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Impacts of temperature regimes and radiation (PAR and UVR) conditions on the possible geographic distribution of desmids

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LIST OF ABBREVIATIONS

1 – qP	excitation pressure, redox poise
Ax (A)	antheraxanthin
CAP	chloramphenicol
Chl	chlorophyll
DTT	dithiothreitol
EPS	epoxidation state
ER	endoplasmic reticulum
Fv/Fm	maximum potential quantum yield of PSII
HPLC	high performance liquid chromatography
HS	heat shock
HSG	heat shock granules
HSP	heat shock proteins
I_k	saturating irradiance
NF (no filter)	unfiltered radiation of a sun simulator
NPQ	non-photochemical quenching
PA	PAR+UVA
PAB	PAR+UVA+UVB
PAR	photosynthetically active radiation (=P)
PCD	programmed cell death
PFR	photon flux (fluence) rate
P-I curve	photosynthesis-irradiance curve
Pmax	maximum rate of photosynthesis
PQ	plastoquinone
PSII	Photosystem II
Q_A	primary electron acceptor
qP	photochemical quenching
$rETR_{max}$	maximum relative electron transport rate
ROS	reactive oxygen species
Rubisco	ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO)
S/V	surface area to volume ratio
SM	streptomycin
UVAR (UVA)	ultraviolet-A radiation
UVBR (UVB)	ultraviolet-B radiation
UVR	ultraviolet radiation

V+A+Z	xanthophyll pool size
VDE	violaxanthin de-epoxidase
VIS	visible light
Vx (V)	violaxanthin
ZEP	zeaxanthin epoxidase
Zx (Z)	zeaxanthin
α	slope of P-I curve, photosynthetic efficiency
λ	wavelength
μ	growth rate

SUMMARY

This study deals with the potential influence of temperature and radiation (photosynthetically active radiation – PAR and ultraviolet radiation – UVR) conditions on patterns of geographic distribution of six *Cosmarium* strains isolated from various climate zones that were grown long-term at constant and relatively low light-temperature regime (16°C and 30 µmol photons m^{-2} s^{-1}): *C. crenatum* var. *boldtianum*– polar climate (Cape Flora, Franz Joseph Land, Russia), *C. punctulatum* var. *subpunctulatum* (SVCK No. 571) – lowland, polar climate (Skarsvåg at 80 m a.s.l, the North Cape, Norway), *C. regnesii* var. *polonicum* – continental (microthermal) climate zone (pool in East Hokkaidō, Japan), *C. meneghinii* – continental (microthermal) climate zone (fountain in Prague, the Czech Republic), *C. punctulatum* var. *subpunctulatum* (SVCK No. 570) – alpine, tropical area (pool on Mt. Cotopaxi at 1600 m a.s.l., Ecuador; 'highlands' subgroup of the temperate (mesothermal) climatic group), *C. beatum* – tropical climate zone (marshy area near Ol Bolossat Lake, Kenya).

The algae were treated under a set of various radiation and temperature conditions *in vitro* in order to estimate their possible geographic distribution patterns according to the obtained physiological responses and resistance during various stress experiments. All of the *Cosmarium* strains investigated were treated under a set of various temperature regimes (0.6, 7, 16, 21, 25, 28, 32, 35, and 37°C) which cover a broad, biologically relevant range (from 0 to 40°C, or 50°C), under a constant light regime (30 µmol photons m^{-2} s^{-1}). Various growth parameters were assessed during the logarithmic growth of algal cultures: growth rate (µ), division time per day, generation time, cell volume, and surface area to volume (S/V) ratio. In addition, the photosynthetic parameters were measured as a variable fluorescence of PSII: maximum quantum yield (Fv/Fm), maximum relative electron transport rate ($rETR_{max}$), electron transport efficiency (slope of P-I curve – α), and saturating irradiance for electron transport (I_k). The values of nonphotochemical quenching (NPQ) and photochemical quenching (qP) as well as the estimation of PSII excitation pressure (1–qP) were derived from P–I curves, which were constructed during the temperature gradient experiments. Ultrastructural changes of the *Cosmarium* cells during and after the low-temperature stress (at 0.6°C) or the high-temperature stress (at 35°C) were observed by means of transmission electron microscope (TEM) images.

Four types of cut-off filters were used to obtain specific radiation conditions: photosynthetically active radiation (PAR 400–700 nm), PAR + UV-A radiation (i.e. PA, UV-A 320–400 nm), PAR + UV-A + UV-B (PAB, UV-B 280–320 nm), UV-A + UV-B (AB), or unfiltered radiation of a sun simulator (NF, no filter). To investigate effects of photoinhibitory PAR on the photosynthetic efficiency of the *Cosmarium* strains the following light intensities were applied: 350, 700, 1200, and 2500 µmol photons m^{-2} s^{-1}. The photosynthetic efficiency (Fv/Fm) and the content of photosynthetic pigments were measured in time-series during the photoinhibitory treatments (1, 4 and 6 h) and during recovery under 30 µmol photons m^{-2} s^{-1} (after 15 min, 1, 2, 4, and 24 h). In addition, rates of the total oxygen evolution were measured in synchrony with

measurements of Fv/Fm in order to observe effects of radiation treatments on total cell metabolism of the *Cosmarium* strains. The activity of violaxanthin de-epoxidase was assessed by an inhibitor dithiothreitol (DTT), estimated as differences in Fv/Fm between DTT-treated and untreated samples of the *Cosmarium* strains. To assay the influence of chloroplast-encoded protein synthesis on the sensitivity to photoinhibition, D-chloramphenicol (CAP) and streptomycin (SM) were added to samples before the photoinhibitory experiments started.

Remarkably, even after a long-term cultivation (more than 15 years) under constant ordinary cultivating conditions (16°C, 30 µmol photons m^{-2} s^{-1}) species- and strain-specific physiological differences were revealed and those were in accordance with the climate prevailing at their sampling sites. The arctic species, *C. crenatum*, exhibited the highest saturating irradiance and photosynthetic capacity under the unchanged, relatively low light-temperature conditions of a climate chamber, as typical for algae adapted to high light intensities. This characteristic was concomitant with what was observed for arctic microalgae exposed to high solar irradiance due to the albedo from snow and ice surfaces. Conversely, the ordinary conditions of a climate chamber appeared to be fairly low for the cultivation of the typical tropical species, *C. beatum*, as judged from the distinctly low Fv/Fm, NPQ, α, and growth rates. In addition, *C. beatum* showed the physiological characteristics which clearly suggested an adaptation to warm-water habitats, such as the highest optimum growth temperature (28°C), and considerable recovery percentage of growth rates and photosynthetic parameters after short-term heat shock treatments. Both *Cosmarium* strains collected from the polar region (*C. crenatum* and *C. punctulatum* No. 571) exhibited relatively high growth rates and photosynthetic parameters (Fv/Fm, $rETR_{max}$, and I_k) at temperatures lower than 21°C, while photosynthetic efficiency (α) increased as the temperatures declined.

Astonishingly, all of the *Cosmarium* strains investigated exhibited exceptionally high values of photosynthetic capacity ($rETR_{max}$: 150–320) and saturating irradiance (I_k: 800–1400 µmol photons m^{-2} s^{-1}) at 21°C, which repeatedly exceeded values recorded for other algae indicating that these desmid strains are adapted to high light. This fact, as well as the relatively high growth temperature optima for all of the *Cosmarium* strains (21–28°C), are further evidence in support of Coesel's hypothesis on the origin of desmids in the tropical zone. Interestingly, the *Cosmarium* strains demonstrated not only adaptive characteristics in accordance with the temperature prevailing at their sampling sites, but also with regard to their evolutionary origin. In addition, chloroplasts of all of the *Cosmarium* strains correspond to the sun-adapted type, which is concomitant with the statements that these strains are rendered as high-light adapted algae. The appearance of cubic membranes and increased number of plastoglobules may represent the first line in protection from reactive oxygen species induced by excessive temperatures, which is accompanied by the alteration of protein synthesis and the occurrence of stress granules in order to preserve the cell homeostasis during the heat influence. However, the prolonged stress of warm and cold temperatures may initiate programmed cell death, as observed in all of the *Cosmarium* strains.

In general, all of the *Cosmarium* strains studied exhibited a high resistance under the relatively high PAR intensities (350 – 1200 μmol photons m^{-2} s^{-1}) applied during the prolonged treatments. Remarkably, by the addition of inhibitors of chloroplast-encoded protein synthesis and an inhibitor of violaxanthin de-epoxidase it was demonstrated that the *Cosmarium* strains developed the 'sun- or shade-plant' protection strategies, in accordance with the climate of their sampling sites. *C. crenatum* and the polar strain of *C. punctulatum* exhibited rather the 'shade-plant strategy' – to suffer some photoinhibition, but acquire increasing protection from photoinhibited PSII centres, while *C. beatum* and the high-mountain strain of *C. punctulatum* displayed the 'sun-plant strategy' – to counteract photoinhibition of PSII by a high rate of repair of photoinhibited PSII reaction centres and a high xanthophyll cycle turnover.

The composition of photosynthetic pigments of the *Cosmarium* strains investigated grown at optimal laboratory conditions (21°C, 30 μmol photons m^{-2} s^{-1}) basically corresponded to that of high-light plants, taking into account the relatively high content of violaxanthin and β-carotene, and xanthophyll-cycle pool size. Strikingly, this study unveiled two different mechanisms of the xanthophyll cycle in the *Cosmarium* strains collected from the polar region, as adaptations to occasional high irradiances which may occur due to the albedo. *C. crenatum* possesses a fast violaxanthin/antheraxanthin turnover, so far known only in some Prasinophycean representatives and in a few arctic-alpine Chrysophyta. Antheraxanthin actively participated in the heat dissipation from PSII in *C. crenatum*, as concluded from the significant negative correlation between maximum quantum yield and antheraxanthin content expressed per xanthophyll-cycle pool. The polar strain of *C. punctulatum*, however, displayed a complete and intensive violaxanthin de-epoxidase action, as judged from the distinctly high production of zeaxanthin during the high light treatment.

It seemed that moderate ultraviolet-B radiation (UVBR) did not exert large damages to PSII in all of the *Cosmarium* strains investigated, compared to UVAR (PA) treatment at 21°C. Strikingly enough, an ameliorating effect of UVBR at 21°C was observed in the tropical species, *C. beatum*, as concluded from higher rates of recovery of maximum quantum yield after the moderate UVBR treatment in comparison to that after only UVAR application. However, high UVBR:PAR ratio appeared as exceedingly detrimental for all of the *Cosmarium* strains studied, as concluded from the drastic decrease of Fv/Fm and total oxygen evolution in all of the *Cosmarium* strains studied, which may indicate the drastic consequences to peat bogs which are particularly poor in oxygen, as native habitats of desmids.

In conclusion, all of the *Cosmarium* strains investigated indeed exhibited the preference for specific ecological niches regarding radiation (PAR and UVR) and temperature regimes, as judged from their growth and photosynthetic behaviour, and ultrastructural changes during experiments applied *in vitro*. Various stressful temperatures and radiation conditions provoked the native physiological responses of the *Cosmarium* strains despite the long-term cultivation (more than 15 years) under constant and relatively low temperature-light conditions, thus these responses appeared as genetically preserved characteristics of the strains. Yet, the fair acclimation possibilities and the ability to undergo programmed cell death in order to save the population certainly favour the cosmopolitan distribution of the genus *Cosmarium*.

ZUSAMMENFASSUNG

Die weltweite geographische Verbreitung der Arten hängt u.a. von verschiedenen abiotischen Umweltfaktoren ab. Daher wurde der Einfluss bestimmter Temperatur- und Strahlungbedingungen (photosynthetische aktive (PAR) und ultravioletter Strahlung (UVR)) in Bezug auf die geographische Verteilung von sechs *Cosmarium* Arten aus verschiedenen Klimazonen untersucht. Diese wurden über etliche Jahre in der Kulturensammlung (SVCK) bei relativ niedrigen Temperatur- und Lichtverhältnissen (16°C and 30 µmol Photonen $m^{-2}s^{-1}$) angezogen: *C. crenatum* var. *boldtianum* – Polarklima (Cape Flora, Franz Joseph Land, Russland), *C. punctulatum* var. *subpunctulatum* (SVCK No. 571) – Flachland, Polarklima (Skarsvåg 80 m ü.*NN*, Nordkap, Norwegen), *C. regnesii* var. *polonicum* – Kontinentale (mikrothermale) Klimazone (Teich in East Hokkaidō, Japan), *C. meneghinii* – Kontinentale (mikrothermale) Klimazone (Prag, Tschechien), *C. punctulatum* var. *subpunctulatum* (SVCK No. 570) – Alpine, tropische Region (Teich, Mt. Cotopaxi auf 1600 m ü.*NN*, Ecuador; 'Hochland' Untergruppe der temperierten (mesothermalen) Klimazone), *C. beatum* – Tropische Klimazone (Marsch nahe Ol Bolossat Lake, Kenia).

Unterschiedliche Stressexperimente wurden durchgeführt, um die mögliche geographische Verteilung der Algen in Abhängigkeit von ihrer Physiologie und Stressresistenz zu ermitteln. Hierbei wurden die *Cosmarium* Arten einem breitem Temperaturspektrum (0.6, 7, 16, 21, 25, 28, 32, 35, und 37°C) unter zunächst konstanten Lichtbedingungen (30 µmol Photonen m^{-2} s^{-1}) ausgesetzt was einen Großteil des biologische relevanten Bereichs an ihren natürlichen Standorten abdeckt (0 bis 40°C oder 50°C). Unterschiedliche Wachstumsparameter (Teilungsrate (µ), Zellteilungen pro Tag, Verdopplungszeit, Zellvolumen und Oberflächen zu Volumen Verhältnis (S/V)) wurden während der exponentielle Wachstumsphase in Batch-Versuchen ermittelt, ebenso wie die photosynthetischen Parameter (maximaler Quanten-Ertrag (Fv/Fm), maximale relative Elektronen-Transport-Rate ($rETR_{max}$), Photosynthese-Effizienz (Steigung der P-I Kurve (α)) und den Sättigungspunkt der Elektronen-Transport-Rate (I_k). Die Werte des nicht-photochemischen „quenching/Löschung" (NPQ) und des photochemischen „quenching" (qP), wie auch die Abschätzung des Exzitations-Drucks auf PSII (1-qP) wurde durch ermittelte P-I Kurven bei verschiedenen Temperaturen errechnet. Bilder der Transmissions-Elektronen-Mikroskopie (TEM) wurden verwendet, um Veränderungen in der Ultrastruktur der *Cosmarium* Zellen während und nach dem Tieftemperatur-Stress (0.6°C) bzw. Hoch-Temperaturstress (35°C) zu untersuchen.

Vier Filtertypen ermöglichten unterschiedliche Strahlungsbedingungen: (PAR 400–700 nm), PAR + UV-A Strahlung (PA, UV-A 320–400 nm), PAR + UV-A + UV-B (PAB, UV-B 280–320 nm), UV-A + UV-B (AB) und ungefilterte Strahlung durch einen Sonnensimulator (kein Filter (NF)). Die PAR Intensitäten 350, 700, 1200, und 2500 µmol Photonen m^{-2} s^{-1} wurden verwendet, um den Photoinhibitionsanfälligkeit der *Cosmarium* Arten zu testen. Der maximale Quanten-Ertrag und die Konzentrationen an photosynthetischen Pigmenten wurde in Zeitreihen (1, 4 und 6 Stunden) währende der Lichtstress-Applikation und einer anschließenden Regenerationsphase (15 min, 1, 2, 4 und 24 Stunden) unter Schwachlichtbedingungen bei 30

µmol Photonen m^{-2} s^{-1} bestimmt. Zusätzlich wurde die Sauerstoffproduktionsrate parallel zu den Fluoreszenzwerten ermittelt, was Rückschlüsse auf die Effekte des gesamten Zellmetabolismus zuließ. Die Aktivität der Violaxanthin-Deepoxidase wurde aufgrund der Differenz zwischen den Fv/Fm Werten zwischen DDT behandelten und unbehandelten *Cosmarium* Arten bestimmt. Um die Sensitivität der Chloroplast-kodierten Proteinsynthese auf Photoinhibition zu testen, wurde D-Chloramphenicol (CAP) und Streptomycin (SM) vor der Lichtexposition zu den Proben gegeben.

Bemerkenswerter Weise wurde auch nach einer Langzeitkultivierung (für mehr als 15 Jahren unter konstanten Bedingungen (16°C, 30 µmol Photonen m^{-2} s^{-1})) art- und stammspezifische physiologische Unterschiede gezeigt, die auf die vorherrschenden klimatischen Bedingungen der ursprünglichen Fundorte hinweisen. Die arktische Art *C. crenatum* zeigte eine Adaptation an hohe Lichtintensitäten durch den höchste Sättigungspunkt und höchste photosynthetische Kapazität trotz der Vorkultur unter niedrigen Licht- und Temperaturbedingungen in der Klimakammer an. Dieses Charakteristikum entspricht dem von arktischen Mikroalgen, die an hohe solare Strahlungen bedingt durch den Albedo-Effekt von Schnee- und Eisflächen angepasst sind. Im Gegensatz dazu, waren die Klimakammerbedingungen der Vorkultur für die tropisch Art *C. beatum* verhältnismäßig zu niedrig, was die niedrigen Werte der Wachstumsrate, von Fv/Fm, NPQ sowie α bestätigen. Die Anpassung an Warmwasser-Habitate von *C. beatum* zeigte sich ebenfalls durch die höchste optimale Wachstums-temperatur (28°C) und durch die beträchtliche Erholung der Wachstumsrate und der photosynthetischen Parameter nach einer kurzzeitigen Hitzeschock-Behandlung. Die *Cosmarium* Arten der polaren Region (*C. crenatum* and *C. punctulatum* No. 571) zeigten relativ hohe Wachstumsraten und photosynthetische Parameter (Fv/Fm, $rETR_{max}$ und I_k) unterhalb einer Temperatur von 21°C, weil die Photosynthese-Effizienz (α) mit abnehmender Temperatur anstieg.

Erstaunlicherweise zeigten alle *Cosmarium* Arten bei 21°C außergewöhnlich hohe Werte der Photosynthese-Kapazität ($rETR_{max}$: 150–320) und des Sättigungspunktes (I_k: 800–1400 µmol photons m^{-2} s^{-1}), welche mehrfach die in der Literatur angegebenen Werte für andere Algen überstieg. Dies deutet darauf hin, dass die Desmidiaceen-Stämme an hohe Lichtintensitäten adaptiert sind. Dieser Befund, in Verbindung mit den relativ hohen optimalen Wachstumstemperaturen aller *Cosmarium* Arten (21–28°C), unterstützt Coesels Hypothese über den Ursprung der Desmidiaceen in tropischen Zonen. Interessanterweise sind sowohl charakteristische Adaptation an die Bedingungen des Fundortes als auch die des ursprünglichen Verbreitungsgebietes bei den Arten zu finden. Die Chloroplasten aller *Cosmarium* Arten konnten dem Sonnen angepasstem Typ zugeordnet werden, was ein weiteres Beweis dafür ist, dass die Algen starklichtadaptiert sind. Das Auftreten kubisch geformter Membranen und eine erhöhte Anzahl von Plastoglobulis könnte eine erste Schutzreaktion gegen reaktive Sauerstoffradikale darstellen, was wiederum durch hohe Temperaturen induziert sein könnte. Dieses wird begleitet durch eine Veränderung der Proteinsynthese und dem Auftreten von „Stress-Granula“, die die Zellhomöostase aufrechterhalten sollen. Jedoch könnte anhaltender Stress von hohen und tiefen Temperaturen auch zum programmierten Zelltod führen, wie es bei allen *Cosmarium* Arten beobachtet wurde.

Generell weisen aber alle *Cosmarium* Arten hohe Stressresistenz gegenüber den PAR Intensität (350–1200 µmol photons m^{-2} s^{-1}) während der Langzeitversuche auf. Durch Zugabe von Inhibitoren der Chloroplast-kodierten-Proteinsynthese und des Inhibitors der Violaxanthin-Deepoxidase wurde gezeigt, dass die *Cosmarium* Arten, entsprechend des Klimas ihrer Fundorte, Schutzstrategien der „Sonnen- oder Schattenpflanzen“ entwickeln. Während *C. crenatum* und die polare Art *C. punctulatum* eher die „Schattenpflanzen-Strategie“ verfolgen (geringere Photoinhibition, jedoch erhöhter Schutz durch die photoinhibierten PSII-Reaktionszentren), zeigen die tropische Art *C. beatum* und der Hoch-gebirgs-stamm von *C. punctulatum* eher die „Strategie der Sonnenpflanzen“, indem eine höhere Photoinhibition des PSII, begleitet von einer hohen Reparaturrate von photoinhibierten PSII-Reaktionszentren und einer hohen Kapazität des Xanthophyll-Zyklus, ausgeglichen wird.

Die Zusammensetzung der Photosynthesepigmente der *Cosmarium* Arten unter optimalen Laborbedingungen (21°C, 30 µmol photons m^{-2} s^{-1}) stimmte grundsätzlich mit denen von Starklicht-Pflanzen überein, da ein relativ hoher Gehalt an Violaxanthin, β-Carotin und ein großer Xanthophyll-Zyklus-Pools vorkommt. Diese Studie demonstriert das Vorkommen zweier unterschiedliche Mechanismen im Xanthophyll-Zyklus der *Cosmarium* Arten aus den polaren Region als Adaptation an plötzlich auftretenden hohe Strahlungsverhältnisse aufgrund des Albedos. *C. crenatum* zeigt einen schnellen Violaxanthin-Antheraxanthin Umsatz, soweit vergleichbar nur mit einigen Vertretern der Prasinophyceen und einigen arktisch/alpinen Chrysophyten. Antheraxanthin spielt bei *C. crenatum* eine aktive Rolle bei der Wärmedissipation am PSII, was sich aus der signifikant negativen Korrelation zwischen dem maximalen Quanten-Ertrag und dem Antheraxanthin-Gehalt im Xanthophyll-Zyklus-Pool ergibt. Der polare Stamm *C. punctulatum* weist dagegen eine vollständige und intensive Violaxanthin-Deepoxidase-Aktivität auf, was sich in der hohen Produktion von Zeaxanthin während der Starklichtexposition zeigte.

Die Ergebnisse bei 21°C deuten darauf hin, dass Ultraviolette-B-Strahlung (UVBR) das PSII aller *Cosmarium* Arten nicht so stark schädigt wie eine vergleichbare Bestrahlung mit UVAR (PA). Interessanterweise wurde für das tropische *C. beatum* bei 21°C ein abmildernder UVB-Effekt beobachtet, was sich auf eine höhere Regenerationsrate des PSII nach mäßiger UVB-Bestrahlung im Vergleich zur alleinigen Bestrahlung mit UVAR begründet. Jedoch hatte eine hohes Verhältnis von UVBR:PAR auch eine zerstörerische Wirkung auf alle untersuchten *Cosmarium* Arten was sich aus einer drastischen Verringerung des Fv/Fm und der Sauerstoffproduktion herleitete. Dies hätte drastische Folgen eines anthropogenen UV-Anstiegs auf das natürliche Verbreitungsgebiet der Desmidiaceen in Torfmooren, die besonders geringe Sauerstoffkonzentrationen aufweisen.

Abschließend kann gesagt werden, dass alle untersuchen *Cosmarium* Arten aufgrund ihres *in vitro* Wachstums- und Photosynthese Verhaltens und der Veränderung in der Ultrastruktur spezifische Anpassungen an die ökologische Nische in Bezug auf die Strahlungs- (PAR und UVR) und Temperaturbedingungen zeigten. Verschiedener Stress, der durch Temperatur und Bestrahlung ausgelöst wurde, bewirkte eine typisch natürliche physiologische Reaktion der *Cosmarium* Arten sogar nach langer

Kultivierungszeit (mehr als 15 Jahre unter konstant niedrigen Temperatur- und Lichtbedingungen), sodass diese Charakteristika als genetisch konserviert angesehen werden müssen. Die hohe Anpassungsfähigkeit und die Möglichkeit zum programmierten Zelltod, begünstigen die Aufrechterhaltung der Population und die globale Verbreitung der Gattung *Cosmarium*.

1. General introduction and thesis outline

1.1 A view on the geographic distribution of desmids

Desmids are a group of exclusively freshwater microalgae which are named so 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). Desmids are members of the algal class Zygnematophyceae, characterised by conjugation mediated sexual reproduction and the total absence of flagellated life cycle stadia (Palamar-Mordvintseva 1982; Coesel and Meesters 2007). Elegance and cell wall decorations of desmids are unique and they have always attracted attention of scientist and amateur microscopists. Two of the pioneer desmidiologists, W. West and G. S. West, stated: "Every person who for the first time examines a varied collection of desmids is astonished at their wonderful symmetry and their elegance of form. Diatoms are admitted by all to be very beautiful microscopic objects, but they are far surpassed in elegance by desmids" (West and West 1904).

In general, micro-organisms tend to have a ubiquitous distribution, as stated by many protistologists who improved the famous Beijerinck's metaphor "in micro-organisms, everything is everywhere, the environment selects" (Fenchel 1993; Fenchel and Finlay 2004). However, the occurrence of numerous endemic and flagship taxa in microalgal groups such as dinoflagellates, diatoms, coccolithophores, chrysophytes, and desmids repeatedly pointed out the flaws of the hypothesis on protist ubiquity (Coleman 2001, 2002; Foissner 2006). Due to the fact that the majority of desmid taxa place high demands on environmental conditions and are regarded as K-strategists (Coesel and Kooijman-Van Blokland 1991; Spijkerman and Coesel 1998; Spijkerman et al. 2004), general geographic distribution areas could be drawn for most of the desmid taxa that do not express a large morphological variability and/or synonymy with the other taxa.

Coesel (1996) refined Krieger's classification system of desmid floral regions, revealing a high number of desmid taxa confined exclusively to certain climatic regions. The circumequatorial zone, comprising the desmid floras of Indo-Malaysia/Northern Australia, tropical America and central Africa, showed the highest number of potentially endemic taxa confined only to the tropical climate (Fig. 1a, 1b). Remarkably, a number of tropical endemic desmid genera, such as *Ichthyodontum* Scott & Prescott, *Ichthyocercus* W. & G. S. West, *Triplastrum* Iyengar & Ramanathan, *Allorgeia* Gauthier-Lièvre and *Amscotia* Grönblad, are exclusive inhabitants of the circumequatorial zone. In addition, the region of Southern Australia/New Zealand is characterised by a number of supposed Australian endemics rather than by pronounced tropical species (Thomasson 1980; Ling and Tyler 1986; Tyler and Wickham 1988). Somewhat less number of endemic desmid species is found in Southern Africa and extratropical South America, and this can be explained by the fact that recolonisation from tropical regions is hampered by mountain chains and deserts in between (Coesel 1996). Although the temperate Eurasia has markedly less

number of endemic species when compared to the pantropic region, many desmid taxa are confined to specific climatic areas, e.g. *Euastrum vigrense* Ryppowa – sub-arctic taxon (Engels and Handke 1994), *Staurastrum verticillatum* Archer – atlantic-climate adapted species, *Cosmarium insigne* Schmidle, *C. perforatum* Lundell, *C. protractum* (Nägeli) de Bary and *C. regnesii* Reinsch var. *polonicum* (Eichler & Gutwinski) Compère (Fig. 1c) – continental-climate adapted taxa, etc. (Coesel 1996, Coesel and Meesters 2007; Stamenković and Hanelt 2011). The distribution of the representatives of arctic-alpine desmid flora appeared to be determined microclimatologically, caused by the low environmental temperature. Hence, except for a few 'genuine' cosmopolitan representatives (Fig. 1d), desmids are principally known for their preference for specific habitats and climatic regions (Coesel 1996; Spijkerman and Coesel 1998; Coesel and Krienitz 2008).

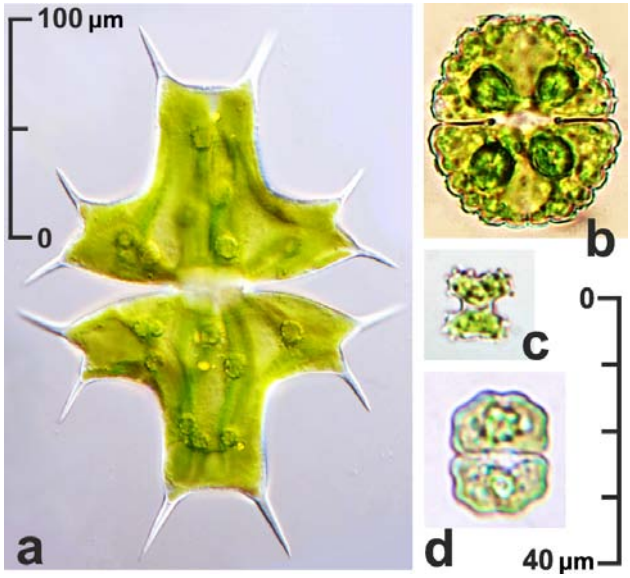

Fig. 1. Two desmid species confined to the tropical zone (**a**) *Micrasterias ceratofera* and (**b**) *Cosmarium beatum*. (**c**) *C. regnesii* var. *polonicum*– subcosmopolitan, continental-climate adapted taxon. (**d**) *C. meneghinii* – cosmopolitan, widely spread species. a – image by Dr. M. Engels; b–d – originals.

So far, climatically induced geographical distribution patterns of macroalgae have been demonstrated considering their physiological behaviour *in vitro* during and after various temperature or radiation treatments (Van de Hoek 1982; Lüning 1990; Lobban and Harrison 1994; Hanelt et al. 2003; Bischof et al. 2006). Accordingly, recognising the different light regime and temperature conditions in various latitudes and altitudes, it is assumed that microalgal clones isolated from various climate zones may show dissimilar growth and photosynthetic behaviour under a set of various radiation and temperature regimes *in vitro*. Taking into account the mentioned observations on the desmid distribution, it appears to be justified to use geographically different desmid isolates to study differences in the growth and photosynthetic behaviour caused by the climate prevailing at their sampling sites.

1.2 Photosynthetically active and ultraviolet radiation

In the broad sense, solar radiation is the total spectrum of the electromagnetic radiation given off by the sun. Regions in the electromagnetic spectrum include (in order of decreasing wavelength – λ): radio waves, microwaves, infrared, visible (VIS) light, UV radiation (UVR), X-rays, and gamma-rays. Visible light corresponds to photosynthetically active radiation (PAR; 400–700 nm) which is utilised in photosynthesis by the photoautotrophs (Huddart and Stott 2010). Compared to equatorial regions, incoming

solar radiation of the polar regions is less intense for two reasons: (i) the solar radiation arrives at an oblique angle nearer the poles, so that the energy spreads over a larger surface area, lessening its intensity; (ii) the radiation travels a longer distance through the atmosphere, which absorbs, scatters and reflects the solar radiation (Przybylak 2003; Huddart and Stott 2010). Tropical areas (i.e. lower latitudes) receive solar radiation which is closer to vertical.

The angle of incidence of the rays, combined with the albedo of the surface has a strong influence on the amount of energy being absorbed (or reflected) at the surface. In the snow- or ice-covered polar zones, almost all direct energy from the sun is reflected because of the extremely high albedo and the small incident angle (Gueymard and Myers 2008). The earth's atmosphere is filled with water vapour, particulate matter, and gases that filter the radiation before reaching the surface. Hence, the summits of mountains and higher elevations receive much more intense radiation than the basal plains (McKnight and Hess 2001). PAR can reach values of up to 2300 µmol photons m^{-2} s^{-1} in mountainous regions during clear weather, as measured at nival and subnival levels (Gorton et al. 2001).

UVR is divided into three types depending on the λ. According to the definition put forward at the Copenhagen meeting of the Second International Congress on Light, UVA is emitted at λ between 315 and 400 nm, UVB at 280–315 nm and UVC at 100–280 nm. On the other hand environmental and dermatological photobiologists normally define UVR regions as UVA 320–400 nm, UVB 290–320 nm and UVC 200–290 nm (Diffey 2002). Due to the short λ, UVR often possesses 'extra' energy causing changes in the chemical structure of molecules which can be detrimental to living organisms. Apart from a small portion near the UVA waveband, UVR is not photosynthetically active (Holzinger and Lütz 2006).

The ozone layer protects the organisms from harmful UVR. Ozone (O_3) is generated when UVR from the sun reacts with the stratospheric atmosphere and oxygen molecule (O_2) and forms a layer of about 15–45 km above the Earth's surface (Peter 1994). As sunlight passes through the atmosphere, all UVC and approximately 90% of UVB radiation is absorbed by ozone, water vapour, oxygen and carbon dioxide. UVA radiation is less affected by the atmosphere. Therefore, the UV radiation reaching the Earth's surface is largely composed of UVA with a small UVB component (Diffey 2002).

However, the absorption of UVBR has decreased due to the thinning and destruction of ozone layer from the release of ozone-depleting substances (organohalogen compounds) that have been widely used in industry (Solomon 1990; Schauffler et al. 1993; Ricaud and Lefévre 2006). Generally, each 1% reduction in ozone causes an increase of 1.3–1.8% in UVB radiation reaching the biosphere (Hollósy 2002). Stratospheric ozone depletion over Antarctica was first observed and reported in the early 1980s (Farman et al. 1985). Net springtime stratospheric ozone loss of up to 60–70% was since observed a yearly recurring phenomenon over Antarctica that intensifies ambient UV-B radiation (Crutzen 1992; Herman et al. 1996). The stratospheric ozone depletion was also detected in the Arctic, at a less severe loss of up to ~ 20–25% (Müller et al. 1997; Dahlback 2002). Ozone loss of ~ 6% has also been reported in the mid latitudes (Smith

et al. 1992; Pearce 1996; WMO 1998; Staehelin et al. 2001). In addition, the other environmental factors may influence the UVR intensity, such as:

sun height – UV radiation varies with time of day and time of year, with maximum levels occurring when the Sun is at its maximum elevation, at around midday during the summer months;

latitude – the closer the Equator, the higher the UV radiation levels;

altitude –with every 1000 metres increase in altitude UVR levels increase by 10% to 12%;

cloud cover – UVR levels are highest under cloudless skies. Even with cloud cover, UVR levels can be high due to the scattering of UV radiation by water molecules and fine particles in the atmosphere;

air pollutants – can reduce UV radiation levels;

ground reflection – UVR is reflected or scattered to varying extents by different surfaces, e.g. snow and white ice can reflect as much as 80% of UV radiation, sea foam about 25%, soil 4–6%, lawn grass 2% (Wängberg et al. 1996; Blumthaler et al. 1997; WMO 1998; Vincent and Neale 2000; WHO 2002).

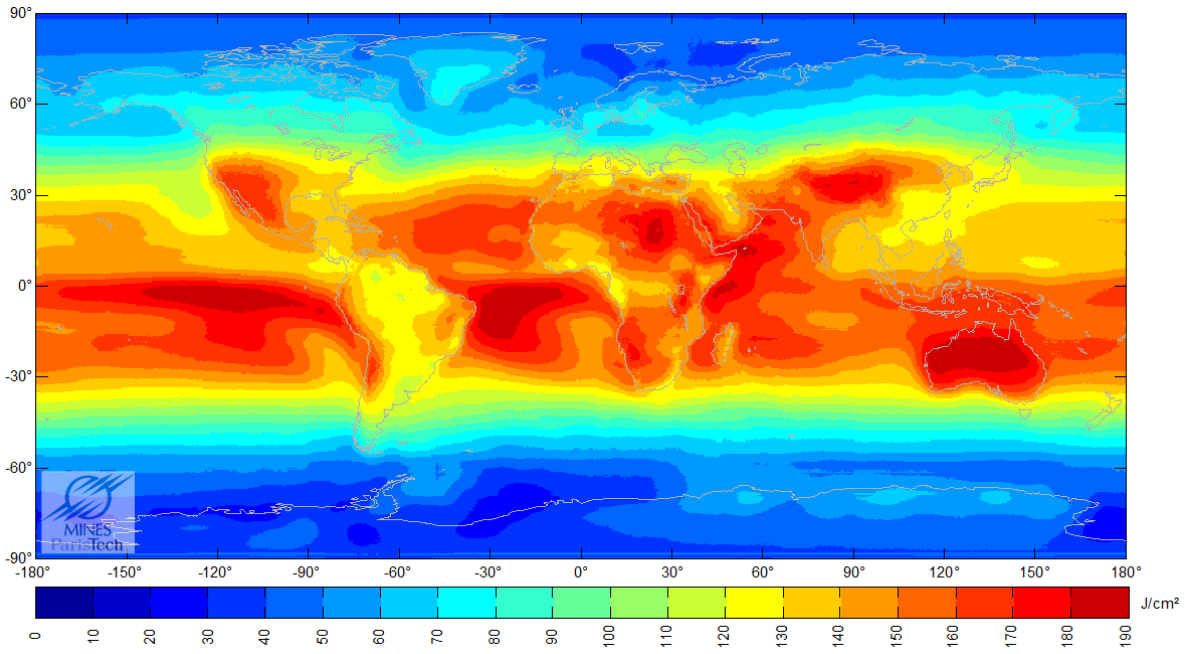

Fig. 2. Yearly mean of daily irradiation in UVR in the world. The displayed quantity is the irradiation for a day averaged over fifteen years (1990–2004), expressed in J/cm^2 (copyright Mines ParisTech/Armines 2008; www.soda-is.com/eng/map/index.html).

The penetration of the UVR into natural waters (oceans, lakes, rivers, etc.) is dependent upon the incident solar radiation, the state of the wind-blown surface, bottom reflectance in shallower waters, and the inherent optical properties of the water body. The optical properties are dependent upon the absorption coefficient and the volume scattering function of the dissolved and suspended material within the water (Smith and Mobley 2007).

As typical inhabitants of various shallow freshwater habitats, i.e. peat bogs, peat ditches, fens, marshes, puddles, pools, and ponds, desmids can be particularly exposed to excessive solar radiation, especially at low latitudes and high altitudes. Presumably, they developed numerous physiological and

ultrastructural mechanisms for the protection under the influences of sudden temperature and radiation changes, which occur during daily and seasonal fluctuations. Yet, water of peat bogs and puddles is significantly enriched with various humic and other organic substances which may greatly absorb UVR (Keddy 2010), thus providing protection to desmid cells against UVR.

1.3 Main climatic zones

Much of the energy from the sun arrives on the earth's surface in the form of infrared radiation. Sunlight at zenith provides an irradiance of over 1 kilowatt per square meter at sea level: 527 watts is infrared radiation, 445 watts is visible light, and 32 watts is ultraviolet radiation (Muneer 1997). The balance between absorbed and emitted infrared radiation has a critical effect on the earth's climate. Infrared radiation is popularly known as 'heat radiation', but light and electromagnetic waves of any frequency will heat surfaces that absorb them. Infrared radiation from the sun accounts for 49% of the heating of the earth, with the rest being caused by visible light that is absorbed and re-radiated at longer wavelengths (Gueymard and Myers 2008).

Generally, the earth's climate can be divided into three distinct climate zones: polar, temperate, and tropical zones. Based on average annual and monthly temperatures and precipitation, and the seasonality of precipitation, a new system was developed – Köppen–Geiger climate classification system (McKnight and Hess 2001; Peel et al. 2007). The Köppen climate classification scheme divides climates into five main groups, each having several types and subtypes.

Group A: Tropical (megathermal) climates are characterised by a constant high temperature considering that all twelve months of the year have average temperatures of 18°C or higher. Tropical climate is distributed equally in circumequatorial latitudes (approximately 0–30°) (McGregor and Nieuwolt 1998).

Group B: Dry (arid and semiarid) climates are characterised by the fact that precipitation is less than potential evapotranspiration. This group includes desert and steppe climates, involving both low latitude climates (average annual temperature above 18°C) and middle latitude climates (average annual temperature below 18°C).

Group C: Temperate (mesothermal) climate has an average temperature above 10°C in their warmest months, and a coldest month average between –3°C and 18°C. This group includes Mediterranean climates, humid subtropical climates, maritime temperate and subarctic climates.

Group D: Continental (microthermal) climates are characterised by the average temperature above 10°C in their warmest months, and a coldest month average below –3°C (or 0°C). These climates usually occur in the interiors of continents, or on their east coasts, north of 40° North latitude. This group includes hot and warm summer (hemiboreal) continental climates, continental subarctic (boreal, taiga) climates, and continental subarctic climates with extremely severe winters (McKnight and Hess 2001; Bonan 2008).

Group E: Polar climates are characterised by average temperatures below 10 C in all twelve months of the year. Polar circles are found both in north and south hemispheres and are defined as the lines of the globe at which sun does not set for at least one night of the year. The polar climate includes tundra and ice cap climates (McKnight and Hess 2001; Przybylak 2003; Peel et al. 2007).

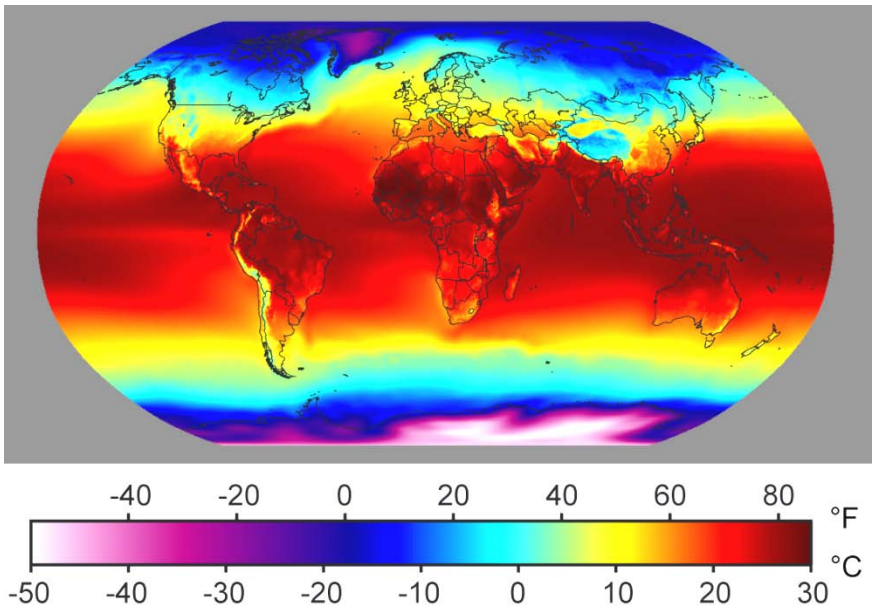

Fig. 3. Map of annual average temperatures as a function of location (copyright Dr. R. A. Rohde).

Although the alpine climate is regarded as a part of the Group E of the Köppen climate classification, many authors suggest separating it into a special group (Allan 1986; Stadel 1990). Thus, the alpine climate represents the average climate for a region above the tree line, and is also referred to as mountain climate or highland climate (Barry 2008). Decreasing air temperature in alpine climate coincides with increasing elevation, and the temperature decrease most directly influences the length of frost days at different altitudes of the mountain. The altitudinal zones of vegetation are particularly well-defined in the high-mountain ranges such as the Rocky Mountains, the Alps, the Pyrenees, the Andes, and the Himalayas (Allan 1986).

Global warming is the rise in the average temperature of Earth's atmosphere and oceans since the late 19th century. It is primarily caused by increasing concentrations of greenhouse gases (e.g. water vapor, carbon dioxide, methane, and nitrous oxide) produced by human activities such as the burning of fossil fuels and deforestation (Huddart and Stott 2010). The global mean temperature increased by 0.6°C from 1990 to 2000 and is projected to increase by another 1.4° C to over 5°C by 2100 (Houghton et al. 2001). This fact provoked a special attention towards investigating temperature influences on desmid physiology, considering that desmids are constituents and important primary producers of fragile oligotrophic and peat-containing ecosystems widely distributed in circumpolar regions.

1.4 The effects of high and low temperatures, high light stress and UV radiation on photosynthesis

Heat stress is one of the main abiotic stresses that limit plant biomass production, especially in tropical and subtropical countries (Boyer 1982). There are several target sites for elevated temperature-induced damage such as the CO_2 fixation system, photophosphorylation, the electron transport chain, and

PSII with its oxygen-evolving complex (OEC) (Feller et al. 1998; Bukhov and Mohanty 1999; Carpentier 1999; Sharkey 2005). The reversible effects of moderate heat stress on PSII likely represent regulation to match reductions in the capacity of downstream reactions. It is has been confirmed that moderate heat stress normally encountered by most plants does not damage PSII, but does substantially reduce the rate of photosynthesis (Sharkey 2005). The decline in Rubisco activity by moderate thermal stress correlated with the loss in photosynthesis (Law and Crafts-Brandner 1999). Rubisco of higher plants is heat stable but the loss in activity at elevated temperature is due to Rubisco activase, which is extremely sensitive to elevated temperature (Salvucci and Crafts-Brandner 2004; Sharkey 2005). In addition, the rate of oxygenation by Rubisco is increased more than that of carboxylation at higher temperatures. Consequently, the photorespiration/net assimilation ratio is larger at higher temperatures (Berry and Raison 1981; Lawlor 2000). As high temperature causes an increase in the fluidity of membranes and lipid saturation, this may significantly decrease electron transport rates and photophosphorylation (Allakhverdiev et al. 2008) or in some cases to initiate zeaxanthin production (Havaux and Tardy 1996). It has been demonstrated that cyclic electron flow was enhanced in chloroplasts from plants which recovered from heat shock. These observations are in line with earlier suggestions that cyclic electron flow could provide additional ATP required for protein synthesis and protect PSII from photoinhibition (Havaux et al. 1991; Allakhverdiev et al. 2005). PSI mediated cyclic electron flow (which would generate the transthylakoid energy gradient needed to regulate PSII) could be a mechanism for limiting PSII activity and so limit the production of activated oxygen species (Heber 2002; Sharkey 2005).

Thermal stress to chloroplasts causes the release of PsbO (33 kDa), PsbP (23 kDa) and PsbQ (17 kDa) proteins and loss of cofactors. Loss of cofactors, especially PsbU, induces the inactivation of PSII, thermal damage of D1 protein, and the production of ROS both in light and in dark when thermal stress persists (Allakhverdiev et al. 2008). *In vitro* experiments suggested that thermal stress at high enough temperatures produces ROS such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide at the PSII reaction centre (Bukhov and Mohanty 1999). Moderate heat exposure of spinach thylakoids could cleave D1 protein producing 9-kDa C-terminal and 23-kDa N-terminal fragments, while the proteins D2, CP43, or CP47 remained intact (Yoshioka et al. 2006). Degradation of phosphorylated D1 was affected by a FtsH protease located in the stroma, which was assumed to diffuse to the granal area as a result of heat-triggered swelling of thylakoids (Yamamoto et al. 1999; Komayama et al. 2007). Hence, it is assumed that degradation of thermally damaged (heat inhibited) and high light damaged (photoinhibited) D1 follows the same route (Allakhverdiev et al. 2008). The upper thermal tolerance of photosynthesis appears to be set by the thermal stability of PSII, which, in turn, is controlled by the degree of saturation of thylakoid membrane fatty acids (Lynch and Thompson 1984). In addition, gene expression, protein synthesis and assembly of proteins into the correct forms may be affected and heat shock responses with accumulation of chaperonin proteins may occur (Vierling 1991; Sørensen et al. 2003).

Slower metabolism at low temperatures decreases the use of light reaction products and thus increases the possibility of creating of ROS and photochemical damage (Falk et al. 1990). Low temperature acclimation enhances thylakoid fatty acid unsaturation (Los and Murata 2004) and causes an increase of the concentration of carotenoids, increase of the total xanthophylls pool, and conversion of violaxanthin to zeaxanthin in microalgae (Huner et al 1998; Anning et al. 2001, Savitch et al. 2001). These observations are consistent with research indicating that low-temperature acclimated microalgae display characteristics of high-light acclimated algae (Maxwell et al. 1994).

Excessive amounts of photosynthetically active radiation (PAR) and UV radiation (UVR) cause a broad spectrum of photochemical, genetic and other damaging effects in aquatic organisms. PAR and UVR are basically considered as non-ionising, i.e. they contain sufficient energy to cause excitation and induce photochemical reactions or accelerate radical reactions. However, UVR can ionise certain types of molecules under specific conditions (Kovács and Keresztes 2002).

Enhanced ultraviolet-B radiation due to stratospheric ozone depletion has several effects on the physiology and productivity of plants and algae. The negative impact of exposure to UVBR includes (i) photoinhibition and eventual photodamage to the photosynthetic apparatus; (ii) protein breakdown and the loss of specific enzymatic or biological function; (iii) formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6–4) pyrimidone photoproducts (6–4PPs) in the DNA; (iv) absorption by aromatic sulfhydryl-containing biomolecules causing direct molecular damage; and (v) production of ROS responsible for oxidative damage within the cell (Vass 1997; Häder 2000; Rijstenbil et al. 2000; Sinha and Häder 2002; Britt 2004; Xue et al. 2005; Holzinger and Lütz 2006; Roleda et al. 2007). Even though it has less energy than UVB, UVA is also able to induce comparable damaging effects as UVB (Turcsányi and Vass 2000; Vass et al. 2002). In some environments, UVA has been reported to be responsible for a major fraction of photoinhibition, but UVA may also be beneficial for attenuating UVB effects (Quesada et al. 1995; Han et al. 2001; Joshi et al 2007).

An excess of energy causes the inactivation of photosynthesis, a phenomenon known as photoinhibition (Powles 1984; Adir et al. 2003; Takahashi and Murata 2008). Photoinhibition is defined as the light-dependent reduction in photosynthetic efficiency occurring when the absorbed energy is in excess of that which can be used for photosynthesic process (Powles 1984; Osmond 1994). The extent to which the photosynthetic apparatus is affected by light stress is explained by two types of photoinhibition: dynamic and chronic photoinhibition. Dynamic photoinhibition (photoprotection) is a down-regulation process of photosynthetic apparatus associated with dissipation of excess energy as heat and which is reversible within few hours (Osmond 1994; Osmond and Grace 1995; Hanelt 1996). On the other hand, chronic photoinhibition (photodamage) is characterised by a slowly reversible loss of PSII reaction centres (primarily D1 protein) in which repair and recovery takes many hours or days (Osmond 1994; Osmond and Grace 1995). It is noteworthy to emphasise that UVR cannot be regarded as being an ‘excessive energy

input' in a proper sense since the maximal irradiance of UVR is much smaller than PAR and the UV wavebands do not contribute considerably to the photosynthetic energy supply (Hanelt et al. 2003).

There are two well-characterised mechanisms that explain the photoinhibition by PAR. In the acceptor-side photoinhibition, impairment of Q_A and Q_B electron acceptors promotes the formation of P680 triplet from which singlet oxygen ($^1O_2$) is formed, with the subsequent induction of oxidative damage to the D1 protein (Vass et al. 1992). Donor-side photoinhibition, on the other hand, results from the impairment of electron transfer between the manganese cluster of OEC with $P680^+$ leading to accumulation of highly oxidising cations such as $P680^+$ and/or $TyrZ^+$ which can provide driving force for direct photochemical cleavage of the protein (Aro et al. 1993). Tyystjärvi (2008) has suggested that the manganese cluster could be impaired by PAR in a low efficiency process *via* the same mechanism as induced by UV.

An essential part of the protective mechanisms against inhibitory PAR relies on the presence of carotenoids. In most of the photosynthetic organisms a process known as non-photochemical (NPQ) mechanism is activated to quench singlet-excited Chl and harmlessly dissipate excess excitation energy as heat (Müller et al. 2001). It is believed that this process is activated *via* several reversible pathways that utilise conversion of epoxidized xanthophyll (X), an oxygenated carotene, to its deepoxidized states (Demmig-Adams and Adams 2006). For instance, in the violaxanthin (Vx) cycle that is dominant in higher plants, green and brown algae, Vx is deepoxidized to anteraxanthin (Ax) then to zeaxanthin (Zx) by Vx deepoxidase (VDE) (Havaux and Niyogi 1999; Jin et al. 2003; Masojídek et al. 2004). Although it is known that Zx participates in the heat dissipation from PSII, it has also been observed that Ax can actively participate in non-photochemical quenching (Goss and Jakob 2010). However, thermal energy dissipation by the xanthophyll cycle might not effectively protect the algae against harmful UV effects (Bischof et al. 1999).

When the rate of damaged D1 protein exceeds that of its repair process, photodamage will occur (Aro et al. 1993; Hanelt 1996). Thus, the extent of recovery from chronic photoinhibition depends on the synthesis of new protein D1 and on the reassembly of repaired PSII centres (Aro et al. 1993). The D1 'damage-repair cycle' constitutes of photodamage to D1 followed by, a prompt and partial disassembly of the PSII holocomplex; exposure of the photodamaged PSII core to the stroma of the chloroplast; degradation of photodamaged D1; *de novo* D1 synthesis and insertion in the thylakoid membrane and finally, re-assembly of the PSII holocomplex, followed by activation of the electron-transport process through the reconstituted D1/D2 heterodimer (Melis 1999). The rates of *de novo* protein synthesis during and after photoinhibition have been estimated by the addition of inhibitors of chloroplast-encoded proteins (e.g. chloramphenicol or streptomycin) before PAR or UVR inhibitory experiments started, in many studies (Samuelsson et al. 1985; Schnettger et al. 1994; Häder et al. 2002).

The primary target of UVA and UVB is at the donor-side of PSII, at the level of the manganese cluster of water oxidation (Vass et al. 1996; Turcsányi and Vass 2000). In addition, UVR affects the Tyr-Z and Tyr-D electron donors, thus both D1 and D2 proteins of PSII can become inactivated (Melis et al. 1992;

Friso et al. 1994a, b; Vass et al. 2002), as well as the quinone electron acceptors Q_A and Q_B (Vass et al. 1999). Increased degradation of D1 protein is a major effect of UVBR photodamage, but UVBR has multiple effects on the photosynthetic apparatus, including loss of plastoquinones, photosynthetic pigments, Rubisco, and membrane components (Lao and Glazer 1996; Rinalducci et al. 2006; Melis et al. 1992; He and Häder 2002; Bischof et al. 2000; Bischof et al. 2002). The activity of UV-photoinhibited PSII is restored *in vivo via* a similar repair process that restores the activity of PSII after PAR photoinhibition. In addition, protein repair of PSII is induced and regulated by both UVB and PAR, and can potentially influence each other's effects in PSII repair (Máté et al. 1998; Sicora et al. 2003). Ameliorating effects of UVBR have been reported in a Mediterranean brown alga (Flores-Moya et al. 1999) several aquatic plants of New Zealand (Hanelt et al. 2006), in macrophytes growing on the coastal barrier reef of Belize (Hanelt and Roleda 2009) and in several Antarctic microalgae (Thomson et al. 2008), indicating that this interesting phenomenon should be thoroughly investigated.

Interestingly, despite the fact that desmids are inhabitants of shallow freshwater bodies which are particularly exposed to excessive PAR and UVR there have been no detailed studies on their protection mechanisms and strategies. So far, the only evidence of 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 (Remias et al. 2012).

1.5 Objectives of the study

The objectives of this study are:

- to determine if microalgal isolates from various regions demonstrate different physiological behaviour which is in accordance with the prevailing climate at the site of clonal origin;
- to observe if such responses are also expressed under a set of varying temperature and radiation (PAR and UVR) conditions, or adaptation occurred due to the influence of long-term constant laboratory conditions;
- to observe if stress at different warm and cold temperatures causes diverse extents of change in the ultrastructure of the *Cosmarium* strains originating from different geographic zones;
- to survey the protective ultrastructural responses of the *Cosmarium* strains against low or high temperatures which may occur in their native habitats and to estimate their possibility to survive at stressful warm and cold temperatures;
- to estimate resistance of the desmid strains under enhanced UV radiation or at warm temperatures, in accordance with the climate change (the thinning of the ozone layer and global warming);

- to estimate the protection strategies of the *Cosmarium* strains isolated from different climatic zones against high irradiance, by means of an inhibitor of violaxanthin de-epoxidase (dithiothreitol) and inhibitors of chloroplast-encoded protein synthesis (chloramphenicol and streptomycin), i.e. if the *Cosmarium* strains demonstrate 'sun- or shade-plant' protection strategies in accordance with the climate of their sampling sites;

- to investigate if the composition and changes of the photosynthetic pigments of the *Cosmarium* strains under cultivating and photoinhibitory PAR applied *in vitro* are in accordance with their source location.

1.6 Thesis outline and declaration of contributions to the publications

The possible geographic distribution patterns of the *Cosmarium* strains isolated from various climatic zones and long-term grown under constant and relatively low light-temperature conditions were estimated for the first time *in vitro*. Physiological responses and ultrastructural characteristics of the desmid strains during and after various temperature or radiation conditions were taken into account to determine their preference to specific climates. In addition, protection strategies and xanthophyll cycle characteristics of desmids under excessive photosynthetically active radiation were studied for the first time. The thesis is divided in six publications.

Publication 1 examines the growth and photosynthetic differences between the six *Cosmarium* clones isolated from various geographic areas under cultivating, relatively low light-temperature regime of a climate chamber (16°C, 30 µmol photons m^{-2} s^{-1}). It is speculated that the polar taxon, *C. crenatum* var. *boldtianum*, is more optimally suited to the low cultivation temperature, than the tropical species, *C. beatum*, as evidenced from their growth rates, maximum quantum yield and photosynthetic efficiency measured. In addition, this paper deals with the morphological and physiological differences between two clones belonging to a cosmopolitan taxon, *C. punctulatum* var. *subpunctulatum*, collected from a lowland, polar zone and from highland, tropical region, under unstressed (standard) cultivation conditions.

Publication 2 deals with a more thorough examination of the differences between six *Cosmarium* clones under a set of various temperature conditions (0.6, 7, 16, 21, 25, 28, 32, 35, and 37°C) at a constant light regime, i.e. with the potential influence of temperature on patterns of geographic distribution of the desmid strains. This research examines if the photosynthetic responses of the *Cosmarium* strains are genetically preserved, or adaptation (i.e. genetic changes) occurred due to the long-term cultivation at relatively low temperature-light regime.

Publication 3 investigates if stress at different temperatures causes diverse extents of change in the ultrastructure of the *Cosmarium* strains originating from different geographic zones. Taking into account the rapid changes of temperature in the habitats of desmids, it is of the interest to explore the role of

ultrastructural protective mechanisms to cope with sudden temperature stress which may occur in shallow freshwater bodies.

Publication 4 deals with the effects of photosynthetically active radiation on photosynthetic performance (estimated as chlorophyll fluorescence and total oxygen evolution rates) of four medium-sized *Cosmarium* strains, with regard to the pattern of their geographic distribution. The protection strategies against high light (i.e. 'sun- or shade-plant' protection strategies) of the desmid strains were estimated by the addition of inhibitors of chloroplast-encoded protein synthesis (chloramphenicol and streptomycin) and an inhibitor of violaxanthin de-epoxidase (dithiothreitol).

As a supplement to Publication 4, **Publication 5** examines the composition of photosynthetic pigments of the *Cosmarium* strains under moderate and photoinhibitory white light by means of high-performance liquid chromatography. Furthermore, this research intends to reveal the contribution of antheraxanthin and zeaxanthin to the heat dissipation from PSII, as judged from significant negative correlations between maximum quantum yield and antheraxanthin (or zeaxanthin) content expressed per xanthophyll-cycle pool.

Publication 6 deals with the sensitivity and photosynthetic protective strategies (estimated by means of an inhibitor of chloroplast-encoded protein synthesis – streptomycin) of four *Cosmarium* strains treated under ultraviolet-A radiation, and moderate and increased ultraviolet-B radiation. In addition, this research examines the role of mucilaginous sheaths of the desmid strains against intensive PAR and UV radiation.

Conceptualisation and implementation of the experimental designs, data gathering and analyses, and manuscript writing of publications 1, 2, 3, 4, 5 and 6 were initiated and conducted by M. Stamenković. Prof. Dr. Dieter Hanelt is also responsible for the research theme and supervision of this dissertation, and he is a co-author for all of the publications. Prof. Dr. Kai Bischof is the second supervisor of the thesis and a co-author for the publication 5.

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2. Growth and photosynthetic characteristics of several *Cosmarium* strains (Zygnematophyceae, Streptophyta) isolated from various geographic regions under a constant light-temperature regime

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Abstract

Numerous detailed studies have been made of climatically and environmentally influenced macroalgal geographic distribution patterns. However, so far, there have been only a few intrinsic investigations of the geographic distributions of microalgae. In order to investigate the physiological differences among geographically different microalgal strains, six *Cosmarium* strains were collected from various climate areas and studied. They were grown under a constant light-temperature regime (16°C and 30 µmol photons m^{-2} s^{-1}) and nutrient supply. The arctic representative, *C. crenatum* var. *boldtianum*, and the typical tropical desmid, *C. beatum*, behaved like algae adapted to high light intensities, as judged from the distinctly high values of photosynthetic capacity and saturating irradiance measured, in accordance with the high solar radiation prevailing in their sampling areas. The arctic taxon appeared more optimally suited to the low cultivation temperature, as evidenced by the relatively high values of growth rates, maximum quantum yield and photosynthetic efficiency measured. The cosmopolitan taxa, *C. meneghinii* and *C. punctulatum* var. *subpunctulatum*, exhibited a high maximum quantum yield and photosynthetic efficiency concomitantly during growth, which explained their ubiquitous distribution. Nevertheless, two clones belonging to *C. punctulatum* var. *subpunctulatum*, collected from polar and mountainous tropical regions, differed significantly with regard to cell volume, growth rates, surface area to volume ratio and photosynthetic parameters. The physiological differences between the *Cosmarium* strains were in accordance with their geographic origin; they are discussed in detail in this study. Moreover, these differences were maintained despite the long-term cultivation under identical and constant laboratory conditions.

Keywords: *Cosmarium*, growth rate, cell volume, maximum quantum yield, photosynthetic capacity and efficiency, saturating irradiance.

Abbreviations: S/V – surface area to volume ratio, P-I curve – photosynthesis-irradiance curve, PSII – Photosystem II, $rETR_{max}$ – maximum relative electron transport rate, I_k – saturating irradiance, α – slope of P-I curve (photosynthetic efficiency), Fv/Fm – maximum potential quantum yield of PSII, PFR – photon flux (fluence) rate, µ – growth rate

Introduction

Previous ecophysiological studies on desmids revealed possible distribution patterns of desmid species and strains concerning the amount of nutrients and chemical condition of habitats (Tassigny 1971; Soeder et al. 1971; Spijkerman and Coesel 1996a, 1996b, 1998; Spijkerman et al. 2004, 2005). These investigations considered the physiological behaviour of desmid isolates from areas belonging to the same climate zone. It is supposed, however, that differences in physiological behaviour of strains belonging to the same species may be linked with the natural climate prevailing at the site of clonal origin (Manhart and McCourt 1992). Wood and Leatham (1992) noted that physiological characteristics in phytoplankton communities may show considerable variations with regard to the climate, even when clones of a single species are compared. Several strains of marine planktonic diatoms isolated from subtropical waters showed higher optimal growth temperatures than those of the same morphological species which originated from cold water (Guillard and Kilham 1977). Concerning desmids, morphological and physiological differences among clones belonging to the same species, *Closterium ehrenbergii* Meneghinii ex Ralphs, have been extensively studied (Ichimura 1985; Kasai and Ichimura 1986). The temperature optima for three mating groups of this species (marked as A, B and P) were determined in a temperature gradient experiment, and they corresponded to the average temperature of their sampling sites (Kasai and Ichimura 1990). Clones of A and B group, isolated from paddy fields and small ephemeral puddles, grew best at 20–30°C; whereas group P clones, originating from cold-water streams and ponds, grew best at 10–15°C. The investigation revealed that these isolates were capable of preserving the natural physiological behaviour in *in vitro* conditions: group P clones are adapted to the cold, seasonally stable environments, while groups A and B clones are adapted to grow during the period when paddy fields are flooded with 20–30°C water, subsequently surviving the drought period or winter in a zygospore stage. Similarly, several strains of *Staurastrum planctonicum* Teil., originating from habitats of various pH values, exposed different photosynthetic rates and affinity constants for inorganic carbon although they had been previously grown under identical laboratory conditions (Spijkerman et al. 2005).

So far, detailed investigations of physiological differences among the specimens originating from various geographic zones have been carried out on macroalgae. From numerous investigations on photosynthetic characteristics of kelps treated under various light-temperature conditions, it was recognised that macroalgae might behave as ‘sun’ or ‘shade’ adapted plants (Lüning 1981; Lobban and Harrison 1994). In all of the investigated seaweed species, the decrease of photosynthetic capacity and saturating irradiance (I_k) from medium to high latitudes corresponded to the decrease of solar irradiance from the Equator to polar regions. On the other hand, the photosynthetic efficiency (estimated as a slope of P-I curve) appeared to be higher in seaweeds growing in circumpolar areas, confirming their better utilisation of solar radiation in such low-light environments (Hanelt et al. 2003). This relationship has been demonstrated in adult macrothalli of various macroalgae from polar to tropical regions (Lüning 1990; Wiencke et al. 1993; Weykam et al. 1996). Interestingly, kelp zoospores exhibited similar changes in saturating irradiance, photosynthetic capacity and

efficiency in accordance with latitudes and depth distribution in a water column (Amsler and Neushul 1991; Roleda et al. 2005, 2006).

Accordingly, recognising the different light regime and temperature conditions in various latitudes and altitudes, it is assumed that microalgal clones isolated from various climate zones may show dissimilar growth and photosynthetic behaviour under constant laboratory conditions. The cosmopolitan distribution of freshwater microalgae may represent a great disadvantage for this kind of investigation. However, desmids are known for a high number of exceptions to this general rule. Due to the fact that the majority of desmid taxa have high demands upon environmental condition and are regarded as K-strategists (Coesel and Kooijman-Van Blokland 1991; Spijkerman and Coesel 1998; Spijkerman et al. 2004), general geographic distribution areas could be drawn for most of the desmid taxa that do not express a large morphological variability and/or synonymy with the other taxa. Coesel (1996) refined Krieger's classification system of desmid floral regions revealing a high number of desmid taxa confined exclusively to certain climatic regions. The circumequatorial zone, comprising the desmids floras of Indo-Malaysia/Northern Australia, tropical America and central Africa, showed the highest number of potentially endemic taxa confined only to the tropical climate. On the contrary, the distribution of the representatives of arctic-alpine desmid flora appeared to be determined microclimatologically, caused by the low environmental temperature. Taking these observations into account, it appears to be justified to use geographically different desmid isolates to study differences in the growth and photosynthetic behaviour caused by the climate prevailing at the sampling sites.

The present study is focused on growth rates, changes of cell volume, and photosynthetic performance during the growth of six *Cosmarium* strains collected from various climate zones and maintained under the constant light-temperature regime (16°C and 30 µmol photons $m^{-2}s^{-1}$) and nutrient supply. The results of this investigation may contribute to further research on the influences of various light and temperature conditions to *Cosmarium* isolates and monitoring of physiological changes.

Materials and Methods

Algal strains

The six *Cosmarium* clones dealt with in this study were isolated from various parts of the world within approximately the same period, in order to exclude the influences of sampling time and, therefore, the influences of constant nutrient, light and temperature regime in laboratory conditions (Table 1). The exception was made for *C. meneghinii*, a typical cosmopolitan desmid, because of the special interest in comparing its physiological activity with that of the other ubiquitous taxa. In addition, all of the strains originated from habitats of a similar trophic condition so as to avoid the influences of the environmental trophic condition on growth and photosynthetic performance. According to the database of the Culture Collection of Autotrophic Organisms (CCALA, Academy of Sciences of the Czech Republic) and reports from the collectors, all of the strains were collected from oligo-mesotrophic habitats. Three *Cosmarium* taxa

of approximately comparable size (medium-sized taxa, *C. punctulatum* var. *subpunctulatum*, *C. beatum* and *C. crenatum* var. *boldtianum*) and two small-sized taxa (*C. meneghinii* and *C. regnesii* var. *polonicum*) (Fig. 1) were selected. Thus, the influence of cell size on the growth rate is minimised within these two groups.

Climate zone	Taxon	No. strain (SVCK)	Original No. of strain	Sampling area	Locality coordinates	Year of isolation	Isolator
Polar	*C. crenatum* Ralfs var. *boldtianum* (Gutw.) W. & G.S. West	561	ASW Wien # 07 141	Cape Flora, Northbrook Island, Franz Josef Land, Russia	79°57′N 50°05′E	1995	Kusel-Fetzmann
Continental (microthermal)	C. *regnesii* Reinsch var. *polonicum* (Eichl. & Gutw.) Comp.	465		pool in East Hokkaidō, Japan	42°54′N, 140°45′E	1998	Gontcharov
Tropical	*C. beatum* W. & G.S. West	533		marshy area nearby Ol Bolossat Lake, Kenya	00°09′S 36°26′E	2001	Engels
Continental (microthermal)	*C. meneghinii* Ralfs	59	1927/Praha Ac. A 231	fountain in Prague, the Czech Republic	50°05′N, 14°25′E	1927	Czurda
Alpine, tropical zone	*C. punctulatum* Bréb. var. *subpunctulatum* (Nordst.) Børgesen	570	ASW Wien # 07 182	pool on Mt. Cotopaxi, 1600 m a.s.l., Ecuador	00°40′S 78°26′W	1996	Kusel-Fetzmann
Lowland, polar zone	*C. punctulatum* Bréb. var. *subpunctulatum* (Nordst.) Børgesen	571	ASW Wien # 07 136	pool near Skarsvåg, 80 m a.s.l, the North Cape, Norway	71°06′N 25°49′ E	1992	Kusel-Fetzmann

Table 1. Data on the *Cosmarium* strains used for the investigation of growth and photosynthetic behaviour under constant light-temperature regime (SVCK – Sammlung von Conjugaten Kulturen, the culture collection of Conjugatophycean algae of the University of Hamburg).

The investigation was based mainly on the geographic origin of the individual isolates; however, we tried to make a narrow link between the known general distributions and the origins of the clones for the selected *Cosmarium* taxa. Generally, the identification of *Cosmarium* taxa is impeded by the presence of synonymy, polymorphism and the existence of so-called 'cryptic' species, which possess similar morphology as a result of the influence of the same environmental conditions. The choice for these experimental strains was made by considering those clones which corresponded most to the description in literature. In addition, the morphology of desmid cells may exhibit some changes in relation to the chemical composition of medium, light and temperature conditions during cultivation, creating difficulties in the determination. Therefore, the identification of the *Cosmarium* strains in cultures should be considered carefully, taking into account a great variability among the taxa of this genus.

Considering that *C. crenatum* var. *boldtianum* was essentially characterised as an arctic-alpine taxon (Coesel 1996; Brook and Johnson 2003), it was chosen as a representative of the polar region. In addition,

this taxon has been recorded in alpine climate region of the mountain ranges situated in Europe, Asia and America (Hirano 1966; Suxena 1978; Alvárez Cobelas 1984; Abdelahad et al. 2003). The clone SVCK 561 corresponds entirely to the description given by West and West (1912) and Hirano (1966), i.e. it has five distinct crenae positioned on the lateral margin; the upper two crenae are somewhat larger than the others. Semicells with an inflation above the isthmus are furnished with 4–6 vertical rows of granules (occasionally grouped into costae). *C. regnesii* var. *polonicum* (syn. *C. regnesii* var. *montanum* Schmidle) is designated as a subcosmopolitan and acidophilic taxon; it spreads in temperate and continental climate zone as an inhabitant of boggy edges of pools and lakes, shallow peat pits and ditches (Coesel and Meesters 2007). It has also been recorded in moderately cold mountainous terrains located in the southern areas of Europe and Asia, growing in fens and moorland pools (Palamar-Mordvintseva 1982; Kouwets 1987).

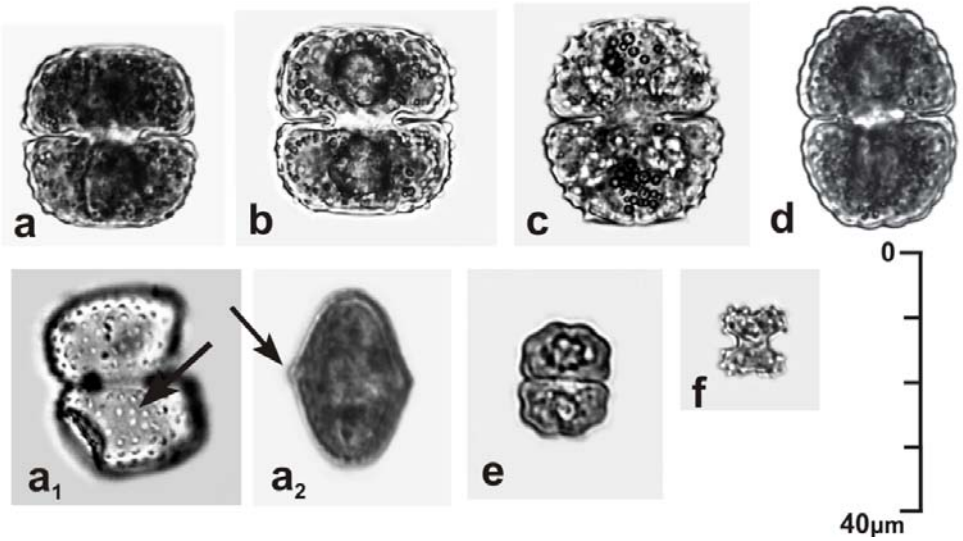

Fig. 1. The *Cosmarium* strains isolated from various climate zones and selected for the investigation of growth and photosynthetic behaviour under a constant light-temperature regime: (**a, a$_1$, a$_2$**) *C. punctulatum* var. *subpunctulatum* (No. 570), (**b**) *C. punctulatum* var. *subpunctulatum* (No. 571), (**c**) *C. beatum*, (**d**) *C. crenatum* var. *boldtianum*, (**e**) *C. meneghinii*, (**f**) *C. regnesii* var. *polonicum*. The arrows show the centrally positioned circle of granules on semicells, characteristic to the variety *subpunctulatum*; (**a$_2$**) – apical view.

C. beatum is a typical tropical desmid, known from Africa (West and West 1895; Rich 1935; Rino 1972; Couté and Rousselin 1975; Compère 1977; Williamson 1994), and India (Agarkar 1971). Recent desmidiological investigations revealed new discoveries of this taxon in Chile (Williamson 2004), and northern Thailand (Coesel et al. 2009). The clone dealt with in this study (SVCK 533) corresponds to the description given by Williamson (2004) with regard to the cell size, position and number of the marginal bidentate crenations and central granules, as well as the truncate apex which is equipped with 4–5 marginal granules. However, the investigated clone is characterised by more transversely elongate pyramidal semicells and a narrow sinus, in contrast to the specimens depicted by Williamson (2004) or Coesel et al. (2009). *C. meneghinii* is a cosmopolitan, distinctly widespread desmid, an inhabitant of exceedingly various

freshwater ecosystems, from oligotrophic ponds, and dystrophic, acidic peat bogs to alkaline and nutrient-rich pools. *C. punctulatum* var. *subpunctulatum* is a cosmopolitan taxon, commonly distributed in temperate region in a wide range of habitats. Mostly recorded in slightly acidic puddles and ditches, it can also be found in moderately alkaline and meso- to eutrophic waters (Brook and Johnson 2003).

Some general climatic (light and temperature) characteristics of the desmid sampling localities

The climate of the greater part of the Franz Joseph Land territory has been classified as EF group (ice cap climate), according to the Köppen–Geiger climate classification system (McKnight and Hess 2001; Peel et al. 2007), considering that all twelve months have average temperatures below 0°C. Cape Flora is a small peninsula of Northbrook Island, located at the southern edge of the Franz Joseph Archipelago. Although the 'midnight sun' is located at a low angle above the horizon in the Arctic Circle, due to the steep slopes of the Cape Flora coast the incident angle of solar rays can be increased as far as 90° and, thus, solar irradiation is largely amplified (Höbenreich 2007; Umbreit 2009). In addition, the remaining snow surfaces may significantly increase solar irradiance (over 1500 µmol photons $m^{-2}s^{-1}$) due to the albedo during clear weather (Stibal et al. 2007).

The climate of northern Norway (Skarsvåg, Magerøya Island, Finnmark), typified as tundra climate (ET), is milder as compared to that of Franz Joseph Land, and in contrast to the main Arctic climate characteristics (McKnight and Hess 2001; Przybylak 2003). Due to the North Atlantic Current, the temperature along the coast is approximately 15°C higher than in the other areas at this latitude (71°). Low clouds and fog are also prominent and a typical phenomenon for the coastal Finnmark region during all seasons (Tollesfrud et al. 1991; Moen 1998).

Both sampling localities which belong to the continental (microthermal) climate (eastern Hokkaidō and Prague) are classified as a warm summer continental (hemiboreal, Dfb) subgroup (McKnight and Hess 2001). Summer in eastern Hokkaidō is breezy and foggy due to the maritime influence, while winter can be severely cold (Kira 1977). Prague (197 m a.s.l.) is characterised by mild continental weather with warm, wet summers and moderately cold winters (Bodri and Čermák 1995).

Lake Ol Bolossat is located in the Nyandarua district of the Central province in Kenya which belongs to the Aw (tropical wet and dry, savanna) subgroup of the tropical climates (McKnight and Hess 2001). Although Lake Ol Bolossat is situated at an elevation of around 2000 m a.s.l., its drainage basin is placed at lower altitudes, comprising a large area of swamps and marshes (Krhoda 1992). The sampling site of the algal material used for the isolation is situated at an elevation not higher than 200 m a.s.l.

Cotopaxi Mt. is a stratovolcano in the Andes Mountains, located about 28 km south of the Equator. The algal samples were taken at approximately 1600 m a.s.l., where the climate differs significantly from the tropical regime predominating at the base of the mountain. The climate at this altitude corresponds to the Cfb (maritime temperate climate; in this case referred as 'highlands') subgroup, belonging to the temperate (mesothermal) group (Peel et al. 2007). According to the climate classification system of the Ands, the

sampling area belongs to the montane level (Tierra templada) (Allan 1986; Stadel 1990). A decrease in air temperature in alpine climate coincides with the increased elevation, whereas the photosynthetically active radiation can reach values up to 2300 µmol photons $m^{-2}s^{-1}$ during clear weather, as measured at nival and subnival levels (Gorton et al. 2001).

Culture conditions

All of the investigated strains were grown in non-axenic batch cultures in a climate chamber. The mineral medium based on Kattner et al. (1977) was prepared with double-distilled water for the cultivation of desmids (L-d medium). The tested *Cosmarium* strains were adapted to this medium at least 9 months before the experiment started. The strains were cultured in modified 1L Erlenmeyer flasks which were filled up with 500 ml of medium. In order to obtain duplicate data, two flasks with medium were prepared for each desmid strain. Sterilised flasks with medium were inoculated with the sample taken from pre-cultured strains in the exponential growth phase, to a final concentration of 1500 cells ml^{-1}. Cultures were bubbled with humidified air at a rate of about 10 l/h so as to prevent CO_2 limitation. The cultures were regularly mixed using a magnetic stirrer in order to prevent the self-shading of cells. Algal clones were grown at 16°C, using a daily light regime of 14 h light and 10 h darkness. The light in a climate chamber was provided by white fluorescent tubes (Osram, L65 Watt/25S, Munich, Germany). Using a cosine quantum sensor (LI-COR, LI–188B), the light intensity was adjusted to 30 µmol photons m^{-2} s^{-1}. For each desmid clone studied, growth was assessed during an incubation period of 4–5 weeks, whereby cell growth, cell volume and photosynthetic performance wre measured every second day. The algal suspensions in Erlenmeyer flasks were homogenised by a magnetic stirrer before sampling.

Determination of cell number, growth rate and cell volume

Cell counting was done by means of an electronic particle counter (Beckman Coulter Electronics model ZB) using an aperture of 100 µm. As an electrolyte solution, 1% NaCl (including 0.5% formaldehyde) was used. Reliability of the counter was checked against cell counts with the inverted microscope (Utermöhl 1958). Specific growth rate per day (µ) was calculated by the formula:

$$\mu = \frac{\ln(N_1/N_0)}{t_1 - t_0}$$

in which N_1 and N_0 are the cell concentrations at the end and beginning of a period of time t days. The generation time (number of divisions per day) was estimated using the formula: $G = \mu/ln(2) = \mu/0.6913$; the doubling time was estimated using the formula: $d = ln(2)/\mu = 1/G$ (Guillard 1973).

The data for cell volume (values of mean volumes) obtained from a Coulter counter were also taken into consideration in this study. Mean cell volumes obtained electronically were compared with volume calculations from linear dimensions of 50 randomly selected cells using formulae for stereometric shapes as suggested by Roth (1981), by means of a light microscope (Zeiss IM, Germany). The same stereometric

shapes were used for the calculation of mean cell surfaces. In addition, the principle of calculation of cell volume for the genus *Cosmarium* according to Hillebrand et al. (1999) was also considered in this study. The authors suggest that cells of the *Cosmarium* species should be considered as two half ellipsoids, where the ellipsoid is represented as a prolate spheroid with elliptical cross-sections. When applied to the linear measurements obtained by a microscope, the test for normality of data (the Kolmogorov-Smirnov test) confirmed normal distribution, so it was justified to use the mean values from a Coulter counter.

The morphological shape of each investigated *Cosmarium* strain was suited to the corresponding stereometric shape as much as possible. Thus, *C. crenatum*, *C. beatum* and *C. punctulatum* var. *subpunctulatum* were basically treated as hexagonal-shaped cells (regular hexagonal prism). However, due to relatively large differences in semicell size of each cell, as observed in old and young semicells, the formula of hexagonal prism volume was not applied. The volume of semicell was calculated as a prism on trapezoid surface and then the average value from both semicells was calculated for each cell. The mean of the surface was calculated by the same means and reduced by the surface of the isthmus. The cell of *C. meneghinii* was treated as a prism on an irregular octagonal base; the surface of this shape was also reduced by the surface of the isthmus. The basically rectangular (or quadratic) shape of C. *regnesii* var. *polonicum* had to be diminished by a deep, widely open sinus (semicylindrical shape).

The thickness of the mucilaginous cell sheath was measured microscopically in fifty randomly chosen cells in cell suspension stained by Indian ink, measured at the cellular apex.

Chlorophyll fluorescence measurements

Photosynthetic efficiency was measured as variable fluorescence of PSII determined 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 equalised by adding a quantity of L-d medium to be approximately 4000 cells ml^{-1} for medium-sized strains, or 9000 cells ml^{-1} for small-sized taxa. Immediately after sampling, the algal suspension was adapted in darkness for 10 min and filled into 5 ml Quartz cuvettes. The maximum quantum yield (Fv/Fm; the ratio of variable to maximum chlorophyll fluorescence from photosystem II) was measured at time zero (n = 5) as described by Hanelt (1998). After dark incubation, the initial fluorescence (Fo) was measured with red measuring light (~0.3 µmol photon m^{-2} s^{-1}, 650 nm), and the maximal fluorescence (Fm) was determined with 600 ms completely saturating white light pulse (~3500 µmol photon m^{-2} s^{-1}).

Photosynthesis (in terms of the relative electron transport rate, rETR = PFR * ΔF/Fm') versus irradiance (P-I) curves were also measured (n = 3, chosen at random from the five replicates) as described by Bischof et al. (1998). PFR refers to photon fluence rate, Fm' is the maximum fluorescence from a light-adapted sample, ΔF (or Fq') refers the difference in fluorescence between Fm' and F'; F' is the fluorescence emission from an irradiated sample (Baker 2008).

The hyperbolic tangent model of Jassby and Platt (1976) was used to estimate P-I curve parameters described as:

$$rETR = rETR_{max} * \tan h(\alpha * I_{PAR} * rETR^{-1}_{max})$$

where $rETR_{max}$ is the maximum relative electron transport rate, tan h is the hyperbolic tangent function, α is the electron transport efficiency and I is the photon fluence rate of PAR. The saturation irradiance for electron transport (I_k) was calculated as the light intensity at which the initial slope of the curve (α) intercepts the horizontal asymptote ($rETR_{max}$). The curve fit was calculated with the Solver Module of MS-Excel using the least squares method and comparing differences between measured and calculated data.

Statistical analysis

Data were tested for normality (Kolmogorov-Smirnov test) and for homogeneity of variance (Levene statistics). Within subjects (repeated measures) ANOVA was performed to find significant differences between the cell numbers estimated by means of a Coulter counter and cell counts obtained by an inverted microscope. rmANOVA was also used to find significant differences between cell volume values obtained microscopically and electronically, and to estimate the differences between cell volume values obtained by the formulas from Roth (1981) and after Hillebrand et al. (1999). The t- test was applied to estimate the differences in cell number obtained by the Coulter counter among the samples taken from duplicates of Erlenmayer flasks. Correlations and simple bivariate regression were performed to determine relationships between growth rates, cell volume, surface area to volume ratio, volume to surface area ratio and photosynthetic parameters. The significance (p) is given as 1-tailed for the Pearson correlation coefficients. Degrees of freedom are presented in parentheses for the rmANOVA results. All the statistical analyses were conducted using PASW (SPSS) program (PASW, Chicago, IL; USA).

Results

Growth and cell volume characteristics of the selected Cosmarium strains under the constant light-temperature regime

The electronic counter provided reliable data concerning the cell number, for all of the investigated strains. rmANOVA, performed to determine the significant differences between the results obtained electronically and those from microscopic counts, showed reproducible data from the Coulter counter, for all of the investigated strains (*C. punctulatum* No. 570 $F(1, 26) = 32.1$, $p=0.121$; *C. punctulatum* No. 571 $F(1, 14) = 8.11$, $p = 0.684$; *C. beatum* $F(1, 22) = 11.51$, $p = 0.19$; *C. crenatum* $F(1, 42) = 15.07$, $p = 0.098$; *C. meneghinii* $F(1, 32) = 25.07$, $p = 0.135$; *C. regnesii* $F(1, 16) = 4.27$, $p = 0.275$). Variation in the cell number within duplicates from Erlenmeyer flasks was less than 2%, and the t-test proved that, among all the investigated strains, there was no significant difference between the samples taken from both flasks (not shown). The mean cell volumes obtained from a Coulter counter exhibited slightly higher values as

compared to the microscopically obtained cell volume values. When applied, however, rmANOVA did not show statistically significant differences between these two types measurements (not shown). The counter is not able to provide the data on the surface area of particles which may represent a large disadvantage in the desmid physiology investigation. For this reason, cell volumes and surface areas obtained from microscopic linear measurements were taken into consideration in this study. There was no significant statistical difference between the values of cell volume obtained by the formula from Hillebrand et al. (1990), and formulas used in this investigation for medium-sized taxa (*C. punctulatum* No. 570 $F(1, 42) = 2.21$, $p = 0.156$; *C. punctulatum* No. 571 $F(1, 22) = 30.71$, $p = 0.18$; *C. beatum* $F(1, 18) = 34.62$, $p = 0.295$; *C. crenatum* $F(1, 22) = 13.7$, $p = 0.189$) and *C. meneghinii* ($F(1, 42) = 18.01$, $p = 0.25$). However, the approximation of the *Cosmarium* shape according to Hillebrand et al. (1990) significantly differed from the approximation of the *C. regnesii* var. *polonicum* shape as a quadratic cell ($F(1, 16) = 4.97$, $p < 0.05$).

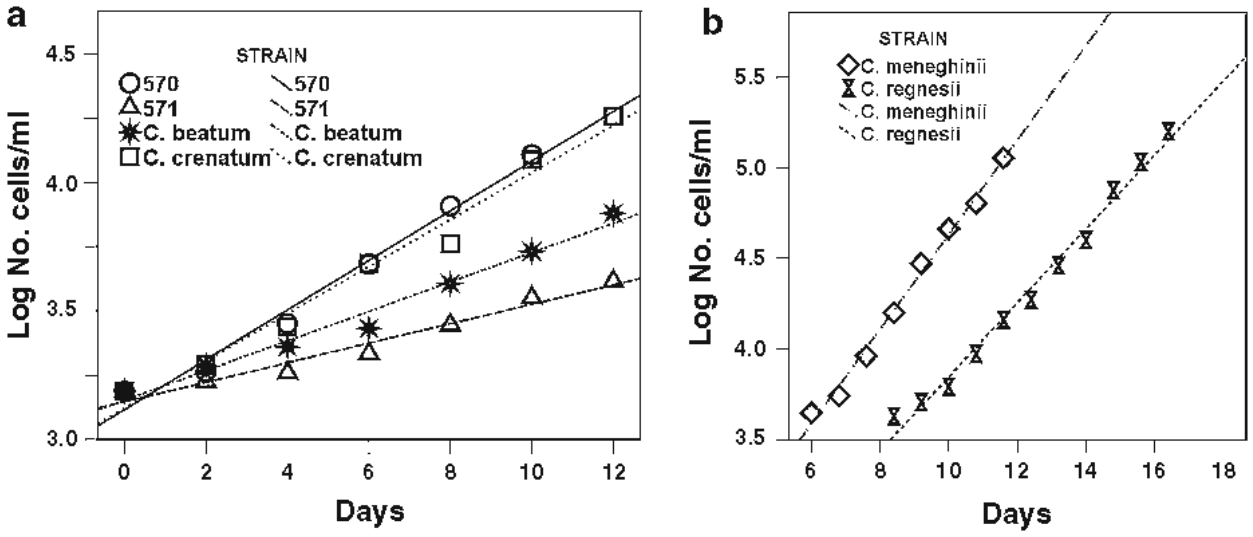

Fig. 2. Linear regression calculated from the changes of log cell number/ml of the investigated *Cosmarium* strains (**a**) medium-sized taxa: ○ – *C. punctulatum* var. *subpunctulatum* (No. 570), △ – *C. punctulatum* var. *subpunctulatum* (No. 571), ✷ – *C. beatum*, □ – *C. crenatum* var. *boldtianum*; (**b**) small-sized taxa: ◇ – *C. meneghinii*, ⧖ – *C. regnesii* var. *polonicum*; cultured under constant light-temperature regime (16°C, 30 µmol photon m^{-2} s^{-1}). Weak increases of cell numbers at the beginning and end of the logarithmic phases of small-sized taxa were excluded in order to find the best fit for the exponential phase.

The cosmopolitan strain collected from the high-mountain, tropical zone, *C. punctulatum* var. *subpunctulatum* (No. 570), was characterised by the steepest logarithmic phase among all of the investigated medium-sized taxa (Fig. 2a, Table 2) and the highest maximum measured growth rate (Table 3). A slightly lower value of the maximum measured µ was observed for *C. crenatum*, while the tropical species, *C. beatum*, showed noticeably low growth rates although both species were characterised by a similar value of mean cell volume, thus showing its poor adaptability under the present experimental conditions. In addition, the need to develop relatively complicated cell wall sculptures, represented as marginal bidentate crenations

and centrally positioned granules on each semicell, may also influence the low growth rates of the tropical species.

Strain						
Regression parameters	*C. punct.*(No. 570)	*C. punct.* (No. 571)	*C. beatum*	*C. crenatum*	*C. menegh.*	*C. regnesii*
R (r)	0.9916	0.9827	0.9888	0.9895	0.9973	0.9949
R^2 (r^2)	0.9832	0.9657	0.9777	0.9791	0.9947	0.9899
Regression equation	y' = 2.97 +2.92*x	y' = 3.07 +2.48*x	y' = 3.09 +2.66*x	y' = 2.99 +2.86*x	y' = –6.05 +4.67*x	y' = –5.62 +4.58*x
β coefficient (standardized coefficient)	0.9916	0.9827	0.9891	0.9895	0.9973	0.9939

Table 2. Parameters of the linear regression analysis of the changes of log cell number/ml for the investigated *Cosmarium* strains (y' – log cell number/ml; x – days).

The small-sized taxa were attributed by higher growth rates compared to the medium-sized taxa. Interestingly, the highest maximum measured μ was noted for *C. meneghinii*, although it was characterised by a higher cell volume compared to that recorded for *C. regnesii* var. *polonicum*.

Strain						
Growth parameters	*C. punct.* (570)	*C. punct.* (571)	*C. beatum*	*C. crenatum*	*C. menegh.*	*C. regnesii*
Maximum measured growth rates (day^{-1})	0.26 (±0.01)	0.15 (±0.02)	0.17(±0.01)	0.23 (±0.03)	0.34 (±0.03)	0.31 (±0.03)
Maximum division (generation) time (day^{-1})	0.38	0.21	0.24	0.32	0.49	0.45
Minimum doubling time (day)	2.62	4.77	4.09	3.08	2.05	2.22
Cell volume (μm^3)	7575 (±88)	12914 (±101)	9686 (±58)	9224 (±35)	1353 (±30)	594 (±19)
Mean cell volume (μm^3)	8090 (±89)	11368 (±92)	9796 (±50)	9378 (±36)	1151 (±25)	519 (±22)
Surface/Volume (μm^{-1})	0.325	0.274	0.291	0.23	0.431	0.538

Table 3. Maximum measured growth rates (±95% confidence intervals), division times, minimum doubling times, cell volume estimated at the maximum measured μ, surface/volume ratio estimated at the maximum measured μ, and averaged cell volume of the logarithmic phase; for the investigated *Cosmarium* strains under constant laboratory conditions (16°C, 30 μmol photon m^{-2} s^{-1}).

When maximum measured μ values of all the investigated *Cosmarium* strain were plotted against the cell volume (dimensions at the maximum measured μ) a significant negative linear correlation was observed ($r = -0.946$, $p < 0.001$) (Fig. 3a). In addition, maximum measured μ and S/V ratio had a significantly positive correlation ($r = 0.771$, $p < 0.05$) (Fig. 3b).

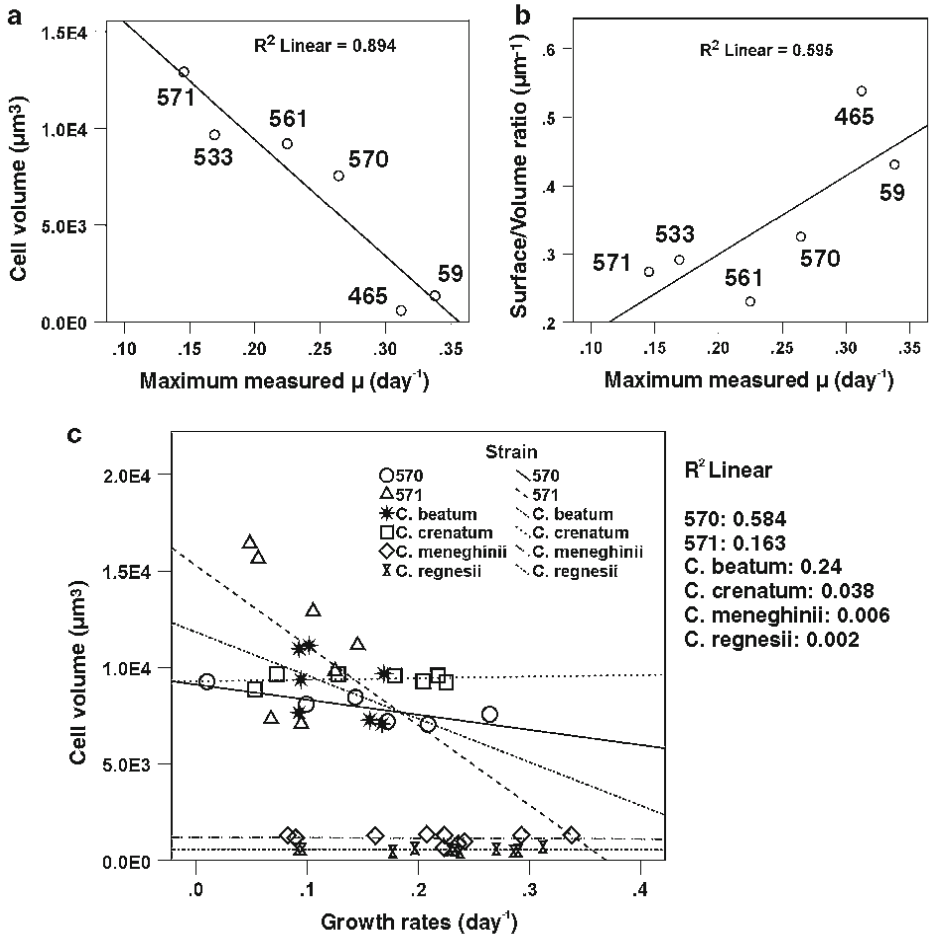

Fig. 3. Relationships between maximum measured µ of the investigated *Cosmarium* strains and: (**a**) cell volume estimated from the maximum measured µ (equation: cell volume = 21517.49 – 60355.63 * maximum measured µ); (**b**) surface area to volume ratio (equation S/V = 0.168 + 1.156 * maximum measured µ), numbers refer to SVCK strain numbers, as given in Table 1; (**c**) relationships between growth rates and cell volume values, during the logarithmic growth of the investigated *Cosmarium* strains: ○ – *C. punctulatum* var. *subpunctulatum* (No. 570), △ – *C. punctulatum* var. *subpunctulatum* (No. 571), ✸ – *C. beatum*, □ – *C. crenatum* var. *boldtianum*, ◇ – *C. meneghinii*, ⧖ – *C. regnesii* var. *polonicum*.

As expected, desmid strains having high maximum measured growth rates were characterised by a high S/V ratio, as was found for both small-sized taxa. Interestingly, this relationship was somewhat disturbed in the medium-sized taxa. *C. punctulatum* (No. 570) was characterised by the highest growth rates and the highest S/V ratio. The next strain that expressed a large S/V value was *C. beatum*, although it was characterised by the relatively low maximum measured µ. The lowest values for the S/V ratio were noted for the arctic species, *C. crenatum*, and the polar strain of *C. punctulatum* (No. 571). Remarkably, the polar strain of *C. punctulatum* (No. 571) was characterised by higher cell volume and consequently lower S/V ratio and lower µ, compared to the high-mountain, tropical strain of the same species.

The changes in cell volume were not correlated with changes in growth rates during the logarithmic growth phase for the small-sized taxa and *C. crenatum*, but this correlation was slightly negative for the other medium-sized taxa (Fig. 3c).

At the beginning of the logarithmic phase, the thickest mucilaginous envelope was found in the arctic representative *C. crenatum*, reaching around 40 µm and forming a copious shield around cells. The polar strain of *C. punctulatum* (No. 571) was also characterised by the expressively developed mucilaginous

sheath (35 µm), while the tropical species *C. beatum* possessed a cottonous mucilaginous sheath (mean thickness 25 µm). *C. punctulatum* (No. 570) showed the lowest thickness of the mucilaginous envelope among the medium-sized taxa, reaching approximately 10 µm. *C. regnesii* var. *polonicum* was characterised by a soft sheath, holding the cells together into mucilaginous clumps (average thickness: 10 µm). All of the desmid taxa mentioned increased the thickness of the mucilaginous envelope during the logarithmic phase. At the beginning of the stationary phase, the thickest mucilaginous sheath was found in *C. crenatum* (50 µm), followed by *C. punctulatum* (No. 571) (45 µm), *C. beatum* (35 µm), *C. punctulatum* (No. 570) (20 µm), and *C. regnesii* (13 µm). On the other hand, *C. meneghinii* did not expose any trace of mucilaginous sheath during the whole growth period.

Photosynthetic characteristics of the selected Cosmarium strains under the constant light-temperature regime

Photosynthetic characteristics of the investigated *Cosmarium* strains under the constant light-temperature regime (16°C, 30 µmol photon m^{-2} s^{-1}) are shown in Table 4. The highest value of maximum quantum yield (Fv/Fm) was measured for the cosmopolitan strain *C. meneghinii*; additionally, it was characterised by the highest mean of maximum quantum yield (derived from the logarithmic growth phase). Furthermore, *C. punctulatum*, collected from the high-mountain, tropical zone exhibited distinctly high Fv/Fm values, followed by the polar strain of the same species and the arctic species, *C. crenatum*. The typical tropical desmid, *C. beatum*, showed the lowest measured values of Fv/Fm among all of the investigated strains. Distinctly high values for $rETR_{max}$ were noted for the arctic strain, *C. crenatum*, pointing to its large photosynthetic capacity under the investigated conditions. The high-mountain strain of *C. punctulatum* exhibited somewhat lower values of $rETR_{max}$, followed by *C. beatum* and *C. regnesii* var. *polonicum*.

As an indicative of the light adaptation of the photosynthetic process, the saturating irradiance (I_k) values revealed additional differences between the *Cosmarium* strains. The strains characterised by the highest maximum and mean I_k values were the arctic representative (*C. crenatum*, Fig. 4a) and the tropical species (*C. beatum*). Somewhat lower I_k values were recorded for the high-mountain strain of *C. punctulatum* and *C. regnesii*, while the Norwegian strain of *C. punctulatum* showed lower I_k values as compared to these two isolates.

By far the highest photosynthetic efficiency, estimated as a slope of P-I curve, was noted for *C. meneghinii* (Fig. 4b). In addition, relatively high photosynthetic efficiency was recorded for the polar strain of *C. punctulatum* and *C. crenatum*. On the other hand, *C. beatum* demonstrated the lowest photosynthetic efficiency, estimated according to the lowest α value recorded.

Photosynthetic parameters		Strain					
		C. punc.(570)	*C. punc.* (571)	*C. beatum*	*C. crenatum*	*C. menegh.*	*C. regnesii*
Fv/Fm	Maximum recorded	0.662	0.63	0.555	0.624	0.688	0.603
	Mean	0.637 (±0.011)	0.594 (±0.019)	0.506 (±0.023)	0.587 (±0.015)	0.647 (±0.021)	0.552 (±0.019)
$rETR_{max}$	Maximum	153.2	125.3	144.5	203.8	133.6	145.4
	Mean	140 (±15)	104.1 (±10)	129 (±11)	179.4 (±12)	115.7 (±10)	113.4 (±16)
I_k (µmol photon $m^{-2}s^{-1}$)	Maximum	754.4	695.6	860.7	975.3	531.7	732
	Mean	688 (±31)	625.3 (±42)	784.5 (±59)	830.6 (±48)	485.9 (±38)	640.3 (±41)
α (slope of P-I curve)	Maximum	0.225	0.236	0.189	0.229	0.248	0.203
	Mean	0.22 (±0.008)	0.229 (±0.002)	0.18 (±0.009)	0.215 (±0.004)	0.235 (±0.004)	0.190 (±0.010)

Table 4. Photosynthetic parameters of the investigated *Cosmarium* strains; maximum – maximum values recorded, mean – average values, derived from the logarithmic growth phase for each strain (± 95% confidence interval). Fv/Fm – maximum recorded quantum yield, $rETR_{max}$ – maximum relative electron transport rate, I_k – saturating irradiance, the light intensity at which the initial slope of curve (α) intercepts the horizontal asymptote ($rETR_{max}$), α – slope of P-I curve, determined using the hyperbolic tangent equation from Jassby and Platt (1976).

To determine significant relationships between growth and photosynthetic characteristics among the investigated strains, maximum measured µ values were plotted against the investigated photosynthetic parameters for all *Cosmarium* strains. Slightly positive correlations were observed between the maximum measured µ values, on the one hand, and Fv/Fm or α values on the other hand. This suggests that desmids characterised by high growth rates exhibit better photosynthetic efficiency; however, these relationships were not statistically significant (maximum measured µ and max. Fv/Fm: $r = 0.569$, $P = 0.119$; maximum measured µ and max. α: $r = 0.382$, $P = 0.294$). With regard to the investigated desmid strains, no correlation was observed between the maximum measured µ and photosynthetic capacity ($rETR_{max}$) or saturating irradiance (I_k). Correlations between cell volume (estimated at the maximum measured µ or as a mean value) and values for Fv/Fm, $rETR_{max}$ and I_k did not exist, thus showing the independence of photosynthetic activity, capacity and saturating irradiance with regard to the cell volume of the *Cosmarium* strains.

The correlation between the mean values of Fv/Fm and α was significantly positive ($r = 0.77$, $p < 0.05$), showing that the desmid strains characterised by a high photosynthetic yield were simultaneously attributed by a high photosynthetic efficiency. Although a slightly negative correlation was observed in the plot between the mean of Fv/Fm and I_k values, this relation was not statistically significant ($r = –0.498$, $p = 0.157$). On the other hand, positive correlations in the relationship between $rETR_{max}$ and I_k values (with regard to both maximum and mean values) were observed, and they were statistically significant (max. $rETR_{max}$ and max. I_k: $r = 0.767$, $p < 0.05$; mean $rETR_{max}$ and mean I_k: $r = 0.734$, $p < 0.05$).

No significant correlations were observed when growth rates and photosynthetic parameters of the investigated strains were plotted over the logarithmic growth phase (not shown). This suggested that photosynthetic parameters were consistent during the investigation period and independent on the growth kinetics. Therefore, it is recommended that they may be used as valid for determining differences between the strains. The photosynthetic characteristics of the *Cosmarium* strains appeared both species- and strain-specific during the whole period of measurements.

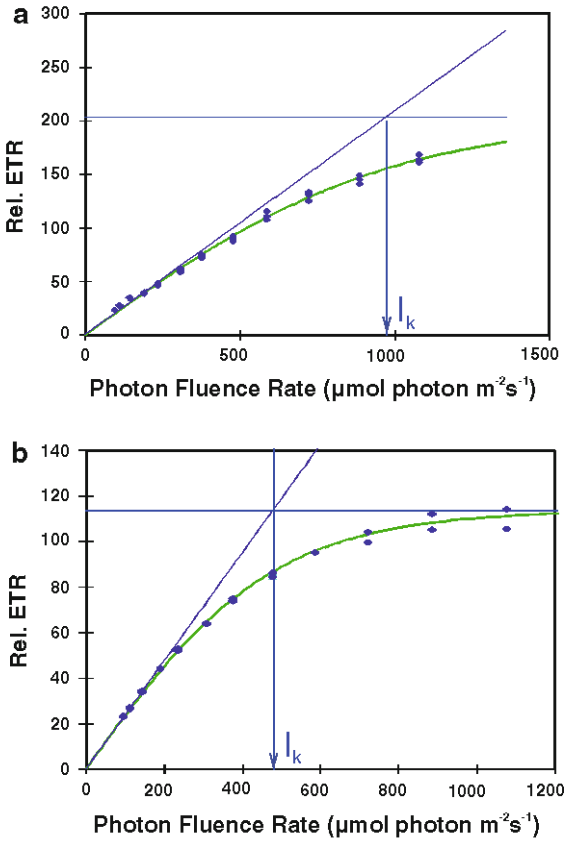

Fig. 4. Photosynthetic performance (P-I curves) of two investigated *Cosmarium* strains, from the middle of the logarithmic growth phase (**a**) *C. crenatum* var. *boldtianum*: $rETR_{max}$ 203.8, slope 0.209, and I_k 975.3 µmol photon $m^{-2}s^{-1}$ (**b**) *C. meneghinii*: $rETR_{max}$ 116.6, slope 0.248, and I_k 478.1 µmol photon $m^{-2}s^{-1}$.

Discussion

The distinctly long minimum doubling times (2–4 days) for the medium-sized taxa were in accordance with the remark that growth of desmid isolates was mainly limited to temperatures below 15°C (Coesel and Wardenaar 1990). The small-sized strains exhibited higher intrinsic growth rates as compared to the medium-sized taxa, which was comparable with observations of several microalgal species when studied in phytoplankton communities and under culture conditions (Fogg 1975; Reynolds 1984). Remarkably, the cosmopolitan taxon, *C. meneghinii*, exhibited the highest maximum measured µ, although it was characterised by a twofold larger cell volume as compared to *C. regnesii* var. *polonicum*. Such a high maximum growth rate may be expected, considering that *C. meneghinii* is one of the desmid species confined not only to oligo-mesotrophic ecosystems, but also to distinctly eutrophic habitats. Coesel and Wardenaar (1994) pointed out that the maximum growth rates in desmids originating from eutrophic habitats (i.e. in *Staurastrum chaetoceras* (Schr.) G.M. Smith) were around 50% higher than those found in the typical oligo-mesotrophic representatives, such as *C. abbreviatum* Rac. var. *planctonicum* W. & G.S. West. Due to this feature, eutrophic planktonic desmids can compete even with fast-growing green microalgae and diatoms. Strikingly enough, although *C. meneghinii* was isolated from a nutrient-poor (oligotrophic) fountain pond and cultivated for more than 80 years under the constant laboratory conditions, it still preserved its native responses, as expected from a eutrophic desmid representative.

The high growth rates of *C. meneghinii* certainly appeared to be due to the absence of a cellular mucus layer in this species, comparable with the observations given by Coesel (1994) and Coesel and Wardenaar (1994). *C. regnesii*, being a typical meso-oligotrophic representative, obviously has to invest photosynthetic energy into the mucilaginous sheath production, which explains its somewhat lower growth efficiency as compared to *C. meneghinii*. Taking into account that *C. meneghinii* had been isolated from a nutrient-poor habitat and exhibited no mucus production during the long-term period of cultivation (even in a nutrient-depleted stationary phase), this characteristic may be considered as genotypically preserved. All the other *Cosmarium* strains gradually increased the thickness of their mucilaginous sheaths during the logarithmic phase, reaching the maximum thickness value at the beginning of a stationary phase. This may be considered as a consequence of the decreased nutrient amount in batch vessels, as several authors suggested that the extracellular matrix had an important role in the trapping and concentration of nutrients (e.g. Yeh and Gibor 1970). The application of the electronic paramagnetic resonance (EPR) technique to a few desmid strains confirmed that the mucilaginous capsule functioned as a selective diffusion medium, concentrating molecules characterised by small and hydrophobic spin labels into the interior of the cell (Freire-Nordi et al. 1998, 2006). Interestingly, *C. crenatum* possesses the thickest mucilaginous envelope among all the *Cosmarium* strains, which might be linked to the role of the extracellular matrix as a protection from freezing and UV radiation in a harsh arctic environment (Boney 1982; Shephard 1987; Komárek and Nedbalová 2007).

The cell volume of medium-sized taxa concomitantly decreased during the logarithmic phase as growth rates increased (Fig. 3c). This coincided with the fact that large and medium cell-sized microalgal clones tend to display strongly reduced cell volumes with an increasing μ (Coesel and Wardenaar 1990). On the other hand, no correlation was found between these parameters during the logarithmic growth of the small-sized taxa, which contradicted the investigations conducted with other small desmid taxa (Coesel and Wardenaar 1994) or small-sized green algae (Prokop and Řičica 1968; Gons and Mur 1980), grown in continuous-flow cultures. The fact that the small-sized taxa wasted nutrients rapidly during the logarithmic phase in batch assays and/or the low experimental temperature, may contribute to the explanation of this discrepancy.

The application of the formula for the *Cosmarium* cell volume calculation given by Hillebrand et al. (1999) showed no significant deviation from the approximation of the *Cosmarium* shapes as described in the section on Materials and Methods, with the only exception of *C. regnesii* var. *polonicum*. Since it is more simplified and practical than the cell volume approximations used in this research, it may be recommended for routine phytoplankton analyses. Unfortunately, the authors did not provide a formula for calculating the ellipsoid surface, as it is rather complicated and may yield an error in the calculation (Bowman 1961).

The arctic species (*C. crenatum*) and the polar representative of *C. punctulatum* (No. 571) were characterised by the lowest surface area to volume ratio as compared to the other *Cosmarium* medium-sized isolates. Taking into account that arctic-alpine desmids are characterised by simple and compact cell shapes

with shallow sinuses, which greatly decreases S/V ratio and prevents desiccation (Coesel 1996; Brook 2001), both *C. crenatum* and *C. punctulatum* (No. 571) morphologically appeared well suited to polar climate conditions. Remarkably, although both strains were cultivated for more than 15 years under identical laboratory conditions, still they showed the cell organisation characteristic to that of the arctic-alpine desmid group. Yet, these characteristics were better expressed in the typical arctic taxon than in the cosmopolitan strain collected from the polar region of northern Norway.

From the distinctly high values of I_k and $rETR_{max}$ obtained from P-I curves, the *Cosmarium* strains can be categorised as algae adapted to rather high light intensities. Considering that desmids are inhabitants of various shallow freshwater habitats such as peat pits, ditches, puddles, ponds, also including semi-aerophilic manner of life (on wet surfaces of rocks, soil, mosses, ephemeral pools, etc.) it is expected that this algal group is well adapted to high solar radiation.

Among all the investigated strains, the arctic strain, *C. crenatum*, appeared best adapted to high light intensities, based on the highest values of saturating irradiance measured. Such large I_k values might be expected, considering that it was collected from mosses growing among the permafrost of Cape Flora, where solar irradiance might be largely amplified due to the steep slope of the peninsula and the albedo from remaining snow surfaces. The I_k and $rETR_{max}$ values obtained for *C. crenatum* corresponded to those measured in vegetative cells of *Chlamydomonas nivalis* (Bauer) Wille, which were collected from snow surface and fluorescence measurement was performed directly after the sampling (Stibal et al. 2007). Additionally, several authors (e.g. Mosser et al. 1977; Remias et al. 2005) recorded the distinctly high I_k values for algae collected from polar and alpine areas. Although it was cultivated for a long period under low irradiances, *C. crenatum* still exhibited the high saturating irradiance and photosynthetic capacity typical for algae adapted to high light intensities. On the other hand, relatively low I_k and $rETR_{max}$ values were noted for the polar strain of *C. punctulatum*. These attributes obviously appeared to be due to the influence of the coastal climate of northern Norway, which is particularly milder than the severe arctic climate of Franz Joseph Land, and characterised by foggy weather which largely screens the ground from intensive solar irradiation. In addition, *C. punctulatum* var. *subpunctulatum* is known as a taxon distributed mainly in temperate and continental climate zones, hence, it is not a typical representative of the arctic-alpine desmid flora, which may additionally contribute to the relatively low photosynthetic parameters measured in this isolate. The noticeably high I_k and $rETR_{max}$ recorded for *C. beatum* coincided with high solar irradiance in circumequatorial region, in accordance with what was demonstrated for various tropical macroalgae (Lüning 1981, 1990; Lobban and Harrison 1994).

This study revealed not only the differences in growth rates and S/V ratios of the isolates belonging to the same taxon (*C. punctulatum* var. *subpunctulatum*), but also the dissimilarity in photosynthetic behaviour. The relatively high photosynthetic capacity and saturating irradiance characterised the high-mountain, tropical strain showing its photosynthetic adaptability to intense solar irradiation, while the low

I_k and $rETR_{max}$ values were recorded for the polar strain, as is generally typical for low-light adapted algae (Gómez 2001; Hanelt et al. 2003). The question whether these two strains of *C. punctulatum* var. *subpunctulatum* have to be considered as ecotypes or ecospecies, can only be proved by hybridisation experiments. If a free gene exchange would be prevented between these two strains, they should be considered as two ecospecies, rather than ecotypes (Coesel 2003). Although the hybridisation was not performed during this study, the morphological and physiological differences noted were rather small and evidently the result of adaptation to environmental conditions. Therefore, it is suggested that the investigated strains (Nos. 570 and 571) represent two ecotypes belonging to one cosmopolitan and widely spread species. Kouwets (1987) noticed quite various ornamentation of the central part of semicells in specimens of *C. punctulatum* var. *subpunctulatum* collected from peat ponds in central France, yet all of the specimens conformed to the original description of the species. Even the samples collected from a single habitat within 1 day may reveal an enormous number of specimens showing a wide range of polymorphism but still belonging to the same species, as it was noted in *C. taxichondrum* Lund. (Gerrath 1979).

The cosmopolitan taxon, *C. meneghinii*, was characterised by the highest photosynthetic efficiency and maximum quantum yield among all the *Cosmarium* taxa studied and, therefore, it appeared most optimally suited for light utilisation under experimental conditions. However, the photosynthetic capacity of this species was not as high as expected. It was previously found that desmids possessing high growth rates (originating from eutrophic environment) also have a high photosynthetic capacity (Coesel and Wardenaar 1994; Spijkerman and Coesel 1998, Spijkerman et al. 2004). The deviation found in *C. meneghinii* might appear as a consequence of acclimation to the relatively cold temperature (16°C), which caused the decrease of $rETR_{max}$ and subsequently the increase of the slope of the P-I curve (i.e. photosynthetic efficiency increased) (compare Figs. 4a and b).

Interestingly, positive correlations were observed between maximum measured μ and Fv/Fm or α values, in all the investigated *Cosmarium* strains. Although these correlations were not statistically significant, it appeared that photosynthetic efficiency was higher in the smaller and fast-growing *Cosmarium* strains. In connection with this, those species possessing high yield values (such as *C. meneghinii* and both strains of *C. punctulatum*) also have high photosynthetic efficiency, which may additionally contribute to the explanation of their cosmopolitan distribution.

The *Cosmarium* strains characterised by a large photosynthetic capacity may be concomitantly attributed as algae adapted to high solar irradiation, according to the statistically positive correlations between $rETR_{max}$ and I_k. For instance, two strains originating from clearly different climate zones (*C. crenatum* and *C. beatum*) were characterised by markedly high and similar values for I_k and $rETR_{max}$ and, thus, both behaved as algae adapted to the high light intensities (e.g. Hanelt et al. 2003). These observations can be considered as expected, taking into account the occasional intensive solar radiation in their habitats. However, the maximum quantum yield and photosynthetic efficiency of these two species are rather

different, revealing better photosynthetic competence of the arctic taxon under the relatively low laboratory temperature.

The apparent geographic distribution patterns of numerous kelp taxa were recognised, e.g. for the species belonging to genera *Laminaria*, *Desmarestia*, *Himantothalus*, *Ascoseira*, *Acrosiphonia*, *Urospora*, and *Gigartina*; moreover, the occurrence of climate-induced endemic species belonging to some of these genera was demonstrated (Lüning and Neushul 1978; Chapman and Lindley 1980; Lüning 1990; Wiencke et al. 1993; Wiencke 1996; Bartsch et al. 2008). However, only a few similar observations on the possible climatic zonation of microalgal species have been published. Hulburt and Guillard (1968) pointed to the different geographic distribution of two species belonging to the genus *Skeletonema*. The tropical species *S. tropicum* Cleve has no cold-water races and is confined exclusively to tropical climate, whereas the species *S. costatum* (Greville) Cleve comprises both northern and southern races, and hence exhibits cosmopolitan and wide-spread distribution. Several strains of *Xanthonema* sp. from various localities situated in Antarctica, Europe and New Zealand showed dissimilar responses to salinity stress in experimental conditions, revealing clones from the alpine European areas, and several isolates from Antarctica as the most sensitive to high salinity (Broady et al. 1997).

It is worth noting that the genus *Cosmarium* has always been regarded as an artificial genus and, thus, taxonomically problematic (West and West 1905, 1908; Prescott et al. 1981; Brook and Johnson 2002); the polyphyly of this genus has subsequently been confirmed by molecular tools (Gontcharov and Melkonian 2008, 2011). Three out of the six investigated *Cosmarium* strains (No. 59, 465 and 570) are classified into three unrelated lineages, according to the results of the sequencing of two ribosomal genes (nu SSU rDNA and cp LSU rDNA), the protein-coding *rbcL*, and the noncoding 1506 group I intron of the SSU rDNA, which additionally contributes to the explanation of the physiological differences between the strains studied (Gontcharov and Melkonian 2008, 2011).

Reviewing the attributes of main desmid floral regions, Coesel (1996) stated a hypothesis that the evolutionary origin of desmidiaceous algae was confined to the tropical region. As evidence for the hypothesis, he presented several arguments regarding this algal group: desmid flora is morphologically more diversified in tropical regions as compared to that in (sub)polar areas; the contribution of desmids to the algal biomass seems to play a more important role in tropical aquatic systems than in cold-temperate ones; desmids are quantitatively a dominant group of primary producers in tropical aquatic ecosystems; the experimentally determined optimal growth temperature for several desmid strains ranges from 25–30°C. Our study may be considered as a supplement to this hypothesis given that desmids appear as algae adapted to high light intensities, characteristic not only for desmids in shallow water bodies, but also at low latitudes. The relatively low growth rates noted for the *Cosmarium* strains are obviously caused by the low cultivating temperature.

The preliminary study shows that differences in morphological and physiological characteristics of the desmid isolates collected from various climates exist, despite the long-term cultivation. Moreover, this is

the first detailed report on the photosynthetic performance of desmids collected from various climate regions, during their growth under ordinary laboratory conditions. The next step of investigation, which includes the application of various light and temperature regimes to the desmid strains, will try to clarify whether the distribution of microalgae follows the geographic pattern as has been observed for certain macroalgae.

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3. Adaptation of growth and photosynthesis to certain temperature regimes is an indicator for the geographic distribution of *Cosmarium* strains (Zygnematophyceae, Streptophyta)

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Abstract

Climatically induced geographical distribution patterns of macroalgae were demonstrated considering their physiological behaviour *in vitro* during and after various temperature or light treatments. In contrast to numerous investigations on possible macroalgal geographic distributions, similar studies regarding microalgae lack. This study deals with the potential influence of temperature on patterns of geographic distribution of six *Cosmarium* strains isolated from various climate zones that were grown long-term at relatively low temperature-light regime. Growth and photosynthetic parameters, obtained from PAM fluorometry, were used to estimate the physiological characteristics of the strains during and after various temperature treatments. Long-term acclimation at constant temperature-light condition tended to affect photosynthetic parameters more than algal growth characteristics. However, all of the *Cosmarium* strains demonstrated physiological responses that were consistent with their source location under both low and high temperature conditions, confirming that such responses are genetically preserved. *Cosmarium* strains displayed photosynthetic capacity and levels of the onset of saturation that repeatedly exceeded values recorded for other microalgae and seaweeds, indicating that these desmid strains are adapted to high light. This fact, as well as the relatively high growth temperature optima for all of the *Cosmarium* strains, are further evidence in support of Coesel's hypothesis on the origin of desmids in the tropical zone. Interestingly, the *Cosmarium* strains demonstrated not only adaptive characteristics in accordance with the temperature prevailing at their sampling sites, but also with regard to their evolutionary origin.

Key words: *Cosmarium,* excitation pressure, growth rate, maximum quantum yield, nonphotochemical quenching, photosynthetic capacity and efficiency, onset of saturation, temperature gradient.

Abbreviations

P-I curve – photosynthesis-irradiance curve, PSII – photosystem II, $rETR_{max}$ – maximum relative electron transport rate, Pmax – maximum rate of photosynthesis, I_k – onset of saturation, α – slope of P–I curve, photosynthetic efficiency, Fv/Fm – maximum quantum yield of PSII, qP – photochemical quenching, 1 – qP – excitation pressure, NPQ – non-photochemical quenching, μ – growth rate, HS – heat stress, Q_A – primary electron acceptor, ROS – reactive oxygen species

Introduction

Numerous investigations on physiological features of seaweeds treated *in vitro* under various light-temperature conditions revealed that macroalgae demonstrate photosynthetic characteristics in accordance with the climate regime of their sampling sites (i.e. seaweeds may behave as sun- or shade-adapted plants and as warm- or cold-environment adapted plants) (Lüning 1990; Hanelt et al. 2003). Generally, the results of these investigations indicated that seaweed species exhibit consistent geographic and depth distribution patterns, as judged from their physiological behaviour and resistance under a set of experimental temperature-light gradients (Lobban and Harison 1994).

In contrast to macroalgae, micro-organisms tend to have a ubiquitous distribution, as stated by many protistologists who improved the famous Beijerinck's metaphor "in micro-organisms, everything is everywhere, the environment selects" (Fenchel 1993; Fenchel and Finlay 2004). However, the occurrence of numerous endemic and flagship taxa in microalgal groups such as dinoflagellates, diatoms, coccolithophores, chrysophytes, and desmids repeatedly pointed out the flaws of the hypothesis on protist ubiquity (Coleman 2001, 2002; Foissner 2006). Except for a few cosmopolitan representatives, desmids are principally known for their preference for specific habitats and climatic regions (Coesel 1996; Spijkerman and Coesel 1998; Coesel and Krienitz 2008).

Nevertheless, only a few experimental and molecular (gene sequencing) approaches have been performed to investigate to what extent climatic factors are involved in the shaping of microalgal geographic patterns (summarised by Coleman 2002; Šlapeta et al. 2005; Foissner 2006). Previous observations on physiology and climate-induced occurrence of microalgae lead to the following questions. Do microalgal isolates from various regions demonstrate different physiological behaviour which is in accordance with the prevailing climate at the site of clonal origin? Are such responses also expressed under a set of varying temperature conditions, or acclimation occurred due to the influence of long-term constant laboratory conditions? Accordingly, is it possible to estimate microalgal geographical patterns considering the physiological responses under a set of various temperature (or light) conditions, similar to what has been observed for macroalgae and vascular plants? Regarding these issues, two important terms should be recognised: adaptation – a genotypic response to long-term environmental changes (the alterations in the genome are stable and remain in the population over generations); acclimation – a response induced by an environmental change that causes a phenotypic alteration with no change in genetic complement (Davison 1991; Huner et al. 1996).

A previous study showed that cells of *Cosmarium* isolates, collected from various climate zones, indeed exhibited native growth and photosynthetic responses under unchanged conditions of a climate chamber (Stamenković and Hanelt 2011). Interestingly, these physiological differences appeared despite the long-term cultivation (more than 15 years) under identical and constant laboratory conditions. Likewise, several authors noted that temperature-induced responses in cultivated microalgae can be species- and even clone-specific, depending on the geographic region from which they had been isolated (Eppley 1972; Kasai

and Ichimura 1990; Suzuki and Takahashi 1995; El-Sabaawii and Harrison 2006). Vidyavati et al. (1983) observed that tropical Indian isolates of *Closterium*, *Cosmarium*, and *Euastrum* were seriously damaged at 0°C and appeared more resistant to short-term exposure to high temperatures (40 and 45°C). A distinctly higher optimum growth temperature (35°C) was noted for a tropical *Cosmarium* isolate, compared to that recorded for desmids from a temperate climate zone (25–30°C) (Bouterfas et al. 2002; Coesel and Wardenaar 1990). These findings warrant further investigation of the strength of the relationship between temperature adaptation and patterns of geographic distribution in desmid strains.

Growth rates and photosynthetic parameters were used to estimate the physiological behaviour of six *Cosmarium* strains collected from various climate areas and grown at different, constant temperatures from 0 to 37°C, temperatures that cover a broad, biologically relevant range (i.e. from 0 to 40°C (Alexandrov 1977) or 0 to 50°C (Maxwell et al. 1995)). Although differences in temperature are often accompanied by changes in light intensity and, thus, these two parameters should be considered in parallel (Maxwell et al. 1995), the present study quantified the effect of temperature at non-saturating irradiance level which had been applied long-term to the desmid cultures. Furthermore, it is known that low- or high-temperature applications combined with light stress cause inactivation of PSII more rapidly than when the two stressors are applied separately, as temperature stress intensifies the inhibition of PSII repair (Murata et al. 2007; Allakhverdiev et al. 2008).

Materials and Methods

Algal strains

The six *Cosmarium* clones examined in this study were isolated from various parts of the world within approximately the same time period, in order to exclude the influences of sampling time and, therefore, the influences of constant nutrient, light and temperature regime under laboratory conditions. One exception was *C. meneghinii*, a typical cosmopolitan desmid, because of the special interest in comparing its physiological activity with that of other ubiquitous taxa (Table 1).

All the strains originated from habitats of a similar trophic condition (oligo-mesotrophic), to avoid the influences of the environmental trophic condition on growth and photosynthetic performance (see Stamenković and Hanelt 2011). Three *Cosmarium* taxa of approximately comparable size (medium-sized taxa, *C. punctulatum* var. *subpunctulatum*, *C. beatum* and *C. crenatum* var. *boldtianum*) and two small-sized taxa (*C. meneghinii* and *C. regnesii* var. *polonicum*) were selected. The details related to taxonomic, ecological and distributional attributes of the taxa investigated, as well as the climate characteristics of the algal sampling locations have been published elsewhere (Stamenković and Hanelt 2011); additionally, a compilation including average surface temperature ranges of the sampling localities (based on data from McKnight and Hess 2001) is shown in Table 1.

Climate zone	Taxon	No. strain (SVCK)	Original No. of strain	Sampling area	Locality coordinates and average temperature ranges	Year of isolation
Polar	*C. crenatum* Ralfs var. *boldtianum* (Gutwinski) W. & G.S. West	561	ASW Wien # 07 141	Cape Flora, Northbrook Island, Franz Joseph Land, Russia	79°57′N 50°05′E -35 – 2°C	1995
Lowland, polar zone	*C. punctulatum* Brébisson var. *subpunctulatum* (Nordstedt) Børgesen	571	ASW Wien # 07 136	pool near Skarsvåg, 80 m a.s.l, the North Cape, Norway	71°06′N 25°49′ E -25 – 10°C	1992
Continental (microthermal)	C. *regnesii* Reinsch var. *polonicum* (Eichler & Gutwinski) Compère	465		pool in East Hokkaidō, Japan	42°54′N, 140°45′E -12 – 21°C	1998
Continental (microthermal)	*C. meneghinii* Ralfs	59	1927/Praha Ac. A 231	fountain in Prague, the Czech Republic	50°05′N, 14°25′E -5 – 23°C	1927
Alpine, tropical zone	*C. punctulatum* Brébisson var. *subpunctulatum* (Nordstedt) Børgesen	570	ASW Wien # 07 182	pool on Mt. Cotopaxi, 1600 m a.s.l., Ecuador	00°40′S 78°26′W 10 – 22°C	1996
Tropical	*C. beatum* W. & G.S. West	533		marshy area nearby Ol Bolossat Lake, Kenya	00°09′S 36°26′E 20 – 31°C	2001

Table 1. Data on the *Cosmarium* strains used for the investigation of growth and photosynthetic behaviour at different temperatures and a constant light regime (30 µmol photons m^{-2} s^{-1}). (MZCH-SVCK – Sammlung von Conjugaten Kulturen, the culture collection of Conjugatophycean algae of the University of Hamburg)

Culture conditions and experimental set-up

All of the *Cosmarium* strains were grown under constant laboratory conditions (16°C, ~30 µmol photons m^{-2} s^{-1}) in a climate chamber of the MZCH-SVCK collection (Sammlung von Conjugaten-Kulturen) over a period of several years. A preliminary investigation revealed that 16°C was sub-optimal for the tropical species (*C. beatum*), as concluded from its low growth rates and poor photosynthetic behaviour (Stamenković and Hanelt 2011). On the other hand, temperatures above 22 or 25°C were sub-optimal (growth-inhibiting) for polar microalgal representatives (Fiala and Oriol 1990; Suzuki and Takahashi 1995). Taking these facts into consideration, all of the experimental *Cosmarium* strains were pre-cultured at 21°C, which was considered as a roughly compromising optimal temperature for both of the tropical and polar strains studied. The mid-range temperature (19°C) appeared as too low temperature for the acclimation of the tropical species, *C. beatum*.

All of the strains were grown in non-axenic batch cultures. The mineral medium based on Kattner et al. (1977) was prepared with double-distilled water for the cultivation of desmids (L-d medium). The *Cosmarium* strains tested had been acclimated to 21°C and L-d medium at least 12 months before the temperature-gradient experiments began.

Sterilised 1 l Erlenmeyer flasks with 500 ml of medium were inoculated to a final concentration of 1500 cells ml^{-1} with the sample taken from pre-cultured strains in the exponential growth phase. Cultures

were bubbled with humidified air at a rate of about 10 l h^{-1} to prevent CO_2 limitation. The cultures were mixed regularly by means of a magnetic stirrer to prevent self-shading of cells. Algal clones were cultured in a climate chamber at 7, 16, 21, 25 and 28°C with a daily light regime of 14 h of light and 10 h of darkness. The light in the climate chamber was provided by white fluorescent tubes (Osram, L65 Watt/25S, Munich, Germany). Using a cosine quantum sensor (LI-COR, LI–188B) the light intensity was adjusted to 30 µmol photons m^{-2} s^{-1}. For each desmid clone tested, growth was assessed during an incubation period of 3–5 weeks, conducting measurements on cell growth and photosynthetic performance every second day except for 25 and 28°C treatments which were measured every day.

To investigate the growth and photosynthetic behaviour of the *Cosmarium* strains at low temperature, a set of inoculated Erlenmeyer flasks was placed directly on plates filled with ice and put in a climate chamber at 0.9°C and 30 µmol photons m^{-2} s^{-1}. The temperature of the medium with cells was 0.6°C (±0.1°C); the cultures were regularly mixed by means of a magnetic stirrer, and the melting ice was replaced each day. After 14 days of the low-temperature treatment, the cultures were transferred to 21°C for recovery.

As temperatures higher than 30°C cause various ultrastuctural changes and/or aberrant morphological growth forms of desmids (Coesel and Wardenaar 1990; Meindl 1990), 32, 35 and 37°C treatments were marked as “heat stress” (HS) experiments. Flasks filled with 50 ml of L-d medium were inoculated with the sample taken from pre-cultured strains at 21°C to a final concentration of about 9000 cells ml^{-1} and placed in the chamber at the test temperature. The cultures were regularly mixed to prevent cell-shading. Duration of the HS experiments was dependent on the desmid cell viability, which was estimated by observation of cytoplasmic streaming (using a light microscope IM-Zeiss) and by chlorophyll fluorescence measurements (using a PAM fluorometer; see below). At the end of the treatments, the flasks with desmids cells were placed in a climate chamber at 21°C for recovery. The temperature of the warmed up medium with cells decreased to around 21°C within 2 h after the transfer. Afterwards, 10 ml of culture medium was replaced with fresh L-d medium, to supplement the nutrient reserves which had been spent during the HS treatments.

Most of the *Cosmarium* strains showed relatively good cell viability a few days after the beginning of cultivation at 32°C, and so this heat stress application was prolonged for 7 days. Temperatures of 35 and 37°C caused greater stress to *Cosmarium* cells; therefore, these experiments lasted only 2 days (at 35°C) or 30 h (at 37°C) and were marked as short-term treatments. To investigate the endurance of *Cosmarium* strains at these temperatures, the treatments were prolonged up to 7 days (35°C) or 4 days (37°C) (marked as long-term treatments), after which the flasks with cells were transferred to 21°C. Growth was measured every day during the HS experiments and during recovery, whereas the desmid photosynthetic characteristics were measured 5 h after the beginning of the experiments and then every day at the same time. All of the temperature-gradient experiments were repeated 3 times.

Determination of cell number and growth rate

Cell counting was done by means of an electronic particle counter (Beckman Coulter Electronics model ZB) using an aperture of 100 µm. 1% NaCl (including 0.5% formaldehyde) was used as an electrolyte solution. Specific growth rate per day (µ) was calculated by the formula (Guillard 1973):

$$\mu = \frac{\ln\left(\frac{N_1}{N_0}\right)}{t_1 - t_0}$$

where N_1 and N_0 are the cell concentrations on days t_1 and t_0, respectively.

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 ml^{-1} (for medium-sized strains), or 9000 cells ml^{-1} (for small-sized taxa) by adding a quantity of (thermally adjusted) L-d medium. Immediately after sampling, the algal suspension was subjected to 10 min of dark adaptation in a water bath at the experimental temperature and filled into 5 ml Quartz cuvettes. A pulse of weak, far red light was applied to empty the electron pool from Q_A. The maximum quantum yield (Fv/Fm) was measured at time zero as described by Hanelt (1998). Initial fluorescence (Fo) was measured with red measuring light (~0.3 µmol photon m^{-2} s^{-1}, 650 nm), and maximal fluorescence (Fm) was determined using 600 ms of completely saturating white light pulse (~3500 µmol photon m^{-2} s^{-1}).

Photosynthesis (in terms of the relative electron transport rate, rETR = PFR * ΔF/Fm′) versus irradiance (P–I) curves were also measured as described by Bischof et al. (1998), where PFR refers to the photon fluence rate; Fm′ is the maximum fluorescence from a light-adapted sample; ΔF (or Fq′) refers to the difference in fluorescence between Fm′ and F′; F′ is the fluorescence emission from an irradiated sample (Baker 2008). 13 irradiances from white light LED (Nichia NSPW500S), ranging from 5 to 1078 µmol photon m^{-2} s^{-1}, were used to create P–I curves; the duration of each irradiance was 30 s. The temperature of the samples was adjusted and preserved by means of a water bath during fluorescence measurements.

The hyperbolic tangent model of Jassby and Platt (1976) was used to estimate P–I curve parameters described as:

$$rETR = rETR_{max} * \tan h(\alpha * PFR * rETR^{-1}_{max})$$

where $rETR_{max}$ is the maximum relative electron transport rate, tan h is the hyperbolic tangent function, and α is the electron transport efficiency. The saturating irradiance for electron transport (I_k) was calculated as the light intensity at which the initial slope of the curve (α) intercepts the horizontal asymptote ($rETR_{max}$). The curve fit was calculated with the Solver Module of MS-Excel, using the least squares method and comparing differences between measured and calculated data.

Non-photochemical quenching (NPQ), as a measure for heat dissipation from PSII, was estimated according to the formula NPQ = (Fm / Fm′) – 1 (Baker 2008). NPQ values were acquired from the end of the P–I curve (at 1078 µmol photon m^{-2} s^{-1}), provided that heat dissipation is largest at high light intensities.

Excitation pressure on PSII reflects the proportion of Q_A in the reduced state (Q_A)red / [(Q_A)red + (Q_A)ox]. It is calculated as 1 – qP, where qP is the coefficient of photochemical quenching, representing the number of open PSIIs (Maxwell et al. 1995). qP values were taken from the end of the P–I curve, considering that photochemical quenching decreases concomitantly with the irradiance increase. Conversely, excitation pressure increases as light intensity increases at a given temperature (Huner et al. 1996).

Statistical analysis

Data were tested for normality (Kolmogorov-Smirnov test) and for homogeneity of variance (Levene statistics). The two-factor (factorial between subjects) ANOVA was used to find significant differences between the means of growth and photosynthetic parameters of the investigated *Cosmarium* strains. Degrees of freedom (for the effect of the model and residuals of the model) are given in parentheses for the two-factor ANOVA test. Differences between the strains and temperatures with regard to the changes of growth rates and photosynthetic parameters were estimated by the Tukey HSD post-hoc test. All of the statistical analyses were conducted using the SPSS program (SPSS, Chicago, IL; USA).

Results

Growth characteristics

All of the investigated *Cosmarium* strains exhibited relatively high growth rates at 25 and 28°C, with the exception of *C. crenatum* and *C. meneghinii*, which showed the highest growth rates (µ) at 21°C (Table 2). Strikingly, the tropical species, *C. beatum*, exhibited the highest µ at 28°C among all of the strains investigated, even compared to the small-sized taxa. The growth rate of the tropical species drastically decreased at lower temperatures, and this species was almost completely unable to divide at 7°C. Conversely, the arctic species, *C. crenatum*, grew most rapidly at 7°C when compared to all of the investigated strains, while temperatures higher than 25°C noticeably decreased µ of this species.

By far the highest growth rate (0.586 day^{-1}) was recorded for the smallest desmid taxon, *C. regnesii*, at 25°C. Unexpectedly, the cosmopolitan species, *C. meneghinii*, had higher growth rates at all of the other temperatures (including the recovery phase after the HS treatments) despite of its twofold larger cell size (see Stamenković and Hanelt 2011).

The short-term treatments at 32, 35 and 37°C caused growth inhibition in all of the *Cosmarium* strains, with the exception of *C. beatum* which showed poor growth rates at 32°C. This tropical species was the only medium-sized strain capable of cell-division during the recovery period, after short-term exposure to 35°C and 37°C, thus showing its fair resistance under HS treatments. Surprisingly, both of the small-sized taxa tested here exhibited considerable endurance in all HS treatments, displaying considerably growth rate

recovery after exposure, even when compared to the tropical representative. On the other hand, *C. crenatum* demonstrated the highest recovery of growth rate after the prolonged treatment at 0.6°C, whereas this treatment was lethal for the tropical species, *C. beatum* (Table 2).

		Strain					
		C. crenatum	*C. punct.* No. 571	*C. regnesii*	*C. meneghinii*	*C. punct.* No. 570	*C. beatum*
Temperatures (°C)	Sign.	Maximum measured growth rates (day^{-1})					
0.6, 14 days treatment	*	0.037	0.006	0.001	0.006	0.033	0.001
7 days recovery (% of control)[a]		276.5	178.4	210.9	259.9	134	0
7	*	0.119	0.094	0.07	0.087	0.084	0.011
16	*	0.225	0.145	0.312	0.338	0.264	0.169
21	*	**0.265**	0.222	0.509	**0.517**	0.271	0.268
25	*	0.258	**0.314**	**0.586**	0.31	**0.458**	0.322
28	*	0.099	0.057	0.179	0.276	0.099	**0.329**
32, 7 days treatment	*	0.052	0.045	0.079	0.058	0.05	0.094
7 days recovery (% of control)[a]		110.2	61.8	328.6	334.1	63	177.5
35, 2 days treatment	ns	0.011	0.009	0.073	0.049	0.049	0.106
7 days recovery (% of control)		82.6	69.5	167.5	253.8	83.6	170.2
37, 30h treatment	ns	0	0	0.056	0.006	0	0
7 days recovery (% of control)		99.6	58.5	132.1	147.1	88.4	162.3

Table 2. Maximum measured growth rates (n = 3, SD typically < 5% of mean) for the investigated *Cosmarium* strains at different temperatures and a constant light regime (30 µmol photons m^{-2} s^{-1}). [a]Control designates the beginning number of cells at the start of the temperature-stress experiment. The maximum growth rates (μ_{max}) for each strain are highlighted in bold. An asterisk (*) denotes significant ($p < 0.05$) differences in growth rates among strains at specific test temperatures; (ns) – not significant; comparisons are given only for the specific temperature and higher neighbouring degree, according to the Tukey HSD post-hoc test. The strains are ordered according to increasing temperature of their original collection site.

The temperature treatments significantly influenced growth rates of the *Cosmarium* strains investigated (temperature factor): $F(8, 108) = 31.5$; $p < 0.001$. In addition, µ values of the *Cosmarium* strains differed significantly under given experimental conditions (strain factor): $F(5, 108) = 190.8$; $p < 0.001$. A significant interaction of between-subject factors (temperature x strain) was found for growth rate: $F(40, 108) = 192.3$; $p < 0.001$.

Photosynthetic characteristics

The cosmopolitan taxa (*C. punctulatum* var. *subpunctulatum* and *C. meneghinii*) exhibited relatively high Fv/Fm values from 16 to 25°C (Fig. 1). The application of lower temperatures (0.6 and 7°C) caused a decrease of maximum quantum yield for all of the *Cosmarium* taxa studied. Yet, the low-temperature treatments appeared less stressful for both polar strains (*C. punctulatum* No. 571 and *C. crenatum*) as judged from the relatively high Fv/Fm values. On the contrary, the high Fv/Fm values were recorded from 25 to

32°C for the typical tropical species, *C. beatum*, demonstrating the high photosynthetic efficiency and preference of this species to warm-water habitats.

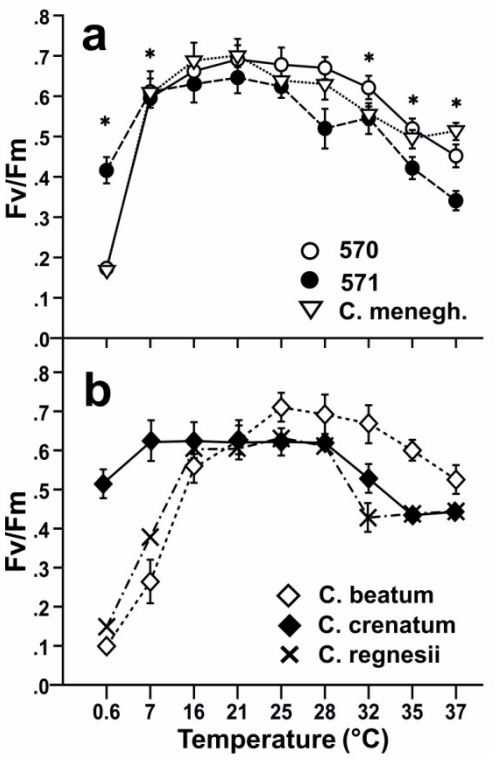

Fig. 1. Maximum recorded Fv/Fm (n = 3; ± SD) for the investigated *Cosmarium* strains at different temperatures and a constant light regime (30 µmol photons m^{-2} s^{-1}). (a) ○ – *C. punctulatum* var. *subpunctulatum* No. 570, ● – *C. punctulatum* var. *subpunctulatum* No. 571, △ – *C. meneghinii*; (b) ◇ – *C. beatum*, ◆ – *C. crenatum*, × – *C. regnesii*. Fv/Fm was measured one week after the beginning of cultivation at 0.6°C, 2 days after the beginning at 32°C and 35°C treatment, or 30 h after the beginning at 37°C treatment. An asterisk (*) refers to the significant temperature influence on the changes of Fv/Fm of the investigated *Cosmarium* strains at the 0.05 level (across both panels); comparisons are given only for the specific temperature and higher neighbouring degree, according to the Tukey HSD post-hoc test.

The short-term HS treatments caused a decrease in Fv/Fm for all the *Cosmarium* strains studied, pointing to inhibitory effects to the photosynthetic machinery and, consequently, growth cessation (compare Table 2 and Table 3). Only the tropical species, *C. beatum*, completely recovered the maximum quantum yield after all of the short-term HS treatments, compared with the medium-sized strains. Interestingly, the typical polar species, *C. crenatum*, showed considerable Fv/Fm recovery after the short-term HS treatments. However, the long-term treatments at 35 or 37°C resulted in severe damage to photosynthetic apparatus of all of the medium-sized *Cosmarium* strains (Fv/Fm values decreased rapidly to 0), which were unable to enhance Fv/Fm during the entire recovery period.

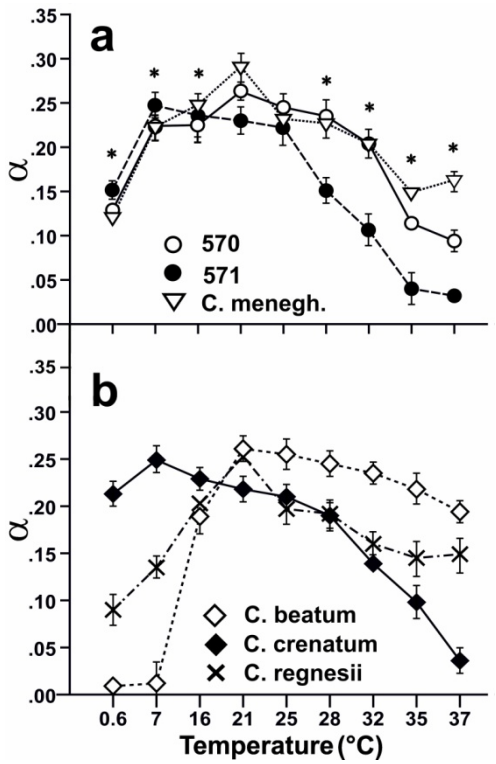

Fig. 2. Maximum photosynthetic efficiency (α) (n = 3; ± SD) for the investigated *Cosmarium* strains (for explanations, refer to Fig. 1).

Surprisingly, both small-sized taxa showed a milder decrease in yield under the long-term HS treatments, and achieved a considerably high recovery percentage. The changes in photosynthetic efficiency (α), capacity ($rETR_{max}$) and in the onset of saturation (I_k) followed the same trend during and after HS and low-

temperature treatments for all of the *Cosmarium* strains as was observed for Fv/Fm changes (data not shown).

	Strain					
	C. crenatum	*C. punct.* No. 571	*C. regnesii*	*C. meneghinii*	*C. punct.* No. 570	*C. beatum*
Temperatures (°C)	Maximum recorded Fv/Fm					
0.6, 14 days treatment	0.37	0.296	0.109	0.172	0.092	0
7 days recovery (% of control)[a]	110.2	100	94.8	100.3	93.7	0
32, 7 days treatment	0.401	0.341	0.24	0.521	0.493	0.63
7 days recovery (% of control)	81.5	80.6	96	100	88.5	119.4
35, 2 days treatment	0.316	0.130	0.431	0.445	0.447	0.594
7 days recovery (% of control)	81	51.6	86.6	100.8	95.6	107.6
35, 7 days treatment	0.011	0.006	0.136	0.265	0.015	0.087
4 days recovery (% of control)	0	0	27.3	72.3	0	0
37, 30h treatment	0.209	0.151	0.444	0.514	0.191	0.525
7 days recovery (% of control)	54.3	9.7	69.8	85.8	51.3	100.1
37, 4 days treatment	0	0.002	0.075	0.302	0.004	0.006
4 days recovery (% of control)	0	0	21	36.3	0	0

Table 3. Maximum recorded values of Fv/Fm at the end of the temperature-stress treatment and recovery percentages (n = 3, SD typically < 5% of mean). [a]Control designates the Fv/Fm value at the moment when the HS experiment started (at 21°C). The strains are ordered according to increasing temperature of their original collection site.

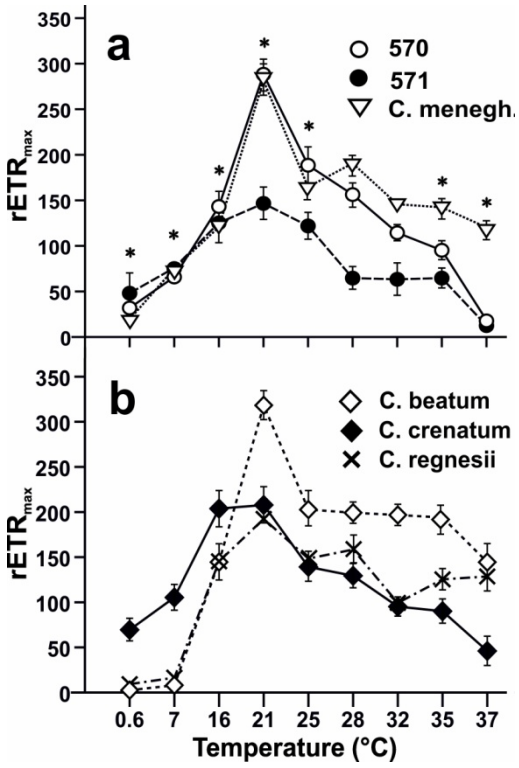

Fig. 3. Maximum photosynthetic capacity ($rETR_{max}$) (n = 3; ± SD) for the investigated *Cosmarium* strains (for explanations, refer to Fig. 1).

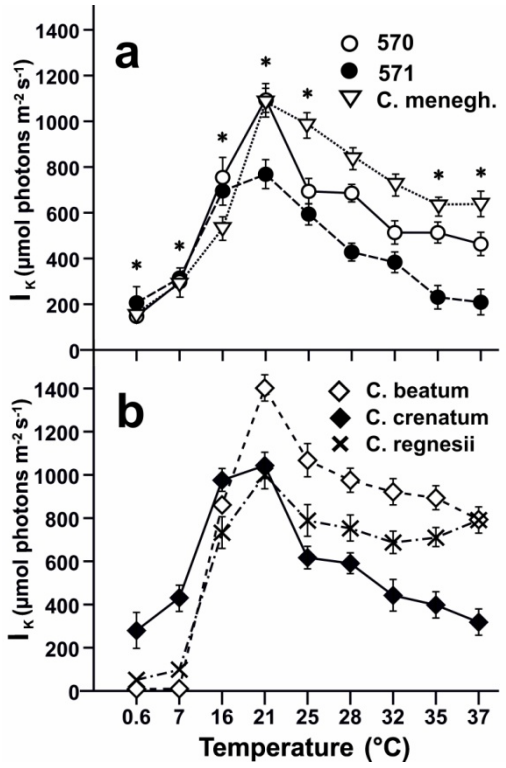

Fig. 4. Maximum saturating irradiance (I_k) (n = 3; ± SD) for the investigated *Cosmarium* strains (for explanations, refer to Fig. 1).

Remarkably, the strains collected from a polar region (*C. punctulatum* No. 571 and *C. crenatum*) gradually increased photosynthetic efficiency parallel to a decrease in temperature below 25°C, reaching the highest α value at 7°C (Fig. 2). All of the other desmid strains studied exhibited the highest photosynthetic efficiency, capacity and the onset of saturation at 21°C (Figs 3 and 4). By far the highest photosynthetic capacity and the onset of saturation at this temperature were recorded for *C. beatum* and the high-mountain strain of *C. punctulatum*, indicating the high-light adapted photosynthesis of these strains. However, $rETR_{max}$ and I_k of *C. beatum* decreased to about half at 16°C, and further decreased at 7 and 0.6°C, demonstrating the poor photosynthetic acclimation of this species at low temperatures applied.

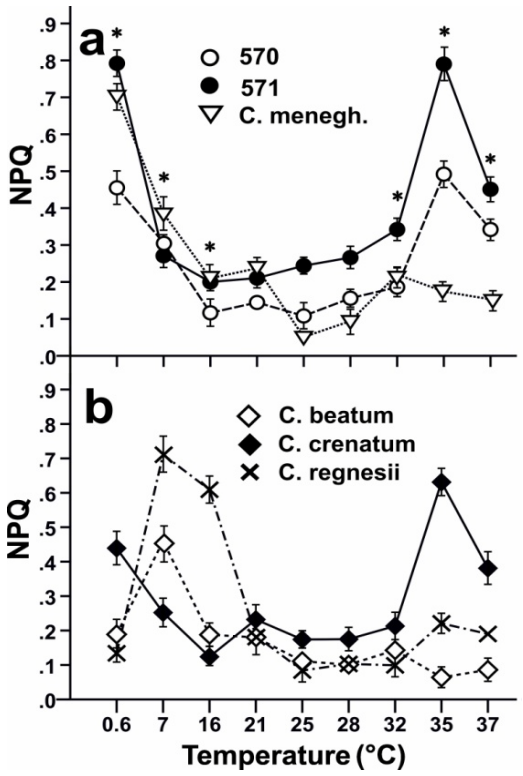

Fig. 5. Average nonphotochemical quenching (NPQ) (n = 9; ± SD) for the investigated *Cosmarium* strains (for explanations, refer to Fig. 1).

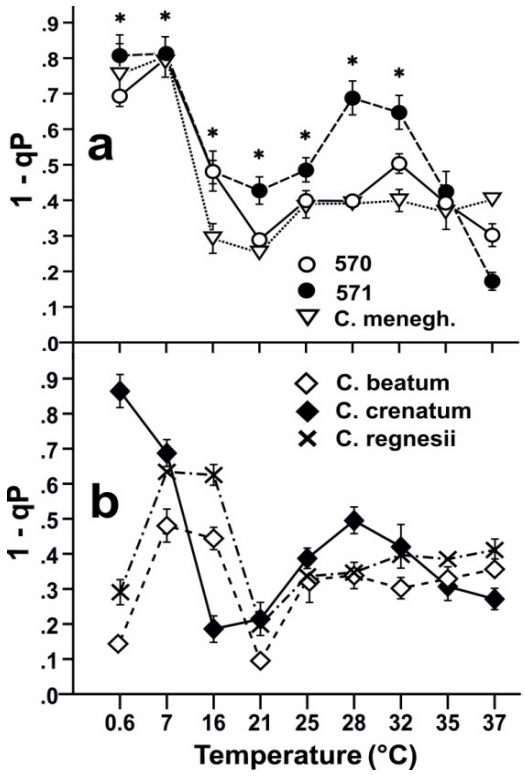

Fig. 6. Average PSII excitation pressure (1 - qP) (n = 9; ± SD) for the investigated *Cosmarium* strains (for explanations, refer to Fig. 1).

The *Cosmarium* strains were characterised by the relatively low non-photochemical quenching in the temperature range between 21 and 28°C (Fig. 5). The PSII excitation pressure was the lowest at 21°C for all of the desmid strains investigated (Fig. 6), in parallel to the highest photosynthetic capacity and the onset of saturation measured at this temperature (Figs 3 and 4). Lower temperatures caused a significant increase of NPQ and 1 – qP in all of the cosmopolitan species and *C. regnesii* var. *polonicum*, concomitantly with Fv/Fm decrease (Fig. 1). However, lower NPQ values, along with relatively high Fv/Fm and moderate 1 – qP rates, were noted for the typical arctic species, *C. crenatum*, showing its fair photosynthetic adaptation and resistance at low-temperature conditions. Temperature treatments above 28°C caused noticeable increases in NPQ rates for both of the polar strains studied (*C. punctulatum* No. 571 and *C. crenatum*),

followed by the increase in 1 – qP and the decrease in Fv/Fm, revealing rather damaging effects to PSII and the photosynthetic electron-transport system.

Table 4. Results of the two-factor ANOVA test for the factors *temperature* and *strain*. F – F ratio, df – degrees of freedom (for the effect of the model and residuals of the model), p – significance.

Between-subjects factors	ANOVA parameters		
Temperature	F	df	p
Fv/Fm	124.5	8, 108	<0.001
$rETR_{max}$	70.2	8, 108	<0.05
I_k	59.1	8, 108	<0.05
α	42.1	8, 108	<0.001
NPQ	38.7	8, 108	<0.001
1 - qP	120.5	8, 108	<0.001
Strain	F	df	p
Fv/Fm	80.4	5, 108	<0.05
$rETR_{max}$	124.2	5, 108	<0.001
I_k	42.2	5, 108	<0.001
α	50.7	5, 108	<0.05
NPQ	111.6	5, 108	<0.001
1 - qP	98.2	5, 108	<0.001

The temperature treatments significantly influenced the photosynthetic parameters in all of the *Cosmarium* strains investigated. In addition, there was a significant difference between the *Cosmarium* strains in all of the photosynthetic parameters during temperature-gradient experiments (Table 4). The Tukey HSD post-hoc test did not demonstrate a significant difference between *C. crenatum* and *C. punctulatum* No. 571 with regard to the maximum recorded Fv/Fm and α values obtained during temperature treatments (Fv/Fm: $p = 0.099$; α: $p = 0.087$). All of the other *Cosmarium* strains differed significantly beyond the 0.05 level with regard to all of the photosynthetic parameters (data not shown).

Discussion

When tested within a gradient of temperatures, the *Cosmarium* isolates collected from various geographic regions demonstrated physiological characteristics that were consistent with climate conditions of their source location, as hypothesised in the earlier study (Stamenković and Hanelt 2011). The cells of the *Cosmarium* strains indeed exhibited the preference for specific temperature niches, as concluded from their growth and photosynthetic behaviour *in vitro* conditions. Low and high temperatures applied provoked the native physiological responses of the *Cosmarium* strains despite the long-term cultivation (more than 15 years) under identical and constant conditions.

The typical tropical taxon, *C. beatum*, showed the physiological characteristics which clearly suggested adaptation to warm-water habitats, such as the highest optimum growth temperature (28°C), and considerable recovery percentage of growth rates and photosynthetic parameters after the short-term HS treatments. In accordance with what has been observed for tropical macroalgae (Lüning 1990; Hanelt 1992), *C. beatum* achieves the distinctly low α and Fv/Fm values at temperatures lower than 21°C which helps to explain why its distribution is limited to several sites in the circumequatorial region (Compère 1977; Williamson 1994; Williamson 2004; Coesel et al. 2009). At temperatures characteristic of higher latitudes, this species exhibits low maximum quantum yields and photosynthetic efficiencies, demonstrating that this species is not capable of using higher amounts of photosynthetic energy at low temperatures. Consequently,

C. beatum displays low growth rates and, hence, cannot compete with various other microalgae adapted to temperate and continental climate zones.

In addition, the photosynthetic characteristics expressed as a possibility to protect PSII from reactive oxygen species (ROS) by heat dissipation may be used to explain the distributional patterns of a typical tropical species (*C. beatum*), and a subcosmopolitan, continental-climate adapted taxon (*C. regnesii* var. *polonicum*). *Cosmarium regnesii* demonstrated distinctly high NPQ and 1 – qP at 16 and 7°C which suggests that this species would be highly competitive in moderately cold freshwater habitats; damage to PSII by ROS is prevented due to efficient heat dissipation (Huner et al. 1998; Wilhelm and Selmar 2011). On the other hand, *C. beatum* exhibited rather limited PSII protection at low temperatures, as judged from the low NPQ and 1 – qP rates as well as low maximum quantum yields and photosynthetic capacities.

The photosynthetic performance of the typical arctic taxon, *C. crenatum* var. *boldtianum*, was remarkably adapted to low temperatures as judged from the relatively low non-photochemical quenching and low excitation pressure, in parallel with the high Fv/Fm and P–I curve parameters. These photosynthetic characteristics are similar to those observed in sub-polar and polar microalgae (Morgan-Kiss et al. 2002; El-Sabaawi and Harrison 2006) or polar macroalgae (Wiencke 1996), when grown at low temperatures in the laboratory. However, a rapid conversion of violaxanthin to zeaxanthin (measured as the NPQ increase) in the cosmopolitan taxa (*C. punctulatum* and *C. meneghinii*), occurred at low temperatures and relatively short, high-light radiation (Wilson et al. 2006; Baker 2008). The protection of PSII by the efficient, non-photochemical quenching may allow cosmopolitan and polar taxa to acclimate to cold temperatures. These taxa remained vital even two weeks after the beginning of cultivation at 0.6°C (Table 2 and 3).

Remarkably, both *Cosmarium* strains collected from the polar region exhibited an increase of photosynthetic efficiency as temperatures declined. So far, it has been observed that α might be enlarged in Arctic and Antarctic macroalgae as a result of low temperatures and low levels of solar radiation in the polar zone (Wiencke et al. 1993; Wiencke 1996). Apparently, the decrease in temperature under laboratory conditions caused a similar effect to α of the polar desmid strains, taking into account that irradiance was also relatively low (30 µmol photons m^{-2} s^{-1}). Palmisano et al. (1987) noted that α declined at increasing incubation temperature in arctic sea-ice diatoms, while dark respiration rates increased, which caused a decrease in net photosynthesis at sub-saturating light levels. On the other hand, the heat-induced thylakoid leakiness could be counteracted by the induction of zeaxanthin synthesis (Havaux and Tardy 1996), and might explain why high NPQ values were measured in the polar strains after the short-term HS treatments.

It appeared that the subcosmopolitan taxon, *C. regnesii* var. *polonicum*, achieved a fairly positive balance between photosynthetic rates and utilisation of photosynthates merely at 25°C, which was reflected in its rapid growth at this temperature. This particular temperature demand may help explain the relative rare occurrence of *C. regnesii* var. *polonicum* in situ (Palamar-Mordvintseva 1982; Kouwets 1999; Coesel and Meesters 2007). On the contrary, the other small-sized species, *C. meneghinii*, exhibited higher photosynthetic capacity, efficiency and growth rates at all of the other temperature treatments which might

explain its rather broad ecological niche (Brook and Johnson 2003; Coesel and Meesters 2007). *C. meneghinii* demonstrated a distinctly high photosynthetic capacity when grown at its optimal growth temperature (21°C) which is in accordance with the fact that fast-growing, eutrophic desmid taxa are characterised by a high Pmax and, consequently, may predominate over chlorococcean algae and Cyanoprokariota in nutrient-rich habitats (Coesel and Wardenaar 1994; Spijkerman and Coesel 1998; Spijkerman et al. 2004).

Astonishingly, all of the *Cosmarium* strains investigated exhibited exceptionally high values of photosynthetic capacity and the onset of saturation at 21°C. By far the highest $rETR_{max}$ and I_k values were recorded for the tropical species, *C. beatum*, and the cosmopolitan taxon, *C. punctulatum* var. *subpunctulatum*, collected from the high-mountain, tropical region. The high levels of photosynthetic capacity and the onset of saturation have been observed in various species of both kelp (Lüning 1981, 1990; Hanelt et al. 2003) and microalgae (Robarts and Zohary 1992) collected from the circumequatorial zone, which receives highest amount of solar radiation. In many cases, the recorded maximum I_k values of the *Cosmarium* strains repeatedly exceeded the observed I_k range for seaweeds, 1.6–500 µmol photons m^{-2} s^{-1} (Wiencke 1996; Saroussi and Beer, 1997; Hanelt et al. 2003) and phytoplankton, i.e. 10–480 µmol photons m^{-2} s^{-1} (Jones 1998; Gilbert et al. 2000; Cruz and Serodio 2008). The recorded I_k range of the *Cosmarium* strains was rather comparable to those recorded for snow algae, which are exposed to high light intensities due to the increased albedo from snow cover (Remias et al. 2005; Stibal et al. 2007), or desert shrubs that have an I_k of about 1000 µmol photons m^{-2} s^{-1} (Wild 1979). As inhabitants of various shallow, freshwater habitats, it is expected that desmids demonstrate photosynthetic characteristics typical for algae adapted to high light, which appeared as their adaptive characteristic.

At the first, it appears reasonable to estimate microalgal geographical patterns based upon physiological responses to different temperature conditions, an approach taken for numerous kelp species (e.g. van de Hoek, 1984; Hanelt et al. 1994; Wiencke 1996). However, a large deviation from the observed temperature-induced geographic distribution pattern of the desmid strains was noted in the arctic taxon, *C. crenatum* var. *boldtianum*. The optimum growth temperature of this taxon (21°C) was clearly higher than that of most arctic microalgae (6 – 8°C) (Fiala and Oriol 1990; Suzuki and Takahashi 1995) or sub-arctic diatoms (~14°C) (Eppley 1972; El-Sabaawi and Harrison 2006). Additionally, considering that *C. crenatum* showed a marked photosynthetic recovery after all of the short-term HS treatments, it cannot be merely considered a typical cryosestic (psychrophilic) alga; presumably, it is secondarily adapted to the polar environment. These features observed in *C. crenatum*, as well as the relatively high optimum growth temperatures (25–28°C) for the other *Cosmarium* strains examined, are consistent with the remark that desmids have higher growth temperature optima than phytoplankton species in temperate regions (Coesel and Wardenaar 1990). Hence, the preference of all of the desmid strains studied to warm freshwater habitats may supplement the assumption on the evolutionary origin of desmids in tropics (Coesel 1996). The exceptionally high values for photosynthetic capacity and the onset of saturation, measured at the relatively

high temperature range (16–25°C), indicated that the desmid strains can be regarded as algae adapted to high light intensities – characteristic not only for shallow freshwater bodies, but also for low latitudes; thus providing additional evidence to Coesel's hypothesis. Therefore, the *Cosmarium* strains demonstrate not only adaptive physiological characteristics in accordance with the temperature prevailing at their sampling sites, but also with regard to their evolutionary (intrinsic) origin. These physiological characteristics appear stable, remaining in cultures over generations. Taking into account that all of the *Cosmarium* strains studied were not cryopreserved and that the conjugation did not occur during the cultivation under (sub)optimal laboratory conditions, the chances for genomic alterations were minimised even during the long-term cultivation (Müller et al. 2007; Griffiths et al. 2000).

The fact that *C. punctulatum*, *C. beatum*, and *C. regnesii* exhibited the highest photosynthetic capacity and the onset of saturation at 21°C, while higher growth rates were measured at higher temperatures, might be explained by the pre-acclimation of cultures to 21°C. Kuebler et al. (1991), Davison (1991) and Coles and Jones (2000) pointed out that lower P–I curve parameters (i.e. Pmax, I_k and α) displayed in cultured versus freshly collected microalgae can arise from acclimation to low light-temperature conditions in laboratory culture. Possibly, processes such as cell division cannot acclimate as thoroughly as photosynthesis to changed temperature conditions (Weiß et al. 1999; Anning et al. 2001). The decrease in $rETR_{max}$ and I_k at 25°C is presumably not due to damage of photosynthetic apparatus, as this temperature does not cause damage to PSII (Çjánek et al. 1998); instead, it is a protective response of desmids for limiting PSII activity to intensified unfavourable conditions (Sharkey 2005; Allakhverdiev et al. 2008).

Interestingly, both of the small-sized *Cosmarium* taxa displayed relatively rapid acclimation to the large temperature increases within the short time. A few previous experiments also demonstrated that small-celled desmids were exceedingly resistant to high temperatures (Vidyavati et al. 1983; Vidyavati 1985). A surprising finding was that *C. meneghinii* displayed by far the highest degree of recovery after the long-term HS treatments (treatments that completely inhibited tropical species), despite having been cultivated for more than 80 years under a constant, relatively low temperature regime. This indicates genetic preservation (and maintenance of expression) of the physiological response of this cosmopolitan species to stressful temperature conditions.

Conclusion

This study revealed clear physiological (growth and photosynthetic) adaptations to temperature among the *Cosmarium* strains collected from polar, temperate, and tropical zones. However, all of the desmid strains studied exhibited the preference to relatively warm habitats, compared to other microalgae from temperate zones. Small-sized desmids exhibited broader potential to acclimate to both relatively low and high temperatures than medium-sized taxa. These findings agree with the idea that small micro-organisms tend to have wider distribution than larger micro-organisms (Fenchel and Finlay 2004).

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4. Ultrastructural differences between six *Cosmarium* strains (Zygnematophyceae, Streptophyta) collected from various geographic zones at optimal, warm and cold temperatures

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Abstract

Numerous studies have revealed that plants collected from different geographic zones and stressed *in vitro* at various temperature and/or light regimes expose changes in ultrastructure which are in accordance with the climate of their sampling sites. This fact initiated the investigation if stress at different temperatures causes diverse extents of change in the ultrastructure of the *Cosmarium* strains originating from different geographic zones. This study revealed that six *Cosmarium* strains demonstrated ultrastructural characteristics that were consistent with their source location under optimal, low and high temperature conditions, confirming that such responses are genetically preserved. Interestingly, chloroplasts of all of the *Cosmarium* strains correspond to the sun-adapted type, which is concomitant with previous statements that these strains are rendered as high-light adapted algae. The *Cosmarium* strains developed multiple ultrastructural responses for the protection from reactive oxygen species (ROS) induced by excessive temperatures, which may occur in desmids natural habitats. The appearance of cubic membranes and increased number of plastoglobules represent the first line in protection from ROS, which is accompanied by the alteration of protein synthesis and the appearance of stress granules in order to preserve the cell homeostasis during the heat influence. However, the prolonged warm- or cold-temperature stress may initiate programmed cell death, as observed in all of the *Cosmarium* strains. The fair acclimation possibilities and the ability to undergo programmed cell death in order to save the population, certainly favour the cosmopolitan distribution of the genus *Cosmarium*.

Key words: Chilling, *Cosmarium*, cubic membranes, heat, heat shock granules, mucilaginous inclusions, programmed cell death, ultrastructure.

Abbreviations

ER – endoplasmic reticulum, HS – heat shock, ROS – reactive oxygen species, HSP – heat shock proteins, HSG – heat shock granules, PSII – photosystem II, $rETR_{max}$ – maximum relative electron transport rate (photosynthetic capacity), I_k – saturating irradiance, PCD – programmed cell death

Introduction

Earlier studies dealt with physiological differences between the *Cosmarium* strains, collected from various climate zones, at optimal (cultivating) conditions and after cold and warm temperatures

(Stamenković and Hanelt 2011, 2012). Therefore, microalgal geographic patterns based upon physiological responses to different temperature conditions were estimated, an approach taken for numerous kelp species (van de Hoek 1984; Wiencke 1996; Hanelt et al. 2003). Remarkably, despite the long-term cultivation (more than 15 years) under constant laboratory conditions, the native physiological responses of the *Cosmarium* strains remained genetically preserved (Stamenković and Hanelt 2011, 2012). A thorough investigation of the influences of temperatures, which exceeded the well-known desmids temperature optima range (25–30°C, Coesel and Wardenaar 1990), revealed clear photosynthetic adaptations to temperatures among the *Cosmarium* strains collected from polar, temperate, and tropical zones. Interestingly, the stronger temperature (cold or warm) stress applied to the *Cosmarium* strains, the larger physiological differences appeared (Stamenković and Hanelt 2012).

Numerous investigations have shown that tropical and chilling-sensitive plants display numerous ultrastructural damages when exposed at low temperatures (Taylor and Craig 1971; Forde et al. 1975; Wise et al. 1983; Ma et al. 1990). The decrease of photosynthetic activity which is accompanied with chilling-induced ultrastructural injuries (as observed in thylakoids, dictyosomes, endoplasmic reticulum and mitochondria) pointed to the poor acclimation possibilities of the tropical plant species at low temperatures, therefore, determining their circumequatorial geographic position (Wise et al. 1983; Kosakivska et al. 2008; Barton 2010). Even cultivars of the same species, with different degree of chilling sensitivity, exhibit differences in cell ultrastructure when exposed to relatively low temperature; revealing dilated and curved thylakoids in chilling-sensitive strains as a result of an elevated reactive oxygen species (ROS) production (Xu et al. 2008). Conversely, plants originated from circumpolar or alpine regions are not capable to acclimate at temperatures higher than approximately 10°C (Raison et al. 1980; Murata and Yamaya 1984). Therefore, most plants have well-established climatic distributional patterns and they developed strategies to cope with specific temperature characteristics of a certain area (Osmond et al. 1987).

Interestingly, a comparative investigation on the temperature influence on the ultrastructural responses of microalgae collected from various climatic areas lacks. This fact requests the continuation of previous investigations on the possible geographic distribution pattern of the six *Cosmarium* strains. The strains were collected from different climatic areas and long-term grown at relatively low light-temperature conditions of a climate chamber, and investigated regarding ultrastructural responses and differences as result of excessive cold and high temperatures.

This study aims to reveal if differences in ultrastructural responses occur between the *Cosmarium* strains from different geographical locations induced by high- or low-temperatures stress. The main ultrastructural changes after warm or cold temperature applications should help in the estimation of possible geographic distribution patterns of the desmid strains, as it has been noted for plants.

In addition, desmids are mainly inhabitants of shallow water bodies (such as peat pits, ditches and puddles) where daily and seasonal temperature changes can be extreme, so it is assumed that they developed numerous ultrastructural and physiological adaptive strategies to survive in such habitats. So far, there are

only a few studies dealing with the temperature influence on the ultrastructure of desmids (Morris et al. 1986; Meindl 1990) which revealed species-specific responses at freezing, cold and warm temperatures. Thus, this study may provide an important view on the possibility of the *Cosmarium* strains to cope with sudden and inconvenient temperature influences which may occur in their natural environment.

Materials and methods

Algal strains

The six *Cosmarium* clones examined in this study were isolated from various parts of the world within approximately the same time period, in order to exclude the influences of sampling time and, therefore, the influences of constant nutrient, light and temperature regime under laboratory conditions. One exception was *C. meneghinii* (isolated 1927), because of the special interest in comparing its physiological activity with that of other ubiquitous taxa:

C. crenatum Ralfs var. *boldtianum* (Gutwinski) W. & G.S. West (No. 561) – isolated 1995; polar climate zone (Cape Flora, Northbrook Island, Franz Joseph Land, Russia), 79°57′N 50°05′E;

C. regnesii Reinsch var. *polonicum* (Eichler & Gutwinski) Compère (No. 465) – isolated 1998; continental (microthermal) climate zone (pool in East Hokkaidō, Japan), 42°54′N 140°45′E;

C. meneghinii Ralfs (No. 59) – isolated 1927; continental (microthermal) climate zone (fountain in Prague, the Czech Republic), 50°05′N 14°25′E;

C. beatum W. & G.S. West (No. 533) – isolated 2001; tropical climate zone (marshy area near Ol Bolossat Lake, Kenya), 00°09′S 36°26′E;

C. punctulatum Brébisson var. *subpunctulatum* (Nordstedt) Børgesen (No. 570) – isolated 1996; alpine, tropical area (pool on Mt. Cotopaxi at 1600 m a.s.l., Ecuador; 'highlands' subgroup of the temperate (mesothermal) climatic group), 00°40′S 78°26′W;

C. punctulatum var. *subpunctulatum* (SVCK No. 571) – isolated 1992; lowland, polar climate (pool near Skarsvåg at 80 m a.s.l, the North Cape, Norway), 71°06′N 25°49′E.

The details on taxonomic, ecological and distributional attributes of the investigated taxa, as well as the climate characteristics of the algal sampling locations are published elsewhere (Stamenković and Hanelt 2011).

Cultivation and temperature treatments

All of the investigated *Cosmarium* strains were grown under standard laboratory conditions (16° C; ~30 µmol photons m^{-2} s^{-1}) in a climate chamber of the SVCK (Sammlung von Conjugaten-Kulturen) collection over a period of several years. Taking into account that 16°C was sub-optimal for the tropical species (*C. beatum*) (Stamenković and Hanelt 2011), while temperatures above 22 or 25°C were recognised as sub-optimal for polar microalgal representatives (Fiala and Oriol 1990; Suzuki and Takahashi 1995), all

of the experimental *Cosmarium* strains were pre-cultured at 21°C, which was considered as a roughly compromising optimal temperature for both of the tropical and polar strains studied.

All of the investigated strains were grown in the mineral medium based on Kattner et al. (1977), which was prepared with double-distilled water (L-d medium). The tested *Cosmarium* strains had been acclimated to 21°C and L-d medium at least 12 months before the cold- and warm-temperature experiments began. Sterilised 1 l Erlenmeyer flasks with 500 ml of medium were inoculated to a final concentration of 1500 cells ml^{-1}. Cultures were bubbled with humidified air at a rate of about 10 l h^{-1} to prevent CO_2 limitation. The cultures were mixed regularly by means of a magnetic stirrer to prevent self-shading of cells. Cells of the *Cosmarium* strains grown at 21°C are regarded as "control" samples.

To investigate ultrastructural changes in the *Cosmarium* strains at the cold temperature, a set of inoculated Erlenmeyer flasks was placed directly on plates filled with ice and put in a climate chamber at 0.9°C and 30 µmol photons m^{-2} s^{-1}; daily light regime 14 h light/10 h darkness. The temperature of the medium with cells was 0.6°C (±0.1°C); the melting ice was replaced each day. To prevent nutrient depletion in cultures, 100 ml of old medium was replaced with new medium (adjusted to 0.6°C) every week. Desmid cells were settled approximately 1 h prior to this exchange. The cold-temperature experiment lasted for 32 days, after which flasks were placed at 21°C and 30 µmol photons m^{-2} s^{-1} to recover for 8 days.

As it was observed that the prolonged 32°C treatment did not cause large physiological changes to the *Cosmarium* strains studied, while the application of 37°C rapidly inhibited photosynthesis in all of the medium-sized desmid strains (Stamenković and Hanelt 2012), 35°C was chosen as an experimental temperature for the estimation of ultrastructural changes (HS treatment). Flasks filled with 50 ml of L-d medium were inoculated with the sample taken from pre-cultured strains at 21°C and placed in a chamber at 35°C (light intensity 30 µmol photons m^{-2} s^{-1}). Cells were treated for 48 h (additionally, *C. beatum* for 7 days, due to its high resistance at warm temperatures), after which the flasks with cells were placed again at 21°C and 30 µmol photons m^{-2} s^{-1} to recover for 34 h.

Light and electron microscopy

Morphological appearance of cells of the *Cosmarium* strains was observed by means of a light microscope (IM Zeiss) equipped with a digital camera (Olympus C5060).

Cosmarium cells grown at 21°C (marked as controls) were fixed 7 days after the beginning of the cultivation (in the middle of the logarithmic phase) following the method of Quader (1985). Heat-stressed cells were fixed immediately after 5, 10, 24 and 48 h of the 35°C HS treatment, and 34 h after recovery at 21°C began. Cells of the typical tropical species, *C. beatum*, were fixed also after 7 days of 35°C treatment. Cells treated at the low-temperature treatment were fixed after 14 and 32 days, and during recovery after 4 and 8 days. Fixation was done with 2% glutaraldehyde in 75 mM cacodylate buffer (pH 7.0); fixed samples were stored at room temperature for 1 h, and later placed on ice. Afterwards the cells were rinsed three times

with 75 mM cacodylate buffer and post fixed with 1% OsO_4 in 75 mM cacodylate buffer and placed overnight at 4°C. After rinsing in cacodylate buffer, the samples were dehydrated in a graded acetone series.

A special fixation procedure with osmium tetroxide-potassium fericyanide (OsFeCN) (Hepler 1981) was used for selectively staining of the endoplasmic reticulum of the heat-stressed *Cosmarium* cells. The cells were fixed in 2% glutaraldehyde in 75 mM cacodylate buffer containing 5 mM calcium chloride. After the cells had been washed in buffer plus $CaCl_2$ they were post-fixed in a buffered mixture of 1% OsO_4 and 0.8% $K_3Fe(CN)_6$ for 2 h. The cells were then washed with 75 mM cacodylate buffer containing 5 mM $CaCl_2$ and placed at 4°C. Subsequently, cells were washed with double-distilled water and poststained with 2% aqueous uranyl acetate for 2 h.

The cells were embedded in resin according to Spurr (1964), sectioned on a ultramicrotome (Leica-Reichert-Jung, Ultracut E) and stained with 2% uranyl acetate and 2% lead citrate. The sections were viewed on a transmission electron microscope LEO 906E (LEO, Oberkochen, Germany) at 100kV, and photographed by a Gatan CCD camera MultiScan Typ 794 connected to a PC equipped with Software Digital Micrograph 3.4.4.

DAPI staining

To observe changes in the nucleus position and chromatin condensation during the heat-shock treatment at 35°C, the *Cosmarium* cells were harvested after 10, 24 and 48 h of the HS treatment. Cells were fixed in 2% PFA (paraformaldehyde; Merck) in 50 mM MSB buffer for 1 h (MSB buffer: 100 mM PIPES) (Sigma P-6757), 10 mM EGTA (Sigma E-4378) and 5 mM $MgSO_4$ at pH 6.8. Cells were rinsed three times with 50 mM MSB and stained in 10 µM DAPI (4'-6-diamidino-2-phenylindole; Sigma D-9542), the final concentration. Bleaching was prevented with DABCO (Sigma D-2522): 2.5 mg ml^{-1} DABCO (1,4-diazabicyclo(2,2,2) octane) in a mixture consisting of 70% glycerine and 30% 25 mM MSB.

The morphology of cell nuclei was observed using a fluorescence microscope Zeiss Axiovert 200, equipped with the Nikon camera Digital sight DS-U1 and connected to a PC with the Eclipse Net software, at excitation wavelength 350 nm. Nuclei are considered to have the normal phenotype when glowing bright and homogenously.

Results

Ultrastructural differences between the Cosmarium strains collected from various climatic areas and grown under standard laboratory conditions (21°C, 30 µmol photons m^{-2} s^{-1})

All of the investigated *Cosmarium* strains have an axial type of the chloroplast. *C. punctulatum*, *C. crenatum*, *C. meneghinii* and *C. regnesii* possess a furcoid, monocentric chloroplast in each semicell, while *C. beatum* has a furcoid, dicentric chloroplast in a semicell (after Teiling 1952). The main differences in the chloroplast structure between the investigated strains are given in Table 1.

Strain	Range of number of thylakoids per granum	Evenness of a margin distribution (%)	Grana width (µm); SD < 5% of mean	Average number of plastoglobules per frontal section; SD < 5% of mean	Plastoglobules diameter (µm); SD < 5% of mean
C. punct. (570)	3 – 8	85 evenly	0.746	39	0.127
C. punct. (571)	8 – 40	40 evenly	0.781	38	0.11
C. beatum	5 – 15	75 evenly	0.759	75	0.1
C. crenatum	3 – 9	20 evenly	0.851	32	0.08
C. meneghinii	3 – 10	20 evenly	0.56	29	0.122
C. regnesii	5 – 20	20 evenly	0.624	19	0.121

Table 1. Some characteristics of the chloroplast ultrastructure of the investigated *Cosmarium* strains grown under standard laboratory conditions (21°C, 30 µmol photons m^{-2} s^{-1}); measurements from at least 40 cells. Measurements were performed according to the instructions given by Lichtenthaler et al. (1981).

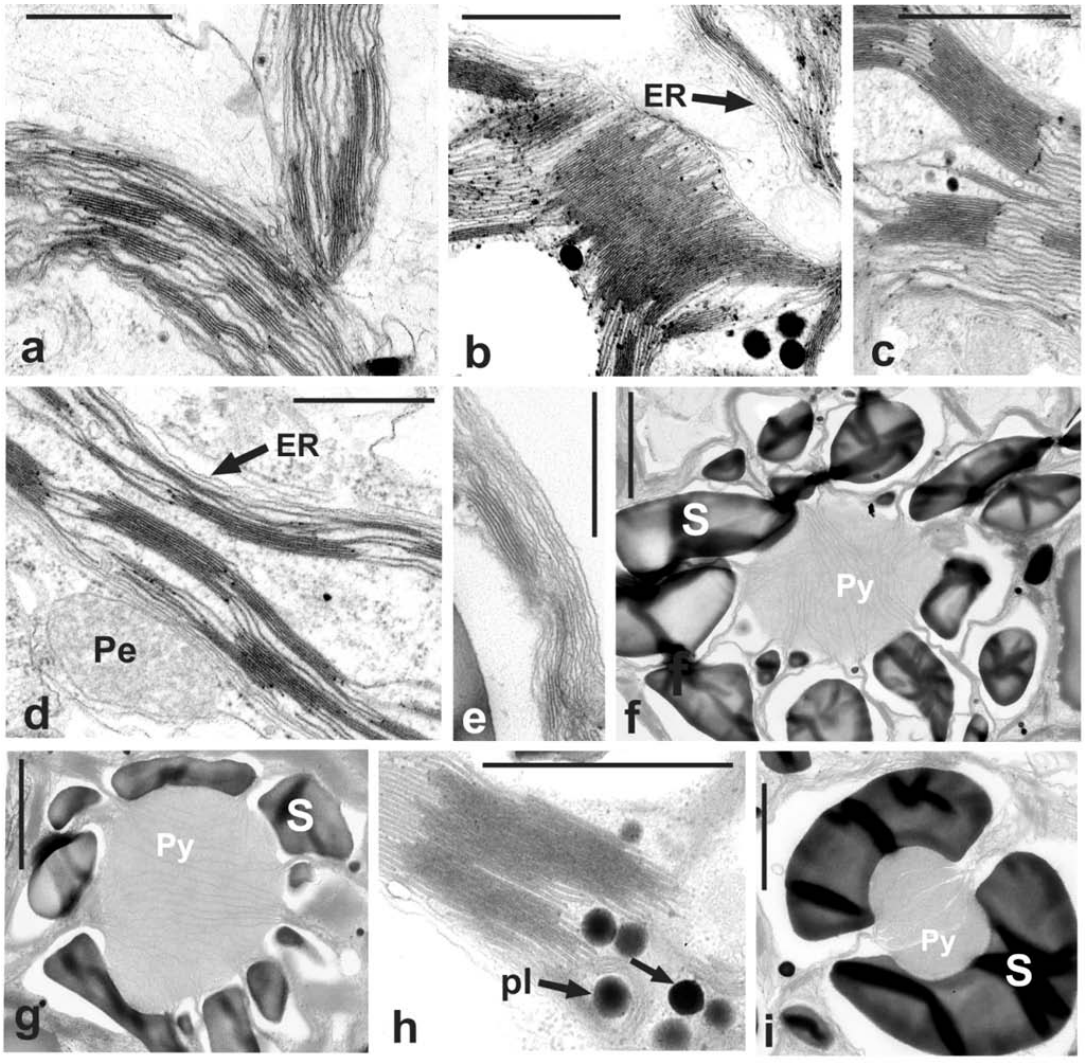

Fig. 1. a-i Ultrastructure details of the *Cosmarium* strains grown at standard laboratory conditions (21°C, 30 µmol photons m^{-2} s^{-1}). **a** Grana and stroma thylakoids of *C. punctulatum* No. 570. **b** Densely arranged grana thylakoids of *C. punctulatum* No. 571. **c** Grana thylakoids of *C. beatum*. **d** Grana thylakoids of *C. crenatum*. **e** Thylakoids of *C. meneghinii*. **f** Pyrenoid of *C. punctulatum* No. 570. **g** Pyrenoid of *C. punctulatum* No. 571. **h** Thylakoids of *C. regnesii*

var. *polonicum*. **i** Pyrenoid of *C. meneghinii*. S – starch particles, Pe – peroxisome, Py – pyrenoid, pl – plastoglobules, ER – endoplasmic reticulum. Bar = 1 μm.

Chloroplasts of the high-mountain tropical strain of *C. punctulatum* (No. 570) exhibit characteristics of a sun-type chloroplast having approximately 3–8 thylakoids per chloroplast section and relatively long stroma lamellae (Fig. 1a). Markedly large starch grains encircle a single pyrenoid within each chloroplast, and large grains are also distributed in stroma. In contrast, the polar strain of *C. punctulatum* (No. 571) has a large number of thylakoids per chloroplast section (approximately 20-40) which are rather unevenly distributed (Fig 1b). Ratio of appressed to non-appressed thylakoid membrane is the highest in this polar *Cosmarium* strain, displaying the low-light-adapted structure of thylakoids. Furthermore, this strain has broader grana stacks compared to the high-mountain one, and less prominent circle of starch granules around pyrenoids (Fig. 1g).

Chloroplasts of the typical tropical species, *C. beatum*, demonstrate characteristics corresponding to the sun-adapted plants, having approximately 5–15 thylakoids per granum, while grana stacks are relatively narrow (Fig. 1c). Interestingly, chloroplasts of the other polar strain, *C. crenatum*, have approximately 3–10 thylakoids per granum, thus approaching to the sun-adapted type (Fig. 1d). Chloroplasts of both of the small-sized taxa (*C. meneghinii* and *C. regnesii*) have relatively low number of thylakoids per granum; i.e. relatively low appressed to non-appressed membrane ratio (Figs 1e, 1h). Both strains originating from the tropical regions (*C. punctulatum* No. 570 and *C. beatum*) have more than 70% of thylakoids evenly positioned; thus additionally confirming the sun-adapted chloroplast structure.

All of the *Cosmarium* strains studied possess numerous plastoglobules; yet, *C. beatum* has by far the highest number of plastoglobules per frontal section, among all of the *Cosmarium* strains. On the contrary, the typical arctic species, *C. crenatum*, has the lowest number of plastoglobules per frontal section (Table 1), which are also the smallest among all of the strains studied.

Thylakoids of pyrenoids are connected to the lamellae system of the rest of the chloroplast through grana-like bridges, and are continuous with the chloroplast lamellae. The small-sized species have 3–5 non-appressed pyrenoid thylakoids (Fig. 1i) while the medium-sized *Cosmarium* strains have approximately 15 to 30 thylakoids intercepting the pyrenoid (Figs. 1f, 1g). The pyrenoid thylakoids are straighten and continuous in all of the *Cosmarium* strains.

All of the *Cosmarium* strains have large peroxisomes attached to the endoplasmic reticulum (ER) cisternae which encircle the chloroplasts (Fig. 1d). The interphase nucleus of the *Cosmarium* cells is large and placed on the isthmic region of cells. Chloroplast's outer membrane is in intimate connection with the nucleus in some parts (Fig. 2a). Mitochondria of the *Cosmarium* cells studied correspond to the typical mitochondria of vascular plants, with large intracristal compartments and a dense matrix (Fig. 2c). Majority of mitochondria are quite closely attached to chloroplasts or the nucleus.

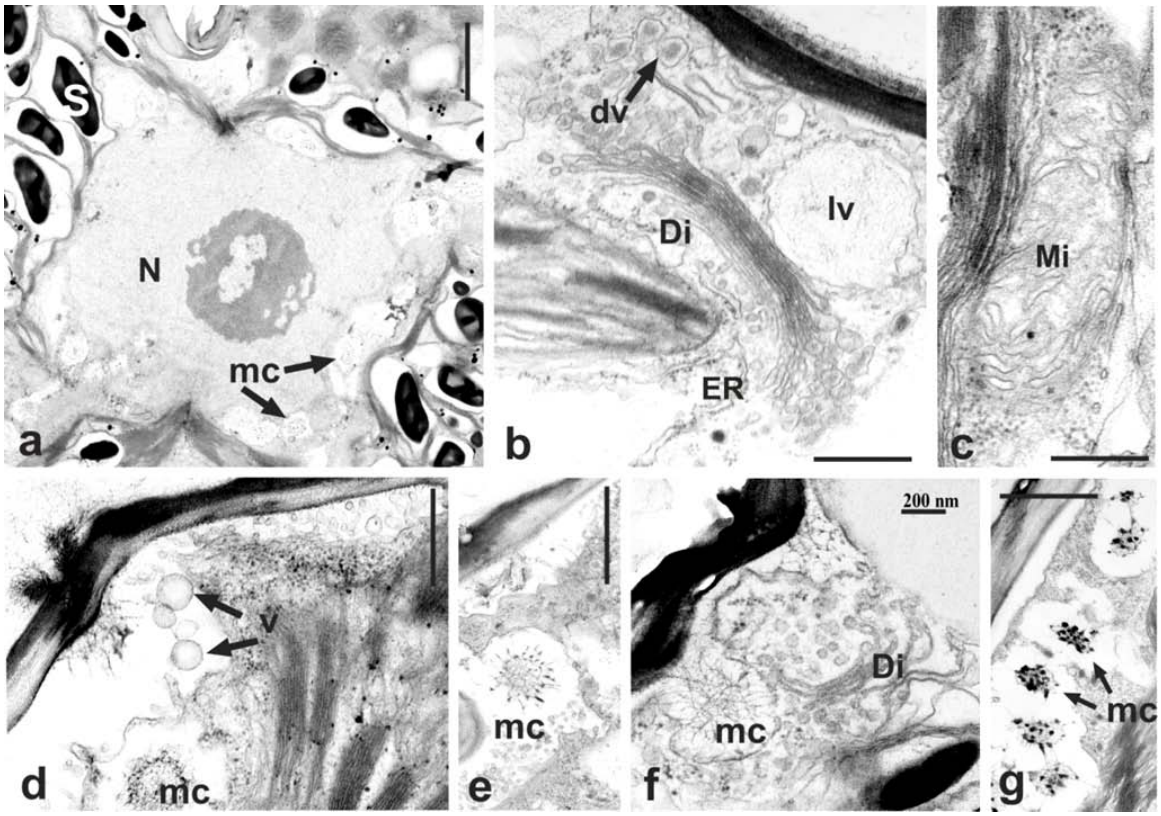

Fig. 2. a-i Ultrastructure details of the *Cosmarium* strains grown at standard laboratory conditions (21°C, 30 µmol photons m^{-2} s^{-1}). **a** Nucleus of *C. beatum* encircled by chloroplast lobes and mucilage clumps. **b** Golgi apparatus of *C. punctulatum* No. 570. **c** Mitochondrion of *C. punctulatum* No. 571. **d** Mucilage vesicles and pore of *C. beatum*. **e** Polyhedral mucilage clump (granule) in *C. beatum*. **f** Mucilage clump in the protoplast of *C. meneghinii*. **g** Mucilaginous clumps in the protoplast of *C. crenatum*. N – nucleus, mc – mucilage clump (granular inclusions), Di – dictyosome, lv – large vesicle, dv – dark vesicle, ER – endoplasmic reticulum, Mi – mitochondrion, S – starch granule, v – vesicle. Bar = 1 µm.

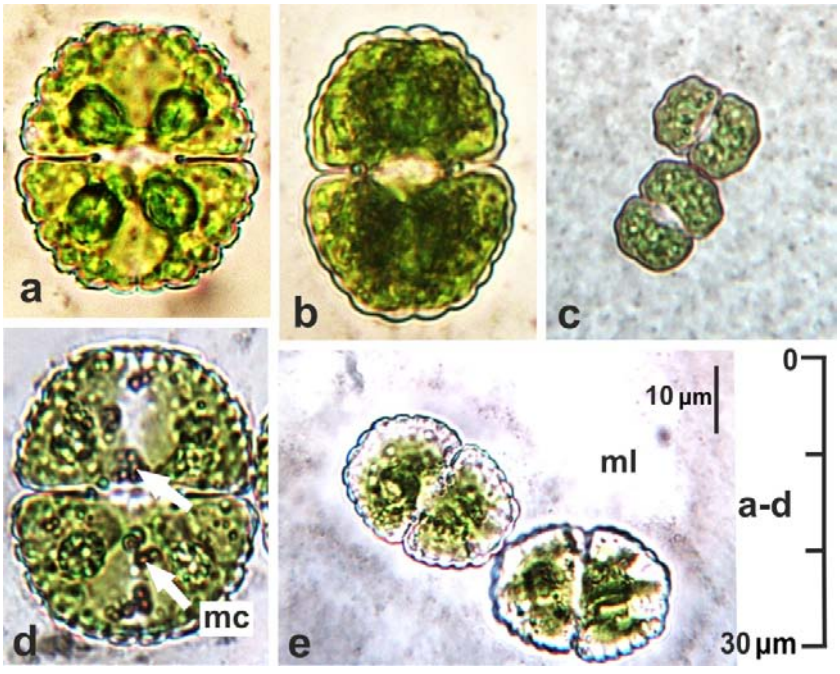

Fig. 3. a-c Cells of the *Cosmarium* strains studied grown at control laboratory conditions (21°C, 30 µmol photons m^{-2} s^{-1}). **a** *C. beatum*. **b** *C. crenatum*. **c** *C. meneghinii* after staining with Indian ink. **d-e** Cells of the *Cosmarium* strains studied grown at 35°C and 30 µmol photons m^{-2} s^{-1}, showing different degrees of plasmolysis. **d** *C. beatum* (24 h treatment). **e** Heavily plasmolysed cells of *C. crenatum* (5 h treatment). mc – mucilaginous clumps (granular inclusions), ml – residues of a mucilaginous layer.

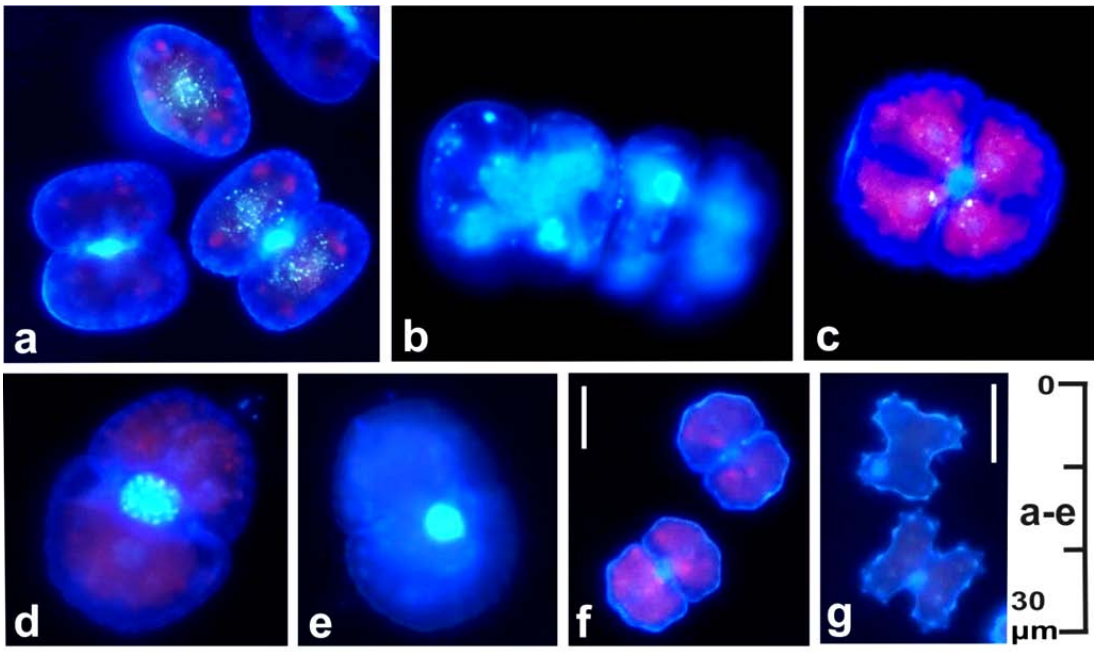

Fig. 4. a-g DAPI stained cells of the *Cosmarium* strains after the 48 h HS treatment at 35°C and 30 µmol photons m^{-2} s^{-1}, showing the position and the structure of the nucleus. **a** *C. punctulatum* No. 570. **b** Dislocation of the nucleus in *C. punctulatum* No. 571. **c** *C. beatum*. **d** Chromatin condensation in *C. crenatum*. **e** Dislocation of the nucleus in *C. crenatum*. **f** *C. meneghinii*. **g** Dislocation of the nucleus in *C. regnesii*.

The *Cosmarium* strains have well-developed Golgi complexes with 8–20 dictyosomes and numerous vesicles spreading in the cytoplasm (Figs 2 b, 2f). All of the known types of dictyosome-associated vesicles are observed in cells of the *Cosmarium* strains: large, dark, flat and pore vesicles (Dobberstein and Kiermayer 1972). Both strains of *C. punctulatum* and *C. regnesii* produce bulky 'large' vesicles (Fig. 2b) which are involved in the transportation of mucilage through pores, to form a mucilaginous sheath. However, a considerable number of these vesicles empties into protoplast compartments, giving the impression that the protoplast is filled with fibrous mucus. Numerous 'large' vesicles in *C. beatum* release their content into compartments inside the cytoplasm. Mucilage in these compartments obtains a needle-shaped formation forming polyhedral mucilaginous clumps (Figs 2d, 2e). These clumps are visible by means of a light microscopy as granules vigorously circulating, and can be so numerous that produce brownish cover over chloroplast and other organelles (Fig. 3d). The mucilaginous polyhedral clumps are placed outside of the cell vacuole (i.e. outside of tonoplast). Likewise, the protoplast of *C. crenatum* is filled with numerous compartments filled with mucilage inclusions arranged into polyhedral formations (Fig. 2g). Interestingly, all of the medium-sized *Cosmarium* strains and *C. regnesii* occasionally develop cytoplasmatic compartments with mucilaginous clumps even in the nearest surrounding of the nucleus (Fig. 2a).

Strikingly enough, mucilage inclusions were observed in the protoplast of a small-sized eutrophic species, *C. meneghinii*, (Fig. 2f) although no trace of the mucilaginous sheath was observed during the logarithmic growth of this species at 16°C (Stamenković and Hanelt 2011) and at 21°C (as noted after the staining of algal suspensions by means of an Indian ink) (Fig. 3c).

Ultrastructural differences between the Cosmarium strains collected from various climatic areas and treated at the heat shock temperature (35°C, 30 μmol photons m^{-2} s^{-1})

All of the *Cosmarium* strains appeared slightly plasmolysed after the 5 h treatment at 35°C, as estimated by means of a light microscope. However, cells of *C. crenatum* were considerably shrunken already after this short treatment (Fig. 3e).

Position of the nucleus appeared unchanged for all of the strains after the 5 and 10 h HS treatment. Longer treatment (48 h) caused the retardation of nuclear migration in both of the strains of *C. punctulatum*, *C. crenatum*, and *C. regnesii* (Figs 4a, b, d, e, g). Interestingly, the position of nucleus remained normal in *C. meneghinii* and *C. beatum* during the entire HS treatment (Figs 4f, 4c). A slight chromatin condensation occurred in both of the polar strains (*C. punctulatum* No. 571 and *C. crenatum*) already after 5 h (Figs 4d, 5a), while longer treatments caused further development of the heterochromatin and abnormal connection of the nucleolar portions. The nuclear shrinkage and similar changes in the nucleolus occurred in *C. beatum* and *C. punctulatum* No. 570 after the HS application longer than 24 h, pointing to the higher resistance at warm temperatures (Fig. 5b).

Several indications of the programmed cell death (PCD) appeared in the *Cosmarium* strains after the HS application longer than 5 h, such as numerous invaginations of the tonoplast (Fig. 6b), and the appearance of irregular circular thylakoid formations in chloroplasts (Fig. 6c).

Both grana and stroma thylakoids were dilated and folded at the beginning of the HS treatment, in all of the *Cosmarium* strains. Remarkably, chloroplasts of the polar strain of *C. punctulatum* were characterised by the densely arranged grana thylakoids while stroma thylakoids were circularly folded, closely surrounding plastoglobules, already after 5 h (Fig. 5d). The identical 'wavy' thylakoid arrangement was observed in *C. crenatum* after 24 h (Fig. 5f, 5h), *C. punctulatum* No. 570 after 48 h, and in *C. beatum* after 7 days of the HS treatment. Interestingly, both of the small-sized taxa did not demonstrate similar thylakoid structure during the entire 35°C HS application (Fig. 6e). In addition, a tendency to increase the stacking of grana thylakoids was observed in all of the desmid strains during the prolonged HS treatment (Table 2). Pyrenoid thylakoids of all of the medium-sized taxa were markedly circularly folded, obtaining the identical structure as described for stroma thylakoids (Fig. 5e). The described thylakoid lattices corresponded to the cubic membrane structures which were formerly called the quasi-crystalline lamellar lattices (McLean and Pessoney 1970; Landh 1995). On the contrary, pyrenoid thylakoids of the small-sized taxa did not exhibit such changes, or they were only slightly swollen.

The average number of plastoglobules per frontal section increased in all of the medium-sized taxa; however, the average diameter of plastoglobules remained almost the same as in control cells (Table 2).

No changes in structure and abundance of starch granules were observed after 5 and 10 h treatments in all of the desmid strains, with the exception of *C. crenatum* which possessed degraded stroma starch

during these treatments (Fig. 6a). Both stroma and pyrenoid starch granules were considerably disintegrated after 48 h HS treatment in all of the desmid strains, with the exception of *C. beatum* and *C. meneghinii*.

The HS treatments longer than 10 h initiated progressive vesiculation and lower stacking of dictyosomes in both of the polar strains (*C. punctulatum* No. 571 and *C. crenatum*) (Fig. 6d). In contrast, dictyosomes of *C. beatum* and the small-sized taxa remained almost intact during the entire 48 h HS application (Fig. 6f).

HS treatments longer than 10 h caused tearing of ER cisternae into smaller and narrower pieces and aggregation of those into sheets. These smooth or rough ER cisternae surround assemblies of dark granules frequently found in the area of nucleus but also in other regions of the cell, in all of the *Cosmarium* strains studied (Figs 5g, 5i). Diameter of dark granules ranged from 33 to 49 nm, and they corresponded to those formed in heat-stressed cells of *M. denticulata* (Meindl 1990), and referred as "heat shock granules" (hsg). Structures known as "rod containing vesicles" (S-vesicles – SV, Kiermayer 1971) are also abundant within these hsg aggregations. Both of the small-sized taxa were characterised by the relatively low amount of hsgs during the entire HS treatment period.

Strain	Range of number of thylakoids per granum	Evenness of a grana margin distribution (%)	Grana width (µm); SD < 5% of mean	Average number of plastoglobules per frontal section; SD < 5% of mean	Plastoglobules diameter (µm); SD < 5% of mean
C. punct. (570)	8 – 50	90 evenly	0.657*	42*	0.142*
C. punct. (571)	14 – 60	60 evenly	0.705^{ns}	64*	0.13^{ns}
C. beatum	5 – 20	65 evenly	0.799^{ns}	95*	0.081^{ns}
C. crenatum	8 – 45	60 evenly	0.608*	89*	0.081^{ns}
C. meneghinii	5 – 30	60 evenly	0.761*	30^{ns}	0.114^{ns}
C. regnesii	5 – 30	60 evenly	0.644^{ns}	19^{ns}	0.113^{ns}

Table 2. Some characteristics of the chloroplast ultrastructure of the investigated *Cosmarium* strains grown at the heat-shock conditions (35°C and 30 µmol photons m^{-2} s^{-1} for 48 h); measurements from at least 40 cells. Measurements were performed according to the instructions by Lichtenthaler et al. (1981). Asterisks (*) refer to the significant difference compared to control cells (21°C, 30 µmol photons m^{-2} s^{-1}); (ns) – not significant (t-test; $p<0.05$).

Mitochondrial cristae formed peculiar circular structure, consisting of up to four lamellar rings, in cells of *C. punctulatum*, *C. crenatum*, and *C. beatum* after the HS treatment longer than 10 h (so-called onion-like cristae structure; John et al. 2005) (Fig. 5c). These structures were not found in the small-sized strains which had rather unaffected mitochondrial organisation. Mitochondria were heavily damaged in the polar strain of *C. punctulatum* and in *C. crenatum* after the 48 h HS treatment; cristae were disintegrated revealing the unstructured matrix (Fig. 5j).

Recovery at 21°C (34 h) led to the relaxation of ultrastructural changes in all of the *Cosmarium* strains studied (data not shown). Still, both of the strains of *C. punctulatum* had considerably damaged and involuted tonoplasts, cubic thylakoid structure, and damaged mitochondrial cristae. Interestingly, the arctic species, *C. crenatum*, showed the rapid revitalisation, as concluded from the fast recovery of thylakoids,

dictyosomes, mucilage granules, starch bodies, ER cisternae and mitochondria. As expected, *C. beatum* and both of the small-sized taxa recovered the cell ultrastructure completely after 34 h of recovery. Only the high mountain strain of *C. punctulatum* had a copious amount of hsgs in the cytoplasm during recovery; hsgs in all of the other strains disappeared.

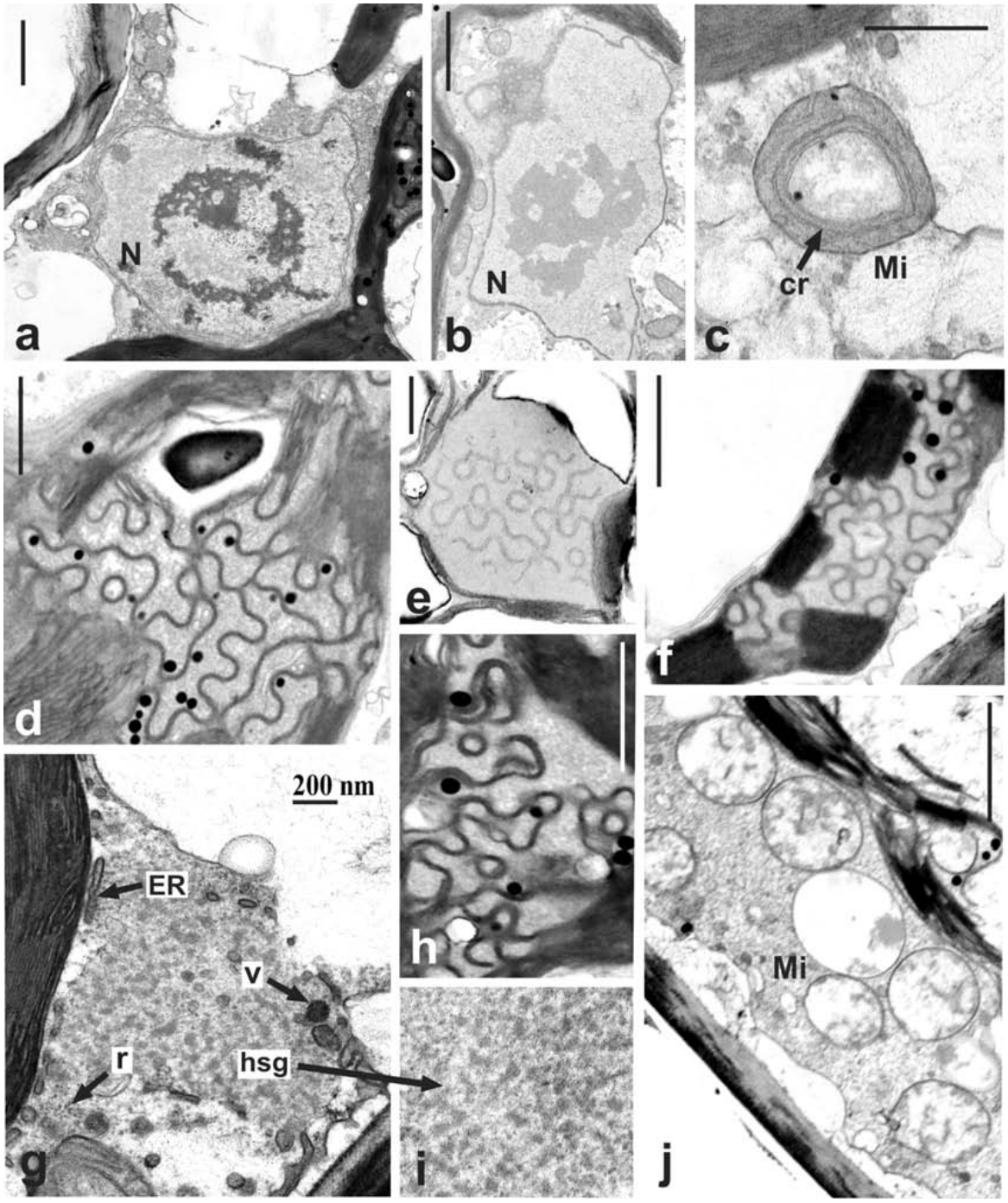

Fig. 5. a-i Ultrastructural changes in the *Cosmarium* strains treated at 35°C and 30 µmol photons m^{-2} s^{-1}. **a** Chromatin condensation in *C. punctulatum* No. 571 after 5 h treatment. **b** Nuclear shrinkage and chromatin condensation in *C. beatum* after 24 h. **c** Onion-like cristae in the mitochondrion of *C. punctulatum* No. 570 after 24 h treatment. **d** Cubic membranes in the chloroplast of *C. punctulatum* No. 571 after 24 h treatment. **e** Cubic membranes in the pyrenoid of *C. punctulatum* No. 571 after 24 h treatment. **f** Cubic membranes in the chloroplast *C. crenatum* after 24 h treatment. **g-i** Heat shock granules in *C. punctulatum* No. 570 after 24 h treatment. **h** Cubic membranes in the chloroplast of *C.*

crenatum after 48 h treatment. **j** Disintegrated mitochondria in *C. crenatum* after 48 h treatment. N – nucleus, Mi – mitochondrion, cr – cristae, ER – endoplasmatic reticulum, v – highly contracted vesicles, r – ribosomes, hsg – heat shock granules. Bar = 1 μm.

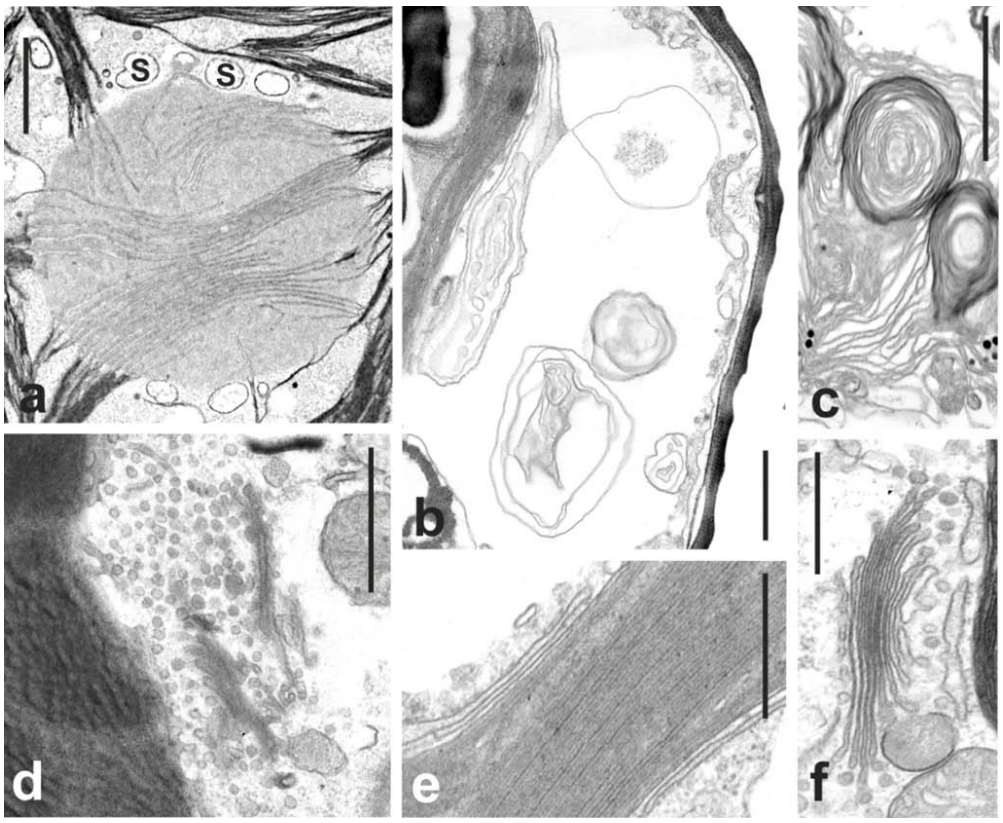

Fig. 6. a-f Ultrastructural changes in the *Cosmarium* strains treated at 35°C and 30 μmol photons m^{-2} s^{-1}. **a** Destroyed starch granules in *C. crenatum* after 24 h. **b** Multivesicular (membranous) bodies in *C. meneghinii* after 24 h. **c** Multivesicular bodies in the chloroplast of *C. beatum* after 7 days. **d** Disintegrated dictyosomes in *C. punctulatum* No. 571 after 24 h. **e** Densely arranged thylakoids in *C. meneghinii* after 24 h. **f** Normally developed Golgi complex in *C. regnesii* after 10 h. S – destroyed starch granules. Bar = 1 μm.

Ultrastructural differences between the Cosmarium strains collected from various climatic areas and treated at the low temperature (0.6°C, 30 μmol photons m^{-2} s^{-1})

No any change in cell differentiation was observed during the entire 32-day treatment at 0.6°C in all of the *Cosmarium* strains studied. Cells of the desmid strains were slightly plasmolysed during several hours when transferred from 21 to 0.6°C. After this initial temperature stress, both of the polar strains (*C. punctulatum* No. 571 and *C. crenatum*) preserved an intact appearance during the entire low-temperature treatment (Figs 7b, e). Interestingly, all of the desmid strains studied had a bright green chloroplast colour (except *C. beatum*), which pointed to fair preservation of chlorophylls at low temperatures (Figs 7a, d, f).

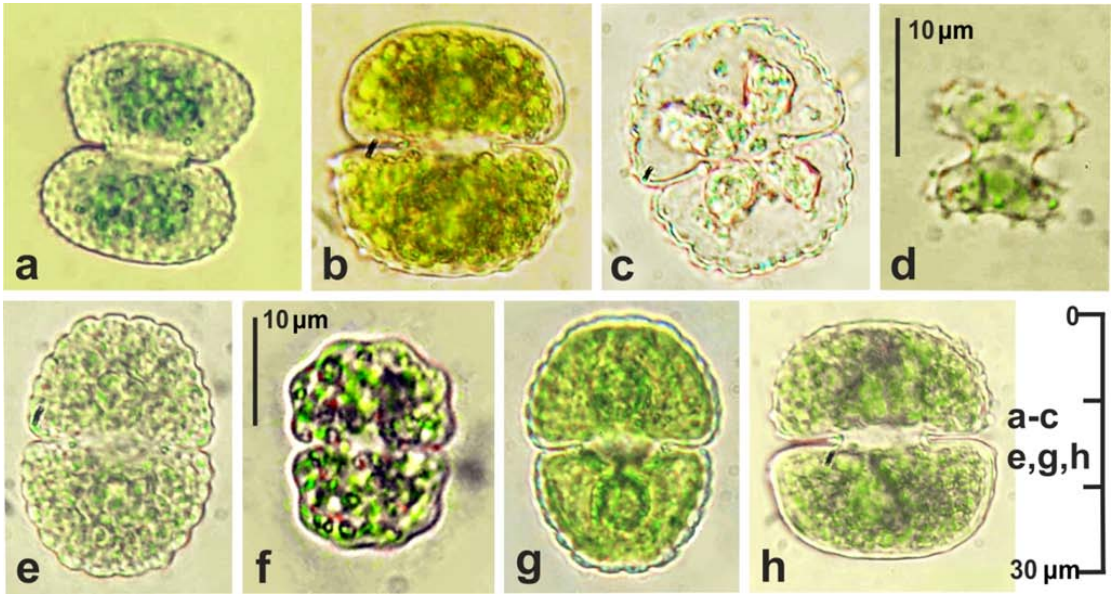

Fig. 7. a-f Cells of the *Cosmarium* strains studied grown at 0.6°C and 30 µmol photons m^{-2} s^{-1} after 32 days. **a** *C. punctulatum* No. 570. **b** *C. punctulatum* No. 571. **c** *C. beatum*. **d** *C. regnesii* var. *polonicum*. **e** *C. crenatum* var. *boldtianum*. **f** *C. meneghinii*. **g-h** Cells of the *Cosmarium* strains studied which were completely recovered within 4 days at 21°C after the low-temperature treatment. **g** *C. crenatum*. **h** *C. punctulatum* No. 571.

The 32-day treatment at 0.6°C was lethal for the typical, tropical species, *C. beatum*, which organelles were destroyed during this experiment (Figs 7c, 9e). This species was unable to recover when transferred to 21°C. Position of nucleus was unchanged during the entire 32-day treatment at 0.6°C in all of the investigated *Cosmarium* strains. All of the medium-sized strains had slightly condensed chromatin during the entire low-temperature treatment (Fig. 8a).

Stroma and grana thylakoids were markedly inflated in chloroplasts of *C. punctulatum* No. 570 and *C. regnesii* after the 2-week cold treatment (Fig. 8b). Initiations of cubic membranes, observed as circularly curved stroma thylakoids, appeared in the polar strain of *C. punctulatum*, *C. crenatum* and *C. meneghinii* already after the 2-week cold treatment (Fig. 8c, d). Interestingly, the prolonged treatment provoked the development of cubic membranes in chloroplasts of *C. punctulatum* No. 570, and *C. regnesii* (Fig. 8e), while the arctic species, *C. crenatum*, regained normal thylakoid structure. Number of plastoglobules was significantly increased in *C. crenatum* after the 4-week cold treatment. Pyrenoid thylakoids obtained the cubic membranous structure in both of the polar strains (*C. punctulatum* No. 571 and *C. crenatum*) after the 2-week treatment. Pyrenoids of both of the small-sized taxa did not display changes compared to control cells, while pyrenoids of the high-mountain strain of *C. punctulatum* were considerably damaged (Fig. 8h).

The cosmopolitan species, *C. meneghinii*, had unchanged abundance and consistency of starch granules during the entire low-temperature application. In contrast, starch granules in the other small-sized taxon, *C. regnesii*, were almost completely disintegrated already after 2-week treatment (Fig. 8f).

Both of the small-sized taxa had involuted and inactive dictyosomes after the two-week treatment, with decreased number of vesicles (Figs 9a, b). The high-mountain strain of *C. punctulatum* had

considerably swollen ER cisternae and damaged dictyosomes during the entire cold treatment (Fig. 9c). *C. regnesii* possessed large aggregations of swollen ER cisternae after the 2-week cold treatment, while the longer treatment provoked the development of considerably bloated (balloon-like) cisternae, encircled with ribosomes (Figs 9d, 9i).

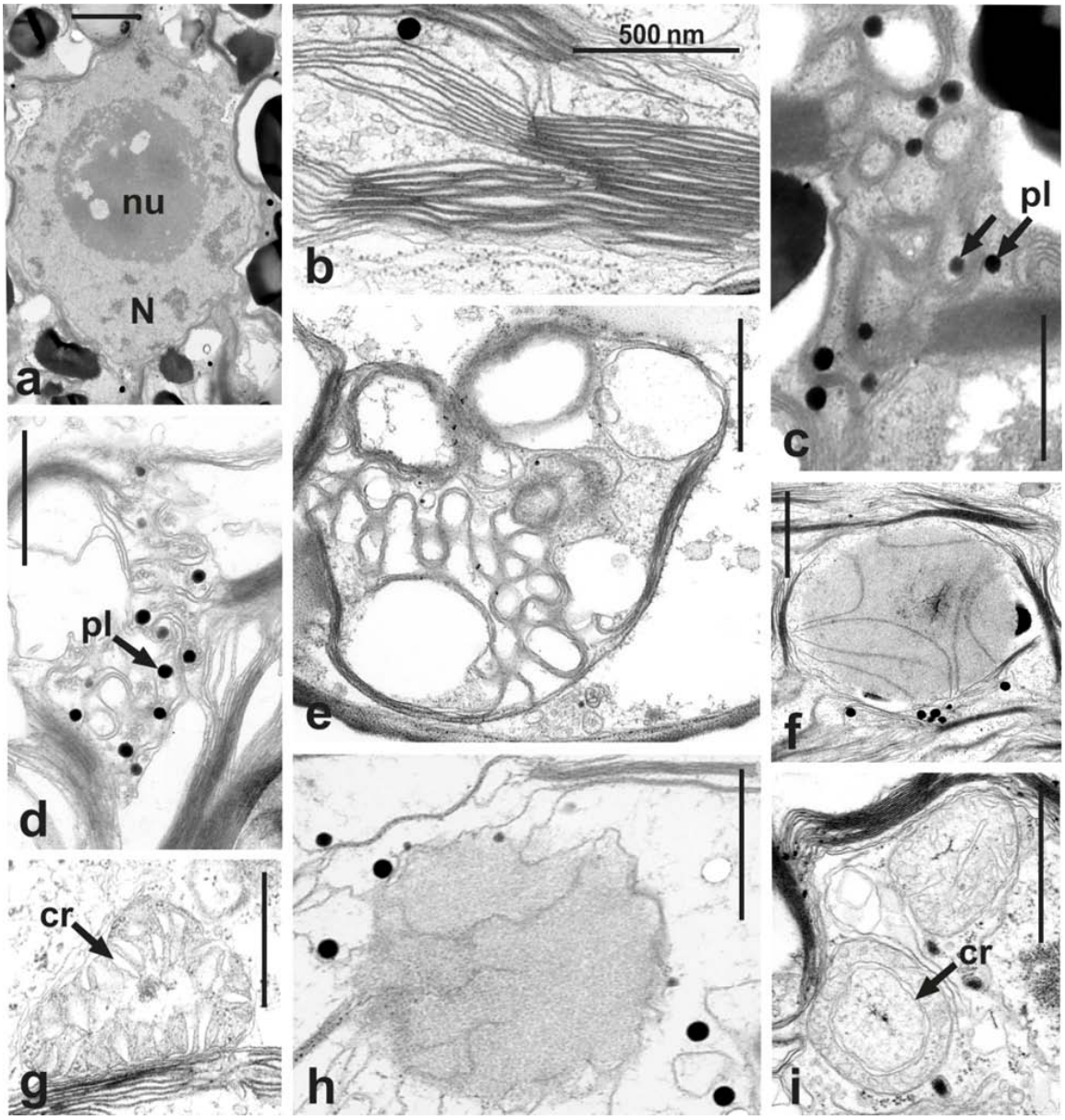

Fig. 8. a-i Ultrastructural changes of cells of the investigated *Cosmarium* strains grown at 0.6°C and 30 µmol photons m^{-2} s^{-1}. **a** Condensed chromatin and enlarged nucleolus in *C. crenatum* after 2 weeks. **b** Dilation of thylakoids in *C. regnesii* after 2 weeks. **c** Cubic membranes in chloroplasts of *C. crenatum* after 2 weeks. **d** Cubic membranes in chloroplasts of *C. meneghinii* after 2 weeks. **e** Cubic membranes in chloroplasts of *C. regnesii* after 32 days. **f** Destroyed starch granules in *C. regnesii* after 2 weeks. **g** Normal mitochondrion in *C. regnesii* after 2 weeks. **h** Damaged pyrenoid in *C. punctulatum* No. 570 after 2 weeks. **i** Onion-like mitochondrial cristae in *C. meneghinii* after 2 weeks. N – nucleus, nu – nucleolus, pl – plastoglobules, cr – cristae. Bar = 1 µm.

Abnormally long mitochondrial cristae emerged in *C. punctulatum* No. 570 and *C. meneghinii* after the 2-week cold treatment, which obtained the onion-like structure in most of the cells at the end of the experiment (Fig. 8i). The cold-temperature treatment did not cause large changes in mitochondrial structure in both of the strains collected from the polar zone and in *C. regnesii* (Fig. 8g).

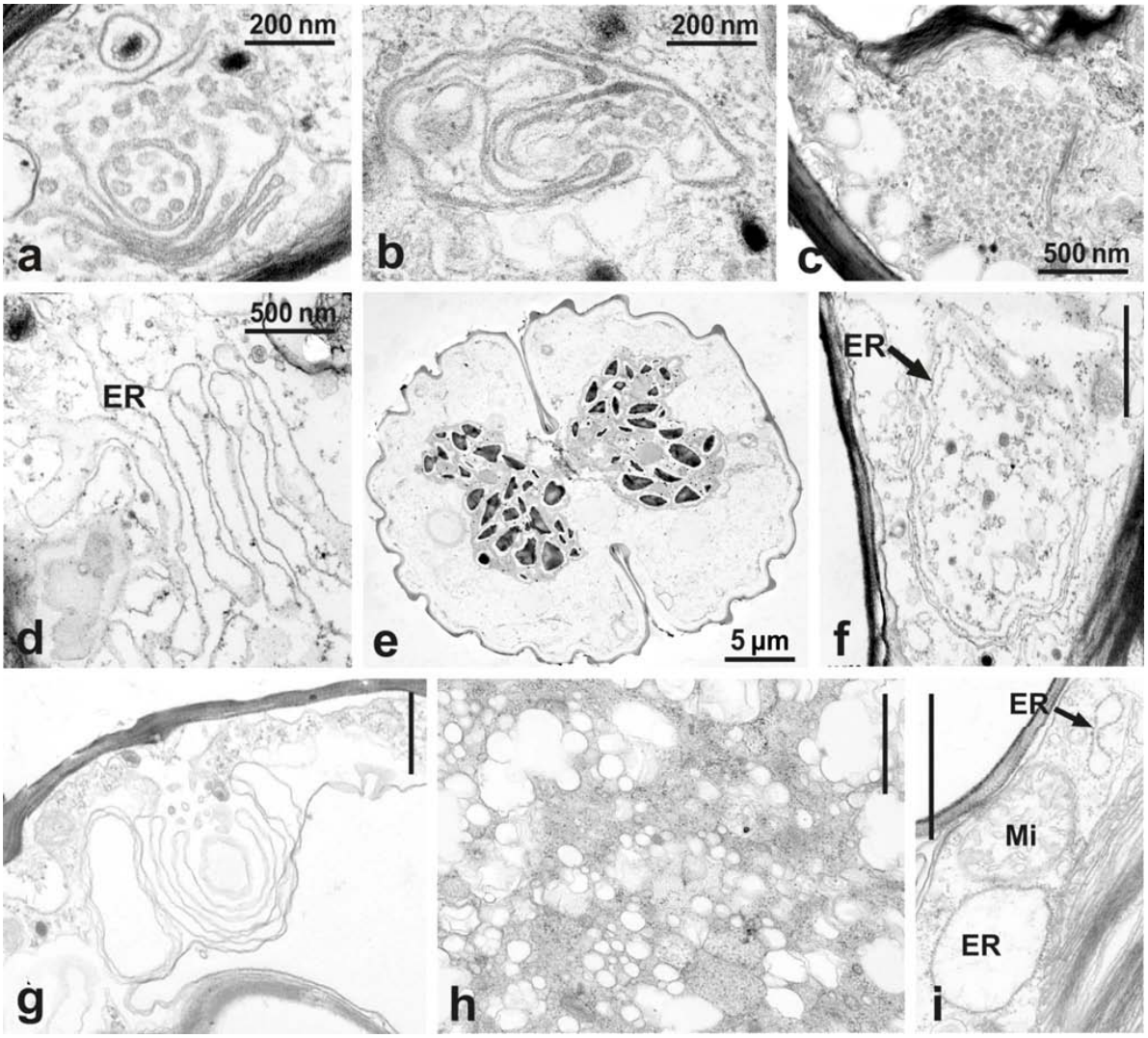

Fig. 9. a-i Ultrastructural changes in cells of the investigated *Cosmarium* strains grown at 0.6°C and 30 µmol photons m^{-2} s^{-1}. **a** Involuted and inactive dictyosomes in *C. meneghinii* after 2 weeks. **b** Involuted and damaged dictyosomes in *C. regnesii* after 2 weeks. **c** Vesiculated and damaged dictyosomes in *C. punctulatum* No. 570. **d** Large aggregations of swollen ER cisternae in *C. regnesii* after 2 weeks. **e** Dead cell of *C. beatum* after 2 weeks. **f** Autophagosome in *C. regnesii* after 32 days. **g** Multivesicular (membranous) bodies as a hallmark of PCD in *C. menenghinii* after 32 days. **h** Foam-like vacuolation of the cytoplasm in *C. punctulatum* No. 570 after 32 days. **i** Heavily bloated (balloon-like) ER cisternae in *C. regnesii* after 2 weeks. ER – endoplasmic reticulum, Mi – mitochondrion. Bar = 1 µm (f – i).

Beside the characteristics described above, the other hallmarks of PCD could have been noted during the 2-week cold experiment, such as the tonoplast involution in all of the investigated *Cosmarium* strains leading to the formation of multivesicular bodies (Fig. 9g). Yet, tonoplast damages were less pronounced in both of the polar strains studied. Signs of autophagy were noted in *C. regnesii*, as indicated by the

surrounding of organelles with ER cisternae and formation of autophagosomes (Fig. 9f). The 32-day treatment caused the foam-like vacuolation of the protoplast in the high-mountain strain of *C. punctulatum* and *C. regnesii* (Fig. 9h).

As expected, cells of both of the polar strains (*C. punctulatum* No. 571 and *C. crenatum*) recovered completely after 4 days of the beginning of recovery at 21°C (Figs 7g, h). Somewhat slower recovery was observed in the high-mountain strain of *C. punctulatum*, as judged from the presence of curved and swollen thylakoids, narrow dictyosome cisternae, the involuted tonoplast, and the relatively low amount of starch granules. Both of the small-celled taxa were almost fully recovered after 4 days of recovery; yet, numerous membranous bodies could have been observed in cells of these strains. All of the *Cosmarium* strains studied (except *C. beatum*) completely regained a normal ultrastructural appearance after 8 days of recovery.

Discussion

Notes on the ultrastructural differences between the Cosmarium strains collected from various climatic areas and grown under standard laboratory conditions (21°C, 30 µmol photons m^{-2} s^{-1})

The ultrastructure of *Cosmarium* chloroplasts corresponds to that of vascular plants. Chloroplasts of desmids consist of a series of lamellae composed of basic membrane units, thylakoids, each of which is a flattened sac composed of a single membrane. TEM studies show that structures analogous to grana of plant chloroplast are present in the *Cosmarium* strains, grana being used specifically to refer to a stack of appressed thylakoids (Gibbs 1970; Brook 1981a; Lütz et al. 1997).

Despite the long-term cultivation (over 15 years) under relatively low light-temperature conditions (16°C and 30 µmol photons m^{-2} s^{-1}) and subsequent acclimation at 21°C, all of the *Cosmarium* cells exhibited several characteristics corresponding to those of high-light adapted plants (i.e. sun plants). These characteristics are demonstrated as: (i) exposed chloroplast membranes (i.e. chloroplasts are furcoid); (ii) relatively low number of thylakoids per granum (low stacking degree, with the exception of *C. punctulatum* No. 571); (ii) long and exposed stroma lamellae; (iv) numerous and moderately large plastoglobuli; (v) markedly large starch grains, situated around pyrenoids (pyrenoid starch) and also between thylakoids (stroma starch) (Lichtenthaler 1984; Lichtentaler et al. 1981, 1982; Meier and Lichtentaler 1981).

Low grana stacking in high-light chloroplasts decreases the ratio of appressed to non-appressed membranes, and such chloroplasts have smaller, though more PSII photosynthetic units and smaller PSII antennae to minimise the deleterious effects of photoinhibition (Anderson 1986; Mustardy and Garab 2003). Accordingly, sun-type chloroplasts have higher content of the cyt b/f complex, plastoquinone, plastocyanin and ferredoxin as well as ATP synthase which support faster rates of electron transport (Anderson et al. 1988). Due to these features, high-light adapted plants have much greater photosynthetic capacities, which saturate at higher irradiance than those of plants growing in shaded habitats (Björkman 1981; Anderson and Osmond 1987). The observed sun-type structure of chloroplasts is in accordance with the distinctly high photosynthetic capacities (measured as $rETR_{max}$), and saturating irradiances noted at 21°C in all of the

investigated *Cosmarium* strains (Stamenković and Hanelt 2012), which undoubtedly rendered them as high-light adapted algae.

However, the polar strain of *C. punctulatum* has the high appressed to non-appressed membrane ratio typical to the low-light adapted plants which possess larger, though fewer PSII photosynthetic units with large antennas and are characterised by lower photosynthetic capacity and saturating irradiance (Anderson et al. 1988). Strikingly, the shade-type chloroplast structure in this strain is in accordance with relatively low values of $rETR_{max}$ and I_k measured at 21°C, when compared to the other *Cosmarium* strains studied (Stamenković and Hanelt 2012). Yet, the polar strain of *C. punctulatum* has well-developed starch granules and numerous plastoglobules, which contribute to the high-light appearance of chloroplast in this strain, as stated above.

Interestingly, the typical tropical species, *C. beatum*, possesses the highest number of plastoglobules per frontal section, which is in accordance with what was observed for tropical plants (Bréhélin and Kessler 2008).

Species- and strain-specific differences in the chloroplast structure exist and they are in accordance with the light prevailing of the sampling sites; yet, chloroplasts of all of the *Cosmarium* strains studied could be roughly typified as that of high-light-adapted plants. This observation is concomitant with the previous study which revealed that all of the *Cosmarium* strains are adapted to high light, confirming the Coesel's hypothesis on the origin of desmids in tropics (Coesel 1996; Stamenković and Hanelt 2011, 2012). However, all of the desmid strains, except the high-mountain strain of *C. punctulatum* and *C. beatum*, have rather unevenly distributed grana margins. This probably appeared as a result of acclimation at the low cultivating irradiation (30 μmol photons m^{-2} s^{-1}), comparable with the fact that grana of shade plants are often irregularly orientated to increase capture of diffuse or variably orientated light (Lawlor 2001).

A few desmidiologists (Carter 1919; Korn 1969) emphasised a very intimate connection between chloroplasts and the cell nucleus. Possibly, there is an essential role in communication between these organelles, as many enzymes of chloroplasts are encoded in the nucleus (Jarvis 2008). This fact, as well as the closely positioned mitochondria, peroxisomes and ER cisternae to the outer chloroplast membrane, enable a rapid response to rapidly changing conditions in desmid habitats.

It has been already stated by West and West (1904) that the protoplasm of many desmids contains numerous granules which exhibit a well-marked 'circulatory movement'. The granular inclusions observed by light and TEM microscopy in all of the medium-sized *Cosmarium* taxa (the most remarkable in *C. beatum* and *C. crenatum*) correspond to those found in the protoplasm of *Bambusina brebissonii* Kützing and *Gonatozygon brebissonii* De Bary (Brook 1981a). The TEM images revealed the polyhedral structure of these electron-dense inclusions, which are well-visible as brownish, vigorously jiggling granules by a light microscope. By means of X-ray analysis, Brook (1981b) disclosed that the granular inclusions of *B. brebissonii* and *G. brebissonii* consist of calcium sulphate, while those in terminal vacuoles of species belonging to *Closterium*, *Pleurotaenium* and *Penium* contain barium sulphate. Since the L-d medium does

not contain Ba, the mucilage polyhedral clumps in the *Cosmarium* strains studied have been probably incrusted by $CaSO_4$, observable as electron-dense inclusions. Brook (1981a) noted that these small inclusions can be so abundant that form a swarming mass of granular material, visible as brownish patches around chloroplast and nucleus. Since the abundance of cytoplasmatic granules increases after certain stress treatments (e.g. after high PAR or UV radiation, pers. observation) this may point to their role in screening of organelles.

Astonishingly, although it has been stated that small-sized, eutrophic desmids never develop mucilaginous sheath, which explains their distinctly high growth rates – comparable to those of fast-growing green algae (Spijkerman and Coesel 1998, Stamenkovic and Hanelt 2011), traces of mucilage were observed in the cytoplasm of a eutrophic species, *C. meneghinii*. This species has a rather low amount of mucilage in the protoplast, and, most likely, only limited (or none) amount of mucus is being transported through cell-wall pores. Possibly, the further evolutional trend will favour the development of small-sized, planktonic desmid taxa with the low mucilage production, which can easily compete with other microalgae (so-called r-strategists) (Pianka 1970; Spijkerman and Coesel 1998). This trend is nowadays greatly favoured by the anthropogenic pollution of freshwater ecosystems, rendering such habitats as greatly unfavourable for the majority of desmids, but not for the small-sized, eutrophic taxa.

Notes on the ultrastructural differences between the Cosmarium strains collected from various climatic areas and treated at the heat shock temperature (35°C, 30 µmol photons m^{-2} s^{-1})

Ultrastructural differences between the *Cosmarium* strains, which arose after the 35°C HS treatment, were revealed in accordance with their geographic origin, concomitantly with what was observed for the differences in photosynthetic and growth features (Stamenković and Hanelt 2012).

The typical tropical species, *C. beatum*, appeared the most resistant to the HS treatment among the medium-sized strains studied, considering that the thylakoid arrangement and the structure of dictyosomes and mitochondria were rather unchanged during the 48 h HS treatment. In contrast, cells of the arctic species, *C. crenatum*, were heavily plasmolysed already 5 h after the beginning of the HS treatment, while thylakoids were dismantled, and dictyosomes and mitochondria were severely damaged after the 48 h HS treatment, showing the sensitivity of this strain to high temperatures. From numerous literature data it has been recognised that polar algae and plants exhibit not only photosynthetic deterioration when exposed at warm-temperature treatments, but also abundant ultrastructural changes (Lüning 1990; Lobban and Harrison 1994; Wiencke 1996; Barton 2010).

A striking find was the presence of cubic membranes in chloroplasts and pyrenoids of the medium-sized *Cosmarium* strains after a certain period of the HS treatment. Interestingly, cubic membranes were not found in *Micrasterias denticulata* cells neither after the HS stress (2–24 h at 35° and 36°C) nor after the application of H_2O_2, KCl and NaCl in order to cause PCD (Darehshouri et al. 2008; Affenzeller et al. 2009). The observed cubic membranes found in the *Cosmarium* strains likely correspond to the gyroid (G) type

which was observed in *Zygnema* filaments (McLean and Pessoney 1970), out from the three fundamental types of cubic membranes: gyroid, double diamond and primitive (Landh 1995; Almsherqi et al. 2009).

The appearance of cubic membranes during the HS application in the *Cosmarium* strains might correspond to the hypothesis that the cubic structural transition represents a protective mechanism, reducing oxidative damage by enhancing the efflux of hydrogen peroxide and free-radical reactive oxygen species (ROS), and by reducing the susceptibility of membrane lipids to the oxidants (Li et al. 2000; Han et al. 2001; Deng et al. 2002; Almsherqi et al. 2009). Moderate heat stress may cause an abundant ROS production in chloroplasts, as it has been shown in numerous studies (see Sharkey 2005). In addition, Rubisco can produce hydrogen peroxide as a result of oxygen side reactions and H_2O_2 production was shown to increase substantially with temperature increase (Allakhverdiev 2008; Sharkey 2005). This fact may explain the heat-induced occurrence of cubic membranes in pyrenoids, which possess markedly high amount of Rubisco (Raven et al. 2008). Interestingly, cubic membranes were noted in both of the polar strains (*C. punctulatum* No. 571 and *C. crenatum*) within the shorter HS treatment time, while the typical tropical species, *C. beatum*, obtained the cubic membrane structure in chloroplasts only after 7 days of the HS treatment. This fact additionally revealed the sensitivity of the polar desmid strains at warm temperatures; possibly, the HS treatment provoked the rapid ROS production in these strains, which initiated the cubic membrane development (see Deng et al. 2002).

A significant increase in the number of plastoglobules in the medium-sized *Cosmarium* strains additionally demonstrated their ability to cope with elevated ROS production at high temperatures. Plastoglobules contain α-tocopherol, phylloquinone and plastoglobulins, and serve in phytol sequestration, thus protecting plastid membrane lipids against oxidative damage (Vidi et al. 2006; Bréhélin and Kessler 2008).

However, the prolonged heat stress provoked the first steps of PCD in the *Cosmarium* strains like it was observed in heat-stressed cells of *Chlorella saccharophila* (Krüger) Migula (Zuppini et al. 2007), soybean-cultured cells (Zuppini et al. 2006) and tobacco leaves (Vacca et al. 2006). A slight chromatin condensation, particular ultrastructural changes in mitochondria, the nucleolus, and the endomembrane system are PCD hallmarks (Polverari et al. 2000; Bidle and Falkowski 2004; Selga et al. 2005) which were found in the *Cosmarium* strains, during the treatment-times longer than 10 h. The presence of the so-called onion-like inner mitochondrial membrane in the HS treated medium-sized *Cosmarium* cells, indicated a serious damage of mitofilin, a protein responsible for maintaining of normal cristae morphology, as it was noted that down-regulation of mitofilin in HeLa cells results in initiation of apoptosis and the formation of this particular structure (John et al 2005).

The retardation of nuclear remigration to the isthmus region at the end of cell development, as observed in all of the *Cosmarium* cells, except *C. beatum* and *C. meneghinii*, was comparable with what was observed for the heat-stressed *Micrasterias* cells (Meindl 1990). Possibly, it is linked with high-temperature effects on actin microfilaments, via an influence on the Ca^{2+} level, as suggested by Joyce et al. (1988) and

Meindl (1990). *C. beatum* and *C. meneghinii* appeared more persistent to the HS treatment, probably as a result of a better preservation of the intracellular ion homeostasis.

The heat-induced inhibition of normal protein synthesis via block of pre-rRNA synthesis in plants usually coincides with the synthesis of a new set of proteins (heat shock proteins – HSPs) which are either not present or present at a low level in untreated cells (Miroshnichenko et al. 2005). The presence of HSPs in all of the *Cosmarium* strains is suggested by the observation of heat shock granules (stress granules) in the cytoplasm, similarly to what was observed for heat-stressed *M. denticulata* cells and several plant cell cultures (Neumann et al. 1984; Nover and Sharf 1984; Meindl 1990). In addition to the RNA preservation, hsgs represent multifuctional complexes, simultaneously combining enhanced chaperone activity by themselves as well as storage and release of HSPs and other essential compounds during stress response and recovery (Smýkal et al. 2000; Miroshnichenko et al. 2005).

Strikingly enough, although both of the small-sized taxa displayed but minor protective changes during the entire HS treatment (i.e. they did not develop cubic membranes or increased number of plastoglobules, and the abundance of hsgs was relatively low) they still revealed the highest photosynthetic and growth recovery after the prolonged HS treatments, among all of the strains studied (Stamenković and Hanelt 2012). Possibly, the small-sized taxa posses some other defending features, such as the heat-stress induced synthesis of HSPs which do not participate in hsg formation or other ROS-protective paths (Allakhverdiev et al. 2008), but this assumption should be thoroughly investigated.

Notes on the ultrastructural differences between the Cosmarium strains collected from various climatic areas and treated at the cold temperature (0.6°C, 30 µmol photons m^{-2} s^{-1})

Comparably with what was observed during the HS treatment at 35°C, numerous ultrastructural differences between the *Cosmarium* strains collected from various climatic areas, were revealed during the application of the cold temperature (0.6°C).

The long-term application of 0.6°C was lethal for the typical tropical species, *C. beatum*, which organelles were almost completely destroyed during the 2-week application. This finding is in accordance with the previously displayed intolerance of this tropical species at temperatures lower than 7°C, as concluded from the rapid deterioration of all of the photosynthetic parameters and growth (Stamenković and Hanelt 2012). The physiological incapability of *C. beatum* to acclimate at low temperatures is in agreement with studies which showed that tropical plants and macroalgae display extreme deterioration of all physiological processes at low temperatures of high latitudes (Lüning 1990; Hanelt et al. 2003; Barton 2010).

In contrast, both of the polar strains revealed a fair durability at the cold-temperature treatment, as judged from relatively small changes in the structure of thylakoids, pyrenoids, Golgi complex and ER cisternae during the entire cold-temperature treatment. Astonishingly, *C. crenatum* completely recovered the maximum quantum yield within only 5 h after the beginning of recovery at 21°C (data not shown) and fully

recovered the normal ultrastructural appearance after 4 days of recovery. These features undoubtedly pointed to high acclimation possibilities of this taxon at low temperatures, comparably with the high recovery rates in several microalgae collected from the polar region and transferred to warmer culture conditions (Priscu and Neale 1995; Ralph et al. 2005; Morgan-Kiss et al. 2006).

In addition, the cosmopolitan species, *C. meneghinii*, successfully acclimated at the prolonged low-temperature treatment, as concluded from rather small ultrastructural changes of pyrenoids and starch granules pointing to well-preserved function of Rubiso and other carboxylating enzymes under such inconvenient conditions. The normal Rubisco activity was found in several psychrophilic and cold-tolerant unicellular algae when grown at cold temperatures, while the temperature increase inhibited carboxylation in these strains (Devos et al. 1998). Conversely, the high-mountain tropical species of *C. punctulatum* and the subcosmopolitan taxon, *C. regnesii* var. *polonicum*, were more sensitive to the prolonged cold-temperature treatment given that starch granules, dictyosomes and ER cisternae were considerably damaged during the entire treatment. These severe injuries corresponded to the H_2O_2-induced hallmarks of PCD in *M. denticulata* cells (Darehshouri et al. 2008), showing the obvious poor-acclimation possibility of these strains at cold temperatures.

Thus, low-temperature investigation additionally supplemented the hypothesis on the possible geographic pattern of the *Cosmarium* strains (Stamenković and Hanelt 2011, 2012), taking into account that the more sensitive a plant is to chilling, the sooner and more extensive are the ultrastructural changes (Wise et al. 1983; Ma et al. 1990).

Remarkably, it appeared that all of the desmid strains (with the exception of *C. beatum*) could have withstood a distinctly prolonged (32-days) cold-temperature regime despite the numerous ultrastructural changes. Considering that the conjugatophycean group of algae is assumed to have an origin in tropics (Coesel 1996; Stamenković and Hanelt 2011, 2012), such a high resistance of the *Cosmarium* strains at the cold temperature is not expected. However, taking into account that majority representatives of this genus have cosmopolitan distribution (Coesel 1996), they certainly developed numerous protective features to endure drastic daily and seasonal temperature decreases at high altitudes and latitudes. A study of the metaphyton of a tundra pond in the Canadian Arctic indicated that three of the five most abundant species were representatives of the genus *Cosmarium*, showing a bimodal periodicity at temperatures lower than 8°C (Brook 1981a). This pointed to the high resistance to low or freezing temperatures in most of the *Cosmarium* taxa which is experimentally proven in this study. Moreover, the cold-temperature resistance may benefit to surviving at epilimnion temperatures below the ice cover (approximately 1°C), during winter in moderate climate zones (Coesel 1996).

Obviously, the *Cosmarium* strains developed multiple protective adaptations against chilling-induced stress (Wise 1995) – one of the first is the formation of cubic membrane structures in chloroplasts. It seems that the initial thylakoid dilation represents an early response to the elevated ROS amount (Wise 1995), as it was observed as a common symptom in several plants during chilling in the light (Taylor and

Craig 1971; Murphy and Wilson 1981; Wise et al. 1983). Presumably, this initial thylakoid swelling provokes the development of cubic membranes, as it was observed in chloroplasts of *Zygnema* filaments during the stationary phase (McLean and Pessoney 1970). A chilling-induced appearance of cubic membranes is well-known in numerous plants, as noted by many authors who referred them as 'serpentine-like' thylakoids (Wise et al. 1983; Gemel et al. 1986; Yun et al. 1996; Kratsch and Wise 2000). However, this study represents the first report of the heat- and chilling-induced cubic membranes in microalgae.

Similarly to what was observed for the prolonged heat stress, the prolonged low-temperature treatment initiated PCD in all of the *Cosmarium* strains, in accordance with the noted chilling-induced PCD in plant cells (Yun et al. 1996; Ishikawa 1996; Kratsch and Wise 2000). Among the typical PCD hallmarks, noticeable tonoplast damages were observed in all of the desmid strains as well as the autophagy in *C. regnesii*. The formation of autophagosomes was a common PCD hallmark observed in *M. denticulata* cells, when stressed with relatively high quantities of NaCl and KCl (Darehshouri et al. 2008). In addition, the foam-like vacuolation of cytoplasm in *C. punctulatum* No. 570 and *C. regnesii* was one of the main PCD hallmarks in *M. denticulata* cells treated with KCl (Affenzeller et al. 2009) and also in some plant species (Mittler et al. 1997). Interestingly, chilling at 3°C or 7°C did not provoke PCD signs in *M. denticulata* (Meindl 1990), in contrast to all of the *Cosmarium* strains studied.

Under extreme abiotic changes in desmid natural habitats PCD of a larger number of cells may guarantee the survival of the population, as surviving cells can use dead cells and mucilage produced by them to protect themselves from further environmental impact (Bidle and Falkowski 2004; Darehshouri et al. 2010). This fact, as well as a considerably high endurance of the *Cosmarium* strains at both warm and cold temperatures may certainly favour the cosmopolitan distribution of this genus, when compared to other desmid genera (e.g. *Micrasterias*).

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5. Protection strategies of *Cosmarium* strains (Zygnematophyceae, Streptophyta) isolated from various geographic regions against excessive photosynthetically active radiation

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Abstract

Photoinhibitory effects of photosynthetically active radiation were examined on four *Cosmarium* strains collected from various climatic zones. Algal cultures were long-term maintained at a relatively cold temperature and low light irradiance. Quantum yield measured by fluorescence (PAM) and oxygen evolution rates were used to estimate the physiological characteristics of the strains during and after various high-light treatments at two temperatures. All of the investigated *Cosmarium* strains demonstrated physiological responses that were consistent with the irradiance prevailing at their source location, confirming that these responses are genetically preserved. In addition, all of the desmid strains exhibited both photoprotection and photodamage at high light intensities which may occur in their natural, shallow freshwater habitats. Remarkably, by the addition of inhibitors of chloroplast-encoded protein synthesis (chloramphenicol and streptomycin) and violaxanthin de-epoxidase (dithiothreitol) it was demonstrated that the *Cosmarium* strains developed the 'sun- or shade-plant' protection strategies, in accordance with the climate of their sampling sites. *C. crenatum* and the polar strain of *C. punctulatum* exhibited rather the 'shade-plant strategy' – to suffer some photoinhibition, but acquire increasing protection from photoinhibited PSII centres, while *C. beatum* and the high-mountain strain of *C. punctulatum* displayed the 'sun-plant strategy' – to counteract photoinhibition of PSII by a high rate of repair of photoinhibited PSII reaction centres and a high xanthophyll cycle turnover. Increased mucilage production in cultures acclimated at cold temperature and treated under high irradiance possibly had a regulatory function in all of the *Cosmarium* strains, to exclude excess photosynthates.

Keywords: *Cosmarium*, distribution pattern, photoinhibition, maximum quantum yield, light adaptation

Abbreviations

PAR – photosynthetically active radiation, PSII – photosystem II, Fv/Fm – maximum quantum yield of PSII, CAP – chloramphenicol, SM – streptomycin, DTT – dithiothreitol, $rETR_{max}$ – maximum relative electron transport rate (photosynthetic capacity), I_k – saturating irradiance

Introduction

A previous study demonstrated that desmid strains were capable of occupying specific temperature (climatic) zones (Stamenković & Hanelt, 2012), in accordance with what has been observed for numerous macroalgae (Lüning, 1990; Hanelt, Wiencke & Bischof, 2003). Based upon those findings, a second experiment was designed to test responses to photosynthetically active radiation (PAR; 400 – 700 nm) to examine whether that factor could act as a factor limiting the geographical distribution of these desmid strains. Generally, the investigated *Cosmarium* strains have been typified as high-light adapted algae, according to their exceptionally high values of photosynthetic capacity and saturating irradiance at moderate temperatures (Stamenković & Hanelt, 2011, 2012). As inhabitants of various shallow freshwater habitats, it is assumed that desmids can easily cope with high PAR intensities. However, no previous study has examined the resistance and photosynthetic protective responses of desmids to high irradiance.

Investigations on photosynthetic behaviour of macroalgae treated under various photoinhibitory light conditions *in vivo* and *in vitro* suggested that they might behave as 'sun' or 'shade' adapted plants (Lüning, 1981; Lobban & Harrison, 1994), and most of them have consistent geographic distribution patterns (Van de Hoek, 1982). Although the photosynthetic behaviour of microalgae under photoinhibitory light has been extensively studied (summarised by Han *et al*., 1999), there have been no comparative studies to confirm if microalgal species are adapted to light conditions that are prevalent at the site of their origin. Taking into account the different PAR irradiance occurring at various latitudes (i.e. the tropical zone receives the highest solar radiation due to the perpendicular position of the sun) and altitudes (high-mountain areas receive higher solar radiation than lowland regions) (Gorton, Williams & Vogelmann, 2001; McKnight & Hess, 2001), it was hypothesised that the desmid strains collected from areas with markedly different light environments might display adaptation strategies to such environments also *in vitro* conditions.

It has been shown that the extent of 'shade- and sun-plant' strategies of vascular plants tends to correspond with the amount of available energy in the environment (Öquist *et al.*, 1992). The shade-plant strategy comprises the maintenance of photoinhibited PSII reaction centres for the controlled non-photochemical dissipation of absorbed excess light, while the sun-plant strategy involves an active degradation and repair of photoinhibited PSII reaction centres and a high non-photochemical quenching especially due to a high xanthophyll cycle capacity (Öquist *et al*., 1992; Bertamini, Muthuchelian & Nedunchezhia, 2004; Wilhelm & Selmar, 2010). Development under high light provides abundant energy for both the PSII repair cycle and growth, even though the energy cost of *de novo* D1 protein synthesis is high (Raven & Samuelsson, 1986). On the other hand, in shade-adapted plants inhibited PSII reaction centres accumulate, since the turnover of the repair cycle is low in comparison with the rate of photoinhibition. Thus, photoinhibition provides a state of stabile down-regulation of PSII, which from a functional view-point of photosynthesis, is indistinguishable from the down-regulation exerted by the build-up of a ΔpH gradient across the thylakoids in high light (Raven & Samuelsson, 1986; Öquist *et al*., 1992).

Under low irradiance it would be wasteful to run a costly repair process to counteract a necessary down-regulation of PSII photochemistry.

This study investigated whether *Cosmarium* strains isolated from various geographic regions demonstrated photosynthetic behaviour in accordance with the light prevailing at their source habitat (under photoinhibitory stress applied *in vitro*), or whether adaptation occurred due to the cultivation at relatively low irradiance and cold temperature conditions in the laboratory. Taking into account that only a few investigations have been conducted to determine if microalgae possess 'shade-plant' and 'sun-plant' strategies (Samuelsson *et al*., 1985, 1987; Lee & Ding, 1992), a special interest of the present study was to reveal light-adaptive strategies of the desmid strains, by the application of an inhibitor of the violaxanthin de-epoxidase and inhibitors of chloroplast-encoded protein synthesis. Considering that the *Cosmarium* strains are particularly sensitive to temperature decrease (Stamenković & Hanelt, 2012) the photoinhibitory treatments were done at both permissive and cold temperatures, to observe the protective strategies of strains when both of the stressors were present.

Materials and methods

Algal strains and culture conditions

The four *Cosmarium* clones examined in this study were isolated from various parts of the world within approximately the same time period, in order to exclude the influences of sampling time and, therefore, the influences of constant nutrient, light and temperature regime under laboratory conditions.

Climate zone	Taxon	No. strain (SVCK)	Sampling area	Locality coordinates and annual mean PAR (µmol photons m^{-2} s^{-1})	Year of isolation
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 ~ 200	1995
Lowland, polar zone	*C. punctulatum* Brébisson var. *subpunctulatum* (Nordstedt) Børgesen	571	pool near Skarsvåg, 80 m a.s.l, the North Cape, Norway	71°06′N 25°49′ E ~ 350	1992
Alpine, tropical zone	*C. punctulatum* var. *subpunctulatum*	570	pool on Mt. Cotopaxi, 1600 m a.s.l., Ecuador	00°40′S 78°26′W ~ 980	1996
Tropical	*C. beatum* W. & G.S. West	533	marshy area nearby Ol Bolossat Lake, Kenya	00°09′S 36°26′E ~ 1300	2001

Table 1. Data on the *Cosmarium* strains used for the investigation of photosynthetic behaviour under 350 – 2500 µmol photons m^{-2} s^{-1}, at 7 and 21°C. (SVCK – Sammlung von Conjugaten Kulturen, the culture collection of Conjugatophycean algae of the University of Hamburg).

The details on taxonomic, ecological and distributional attributes of the investigated taxa, as well as the climate characteristics of the algal sampling locations are published elsewhere (Stamenković & Hanelt, 2011). A compilation including annual average PAR of the sampling localities (based on data from Pinker & Laszlo, 1992a, 1992b; Frouin & Pinker, 1995) is shown in Table 1.

All of the investigated *Cosmarium* strains were grown under cultivating conditions (16°C; ~30 µmol photons m^{-2} s^{-1}) in a climate chamber of the SVCK (Sammlung von Conjugaten-Kulturen) collection over a period of several years. All of the investigated strains were grown in the mineral medium (L-d) based on Kattner *et al*. (1977). The tested *Cosmarium* strains had been acclimated to 21°C (compromising optimal temperature, see Stamenković & Hanelt, 2012) and L-d medium 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}). Sterilised 1 l Erlenmeyer flasks with 500 ml of medium were inoculated to a final concentration of 1500 cells ml^{-1}. Cultures were bubbled with humidified air at a rate of about 10 l h^{-1} to prevent CO_2 limitation. The cultures were mixed regularly by means of a magnetic stirrer to prevent self-shading of cells. Another set of inoculated Erlenmeyer flasks was grown 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 – 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 (PAR) was provided by a sun simulator (SonSi, iSiTEC GmbH, Germany), as described by Hanelt *et al*. (2006). 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 irradiance coming from a stabilised 400 W Metallogen lamp (Philips MSR 400 HR, Germany) consisting of a number of lanthanide which emanate a solar-like continuum. A filter GG400 (Schott Glas, Germany) ($\lambda > 400$ nm) was used to exclude UV radiation. Wire meshes acted as filters reducing the irradiance without changing the spectrum: 350 and 700 µmol photons m^{-2} s^{-1} (moderate radiation), 1200 and 2500 µmol photons m^{-2} s^{-1} (high radiation). The light intensity was adjusted using a cosine quantum sensor (LI-COR, LI–188B, USA). Desmids samples were exposed to the PAR irradiances in a 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 of the chlorophyll fluorescence and oxygen evolution were performed after 1, 4 and 6 h of inhibition and after 15 min, and 1, 2, 4, 24 h of recovery. All of the PAR 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 ml^{-1} by adding a quantity of thermally adjusted L-d medium. For the samples treated with inhibitors, L-d medium was enriched with a respective concentration of each inhibitor to preserve the desired concentration (see below). Immediately after sampling, the algal suspension was subjected to 3 min of dark adaptation in a water bath at the experimental temperature and filled into 5 ml Quartz cuvettes. A pulse of weak, far red light was applied to empty the electron pool from plastoquinone. The maximum quantum yield (Fv/Fm) was measured at time zero as described by Hanelt (1998). Initial fluorescence (Fo) was measured with red measuring light (~0.3 µmol photons m^{-2} s^{-1}, 650 nm), and maximal fluorescence (Fm) was determined using 600 ms of completely saturating white light pulse (~3500 µmol photon m^{-2} s^{-1}).

To eliminate the possible handling effect due to repeated measurements, chlorophyll fluorescence was also measured for control samples at time-series in synchrony with recovery of treated samples, and designated as a disturbed control. Another set of controls in parallel to each replicate was separately prepared and cultured at 21 or 7°C and 30 µmol photons m^{-2} s^{-1}, and designated as undisturbed controls. Photosynthetic efficiency of undisturbed controls was measured at the end of the recovery period (24 h) of the experiment. Time-series recovery in maximum quantum yield of the *Cosmarium* strains after exposure to different irradiances was expressed as a percent recovery of the disturbed control.

Inhibitor studies

Dithiothreitol (DTT; Sigma, Germany) is a frequently used inhibitor of violaxanthin de-epoxidase in investigations on activity of algal xanthophyll cycle (Uhrmacher, Hanelt & Nultsch, 1995; Häder *et al*., 2002). A set of *Cosmarium* samples with the same number of cells per ml as in control samples was treated with 0.65 mM DTT (the final concentration in a sample) 1 h before photoinhibitory experiments started, at 7 or 21°C.

To assay the influence of chloroplast-encoded protein synthesis on the sensitivity to photoinhibition, D-chloramphenicol (CAP) and streptomycin (SM) (Sigma, Germany) were added to samples 1 h before the photoinhibitory experiments started. Additionally, assays with a chloramphenicol isomer which does not inhibit protein synthesis, L-CAP (Sigma, Germany), were done in order to estimate if the inhibition of photosynthesis by a nitro group of the benzene ring occurs (Okada, Satoh & Katoh, 1991). The final concentrations of inhibitors in samples were 100 µg ml^{-1} for CAP and 20 µg ml^{-1} for SM; 0.5% ethanol was used to enhance the absorption of the antibiotics (Samuelsson *et al*., 1985; Lidholm, Gustafsson & Öquist, 1987; Han, Sinha & Häder, 2003). These concentrations of inhibitors had no effects on fluorescence parameters of the *Cosmarium* strains exposed to dim white light, at 21 and 7°C (control experiments).

Oxygen evolution

In synchrony with measurements of maximum quantum yield, the changes in oxygen production were measured using a fiber-optic oxygen meter – Presens Fibox 3 (Precision Sensing GmbH, Germany), attached to a PC with the software OxyView PS3. After different times of photoinhibition, 5 ml of the homogenised 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 certify saturating carboxylating conditions. The beginning amount of oxygen in cuvettes before each measurement was around 10%, and the cuvettes were tightly closed by rubber stoppers during measurements. Light source was a projector containing a halogen lamp (Xenophot, Osram, Germany), and samples were irradiated with a light intensity of 100 µmol photons m^{-2} s^{-1}, using a 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 continually stirred by means of a small magnetic bean during measurements. The temperature of the sample was maintained to the desired temperature (21 or 7°C) by means of a thermostated water jacket. Oxygen evolution of each non-photoinhibited control was standardised to 100% and the degrees of photoinhibition after different treatments were related to these controls.

Mucilage thickness

Thickness of the mucilaginous cell sheath was measured microscopically in fifty randomly chosen cells in cell suspension stained by Indian ink, measured at the cellular apex. Measurements were done for control (non-exposed) samples, after all of the PAR intensities and treatment times and at the end of recovery, at 7 and 21°C.

Statistical analysis

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 of disturbed and undisturbed controls. Photosynthetic responses to varying irradiance, exposure time and interaction effect were tested using the multiple analyses of variance (MANOVA). The one-factor between subjects (one-way) ANOVA was applied to find differences in the thickness of mucilaginous layers of the *Cosmarium* strains treated under PAR treatments compared to that of untreated samples. Correlations were performed to determine relationships between Fv/Fm and oxygen values (expressed as % of controls) at the end of photoinhibitory treatments (at 7 and 21°C), for all of the strains and PAR intensities. Correlations were also done for values of Fv/Fm and the mucilage thickness after 6 h of photoinhibitory treatments and at the end of recovery, at 7 and 21°C. The significance (p) is given as 1-tailed for the Pearson correlation coefficients. Degrees of freedom (for the effect of the model and residuals of the model) are presented in parentheses for the ANOVA results. All of the statistical analyses were conducted using the SPSS program (SPSS, Chicago, IL, USA).

Results

Chlorophyll fluorescence

Time-series measurements of the 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).

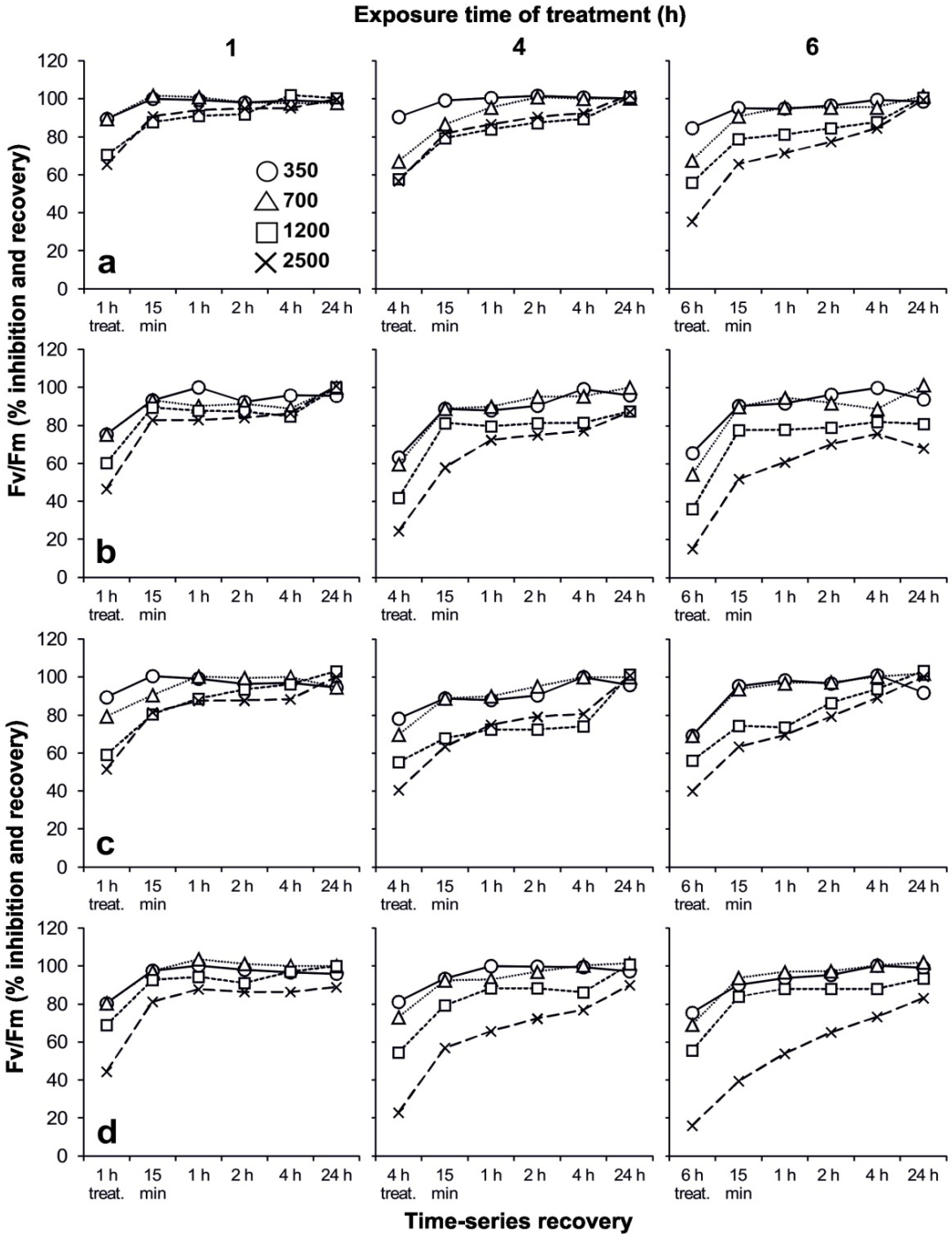

Fig. 1. Inhibition and time-series recovery in the mean of maximum quantum yield (Fv/Fm) of the *Cosmarium* strains collected from various geographic areas, after exposure to photosynthetically active radiation (350, 700, 1200, and 2500 µmol photons m^{-2} s^{-1}) during different treatment times (1, 4, and 6 h) at 21°C, expressed as a percent of disturbed

control. (**a**) *C. punctulatum* No. 570, (**b**) *C. punctulatum* No. 571, (**c**) *C. beatum*, (**d**) *C. crenatum*. Controls were untreated samples cultured at 21°C and 30 µmol photons m^{-2} s^{-1}. ○ – 350, △ – 700, □ – 1200, × – 2500 µmol photons m^{-2} s^{-1}. Standard deviations (SD) are less than 10% of mean (n = 9); not shown for the sake of clarity.

Exposure to increasing time of photoinhibiting PAR at 21°C reduced maximum quantum yield (Fv/Fm) of all of the strains investigated (Fig. 1). The high-mountain strain of *C. punctulatum* (No. 570) demonstrated the smallest depression of Fv/Fm after all of the PAR intensities and treatment times applied, among all of the *Cosmarium* strains studied. Application of 350 and 700 µmol photons m^{-2} s^{-1} for 1 h caused minor Fv/Fm decrease (around 90% of control) after which cells rapidly recovered to 100%, within 15 minutes. Stronger PAR intensities (1200 and 2500 µmol photons m^{-2} s^{-1}) caused more pronounced inhibition in this strain after 6 h treatments. However, a full recovery was achieved during 24 h, after both of the high PAR intensities applied. Likewise, the typical tropical species, *C. beatum*, successfully recovered Fv/Fm even after the prolonged high PAR stress (6 h at 2500 µmol photons m^{-2} s^{-1}), during the 24 h of recovery. Strong PAR irradiances (1200 and 2500 µmol photons m^{-2} s^{-1}) applied for 4 and 6 h caused the deep inhibition of Fv/Fm in the polar strain of *C. punctulatum* (No. 571) (i.e. 17% of the control after 6 h at 2500 µmol photons m^{-2} s^{-1}), causing an incomplete recovery after 24 h.

When compared to *C. punctulatum* No. 570 and *C. beatum*, the arctic species, *C. crenatum* var. *boldtianum*, demonstrated steeper inhibition of Fv/Fm after 1 h treatment at 350 and 700 µmol photons m^{-2} s^{-1} (80% of control) and fully recovered after 1 h. This strain did not completely recover Fv/Fm after 6 h treatment at 1200 and 2500 µmol photons m^{-2} s^{-1}; the latter caused a pronounced inhibition of maximum quantum yield (18% of control).

Table 2. Multiple analysis of variance (MANOVA) and significance values for the main effects and interactions of PAR intensity (350, 700, 1200, and 2500 µmol photons m^{-2} s^{-1}) and exposure time on photosynthetic efficiency of the *Cosmarium* strains studied, grown at 21°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. punctulatum (570)	PAR intensity (*A*)	3	856.2	<0.05*
	Exposure time (*B*)	2	52.3	<0.05*
	*A*B*	6	10.2	<0.05*
C. punctulatum (571)	PAR intensity (*A*)	3	201.5	<0.001*
	Exposure time (*B*)	2	80.1	<0.001*
	*A*B*	6	9.9	<0.001*
C. beatum	PAR intensity (*A*)	3	110.2	<0.05*
	Exposure time (*B*)	2	2.3	0.124^{ns}
	*A*B*	6	1.9	0.227^{ns}
C. crenatum	PAR intensity (*A*)	3	71.5	<0.001*
	Exposure time (*B*)	2	83.7	<0.05*
	*A*B*	6	31.4	<0.001*

Multiple analyses of variance (MANOVA) demonstrated significant effects of PAR intensities and exposure time at 21°C in *C. punctulatum* No. 570, *C. punctulatum* No. 571 and *C. crenatum*; in addition, interactions of these variables were significant (Table 2). However, the duration of treatments had no significant influence on the photosynthetic behaviour of *C. beatum*.

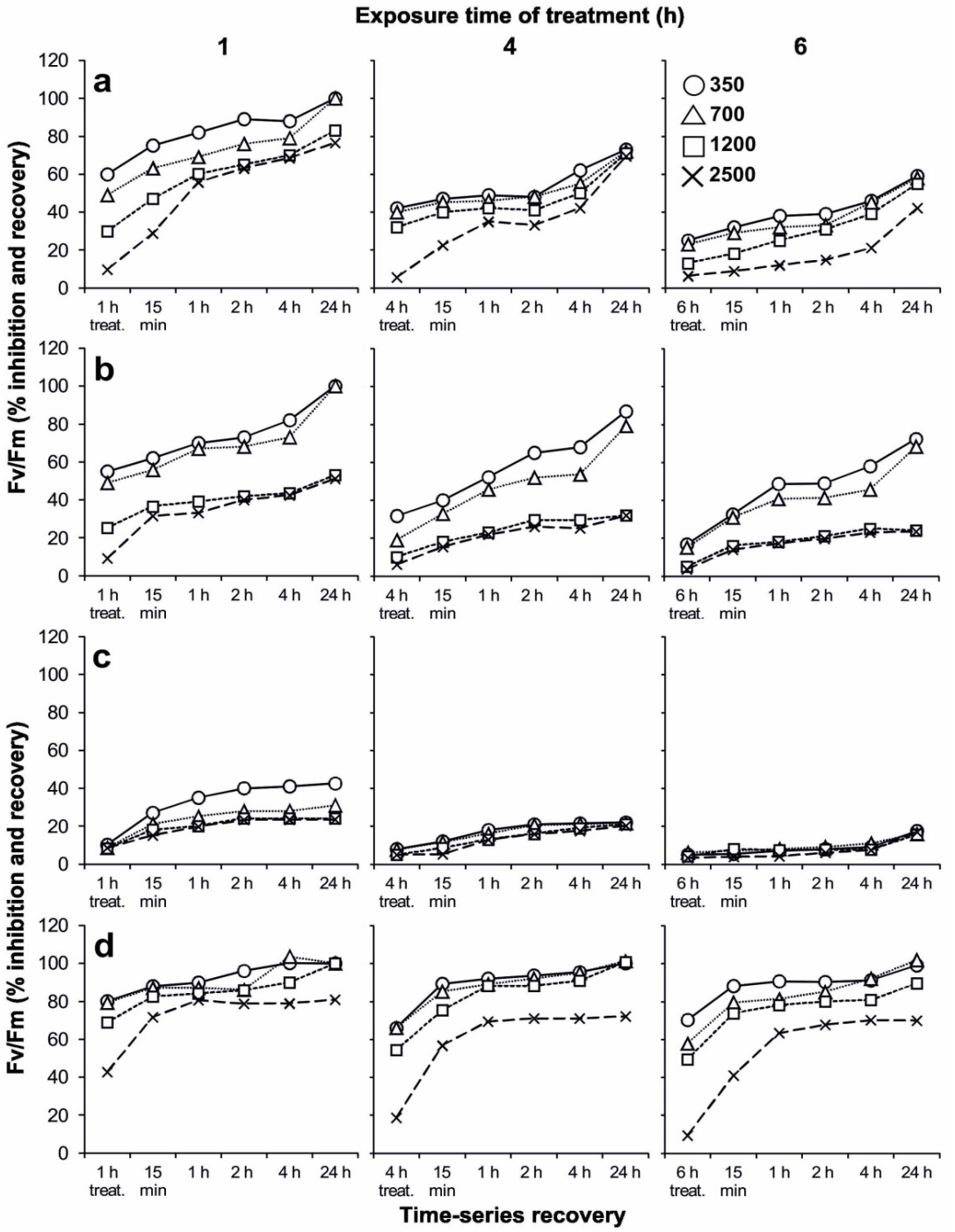

Fig. 2. Inhibition and time-series recovery in the mean of maximum quantum yield (Fv/Fm) of the *Cosmarium* strains collected from various geographic areas, after exposure to photosynthetically active radiation (350, 700, 1200, and 2500 µmol photons m^{-2} s^{-1}) during different treatment times (1, 4, and 6 h) at 7°C, and expressed as a percent of disturbed control. (**a**) *C. punctulatum* No. 570, (**b**) *C. punctulatum* No. 571, (**c**) *C. beatum*, (**d**) *C. crenatum*. Controls were untreated samples pre-acclimated at 7°C and 30 µmol photons m^{-2} s^{-1}. ○ – 350, △ – 700, □ – 1200, × – 2500 µmol photons m^{-2} s^{-1}. Standard deviations (SD) are less than 10% of mean (n = 9); not shown for the sake of clarity.

The application of photoinhibitory PAR caused deeper inhibition of maximum quantum yield in all of the *Cosmarium* strains pre-acclimated at 7°C (Fig. 2). The only exception to this observation was found for the typical arctic taxon, *C. crenatum*, which did not demonstrate larger inhibition of Fv/Fm under 350,

700 and 1200 µmol photons m^{-2} s^{-1} at 7°C, compared to that at 21°C. Maximum quantum yield of the typical tropical strain, *C. beatum*, rapidly decreased after 1 h of the moderate PAR stress, while the prolonged strong PAR application dropped Fv/Fm down to 0. No recovery was observed in this species after all of the PAR intensities applied at 7°C. The polar strain of *C. punctulatum* displayed somewhat higher resistance at moderate PAR intensities (350 and 700 µmol photons m^{-2} s^{-1}) applied at 7°C compared to the high-mountain one, while 4 and 6 h treatments delayed the recovery. Yet, higher PAR intensities caused a strong Fv/Fm inhibition in this strain after which recovery was limited up to around 50% or 22%, for 1 and 6 h treatment, respectively.

Table 3. Multiple analysis of variance (MANOVA) and significance values for the main effects and interactions of PAR intensity (350, 700, 1200, and 2500 µmol photons m^{-2} s^{-1}) and exposure time on photosynthetic efficiency of the *Cosmarium* strains studied, pre-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. punctulatum (570)	PAR intensity (*A*)	3	141.3	<0.001*
	Exposure time (*B*)	2	36.7	<0.001*
	*A*B*	6	41.8	<0.001*
C. punctulatum (571)	PAR intensity (*A*)	3	198.0	<0.05*
	Exposure time (*B*)	2	57.3	<0.001*
	*A*B*	6	35.4	<0.001*
C. beatum	PAR intensity (*A*)	3	15.6	0.199^{ns}
	Exposure time (*B*)	2	2.3	0.287^{ns}
	*A*B*	6	18.3	0.113^{ns}
C. crenatum	PAR intensity (*A*)	3	132.8	<0.05*
	Exposure time (*B*)	2	83.9	<0.05*
	*A*B*	6	61.5	<0.05*

MANOVA demonstrated significant effects of PAR intensity and exposure time in both of the strains of *C. punctulatum* and *C. crenatum*, which were pre-acclimated and treated at 7°C (Table 3). However, PAR intensities and treatment times had not significant influence on the photosynthetic behaviour of *C. beatum* pre-acclimated at 7°C.

Oxygen evolution

The average O_2 production of the *Cosmarium* strains studied (expressed in µMolO_2 mgChl^{-1} min^{-1}) grown at 7 and 21°C is shown in Table 4. Acclimation at 7°C decreased oxygen production in all of the *Cosmarium* strains; yet, *C. crenatum* exhibited the highest oxygen production at this temperature, thus demonstrating the highest photosynthetic activity among all of the *Cosmarium* strains studied.

In parallel with the measurements of Fv/Fm during the time-series of inhibition and recovery, total oxygen evolution was measured. In order to observe effects of PAR on total cell metabolism of the *Cosmarium* strains, the measurement of oxygen evolution included photosynthesis as well as respiration rates. Gross oxygen and Fv/Fm values for inhibitions were expressed as percents of controls, and these percentages were plotted for all of the PAR intensities (Fig. 3) – in order to gain a view on the PAR influences on the entire cell metabolism compared to PSII inhibition (estimated by chlorophyll fluorescence).

Table 4. Average O_2 production of the *Cosmarium* strains studied, grown at 21°C or acclimated at 7°C (30 µmol photons m^{-2} s^{-1}). Standard deviations (SD) are less than 5% of mean (n = 3).

	Average O_2 production (µMolO_2 mgChl^{-1} min^{-1})			
Temperature	*C. punct.* (570)	*C. punct.* (571)	*C. beatum*	*C. crenatum*
21°C	2.55	2.49	2.43	3.52
7°C	1.46	1.59	0.31	2.99

Taking into consideration inhibitions after all of the PAR irradiances and treatment times at 21°C, a significant positive correlation was found ($r = 0.915$, $p < 0.001$). However, when correlations were done for total oxygen and Fv/Fm percentage values for each strain separately, significant linear correlations were found for *C. punctulatum* No. 570 and *C. beatum* ($r = 0.86$, $p < 0.001$; $r = 0.894$, $p < 0.001$) (Fig. 3a), while this relationship corresponded rather to the quadratic approximation for both of the polar strains (*C. punctulatum* No. 571 and *C. crenatum*) (Fig. 3b).

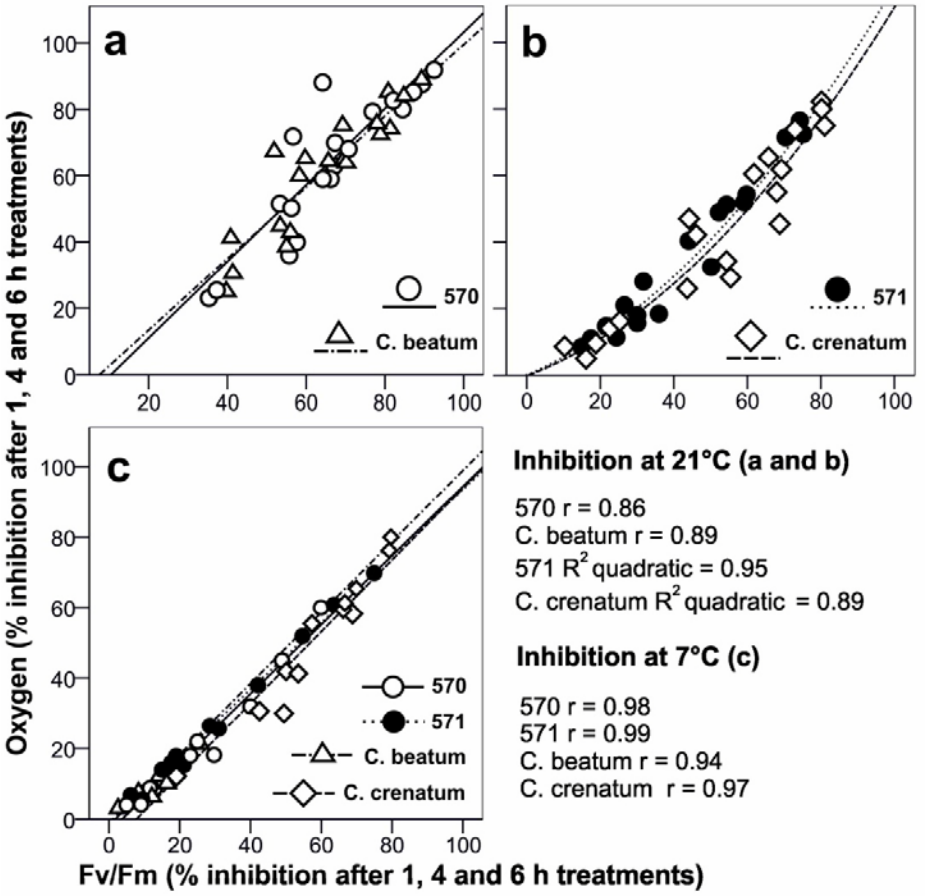

Fig. 3. a-b Relationships between gross oxygen evolution rates and Fv/Fm values (expressed as percentages of control) regarding inhibitions after all of the treatment times and PAR intensities applied at 21°C for (**a**) *C. punctulatum* No. 570 and *C. beatum* (**b**) *C. punctulatum* No. 571 and *C. crenatum*. **c** Relationships between gross oxygen evolution rates and Fv/Fm values (expressed as percentages of control) regarding all of the treatment times and PAR intensities applied at 7°C, for all of the *Cosmarium* strains studied. ○ – *C. punctulatum* No. 570, ● – *C. punctulatum* No. 571, △ – *C. beatum*, ◇ – *C. crenatum*.

When oxygen percentages are plotted with those for Fv/Fm regarding inhibitions at 7°C, significant positive correlations were found considering all of the treatment times and PAR intensities, for all of the *Cosmarium* strains studied (Fig. 3c).

Effects of DTT

The addition of DTT prior to photoinhibitory treatments caused a marked inhibition of Fv/Fm depression during PAR treatments, i.e. Fv/Fm values of the DTT treated samples were noticeably higher than those of untreated samples.

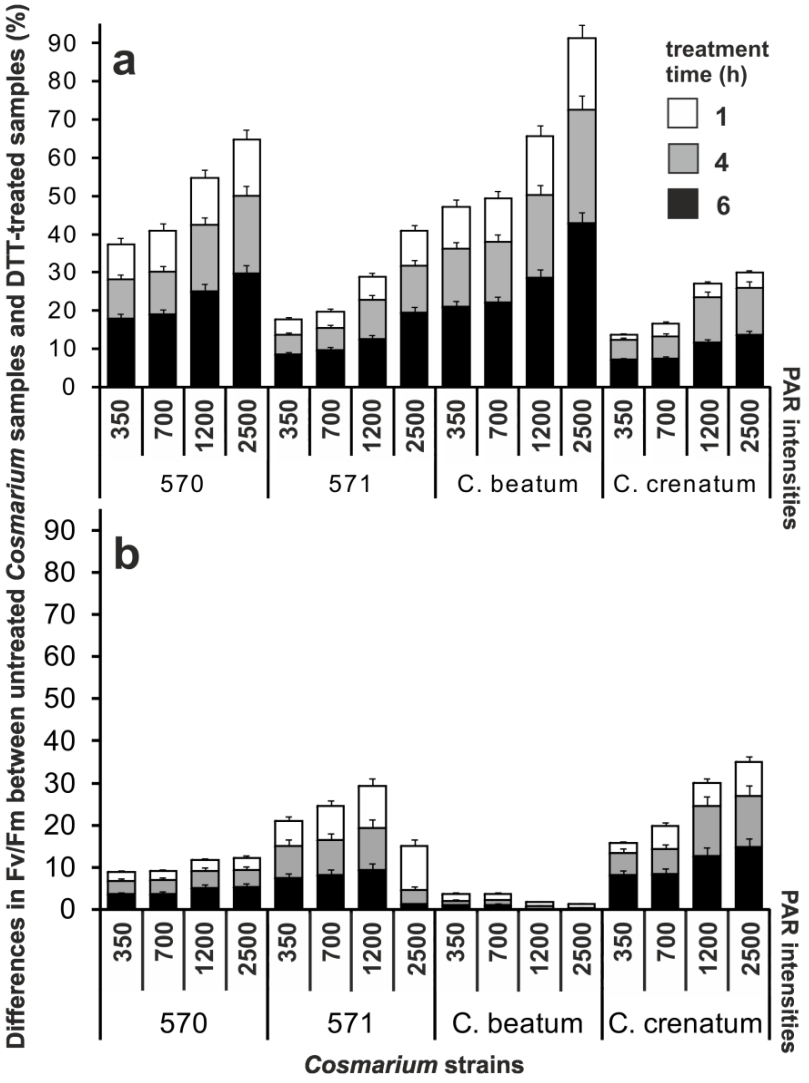

Fig. 4. Differences in Fv/Fm between DTT-treated samples and untreated samples (expressed as percentages of controls) at the end of photoinhibitory PAR treatments (350 – 2500 µmol photons m^{-2} s^{-1} for 1, 4 and 6 h), for all of the *Cosmarium* strains studied. (**a**) 21°C, (**b**) 7°C (vertical bars – SD, n = 3).

Values for Fv/Fm of DTT-treated and untreated samples (at the end of PAR treatments) were expressed as percentages of controls. Percentages of untreated samples were subtracted from percentages of DTT-treated samples for all of the strains, PAR intensities, and treatment times (Fig. 4). The normally strong decrease of Fv/Fm, which is higher at longer exposition time and stronger irradiance, was prevented by DTT and, thus, caused a higher difference during longer and stronger PAR treatments, especially at 21°C. This indicated a large de-epoxidase effect under prolonged high PAR intensities (e.g. 6 h under 2500 µmol photons m^{-2} s^{-1}), which was successfully blocked by DTT and, thus, caused a smaller decrease of the quantum yield in the DTT-treated samples at 21°C.

The tropical species, *C. beatum*, exhibited by far the highest Fv/Fm difference for all of the treatment times and PAR intensities, indicating a high activity of de-epoxidase which was blocked by the inhibitor. On the contrary, the arctic species, *C. crenatum*, showed the lowest de-epoxidase activity after all of the treatment times and PAR intensities, as judged from the lowest de-epoxidase inhibition by DTT (Fig. 4a). Interestingly, the high-mountain strain of *C. punctulatum* (No. 570) exhibited larger de-epoxidase inhibition by DTT after all of the treatment times and PAR intensities at 21°C, compared to that of the polar strain (No. 571); hence indicating a marked de-epoxidase action and an intensive PSII protection by the xanthophyll cycle.

Acclimation at 7°C caused a limited activity of de-epoxidase in all of the *Cosmarium* strains, as judged from the lower differences in Fv/Fm between DTT-treated and untreated samples (Fig. 4b). The only exception was the arctic species, *C. crenatum*, which exhibited almost the equal xanthophyll cycle activity as those observed at 21°C, concerning all of the treatment times and PAR intensities. The tropical species, *C. beatum*, displayed no de-epoxidase activity at 7°C.

Effects of translation inhibitors

Fv/Fm values were measured for CAP- and SM-treated samples in parallel with untreated samples during photoinhibitory PAR treatments and in recovery, at 7 and 21°C. The gained values were expressed as percentages of controls; afterwards, Fv/Fm percentages of treated samples were subtracted from untreated samples. These differences (for 1 and 6 h treatment-times at 21°C) are shown in Fig. 5. L-CAP (+ 0.5% ethanol) did not cause as large a decrease in the Fv/Fm ratio as D-CAP (data not shown), indicating that CAP was not having any toxic effect in the light. This was important to establish in view of the suggestion that the nitro group attached to the benzene ring of the molecule may inhibit photosynthesis by electron acceptance in PSI (Okada et al. 1991).

All of the antibiotic-treated *Cosmarium* strains demonstrated a rapid decrease of Fv/Fm during the photoinhibitory PAR treatments at 21°C, which was considerably lower compared to that of untreated samples. The largest deviation of CAP-treated samples from untreated samples was observed after 15 min of recovery in all of the *Cosmarium* strains, indicating a rapid *de novo* D1 protein synthesis during the photoinhibitory treatment and immediately after PAR stress. The protein synthesis afterwards decreases during recovery, reaching the lowest value after 24 h. In contrast, the inhibition caused by SM significantly increased during recovery, being the highest after 24 h.

All of the inhibitory PAR treatments which lasted for 1 h caused a fair regeneration of maximum quantum yield, concerning both of the antibiotic treatments, as judged from the relatively low difference between Fv/Fm of treated and untreated samples during recovery (Fig. 5). The prolonged treatments (4 and 6 h) caused a much lower regeneration of the antibiotic-treated samples, which undoubtedly indicated an obstruction of *de novo* protein synthesis, caused by both of the antibiotics applied. Remarkably, the tropical species, *C. beatum*, exhibited by far the highest depression of Fv/Fm in CAP- and SM-treated samples compared to that of untreated samples, thus indicating an exceedingly high involvement of D1 protein turnover. In contrast, the typical arctic strain, *C. crenatum*, displayed minor Fv/Fm differences in the antibiotic-treated samples, for all of the treatment times and PAR intensities at 21°C. Interestingly, this species appeared rather insensitive to the quantity of SM which drastically prevented the *de novo* protein synthesis in *C. beatum*. These facts pointed to a rather small amount of the protein synthesis in *C. crenatum*, during recovery after photoinhibitory PAR influence. Both strains of the cosmopolitan species, *C. punctulatum*, were in the middle between these two extremes, regarding the action of *de novo* protein synthesis after the photoinhibitory treatments.

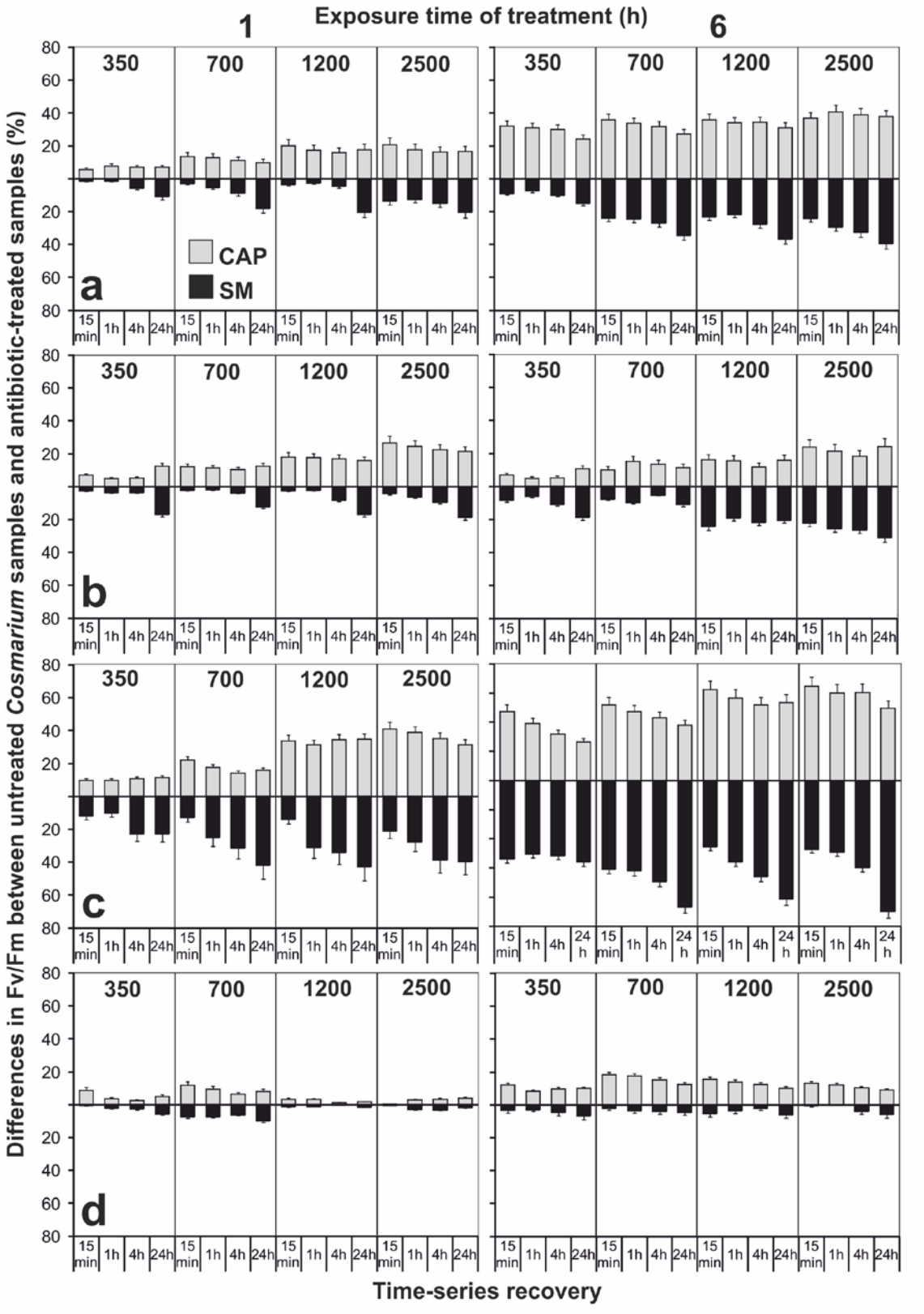

Fig. 5. Differences in Fv/Fm between untreated samples and CAP-treated (grey) or SM-treated samples (black) (expressed as percentages of controls) during the period of recovery after all of the PAR inhibitory treatments (350, 700, 1200 and 2500 µmol photons m^{-2} s^{-1}; for 1 and 6 h) at 21°C: (**a**) *C. punctulatum* No. 570, (**b**) *C. punctulatum* No. 570, (**c**) *C. beatum*, (**d**) *C. crenatum* (vertical bars – SD, n = 3).

Application of CAP and SM to samples acclimated at 7°C and treated under photoinhibitory PAR intensities caused no significant decrease of Fv/Fm compared to that of untreated samples, in *C. beatum* and both of the strains of *C. punctulatum*. This fact indicated that limited (or none) protein synthesis occurs at the low temperature in these strains. A slight depression of Fv/Fm was observed for the CAP-treated samples of *C. crenatum*; however, it was almost insignificant compared to those at 21°C (data not shown).

Mucilage production

Acclimation of cultures at 7°C caused a marked increase of the mucilage thickness in all of the *Cosmarium* strains. The application of photoinhibitory PAR intensities (350 – 2500 µmol photons m^{-2} s^{-1}) increased the mucilage thickness in all of the *Cosmarium* strains; still, the increase of the mucilage thickness was larger in cultures pre-acclimated at 7°C (an example is shown in Fig. 6a). Thickness of mucilaginous sheaths decreased during the recovery period, having somewhat higher values than in control cells for all of the *Cosmarium* strains studied, at 7 and 21°C (data not shown). Additionally, significant negative correlations were found in plots between Fv/Fm values and the thickness of mucilaginous layers after 6 h of photoinhibitory PAR treatments (700, 1200 and 2500 µmol photons m^{-2} s^{-1}) at 21°C for all of the desmid strains studied (Fig. 6b): *C. punctulatum* No. 570 ($r = 0.89$, $p < 0.05$), *C. punctulatum* No. 571 ($r = 0.93$, $p < 0.001$), *C. beatum* ($r = 0.9$, $p < 0.05$) and *C. crenatum* ($r = 0.94$, $p < 0.001$).

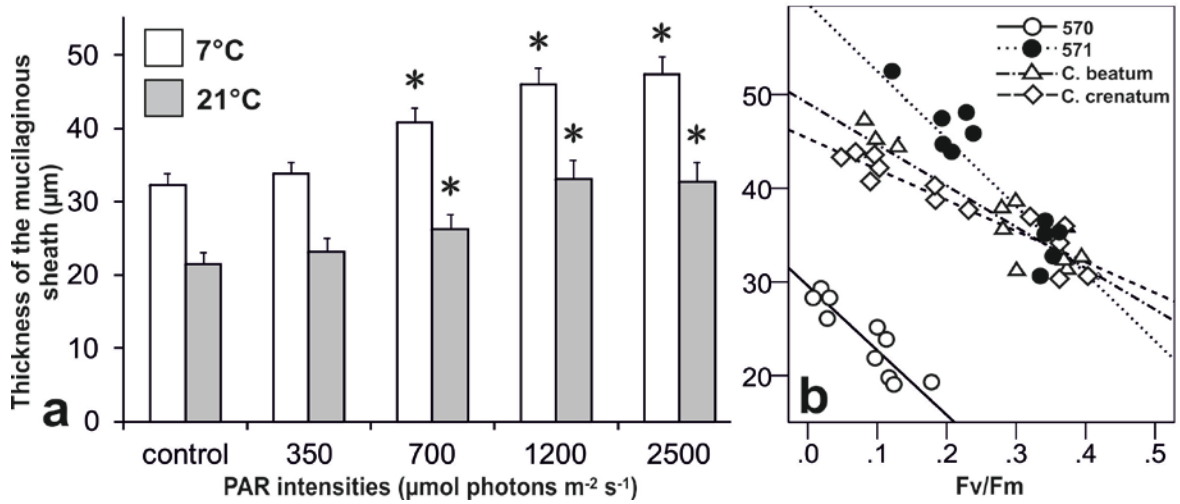

Fig. 6. (**a**) Changes of the thickness of the mucilaginous sheath in *C. punctulatum* No. 571 after 6 h of photoinhibitory PAR (350, 700, 1200; and 2500 µmol photons m^{-2} s^{-1}) at 7 and 21°C (vertical bars – SD, n = 3). (**b**) Relationship between values of Fv/Fm and the mucilage thickness measured after 6 h inhibition under photoinhibitory PAR (700, 1200 and 2500 µmol photons m^{-2} s^{-1}) at 21°C. ○ – *C. punctulatum* No. 570, ● – *C. punctulatum* No. 571, △ – *C. beatum*, ◇ – *C. crenatum*. Asterisks represent significant differences in the mucilage thickness of treated samples compared to control samples (one-way ANOVA, Tukey HSD test, $p < 0.05$).

Discussion

Remarkably, this study revealed that strain- and species-specific differences in the photosynthetic behaviour among *Cosmarium* strains to a set of various PAR intensities agree well with the adaptation required for these clones to effectively respond to the prevailing light environment at their source localities. Thus, *Cosmarium* isolates may inhabit certain environmental niches based on adaptation to both certain temperature regimes (Stamenković & Hanelt, 2012), as well as to specific PAR intensities (this study). These responses appeared as genetically preserved characteristics of the strains, despite the long-term cultivation (more than 15 years) under constant, relatively low temperature-light conditions (16°C, 30 µmol photons m^{-2} s^{-1}).

In general, all of the *Cosmarium* strains studied exhibited a high resistance under the relatively high PAR intensities (350 – 1200 µmol photons m^{-2} s^{-1}) applied during the prolonged treatments. This observation is in accordance with the fact that the *Cosmarium* strains are regarded as high-light adapted algae, as judged from the exceptionally high values for saturating irradiance and photosynthetic capacity at 21°C (I_k: 800–1400 µmol photons m^{-2} s^{-1}, $rETR_{max}$: 150–330), as found during the earlier study (Stamenković & Hanelt, 2012). The desmid strains demonstrated rather rapid recovery after moderate PAR treatments, similarly with what was observed for *Chlamydomonas reinhardtii* Dangeard (Ohad, Kyle & Arntzen, 1984; Lidholm *et al*., 1987) and *Anacystis nidulans* (Richter) Drouet & Daily (Samuelsson *et al*., 1985), and considerably faster than most vascular plants (Ögren, Öquist & Hällgren, 1984; Greer, Berry & Björkman, 1986; Kyle, Osmond & Arntzen, 1987). Moreover, the *Cosmarium* strains withstood the distinctly prolonged photoinhibitory PAR treatments (6 h), compared to those used in previous investigations with the other microalgae.

All of the *Cosmarium* strains revealed both fast and slow phases of recovery at 21°C after high light stress, thus demonstrating that both photoprotection and photodamage have roles as strategies to cope with enhanced PAR (as in macroalgae (Lüning, 1990)). Considering that the *Cosmarium* strains are considered to be high-light adapted algae, they are expected to have a well-developed photoprotection process (also known as dynamic photoinhibition), which involves dissipation of excessive radiation (Osmond, 1994; Demmig-Adams & Adams, 2006), as it has been noted in numerous high-light adapted algae and plants (Demmig-Adams & Adams, 1992; Hanelt *et al*., 2003). This process may be modulated by an increase in the zeaxanthin content of the PSII antenna (Demmig-Adams & Adams, 2006) and/or by increasing the amount of inactive centers thereby protecting the photosynthetically active centers (Öquist & Chow, 1992). In addition, impairment of PSII protein D1, the major target of oxidative damage in PSII, induces photodamage (chronic photoinhibition) after which a *de novo* synthesis of D1 occurs (Kato & Sakamoto, 2009). The pronounced photodamage is also known as a protective strategy of sun-adapted plants (Öquist *et al*., 1992; Bertamini *et al*., 2004).

Treatments under high irradiances at 7°C provoked the decrease in maximum quantum yield and oxygen evolution in all of the investigated *Cosmarium* strains, correspondingly with the preference of

desmids to relatively warm temperatures (21–28°C) (Coesel & Wardenaar, 1990; Stamenković & Hanelt, 2012). Yet, the highest resistance at this temperature was observed in the typical arctic species, *C. crenatum*, as judged from the highest oxygen production and the highest Fv/Fm recovery rates after strong PAR applications, among all of the strains studied. Increased susceptibility of chilling-sensitive plants and macroalgae to high-light stress at low temperatures is a consequence of the slow energy-consuming carbon metabolism and low rates of repair processes in the chloroplast (Greer *et al*., 1986; Öquist *et al*., 1987).

In accordance with observations that fluorescence (as an energy dissipating mechanism) competes with thermal energy dissipation (Ruban, Andrew & Horton, 1993; Uhrmacher *et al*., 1995), the addition of DTT to algal cultures did not prevent the decrease of Fv/Fm but shifted it to higher fluences, as observed in all the *Cosmarium* strains studied. Chilling conditions inhibit the enzymatic conversion of violaxanthin to zeaxanthin (Demmig-Adams *et al*., 1989; Demmig-Adams & Adams, 1992), which caused rather small difference in Fv/Fm of DTT-treated and untreated *Cosmarium* samples under photoinhibitory PAR applied at 7°C, with the exception of the arctic species, *C. crenatum*. The assay with translation inhibitors clearly show that protein synthesis is required for reactivation of photosynthesis following photoinhibitory damage in all of the *Cosmarium* strains. CAP and SM added to algal samples prior to photoinhibitory treatments caused more than double the rate of photoinhibition during the initial phase and strongly delayed recovery, which was consistent with previous reports regarding green algae and cyanoprokariota (Ohad *et al*., 1984; Samuelsson *et al*., 1985, 1987; Lidholm *et al*., 1987; Kim & Melis, 1992). This observation is in accordance with the view that a repair mechanism is operating during the photoinhibitory treatment and that a quasi steady level of inhibition obtained at a certain PAR reflects the net photoinhibitory effect as a function of the balance between the processes of inhibition and repair (Samuelsson *et al*., 1985; Schnettger *et al*., 1994).

Although both of the antibiotics used are well-known to successfully prevent *de novo* protein synthesis after photoinhibition, as observed in numerous investigations on plant and algal photosynthesis (Lidholm *et al*., 1987; Öquist *et al*., 1987; Franklin *et al*., 1992), their physiological action differs. Chloramphenicol binds to the 23S rRNA of the 50S ribosomal subunit and prevents peptide bond formation (Jardetzky 1963), while streptomycin binds to the small 16S rRNA of the 30S subunit of the ribosome, interfering with the binding of formyl-methionyl-tRNA to the 30S subunit, which leads to inhibition of protein synthesis (Sharma et al. 2007). It appeared that CAP had more pronounced influence on the *de novo* D1 protein synthesis during the photoinhibitory treatment and in recovery (after 15 min), while SM had a more expressive effect during the course of recovery (the strongest effect after 24 h). Correspondingly, SM applied to several macroalgae (*Ulva*, *Dictyota* and *Porphyra*) caused a higher recovery of Fv/Fm compared to that of CAP-treated samples, indicating that inhibition of *de novo* protein synthesis was weaker when induced by SM (Häder *et al*., 2002). These remarks may suggest that, in accordance with their different physiological actions, SM and CAP have different effect on the *de novo* D1 protein synthesis. Supposedly, CAP acts faster than SM and more specifically inhibits D1 protein synthesis during the photoinhibition and

at the beginning of recovery, whereas, due to the slower action, SM possibly inhibits the synthesis of proteins which are damaged by reactive oxygen species (ROS), which might appear during photoinhibition.

The typical tropical species (*C. beatum*) exhibited all of the strategies available to help cope with excessive PAR, as noted for sun-adapted plants and algae (Öquist *et al*., 1992; Häder *et al*., 2002): (i) high de-epoxidase activity at 21°C, pointing to the rapid conversion of violaxanthin to zeaxanthin; (ii) by far the highest rate of D1 turnover and *de novo* protein synthesis; (iii) impossibility to acclimate at 7°C, as concluded from the rapid and irreversible decrease of Fv/Fm under photoinhibitory PAR applied to cultures pre-acclimated at 7°C, hence showing rather damaging effects of PAR to PSII. On the contrary, the arctic species, *C. crenatum*, displayed characteristics typical to that of shade-plant strategists (Öquist *et al*., 1992; Bertamini *et al*., 2004) and polar macroalgae (Lüning, 1990; Lobban & Harrison, 1994): (i) lower de-epoxidase activity at 21°C, when compared to the other *Cosmarium* strains (ii) a low reliance on chloroplast-encoded protein synthesis to resist photoinhibition (iii) a relatively slow rate of protein-synthesis-dependent recovery at 21°C. These facts are consistent with the hypothesis that shade-adapted plants in their photoinhibited state maintain a high level of photoinhibited, though presumably still physically intact, PSII centres (Öquist *et al*., 1992; Roleda, Hanelt & Wiencke, 2006). Such photoinhibited PSII reaction centres would be of physiological significance for the controlled dissipation of light energy absorbed in excess to the capacity of photosynthesis, enabling the polar microalgae, such as *C. crenatum*, to survive in conditions of low temperature and occasionally high irradiance (Cleland, Melis & Neale, 1986; Allakhverdiev *et al*., 1987; Setlik *et al*., 1990; Styring *et al*., 1990).

The distinctly low protein synthesis rates in *C. crenatum* after the photoinhibitory treatments agrees with observations made on shade-adapted plants or plants and algae acclimated to low-light conditions, plants that demonstrate low or no D1 protein degradation after photoinhibition (Samuelsson *et al*., 1987; Cleland, 1988; Lönneborg *et al*., 1988; Bertamini *et al*., 2004). Actually, habitats where the shade-plant strategy would be appropriate are those with relatively cold temperatures and occasionally high solar radiation (i.e. arctic-alpine environments) (Post, Adamson & Adamson, 1990; Öquist & Huner, 1991; Ottander & Öquist, 1991; Ball, Hodges & Laughlin, 1991). Low temperatures slow down the PSII repair cycle, as judged from a retarded D1 protein degradation upon photoinhibition (Chow, Osmond & Huang, 1989; Gong & Nilsen, 1989; Aro *et al*., 1990) and also from the relative insensitivity of photoinhibition to chloroplast-encoded *de novo* protein synthesis (Greer, Ottander & Öquist, 1991). Despite the fact that *C. crenatum* has a high saturating irradiance at relatively low temperatures and is characterised as a high-light adapted alga (Stamenković & Hanelt, 2011, 2012), it developed the 'shade-plant' strategy to cope with the influences of cold temperatures of the polar climate. Furthermore, it has been recognised that the turnover of the D1 protein does not play a significant protective role in either photoinhibition or recovery of photosynthesis in macroalgae adapted to low temperatures (Hanelt *et al*., 1994; Hanelt, 1998; Hanelt *et al*., 2003). Therefore, the arctic microalga, *C. crenatum*, appears to possess adaptive characteristics typical to that of polar macroalgae, which enable its survival at low temperatures.

The photosynthetic behaviour of the cosmopolitan species, *C. punctulatum*, during and after the photoinhibitory stress was between these two extreme types of sun- and shade-plant strategies. Yet, the high-mountain strain appeared better suited to high light intensities at 21°C compared to the polar one, as judged from the milder depression of Fv/Fm and faster recovery, for all of the PAR intensities and treatment times. In addition, de-epoxidase activity and *de novo* D1 protein synthesis were significantly higher at 21°C in the high-mountain strain of *C. punctulatum* (compared to that of the polar strain), thereby displaying 'sun-plant adaptation' strategies of this strain.

The measured values of gross oxygen for unstressed controls of the *Cosmarium* strains at 21°C corresponded to those measured for *Micrasterias denticulata* Brébisson ex Ralfs cells at the same temperature (Lütz, Seidlitz & Meindl, 1997). Changes in total oxygen production rates and Fv/Fm showed a significant correlation during PAR treatments at 7 and 21°C, similarly as it was observed during photoinhibitory investigations on macroalgae (Hanelt, Uhrmacher & Nultsch, 1995; Uhrmacher *et al.*, 1995). However, both of the polar strains (*C. punctulatum* No. 571 and *C. crenatum*) displayed a high sensitivity under the prolonged (6 h) strong PAR treatments (1200 and 2500 µmol photons m^{-2} s^{-1}) which caused a deeper inhibition of gross oxygen production than Fv/Fm at 21°C (Fig. 3b). Possibly, the increased dark respiration and/or photorespiration rates at higher PAR intensities caused the decrease in oxygen production in the polar strains. Similar observations have been observed in several seagrasses and macroalgae (Beer & Björk, 2000; Beer & Axelson, 2004).

At cold temperatures, algal growth is thought to be principally limited by the light-independent reaction, resulting in an excess of photosynthates at high irradiance (Reynolds, 1989). In addition to the numerous functions (Spijkerman & Coesel 1998; Stamenković & Hanelt, 2011), the production of mucilage by desmids likely serves a regulatory function – to exclude a mass of excess photosynthates. This may be especially important at low temperatures which may impede the growth of desmids even after a small decrease (Stamenković & Hanelt, 2012). Mucilaginous sheath of desmids is transparent, but increased amount of mucilage granules inside cells may form brownish patches which screen organelles (Brook, 1981) and thereby may serve in a protection from excessive PAR intensities.

In conclusion, as observed for vascular plants (Öquist *et al*., 1992; Bertamini *et al*., 2004), the *Cosmarium* strains examined in this study tended to develop two defensive strategies against photoinhibitory light in accordance with the light intensity and temperatures prevailing at their native habitats: the shade-plant strategy, as in *C. crenatum* and *C. punctulatum* No. 571 (with maintenance of photoinhibited PSII reaction centres for the controlled non-photochemical dissipation of absorbed excess light), and the sun-plant strategy, as in *C. beatum* and *C. punctulatum* No. 570 (besides the high xanthophyll cycle activity, an intensive degradation and repair of photoinhibited PSII reaction centres occurs). Yet, all of the desmid strains studied demanded relatively high temperature to achieve a thorough recovery after the PAR stress, which was in accordance with the noted preference of desmids to warm-water habitats (Coesel & Wardenaar, 1990; Stamenković & Hanelt, 2012).

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6. Composition of xanthophylls in several *Cosmarium* strains (Zygnematophyceae, Streptophyta) is related to their geographic distribution pattern

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Abstract

Changes in the composition of xanthophylls of four *Cosmarium* strains collected from different geographic areas was examined under moderate and photoinhibitory white light by means of high-performance liquid chromatography. Astonishingly, all of the *Cosmarium* strains demonstrated differences in the pigment composition which arose due to the influence of the climate prevailing at their sampling sites, both under optimal conditions and after photoinhibitory light treatments. These differences appeared despite a long-term cultivation at relatively low light-temperature regime, and are regarded as adaptations (genotypically preserved features) to light characteristics of the sampling sites. Strikingly, this study unveiled two different mechanisms of the xanthophyll cycle in the *Cosmarium* strains collected from the polar region, as adaptations to occasional high irradiances which may occur due to the albedo. The typical arctic species, *C. crenatum*, displayed an incomplete violaxanthin cycle yielding an accumulation of high antheraxanthin amounts during the high irradiance stress, which rapidly converted back to violaxanthin in recovery period. Therefore, *C. crenatum* possesses a fast violaxanthin/antheraxanthin turnover, so far known only in some Prasinophycean representatives and in a few arctic-alpine Chrysophyta. Antheraxanthin actively participated in the heat dissipation from PSII in *C. crenatum*, as concluded from the significant negative correlation between maximum quantum yield and antheraxanthin content expressed per xanthophyll-cycle pool, while the other *Cosmarium* strains relied rather on the zeaxanthin-dependent energy quenching. The polar strain of *C. punctulatum*, however, displayed a complete and intensive violaxanthin de-epoxidase action, as judged from the distinctly high production of zeaxanthin during the high light treatment.

Keywords: *Cosmarium*, distribution pattern, carotenoid composition, xanthophyll cycle, antheraxanthin accumulation, dithiothreitol, maximum quantum yield

Abbreviations

Chl – chlorophyll, Vx (V) –violaxanthin, Ax (A) – antheraxanthin, Zx (Z) – zeaxanthin, V+A+Z – xanthophyll pool size, VDE – violaxanthin de-epoxidase, ZEP – zeaxanthin epoxidase, DTT – dithiothreitol, PAR – photosynthetically active radiation, PSII – photosystem II, Fv/Fm – maximum quantum yield of PSII, NPQ – non-photochemical quenching, EPS – epoxidation state, $rETR_{max}$ – maximum relative electron transport rate, PFR – photon flux (fluence) rate

Introduction

Previous investigations revealed that several *Cosmarium* strains, isolated from various geographic areas, are regarded as rather high-light adapted algae according to the exceptionally high values of photosynthetic capacity (expressed as $rETR_{max}$) and saturating irradiance (Ik) (Stamenković and Hanelt 2011, 2012). In accordance with the fact that desmids are typical inhabitants of shallow freshwater habitats and cope with occasional high irradiances, forceful protective adaptations, both concerning photoprotection and photodamage, are hypothesised. All of the investigated *Cosmarium* strains displayed physiological responses that were consistent with the light prevailing at their source location, confirming that such responses are genetically preserved despite the long-term cultivation under the constant and relatively low light-temperature conditions (Stamenković and Hanelt 2011). These observations initiated the investigation of the composition of photosynthetic pigments of the *Cosmarium* strains, collected from different climatic zones, when grown at optimal laboratory conditions and after a photoinhibitory PAR stress. The topic of this study is to investigate if the composition of photosynthetic pigments of several *Cosmarium* strains can be related to the light prevailing at their sampling sites. Furthermore, as it is known that changes of the composition of photosynthetic pigments may occur during the cultivation under constant laboratory conditions (Osmond et al. 1993), there is an interest to observe if such an acclimation appeared in the long-term cultivated *Cosmarium* strains.

So far, the composition of pigments of desmids dealt with strains which were grown under optimal conditions (Herrmann 1968; Fawley 1991) or fresh-isolated desmid samples (Züllig 1982; Van Heukelem et al. 1992). Lütz et al. (1997) observed changes of the photosynthetic pigment composition in *Micrasterias denticulata* Brébisson ex Ralfs stressed under UVB radiation and found changes of Chl a and b contents and almost complete decomposition of β-carotene. To date, there have been no data on influences of photoinhibitory PAR on photosynthetic pigments of desmids.

The extent to which the photosynthetic apparatus is affected by light stress is explained by two types of photoinhibition, dynamic and chronic photoinhibition (i.e. photoprotection and photodamage) (Long et al, 1994; Osmond, 1994). Photoprotection by thermal energy dissipation controlled by carotenoids enables plants and algae to recover rapidly after the offset of the stressful condition (Osmond 1994). The xanthophyll cycle, or more specifically the de-epoxidized form – zeaxanthin (Zx), is involved in the protection of the photosynthetic apparatus against damage by excessive light absorbed by chlorophyll (Demmig-Adams and Adams 1992b, 2006). Subsequently, it has been demonstrated that antheraxanthin (Ax) may also have a significant role in the thermal dissipation, as it has been proven for Zx (Goss et al. 1998, 2006; Goss and Jakob 2010). Demmig-Adams (1990) concluded that the larger the xanthophyll cycle pool is, the greater is the capacity to form zeaxanthin, and presumably the capacity for the photoprotective process associated with zeaxanthin.

The light conditions can be regarded as the major factor that triggers the potential of photoprotection by non-photochemical energy dissipation (Goss and Jacob 2010). Additionally, it has long been recognised

that adaptation and acclimation of plants and algae to different light environments involves alterations in their capacity to utilise light through photosynthesis and efficiently dissipate the excess energy (Björkman 1981). Sun leaves and tropical plants exposed to high light irradiances contain larger pools of xanthophyll cycle pigments (V+A+Z) and β-carotene and accumulate greater Zx+Ax levels under high light stress, enabling them to achieve high levels of NPQ, compared to those which develop in low-light environments (Demmig-Adams and Adams 1992, 1994; Osmond et al. 1993; Brugnoli et al. 1994; Demmig-Adams et al. 1995; Adams and Demmig-Adams 1996). Moreover, clear relationships between the NPQ capacity (in the context of the Zx and Ax production) on one hand and the spatial and temporal distribution of macro- and microalgae on the other hand, have been displayed in numerous studies (Lüning 1981, 1990; Henley et al. 1991; Lobban and Harison 1994; Meyer et al. 2000; Fujiki et al. 2003; Serodio et al. 2005). Lavaud et al. (2007) suggested that geographic and seasonal distributions of diatoms and other phytoplankton species might be influenced by their ability to cope with rapid fluctuations in light intensity, as concluded from their capacity and activity of carotenoids involved in the dissipation of excess energy.

The present study investigates a relationship between the amount and action of photosynthetic pigments and potential geographic distribution patterns of desmid strains. The carotenoid composition of four *Cosmarium* strains collected from different climatic areas, and treated under optimal growth conditions or under photoinhibitory light treatments, suppose to provide an answer if photoprotective adaptations to specific light conditions exist among the strains.

Materials and methods

Algal strains and culture conditions

The four *Cosmarium* clones examined in this study were isolated from various parts of the world within approximately the same time period, in order to exclude the influences of sampling time and, therefore, the influences of constant nutrient, light and temperature regime under laboratory conditions:

C. crenatum Ralfs var. *boldtianum* (Gutwinski) W. & G.S. West (SVCK No. 561) – isolated 1995; polar (arctic) climate zone (Cape Flora, Northbrook Island, Franz Joseph Land, Russia), 79°57′N 50°05′E;

C. beatum W. & G.S. West (SVCK No. 533) – isolated 2001; tropical climate zone (marshy area near Ol Bolossat Lake, Kenya), 00°09′S 36°26′E;

C. punctulatum Brébisson var. *subpunctulatum* (Nordstedt) Børgesen (SVCK No. 570) – isolated 1996; alpine, tropical area (pool on Mt. Cotopaxi at 1600 m a.s.l., Ecuador; ‘highlands’ subgroup of the temperate (mesothermal) climatic group – Tierra templada), 00°40′S 78°26′W;

C. punctulatum var. *subpunctulatum* (SVCK No. 571) – isolated 1992; lowland, polar climate (pool near Skarsvåg at 80 m a.s.l, the North Cape, Norway), 71°06′N 25°49′E.

The details on taxonomic, ecological and distributional attributes of the investigated taxa, as well as the climate characteristics of the algal sampling locations are published elsewhere (Stamenković and Hanelt 2011).

All of the investigated *Cosmarium* strains were grown under ordinary laboratory conditions (16° C; ~30 µmol photons m^{-2} s^{-1}) in a climate chamber of the MCZH-SVCK (Sammlung von Conjugaten-Kulturen) collection over a period of several years. Taking into account that 16°C was a sub-optimal temperature for the tropical species (*C. beatum*) (Stamenković and Hanelt 2011), while temperatures above 22 or 25°C were recognised as sub-optimal for polar microalgal representatives (Fiala and Oriol 1990; Suzuki and Takahashi 1995), all of the experimental *Cosmarium* strains were pre-cultured at 21°C, which was considered as a roughly compromising optimal temperature for both of the tropical and polar strains studied. All of the investigated strains were grown in the mineral medium based on Kattner et al. (1977) (L-d medium). The tested *Cosmarium* strains had been acclimated to 21°C and L-d medium at least 12 months before the photoinhibitory experiments began (at a daily light regime of 14 h of light and 10 h of darkness). Sterilised 1 l Erlenmeyer flasks with 500 ml of medium were inoculated to a final concentration of 1500 cells ml^{-1}. Cultures were bubbled with humidified air at a rate of about 10 l h^{-1} to prevent CO_2 limitation. The cultures were mixed regularly by means of a magnetic stirrer to prevent self-shading of cells.

Photoinhibition and recovery

Photosynthetically active radiation (PAR) was provided by a sun simulator (SonSi, iSiTEC GmbH, Germany), as described by Hanelt et al. (2006). 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 (±0.5°C) by a thermostated water jacket. The samples were irradiated with irradiance coming from a stabilized 400 W Metallogen lamp (Philips MSR 400 HR) consisting of a number of lanthanide which emanate a solar-like continuum. A filter Schott GG400 (λ > 400 nm) was used to exclude UV radiation, and it was followed by a diffuser plate. Wire meshes acted as filters reducing the irradiance without changing the spectrum: 700 and 1200 µmol photons m^{-2} s^{-1}. The light intensity was adjusted using a cosine quantum sensor (LI-COR, LI–188B). Desmids samples were exposed to high PAR irradiances for 4 or 6 h (n = 3 per treatment combination) at 21°C. Afterwards, the samples were returned to a climate chamber at 21°C and 30 µmol photons m^{-2} s^{-1} for recovery. Measurements on the chlorophyll fluorescence and sampling for the high-performance liquid chromatography (HPLC) analysis were performed after 2, 4 and 6 h of inhibition and after 15 min, and 1, 2, 4 h of recovery. The number of cells was equalised to be approximately 15000 cells ml^{-1} during the light treatments. All of the PAR 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 ml^{-1} by adding a quantity of thermally adjusted L-d medium. For the samples treated with dithiothreitol (DTT), L-d medium was enriched with a respective concentration of DTT to preserve the desired concentration (see below). Immediately after sampling, the algal suspension was subjected to 3 min of dark adaptation in a water bath at 21°C and filled into 5 ml Quartz cuvettes. The maximum quantum yield (Fv/Fm) was measured at time zero as described by Hanelt (1998). A pulse of weak, far red light was applied to empty the electron pool from plastoquinone. Initial fluorescence (Fo) was measured with red measuring light (~0.3 µmol photons m^{-2} s^{-1}, 650 nm), and maximal fluorescence (Fm) was determined using 600 ms of a completely saturating white light pulse (~3500 µmol photon m^{-2} s^{-1}).

High-performance liquid chromatography (*HPLC) analysis*

15 ml of the *Cosmarium* cultures were filtered onto 15 mm Whatman glass fiber filters, which were immediately frozen in liquid nitrogen and stored at –80°C until analysis. After 4 h of lyophilisation (Christ Alpha 1-4 LD plus, Germany), ice-cold 90% acetone and glass beads (2 and 4 mm diameter) were added and the filters were homogenised in a cell mill (Vibrogen IV; Edmund Bühler, Tübingen, Germany) at 0°C for 3 min. The filtrates were extracted for 24 h at 4°C in the dark. Subsequently, the extract was centrifuged at 16 000 *g* and 4°C for 5 min and filtered through a nylon syringe filter (pore size: 0.45 µm, Nalgene®; Labware, Rochester, NY). Pigment analysis was performed by reversed phase HPLC on a LaChromElite® system equipped with a chilled autosampler L-2200 and a DAD detector L-2450 (VWR-Hitachi International GmbH, Darmstadt, Germany). A Spherisorb ODS-2 column (25 cm × 4.6 mm, 5 µm particle size) (Waters, Milford, MA) with a LiChrospher® 100 RP-18 guard cartridge was used for the separation of pigments. Peaks were detected at 440 nm and identified as well as quantified by co-chromatography with pigment standards for Chl a, Chl b, neoxanthin, lutein, xanthophyll-cycle pigments and β-carotene obtained from DHI Lab Products (Hørsholm, Denmark) using the software EZChrom Elite 3.1.3. Pigment concentrations other than Chl a were normalised to Chl a.

Action of dithiothreitol (DTT) on violaxanthin de-epoxidase

To investigate the inhibitory effect of DTT on the rate of violaxanthin de-epoxidation, the *Cosmarium* strains were treated with 0.65 mM DTT (the final concentration in a sample) and exposed 4 h under 700 µmol photons m^{-2} s^{-1} at 21°C. Additionally, DTT-untreated samples were exposed under the identical experimental condition. In parallel with the photoinhibitory light treatments, DTT-untreated samples and samples treated with 0.65 mM DTT were cultivated 4 h under dim-light (30 µmol photons m^{-2} s^{-1}) at 21°C. All of the experiments were repeated three times.

Statistical analysis

Data were tested for normality (Kolmogorov-Smirnov test) and for homogeneity of variance (Levene statistics). A set of the one-factor between subjects (one-way) ANOVAs was performed to find significant

differences between the *Cosmarium* strains regarding the concentrations of carotenoids (neoxanthin, violaxanthin, antheraxanthin, zeaxanthin, lutein, β-carotene), when grown at optimal laboratory conditions (21°C, 30 µmol photons m^{-2} s^{-1}). In addition, one-way ANOVA tests were applied to estimate differences in the xanthophyll-pool size between control samples and samples treated under photoinhibitory PAR treatments, for all of the *Cosmarium* strains studied. One-way ANOVA tests were also applied to find significant differences in the amount of xanthophyll cycle pigments of DTT-treated and untreated samples exposed under 700 µmol photons m^{-2} s^{-1} treatment, compared to control samples (grown under 30 µmol photons m^{-2} s^{-1}, at 21°C).

Results

The composition of photosynthetic pigments of the Cosmarium strains grown under control conditions (30 µmol photons m^{-2} s^{-1}, 21°C)

The pigment composition of the investigated *Cosmarium* strains grown under constant laboratory conditions (21°C, 30 µmol photons m^{-2} s^{-1}) is shown in Table 1, while percentages of carotenoid composition are presented in Fig. 1.

	Strains			
	C. punctulatum (No. 570)	*C. punctulatum* (No. 571)	*C. beatum*	*C. crenatum*
Pigments	mean	mean	mean	mean
Neoxanthin	56.11	14.67	34.18	13.40
Violaxanthin	49.99	40.92	66.82	33.49
Antheraxanthin	21.62	25.13	17.56	13.76
Zeaxanthin	7.99	10.33	14.03	7.07
Xanth. pool (V+A+Z)	79.59	76.37	98.41	54.33
Lutein	207.44	232.5	157.97	242.25
β-carotene	102.05	76.64	99.86	74.97
V/ (V+A+Z)	0.63	0.54	0.68	0.61
Z/ (V+A+Z)	0.1	0.13	0.14	0.13
Chl a	$4.75*10^{-11}$	$6.65*10^{-11}$	$7.79*10^{-11}$	$4.97*10^{-11}$
Chl b	$1.54*10^{-11}$	$2.80*10^{-11}$	$2.71*10^{-11}$	$2.25*10^{-11}$
Chl a/b	3.07	2.37	2.87	2.2

Table 1. Pigment composition of the investigated *Cosmarium* strains grown under control laboratory conditions (21°C, 30 µmol photons m^{-2} s^{-1}). The concentrations of carotenoids are expressed in mmol $(molChl\ a)^{-1}$. The amounts of chlorophyll a and b are expressed in mol $cell^{-1}$. All values are the means from at least 3 samples (SD typically less than 5% of mean).

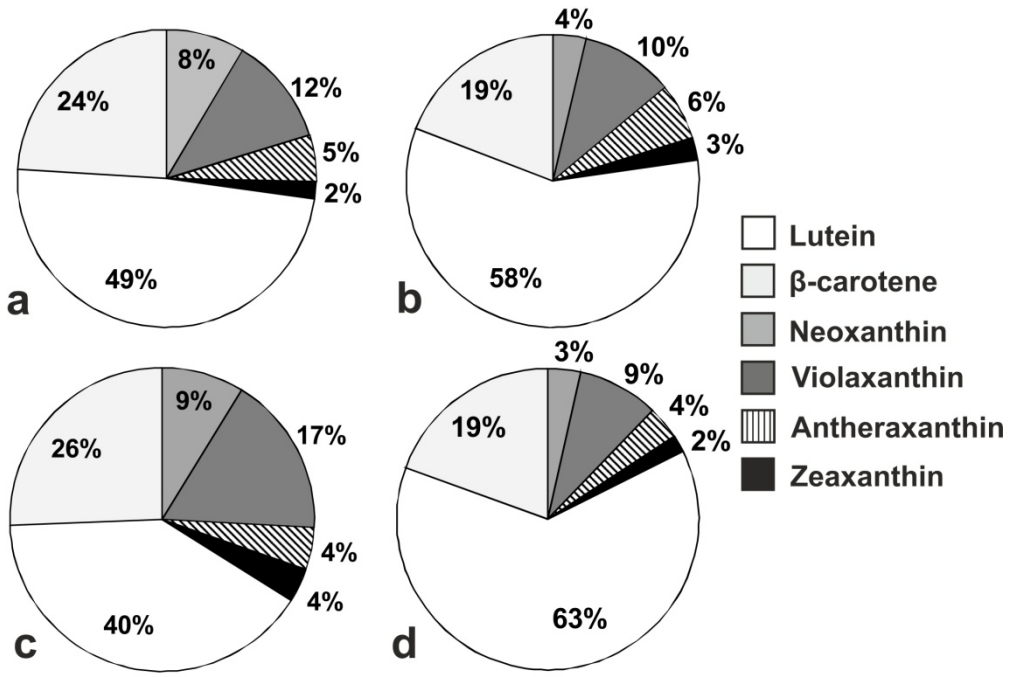

Fig. 1. Carotenoid composition of the investigated *Cosmarium* strains, grown under control laboratory conditions (30 μmol photons m^{-2} s^{-1}, 21°C): (**a**) *C. punctulatum* No. 570, (**b**) *C. punctulatum* No. 571, (**c**) *C. beatum*, (**d**) *C. crenatum*. The relative sizes of the pie diagrams reflect the difference in the total carotenoid content; concentrations of carotenoids were previously standardised to Chl a basis. Values are the means from 3 samples.

The typical tropical species (*C. beatum*) was characterised by the highest amount of xanthophyll cycle pigments (25% of the total carotenoids), as well as the highest quantities of violaxanthin (Vx), zeaxanthin (Zx) and β-carotene. The violaxanthin content relative to the xanthophyll-pool size (V/V+A+Z) was also by far the highest in this species, compared to that of the other investigated desmids. In addition, the tropical species was characterised by the moderately high Chl a/b ratio. In contrast, the arctic species, *C. crenatum*, was characterised by the lowest amount of Vx and β-carotene among all the *Cosmarium* strains, as well as the lowest total xanthophyll-pool size and Chl a/b ratio. Conversely, the lutein amount was the highest in this species.

The quantities of Vx and β-carotene, as well as the xanthophyll-pool size, were larger in the high-mountain tropical strain of *C. punctulatum* (No. 570) compared to the polar one (No. 571). The high-mountain strain was also characterised by the highest Chl a/b ratio among all the investigated desmids. All of these facts pointed to a fair adaptation of the high-mountain strain of *C. punctulatum* to high light intensities.

A set of one-way ANOVAs showed that the *Cosmarium* strains studied did not differ significantly regarding the content of antheraxanthin: $F(3, 26) = 19.7$, $p = 0.325$, and zeaxanthin: $F(3, 32) = 28.5$, $p = 0.653$, when grown at standard laboratory conditions (21°C, 30 μmol photons m^{-2} s^{-1}). However, significant differences between the *Cosmarium* strains were found regarding the composition of other carotenoids, as

concluded from the one-way ANOVA results: violaxanthin $F(3, 37) = 48.1$, $p < 0.05$; neoxanthin $F(3, 32) = 18.06$, $p < 0.05$; lutein $F(3, 26) = 10.4$, $p < 0.05$; β-carotene $F(3, 30)$, $p < 0.05$.

Changes of the composition of xanthophyll-cycle pigments in the Cosmarium strains during and after photoinhibitory PAR treatments

The application of 6 h photoinhibitory PAR (700 and 1200 µmol photons m^{-2} s^{-1}, at 21°C) to algal samples caused pronounced changes of the composition of xanthophyll-cycle pigments during and after the treatments in all of the *Cosmarium* strains (Fig. 2)

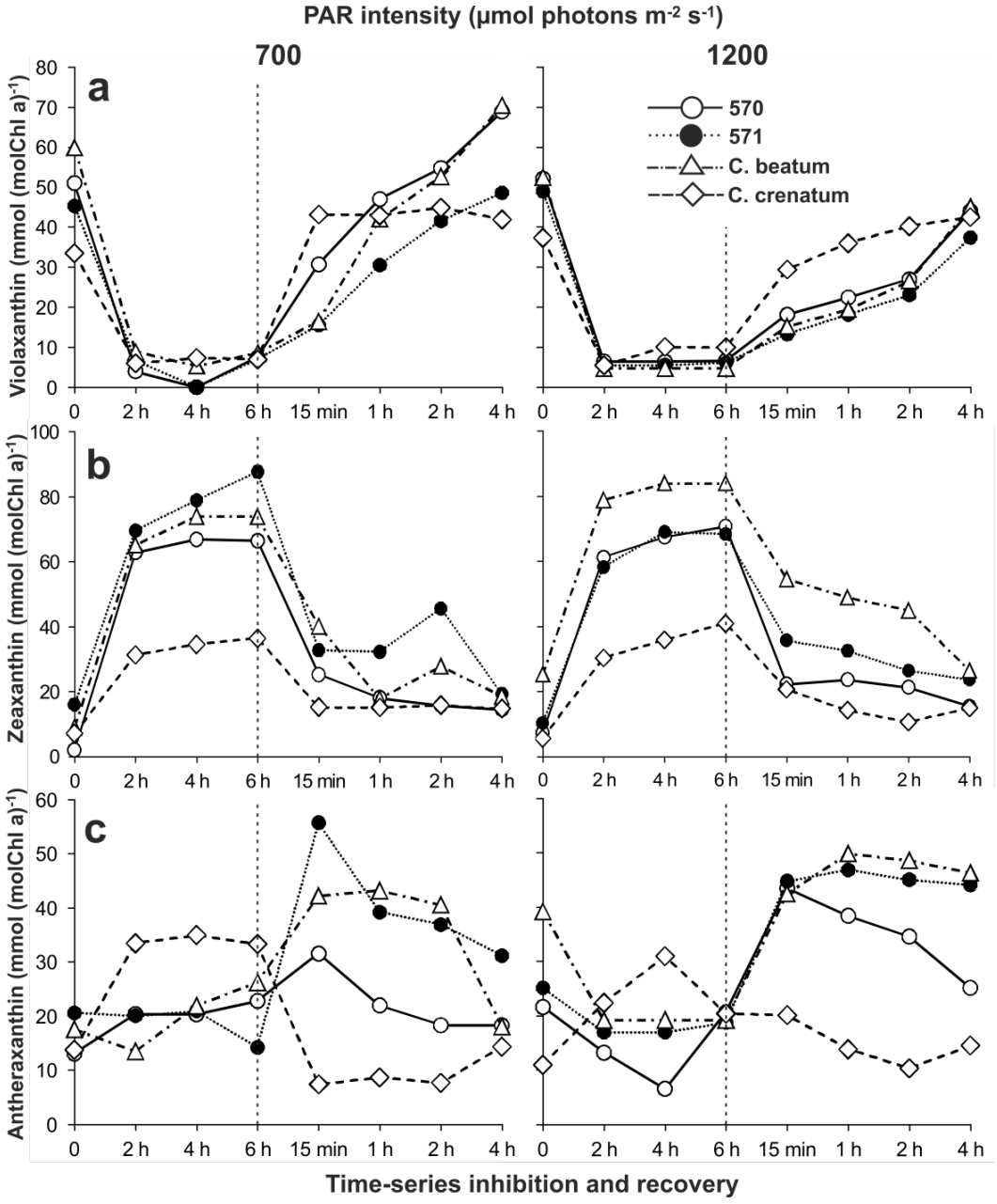

Fig. 2. Kinetics of changes of xanthophyll-cycle pigments during and after 6 h of photoinhibitory PAR treatments (700 and 1200 µmol photons m^{-2} s^{-1}, 21°C) for the investigated *Cosmarium* strains: (**a**) violaxanthin, (**b**) zeaxanthin, (**c**) antheraxanthin. ○ – *C. punctulatum* No. 570, ● – *C. punctulatum* No. 571, △ – *C. beatum*, ◇ – *C. crenatum*. Values are standardised to Chl a content. Standard deviations (SD) are less than 5% of mean (n = 3).

The content of Vx markedly decreased during the first 2 h of PAR treatments, reaching values below 10 mmol (mol Chl a)$^{-1}$, in all of the *Cosmarium* strains studied (Figs 2a and 2b). The recovery of the *Cosmarium* samples under 30 μmol photons m^{-2} s^{-1} at 21°C caused the return of the Vx amount to the values of control samples (grown at 30 μmol photons m^{-2} s^{-1}at 21°C) within 4 h.

The kinetics of the Zx amount was inversely related to that of Vx during the PAR treatments. The content of Zx rapidly decreased within 15 min of recovery under 30 μmol photons m^{-2} s^{-1}, in all of the *Cosmarium* strains (Fig. 2b). Stronger PAR irradiation treatment (1200 μmol photons m^{-2} s^{-1}) caused a more intensive development of Zx quantity after 6 h treatment in the high-mountain strain of *C. punctulatum* (No. 570) (up to 70.75 mmol (mol Chl a)$^{-1}$), compared to that after the application of the lower irradiation (up to 66.42 mmol (mol Chl a)$^{-1}$).

Interestingly, the polar strain of *C. punctulatum* (No. 571) produced the highest Zx amount after 6 h exposure under 700 μmol photons m^{-2} s^{-1} (87.66 mmol (mol Chl a)$^{-1}$) among all of the investigated desmids. However, the Zx content was lower after the application of 1200 μmol photons m^{-2} s^{-1}, obviously pointing to a larger stress caused by the strong PAR irradiation. The Zx content in the tropical species, *C. beatum*, was distinctly high after both of the photoinhibitory treatments, reaching 83.96 mmol (mol Chl a)$^{-1}$ after exposure under 1200 μmol photons m^{-2} s^{-1}. The arctic species, *C crenatum*, was characterised by the lowest Zx amount not only in control samples but also after the PAR treatments. Remarkably, this species was attributed by the rapid increase of Vx quantity during the recovery period, achieving the control value within 15 min after the weaker PAR treatment.

The amount of antheraxanthin (Ax) remained equalised or declined during the photoinhibitory treatments in both of the strains belonging to *C. punctulatum* and in *C. beatum*, probably due to the fast conversion of Vx to Zx (Figs 2c). Strikingly, the Ax quantity increased noticeably during both of the PAR treatments in the arctic species, *C. crenatum*, reaching 34.95 mmol (mol Chl a)$^{-1}$ after 700 μmol photons m^{-2} s^{-1} treatment. The Ax quantity decreased strongly during the 15 min of recovery in this strain, in contrast to all of the other *Cosmarium* strains.

No changes in the amount of chlorophylls (chlorophyll bleaching), lutein and β-carotene were observed during and after the photoinhibitory PAR treatments, for all of the *Cosmarium* strains studied. The amount of neoxanthin slightly decreased during the recovery period, between 15 min and 1 h of recovery.

The content of the xanthophyll-pool size increased during both PAR treatments in all of the *Cosmarium* strains, confirming the *de novo* synthesis of xanthophylls (Fig. 3). The high-mountain strain of *C. punctulatum* and *C. beatum* showed larger increases of the xanthophyll-pool size compared to the polar strains, pointing to a fair adaptability under the relatively high PAR stress (the xanthophyll-pool size of *C. punctulatum* No. 570 increased by 36.5%, while that of *C. beatum* increased by 31%). The

typical tropical species was attributed by the highest content of the xanthophyll-pool size after 1200 µmol photons m^{-2} s^{-1} irradiation, among all the *Cosmarium* strains studied (131.8 mmol (mol Chl a)$^{-1}$).

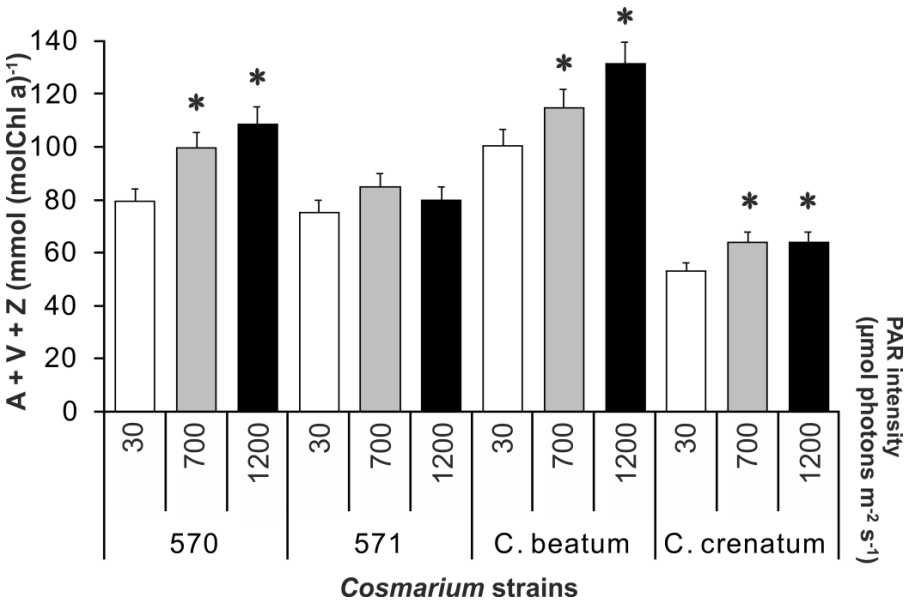

Fig. 3. Changes of the xanthophyll-pool size estimated for control samples (grown under 30 µmol photons m^{-2} s^{-1}, 21°C) and after 6 h treatments under 700 and 1200 µmol photons m^{-2} s^{-1}, for all of the investigated *Cosmarium* strains. Vertical bars represent SD; n = 3. Asterisks represent significant increases of the xanthophyll-pool size compared to controls (Tukey HSD test, $p < 0.05$).

On the contrary, insignificant increases of the xanthophyll-pool size were observed for the polar strain of *C. punctulatum*: up to 12.8% after 700 µmol photons m^{-2} s^{-1} exposure, or up to 6.4% after the stronger PAR treatment; thereby showing that *de novo* synthesis of xanthophylls was very limited in this strain. Additionally, the arctic species, *C. crenatum*, demonstrated rather low *de novo* synthesis of xanthophylls after both of the PAR treatments (up to 20.5% of the control value).

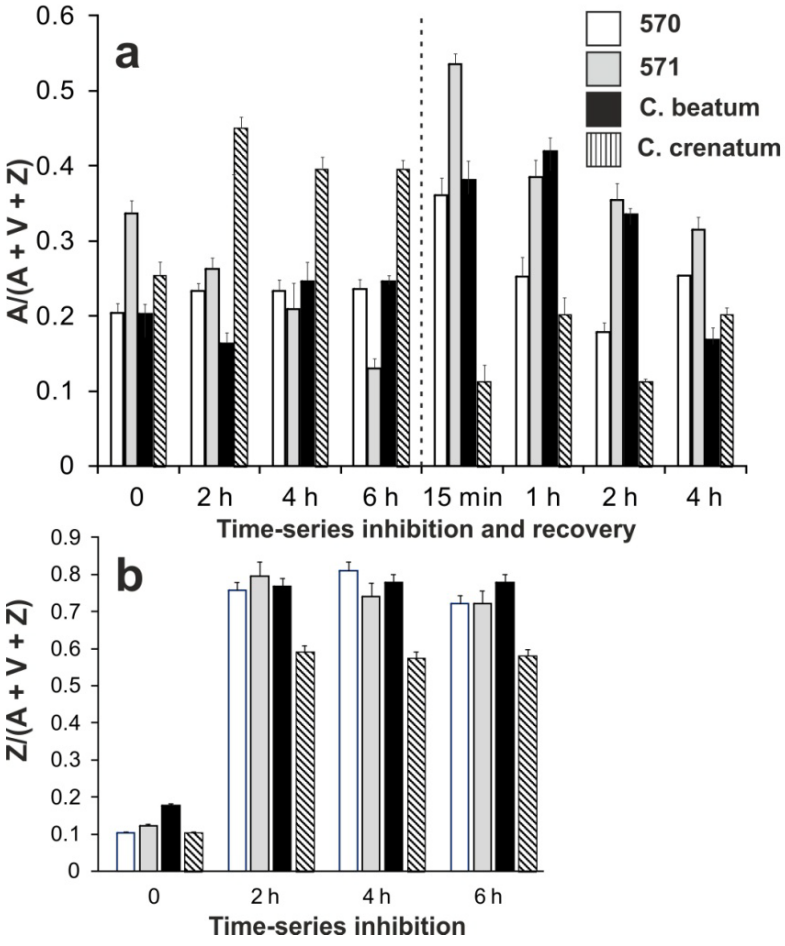

Fig. 4. (**a**) The kinetics of changes of antheraxanthin content standardised to xanthophyll pool size (V+A+Z) during the 6 h treatment under 700 µmol photons m^{-2} s^{-1} irradiation and in recovery, at 21°C. (**b**) The kinetics of changes of zeaxanthin content standardised to xanthophyll pool size (V+A+Z) during the 6 h treatment under 1200 µmol photons m^{-2} s^{-1} irradiation, at 21°C. (0 – values for controls, grown under 30 µmol photons m^{-2} s^{-1}, 21°C). Vertical bars represent SD; n = 3.

The one-way ANOVA demonstrated a significant increase of the xanthophyll-pool size for the high-mountain strain of *C. punctulatum*, *C. beatum*, and *C. crenatum*, taking into account both PAR treatments: *C. punctulatum* No. 570 $F(2, 20) = 30.6$, $p < 0.05$; *C. beatum* $F(2, 16) = 26.7$, $p < 0.05$; *C. crenatum* $F(2, 32) = 18.3$, $p < 0.05$. The increase of the xanthophyll-pool size was not statistically significant for the polar strain of *C. punctulatum*: $F(2, 47) = 56.1$, $p = 0.224$.

The rapid increase in the Zx content (expressed per xanthophyll-pool size) was observed for all of the investigated desmids during the first 2 hours of PAR treatments; afterwards the Zx amount remained equalised, ranging around 0.8 (Fig. 4b). The amount of Ax (standardised to xanthophyll pool size) decreased in the polar strain of *C. punctulatum* during both PAR treatments, or remained equalised in the high-mountain strain of *C. punctulatum* and *C. beatum* (Fig. 4a). In contrast, A/(V+A+Z) ratio increased significantly in *C. crenatum* during the treatment and decreased rapidly during 15 min of recovery.

In accordance with the previous observations regarding the changes of xanthophyll-cycle pigments during and after photoinhibitory PAR treatments, changes in the epoxidation state (EPS) were also noted for all of the investigated *Cosmarium* strains (Fig. 5). The epoxidation state diminished strongly after 2 h exposure at 700 and 1200 µmol photons m^{-2} s^{-1} irradiation treatments in *C. punctulatum* No. 570 and *C. beatum*, pointing to a fair efficiency of VDE. On the contrary, *C. crenatum* exhibited quite milder depression in EPS curves after the PAR treatments (i.e. only up to 0.314 after 700 µmol photons m^{-2} s^{-1}). Interestingly, the polar strain of *C. punctulatum* was characterised by a deeper depression of EPS after 700 µmol photons m^{-2} s^{-1} treatment, compared to that after the stronger irradiation experiment. Apparently, VDE activity was to some extent impeded by the higher irradiance treatment, and/or the cells were already considerably damaged.

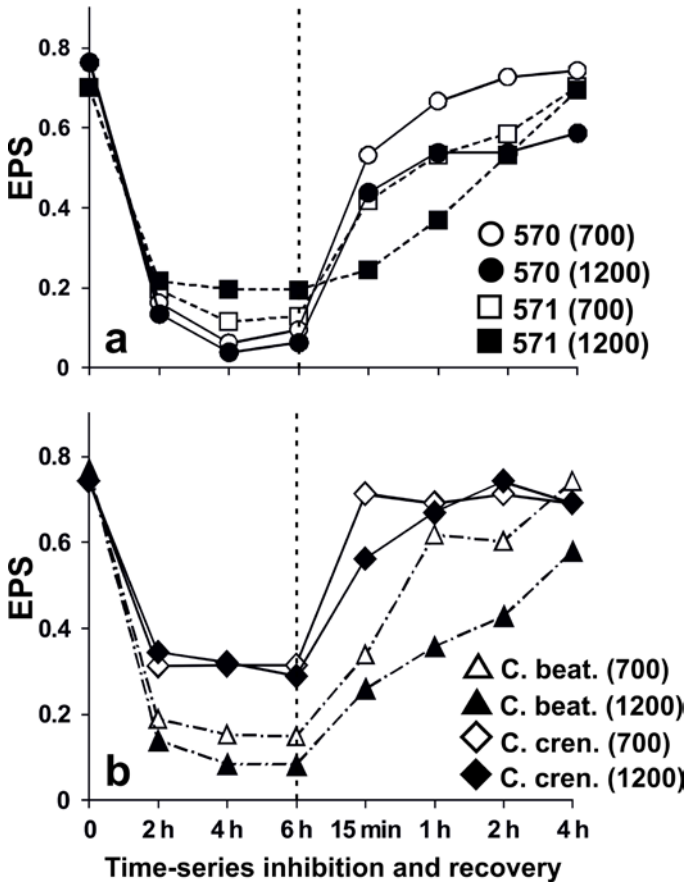

Fig. 5. The changes of the epoxidation state of the xanthophyll cycle [EPS=(V+0.5A)/(V+A+Z)] during and after 6 h treatment under 700 and 1200 µmol photons m^{-2} s^{-1} for all of the *Cosmarium* strains. (**a**) *C. punctulatum* Nos. 570 and 571 (**b**) *C. crenatum* and *C. beatum*. Standard deviations (SD) are less than 5% of mean (n = 3).

The fluorescence ratio Fv/Fm of dark-acclimated algae is indicative of the photoinhibitory degree of photosynthesis (Krause and Weis 1991). Taking into account

that fluorescence, as an energy dissipating mechanism, competes with thermal energy dissipation, the negative correlations in plots between Fv/Fm and Z/(V+A+Z) indicate that an increase of Zx content could be responsible for an increase of thermal dissipation, which causes a decrease of the fluorescence signal (Uhrmacher et al. 1995). Several authors (Duval et al. 1992; Benet et al. 1994; Förster et al. 2009) showed that a relationship between the formation of Zx and a decrease in the chlorophyll fluorescence in several macroalgae and plants exists.

To investigate relationships between changes of Fv/Fm and ratios of Zx, Ax, or Zx+Ax expressed per xanthophyll pool size (V+A+Z), correlations were done for these parameters for all of the investigated *Cosmarium* strains, taking into account the PAR exposure (6 h under 700 or 1200 µmol photons m^{-2} s^{-1}) and recovery period (4 h under 30 µmol photons m^{-2} s^{-1}), at 21°C (Table 2).

		Fv/Fm * Z/(V+A+Z)		Fv/Fm * A/(V+A+Z)		Fv/Fm * Z+A/(V+A+Z)	
Strain	PAR (µmol photons m^{-2} s^{-1})	r	p	r	p	r	p
C. punct. (570)	700	–0.966	<0.001	0.016	0.585^{ns}	–0.949	<0.001
	1200	–0.953	<0.001	0.484	0.164^{ns}	–0.922	<0.001
C. punct. (571)	700	–0.941	<0.001	0.679	<0.05	–0.778	<0.05
	1200	–0.864	<0.05	0.649	<0.05	–0.745	<0.05
C. beatum	700	–0.96	<0.001	0.273	0.256^{ns}	–0.879	<0.05
	1200	–0.968	<0.001	0.387	0.357^{ns}	–0.862	<0.05
C. crenatum	700	–0.987	<0.001	–0.893	<0.05	–0.967	<0.001
	1200	–0.949	<0.001	–0.884	<0.05	–0.949	<0.001

Table 2. Pearson's correlation coefficients (r) for the relationships between maximum quantum yield and zeaxanthin, antheraxanthin or zeaxanthin + antheraxanthin standardised to xanthophyll poll size (V+A+Z), for all of the *Cosmarium* strains. The values were taken during 700 and 1200 µmol photons m^{-2} s^{-1} treatments and during 4 h recovery under 30 µmol photons m^{-2} s^{-1}at 21°C. (ns – not significant).

Significant negative linear correlations between the values of maximum quantum yield and Z/(V+A+Z) were found for all of the investigated *Cosmarium* strains, taking into account both PAR irradiances. Plots between Fv/Fv and A/(V+A+Z) ratio displayed no significant correlation for the high-mountain strain of *C. punctulatum* and *C. beatum*, or the correlation was statistically positive for the polar strain of *C. punctulatum*. Interestingly, this correlation was significantly negative for the arctic species, *C. crenatum*, pointing to the possible efficient thermal dissipation by antheraxanthin, i.e. non-photochemical quenching (NPQ) increased by Ax, which was concomitant with the decrease of Fv/Fm. Correlations between Fv/Fm and Z+A/(V+A+Z) were significantly negative for all of the investigated *Cosmarium* strains regarding both of the PAR intensities applied. Yet, this correlation was weaker in the polar strain of *C. punctulatum* compared to that between Fv/Fm and Z/(V+A+Z) which pointed that this strain does not significantly rely on the thermal dissipation by antheraxanthin.

Changes of the composition of xanthophyll-cycle pigments in the Cosmarium strains after the treatment under 700 µmol photons m^{-2} s^{-1}, using dithiothreitol (DTT) as a de-epoxidase inhibitor

To observe the action of VDE during a PAR treatment in the *Cosmarium* strains collected from different geographic areas, 0.65 mM dithiothreitol (DTT) was added prior to the photoinhibitory experiment (700 µmol photons m^{-2} s^{-1}).

The addition of DTT during control conditions (30 µmol photons m^{-2} s^{-1}, 21°C) did not influence on the change of the composition of xanthophyll-cycle pigments of the *Cosmarium* strains, compared to that of untreated samples (Fig. 6). However, the addition of 0.65 mM DTT prior to the photoinhibitory treatment prevented the Zx increase and, moreover, caused an increase of Vx content in both of the strains of *C. punctulatum* and *C. crenatum*, compared to control samples (*C. punctulatum* No. 570: by 30.5% of control, *C. punctulatum* No. 571: 27.8%, *C. crenatum*: 21.1%). The Vx content distinctly increased in the tropical species, *C. beatum*, which was characterised by the highest amount of Vx, among all of the strains studied (up to 99.01 mmol (mol Chl a)$^{-1}$).

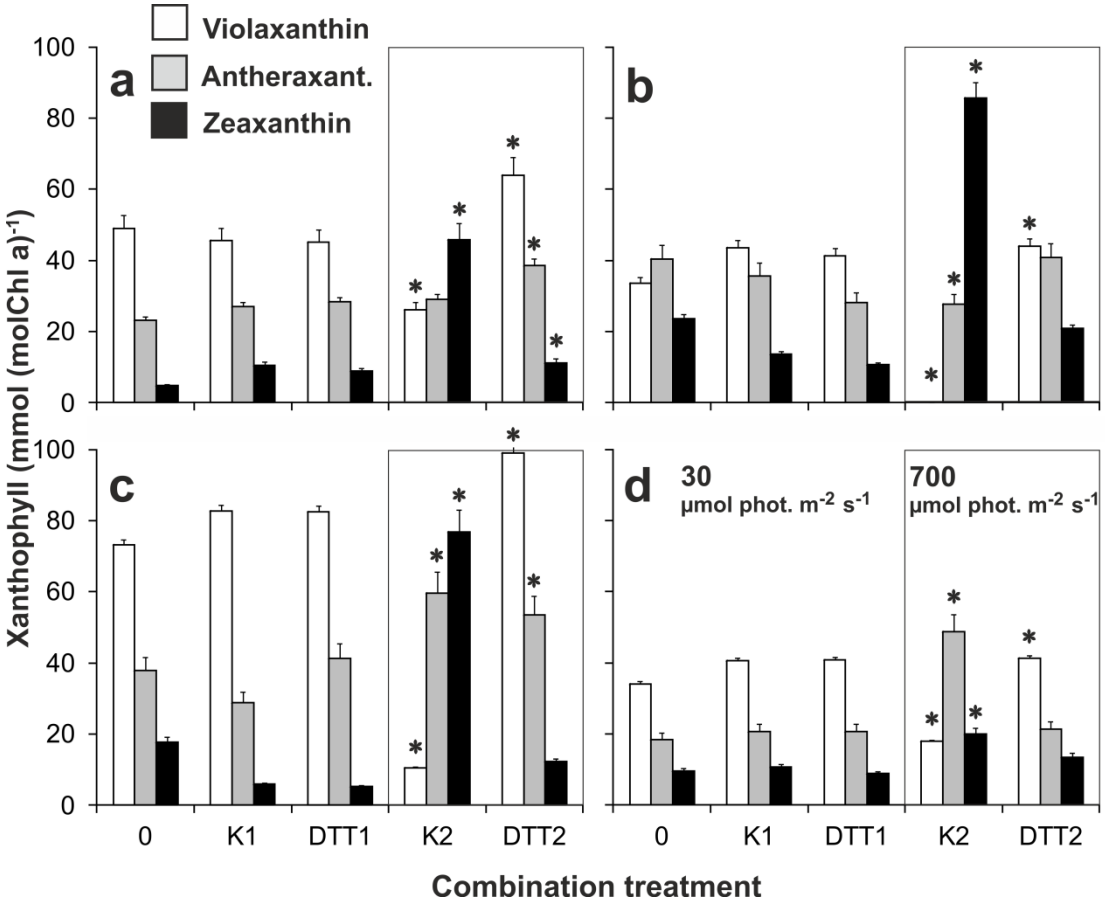

Fig. 6. Effects of the photon fluence rate and dithiothreitol (DTT) on the composition of xanthophyll-cycle pigments (in mmol(mol Chl a)$^{-1}$) for all of the investigated *Cosmarium* strains: (**a**) *C. punctulatum* No. 570 (**b**) *C. punctulatum* No. 571, (**c**) *C. beatum* (**d**) *C. crenatum*. 0 – (control) untreated *Cosmarium* samples (grown at 30 µmol photons m^{-2} s^{-1}, 21°C; measurements at the beginning of the experiment); K1 – untreated *Cosmarium* samples; after 4 h cultivation at optimal laboratory conditions (21°C, 30 µmol photons m^{-2} s^{-1}); DTT1 – samples treated with 0.65 mM DTT, after 4 h cultivation at optimal laboratory conditions; K2 – untreated *Cosmarium* samples, after 4 h under 700 µmol photons m^{-2}

s^{-1}, at 21°C; DTT2 – samples treated with 0.65 mM DTT, after 4 h treatment under 700 μmol photons m^{-2} s^{-1}, at 21°C. Vertical bars represent SD; n = 3. Asterisks represent significant differences in the xanthophyll amount of K1, DTT1, K2 and DTT2 samples compared to control (0) samples (Tukey HSD test, $p < 0.05$).

In accordance with previous observations, the polar strain of *C. punctulatum* exhibited an intensive VDE activity, as concluded from the highest Zx quantity measured in the DTT-untreated samples after 700 μmol photons m^{-2} s^{-1} treatment, among all the investigated strains (85.67 mmol (mol Chl a)$^{-1}$). Conversely, the amount of Vx dropped down to zero in the DTT-untreated samples under 700 μmol photons m^{-2} s^{-1} treatment. Additionally, *C. beatum* was characterised by the distinctly high zeaxanthin amount in the DTT-untreated samples after 700 μmol photons m^{-2} s^{-1} treatment; the application of DTT strongly inhibited VDE as concluded from the exceptionally high Vx quantity measured after the PAR treatment. The other carotenoids and chlorophylls demonstrated no significant differences between control and DTT-treated samples in any of the *Cosmarium* species studied.

Table 3. Results of one-way ANOVA tests for Vx, Ax and Zx factors, for each *Cosmarium* strain studied. F – F ratio, df – degrees of freedom (for the effect of the model and residuals of the model), p – significance.

Strain	ANOVA factors	ANOVA parameters		
		F	df	p
570	Vx	84.5	4, 48	< 0.001
	Ax	24.2	4, 27	< 0.05
	Zx	53.7	4, 32	< 0.05
571	Vx	80.3	4, 41	< 0.001
	Ax	23.9	4, 26	< 0.05
	Zx	76.1	4, 34	< 0.001
beatum	Vx	91.5	4, 25	< 0.001
	Ax	76.3	4, 27	< 0.001
	Zx	84.5	4, 30	< 0.001
crenatum	Vx	87.6	4, 37	< 0.001
	Ax	91.2	4, 42	< 0.001
	Zx	42.4	4, 23	< 0.05

The DTT-untreated samples of *C. crenatum* exposed to 700 μmol photons m^{-2} s^{-1} displayed a marked accumulation of Ax (up to 48.65 mmol (mol Chl a)$^{-1}$), while the Zx content slightly increased compared to a control sample. The addition of DTT successfully prevented the synthesis of Ax and Zx in DTT-treated samples of *C. crenatum* during the PAR treatment, while the Vx amount increased compared to the control sample (by 21.1%).

A set of one-way ANOVA tests was applied to find differences in Vx, Ax, and Zx amounts of samples labelled as K1, DTT1, K2 and DTT2 (for the explanation of experimental conditions refer to Fig. 6) compared to control samples (untreated samples grown at 30 μmol photons m^{-2} s^{-1}, 21°C). Results of ANOVA tests displayed significant changes of all of the xanthophyll-cycle pigments, for all of the *Cosmarium* strains studied (Table 3).

Discussion

The composition of photosynthetic pigments of the investigated *Cosmarium* strains grown at standard laboratory conditions (21°C, 30 μmol photons m^{-2} s^{-1}) basically corresponded to that of sun plants (i.e. high-light plants) or sun-acclimated plants (Czeczuga 1987; Anderson and Osmond 1987; Demmig-

Adams and Adams 1996; Demmig-Adams and Adams 2006), taking into account relatively high values of Vx (expressed per Chl a) and β-carotene, and a moderately high xanthophyll-cycle pool (A+V+Z) size (up to 25% of all carotenoids). In addition, no α-carotene was found in all of the *Cosmarium* strains, including also the previous investigations on desmid pigments (Herrmann 1968; Züllig 1982; Van Heukelem et al. 1992; Lütz et al. 1997). This additionally pointed to the sun-adapted pigment composition, as α-carotene is known as a usual component of low-light grown leaves (Thayer and Björkman 1990; Demmig-Adams and Adams 1992).

When stressed under photoinhibitory light intensities, the *Cosmarium* strains (with the exception of *C. crenatum*) produced a markedly high amount of Zx expressed per Chl a (66 – 88 mmol (mol Chl a)$^{-1}$) which corresponded to that of sun-adapted plants, e.g. perennial shrubs, vines and some crop species grown at a PFR of 2000 μmol photons m^{-2} s^{-1} (70.4 – 95.9 mmol (mol Chl a+b)$^{-1}$) (Demmig-Adams and Adams 1992a). Furthermore, all of the *Cosmarium* strains, except *C. crenatum*, possess a large xanthophyll-cycle pool size (76 – 98 mmol (mol Chl a)$^{-1}$), which is in the range of the high-light adapted plants grown in full sunlight (109 – 119 mmol (mol Chl a+b)$^{-1}$) (Demmig-Adams and Adams 1992a; Demmig-Adams 1998). Concomitantly, the markedly high ratio of Z/(V+A+Z) in the high-light stressed *Cosmarium* corresponds to that of sun-adapted plants under high PAR irradiance (Hager 1980; Thayer and Bjorkman 1990; Demmig-Adams 1998), pointing to a high efficiency of VDE. The ratio Z/(V+A+Z) of the *Cosmarium* strains was distinctly higher than that of macroalgae collected from eulittoral zone such as *Dictyota*, *Pelvetia*, *Laminaria* and *Ulva* (Z/(V+A+Z) ranged between 0.3 – 0.58) (Franklin et al. 1991; Duval et al. 1992; Benet et al. 1994; Uhrmacher et al. 1995). The fact that all of the *Cosmarium* strains showed both rapid and strong increases and decreases in zeaxanthin content (Fig. 2), furthermore confirmed the high-light adaptation of the *Cosmarium* cells, as sun-plants and high-light acclimated leaves are known for the distinct Zx-dependent non-photochemical quenching (NPQ) (Czeczuga 1987; Demmig-Adams 1998).

Interestingly, several species- and strain-specific differences in the pigment composition of the *Cosmarium* strains have been revealed despite a long-term cultivation (more than 15 years) under the relatively low light-temperature regime (16°C, 30 μmol photons m^{-2} s^{-1}). The differences in the pigment composition between the strains originated from various geographic regions were not as expressive as it had been found for numerous low- and high-light adapted vascular plants and macroalgae (Adams and Demmig-Adams 1992; Gamon et al. 1990; Lüning 1990; Thayer and Björkman 1990; Henley et al. 1991; Lobban and Harison 1994; Hanelt et al. 2003); yet, these differences were statistically significant. This fact can be certainly explained by the long-term cultivation of the *Cosmarium* strains under the identical and relatively low light-temperature conditions (16°C, 30 μmol photons m^{-2} s^{-1}), which may cause the acclimation of photosynthesis to the conditions of a climate chamber. Yet, when pre-acclimated at 21°C and 30 μmol photons m^{-2} s^{-1}, the typical tropical species, *C. beatum*, and the high-mountain tropical strain of *C. punctulatum*, displayed characteristics of high-light adapted plants, such as the relatively high Chl a/b ratio and A+V+Z size, high amounts of β-carotene and Vx, as measured in perennial shrubs and several crop

species (Demmig-Adams and Adams 1992a; Demmig-Adams 1998). On the other hand, the polar strains, *C. punctulatum* No. 571 and *C. crenatum*, demonstrated somewhat lower amounts of Vx and β-carotene, lower sizes of V+A+Z (up to 15% of total carotenoids in *C. crenatum*), lower Chl a/b ratio (which means a larger antenna size) and V/(V+A+Z) ratio compared to that of the tropical strains. These values were not as low as in e.g. shade-grown rainforest plant species, but belonged to the upper level of ranges observed for the shade-adapted plants (Demmig-Adams and Adams 1992a, 1992b).

Photoinhibitory PAR treatments (700 and 1200 μmol photons m^{-2} s^{-1}) at 21°C provoked more obvious strain-specific differences in the pigment composition of the *Cosmarium* isolates, in accordance with the climate of their sampling sites. Both of the strains collected from the tropical zone, *C. punctulatum* No. 570 and *C. beatum*, displayed an intensive action of VDE as concluded from the extremely high degree of conversion of Vx into Zx and the low EPS values (V/(V+A+Z) reached below 0.1, while EPS was below 0.09 during 6 h under 1200 μmol photons m^{-2} s^{-1}) correspondingly to what was observed for sun-acclimated plants, tropical macroalgae, and plants growing in forest canopies of the tropical region (Lüning 1981, 1990; Thayer and Björkman 1990; Demmig-Adams and Adams 1996; Demmig-Adams 1998; Lütge 2008).

Remarkably, the Norwegian strain of *C. punctulatum* appeared very efficient in conversion of Vx to Zx. It synthesised 87.66 mmol (mol Chl a)$^{-1}$ of Zx when treated 6 h under 700 μmol photons m^{-2} s^{-1}, and thus appeared more efficient even when compared with the high-mountain strain of *C. punctulatum* and *C. beatum*. Since the Norwegian polar climate is mainly foggy and cloudy (Tollefsrud et al. 1991; Moen 1998), the *de novo* synthesis of xanthophylls might be rather limited in this strain in order to save energy necessary for the cell maintenance under lower-light conditions. Hence, the rapid action of VDE in *C. punctulatum* No. 571 might be considered as a strategy to cope with occasionally excessive PAR irradiation in such an environment. In addition, it is worth emphasising that the polar strain of *C. punctulatum* was characterised by the lowest capacity for the electron transport rate at 21°C, compared to that of the other *Cosmarium* strains studied (Stamenković and Hanelt 2012), i.e. values of $rETR_{max}$ values for this strain reached up to 156, while $rETR_{max}$ of the tropical species, *C. beatum*, was around 318. The fact that plants with lower rates of photosynthesis may exhibit larger de-epoxidation rates, i.e. they produce high Zx amounts and have a lower EPS (Demmig-Adams and Adams 1992a; Demmig-Adams and Adams 1992b; Demmig-Adams 1998) explains the high conversion to Zx in this strain, as well as the low value of EPS recorded during the 700 μmol photons m^{-2} s^{-1} treatment. The same phenomenon was noted for the perennial shrubs and vines, which had relatively low photosynthesis rates but accumulated more Zx in full sunlight, despite the smaller xanthophyll cycle pools compared to that of the annual crop species and perennial mesophytes (Demmig-Adams and Adams 1992a).

The arctic species, *C. crenatum*, displayed the lowest Zx conversion during PAR treatments among all of the *Cosmarium* strains; however, the Ax amount markedly increased reaching values between 34.9 – 48.65 mmol (mol Chl a)$^{-1}$, while A/(V+A+Z) ratio increased up to 0.45. Strikingly, the accumulation of Ax in *C. crenatum* during photoinhibitory PAR treatments corresponds to that noted in representatives of

Prasinophyceae (Chlorophyta). The particular attribute of some Prasinophyceaen species (*Mantoniella squamata* Manton & Parke and *Micromonas pusilla* (Butcher) Manton & Parke) is an incomplete Vx cycle, leading to an accumulation of antheraxanthin instead of zeaxanthin. Thus, the arctic species, *C. crenatum*, may show Vx/Ax cycle typical for primitive green algae, in contrast to the other *Cosmarium* strains which displayed a normal Vx/Zx cycle. The Zx-depleted Vx/Ax cycle of Prasinophyceae is the result of an extremely slow de-epoxidation step from Ax to Zx and a fast epoxidation from Ax back to Vx in the light (Frommolt et al. 2001; Goss and Jakob 2010), similarly as it has been observed in *C. crenatum* (Figs 2 and 4). Indeed, *C. crenatum* exhibited the fastest recovery of the Vx amount (per Chl a, and per V+A+Z), among all the investigated desmids; it successfully regained the beginning Vx quantity within only 15 minutes.

It is of great importance to emphasise that Ax can completely replace Zx in the mechanism of enhanced thermal dissipation of excitation energy (Goss et al. 1998; Goss and Jakob 2010). Intact *M. squamata* cells that are able to synthesise high amounts of Ax quench chlorophyll fluorescence very efficiently, thus showing a good correlation between Ax contents and the extent of NPQ (Goss et al. 1998). Correspondingly, a significant negative correlation was observed between Fv/Fm and A/(V+A+Z) in *C. crenatum*, which indicated a possible high thermal dissipation by Ax in this strain, which was concomitant with the decrease of chlorophyll fluorescence. In contrast, such a correlation was not observed for the other *Cosmarium* strains, which pointed that there was no high reliance on Ax in the non-photochemical quenching for these strains. It is worth emphasising that, in contrast to *M. squamata*, high-light stressed cells of *C. crenatum* synthesised also Zx in a smaller amount, and significant negative correlations between Fv/Fm and both Z/(V+A+Z) and A+Z/(V+A+Z) were displayed, showing that Zx also participated in non-photochemical quenching. The addition of DTT to *C. crenatum* samples successfully prevented VDE action, as no increase in Zx and Ax was observed during the photoinhibitory PAR treatment, similarly to what had been observed for DTT-treated *M. squamata* cells (Goss et al. 1998). Holt et al. (2005) and Goss et al. (2006) proposed that Ax, as it has been described for Zx in *Arabidopsis thaliana* (Linnaeus) Heyhnhold, is able to form a heterodimer with a Chl a molecule, which, after excitation, performs charge separation and dissipation of the excitation energy as heat. Our results are consistent with previous numerous findings that Zx and/or Ax actively participate in the heat dissipation from PSII (dynamic photoinhibition), concomitantly with the decrease in fluorescence signal (Duval et al. 1992; Benet et al. 1994; Uhrmacher et al. 1995; Förster et al. 2009).

The accumulation of Ax and limited Zx synthesis, were observed in the snow algae, *Ochromonas itoi* and *Ochromonas smithii* (Chrysophyta), which had been collected from the ice surface of a high-mountain region, and treated 6 h under 1500 μmol photons m^{-2} s^{-1} at 4°C (Koyama et al. 1996; Tanabe et al. 2001). The 'short' Vx/Ax cycle in *O. itoi*, *O. smithii* and *C. crenatum* may represent the prerequisite to survive under conditions of low temperature and strong light (due to the albedo from snow and ice surfaces) in harsh polar and alpine climates, where algal photoprotective mechanisms must react rapidly. The faster kinetics of Vx de-epoxidation can easily be explained if one considers that VDE of Vx/Ax cycle has to

convert the pool of Vx only once, compared with the two reaction cycles that are necessary to synthesise Zx in plants and green algae (Goss et al. 1998). The presence of Vx/Ax cycle may also be of a great importance for *C. crenatum*, which is known as a hemi-atmophytic species (West and West 1912; Hirano 1966), enabling it to cope with sudden and strong light intensities at wet surfaces of mosses and dripping rocks.

Taking into account that *C. punctulatum* is not a typical arctic-alpine desmid (Coesel and Meesters 2007) contributes to the explanation why Vx/Zx cycle is a mechanism to cope with high light intensities in this species, instead of Vx/Ax cycle, as found in the typical polar microalgae – *C. crenatum*, *O. itoi* and *O. smithii*. Although both of the polar strains displayed rather a low *de novo* xanthophyll synthesis during the photoinhibitory light intensities, which possibly appeared a consequence of the adaptation to severe polar conditions, larger *de novo* synthesis of the xanthophyll-cycle pigments was found for *C. crenatum*, demonstrating its better adaptability under high light stress.

Both of the *Cosmarium* strains collected from the tropical region, *C. punctulatum* No. 570 and *C. beatum*, displayed a significant *de novo* synthesis of xanthophyll-cycle pigments, which was somewhat higher during the stronger irradiance (1200 μmol photons m^{-2} s^{-1}). This fact, in addition to the high resistance under the strong PAR intensities confirmed the fair adaptation to high light intensities prevailing at their sampling sites. Considering that *C. punctulatum* No. 570 and *C. beatum* are characterised by distinctly high photosynthetic capacities at 21°C, typical of sun-adapted plants (Stamenkovic and Hanelt 2012), this feature may enable them to synthesise a large amount of carotenoids (Demmig-Adams and Adams 1992a; Demmig-Adams 1998). In addition, both of these strains possess large pools of β-carotene which may facilitate a forceful synthesis of xanthophyll-cycle pigments and large turnover rates of carotenoid metabolism (Thayer and Björkman 1990). This fact is in accordance with the observation that phytoplankton assemblages increase the xanthophyll-pool size rapidly to variation in ambient irradiance on a time scale of hours to days (Casper-Lindley and Björkman 1998; Moisan et al. 1998; Fujiki et al. 2003). Van Leeuwe et al. (2008) revealed that larger xanthophyll-pool sizes appeared in microphytobenthic communities in southern European estuaries, which might be explained by that higher incident irradiance induced the synthesis of a constitutively larger xanthophyll pool. Moreover, the significant increase of Vx in the *Cosmarium* strains from the tropical zone, when VDE was blocked by DTT prior to the photoinhibitory irradiance, furthermore confirmed the potent *de novo* synthesis of xanthophylls during the high light influence in these strains.

It has been previously found that the development of macro- and microalgal species at different depths below the water surface and their geographic distribution are associated with their ability to adapt to certain light conditions (Lüning 1981, 1990; Lobban and Harison 1994; Rodrigues et al. 2002; Cardol et al. 2008; Goss and Jacob 2010). The Prasinophycean species from estuarine areas (e.g. high-light adapted *Ostreococcus* strains), showed a significantly increased Vx-cycle pigment pool accompanied by a higher Vx de-epoxidation and a stronger NPQ, in contrast to the deep-sea ecotype of *Ostreococcus* (Six et al. 2009). Similarly, several diatomologists (Meyer et al. 2000; Dimier et al. 2007; Lavaud et al. 2007) noted that a

relationship between NPQ capacity (including both violaxanthin- and diadinoxanthin-cycle dependent photoprotection) and diatom diversity in ecosystems differs not only in their seasonal distribution, but also in their geographic habitats. Therefore, the NPQ capacity of microalgal species has been regarded as an adaptive response to ecological conditions and as an indicator of their photoprotective strategy (Lavaud et al. 2007; Dimier et al. 2009).

To the authors' knowledge, this study represents the first comparative report on differences of the xanthophyll composition of desmid strains which arose as a consequence of different climate (light) dominance at their sites of origin, despite the long-term acclimation. Taking into account the photosynthetic pigment composition and the rapid xanthophyll-cycle turnover during photoinhibitory PAR irradiances, it is apparent that all of the *Cosmarium* strains are fairly equipped to cope with sudden and strong light intensities which may occur in their environment. As desmids are typical inhabitants of various shallow freshwater habitats, the sun-type adaptations of the photosynthetic pigment composition as well as the rapid action of the xanthophyll cycle are expected.

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

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Abstract

Photoinhibitory effects of ultraviolet radiation (UVR) on four *Cosmarium* strains were studied with respect to the their geographic distribution pattern. Physiological characteristics of the strains during and after various UVR spectral combinations at two temperature gradients were determined by fluorescence and oxygen evolution rates and with an inhibitor of chloroplast-encoded protein synthesis (streptomycin). In accordance with the UVR prevailing at their sampling sites, all of the *Cosmarium* strains investigated exhibited consistent geographic distribution patterns, despite a long-term cultivation under constant laboratory conditions. It appeared that moderate ultraviolet-B radiation (UVBR) did not exert large damages to PSII in all of the *Cosmarium* strains, compared to ultraviolet-A radiation (UVAR) treatment at 21°C. Strikingly enough, an ameliorating effect of UVBR at 21°C was observed in the typical tropical species, *C. beatum*. This is concluded from higher rates of recovery of maximum quantum yield after moderate UVBR treatment, compared to that after UVAR application. UVBR did not trigger the production of mucilaginous envelopes of the *Cosmarium* strains; however, intensive solar radiation (i.e. increased PAR) may induce the production of mucilage of desmids. Concomitantly with this observation, mucilage of desmids has rather a limited role in the protection against UVR, as demonstrated by the measurements of absorption in the UVR range. Increased UVB (i.e. high UVBR:PAR ratio) severely decreases oxygen evolution in all of the *Cosmarium* strains, which may indicate drastic consequences to peat bogs which are particularly poor in oxygen, as native habitats of desmids.

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

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, NF (no filter) – unfiltered radiation of a sun simulator, PSII – photosystem II, Fv/Fm – maximum quantum yield of PSII, SM – streptomycin, PFR – photon flux rate, PQ – plastoquinone

Introduction

Earlier studies demonstrated that desmid strains are capable to occupy specific geographic patterns regarding temperature or PAR regimes (Stamenković and Hanelt 2011, 2012) – an approach that has also been shown for numerous kelp species (Lüning 1981, 1990; Lobban and Harrison 1994; Hanelt et al. 2003). The previous findings initiated the investigation if UV radiation (UVR; 280–400 nm), as a limiting factor, may also have an influence on the possible geographic pattern of desmids.

So far, extensive investigations on UVR-induced geographic and depth distribution patterns of seaweeds have been performed (summarised by Bischof et al. 2006), revealing that majority of macroalgae occupy specific ecological niches in accordance with their resistance under UVR. Although numerous investigations of UVR effects on microalgae have been performed at ecosystemic, physiological and ultrastructural levels (Harrison and Smith 2009; Holzinger and Lütz 2006; Häder et al. 2007), comparative studies on the impacts of UVR on the possible geographic pattern of microalgae have been a topic of only a few investigations (Helbling et al. 2001, Bouchard et al. 2005; Doyle et al. 2005; Halac et al. 2010; Williamson et al. 2010). Except for a few cosmopolitan representatives, desmids are principally known for their preference for specific habitats and climatic regions (Coesel 1996; Spijkerman and Coesel 1998; Coesel and Krienitz 2008). 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. Therefore, these facts provoked the study to unveil if 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*. In addition, 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 (Brook 1981; Coesel and Meesters 2007). Taking into account that the *Cosmarium* strains produce a vast amount of mucilage (Stamenković and Hanelt 2011) which is hypothesised to have a role in screening cells against UVBR as it has been observed in cyanoprokariota (Garcia-Pichel and Castenholz 1991; Singh et al. 2010), the measurements on the absorption of mucilage in the UVR range are performed to explore this assumption.

Conjugatophycean algae represent abundant and frequently predominant organisms in shallow freshwater habitats and are exposed to intensive and varying UVR conditions. 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 (Häder 1987). On the other hand, freshly isolated *Micrasterias denticulata* Brébisson ex Ralfs demonstrated a marked resistance against UVR wavelengths down to 284 nm (Meindl and Lütz 1996; Lütz et al. 1997).

Although one of the main effects of UVR on algae is photoinhibition (Cullen et al. 1992; Helbling et al. 1992; Holm-Hansen et al. 1993; Helbling et al. 2001), 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 PAR and the

UV wavebands do not contribute significant energy supply for photosynthetic chemistry (Hanelt and Roleda 2009). 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 (Flores-Moya et al. 1999; Hanelt et al. 2006; Hanelt and Roleda 2009). Hence, 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.

As UVR increases with increasing solar intensity it is assumed that the *Cosmarium* strains may develop a considerable *de novo* protein synthesis under UVR stress – as observed in so-called 'sun-plant' strategists (Raven and Samuelsson 1986; Öquist et al. 1992), which has been estimated by an inhibitor of chloroplast-encoded protein synthesis (Schnettger et al. 1994; Häder et al. 2002). Considering that the *Cosmarium* strains are particularly sensitive to temperature decrease (Stamenković and Hanelt 2012) the UVR photoinhibitory treatments were done at both permissive and cold temperatures, to observe the protective strategies of geographically different strains when both of the stressors were present.

Materials and methods

Algal strains and culture conditions

The four *Cosmarium* clones examined in this study were isolated from various parts of the world within approximately the same time period, in order to exclude the influences of sampling time and, therefore, the influences of constant nutrient, light and temperature regime under laboratory conditions:
C. crenatum Ralfs var. *boldtianum* (Gutwinski) W. & G.S. West (SVCK No. 561) – isolated 1995; polar (arctic) climate zone (Cape Flora, Northbrook Island, Franz Joseph Land, Russia), 79°57′N 50°05′E;
C. beatum W. & G.S. West (SVCK No. 533) – isolated 2001; tropical climate zone (marshy area near Ol Bolossat Lake, Kenya), 00°09′S 36°26′E;
C. punctulatum Brébisson var. *subpunctulatum* (Nordstedt) Børgesen (SVCK No. 570) – isolated 1996; alpine, tropical area (pool on Mt. Cotopaxi at 1600 m a.s.l., Ecuador; 'highlands' subgroup of the temperate (mesothermal) climatic group – Tierra templada), 00°40′S 78°26′W;
C. punctulatum var. *subpunctulatum* (SVCK No. 571) – isolated 1992; lowland, polar climate (pool near Skarsvåg at 80 m a.s.l, the North Cape, Norway), 71°06′N 25°49′E.

The details on taxonomic, ecological and distributional attributes of the investigated taxa, as well as the climate characteristics of the algal sampling locations are published elsewhere (Stamenković and Hanelt 2011).

All of the investigated *Cosmarium* strains were grown under ordinary laboratory conditions (16° C; ~30 µmol photons m^{-2} s^{-1}) in a climate chamber of the SVCK (Sammlung von Conjugaten-Kulturen) collection over a period of several years. Taking into account that 16°C was a sub-optimal temperature for the tropical species (*C. beatum*) (Stamenković and Hanelt 2011), while temperatures above 22 or 25°C were

recognised as sub-optimal for polar microalgal representatives (Fiala and Oriol 1990; Suzuki and Takahashi 1995), all of the experimental *Cosmarium* strains were pre-cultured at 21°C, which was considered as a roughly compromising optimal temperature for both of the tropical and polar strains studied. All of the investigated strains were grown in a mineral medium based on Kattner et al. (1977) (L-d medium). The *Cosmarium* strains had been acclimated to 21°C and L-d medium at least 12 months before the photoinhibitory experiments began (at a daily light regime of 14 h of light and 10 h of darkness). Sterilised 1 l Erlenmeyer flasks with 500 ml of medium were inoculated to a final concentration of 1500 cells ml^{-1}. Cultures were bubbled with humidified air at a rate of about 10 l h^{-1} to prevent CO_2 limitation. The cultures were mixed regularly by means of a magnetic stirrer to prevent self-shading of cells. Another set of inoculated Erlenmeyer flasks was grown 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 – 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 et al. (2006). 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 irradiance coming from a stabilised 400 W Metallogen lamp (Philips MSR 400 HR) consisting of a number of lanthanide which emanate 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 at 21°C had no large damaging effect to the *Cosmarium* strains studied, as judged from the relatively small depression of Fv/Fm during 6 h treatment, this irradiance was selected as a 'background' for the study of effects of UVR spectral combinations. To investigate effects of PAR and/or UV radiation 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 1). PAR and UV irradiation 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 (1981). 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, FRG). 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

program. 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) (LI-COR Biosciences, USA) for PAR measurement; and, 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 erythermal-weighted energy (µW cm^{-2}). Data were converted to unweighted UVB irradiance according to McKenzie et al. (2004).

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

Table 1. Irradiation conditions of the different spectral ranges of the sun simulator with the optical filters WG295, WG320, GG400 and UG5 and without filters.

Desmids samples were exposed to the spectral combinations in a 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 ml^{-1} by adding a quantity of thermally adjusted L-d medium. For the samples treated with streptomycin, L-d medium was enriched with a respective concentration to obtain the desired concentration (see below). Immediately after sampling, the algal suspension was subjected to 3 min of dark adaptation in a water bath at the experimental temperature and filled into 5 ml Quartz cuvettes. A pulse of weak, far red light was applied to oxidise the plastoquinone pool. The maximum quantum yield (Fv/Fm) was measured at time zero as described by Hanelt (1998). Initial fluorescence (Fo) was measured with red measuring light (~0.3 µmol photons m^{-2} s^{-1}, 650 nm), and maximal fluorescence (Fm) was determined using 600 ms of completely saturating white light pulse (~3500 µmol photon m^{-2} s^{-1}). To eliminate the possible handling effect due to repeated measurements, chlorophyll fluorescence was also measured in time zero control at time-series in synchrony with recovery of treated samples, and designated as a disturbed control. Another set of controls in parallel to each replicate

was separately prepared and cultured at 21 or 7°C and 30 µmol photons m^{-2} s^{-1}, and designated as undisturbed controls. Photosynthetic efficiency of undisturbed controls was measured at the end of the recovery period (24 h) of the experiment. Time-series recovery in maximum quantum yield of the *Cosmarium* strains after exposure to different spectral irradiance was expressed as a percent recovery of the disturbed control.

Inhibitor studies

To assay the influence of chloroplast-encoded protein synthesis on the photoinhibition degree, streptomycin (SM) (Sigma, Germany) was added to samples 1 h before photoinhibitory experiments started. The final concentration of SM in samples was 20 µg ml^{-1}; 0.5% ethanol was used to enhance the absorption of the antibiotic (Han et al. 2003). This concentration had no effects on fluorescence parameters of the *Cosmarium* strains exposed to dim white light, at 21 and 7°C (control experiments). Absorptions of UV radiation by L-d medium and SM solution, measured by a UVPC-2101 UV-VIS spectrophotometer (Shimadzu, Japan), were negligible (data not shown).

Oxygen evolution

In synchrony with measurements of maximum quantum yield, the changes in oxygen production were measured using a fiber-optic oxygen meter – Presens Fibox 3 (Precision Sensing GmbH, Germany), attached to a PC with the software OxyView PS3. After different times of photoinhibition, 5 ml of the homogenised 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 certify saturating carboxylating conditions. The concentration of oxygen in the cuvettes before each measurement was around 10% of saturation level, and the cuvettes were tightly closed by rubber stoppers during measurements. Light source was a projector equipped with a halogen lamp (Xenophot, Osram, Germany), and samples were irradiated with 100 µmol photons m^{-2} s^{-1}, using red measuring light (650 ± 20 nm). The measurements lasted 10 min, until a steady state level of oxygen evolution was achieved. The sample was continually stirred by means of a small magnetic bean during measurements. The temperature of the sample (21 or 7°C) was maintained by means of a thermostated water jacket. Oxygen evolution of each non-photoinhibited control was set to 100% and the degrees of photoinhibition after different treatments were standardised to these controls.

Mucilage thickness and absorption of PAR and UV radiation

Thickness of the mucilaginous cell sheath was measured microscopically in fifty randomly chosen cells in cell suspension stained by Indian ink, measured at the cellular apex. Measurements were done for control (non-exposed) samples and after all of the spectral combinations (6 h exposure) at 7 and 21°C. To

investigate the absorption of UV radiation of mucilaginous layers of desmids 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 PAB and AB spectral combinations. 10 ml of a treated 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 homogenised 5 min by means of a small laboratory shaker. Afterward the filtrates were placed in 5 ml quartz cuvettes and UV absorption was estimated by means of a UVPC-2101 UV-VIS spectrophotometer (Shimadzu, Japan).

Statistical analysis

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 of disturbed and undisturbed controls. Photosynthetic responses to varying irradiance, exposure time and interaction effect were tested using the multiple analyses of variance (MANOVA). 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. The three-factor (factorial between subjects) ANOVA was used to estimate differences in Fv/Fm of SM-treated *Cosmarium* strains (expressed as % of control) between two temperature degrees (7 and 21°C) and spectral irradiances (PA, PAB, AB, and NF). The one-factor between subjects (one-way) ANOVA was applied to find differences in the thickness of mucilaginous layers of the *Cosmarium* strains treated under various spectral irradiances compared to that of untreated samples. Differences between the strains and UVR spectral compositions with regard to changes of the mucilage thickness were estimated by the Tukey HSD post-hoc test. Degrees of freedom (for the effect of the model and residuals of the model) are presented in parentheses for ANOVA results. All of the statistical analyses were conducted using the SPSS program (SPSS, Chicago, IL; USA).

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).

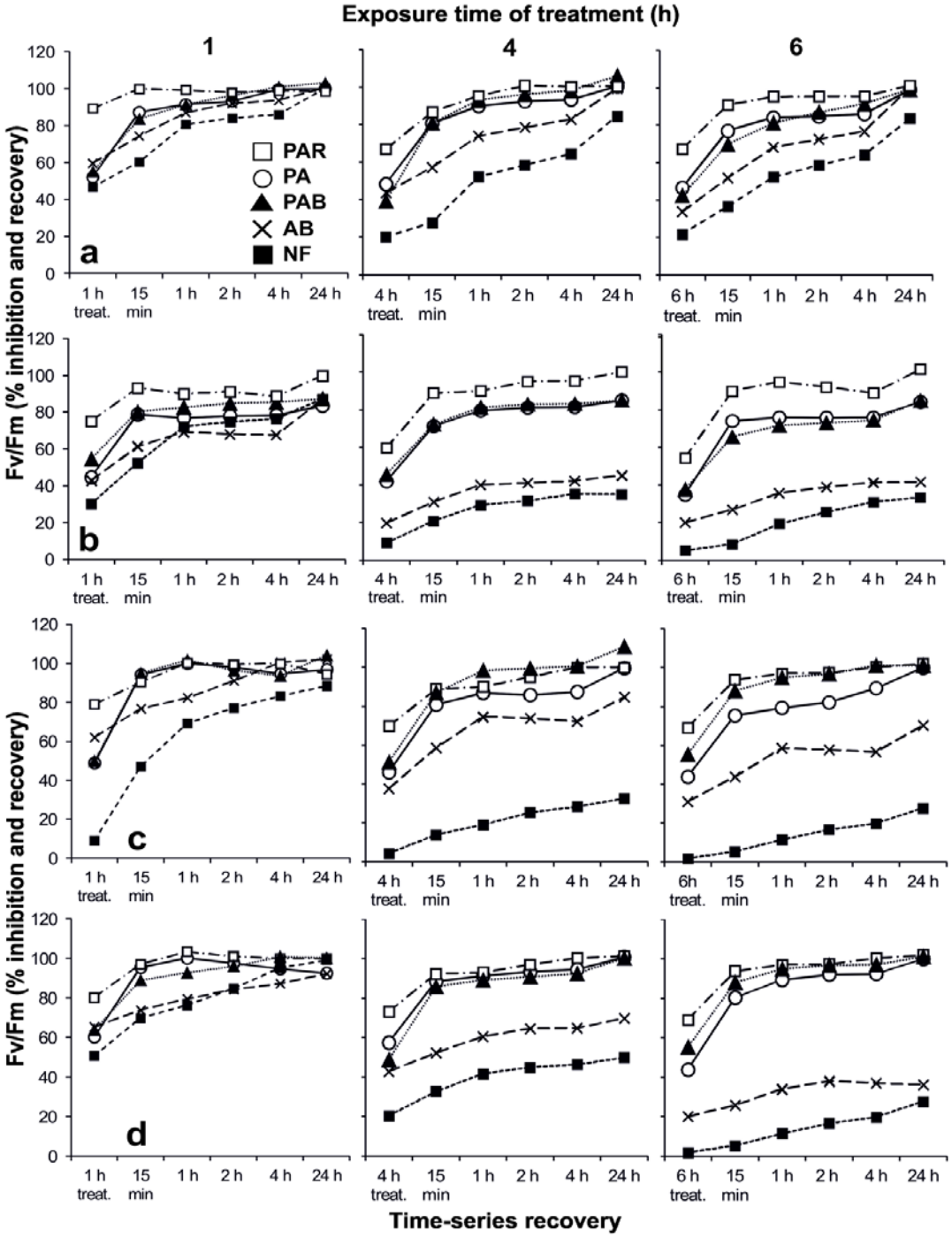

Fig. 1. Inhibition and time-series recovery in the mean of maximum quantum yield (Fv/Fm) of the *Cosmarium* strains collected from various geographic areas, after exposure to photosynthetically active radiation (700 µmol photons m^{-2} s^{-1}, PAR = P), PAR + UV-A (PA), PAR + UV-A + moderate UV-B (PAB),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

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. Short (1 h) PA, PAB and AB treatments caused inhibition of around 50% of the control value, after which full recovery was achieved within 4 h (for PA and PAB treatments), or after 24 h (for AB and NF treatments). Remarkably, the high-mountain strain of *C. punctulatum* showed a complete recovery of Fv/Fm within 24 h even after the prolonged (6 h) stress under PA, PAB and AB spectral combinations, while the recovery after NF treatment achieved 84% – the highest recovery value for this treatment among all of the *Cosmarium* strains studied. Conversely, even short-term treatments under any of UVR combinations provoked an incomplete recovery of the polar strain of *C. punctulatum* (No. 571, Fig. 1b). There was no difference between PA and PAB treatments, both with regard inhibition and recovery, in both of the strains of *C. punctulatum* (for all of the treatment times).

The typical tropical species, *C. beatum* (Fig. 1c), achieved a full recovery of Fv/Fm within 1 h after the short-term PA and PAB applications; thus showing its insensitivity under moderate UVA and UVB radiation. Remarkably, the addition of a moderate UVB intensity (0.89 W m^{-2}) during 4 and 6 h treatments caused a faster recovery of Fv/Fm, compared to that after the only UVA (PA) application. The arctic taxon, *C. crenatum* var. *boldtianum* (Fig. 1d), demonstrated a rather low sensibility under 1 h PA and PAB treatments, as concluded from a small depression of Fv/Fm during these treatments (around 60%), after which a full recovery was attained within 1 h or 4 h for PA and PAB treatments, respectively. This species appeared rather sensitive under 6 h AB treatment, as concluded from the strong Fv/Fm decrease (20%) which caused rather limited recovery. The maximum quantum yield dropped down to zero during 6 h NF application in both tropical and arctic species, after which recovery reached only up to around 26%.

Table 2. Multiple analysis of variance (MANOVA) and significance values for the main effects and interactions of radiation treatment (spectral irradiance compose of P, PA, PAB, AB, and NF) and exposure time on photosynthetic efficiency of the *Cosmarium* strains studied, grown at 21°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. punctulatum (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. punctulatum (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*

Multiple analyses of variance (MANOVA) demonstrated significant effects of irradiance and exposure time at 21°C for all of the strains studied (Table 2). Interactions of these variables were significant for *C. punctulatum* No. 571 and *C. crenatum*.

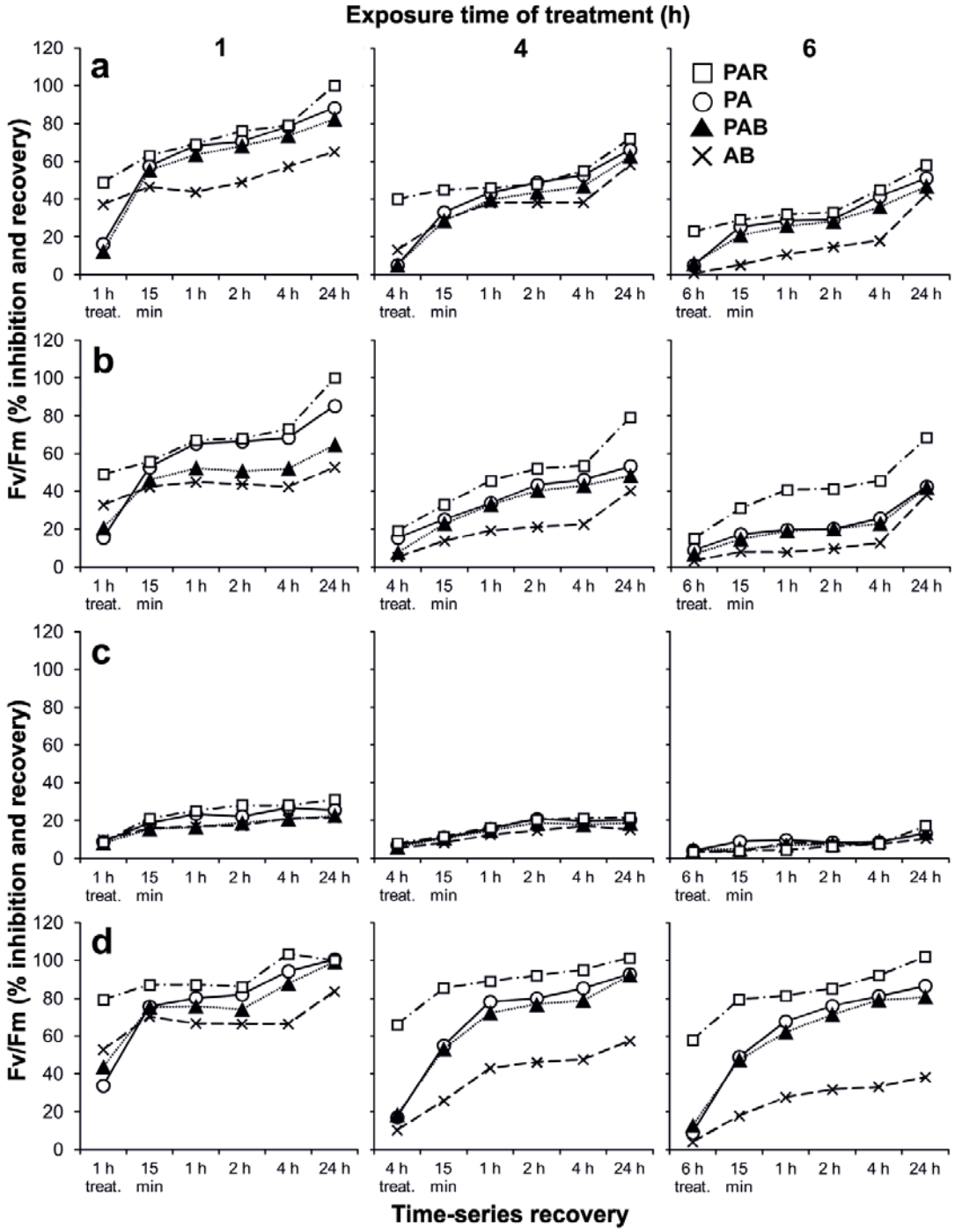

Fig. 2. Inhibition and time-series recovery in the mean of maximum quantum yield (Fv/Fm) of the *Cosmarium* strains collected from various geographic areas, acclimated and treated at 7°C. (**a**) *C. punctulatum* No. 570, (**b**) *C. punctulatum* No. 571, (**c**) *C. beatum*, (**d**) *C. crenatum*. For explanations, refer to Fig. 1.

UVR caused deeper inhibition of maximum quantum yield in all of the *Cosmarium* strains pre-acclimated at 7°C (Fig. 2). The typical arctic taxon, *C. crenatum* (Fig. 2c), displayed relatively high resistance to PA and PAB combinations during 1 h treatment at 7°C, achieving a complete recovery after 24 h. Longer treatments under these spectral combinations caused a marked inhibition of Fv/Fm (below 20%), causing an incomplete recovery for this taxon. Both of the strains of *C. punctulatum* (Fig. 2a, b) showed noticeably stronger inhibition when treated under PA or PAB at 7°C for all of the treatment times, compared

to that at 21°C. Maximum quantum yield of *C. beatum* (Fig. 2c) rapidly decreased under all of the UVR treatment combinations at 7°C, indicating severe cell damages of this tropical species.

Table 3. Multiple analysis of variance (MANOVA) and significance values for the main effects 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. punctulatum (570)	Spectral irradiance (*A*)	3	176.3	<0.001*
	Exposure time (*B*)	2	124	<0.001*
	*A*B*	6	96.9	<0.001*
C. punctulatum (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*

MANOVA demonstrated significant effects of UV spectral combinations and exposure time in both of the strains of *C. punctulatum* and *C. crenatum*, which were pre-acclimated and treated at 7°C (Table 3). However, UV spectral combinations and treatment times had not a significant influence on the photosynthetic behaviour of *C. beatum* pre-acclimated at 7°C.

Oxygen evolution

The average O_2 production of the *Cosmarium* strains studied (expressed in µMolO_2 mg Chl^{-1} min^{-1}) grown at 7 and 21°C is shown in Table 4. Acclimation at 7°C decreased oxygen evolution in all of the *Cosmarium* strains; yet, *C. crenatum* exhibited the highest oxygen production at this temperature.

Table 4. Average O_2 production at 100 µmol photons m^{-2} s^{-1} (650nm) of the *Cosmarium* strains studied, grown at 21°C or acclimated at 7°C (30 µmol photons m^{-2} s^{-1}). Standard deviations (SD) are less than 5% of mean (n = 3).

	Average O_2 production (µMolO_2 mg Chl^{-1} min^{-1})			
Temperature	*C. punct.* (570)	*C. punct.* (571)	*C. beatum*	*C. crenatum*
21°C	2.47	2.51	2.48	3.49
7°C	1.39	1.6	0.28	2.87

Concomitantly with measurements of Fv/Fm during the time-series of inhibition and recovery, oxygen evolution was measured. In order 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 et al. 1997). Oxygen and Fv/Fm values measured during inhibition under UVR treatments were expressed as percents of controls (for both temperature grades) and these percentages were plotted for all of the spectral combinations (Fig. 3).

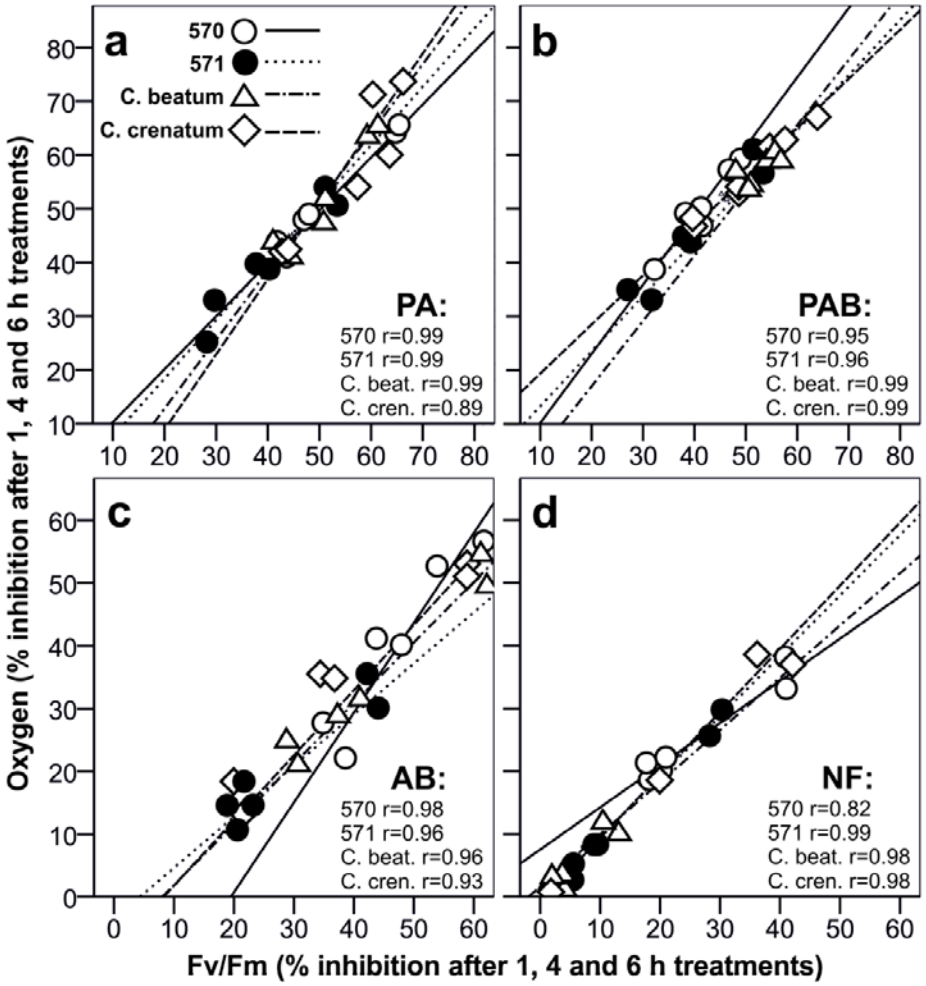

Fig. 3. Relationships between gross oxygen evolution rates and Fv/Fm values (expressed as % of control samples) regarding inhibitions under PA, PAB, AB and NF spectral combinations at 21°C, for all of the *Cosmarium* strains: (**a**) PA (**b**) PAB (**c**) AB (**d**) NF treatment. ○ – *C. punctulatum*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

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 (0.58 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 application of 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). Still, oxygen evolution rates during both NF and AB treatments fitted well to changes of Fv/Fm, when the *Cosmarium* strains were stressed at 21°C (Fig. 3c, d).

Relationships between total oxygen evolution rates and Fv/Fm for the *Cosmarium* samples treated under UVR spectral combinations at 7°C were significantly positive in 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. Measured values were expressed as percents of controls; afterwards Fv/Fm percentages of SM-treated samples were subtracted from that of untreated samples. These differences (for 1 and 6 h treatment-times at 21°C) are shown in Fig. 4.

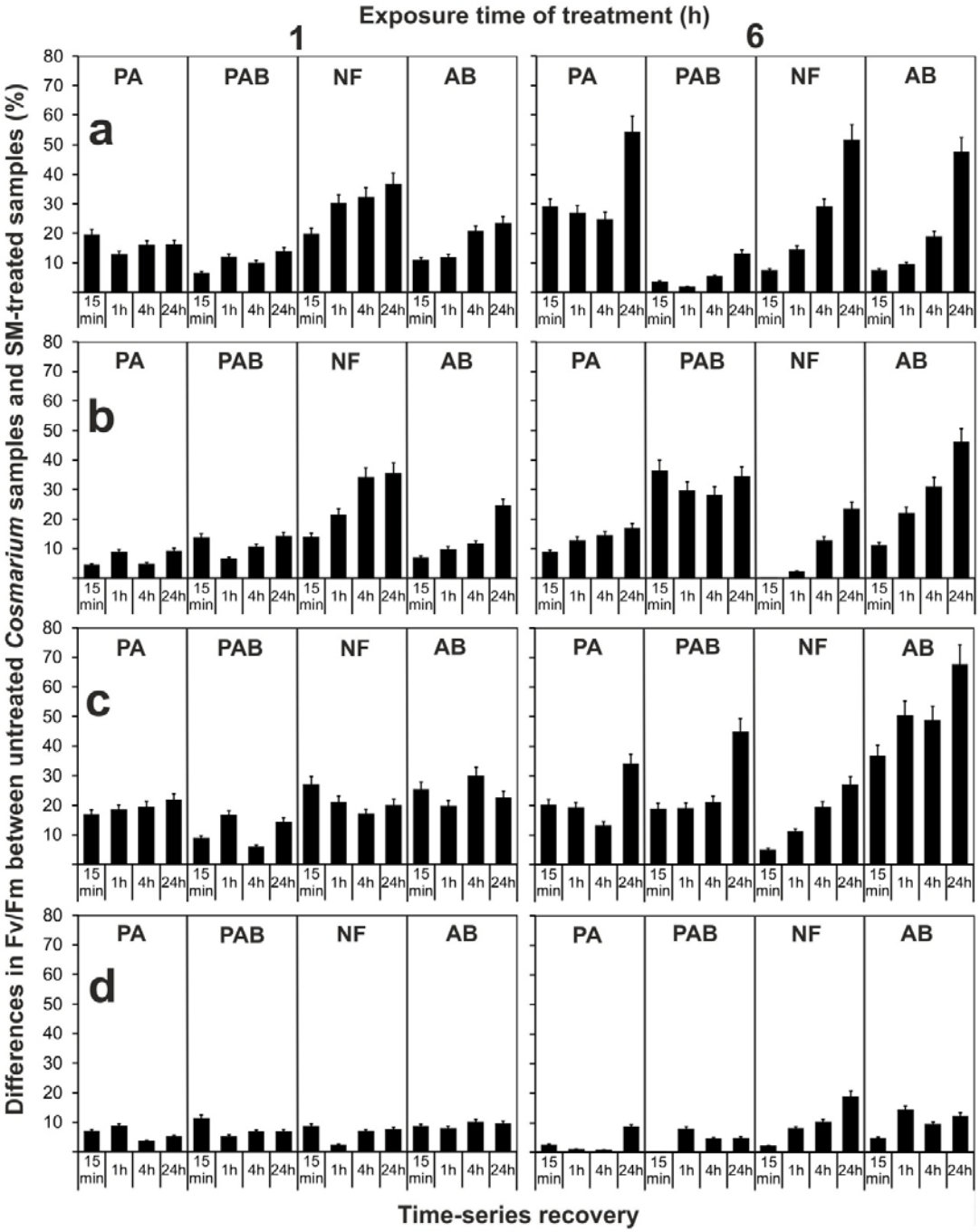

Fig. 4. Differences in Fv/Fm between untreated samples and SM-treated *Cosmarium* samples (expressed as percents of controls) during the period of recovery after all of the UVR spectral combinations (PA, PAB, NF, and AB), at 21°C. (vertical bars – SD, n = 3). (**a**) *C. punctulatum* No. 570, (**b**) *C. punctulatum* No. 571, (**c**) *C. beatum*, (**d**) *C. crenatum*.

All of 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, as it was previously found that SM-induced inhibition of *de novo* synthesis of chloroplast-encoded proteins was correlated with increase of inhibition in several SM-treated plant samples (Schnettger et al. 1994).

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 application under PAB 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), showing an ameliorating effect of UVBR on the rate of *de novo* protein synthesis in this strain. 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 of this strain under PAB. Yet, the 6 h NF treatment appeared rather detrimental to protein synthesis in both strains of this cosmopolitan species, as concluded from the slight inhibition of *de novo* protein synthesis in SM-treated samples.

The typical tropical species, *C. beatum* (Fig. 4c), showed markedly high Fv/Fm depressions of SM-treated samples compared to that of untreated samples under all of UVR treatments, thus pointing to the distinctly high protein synthesis during recovery. On the contrary, the arctic species, *C. crenatum* (Fig. 4d), displayed minor Fv/Fm inhibition in SM-treated samples compared to that of untreated samples, for all of the treatment times and UVR spectral combinations. This pointed to a noticeably small degree of 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 significant higher 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 (or none) *de novo* protein synthesis occurs at the low temperature in these strains, whereas only minor *de novo* protein synthesis rates were observed in *C. crenatum* (data not shown).

Three-way (between subjects) ANOVA revealed that differences in Fv/Fm percentages (obtained during recovery) of the investigated strains varied significantly beyond 0.05 level, after the application of SM at two temperature grades (strain factor): $F(3, 98) = 62.1$, $p < 0.001$. Spectral UVR combinations and temperature grades significantly influenced on the differences in Fv/Fm percentages for all of the *Cosmarium* strains: (UVR treatment factor) $F(3, 98) = 32.2$, $p < 0.05$; (temperature factor) $F(1, 98) = 46.5$, $p < 0.001$.

Mucilage production and UVR absorption

Acclimation of the *Cosmarium* cultures at 7°C caused an increase of the mucilage thickness in all of the strains studied (Fig. 5). In addition, 6 h PA treatment caused a significant increase of the mucilage

thickness in the high-mountain strain of *C. punctulatum* and *C. beatum*, while PAR (700 µmol photons m^{-2} s^{-1}) and PAB increased the mucilage thickness in all of the desmid strains. These observations pointed to rather low damaging effects of moderate UVA and UVB treatments on the mucilage production in the *Cosmarium* strains. In contrast, both NF and AB treatments significantly decreased the thickness of the mucilage envelope in both of the polar strains, *C. punctulatum* No. 571 and *C. crenatum*.

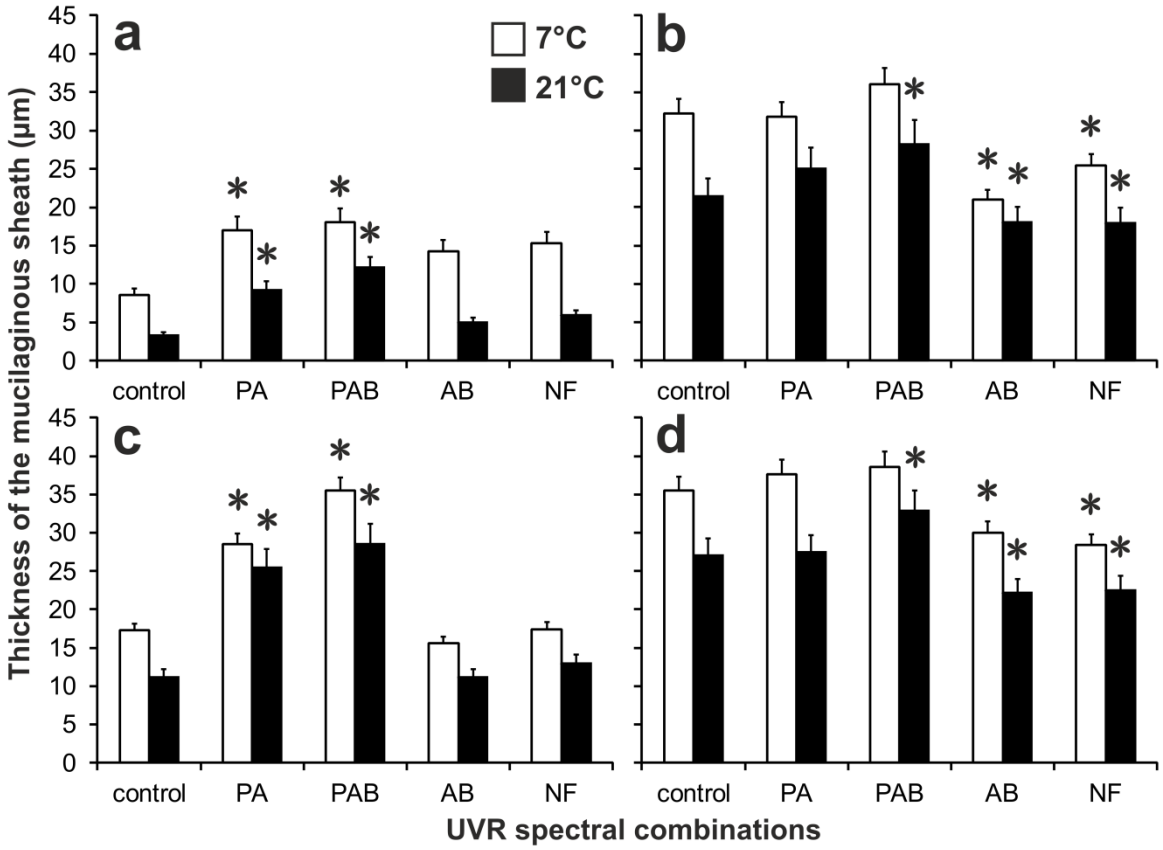

Fig. 5. Changes of the mucilage thickness after 6 h under PAR (700 µmol photons m^{-2} s^{-1}) and UVR treatments (PA, PAB, AB, and NF) at 7 and 21°C (vertical bars – SD, n = 3). (**a**) *C. punctulatum* No. 570, (**b**) *C. punctulatum* No. 571, (**c**) *C. beatum*, (**d**) *C. crenatum*. Asterisks represent significant differences in the mucilage thickness compared to control samples (Tukey HSD test, $p < 0.05$).

Results of the one-way ANOVA test displayed significant changes (decreases or increases) of the thickness of the mucilaginous sheath after the UVR spectral treatments (PA, PAB, NF, and AB) in all of the *Cosmarium* strains investigated: *C. punctulatum* No. 570 $F(17, 68) = 86.3$, $p < 0.001$; *C. punctulatum* No. 571 $F(17, 68) = 67.2$, $p < 0.001$; *C. beatum* $F(17, 68) = 76.9$, $p < 0.001$; *C. crenatum* $F(17, 68) = 86.3$, $p < 0.001$.

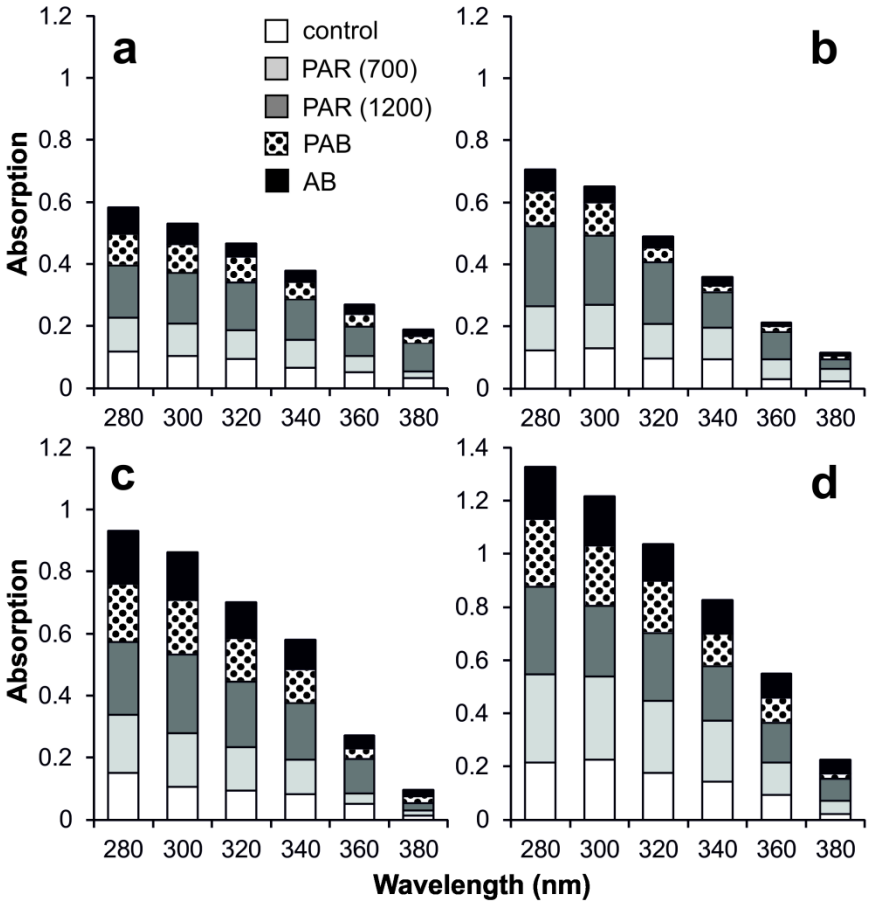

Fig. 6. Absorption of UVA and UVB radiation spectrum (280 – 400 nm) by isolated mucilaginous envelopes of the *Cosmarium* strains treated 6 h under a set of PAR (700 and 1200 µmol photons m^{-2} s^{-1}) or PAB and AB spectral combinations, at 21°C. (**a**) *C. punctulatum* No. 570, (**b**) *C. punctulatum* No. 571, (**c**) *C. beatum*, (**d**) *C. crenatum*.

All of the *Cosmarium* strains grown at 21°C exhibited a relatively low UV absorption by their mucilaginous envelopes in the range 280 – 400 nm, pointing to an insignificant role in the protection from UV radiation (Fig. 6). Absorption was larger in the UVB range (280 – 320 nm), while it decreased greatly towards longer wavelengths. UVR absorption increased concomitantly with the increase of the thickness of a mucilaginous layer in all of the *Cosmarium* strains treated under photoinhibitory PAR intensities (700 and 1200 µmol photons m^{-2} s^{-1}) (Figs 5 and 6). Additionally, PAB treatment caused a slightly higher UVR absorption compared to that of control samples, while AB treatment appeared detrimental to the mucilage development in all of the *Cosmarium* strains, leading to the decrease of UVR absorption.

The UVR absorption of mucilaginous shields of all of the *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

UVR exerted an additional stress on the photosynthetic apparatus of the *Cosmarium* strains when compared to the relatively moderate PAR intensity (700 µmol photons m^{-2} s^{-1}). Consistently with the

observed geographic distribution patterns of the *Cosmarium* strains regarding temperature or PAR regimes (Stamenković and Hanelt 2012) the impact of experimentally applied UVR on desmid physiology furthermore revealed that the *Cosmarium* strains prefer specific ecological niches regarding the prevailing solar radiation.

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 (Grzymski et al. 2001) affecting electron transport both at the water-oxidizing complex and the binding site of the Q_B quinone electron acceptor (Turcsányi and Vass 2000). 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 more than UVB, and UVA is not attenuated by the ozone layer (Holm-Hansen et al. 1993; Dring et al. 1996; Figueroa et al. 2003). 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 (Holm-Hansen et al. 1993; Helbling et al. 2001).

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 (Häder et al. 2002; Gao et al. 2008; Hanelt et al. 2009). Generally, this treatment did not provoke larger damage to PSII compared to that during PA in all of the *Cosmarium* strains studied, as concluded from approximately the same range of inhibition and recovery of PSII at 21°C. Although UVBR has stronger detrimental effects on the photosynthetic apparatus than UVAR (Vass et al. 2005), it has been observed that protein repair capacity in intact cells is enhanced when UVB is accompanied by a moderate intensity of visible light and provides protection against photodamage (Teramura et al. 1980; Jordan et al. 1991; Dring et al. 2001; Figueroa et al. 2003). This UVBR-induced positive effect becomes non significant at high light intensity characteristic of strong sunlight (Sicora et al. 2003).

Strikingly enough, moderate UVB radiation caused ameliorating effect to photosynthesis of the tropical species, *C. beatum*. The UVB ameliorating phenomenon is demonstrated by the lower Fv/Fm recovery kinetics under PA- compared to PAB-treatment (Hanelt and Roleda 2009), as it was noted in *C. beatum*. So far, this phenomenon was described in a few macrophytes that had been previously adapted to a high UV environment and the studies were conducted at high PAR and UV ratio (Flores-Moya et al. 1999; Hanelt et al. 2006; Hanelt and Roleda 2009). Taking into account that the ameliorating effect of UVBR was exclusively found in the typical tropical *Cosmarium* species, this study furthermore confirmed the previous observations on macroalgae. Possibly, this phenomenon might 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

(Hanelt and Roleda 2009). Máté et al. (1998) 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. In contrast to our study and observations on high-light adapted macrophytes, Thomson et al. (2008) observed positive effects of intermediate fluxes of UVBR on some Antarctic microalgae, which pointed that this interesting phenomenon should be thoroughly investigated.

However, unfiltered radiation from a sun simulator considerably increased UVBR intensity (1.98 W m^{-2}) and UVBR:PAR ratio (Table 1), which reached more than a twofold value compared to that of temperate or (sub)tropic zones (Lesser 1997; Häder et al. 2002; Gao et al. 2008; Hanelt and Roleda 2009). 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 (Richter et al. 1990), direct molecular damage by absorption by aromatic and sulfhydryl-containing biomolecules (Vass 1997), DNA lesions (Sinha and Häder 2002) and induction of reactive oxygen species (Nishiyama et al. 2006), and so apparently this treatment provoked multiple damages to the *Cosmarium* cells.

Streptomycin binds to the small 16S rRNA of the 30S subunit of the prokaryotic ribosome, interfering with the binding of formyl-methionyl-tRNA to the 30S subunit. This leads to codon misreading and inhibition of protein synthesis (Sharma et al. 2007); hence, SM is commonly used as an inhibitor in the estimation of the turnover of the chloroplast-encoded protein synthesis (principally D1 protein) (Samuelsson et al. 1985; Häder et al. 2002). Schnettger et al. (1994) 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. The authors revealed a moderate positive correlation between the changes of Fv/Fm and relative D1 protein content in SM-treated and untreated plant species during and after the photoinhibitory PAR influence at permissive temperatures. 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 synthesis of other proteins damaged by reactive oxygen species (ROS), which may be produced during the UVR treatments.

The high-mountain, tropical strain of the cosmopolitan species, *C. punctulatum*, exposed the highest rate of *de novo* protein synthesis after the prolonged PA treatment and high protein synthesis rates under NF and AB treatments at 21°C, revealing a high reliance on the *de novo* protein synthesis under such spectral combinations. Remarkably, the high-mountain strain of *C. punctulatum* displayed the lowest *de novo* protein synthesis after the prolonged application of a moderate UVBR intensity (0.89 W m^{-2}; i.e. PAB treatment), compared to the other treatments (PA, NF, AB), which indicated a high resistance of this strain at moderate UVBR intensities. It has been observed 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 (Helbling et al. 2001) and possess fairly developed DNA- and

photosynthesis-repair mechanisms (Sancar and Sancar 1988; Mitchell and Karentz 1993). Still, 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 (Chaturverdi and Shyam 2000; Vass et al. 2005).

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 possess exceedingly high rates of D1 turnover and *de novo* protein synthesis (Öquist et al. 1992; Häder et al. 2002). The relatively high resistance to UV radiation, accompanied with potent DNA- and PSII-repair mechanisms and photoprotection strategies, is well-known characteristics of numerous macroalgae growing in (sub)tropic areas (Wood 1989; Van de Poll 2002; Häder et al. 2011; Lesser 2012).

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 to what was observed for shade-plant strategists and polar macroalgae (Öquist et al. 1991; Hanelt et al. 2003). Protein synthesis may 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 (Chow et al. 1989; Gong and Nilsen 1989; Aro et al. 1990). 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, considering that an increase in temperature results in faster metabolism activity and thus a fast turn-over of the D1 protein, as seen in many studies (Greer et al. 1986; Rae et al. 2000; Bouchard et al. 2005; Helbling et al. 2010).

The increase of the thickness of the mucilaginous sheath in all of the *Cosmarium* strains acclimated at 7°C and treated under 700 µmol photons m^{-2} s^{-1} could be explained by the fact that mucilage possibly has a regulatory function, to exclude a mass of excess photosynthates (Reynolds 1989). As growth of most desmids is limited at regimes of low temperature and moderate or high irradiance (Coesel and Wardenaar 1990), such conditions may result in the high production of photosynthates which could be excluded as mucilage through cell wall pores. Strong UVBR accompanied by moderate or low PAR intensity (i.e. high UVBR:PAR ratio) diminished the thickness of the mucilaginous layer in all of the desmid strains, pointing to severe damages of secretory organelles of the *Cosmarium* strains. Structural damages of dictyosomes and ER cisternae appeared in *M. denticulata* treated at low UVBR wavelengths (below 287 nm), while the production of mucilaginous secretory vesicles was reduced or completely inhibited (Meindl and Lütz 1996). Possibly, the prolonged and unfiltered UVBR of a sun-simulator caused the same effect to the *Cosmarium* cells.

Surprisingly, the *Cosmarium* strains do not place a high reliance on the protection against UVR by means of mucilaginous sheaths. Therefore, this study refuted earlier assumptions that the production of vast amounts of slime may elicit protective function leading to UVR tolerance in desmids (Meindl and Lütz 1996; Lütz et al. 1997). However, moderately high PAR intensities of solar radiation may trigger the extensive mucilage production, as well as the formation of brownish mucilaginous clumps inside cells (probably incrusted with minerals (Brook 1981)) which can also serve in the protection from UVR. The *Cosmarium* strains obviously produced no UVR-screening compounds inside cells or as components of the mucilaginous layer, although mycosporine-like amino acids (MAAs) have been detected in some Streptophycean green algae (Karsten et al. 2005).

In order to demonstrate the influence of UVR on the total energy balance of desmid cells, the measurements of oxygen evolution were not corrected for respiratory losses. The alterations of the gross oxygen evolution were significantly positively correlated with changes of maximum quantum yield, during application of all of the UVR spectral combinations. Turcsányi and Vass (2000) noted that the time course of variable fluorescence of isolated spinach thylakoids is much less affected by UVA than oxygen evolution; similarly to that observed with UVB radiation and opposite to that seen under photoinhibition by visible light (Vass et al. 1996). 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 significantly influence on respiration rates (also observed by Teramura et al. 1980), and/or damages of the electron transport occurred, considering that variable fluorescence reflects the capacity of PSII to reduce the PQ pool and Q_A (Turcsányi and Vass 2000).

The intensive UVBR from the unfiltered spectrum of the sun simulator (i.e. high UVBR:PAR ratio) caused a drastic decrease of oxygen evolution in all of the investigated *Cosmarium* strains, indicating that desmids are noticeably sensitive to increased UV radiation. Considering that each 1% reduction in ozone layer causes an increase of 1.3–1.8% in UVBR reaching the biosphere (Hollósy 2002), the amount of UVBR reaching the earth's surface may be particularly increased in polar regions due to the thinning of the ozone layer (Smith et al. 1992; Booth and Madronich 1994; Dahlback 2002; Kane 2008). This may have particularly negative consequences for desmids growing in circumpolar peat bog ecosystems, as this study revealed large detrimental effects of UVBR combined with cold temperature. Since desmids have a precious role as primary producers in peat bogs, which can be completely anoxic at the depth of a few centimetres (Keddy 2010), the destruction of such ecosystems may occur as a consequence of the increased UVR.

All of the *Cosmarium* strains exhibited the relatively high sensitivity under UVR spectral combinations during 4 and 6 h treatments, which was in contrast to the marked resistance of *M. denticulata* against strong UVBR (Lütz et al. 1997). It is worth emphasising that *M. denticulata* was cultured in a diluted 'desmid medium' with soil extract (Schlösser 1982) 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 are fairly protected in such habitats – which may explain the sensitivity of the *Cosmarium* strains under UVR 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 (Montecino and Pizarro 1995; Bischof et al. 1998, 1999; Figueroa and Gómez 2001). Yet, strain- and species-specific differences were exposed 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.

To the authors' knowledge this is the first comparative study on the influences of UVR as a climatic factor on the possible geographic distribution pattern 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, with regard to all of the life stages (Bischof et al. 2006). 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 (Fenchel 1993; Fenchel and Finlay 2004).

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8. Future perspectives

This preliminary study revealed that the *Cosmarium* strains collected from various geographic regions demonstrated different physiological behaviour which is in accordance with the climate prevailing at the site of the clonal origin. The species- and strain-specific differences were expressed under a set of varying temperature and radiation (PAR and UVR) conditions, demonstrating that the responses are genetically preserved (i.e. such physiological performances can be considered as adaptations – genotypic responses to long-term environmental changes). Acclimation under relatively low light-temperature conditions of a climate chamber affected more photosynthetic parameters than morphological or growth parameters.

Yet, further analyses, which will include more taxa of this cosmopolitan genus, are needed to supplement the previous observations. Taking into account that the genus *Cosmarium* includes over 1000 species, the extension of the study seems to be justified. In addition, investigations on the influence of temperature and radiation regimes on the possible geographic distribution patterns of desmids should concern representatives of the other cosmopolitan desmid genera such as *Closterium*, *Staurastrum* and *Micrasterias*. So far, there is only a limited amount of data on the photosynthetic behaviour of 'genuine' arctic-alpine desmids (i.e. snow desmids), such as *Mesotaenium berggrenii* and *Ancylonema nordenskioeldii*, which indicates that their physiological responses should be thoroughly investigated under a set of various temperature and radiation conditions *in vitro*. Concurrently, there are no data on the physiological behaviour *in vitro* of the typical tropical desmid representatives such as *Ichthyodontum*, *Ichthyocercus*, *Allorgeia*, and *Amscotia*. Taking these facts into consideration, further investigations on the temperature optima of desmids are necessary in order to supplement (or refute) the Coesel's hypothesis on the origin of desmids in the tropical zone.

The acclimation or even adaptation of microalgae in cultures should be studied in details, as it has been noted that the phenomenon of the so-called 'evolution in a bottle' may appear in microalgal (or bacterial) cultures due to the long-term subcultivation. This should be estimated by the comparison of physiology of old desmid isolates versus freshly isolated desmid strains under a set of permissive and stress laboratory conditions. A special attention should also be directed towards the mutual combinations of various temperature and radiation regimes, as well as nutrient composition of the algal medium. Furthermore, competitive abilities of desmid species in respect to the other microalgae should be examined *in vitro* conditions.

Role of the mucilaginous sheath and brownish mucilaginous clumps in desmids should be thoroughly investigated in numerous desmid taxa, as this study revealed that they might have a regulatory function (to exclude a mass of photosynthates) and in the protection against high light intensities.

Finally, taking into account that all of the *Cosmarium* strains exhibited distinctly high resistance under high PAR, moderate UVB radiation, and sudden temperature changes, this indicated fair protection strategies against stressors. It is of the interest to investigate the ROS-protection strategies by means of

carotenoids and antioxidants, which may include both non-enzymatic and enzymatic pathways for detoxification or scavenging of ROS. In connection with this, the appearance and role of cubic membranes and heat shock granules should be investigated in more details. So far, the presence of UVR-screening compound of tannin nature was exclusively determined in the desmid inhabiting snow surfaces, *Mesotaenium berggrenii*, but it is of the interest to explore if other desmids have similar phenolic compounds, which may have protective or antimicrobial properties. Possibly, some of the secondary products of desmids such as lipids, proteins, pectins, mucilage (as a source of simple carbohydrates) may be economically used, if specific and 'fastidious' demands of desmids are satisfied.

A famous phycologist, Dr. John Lund stated: "...desmids are very attractive green algae... but because they never upset fishing or offend the public, interest in them is considered to be 'academic' and without much 'cost benefit' (Lund 1973, cited in Brook 1981, see Introduction). Contrastingly, this preliminary study indeed demonstrated that desmids are still unexplored group of algae at physiological, ecological and taxonomic levels, but also at an economic view – as some of the desmid taxa could be potentially used for the mass production of relevant products for mankind.

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