly high rates of *de novo* protein synthesis after all of the UVR treatments at 21°C, which was in accordance with the fact that plant and algal species adapted to high-light intensities (such as numerous tropical plants) possess exceedingly high rates of D1 turnover and *de novo* protein synthesis.[25,27,36] The high resistance to UV radiation, accompanied with strong DNA- and PSII-repair mechanisms is well-known characteristics of numerous macroalgae growing in (sub)tropic areas.[66–69] On the contrary, the arctic species, *C. crenatum*, exhibited a noticeably low reliance on chloroplast-encoded protein synthesis under all of the UVR spectral combinations applied, in accordance with what was observed for shade-plant strategists and polar macroalgae.[25,70] Protein synthesis might represent a large burden for polar macro- and microalgae since low temperatures slow down the PSII repair cycle, as judged from retarded D1 protein degradation upon photoinhibition.[71–73] Moreover, *C. crenatum* exhibited by far the highest resistance under all of the UVR treatments applied at 7°C, which additionally confirmed its fair acclimation at low temperatures. In contrast, UVR applied at low temperature (7°C) appeared lethal to the tropical species, *C. beatum*, and caused severe damages to photosynthesis of both strains belonging to the cosmopolitan species, *C. punctulatum*. Therefore, all of the desmid strains studied

demand a relatively high temperature for the complete recovery after UVR treatments, taking into account that an increase in temperature results in faster turnover of the D1 protein,[7,9,74,75] and in accordance with the noted preference of desmids to warm temperatures.[28,76]

Surprisingly, the *Cosmarium* strains studied do not place a high reliance on the screening of UVR by means of mucilaginous sheaths. Hence, this study refuted earlier assumptions that the production of vast amounts of slime may elicit protective function leading to UVR tolerance in desmids.[17,18] So far, the only evidence on a UVR-screening compound in desmids has been revealed in the typical arctic-alpine taxon, *Mesotaenium berggrenii* (Wittrock) Lagerheim, in the form of brownish vacuolar pigment of tannin nature.[75] Yet, the *Cosmarium* strains obviously produced no UVR-screening compounds inside cells or as components of mucilaginous layers, although mycosporine-like amino acids (MAAs) have been detected in some Streptophycean algae.[78] Moreover, strong UVBR accompanied by moderate or low PAR intensities (i.e. high UVBR:PAR ratio) ceased the production of mucilaginous layers in all of the desmid strains, as mucilaginous layers detached from cells which were treated under strong UVBR (data not shown). It is known that UVBR may cause severe damages of secretory organelles (dictyosomes and ER cisternae), as observed in *M. denticulata*,[17] leading to the decrease of mobility of desmids and reducing their ability to escape from influences of enhanced solar radiation.[16,79]

Correlations between rates of the gross oxygen evolution and maximum quantum yield were significantly positive during application of all UVR spectral combinations. Turcsányi and Vass noted that the time course of variable fluorescence of isolated spinach thylakoids is much less affected by UVA than oxygen evolution;[46] similarly to that observed with UVB radiation and opposite to that seen under photoinhibition by visible light.[80] The discrepancy between these investigations and our study may indicate that moderate UVAR and UVBR applied during this study (i.e. PA and PAB treatments) did not have a significant influence on respiration rates (also observed by Teramura and co-workers[51]), and/or damages of the electron transport occurred, considering that variable fluorescence reflects the capacity of PSII to reduce Q_A and the PQ pool.[46] The gross oxygen measurement is widely used *in situ* investigations on microalgae, where the photoinhibition of various kelp species was

estimated under unfiltered solar radiation and in most cases a correlation between Fv/Fm (and/or ΔFm/Fm') and gross photosynthesis at different levels of photoinhibition was demonstrated.[81–83] The intensive UVBR from the unfiltered spectrum of the sun simulator caused a drastic decrease of oxygen evolution in all of the investigated *Cosmarium* strains, indicating that desmids are noticeably sensitive to high UV radiation. Considering that each 1% reduction in ozone layer causes an increase of 1.3–1.8% in UVBR reaching the biosphere,[84] the amount of UVBR reaching the earth's surface may be increased in polar regions due to the thinning of the ozone layer.[30,85–87] This may have particularly negative consequences for desmids growing hemi-atmophytically on wet surfaces of moss-cushions and hummocks of arctic and subarctic peat bogs (where they can be directly exposed to solar radiation) as this study revealed large detrimental effects of UVBR combined with cold temperature. Given that desmids have a precious role as primary producers in peat bogs, which can be completely anoxic at the depth of a few centimetres,[88] damages of such ecosystems may occur as a consequence of the increased UVR.

In contrast to the high sensitivity of the *Cosmarium* strains studied under the prolonged AB and NF spectral combinations, cells of *M. denticulata* demonstrated a significant resistance *in vitro* against strong UVBR.[18] It is worth emphasizing that *M. denticulata* was cultured in a diluted 'desmid medium' with soil extract[89] which possibly possessed some absorption in the UVBR range, while L-d medium (as a purely mineral medium) demonstrated no absorption in the UVR range. Taking into account that water of desmid natural habitats (peat bogs, fens, marshes, puddles, and ponds) contains a vast amount of dissolved organic compounds and particles which may greatly attenuate UVR penetration, desmids can be fairly protected in deeper water layers of such habitats – which may explain the sensitivity of the *Cosmarium* strains under high UVBR in laboratory conditions. Furthermore, the long-term acclimation in laboratory conditions (i.e. no UVR stress applied) may increase the sensitivity of the *Cosmarium* strains to UVR, as it is known that some algae are capable to acclimate under moderate UVR intensities.[90–93] Yet, strain- and species-specific differences were displayed under a set of experimentally applied UVR spectral combinations at both temperature

grades, confirming that such responses are genotipically preserved and expressed despite the long-term cultivation.

Conclusions

To the authors' knowledge this is the first comparative study on the influences of UVR as a climatic factor on possible geographic distribution patterns of desmid strains, as judged from their physiological responses *in vitro* conditions. Numerous studies on the UVR-induced geographic and depth zonation of seaweeds have been done, revealing consistent distribution models for the most of macroalgae taking into account all their life stages.[1] Comparably, our study revealed that microalgae are capable to occupy specific geographic areas in relation to prevailing UVR conditions, which additionally contributed to the negation of a hypothesis on the global dispersion of microorganisms.[94–96] With the exception of the high-mountain strain of the cosmopolitan taxon, *C. punctulatum* var. *subpunctulatum*, all of the *Cosmarium* strains studied displayed a high sensitivity under the strong UVB-radiation, which may indicate consequences to the primary production in circumpolar peat-containing ecosystems (as typical habitats of desmids), due to the increase of UVB-radiation. Unexpectedly, the *Cosmarium* strains do not place a high reliance on the UVR screening by the well-developed mucilaginous layers which envelop cells, in contrast to many cyanobacteria.[97]

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806 **Table 1.** Data on the *Cosmarium* strains used for the investigation of photosynthetic behaviour under
807 UVR spectral combinations, at 7 and 21°C. MZCH – Microalgae and Zygnematophyceae Collection
808 Hamburg.

Climate zone	Taxon	No. strain (MZCH)	Sampling area	Locality coordinates and yearly mean of daily irradiation in UV (280–400 nm) (J cm^{-2})[1]	Year of isolation
Alpine, tropical zone	*C. punctulatum* Brébisson var. *subpunctulatum* (Nordstedt) Børgesen	570	pool on Mt. Cotopaxi, 1600 m a.s.l., Ecuador	00°40′S 78°26′W ~ 160	1996
Lowland, polar zone	*C. punctulatum* var. *subpunctulatum*	571	pool near Skarsvåg, 80 m a.s.l, the North Cape, Norway	71°06′N 25°49′ E ~ 25	1992
Tropical	*C. beatum* W. & G.S. West	533	marshy area nearby Ol Bolossat Lake, Kenya	00°09′S 36°26′E ~ 175	2001
Polar	*C. crenatum* Ralfs var. *boldtianum* (Gutwinski) W. & G.S. West	561	Cape Flora, Northbrook Island, Franz Joseph Land, Russia	79°57′N 50°05′E ~ 20	1995

809 [1] Copyright: Mines ParisTech / Armines 2008 (www.soda-is.com/eng/index.html).
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Table 2. Irradiation conditions of the different spectral ranges of the sun simulator with the optical filters WG295, WG320, GG400 and UG5, and without filters.

Filter	Radiation condition	PAR (µmol photons m^{-2} s^{-1})	PAR (W m^{-2})	UVA (W m^{-2})	UVB (W m^{-2})	Ratio PAR:UVA:UVB
GG400	PAR	700	152.2	0.1	0.00	100 : 0.07 : 0
WG320	PA	699	152	27.5	0.23	100 : 18.1 : 0.15
WG295	PAB	700	152.2	28.7	0.89	100 : 18.9 : 0.58
UG5	AB	32	6.9	24.9	1.34	1 : 3.6 : 0.2
no filter	NF	707	153.7	28.9	1.98	100 : 18.8 : 1.28

838 **Table 3.** Tests of between-subjects effects of MANOVA and interactions of radiation treatment
839 (spectral irradiance compose of P, PA, PAB, AB, and NF) and exposure time on photosynthetic
840 efficiency of the *Cosmarium* strains studied, grown at 21°C and 30 µmol photons m^{-2} s^{-1}. df – degrees
841 of freedom (for the effect of the model), F – F ratio, p – significance (* – significant; ns – not
842 significant).

Strain	Source of variation	df	F-value	p-value
C. punct. (570)	Spectral irradiance (*A*)	4	914.2	<0.001*
	Exposure time (*B*)	2	87.9	<0.001*
	*A*B*	8	1.1	0.104^{ns}
C. punct. (571)	Spectral irradiance (*A*)	4	218	<0.001*
	Exposure time (*B*)	2	175.5	<0.001*
	*A*B*	8	186.7	<0.001*
C. beatum	Spectral irradiance (*A*)	4	100.7	<0.001*
	Exposure time (*B*)	2	96.4	<0.001*
	*A*B*	8	1.3	0.218^{ns}
C. crenatum	Spectral irradiance (*A*)	4	161.5	<0.001*
	Exposure time (*B*)	2	80.9	<0.001*
	*A*B*	8	93.8	<0.001*

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Table 4. Tests of between-subjects effects of MANOVA and interactions of radiation treatment (spectral irradiance compose of P, PA, PAB, and AB) and exposure time on photosynthetic efficiency of the *Cosmarium* strains studied, acclimated at 7°C and 30 µmol photons m^{-2} s^{-1}. df – degrees of freedom (for the effect of the model), F – F ratio, p – significance (* – significant; ns – not significant).

Strain	Source of variation	df	F-value	p-value
C. punct. (570)	Spectral irradiance (*A*)	3	176.3	<0.001*
	Exposure time (*B*)	2	124	<0.001*
	*A*B*	6	96.9	<0.001*
C. punct.(571)	Spectral irradiance (*A*)	3	87.6	<0.001*
	Exposure time (*B*)	2	99	<0.001*
	*A*B*	6	104.3	<0.001*
C. beatum	Spectral irradiance (*A*)	3	36.2	0.217^{ns}
	Exposure time (*B*)	2	8.7	0.163^{ns}
	*A*B*	6	11.6	0.181^{ns}
C. crenatum	Spectral irradiance (*A*)	3	90.8	<0.001*
	Exposure time (*B*)	2	101.1	<0.001*
	*A*B*	6	163.6	<0.001*

870 **Table 5.** Average O_2 production of the *Cosmarium* strains studied, grown at 21°C or acclimated at
871 7°C (30 µmol photons m^{-2} s^{-1}). Standard deviations (SD) are less than 5% of mean (n = 3).

Average O_2 production ($\mu MolO_2$ mg Chl^{-1} min^{-1})				
Temperature	*C. punct.* (570)	*C. punct.* (571)	*C. beatum*	*C. crenatum*
21°C	2.57	2.43	2.48	3.49
7°C	1.32	1.6	0.28	2.87

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894 **Figure Legends**

895 **Fig. 1.** Inhibition and time-series recovery in the mean of maximum quantum yield (Fv/Fm) of the
896 *Cosmarium* strains collected from various geographic areas, after exposure to photosynthetically
897 active radiation (700 μmol photons m^{-2} s^{-1}, PAR), PAR + UVA (PA), PAR + UVA + moderate UVB
898 (PAB), UVA + UVB (AB) and unfiltered radiation (NF) during different treatment times (1, 4, and 6
899 h) at 21°C, expressed as percentages of disturbed controls. (a) *C. punctulatum* No. 570, (b) *C.*
900 *punctulatum* No. 571, (c) *C. beatum*, (d) *C. crenatum*. Controls were untreated samples cultured at
901 21°C and 30 μmol photons m^{-2} s^{-1} (Fv/Fm values are shown for each strain). □ –700 μmol photons m^{-2} s^{-1}, ○ – PA, ▲ – PAB, × – AB, ■ – NF. Standard deviations (SD) are less than 10% of mean (n =
903 9); not shown for clarity.

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905 **Fig. 2.** Inhibition and time-series recovery in the mean of maximum quantum yield (Fv/Fm) of the
906 *Cosmarium* strains collected from various geographic areas, acclimated and treated at 7°C. (a) *C.*
907 *punctulatum* No. 570, (b) *C. punctulatum* No. 571, (c) *C. beatum*, (d) *C. crenatum*. Controls were
908 untreated samples acclimated at 7°C and 30 μmol photons m^{-2} s^{-1} (Fv/Fm values are shown for each
909 strain). □ –700 μmol photons m^{-2} s^{-1}, ○ – PA, ▲ – PAB, × – AB. Standard deviations (SD) are less
910 than 10% of mean (n = 9); not shown for clarity.

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912 **Fig. 3.** Relationships between gross oxygen evolution rates and Fv/Fm values (expressed as % of
913 control samples) regarding inhibitions under PA, PAB, AB and NF spectral combinations at 21°C, for
914 all of the *Cosmarium* strains: (a) PA (b) PAB (c) AB (d) NF treatment. ○ – *C. punctulatum* No. 570,
915 ● – *C. punctulatum* No. 571, △ – *C. beatum*, ◇ – *C. crenatum*. Pearson correlation coefficients (r)
916 for each strain are given in panels which represent different spectral treatments.

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918 **Fig. 4.** Differences in Fv/Fm between untreated samples and SM-treated *Cosmarium* samples
919 (expressed as percents of controls) during the period of recovery after all of the UVR spectral

combinations (PA, PAB, NF, and AB), at 21°C. (vertical bars – SD, n = 3). (a) *C. punctulatum* No. 570, (b) *C. punctulatum* No. 571, (c) *C. beatum*, (d) *C. crenatum*.

Fig. 5. Absorption of UVA and UVB radiation (280, 320 and 400 nm) by isolated mucilaginous envelopes of the *Cosmarium* strains treated 6 h under PAR (700 and 1200 µmol photons m^{-2} s^{-1}), PAB or AB spectral combinations, at 21°C. (a) *C. punctulatum* No. 570, (b) *C. punctulatum* No. 571, (c) *C. beatum*, (d) *C. crenatum*. Vertical bars are SDs; n = 3. Asterisks represent significant changes of the UVR absorption by desmid mucilaginous sheaths isolated from PAR, PAB and AB treated samples, compared to control samples (30 µmol photons m^{-2} s^{-1}) (Tukey HSD test, $p < 0.05$).

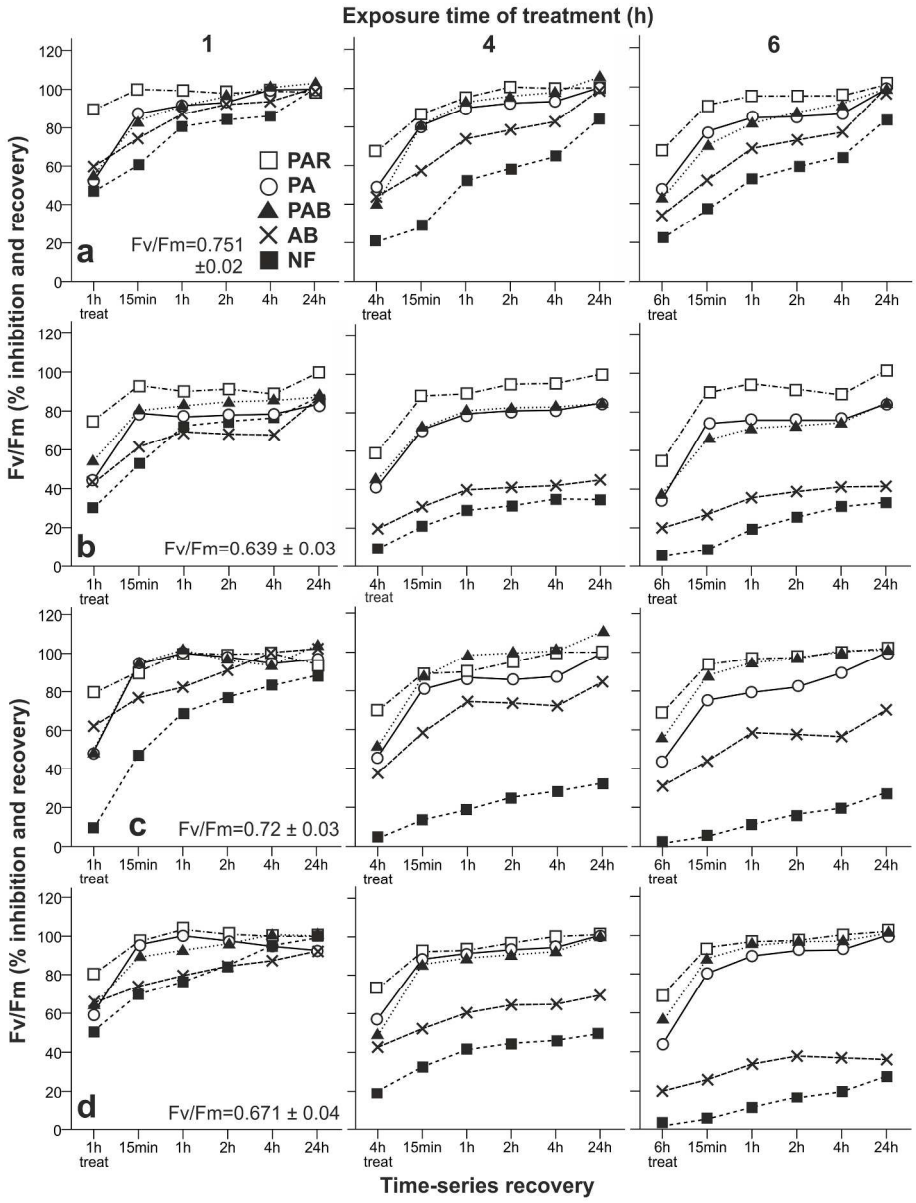

Figure 1
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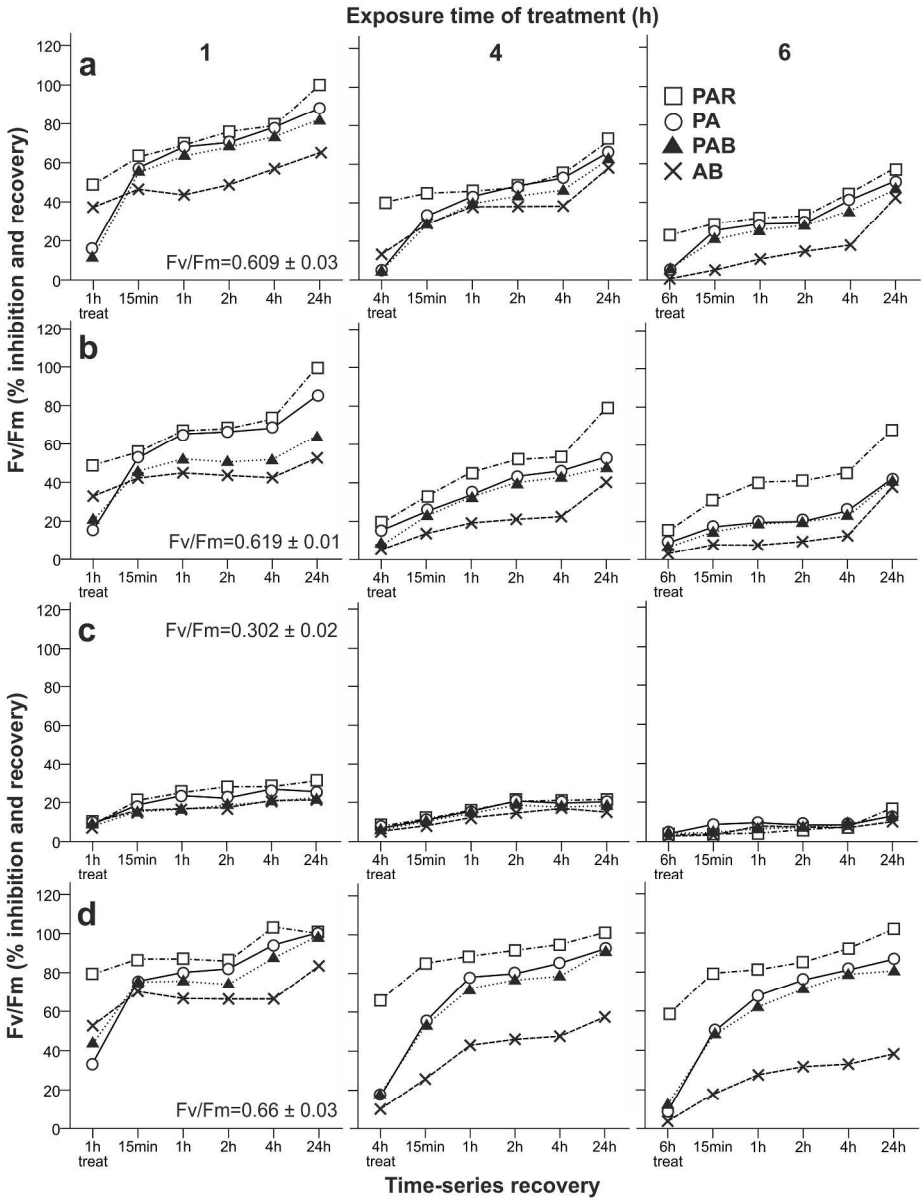

Figure 2
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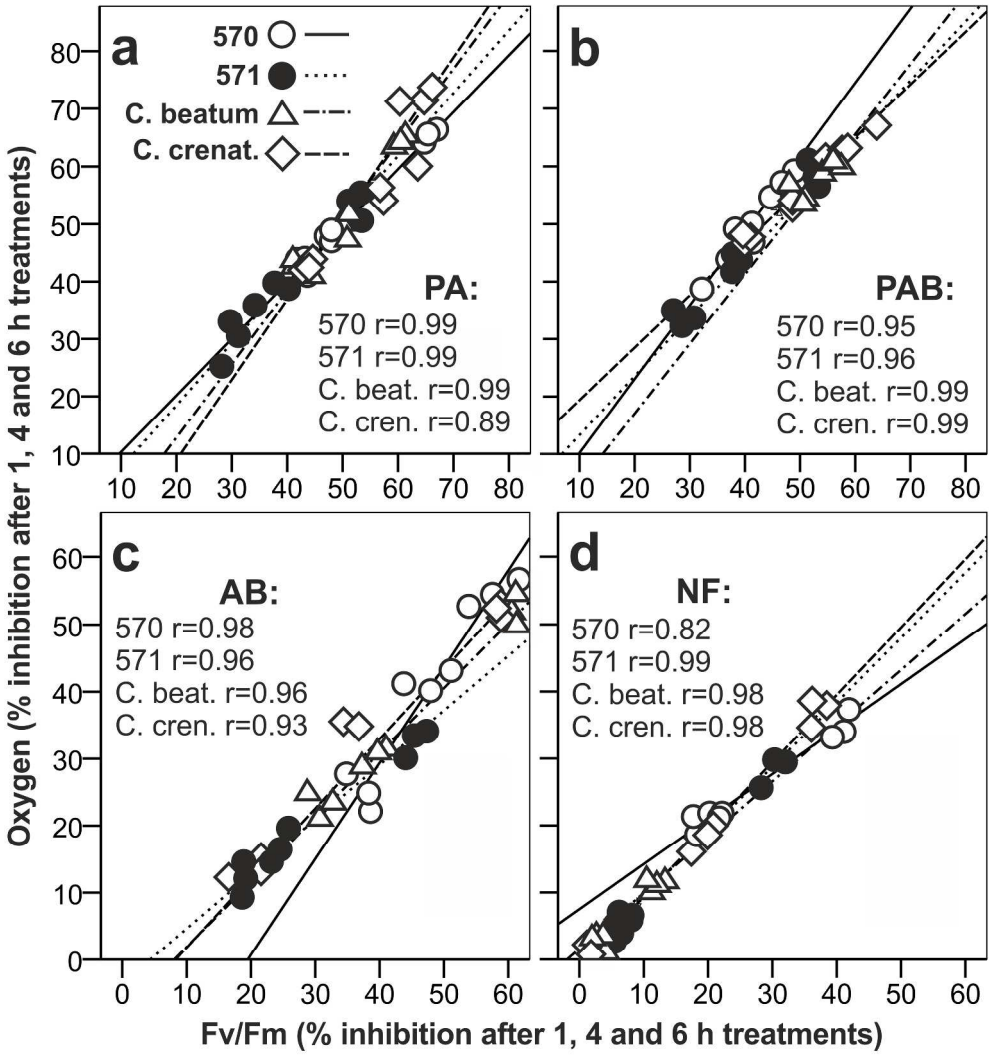

Figure 3
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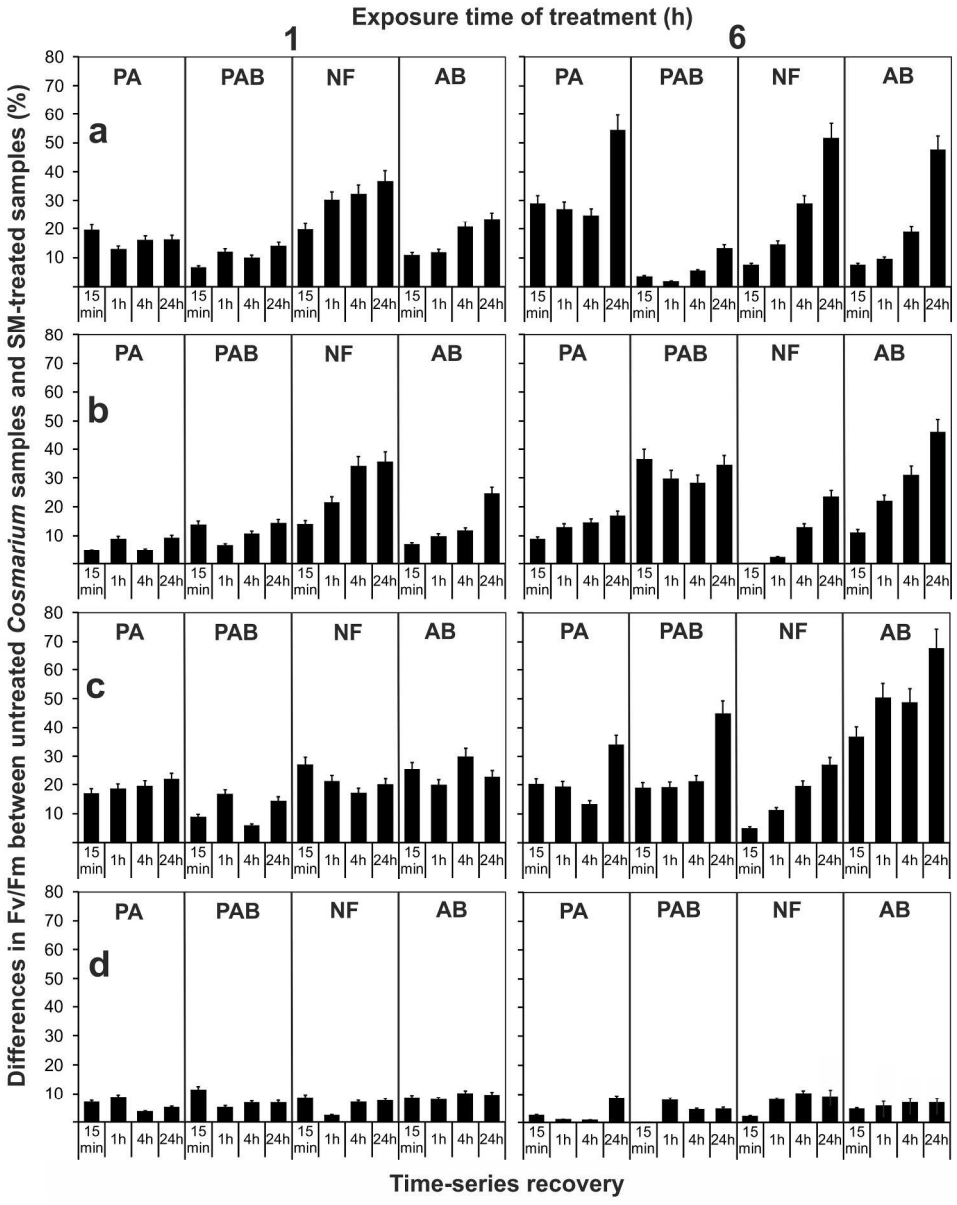

Figure 4
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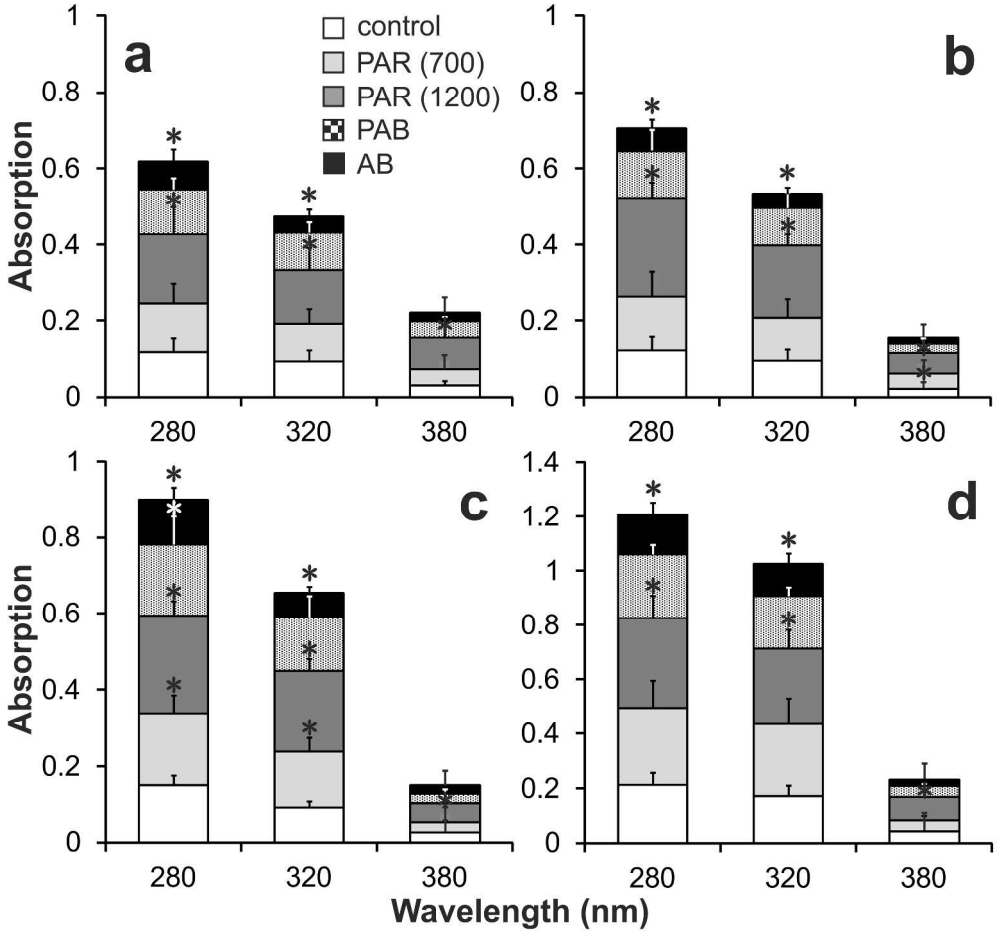

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