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Characterization of the Community of Snow Algae and Their Photochemical Performance *in situ* in the Giant Mountains, Czech Republic

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Abstract

The Czech Republic's Giant Mountains are a unique locality for studying snow algae because representatives of both significant genera, *Chlamydomonas* and *Chloromonas* (Chlorophyta), are regularly found there and are capable of completing their life cycle in several weeks. Physical and chemical environmental characteristics of two sites were measured in June 2008 and the photochemical processes of the snow algae were studied using variable chlorophyll fluorescence techniques. Correlations between the environmental conditions and the rapid light curve parameters were evaluated. The environment was characterized by stable snow temperature (T_{snow}; −0.6 to −0.3 °C) but variable air temperature (T_{air}; 4.3 to 15.3 °C), photosynthetically active radiation (PAR; maximum of approximately 2000 μmol m^{-2} s^{-1}), and ultraviolet radiation (UVR; 0.135 to 2.27 mW cm^{-2}). The snow chemical composition at both experimental sites was similar, regardless of whether snow algae were present or not, and the nutrient concentrations resembled mesotrophic to eutrophic water. Only concentration of $P\text{-}PO_4^{3-}$ was significantly higher in the presence of algae. Vegetative and mating cells of *Chloromonas* cf. *nivalis* were observed on the snow surface down to a depth of 6 cm. The maximum quantum yield in a range from 0.479 to 0.624 indicated only minor or no stress conditions. While the relative electron transfer rate (142 to 241) and the initial slope (0.287 to 0.505 μmol^{-1} m^2 s^1) were negatively related to T_{air}, PAR, and UVR, the saturation irradiance values were very stable (350 to 489 μmol m^{-2} s^{-1}). Various strategies of acclimation to high PAR and/or UVR at different stages of the life cycle are proposed.

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Introduction

Snow algae occur in mountains and the polar regions and they are potentially important primary producers in the snow ecosystem, and at favorable conditions they could be considered as an important CO_2 sink (Painter et al., 2001; Williams et al., 2003). Studies of snow algae have focused mainly on their ecology and taxonomy (e.g. reviews of Kol, 1968; Hoham and Ling, 2000; Hoham and Duval, 2001; Komárek and Nedbalová, 2007). Despite their representing a unique model system for investigating the survival of photosynthetic microorganisms at low temperatures and high irradiances, only little attention has been given to their physiology. Detailed study of their adaptation and acclimation response to such extreme environments began when scientific interest in extremophilic microorganisms increased in the last decades (Bidigare et al., 1993; Müller et al., 1998b; Hoham and Ling, 2000; Gorton and Vogelmann, 2003; Remias et al., 2005; Řezanka et al., 2008). Photosynthesis of the snow has been measured *in situ* using either $^{14}CO_2$ fixation (Fogg, 1967; Thomas, 1972; Javornický, 1973; Komárek et al., 1973; Mosser et al., 1977; Felip et al., 1995), gas exchange (Williams et al., 2003) or variable chlorophyll fluorescence (Kvíderová et al., 2005; Stibal et al., 2007) techniques.

The variable chlorophyll fluorescence allows measurement of the rapid light curves (RLCs) and provides the most appropriate means for screening of dynamic processes occurring in the photosystem II reaction centers, i.e. efficiency of light energy utilization, diurnal changes in the photosystem II photochemistry, or electron transfer rates (e.g. Beer et al., 1998; Ralph and Gademann, 2005; Herlory et al., 2007; Cruz and Serodio, 2008). The first measurements of RLCs in cysts of the snow alga *Chloromonas brevispina* suggested that the photochemical activity could be measured in natural samples of the cryosestic communities (Kvíderová et al., 2005), and its seasonal and diurnal changes in vegetative cells and cysts of the snow alga *Chlamydomonas nivalis* were measured in Svalbard (Stibal et al., 2007).

The Giant Mountains in the Czech Republic represent a unique locality for the study of snow algae because representatives of both significant genera, *Chlamydomonas* and *Chloromonas* (Chlorophyta), are regularly found there. Snow algae were recorded there in 1976 (Fott et al., 1978) and in 1986 and 1987 (Kociánová et al., 1989), and continual observations have been performed since 2005. The "algal blooms" occur usually in small-scale (measured in dimensions of meters to tens of meters) temporal snowfields during May and June, and species distribution depends on altitude. While *Chloromonas brevispina* and *Chloromonas rosae* are observed at lower elevations of about 1000 to 1200 m a.s.l., *Chloromonas nivalis* and *Chlamydomonas nivalis* are found at higher sites (Nedbalová et al., 2008). The algae could be subjected to low temperatures, freeze-thaw cycles, high and variable photosynthetically active radiation (PAR), and ultraviolet radiation (UVR) and must complete their life cycles in several

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weeks before the snowfield melts completely. These dynamic changes provide excellent conditions for study of acclimatization processes in zoospores as well as in zygospores and the changes of these processes during transition from zoospores to zygospores. While physiological studies in the field in *Chlamydomonas* or *Chloromonas* zygospores are common (Williams et al., 2003; Kvíderová et al., 2005; Stibal et al., 2007), those in zoospores are rare (Thomas and Duval, 1995). The photochemical activity of *Chloromonas* zoospores has not been measured in the field yet and could be performed in the Giant Mountains.

For this study, two sites had been selected at the southern and northern side of the Luční hora mountain in the Czech Republic's Giant Mountains where different irradiance conditions were expected. The aims of this study were the following: (1) physical conditions—snow and air temperatures (T_{snow} and T_{air}), as well as the PAR and the UVR—were measured at the experimental sites to get data for correlation of these factors to the variable chlorophyll fluorescence parameters. These relationships were examined to determine the possible influence of freeze-thaw cycles on algal physiology, and to determine cultivation conditions in laboratory experiments in which the snow algae isolated from these localities will be used. In addition, (2) the chemical composition of the snow in the presence and absence of the algae was analyzed to evaluate possible nutrient stress that could also decrease the parameters of the variable chlorophyll fluorescence. (3) The species composition, cell density and depth in which the cells were observed, were determined. Finally, (4) the algae's photochemical response to actual environmental conditions was studied and correlations between the fluorescence and RLC parameters and the environmental factors were found.

Material and Methods

STUDY SITE

The research was carried out at Luční hora mountain (see Hejcman et al., 2006, for a detailed description of the mountains). The sampling sites were located on southern (South Site; 50°43′36.7″N, 15°41′04.2″E, 1534 m a.s.l.) and northern (North Site; 50°43′43.7″N, 15°41′06.9″E, 1511 m a.s.l.) slopes of the mountain. The measurements were performed four times a day between 18 and 20 June 2008, in order to compare differing PAR. During this period, the community was visible and sampling was easier. Night measurements could not be made for safety reasons.

TEMPERATURE, PAR, AND UVR

Two Minikin QT data loggers measuring T_{air} and PAR (EMS, Brno, Czech Republic) were positioned in the vicinity (less than 3 m distant) of massive (i.e. macroscopically visible) patches of snow algae at each site. The data were recorded continuously, averaged, and recorded every 10 minutes. The actual total UVR was measured using a digital YK-34UV UVR meter (Lutron, Taiwan) and the T_{snow} by a Multi-A digital thermometer (Fisher, U.S.A.) before sampling for the fluorescence measurements.

SNOW CHEMISTRY

For chemical analyses, snow samples of approximately 100 mL were collected once at each site on 20 June 2008. One sample was collected from the green patches where the green zoospores had formed dense communities, and a second from white snow. The samples were kept cold (<5 °C) during transport to the Analytical Laboratory of the Institute of Botany of the Academy of Sciences of the Czech Republic in Třeboň. The pH of the snow samples was measured using a CPH51 laboratory pH-meter (Crytur, Czech Republic). Conductivity was measured using a CDM3 laboratory conductometer (Radiometer, Denmark). Alkalinity was estimated from potentiometric titration by 0.1 M HCl to pH 4. Dissolved inorganic carbon (DIC) and its forms (CO_2-C, HCO_3^--C, and CO_3^{2-}-C) were calculated according to equations from Stumm and Morgan (1981). The concentration of nitrogen (as dissolved inorganic nitrogen in the forms NH_4^+-N, NO_2^--N, and NO_3^--N, and total nitrogen, N_{total}) and phosphorus (as dissolved reactive phosphorus, PO_4^{3-}-P, and total phosphorus, P_{total}) were measured by a flow injection analyzer (FIA, Tecator, Sweden; Růžička and Hansen, 1981). For N_{total} and P_{total} determination, persulfate mineralization treatment was applied at 151 °C for 30 min. The NH_4^+-N concentration was determined by gas diffusion method (detection limit 10 μg L^{-1}) and NO_3^--N and NO_2^--N concentrations by reaction with sulfonamide (detection limit 10 μg L^{-1}). The PO_4^{3-}-P and P_{total} concentrations were determined from reaction of PO_4^{3-} with ammonium molybdate and reduction by stannous chloride to phosphomolybdenum blue (detection limit 10 μg L^{-1}). The concentration of Cl^- ions was determined by reaction with mercury thiocyanate and ferric ions (detection limit 5 μg L^{-1}). The chlorophyll *a* (Chl *a*) concentration was determined using a Shimadzu UV-1650PC spectrophotometer according to Pechar (1987).

OCCURRENCE OF SNOW ALGAE IN THE SNOWFIELD

The presence or absence of algae was checked visually early in the morning, and the green patches were marked for easier sampling during the day. A small hole (10 × 10 cm) was dug and a conventional ruler was used to measure the depth at which the cells were found before the individual sampling for the fluorescence measurements.

The melting rate was estimated from the shift of the snowfield margin relative to surrounding structures (e.g. stones) and from the change in the actual depth to the bottom of an experimental chamber in the snow that was being tested. The chamber was completely buried in the morning with its top at the same level as the snow's surface. When the snow melted, the sides of the chamber appeared above the snow's surface and served for the measurements.

SPECIES COMPOSITION AND CELL DENSITIES

A sample of approximately 5 mL of snow water equivalent was collected for determining the species composition and cell counts before the first fluorescence measurement and was fixed by Lugol's solution. The cell number was counted in a Bürker chamber under an Olympus BX-51 light microscope (Olympus C&S, Japan). The cell counts in 144 large squares corresponding to a volume of 0.004 mm^3 were recorded. The cell number was calculated twice for each sample. Microphotographs of the fixed samples were taken using an Olympus DP71 digital camera (Olympus C&S, Japan) and processed using QuickPhoto Camera 2.3 software (Promicra, Czech Republic). An immersion objective of 100× and eyepiece of 10× magnification were used for identification.

PHOTOCHEMISTRY

The snow samples (approximately 10 mL) for the RLC measurements were collected at the layer of maximum cell density into a sterile test tube. The samples were kept in the dark during

TABLE 1

Site physical environmental characteristics at the time of the fluorescence measurements used for the correlation and ordination analysis (paired *t*-test, South Site $n = 8$, North Site $n = 7$). PAR—photosynthetically active radiation, UVR—ultraviolet radiation.

	South Site			North Site		
	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum
T_{snow} (°C)	−0.5 ± 0.1	−0.6	−0.3	−0.443 ± 0.098	−0.5	−0.3
T_{air} (°C)	10.3 ± 2.7	7	14	10.1 ± 2.2	7.1	13.3
PAR (μmol m^{-2} s^{-1})	632 ± 606	85	1595	591 ± 645	99	1908
UVR (mW cm^{-2})	0.758 ± 0.767	0.135	2.27	0.665 ± 0.476	0.18	1.56

transfer to the temporary field laboratory located in the Luční Bouda Hotel, where the fluorometric measurements were performed. The transfer lasted usually 40 min, but the sample temperature did not exceed 0 °C.

The fluorescence measurements of the light dependency of the relative electron transfer rate (rETR) were performed using a Dual Modulation Fluorometer (Photon Systems Instruments, Czech Republic). A subsample of volume 1.2 mL was poured into the plastic cuvette. The quantum yield was measured in dark and under actinic blue light of 10 different irradiances. The protocol for Q_A-reoxidation was applied (Stibal et al., 2007). The period of dark or the individual actinic light lasted 20 s. The rETR was calculated according to Maxwell and Johnson (2000):

$$\mathrm{rETR} = 0.5 \times \Phi_{PSII} \times \mathrm{PFD}, \quad (1)$$

where PFD is the incident light and Φ_{PSII} is the quantum yield of photosystem II.

The actual quantum yield for the given actinic light (Φ_{PSII}) is calculated as

$$\Phi_{PSII} = \frac{F'_m - F_t}{F'_m}, \quad (2)$$

where F_t is the steady-state fluorescence level, and F'_m is the maximum fluorescence level when the saturating pulse is applied. The maximum quantum yield (F_v/F_m), measured in the dark-adapted stage, is calculated as

$$\frac{F_V}{F_m} = \frac{F_m^0 - F_0}{F_m^0}, \quad (3)$$

where F_0 is the minimum fluorescence level and F_m^0 is the maximum fluorescence level when the saturating pulse is applied.

The values of maximum relative electron transfer rate ($rETR_{max}$) and initial slope (α) were estimated from the rectangular hyperbolic curve parameters (Jassby and Platt, 1976) using SigmaPlot software (SPSS, U.S.A.):

$$\mathrm{rETR} = \mathrm{rETR}_{max} \tanh \frac{\alpha \times I}{\mathrm{rETR}_{max}}, \quad (4)$$

where rETR is the actual relative electron transfer rate, $rETR_{max}$ is the maximum relative electron transfer rate, α is the initial slope of the RLC, and I is the actinic light value.

The saturation irradiance (E_k), i.e. the point where the photosynthesis stops to be light-limited, was calculated as

$$E_k = \frac{\mathrm{rETR}_{max}}{\alpha}. \quad (5)$$

STATISTICAL ANALYSES

Statistical significances of observed differences and correlation analyses were evaluated using Statistica software (StatSoft, U.S.A.). Differences in T_{air}, T_{snow}, PAR, and UVR between the sites as well as differences in the snow chemical composition at both sites and in the samples with and without algae were evaluated by paired *t*-test. Differences in the cell counts at the South and North Sites were evaluated by *t*-test. To evaluate the relationships in the photochemical parameters (F_v/F_m, $rETR_{max}$, α, and E_k) and environmental variables (T_{snow}, T_{air}, PAR, and UVR), correlation analysis was performed. The results were considered significant if the *p*-value was less than 0.05.

Results

TEMPERATURE, PAR, AND UVR

T_{snow} was very stable while T_{air} changed gradually during the day. T_{air} did not, however, drop below 0 °C, indicating that the cells were not exposed to freeze-thaw cycles which might have influenced fluorescence parameters. The PAR and UVR varied due to unstable cloud cover ranging from medium rainfall to full sunlight (Table 1, Fig. 1). No significant differences were found between the sites in any physical parameters.

SNOW CHEMISTRY

The snow chemical composition was similar at both sites (Table 2), and there were no significant differences between samples with or without the algae, with the exception that the PO_4^{3-}-P concentration was increased at both sites in samples containing the algae ($n = 4$, $p = 0.004$). The high N:P ratio in the sample with algae from the South Site was caused by extremely high $N\text{-}NH_4^+$ concentration. The DIC concentration and the concentrations of its forms (CO_2-C, HCO_3^--C, and $C\text{-}CO_3^{2-}$-C) show that dissolved CO_2 is the main available inorganic carbon source for the algae. No significant difference in Chl *a* concentration in samples with and without algae was observed, probably due to variability in the Chl *a* concentration in samples containing algae at individual sites (Table 2).

OCCURRENCE OF SNOW ALGAE IN SNOWFIELDS

The algae were found approximately 20 to 50 cm from the margin of the snowfields and no algae were observed in their central parts, but the algae did not occur in a continuous line along the margin. Snow algae formed patches in dimensions of tens to hundreds of cm^2, and these patches moved downhill during melting, maintaining stable positions in relation to the snowfield margin. The depth at which the cells were observed and collected for the RLC measurements ranged from the snow surface to 6 cm down (Table 3) in a band 1–2 cm thick. The depth was affected by melting (Fig. 2), and approximately 20 cm of snow melted daily.

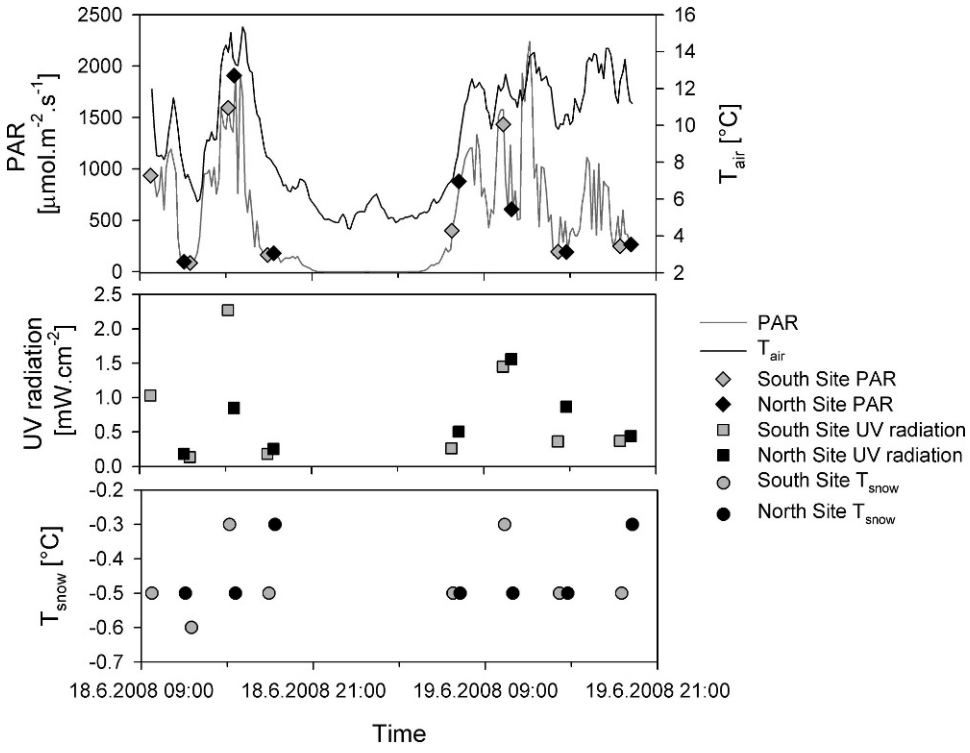

FIGURE 1. The time course of physical variables (T_{air}, T_{snow}, PAR, and UVR) at both sites during the experiment.

SPECIES COMPOSITION AND CELL DENSITIES

Both sites were inhabited by vegetative green cells of *Chloromonas* cf. *nivalis* with two or four flagella indicating the beginning of mating (Fig. 3) and zygospore formation. The cell numbers were 2.59 ± 0.00 $\times 10^5$ cells mL^{-1} (mean ± standard deviation, $n = 2$, 144 squares counted in each case) at the South Site and 1.61 ± 0.45 $\times 10^5$ cells mL^{-1} (mean ± standard deviation, $n = 2$, 144 squares counted in each case) at the North Site. The actual cell density in the interstitial water could be higher because melted snow samples were used. The site differences were not statistically significant.

TABLE 2

Chemical properties of the snow. Statistically significant differences are marked by asterisk (*t*-test, * *p*-value < 0.05, ** *p*-value < 0.01; *n* = 4, S-N—site comparison, A—algal presence/absence comparison).

Parameter	South Site		North Site		
	− algae	+ algae	− algae	+ algae	Significance
pH	5.53	5.56	5.04	4.96	
Conductivity (µS cm^{-1})	5.1	6.2	5.4	8.1	
Alkalinity (mmol L^{-1})	0.27	0.27	0.18	0.16	
NH_4^+-N (µg L^{-1})	148	729	132	195	
NO_2^--N (µg L^{-1})	2.42	2.77	1.60	8.36	
NO_3^--N (µg L^{-1})	69.6	34.2	60.2	73.0	
N_{total} (mg L^{-1})	0.68	1.60	0.65	1.27	
PO_4^{3-}-P (µg L^{-1})	21.9	46.2	20.3	47.6	*A
P_{total} (µg l^{-1})	60.9	78.4	62.4	87.8	
Cl^- (mg L^{-1})	1.61	2.07	1.41	1.98	
Chlorophyll *a* (µg L^{-1})	10.3	219.2	26.1	320	
N:P inorganic molar	22.24	36.64	21.25	12.86	
DIC (mmol L^{-1})	6.85	7.41	3.38	3.17	
CO_2-C (mmol L^{-1})	6.66	7.24	3.11	2.90	
HCO_3^--C (mmol L^{-1})	0.19	0.17	0.27	0.27	
CO_3^{2-}-C (mmol L^{-1})	0.00	0.00	0.00	0.00	

PHOTOCHEMISTRY

Any decrease in the quantum yield (F_v/F_m and Φ_{PSII}) values would have indicated stress conditions. The minimum values were observed after a prolonged exposition to the high PAR and UVR lasting tens of minutes to hours (Fig. 4), but the PAR and UVR did not appear to cause severe damage to the photosynthetic apparatus because of 19 and 23% decline in F_v/F_m compared to its maximum values at the South and the North Sites, respectively (Table 3). The $rETR_{max}$ and α are derived from the dependency of the rETR (calculated from F_v/F_m and Φ_{PSII}; Equation 1) on irradiance (Equation 4) and the stress conditions would have influenced the $rETR_{max}$ and/or α that could have been seen from the changes of shape of the RLCs. The resulting E_k would have been directly proportional to $rETR_{max}$ and inversely proportional to α (Equation 5) and would have reflected the photoacclimation of the photosynthetic apparatus to incoming irradiance. The decrease of the RLC parameters after the exposition to higher PAR and UVR was observed at both experimental sites (Fig. 4). The $rETR_{max}$ and E_k were significantly higher at the South Site (Table 3, $n = 14$, $p = 0.013$ for $rETR_{max}$ and $p = 0.008$ for E_k), probably due to acclimatization to the possible long-term higher PAR and UVR.

T_{snow}, T_{air}, PAR, and UVR were significantly positively correlated at the South Site but these correlations were not observed at the North Site, probably due to the different microclimatic conditions at the North Site. The stable T_{snow} did not affect any RLC parameters at both sites but the negative effects of the T_{air}, PAR, and UVR on the snow algae photochemistry were proved at the North Site only. The E_k was not affected by environmental variables at both sites. F_v/F_m, the $rETR_{max}$, and α reacted similarly to the changes in PAR and UVR at both sites, suggesting integrated photoacclimation processes for stabilization of the E_k.

The average depth at which the community was observed and collected in a given sampling was not related to either

TABLE 3

The ranges of the depth at which the snow algae were observed, and of the photochemical parameters (F_v/F_m, $rETR_{max}$, α, E_k) at both sites. The significant differences between the sites are marked by asterisk (paired *t*-test, * *p*-value < 0.05, ** *p*-value < 0.01, * *p*-value < 0.001; South Site *n* = 8, North Site *n* = 7).**

	South Site			North Site			
	Mean ± SD	Minimum	Maximum	Mean ± SD	Minimum	Maximum	Significance
Depth (cm)	2.06 ± 1.02	1	4	2.86 ± 1.21	1	5	
F_v/F_m	0.568 ± 0.049	0.507	0.624	0.548 ± 0.051	0.479	0.624	
$rETR_{max}$	184 ± 34	142	241	146 ± 22	122.1	178	*
α ($\mu mol^{-1}\ m^2\ s$)	0.408 ± 0.053	0.350	0.505	0.369 ± 0.052	0.287	0.445	
E_k ($\mu mol\ m^{-2}\ s^{-1}$)	450 ± 37	372	489	396 ± 26	350	425	***

environmental variables or to the RLC parameters at both sites (Table 4).

Discussion

SNOW ALGAE COMMUNITY ENVIRONMENT

The physical and chemical environmental conditions at the experimental sites did not differ from those at other localities (Kol, 1968; Komárek et al., 1973; Newton, 1982; Kociánová et al., 1989; Nedbalová et al., 2008). The daytime PAR and UVR was highly variable during the experiment, and no prolonged exposure (in terms of hours at a time) to photoinhibiting irradiances above ca 1000 $\mu mol\ m^{-2}\ s^{-1}$ were encountered. (Such irradiances are generally used to study photoinhibition damage in plant physiology, e.g. by Baroli and Melis, 1996; Gordillo et al., 2001.) However, minor short-term photoinhibitory effects cannot be ruled out completely.

The conductivity values were similar to those of other localities where the snow algae had been observed (Newton, 1982; Hoham et al., 1989; Müller et al., 1998a; Hoham and Ling, 2000; Hoham and Duval, 2001; Komárek and Komárek, 2001), but, probably due to a high melting rate, significantly higher conductivity in the presence of algae was not observed in the samples. The values of alkalinity, temperature, and pH indicate that the main available inorganic carbon source for the algae should be CO_2 (Stumm and Morgan, 1981). Moreover, the possibility of CO_2 limitation in dense populations can lead to competition between different species with various carbon concentrating mechanisms (Hoham and Duval, 2001), especially in the lower elevations where different species are observed (Nedbalová et al., 2008).

The nutrient concentrations were similar to those found at this locality in previous years (Nedbalová et al., 2008). According to the classification of OECD (1982), the observed phosphorus concentration corresponds to mesotrophic to eutrophic water while the snow is oligotrophic in other localities (Hoham and Duval, 2001), indicating that the nutrient limitation could not affect the fluorescence parameters. The optimum N:P molar ratio can range from 11.8 to 36.6 in different algal species and is affected by environmental conditions (Wynne and Rhee, 1986). The observed N:P ratio fell within this range, but laboratory evaluation of the optimum and limiting N:P ratios for isolated *Chloromonas* cultures will be necessary.

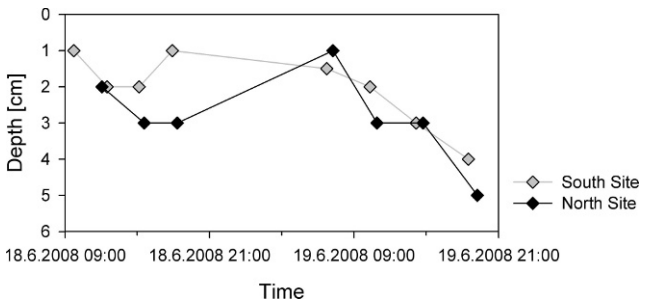

FIGURE 2. Time course of middle depth of the band in which the algal community was observed at both sites.

COMMUNITY OF THE SNOW ALGAE

The snowfields in the Giant Mountains are not perennial and the life cycle of the snow algae should be completed within several weeks. At Luční hora mountain, the conditions at a given part of a temporal snowfield are very dynamic; melting occurs rapidly and the algae are washed out of the snowfield. In my snow samples, the two- and four-flagellate cells of *Chloromonas* cf. *nivalis* were observed. This is one of four species regularly found in the Giant Mountains and is adapted to a broad range of environmental factors (Nedbalová et al., 2008). The presence of the four-flagellate cells in samples indicates that the algae probably had started to mate and to form resting stages (Hoham and Mullet, 1977). However, snowfields could melt away before the orange or red zygospores are formed, and perhaps formation of the resting stages occurs in the soil.

Small green patches were observed only at the margins of the snowfield. The higher phosphorus concentration possibly originating from wetted grass litter and high water content of the snow could promote cell division (Hoham and Duval, 2001). Extracts of coniferous litter have been shown to increase the growth rate of *Chloromonas pichinchae* probably due to high phosphorus concentration (Hoham, 1976).

The depth at which the cells were found ranged from the surface to 6 cm and did not differ from other temporal or perennial snowfields (Hindák, 1969; Thomas, 1972; Komárek et al., 1973; Thomas and Duval, 1995). The observed migration of the flagellates in snow is a reaction to changing irradiance (Kociánová et al., 1989; Hoham et al., 1998; Hoham and Ling, 2000; Hoham et al., 2000; Komárek and Komárek, 2001; Novis, 2002; Komárek and Nedbalová, 2007), but no significant correlations with the physical environmental factors were found during my observations because the migration pattern was potentially affected by rapid melting of the snowfield.

PHOTOCHEMISTRY OF THE SNOW ALGAE

The measured F_v/F_m parameter corresponded to values measured for algae in similar environments, i.e. with low temperature and relatively high PAR (Barták et al., 2003; Kvíderová et al., 2005; Stibal et al., 2007), and its values indicated a good physiological state of the snow algae. The minimum values

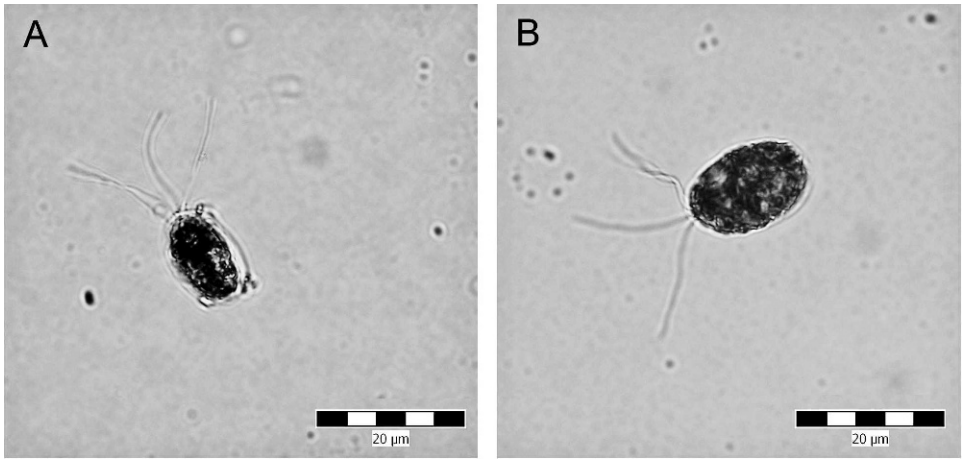

FIGURE 3. Microphotographs of the four flagellate cells of *Chloromonas* cf. *nivalis*. The samples were fixed by Lugol's solution. The black color indicates that the cells are full of starch. Immersion oil, 100× objective magnification. A = South Site, B = North Site.

show that only minor stress was encountered (Krause and Weis, 1991; Maxwell and Johnson, 2000; Roháček, 2002; Kromkamp and Forster, 2003), usually after a longer exposure to the PAR and UVR radiation. The T_{snow} was very stable and did not influence the algae's photochemical performance. Ice nucleation activity in *Chloromonas nivalis* at similar cell densities had been recorded at temperatures in a range from −7 to −4 °C, so the negative effects of freeze-thaw cycles would not be expected even at lower temperatures (Hájek et al., 2009). Surprisingly, T_{air} was negatively correlated with the F_v/F_m and RLC parameters at the North Site. T_{air} could reflect the mean PAR conditions in the previous hours, cumulative negative effects of high UVR, or the diurnal rhythms of the photochemical processes, since the values of PAR and UVR used in the correlation analyses referred to actual PAR and UVR conditions at the time of sampling.

The changes in F_v/F_m and RLC parameters followed the diurnal course of the irradiance and its variability during the experiment. These diurnal rhythms are well-documented in *Chlamydomonas nivalis* cysts (Stibal et al., 2007) and in other autotrophic microorganisms growing at low temperatures (e.g. Suzuki et al., 1997; McMinn et al., 2003). The lowest RLC parameters values after the exposure to high PAR and UVR possibly reflected dynamic regulation of the photochemical processes and slight damage to the photosynthetic apparatus. Detailed physiological experiments will be necessary to reveal the photochemical response dynamics and recovery after the PAR/

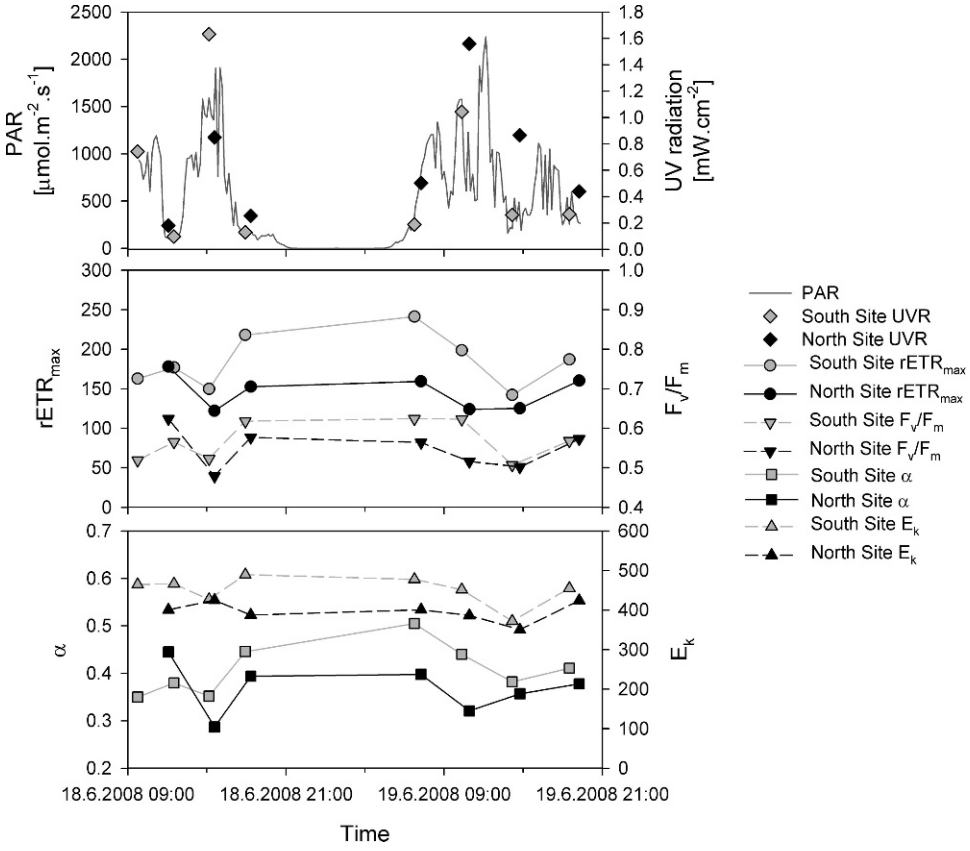

FIGURE 4. The diurnal changes of F_v/F_m and RLC parameters ($rETR_{max}$, α, E_k) and their relevance to PAR and UVR at both sites.

TABLE 4

The correlations of the environmental variables (T_{snow}, T_{air}, PAR, UVR), depth, and the photochemical parameters (F_v/F_m, $rETR_{max}$, α, E_k) at the South Site (normal font) and the North Site (italics). The significant differences between the sites are marked by asterisk (paired *t*-test, * *p*-value < 0.05, ** *p*-value < 0.01, * *p*-value < 0.001; *n* = 8 for the South Site, *n* = 7 for the North Site).**

	T_{snow}	T_{air}	PAR	UVR	Depth	F_v/F_m	$rETR_{max}$	α	E_k
T_{snow}		0.718*	0.906**	0.884**	−0.025	0.058	−0.134	−0.054	−0.225
T_{air}	*−0.192*		0.774*	0.802*	0.301	−0.492	−0.531	−0.522	0.302
PAR	*−0.340*	*0.704*		0.966***	−0.214	−0.110	−0.245	−0.269	−0.119
UVR	*−0.456*	*0.672*	*0.333*		−0.113	−0.285	−0.415	−0.429	−0.245
Depth	*0.642*	*0.419*	*−0.157*	*0.103*		−0.258	−0.320	−0.126	−0.512
F_v/F_m	*0.370*	*−0.860**	*−0.645*	*−0.742*	*−0.154*		0.931**	0.880**	−0.259
$rETR_{max}$	*0.323*	*−0.772**	*−0.500*	*−0.821**	*−0.189*	*0.966****		0.929**	0.753*
α	*0.227*	*−0.925***	*−0.722*	*−0.765**	*−0.305*	*0.935***	*0.915***		0.455
E_k	*0.249*	*0.275*	*0.504*	*−0.246*	*0.229*	*0.160*	*0.298*	*−0.111*	

UVR exposure in the snow algae at individual stages of their life cycle (zoospores, zygospores) to different irradiance fluctuation patterns.

Photoinhibition in *Chlamydomonas nivalis* does not occur even at PAR above 1000–1500 μmol m^{-2} s^{-1} (Mosser et al., 1977; Williams et al., 2003), but the photoinhibition in *Chloromonas* cf. *nivalis* could occur at lower PAR, because this genus is found in lower altitudes and in shaded localities in the Giant Mountains (Nedbalová et al., 2008).

The negative effect of UVR and protective mechanisms of *Chlamydomonas nivalis* have been suggested in laboratory studies (Duval et al., 2000) and field observations (Thomas and Duval, 1995), but the effects of the UVR on snow representatives of the genus *Chloromonas* have not yet been documented. The amount of UVR in the Giant Mountains is ca. one-fifth of that recorded in Sierra Nevada (Thomas and Duval, 1995), but the algae were found at depths where UVR could penetrate (Gorton and Vogelmann, 2003) and may potentially decrease the photosynthetic capacity of the snow algae. The observed negative effects of the UVR did not cause serious damage to flagellates, probably due to the lower doses of the UVR and migration of the zoospores deeper into the snow. The massive production of protective secondary carotenoids resulting in orange or red cell color (Bidigare et al., 1993) was not observed because the snowfield melted before the zygospores could be formed and could initiate their synthesis in high light conditions.

Both parameters $rETR_{max}$ and α change in response to incoming PAR, and the E_k is always the result of their interplay. Although both $rETR_{max}$ and α reacted dynamically to the changing light environment, E_k remained relatively stable. Higher sensitivity of algae to irradiance resulted in lower E_k values being observed at the North Site. The E_k variability of *Chlamydomonas nivalis* cysts was much greater, and similar variability was also observed in its vegetative cells (Stibal et al., 2007). Those experiments were carried out in a natural community, however, and so the results could reflect the response of the cell type prevailing in the samples, in this case the orange spores. It is possible that the cells use different mechanisms for acclimation to high PAR and/or UVR according to the stage of their life cycle. The preliminary results indicate that the flagellate cells adjust their photosynthetic apparatus to prevailing/average light conditions and migrate, but the cysts protect themselves by rapidly adjusting their photochemistry to actual incident irradiance and by production of red and orange screening compounds (Bidigare et al., 1993; Remias et al., 2005; Kvíderová, 2009). The change of photoacclimation strategy could be one of the first steps of resting stage formation when morphological changes are not detectable. Laboratory experiments with pure cultures of flagellates and cysts will be necessary to confirm this hypothesis.

Conclusions

Snow is an environment characterized by low temperatures, freeze-thaw cycles, high PAR and UVR, and low nutrient concentrations. Permanent snowfields, e.g. at high altitudes in mountainous regions or in the polar regions, could be among "objectively extreme, but stable environments" where the conditions and their changes could be predictable (Elster, 1999). In contrast to these permanent snowfields, the typical snowfields of the Giant Mountains last only several weeks, and the conditions at a snowfield could change completely over just several days. Although the snow temperature remains stable, within two or three hours the PAR and UVR could range from full sunlight to dim light during a thunderstorm. The snow chemistry indicates possible phosphorus limitation and its supply from surrounding vegetation results in the development of dense algal populations at the margins of snowfields. The snowfields in the Giant Mountains should be regarded as a marginal, unstable type of extreme environment (Elster, 1999), and isolates from such habitats should adapt to relatively broad ranges of conditions.

The variable chlorophyll fluorescence can provide a deep insight into photochemical processes in snow algae and their response to dynamic environmental conditions. The photosynthetic apparatus of the *Chloromonas* cf. *nivalis* zoospores is adapted to prevailing incoming PAR of approximately 350–500 μmol m^{-2} s^{-1} indicated by relatively stable E_k in spite of dynamic light conditions encountered in the field. According to actual PAR conditions, the flagellates move actively in the snow to reach favorable irradiance. The migration provides rapid and effective protection of the photochemical processes against high PAR and UVR, which could negatively affect algal physiology and biochemistry, not only at the level of photosystem 2 photochemistry. Even if the high PAR and UVR caused only minor damage to the cells, it still could be detected by fluorescence methods and negative correlations between the RLC parameters, and environmental factors could be found.

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