CHAPTER FIVE

How to survive winter?

Adaptation and acclimation strategies in eukaryotic algae from polar terrestrial ecosystems

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5.1 Introduction

The polar regions are of outstanding international scientific and environmental significance as they support important components of the global biogeochemical cycles. They comprise a whole range of habitats with extreme environmental conditions, which challenge living organisms with multiple environmental stresses. At the same time, they are vulnerable to disturbances and have long recovery times (Robinson et al., 2003; Elster & Benson, 2004; Thomas et al., 2008). Moreover, the Arctic is especially undergoing a particularly rapid climate change compared to the rest of the planet, including changes in temperature and precipitation (Thomas et al., 2008). However, predicting the impacts of climate change on arctic ecosystems is difficult (Bokhorst et al., 2015), because (i) climate change is not uniform across the Arctic (AMAP, 2011), and (ii) at local and regional scales, ecosystem responses to warming are not necessarily the same due to variations driven by other biotic and climatic factors (Post et al., 2009; Callaghan et al., 2013). Warming of the Arctic is also expected to result in an increasing frequency of stochastic climatic events (Saha et al., 2006; Bokhorst et al., 2009, 2011, 2015; Callaghan et al., 2013; Bjerke et al., 2014), such as extreme winter warming.

Photosynthetic microorganisms are major primary producers in the most hostile polar habitats and have successfully adapted to a wide range of extreme conditions, including winter freezing and desiccation (Thomas et al., 2008). A thorough understanding of their adaptation and acclimation strategies is crucial for evaluating and predicting their response to environmental changes. However, polar terrestrial eukaryotic microalgae, in contrast to cyanobacteria, usually form extensive, yet only annual, mats (Pichrtová et al., 2016b). It seems that, despite various stress tolerance mechanisms, winter conditions have a strong effect on the survival of whole populations, which appear to depend on a small fraction of surviving cells (Hawes, 1990; Pichrtová et al., 2016b).

In this chapter, we review the present knowledge on winter survival strategies of photosynthetic micro-eukaryotes in polar terrestrial ecosystems. Throughout this review, the term terrestrial also includes hydroterrestrial environments as defined by Elster (2002) unless further specified. Hydroterrestrial habitats have liquid water available almost throughout the entire growing season, but freeze-dry during winter (Elster, 2002). They include not only wetlands, shallow pools, snow-fed streams and rivers, seepages and springs, but also supraglacial and snow field habitats (Elster, 2002).

5.2 Physical settings

The most important feature limiting life in the polar regions is the frigid climate. The severity and complexity of polar environments are a consequence of the Earth's geometry. The North and South Poles, instead of having a daily alternation of diurnal cycles, have nightless summer months, followed by sunless winters and receive on average less solar radiation than the equatorial regions. However, this is not the only reason for the frigidity of the polar regions. Reflection losses in areas covered by ice or snow are higher in comparison with a snow-free terrestrial landscape or open waters (e.g. Youssouf et al., 2016; Lembo et al., 2017). Nevertheless, these properties affecting the heat balance in the polar regions have changed over geological time (Thomas et al., 2008).

Frost-free periods are usually quite short and diurnal fluctuations in temperature causing repeated freeze–thaw cycles can occur at any time (Davey & Rothery, 1992; Elster & Komárek, 2003). This overnight freezing is restricted to the vegetation surface, with the temperature dropping only to a few degrees below zero. In winter, air temperatures fall far below 0°C. The ground surface temperature of a study site on Svalbard reached down to temperatures between −30 and −35°C several times during winter (Láska et al., 2012). Hence, the terrestrial algal communities are completely desiccated and frozen until liquid water is available again when spring returns. However, terrestrial algae can be protected by

Figure 5.1 Stream with well acclimated (hardened) dry and frozen biomass of viable *Klebsormidium* sp. Central Svalbard, Endalen, late autumn. Photo J. Elster. (A black and white version of this figure will appear in some formats. For the colour version, please refer to the plate section.)

insulating snow cover and do not experience temperatures far below zero, even during winter (Hawes, 1989; Davey, 1991).

Seasonal and less pronounced diurnal variations of the polar terrestrial environments result in a series of water availability and freezing/melting gradients ranging from aquatic and semiaquatic to dry/ice habitats. These gradients result in different patterns, which change over geological time and are also influenced by current climate change (Elster, 2002). The widespread warming and increasing or decreasing winter precipitation in the Arctic and in some areas in the Antarctic have resulted in different regional snow-cover responses (Callaghan et al., 2011). Snow cover and its properties are principal ecological parameters for the terrestrial polar environment and influence their surface energy balance, water balance and thermal regimes.

Three different habitat categories were described in polar non-marine environments based on the variability in their ecological characteristics, mainly the availability of liquid water during the growing season (Broady, 1996; Vincent, 1988; Vincent & James, 1996; Elster, 2002) (Figures 5.1, 5.2 and 5.3). Terrestrial and hydroterrestrial environments are regarded as unstable; the third category is represented by stable lacustrine habitats.

5.3 Stress factors in the polar terrestrial environment

The polar environment is characterised by several interrelated stress factors. Low temperatures have several effects on living organisms. Membrane fluidity decreases with decreasing temperatures, and the rate of metabolic reactions slows down as described by the Arrhenius

Figure 5.2 Moss stream with dead *Tribonema* sp. biomass, central Svalbard, Endalen, beginning of July. Because of water erosion during spring melt, the stream started to flow in a different direction resulting in *Tribonema* biomass drying and not surviving the summer period. Photo J. Elster. (A black and white version of this figure will appear in some formats. For the colour version, please refer to the plate section.)
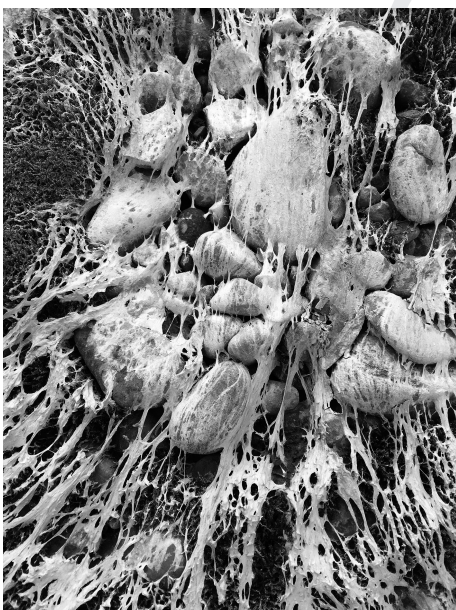

Figure 5.3 Detail of moss stream with dead *Tribonema* sp. biomass. Photo J. Elster. (A black and white version of this figure will appear in some formats. For the colour version, please refer to the plate section.)

equation. This slowdown also affects repair processes, which makes various stress factors more harmful at low temperatures (Roos & Vincent, 1998). Freezing itself is stressful in two different ways. First,

formation of ice crystals mechanically disrupts the cells, particularly if the crystals are formed intracellularly (Hawes, 1990; Fuller, 2004). Second, extracellular freezing increases intracellular solute concentrations, which leads to osmotic stress. This phenomenon makes the physiological effect of freezing similar to that of desiccation or salt stress; they all lead to osmotic dehydration and a lower intracellular water potential (Bisson & Kirst, 1995). Therefore, these stressors have to be discussed together since the adaptation mechanisms of algae are similar.

Algae are poikilohydric organisms that are able to tolerate desiccation to different capacities. The external environment directly manages their metabolic activity by affecting the presence or absence of water. In addition to poikilohydry, on a physiological level, changes in temperature and water status provoke a series of adaptive responses such as developing resistance and tolerance to cold, freezing, drought, desiccation and salinity stress (Elster & Benson, 2004)

Naturally, other stresses play important roles in polar habitats that are not addressed here in detail – namely, high solar irradiance (including deleterious effects of UV radiation) during the polar days, long periods of darkness during the polar nights, the short vegetation season or low nutrient availability.

5.4 Life strategies and adaptation mechanisms

Polar organisms have developed a wide range of adaptive strategies that allow them to avoid, or at least minimise, the injurious effects of extreme and fluctuating environmental conditions. However, such strategies are not exclusive to polar algae, as most of them can also be found in selected habitats at other latitudes (e.g. various aeroterrestrial and mountainous habitats). Nevertheless, Elster & Benson (2004) suggested that the physical and chemical conditions that occur uniquely in polar environments have selected for specific and resilient sets of adaptive biological characteristics.

Therefore, we present an overview of the most important adaptive strategies enabling winter survival of polar terrestrial algae together with specific examples from various studies performed on polar microalgae. First, we focus on the formation of specialised cells. The three main phylogenetic groups of algae using this strategy in polar terrestrial environments are introduced. Then, we present examples of algae which, in contrast to the previous subchapter, survive stress conditions in a vegetative state. In addition, we describe the phenomena of acclimation and mixotrophy. Finally, various biochemical adaptations are presented.

5.4.1 Production of specialised stages

Complex life cycles are a major avoidance strategy and usually involve the development of specialised stages that enable algae in general to survive the most stressful periods. Agrawal (2009) distinguished between dormant cells that require a period of dormancy before germination and other types of specialised cells that are also resistant but not dormant and thus can germinate immediately after formation. Both types of specialised cells are, however, important not only for stress tolerance, but also for dispersal in time and space (Rengefors et al., 1998).

5.4.1.1 Snow algae (Chlamydomonadaceae)

Snow algae are a diverse group of extremophilic microorganisms that form massive red, pink, orange or green blooms on snow in the polar and mountain regions worldwide. They are mostly represented by species from the genera *Chloromonas*, *Chlamydomonas* and *Chlainomonas*, from the green algal order Chlamydomonadales, that have optimised their physiology to the snow environment (Komárek & Nedbalová, 2007). The key feature of their life cycle that has been described in detail in many species is the presence of both flagellated and immotile stages (e.g. Hoham et al., 1979, 1983).

The formation of resistant coccoid stages (cysts or aplanozygotes) can be viewed as one of the major adaptations to the snow habitat, which allows them to survive long periods that are not suitable for active growth. As the nature of these cells is frequently not clear, we use the term cysts through the following text. In contrast to flagellates, cyst-like stages are believed to be resistant to freezing, high irradiances and any environmental changes in general (Remias, 2012). They are characterised by a resistant cell wall. The detailed structure of the ornamented secondary cell wall of *Chloromonas* cysts is used for determination on a fine scale taxonomic level (Procházková et al., 2018; Matsuzaki et al., 2019). For
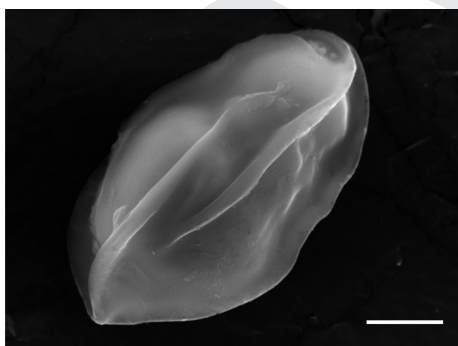

Figure 5.4 Scanning electron microscopy picture of a snow alga identified as a cyst of *Chloromonas* cf. *nivalis*. The field sample was collected in Nathorst Land, Svalbard. Scale bar, 5 μm. Courtesy of L. Procházková.

example, the cysts of the Antarctic species *Chloromonas polyptera* are characterised by many flanges-like structures, which enables to differentiate them from other taxa (Remias et al., 2013). A detailed study of arctic species of *Chloromonas* is still at its beginning (Figure 5.4). In an extensive field study performed in Svalbard, Müller et al. (1998) showed that cysts of snow algae contained high amounts of lipids and starch that serve as food reserves.

Mature cysts of different species were repeatedly shown to be metabolically active, which contradicts the view that they are solely dormant stages (Remias et al., 2005, 2010). In a study performed in Svalbard, Stibal et al. (2007) observed detailed dynamics of photosynthetic activity of different types of snow algal cysts which were associated with red snow. However, these measurements were performed during the summer period when light for photosynthesis is available. To our knowledge, the physiological status of snow algal cysts during winter has not been studied yet.

5.4.1.2 Zygnematophyceae

Zygnematophyceae is a class of streptophyte algae closely related to land plants (Embryophyta) (de Vries et al., 2016, 2018). Therefore, understanding their stress tolerance mechanisms is also important for research related to the transition of plants to life on land. The Zygnematophyceae represent the most species-rich lineage of charophyte algae and are characterised by a unique mode of sexual reproduction, namely conjugation, which leads to the formation of highly specialised, stress-resistant zygospores containing algaenan (a sporopollenin-like substance) in their cell walls (Poulíčková et al., 2007). In addition to zygospores, other specialised cell types have been described in Zygnematophyceae, namely parthenospores, aplanospores and akinetes.

Members of this class are very common in polar terrestrial habitats. Several filamentous genera form extensive mucilaginous mats in slow-flowing meltwater streamlets or shallow puddles both in the Arctic (Sheath et al., 1996; Kim et al., 2008; Holzinger et al., 2009) and Antarctica (Hawes, 1989, 1990; Davey, 1991; Skácelová et al., 2013). Other Zygnematophyceae, such as *Mesotaenium berggrenii* and *Ancylonema nordenskioeldii*, are typical inhabitants of bare glacier surfaces.

Despite their abundance in the polar regions, the conjugation process and formation of highly resistant zygospores in these regions has only very rarely been observed nor are there reports of other types of specialised cells. Elster et al. (1997) reported zygospore formation of *Zygnema* cf. *leiospermum* occurring in multiple sites of a glacial stream in Central Ellesmere Island. Pichrtová et al. (2016b) observed conjugation only

twice (species *Zygnemopsis lamellata* and *Zygnema* cf. *calosporum*) in Svalbard, despite intensive research (Pichrtová et al., 2018). Remias et al. (2011) reported the conjugation process and young zygotes in *Ancylonema nordens-kiöldii*, which were collected from the Midtre Lovenbreen glacier in Svalbard. Therefore, it has been suggested that a trade-off between sexual reproduction and growth plays an important role in these extreme environments (Holzinger et al., 2009).

5.4.1.3 *Diatoms*

Diatoms (Bacillariophyceae) are a group of algae which are characterised by a golden-brownish pigmentation and a siliceous shell wall (Round et al., 1990). They represent one of the most abundant algal groups in many polar fresh-water and terrestrial habitats (Jones, 1996; Van de Vijver & Beyens, 1999; Van de Vijver et al., 2002). Diatoms are known to be able to form two types of resting stages, namely morphologically distinct resting spores and vegetative-looking resting cells. Both stages are characterised by reduced metabolic activity and differences in their cellular components compared with vegetative cells. During this resting state only small amounts of cellular carbon are required to survive (Kuwata et al., 1993; Jewson et al., 2008). The resting stages are characterised by dense and dark cytoplasmic matter, rounder plastids (Round et al., 1990), condensed organelles, larger vesicles of storage products, granular cytoplasm, enlarged vacuoles or oil droplets and contracted chloro-plasts (McQuoid & Hobson, 1996).

Resting spores are morphologically different from actively growing or vege-tative cells. They are characterised by a rounder shape, thicker cell wall and different ornamentation (McQuoid & Hobson, 1995, 1996). Spore formation is usually associated with limiting nutrient concentrations and winter survival (McQuoid & Hobson, 1995, 1996; Kuwata & Takahashi, 1999). The most com-mon occurrence of diatom resting spores is in centric marine diatoms from temperate neritic habitats (McQuoid & Hobson, 1996; Kuwata and Takahashi, 1999) and are generally relatively rare in non-marine (observed mostly in centric) species (Edlund et al., 1996; McQuoid & Hobson, 1996; Jewson et al., 2008). To our knowledge, resting spores have not yet been observed in terres-trial diatoms.

5.4.2 Survival in the vegetative state

As mentioned above, many algae survive stress conditions in a vegetative state without the production of any morphologically distinct cells (Agrawal, 2009). In this case, the vegetative cells, similarly to dormant cells, have reduced physiological activity combined with other adapta-tions, e.g. biochemical. Sheath et al. (1996), who studied tundra stream algae in the Arctic, including members of the Chlorophyta,

Streptophyta, Rhodophyta and stramenopiles (e.g. diatoms), observed that most species do not form specialised cells, but instead, their vegetative cells are adapted to withstand prolonged freezing. They possess however thick cell walls and accumulate storage materials (Sheath et al., 1996).

Recently, stress tolerance of polar hydroterrestrial algae of the genera *Zygnema* and *Zygnemopsis* (class Zygnematophyceae) has been intensively studied. Since these algae rarely form truly specialised stages, they are a typical example of algae with the ability to survive multiple stress conditions in a vegetative state: the modified, resistant vegetative cells are called pre-akinetes (McLean & Pessoney, 1971; Pichrtová et al., 2014a). Pre-akinetes are formed directly from young vegetative cells that stop dividing and gradually accumulate storage compounds and thicken their cell walls; their chloroplasts are structurally reduced, and their physiological activity is diminished (Pichrtová et al., 2014b, 2016a; Herburger et al., 2015, Figure 5.5). These cells are not dormant and gradually recover their vegetative appearance and physiological activity immediately after transfer to favourable conditions, which enables rapid growth of *Zygnema* populations during early spring (Fuller, 2013; Pichrtová et al., 2016b).

Such pre-akinetes in *Zygnema* were observed at the end of summer in Svalbard (Pichrtová et al., 2014a, 2016b) and viable pre-akinetes were even found frozen in solid ice during winter (Pichrtová et al., 2016b). After
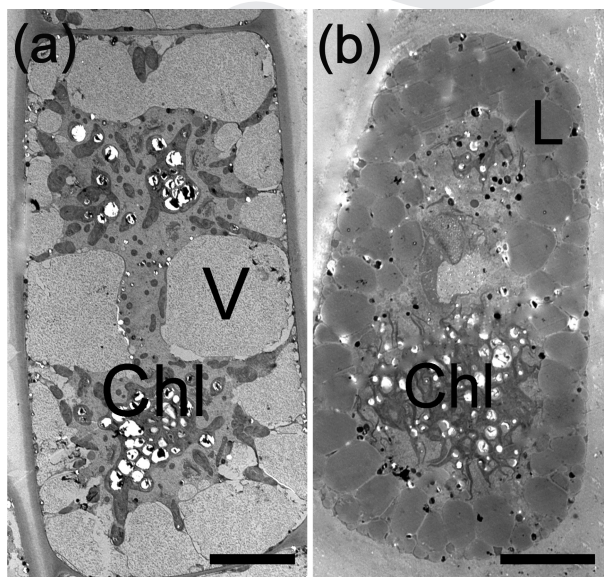

Figure 5.5 Transmission electron microscopy picture of Antarctic *Zygnema* sp. cells. (a) An overview of a young vegetative cell with a high degree of vacuolisation and stellate chloroplasts, and (b) an overview of a pre-akinete with reduced chloroplasts and massive accumulation of lipid bodies. Chl, chloroplast; L, lipid body; V, vacuole. Scale bar, 10 μm. Reprinted from Pichrtová et al., (2016a).

melting and transfer into a culture medium, they immediately restored their photosynthetic activity and gradually developed back into young and dividing vegetative cells (Pichrtová et al., 2016b). Pre-akinetes can also be found as senescent or stationary-phase-like cells in old cultures with depleted nutrients. Thus, it has been suggested that nutrient limitation is involved in pre-akinete formation (Fuller, 2013; Pichrtová et al., 2014a). This hypothesis was later supported experimentally in which pre-akinetes were formed much faster in medium lacking any form of nitrogen (Pichrtová et al., 2014b). Such experimentally induced pre-akinetes were tolerant to desiccation, whereas young vegetative cells or intermediate stages did not survive any of the desiccation treatments (Pichrtová et al., 2014b). The phenomenon of stress tolerance in response to nutrient depletion is also well known for bacteria (Siegele & Kolter, 1992) and eukaryotic algae from non-polar regions (McLean & Pessoney, 1971; Nagao et al., 1999).

Hawes (1990) conducted a series of freezing experiments with an Antarctic *Zygnema* species. He used both newly collected material from the field described as 'resembling pre-akinetes' as well as cultures in the exponential growth phase and hence young vegetative cells. The newly collected samples showed no decrease in photosynthetic rate during repeated freeze–thaw cycles (temperature ranging between +5 and −4°C), and several cells were still alive even after 120 days at −20°C. In addition, the cultures were able to survive at −15°C for 60s when very slow cooling rates were applied, which indicates a certain level of frost tolerance even in young cells (Hawes, 1990). This was confirmed by our observations with an Arctic *Zygnema* species. Young cells survived slightly subzero temperatures and were not able to recover from frost damage only when exposed to −8°C. By contrast, pre-akinetes survived even at −70°C during 8 hours of exposure (Trumhová et al., 2019).

Klebsormidium is a globally distributed algal genus which is ecologically important in the polar terrestrial environment (Ryšánek et al., 2016). No specialised dormant stages are known in members of this genus, yet they were repeatedly shown to be outstandingly tolerant of stress in the vegetative state, being able to survive both desiccation and freezing (Elster et al., 2008; Nagao et al., 2008; Karsten et al., 2010). Freezing to −4°C had no effect on the survival of the investigated strains, and they still retained 80% viability at −40°C (Elster et al., 2008).

Šabacká & Elster (2006) studied the effect of freezing and desiccation on several strains of antarctic terrestrial microalgae from the genera *Chlorella, Chlorosarcina, Pseudococcomyxa* and *Klebsormidium*. Whereas minor subzero temperatures (–4°C) during experimentally induced summer diurnal freeze–thaws did not cause significant damage, low subzero temperatures representing an annual winter freeze (–40°C) were fatal for more than 50% of the

populations. However, individual members survived also in −100°C and −196°C treatments, but with markedly reduced viability (Šabacká & Elster, 2006).

Most diatom species do not form morphologically different resting spores, but to ensure survival under stress conditions, they create physiologically adapted resting cells (Figure 5.6). They are morphologically identical to vegetative cells (Lund, 1954; Anderson, 1975; Sicko-Goad et al., 1986), yet are characterised by dense and dark cytoplasmic matter and rounder plastids (Round et al., 1990) as well as lower pigmentation and contracted chloroplasts (Kuwata et al., 1993). Resting cells have been observed in many pennate diatoms (McQuoid & Hobson, 1996), including terrestrial and freshwater species (Souffreau et al., 2013). It has been suggested that resting cell formation in terrestrial diatoms might be important for their survival during freezing and desiccation, which might represent a specific adaptation to the varying environmental conditions in terrestrial habitats (Souffreau et al., 2013). By contrast, both vegetative and laboratory-induced resting cells of freshwater benthic diatoms were shown to be highly sensitive to desiccation, abrupt heating or freezing (Souffreau et al., 2010; Hejduková et al., 2019). A recent study of diatom survival in freshwater and hydroterrestrial habitats in the High Arctic observed remarkable viability of diatoms during the winter season with more than 20% of the cells on
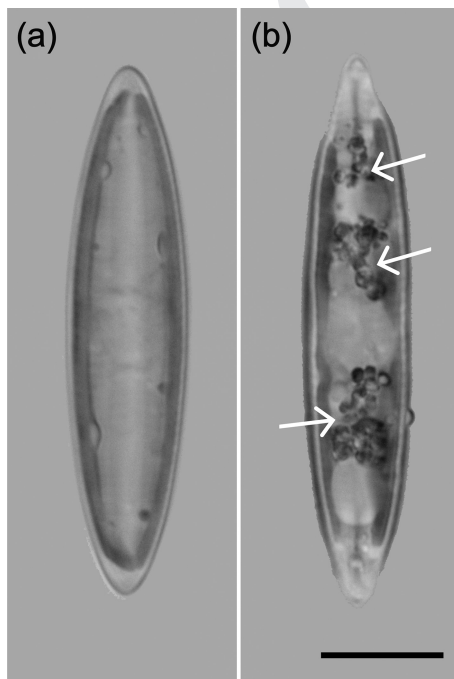

Figure 5.6 Vegetative (a) and resting (b) cells of the diatom *Navicula radiosa*. The laboratory strain was isolated from a sample collected on Svalbard. Note the shrinkage of the chloroplast and numerous lipid droplets (marked by arrows) in the resting cell that was induced by a 2-week incubation in dark at 5°C. Scale bar, 10 μm. Photo: E. Hejduková.

average found in a resting stage and almost 5% becoming active after thawing. The populations were, however, dominated by dead or damaged cells (Hejduková, in press).

5.4.3 Acclimation to cold conditions and desiccation

Acclimation also plays an important role in the development of tolerance for winter survival. The period of declining temperatures during autumn may be important in promoting cold-hardiness. Various antarctic hydroterrestrial algae were found to be psychrotrophs rather than psychrophiles, which might suggest that their ability to grow at cold temperatures is not genetically fixed but a result of acclimation (Seaburg et al., 1981).

The positive effect of acclimation has already been described in a non-polar strain of *Klebsormidium flaccidum*, in which survival rates increased by 15% after exposure to 2°C for 7 days (Nagao et al., 2008). Unfortunately, this phenomenon has not been experimentally studied on polar isolates of *Klebsormidium*. Nevertheless, a recent phylogenetic analysis demonstrated unlimited dispersal and intensive gene flow, together with remarkable ecological plasticity, of this genus (Ryšánek et al., 2016). Moreover, laboratory strains of *Klebsormidium* were shown to be highly resistant to both freezing and desiccation injuries and no statistical differences were observed between strains originating from different regions and habitats (Elster et al., 2008). All these findings support the ecological role of acclimation.

The effect of acclimation, in this case to osmotic stress and desiccation, was also studied in Arctic *Zygnema* pre-akinetes (Pichrtová et al., 2014a, 2014b). Populations sampled from partly desiccated environments were much more resistant to osmotic stress than populations from pools, even though all samples consisted of well-developed pre-akinetes (Pichrtová et al., 2014a). This finding, later confirmed by a laboratory study, shows that acclimation plays an important role in stress tolerance of *Zygnema* pre-akinetes (Pichrtová et al., 2014b). As mentioned above, the pre-akinetes were induced by nitrogen depletion and subsequently experimentally desiccated (Pichrtová et al., 2014b). In general, the pre-akinetes tolerated desiccation at 86% relative humidity, but survival at 10% relative humidty only occurred after additional acclimation induced either by very slow experimental desiccation or precultivation on agar plates (Pichrtová et al., 2014b).

Acclimation is also known to increase the tolerance of diatoms to unfavourable conditions (Mock & Valentin, 2004). Resting cells of terrestrial diatoms, induced in laboratory conditions by acclimation at a lower temperature and in dark conditions as well as under nitrogen limitation, showed a higher degree of stress tolerance, especially to desiccation (Souffreau et al., 2013). The same methodological approach for the induction of resting cells was applied by Hejduková et al. (2019), who emphasised their importance for the survival of

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mild freezing (–4 C treatment) in a set of polar and temperate diatom strains originating from terrestrial and freshwater environments.

Acclimation might also be involved in speciation. Physiological differences among various populations of stress-acclimatised organisms might be related to life cycle timing, germination, dormancy, frost resistance and many other characteristics. As there are many environmental gradients, so there are gradients of evolutionary progression from induced acclimation, to temporary ecoforms, to more stable ecotypes and finally to a new species (Svoboda, 2009).

5.4.4 Mixotrophy

The shift from photoautotrophy towards mixotrophy or heterotrophy can be an important process for the successful survival of algal populations in low nutrient and low-light polar environments. During long periods of darkness, mixotrophic algae can remain metabolically active and rapidly resume photosynthetic activity when light becomes available. The utilisation of organic compounds has been documented in many algal taxa belonging to most of the ecologically important taxonomic groups (e.g. diatoms, chrysophytes, green algae, cryptophytes, dinoflagellates). Actually, photosynthetic eukaryotes have a wide range of strategies for mixotrophy, ranging from phagotrophy to the uptake of dissolved organic carbon (Jones, 2000).

Among polar lacustrine communities, the phenomenon of mixotrophy was shown to be a widely used survival strategy that plays a major role in carbon cycling (Laybourn-Parry et al., 2000). Decades ago it was found that the high proportion of mixotrophic algae in boreal humic lakes might compensate for the poor light environment (Jansson et al., 1996). The current climate warming in both polar regions is associated with increased snowmelt that can occur even during winter months. Such events could support protists capable of mixotrophic growth whose adaptive capacity is supposed to shape the structure and function of microbial communities (Vrionis et al., 2013). However, in contrast to freshwater and marine environments, the possible role of mixotrophic nutrition in polar terrestrial habitats is still poorly understood.

5.4.5 Biochemical acclimation and adaptation mechanisms

The above discussed acclimation and adaptation mechanisms, production of resting stages and acclimation, are closely connected with a shift in the biochemical composition of the cells. In the following, we introduce the most widespread strategies against desiccation and cryoinjury stresses.

5.4.5.1 *Lipids*

Lipid accumulation is considered as a general response of algae to unfavourable conditions, such as nutrient depletion and other stresses, e.g. high light, alkaline pH or heavy-metal exposure (Guschina & Harwood, 2006). The

accumulation of nitrogen free lipids under stress conditions is thought to be the result of a shift in photosynthate allocation from growth to energy storage (Vítová et al., 2014). For example, an extensive occurrence of lipid bodies was observed in the terrestrial green alga *Prasiola crispa* from Antarctica when exposed to desiccation (Jacob et al., 1992). High lipid content is a major feature of specialised and vegetative cells that are resistant to seasonal changes of environmental factors, and play a crucial role in over-wintering of populations. This strategy is shared by various unrelated groups of algae as lipid accumulation is typical for resting cells of diatoms, *Zygnema* pre-akinetes (Pichrtová et al., 2016a), snow algal cysts (Remias et al., 2010) and various other algae that survive unfavourable conditions in the vegetative state (Sheath et al., 1996). This lipid accumulation serves as an energy supply and carbon source, and enables rapid growth early in the spring, which gives these species a competitive advantage (Davey, 1988; Pichrtová et al., 2016a).

In snow algae, the transformation into cysts and lipid production is accompanied by progressive incorporation of esterified secondary carotenoids, namely astaxanthin (Remias et al., 2010). The decrease in water content because of the high content of lipid bodies containing astaxanthin is considered as an important mechanism which protects the cysts from the deleterious effects of subzero temperatures. The accumulation of astaxanthin is particularly high in the red snow forming species *Chlamydomonas nivalis*, where the astaxanthin/chlorophyll a ratio frequently reaches values of around 20 (Remias et al., 2005). Large amounts of astaxanthin diglukoside diesters were detected in cysts of this species suggesting that astaxanthin accumulation also has an additional role in carbon and hence energy storage (Řezanka et al., 2008).

Apart from the total lipid content, the changes in lipid and fatty acid composition also represent an important process in enhancing cell survival under stress conditions in polar habitats. Perhaps the best studied metabolic adaptation of microorganisms to low temperatures is the desaturation of fatty acids in membrane lipids that ensures the maintenance of membrane fluidity (Morgan-Kiss et al., 2006). A high proportion of polyunsaturated fatty acids, reaching up to 70% of total fatty acids, was repeatedly reported in field samples of psychrophilic algae collected from various cold habitats (e.g. Procházková et al., 2018), as well as in their laboratory cultures (Teoh et al., 2004). Several long-chain polyunsaturated fatty acids were detected in antarctic *Zygnema* sp., although in low concentrations, likely because their content was not investigated at low cultivation temperatures (Pichrtová et al., 2016a). A high proportion of unsaturated fatty acids enhancing physiological performance of snow algal cysts at low temperatures is also often detected. For example, 80% of the total fatty

acid pool consisted of monounsaturated fatty acids in red cells of *Chlamydomonas nivalis* from Antarctica (Bidigare et al., 1993). Spijkerman et al. (2012) found that between 45 and 55% of total fatty acids were polyunsaturated fatty acids in field samples from Svalbard that contained red and orange cysts, which were also attributed to *Chlamydomonas nivalis.*

The complex adaptation of polar algae to their extreme habitats makes them suitable candidates for low temperature biotechnologies. Low requirements for cultivation temperature and light, combined with relatively high growth rates and a high lipid/polyunsaturated acid content were demonstrated for example in a cold-adapted green alga belonging to the genus *Monoraphidium,* which was isolated from Antarctica (Řezanka et al., 2017).

5.4.5.2 *Accumulation of inorganic and organic osmolytes*

One of the most widespread protective adaptations is the accumulation of osmolytes. These compounds prevent water loss and thus help to maintain homeostasis (Bisson & Kirst, 1995). As such, they contribute to tolerance to desiccation (Crowe et al., 2002), freezing-drying (Tanghe et al., 2003) and osmotic stress (Reed et al., 1984). Osmolytes are either simple inorganic ions or various organic substances, such as sugars, polyols or amino acids. Some organic osmolytes, termed compatible solutes, play different additional roles in cellular protection as they protect and stabilise membranes, proteins and DNA (Crowe et al., 2002, 2004; Yancey, 2005). They can prevent intracellular freezing by decreasing the freezing point as well as through water replacement. This process is termed vitrification and is involved in both desiccation and freezing tolerance (Welsh, 2000; Yancey, 2005; Clarke et al., 2013). It is generally accepted that especially sucrose and trehalose bind to dried membranes, proteins and nucleic acids as water substitutes, and as such preserve the native structures of the molecules (Crowe et al., 2002).

Several compounds (glucose, sucrose, an additional unidentified sugar and several amino acids) were shown to accumulate during cold acclimation in *Klebsormidium flaccidum* (Nagao et al., 2008). Moreover, a recent transcriptomics study on *Klebsormidium crenulatum* showed the up-regulation of enzymes connected with the biosynthesis of the sucrose and raffinose family of oligosaccharides in response to desiccation stress (Holzinger et al., 2014).

In an antarctic *Zygnema* sp., sucrose was identified as the main carbohydrate with traces of glucose, fructose and mannitol (Hawes, 1990). Moreover, Kaplan et al. (2013) revealed quite low (negative) values of cellular osomotic potentials in arctic and antarctic *Zygnema* spp., but the composition of soluble carbohydrates was not investigated. Comparison of metabolic composition of

vegetative cells and pre-akinetes showed accumulation of several sugars and sugar alcohols, mainly raffinose, in pre-akinetes. Sucrose was one of the most abundant sugars, but only showed a slight increase during pre-akinete formation (Arc et al., 2020). Furthermore, a recent transcriptomics study confirmed a strong up-regulation of sucrose synthesis in response to desiccation stress in *Zygnema* (Rippin et al., 2017).

Prasiola (Trebouxiophyceae) synthesises free proline as a cryoprotectant, and accumulates it at the onset of winter (Jackson & Seppelt, 1995). Therefore, it can continue to photosynthesise down to -7°C (Davey, 1989) or -15°C (Becker, 1982), even though the thallus appears to be frozen. In addition, *Prasiola* is also tolerant to salt (Jacob et al., 1992) and desiccation stress (Kosugi et al., 2010).

5.4.5.3 Secretion of extracellular polysaccharides

The secretion of extracellular polysaccharides (EPS) is typical for cyanobacteria where it helps in increasing their freezing and desiccation tolerance (Tamaru et al., 2005). However, the production of hydrophilic polysaccharides, i.e. mucilage, is also widespread in eukaryotic algae (Domozych, 2011). Mucilage helps to retain water around the cells and protects them from freezing and desiccation stress. In several habitats across the Arctic, there are species which produce mucilage in quite high quantity e.g. *Hydrurus foetidus* belonging to the Chrysophyceae (Klaveness et al., 2011). Also *Zygnema* sp. pre-akinetes are often embedded in thick mucilaginous sheaths. Fuller (2013) showed that these secondary pectic layers of the cell walls act as a sponge and provide extracellular protection against desiccation. Recently, homogalactouronan was revealed as the major component of these pectic layers and its crucial role in desiccation resistance was confirmed (Herburger et al., 2019).

Apart from desiccation avoidance, mucilage layers also enable the algae to aggregate into mats, biofilms and crusts. In these structures, numerous other microorganisms are embedded. As such, these algae are true ecosystem engineers, because their mucilage layers protect whole communities against multiple environmental stresses (Knowles & Castenholz, 2008). In general, the formation of these aggregates is itself considered as a stress avoidance strategy (Holzinger & Pichrtová, 2016). In addition, the secretion of EPS may serve as a source of organic matter for bacteria (Bashenkhaeva et al., 2015).

5.4.5.4 Ice-binding proteins (IBPs) and ice nucleation agents

Ice-binding proteins (IBPs) are a diverse group of proteins that bind to ice surfaces and control ice growth to avoid freezing injury. They have been detected in many cold-adapted organisms including bacteria, fungi, algae,

vascular plants and animals (Dolev et al., 2016). In polar algae, IBPs that are able to distort the shape of growing ice crystals and thus prevent mechanical damage to the cells were first discovered in sea-ice diatoms (Raymond, 2000; Janech et al., 2006). Also another type of IBP that is secreted by sea-ice-associated chlamydomonad algae helps to maintain a liquid environment in brine pockets by structuring the external ice (Raymond et al., 2009). Reports on the occurrence of IBPs from terrestrial cold-adapted algae are limited to species associated with snow. Genes for more than 20 isoforms of IBP were identified in *Chloromonas brevispina* indicating the importance of this protein for this species. Their close relationship to bacterial and fungal proteins provided evidence that they were possibly acquired by horizontal gene transfer (Raymond, 2014). A screening of snow algal strains from the CCCryo Culture Collection of Cryophilic Algae (Fraunhofer, Postdam-Golm, Germany), which contains mainly isolates from the Arctic, revealed that only psychrophilic strains from the Chlamydomonadales were able to produce IBPs. Tests of recrystallisation activity revealed a rather high variability of active concentrations of IBPs, even within this small taxonomic group (Leya, 2013). It can be expected that the secretion of IBPs is common not only in snow algae, but also in other terrestrial groups of polar eukaryotic algae that have to cope with freezing.

Another way for preventing ice crystal formation is controlled freezing at relatively high temperatures due to ice nucleation agents. Ice nucleation proteins can be considered as a subset of IBPs (Dolev et al., 2016). However, current knowledge on ice nucleation activity in algae is limited. It was suggested that ice nucleation agents are produced by fungi and bacteria that are associated with the algae in field samples. For example, field samples of the green alga *Prasiola crispa* had a mean freezing point that was 10°C higher compared to axenic culture (Worland & Lukešová, 2001).

5.5 Conclusions

Even though eukaryotic algae are the most important primary producers in many polar terrestrial habitats, their stress tolerance with respect to winter survival has hardly been described. In this chapter, we give a complex overview of the present knowledge about stress tolerance strategies investigated in polar terrestrial algae. Different mechanisms have been described that enable the cells to survive extreme conditions and several of these mechanisms are present in phylogenetically unrelated taxa. These include survival in only a slightly modified vegetative state. This is a very advantageous strategy because it is energetically less

demanding than sexual reproduction connected with the formation of true specialised stages.

Due to the lack of studies, it is often difficult to state which strategies have developed as unique traits of polar organisms and which are shared also by their non-polar relatives. Nevertheless, the evaluation of winter survival strategies is important to predict the effect of global climate change on polar terrestrial microbial communities, their biodiversity and ability to cope with potential invasive species.

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