Antarctic strain of green filamentous alga *Zygnema* sp. shows a high resistance to photoinhibition under simulated polar conditions

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Abstract

This study deals with treatment-dependent differences in sensitivity of Antarctic filamentous alga *Zygnema* sp. to photoinhibition. *Zygnema* sp. (strain EEL201) was collected at the James Ross Island, Antarctica (57° 52′ 57′′ W, 63° 48′ 02′′ S). In a laboratory, the alga was cultivated on agar first and then innoculated to liquid medium. They were exposed to a short-term (30 min.) high light (HL) treatments. Particular treatments comprised 600, 1 400 and 2 100 and 3 500 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR). Photosynthetic efficiency of *Zygnema* sp. in individual HL treatments was monitored by chlorophyll fluorescence parameters, potential (F_V/F_M) and actual (Φ_{PSII}) quantum yield of photochemical processes in photosystem II in particular. *Zygnema* sp. showed a high resistance to HL since it both chlorophyll fluorescence parameters recovered to about 70% of initial values after 4 h in dark. Chlorophyll fluorescence measured immediately after particular treatment, showed HL-dependent decrease in absolute values of chlorophyll fluorescence signal and consequent uncompleted recovery as well. Quenching of F_0, an indicator of changes in light-harvesting complexes of photosystem II, did not show dose-dependent response, however, general trend was a decrease found immediately HL treatment with consequent uncompleated recovery. In general, *Zygnema* sp. exhibited high resistance to PAR doses that the species can whitness in the field during austral summer. Thus the species could be considered highly adapted for high light and has effective mechanisms to cope with photoinhibition. Involvement of particular photoprotective mechanism, their activation and share in natural environment is a topic for future studies.

Key words: *Zygnema* sp., high light treatment, chlorophyll fluorescence, quantum yield, potential yield

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Introduction

Microalgae are widely known as aquatic organisms. Inspite of that, the numerous microalgal representatives of various taxonomic groups have adapted to life in aeroterrestrial habitats, such as soil crusts, tree bark, rocks or man made surfaces. In polar regions, many other microalgae live in hydroterrestrial environments where they are exposed to dry atmospheres like shallow pools or wetlands (Pichrtova et al. 2014). In aero- or hydroterrestrial environments, green microalgae belong to the most abundant algal groups (Rindi 2007). Green microalgae of subgroup *Streptophyta* are of particular because it also comprises all land vascular plants (embryophytes; *e.g.* Becker et Marin 2009). Several members of this group are remarkably desiccation tolerant (*e.g.* Elster et al. 2008, Holzinger et al. 2010, Graham et al. 2012, Karsten et Holzinger 2012, Aigner et al. 2013).

In this study, the alga *Zygnema* sp. (Zygnematophyceae, Streptophyta) from natural Antarctic habitats was used. Microalgae isolated from such an extreme environment represent good model organisms for studying adaptive strategies (Elster et Benson 2004). *Zygnema* sp. is one of the most common streptophyte algae in the Arctic and Antarctica, usually forming extensive mats in shallow pools or on wet soil, living at the transition between aquatic and also aeroterrestrial environments (Hawes 1990, Kang et al. 2007, Kim et al. 2008, Holzinger et al. 2009). Vegetative filaments of *Zygnema* sp. lack constitutive desiccation tolerance (McLean et Pessoney 1971), and the effect of field acclimation (hardening) or dormant stages production is assumed. The formation of various resistant cell types has been frequently reported in *Zygnema* sp., namely zygospores, parthenospores, and akinetes (Kadlubowska 1984, Poulíčková et al. 2007, Stancheva et al. 2012); their role in stress resistance is not yet fully understood. *Zygnema* sp. commonly forms green algal akinetes as described earlier – developed from vegetative cells, with thick cell walls and accumulated storage products. Akinetes were described as individual cells that form from stationary- phase cells in starved cultures (McLean et Pessoney 1971). *Zygnema* sp. akinete production can occur in naturally desiccated sites, and akinetes were found that could survive experimental desiccation in a *Zygnema* sp. from Texas (McLean et Pessoney 1971) and *Zygnema stellinum* from Belarus (Genkel et Pronina 1979).

Physiological effects of high light to photosynthetic apparatus of lichens are similar to those of desiccation (Jensen et Feige 1991). Both stresses lead to production of reactive oxygen species (ROS) in core of photosystem II (PS II) and neighbouring pigment-protein complexes of light harvesting complexes (LHCs). High light-induced ROS act as strong oxidative agens that cause negative changes in thylakoidal photosynthetic apparatus. Oxidation on donor side of PS II, excessive reduction on the acceptor side of PS II, and consequently, destruction of D1 protein is major damages to PS II (Anderson et Barber 1996). Therefore, under both high light stress and dehydration, reactive oxygen species are considered major cause of damage in photosynthetic organisms. During photoinhibition, however, several mechanisms are activated that minimize such damages to photosynthetic apparatus. Such protective mechanism comprise mainly thermal dissipation of absorbed light energy. The mechanisms are present in higher plants (Adams III et al. 2006) as well as in lichens.

Majority of studies of photoinhibition in the lichens and mosses from polar regions are made under controlled laboratory conditions. Several field experiments, however, have been made to study photoinhibition in Antarctica using both gas ex-

change and chlorophyll fluorescence approach in the field (*e.g.* Kappen et al. 1998). Among them, the study made on Antarctic mosses (Lovelock et al. 1995) pointed out reversible photoinhibition in Antarctic moss measured at wet state. Field studies made on Antarctic lichens could hardly distiguish between limitation of photosynthetic processes related to thallus dehydration and progressive photoinhibition because the processes co-occur simultaneously. Last studies showed large capacity of lichens to cope with short-term high light stress. For *Usnea antarctica*, Barták et al. (2003) reported substantial decrease of chlorophyll fluorescence parameters found immediatelly after photoinhibitory treatment, as well as their fast recovery. In the study, fast phase of recovery (lasting typically 30 min.) was attributed to structural changes in PS II and LHC and the effects of antioxidative mechanisms. Slow phase of recovery (lasting from tens to hundreds of minutes) was attributed to resynthetic processes that repair damaged components of PS II an LHCs. Long-term photoinhibition exploiting the exposition of wet lichen thalli to high light for the periods longer than 1 h, has been applied in Central European (Barták et al. 2008) but not Antarctic lichens. During short term high-light stress, fast recovery of chlorophyll fluorescence parameters towards prephotoinhibition values indicated that *U. antarctica* had low susceptibility to photoinhibition (Očenášová et al. 2014).

In this paper, we bring the results of photoinhibition of PS II and analyze time- and light dose-dependent changes of F_V/F_M and Φ_{PSII}.

Material and Methods

Zygnema collection and handling

In this study, a *Zygnema* strain EEL201 (Collection of Extreme Environment Life Laboratory, Masaryk University, Brno, Czech Republic) was used for experiments. The species was collected in January 2015 at James Ross Island from a long-term research plot (LTRP) located close to Mendel station (63° 48′ 03′′ S, 57° 52′ 50′′ W). The samples were taken from temporaly streams running through the LTRP. The collected samples of almost monospecific clusters were placed into 30 ml plastic tubes, then stored in a refrigerator at 5°C. Samples were then transported to a laboratory in a portable box maintaining low temperature of 7°C thanks to several freeze packs inserted into the box. In a laboratory, *Zygnema* sp. was inoculated to a BBM agar and cultivated on Petri dishes for 8 weeks at 22°C and 60 μmol m^{-2} s^{-1} PAR. Then, the alga was put into a liquid medium and cultivated in 100 ml glass flasks for following 14 days.

High light exposition

In our experiment, the *Zygnema* sp. were exposed to different irradiances: 600 (LL), 1 400 (ML), 2 100 (HL), and 3 500 μmol m^{-2} s^{-1} (SHL). Short term exposition (30 min.) was used in order to evaluate capacity of photoprotective mechanisms activated during short-term stress. During exposition, the microbial plate with *Zygnema* sp. were kept on a thermostat metal block (Peltier cooling unit, Con Brio, Czech Republic), surrounded by melting ice to reach constant low temperature of

Zygnema sp. (5°C, checked by a infrared thermometer) throughout the experiment. Light was provided by a source consisting of several white bright LEDs. In these treatments, the extent of treatment-induced photoinhibition was evaluated as a change in chlorophyll fluorescence parameters.

Chlorophyll fluorescence measurements

Light treatment-induced changes in photosynthetic apparatus of *Zygnema* sp. were evaluated by several chlorophyll fluorescence parameters. In this study, the chlorophyll fluorescence parameters (i) before photoinhibitory treatment, (ii) immediately after, (iii) after 20 min. and (iv) after 60 min. of dark recovery were recorded. For chlorophyll fluorescence measurements, a FluorCam (HFC-010, Photon Systems Instruments, Czech Republic) was used. The chlorophyll fluorescence parameters were evaluated on dark-adapted *Zygnema* sp. (10 min.) using the measurement of slow Kautsky kinetics supplemented with quenching analysis. The method consisted of determination of basic fluorescence (F_0), maximum fluorescence (F_M) in dark adapted state, steady-state fluorescence recorded on the sample exposed to actinic light for 5 min. (F_S), maximum fluorescence measured on actinic light-adapted sample ($F_M´$) and maximum chlorophyll fluorescence ($F_M´´$) measured after 1 min. of dark relaxation. For each treatment, at least three replicates were measured. Finally, the following chlorophyll fluorescence parameters were evaluated: (1) F_V/F_M, (2) Φ_{PSII}, and (3) qF_0 (Bilger et Schreiber 1986).

Results

Exposure of *Zygnema* sp. to PAR led to a short-term photoinhibition demonstrated as a decrease in F_V/F_M (Fig. 1). Typically, F_V/F_M droped to the value about 0.2 immediately after the end of exposition period. Irrespective of PAR dose, F_V/F_M recovered to about 50-70% of initial maximum after 20 min. (fast phase of recovery). Then, much slower increase in F_V/F_M recovery followed within hundreds of minutes indicating slow phase of recovery. Recovery after 4 h of recovery, 92, 75% recovery was achieved in LL, and ML photoinhibitory treatmens, respectively. Only 50% recovery in F_V/F_M was achieved in SHL treatment after 4 h recovery. For full recovery, 24 h was necessary in SHL treatment (*see* Fig. 1).

Thanks to generally low initial values of Φ_{PSII}, the parameter response to LL, ML, and HL photoinhibitory treatment was somewhat different from F_V/F_M. Slight but insignificant decrease in Φ_{PSII} was found immediately after photoinhibitory treatment followed by more or less constant Φ_{PSII} values fluctuating around pretreatment value. The only exception was SHL treatment which resulted in a 35% decrease of Φ_{PSII}. Such SHL treatment-induced photoinhibition of PS II lasted for some 40 min. Then, *Zygnema* sp. showed 96% (*see* Fig. 1). The same was true for other chlorophyll fluorescence parameters such as *e.g.* F_0, that did not show any response to LL, ML, HL, and SHL treatments.

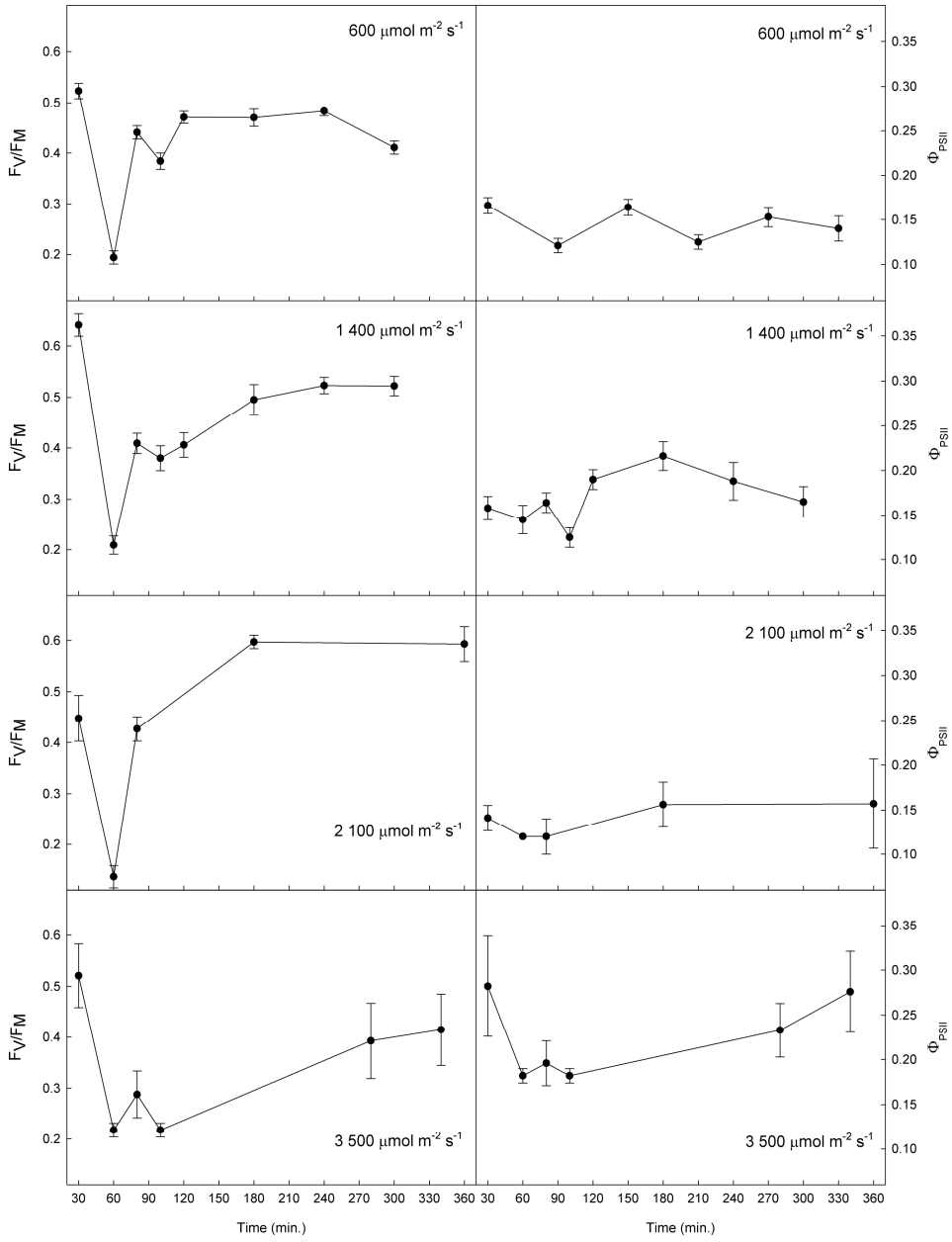

Fig. 1. Time courses of potential (F_V/F_M - left) and effective quantum yield (Φ_{PSII} - right) recorded before and after 30 min. photoinhibitory treatment with consequent recovery. In SHL treatment (3 500 μmol m^{-2} s^{-1}), full recovery was achieved after 24 h (F_V/F_M = 0.530±0.04, Φ_{PSII} = 0.278±0.03).

Quenching of F_0 showed positive values since in all cases $F_0 > F_0'$ as documented earlier for several algae species (Komárek et al. 2010, Masojídek et al. 2001). Due to low chlorophyll fluorescence signals, variability of qF_0 was high and qF_0 did not show the same trend in all treatments. Generally a decrease was found immediately after treatment followed with less pronounced increase during recovery period (Fig. 2).

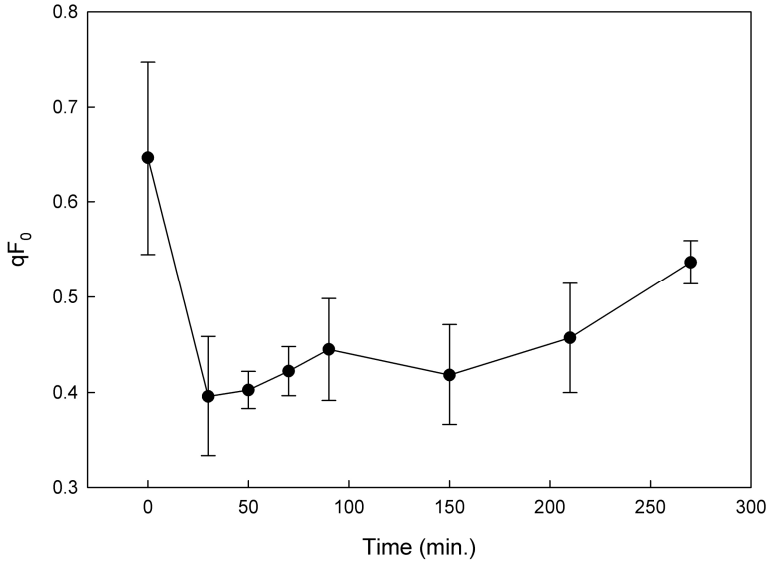

Fig. 2. Time course of F_0 quenching coefficient (qF_0) recorded before and after photoinhibitory treatment (1 400 μmol m^{-2} s^{-1}) and during consequent recovery.

Discussion

PAR-induced decrease in F_V/F_M found after 1 h treatment was dose-dependent. Similarly, recovery to pre-treatment initial values was dose dependent as well. Decrease in F_V/F_M found even after LL photoinhibitory treatment could be explained by the fact that *Zygnema* sp. from polar regions shows (i) light-saturated photosynthesis at PAR of about 200 μmol m^{-2} s^{-1}, and (ii) gradually increased inhibition photosynthetic electron transport with PAR rising from 500-3000 μmol m^{-2} s^{-1} (Kaplan et al. 2013).

Zygnema sp. showed a high resistence to photoinhibitory treatment induced by both physiological and unrealistically high PAR doses. Such resistence should be a constitutive characteristic of the species since it grows in fully lit freshwater Antarctic habitats during austral summer. *Zygnema* sp. grows typically in shallow pools, streamlets or on the surface of wet soils. The *Zygnema* strain EEL201 exploited in this study, was collected from James Ross Island after several fully sunny days. It is, therefore, expected that natural photoprotective mechanisms were fully developed and activated at the time of collection. Among them, those related to UV-B protection comprise polyphenolic compounds

(Pichrtová et al. 2013) and other secondary metabolites that provide an effective UV-B protection (Germ et al. 2009, Holzinger et al. 2009). Mechanisms involved into *Zygnema* sp. photoprotection against PAR have been studied only in limited extent and remained generally unknown. However, involvement of xanthophyll cycle pigments comprising viola- to zeaxanthin conversion might be hypothesised since it is a general mechanism in majority of green algae (Erikson et al. 2015) and well documented *e.g.* for *Z. circumcarinatum* (Orosa et al. 2000) and polar *Zygnema* strains (Pichrtová 2014). Similarly, Korean group indicated involvement of antioxidative enzymes into *Zygnema* sp. protection against a short-wave radiation (Choi et al. 2014). The same mechanisms, *i.e.* increased amounts of superoxid dismutase, catalase, and altered protein expression might be expected *Zygnema* sp. in response to PAR stress.

F_0 is related to LHC II not to chlorophylls in reaction centre, therefore increased F_0 quenching results from increases in energy dissipation in PS II antennae (Roháček 2002). In mosses, qF_0 was found fast responsive to hydration/dehydration (Mayba et al. 2001). In our study, however, qF_0 did not recover to pretreatment values indicating that rearrangements of LHC II to initial state was not completed after 4 h recovery. Generally, *Zygnema* strain EEL201 showed a high resistence to high light stress similarly to other species from polar regions studied under *in vitro* conditions (*e.g. Cosmarium* sp.– Stamenkovic et Hanelt 2013). *Zygnema* strain EEL201 demonstrated physiological responses in photosynthetic apparatus that were consistent with the light intensities prevailing at the source location at James Ross Island. This may suggest that photoprotective mechanisms are constitutive and genetically preserved. In general, *Zygnema* strain EEL201 exhibited fast recovery of F_V/F_M to pre-photoinhibitory values even when exposed to relatively high PAR intensities. Therefore, the strain EEL201 from James Ross Island might be regarded as high light–adapted algae.

References

ADAMS III, W. W., ZARTER, C. R., MUEH, K. E., AMIARD, V. and DEMMIG-ADAMS, B. (2006): Energy dissipation and photoinhibition: A continuum of photoprotection. *In*: B. Demmig-Adams, William W. Adams III and A. K. Mattoo (eds): Photoprotection, photoinhibition, gene regulation, and environment. Springer, The Netherlands, pp. 49-64.

AIGNER, S., REMIAS, D., KARSTEN, U. and HOLZINGER, A. (2013): Unusual phenolic compounds contribute to ecophysiological performance in the purple-colored green alga *Zygogonium ericetorum* (Zygnematophyceae, Streptophyta) from a high-alpine habitat. *Journal of Phycology*, 49: 648-660.

ANDERSON, B., BARBER, J. (1996): Mechanism of photodamage and protein degradation during photoinhibition of photosystem II. *In*: Baker N. R. (ed.): Photosynthesis And The Enviroment. Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 101-121.

BARTÁK, M., VRÁBLÍKOVÁ, H. and HÁJEK, J. (2003): Sensitivity of photosystem 2 of antarctic lichens to high irradiance stress: fluorometric study of fruticose (*Usnea antarctica*) and foliose (*Umbilicaria decussata*) species. *Photosynthetica*, 41: 497-504.

BARTÁK, M., VRÁBLÍKOVÁ-CEMPÍRKOVÁ, H., ŠTEPIGOVÁ, J., HÁJEK, J., VÁCZI, P. and VEČEŘOVÁ, K. (2008): Duration of irradiation rather than quantity and frequency of high irradiance inhibits photosynthetic processes in the lichen *Lasallia pustulata*. *Photosynthetica*, 46:161-169.

BECKER, B., MARIN, B. (2009): Streptophyte algae and the origin of embryophytes. *Annals of Botany*, 103: 999-1004.

BILGER, W., SCHREIBER, U. (1986): Energy-dependent quenching of dark-level chlorophyll fluorescence in intact leaves. *Photosynthesis Research*, 10: 303-308.

CHOI, J-I., YOON, M., LIM, S., KIM, G. H. and PARK, H. (2014): Effect of gamma irradiation on physiological and proteomic changes of Arctic *Zygnema* sp. (Chlorophyta, Zygnematales). *Phycologia*, 54: 333-341.

DEMMIG-ADAMS, B., ADAMS III, W. W. (2000): Harvesting Sunlight Safely. *Nature*, 403: 371-374.

ELSTER, J., BENSON, E. E. (2004): Life in the polar environment with a focus on algae and cyanobacteria. *In*: B. Fuller, N. Lane, E. Benson (eds.): Life in the Frozen State, Taylor and Francis, London, pp. 109-150.

ELSTER, J., DEGMA, P., KOVÁČIK, L'., VALENTOVÁ, L., ŠRÁMKOVÁ, K. and BATISTA PEREIRA, A. (2008): Freezing and desiccation injury resistance in the filamentous green alga *Klebsormidium* from the Antarctic, Arctic and Slovakia. *Biologia*, 63: 843-851.

ERIKSON, E., WAKAO. S. and NIYOGI, K. K. (2015): Light stress and photoprotection in *Chlamydomonas reinhardtii*. *The Plant Journal*, 82: 449-465.

GENKEL, P. A., PRONINA, N. D. (1979): Ecology of *Zygnema stellinum* Vauch. during desiccation of a shallow body of water. *Biology bulletin of the Academy of Sciences of the USSR*, 6: 504-509.

GERM, M., KREFT, I. and GABERŠČIK, A. (2009) UV-B radiation and selenium affected energy availability in green alga *Zygnema*. *Biologia*, 64: 676-679.

GRAHAM, L.E., ARANCIBIA-AVILA, P., TAYLOR, W.A., STROTHER, P. K. and COOK, M. E. (2012): Aeroterrestrial Coleochaete (Streptophyta, Coleochaetales) models early plant adaptation to land. *American Journal of Botany*, 99: 130-144.

HAWES, I. (1990): Effects of freezing and thawing on a species of *Zygnema* (Chlorophyta) from the Antarctic. *Phycologia*, 29: 326-331.

HOLZINGER, A., ROLEDA, M. Y. and Lütz, C. (2009): The vegetative arctic freshwater green alga *Zygnema* is insensitive to experimental UV exposure. *Micron*, 40: 831-838.

HOLZINGER, A., TSCHAIKNER, A. and REMIAS, D. (2010): Cytoarchitecture of the desiccation-tolerant green alga *Zygogonium ericetorum*. *Protoplasma*, 243: 15-24.

JENSEN, M., FEIGE, G. B. (1991): Quantum efficiency and chlorophyll fluorescence in the lichens *Hypogymnia psysodes* and *Parmelia sulcata*. *Lichen Biology*, 11: 2-3.

KADLUBOWSKA, J. Z. (1984): Conjugatophyceae I: Chlorophyta VIII: Zygnemales. *In*: H. Ettl, J. Gerloff, H. Heynig, D. Mollenhauer (eds.): Süsswasserflora von Mitteleuropa, Vol. 16, pp. 1-532.

KANG, S. H., JOO, H. M., PARK, S., JUNG, W., HONG, S. S., SEO, K-W., JEON, M. S., CHOI, H-G. and KIM, H. J. (2007): Cryobiological perspectives on the cold adaptation of polar organisms. *Ocean Polar Research*, 29: 263-271.

KAPLAN, F., LEWIS, L. A., HERBURGER, K. and HOLZINGER, A. (2013): Osmotic stress in Arctic and Antarctic strains of the green alga *Zygnema* (Zygnematales, Streptophyta): Effects on photosynthesis and ultrastructure. *Micron*, 44: 317-330.

KAPPEN, L., SCHROETER, B., GREEN, T. G. A. and SEPPELT, R. D. (1998): Chlorophyll a fluorescence and CO_2 exchange of *Umbilicaria aprina* under extreme light stress in the cold. *Oecologia*, 113: 325-331.

KARSTEN, U., HOLZINGER, A. (2012): Light, temperature, and desiccation effects on photosynthetic activity, and drought-induced ultrastructural changes in the green alga *Klebsormidium dissectum* (Streptophyta) from a high alpine soil crust. *Microbial ecology*, 63: 51-63.

KIM, G. H, KLOCHKOVA, T. A. and KANG, S. H. (2008): Notes on freshwater and terrestrial algae from Ny-Alesund, Svalbard (high Arctic sea area). *Journal of Environmental Biology*, 29: 485-491.

KOMÁREK, O., FELCMANOVÁ, K., ŠETLÍKOVÁ, E., KOTABOVÁ, E., TRTÍLEK, M. and PRÁŠIL, O. (2010): Microscopic measurements of the chlorophyll a fluorescence kinetics. *In*: D. J. Suggett, M. Borowitzka, O. Prášil (eds.): Chlorophyll a fluorescence in aquatic sciences: Methods and applications, developments in applied phycology. pp. 91-101.

LOVELOCK, C. E., JACKSON, A. E., MELICK, D. R. and SEPPELT, R.D. (1995): Reversible photoinhibition in antarctic moss during freezing and thawing. *Plant Physiology*, 109: 955-961.

MASOJÍDEK, J., PECHAR, L., KOBLÍŽEK, M., ADAMEC, L. and KOMENDA, J. (2001): Affinity of surface phytoplankton populations to high irradiance in hypertrophic fish ponds: implications of the competition between chlorococcal algae and cyanobacteria. *Nova Hedwigia*, 123: 255-273.

MAYBA, N., BECKETT, R. B., CSINTALAN, Z. and TUBA, Z. (2001): ABA increases the desiccation tolerance of photosynthesis in the afromontane understorey moss *Atrichum androgynum*. *Annals of Botany*, 88: 1093-1100.

MCLEAN, R. J, PESSONEY, G. F. (1971): Formation and resistance of akinetes of *Zygnema*. *In*: B. C. Parker, R. M. Brown Jr. (eds.): Contributions in Phycology, pp. 145-152.

OČENÁŠOVÁ, P., BARTÁK, M. and HÁJEK, J. (2014): Photoinhibition of photosynthesis in Antarctic lichen *Usnea antarctica*. II. Analysis of non-photochemical quenching mechanisms activated by low to medium light doses. *Czech Polar Reports*, 4: 90-99.

OROSA, M., TORRES, E., FIDALGO, P. and ABALDE, J. (2000): Production and analysis of secondary carotenoids in green algae. *Journal of Applied Phycology*, 12: 553-556.

PICHRTOVÁ, M. (2014): Stress resistance of polar hydro-terrestrial algae *Zygnema* spp. (Zygnematophyceae, Streptophyta). Ph.D. Thesis, Charles University, Prague, 146 p.

PICHRTOVÁ, M., REMIAS, D., LEWIS, L. A. and HOLZINGER, A. (2013): Changes in phenolic compounds and cellular ultrastructure of arctic and antarctic strains of *Zygnema* (Zygnematophyceae, Streptophyta) after exposure to experimentally enhanced uv to PAR ratio. *Microbial Ecology*, 65: 68-83.

PICHRTOVÁ, M., HÁJEK, T. and ELSTER, J. (2014): Osmotic stress and recovery in field populations of *Zygnema* sp. (Zygnematophyceae, Streptophyta) on Svalbard (High Arctic) subjected to natural desiccation. *FEMS microbiology ecology*, 89: 270-280.

POULÍČKOVÁ, A., ŽIŽKA, Z., HASLER, P. and BENADA, O. (2007): Zygnematalean zygospores: morphological features and use in species identification. *Folia microbiologica*, 52: 135-145.

RINDI, F. (2007): Diversity, distribution and ecology of green algae and cyanobacteria in urban habitats. *In*: J. Seckbach (ed.): Algae and Cyanobacteria in Extreme Environments. Springer, Berlin, pp. 619-638.

ROHÁČEK, K. (2002): Chlorophyll fluorescence parameters: The definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica*, 40: 13-29.

STANCHEVA, R., SHEATH, R. G. and HALL, J. D. (2012): Systematics of the genus *Zygnema* (Zygnematophyceae, Charophyta) from Californian watersheds. *Journal of Phycology*, 48: 409-422.

STAMENKOVIC, M., HANELT, D. (2013): Protection Strategies of Cosmarium strains (Zygnematophyceae, Streptophyta) Isolated from Various Geographic Regions Against Excessive Photosynthetically Active Radiation. *Photochemistry and Photobiology*, 89: 900-910.