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Freezing tolerance of freshwater diatoms as a key to their success in polar regions

Mrazová tolerance sladkovodních rozsivek jako klíč k jejich úspěchu v polárních oblastech

Doctoral Thesis

Supervisor: doc. RNDr. Linda Nedbalová, Ph.D.

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DECLARATION

I hereby declare that this doctoral thesis has not been submitted to obtain the same or any other academic degree earlier or at any other institution. I have written this thesis independently, using the references listed.

Eva Hejduková

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Abstract (English version)

Diatoms are microalgae characterised by a golden-brown colour and ornamented silica shells. They thrive in various environments worldwide ranging from aquatic marine and freshwater to terrestrial. Together with cyanobacteria and green algae, they are important primary producers in both polar regions (Arctic and Antarctica). The polar environment is characterised by extreme natural conditions, which microalgae have to overcome, such as low and freezing temperatures, desiccation, long periods of dark and irregular nutrient and liquid water availability. Many microorganisms overwhelmed by unfavourable conditions use dormancy and create stress resistant stages. Yet, freshwater pennate diatoms are not known to form such morphologically distinct stages. Despite this fact, they prosper well in many polar habitats.

This doctoral thesis presents a summary of the challenges of life in polar environments and reviews the current knowledge of survival strategies of microalgae with the focus on freezing stress. The conducted research provides a deeper insight into survival of freshwater pennate diatoms in the severe conditions of the polar environment. Their tolerance to freezing stress was evaluated experimentally under laboratory conditions and by field observations over a one-year period in Svalbard (High Arctic). Multiparameter fluorescent staining was introduced for the evaluation of the physiological status at a single-cell level. The studies found that diatoms originating from freshwater polar environments are able to survive mild freezing ($-4\,°C$) without any harm, though they appeared to be rather sensitive to lower temperatures ($-20\,°C$). The very low survival of severe freezing ($-40\,°C$ and liquid nitrogen) suggests that the freezing conditions in natural habitats are less extreme, which was supported by data obtained during field study temperature measurements. Furthermore, formation of resting cells was evaluated. No morphologically distinct stress resistant stages were detected. Nevertheless, vegetative-looking resting cells were induced by nutrient starvation under dark and cold conditions. Their importance for freezing survival was proved for the mild and middle freezing temperatures. Diatoms withstand laboratory freezing successfully also as common vegetative cells. Both the laboratory experiments and the field study suggested that the diatom overwintering strategy in polar environments seems to be associated with survival of a small number of vegetative cells, which later provide an inoculum for population growth in the next vegetative season.

Abstrakt (Czech version)

Rozsivky jsou mikroskopické řasy charakteristické zlatohnědou barvou a tvorbou vzorovaných křemičitých schránek. Vyskytují se po celém světě v nejrůznějších prostředích od mořských a sladkovodních po terestrická. V obou polárních oblastech (Arktidě a Antarktidě) jsou společně se sinicemi a zelenými řasami významnými primárními producenty. Polární prostředí se vyznačuje extrémními přírodními podmínkami, jako jsou nízké a mrazivé teploty, vysychání, dlouhá období tmy, nepravidelný příjem živin a nedostatek vody v kapalném skupenství, jež musí být mikroskopické řasy schopny překonat. Mnoho mikroorganismů přežívá nepříznivé podmínky prostřednictvím dormance a vytváří stádia odolná vůči stresu. Nicméně není známo, že by byly sladkovodní penátní rozsivky schopny taková morfologicky odlišná stadia tvořit. Navzdory této skutečnosti však velmi dobře prosperují v mnoha polárních habitatech.

Disertační práce se zaměřuje na rizika spojená s životem v polárním prostředí a shrnuje současné poznatky o strategiích přežití mikroskopických řas v souvislosti se stresem způsobeným mrazem. Provedený výzkum detailněji nahlíží na přežití sladkovodních penátních rozsivek v nehostinných podmínkách polárního prostředí. Jejich tolerance vůči vymrzání byla posuzována experimentálním testováním v laboratorních podmínkách a sledováním v terénu po dobu jednoho roku na Špicberkách (Vysoká Arktida). Pro vyhodnocení fyziologického stavu na úrovni jednotlivých buněk bylo zavedeno multiparametrické fluorescenční barvení. Studie dospěly k závěru, že jsou polární sladkovodní rozsivky schopny téměř bez poškození přežít mírné vymrzání ($-4\ °C$). Ukázalo se však, že jsou poměrně citlivé vůči nižším teplotám ($-20\ °C$). Nízká schopnost přežití hlubokého mrazu ($-40\ °C$ a kapalný dusík) naznačuje, že mrazové podmínky na přírodních stanovištích nejsou tak extrémní, což potvrzují i data teplotních měření získaná během terénní studie. Zároveň byla sledována schopnost tvorby klidových buněk. Nebyla pozorována žádná morfologicky odlišná stádia, která by mohla být odolná vůči stresu. Nicméně tvorba vegetativně vypadajících klidových buněk byla podnícena nedostatkem živin, tmou a chladem. Jejich význam pro přežívání byl prokázán pro mírné a střední vymrzání. Rozsivky však také úspěšně přežívají experimentální zmrazení jako běžné vegetativní buňky. Jak laboratorní, tak terénní studie naznačily, že by strategie přezimování rozsivek v polárním prostředí mohla být spojena s přežitím malého počtu vegetativních buněk, které později poskytují inokulum pro růst populace v následujícím vegetativním období.

ACKNOWLEDGEMENT

1 INTRODUCTION

1.1 DIATOMS

Diatoms (Bacillariophyceae) are photosynthetic microscopic algae belonging to the eukaryotic group Stramenopila (Julius and Theriot 2010; Adl *et al.* 2012). They are basically unicellular although some species form colonies. They are characterized among other microalgae by a golden-brown colour and ornamented shell composed of silicon dioxide (SiO_2), which is typical for them (Round *et al.* 1990; Lee 2008; Julius and Theriot 2010). Estimates of total diatom diversity are 30,000–100,000 species (Mann and Vanormelingen 2013), which makes them one of the most numerous groups of eukaryotic photosynthetic microorganisms on Earth (Mann 1999).

1.1.2 MORPHOLOGY AND REPRODUCTION

The term diatoms is derived from the Greek "*diatomos*" meaning "cut in half " indicating their two-part silica shell (Armbrust 2009), termed a frustule, which consists of two almost identical halves fitting together: a slightly bigger epitheca and smaller hypotheca. Each theca consists of a base part – the valve (epivalve, hypovalve) and a valve mantle – the cingulum (epicingulum, hypocingulum). The cingula together make siliceous bands or belts termed the girdle (Round *et al.* 1990; Julius and Theriot 2010). Diatom morphology is highly variable: a range of frustule shapes was described depending on the principal axes, girdle and valval view, the endings etc. Striation, the pattern on the valve surface, is an important and often species-specific characteristic (Fig. 1). The striae could appear as a continual groove or consist of many fine pores or cuts. The density of rows varies as well. In some groups, an isolated pore (stigma) in the central area and/or a raphe slit along the frustule, which serves for moving, can be distinguished (Fig. 1).
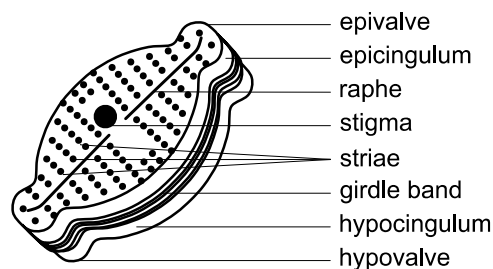
Fig. 1. Diatom frustule morphology.

The diatom life cycle is quite unique. They can reproduce both sexually and asexually, although the vegetative mitotic way of reproduction predominates as with many other microalgae while sexual reproduction is quite rare. Normally the one maternal cell divides and its epitheca and hypotheca become the epithecae of the two daughter cells, which then produce new hypothecae. As a result of cell division, one of the daughter cells is always smaller than the maternal, which leads to decreasing average cell size within the population (Fig. 2). Cell size is restored via

sexual reproduction when two gametes create an auxospore which matures into one diatom cell (Round *et al.* 1990; Lee 2008; Julius and Theriot 2010).
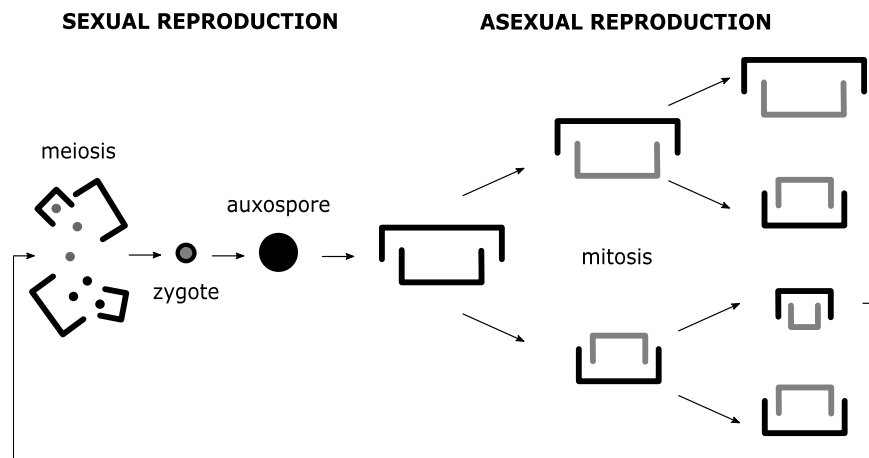
Fig. 2. Diatom cell cycle, the newly formed thecae shown in grey (modified according to Round et al. 1990 and Kale and Karthick 2015).

1.1.3 TAXONOMY

The diatom taxonomic classification is traditionally built on light microscopy observation of silica shell morphology. The development of scanning electron microscopy in the 1970s allowed for the study of the morphological structures more deeply and led to the description of a number of new species (Mann 1999). The key characteristics of diatom determination are: symmetry, valve shape, presence of a raphe slit, its form and type, striae pattern and density, and other features (Round *et al.* 1990; Mann 1999). Two main diatom groups can be distinguished: radially symmetrical centric diatoms and bilaterally symmetrical pennate. Diatoms can also be classified as pennate raphid and araphid (without a raphe slit) depending on the presence of the raphe (Round *et al.* 1990). Nevertheless, recent studies focused on molecular data revealed that the taxonomy is much more complicated, suggesting the existence of two clades, none of which clearly corresponds to the traditional centric and pennate categories (Medlin *et al.* 1996; Medlin and Kaczmarska 2004).

In the past, differences in valve morphology were used to define taxa without understanding how the differences are generated and evolutionarily developed. Thus, many mistakes were made because of poor knowledge of the diatom life cycle, population variation within species and phenotypic plasticity, which all seem to be much more significant than previously expected (Mann 1999, 2010). It has been suggested that taxonomists largely underestimated the number of diatom species, even between already-described organisms (Mann 1999; Mann and Vanormelingen 2013). With the introduction of molecular methods, along with ecological and/or sexual reproduction data, it has become increasingly obvious that many diatom morphospecies are species complexes (Sarno *et al.* 2005; Souffreau *et al.* 2013b; Pinseel *et al.* 2017a) and that a high-frequency of cryptic diversity occurs within diatom genera, which makes species-level determination based on morphological description very difficult (Vanormelingen

et al. 2007, 2013; Evans *et al.* 2009; Souffreau *et al.* 2013b; Pinseel *et al.* 2017b). Future revisions of diatom species taxonomy based on the multidisciplinary approach, which combines crossing experiments and genetic assessments with morphological analysis, is increasingly required.

1.1.4 DIATOM CULTURING AND MATERIAL PRESERVATION

The techniques of diatom isolation and cultivation are almost identical to those for other algae. Single or a small number of cells could be isolated from natural samples to establish cultures. Cultivation is possible in both agar gel or liquid medium, in well-plates or Petri dishes (Round et al. 1990). Silica rich media, such as Wright's cryptophyte (WC) medium (Guillard and Lorenzen 1972) for freshwater taxa and f/2 medium reduced by half from the original f medium (Guillard and Ryther 1962) for marine taxa, are often used.

To fix the material for observation of the frustule characteristics (Fig. 3), several methods of oxidation, resulting in removal of organic material, exist (Blanco *et al.* 2008). The protocols vary in e.g., incubation period, temperature, concentration of the oxidants – mostly nitric acid (HNO_3) or hydrogen peroxide (H_2O_2). The suspensions need to be repeatedly rinsed with distilled water (Blanco *et al.* 2008; Julius and Theriot 2010). A cleaned portion of the material should be dried before preserving for light or scanning electron microscope (SEM) observation. For light microscopy, embedding drops of dried and cleaned material in Naphrax or Meltmount is used. For SEM, cell suspensions are usually filtered through polycarbonate membrane filters and fixed on aluminium stubs after air-drying. The stubs are sputter-coated with gold or platinum layer. Ethanol-based preservatives are recommended when archiving diatoms for future molecular studies (Julius and Theriot 2010).

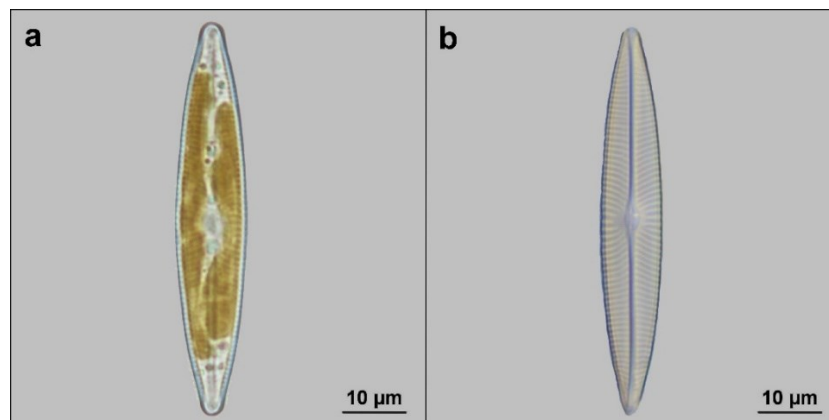
Fig. 3. *Navicula radiosa* Kützing (a) before and (b) after the oxidation process.

1.1.5 ECOLOGY AND BIOGEOGRAPHY

Diatoms can be found in almost all terrestrial, marine and freshwater environments (Round *et al.* 1990; Mann 1999; Vanormelingen *et al.* 2008). They inhabit permanent water bodies like lakes and rivers as well as temporary biotopes as pools, seepages, wetlands or moist soils

(Round *et al.* 1990). They live freely in the water column as plankton, or, as a significant part of the benthos, they create typical brownish scums and growths on various types of substrata including plants (Round *et al.* 1990; Lee 2008). In general, the centric diatoms occur predominantly in the marine environment, while the pennate are found roughly equally in freshwater and marine habitats (Lee 2008). In marine ecosystems, diatom populations are greater in cooler water (Lee 2008). Both single cells and colonies can be found in both environments (Round *et al.* 1990; Julius and Theriot 2010). Diatom occurrence is species specific and depends on environmental characteristics. Diatom species are found in a wide range of chemical and physical conditions (e.g., nutrient, acidity, salinity, temperature). The many combinations of these characteristics provide a large diversity of niches for the thousands of described diatom species (Round *et al.* 1990; Julius and Theriot 2010).

Diatom distribution is worldwide including the Arctic and Antarctic regions. According to the formulation of ubiquity hypothesis, the distribution of diatoms and many other microbial species of cell size less than 1 mm were believed to be cosmopolitan and affected by the prevailing ecological conditions, since their dispersal was not supposed to be limited by geographic boundaries (Finlay 2002; Vanormelingen *et al.* 2008). Nevertheless, later analyses of freshwater diatom dispersal revealed that isolation processes explain the patterns in regional genus richness more than environmental conditions, contradicting the unlimited dispersal of the ubiquity theory (Vyverman *et al.* 2007). This conclusion is supported by the high level of endemism reported in some isolated Antarctic regions (Cremer *et al.* 2004; Van de Vijver *et al.* 2005; Vanormelingen *et al.* 2008; Vyverman *et al.* 2010; Kopalová *et al.* 2015), which is estimated to be an incredible 44 % of all freshwater species (Verleyen *et al.* 2021). Moreover, recent findings suggest that diatom sensitivity to dispersion related stress factors is high (Souffreau *et al.* 2010, 2013a).

1.1.6 DIATOM APPLICATION

Due to the widespread diatom distribution, their specific demands, quick reflection of environmental changes and relatively easy determination, diatoms are ideal organisms for environmental conditions assessment. For biomonitoring in water management, several evaluation indexes of trophic levels or ecological state are applied (Sládeček 1986; Kelly and Whitton 1995; Rott *et al.* 1997, 1999; Kelly 1998). Diatom silica frustules are also well preserved and easily detected for long periods of time in sediments. Analyses of such communities are used in paleolimnological and paleoecological reconstructions of past environments (Dixit *et al.* 1992; Smol and Cumming 2000; Hodgson and Smol 2008). Diatoms provide valuable information to document the causes and implications of natural condition changes such as temperature, light availability, ice cover and various water characteristics (e.g., transparency, turbulency, flow rate, precipitation, evaporation). They also indicate chemical conditions, such as nutrient availability, acidity, salinity etc. (Birks *et al.* 1990; Round 1991; Dixit *et al.* 1992; Smol and Cumming 2000; Hodgson and Smol 2008). Paleolimnological diatom analyses became especially important for high latitude areas studies of global

environmental change whilst applicability of other techniques (dendroecology, palynology) is limited there (Douglas *et al.* 2004; Hodgson and Smol 2008; Douglas and Smol 2010; Spaulding *et al.* 2010).

Interestingly, diatom analyses are commonly used in forensic science, mainly in investigations of drowning cases (Pollanen 1998; Verma 2013; Rana and Manhas 2018). Diatom composition in the bone marrow of deceased bodies or on clothes, shoes or other items provides evidence of place of the act and therefore plays a significant role in the diagnosis of death in criminal investigations (Pollanen 1998; Krstic *et al.* 2002; Vinayak *et al.* 2013; Kaushik *et al.* 2017). Diatom fossils, which settled in freshwater and marine waters all over the world, form a sedimentary rock – diatomite. Its unique pore structure is important for practical applications in diverse industries: for example as an isolation, abrasive and filtration material, various forage, sorbents, or fillers (Harwood 2010).

1.2 SETTING THE SCENE: THE CHALLENGING ENVIRONMENT OF POLAR REGIONS

Polar regions are generally defined as areas surrounding the geographical poles (the North Pole or the South Pole) lying within the Arctic and Antarctic Circles. These are parallels of 66°33′ north and south latitudes. Such high latitude areas are defined by terms of solar radiation where there is at least one day when the Sun does not rise above the horizon in the winter or fall below in the summer, respectively (Thomas *et al.* 2008a). Beyond these geographical definitions, the boundaries of the polar and subpolar regions are delineated in various other ways depending on the field of scope by e.g., climatologists, ecologists, biologists, geographers, administrators, or politicians. Some simple definitions according to ecological and climatological aspects are: the 10 °C summer isotherm (the mean temperature of the warmest month corresponding to July and February respectively does not exceed 10 °C) and the position of the treeline, which usually coincides due to tree growth limits (Pienitz *et al.* 2004; Thomas *et al.* 2008a) (Fig. 4). Beneficially, the same criteria could be used for both hemispheres, which provides a solid basis for comparison (Pienitz *et al.* 2004). Another definition is the oceanographic boundary, which is, for example for Antarctica, defined by the convergence of oceanic waters, which roughly corresponds to the 10 °C February isotherm (Pienitz *et al.* 2004). On the other hand, the regions also differ in many aspects. Antarctica is a continent of 13.3 million km^2 almost completely covered by ice sheets enclosed by the Southern Ocean, which largely freezes over every year. In contrast, most of the Arctic includes the Arctic Ocean which is surrounded by islands and the northern-most parts of continents and is covered by pack ice that can persist for years. (Thomas *et al.* 2008a).

The geographic difference is of key importance with many consequences for biodiversity, energy balances, climatic conditions etc. (Elster and Benson 2004; Thomas *et al.* 2008a). The Arctic Ocean is connected to temperate oceans and the region has several land connections with other climate zones, while Antarctica is cut off from the rest of the world due to the barrier of

the ocean (Elster and Benson 2004; Thomas *et al.* 2008a). Biogeographically, four main zones are generally recognized in both polar areas: tundra, sub-polar desert, polar desert and cold desert. The distribution of these zones varies in the Arctic and Antarctica and is further categorized in detail on a geographical scale. Nevertheless, both polar regions are known as "frozen deserts at the ends of the Earth" for their extreme environment represented by temperatures below zero, lot of ice and snow, continuous daylight, and long periods without light, where hardly anything survives (Thomas *et al.* 2008a).
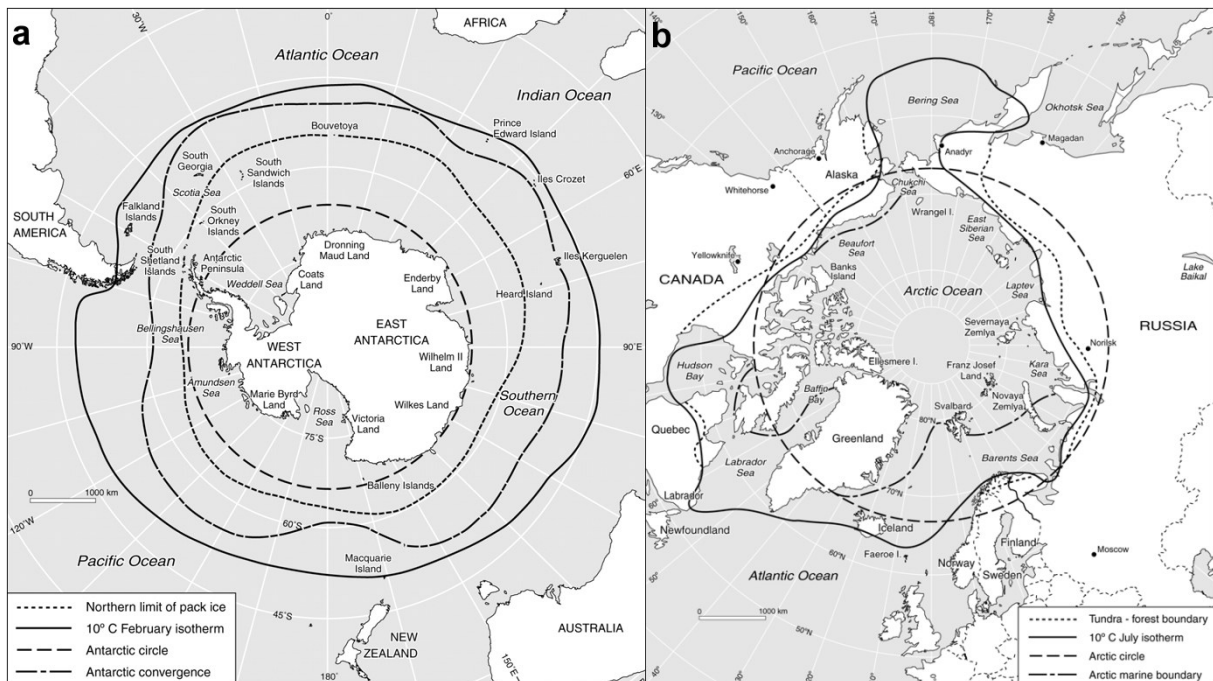
Fig. 4. Map of (a) Antarctica and (b) the Arctic (Pienitz *et al.* 2004).

1.2.1 THE KEY STRESS FACTORS

In polar habitats, there are several crucial factors that influence living organisms including microbial communities. Extended winter freezing, at times under thick snow or ice cover, and the unavailability of liquid water until the onset of spring are probably the key environmental stress factors affecting the structure and biomass of microbial assemblages, even though many others, such as seasonality, nutrient limitation, high irradiance and desiccation, also occur (Davey 1989; Hawes 1990; Davey *et al.* 1992; Elster and Benson 2004).

LOW TEMPERATURE AND FREEZING

In the Köppen-Geiger climate classification, the polar climate category reflects the 10 °C summer isotherm and globally represents 12.8 % of land mass. The prevailing climate types in the Arctic and Antarctica are, according to the mean temperature of the hottest month, defined as polar tundra, where the temperature does not drop below 0 °C, and the polar frost climate, where it equals zero or less (Peel *et al.* 2007). Winter freezing is suggested to be the major event causing significant mortality in microbial communities (Hawes 1990). However, a wide range

of temperatures appear according to the geographical location and the type of hydro-terrestrial environment.

For example, the winter air temperature measured in the study of a stream and ponds in the Maritime Antarctic fell to almost −30 and −40 °C, respectively, while the under-ice temperature in the water bodies was only −4 and −13 °C (Hawes 1989; Schmidt *et al.* 1991). Air and soil temperatures naturally vary more in comparison to aquatic environments, which are more buffered to temperature oscillations. In the summer days, air and/or soil temperatures may reach 15 °C or even more (Table 1). Overnight freezing is limited to the soil or vegetation surface and hardly ever falls deeper than a few degrees below zero (Davey *et al.* 1992; Elster and Benson 2004). During autumn, temperatures are usually more stable (around zero) and short-term freeze-thaw cycles frequently occur (Davey *et al.* 1992).

In contrast, most of the aquatic polar habitats experience water temperatures close to zero for much of the year. Shallow ice-free lakes can even heat to 10 °C or more in summer (Table 1) and their surface waters may reach 15 °C due to light absorption (Vincent *et al.* 2008). Temperature difference depends a lot on the character of the water bodies. There is a sharp contrast between shallow and deep lakes. For example, summer measurements on James Ross Island revealed many oscillations in the shallow coastal lake Lachman 1 while the conditions in the deep and frozen Rožmberk Lake nearby were stable (Nedbalová *et al.* 2013). Moreover, some of the lakes are known to be covered by thick perennial ice that does not melt or to have a liquid brine layer along the bottom overlaid by an ice layer (Vincent *et al.* 2008). These also contrast to streams and wetlands, which experience periodic freezing and desiccation each season (Hawes 1989).

Table 1. Temperature extremes measured at various sites in the Arctic and Antarctica.

Study (author, year)		Summer min	Summer max	Winter min	Winter max
Ross Island (Schmidt *et al.* 1991)	air	around −8 °C	5 °C	almost −40 °C	around −7 °C
Signy Island (Hawes 1989)	air	−3 °C	12 °C	less than −30 °C	0 °C
Vostok Station (Comiso 2000)	air		−30 °C	−79 °C	
Svalbard climate statistics (Førland *et al.* 1997)	air	0 °C	15 °C	−30 °C	−10 °C
North Pole Station (Rigor *et al.* 2000)	air	−2 °C	2 °C	−50 °C	−7 °C
Signy Island (Davey *et al.* 1992)	fellfield soils	−1 °C	16 °C	−8 °C	−2 °C
Signy Island (Davey and Rothery 1992)	fellfield soils	−1 °C	12 °C		
James Ross Island (Váczi *et al.* 2011; Váczi and Hájek 2013)	Dulánek Lake	around 0 °C	around 5 °C	around −13 °C	around −3 °C

James Ross Island (Váczi *et al.* 2011; Váczi and Hájek 2013)	Lachman Lake	around −2 °C	around 10 °C	−23 °C	around −5 °C
Ross Island (Schmidt *et al.* 1991)	ponds	around −2 °C	around 5 °C	−13 °C	around −5 °C
Signy Island (Hawes 1989)	stream			− 4 °C	
Svalbard (Tashyreva and Elster 2016)	stream and pond	2 °C	around 16 °C		
Kuparuk River basin, Alaska (Taras *et al.* 2002)	snow–ground interface			−24 °C	−5 °C

Temperature shifts below zero bring dramatic changes to cells. If the biological water is cooled below its equilibrium melting point, it becomes unstable and tends to the crystalline state – freezing, which is accompanied by ice formation and changes in water-soluble components. Ice crystals begin to form first in the extracellular environment from one or a small number of nuclei before it occurs inside the cells (Karlsson and Toner 1996; Thomas *et al.* 2008b). As ice crystals are formed, the rising osmotic gradient causes cell water loss. As a consequence, the cell volume is reduced although the content still remains unfrozen (Meryman 1974; Mazur 1984). The natural slow rate of cooling ensures gradual changes in osmotic equilibrium, while extreme rates could cause cell dehydration and perhaps internal freezing, which is almost always related to cell injuries or lethal damages. Several models of intracellular ice formation has been described but are still not completely clear (Toner *et al.* 1990; Muldrew and McGann 1994; Zhao *et al.* 2006; Acker 2015).

Cell damages during freezing occur for a number of different reasons: e.g. damages of membranes, mechanical deformation, imbalances in metabolism or dehydration via high osmotic activity (Mazur *et al.* 1972; Meryman 1974; Muldrew and McGann 1990; Karlsson and Toner 1996; Thomas *et al.* 2008b). Moreover, the process of thawing represents a further danger for the cells through the process of recrystallization representing fusion of small intracellular ice particles into larger crystals with potentially damaging effects (Mazur *et al.* 1972; Mazur 1984; Cañavate and Lubian 1997; Hájek *et al.* 2012).

DESICCATION

Drought and desiccation are other factors related to freezing which in many aspects result in a similar stress: increased osmotic pressure through the loss of water (Mazur 1984; Welsh 2000; Thomas *et al.* 2008b). Yet, they also represent temperature-independent stress factors. Low humidity (10 % and less) at 0 °C could cause a water-potential deficit of about 800 bars. In dry valleys of Antarctica, the relative humidity often falls due to down-valley blowing and little moisture-containing katabatic winds (Thomas *et al.* 2008b). Water unavailability is generally more limiting than low temperature with soil and other terrestrial biotas being potentially subject to extreme environmental stresses (Arnold *et al.* 2003; Thomas *et al.* 2008b). However, desiccation is not uncommon in many freshwater and temporarily flooded polar habitats where

liquid water availability changes during the year (McKnight *et al.* 1999; Elster 2002; Pichrtová *et al.* 2014a).

SNOW AND ICE COVER

Ice and snow are accumulated due to intensive winter freezing and precipitation, which plays an important role in the temperature and light regimes. Light transmittance is naturally reduced by increased snow depth. Even thin snow layers reflect a significant portion of the light signal with the amount of reduction depending on the snow structure and wavelength (Beaglehole *et al.* 1998; Perovich 2007). Despite a relatively transparent ice layer on perennially ice-covered lakes, the quantities of transmitted solar radiation could be severely attenuated by a massive snow cover and/or other specific characteristics such as wind-blown debris from the surroundings (Howard-Williams *et al.* 1998; Vincent *et al.* 1998; Thomas *et al.* 2008c). In contrast, the snow cover does not accumulate in some lakes as it is quickly blown off by wind (Henshaw and Laybourn-Parry 2002).

However, snow and/or ice cover could provide an effective thermal insulation for microbial populations from fluctuations and low air temperatures (Hawes 1989; Fahnestock *et al.* 1998; Morgner *et al.* 2010). Deep snow cover results in significantly higher winter soil temperatures and increases soil respiration (Elberling 2007; Morgner *et al.* 2010). Heterogeneity in snow depth and its spatial distribution leads to local variations in minimum temperatures (Davey *et al.* 1992; Elberling 2007) and early and deep snow cover sustains higher levels of microbial activity throughout winter compared to later developed snow cover (Fahnestock *et al.* 1998). The responses of soil respiration and plants to changes in snow regimes in the High Arctic are widely studied (Elberling 2007; Morgner *et al.* 2010; Preece and Phoenix 2014; Rumpf *et al.* 2014). Additionally extensive winter snow and ice accumulation permits high meltwater discharges in spring (Hawes 1989).

SOLAR RADIATION (LIGHT AND/OR DARK)

Due to their geographical location, polar regions around mid-summer, when there is Sun for most of the day, receive more solar radiation than areas on the equator. Despite this, the total year amounts of incident solar radiation delivered are reduced compared to lower latitudes, because the Sun disappears in winter and the angular height of the Sun is low (Thomas *et al.* 2008a; Vincent *et al.* 2008). Areas around the North Pole receive 43 % less light than at the equator (Thomas *et al.* 2008a). The process of photosynthesis is therefore strongly influenced: seasonality plays an essential role instead of the daily light and dark cycles in lower latitudes. It ends with the onset of winter darkness and resumes in spring when the sunlight returns, which limits total annual production (Vincent *et al.* 2008). Irradiance exposure is also affected by the presence of environmental factors possibly decreasing light penetration such as snow cover, ice layers, clouds or the water column (Karentz 1991; Kelley 1997).

The maximum solar irradiance in the Arctic and Antarctica reaches values around 1500 µmol photons m^{-2} s^{-1} (Davey 1991; Stibal *et al.* 2007). Exposure to high light is connected to many risks. The rate of photosynthesis may decrease due to the process of photoinhibition when light energy absorption exceeds the capacity of chemical reactions (Thomas *et al.* 2008b). For organisms performing oxygenic photosynthesis, this danger is represented, for example, by the production of potentially damaging reactive oxygen species which can be produced from accumulated molecular oxygen by the excess energy (Apel and Hirt 2004; Karsten *et al.* 2009). Biological injuries are mostly associated with ultraviolet (UV) radiation in the short wavelengths (200–400 nm). DNA is one of the targets for UV-induced damages in organisms ranging from microorganisms to humans, since UV is capable of damaging its structure via mutagenic and cytotoxic effects (Karentz 1991; Sinha and Häder 2002; Pfeifer *et al.* 2005; Holzinger and Lütz 2006). Other cell components and processes, such as proteins, the photosynthetic apparatus and reproduction could also be affected (Sinha *et al.* 1996; Holzinger and Lütz 2006; Thomas *et al.* 2008b; Karsten *et al.* 2009).

Weakening of the stratospheric ozone layer, which protects the Earth from biologically harmful short-wavelength solar radiation, is an on-going concern. Dramatic changes in the ozone thickness correspond to variations in solar UV radiation intensities (Thomas *et al.* 2008b). Ozone depletion was first noticed in Antarctica in the mid-1980s, but also detected in the Arctic since 1992 where it is more variable since the Arctic stratosphere is warmer and more mixed than that of Antarctica (Huntington *et al.* 2005). The presence of ozone holes represents a further environmental stress that probably modified the taxonomic structure of communities in polar areas (Karentz 1991).

NUTRIENT SUPPLY

Low nutrient supply represents another stress factor in polar environments, being important for biological production. Low temperature, freezing and desiccation inhibit microbial activity and other geo-chemical processes (e. g. soil weathering), which reduces nutrient release into the surface and groundwater (Vincent *et al.* 2008). Polar water bodies are globally known as being low-productive or oligotrophic, with the exception of areas where enrichment by the activity of seal or seabird colonies occurs (Hawes 1983; Hullar and Vestal 1989; Sheath *et al.* 1996; Vincent *et al.* 2008). Low delivery of nitrogen (N) and/or phosphorus (P) limits polar microorganisms from terrestrial (Davey and Rothery 1992; Arnold *et al.* 2003) and freshwater habitats, both from lakes (Levine and Whalen 2001; Brutemark *et al.* 2006; Symons *et al.* 2012; Hogan *et al.* 2014) and streams (Peterson *et al.* 1983; Hullar and Vestal 1989; Sheath *et al.* 1996). Furthermore, nutrients mixing in water by wind is inhibited in polar lakes due to the long ice cover period (Vincent *et al.* 2008).

Globally, primary production of freshwater, marine and terrestrial ecosystems is N+P co-limited in most cases (Elser *et al.* 2007). However, it is not easy to provide a clear judgement for polar environments. Field studies mostly focused on lake phytoplankton propose several

possible nutrient limitation categories: concurrent N+P limitations are mentioned as frequent (Levine and Whalen 2001; Symons *et al.* 2012; Hogan *et al.* 2014) together with N limitations (Hawes 1989; Levine and Whalen 2001; Symons *et al.* 2012; Nedbalová *et al.* 2013; Hogan *et al.* 2014) and less frequently P limitations are reported (Brutemark *et al.* 2006; Symons *et al.* 2012; Hogan *et al.* 2014). Interestingly, nitrogen variation was one of the environmental variables which best explained diatom species distributions in a study from the Canadian High Arctic (Lim *et al.* 2001). Moreover, 38 % of lakes and ponds included in a subarctic study from Canada were shown not to be limited by nitrogen or phosphorus (Symons *et al.* 2012). Secondary limitations by some micronutrients, such as iron, are also suggested (Levine and Whalen 2001).

In contrast, streams are either P limited and/or N+P co-limited rather than only N limited (Peterson *et al.* 1983; Sheath *et al.* 1996). Such studies must also consider year to year variability depending on drainage and discharge from the surroundings into the stream (Hullar and Vestal 1989). Evidences from fellfield soils suggest that the growth of terrestrial microalgal communities is nitrogen-limited (Davey and Rothery 1992; Arnold *et al.* 2003).

Variability in nutrient limitations of arctic lakes could also be associated with the fact that some have recently faced anthropogenically enhanced nutrient enrichment (e.g. warming-induced permafrost melting, atmospheric N deposition) resulting in a shift in nutrient limitation of oligotrophic lakes from N to P (Elser *et al.* 2009; Hogan *et al.* 2014). However, growth may also be controlled by other factors than nutrients and light (Howard-Williams and Vincent 1989; Symons *et al.* 2012).

1.2.2 CLIMATE CHANGE

In the twentieth century, a warming trend has been documented and polar areas captured the interest of scientists and the public in the context of global warming and changing climate conditions. A more rapid increase of the average air surface temperature than in the rest of the world was documented in the Arctic (certain parts averaged an increase of 5–7 °C over the past four decades), and it is likely that the temperature will rise dramatically in the future (Huntington *et al.* 2005; Meredith *et al.* 2019).

Reduction of snow and ice cover and a longer open-water season are very likely to change the thermal regimes of freshwater ecosystems (stratification, circulation in lakes), to increase primary production and affect biodiversity across most of the Arctic (Wrona *et al.* 2005). Permafrost thawing brings both positive and negative effects on freshwaters: e.g. increased nutrients, sediment deposition and lake drainage (Wrona *et al.* 2005), with likely changes to the carbon cycle (Wrona *et al.* 2005; McGuire *et al.* 2009; Semenchuk *et al.* 2016). The reduced number of days with temperatures below the freezing point would naturally influence the character of winter precipitation, with a higher proportion falling as rain instead of snow. Such rain-on-snow events lead to more shallow snow cover, an earlier spring thaw, destabilization of the snowpack and sub-snowpack temperature increase (Putkonen and Roe 2003; Rennert *et*

al. 2009; Cooper 2014). As the latent heat of rainwater warms the snow and soil layer, and freezes within the snowpack (Rennert *et al.* 2009), ice layers in the snowpack are created and ice encasement of vegetation and soil animals could occur, building a strong barrier with low thermal protection (Coulson *et al.* 2000; Bale and Hayward 2010; Cooper 2014; Preece and Phoenix 2014) and make the nutrient-rich surface difficult to penetrate for ungulates (Rennert *et al.* 2009; Cooper 2014).

Marine ecosystems would also be affected. Sea ice extent, its thickness and duration would be reduced (Notz 2009; Meredith *et al.* 2019), the perennial ice layer would become seasonal, and the amount of sunlight and sea surface temperature increase and thermal regimes modified. Altogether, these would bring significant consequences for primary productivity in the upper ocean (Wrona *et al.* 2005; Perovich and Polashenski 2012; Meredith *et al.* 2019).

1.3 MICROALGAE IN POLAR ENVIRONMENT: THEIR HABITATS AND SURVIVAL STRATEGIES WITH EMPHASIS ON DIATOMS

Polar microalgae undoubtedly significantly contribute to primary production and are an integral component of the food webs in high latitude regions. Their importance is also noted as a bioindicator of environmental conditions as they could promptly act in response to broad spectrum of environmental changes. Due to their great abundance, diversity, dispersal rates and short life cycles, they are used for reconstructions of past environments (Douglas *et al.* 2004). Groups of algal indicators are often applied in the paleolimnology of high latitude regions to reconstruct various parameters: not only physical factors like temperature, snow and ice layers, but also water chemistry as including pH, salinity and trophic levels (Birks *et al.* 1990; Hodgson and Smol 2008), which participate in processes related to climate change (Dixit *et al.* 1992; Douglas *et al.* 2004; Spaulding *et al.* 2010).

Algae and cyanobacteria both occur in most of the freshwater, terrestrial and marine habitats of high latitude regions, where at least some light is available. In various types of inland waters, microalgae compose benthic mats and planktonic communities (Thomas *et al.* 2008c). An extraordinary diversity of lake types is known from both the Arctic and Antarctica and range from small shallow ponds to large deep lakes varying in chemical and physical parameters. Remarkably, several lake types are known exclusively from polar areas (Thomas *et al.* 2008c; Vincent *et al.* 2008; Nedbalová *et al.* 2013). The Arctic has more rivers and streams in comparison to Antarctica, where rivers are quite rare (Thomas *et al.* 2008c). These are characterized by highly seasonal vegetation (predominantly algal) and maximum discharge in spring associated with snow-melt (Hawes 1989).

Terrestrial and inland waters, forming overlapping hydro-terrestrial ecosystems, could be roughly categorized as stable and unstable (Elster 2002; Elster and Benson 2004) based on temperature conditions and water availability. Stable habitats with relatively consistent conditions include frozen ground (permafrost), glacial ice, melting glacial surfaces, lakes and soil within subglacial systems, and temporary snowfields, which are stable for a particular

period of time (Elster and Benson 2004). Even such habitats are colonised by several genera of microalgae (Remias 2012; Hoham and Remias 2020). For example, the phenomenon of macroscopically visible communities of snow and glacial algae and their interesting life strategies has recently captured attention (Remias *et al.* 2010, 2018; Procházková *et al.* 2019), mostly because of their impact on the acceleration of glacier melting (Ganey *et al.* 2017; Stibal *et al.* 2017).

Unstable ecosystems are affected by periodic environmental changes from the diurnal to seasonal scales (e.g., desiccation-rehydration, freeze-thaw cycles). The terrestrial environment, where liquid water is accessible as air humidity or for a short period of time, is represented by biotic surface crust layers held together by algae, fungi, lichens, and mosses ("soil/cryptogamic crusts") and soil (Elster 2002; Elster and Benson 2004). An enhanced tolerance to environmental fluctuations is crucial for the survival of microalgae in such habitats (Fig. 5). Repeated freezing and low temperatures are much more harmful than a single freezing event (Davey 1991; Trumhová *et al.* 2019) thus desiccation is probably a major factor influencing the colonization of fellfield soils (Davey 1991). With the onset of winter, the temperatures fall far below 0 °C, the water level drops, and mats become desiccated and frozen (Davey *et al.* 1992). Interestingly, an annual Arctic field study showed that soil respiration occurs even at near-surface temperatures of incredible −12 °C (Elberling 2007).
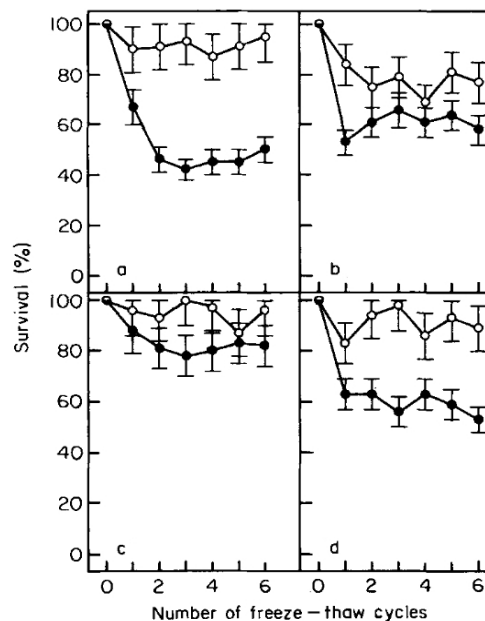
Fig. 5. Survival of repeated freeze-thaw cycles down to −3 °C (○) or −10 °C (●) by various polar terrestrial algae: (a) *Phormidium autumnale* (Agardh) Gomont, (b) *Zygnema* sp., (c) *Pinnularia borealis* var. *rectangularis* Carlsonand, (d) *Planktosphaerella terrestris* Reisigl (Davey 1991).

Hydro-terrestrial habitats, characterized by liquid water availability for almost the entire vegetative season, include for example: wetlands, damp soils, seepages, shallow lakes and

pools, glacial and snow-fed streams and their periodically flooded surroundings, moist rock walls, wet slopes and meadows (Elster 2002). Several field studies and laboratory experiments revealed that many polar cyanobacteria and algae from unstable environments are known to survive prolonged desiccation (Davey 1989, 1991; Hawes *et al.* 1992; Potts 1999; Šabacká and Elster 2006; Pichrtová *et al.* 2014b, a) and freezing (Davey 1989, 1991; Hawes 1990; Šabacká and Elster 2006; Elster *et al.* 2008; Pichrtová *et al.* 2016; Tashyreva and Elster 2016; Trumhová *et al.* 2019) with almost no harm. Besides this, tolerance seems to be also habitat dependent. Filamentous cyanobacteria originating from continental Antarctica showed a higher freezing resistance than similar types isolated from maritime Antarctica (Šabacká and Elster 2006). Another study, which focused on dehydration stress, demonstrated high mortality rates of a filamentous green alga *Prasiola crispa* (Lightf.) Menegh., which prefers a wet environment and low mortality of a cyanobacterium *Phormidium autumnale* Gomont from exposed fellfield soils (Davey 1989). There are also noticeable differences in stress survival between algal and cyanobacterial species, where stresses are usually less injurious for cyanobacteria (Davey 1989, 1991; Šabacká and Elster 2006).

Microalgae are naturally an integral part of marine ecosystems as well. The sea provides a relatively stable environment: no substantial barriers for migration and nutrients transport, and temperatures do not fall below the freezing point of full saline sea water (−1.9 °C) (Thomas *et al.* 2008d). Cyanobacteria and algae compose no less an important part of the phytoplankton of open oceans and as benthos they colonize surfaces and pools in the intertidal zone, shallow sublittoral, deep waters and under ice shelves (Thomas *et al.* 2008d; Wulff *et al.* 2009; Wang *et al.* 2018; Fredriksen *et al.* 2019). Diatoms, together with dinoflagellates, haptophytes and cryptophytes, dominate in the polar phytoplankton (Bursa 1963; Heimdal 1989; Mcminn and Hodgson 1993) and are one of the most significant microalgal groups producing organic carbon in the oceans. They also participate in the other biogeochemical nutrient cycles, like carbon, nitrogen, phosphorus, silicon and iron, and serve as a basis for marine food cycles (Sarthou *et al.* 2005; Armbrust 2009). As a dominant part of marine snow (rapidly sinking aggregates of organic detritus), they sink to the bottom and become a food source for deep-water organisms (Sarthou *et al.* 2005). An interesting group of marine microalgae that fascinates many scientists are sea ice algae. Their existence is bound to sufficient light conditions and the presence of sea ice, as they inhabit its lower side (Werner *et al.* 2007; Ewert and Deming 2013). Therefore, their occurrence is strongly seasonal; the blooms develop largely only in early spring and autumn (Marquardt *et al.* 2016; Wang *et al.* 2018).

Temperature is one of the essential environment factors determining algal growth and survival. Low temperatures generally result in reduced molecular motion. The Arrhenius equation describes the relationship between reaction rate and temperature, which can be similarly applied to enzyme-catalysed cell processes (Thomas *et al.* 2008b). Interestingly, as long as the liquid environment does not become solid even at temperatures below zero (which might be down to −40 °C due to the process of supercooling), biochemical processes can continue at lower, but

still acceptable, rates (Mazur 1984; Thomas *et al.* 2008b). Temperatures influence the life of microorganisms either directly (growth rate impact, enzyme activity, cell composition, nutrition), or by its effects on membranes, substance solubility, ion transport, diffusion, etc. (Beales 2004; D'Amico *et al.* 2006; Morgan-Kiss *et al.* 2006).

Corresponding to their temperature requirements, microorganisms can be classified as: psychrophiles, mesophiles, thermophiles or hyperthermophiles. Psychrophiles are able to grow well at temperatures around 0 °C. Although they thrive better at higher temperatures (optima ≤ 15 °C), they do not thrive at temperatures above 20 °C. These are mostly isolated from the Arctic and Antarctic environments (Morita 1975; Sigee 2004). Psychrophilic adaptations have been well studied in sea ice diatoms (Morgan-Kiss *et al.* 2006). Mesophiles grow in the range 15–45 °C, having their optima between 20 and 40 °C, while thermophiles succeed at temperatures above 55 °C, with optima between 55 and 65 °C. The hyperthermophiles thrive at temperatures above 90 °C, with growth optima between 80–113 °C, and include the bacteria growing in deep ocean vents (Sigee 2004). There may never be strict limits, because the definition of these terms is artificial and often overlap (Morita 1975; Thomas *et al.* 2008e). For example, cold adapted microorganisms tolerate temperature fluctuations and thus could be classified as psychrotrophs or psychrotolerant organisms (Morita 1975; Thomas *et al.* 2008b). Additionally, differences between separate lineages within the same species complexes are described in, for instance, *Pinnularia borealis* Ehrenberg and *Hantzschia amphioxys* (Ehrenberg) Grunow. Their Antarctic lineages have lower optimal growth temperatures and upper lethal temperatures than those from temperate regions (Souffreau *et al.* 2013b).

However, it is not only cold that microorganisms have to face in polar environments. There are many additional factors they have to overcome in order to colonize and survive, for instance a high level of solar radiation, low nutrient availability, high osmotic pressure and long periods of dehydration or dark (Thomas *et al.* 2008b; De Maayer *et al.* 2014). Therefore, microorganisms had to evolve many environmental, physiological and molecular adaptations (De Maayer *et al.* 2014). The introduction of molecular methods made it possible to make progress concerning metabolic adaptations, evolution and cell biology. Use of the "omics sciences" (genomics, transcriptomics, proteomics, or metabolomics) recently helped to identify, characterize, and quantify many new biomolecules and pathways involved in the cell structure, its function and dynamics in many microalgae including diatoms (Blanc *et al.* 2012; Lyon and Mock 2014; Mock *et al.* 2017; Raymond-Bouchard and Whyte 2017; Falciatore *et al.* 2020).

1.3.1 ANTIFREEZE STRATEGY AND OSMOREGULATION

The consequence of natural freezing and desiccation is intracellular water loss. Since they both result in osmotic stress, similar protective mechanisms are probably required (Mazur 1984; Welsh 2000; Tashyreva and Elster 2012). On the intracellular level microalgae produce a wide array of substances known as compatible solutes (mostly sugars, amino acid, polyols and their

derivates) (Table 2) that balance the osmotic difference between the inner cell and its surroundings (Welsh 2000; Deming and Young 2017). Significant increases of amino acids (predominantly proline, homarine or gamma-aminobutyric acid) were reported in relation to laboratory-induced cold and osmotic stress or with the onset of winter conditions in many microalgae including the Antarctic sea ice diatoms (Table 2). Sugars (sucrose, glucose, trehalose) and polyols (erythritol, glycerol, mannitol) were found to be accumulated by Antarctic soil microalgae as winter proceeded (Arnold et al., 2003) or as a result of cold acclimation that resulted in higher freezing survival (Nagao *et al.* 2008). Interestingly, benthic diatoms isolated from the North sea produced glycerol not only in cold (4 °C), but also at high temperatures (35 °C) (Scholz and Liebezeit 2013). Another substance often found in diatoms is glycine-betaine, which has a similar osmoprotective function as other compatible solutes including the response to low temperatures (Scholz and Liebezeit 2013; Dawson *et al.* 2020). The organosulfur compound dimethylsulfoniopropionate (DMSP) is known as a precursor of dimethyl sulphide (DMS), a gas believed to play an important role in global climate regulation (Charlson *et al.* 1987). Polar regions were found to have high DMS fluxes to the atmosphere (Matrai 1997; Savoca and Nevitt 2014). The major DMSP producers are marine microalgae from the Dinophyceae and Prymnesiophyceae (Keller *et al.* 1989), however other microalgae including diatoms were also found to produce DMSP (Keller *et al.* 1989; Kasamatsu *et al.* 2004; Sheehan and Petrou 2020). Several studies emphasized its cryoprotective and osmoregulation functions due to its increased production in response to low temperatures (Kirst *et al.* 1991; Scholz and Liebezeit 2013; Dawson *et al.* 2020) or higher salinities (Kirst *et al.* 1991; Lyon *et al.* 2016; Dawson *et al.* 2020; Wittek *et al.* 2020).

Various substances are also excreted as a response to freezing temperatures on the extracellular level (Table 2). These compounds were found to interact with the outer environment in order to make brine pockets (Fig. 6) within the ice habitable and/or avoid frost damages. Most of these compounds could be grouped as ice-binding proteins (IBP), antifreeze proteins (AFP), ice-active substances (IAS) or extracellular polymeric substances (EPS). Several functions that overlap range from ice recrystallization inhibition (Raymond and Fritsen 2001; Raymond and Knight 2003; Raymond *et al.* 2009; Bayer-Giraldi *et al.* 2011), maintaining liquid environments via changing the brine viscosity (Krembs *et al.* 2002), decreased freezing point (Bayer-Giraldi *et al.* 2011), ice-binding activity, its reduction and influence on the growth and morphology of single ice crystals (Raymond 2000; Raymond and Fritsen 2000; Gwak *et al.* 2010; Bayer-Giraldi *et al.* 2011). Secretion of such extracellular compounds could ensure that microalgae grow well and successfully divide even at temperatures below zero and/or higher salinities (Krembs *et al.* 2002, 2011; Bayer-Giraldi *et al.* 2010; Aslam *et al.* 2012). Microscopic analyses of pennate diatoms isolated in spacious brine pockets of an ice-core revealed their intact chloroplasts and indicated mucus in diatom-containing pores (Krembs *et al.* 2002). The responses are usually complex, and not limited to only one function or compound secretion: for instance the sea ice diatom *F. cylindrus* produced all types of EPS (soluble, insoluble and frustule-associated) when frozen and under high-salinity conditions (Aslam *et al.* 2018). Some

of these substances have been exclusively found in polar microalgae and diatoms. This restricted production implies its value for freeze protection and survival in cold and icy environments (Janech *et al.* 2006; Raymond and Morgan-Kiss 2013). IASs are associated with many, if not all, sea ice diatoms (Raymond 2000) and many other freshwater and terrestrial photosynthetic taxa from Antarctica such as cyanobacteria, eukaryotic algae and mosses (Raymond and Fritsen 2000). Moreover, the possibility of horizontal gene transport due to separate origins of IBPs in different microalgae was emphasized (Janech *et al.* 2006; Raymond *et al.* 2009; Raymond and Morgan-Kiss 2013). On the contrary, a broad distribution of AFPs was revealed in organisms apparently unrelated to cold environments (Bayer-Giraldi *et al.* 2010).
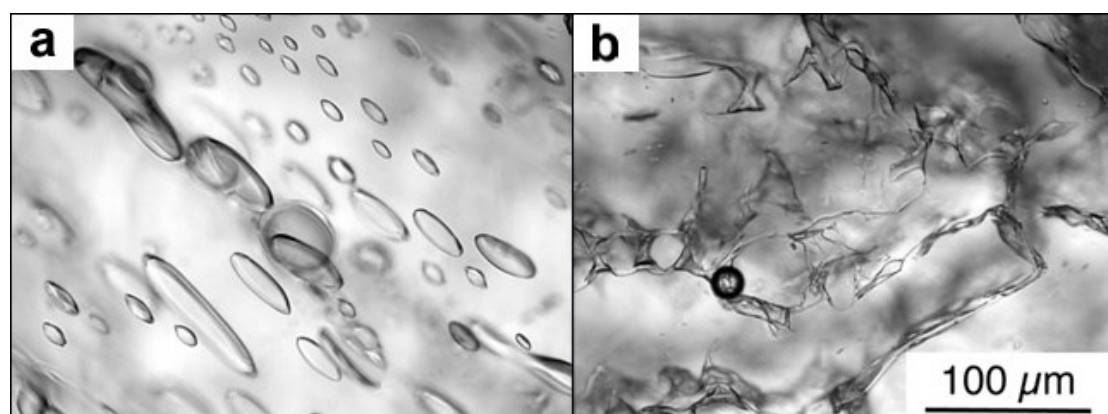
Fig. 6. Microphotographs of artificial sea ice texture at −10 °C (a) without EPS and (b) with added diatom EPS (Krembs *et al.* 2011).

Table 2. Overview of compounds produced by diatoms and some other microalgae and their activity.

Compound (author, year)	Activity suggested	Microorganism	Original habitat
erythritol, glycerol, glucose, sucrose, trehalose (Arnold *et al.* 2003)	cryoprotection	soil microalgae	fellfield soil, Antarctica
glucose, sucrose, amino acids (Nagao *et al.* 2008)	cryoprotection, osmoregulation	*Klebsormidium flaccidum* (Kützing) Silva, Mattox & Blackwell	soil, Japan
homarine, glycine-betaine, DMSP (Scholz and Liebezeit 2013)	cryoprotection, osmoregulation	*Achnanthes brevipes* C.A. Agardh, *Amphora arenaria* Donkin, *Cocconeis peltoides* Hustedt, *Navicula digitoradiata* (Gregory) Ralfs, *Navicula gregaria* Donkin	benthos, Wadden Sea, Netherlands
homarine, proline, ornithine, glutamic acid, DMSP, etc. (Dawson *et al.* 2020b)	cryoprotection, osmoregulation	*Fragilariopsis cylindrus* (Grunow ex Cleve) Helmcke & Krieger, *Navicula* cf. *perminuta*, *Nitzschia lecointei* Van Heurck	sea ice, Antarctica
proline (Krell *et al.* 2007)		*F. cylindrus*	sea ice, Antarctica
proline (Jackson and Seppelt 1995)	cryoprotection	*Prasiola crispa* (Lightfoot) Kützing	moist ground, Antarctica

Compound (Reference)	Function	Species	Location
DMSP (Lyon *et al.* 2016)	osmoregulation	*F. cylindrus*	Southern Ocean, Antarctica
DMSP (Wittek *et al.* 2020)	osmoregulation	*F. cylindrus*	sea-ice, Antarctica
DMSP (Sheehan and Petrou 2020)		diatoms, haptophytes	Southern Ocean, Antarctica
DMSP (Kasamatsu *et al.* 2004)		*Navicula* sp., *Nitzschia* sp.	surface water, Saroma Ko lagoon, Japan
DMSP (Keller *et al.* 1989)		dinophytes, primnesiophytes, chrysophytes prasinophytes, diatoms	various marine phytoplankton
AFP (Bayer-Giraldi *et al.* 2011)	recrystallization inhibition, ice-binding, ice-crystals growth modification	*F. cylindrus*	sea ice, Weddell Sea, Antarctica
AFP (Bayer-Giraldi *et al.* 2010)		*F. cylindrus, Fragilariopsis curta* (Van Heurck) Hustedt 1958	sea ice, Weddell Sea, Antarctica
AFP (Gwak *et al.* 2010)	ice-crystals growth modification	*Chaetoceros neogracile* VanLandingham	marine, Antarctica
EPS (Aslam *et al.* 2012)	cryoprotection	*Synedropsis* sp., *F. curta, F. cylindrus*	sea ice
EPS (Aslam *et al.* 2018)		*F. cylindrus*	Weddell Sea, Antarctica
EPS (Krembs *et al.* 2011)	alter the ice microstructure, desalination	*Melosira arctica* Dickie	sea ice, Chucki Sea, Arctic
EPS (Krembs *et al.* 2002)	maintenance of habitable pore spaces	population of sea ice pennate diatoms	sea ice, Chucki Sea, Arctic
EPS (Wolfstein and Stal 2002)	reserve products storage	population of benthic diatoms	benthos, Westerschelde, Netherlands
EPS (Smith and Underwood 1998)		*Cylindrotheca closterium* (Ehrenberg), *Navicula perminuta* (Grun.) Van Heurck, *Nitzschia sigma* (Kütz.) Grunow	epipelon, Alresford Creek, United Kingdom
EPS (Smith and Underwood 2000)		*C. closterium, N. perminuta, Nitzschia frustulum* (Kütz.) Grunow, *N. sigma, Surirella ovata* (Kütz.) Grunow	epipelon, Alresford Creek, United Kingdom
IAS (Raymond 2000)	ice-binding	sea ice diatoms	sea ice, McMurdo Sound, Antarctica
IAS (Raymond and Fritsen 2000)	ice-binding	various cyanobacteria, *Prasiola* sp.	freshwater, terrestrial, Antarctica
IAS (Raymond and Knight 2003)	recrystallization inhibition, cryoprotection	sea ice diatoms	sea ice, Antarctica
IBP (Raymond *et al.* 2009)	recrystallization inhibition, slow the drainage of brine from sea ice	*Chlamydomonas* cf. sp.	intertidal flats, Antarctica
IBP (Janech *et al.* 2006)		*Navicula glaciei* Van Heurck, *F. cylindrus, Thalassiosira pseudonana* Hasle & Heimdal 1970	sea ice, Cape Evans, Antarctica

1.3.2 MEMBRANE FLUIDITY MAINTENANCE

Low temperatures have been demonstrated to affect membrane lipid bilayers mostly by solidification and permeability loss (Los and Murata 2004; Bakermans 2017). Lipids are important components which sustain membrane structures. Their composition shifts can be found in relation to environmental condition changes and thus physiological status (Nichols *et al.* 1993; Mock and Kroon 2002a; Olofsson *et al.* 2012). Psychrophilic and psychrotrophic membrane adaptations include changes in lipid class composition, shifts of saturated to (poly)unsaturated fatty acids and reductions in lipid head group amounts and sizes (Moyer and Morita 2007; De Maayer *et al.* 2014; Lyon and Mock 2014).

Well documented lipid compounds associated with cold tolerance of microalgae are polyunsaturated fatty acids (PUFA) (Nichols *et al.* 1993; Teoh *et al.* 2013; Lyon and Mock 2014), which have been identified in both isolated cultures and natural communities of polar marine diatoms (Nichols *et al.* 1993; Henderson *et al.* 1998; Teoh *et al.* 2004). An Antarctic strain of *Chlamydomonas* sp. predominantly produced PUFA if compared to its temperate and tropical relatives and the Antarctic diatom *N. glaciei* increased its PUFA level as the temperature decreased (Teoh *et al.* 2013). Sugars were also found to have stabilization effects on phospholipid bilayers (Crowe *et al.* 1987), with natural or artificial membrane vesicles together with trehalose appearing to be the most effective (Welsh 2000). Sugar concentration generally increases with the onset of winter within individual cells of soil microalgae in Antarctica (Arnold *et al.* 2003). Moreover, optimal photosynthetic activity is related to membrane fluidity and its maintenance is essential for photosynthetic reactions at low temperatures. Sea ice diatoms have been described to increase the percentage of the fatty acids monogalactosyldiacylglycerols exclusively present in chloroplasts in order to support the fluidity of the thylakoid membrane (Mock and Kroon 2002b).

1.3.3 ENZYMATIC ACTIVITY

Enzymes are proteins with biocatalytic functions, being able to accelerate chemical reactions without modifying the equilibrium constant of the reaction. Their efficiency is temperature dependent and follows the Arrhenius law, so the reaction rates are greatly reduced at low temperatures (De Maayer *et al.* 2014; Collins and Gerday 2017). Therefore, the maintenance of appropriate rates of essential enzyme-catalysed metabolic processes is one of the challenges of life in cold environments. To compensate for the reduced rates of chemical reactions, psychrophilic organisms have the ability to genetically modify the molecular configurations of enzymes (Gerday *et al.* 1997; Thomas *et al.* 2008b; Feller 2013; Collins and Gerday 2017). These cold-active/cold-adapted enzymes are characterised by a higher specific activity at low temperatures (frequently up to an order higher) than those observed in their mesophilic counterparts (Loppes *et al.* 1996; Gerday *et al.* 1997; D'Amico *et al.* 2006; Peng *et al.* 2021) and are found even in marine Antarctic diatoms (Descolas-Gros and de Billy 1987). With the development and accessibility of molecular methods, variations in allele expressions were

revealed across various environmental conditions such as darkness, nutrient limitations and low or high temperatures (Mock *et al.* 2017; Peng *et al.* 2021). The high specific activity is often associated with heat sensitivity (Loppes *et al.* 1996; Gerday *et al.* 2000), which has been well documented, for example, the RuBisCO enzyme (ribulose-1,5-bisphosphate carboxylase/oxygenase) of two psychrophilic species of *Chloromonas*, despite their temperature optima being comparable to the mesophilic *Chlamydomonas reinhardtii* Dangeard (Devos *et al.* 1998). The structural conformations of some psychrophilic enzymes have been described as being highly similar to their mesophilic and thermophilic homologues (D'Amico *et al.* 2006; Collins and Gerday 2017).

Another type of adaptive mechanism counteracting the effect of low temperature is to increase production of the key enzyme to compensate for the reduced activity (Devos *et al.* 1998; Thomas *et al.* 2008b; Gleich *et al.* 2020). Furthermore, inhibition of denaturation and stabilization of cold sensitive enzymes by compatible solutes, including sugars, glycerol, glycine betaine, proline, EPS and DMSP, has also been suggested (Crowe *et al.* 1987; Welsh 2000; Bayer-Giraldi *et al.* 2011). Interestingly, enzymes of organisms thriving in low temperature environments are already successfully applied in biotechnology in fields requiring activity at mild temperatures, such as bioremediation, the food and textile industries and still have much more to offer (Gerday *et al.* 2000; Feller 2013).

1.3.4 LIGHT PROTECTION

Solar radiation is an essential resource for phototrophs, but, while high latitude regions are commonly known as light limited, summer irradiances can be extremely high. This represents another challenge for life of polar microorganisms due to the potentially damaging effects of, for instance, increased levels of UV radiation, high-energy photosynthetically active radiation or reactive oxygen species (ROS). These negatively affect biological molecules such as DNA and various proteins, lead to the destruction of the cellular structures of chloroplasts, mitochondria, nuclei, cytoplasm etc. and result in inhibition of biological processes including cell division and/or photoinhibition (Sakshaug and Slagstad 1991; Sinha and Häder 2002; Pfeifer *et al.* 2005; Holzinger and Lütz 2006; Karsten *et al.* 2009). For example polar marine diatoms exposed to UV-B were reported to have significantly decreased growth rates and maximum quantum yields of photosystem II indicating damages to the reaction centres (Nilawati *et al.* 1997; Underwood *et al.* 1999).

Therefore, a wide range of defence systems are exhibited by photoautotrophic organisms living in high irradiances. Interestingly, migration was a primary response of intertidal benthic biofilms dominated by diatoms to increasing light (Underwood *et al.* 1999; Perkins *et al.* 2010). This process is usually accompanied by secretion of EPS for movement (Wang *et al.* 2000; Wolfstein and Stal 2002; Bahulikar and Kroth 2007; Prins *et al.* 2020), however its production under different light conditions serves different functions: e.g. as a carbon source (Guihéneuf *et al.* 2008). The excretion of various products is related to photoadaptation including

antioxidant enzymes (e.g. superoxide dismutase, glutathione peroxidase, dehydroascorbate reductase) and other antioxidants (e.g. ascorbate, tocopherol, carotenoids) (Karsten *et al.* 2009). Phenolic compounds may also be involved in UV protection of microalgae (Pichrtová *et al.* 2013). Moreover, production of light shielding pigments also plays an important role in photoprotection (Holzinger and Lütz 2006; Karsten *et al.* 2009). Accumulated UV-sunscreen substances were documented in the upper layer of algal mats in high light environments, while elevated concentrations of chlorophyll a and phycocyanin were detected in the lower layer (Howard-Williams and Vincent 1989). Snow algae have become a quite widely studied group of algae for pigment and ultrastructure changes (Remias *et al.* 2010, 2018; Remias 2012; Procházková *et al.* 2019; Hoham and Remias 2020). However, even diatoms were found to have increased pigment concentrations in relation to light protection and photosynthetic efficiency (Underwood *et al.* 1999; Blommaert *et al.* 2017; Prins *et al.* 2020).

1.3.5 Dark survival and heterotrophic growth

Periods of dark are substantial characteristics of the Arctic and Antarctic polar nights. Microalgae were reported to have different strategies to overcome the prolonged darkness when they are unable to photosynthesize. Some algae produce dormant stages, others modify their metabolic rates, while still others use heterotrophy and/or accumulate energy in various storage products (Smayda and Mitchell-Innes 1974; Fryxell 1989; Zhang *et al.* 1998; McKnight *et al.* 2000). It was also shown that dark survival varies between species and could be temperature dependent (Smayda and Mitchell-Innes 1974; Durbin 1978). Darkness naturally increases diatom cell mortality (Agustí *et al.* 2020). Still, small percentages of temperate marine species survived more than three months of dark (Smayda and Mitchell-Innes 1974; Peters 1996; Veuger and van Oevelen 2011), while polar species were able to survive the same or longer period more readily (Peters and Thomas 1996; Zhang *et al.* 1998; Karsten *et al.* 2019) showing persistent activity and/or the ability to quickly react to increased levels of light (Zhang *et al.* 1998; Wilhelm *et al.* 2006).

Various microalgae sampled in autumn and/or during winter were observed to contain large amounts of energy-rich reserve compounds such as starch or lipids (Fryxell 1989; McKnight *et al.* 2000). In response to dark, diatoms were described to synthetize triacylglycerols (Mock and Kroon 2002a; Jiang and Gao 2004; Mekhalfi *et al.* 2014; Schaub *et al.* 2017), EPS (Smith and Underwood 1998, 2000; Wolfstein and Stal 2002) and PUFAs, often eicosapentaenoic acid (Jiang and Gao 2004; Guihéneuf *et al.* 2008; Pahl *et al.* 2010; Mekhalfi *et al.* 2014; Sayanova *et al.* 2017). Accumulation of such storage products occurs also due to other changes in environmental conditions indicating this to be a general stress response (Smith and Underwood 1998, 2000; Siaut *et al.* 2011; Mekhalfi *et al.* 2014; Sayanova *et al.* 2017). Moreover, even low levels of light lead to production of fatty acids and desaturation of chloroplast membrane lipids to keep its fluidity and thus the velocity of electron flow (Mock and Kroon 2002b).

Use of an external carbon source under dark conditions for heterotrophic growth is no less an important process in the dynamics of phytoplankton under ice covered lakes and seas (Zhang *et al.* 1998; McKnight *et al.* 2000). Various microalgae appeared to metabolize numerous products (Perez-Garcia *et al.* 2011; Morales-Sánchez *et al.* 2015). The results of many studies provide evidence for the ability of diatoms to grow heterotrophically with glucose as the only carbon source and/or to enhance primary production (Lewin 1953; Saks 1983; Tuchman *et al.* 2006). A wider scale of substrates is utilized under dark conditions in comparison to light (Fig. 7) indicating that the transport systems for these molecules may be light activated (Tuchman *et al.* 2006). Interestingly heterotrophic growth in diatoms was suggested to differ from green algae due to it being linked with their ability to maintain photosynthesis under dark conditions using chloro-respiration as a protection from photo injuries after light returns (Wilhelm *et al.* 2006). As higher temperatures in polar regions are expected, it is likely that the requirements for light energy will increase through higher respiration rates while the annual distribution of light and dark will remain unchanged (McMinn and Martin 2013; Schaub *et al.* 2017).
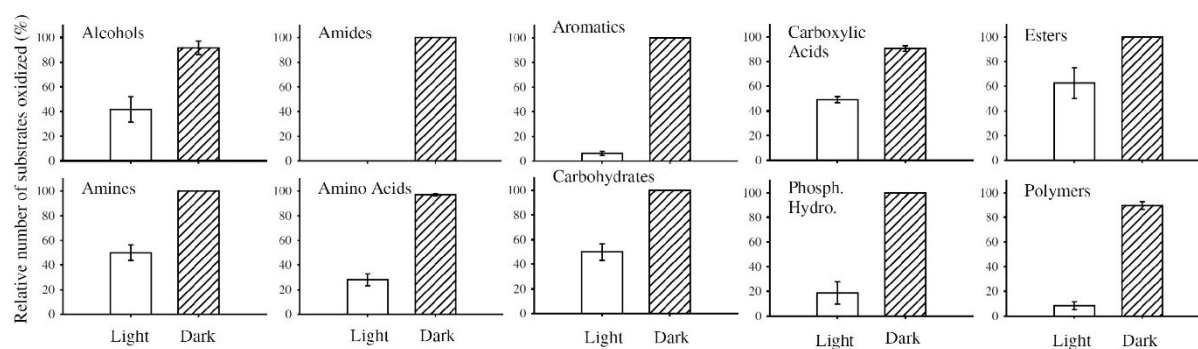
Fig. 7. The number of substrates from various chemical families oxidized by eight diatoms grown in the light and dark measured over 12 days (Tuchman *et al.* 2006).

1.3.6 MIGRATION

Migration is also known as a response to various stresses. Intertidal diatoms are well-known to react to rising light by vertical migration into the sediment (Round and Palmer 1966; Paterson 1986; Underwood *et al.* 1999; Consalvey *et al.* 2004; Perkins *et al.* 2010; Blommaert *et al.* 2017; Prins *et al.* 2020). Tidal activity and photoperiod were suggested to be the main factors affecting their diurnal migration strategy (Happey-Wood and Jones 1988; Haro *et al.* 2019). Diatoms were found to react similarly to changes of many other stimuli: desiccation (Evans 1959; Perkins *et al.* 2010), temperature (Round and Palmer 1966; Consalvey *et al.* 2004), and biological stresses such as grazing (Saburova and Polikarpov 2003; Consalvey *et al.* 2004), nutrient, and salinity changes (Consalvey *et al.* 2004) induce diatom motility as well. Nevertheless, the use of migration strategy related to freezing stress avoidance has not been proven yet. Furthermore, variations in diatom migration may be species-specific (Paterson 1986; Underwood *et al.* 2005; Du *et al.* 2012). Both vertical and horizontal migration has been

described in diatoms (Hay *et al.* 1993; Consalvey *et al.* 2004). A study of locomotion speeds found a higher rate on horizontal surfaces in comparison to vertical movement through the substrata (Hay *et al.* 1993). The maximum vertical depth recorded for diatoms is 8 cm in coarse sands (Saburova and Polikarpov 2003). Diatom movement is usually ensured by the secretion of various EPS components (Smith and Underwood 1998; Wang *et al.* 2000; Wolfstein and Stal 2002; Bahulikar and Kroth 2007; Prins *et al.* 2020).

1.3.7 SPECIALIZED CELL STAGES

A widespread strategy to survive periods of adverse environmental conditions among different groups of algae and other protists is the formation of specialized stress-tolerant cells as a part of their life cycle (McKnight *et al.* 2000; Agrawal 2009; Ellegaard and Ribeiro 2018). Dormant stages exhibiting long-term viability are characterized by a reduced metabolic activity and presence of thick cell walls, and vesicles containing accumulated starch, lipids or other materials such as pigments (Jewson *et al.* 2010; Ellegaard and Ribeiro 2018). The importance of dormant stages in relation to polar and/or winter freezing (often together with desiccation) has been shown for many cyanobacteria (Sutherland *et al.* 1979; Tashyreva and Elster 2012) and microalgae such as *Zygnema* sp. (Pichrtová *et al.* 2014a; Trumhová *et al.* 2019), *Tribonema bombycinum* Derbes et Solier (Nagao *et al.* 1999) and snow algae, for which interestingly some were previously identified as other algae (Remias *et al.* 2010, 2018; Remias 2012; Hoham and Remias 2020).

Diatoms are known to form two types of dormant cells: morphologically discernible resting spores and physiological resting cells morphologically identical to the vegetative cells (Round *et al.* 1990; Kuwata *et al.* 1993; McQuoid and Hobson 1996; Lee 2008). A common strategy to overcome periods of nonoptimal environmental conditions in centric diatoms, both marine (Kuwata *et al.* 1993; Sugie and Kuma 2008; Pelusi *et al.* 2020) and freshwater (Edlund *et al.* 1996; Jewson *et al.* 2008, 2010; Jewson and Granin 2015), is the formation of resting spores, characterised by a different morphology (rounder shape, thicker cell wall and different pattern) and the changes described above (Kuwata *et al.* 1993; McQuoid and Hobson 1995, 1996; Jewson *et al.* 2010). However, the formation of resting spores in pennate diatoms have thus far been observed only rarely and almost always in association to internal valve formation (Schmid 1979; von Stosch and Fecher 1979).

Physiological resting cells (as opposed to resting spores) are morphologically identical to vegetative cells (Anderson 1975; Sicko-Goad 1986; Sicko-Goad *et al.* 1986; Kuwata *et al.* 1993; McQuoid and Hobson 1996), yet the general characteristics of the dormant stages are similar to those of true resting spores: low consumption of energy reserves, low photosynthetic capacities, and different structure of cellular components (dense and dark cytoplasmic mass, rounder plastids, condensed organelles, contracted chloroplasts, larger vesicles of storage products, enlarged vacuoles and oil droplets) (Sicko-Goad 1986; Sicko-Goad *et al.* 1986; Round *et al.* 1990; Kuwata *et al.* 1993; McQuoid and Hobson 1996; Jewson *et al.* 2010; Morin *et al.*

2019). Such vegetative-looking resting cells are known from both pennate (Sicko-Goad *et al.* 1989; Souffreau *et al.* 2013a; Morin *et al.* 2019) and centric diatoms (Sicko-Goad *et al.* 1989; Kuwata *et al.* 1993; Jewson *et al.* 2008). Upon the return of optimal conditions, the transformation to a metabolically active state occurs quickly (Sicko-Goad 1986; Morin *et al.* 2019). Modifications of the vegetative stage are not unusual in freshwater algae to survive considerable periods of drought (Evans 1959) and resting cell formation is beneficial for surviving freezing and desiccation stress in terrestrial diatoms (Evans 1959; Souffreau *et al.* 2013a).

The formation of both resting structures is often influenced by very similar cues: age of cells, pH (von Stosch and Fecher 1979), photoperiod (von Stosch and Fecher 1979; Jewson *et al.* 2010; Jewson and Granin 2015), temperature (Durbin 1978), and nutrient limitations such as iron, nitrogen, phosphorus or silica (Durbin 1978; von Stosch and Fecher 1979; Kuwata *et al.* 1993; Sugie and Kuma 2008; Jewson *et al.* 2010; Jewson and Granin 2015; Pelusi *et al.* 2020). Resting stages are able to persist for long periods of time in dark and anaerobic conditions (up to several years and perhaps decades) (Lund 1954; Sicko-Goad *et al.* 1986; Härnström *et al.* 2011; Pelusi *et al.* 2020) and provide an inoculum for a future recolonization similarly as 'seed banks' in higher plants (Poulíčková *et al.* 2008; Julius and Theriot 2010).

1.3.8 VEGETATIVE CELL SURVIVAL

However, survival of environmentally-related stress in a vegetative state without the production of any specialized cells is not uncommon across various microalgae and cyanobacteria (Sheath et al. 1996; Agrawal 2009). A study of overwintering strategies of the Antarctic phytoplankton from a perennially ice-covered lake found a persistence of vegetative cell populations within the summer photic zone during the months of darkness as one of the main overwintering strategies, together with the formation of resting stages and in situ growth. Typical vegetative cells were described as the most abundant forms for all the species found in the water column (McKnight et al. 2000). Vegetative cell survival appears to be a characteristic also for polar hydro-terrestrial cyanobacteria (Hawes et al. 1992; Sheath et al. 1996; Tashyreva and Elster 2016) and algae (Hawes 1990; Sheath et al. 1996; Elster et al. 2008; Jimel et al. 2021) to withstand prolonged drought, freezing and/or dark. For example, up to 80 % vegetative cells of *Klebsormidium* sp. were viable after a −40 °C freezing treatment and no significant differences between the tested strains which originated from various habitats in the Arctic, Antarctica and Central Europe (Elster et al. 2008). In Antarctic marine diatoms, prolonged darkness also did not induce resting spore formation with all the studied species surviving most presumably in regular vegetative stages (Peters and Thomas 1996). Moreover, in the freshwater *Aulacoseira baicalensis* (K. Meyer) Simonsen, in situ growth was observed during winter (Bondarenko et al. 2006). Likewise, some terrestrial diatoms from temperate habitats were shown to survive adverse conditions as vegetative cells (Souffreau et al. 2013a).

1.3.9 ACCLIMATION AND ACCLIMATIZATION

In contrast to genotypic adaptations, interactions of inherited individual constitutions and environment should be also considered in relation to survival in polar areas. The importance of a field response, termed acclimatization, to autumn subzero temperatures was found for Antarctic fellfield microorganisms, which enhanced their cold-hardiness before the onset of winter freezing temperatures (Davey *et al.* 1992). Besides laboratory-induced cold acclimation, increased freezing tolerance of *T. bombycinum and K. flaccidum* (Nagao *et al.* 1999, 2008) and resting cells of pennate diatoms induced by cold, dark and nitrogen limitation resulted in higher survival to both freezing and desiccation stress (Souffreau *et al.* 2013a). Diatom freezing resistance also appeared to be enhanced by dehydration (Hostetter and Hoshaw 1970). Similarly, natural slow desiccation or nutrient starvation increased the stress tolerance of *Zygnema* sp. (Pichrtová *et al.* 2014a, b; Trumhová *et al.* 2019). In spite of the limited data about pennate diatom cold-acclimation related to freezing, the ability to rapidly modify photosynthetic competence under less favourable light and temperature conditions has been observed several times (Rochet *et al.* 1985; Mock and Valentin 2004; Mock and Hoch 2005; Yoshida *et al.* 2020).

Physiological changes of the protoplast, such as lipid content increase (Sánchez-Saavedra 2006), chloroplast enlargement, vacuole size reduction, number of starch grains and cytoplasmic volume increase, are often described as a result of cold acclimatization/acclimation processes (Nagao *et al.* 2008). Accumulation of callose in the corners of cell walls due to cellular water loss was reported as a self-protective mechanism against mechanical damage during desiccation (Herburger and Holzinger 2015). Its importance for frost survival should also be considered.

2 Publications

2.1 Research objectives

The aim of the doctoral thesis was to achieve a deeper insight into the survival strategy of freshwater pennate diatoms in the harsh conditions of the polar environments. The work could be basically subdivided into two main parts: experimental testing under laboratory conditions and the field study in the High Arctic (Svalbard).

Experimental testing of diatom tolerance limits to freezing related temperature stress:

- Assessment of the freezing survival of polar and temperate strains using different freezing regimes.
- Induction of stress resistant dormant stages by simulation of changes in their natural environment by temperature, light and nutrient availability manipulation.
- Comparison of freezing tolerance between laboratory induced dormant stages and vegetative cells.
- Introduction of a multiparameter fluorescent staining protocol for diatom viability evaluation, which enables a more precise assessment of physiological activity on a single cell level.

Field study of the annual cycle of diatom communities in the polar environments:

- Study the seasonal development of natural diatom populations in Svalbard with a focus on their morphology and physiological activity using the multiparameter fluorescent staining protocol.
- Test the potential effect of repeated freeze-thaw cycles, depth of ice and snow cover on freezing survival.

2.2 LIST OF PUBLICATIONS

This doctoral thesis is based on the following publications:

I.   **Hejduková E**, Pinseel E, Vanormelingen P, Nedbalová L, Elster J, Vyverman W, Sabbe K (2019). Tolerance of pennate diatoms (Bacillariophyceae) to experimental freezing: comparison of polar and temperate strains. *Phycologia* 58:382–392. https://doi.org/10.1080/00318884.2019.1591835

Journal five-year Impact Factor: 2.113

II.   **Hejduková E**, Nedbalová L (online first 2021). Experimental freezing of freshwater pennate diatoms from polar habitats. *Protoplasma*:1–17. https://doi.org/10.1007/s00709-021-01648-8

Journal five-year Impact Factor: 2.772

III. **Hejduková E**, Elster J, Nedbalová L (2020). Annual cycle of freshwater diatoms in the High Arctic revealed by multiparameter fluorescent staining. *Microbial Ecology* 80:559–572. https://doi.org/10.1007/s00248-020-01521-w

Journal five-year Impact Factor: 3.863

IV.   Pichrtová M, **Hejduková E**, Nedbalová L, Elster J (2020). How to survive winter? Adaptation and acclimation strategies of eukaryotic algae in polar terrestrial ecosystems. In: di Prisco G, Edwards HG, Elster J, Huiskes AH (eds) *Life in Extreme Environments: Insights in Biological Capability*. Cambridge University Press, Cambridge, pp 101–125

For the original publications see the appendices.

2.3 Author's contribution

Publication I

Eva Hejduková, Pieter Vanormelingen and Linda Nedbalová designed the study. Eva Hejduková carried out the laboratory experiments, performed microscopy analyses, evaluated the data and wrote the manuscript. Eveline Pinseel contributed to the statistical analyses and helped with manuscript writing. Linda Nedbalová helped with data analyses and writing of the manuscript. Wim Vyverman and Koen Sabbe participated in final improvements of the paper draft.

Publication II

Eva Hejduková designed the study, prepared the experimental cultures, conducted the laboratory experiments, performed microscopy analyses, evaluated the data and wrote the manuscript. Linda Nedbalová was responsible for the statistical analyses and provided comments on the manuscript.

Publication III

All the authors contributed equally on the study design. Eva Hejduková collected the field samples, performed the laboratory and microscopy analyses, evaluated the data and wrote the manuscript. Linda Nedbalová participated in data evaluation and improvement of the manuscript text. Josef Elster helped in the field and commented on the final paper draft.

Publication IV

Martina Pichrtová wrote the general introduction, environmental settings and sections related to life strategies and adaptation mechanisms of the Zygnematophyceae group. Eva Hejduková was responsible for writing the parts linked to diatoms and their life strategies and Linda Nedbalová contributed on snow algae and the introductory paragraphs. Josef Elster wrote the first draft of the whole chapter and he was responsible for the manuscript revision.

I declare the participation of Eva Hejduková in completing the research and writing the papers, as described above.

doc. RNDr. Linda Nedbalová, Ph.D.

3 SUMMARY AND CONCLUSIONS

This doctoral thesis provided new insights into freshwater pennate diatoms freezing tolerance and their survival strategy in relation to polar environments. The data of diatom freezing survival were acquired by experimental testing conducted under laboratory conditions (**Publication I, II)**, and by a field study performed in Svalbard (High Arctic) (**Publication III)**. Furthermore, the stress factors related to polar habitats, microalgal adaptation mechanisms, their life strategies and other issues were summarized in a book chapter (**Publication IV**).

Temperate diatoms previously appeared to be sensitive to several stresses such as desiccation, heat and freezing. Regarding freezing stress, the only surviving strains were those belonging to terrestrial or soil-inhabiting genera and several of them showed quite a high resistance (Souffreau *et al.* 2010, 2013a). Our study comparing $-20\,°C$ freezing survival between temperate and polar diatom strains found a slightly higher tolerance in the polar diatoms. Tolerance was revealed not only in strains originating from terrestrial habitats, but even among freshwater species (**Publication I**). Interestingly, shifts in growth optima and lethal maxima, in contrast to most lineages from more temperate regions, were already observed in Antarctic lineages of two diatom strains, indicating their potential niche differentiation (Souffreau *et al.* 2013b). All the diatoms tested appeared to be capable of surviving mild $-4\,°C$ freezing without any harm. In contrast, survival to extreme freezing ($-40\,°C$ and liquid nitrogen) was shown only in strains belonging to the *Pinnularia borealis* species complex, which is generally regarded as terrestrial (Ettl and Gärtner 2013; Zidarova *et al.* 2016), despite the fact that some of them originated from freshwater habitats and both temperate and polar environments (**Publication I**). Our findings also support the available data on diatom cryopreservation, which appeared to be highly challenging. In contrast to many other microalgae, the success in diatoms largely depends on the choice of cryoprotectant and its concentration, ecology of the species and furthermore seems to be related to individual ecophysiology and is thus highly species-specific (Stock et al. 2018; Hejduková, unpublished data). Additionally, intraspecific variation in stress resistance among temperate diatom strains belonging to the same lineages was previously suggested (Souffreau *et al.* 2013a). Moreover, the results of laboratory studies found that freezing conditions in natural habitats are not necessarily that severe, which is supported by temperature data taken during the field study (**Publication III**).

Formation of vegetative-looking resting cells in pennate diatoms was shown under laboratory conditions when incubated at cool temperatures, under light and nutrient limitations (**Publication I** and **II**). Such resting cells appeared to positively influence the survival of polar diatoms after exposure to freezing in comparison to resting cells induced by just dark and cool, or with a concurrent phosphorus limitation (**Publication II**). Furthermore, the importance for survival was proven for polar and temperate diatoms under mild below-zero temperatures (**Publication I**). No morphologically distinct diatom resting stages were observed (**Publication I, II** and **III**). However, even vegetative cells of diatoms tolerated various freezing

stresses relatively well (**Publication I** and **II**). Vegetative cells of the polar strains were remarkably viable in contrast to formerly tested temperate diatoms, which only three out of 17 morphospecies were able to survive as vegetative cells (Souffreau *et al.* 2013a).

Additionally, it was shown that experimental conditions, namely rates of thawing and freezing, significantly influenced the final survival, while the thawing rate appeared to be of approximately the same or even greater importance as freezing (**Publication I** and **II)**. This supports several previous findings about the effect of the rate of temperature change on microalgae freezing survival (Cañavate and Lubian 1995, 1997; Hájek *et al.* 2012).

The physiological activity detected using multiparameter fluorescence staining allowed for distinguishing the physiological status of diatoms at the single-cell level. It was revealed that, immediately after thawing, only a minority of the cells of surviving diatoms were metabolically active in both the laboratory experiments (**Publication II**) and the field study (**Publication III**). The cells were mostly found as inactive and intact (presumably dormant cells), injured and inactive, or dead.

The fluorescent staining methodology applied on freshwater communities five times throughout the one-year period following key events for algal survival (summer vegetative season, autumn dry-freezing, winter frozen state, spring thawing, and summer again) provided the first detailed evidence of diatom annual development in the extreme conditions of the High Arctic. Understandably, the physiological activity of natural diatom communities reflects the period of the year. The highest level of active cells appeared in their vegetative season in summer while winter freezing significantly increased the population mortality (**Publication III**). A remarkable portion of inactive dormant cells was examined during periods of autumn dry-freezing and after spring thawing. A surprising number of non-active dormant cells appeared after thawing of winter samples as well. However, the thicker the ice layer was, the more dead cells were present. The assumption of the harmful effect of autumn freeze-thaw cycles on spring survival was not confirmed (**Publication III**). To our knowledge, this is the first field study focused on freshwater diatom survival in their natural environment of the polar regions.

Diatoms originating from freshwater polar environments are sensitive to freezing stress as both vegetative and vegetative-looking resting cells (**Publication I and II**). Such stress sensitivity could have an impact on diatom dispersal abilities and could be linked to the occurrence of many endemic species in Antarctica (Cremer *et al.* 2004; Van de Vijver *et al.* 2005; Vanormelingen *et al.* 2008; Vyverman *et al.* 2010; Kopalová *et al.* 2015; Zidarova *et al.* 2016). Diatom success in polar environments seems to be associated with the ability to withstand unfavourable conditions as small numbers of vegetative cells or adapted vegetative-looking resting cells. These overcome the winter periods and provide an inoculum for establishment of new communities each vegetative season (**Publication III**). The lower resistance of algal mats to freeze-thaw cycles was previously suggested to be a predetermination to their annual character (Davey 1989; Hawes *et al.* 1992). Studies focused on diatom survival, their

ecophysiology and development, could help to reveal other possible roles in ecosystems and to better understand the polar environment. Many aspects of life in such extreme environments still await further investigation. Future studies should focus on the molecular mechanisms underlying diatom stress tolerance and acclimation. Further interest in resting cell induction could be beneficial since its role in the diatom life cycle is still not fully understood.

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APPENDICES – ORIGINAL PUBLICATIONS

Publication I:

Tolerance of pennate diatoms (Bacillariophyceae) to experimental freezing: comparison of polar and temperate strains

PUBLICATION II:

EXPERIMENTAL FREEZING OF FRESHWATER PENNATE DIATOMS FROM POLAR HABITATS

PUBLICATION III:

ANNUAL CYCLE OF FRESHWATER DIATOMS IN THE HIGH ARCTIC REVEALED BY MULTIPARAMETER FLUORESCENT STAINING

PUBLICATION IV:

HOW TO SURVIVE WINTER?
ADAPTATION AND ACCLIMATION STRATEGIES OF EUKARYOTIC
ALGAE IN POLAR TERRESTRIAL ECOSYSTEMS