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Formation of lipid bodies and fatty acid composition changes upon pre-akinete formation in arctic and Antarctic *Zygnema* (Zygnematophyceae, Streptophyta) strains.

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Abstract

Filamentous green algae of the genus *Zygnema* (Zygnematophyceae, Streptophyta) are key components of polar hydro-terrestrial mats where they face various stressors including UV irradiation, freezing, desiccation and osmotic stress. Their vegetative cells can develop into pre-akinetes, i.e., reserve-rich, mature cells. We investigated lipid accumulation and fatty acid (FA) composition upon pre-akinete formation in an arctic and an Antarctic *Zygnema* strain using transmission electron microscopy (TEM) and gas chromatography coupled with mass spectrometry (GC-MS). Pre-akinetes formed after nine weeks of cultivation in nitrogen-free medium, which was accompanied by massive accumulation of lipid bodies. The composition of FAs was similar in both strains where α-linolenic acid (C18:3) dominated in young vegetative cells. Pre-akinete formation coincided with a significant change in FA composition. Oleic (C18:1) and linoleic (C18:2) acid increased most (up to 17- and 8-fold, respectively). Small amounts of long-chain polyunsaturated FAs were also detected, e.g. arachidonic (C20:4) and eicosapentaenoic (C20:5) acid. Pre-akinetes exposed to desiccation at 86% relative humidity (RH) were able to recover maximum quantum yield of photosystem II, but desiccation had no major effect on FA composition. The results are discussed with regard to the capability of *Zygnema* spp. to thrive in extreme conditions.

Running title: Fatty acid composition of polar *Zygnema* spp.

Keywords: desiccation stress, fatty acid methyl ester, lipids, nitrogen starvation, polar green microalgae

Introduction

Algae and cyanobacteria can be found in almost all polar terrestrial habitats, including wetlands, snow, ice, soil or rocks (Kim et al. 2008; Sheath et al. 1996; Skácelová et al. 2013; Spijkerman et al. 2012). They represent important primary producers and possess various tolerance mechanisms that enable them to survive the multiple stresses that characterize these environments, such as freezing, freeze–thaw cycles, UV and high irradiation, and desiccation (Elster 2002). Most species of polar freshwater and terrestrial algae do not form spores, but are capable of surviving in the vegetative state (Sheath et al. 1996). Such stress-tolerant vegetative cells differ morphologically and physiologically from unstressed or cultured cells by accumulation of reserves such as lipid droplets, and by forming thick cell walls (Sheath et al. 1996; Kim et al. 2008).

Modifications of vegetative cells were recently investigated in polar filamentous conjugating green algae of the genus *Zygnema* (Pichrtová et al. 2014a, b, 2016). *Zygnema* spp. are very common to both the Arctic and Antarctica, where they form large mats in shallow meltwater streams and pools (Holzinger et al. 2009; Kim et al. 2008; Skácelová et al. 2013). Young vegetative cells of *Zygnema* spp. have two stellar chloroplasts and are highly vacuolated. When resources are limiting they stop growing and gradually develop into "pre-akinetes", which have been defined as modified vegetative cells with thick cell walls and mucilaginous pectic layers, reduced chloroplast lobes, lower physiological activity and accumulated storage compounds (Fuller 2013; Herburger et al. 2015; McLean & Pessoney 1971). Of the latter, lipid bodies are the most abundant and can be readily visualized by light and transmission electron microscopy (Holzinger et al. 2009; Fuller 2013; McLean & Pessoney 1970; Kaplan et al. 2013; Pichrtová et al. 2014b). Importantly, well developed pre-akinetes are more stress tolerant than young vegetative cells. When hardened, pre-akinetes can tolerate osmotic stress (Kaplan et al. 2013; Pichrtová et al. 2014a), slow desiccation (Pichrtová et al. 2014b) and they also serve as overwintering stages (Pichrtová et al. 2016). This is in agreement with reports on other algae, in which mature, starved cells were found to be more stress tolerant than young cells from nutrient-sufficient log-phase cultures (e.g. Nagao et al. 1999). Cells with lipid bodies and thick cell walls were also described from various natural stress environments (Morison & Sheath 1985; Darling et al. 1987; Hoppert et al. 2004). Interestingly, accumulation of lipids was also observed in desiccation tolerant, thick-walled cells in bryophyte protonemata (Rowntree et al. 2007).

Under laboratory conditions the formation of lipid-rich cells can be easily induced by nitrogen deprivation (Pichrtová et al. 2014b; Abe et al. 2014; Goncalves et al. 2013; Ruiz-Domínguez et al. 2015; Zhu et al. 2015). Other stress factors also induce lipid accumulation and influence lipid and fatty acid (FA) content and composition (recently reviewed by e.g. Guschina & Harwood 2006; Vítová et al. 2014). Microalgae from cold environments, including polar and alpine species, have evolved to maintain membrane fluidity at low temperatures, and low temperature stress increases the abundance of polyunsaturated fatty acids (PUFAs) and short-chain or branched FAs (Morgan-Kiss et al. 2006). Desiccation stress has also been shown to promote accumulation of triacylglycerols (TAG) in *Chlorella kesslerii* (Shiratake et al. 2013). Exposure to stress factors, most commonly nitrogen deprivation, is generally applied in algal biotechnology to induce lipid accumulation (Sharma et al. 2012), and there is increasing commercial interest in using algae as promising sources of biofuel or high-value PUFAs (reviewed e.g. by Sharma et al. 2012; Guschina & Harwood 2006). However, our knowledge of FA composition of conjugating green algae is only very limited (Lang et al. 2011). Our major aim was to conduct a detailed analysis of the FA composition of an arctic and an Antarctic *Zygnema* spp. in which pre-akinete formation and lipid accumulation were observed (Pichrtová et al. 2014b; Pichrtová et al. 2016). We tested if FA composition changes upon the formation of pre-akinetes and desiccation stress. The results

are discussed in the context of survival and development under the harsh environmental conditions of the Arctic and Antarctica.

Materials & Methods

Algal material and cultivation

The Arctic *Zygnema* sp. strain "B" (CCALA 976; Pichrtova 2011/1) and the Antarctic *Zygnema* sp. "C" (CCALA 880; Snokhousova et Elster 2009/8) described in Pichrtová et al. (2014b) were used for the experiments. For detailed information including description of collection sites and phylogenetic position of the strains see (Pichrtová et al. 2014b). Cultures were maintained on Bold's Basal Medium (BBM; Bischoff & Bold 1963) solidified with 1.5% agar and incubated under optimal growth conditions, 18 °C and continuous light (35 µmol m^{-2} s^{-1}). Hereafter, these cultures are referred to as "young cultures".

Induction of pre-akinetes, desiccation and harvest of algal cultures

To induce the formation of pre-akinetes and lipid accumulation, the cultures were transferred to agar plates with Bold's Basal Medium without nitrate or any other source of nitrogen, and kept at 18 °C and continuous light (35 µmol m^{-2} s^{-1}) for 9 weeks as previously described (Pichrtová et al. 2014b). Pre-akinete cultures are referred to as "mature cultures".

After nine weeks of nitrogen depletion, thin layers of pre-akinete filaments were spread on glass-fibre filters (4.7 cm in diameter) and placed into desiccation chambers either over saturated KCl (86% relative air humidity, RH) or partly dried silica gel (18% RH). Each chamber was equipped with a small electric fan allowing fast (<12 h) water content equilibration of the samples. After 24 hours of desiccation, the algal material was rehydrated by adding of 2 mL of water, and transferred into another chamber above distilled water (98–100% RH) for another 24 hours, and the proportion of surviving cells was estimated using a light microscope. All experiments were performed at room temperature conditions.

For further analysis the biomass of young, nutrient-sufficient 2-week old cultures and of mature cultures were harvested. The latter were also investigated at five different stages of the desiccation/recovery experiment: (1) prior to desiccation treatment, (2) after desiccation above saturated KCl or (3) silica gel, and after rehydration following the desiccation over (4) saturated KCl solution and (5) silica gel. The filters with biomass were placed into 2 mL Eppendorf tubes, immediately frozen in liquid nitrogen, freeze-dried and stored at –80 °C prior to analyses. For each combination of strain and treatment, five independent replicates were obtained.

Chlorophyll a *fluorescence*

Chlorophyll *a* fluorescence was measured by an imaging modulated fluorimeter FluorCam (PSI, Czech Republic). The minimum fluorescence of dark-acclimated cultures (F_0) and the maximum fluorescence after the application of saturation pulse (F_M) were recorded from each sample spread on a filter. Maximum quantum yield of photosystem II photochemistry ($F_V/F_M = (F_M - F_0) / F_M$) was computed in order to characterize the physiological state of the cultures (Maxwell & Johnson 2000). F_V/F_M of the cultures was measured for all cultures before desiccation and after recovery (n=5).

Gas chromatography coupled with mass spectrometry (GC-MS)

Fatty acids were derivatized to fatty acid methyl esters as described by (Li-Beisson et al. 2010). Briefly, about 5 mg of freeze-dried sample were treated with 2 mL of methanol:toluene:sulphuric acid 10:3:0.25 (v:v:v) containing 0.01% (w:v) butylated hydroxytoluene. 200 µg of heptadecanoic acid (C17:0, dissolved in hexane) were added simultaneously as internal standard. Samples were incubated at 80 °C for 90 min with constant agitation before adding 760 µL of hexane and 2.3 mL of 0.9% NaCl (w:v). Samples were vigorously mixed before centrifugation for 10 min at 3000 × *g*. The supernatant was transferred to autosampler vials and kept at −20 °C if not analyzed immediately. Fatty acid methyl esters were separated using a Trace 1300 gas chromatograph (Thermo-Scientific, USA) on a 30 m BPX70 column (SGE Analytical Science #054622, Melbourne, Australia) and detected using a TSQ 8000 triple quadrupole mass spectrometer (Thermo-Scientific, Waltham, USA) operated in full scan mode (50–550 m/z). The temperature gradient was first set to 110 °C for 1 min, then increased by 9 °C min^{-1} up to 180 °C, and finally increased by 15 °C min^{-1} up to 230 °C, which was held for 5 min with a carrier gas flow of 1.5 mL min^{-1} of helium and a split ratio set to 50. The ion source temperature was set to 250 °C and the transfer line to 240 °C. A commercial fatty acid methyl ester mix (Sigma Aldrich ref. 18919, Missouri, USA) was used to confirm the identities of the FAs. External standards of palmitic, stearic, oleic, linoleic and α-linolenic acid were used in conjunction with the internal standard to determine the total amount of each of these fatty acids. Data analysis was performed using the Xcalibur software (Thermo-Scientific, Waltham, USA).

Transmission electron microscopy

For transmission electron microscopy young *Zygnema* sp. of strains B and C were prepared according to (Aichinger & Lütz-Meindl 2005) with modifications by (Pichrtová et al. 2013). Briefly, samples were high-pressure frozen in 150 mM sucrose and freeze substitution was carried out in 2% OsO_4 in acetone at –80 °C; samples were then gradually transferred via propylene oxide to Agar Low Viscosity Resin (Agar Scientific R 1078). Mature cells were chemically fixed according to (Holzinger et al. 2009). Samples were fixed for 1 h in 2.5% glutaraldehyde in 20 mM cacodylate buffer (pH=6.8), rinsed and postfixed in 1% OsO_4 at 4 °C for 18 h. Samples were dehydrated in increasing ethanol concentrations, embedded in modified Spurr's resin (Ellis 2006). Ultrathin sections were counterstained with uranyl acetate and Reynold's lead citrate and viewed with a Zeiss Libra 120 transmission electron microscope at 80 kV. Digital images were captured with a TRS (Tröndle Restlicht Verstärker Systeme) 2k SSCCD camera controlled by OSIS iTEM software and further processed with Adobe Photoshop Elements 11.

Statistical analyses

F_V/F_M data were analyzed using the general linear model factorial analysis of variance (ANOVA). The two factors "strain" and "treatment" were considered as factors with fixed effects. Additionally, Tukey's test was performed for post hoc pairwise comparisons.

Principal component analysis was used to visualize correlations between relative abundance of individual fatty acids and genotypes/treatments.

Differences in the absolute content of individual fatty acids were tested using two-way ANOVA (factors "genotype" and "culture age"). In case a significant interaction between both factors was detected, differences between the young and mature cultures were compared separately for each genotype using Welch two-sample t-test with Bonferroni correction.

Changes in FA composition during desiccation/rehydration were tested by two-way ANOVA. In cases where no statistical interaction between genotype and treatment was observed, FA levels in both genotypes were normalized to the mean of the respective not

desiccated group and then combined. To compare FA relative abundance between treatment groups and to the non-desiccated condition a one-way ANOVA followed by Dunnett's post hoc tests were performed.

The analyses were performed in Statistica 12 (Statsoft) and R 3.2.1 (including the packages 'beeswarm' and 'multcomp'). All tests were two-sided and the assumption of normal distribution was tested.

Results

Ultrastructure of young and mature cultures

Young vegetative cells of strain B showed a high degree of vacuolization (Fig. 1 a). The two stellate chloroplasts contained pyrenoids with starch grains and were surrounded by a thin layer of cytoplasm (Fig. 1 a). Within the cytoplasm, multiple sections through the chloroplast lobes with parallel-arranged thylakoid membranes were found (Fig. 1 b), and numerous Golgi bodies and long mitochondria indicated that the cells were metabolically active (Fig. 1 b). Cells of strain C also showed a large degree of vacuolization (Fig. 1 c) and the pyrenoids were surrounded by massive starch grains (Fig. 1 d). The cytoplasm contained a centrally arranged nucleus, surrounded by numerous mitochondria and Golgi bodies (Fig. 1 d). The cytoplasm of both strains was dense, and virtually no lipid droplets were detected in young cultures (Fig. 1 b, d).

By contrast, lumina of mature cells of both strains were filled with large (up to 4 µm) lipid bodies with medium electron density and only little vacuolization remained (Fig. 2 a–d). The chloroplasts were reduced, but the pyrenoids still contained starch grains and numerous plastoglobuli were present (Fig. 2 b). In the cytoplasm of both strains many electron-dense particles were found in addition to the lipid bodies (Fig. 2 b, d).

Physiology of young and mature cells, and response to desiccation stress

Young and mature cells also clearly differed in their physiological performance. Cultures that starved for 9 weeks had significantly lower maximum quantum yield of photosystem II (F_V/F_M) than young ones, and this difference was more pronounced in strain C than in B (Fig. 3). After desiccation at 18% RH and rehydration the low F_V/F_M values (Fig. 3) indicated that neither of the strains was able to survive this treatment, and this was confirmed by light microscopy, which showed disintegrated or empty cells (Supp. Fig. 1 b). In contrast, both strains survived desiccation above saturated KCl. Mature cells of strain C fully recovered F_V/F_M, and those of strain B recovered 78% of their initial F_V/F_M values (Fig. 3). In both strains, light microscopy showed that about 50% of the cells survived desiccation over KCl (Supp. Fig. 1 a).

Fatty acid composition of young and mature cultures

Principal component analysis based on the relative FA composition separated all samples into 4 clusters along the first two axes that together account for 78% of total variation (Fig. 4). PC1 separated pre-akinete cultures of *Zygnema* sp. C from all other samples. In addition, PC2 clearly separated all pre-akinete cultures from all young ones while simultaneously grouping

the samples by strain. The young samples of both strains were characterized by high relative proportion of hexadecatrienoic (C16:3, no. 7) and lignoceric (C24:0, no. 21) acid. On the other hand, high relative proportions of oleic (C18:1, no. 9), linoleic (C18:2, no. 10) and γ-linolenic (C18:3g, no. 11) acid discriminated mature cells (Fig. 4).

Absolute quantification of FA content revealed that young cultures of both species shared a very similar overall pattern in the content of dominant fatty acids quantified using external standards, even though their abundance slightly differed (Fig. 5). The dominant fatty acids in young cells of both strains were palmitic (C16:0), hexadecatrienoic (C16:3), linoleic (C18:2) and α-linolenic (C18:3) acid (Fig. 5). In mature cells of both strains, massive increases in contents of oleic (C18:1) and linoleic (C18:2) acids were detected (Fig. 5). In contrast, the content of α-linolenic (C18:3) acid, the most abundant compound in young cells, decreased during starvation.

Proportions of all detected FAs were estimated based on the respective peak areas (Supp. Fig. 2). Palmitic (C16:0), hexadecatrienoic (C16:3), linoleic (C18:2) and α-linolenic (C18:3) acids accounted for more than 80% of detected FAs in young cells (Supp. Fig. 2 a). In pre-akinetes, a similar proportion was made up by the two dominant FAs, oleic (C18:1) and linoleic (C18:2) acid; Supp. Fig. 2 b. Other important FAs in young samples were oleic (C18:1), stearidonic (C18:4) and eicosapentaenoic (C20:5) acids (Supp. Fig. 2 a).

Desiccation and rehydration treatment

No major changes in content of the most abundant fatty acids during desiccation or rehydration could be detected (Fig. 6). The content of some FAs was lower after desiccation and rehydration than in non-desiccated samples. However, this decline was statistically significant only in for hexadecatrienoic (desiccated at 86 and 18% RH) and myristic acid (desiccated at 86% RH; Fig. 6). A similar conclusion could be drawn when comparing relative content of all FAs. All mature samples were clearly separated only by strain (B or C), but samples from different desiccation/rehydration treatments were randomly scattered within those clusters (Fig. 4). No clear separation of desiccated and non-desiccated samples was shown even along other PC axes, only the rehydrated samples clustered together (Supp. Fig. 3).

Discussion

Lipid bodies accumulate in pre-akinetes

As a result of nitrogen starvation polar *Zygnema* sp. strains form pre-akinetes, which are filled with lipid bodies and after hardening, are capable of surviving desiccation (Pichrtová et al. 2014b). In this study we characterized fatty acid composition of young cells and pre-akinetes in response to starvation and desiccation stress.

In both strains, the arctic and the Antarctic one, a high degree of vacuolization of young cells but hardly any lipid bodies or other storage compounds were observed, in agreement with previous work on other *Zygnema* spp. (McLean & Pessoney 1971; Herburger et al. 2015). When these cultures were transferred to nitrate-free agar medium, pre-akinetes with large lipid bodies formed (Pichrtová et al. 2014). Alpine *Zygnema* strains also accumulated lipids after several months of culture in medium in which nutrients became depleted over time (Herburger et al. 2015). Lipid bodies were also found in field samples, for example in *Zygnema* sp. from Svalbard at the end of vegetation season (Holzinger et al. 2009; Pichrtová et al. 2016). In samples from India it was shown that the oil contributed to 8.3% of the dry mass (Sawarkar & Nandkar 2013).

Accumulation of neutral lipids, predominantly triacylglycerols (TAGs), are characteristic of starved or otherwise stressed cells and hence, TAGs are often observed in algal cultures in the stationary phase (Abe et al. 2014; Goncalves et al. 2013). Nutrient deficient algae continue synthesizing fatty acids which, if not needed as building blocks of new membranes, are used for production of TAGs (Thompson 1996). The accumulation of N-free lipids (and carbohydrates) under stress conditions is thought to result from a shift in photosynthate allocation from growth to energy storage (Solovchenko 2012; Vítová et al. 2014). In addition, plastid membranes could be actively degraded and the released fatty acids incorporated into TAGs (Boyle et al. 2012; Miller et al. 2010). Recycling of polar membrane lipids into lipid bodies was also detected by radiolabelling in *Chlorella protothecoides* (Goncalves et al. 2013). Upon pre-akinete formations we observed chloroplast degradation in conjunction with accumulation of plastoglobuli in starved cells of both strains (Fig. 2). Accordingly, F_V/F_M was significantly reduced in pre-akinetes, in agreement with previous studies, which showed that pre-akinete formation coincides with lowered effective quantum yield (Herburger et al. 2015; Pichrtová et al. 2014b).

Oleic acid and linoleic acid increase most upon pre-akinete formation

Fatty acid distribution patterns reflect phylogenetic relationships and can be used as chemotaxonomic markers, and the FAs composition of the two *Zygnema* sp. strains is typical of green algae (Lang et al. 2011; Graeve et al. 2002). The predominant fatty acids in both strains were palmitic (C16:0), hexadecatrienoic (C16:3), oleic (C18:1), linoleic (C18:2) and α-linolenic (C18:3) acid (Fig. 5, Supp. Fig. 2). An extensive screening of FAs composition across various algal groups showed that palmitic acid is a typical compound of cyanobacteria and primary plastid groups (Lang et al. 2011), within which the division Streptophyta is characterized by high proportions of oleic acid (Lang et al. 2011). The FA composition was very similar in both strains, albeit some differences in relative abundance of the less abundant FAs were found, separating the two strains in the PCA analysis (Fig. 4). Variation in individual FAs was also found in closely related species or isolates of the same species by (Lang et al. 2011). For example, some *Closterium* strains (Zygnematophyceae) contained high amounts of γ-linolenic acid, whereas it was absent in others (Lang et al. 2011).

Fatty acids are building blocks of phospholipids, glycolipids and betaine lipids, which are integral parts of membranes (Zhu et al. 2015). Young cultures had virtually no lipid bodies, and palmitic, hexadecatrienoic and α-linolenic acid, typical compounds of membrane lipids (Kumari et al. 2013), were the dominant fatty acids (Fig. 5). Upon pre-akinete formation, typical membrane lipids such as hexadecatrienoic and α-linolenic acid became less abundant, as also found in other nutrient-deficient green algae (Boyle et al. 2012; Arisz et al. 2000), whereas FAs typical of storage lipids increased. The most striking increase was found for oleic (C18:1) and linoleic (C18:2) acid, which rose up to 17-fold and 8-fold, respectively. Accumulation of these two FAs was also found as a result of nitrogen depletion in aeroterrestrial *Coccomyxa* sp. (Abe et al. 2014), *Chlamydomonas moewusii* (Arisz et al. 2000) and *Chlamydomonas reinhardtii* (Boyle et al. 2012). Oleic acid, a typical component of neutral lipids (Arisz et al. 2000; Thompson 1996; Zhu et al. 2015) also accumulated in snow algae (Spijkerman et al. 2012) and *Chlorella zofingiensis* (Zhu et al. 2015). Hence, the changes in FA composition reflects the transition in photosynthate allocation from growth processes to energy storage.

In general, increase in saturated and monounsaturated FA and decrease in PUFA content accompanies nitrogen starvation (Abe et al. 2014). However, the absolute content of PUFAs in our samples did not change consistently with pre-akinete formation. Hexadecatrienoic and α-linolenic acid were more abundant in fresh cultures, and γ-linolenic and arachidonic acid in mature cultures. *Zygnema* spp. also contained stearidonic (C18:4), eicosatrienoic (C20:3), arachidonic (C20:4) and eicosapentaenoic (C20:5) acid. Long-chain PUFAs (C>18) are interesting compounds for biotechnological applications commonly found mainly in red and chromalveolate algae (Graeve et al. 2002; Lang et al. 2011). Markedly, no traces of these compounds were found in the closely related streptophytic alga *Klebsormidium* from Antarctica (Teoh et al. 2004). Other cultivation conditions than nitrogen availability have also an important influence on FA unsaturation, typically low temperature (Morgan-Kiss et al. 2006). In our study the algae were kept at 18 °C and therefore it can be assumed that field collected samples or cultures maintained at low cultivation temperature would have a slightly different FA composition.

Furthermore, we investigated if desiccation stress of pre-akinetes leads to additional changes in FA composition. Air drying was used to stimulate TAG production in biotechnologically interesting strains (Shiratake et al. 2013). For example, when exposed to 98% RH, *Chlorella kessleri* showed increased FAs production. In contrast, we did not find elevated levels of FAs in either of the *Zygnem*a strains exposed to desiccation (Fig. 6). In agreement with (Pichrtová et al. 2014b), pre-akinetes of both strains survived mild desiccation stress at 86% RH. Strain C was described as more tolerant of desiccation than strain B in (Pichrtová et al. 2014b) and here, fully recovered initial F_V/F_M values after desiccation at 86% RH whereas strain B recovered 78% of initial F_V/F_M. However, desiccation at 18% RH was lethal. In both strains, neither desiccation nor rehydration caused substantial changes in FA contents (Fig. 6). Putative separation of rehydrated samples (Supp. Fig. 3) is probably only due to the effect of FA degradation in dead cells, because samples desiccated at 86% RH cluster together with those desiccated at 18% RH.

Zygnema spp. in polar habitats

Massive accumulation of storage compounds in response to nitrogen starvation is not a general feature of all algae, being dependent on the taxon-specific strategies of carbon allocation (Giordano et al. 2015). However, some species including *Zygnema* spp. produce pre-akinetes, which are mature, vegetative cells that develop upon nutrient depletion, accumulate lipids and can be hardened to the stresses that accompany the end of their growing season. Apparently, this ability to accumulate storage compounds upon seasonal changes of

environmental factors is advantageous in the unstable conditions of polar terrestrial environments. Pre-akinete formation allows year-to-year survival of vegetative cells, without production of specialized resistant cells during the short and cold polar summer (Pichrtová et al. 2016). Lipid accumulations serve as source of energy and carbon and as a supply of fatty acids necessary for rearrangements of the membranes (Vítová et al. 2014). Therefore, lipid storage may provide the species with a competitive advantage during early polar summer (Davey 1988) as pre-akinetes may utilize their storage to supplement photosynthetic carbon assimilation and support rapid growth under nutrient abundance.

Conflict of interest:

The authors declare that they have no conflict of interest.

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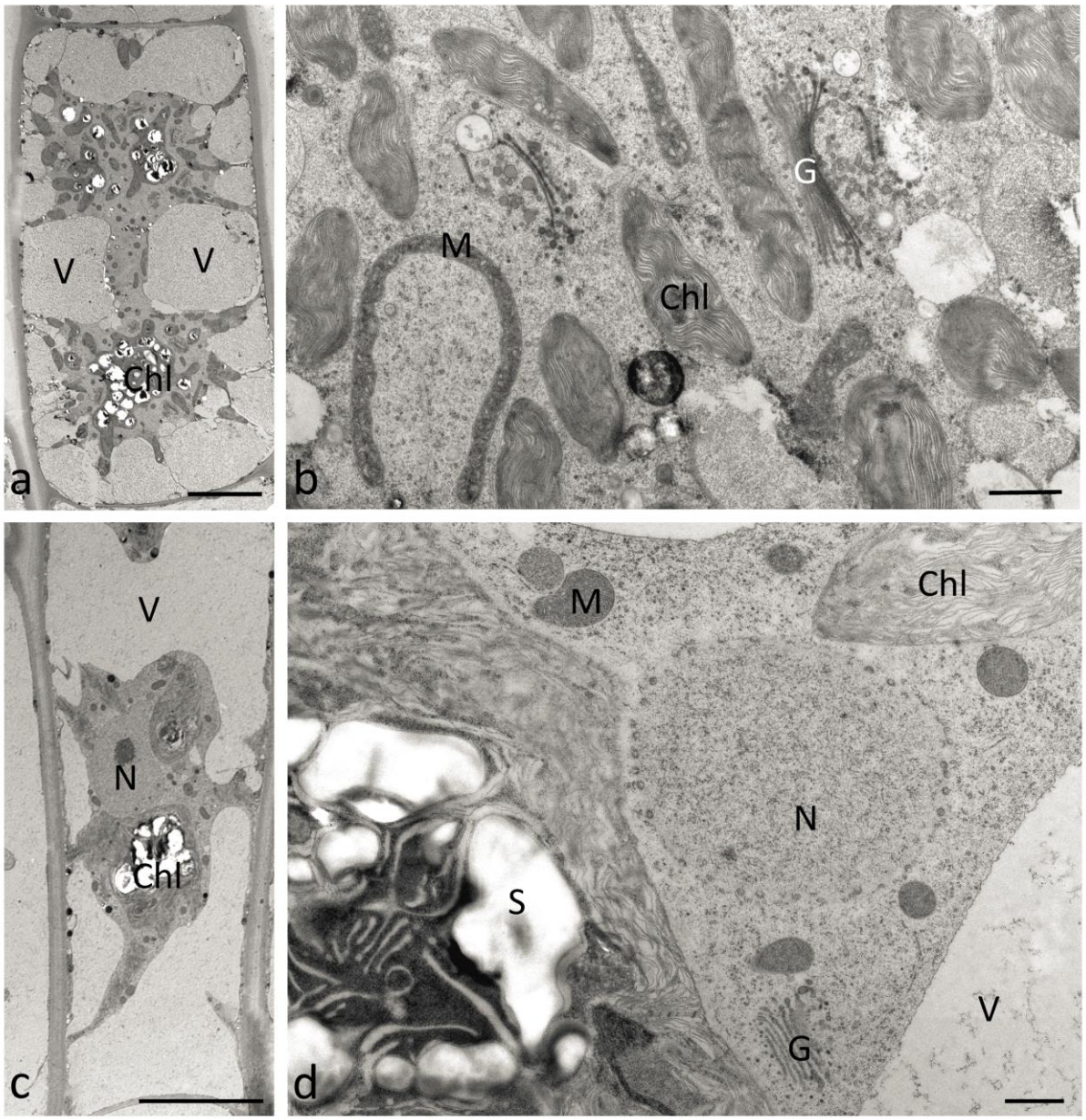


Figure 1. Transmission electron micrographs of young vegetative *Zygnema* sp. cells of strains B (a–b) and C (c–d). (a) Overview showing a high degree of vacuolization and two stellate chloroplasts, (b) cytoplasmic detail with numerous chloroplast lobes, Golgi bodies and mitochondrion, (c) overview with central nucleus and large vacuoles, (d) detail of the cell centre with nucleus, Golgi body, mitochondria, chloroplast with starch grains surrounding the pyrenoid. Abbreviations: Chl chloroplast, G Golgi body, M mitochondrion, N nucleus, S starch, V vacuole. Bars: a, c 10 µm; b, d 1 µm.

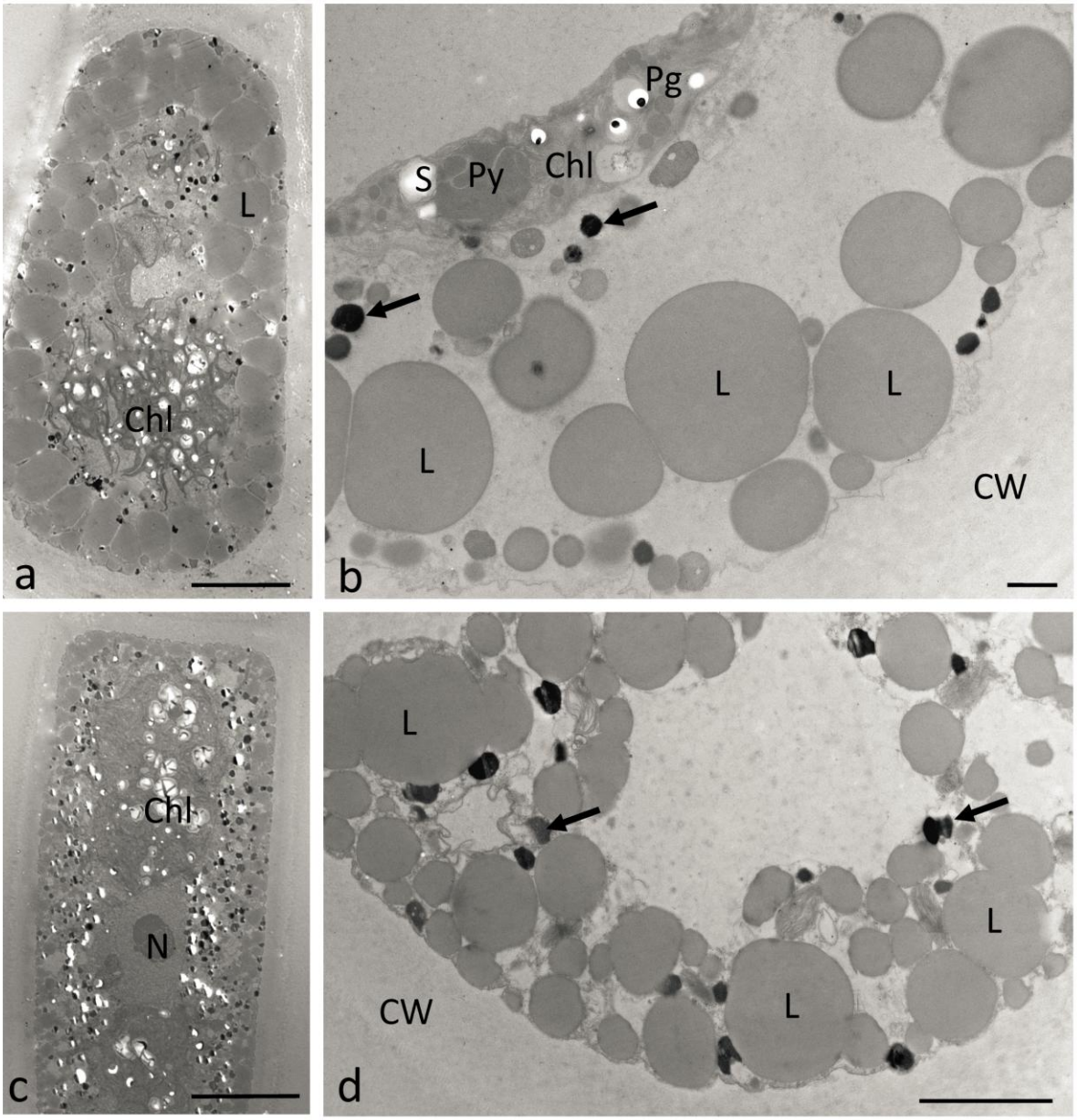


Figure 2. Transmission electron micrographs of mature vegetative cells of *Zygnema* sp. strains B (a–b) and C (c–d). (a) Overview showing the cell periphery filled with lipid droplets, and central chloroplasts; (b) detail of the cell periphery with cell wall, lipid droplets, and electron-dense particles (arrows); the chloroplast contains starch grains and numerous plastoglobules, (c) overview with central nucleus and chloroplasts; lipid droplets and electron-dense particles in the cell periphery, (d) lipid droplets and electron-dense particles (arrows). Abbreviations: Chl chloroplast, CW cell wall, N nucleus, Pg plastoglobule, Py pyrenoid, S starch. Bars: a, c 10 µm; b, d 1 µm

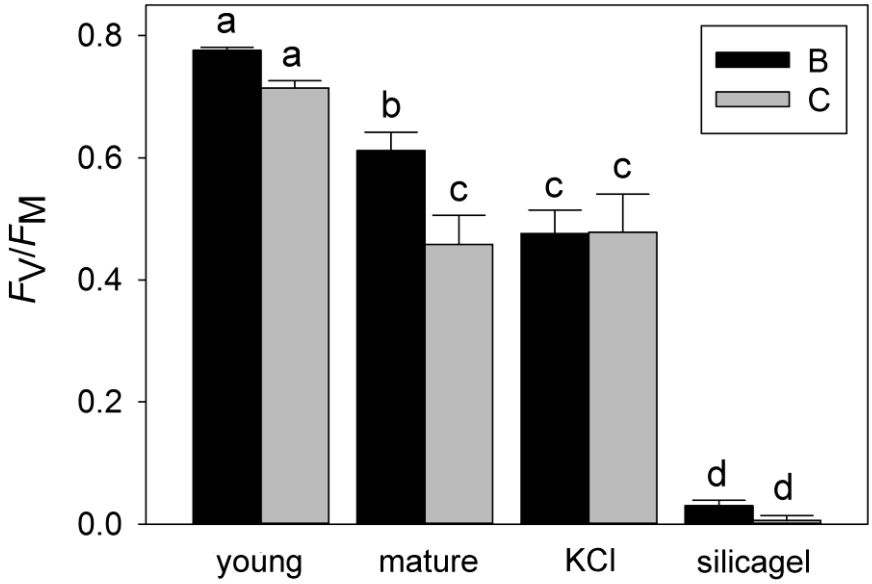
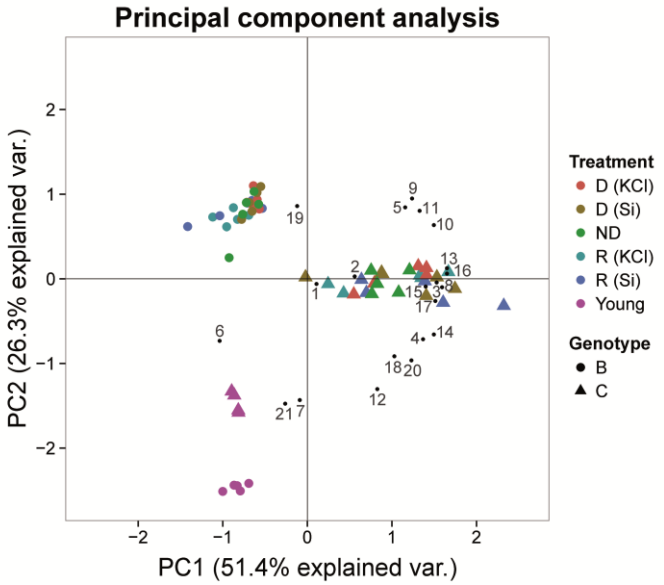


Figure 3: Maximum quantum yield of photosystem II (F_V/F_M) of young and mature cultures of *Zygnema* sp. strains B and C prior to desiccation, and of mature cultures that were rehydrated after desiccation above KCl (86% RH) or silicagel (18% RH); mean + SD, n =5. Different letters represent statistical differences between means ($P < 0.01$; general linear model factorial ANOVA, Tukey's post hoc tests).



Number	C : D	Common name
1		Levulinic acid
2	C12:0	Lauric acid
3	C14:0	Myristic acid
4	C16:0	Palmitic acid
5	C16:1a	Palmitoleic acid
6	C16:1b	(*isomer*)
7	C16:3	Hexadecatrienoic acid
8	C18:0	Stearic acid
9	C18:1	Oleic acid
10	C18:2	Linoleic acid
11	C18:3g	γ-linolenic acid
12	C18:3	Linolenic acid
13	C20:0	Arachidic acid
14	C18:4	Stearidonic acid
15	C20:1	Eicosenoic acid
16	C20:2	Eicosadienoic acid
17	C20:3	Dihomo-γ-linolenic acid
18	C22:0	Behenic acid
19	C20:4	Arachidonic acid
20	C20:5	Eicosapentaenoic acid
21	C24:0	Lignoceric acid

Figure 4: PCA ordination plot of samples from both strains and all experimental treatments based on their relative FA composition. Abbreviations: D (KCl) desiccated over KCl, D (Si) desiccated over silicagel, ND not desiccated, R (KCl) desiccated over KCl and rehydrated, R (Si) desiccated over silicagel and rehydrated, Young: young cultures. The first two axes are shown, for additional two axes see Suppl. Fig. 3.

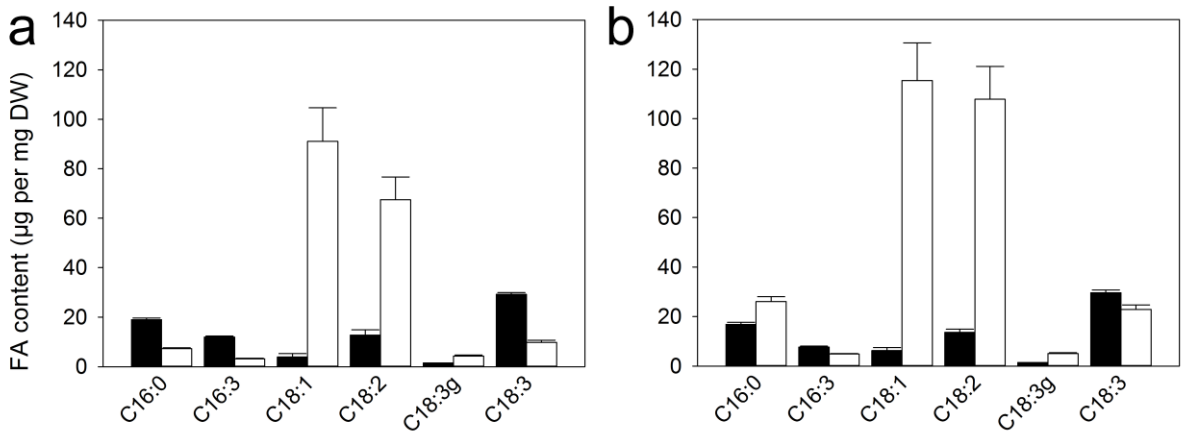


Figure 5: Absolute quantification of fatty acid content in *Zygnema* sp. B (a) and *Zygnema* sp. C (b). Black bars show young cultures and white bars pre-akinetes cultures. The FAs shown here were quantified using external standards; relative quantification data is also available for all other detected compounds (Supp. Fig. 2). Data are means plus standard deviation (n=5). Welch's two sample t-test with Bonferroni correction proved that the content of each fatty acid (within strain) was significantly different between young and mature cultures $p < 0.001$).

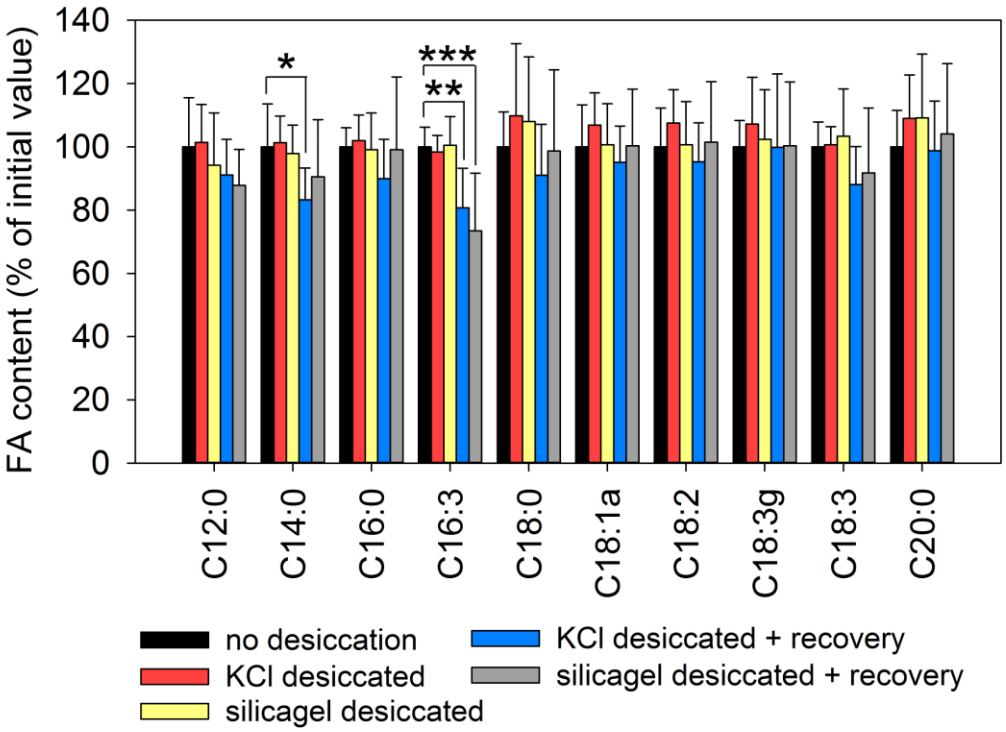


Figure 6: Fatty acid content in different experimental treatments relative to non-desiccated control. Only data for the most abundant fatty acids are shown. As two-way ANOVA did not reveal any significant interaction between strain and treatment, data for both strains were analyzed together (mean + SD, $n = 10$). Significant differences from the initial value (no desiccation) are marked by asterisks ($p < 0.001$***, $0.001 < p < 0.01$**, $0.01 < p < 0.05$*, Dunnett's test).