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ORIGINAL PAPER

Annual development of mat-forming conjugating green algae *Zygnema* spp. in hydro-terrestrial habitats in the Arctic

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Abstract Conjugating green algae of the genus *Zygnema* (Zygnematophyceae, Streptophyta) are dominant eukaryotic components of hydro-terrestrial microbial mats in the Arctic. Considering the harsh environmental conditions, the aim of this study was to elucidate mechanisms that enable *Zygnema* spp. to thrive in this habitat. We hypothesized that changes in morphology, physiological performance, and stress tolerance take place during the annual life cycle of the algae. We thus selected four natural populations of *Zygnema* spp. on Svalbard and investigated them throughout the vegetation season by means of light microscopy and chlorophyll *a* fluorescence. Additionally, we also investigated one overwintering population. No formation of specialized resting stages (e.g., dormant zygospores) was observed. Markedly, *Zygnema* spp. survived harsh periods as modified vegetative cells, i.e., pre-akinetes. Pre-akinetes tolerate both desiccation during summer and freezing in winter. These cells are not dormant and therefore recover their physiological activity immediately after transfer to favorable conditions, undergoing rapid growth in the early spring. Nevertheless, once pre-akinetes begin to grow, these newly produced vegetative cells lose stress tolerance. Such rapid dehardening explains their high mortality due to frequent freeze–thaw cycles in the early spring. Arctic *Zygnema* spp. thus face a phenological trade-off between missing the early growing season and experiencing frost damage.

Keywords Chlorophyll *a* fluorescence · Desiccation · Green algae · Stress tolerance · Svalbard · Zygnematophyceae

Introduction

The lack of liquid water is the major stress factor affecting living organisms in polar regions. Frozen water is present most of the year, and during the short summers, the precipitation level is usually low (Robinson et al. 2003; Rautio et al. 2011). Nevertheless, many terrestrial habitats are supplied by meltwater from snow and glacier-fed streams or melting permafrost throughout nearly the entire vegetation season. Such environments, which are referred to as hydro-terrestrial, include areas such as wetlands, shallow lakes, pools, streams, seepages, and springs (Elster 2002). Hydro-terrestrial habitats are generally small, shallow, and unstable. These habitats can partially or fully dry out during the summer, undergo diurnal freeze–thaw cycles in the spring and autumn, and freeze solid in the winter. Therefore, any inhabitants of such habitats must be well adapted to these annual cycles of unfavorable conditions.

Due to specific climatic conditions including the lack of liquid water, the biodiversity and ground cover of vascular plants is low in high Arctic desert and/or semidesert environments (Thomas et al. 2008). On the other hand, these environments are usually rich in microbial life. In

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many extreme polar habitats, eukaryotic microalgae and cyanobacteria, together with lichens and mosses, represent the most important phototrophs (Aleksandrova 1988; Vincent 2000). Numerous reports show that eukaryotic microalgae are less stress-resistant than cyanobacteria (Davey 1988, 1989; Šabacká and Elster 2006) and that they undergo typical annual development, while cyanobacteria form rather perennial mats (Tang et al. 1997). Winter is regarded as the major stressful season, causing significant declines in algal populations (Hawes1988, 1990; Davey 1991a; Davey and Rothery 1992; Šabacká and Elster 2006). New biomass grows rapidly from small inoculum in the early spring, sometimes even under snow (Davey 1988).

Filamentous conjugating green algae (class Zygnematophyceae), typically represented by the genus *Zygnema*, comprise the most common eukaryotic components of Arctic hydro-terrestrial mats (Sheath et al. 1996; Kim et al. 2008; Holzinger et al. 2009; Pichrtová et al. 2014a). These algae are closely related to land plants (Embryophyta; Leliaert et al. 2012) and are characterized by a special type of sexual reproduction, the process of conjugation, which leads to the formation of highly specialized, stress-resistant zygospores containing algaenan (a sporopollenin-like substance) in their cell walls (Kadlubowska 1984; Poulíčková et al. 2007). Despite the abundance of polar *Zygnema*, the formation of zygospores in these algae has only very rarely been observed (Elster et al. 1997). In addition, other types of specialized cells were described in the genus *Zygnema*, for example akinetes—asexual rectangular cells with cell walls of the same structure as that of the zygospores (Kadlubowska 1984; Stancheva et al. 2012).

However, polar *Zygnema* can survive various stress conditions, e.g., osmotic stress (Pichrtová et al. 2014a), desiccation (Pichrtová et al. 2014b), and freezing (Hawes 1990), by forming modified mature vegetative cells that are formed directly from vegetative cells via the gradual accumulation of storage materials and cell wall thickening (McLean and Pessoney 1971; Herburger et al. 2015). Their chloroplasts have reduced lobes, and their physiological activity is diminished. Here, we call such cells pre-akinetes (Fuller 2013; Pichrtová et al. 2014b) to distinguish them from true akinetes with characteristic cell wall features. The formation of pre-akinetes was previously observed in the Arctic at the end of the vegetation season (Pichrtová et al. 2014a). Thus, pre-akinetes might play an important role in the survival of *Zygnema* in polar hydro-terrestrial environments, but to date, no comprehensive study has been conducted on the annual development of these algae. The aim of the current study was to characterize the physiological performance of *Zygnema* during its entire annual life cycle in hydro-terrestrial habitats of Svalbard (High Arctic), with a focus on the production of specialized cells (zygospores, akinetes), which are hypothesized to assure survival of these algae during unfavorable periods.

Materials and methods

Study site

Green algal mats growing in small pools in the western part of Petunia Bay (Billefjorden, central Svalbard, High Arctic) were selected for this study (Online Resource 1). These mats are dominated by conjugatophytes of the genus *Zygnema* and occur in close proximity to the Petuniahytta field research station (Elster and Rachlewicz 2012). All investigated pools occupy an area of up to 5 m^2 and are very shallow (<5 cm). Therefore, the water temperature can reach as high as 16 °C during sunny summer days. The main sources of water in the summer are melting permafrost and streamlets that bring meltwater from snowfields. The water chemistry of the shallow pools is influenced by limestone inclusions in their catchments and sometimes by seawater. Therefore, these pools have neutral to slightly alkaline pH levels of between 7.2 and 8.4, and conductivity between 502 and 1584 μS cm^{-1} (Komárek et al. 2012). The vegetation season lasts from June to September. The monthly mean air temperature in the area ranges from –17 to 7.2 °C, and during winter, the temperature can occasionally fall below –30 °C or exceed 0 °C (Láska et al. 2012).

Field sampling of natural populations

The seasonal development of algal mats was investigated in the summer seasons of 2010 and 2011. Altogether, four populations forming hydro-terrestrial mats were selected for investigation. Twenty permanent spots that were marked per population served as replicates. The morphology of the cells was studied under an Olympus BX53 light microscope during early (June 28–July 12) and late (August 26–September 8) summer.

In addition, a winter sampling of one of the populations (referred to as population 2) was performed in April 2012 when the biomass was still frozen solid in ice and covered by 30 cm of ice-snow. Population 2 formed the most extensive mats during the summer which made it easy to be localized even under snow and ice cover.

Laboratory experiments with population 2 collected in winter

The frozen winter samples were transported to the university laboratory in Prague for further investigation. After melting, one portion of the biomass was transferred into

fresh liquid BBM medium (Bischoff and Bold 1963) and stored at 5 or 20 °C at a PAR (photosynthetic active radiation) irradiance of 39 µmol photons m^{-2} s^{-1}.

The other portion of the frozen biomass was melted, placed onto agar plates, and cultivated in crossed gradients of light and temperature (Labio, Czech Republic; Fig. 1; Kvíderová and Lukavský 2001) for 1 week. This method enables various parameters (growth rate, pigment content, and chlorophyll fluorescence parameters) to be monitored simultaneously in factorial combinations of light and temperature. Twenty-five positions were defined: five levels of temperature (T1–T5; mean values 7.1–25.0 °C) and five levels of irradiance (I1–I5; mean values 26–368 µmol photons m^{-2} s^{-1}; Fig. 1).

At the end of the cultivation period, biomass grown under a crossed gradient of light and temperature was desiccated together with freshly melted material. The algal filaments were evenly spread on glass fiber filters (Whatman GF/C) without additional moistening and transferred to a closed glass chamber over a saturated sodium chloride solution (relative air humidity of 75 %). After 24 h, the samples were transferred to liquid BBM medium, and after additional 48 h, the percentage of living cells was estimated using light microscopy observation.

Chlorophyll *a* fluorescence

Chlorophyll *a* fluorescence was measured using an imaging modulated fluorometer FluorCam (PSI, Czech Republic). The field-collected samples were transferred to the research station and dark-acclimated for 30 min prior to measurement of F_V/F_M, the maximum quantum yield of photosystem II (PSII). The samples were then kept in darkness for an additional 8 h, followed by a second measurement of F_V/F_M to assess the recovery rate after possible photoinhibitory stress under field conditions.

Chlorophyll *a* fluorescence was also measured on the melted winter material during 80-h recovery in fresh medium and cultivation in crossed gradients of light and temperature. Two parameters were selected, F_V/F_M and steady-state quantum yield of PSII in the light (Φ_{PSII}; measured at the actinic light of 100 µmol photons m^{-2} s^{-1}). Briefly, F_V/F_M characterizes the overall physiological state of PSII, whereas Φ_{PSII} serves as a good estimation of photosynthetic activity (Maxwell and Johnson 2000).

Pigment content

After cultivation, the biomass from nine representative positions was harvested, filtered on glass fiber filters, and extracted in 80 % acetone (combination of positions T1, T3, and T5, and I1, I3, and I5; Fig. 1). The contents of chlorophylls *a* and *b* and total carotenoids were estimated spectrophotometrically according to the equations provided by (Lichtenthaler and Buschmann 2001).

Statistical analyses

The seasonal changes in F_V/F_M were tested by general linear model (GLM) repeated-measures analysis of variance (ANOVA) with nested design. The time of dark acclimation was set as a repeated factor. The other factors tested, "population," "day," and "season," were regarded as fixed factors, with "day" nested in "season." "Season" had two levels: early and late summer. For each population, 20 independent replicate measurements were taken.

The recovery of F_V/F_M and Φ_{PSII} values, measured after melting on eight replicate samples, was described by the asymptotic function of hyperbolic growth: $P = P_{max} \times \text{slope} \times \text{time}/(P_{max} + \text{slope} \times \text{time}) + P_{min}$, where P is the chlorophyll fluorescence parameter, P_{max} is its maximum increase, P_{min} is its minimum value (y-intercept), and

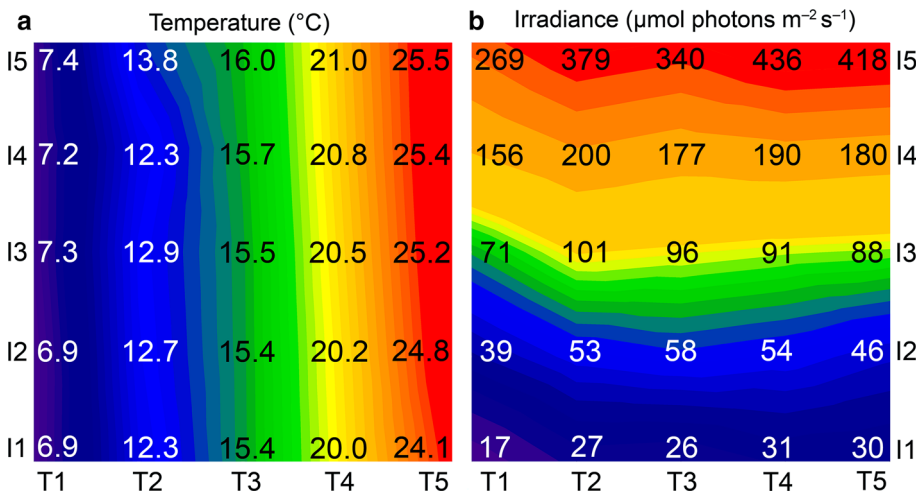

Fig. 1 Temperature (**a**) and irradiance (**b**) measured at different positions of the cultivation unit for a crossed gradient of light and temperature

slope is its initial (maximum) recovery rate in *time* (maximum slope of the curve). The three parameters of the model were estimated, and the differences between both cultivation temperatures were statistically tested using GraphPad Prism 6 software (GraphPad Software, USA).

Data obtained from the one-week cultivation under a crossed gradient of light and temperature (dry weight and pigment content) were not analyzed statistically, because the very slow growth in the lowest temperature did not provide enough biomass for replicate samples.

Results

Seasonal changes in morphology

In the early summer (late June), the investigated sites were full of dead *Zygnema* biomass from the previous year. Nevertheless, new biomass was produced rapidly, and within a few weeks, the pools were filled with new *Zygnema* mats. Light microscopy revealed that the cells had typical vegetative morphology, with two clear, stellate chloroplasts and large, hyaline vacuoles (Fig. 2a).

In the late summer (August), the growth ceased and the cells began to accumulate storage materials. Their morphology gradually changed, becoming mature, stationary-phase-like cells usually referred to as pre-akinetes (Fig. 2b). The filaments also tended to break into short fragments or even single cells.

In April, we collected frozen biomass from under the ice-snow. The biomass was frozen solid in ice but still appeared bright green. After melting, the biomass consisted of viable pre-akinetes that appeared similar to those observed in late summer (Fig. 2c).

We never observed conjugation or zygospore formation at these particular sites. True akinetes with zygospore-like cell walls were also not produced by the investigated populations.

Seasonal changes in chlorophyll fluorescence

A significant rise in F_V/F_M values was detected between 30 min and 8 h of dark acclimation in all cases ($p < 0.0001$), indicating that 30 min was not sufficient for the relaxation of PSII (Fig. 3). However, the significance of the interaction between the dark acclimation time and the season ($p < 0.0001$) indicates that the dynamics of F_V/F_M differed between both parts of the summer season, and that the increase in F_V/F_M after 8 h in the dark was much more pronounced in the early summer.

Notably, population 2 exhibited different seasonal development of F_V/F_M compared to the three other populations (Fig. 3). The values after 30 min and 8 h of dark acclimation were both significantly lower in late summer than in early summer in populations 1 ($p < 0.0001$), 3 ($p < 0.0001$), and 4 ($p = 0.0001$, respectively, $p < 0.0001$). On the other hand, the 8-h dark values remained the same throughout the summer ($p = 1.00$) in population 2, and after only 30 min of darkness, the F_V/F_M was even higher at the end of the season ($p < 0.0001$). A complete list of the results of the GLM ANOVA and Tukey's post hoc tests can be found in Online Resource 2.

Viability and stress tolerance of frozen cells from population 2

Photosynthetic activity began to recover immediately after melting and transfer to fresh liquid BBM medium. At 24 h following rehydration, cell division was already observed. The biomass growth was evident at both temperatures (5 and 20 °C). However, at 20 °C, the recovery rate of both F_V/F_M and Φ_{PSII} (slopes of the hyperbolae; $p = 0.0004$,

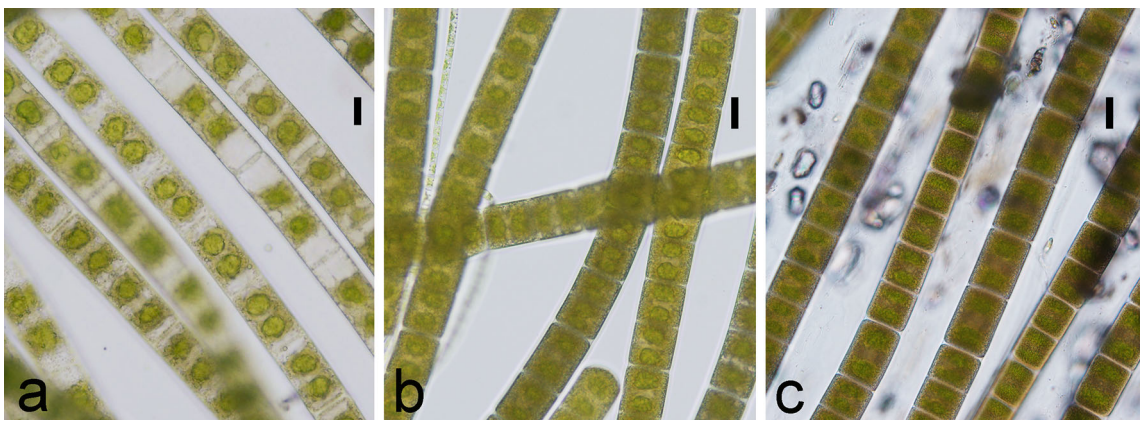

Fig. 2 Typical morphology of field-collected *Zygnema* sp. (population 2) over the course of the season: **a** vegetative cells with stellate chloroplasts (July 6), **b** pre-akinetes (August 24), and **c** overwintering pre-akinetes immediately after melting (April 22). *Scale bars* 20 µm

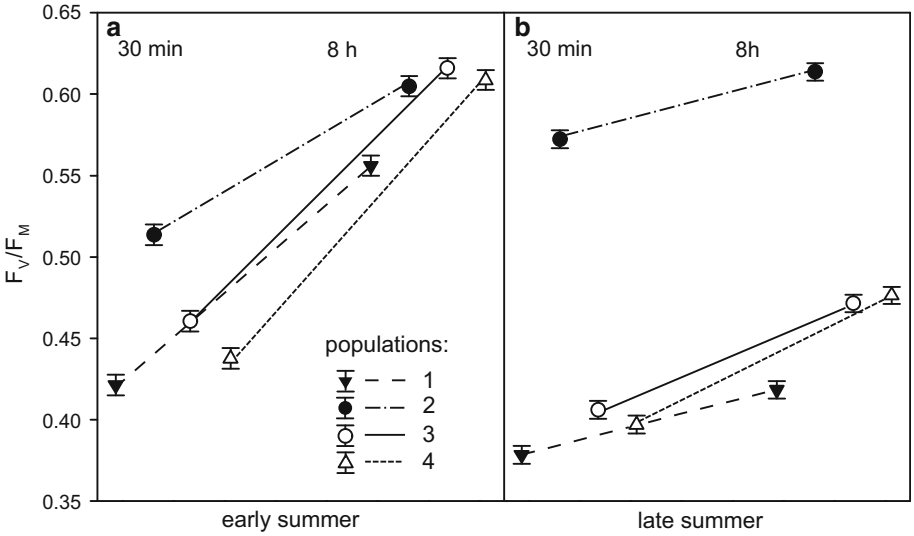

Fig. 3 Maximum quantum yield of photosystem II (F_V/F_M) measured in four natural *Zygnema* sp. populations. Measurements were taken after 30 min and 8 h of dark acclimation. Each value represents the mean of 20 sites on 3 (**a**) and 4 (**b**) different days. Weighted mean ± standard error, $n = 60$ (**a**) and 80 (**b**)

respectively, 0.0061) as well as their estimated maxima (asymptotes of the hyperbolae; $p = 0.0001$, respectively, 0.042) were higher (Fig. 4; Online Resource 2). There were no significant differences in the starting minimum values of the parameters. Fitted parameters of the hyperbolae and complete results of the tests can be found in Online Resource 2.

After 1 week of cultivation, biomass growth and cell division were apparent at all positions of the crossed gradients of light and temperature. At all combinations of conditions, the pre-akinetes transformed back into vegetative cells. The highest values of the fluorescence parameters were reached under various combinations of low irradiance and high temperature (Fig. 5).

Accordingly, the highest growth rate (estimated as an increase in dry weight) was also detected at the highest temperature tested combined with intermediate irradiance (Fig. 6a). In general, the relative pigment content also increased with increasing temperature and decreasing irradiance (Fig. 6b–e). Increases in irradiance led to lower chlorophyll-to-carotenoid ratios as well (Fig. 6f).

The chlorophyll fluorescence parameters decreased to zero within 30 min after the transfer into 75 % relative air humidity, indicating that physiological activity ceased due to the loss of intracellular water. Notably, no single cell from liquid culture survived 24 h of desiccation, whereas in the freshly melted material consisting of pre-akinetes, approximately 5 % of the cells survived these conditions.

Discussion

In this study, we showed that Arctic *Zygnema* spp. are able to survive throughout an entire annual cycle in a vegetative state without forming stress-resistant zygospores or akinetes. Vegetative cells gradually accumulate storage

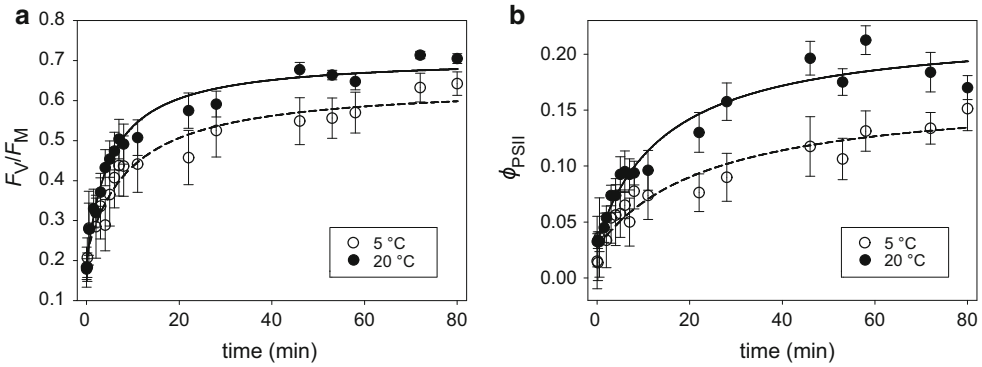

Fig. 4 Recovery of parameters of chlorophyll fluorescence after melting during cultivation at 5 and 20 °C: **a** maximum quantum yield of photosystem II, and **b** steady-state quantum yield of PSII in the light; $n = 8$, mean ± SD. The parameters were fitted by hyperbolic growth function; the initial slope of the curve denotes the maximum recovery rate of the parameters. The fitted curves are shown as dashed (5 °C) and solid (20 °C) lines, respectively

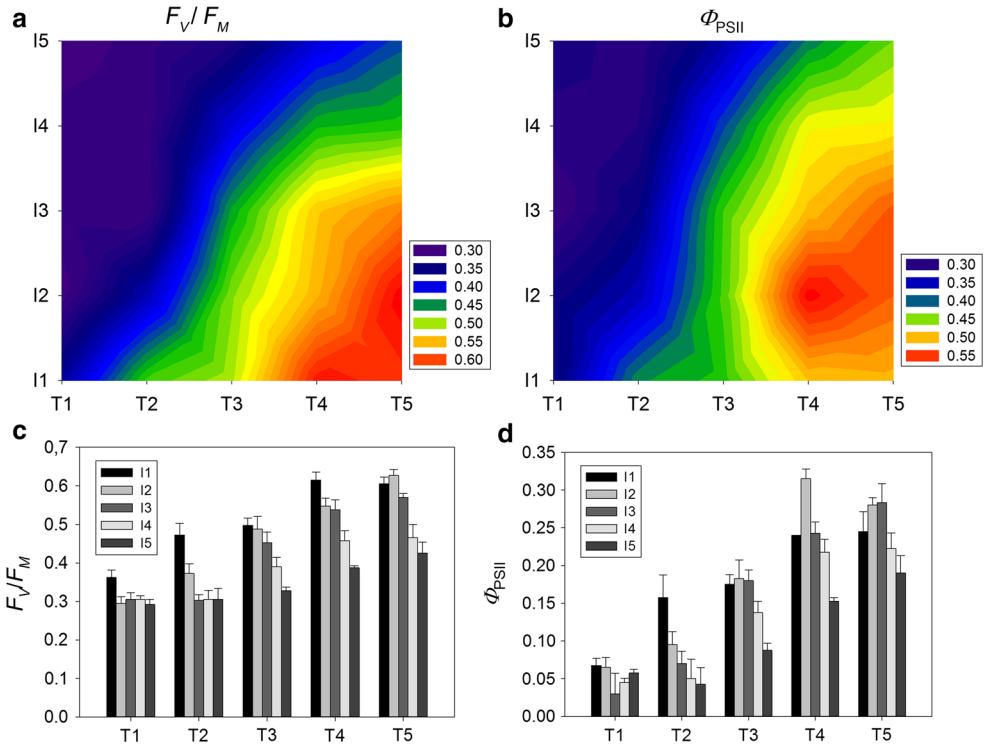

Fig. 5 Fluorescence parameters measured on biomass cultivated for 1 week at different positions of the crossed gradient of light and temperature. Average values of temperature and irradiance were as follows: T1 = 7.1, T2 = 12.8, T3 = 15.6, T4 = 20.5, and T5 = 25.0 °C; and I1 = 26, I2 = 50, I3 = 89, I4 = 181, and I5 = 368 µmol photons m^{-2} s^{-1}; $n = 4$, mean + SD

compounds and reduce their physiological activity during the vegetation season. Such modified vegetative cells (referred to as pre-akinetes) that were naturally hardened by slow desiccation were tolerant to osmotic stress (Pichrtová et al. 2014a) and desiccation (Pichrtová et al. 2014b), and were also able to survive the Arctic winter (this study). These cells are not dormant and recover quickly under favorable conditions, which is important for the rapid biomass growth that occurs soon after snowmelt.

The morphological characteristics of pre-akinetes differ from those of young vegetative cells in several ways. Pre-akinetes contain granules of storage substances and are slightly brownish. Ultrastructural investigations confirmed that pre-akinetes accumulate high levels of lipids and have reduced chloroplast lobes (McLean and Pessoney 1971; Holzinger et al. 2009; Pichrtová et al. 2014b; Herburger et al. 2015). Pre-akinete formation was also accompanied by a significant change in fatty acid composition (Pichrtová et al. 2016). Pre-akinetes also possess thicker cell walls than young vegetative cells but lack the three-layered walls of zygospores containing algaenan (Fuller 2013).

Pre-akinetes formed gradually from young vegetative cells during late summer. Their formation was not induced by desiccation under natural (Pichrtová et al. 2014a) or experimental (Pichrtová et al. 2014b) conditions. However, these cells formed more readily under nitrogen-limited culture conditions, indicating the important effect of starvation (Pichrtová et al. 2014b). We thus hypothesize that nitrogen (or other nutrients) is depleted during extensive growth in polar seepage pools, which in turn causes the cessation of growth and pre-akinete formation. In general, nutrient cycling is relatively slow in polar habitats (Robinson et al. 2003), and nutrient availability is usually a limiting factor (Davey and Rothery 1992; Arnold et al. 2003; Hogan et al. 2014), although exceptions exist, such as near bird colonies (Zwolicki et al. 2013).

In addition, other physicochemical environmental factors that change during the season (e.g., temperature, light intensity/spectral composition, and electric conductivity) can also lead to the accumulation of storage products and pre-akinete formation. For example, the lipid content in the eustigmatophyte *Nannochloropsis oculata* increased in autumn, even with constant nutrient availability (Olofsson et al. 2012). The cessation of growth in dense populations has also been observed in microalgal mass cultures. In addition to nutrient depletion, this observation may reflect the reduced photosynthesis caused by self-shading (Richmond and Hu 2013) or the lack of CO_2; the biomass itself also produces and accumulates growth inhibitors (Park et al. 2014).

Recent studies on polar *Zygnema* spp. also showed that pre-akinetes become more stress-resistant after hardening, e.g., by slow desiccation (Pichrtová et al. 2014a, b). The

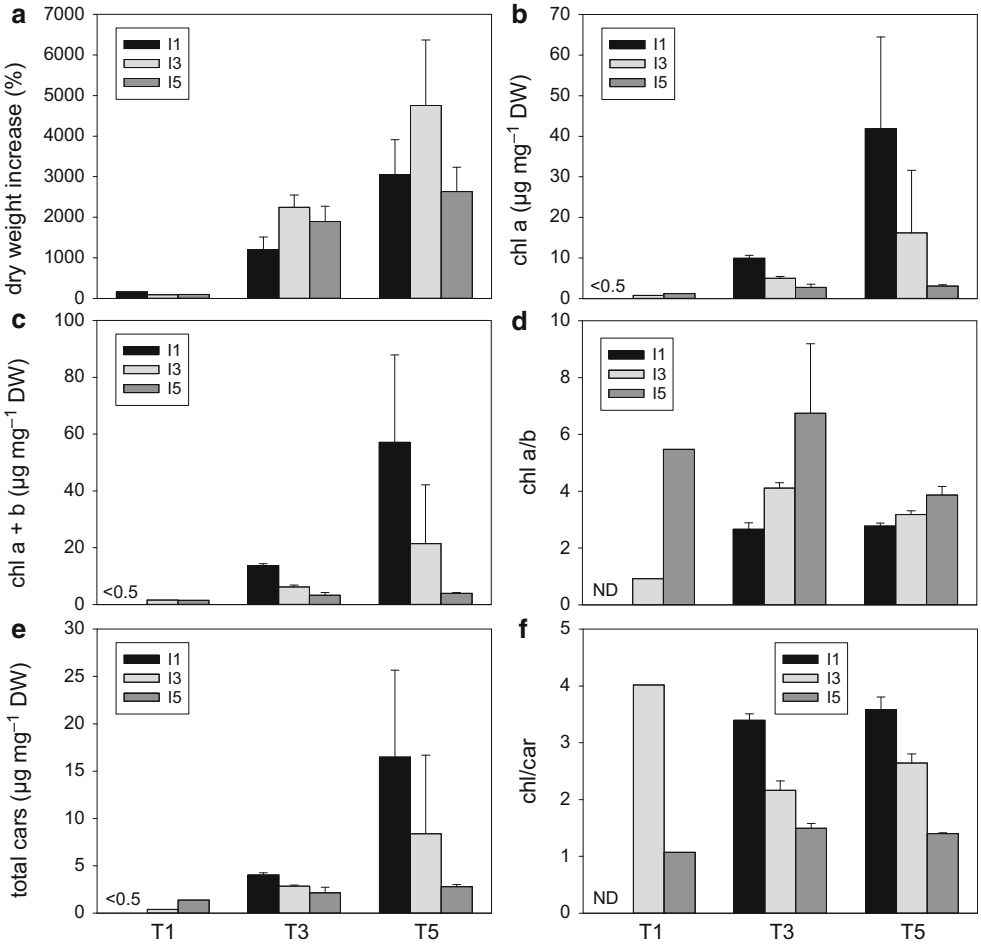

Fig. 6 Dry weight and pigment content of biomass grown for 1 week under a crossed gradient of light and temperature. For T1, $n = 1$, and for T3 and T5, $n = 4$, mean + SD

physiological effects of freezing and desiccation are similar because they both lead to lower intracellular water potential (Bisson and Kirst 1995). Thus, acclimation during exposure to desiccation stress at the end of the summer is expected to result in resistance to freezing. Conversely, we showed that naturally frozen pre-akinetes were able to survive desiccation immediately after melting.

The biochemical nature of such hardening is currently under investigation. Green microalgae accumulate various substances with protective functions against desiccation and freezing stress (Holzinger and Karsten 2013). For example, the Antarctic trebouxiophyte green alga *Prasiola crispa* accumulates proline before winter (Jackson and Seppelt 1995). Similarly, the intracellular concentrations of glycerol and erythritol in Antarctic soil microalgae increase at the end of summer (Arnold et al. 2003). Moreover, changes in cell wall structure and composition accompany pre-akinete formation. The secondary pectin layer of the cell wall allows water to be retained due to its physicochemical properties (Fuller 2013).

Not only do pre-akinetes differ from young vegetative cells in terms of morphology and ultrastructure, but (above all) they also differ in terms of physiological characteristics and stress tolerance. The sustained decline in F_V/F_M indicates either photoinhibitory damage to PSII or downregulation at PSII due to sink limitation, i.e., reduced requirements for CO_2 fixation during, e.g., winter dormancy or nutrient starvation (Adams and Demmig-Adams 2004). The latter interpretation of sink limitation fits our observation that old, stationary-phase cells (pre-akinetes) are usually physiologically less active than young vegetative cells (Coleman 1983; Herburger et al. 2015). Indeed, in the majority of populations, the F_V/F_M values were significantly lower in late summer than in early summer. However, population 2 showed no clear trend throughout the summer season, suggesting that pre-akinetes in this population were exceptionally well adapted to utilizing light throughout the growing season, in contrast to the others whose pre-akinetes lost their PSII potential in late summer. The same population was included in a previous

study by Pichrtová et al. (2014a; also referred to as "population 2"); although it formed pre-akinetes like other populations, its F_V/F_M values were also markedly higher than those of the other populations investigated. This difference in populations is likely associated with genetic variability rather than acclimation to local conditions (Pichrtová et al. 2014a).

The significant increase in F_V/F_M after 8 h of dark acclimation indicates strong photoinhibition under natural conditions, particularly in young vegetative cells (i.e., in early summer). The populations are exposed to continuous daylight throughout the summer without the possibility of full dark recovery of the photosynthetic apparatus. The strong photoinhibition may be thus understood as a constitutive photoprotective mechanism that prevents excess chlorophyll excitation at PSII, and thus reduces oxidative stress and uncontrolled photodamage (Adams and Demmig-Adams 2004). Another excitation-reducing mechanism involves self-screening of the cells by aggregating filaments into multilayered structures. Mat-forming growth is a common strategy used by microorganisms for protection against multiple stresses (Bischof et al. 2002; De los Ríos et al. 2004; Knowles and Castenholz 2008; Holzinger and Pichrtová 2016). In addition, *Zygnema* produce a wide range of phenolic substances that provide screening not only in the UV range, but (partially) also in the PAR region, which can provide an additional layer of protection against excessive irradiation (Pichrtová et al. 2013).

Accordingly, when the algae were cultivated in crossed gradients of light and temperature, the highest values of both fluorescence parameters and the highest biomass production occurred under low irradiance. Low-light adaptation and photoinhibition at higher irradiance were previously reported in Arctic and Antarctic *Zygnema* (Kaplan et al. 2013). Moreover, an Antarctic *Zygnema* sp. was able to grow under very low PAR flux (as low as 1 μmol photons m^{-2} s^{-1}; Davey 1991b). Low-light adaptation is a typical feature of soil and biofilm microalgae that grow under soil particles or under upper biomass layers (Davey 1991b; Gray et al. 2007; Karsten et al. 2013).

In contrast to low irradiance levels, optimal *Zygnema* growth occurred at the highest temperature tested (ca. 25 °C), and recovery was faster at high cultivation temperatures. Similarly, the maximum growth rate of an Antarctic *Zygnema* sp. was achieved at 20 °C (Davey 1991b). A similar phenomenon was previously observed in other Arctic and Antarctic microalgae, which were shown to be psychrotrophs rather than psychrophiles (Seaburg et al. 1981; Davey 1991b). A broad temperature range for growth (20–30 °C units) is important under the highly unstable conditions of polar wetlands (Seaburg et al. 1981).

Markedly, conjugation and zygospore formation was observed only very rarely in the Arctic (Elster et al. 1997). This is in contrast to the findings of Genkel and Pronina (1979) who investigated the populations of *Zygnema stellinum* in the temperate climate of Belarus and concluded that pre-akinetes are able to survive short-term stress events during the summer, but the formation of highly resistant cells (zygospores and parthenospores) is necessary for survival in the winter, the major annual stress event. Nevertheless, Arctic and Antarctic microalgae have repeatedly been shown to survive as vegetative cells with thick cell walls and accumulated reserves (Hawes 1990; Sheath et al. 1996).

The formation of pre-akinetes is advantageous because these cells are not dormant but are instead physiologically active immediately after melting. Similarly, Antarctic vegetative *Prasiola* also recovered photosynthetic activity immediately after collection during winter after being frozen for 3 months (Jackson and Seppelt 1995). Additionally, the above-mentioned adaptation of pre-akinetes to low-light levels enables them to grow even under snow cover. Rapid growth in early spring is an important competitive advantage, as these algae must compete with cyanobacteria and other microalgae in unstable habitats, with only a few weeks of favorable conditions (the presence of liquid water) per year (Davey 1988, 1991b). The survival of only modified vegetative cells may also be due to the low grazing pressure in the Arctic habitats. Potential grazers of microalgae in the Arctic include insects such as chironomids, caddisflies, and mayflies (Sheath et al. 1996), which are low in abundance compared to grazers in temperate regions. Finally, filaments consisting of pre-akinetes tend to break into single cells (Pichrtová et al. 2014a) and can thus serve as airborne propagules.

Even though frozen filaments consist of viable pre-akinetes during the winter, the spring always brings high mortality to the biomass of the previous year; the reason for this remains unknown. A similar phenomenon was observed for other Arctic and Antarctic eukaryotic algae (Davey 1991a; Elster 2002). Here, we showed that immediately after transfer to fresh liquid medium, the pre-akinetes began to grow, but the dehardened young cells lost their stress tolerance. Therefore, we propose that the early-spring diurnal, short-term cycles of freeze–thaw and/or desiccation–rehydration (Láska et al. 2012) represent a bottleneck in the life cycle of Arctic populations of *Zygnema*. For the population to survive, it is crucial that at least a few cells retain their resistance (Hawes 1988, 1990; Tashyreva and Elster 2012).

We conclude that naturally hardened pre-akinetes play a key role in the survival of *Zygnema* spp. in the polar hydroterrestrial environment. However, populations of these species exhibit a phenological trade-off between missing the early growing season and experiencing frost damage. Also, the scarcity of sexual reproduction indicates that

Arctic *Zygnema* populations live at their physiological limits.

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