Desiccation tolerance and osmotic potential of *Zygnema* on Svalbard

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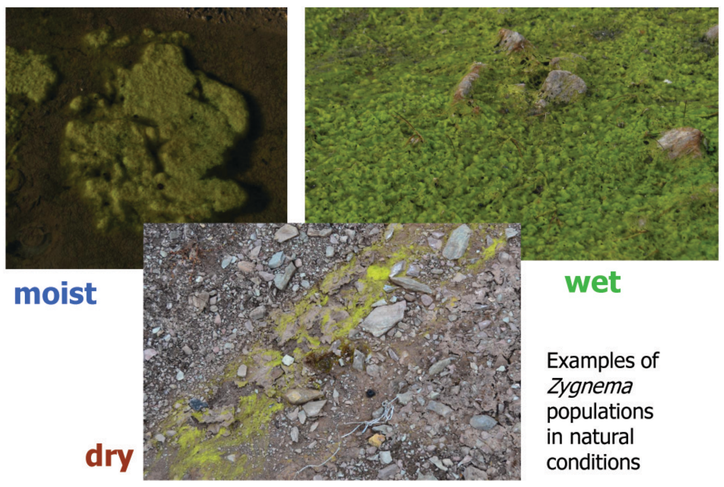
Introduction

Zygnema is a genus of filamentous green algae belonging to the class Zygnematophyceae (Streptophyta). They occur worldwide, including polar regions, and grow typically in shallow pools, streamlets or on the surface of wet soil where they are exposed to a range of environmental stresses, including UV radiation (Pichrtová et al. 2012) and desiccation. It has been suggested that a direct relationship between desiccation tolerance and osmotic potential exists in aeroterrestrial green algae (Kaplan et al. 2011). Moreover, osmotic potential of Arctic and Antarctic strains of *Zygnema* in laboratory conditions has been studied recently (Kaplan et al. 2012). Therefore, the aim of our study was to to evaluate the level of field acclimation of *Zygnema* cells to desiccation n field conditions. We selected populations in different stages of natural desiccation and compared their cell osmotic potential and ability to cope with osmotic stress.



Examples of *Zygnema* populations in natural conditions

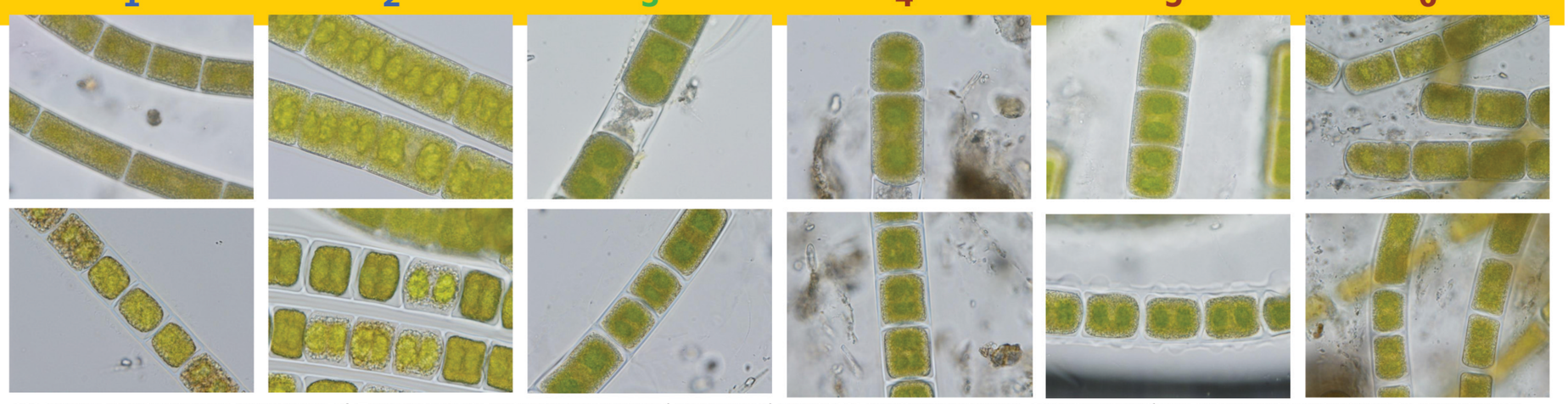
Materials and methods

Field samples were collected in Billefjorden, Svalbard. Six natural populations in 3 stages of desiccation were selected – "**moist**", biomass floating in water, „**wet**", biomass on wet soil surface without free water, and „**dry**", biomass on dry soil surface forming paper-like films. Morphology and viability of the cells was studied with light and epifluorescence microscopy. Physiological state was estimated by measuring chlorophyll a fluorescence parameters - maximum quantum yield of PSII (F_V/F_M) and steady-state quantum yield of PSII in the light (Φ_{PSII}). Osmotic potential (Ψ_s) of the cells at the turgor loss was estimated as the osmotic potential of sorbitol solution in which plasmolysis already occurs. Furthermore, the algae were stressed with 2M sorbitol (Ψ_s in which plasmolysis occurred in all samples) and then their recovery in water was studied.

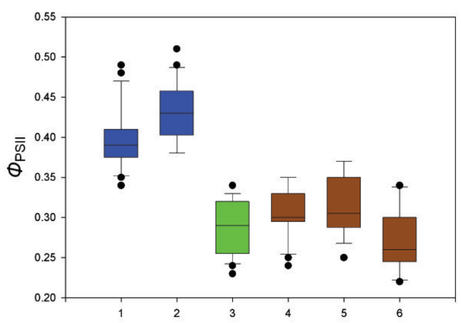
Results

Population	1	2	3	4	5	6
Plasmolysis occurrence in sorbitol solutions:						
300 mM	-	-	-	-	-	-
450 mM	+	+	-	-	-	-
600 mM	++	++	-	-	-	-
750 mM	++	++	+	-	-	+
% viable cells in the experiment with 2M sorbitol:						
before	100	100	100	80	90	100
after	40	25	95	80	90	100

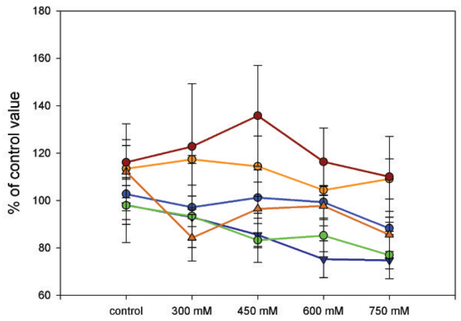
Occurrence of plasmolysis and proportion of viable cells in different molar concentration of sorbitol. + first occurrence of plasmolysis, ++ 50% of the cells plasmolysed. Dead and viable cells were counted before and after the treatment with 2M sorbitol.



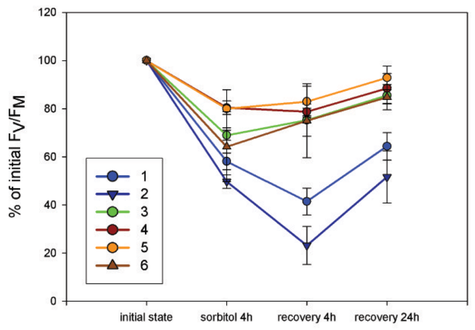
LM images showing normal appearance of *Zygnema* cells in water (upper row) and plasmolysed cells in 2M sorbitol (lower row). Note the very dense cytoplasmic content that is characteristic for forming pre-akinetes (McLean & Pessoney 1971).

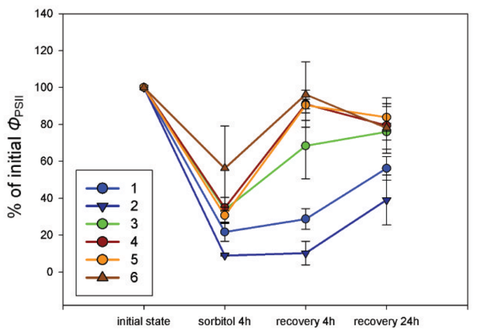


Steady-state quantum yield of PSII in the light (Φ_{PSII}) prior to the experiment. Moist populations show significantly higher photosynthetic activity than populations subjected to various levels of desiccation stress.

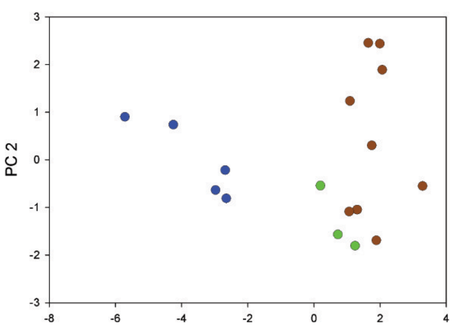


Φ_{PSII} after 24h incubation in different sorbitol concentrations. Relative values compared to the initial state are given. In populations 1, 2 and 3 Φ_{PSII} decreased significantly with increasing sorbitol concentration.

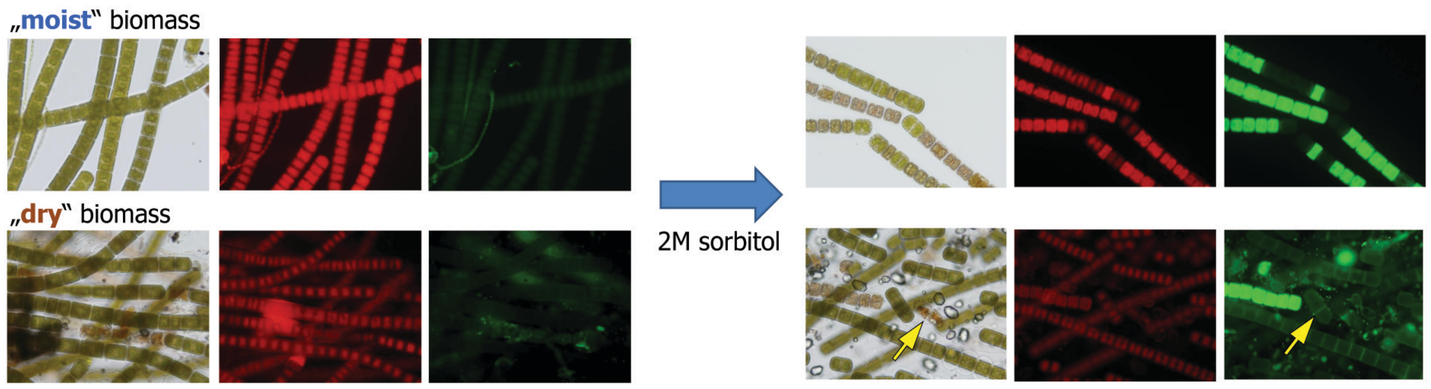




Fluorescence parameters changes during sample incubation in 2M sorbitol. Relative values compared to control samples and to the initial values before the experiment are given. Dry populations recovered initial values of both fluorescence parameters within only 4 hours, whereas moist populations did not fully recover even after 24 hours in water. The wet populations resemble the dry ones; however, their initial values of Φ_{PSII} did not fully recover after 4 hours.



PCA ordination plot of samples stressed i by the incubation in 2M sorbitol solution based on various measured parameters. The first principal component explains 68% of the total variation, the second PC 19%. The moist samples are apparently separated from the others along the first principal component which strongly correlates with recovery rate of the fluorescence parameters. The PC2 is mainly related to initial values of F_V/F_M.



LM and epifluorescence images – chlorophyll autofluorescence and SYTOX green dyed samples. The pictures were taken and the proportion of viable cells was estimated both before the incubation in 2M sorbitol and at the end of the experiment, after subsequent 24h recovery in water. The SYTOX green dye penetrates into cells with damage membranes. However, dead cells without nucleic content are not marked by SYTOX green anymore (see yellow arrows).

Summary

A majority of viable cells was observed in all field samples including fully desiccated biomass suggesting that *Zygnema* is well adapted to its life in semi-terrestrial environment. Even though the overall appearance of the cells with dense cytoplasmic content was similar in all samples, the **moist** populations markedly differed in their reaction to osmotic stress from **wet** and **dry** populations. They plasmolysed sooner (at 450 mM sorbitol) and also showed high mortality after incubation in 2M sorbitol (Ψ_s=−5.9 MPa). Moreover, the recovery of fluorescence parameters thereafter was slower in **moist** populations too. Nevertheless, a significant decline in steady-state quantum yield of PSII in the light with increasing sorbitol concentration was proven in both **moist** and **wet** samples. This indicates that a distinct stress response occurs also in **wet** samples even though plasmolysis cannot be observed under 750 mM sorbitol. In summary, it appears that as the cells are subjected to reduced water potential during slow natural desiccation, they become resistant (hardened) to severe desiccation stress.

ACKNOWLEDGEMENTS: This study was supported by the Grant No. LM2010009 CzechPolar (MSMT CR), and CZ.1.07/2.2.00/28.0190 (EU). Support was granted as a long-term research development project of the Academy of Sciences of the Czech Republic RVO 67985939 and Specific University Research project No. 263212.

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