University of South Bohemia, Faculty of Biological Sciences
Department of Botany
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Master Thesis

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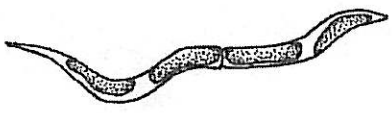
Comparison of Life Strategies in Two Green Algæ from Snow and Soil of the Polar Regions

~or~

Raphidonema nivale in snow – a resident or a guest?



Supervised by: **Josef Elster** & **Ondřej Prášil**

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Annotation: Ecological and physiological characteristics of two algæ from polar terrestrial habitats – *Raphidonema nivale*, isolated from snow, and *R. sempervirens*, isolated from soil - were compared their temperature and light optima for growth and photosynthesis. *In situ* observations were performed in a summer season at a snowfield on Svalbard.

Acknowledgements: This work was supported from the grants EU No. QLRI-CT-2001-01645 (J. Elster) and MŠMT No. 5/2001 (J. Elster and O. Prášil). I am very grateful to J. Elster, O. Prášil and the members of the 9th Czech Expedition on Svalbard for advice and help.

Hereby I declare that I worked up this thesis myself only with a help of literature listed in References and people listed in Acknowledgements.

January 9th, 2004, České Budějovice

1. Introduction

Snowfields and glaciers are inhabited by algæ that have developed special ecological and physiological adaptations to such a harsh environment. Most of the „true" snow algæ belong to the order Chlamydomonadales (Chlorophyceae); they go through a complex life history involving motile vegetative stages with flagella that undergo copulation or thick-walled resting spores and zygotes that allow them to survive the time when snow has completely melted and probably to be spread by wind (Hoham 1974a, b, 1975b, Hoham and Mullet 1977, 1978, Hoham et al. 1979, 1983, Ling and Seppelt 1993, 1998, Ling 2001, 2002, Müller et al. 2001). They also produce secondary carotenoids that provide them protection against excessive light (Bidigare et al. 1993).

However, there is a number of in-snow-found species of the genera *Raphidonema* and *Koliella* that have none of these adaptations available. They are simply-filamentous green algæ, recently proposed to belong to Trebouxiophyceae (Katana et al. 2001), with no sexual reproduction and special stages observed nor shield pigments production recorded. In most cases, they are associated species only present in low amounts, with an exception of *Koliella tatrae* found to be dominant of green snow in Tatra Mts., Slovakia (Komárek et al. 1973).

The commonest representative of them, *Raphidonema nivale* Lagerh., was first described in red-colored snow from Mount Pichincha in Ecuador (Lagerheim 1892). Since that it has been reported in snow from Greenland (Kol 1969), Svalbard (Kol and Eurola 1974, Leya et al. 2000), Europe (Kol 1974, 1975a, b, Kol and Eurola 1973), North America (Kol 1968), South America, Japan (Kobayashi and Fukushima 1952), Antarctica (Kol 1972, Mataloni and Tesolín 1997, Komárek and Komárek 2001), Australia (Marchant 1982) and New Zealand (Thomas and Broady 1997, Novis 2002a). It was also the first species of snow to be transferred into pure culture and treated in laboratory (Hoham 1973).

Bringing into cultures has emerged more questions than answered. Cells of *Raphidonema* and *Koliella* strains showed great pleiomorphism comprising variability in size and shape as well as number of cells in filaments, with many overlapping features: within a single strain of *R. nivale*,

morphotypes resembling *Raphidonema sempervirens*, *Koliella chodatii*, *K. corcontica*, *K. nivalis*, *K. spiculiformis*, *Stichococcus bacillaris* and *Chodatia tetralantoidea* were present (Hoham 1973, Novis 2002). Also, changes in morphology and ultrastructure as a response to changing environmental factors were observed in a marine species *Koliella antarctica* (Zanetti et al. 2001). Therefore, validity of the genera *Raphidonema* and *Koliella* and their species based on morphological features (Hindák 1996) is not broadly accepted.

Moreover, most strains of *Raphidonema* and *Koliella* show different ecophysiological characteristics than those of typical snow algæ like *Chlamydomonas* or *Chloromonas* species (Hoham 1975a, Stibal 2003). Also, as mentioned above, *R. nivale* was never found to be abundant in direct microscopical observations even though isolated from the sample afterwards. This fact brought some authors to speculations whether it could be a soil species reaching snow with soil particles carried by wind such as *Stichococcus bacillaris* (Novis 2002a).

This study was focused on the comparison of two polar *Raphidonema* species – *R. nivale* and *R. sempervirens* - isolated from snow and soil, respectively. In order to learn about their ecological and physiological adaptations and/or acclimations to Arctic environment, growth and photosynthesis characteristics in varied temperature and light were examined. In addition, *in situ* observations on presence, abundance and dynamics of *R. nivale* in snow were made during a summer season on Svalbard. This work provides substantially new information on ecological and physiology of *Raphidonema nivale* and *R. sempervirens* and contributes to the general knowledge on life strategies of microalgæ inhabiting polar terrestrial habitats.

2. Materials and methods

2.1 Description of strains

Raphidonema nivale Lagerheim

Cells solitary or in filaments of indefinite length, cylindrical, with one or both tapered or both rounded apices, width 1-4 µm, length 2-50 µm, 1-2 parietal chloroplasts per cell. Strain isolated from snow collected on Antarctic Peninsula, 65°S.

Raphidonema sempervirens Chodat [Syn.: *Koliella sempervirens* (Chodat) Hindák]

Cells in short fragmentary filaments or solitary, cylindrical, with one tapered and one rounded end, width 2-3 µm, length 5-16 µm. 1-2 parietal chloroplasts in a cell. Strain isolated from soil collected on NW Spitsbergen, 79°N.

2.2 Growth and morphology measurements

In order to find temperature- and light demands of both strains, the method of crossed gradients of temperature and light was used (Kvíderová and Lukavský 2001). The light intensity reached from 50 to 1250 µmol.m^{-2}.s^{-1}, temperature from 0 to 24°C. Suspension of algæ in the concentration of 25,000 cells mL^{-1} was placed into microplates and optical density at 750 nm (so as to avoid chlorophyll absorption) was measured at weekly intervals using an iEMS plate-reader (LabSystems, Finland). Optical density was recalculated to cell concentration according to an empirically appointed conversion curve (Figure 1). Growth rates (R, expressed as the number of cell divisions, in days^{-1}) were calculated using the equation

$$R = \log(N_T/N_0)/(T \times \log 2) \quad (1)$$

where T is time (in days of cultivation), N_0 the initial cell concentration (25,000 cells mL^{-1}) and N_T is the cell concentration after T days.

In samples from temperature- and light extremes (tl - 0°C, 50 µmol.m^{-2}.s-1; Tl - 24, 50; tL - 0, 1250; TL - 24, 1250), cell length and width was measured, and cell apices shape and number of cells in filaments were

recorded. Statistical analysis of morphological data was conducted using the analysis of variance (ANOVA).

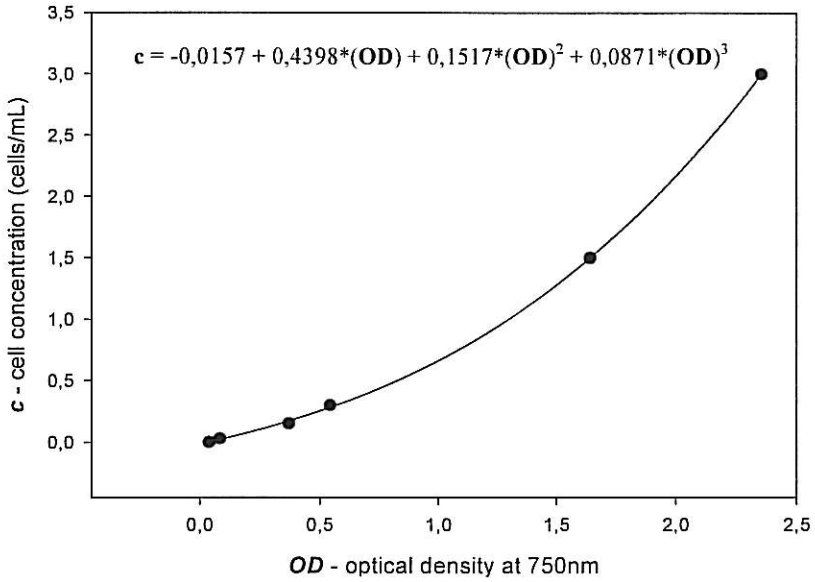

Figure 1: Conversion curve of *Raphidonema nivale* and *R. sempervirens* for recalculations optical density to cell concentrations: $r^2(y_0)=0.62$; $r^2(a)=0.99$; $r^2(b)=0.99$; $r^2(c)=0.99$

2.3 Photosynthesis measurements

For photosynthesis measurements, strains of both *Raphidonema nivale* and *R. sempervirens* were batch cultured at three different temperatures (3, 12 and 22°C) and growth light 80 $\mu mol.m^{-2}.s^{-1}$. Light was provided by Osram Dulux L55W/12-950 compact fluorescent lamps. Cultures were grown in Tamiya culture flasks in BG11 medium, bubbled with air to ensure ample CO_2 supply and mixing and placed in temperature controlled water baths. Cultures were maintained at each growth temperature for at least one week prior to analysis. Prior to each measurement, cultures were acclimated to the measuring temperature (3, 12 or 22°C) for 30 min in the measuring cuvette.

For pigment analyses, 5 mL of cell suspension was collected on a glass fibre filter (GF/F, Whatman) and ground in 90% acetone in a tissue homogenizator. Glass fibre debris was removed by centrifugation and absorption of the clarified acetone extracts was measured against 90% acetone in a dual beam spectrometer (Shimadzu UV 3000). Chlorophyll concentration was calculated according to Jeffrey and Humphrey (1975).

Pigment composition was determined by high performance liquid chromatography (HPLC) using acetone extracts collected in the same manner as those for chlorophyll analyses, but using 100% acetone. Separation was performed using a polymeric C_{18} reversed-phase column (VYDAC 201TP) with a linear gradient from eluent A (methanol: 0.5 M ammonium acetate 80:20) to eluent B (methanol: acetone, 70:30) (Van Heukelem et al. 1992). Pigments were quantified by integration of the respective peak areas and determined after Jeffrey et al. (1997).

The absolute values of the steady-state rate of oxygen evolution were measured with the Clark-type electrode (YSI Model 5793, YSI Inc., Ohio, USA). The measurements were performed at 3, 12 and 22°C in temperature-controlled laboratory-built chamber (Bartoš et al. 1972). The actinic light was provided by the projector with 250W halogen lamp equipped with series of neutral density filters. The intensity of the actinic light was measured using the PAR quantum detector. The signal from the oxygen electrode was digitised using the Oxycorder (Photon System Instruments, Czech Republic) and the OxyWin software. The gross photosynthesis was calculated from the net photosynthesis after a correction for oxygen consumption due to dark respiration.

Chlorophyll variable fluorescence at different intensities of the actinic light was measured in parallel to the oxygen evolution using the PAM-101 fluorometer (Walz, Germany) equipped with fibreoptics light guide (the Multiple Turnover or MT protocol). Before exposure of cell suspensions to each actinic light intensity, the cells were also maintained for 3 minutes in the dark. The following yields of the chlorophyll fluorescence were then measured: F_o - the intrinsic fluorescence yield in the dark, F_m - the maximum fluorescence in the dark, F' - the steady state value of the fluorescence in the light and F_m' - the maximal fluorescence yield in the light. The maximum fluorescence yields were induced by pulse of saturating light of 1 sec duration.

From the measured fluorescence yields, the following parameters were calculated according to Maxwell and Johnson (2000). The maximum quantum yield of photochemistry in Photosystem II is defined as

$$F_v/F_m = (F_m-F_0)/F_m \qquad (2)$$

Appendix 1) simulating freeze-thaw cycles. Two aliquots were desiccated in a desiccator at 0°C and 20°C, respectively.

Viability evaluation was based on controlled cultivation of algal colonies on agar plates (Lukavský 1974, 1975). Cryovials from liquid nitrogen were quickly melted in water bath 40-50°C for ca 2-5min, then the inoculum of 0.1 mL was uniformly spread by a glass rod on an agar plate with the Z nutrient solution solidified with 2% agar. Petri dishes were exposed in identical unit and conditions as pre-cultivation for ca 1 week and evaluated under the light microscope as

$$V = (N_C \times 100)/(N_C + N_D) \quad (5)$$

where V is the viability in %, N_C the number of colonies and N_D the number of dead cells.

Table 1: Used regimes of freezing and desiccation

Process:		freezing	freezing	freezing	desiccation	desiccation	freezing
Starting temperature		20°C	20°C	20°C	20°C	20°C	20°C
Final temperature		-4°C	-40°C	-100°C	20°C	0°C	-196°C
phase 1	start temp	20°C	20°C	20°C	20°C	20°C	20°C
	final temp	0°C	0°C	0°C	20°C	0°C	-196°C
	rate	-4°C/min	-4°C/min	-4°C/min			
	time	5 min	5 min	5 min	1 week	1 week	60 min
phase 2	start temp	0°C	0°C	0°C			-196°C
	final temp	-4°C	-40°C	-40°C			20°C
	rate	-5°C/min	-5°C/min	-5°C/min			
	time	1 min	8 min	8 min			5 min
phase 3	start temp	-4°C	-40°C	-40°C			
	final temp	-4°C	-40°C	-100°C			
	rate	0°C/min	0°C/min	-12°C/min			
	time	5 min	5 min	5 min			
phase 4	start temp	-4°C	-40°C	-100°C			
	final temp	40°C	40°C	-100°C			
	rate			0°C/min			
	time	5 min	5 min	5 min			
phase 5	start temp			-100°C			
	final temp			40°C			
	rate						
	time			5 min			
times repeated		3	3	3	1	1	1

2.5 Study area and collection of algæ

The study site was located right by the forefront of Werenskiöld glacier (77°04.428´N; 15°14.943´E) in altitude about 100 m a.s.l., sloped 20-40° to W-SW. Snow surface showed patchy pink coloration caused by spores and

zygotes of *Chlamydomonas nivalis* and in upper parts was polluted with soil from above (Figure 2 and Appendices 4, 5). During the whole summer season, samples of snow from upper layers (up to 5 cm), soil and meltwater were collected into sterile plastic 50mL flasks and no later than one hour after sampling examined with a field microscope (Leitz HM-Lux), cell shape and size was recorded and cell concentration calculated using a Cyrus-I counting chamber. Individual cells were drawn. Conductivity, pH and temperature of samples were measured with the Testo 252 multimeter (Testo, Germany).

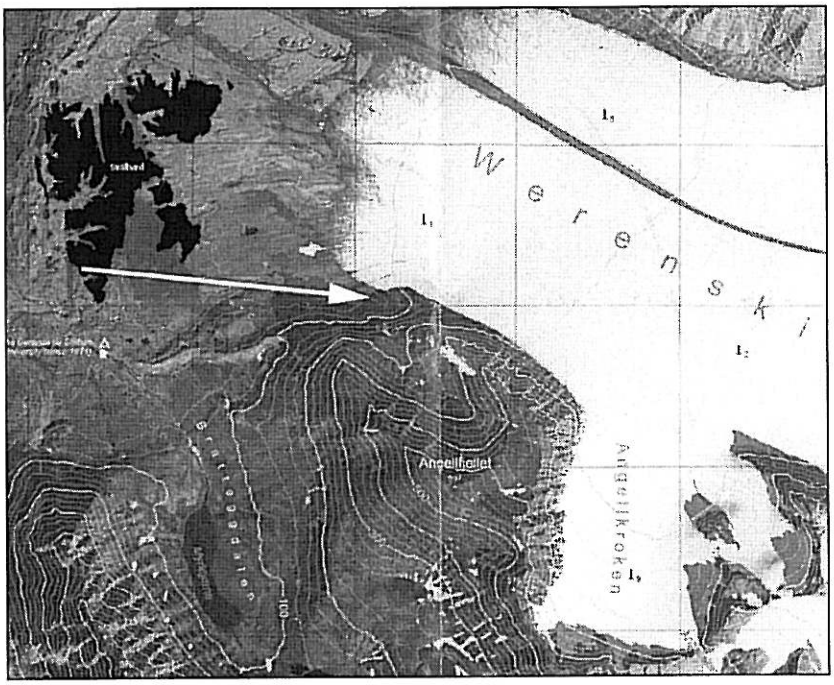

Figure 2: Map of the study area (arrow head) and localisation on Svalbard (arrow tail). The 1990-orthophotomap by Jania et al. (2002).

Used abbreviations:

RN	*Raphidonema nivale*
RS	*Raphidonema sempervirens*
rn0322	*R. nivale* grown at 3°C and measured at 22°C etc.
TL	temperature 24°C, light 1250 μmol.m^{-2}.s^{-1}
Tl	temperature 24°C, light 50 μmol.m^{-2}.s^{-1}
tL	temperature 0°C, light 1250 μmol.m^{-2}.s^{-1}
tl	temperature 0°C, light 50 μmol.m^{-2}.s^{-1}
Chl *a*	chlorophyll a
Chl *b*	chlorophyll b
F_v/F_m	maximum quantum yield of photosynthesis
ETR	electron transport rate
$PSII_{Closure}$	fraction of closed reaction centres of Photosystem II
P_{max}	chlorophyll normalised maximum photosynthetic rate (nmol O_2.mol Chl^{-1}.min^{-1})
α	chlorophyll normalised light-limited photosynthetic efficiency (nmol O_2.mol Chl^{-1}.min^{-1}.μmol^{-1}.m^{-2}.s^{-1})
E_k	minimum irradiance required to saturate photosynthesis (μmol.m^{-2}.s^{-1})

3. Results

3.1 Temperature- and light demands

In *Raphidonema nivale*, after 42 days of cultivation at crossed gradients the maximum cell concentration reached 370,000 cells mL^{-1} (OD^{750} = 0,67) at 12°C and 200 $\mu mol.m^{-2}.s^{-1}$ (Figure 4). This cell concentration corresponds to growth rate 0.09 day^{-1}. Figure 3 shows temporal changes in cell concentrations for temperature and light extremes. In lower temperature, growth rates were considerably higher than in higher temperature.

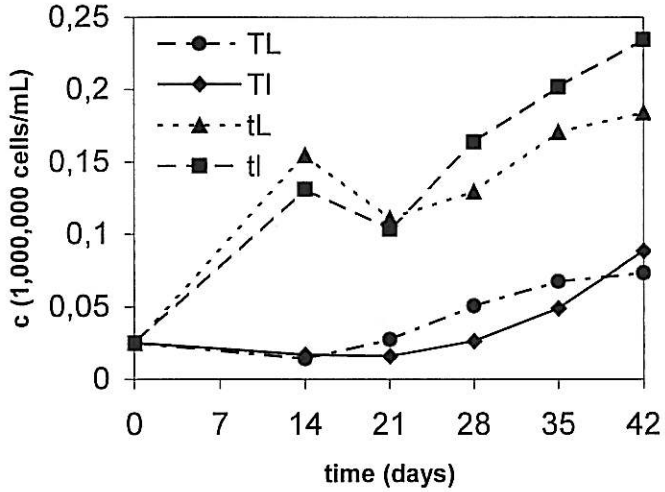

Figure 3: Growth of *R. nivale* cultured at crossed gradients of temperature and light, as the cell concentration for extreme values of temperature and light

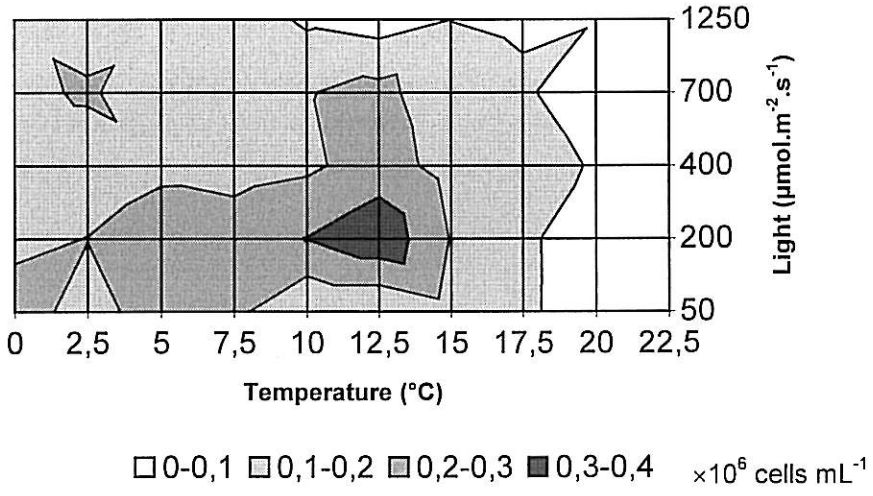

Figure 4: Growth of *Raphidonema nivale* cultured at crossed gradients of temperature and light, as the cell concentration (10^6 cells mL^{-1}, grey scale areas) after 42 days

In *Raphidonema sempervirens*, after 35 days of cultivation at crossed gradients the maximum cell concentration reached 1,270,000 cells mL^{-1} (OD^{750} = 1,50) at 18°C and 50 $\mu mol.m^{-2}.s^{-1}$ (Figure 6). This cell concentration corresponds to growth rate 0.16 day^{-1}. Figure 5 shows temporal changes in cell concentrations for temperature and light extremes. The highest growth was reached at higher temperature and lower light.

Figure 7 shows growth rates of both strains at temperature and light extremes and at optima for each strain.

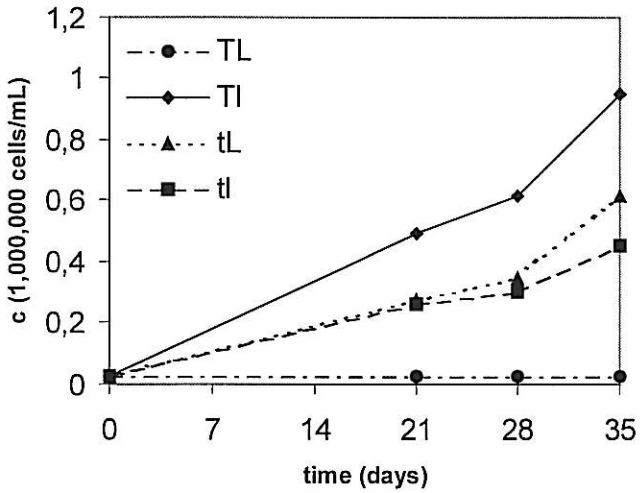

Figure 5: Growth of *R. sempervirens* cultured at crossed gradients of temperature and light, as the cell concentration for extreme values of temperature and light

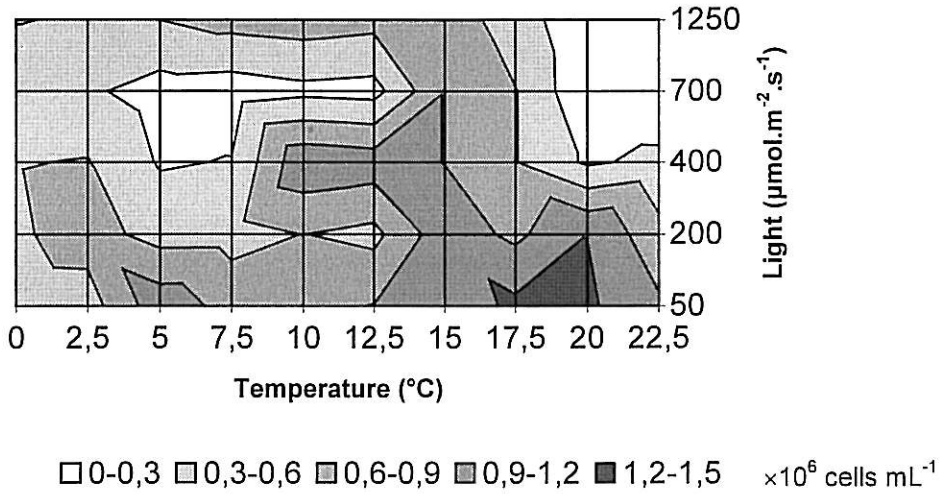

Figure 6: Growth of *Raphidonema sempervirens* cultured at crossed gradients of temperature and light, as the cell concentration (10^6 cells mL^{-1}, grey scale areas) after 35 days

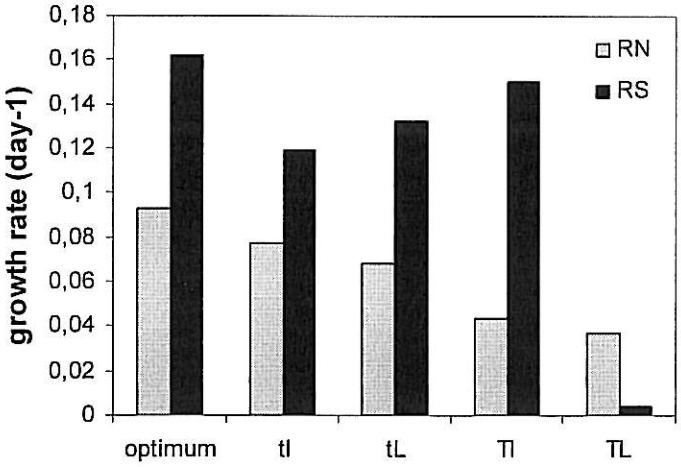

Figure 7: Growth rates (day^{-1}) for temperature and light extremes and optima at crossed gradients of temperature and light. For *R. nivale*, optimum = 12°C and 200 $\mu mol.m^{-2}.s^{-1}$, for *R. sempervirens*, optimum = 18°C and 50 $\mu mol.m^{-2}.s^{-1}$.

3.2 Morphological variability

Morphological modifications were observed as a response to temperature and light variation. Figure 8 shows variability in cell size and shape in *R. nivale*, Figure 9 similar variability in *R. sempervirens*.

In *Raphidonema nivale*, the cells showed significant differences in length ($p<0.01$; $F=23.76$), width ($p<0.01$; $F=20.15$), shape of apices ($p<0.01$; $F=33.43$) and number of cells in filaments ($p<0.01$; $F=18.66$). Cells were longer in higher temperature and within a single temperature value longer in lower light; wider in higher temperature; number of cells in filaments was higher in higher temperature; no trends in shape of cell apices were apparent.

In *Raphidonema sempervirens*, there were significant differences in cell length ($p<0.01$; $F=53.77$), width ($p<0.01$; $F=25.00$) and shape of apices ($p<0.01$; $F=17.23$). Cells were longest in high temperature and high light; wider in higher temperature; there was no trend in the shape of cell ends; differences in number of cells in filaments were not significant ($p=0.24$; $F=1.4$).

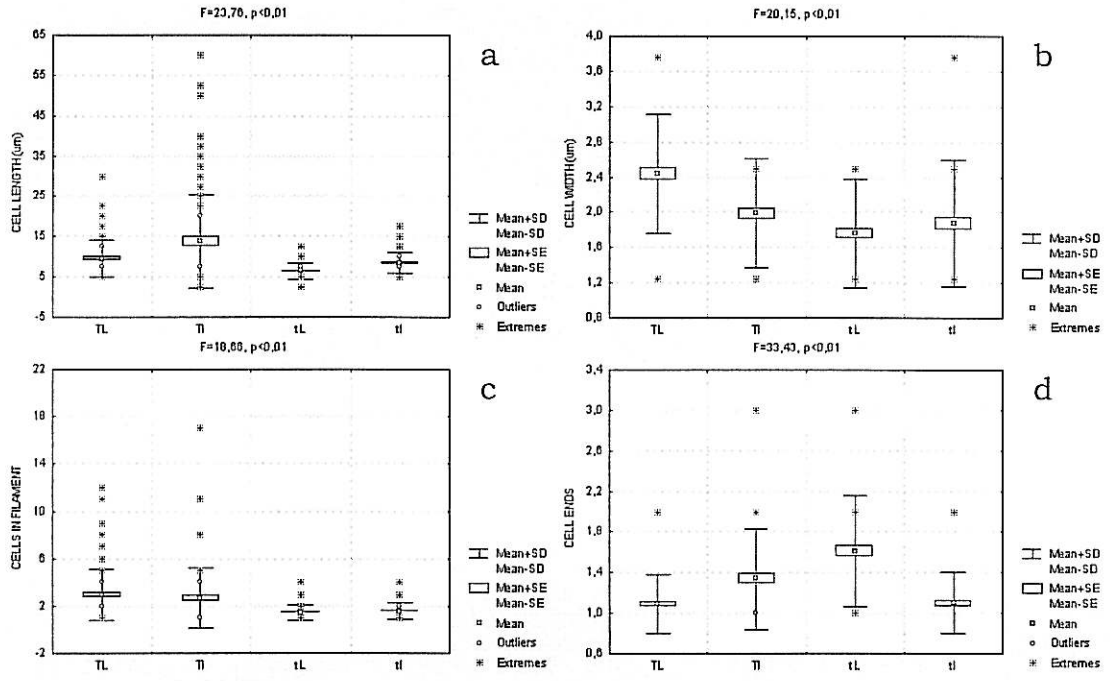

Figure 8: Morphological parameters of *Raphidonema nivale* in extreme values of temperature and light: a) cell length, b) cell width, c) number of cells in filament, d) shape of cell apices – 1=both apices tapered, 2=one apex rounded and one tapered, 3=both apices rounded (n=100).

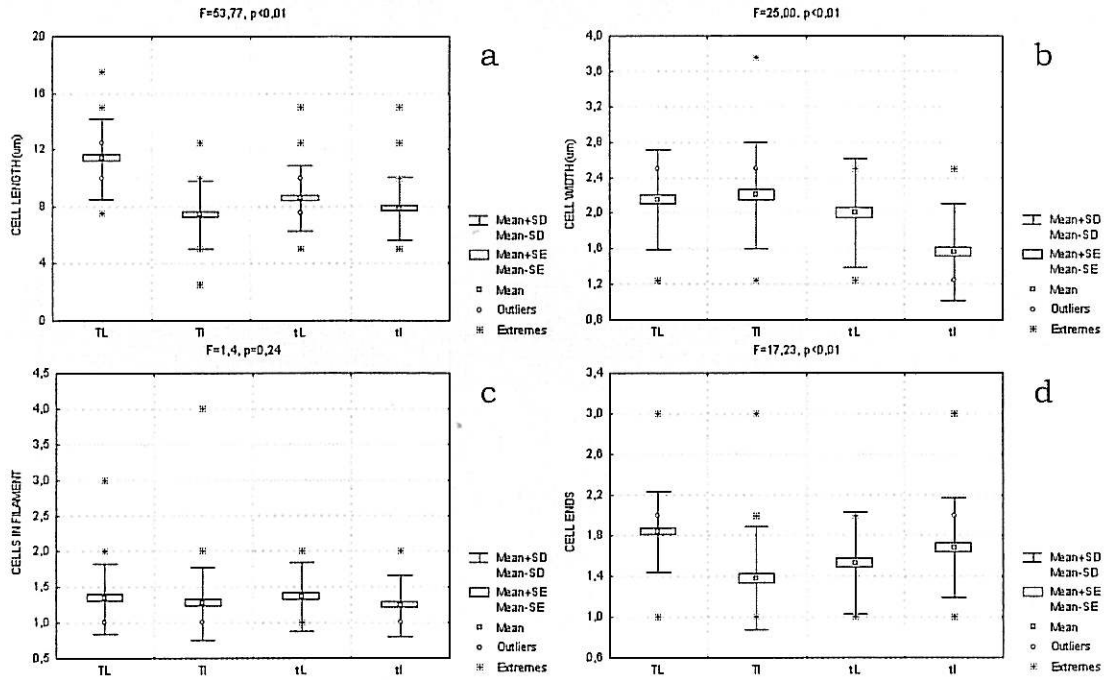

Figure 9: Morphological parameters of *Raphidonema sempervirens* in extreme values of temperature and light: a) cell length, b) cell width, c) number of cells in filament, d) shape of cell apices – 1=both apices tapered, 2=one apex rounded and one tapered, 3=both apices rounded (n=100).

3.3 Pigment composition

Pigment composition of both strains is shown in Table 2. Neither astaxanthin nor other secondary carotenoids were detected. There was no significant difference between pigment composition of *Raphidonema nivale* and *R. sempervirens*.

Table 2: Relative pigment composition of the strains of *R. nivale* and *R. sempervirens*

	Neo xanthin	Viola xanthin	Anthera xanthin	Lutein	Zea xanthin	Chl *b*	Chl *a*	β-carotene
RN	0,059	0,078	0,016	0,219	0,024	0,113	0,422	0,069
RS	0,060	0,100	0,009	0,173	0,048	0,077	0,496	0,037

3.4 Rate and efficiency of photosynthesis

Both strains were successfully grown at 2 and 12°C, whereas only *Raphidonema sempervirens* was also capable of growth at 22°C. All five growth variants were then measured at 2, 12 and 22°C. Photosynthetic activity of *R. nivale* was recorded at all temperatures, even at 22°C.

The kinetics of photosynthetic electron transport was clearly temperature dependent in both species. The amount of reduced electron acceptors was highest and therefore the rate of electron transport was the lowest at 3°C (Figure 10). The values of the maximum quantum yield of photosynthesis in the dark (F_v/F_m) measured by both MT and ST techniques for all variants are shown in Figure 11. In *R. nivale*, it reached from 0.57 to 0.74 when measured by MT, or from 0.52 to 0.61 when measured by ST; in *R. sempervirens* the values were between 0.50 and 0.73, or 0.55 and 0.67, respectively.

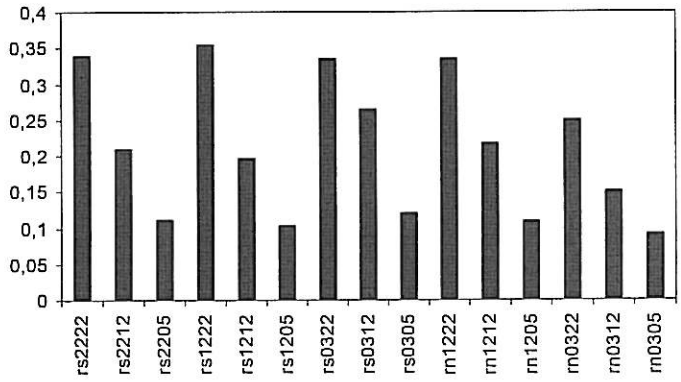

Figure 10: The fraction of reduced electron acceptors of PSII at blue actinic light (72 $\mu mol^{-1}.m^{-2}.s^{-1}$) 6 ms after the ST pulse

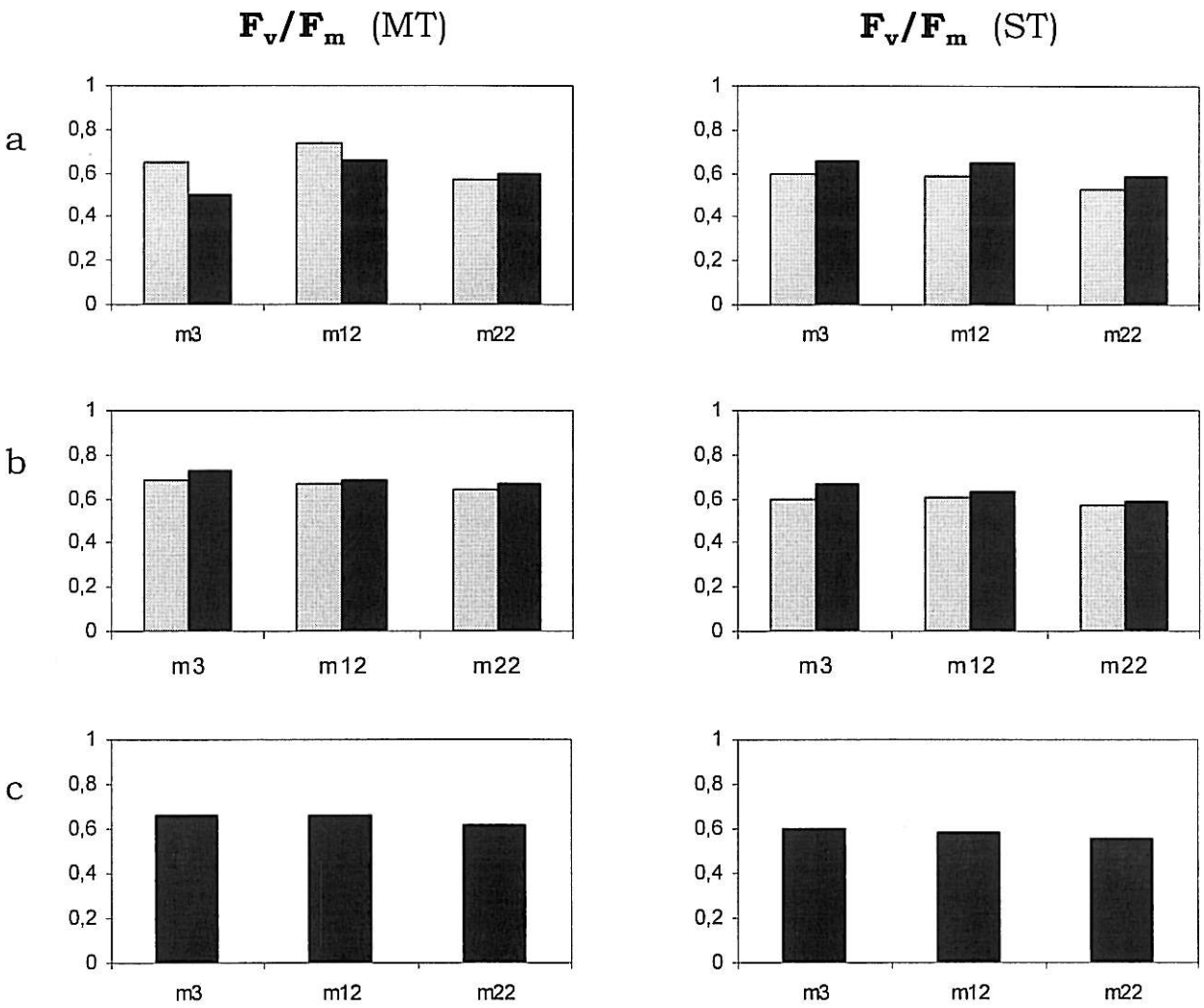

Figure 11: The values of the maximum quantum yield of photosynthesis in the dark (F_v/F_m) measured by MT and ST protocol (grey bars – *R. nivale*, black bars – *R. sempervirens*, a – grown at 3°C, b – grown at 12°C, c – grown at 22°C, m3-m22 – measuring temperature)

Photosynthetic parameters (P_{max}, E_k and α) calculated from $PSII_{Closure}$ and ETR are shown in Figures 12 and 13.

In *R. nivale*, chlorophyll normalised maximum photosynthetic rate was higher when grown at 3°C (reaching 103 nmol O_2.mol Chl^{-1}.min^{-1} when measured at 12°C) than at 12°C (reaching 61 nmol O_2.mol Chl^{-1}.min^{-1} when measured at 3°C). The chlorophyll normalised light-limited photosynthetic efficiency (α) was highest when grown at 12°C and measured at 3°C (0.90 nmol O_2.mol Chl^{-1}.min^{-1}.μmol^{-1}.m^{-2}.s^{-1}). The minimum irradiance required to saturate photosynthesis (E_k) ranged between 24 and 160 $\mu mol.m^{-2}.s^{-1}$.

In *R. sempervirens*, P_{max} increased with measuring temperature and reached 79 nmol O_2.mol Chl^{-1}.min^{-1} when grown at 3°C; similarly, E_k was highest when measured at 22°C and reached 115 µmol.m^{-2}.s^{-1} when grown at 3°C. The chlorophyll normalised light-limited photosynthetic efficiency (α) was highest when measured at 12°C (0.89 nmol O_2.mol Chl^{-1}.min^{-1}.μmol^{-1}.m^{-2}.s^{-1} when grown at 12°C).

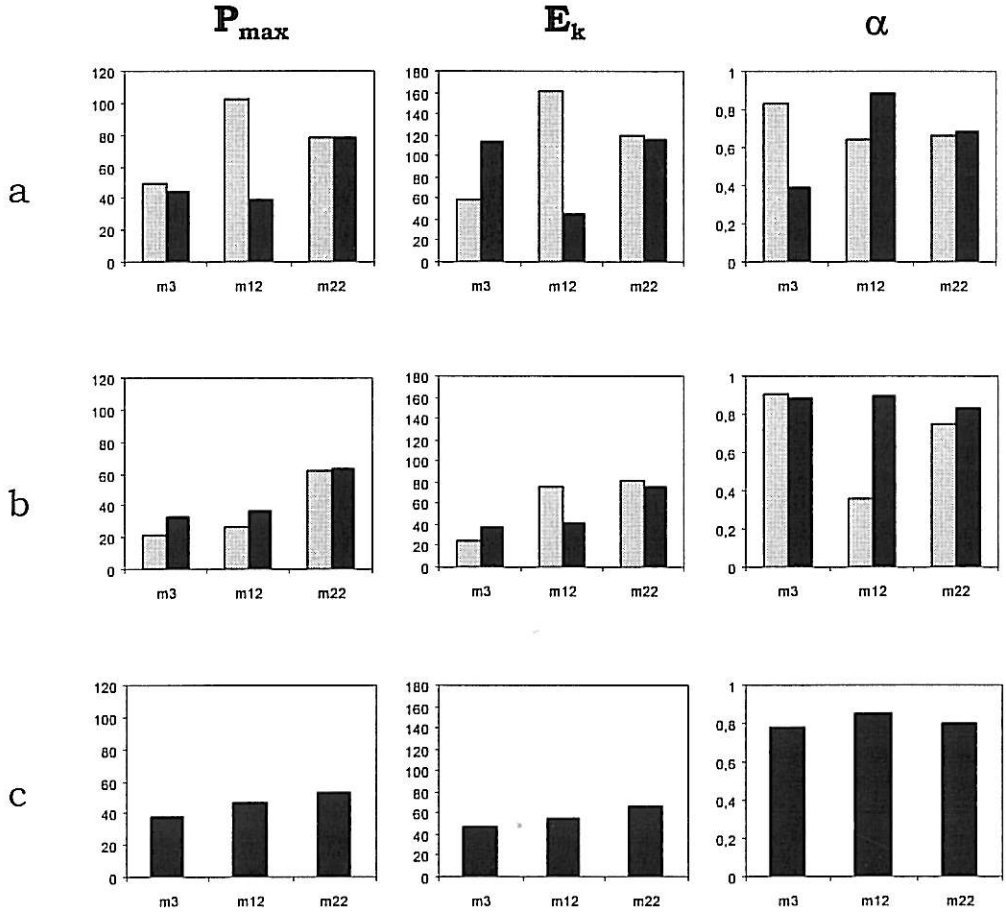

Figure 12: Values of P_{max}, E_k and α calculated from the electron transport rate (grey bars – *R. nivale*, black bars – *R. sempervirens*, a – grown at 3°C, b – grown at 12°C, c – grown at 22°C, m3-m22 – measuring temperature)

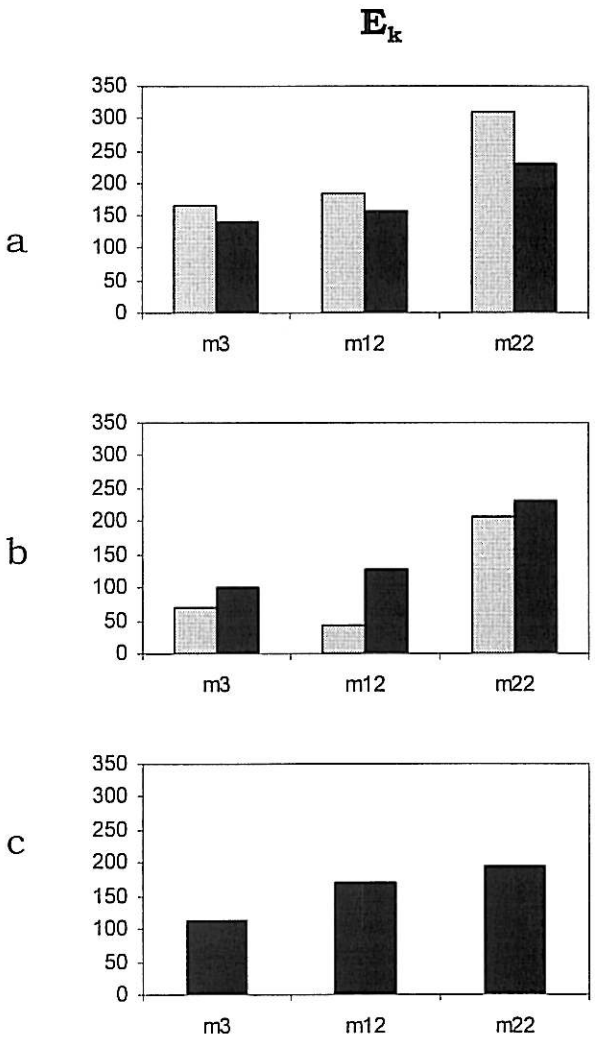

Figure 13: Values of E_k calculated from the $PSII_{Closure}$ (grey bars – *R. nivale*, black bars – *R. sempervirens*, a – grown at 3°C, b – grown at 12°C, c – grown at 22°C, m3-m22 – measuring temperature)

3.5 Viability after exposure to freezing and desiccation

Table 3 shows the absolute values of viability for both strains. Figure 14 shows viabilities of both strains expressed as the percentage of control sample viability.

Raphidonema nivale showed higher viability than *R. sempervirens* after exposure to deeper freezing (-40°C, ~~-~~100°C, liquid nitrogen) and after desiccation at 0°C. After freezing to –4°C and –40°C, viability of *R. nivale* was even higher than that of control samples (110.5% and 105.2% of control, respectively). Cells of *Raphidonema sempervirens* grew better than *R. nivale* after exposure to –4°C and after desiccation at 20°C. Also, viability in control samples was higher than in *R. nivale*. After freezing to -4°C, viability was higher than in control sample (100.4% of control sample).

Table 3: Viability (in %) after exposure to freezing and desiccation in *Raphidonema nivale* and *R. sempervirens*

	frozen -4°C	*frozen -40°C*	*frozen -100°C*	*direct in N_2*	*desic. 0°C*	*desic. +20°C*	*control*
RS	97,6	68,6	23,6	13,3	68,3	72,0	97,2
RN	92,5	91,4	56,1	61,5	76,7	6,3	83,7

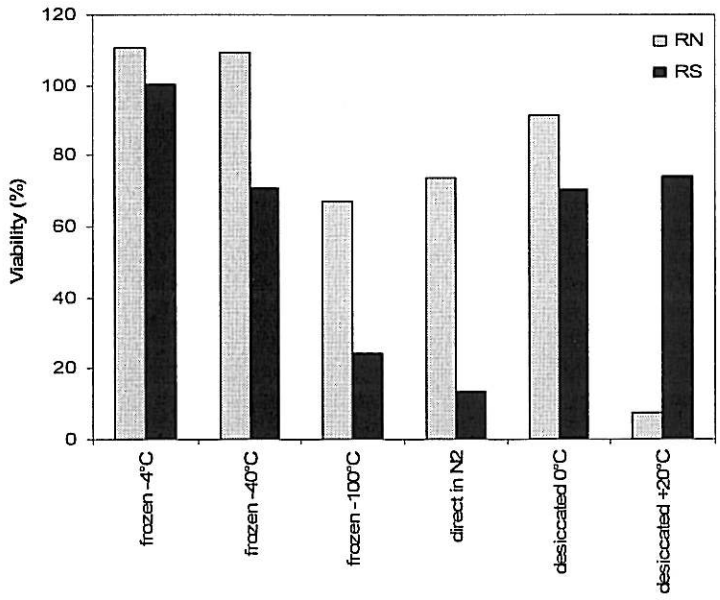

Figure 14: Viability after exposure to freezing and desiccation as the percentage of control sample in *Raphidonema nivale* and *R. sempervirens*

3.6 Field observations

Samples of snow were collected from 9th July, shortly after last snowfall, until 11th August 2003, when the snowfield was already crumbled and melted completely a few days afterwards (Appendix 4). Figure 15 shows the values of pH, conductivity and temperature of snow throughout the sampling period. In all samples, *Chlamydomonas nivalis*, a dominant alga of Arctic snowfields, was present in high abundances (reaching more than 700,000 cells mL^{-1}, see Figure 16), mostly in spores and zygotes. Also, vegetative flagellates were found.

Until 31st July, no cells of *Raphidonema nivale* were found in snow samples. On 29-30th July, a strong katabatic wind blew over Werenskiöld glacier, and brought large quantity of soil particles onto snow surface. In

samples from 31st July, high abundance of cells of *R. nivale* (>1,000,000 cells mL^{-1}) was found, while abundances of *C. nivalis* remained unchanged. Faint green coloration of water-snow-soil mixture was visible. Cells were morphologically very variable, solitary or in short filaments (up to 8 cells), straight or coiled, mostly with tapered apices, size 2-3 × 12-50 µm. Morphotypes resembling *Koliella spiculiformis*, *K. spirulinoides*, *K. nivalis* and *Stichococcus bacillaris* were found (Figure 17), and clear transitions between morphotypes were observed. In samples from the next day, abundance of *R. nivale* decreased to ca 100,000 cells mL^{-1} and most of cells showed signs of damage. On 6th August, another katabatic wind occured and brought more soil on the snowfield. In samples from 7th August, abundance of *R. nivale* was high again (ca 1,000,000 cells mL^{-1}). Since that, it was decreasing until the end of the sampling period (Figure 16).

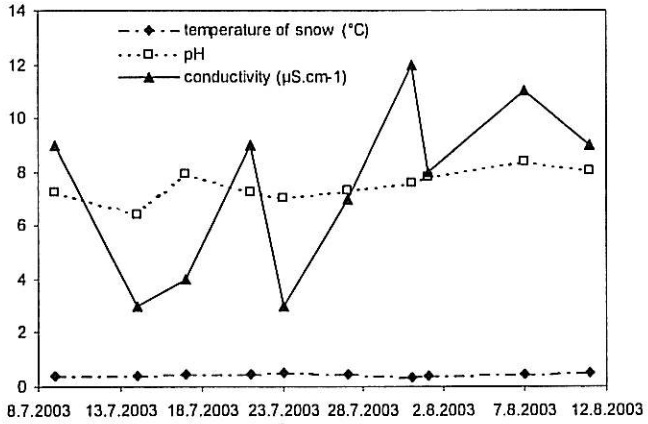

Figure 15: Values of snow temperature, pH and conductivity at the studied snowfield during the entire sampling period

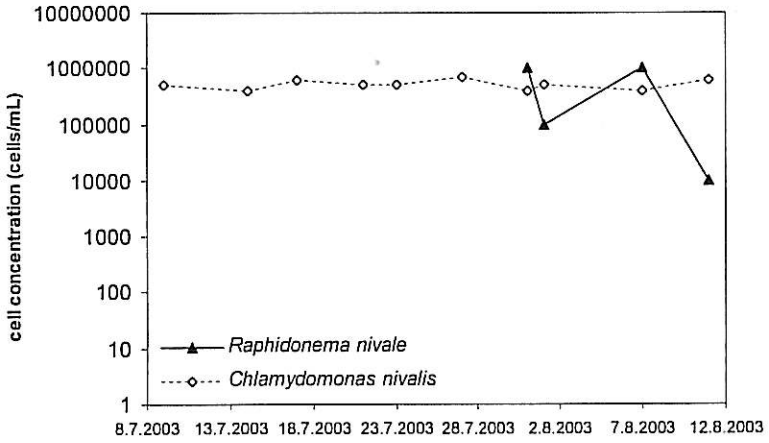

Figure 16: Cell concentrations of *Raphidonema nivale* and *Chlamydomonas nivalis* in snow samples from the Werenskiöld snowfield

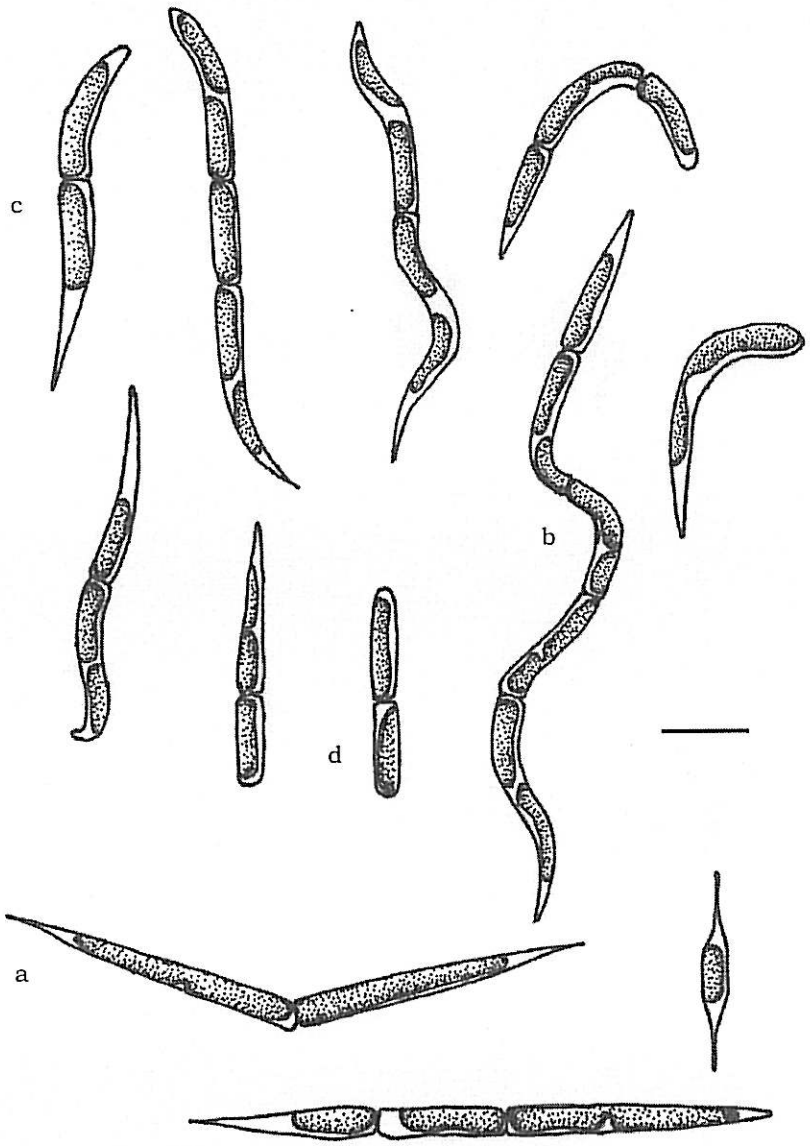

Figure 17: Cells and filaments of *Raphidonema nivale* observed in snow samples from Werenskiöld snowfield (scale bar = 10µm). Morphotypes resembling *Koliella spiculiformis* (a), *K. spirulinoides* (b), *K. nivalis* (c) and *Stichococcus bacillaris* (d)

4. Discussion

Despite being described more than a hundred years ago and reported in snow from all over the world, the species *Raphidonema nivale* still retains its secrets. While the dominant and eye-striking algæ like *Chlamydomonas nivalis* have been paid much attention so far in terms of taxonomy, ecology, physiology or biochemistry, *R. nivale* and its allied species have been more or less avoided.

Morphology and taxonomy

An exception from this avoidance is the long-term discussion on the validity of the genera *Raphidonema* and *Koliella*, both comprising species inhabiting snow and soil in the Polar Regions. After Hindák (1996), cells of *Koliella* should not form filaments with physiological connections and should fall into fragments shortly after cell division, whereas *Raphidonema* does form filaments. This is accepted by some authors (Mataloni and Tesolín 1997, Komárek and Komárek 2001), but some consider them overlapping and place them into *Raphidonema* (Hoham 1973). According to molecular data, even the genus *Koliella* appears polyphyletic, and a branch containing soil and snow species seems to be closely relative to another similar species *Stichococcus bacillaris* (Katana et al. 2001). Moreover, pleiomorphism is a well-known phenomenon in these algæ, induced by variability of physical factors or culture conditions (Hoham 1973, Zanetti et al. 2001) and enabled by high percentage of matrix polysaccharides and a low amount of cellulose (Piro et al. 2000).

All these discrepancies and imperfections make the determination of single species found in both soil and snow very difficult, if not impossible, and limits it often only to generic level, which complicates descriptions and comparisons of algal communities from these habitats. Some authors consider every morphological variation significant and describe rich snow communities: e.g. Komárek and Komárek (2001) found three species of *Koliella* and four species of *Raphidonema* (one of them new) in snow from the King George Island in the maritime Antarctica, without any apparent transitions among them. On the other hand, many authors list only *Koliella* or *Raphidonema* sp., without more detailed descriptions (Yoshimura et al. 1997, Ohtani et al. 1998, Müller et al. 1998, 2001, Takeuchi 2001).

Results of the morphological evaluation of strains cultured in different temperature and light conducted in this study show that particularly cell length and width of *R. nivale* and *R. sempervirens* can be changed by temperature and light. Cell apices of both strains were also changed, this can be however caused by manipulation during experiments, as there was no apparent trend. In *R. nivale*, number of cells in the filament is also influenced by temperature. These variations are most likely caused by changes in growth and division rates. During field observations at Werenskiöld snowfield, several morphotypes resembling different species of *Koliella* and *Raphidonema* were found, with clear transitions between them (unlike at King George Island, Komárek and Komárek 2001). This is in accordance with observations reported by Novis (2002a). No transitions were however found when comparing cultures of *R. nivale* and *R. sempervirens*.

Therefore, it is suggested here that *R. nivale* is a pleiomorphic species responding to environmental changes (e.g. transfer soil-snow) by growth and division modification, and thus changing cell size and shape, which may cause misdeterminations and descriptions as multi-species communities.

Ecology

As summarized by Elster (2002), green algæ represent important components of microbial assemblages in both soil and snow in the Polar Regions. In soil, it is a very diverse group, especially in the Arctic (Elster et al. 1999), and displays high ability to survive freezing and desiccation that occurs very often. In snow, species of the order Chlamydomonadales predominate, completing their whole life-cycles within snow and surviving non-snow periods in resting spores and zygotes. The number of these autochthonous species of the „true" snow algæ is however limited, compared to the total number of species reported from snow. The rest are allochthonous algæ brought onto snow by wind or water, being able to stay alive and even proliferate for a short time. Soils are most likely the pool for such species, as there is close contact with snow and soil for the entire growing season.

The snow species of *Raphidonema* and *Koliella* never predominate in snowfields, with an exception reported from Tatra Mountains, Slovakia

(Hindák and Komárek 1968, Komárek et al. 1973), and in most cases they are only present in low amounts, often attached to soil particles or vegetation, and occur particularly at localities with higher nutrient supply (Ling and Seppelt 1993, Mataloni and Tesolín 1997, Müller et al. 1998, Komárek and Komárek 2001). Therefore, some authors suggest that they are capable of growing in other habitats than snow (Hoham 1975a), or even that they are soil algæ only occasionally brought on snow (Novis 2002a). Results given in this study support the latter suggestion. Cells of *R. nivale* only occured in snow after katabatic wind had blown and a soil-water-snow mixture had formed blackish patches on the snow surface, whereas concentrations in soil were stable, though low, for the entire sampling period. Faint green coloration caused by high cell density of *R. nivale* at Werenskiöld snowfield can be explained by physical concentration of cells, also described by Novis (2002b). Also, the fact that very similar species of *Raphidonema* were recorded in polar soils (Broady 1979, Cavacini 2001) supports this suggestion. The absence of *R. nivale* in soil can be attributed to wrong determinations due to being unexpected.

Growth and photosynthesis

Regarding temperature optima for growth, both *Raphidonema nivale* and *R. sempervirens* can be considered psychrophilic. Having its optimum at 12°C, the strain of *R. nivale* seems adapted/acclimated to lower temperatures than *R. sempervirens*, and it may enable this species to survive in snow for some time and thus be reported as a snow alga. However, when compared with the „true" snow algæ, temperature optima for *R. nivale* obtained in this study appear rather high: Hoham (1975a) reports growth optima as low as 1°C in *Chloromonas pichinchae*, and similar optima were found in *Chloromonas nivalis* (Stibal 2003). The only data on growth of *R. nivale*, given by Hoham (1975a), show that this species is able to grow in a wide range of temperatures (1-15°C) and grows best at 5°C. However, the continuous gradient of temperature was not used in Hoham's study, and therefore 5°C cannot be taken for the exact optimum.

For *R. sempervirens*, no data to compare with are available. Growth demands seem to be similar to those of the closely relative species *Stichococcus bacillaris*, isolated from alpine soil (Stibal 2003), and also of

another soil alga *Cylindrocystis brébisonii* (Hoham 1975a), also found in Arctic and Antarctic soils (Davey and Clarke 1991, Brynychová et al., 2001) and sometimes reported from snow (Kol 1972, 1975b, Yoshimura et al. 1997, Takeuchi 2001).

In terms of photosynthesis, the „true" snow algæ are expected to be set on low temperatures and their sensitivity to temperature higher than 10-15°C is attributed to the membrane lipids and photosystems proteins composition, similar to those described in a *Chlamydomonas* species from an Antarctic lake (Morgan-Kiss 2002a, b). Several *in situ* measurements have been performed (Thomas 1972, Komárek et al. 1973, Williams et al. 2003) showing that snow algæ are well capable of photosynthetic activity in snow. Soil algæ are known to photosynthesize even at –7°C (Davey 1989). However, response of snow and soil algal photosynthesis to environmental alterations is not known.

As reviewed by Davison (1991), temperature effects on photosynthesis can be divided into three groups: short-term changes in light-saturated photosynthesis, short-term changes in light-limited photosynthesis and long-term acclimations. The typical response for light-saturated photosynthesis is to increase progressively with increasing temperature to an optimum, and then to decline rapidly. In light-limited photosynthesis, the light harvesting efficiency (α) tends to decrease with increasing temperature, and so does the rate of net photosynthesis. In algæ grown for some time in lower temperature, the maximum rate of photosynthesis (P_{max}) tends to increase and the photosynthesis optimum shifts downwards. Also, it was observed that the photosynthesis optimum temperature is often higher than the optimum for growth.

Results from photosynthesis experiments gained in this study show that both *Raphidonema nivale* and *R. sempervirens* are well adapted to low temperatures, as evident from F_v/F_m values measured at 3°C. In *R. nivale*, the maximum rate of photosynthesis was higher when grown in lower temperature, and higher in higher incubation temperature. In *R. sempervirens*, P_{max} increased with incubation temperature and was higher in lower cultivation temperatures. The minimum irradiance needed for saturation of photosynthesis consistently increased with incubation temperature, and tended to be higher when grown in lower temperature. This

corresponds with the general knowledge of temperature effects on algal photosynthesis (Davison 1991). Also, it is apparent that photosynthesis optimum in *R. nivale* is higher than optimum for growth, as it was able to photosynthesize even in temperature in which it did not grow.

It is probable that in *R. nivale* low temperature is not the main limiting factor for growth in snow, as it is able to photosynthesize even in low temperature, as well as the soil species *R. sempervirens*. However, because *Raphidonema* and *Koliella* species were only found in snow with high nutrient content, and in nutrient-depleted snow exhibited damage after a short time, it is hypothesized here that due to the lack of photoprotective pigments in their cells, it is the synergical effect of low temperature, high irradiance and nutrient depletion that is fatal for them. In polar soil, inhibitory irradiance is very infrequent, and nutrient supply higher than in snow. Thus, soil algæ are exposed to low temperature, but not with concomitant stress of high irradiance and nutrient depletion. As shown in this study, both strains are quite well adapted to survive freeze-thaw cycles, particularly *R. nivale*, which was also observed by Hoham (1975a). Exposure to subnatural temperature showed that *R. nivale* is well equipped for deep freeze, probably by membrane composition. All these features indicate a good adaptation/acclimation for polar soil environment and support the hypothesis that *R. nivale* is a soil species.

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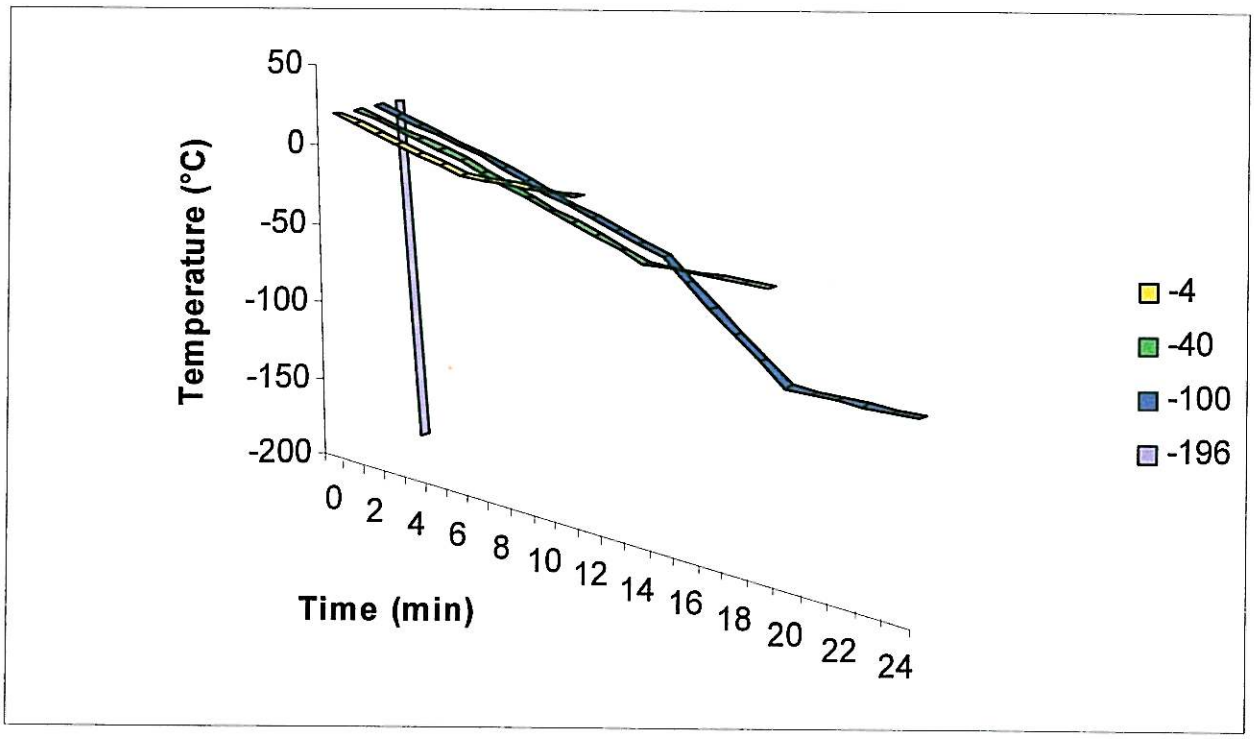
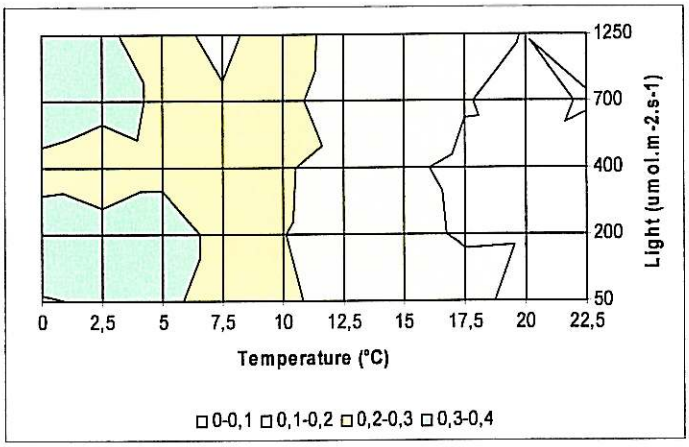
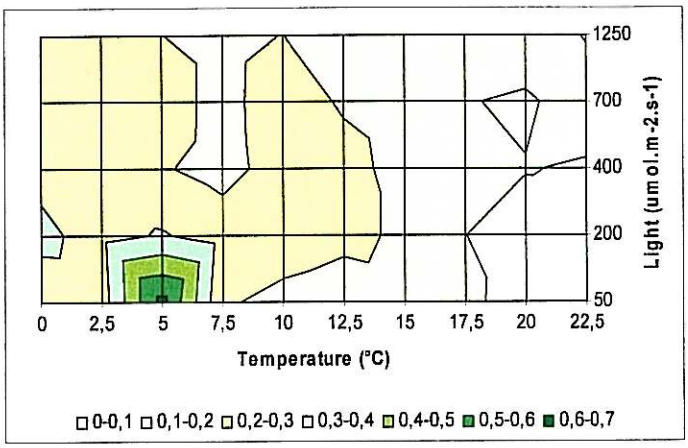
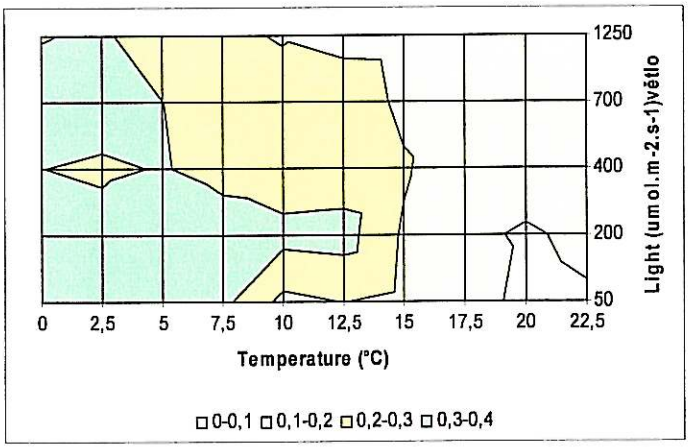
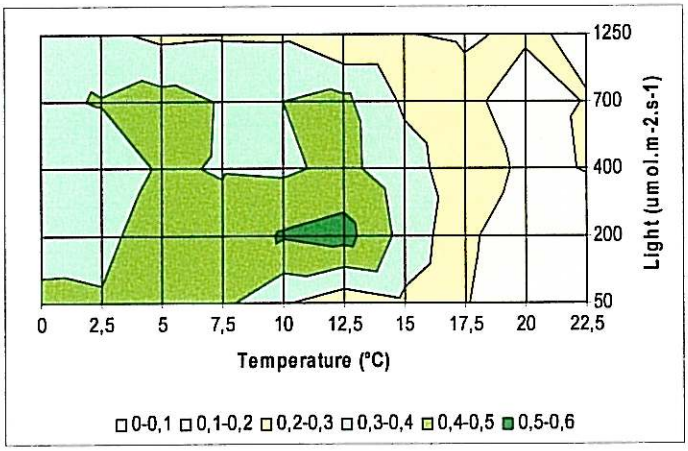


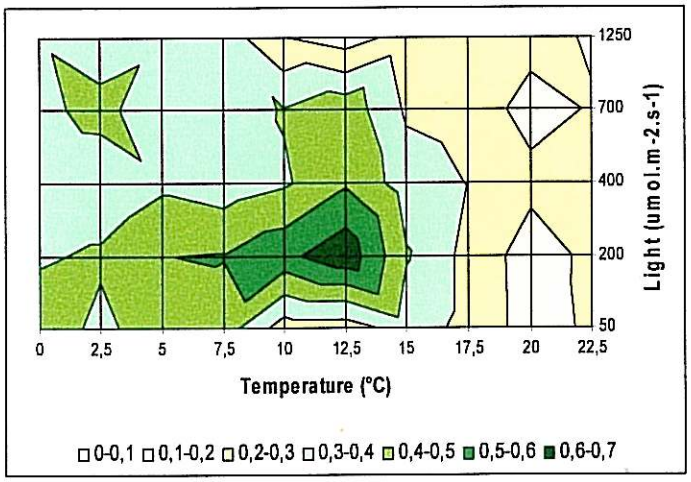
Appendix 1: Visualisation of freezing regimes described in 2.5





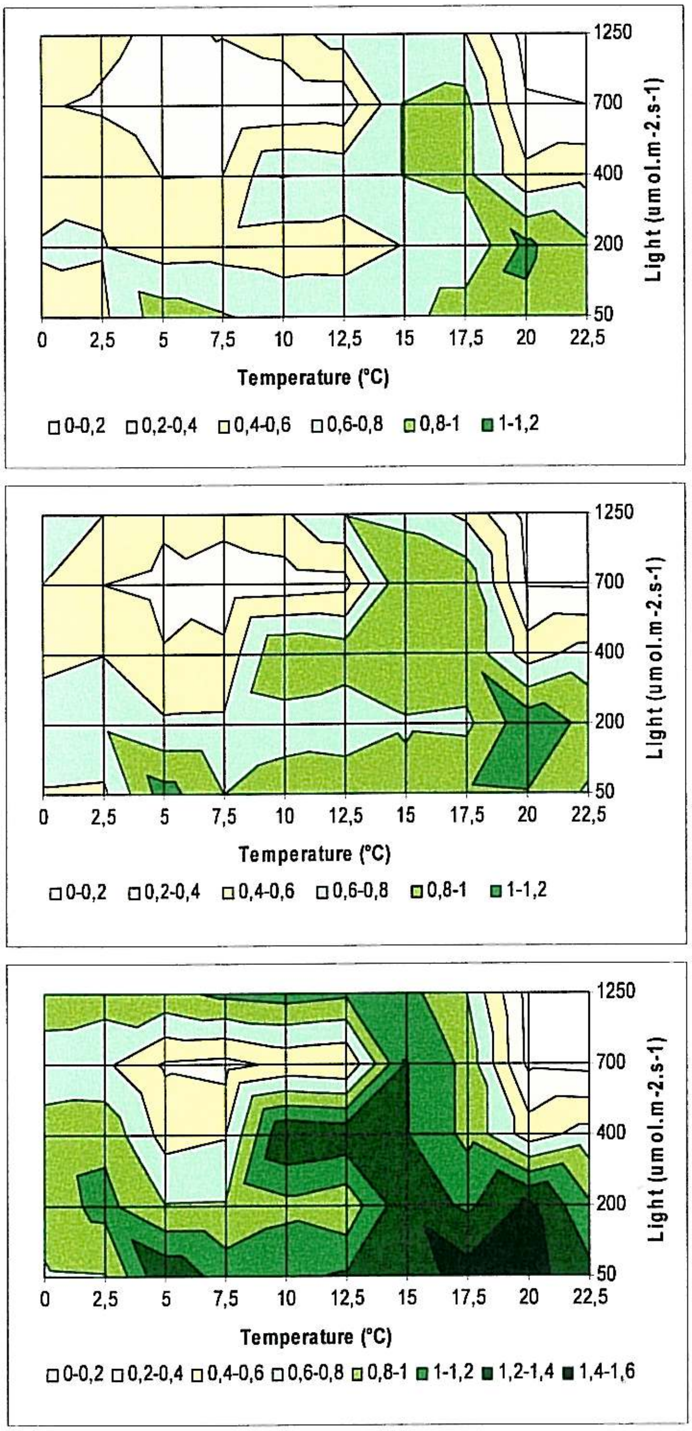






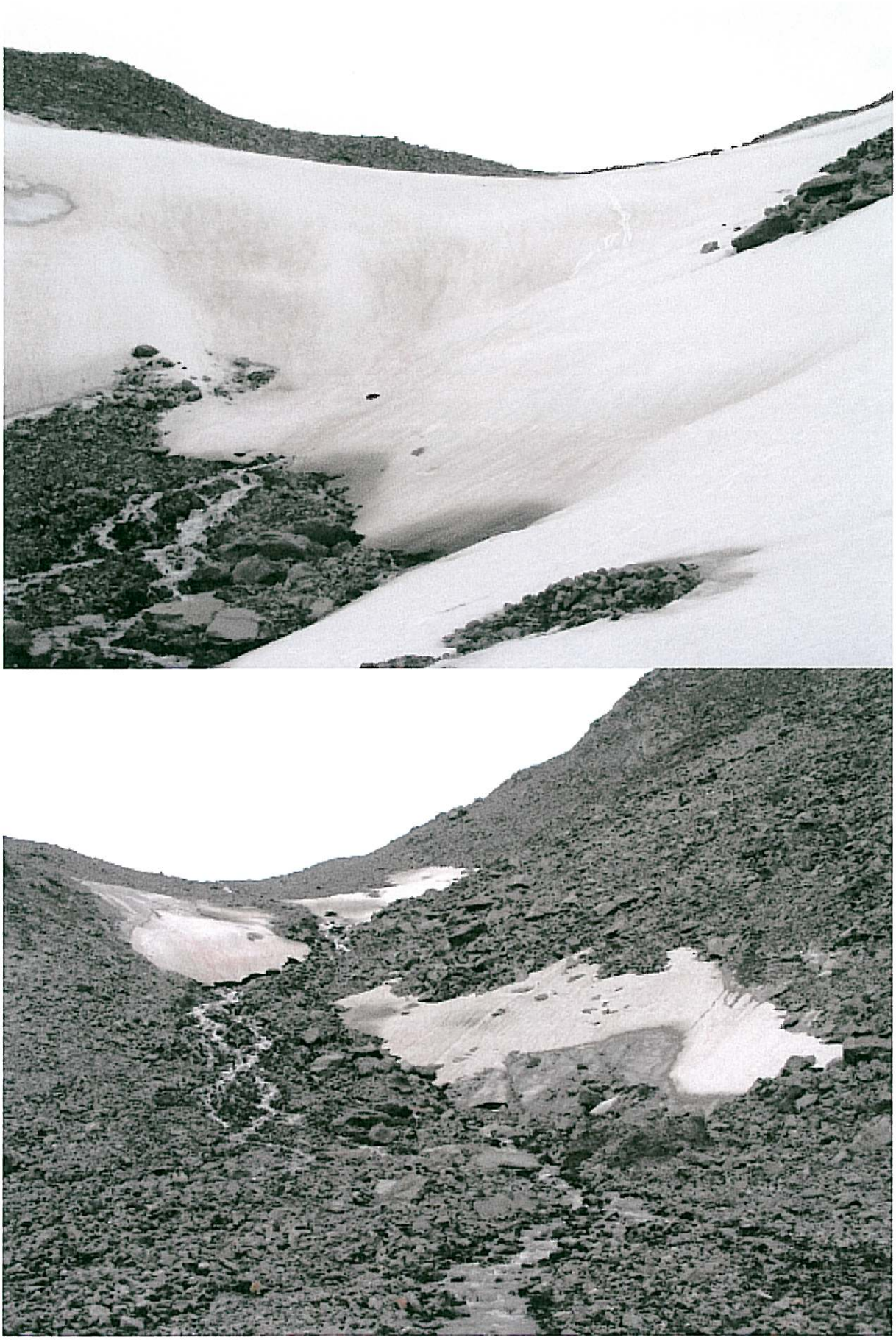
Appendix 2: Crossed gradients diagrams showing OD^{750} values of *R. nivale* from day 14(a), 21(b), 28(c), 35(d) and 42(e).

a b
c d
e



Appendix 3: Crossed gradients diagrams showing OD^{750} values of *R. sempervirens* from day 21(a), 28(b) and 35(c).

a
b
c



Appendix 4: Study site at Werenskiöld glacier. Above the snowfield in mid-July, below in mid-August 2003

Appendix 5: Soil brought onto snow by katabatic wind (föhn). Above a general view, a detail below where slight green coloration caused by *R. nivale* is visible