Chapter 14
Polar Microalgae: Functional Genomics, Physiology, and the Environment

Amanda Hopes, David N. Thomas, and Thomas Mock

Abstract Microalgae underpin most foodwebs in polar regions as terrestrial primary production is too limited to support these complex and productive ecosystems. The success of microalgae in these extreme and highly variable ecosystems is rooted in their evolution and adaptation. The recent application of omics approaches in addition to biochemical and physiological measurements enabled a step change in our understanding of how these important organisms are adapted to their environment and how they have evolved from non-polar anchestors. This chapter is focused on diatoms and green algae as both groups of microalgae are most prevalent in polar regions. First genomes, transcriptomes, and reverse genetic tools have recently become available for representative species from both groups. They serve as important platforms to advance studies on their ecology, evolution, and adaptation. We highlight some of the key findings from these studies and link them with biochemical and physiological data to give insights into how genes and their products have shaped important microalgae in their diverse polar environments such as oceans, sea ice, permanently frozen lakes, snow and glaciers. Data from these studies will pave the way for understanding how these key organisms and their communities are going to respond to global climate change. They already provide novel genetic resources for various different biotechnological applications.

Contents

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14.1 Introduction

Protists inhabiting polar regions have been the subject of intense interest ever since the first explorers ventured into the inhospitable seas of the Arctic and Southern Oceans (Ehrenberg 1841, 1853; Hooker 1847; Sutherland 1852). The first records of microbial biodiversity in extreme environments were made with the most basic of microscopes, and until the mid 1900s (ultimately when scientific programs in polar regions became more common) much of the work on protists remained largely descriptive and restricted to the more robust physiological experiments that could be attempted under unfavorable field conditions. Despite the fact that there has been nearly 170 years of research into algae living in the Arctic and Antarctic it is only in the last 20 years that there has been a revolution in laboratory facilities available at remote sites, and of course the technological advances that allow collection, extraction, and subsequent cultivation of organisms in home laboratories. Coupled to this we now have the sophisticated molecular tools to determine the true extent of this diversity, and in turn the molecular and physiological capabilities that permit life to continue at the extremes of low temperature. That is not to belittle the need to still look down the microscopes as works such as Scott and Marchant (2005) quite eloquently demonstrate.

This review is restricted to a discussion of microalgae found in Arctic and Antarctic regions, and most of the discussion will concentrate on diatoms and green algae (Fig. 14.1) living in sea ice, lake waters and snow because most physiological and molecular studies have been conducted with species from these two groups. However, there are other seasonally ice covered sub-polar regions, such as the Baltic Sea and Sea of Okhotsk where much of our understanding about cryogenic adaptations and microbial ecology are forwarding our understanding (see Granskog et al. 2006). Both in Polar and sub-Polar systems a huge diversity of microalgal species exist that for the purposes of this review are split into either psychrophiles, organisms with an optimal growth temperature at or below 15 °C, and a maximum growth temperature below 20 °C, or psychrotrophes, organisms with the ability to grow at temperatures below 15 °C but exhibiting maximum growth rates at temperature optima above 18 °C (Deming 2002).

Naturally permanently low temperatures combined with strong seasonality of solar irradiance are the most important environmental factors for evolution and life of polar photosynthetic organisms. Despite this a wide range of phylogenetic groups of algae have successfully adapted to these extreme environmental

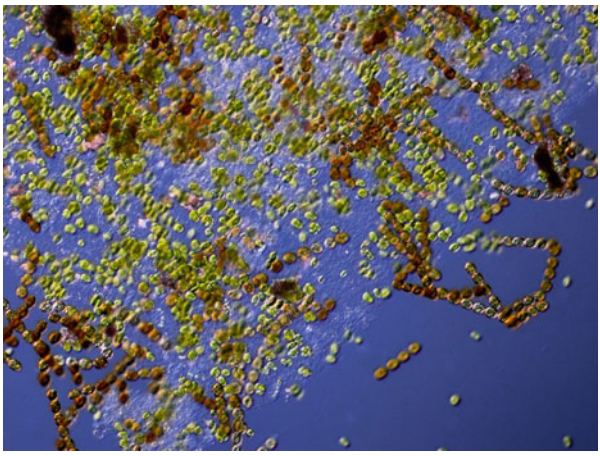

Fig. 14.1 Polar marine microalgal community composed of a green alga (*Chlamydomonas* sp.) and a chain-forming diatom (*Melosira arctica*). Image courtesy by Brian Eddie, Arizona State University, USA

conditions despite the polar regions being a geologically young habitat in the Earth System. The Antarctic continent, with the formation of a permanently cold-water ocean (Southern Ocean), was formed ca. 25 Mio years ago whereas the Arctic Ocean formed ca. 6 Mio years ago (Beil and Thiede 1990; Hansom and Gordon 1998; Thomas et al. 2008). However, most of the algal groups are older than 25 Mio years (Kooistra and Medlin 1996). Thus, the formation of polar environmental conditions was a major radiation event where new species developed that were able to grow under these extreme conditions.

14.2 Environmental Conditions

14.2.1 Light

The strong seasonality of solar irradiance is the major factor that influences the availability of light for photoautotrophic organisms at high latitudes. However, snow and ice thickness very much determine how much of the light is able to penetrate to regions where photosynthetic organisms are living (Eicken 1992). It cannot be forgotten that despite high latitude regions being commonly thought of as light limited systems, in fact during summer periods irradiances on snow and ice surfaces can be extremely high with high doses of harmful ultraviolet radiation being a commonly reported stress factor (reviewed by Brierley and Thomas 2002). Therefore, a wide range of photoadaption is exhibited and is a prerequisite for photoautotrophic organisms living at high latitudes (Kirst and Wiencke 1995). Snow algae, which are mainly chlorophytes, grow on and within snow and ice surfaces and may therefore be exposed to high doses of UV radiation. This is of course true for the microalgal assemblages of glacial lakes, cryoconite holes on glacial systems, and seasonally formed melt features both in terrestrial systems and

on the surfaces of pack ice (Vincent et al. 2000; Säwström et al. 2002; Hodson et al. 2005; Mindl et al. 2007).

In contrast, microalgae growing inside or under sea ice, as well as in or under permanent ice covers of the Antarctic dry valley lakes are photosynthetically active in a light environment almost without UV radiation and less than 1% of incident photosynthetically active radiation (PAR). Beyond the aquatic systems hypoliths—mostly cyanobacteria and chlorophytes—that grow on the underside of stones and rocks in periglacial systems, where they utilize irradiances far less than >0.1% of the incident light for photosynthesis (Cockell and Stokes 2004). Far beyond these habitat-specific light irradiance differences, all photoautotrophs in high latitude regions must have the physiological ability to survive several months of darkness.

14.2.2 Seawater

In high latitudes, the Polar Oceans are the major habitat of microalgae in terms of biomass, abundance, and species diversity. Most of these cold water masses are characterized by seasonal surface-freezing and strong vertical mixing due to katabatic polar winds, convection at frontal zones or deep-water formation (Cottier et al. 2017; Meredith and Brandon 2017). However, the central Arctic Ocean and the Southern Ocean are not similar regarding their physical and chemical conditions. The Arctic Ocean is a Central Ocean surrounded by landmasses with a permanent cover of multi-year sea ice in its central area around the north pole. The Southern Ocean surrounds the Antarctic continent with a series of circumpolar fronts, and different water masses with distinct physical and chemical characteristics occur between these fronts (Tomczak and Godfrey 2003).

Most of the sea ice in the Southern Ocean is seasonal with an advance and retreat of 16 million km^2 in sea ice around the continent within 1 year (Fig. 14.2). Multi-year sea ice only occurs close to the continent in inlets or bays or in major ocean gyre systems in the Weddell and Ross Seas (Stammerjohn and Maksym 2017). This results in Antarctic sea-ice having a mean thickness of about 0.55 m. In contrast, the mean thickness of Arctic sea ice is approximately 3 m, due to 50% of the Arctic pack ice being multiyear ice lasting between 2 and 11 years (Dieckmann and Hellmer 2003). The latitudinal influence of sea ice is greater in the Arctic covering a region that extends from 90°N to 44°N, whereas in the Southern Ocean the region is only 75°S to 55°S, although at the maximum extents Arctic ice covers an area of 16 million km^2 and Antarctic sea ice 19 million km^2 (Meier 2017; Stammerjohn and Maksym 2017).

Overwhelmingly, in the past decade, it has been the reduction in summer sea ice in the Arctic Ocean that has captured the imagination of policy makers, media, and the non-specialized audience. This loss in the sea ice extent and volume in the northern hemisphere has been contrasted by slight increases in overall sea ice extent in the Southern Ocean (Stroeve et al. 2005; Stoeve and Notz 2015; Meier 2017; Stammerjohn and Maksym 2017). This results in the overall global sea ice extent

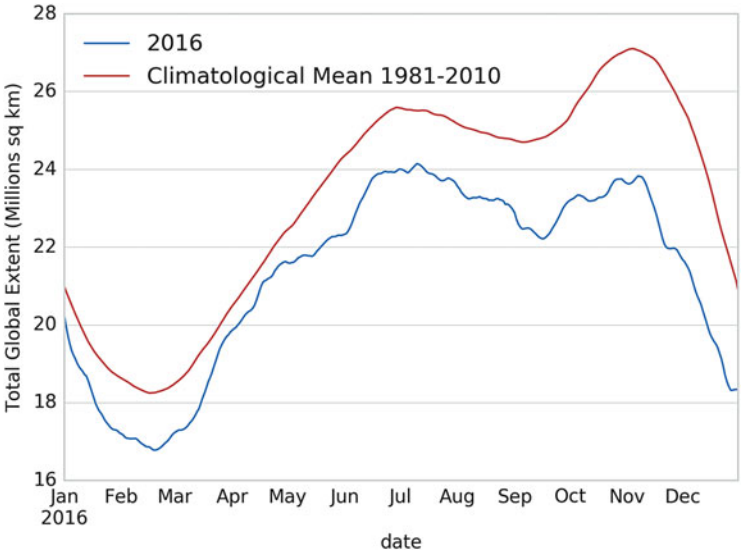

Fig. 14.2 Time series of daily global sea ice extent (Arctic plus Antarctic). It shows the global sea ice extent for 2016 tracking below the 1981–2010 average. Image provided by the National Snow and Ice Data Center, University of Colorado, Boulder, USA (http://nsidc.org/arcticseaicenews/)

varying on average between 18 and 26 million km^2, although in 2016 the range was from 17 to 24 due to the changes in the Arctic sea ice compared to the 1981–2010 climatological mean (Fig. 14.2).

The Southern Ocean has the highest inventory of unused macronutrients in the World Ocean (Fig. 14.3) and is the most important province for the export and burial of biogenic silica from diatoms (Smetacek 1998; Smetacek and Nicol 2005). The discovery of high macronutrient concentrations and relatively low phytoplankton concentrations in the Southern Ocean led to the concept of the "Antarctic Paradox" that was subsequently referred to as high nitrogen-low chlorophyll a region (HNLC). Micronutrients such as iron are considered to be the reason for this Antarctic Paradox, and several international large-scale iron fertilization experiments confirmed this hypothesis (reviewed by Boyd et al. 2007). Thus, the supply of iron to Southern Ocean phytoplankton (iron is a requirement for proteins involved in photosynthetic carbon assimilation) resulted in marked increases in both carbon fixation and nitrate utilization rates. However, it is assumed that many offshore species do have a lower requirement for iron and therefore are well adapted to these conditions.

In contrast, the Arctic Ocean is relatively rich in micronutrients such as iron because of terrigenous sources of micronutrients primarily via river runoff and also dust and sediments deposited in shallow coastal water masses. Thus, macronutrients are more important in limiting phytoplankton biomass in the Arctic Ocean compared to the Southern Ocean. However, the most important factors regulating the large-scale distribution of phytoplankton production and biomass in the Arctic

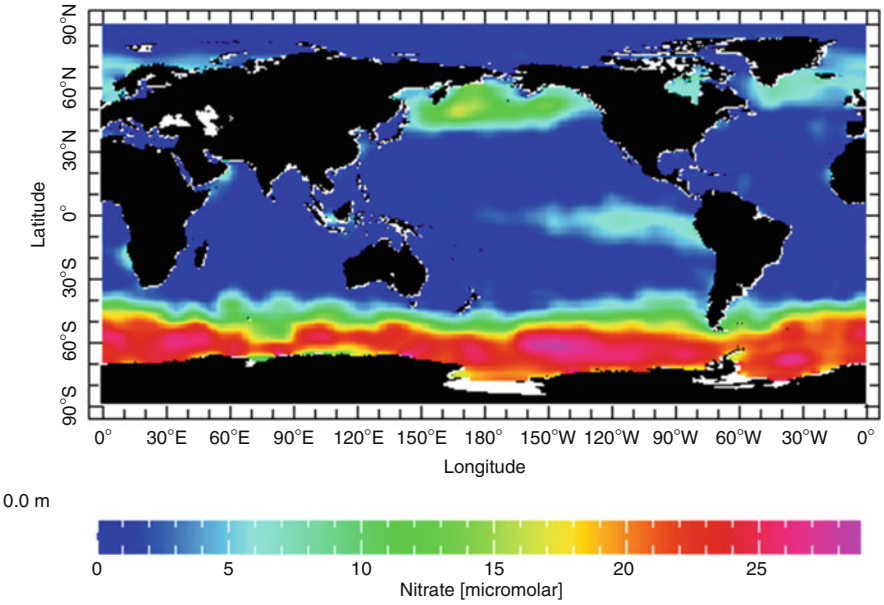

Fig. 14.3 Map of high nutrient-low chlorophyll (HNLC) regions around the world. Measurement in map is of nitrate, with the scale as a gradient of color pictured on the *bottom* (http://www.atmosphere.mpg.de/media/archive/1058.gif)

Ocean are probably the surface ice cover and the depth of the surface mixed layer, thus the availability of light (Sakshaug and Slagstad 1991). The distribution of macronutrients in the Arctic Ocean is highly heterogeneous between basins resulting in significant regional differences in primary production dynamics (Wheeler et al. 1997; Dittmar and Kattner 2003; Jones et al. 2003).

Beside the dominance of diatoms in both polar oceans, Prymesiophytes such as *Phaeocystis* spp. and *Emiliania* spp. are the second most abundant algal group. They even may form large blooms under more stable conditions and therefore outgrow bloom-forming diatoms (e.g., Smith et al. 1991; Merico et al. 2003). Dinoflagellates, Chlorophytes, Prasinophytes, and other algal groups are underrepresented in polar oceans (Kopczynska et al. 1986; Smetacek et al. 2002). However, a study by Lovejoy et al. (2006) who used 18S rRNA clone gene libraries indicated a high diversity of microbial eukaryotes in the Arctic Ocean. This is either indicative of a large number of endemic species or a high number of under-sampled taxa. Nevertheless, the dominance of diatoms in polar oceans makes this group ecologically the most important group of polar microalgae (Lizotte 2003a, b). Diatoms in general are estimated to contribute to at least 50% of the global marine primary production (Nelson et al. 1995).

Due to the presence of glaciers and permafrost, photosynthetic biomass on land in polar regions is negligible compared to that found in the ocean. Consequently, polar diatoms are of interest not only because of their important role as the main

food source for the whole polar food web (terrestrial and aquatic) but also because of their ability to thrive in this extreme ecosystem.

14.2.3 Sea Ice

Sea ice being one of the most extreme and largest habitats in polar oceans is important in structuring the whole polar ecosystem (Eicken 1992; Brierley and Thomas 2002; Arrigo and Thomas 2004). At its maximum, it covers 13% of the Earth's surface (Comiso 2003). The physical characteristics pertinent to the biology living in sea ice have been reviewed by Petrich and Eicken (2017): Sea ice, in contrast to fresh water ice, is not solid but is comprised of a system of brine channels (Fig. 14.4) that provide a habitat characterized by low temperature (ca. −2 to −20 °C), high salinity (35–200), high pH (up to 11), and low irradiances that can be below 1 μmol photons m^{-2} s^{-1} despite the fact the ice is only 1–2 m from the ocean surface (Eicken 1992; Gleitz et al. 1995; Kirst and Wiencke 1995).

Sea water typically containing about 34 g of dissolved salts and ions (mostly sodium, chloride, sulfate, magnesium, calcium, and potassium) does not begin to freeze until temperatures drops below −1.86 °C. At this temperature, ice crystals begin to form and rise to the surface. These initial ice crystals (termed frazil ice) vary in shape, from plates to needles, and size, from ≤ millimeter to centimeter in length. The crystals consolidate by wind and water motion within hours to form loosely aggregated discs (termed pancakes). After a few days of growth by accumulation of more and more ice crystals that form in the upper water column, pancakes can be several meters across and up to 50 cm thick. They freeze together and after 1 or 2 days a closed ice cover has formed (termed pack ice). As temperatures continue to decrease this pack ice thickens, not necessarily by the accumulation of more ice crystals but by the growth of columnar ice at the ice–water interface. This type of ice is formed by the vertical elongation of frazil

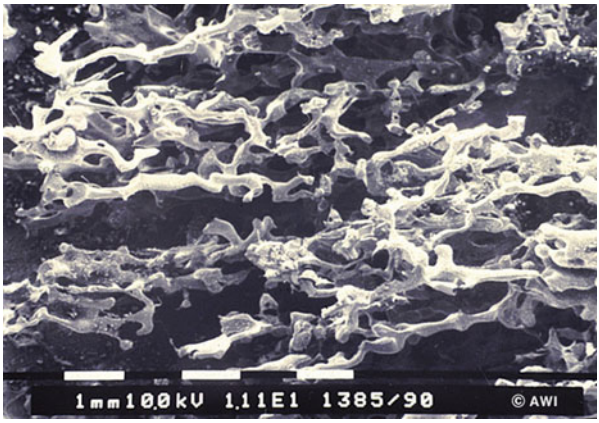

Fig. 14.4 Brine channel system in columnar sea ice made visible by filling the system with epoxy resin under a vacuum. Picture by Alfred-Wegener Institute for Polar and Marine Research, Bremerhaven, Germany, based on the work of J. Weissenberger et al. (1992)

ice crystals. The proportion of frazil ice to columnar ice depends largely on the turbulence of the water in which it was formed. The more turbulent the water the more frazil ice is usually formed. Antarctic sea ice thus contains up to 80% frazil ice as it is formed under more turbulent conditions. In the Arctic, sea ice is formed under more calm conditions containing up to 80% columnar ice (Petrich and Eicken 2017; Fig. 14.5 = ice from Arctic and Antarctic, polarization).

When ice is formed from seawater, salt ions and air in the water cannot be incorporated into the ice crystals and are therefore concentrated as salty brine either into inclusions of pockets and channels (Fig. 14.4) or released into the water below the ice. Thus, sea ice is a solid matrix penetrated by a labyrinth of channels and pores that contain highly concentrated brine and air bubbles (Fig. 14.4). Brine channels vary in size from a few micrometers through several millimeters in diameter and are the main habitat for all microorganisms in sea ice (reviewed by Brierley and Thomas 2002; Deming 2002; Lizotte 2003a; Mock and Thomas 2005). Their volume and the concentration of salt in them is directly proportional to temperature (Fig. 14.5) (Weissenberger et al. 1992; Krembs et al. 2000; Petrich and Eicken 2017). When temperatures decrease, brine volume decreases and salt content increases. Thus, the colder ice contains brine channels with highly salty brines and overall fewer, smaller, and less interconnected channels than warmer ice. Since ice at the sea-ice air interface is usually colder than ice in contact to the underlying water, a temperature gradient exists through the ice, resulting in a gradient in brine salinity and the overall volume of brine in sea ice as well. A host of protists and zooplankton have been recorded from sea ice (Horner 1985; Palmisano and Garrison 1993; Lizotte 2003a; Werner 2006; Bluhm et al. 2017),

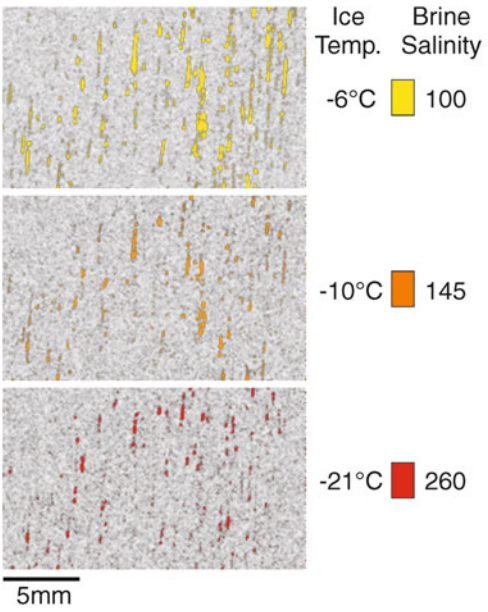

Fig. 14.5 Color-enhanced magnetic resonance images of the same piece of ice shows how the pore space and size of the brine channels and pockets reduces with decreasing temperature, with corresponding increase in salinity of brines contained within the pores. Image after Thomas and Dieckmann (2002), based on the work of Eicken et al. (2000)

Fig. 14.6 Ice floe (*upside down*, about 80 cm thick) with dense populations of pennate diatoms at the sea-ice water interface (indicated by *brown* color that is caused by their main light-harvesting pigment fucoxanthin). Image from David N. Thomas

although among the photoautotrophs the most studied are the diatoms. All organisms living within the sea ice matrix have to have plastic physiologies to cope with these ever-changing physical and chemical conditions of their environment, which are dominated by the temperature and salinity changes.

Microalgae are mainly introduced into the ice as it is forming. They get caught between ice crystals or simply stick to them as crystals rise through the water when it freezes in fall. During the formation of consolidated ice, diatoms become trapped within brine channels. Pennate diatoms are the most conspicuous organisms in sea ice along with other microalgae (e.g., Dinoflagellates, flagellates), heterotrophic protists (e.g., ciliates) and bacteria (Brierley and Thomas 2002; Thomas and Dieckmann 2002; Caron et al. 2017). These micrometer-sized algae, with their main light-harvesting pigment being fucoxanthin, can reach such concentrations in sea ice that they discolor the ice visibly brown (Fig. 14.6). The time for acclimation to the new conditions in sea ice is not very long since day light hours are continually decreasing as winter approaches. Nevertheless, diatoms, especially at the ice–water interface where conditions are most similar to the water below the ice, are often able to photoacclimate rapidly and can accumulate to high biomass even before the winter begins (Gleitz and Thomas 1993). Sea-ice diatoms are very efficient in using solar irradiance and are able to grow at irradiance levels below 1 μmol photons m^{-2} s^{-1} (Mock and Gradinger 1999). Light levels are minimal during high latitude winters, not only due to short days or complete darkness, but also due to snow cover on top of the ice that is a very efficient reflector of solar irradiance (Perovich 2017).

Sea ice is mostly an ephemeral feature since after its formation and consolidation the majority of it melts resulting in the release of all organisms within to the underlying water. Increase in solar irradiance is the most important factor that causes the ice to melt. A common feature and a sign for the beginning of the ice melt is the formation of melt ponds on the surface of the ice (Fig. 14.7). They are more common in the Arctic than Antarctic. One reason for fewer melt ponds in the Antarctic is that more heat is derived from underlying water and melting from

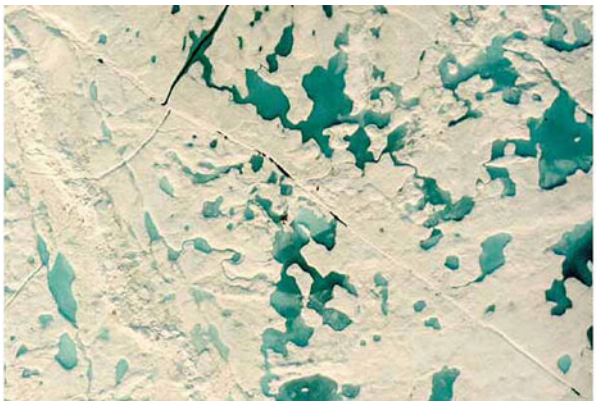

Fig. 14.7 Melt ponds on top of Arctic sea ice. This picture is 100 m across. The melt ponds have different shades of *green-blue*, which is determined by the optical properties of the ice underneath the water in the melt ponds (http://www.arcticice.org/close100m.htm)

above is less significant (Haas 2017). When melting continues due to increasing water temperatures and solar irradiance on top of the ice, the ice gets thinner and more porous. Large pores and brine channels that are filled with seawater characterize warm ice, and the ice itself has very little strength and is easily broken up. However, not all the ice that is formed in fall actually melts during next summer. If it survives the summer, refreezing occurs during the following winter that makes the ice even thicker. The longevity of the ice depends on the geographical location, on the wind, and ocean currents. Sea ice of northern Greenland and the Canadian archipelago can be up to 15 years old with an average thickness of 6–8 m (Haas 2017), although the extent of such thick multiyear ice is considerably reduced in the past 20 years. Such differences in physical properties of the ice also result in differences in the abundance, activity, and composition of the microbial communities within sea ice.

Despite the high diversity of autotrophs within sea ice, that also includes Prasinophytes, autotrophic dinoflagellates and cilliates, two small pennate diatoms, *Fragilariopsis cylindrus* (Grunow) Krieger and *F. curta* (Van Heurck) Hustedt, and the prymnesiophyte *Phaeocystis antarctica* Karsten are the dominant species in blooms in the Antarctic sea ice zone (Leventer 1998; Lizotte 2001). Gleitz et al. (1998) found that at high diatom standing stocks species diversity decreases. This has also been reported by Gleitz and Thomas (1993), who showed that as first-year sea ice grew and high algal standing stocks established, the assemblages were dominated by only a very few small diatom species. Taking into consideration the findings of other studies, Gleitz and Thomas (1993) suggested that pore and channel size was the major factor in the preferential accumulation of a few smaller species within sea ice. However, Gleitz et al. (1998) subsequently concluded that it was the physiological capacity of these species to maintain high growth rates in the spring and summer, in connection with their life history cycles, that may be the key to the prominence of so few diatom species in the ice.

Phaeocystis species are more usually found in sea-ice habitats not constrained by the brine channel systems, such as surface ponds, rotten summer sea ice, or

freeboard/infiltration layers. Especially in the latter, these are situations where the constraints of salinity, temperature, and low light do not inhibit primary production as they do in interior ice assemblages, thereby enabling high standing stocks (including diatoms) to accumulate (Haas et al. 2001; Kennedy et al. 2002; Kattner et al. 2004).

Dense dinoflagellate and chrysophyte assemblages can develop in the upper sea-ice interior, and high rates of primary production have been measured at these sites, especially in spring when the upper sea ice temperature is low and brine salinities are high (Stoecker et al. 1997, 1998, 2000). These algal assemblages are often poorly defined, but they may make an important additional contribution to total sea-ice primary production.

Even psychrophilic, halotolerant *Chlamydomonas* spp. have been isolated from sea ice in both the Arctic and Antarctic (Hsiao 1983; Ikävalko and Gradinger 1997; Krembs and Engel 2001; Eddie et al. 2008). While diatoms have received most of the research attention in sea ice work, other groups of organisms such as *Chlamydomonas* species will increasingly attract effort, especially since similar organisms are routinely isolated from saline and freshwater lakes in Arctic and Antarctic sites.

14.2.4 Snow

In regions where snow persists during the summer such as in high-altitude and in the low-latitude polar regions, its color may change from white to red, pink, green, yellow, or orange. The largest patches are often red and therefore called "blood snow" or "watermelon snow" (Fig. 14.8). These macroscopic expressions are based on massive growth of unicellular psychrophilic green algae. These algae are reviewed by Hoham and Duval (2001). Most snow algae belong to the genera

Fig. 14.8 Watermelon snow pits superimposed with an orange bootprint. The coloration is caused by *Chlamydomonas nivalis* (https://en.wikipedia.org/wiki/Watermelon_snow)

Chlamydomonas and *Chloromonas* (Chlorophyta, Volvocales), and these are most active in spring and summer. The beginning of snow melt in spring provides liquid water between ice crystals that is essential for the vegetative stages. The snow has to be neither too cold nor too dry such as freshly fallen snow. Green flagellated stages are often observed within this wet snow (Fig. 14.9a). They are able to move within the snow layer to reach optimal depths for their light and temperature requirement. They can form massive blooms and color the snow green if enough nutrients are available as is commonly found close to bird colonies or nutrient-rich streams or ponds.

During summer and fall, they have to acclimate to extreme temperature regimes, high irradiance and UV radiation, and low nutrient levels. For instance, in high-altitude regions (above 2500 m), UV radiation can be very high and spherical integrated photosynthetic active radiation (PAR) can often reach 4500 μmol photons m^{-2} s^{-1} and occasionally up to 6000 μmol photons m^{-2} s^{-1}. If less liquid water becomes available and therefore also nutrients, most flagellated stages turn into immotile hypnoblast stages (Fig. 14.9b) because this form is the most resistant to environmental changes. The transformation into hypnoblasts is characterized by a massive incorporation of reserve material, including sugars, lipids, and by formation of esterified extraplastidal secondary carotenoides. Studies have shown that the cells mainly form oxycarotenoides and in particular astaxanthin that has a red color and therefore gave the snow its name "blood snow" (Müller et al. 1998). Theses hypnocygotes and other resting cells have thick cell walls and sometimes mucilaginous envelopes (Müller et al. 1998). They can survive dry and warm periods in a dormant state and tolerate high pressure such as under thick snow. They also tolerate freezing in ice blocks at temperatures down to −35 °C during winter. However, some of these resting stages can remain photosynthetically active even under very high photon flux densities because of well-protected photosystems by secondary carotenoids (Remias et al. 2005).

14.2.5 Rock Surfaces

Most of the Antarctic continent is covered with a several kilometer thick layer of meteoric ice. However, parts of this continent are ice free such as the McMurdo Dry Valleys in southern Victoria Land (Fig. 14.10). With ca. 4500 km^{-2}, this is the largest ice-free area on this continent. Precipitation in this region is below 10 cm $year^{-1}$ that makes it one of the driest deserts on earth, and air temperature ranges from 5 to −55 °C (Priscu 1998).

Periglacial activity, the freezing and thawing of ground water, often sorts rocks and stones into defined patterns in Polar deserts, regions of permafrost, and high altitude stone rubble fields. The sorting of the stones results in a high degree of spatial heterogeneity in the light incident under different parts of the stone/rock patterning and vegetation patterns. In turn, this results in regions of the stone field where light penetrating the stones is sufficient to support photosynthetic carbon

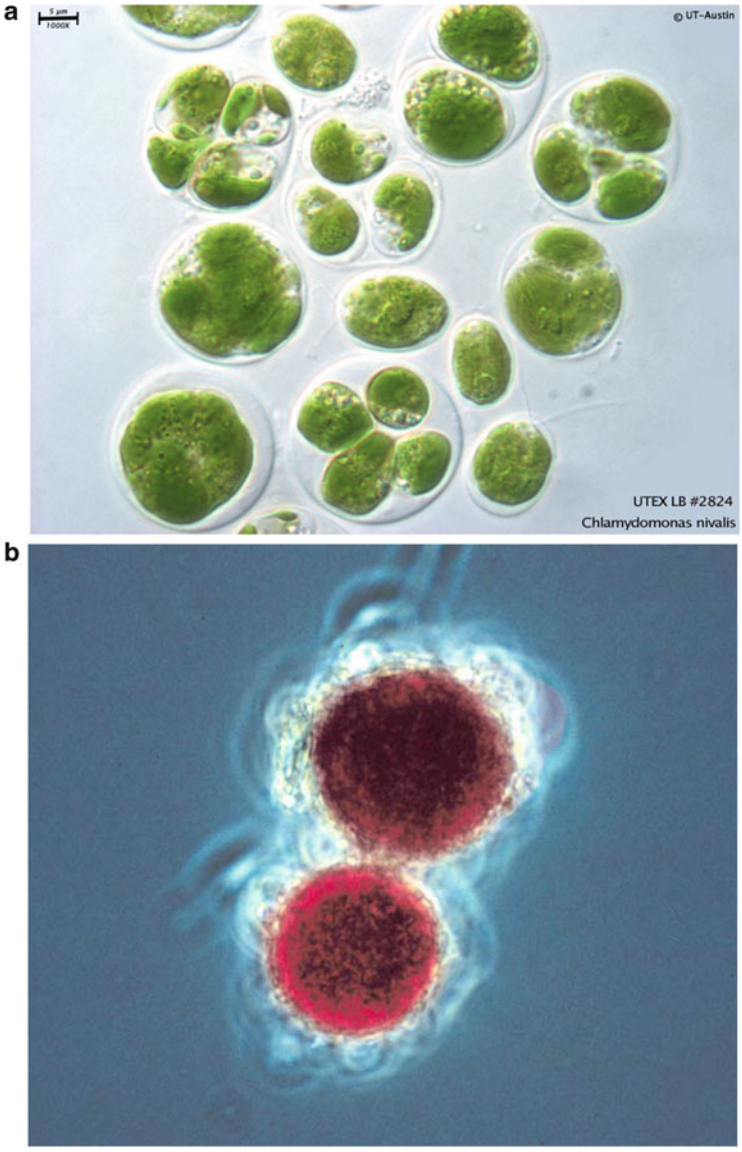

Fig. 14.9 (**a**) Vegetative stages of *Chlamydomonas nivalis*. Some of the single cells show two flagella. Picture from UTEX Image Bank: (http://www.bio.utexas.edu/research/utex/photogallery/c/Chlamydomonas %20nivalis %20LB %202824.htm); (**b**) *Chlamydomonas nivalis* aplanospores filled with the *red* pigment astaxanthin and with attached particles. Image courtesy by Brian Duval and Lynn Rothschild

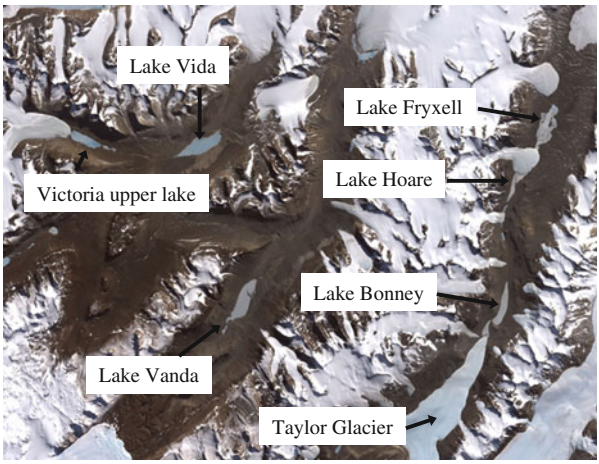

Fig. 14.10 Satellite image (NASA, USA) from McMurdo Dry Valleys. Best studied lakes are Lake Bonney, Hoare, and Fryxell that are located within the Taylor Valley

assimilation by the hypoliths inhabiting the underside of the stones (Cannone et al. 2004; Cockell and Stokes 2004).

The patterning of stone fields influenced by periglacial activity is often polygonal, and both the Arctic and Antarctic rocks at the edges of the polygons support well-developed assemblages of photosynthetic organisms, whereas in the center of the polygons colonization by hypoliths is significantly reduced (Cockell and Stokes 2004). These cyanobacteria and unicellular algae are growing, even thriving, in an extreme environment, where temperatures sink below −30 °C, water is minimal, and light conditions are reduced to virtually nothing. Likewise, there are microalgae associated with cryptoendolithic layers growing within sandstones in regions such as the Dry Valleys. Typically, these communities have layers of fungi and cyanobacteria, but microalgae such as *Hemichloris antarctica* are frequently found in the lowest bands of endolithic systems where the irradiance can be as low as 0.05 µmol photons m^{-2} s^{-1} (Johnston and Vestal 1991; Friedmann et al. 1993).

14.2.6 Permanently Ice Covered Lakes

Besides short-term glacier melt events, most of the liquid water, and therefore accumulation of organisms, is available in the perennial ice-covered lakes that are characteristic for the Dry Valleys and also regions such as the Vestford Hills. However, the source of lake waters is assumed to be glacier meltwater that penetrates the lakes without melting the surface ice cover (Priscu 1995). A sensitive balance of freeze-thaw cycles is assumed to keep the lakes permanently covered with ice but also accessible for water in- and outflow from underneath. There are numerous permanently covered lake systems in the McMurdo Dry Valleys, but the best studied are Lake Bonney, Hoare, and Fryxell that are located within the Taylor Valley (Fig. 14.10).

The major food-web components in all lake systems are unicellular eukaryotes and prokaryotes. The main groups are green algae, diatoms, ciliates, rotifers, heterotrophic nanoflagellates, bacteria, and viruses. Autotrophic phytoplankton play an essential role in functioning of the food web by production of organic carbon. However, photosynthesis is strongly limited by the availability of solar irradiance. Measured irradiance below the ice cover of Lake Bonney never exceeds 50 μmol photons m^{-2} s^{-1}, and the wavelength of maximum transmission through the water column is in the range from 480 to 520 nm with longer wavelengths (>600 nm) being diminished. This light is only available from late September through mid-March and this seasonality is the trigger for spring phytoplankton growth in these non-turbulent waters. Vertical stratification is very pronounced in most of the lakes and is sometimes accompanied with strong gradients in salinity and nutrients. Vertical stratification is also pronounced for many phytoplankton species. The water layer immediately beneath the ice cover in Lake Bonney is dominated by the crytomonad *Chroomonas* sp. and *Chlamydomonas intermedia* whereas *Chlamydomonas raudensis* is confined to the deep saline and low-irradiance layers of the photic zone (Morgan-Kiss et al. 2006).

14.3 Adaptation of Microalgae at High Latitudes

14.3.1 Diatoms (Bacillariophyceae)

Psychrophilic diatoms are one of the most abundant groups of phytoplankton in polar oceans. This is mainly due to the presence of higher silicate concentrations in these waters and to their successful adaptation to strong vertical mixing in polar waters, strong seasonality in solar irradiance, freezing temperatures, and extremes of salinity (Cota 1985; Fiala and Oriol 1990; Boyd 2002; Mock and Valentin 2004; Ryan et al. 2004; Ralph et al. 2005). Due to their importance as primary producers, many physiological studies with polar diatoms were related either to growth and its dependency on nutrients and temperature or to regulation of photosynthesis under typical polar condition. This section aims to provide a comprehensive overview of new data regarding physiological and in particular molecular adaptation for this important group of polar algae.

Maximum growth rates for many polar diatoms are in the range of 0.25–0.75 divisions per day, that is two- to threefold slower than growth at temperatures above 10 °C (Sommer 1989). Many of these diatoms are psychrophilic and not able to live at warmer temperatures (above ca. 15 °C), which is indicative of the presence of specific molecular adaptations that enable these diatoms to grow under freezing temperatures.

14.3.1.1 Functional Genomics

Approaches to uncover the gene repertoire of a polar diatom have been dominated by the genus Fragilariopsis, in particular *Fragilariopsis cylindrus*, a marine indicator species for cold water, found at both poles (von Quillfeldt 2004) and in seasonally cold waters (Hendey 1974; Hällfors 2004).

The first approaches involved constructing and sequencing two expressed sequence tag (EST) libraries, one generated under freezing temperatures (Mock et al. 2005) and the another under increased salinity (Krell 2006). 966 EST were generated from the cold stress library and 1691 from the salt stress library. There are now over 21,000 EST from *F. cylindrus* on the EST-databank at NCBI and about 200 gene-specific oligonucleotides (70mers) from the original EST libraries for functional gene-array experiments (Mock and Valentin 2004). An important addition to algal research, particularly in terms of understanding polar adaptation, is the recent publication of the *F. cylindrus* genome and RNA-sequencing data generated under a range of polar conditions (Mock et al. 2017). This is the third diatom genome to be published and the first polar diatom. There is only one other polar microalga with a published genome, the psychrotolerant freshwater green alga *Coccomyxa subellipsoidea* (Blanc et al. 2012).

All EST-sequences were compared against the genomes of *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*. In addition, 11 algae and plant databanks were consulted to annotate sequences that were not found in the temperate diatom genomes. Nevertheless, over 50% of sequences showed no similarity to known sequences in these databanks and to both diatom genomes even when using a comparatively high e-value of $\leq 10^{-4}$ (Mock et al. 2005).

In the cold-stress EST library, the most abundant functional categories were related to translation, posttranslational modification of proteins, and transport of amino acids and peptides by ABC transporters. Some of these ABC transporters displayed homology to bacterial permeases and others appeared to be involved in translational or posttranslational control. However, most of them could not be assigned a function.

The presence of six different DNA/RNA helicases in the cold-stress library indicated that DNA and RNA coiling and uncoiling are important under freezing temperatures. Minimizing the likely formation of secondary structures and duplexes of mRNAs under low temperature stress is necessary to initiate translation. However, protein domains of DNA/RNA helicases are also the eighth most abundant protein domain in the genome of *T. pseudonana* (Armbrust et al. 2004), and therefore more evidence is necessary to conclude that these enzymes are essential to cope with freezing temperatures. The most abundant sequences in this library in terms of their redundancy were either sequences that were related to energy generation (e.g., fucoxanthin-chlorophyll a, c-binding proteins) or completely unknown sequences (Mock et al. 2005).

In the salt-stress library, the most abundant functional categories of sequences were related to posttranslational modification of proteins (e.g., heat-shock proteins;

hsps) and ion-transport (Krell 2006). Most of them were hsps and different ionic transporter genes reflecting the requirement to reestablish homeostasis under salt stress. Several sequences of different kinds of V-type H+-ATPases and antiporters for various ions such as sodium, potassium and calcium were found in this library. V-type H+-ATPases are of great importance in establishing an electrochemical proton gradient across the tonoplast to drive sodium sequestration into the vacuole (Shi et al. 2003).

One important organic osmolyte under salt stress in diatoms is the amino acid proline. Many genes involved in proline synthesis were found in the salt-stress-EST library indicating that this pathway was active under experimental conditions (Krell 2006). The gene coding for pyrroline-5-carboxylate reductase (P5CR, catalyzing the final step in proline synthesis) could be identified among the most abundant sequences in the salt-stress library (Krell 2006). Furthermore, seven proteins involved in the proline synthesis pathway increased in abundance in response to high salinity (Lyon et al. 2011). This indicates that proline may be important for salt stress acclimation.

One of the interesting aspects of the *F. cylindrus genome* is the high number of divergent alleles. Approximately, 25% of the diploid genome consists of alleles that are highly divergent, particularly in comparison to the temperate diatom genomes of *T. pseudonana* and *P. tricornutum* (Mock et al. 2017).

Differential expression can be seen between divergent alleles under different conditions, many of which are commonplace in the polar environment, including prolonged darkness, freezing and elevated temperatures, iron starvation, and increased CO_2 concentration (Fig. 14.11b). In addition, dN/dS analysis suggests that there may be a positive correlation between allelic differentiation and diversifying selection (Mock et al. 2017).

Copper rather than iron-binding proteins are enriched in the *F. cylindrus* genome as are plastocyanin/azurin-like domains. This may facilitate electron transport during photosynthesis while reducing iron dependence. In terms of photosynthesis, a large number of light-harvesting complex (LHC) proteins are also present including Lhcx, which is involved in stress response. There are also a larger number of methionine sulfoxide reductase (MSR) genes in the *F. cylindrus* genome compared to *T. pseudonana* or *P. tricornutum* that are linked to oxidative stress under cold temperatures (Lyon and Mock 2014).

A large number of zinc-binding proteins can be found in this genome compared to the sequenced temperate diatoms. These contain myeloid-Nervy-DEAF-1 domains (MYND) which are associated with protein–protein interactions and regulation.

Enrichment of specific gene groups can be found within the diverged alleles; these include: catalytic activity, transport, membrane proteins, and metabolic processes (Fig. 14.11a). Furthermore, divergent alleles were found to be differentially expressed under different conditions, suggesting that they may be involved in adaptation to polar conditions. Given the low sequence identity between promoters of divergent alleles and their differential regulation, it is seems likely that individual copies are under different regulatory controls. RNA-seq data focused on

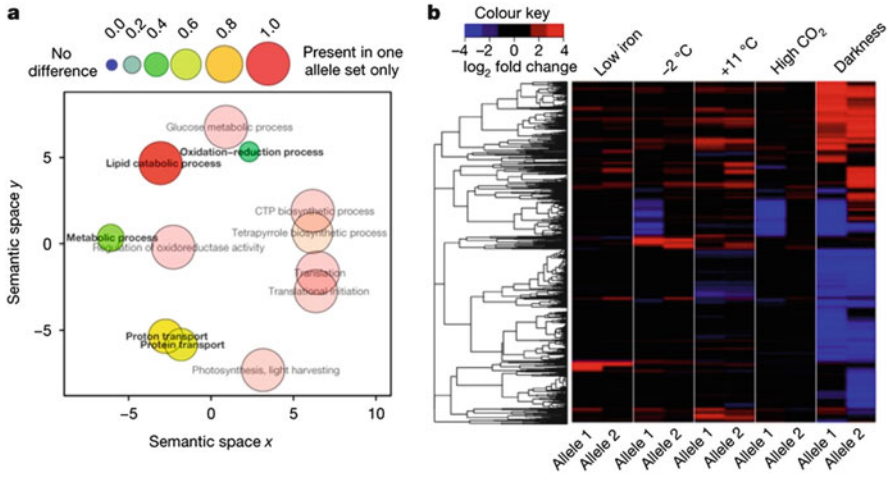

Fig. 14.11 Bi-allelic transcriptome and metatranscriptome profiling. (**a**) REViGO semantic similarity scatterplot of biological process gene ontology terms for *Fragiariopsis cylindrus*-like sequences (*E*-value $\leq 1 \times 10^{-10}$) in Southern Ocean metatranscriptome samples. Gene ontology terms that are overrepresented in the set of diverged alleles compared to non-diverged alleles are shown in bold. (**b**) Hierarchical clustering of 4030 differentially expressed allelic gene pairs in *F. cylindrus* (likelihood ratio test, $P < 0.001$; log2 fold change ≤ -2 or $\geq +2$) under low iron, freezing temperature (−2 °C), elevated temperature (+11 °C), elevated carbon dioxide (1000 ppm CO_2) and prolonged darkness, relative to optimal growth conditions. Each experimental treatment corresponds to two separate columns for both allelic variants and each single-haplotype gene to a single row. Image is taken from Mock et al. (2017)

changes in expression under prolonged (7 days) darkness as this condition gave rise to the highest number of up- and downregulated genes (Fig. 14.11b). Downregulated genes include those involved in photosynthesis, light harvesting, photoprotection, and translation. Genes involved in regulation of gene expression, DNA replication, signal transduction, and starch, sucrose, or lipid metabolism were upregulated (Mock et al. 2017). RNA-seq data suggests that during darkness, photosynthetic activity and supporting processes are reduced while processes such as chrysolaminarin and fatty acid storage are used instead.

Interestingly, as well as displaying the largest differential expression, growth under prolonged darkness also led to double the number of RNA-seq reads (30%) that did not map to predicted genes compared to any other condition. Alleles with the largest dN/dS ratios tended to show strong differences in expression between conditions; in addition, the majority of these alleles have no known function. As mentioned, this suggests a positive correlation between diversifying selection and allelic differentiation. It also highlights the necessity for reverse genetics in polar species to determine the function of these sequences and in turn understand how they are adapted to polar environments.

One of the most interesting discoveries in the *F. cylindrus* EST salt-stress library was a gene involved in antifreeze processes (Krell 2006; Krell et al. 2008). The

presence of ice-binding protein (IBP) genes in this species was verified following sequencing of the genome (Mock et al. 2017). Shortly after, IBPs were identified and characterized in the polar diatom *Navicula glaciei* (Janech et al. 2006). Since then several papers have been produced which explore the function of IBPs in polar diatoms; this is discussed in more detail in the next section. In diatoms, ice-binding proteins have been identified in *F. cylindrus*, *Fragilariopsis curta*, *N. glaciei*, *C. neogracile*, *Attheya* sp., *Amphora* sp., and *Nitzschia stellate* (Janech et al. 2006; Krell et al. 2008; Bayer-Giraldi et al. 2010; Gwak et al. 2010; Raymond and Kim 2012).

The N-terminal sequences of the identified IBPs of *N. glaciei, F. cylindrus,* and each of the *T. ishikariensis* antifreeze isoforms are most likely signal peptides and have low probabilities of being mitochondrial- or chloroplast-targeting peptides (Janech et al. 2006; Fig. 14.12). N-terminal sequences were found in *Attheya* sp. but not *Amphora* sp. or *Nitzschia stellate* and therefore may not be secreted (Raymond and Kim 2012).

Many diatom genes show homology to bacterial or fungal genes suggesting origins from horizontal gene transfer (HGT). *N. glaciei* and *F. cylindrus* IBPs show sequence similarity to several antifreeze isoforms of the Basidomycete fungus *Typhula ishikaiensis* (Figs. 14.12 and 14.13), which is known to inhabit sea ice (Janech et al. 2006). Sorhannus (2011) also found homology between IBPs of *F. cylindrus* and *F. curta* to IBPs from basidomycetes; however, in contrast to findings from Janech et al. (2006), IBPs from *N. glaciei* are placed in a separate clade and are suggested to originate from ancestral genes along with IBPs from *C. neogracile*.

Similarities between *F. cylindrus* and *N. glaciei* IBPs to hypothetical proteins from Gram-negative bacteria such as *Cytophaga hutchinsonii* and *Shewanella denitrificans* (between 43 and 58% amino acid sequence identity) have been observed. These bacteria have frequently been isolated from Arctic and Antarctic sea ice (Junge et al. 2002), and Cytophaga–Flavobacterium–bacteroides, which include *C. hutchinsonii*, are important in well-established sea-ice algal assemblages (Bowman et al. 1997) and the coldest (wintertime) sea ice (Junge et al. 2004). Raymond and Kim (2012) found IBPs from *Attheya* sp., *Amphora* sp., and *Nitzschia stellate* to show greatest homology to bacterial IBPs. These diatom IBPs contain no introns, and furthermore, *Flavobacterium frigoris*, which produces an IBP with 47% amino acid identity to an IBP in *Nitzschia stellate*, was isolated from Antarctic sea ice in the same layer as diatoms.

Expression of IBPs have also been demonstrated in the Antarctic bacterium *Marinomonas primoryensis,* where they aid adherence to ice, allowing *M. primoryensis* to remain near the top of the water column (Guo et al. 2012) and in an Antarctic *Colwellia* sp. where they inhibit ice recrystallization (Raymond et al. 2007). In other organisms, antifreezes appear to have arisen from a variety of proteins with other functions, although some retain the original functions (Cheng 1998). Other genes with homology to bacteria found in the *F. cylindrus* genome include ABC transporters with similarities to bacterial permeases and proton-

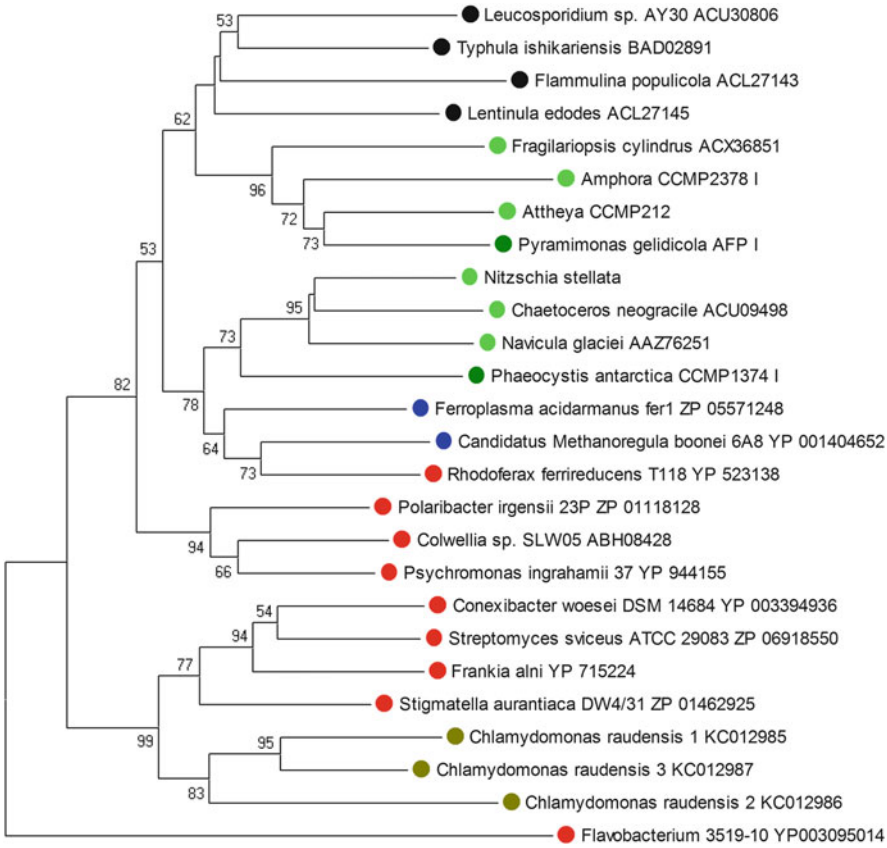

Fig. 14.12 Neighbor-joining tree constructed from amino acid sequences of selected ice-binding proteins (IBP) and IBP-like proteins. The *Chlamydomonas raudensis* IBPs (*olive*) are closest to IBP-like proteins in several bacteria and relatively distant from other algal IBPs. The tree was rooted with the Flavobacterium 3519-10 IBP. *Numbers at nodes* indicate bootstrap values for 500 replications. Values less than 50 are not shown. Colors: *black*, fungi; *light green*, diatoms; *dark green*, prasinophyte and prymnesiophyte; *blue*, archaea; *red*, bacteria; *olive*, *C. raudensis*

pumping proteorhodopsins, for trace-metal-independent ATP synthesis (Strauss et al. 2013).

14.3.1.2 Molecular Physiology

The presence of genes in a genome only indicates the potential for physiological adaptation, but knowledge of the expression and regulation of genes and their respective proteins leads to an actual understanding of how these diatoms cope with the extreme polar conditions. Expression analysis can be done by focusing on

Fig. 14.13 ClustalW alignment of ice-binding proteins from *Navicula glaciei* (Acc. no. DQ062566), *Fragilariopsis cylindrus* (CN212299), and *Typhula ishikariensis* (AB109745), and hypothetical proteins from *Cytophaga hutchinsonii* (ZP_00309837) and *Ferroplasma acidarmanus* (ZP_500309837). Predicted signal peptides are *underlined*. Gaps have been inserted to improve alignment. Conserved residues are *shaded*. The N-terminal sequence of Cytophaga protein and the N- and C-terminal sequences of Ferroplasma protein are truncated. Residue numbers are shown at *right*. Alignment is taken from Janech et al. (2006)

single genes (e.g., northern blots or quantitative PCR) or multiple genes through gene arrays or RNA sequencing. Arrays can be composed of known genes (gene-specific arrays) or the whole genome sequence (tiling arrays).

One of the most dramatic environmental changes in polar marine sea-ice habitats is the freezing of seawater and melting of the ice. The inclusion of organisms into newly formed sea ice represents a strong selective pressure. Only those organisms that are capable of acclimation to the relatively fast-changing conditions of temperature, irradiance, and salinity can survive.

Several experiments have been conducted to investigate gene expression under polar conditions including freezing temperatures, high salinity, high irradiance, and

prolonged darkness. Some study multiple genes using macro-arrays (Mock and Valentin 2004) or RNA-seq (Mock et al. 2017) while others focus on specific genes such as ice-binding proteins (Bayer-Giraldi et al. 2010, 2011).

Data from EST libraries has been used to produce arrays for two polar diatom species, *F. cylindrus* (Mock and Valentin 2004) and *C. neogracile* (Hwang et al. 2008; Park et al. 2010). About 200 70mer oligonucleotides were compiled into a nylon-membrane-based macro-array to study short-, mid-, and long-term acclimation to freezing temperatures under high and low irradiance in *F. cylindrus*. 1400 *C. neogracile* transcripts were analyzed using micro-arrays to observe expression at 4 and 10 °C (Hwang et al. 2008) as well as under high, moderate, low, and changing light intensities (Park et al. 2010).

The short-term response to freezing temperatures, which simulates the incorporation into newly formed sea ice during fall, was characterized by downregulation of genes encoding proteins for photosystem II (psbA and psbC) and carbon fixation (RUBISCO large subunit, rbcL) regardless of light intensity used (3 and 35 μmol photons m^{-2} s^{-1}). However, under higher irradiance (35 μmol photons m^{-2} s^{-1}), upregulation of genes encoding chaperons (hsp 70) and genes for plastid protein synthesis and turnover (elongation factor EfTs, ribosomal rpS4 and plastidial ftsH protease) were observed (Mock and Valentin 2004).

In *Chaetoceros neogracile,* increased irradiance led to both up- and downregulation of particular LHCx proteins and fucoxanthin-chlorophyll a, c-binding proteins (FCPs) (Park et al. 2010). Several genes for cell division, transcription, and signaling were upregulated while many genes for photosynthesis (including LHC, FCPs, and PSII-associated proteins) were downregulated along with some transporter genes including members from the ABC-transporter family.

In *Fragilariopsis cylindrus*, freezing accompanied with a reduction in irradiance (from 35 to 3 μmol photons m^{-2} s^{-1}) showed a typical response to low-light acclimation by upregulation of genes encoding specific FCPs without signs of a cold stress response. FCPs are a diverse gene family composed of genes involved in light harvesting as well as dissipation of light (see Sect. 14.2; Mock and Valentin 2004). Low irradiance in this species also leads to an increase in chloroplast PUFAs which can maintain electron flow by increasing fluidity of the thylakoid membrane (Mock and Kroon 2002a).

Upregulation of stress response genes and genes for protein turnover only under higher light intensities and decreasing temperatures indicates that a decrease in temperature at such light intensities mimics a further increase in light that could be more stressful than the actual decrease in temperature was by itself (Mock and Valentin 2004). This phenomenon is probably part of a cold-shock response that is also known from temperate plants when they get exposed to lower temperatures (Allen and Ort 2001).

Entomoneis kufferathii, a sea-ice diatom, showed high catalase activity, which is linked to protection against oxidative damage, in response to high irradiance and low temperatures (Schriek 2000). Genes for glutathione metabolism, an important antioxidant, were upregulated soon after exposure to high light in *C. neogracile*, although glutathione S-transferase and superoxide dismutase (SOD), two enzymes

involved in scavenging ROS, were downregulated (Park et al. 2010). A gradual increase in heat-shock proteins was observed under the same conditions over 6 h. Shifts in irradiance from either low to high or high to low light resulted in an increase in SOD in *Chaetoceros brevis* (Janknegt et al. 2008). An increase in temperature in *C. neogracile* from 4 °C to 10 °C resulted in the upregulation of several antioxidant genes including monoascorbate reductase, glutaredoxin, glutathione peroxidase, glutathione S-transferase, and alternative oxidase (Hwang et al. 2008). Polar diatoms appear to have tailored multiple resources for dealing with stress caused by the extreme polar environment.

Psychrophilic plants and diatoms are able to acclimate to higher irradiances under low temperatures (Streb et al. 1998; Mock and Hoch 2005; Ralph et al. 2005; Morgan-Kiss et al. 2006; Park et al. 2010). Long-term acclimation experiments to higher irradiances at freezing temperatures, when compared to the same light intensity but higher temperatures (+5 °C), revealed that cells kept at lower temperatures showed a typical response known from high-light acclimation: higher non-photochemical quenching, upregulation of the gene psbA, and upregulation of high-light FCPs that are involved in energy dissipation (Mock and Valentin 2004; Mock and Hoch 2005). A rapid increase in diatoxanthin (Dtx) in *C. neogracile* under high light also demonstrates energy dissipation through NPQ, along with an increase in expression of specific FCPs (Park et al. 2010).

In *F. cylindrus*, a reduction in expression of other photosynthesis-related genes (such as rbcL) was not observed after several months under freezing conditions indicating that long-term acclimation had been achieved.

Temperature effects that are less dependent on adjustments of the energy flow under freezing temperatures could also be identified by gene expression analysis (Mock and Valentin 2004). In the Mock and Valentin (2004) study, genes were selected that were either abundant in the EST libraries (e.g., ABC transporters) or were important for general acclimation to freezing temperatures (e.g., IBP, fatty-acid desaturase). Three unknown but abundant genes (in EST libraries) were also selected to see whether at least one of them is upregulated under freezing temperatures. Expression of these genes was investigated at +5 °C and 9 days after reducing the temperatures to −1.8 °C.

Upregulation of a gene encoding a delta5-desaturase under freezing temperatures indicated the necessity for production of polyunsaturated fatty acids (PUFAs) to maintain membrane fluidity at lower temperatures. Delta-5 desaturases produce omega3-fattyacids such as EPA (20:5 n−3), one of the most abundant fatty acid in diatoms and the main fatty acid in the galactolipids MGDG and DGDG. Thus, it can be assumed that more EPA is necessary under freezing temperatures to keep the thylakoid membrane fluid for electron transport or other membrane-bound processes.

Teoh et al. (2013) also found that PUFA concentration increased in *N. glaciei* with a decrease in temperature. In contrast, a delta-12 desaturase gene also known for producing PUFAs was not upregulated in temperate cyanobacteria (Nishida and Murata 1996). This indicates a different mechanism of gene regulation for this enzyme in psychrophilic diatoms.

An ABC-transporter gene was strongly upregulated at −1.8 °C in *F. cylindrus*, however, the family of ABC transporters is composed of genes with very diverse functions so it unclear of its specific function in response to freezing temperatures (Mock and Valentin 2004).

Extracellular polymeric substances (EPS) are linked to adaptation of diatoms in polar environments as both cryoprotectants and through maintenance of the cells microclimate (Underwood et al. 2010). An example of this can be seen in the sea-ice diatom *Melosira arctica*, in which EPS from the sea-ice diatom *Melosira arctica* altered the microstructure of ice-pore morphologies leading to salt retention (Krembs et al. 2011). EPS can include, but are not limited to substances such as polysaccharides (Aslam et al. 2012), uronic acid, peptides, proteins and glycoproteins (Krembs et al. 2011; Underwood et al. 2013).

Cryoprotectants can also help to maintain both the internal and external environment in polar cells and include solutes such as proline, DMSP and betaine (Lyon and Mock 2014). Proline synthesis genes were enriched in the *F. cylindrus* cold stress EST library (Mock et al. 2005).

DMSP pathway-linked protein concentrations were also increased in response to high salinity as were two protein isoforms with homology to bacterial/archaeal glycine betaine methyltransferase (Lyon et al. 2011). DMSP, which has been found in high concentrations in ice-diatom communities, has been shown to protect enzymes against denaturation in freezing conditions (DiTullio et al. 1998).

Studies on ice-binding proteins in diatoms show that they have antifreeze properties and are able to inhibit ice recrystallization (Gwak et al. 2010; Bayer-Giraldi et al. 2011; Raymond 2011). As several IBPs have similarities to bacterial or fungal sequences, it is hypothesized that they have been acquired through HGT (see Sect. 14.3.1.1) and may have allowed diatoms to colonize sea ice.

An IBP protein in *F. cylindrus* was strongly upregulated (ca. 50-fold) under freezing temperatures (Mock and Valentin 2004), while Bayer-Giraldi et al. (2011) found several isoforms in *F. cylindrus* and *F. curta* to be differentially regulated depending on temperature and salt stress. *F. cylindrus* IBPs in both of these studies have been identified in the recently published genome (Mock et al. 2017). Proteomics studies on *C. neogracile* also showed an increase in concentration of IBPs in response to freezing conditions (Gwak et al. 2010). Furthermore, isolation of IBP transcripts from Arctic and Antarctic sea ice suggests that they are found at similar levels as genes with essential metabolic processes such as photosynthesis (Uhlig et al. 2015). Within the same study, it was found that most IBP transcripts originated from diatoms, haptophytes, and crustaceans; however, many of the IBPs have not been previously characterized (Uhlig et al. 2015). These results support the hypothesis that these proteins are of great importance not only under salt stress but also under freezing temperatures to protect the cells from injury by growing ice crystals.

An important adaptation for polar photosynthetic organisms is the need to survive for periods of prolonged darkness. As discussed in Sect. 14.3.1.1, 7 day darkness in *F. cylindrus* leads to a decrease in photosynthesis and associated processes. Genes which are involved in starch, sugar, and fatty acid metabolism

are upregulated (Mock et al. 2017) suggesting that *F. cylindrus* is able to use existing cellular resources in place of photosynthesis. Diatoms are able to store glucan for use in periods of extended darkness (van Oijen et al. 2003) and are able to uptake molecules such as sugar and starch (Palmisano and Garrison 1993). The urea cycle in diatoms has been suggested as a means to process inorganic carbon and nitrogen, particularly during low nitrogen availability (Allen et al. 2011). All genes for the urea cycle can be found in the *F. cylindrus* genome. Proton-pumping proteorhodopsins, for trace-metal-independent ATP synthesis (Strauss et al. 2013), were upregulated under darkness, suggesting a role in energy production. There are also ATP-independent enzymes available to *F. cylindrus* which may save chemical energy such as pyrophosphate-dependent phospho-fructo-kinase which was elevated during salinity acclimation (Lyon et al. 2011).

Information is steadily becoming available for polar diatoms. New insights are being gained into their adaptations and the importance of their roles in polar communities. Although much has been learned, there are vast numbers of genes with unknown or partially characterized functions in many of these studies. For example, many identified transcripts have no homology to existing sequences (Mock et al. 2005, 2017; Krell 2006), and different FCPs and LHC proteins are both up- and downregulated under the same conditions (Park et al. 2010). Reverse genetics is needed in order to establish the function and roles of these genes and their pathways. A transformation system for *F. cylindrus* has been successfully established—as far as we are aware, this is the first transformation system for any eukaryotic polar species (Hopes and Mock, unpublished). Furthermore, CRISPR-Cas for gene knock-out and gene silencing in the temperate diatoms *Thalassiosira pseudonana* (Hopes et al. 2016; Kirkham and Mock, unpublished) and *Phaeodactylum tricornutum* (Nymark et al. 2016; De Riso et al. 2009) have been established. Work on CRISPR-Cas in *F. cylindrus* is also currently ongoing.

With the establishment of additional, elegant molecular tools for diatoms, there is a much greater scope for potential research and therefore our understanding of these psychrophilic and psychrotolerant organisms and their environment.

14.3.2 Green Algae (Chlorophyceae)

Most polar green algae live in freshwater ecosystems such as snow, permanently ice-covered lakes, or more ephemeral habitats like creeks or melt ponds on top of snow or sea ice. Most species belong either to the genera *Chlamydomonas, Chloromonas*, or *Chlorella*, and many of them are very motile due to the presence of flagella.

Ecologically important species that are physiologically and molecularly well characterized are *Chlamydomonas raudensis, Chlamydomonas nivalis*, and *Chlamydomomas* sp. ICE-L. *C. raudensis* is an abundant species in permanently ice-covered lakes and the clone UWO241 has been studied for decades (see review by Morgan-Kiss et al. 2006). *C. nivalis* is a dominant representative of the snow-

algae community and also intensively studied (Williams et al. 2003). Therefore, this discussion will mainly focus on *Chlamydomonas* sp. There is less research in this area in terms of functional genomics; however, the genome sequencing of *Coccomyxa subellipsoidea* provides some insight into polar adaptations within the Chlorophyceae as does the cold shock EST library for *Pyramimonas gelidicola*.

14.3.2.1 Functional Genomics

Coccomyxa subellipsoidea is a psychrotolerant green alga that has been isolated from dried algal peat in Antarctica, and although it can grow at low temperatures it shows optimal growth at around 20 °C (Blanc et al. 2012). Despite not being a true psychrophile, its genome has some pronounced differences to mesophilic chylorphytes and offers several insights into polar adaptation. Although the genomes of several green algae have been sequenced, this is the first genome to be published from a polar microalga. An EST library has also been generated under cold shock conditions for the psychrophilic *Pyramimonas gelidicola*, a dominant primary producer from Antarctic sea ice (Jung et al. 2012).

In comparison to other sequenced chlorophytes, *C. subellipsoidea* has a large number of mitochondrial and chloroplast sequences integrated into its nuclear genome. GC content of these organelle genomes is also comparatively high. It is important to maintain homeostasis and efficient cellular functions under the extreme conditions found in polar regions. This includes lipid metabolism and membrane fluidity. Four lipid protein families were over-represented in *C. subellipsoidea*: type-I-fatty acid synthases, FA elongases, FA ligases, and type 3 lipases. There were also three fatty acid desaturases present that were not found in temperate counterparts (Blanc et al. 2012). An increase in double bonds in membrane based lipids helps to increase fluidity at cold temperatures (Los and Murata 2004). Within the same species, there were a high number of genes involved in polysaccharide and cell wall metabolism (Blanc et al. 2012). As previously mentioned, both glycoproteins and polysaccharides can act as cryopreservants in microalgae. Two genes involved in cryoprotection with homology to late embryogenesis abundant (LEA) proteins have also been found in *C. subellipsoidea* (Liu et al. 2011).

Structural parts such as the cytoskeleton of the cell also have to be adapted to low temperatures in order to conduct mitosis, meiosis, secretion, and cell motility.

The tubulin alpha chain protein domain was the fifth most abundant in the EST library from *P. gelidicola* (Jung et al. 2012). Willem et al. (1999) showed that alpha-tubulin from two *Chloromonas* spp. had five amino acid substitutions compared to the mesophilic *Chlamydomonas reinhardtii*. Two of these substitutions occurred in the region of inter dimer contacts that could therefore positively influence microtubule assembly under low temperatures.

Translation elongation factor-1a was prominent in ESTs from *P. gelidicola* (Jung et al. 2012). Furthermore, a translation elongation factor-1a was found in the *C. subellipsoidea* genome that is able to functionally replace elongation factor

like EFL found in previously sequenced chlorophytes. Upregulation of an elongation factor involved in protein synthesis has also been observed in cold shock diatoms (Mock and Valentin 2004).

Given that polar species may be exposed to freezing temperatures and high light, many adaptive strategies include proteins involved in stress response and protection against ROS. DOPA-dioxygenase which provides protection against solubilized oxygen was identified in the *C. subellipsoidea* genome, as were two genes with homologs to phospholipase D and chalcone synthase. The former is involved in stress response, while homologs of the latter are involved in metabolites for UV photoprotection and antimicrobial defense in plants (Blanc et al. 2012).

Both heat shock protein 70 (hsp70) and stress-related chlorophyll a/b binding protein were enriched in *P. gelidicola* ESTs. Heat shock protein 70 appears to be a key component involved in adaptation of several polar microalgae species (Mock and Valentin 2004; Krell 2006; Liu et al. 2010).

When comparing *C. subellipsoidea* to temperate chlorophytes, Blanc et al. (2012) found that as well as enrichment of certain gene families and gene additions there were also several key genes missing. This includes PsaN, which is involved in docking plastocyanin to the PSI complex. This leads to a drop in electron transfer from plastocyanin to PSI which may be beneficial in a polar environment as low temperatures create an excess of electrons through this system which in turn leads to an increase in ROS. As PsaN is not crucial for photosynthesis, loss of this gene may protect the cell from oxidative damage (Blanc et al. 2012). *C. subellipsoidea* also has genes for dioxygenase and FA desaturases that utilize dioxygen and therefore may provide further protection against ROS (Blanc et al. 2012).

One gene loss which could reduce cellular efficiency in *C. subellipsoidea*, however, is a pyruvate phosphate dikinase (PPDK), which produces ATP through glycolysis. Function of this gene appears to be replaced by three pyruvate kinases, which potentially produce less chemical energy (Blanc et al. 2012).

In terms of nutrient acquisition, *C. subellipsoidea* has a large number of genes for amino acid permeases and transporters which may enhance uptake of organic nutrients. It also has cobalamin-dependent methionine synthase but lacks the cobalamin-dependent version of this gene MetH (Blanc et al. 2012), suggesting that this species is not dependent on this often bacteria-associated cofactor (Croft et al. 2005) for synthesis of this important amino acid.

There is still much to discover in establishing the function and origins of many genes specific to polar species. There were a higher number of ESTs with unknown functions under freezing conditions in *P. gelidicola* compared to 4 °C (Jung et al. 2012). Furthermore, there are over 2300 genes in the *C. subellipsoidea* genome with no known homologs in sequenced mesophilic chlorophytes. The majority of these genes show homology to Streptophytes and other Eukaryotes, suggesting origins from a common ancestor to chlorophytes. Interestingly rather than displaying homology to green algae, most of the genes involved in defense, detoxification, and carbohydrate metabolism show higher sequence similarity to bacteria, suggesting possible acquisition by HGT.

As discussed in Sect. 14.3.1.1, ice-binding proteins in diatoms appear to have bacterial or fungal origins. Several IBPs have also been identified in psychrophilic or psychrotolerant green algae including *Pyramimonas gelidicola* (Jung et al. 2014), *Chlamydomonas raudensis* (Raymond and Morgan-Kiss 2013), *Chlamydomonas* sp. strain CCMP681 (Raymond et al. 2009), *Chloromonas sp.* (Jung et al. 2016) and *Chloromonas brevispina* (Raymond 2014). Raymond and Morgan-Kiss (2013) separate ice-binding proteins into two different groups: IBP I, a group of similar proteins appearing to have fungal or bacterial origins (Raymond and Morgan-Kiss 2013; Sorhannus 2011; Raymond and Kim 2012; Jung et al. 2014; Raymond 2011) and IBP II. So far all studied algal species have type I IBPs with the exception of *Chlamydomonas* sp. strain CCMP681 which has four type II isoforms isolated from ESTs (Raymond et al. 2009; Raymond and Morgan-Kiss 2013). A polyphyletic origin for IBPs has been suggested given their sequential and structural differences, as well as a lack of IBPs in temperate species (Fig. 14.13; Raymond and Morgan-Kiss 2013).

As more genomes and transcriptomes become available for polar chlorophytes, more light can be shed on their intricacies and adaptations to extreme environments. The genome of an important Antarctic sea ice chlorophyte, *Chlamydomonas* ICE-L, has been recently sequenced (personal communication with Naihao Ye, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao 266071, China) and Raymond and Morgan-Kiss (2013) plan to compare the transcriptome of *C. raudensis* to a temperate counterpart.

14.3.2.2 Molecular Physiology

Maximum growth rates of polar green algae are comparable to those from polar diatoms. They range from 0.2 to 0.4 day^{-1} (Tang et al. 1997). Temperatures above 18 °C are mostly lethal to these algae. *C. raudensis* has its maximum photosynthetic rates at 8 °C, which declines steadily with increasing temperatures (Morgan-Kiss et al. 2006). This indicates maximal efficiency in converting light into photosynthetic energy at low temperatures. The quality of light also plays an important role, and *C. raudensis* is not able to grow under red light (Morgan-Kiss et al. 2005). This is probably a consequence of almost never being exposed to a longer wavelength spectrum in the natural habitat of permanently ice covered lakes where the ice absorbs all longer wavelengths of solar irradiance (Fritsen and Priscu 1999; Morgan-Kiss et al. 2006). However, the majority of this light is reflected on the white surface of ice and scattered while passing through ice. Thus, the environment below the ice is characterized by low intensities enriched in blue-green wavelengths (Lizotte and Priscu 1992).

Many physiological and molecular investigations have been conducted with *C. raudensis* to find the reasons for successful photo adaptation under these extreme conditions. A comparison with the temperate *C. reinhardtii* partly uncovered the mechanisms of photo adaptation in *C. raudensis* (Morgan-Kiss et al. 2005, 2006): In contrast to the temperate *C. reinhardtii*, the psychrophile has lost its ability to

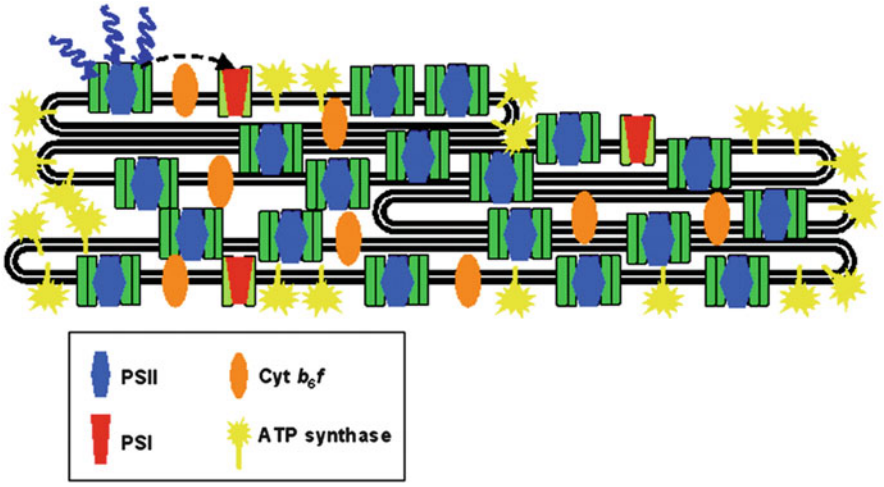

Fig. 14.14 Model for organization of thylakoid pigment–protein complexes of the electron transport chain in the psychrophilic *Chlamydomonas raudensis* UWO 241. In the natural, extremely stable light environment of extreme shade and predominantly *blue-green* wavelengths (*blue lines*), the majority of available light would be preferentially absorbed by PSII. Adaptation in *C. raudensis* to this light environment has led to an unusually high PSII/PSI stoichiometry and highly efficient energy transfer from LHCII to PSII. Conversely, PSI and associated light-harvesting complexes are both structurally and functionally downregulated. Given the severe reduction in light-harvesting capacity of PSI, it is proposed that PSI centers are largely excited via a spillover energy transfer mechanism from PSII (*dotted line*). Photosynthetic membranes may be arranged as loose stacks rather than distinct granal and stromal regions to promote energy spillover between the photosystems. Picture from Morgan-Kiss et al. (2006)

live under high light but increased its efficiency of light harvesting under low light in the blue-green spectrum. This adaptation can be seen in structural changes of the photosynthetic apparatus (Fig. 14.14). For instance, *C. raudensis* has an unusually high ratio of photosystem II to I and significantly higher levels of light-harvesting II complexes than its temperate counterpart *C. reinhardtii*. These changes are probably an adaptive advantage under constant exposure to blue light of low photon flux densities because the light-harvesting apparatus of photosystem II (PSII) utilizes chlorophyll b and short-wavelength-absorbing chlorophyll a to absorb light predominantly in the blue region. Interestingly, most marine algae (e.g., red algae, diatoms), which are also living in a blue-green light environment because of optical properties of the seawater, also show a high ratio of PSII to PSI due to chromatic regulation (Fujita 2001). However, most of them, and even psychrophilic diatoms, have the physiological ability to grow under high irradiance levels.

While the ability to dissipate excess energy through NPQ has been reduced in *C. raudensis* (Morgan-Kiss et al. 2006), other polar species in this genera have retained this ability which allows them to photosynthesize under high-light conditions. *Chlamydomonas* sp. ICE-L shows an upregulation of light-harvesting complex (LHC) genes LhcSR1 and LhcSR2, accompanied by an increase in NPQ

following high light, UV-B radiation, and high salinity. This suggests that these LHC genes play a role in stress response and energy dissipation (Mou et al. 2012). In order to mitigate photoinhibition, a psychrotolerant *Chlorella* sp. isolated from Arctic glacier melt water decreases the size of its light-harvesting complex (Cao et al. 2016).

Another interesting similarity between diatoms (psychrophilic and temperate) and *C. raudensis* is the biochemistry and architecture of the thylakoid membrane. Diatoms, as well as *C. raudensis*, have high concentrations of polyunsaturated fatty acids in their thylakoid lipids classes, and their thylakoid membranes are not organized in grana and stroma (Mock and Kroon 2002a, b; Morgan-Kiss et al. 2006). This possibly means that looser membrane stacks in *C. raudensis* and homogeneously folded membranes in diatom plastids promote energy spill over between photosystems and therefore light energy transfer between photosystems (Morgan-Kiss et al. 2006).

An increase in transcripts for omega-3 fatty acid desaturase (CiFAD3) was measured in a *Chlamydomonas* sp. ICE-L under both high (12 °C) and low temperatures (0 °C) compared to a control at 6 °C, as well as at high salinity (Zhang et al. 2011; An et al. 2013). This suggests that PUFAs may also play a role in heat stress and high salinity acclimation. Consumption of PUFAs was also observed in the same species during darkness (Xu et al. 2014), indicating that PUFAs are an important aspect of adaptation to several extreme conditions found in the polar regions. As with diatoms, antioxidants also play an important role in cold-shock adaptation, as seen in an Antarctic *Chlamydomonas* sp. in which an increase in glutathione S-transferase was observed (Kan et al. 2006).

The snow alga *C. nivalis* is exposed to the full spectrum of solar irradiance (UVC to infrared) and must therefore have a completely different photosynthesis performance compared to the low light adapted *C. raudensis* (Remias et al. 2005). The most striking difference between photosynthesis of both psychrophilic green algae is that *C. nivalis* does not seem to be inhibited by high solar irradiances. Even an exposure of cells to photon flux densities of 1800 μmol photons m^{-2} s^{-1} for 40 min at 1.5 °C did not inhibit net photosynthesis (Remias et al. 2005). This extreme photosynthetic performance is only possible by a change in the life cycle. A combination of factors may trigger the formation of immotile red hypnoblast stages that are most resistant to environmental changes (Müller et al. 1998; Remias et al. 2005).

The transformation into hypnoblasts is characterized by a substantial incorporation of sugars and lipids and by the formation of esterified extraplastidal secondary carotenoids (Hoham and Duval 2001). The most important carotenoid is astaxanthin which is located in cytoplasmatic lipid globuli (Müller et al. 1998; Remias et al. 2005) and is assumed to be responsible for the high photostability and therefore the absence of photoinhibition under strong solar irradiance on top of snow (Remias et al. 2005). Mature hypnoblasts can contain about 20 times more of this pigment than chlorophyll a, where the astaxanthin is possibly acting as a filter to reduce the irradiance that would otherwise be damaging to the photosynthetic activity inside the plastids. Exposure to UV-B in *Chlamydomonas* sp. ICE-L led to

an increase in expression of heat shock protein 70 (Liu et al. 2010) which suggests a role in protection against high irradiance.

High solar irradiance is not the only harsh condition on top of snow. Drought due to freezing of water is another main stress on the hypnoblast stages of *Chlamydomonas nivalis*. Like cacti in the desert, these stages have very rigid cell walls as the outer boundary to an extreme environment (Müller et al. 1998; Remias et al. 2005). Sometimes cells secrete carbohydrates to produce a visible mucilage sheet around them (Müller et al. 1998). These carbohydrates are not only attractive to bacteria that use them as a substrate but they also trap particles transported into the snow by wind. These particle-covered cells increase the absorption of solar irradiance and therefore the production of heat. This heat might cause melting of surrounding snow crystals and therefore provide liquid water to the cells (Takeuchi 2002). Such small spots of melt events around warm bodies (e.g., rock debris, cells) are called cryoconite holes (Takeuchi 2002). However, these adhering particles may also shade and thus protect *C. nivalis* against high irradiance. This is not universal and hypnoblasts from *C. nivalis*, for example, never show such attached structures.

Chemical reactions are influenced by temperature according to the relationship described by Arrhenius. In general, a 10 °C reduction in growth temperature causes biochemical reaction rates to decline 2–3 times. However, doubling times of psychrophilic algae can be comparable to mesophilic algae (Sommer 1989) which means that rates of enzyme catalyzed reactions must be optimized to low temperatures in these organisms (Feller and Gerday 2003). Studies with the enzyme nitrate reductase (NR), for instance, showed that these enzymes from psychrophilic algae possess structural modifications that make them more cold adapted, being more catalytically efficient at lower temperatures but at the same time less thermally stable, than NRs from mesophilic species (Di Martino Rigano et al. 2006). It also appears that light and salinity may influence nitrogen metabolism in *Chlamydomonas* sp. ICE-L (Wang et al. 2015).

In contrast to NR, the temperature maximum for carboxylase activity of ribulose-1-5-bisphosphatecarboxylase/oxygenase (RUBISCO), one of the most critical enzymes for inorganic carbon fixation in photoautotrophes, was not altered in some psychrophilic green algae and the specific activity at low temperatures was actually lower in the psychrophilic if compared to the mesophilic Rubisco (Devos et al. 1998). Decreased catalytic efficiency of these RUBISCOs under low temperature seems to be at least partly compensated by an increased cellular concentration of the protein. This is supported by the presence of RUBISCO as the fifth most abundant EST in *P. gelidicola* (Jung et al. 2012). An increase in ribosomal proteins seen at colder temperatures may counteract reduced efficiencies in translation (Toseland et al. 2013); alternatively, it may help to cope with upregulation of proteins due to reduced activity. Cao et al. (2016) found that a strain of arctic *Chlorella* increased both proteins and lipids at lower temperatures.

Expression and secretion of ice-binding proteins in polar Chlorophytes helps to maintain a fluid environment and reduce damage from ice crystals. Studies which look at IBPs through recombinant proteins and culture supernatant have demonstrated functions including changes in ice morphology, ice pitting, recrystallization

inhibition, and the creation of smaller brine pockets which helps to maintain salinity (Raymond et al. 2009; Raymond and Kim 2012; Raymond and Morgan-Kiss 2013; Jung et al. 2014).

As with diatoms, molecular tools are constantly improving for Chlorophytes, including techniques for activating gene expression with transcription activator-like effectors (TALEs) (Gao et al. 2014) and gene editing with CRISPR-Cas (Shin et al. 2016; Wang et al. 2016). So far only temperate green algae have been selected for targeted gene knock-out, but as molecular tools such as these become available in their polar counter-parts, the potential to discover the function and role of important genes and pathways in polar adaptation drastically increases.

14.4 Conclusions

The application of omics approaches in combination with biochemical and physiological measurements has revealed unique adaptations in polar microalgae. Unsurprisingly, there is evidence that the extreme and highly variable conditions in polar ecosystems were driving those adaptations. While some of these adaptations (e.g., allelic divergence, gene duplications) were the consequence of mutations and subsequent diversification, others were based on biotic interactions that enabled transfer of genes (e.g., ice-binding) between different species and therefore the entire community to thrive under the extreme conditions of polar ecosystems. These mechanisms of adaptive evolution are not unique to polar microalgae but how they are used to produce unique phenotypes required to survive temperatures below freezing, long periods of darkness, strong seasonality, and fluctuations in nutrients and salinity is still unknown. Once we have obtained genetically tractable model species such as *Fragilariopsis cylindrus* and *Chlamydomonas* sp. ICE, we'll be able to better understand how genotypes impact phenotypes that matter to thrive under polar conditions. With these model species, we will be able to test, through experimental evolution approaches, how their populations respond to global warming, which is still largely unknown. Results from these model species can be used to inform studies on natural populations (e.g., barcoding, metatranscriptomes, and metagenomes) in terms of identifying their standing pool of genetic variation and evolutionary potential to respond to global warming. Identification of genetic diversity in these organisms not only provides new insights into their evolution and adaptation but also contributes to extend the pool of marine genetic resources, which so far is dominated by genes and their products from non-polar organisms.

References

Allen DJ, Ort DR (2001) Impacts of chilling temperatures on photosynthesis in warm-climate plants. Trends Plant Sci 6:36–42. doi:10.1016/S1360-1385(00)01808-2

Allen AE, Dupont CL, Oborník M et al (2011) Evolution and metabolic significance of the urea cycle in photosynthetic diatoms. Nature 473:203–207

An M, Mou S, Zhang X et al (2013) Expression of fatty acid desaturase genes and fatty acid accumulation in Chlamydomonas sp. ICE-L under salt stress. Bioresour Technol 149:77–83. doi:10.1016/j.biortech.2013.09.027

Armbrust EV, Berges JA, Bowler C, Green BR, Martinez D, Putnam NH, Zhou S, Allen AE, Apt KE, Bechner M, Brzezinski MA, Chaal BK, Chiovitti A, Davis AK, Demarest MS, Detter JC, Glavina T, Goodstein DS (2004) The genome of the diatom Thalassiosira pseudonana: ecology, evolution, and metabolism. Science 306:79–86

Arrigo KR, Thomas DN (2004) Large scale importance of sea ice biology in the Southern Ocean. Antarct Sci 16:471–486. doi:10.1017/S0954102004002263

Aslam SN, Cresswell-Maynard T, Thomas DN, Underwood GJC (2012) Production and characterization of the intra- and extracellular carbohydrates and polymeric substances (Eps) of three sea-ice diatom species, and evidence for a cryoprotective role for Eps. J Phycol 48:1494–1509. doi:10.1111/jpy.12004

Bayer-Giraldi M, Uhlig C, John U et al (2010) Antifreeze proteins in polar sea ice diatoms: diversity and gene expression in the genus *Fragilariopsis*. Environ Microbiol 12:1041–1052. doi:10.1111/j.1462-2920.2009.02149.x

Bayer-Giraldi M, Weikusat I, Besir H, Dieckmann G (2011) Characterization of an antifreeze protein from the polar diatom *Fragilariopsis cylindrus* and its relevance in sea ice. Cryobiology 63:210–219. doi:10.1016/j.cryobiol.2011.08.006

Beil U, Thiede J (1990) Geophysical history of polar oceans: Arctic versus Antarctic. Kluwer Academic Publishers, Netherlands

Blanc G, Agarkova I, Grimwood J et al (2012) The genome of the polar eukaryotic microalga Coccomyxa subellipsoidea reveals traits of cold adaptation. Genome Biol 13:R39. doi:10.1186/gb-2012-13-5-r39

Bluhm BA, Swadling KM, Gradinger R (2017) Sea ice as a habitat for macrograzers. In: Thomas DN (ed) Sea ice, 3rd edn. Wiley-Blackwell, Oxford, pp 394–414

Bowman JP, Sa MC, Brown MV et al (1997) Diversity and association of psychrophilic bacteri in Antarctic sea ice. Appl Environ Microbiol 63:3068–3078

Boyd PW (2002) Review of environmental factors controlling phytoplankton processes in the Southern Ocean 1. J Phycol 38:844–861. doi:10.1046/j.1529-8817.2002.t01-1-01203.x

Boyd PW, Jickells T, Law CS et al (2007) Mesoscale iron enrichment experiments 1993-2005: synthesis and future directions. Science 315:612–617. doi:10.1126/science.1131669

Brierley AS, Thomas DN (2002) Ecology of Southern Ocean pack ice. Adv Mar Biol 43:171–276

Cannone N, Guglielmin M, Gerdol R (2004) Relationships between vegetation patterns and periglacial landforms in northwestern Svalbard. Polar Biol 27:562–571. doi:10.1007/s00300-004-0622-4

Cao K, He M, Yang W et al (2016) The eurythermal adaptivity and temperature tolerance of a newly isolated psychrotolerant Arctic *Chlorella* sp. J Appl Phycol 28:877–888. doi:10.1007/s10811-015-0627-0

Caron DA, Gast RJ, Garneau M-E (2017) Sea ice for a habitat for micrograzers. Sea ice. In: Thomas DN (ed) Sea ice, 3rd edn. Wiley-Blackwell, Oxford, pp 370–393

Cheng CHC (1998) Evolution of the diverse antifreeze proteins. Curr Opin Genet Dev 8:715–720. doi:10.1016/S0959-437X(98)80042-7

Cockell CS, Stokes MD (2004) Widespread colonization by polar hypoliths H. Nature 431:414. doi:10.1038/nature03019

Comiso J (2003) Large-scale characteristics and variability of the global sea ice cover. In: Thomas DN, Dieckmann G (eds) Sea ice: an introduction to its physics, chemistry, biology and geology. Blackwell Science, Oxford, pp 112–142

Cota GF (1985) Photoadaptation of high Arctic ice algae. Nature 315:219–222

Cottier F, Steele M, Nilsen F (2017) Sea ice and Arctic Ocean oceanography. In: Thomas DN (ed) Sea ice, 3rd edn. Wiley-Blackwell, Oxford, pp 197–215

Croft MT, Lawrence AD, Raux-Deery E et al (2005) Algae acquire vitamin B12 through a symbiotic relationship with bacteria. Nature 438:90–93. doi:10.1038/nature04056

De Riso V, Raniello R, Maumus F et al (2009) Gene silencing in the marine diatom Phaeodactylum tricornutum. Nucleic Acids Res 37:e96. doi:10.1093/nar/gkp448

Deming JW (2002) Psychrophiles and polar regions. Curr Opin Microbiol 5:301–309

Devos N, Ingouff M, Loppes R, Matagne RF (1998) Rubisco adaptation to low temperatures: a comparative study in psychrophilic and mesophilic unicellular algae. J Phycol 34:655–660. doi:10.1046/j.1529-8817.1998.340655.x

Di Martino Rigano V, Vona V, Lobosco O et al (2006) Temperature dependence of nitrate reductase in the psychrophilic unicellular alga *Koliella antarctica* and the mesophilic alga *Chlorella sorokiniana*. Plant Cell Environ 29:1400–1409. doi:10.1111/j.1365-3040.2006.01523.x

Dieckmann GS, Hellmer HH (2003) The importance of sea ice: an overview. In: Thomas DN, Dieckmann GS (eds) Sea ice – an introduction to its physics, chemistry, biology and geology. Blackwell Science, Oxford, pp 1–21

Dittmar T, Kattner G (2003) The biogeochemistry of the river and shelf ecosystem of the Arctic Ocean: a review. Mar Chem 83:103–120. doi:10.1016/S0304-4203(03)00105-1

Ditullio GR, Garrison DL, Mathot S (1998) Dimethylsulfonopropionate in sea ice algae from the Ross Sea polynya. In: Lizotte M, Arrigo K (eds) Antarctic sea ice: biological processes, interaction and variability, Antarctic research series, vol 73, pp 139–146

Eddie B, Krembs C, Neuer S (2008) Characterization and growth response to temperature and salinity of psychrophilic, halotolerant *Chlamydomonas* sp. ARC isolated from Chukchi Sea ice. Mar Ecol Prog Ser 354:107–117. doi:10.3354/meps07243

Ehrenberg CG (1841) Einen Nachtrag zu dem Vortrage über Verbreitung und Einfluß des mikroskopischen Lebens in Süd- und Nordamerika. Berichte über die zur Bekanntmachung geeigneten Verhandlung der K Akad der Wissenschaften zu Berlin. Monatsberichte 1841:202–207

Ehrenberg CG (1853) Über neue Anschauungen des kleinstein nördlichen Polarlebens. Berichte über die zur Bekanntmachung geeigneten Verhandlung der Königlich-Preussischen Akad der Wissenschaften zu Berlin. Monatsberichte 1853:522–529

Eicken H (1992) The role of sea ice in structuring Antarctic ecosystems. Polar Biol 12:3–13

Eicken H, Bock C, Wittig R et al (2000) Magnetic resonance imaging of sea-ice pore fluids: methods and thermal evolution of pore microstructure. Cold Reg Sci Technol 31:207–225. doi:10.1016/S0165-232X(00)00016-1

Feller G, Gerday C (2003) Psychrophilic enzymes; hot topics in cold adaptation. Nat Rev Microbiol 1:200–208

Fiala M, Oriol L (1990) Light-temperature interactions on the growth of Antarctic diatoms. Polar Biol 10:629–636

Friedmann EI, Kappen L, Meyer MA et al (1993) Long-term productivity in the cryptoendolithic microbial community of the Ross Desert, Antarctica. Microb Ecol 25:51–69

Fritsen CH, Priscu JC (1999) Seasonal change in the optical properties of the permanent ice cover on Lake Bonney, Antarctica: consequences for lake productivity and phytoplankton dynamics. Limnol Oceanogr 44(2):447–454

Fujita Y (2001) Chromatic variation of the abundance of PSII complexes observed with the red alga Prophyridium cruentum. Plant Cell Physiol 42:1239–1244. doi:10.1093/pcp/pce164

Gao H, Wright D, Li T et al (2014) TALE activation of endogenous genes in Chlamydomonas reinhardtii. Algal Res 5:52–60. doi:10.1016/j.algal.2014.05.003

Gleitz M, Thomas DN (1993) Variation in phytoplankton standing stock, chemical composition and physiology during sea-ice formation in the southeastern Weddell Sea, Antarctica. J Exp Mar Biol Ecol 173:211–230. doi:10.1016/0022-0981(93)90054-R

Gleitz M, v.d. Loeff MR, Thomas DN et al (1995) Comparison of summer and winter inorganic carbon, oxygen and nutrient concentrations in Antarctic sea ice brine. Mar Chem 51:81–91. doi:10.1016/0304-4203(95)00053-T

Gleitz M, Bartsch A, Dieckmann GS, Eicken H (1998) Composition and succession of sea ice diatom assemblages in the eastern and southern Weddell Sea, Antarctica. Antarct Res Ser 73:107–120

Granskog M, Kaartokallio H, Kuosa H et al (2006) Sea ice in the Baltic Sea – a review. Estuar Coast Shelf Sci 70:145–160. doi:10.1016/j.ecss.2006.06.001

Guo S, Garnham CP, Whitney JC, Graham LA, Davies PL, Hensel M (2012) Re-evaluation of a bacterial antifreeze protein as an adhesin with ice-binding activity. PLoS One 7(11):e48805

Gwak IG, sic Jung W, Kim HJ et al (2010) Antifreeze protein in Antarctic marine diatom, *Chaetoceros neogracile*. Mar Biotechnol 12:630–639. doi:10.1007/s10126-009-9250-x

Haas C (2017) Sea ice thickness distribution. In: Thomas DN (ed) Sea ice, 3rd edn. Wiley-Blackwell, Oxford, pp 42–64

Haas C, Thomas DN, Bareiss J (2001) Surface properties and processes of perennial Antarctic sea ice in summer. J Glaciol 47:613–625. doi:10.3189/172756501781831864

Hällfors G (2004) Checklist of Baltic Sea phytoplankton species (including some heterotrophic protistan groups). Balt Sea Environ Proc 95:208

Hansom J, Gordon J (1998) Antarctic environments and resources – a geographical perspective. Addison Wesley Longman, Harlow

Hendey NI (1974) A revised checklist of British diatoms. J Mar Biol Assoc UK 54:277–300

Hodson AJ, Mumford PN, Kohler J, Wynn PM (2005) The High Arctic glacial ecosystem: new insights from nutrient budgets. Biogeochemistry 72:233–256. doi:10.1007/s10533-004-0362-0

Hoham RW, Duval B (2001) Microbial ecology of snow and freshwater ice with emphasis on snow algae. In: Jones HG, Pomeroy JW, Walker DA, Hoham RW (eds) Snow ecology: an interdisciplinary examination of snow-covered ecosystems. Cambridge University Press, Cambridge, pp 168–228

Hooker J (1847) The botany of the Antarctic voyage of H.M. Discovery ships Erebus and Terror in the years 1838–1843 Part 1. Flora Antarctica. Reeve Brothers, London

Hopes A, Nekrasov V, Kamoun S, Mock T (2016) Editing of the urease gene by CRISPR-Cas in the diatom *Thalassiosira pseudonana*. Plant Methods 12:49–60. doi:10.1186/s13007-016-0148-0

Horner R (1985) Sea ice biota. CRC Press, Baco Raton, FL

Hsiao S (1983) A checklist of marine phytoplankton and sea ice microalgae recorded from Arctic Canada. Nova Hedwigia 37:225–314

Hwang YS, Jung G, Jin E (2008) Transcriptome analysis of acclimatory responses to thermal stress in Antarctic algae. Biochem Biophys Res Commun 367:635–641. doi:10.1016/j.bbrc.2007.12.176

Ikävalko J, Gradinger R (1997) Flagellates and heliozoans in the Greenland Sea ice studied alive using light microscopy. Polar Biol 17:473–481. doi:10.1007/s003000050145

Janech MG, Krell A, Mock T et al (2006) Ice-binding proteins from sea ice diatoms (Bacillariophyceae). J Phycol 42:410–416. doi:10.1111/j.1529-8817.2006.00208.x

Janknegt PJ, Van De Poll WH, Visser RJW et al (2008) Oxidative stress responses in the marine antarctic diatom *Chaetoceros brevis* (Bacillariophyceae) during photoacclimation. J Phycol 44:957–966. doi:10.1111/j.1529-8817.2008.00553.x

Johnston CG, Vestal JR (1991) Photosynthetic carbon incorporation and turnover in Antarctic cryptoendolithic microbial communities: are they the slowest-growing communities on earth? Appl Environ Microbiol 57:2308–2311

Jones EP, Swift JH, Anderson LG et al (2003) Tracing Pacific water in the North Atlantic Ocean. J Geophys Res 108:1–10. doi:10.1029/2001JC001141

Jung W, Lee SG, Kang SW et al (2012) Analysis of expressed sequence tags from the Antarctic psychrophilic green algae, *Pyramimonas gelidicola*. J Microbiol Biotechnol 22:902–906. doi:10.4014/jmb.1201.01002

Jung W, Gwak Y, Davies PL et al (2014) Isolation and characterization of antifreeze proteins from the Antarctic marine microalga *Pyramimonas gelidicola*. Mar Biotechnol 16:502–512. doi:10.1007/s10126-014-9567-y

Jung W, Campbell RL, Gwak Y et al (2016) New cysteine-rich ice-binding protein secreted from antarctic microalga, chloromonas sp. PLoS ONE 11:1–26. doi:10.1371/journal.pone.0154056
Junge K, Imhoff F, Staley T, Deming JW (2002) Phylogenetic diversity of numerically important Arctic sea-ice bacteria cultured at subzero temperature. Microb Ecol 43:315–328. doi:10.1007/s00248-001-1026-4
Junge K, Eicken H, Deming JW (2004) Bacterial Activity at -2 to -20 degrees C in Arctic wintertime sea ice. Appl Environ Microbiol 70:550–557. doi:10.1128/AEM.70.1.550
Kan GF, Miao JL, Shi CJ, Li GY (2006) Proteomic alterations of antarctic ice microalga Chlamydomonas sp. under low-temperature stress. J Integr Plant Biol 48:965–970. doi:10.1111/j.1744-7909.2006.00255.x
Kattner G, Thomas DN, Haas C et al (2004) Surface ice and gap layers in Antarctic sea ice: highly productive habitats. Mar Ecol Prog Ser 277:1–12. doi:10.3354/meps277001
Kennedy H, Thomas DN, Kattner G et al (2002) Particulate organic matter in Antarctic summer sea ice: concentration and stable isotopic composition. Mar Ecol Prog Ser 238:1–13. doi:10.3354/meps238001
Kirst GO, Wiencke C (1995) Ecophysiology of polar algae. J Phycol 31(2):181–199
Kooistra WHCF, Medlin LK (1996) Evolution of the diatoms (Bacillariophyta). Mol Phylogenet Evol 6:391–407
Kopczynska EE, Weber LH, EI-Sayed SZ (1986) Phytoplankton species composition and abundance in the Indian sector of the Antarctic Ocean. Polar Biol 6:161–169
Krell A (2006) Salt stress tolerance in the psychrophilic diatom *Fragilariopsis cylindrus*. Dissertation, University of Bremen, Germany
Krell A, Beszteri B, Dieckmann G et al (2008) A new class of ice-binding proteins discovered in a salt-stress-induced cDNA library of the psychrophilic diatom *Fragilariopsis cylindrus* (Bacillariophyceae). Eur J Phycol 43:423–433. doi:10.1080/09670260802348615
Krembs C, Engel A (2001) Abundance and variability of microorganisms and transparent exopolymer particles across the ice-water interface of melting first-year sea ice in the Laptev Sea (Arctic). Mar Biol 138:173–185. doi:10.1007/s002270000396
Krembs C, Gradinger R, Spindler M (2000) Implications of brine channel geometry and surface area for the interaction of sympagic organisms in Arctic sea ice. J Exp Mar Biol Ecol 243:55–80. doi:10.1016/S0022-0981(99)00111-2
Krembs C, Eicken H, Deming JW (2011) Exopolymer alteration of physical properties of sea ice and implications for ice habitability and biogeochemistry in a warmer Arctic. Proc Natl Acad Sci U S A 108:3653–3658. doi:10.1073/pnas.1100701108
Leventer A (1998) The fate of Antarctic "Sea ice diatoms" and their use as paleoenvironmental indicators. Antarct Res Ser 73:121–137
Liu S, Zhang P, Cong B et al (2010) Molecular cloning and expression analysis of a cytosolic Hsp70 gene from Antarctic ice algae *Chlamydomonas* sp. ICE-L. Extremophiles 14:329–337. doi:10.1007/s00792-010-0313-8
Liu X, Wang Y, Gao H, Xu X (2011) Identification and characterization of genes encoding two novel LEA proteins in Antarctic and temperate strains of *Chlorella vulgaris*. Gene 482:51–58. doi:10.1016/j.gene.2011.05.006
Lizotte MP (2001) The contributions of sea ice algae to Antarctic marine primary production. Am Zool 41:57–73. doi:10.1668/0003-1569(2001)041[0057:TCOSIA]2.0.CO;2
Lizotte M (2003a) Microbiology of sea ice. In: Thomas DN, Dieckmann GS (eds) Sea ice – an introduction to its physics, chemistry, biology and geology. Blackwell Science, Oxford, pp 184–210
Lizotte M (2003b) The influence of sea ice on Ross Sea biogeochemical processes. Antarct Res Ser 78:107–122
Lizotte MP, Priscu J (1992) Spectral irradiance and biooptical properties in perennial ice-covered lakes of the dry valleys (McMurdo Sound Antarctica). Antarct Res Ser 57:1–14
Los D, Murata N (2004) Membrane fluidity and its roles in the perception of environmental signals. Biochim Biophys Acta Biomembr 1666:142–157. doi:10.1016/j.bbamem.2004.08.002

Lovejoy C, Massana R, Pedro C (2006) Diversity and distribution of marine microbial eukaryotes in the Arctic Ocean and adjacent seas diversity. Appl Environ Microbiol 72:3085–3095. doi:10.1128/AEM.72.5.3085

Lyon B, Mock T (2014) Polar microalgae: new approaches towards understanding sdaptations to an extreme and changing environment. Biology (Basel) 3:56–80. doi:10.3390/biology3010056

Lyon BR, Lee P, Bennett JM et al (2011) Proteomic analysis of a sea-ice diatom: salinity acclimation provides new insight into the dimethylsulfoniopropionate production pathway. Plant Physiol 157:1926–1941. doi:10.1104/pp.111.185025

Meier W (2017) Losing Arctic sea ice: observations of th erecent decline and the long-term context. In: Thomas DN (ed) Sea ice, 3rd edn. Wiley-Blackwell, Oxford, pp 261–289

Meredith MP, Brandon M (2017) Oceanography and sea ice in the Southern Ocean. In: Thomas DN (ed) Sea ice, 3rd edn. Wiley-Blackwell, Oxford, pp 216–238

Merico A, Tyrrell T, Brown CW et al (2003) Analysis of satellite imagery for *Emiliania huxleyi* blooms in the Bering Sea before 1997. Geophys Res Lett 30:1337. doi:10.1029/2002GL016648

Mindl B, Anesio AM, Meirer K et al (2007) Factors influencing bacterial dynamics along a transect from supraglacial runoff to proglacial lakes of a high Arctic glacieri. FEMS Microbiol Ecol 59:307–317. doi:10.1111/j.1574-6941.2006.00262.x

Mock T, Gradinger R (1999) Determination of Arctic ice algal production with a new in situ incubation technique. Mar Ecol Prog Ser 177:15–26. doi:10.3354/Meps177015

Mock T, Hoch N (2005) Long-term temperature acclimation of photosynthesis in steady-state cultures of the polar diatom *Fragilariopsis cylindrus*. Photosynth Res 85:307–317. doi:10.1007/s11120-005-5668-9

Mock T, Kroon BM (2002a) Photosynthetic energy conversion under extreme conditions—I: important role of lipids as structural modulators and energy sink under N-limited growth in Antarctic sea ice diatoms. Phytochemistry 61:41–51. doi:10.1016/S0031-9422(02)00216-9

Mock T, Kroon BM (2002b) Photosynthetic energy conversion under extreme conditions—II: the significance of lipids under light limited growth in Antarctic sea ice diatoms. Phytochemistry 61:53–60. doi:10.1016/S0031-9422(02)00216-9

Mock T, Valentin K (2004) Photosynthesis and cold acclimation: molecular evidence from a polar diatom. J Phycol 40:732–741. doi:10.1111/j.1529-8817.2004.03224.x

Mock T, Thomas D (2005) Sea ice – recent advances in microbial studies. Environ Microbiol 7:605–619

Mock T, Krell A, Glöckner G et al (2005) Analysis of expressed sequence tags (ESTS) from the polar diatom *Fragilariopsis cylindrus*. J Phycol 42:78–85. doi:10.1111/j.1529-8817.2005.00164.x

Mock T, Otillar RP, Strauss J et al (2017) Evolutionary genomics of the cold-adapted diatom *Fragilariopsis cylindrus*. Nature 541:536–540. doi:10.1038/nature20803

Morgan-Kiss RM, Ivanov AG, Pocock T et al (2005) The antarctic psychrophile, *Chlamydomonas raudensis* Ettl (UWO241) (Chlorophyceae, Chlorophyta), exhibits a limited capacity to photoacclimate to red light. J Phycol 41:791–800. doi:10.1111/j.1529-8817.2005.04174.x

Morgan-Kiss RM, Priscu JC, Pocock T et al (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. Microbiol Mol Biol Rev 70:222–252. doi:10.1128/MMBR.70.1.222-252.2006

Mou S, Zhang X, Ye N et al (2012) Cloning and expression analysis of two different LhcSR genes involved in stress adaptation in an Antarctic microalga, *Chlamydomonas* sp. ICE-L. Extremophiles 16:193–203. doi:10.1007/s00792-011-0419-7

Müller T, Bleiß W, Martin CD et al (1998) Snow algae from northwest Svalbard: their identification, distribution, pigment and nutrient content. Polar Biol 20:14–32. doi:10.1007/s003000050272

Nelson DM, Tréguer P, Brzezinski MA et al (1995) Production and dissolution of biogenic silica in the ocean: revised global estimates, comparison with regional data and relationship to biogenic sedimentation. Glob Biogeochem Cycles 9:359–372

Nishida I, Murata N (1996) Chilling sensitivity in plants and cyanobacteria: the crucial contribution of membrane lipids. Annu Rev Plant Physiol Plant Mol Biol 47:541–568. doi:10.1146/annurev.arplant.47.1.541

Nymark M, Sharma AK, Sparstad T et al (2016) A CRISPR/Cas9 system adapted for gene editing in marine algae. Sci Rep 6:24951. doi:10.1038/srep24951

Palmisano AC, Garrison DL (1993) Microorganisms in Antarctic sea ice. In: Friedmann E (ed) Antarctic microbiology. Wiley-Liss, New York, NY, pp 167–218

Park S, Jung G, Hwang YS, Jin E (2010) Dynamic response of the transcriptome of a psychrophilic diatom, *Chaetoceros neogracile*, to high irradiance. Planta 231:349–360. doi:10.1007/s00425-009-1044-x

Perovich D (2017) Sea ice and sunlight. In: Thomas DN (ed) Sea ice, 3rd edn. Wiley-Blackwell, Oxford, pp 110–137

Petrich C, Eicken H (2017) Overview of sea ice growth and properties. In: Thomas DN (ed) Sea ice, 3rd edn. Wiley-Blackwell, Oxford, pp 1–41

Priscu JC (1995) Phytoplankton nutrient deficiency in lakes of the McMurdo Dry Valleys, Antarctica. Freshw Biol 34:215–227

Priscu JC (1998) Ecosystem dynamics in a Polar desert: the McMurdo Dry Valleys, Antarctica. American Geophysical Union, Washington, DC

Ralph PJ, McMinn A, Ryan KG, Ashworth C (2005) Short-term effect of temperature on the photokinetics of microalgae from the surface layers of Antarctic pack ice. J Phycol 41:763–769. doi:10.1111/j.1529-8817.2005.00106.x

Raymond J (2011) Algal ice-binding proteins change the structure of sea ice. Proc Natl Acad Sci 108:E198. doi:10.1073/pnas.1106288108

Raymond J (2014) The ice-binding proteins of a snow alga, *Chloromonas brevispina*: probable acquisition by horizontal gene transfer. Extremophiles 18:987–994. doi:10.1007/s00792-014-0668-3

Raymond J, Kim HJ (2012) Possible role of horizontal gene transfer in the colonization of sea ice by algae. PLoS ONE 7:35968. doi:10.1371/journal.pone.0035968

Raymond J, Morgan-Kiss R (2013) Separate origins of ice-binding proteins in Antarctic *Chlamydomonas* species. PLoS ONE 8:e59186. doi:10.1371/journal.pone.0059186

Raymond J, Fritsen C, Shen K (2007) An ice-binding protein from an Antarctic sea ice bacterium. FEMS Microbiol Ecol 61:214–221. doi:10.1111/j.1574-6941.2007.00345.x

Raymond J, Janech MG, Fritsen CH (2009) Novel ice-binding proteins from a psychrophilic antarctic alga (Chlamydomonadaceae, Chlorophyceae). J Phycol 45:130–136. doi:10.1111/j.1529-8817.2008.00623.x

Remias D, Lutz-Meindl U, Lutz C (2005) Photosynthesis, pigments and ultrastructure of the alpine snow alga *Chlamydomonas nivalis*. Eur J Phycol 40:259–268. doi:10.1080/09670260500202148

Robinson DH, Kolber Z, Sullivan CW (1997) Photophysiology and photoacclimation in surface sea ice algae from McMurdo Sound, Antarctica. Mar Ecol Prog Ser 147:243–256. doi:10.3354/meps147243

Ryan KG, Ralph P, McMinn A (2004) Acclimation of Antarctic bottom-ice algal communities to lowered salinities during melting. Polar Biol 27:679–686. doi:10.1007/s00300-004-0636-y

Sakshaug E, Slagstad D (1991) Light and productivity of phytoplankton in polar marine ecosystems: a physiological view. Polar Res 10:69–86. doi:10.1111/j.1751-8369.1991.tb00636.x

Säwström C, Mumford P, Marshall W et al (2002) The microbial communities and primary productivity of cryoconite holes in an Arctic glacier (Svalbard 79 degrees N). Polar Biol 25:591–596. doi:10.1007/s00300-002-0388-5

Schriek R (2000) Effects of light and temperature on the enzymatic antioxidative defense systems in the Antarctic ice diatom *Entomoneis kufferathii* Manguin. Rep Polar Res 349:1–130

Scott FJ, Marchant H (eds) (2005) Antarctic marine protists. Australian Biological Resources Study/Australian Antarctic Division, Canberra

Shi H, Lee B, Wu S-J, Zhu J-K (2003) Overexpression of a plasma membrane Na+/H+ antiporter gene improves salt tolerance in *Arabidopsis thaliana*. Nat Biotechnol 21:81–85. doi:10.1038/nbt766

Shin S-E, Lim J-M, Koh HG et al (2016) CRISPR/Cas9-induced knockout and knock-in mutations in *Chlamydomonas reinhardtii*. Sci Rep 6:27810. doi:10.1038/srep27810

Smetacek V (1998) Diatoms and the silicate factor. Nature 391:224–225. doi:10.1111/j.1600-0579.1998.tb00036.x

Smetacek V, Nicol S (2005) Polar ocean ecosystems in a changing world. Nature 437:362–368. doi:10.1038/nature04161

Smetacek V, Klaas C, Menden-Deuer S, Rynearson TA (2002) Mesoscale distribution of dominant diatom species relative to the hydrographical field along the Antarctic Polar Front. Deep Res Part II Top Stud Oceanogr 49:3835–3848. doi:10.1016/S0967-0645(02)00113-3

Smith WO Jr, Codispoti LA, Nelson DM et al (1991) Importance of Phaeocystis blooms in the high-latitude ocean carbon cycle. Nature 352:514–516

Sommer U (1989) Maximum growth rates of Antarctic phytoplankton: only weak dependence on cell size. Limnol Oceanogr 34:1109–1112

Sorhannus U (2011) Evolution of antifreeze protein genes in the diatom genus *Fragilariopsis*: evidence for horizontal gene transfer, gene duplication and episodic diversifying selection. Evol Bioinforma 2011:279–289. doi:10.4137/EBO.S8321

Stammerjohn S, Maksym T (2017) Gaining (and losing) Antarctic sea ice: variability, trends and mechanisms. In: Thomas DN (ed) Sea ice, 3rd edn. Wiley-Blackwell, Oxford, pp 290–303

Stoecker DK, Gustafson DE, Merrell JR et al (1997) Excystment and growth of chryophytes and dinoflagellates at low temperatures and high salinities in Antarctic sea-ice. J Phycol 33:585–595

Stoecker DK, Gustafson DE, Black MMD, Baier CT (1998) Population dynamics of microalgae in the upper land-fast sea ice at a snow-free location. J Phycol 34:60–69. doi:10.1046/j.1529-8817.1998.340060.x

Stoecker DK, Gustafson DE, Baier CT, Black MMD (2000) Primary production in the upper sea ice. Aquat Microb Ecol 21:275–287

Stoeve J, Notz D (2015) Insights on past and future sea-ice evolution from combining observations and models. Glob Planet Chang 135:119–132

Strauss J, Gao S, Morrissey J, et al (2013) A light-driven rhodopsin proton pump from the psychrophilic diatom *Fragilariopsis cylindrus*. In: Proceeding of EMBO workshop: the molecular life of diatoms, Paris, France, 25–28 June 2013

Streb P, Shang W, Feierabend J, Bligny R (1998) Divergent strategies of photoprotection in high-mountain plants. Planta 207:313–324. doi:10.1007/s004250050488

Stroeve JC, Serreze MC, Fetterer F et al (2005) Tracking the Arctic's shrinking ice cover: another extreme September minimum in 2004. Geophys Res Lett 32:1–4. doi:10.1029/2004GL021810

Sutherland PC (1852) Journal of a voyage in Baffin's Bay and Barrow Straits in the years 1850–51, performed by H.M. ships "Lady Franklin" and "Sophia," under the command of Mr.William Penny in search of the missing crews of H.M. ships "Erebus" and "Terror", vol 1 and 2. Longman, Brown, Green, and Longmans, London

Takeuchi N (2002) Optical characteristics of cryoconite (surface dust) on glaciers: the relationship between light absorbency and the property of organic matter contained in the cryoconite. Ann Glaciol 34:409–414

Tang EPY, Vincent WF, Proulx D et al (1997) Polar cyanobacteria versus green algae for tertiary wast-water treatment in cool climates. J Appl Phycol 9:371–381

Teoh ML, Phang SM, Chu WL (2013) Response of Antarctic, temperate, and tropical microalgae to temperature stress. J Appl Phycol 25:285–297. doi:10.1007/s10811-012-9863-8

Thomas DN, Dieckmann GS (2002) Antarctic sea ice–a habitat for extremophiles. Science 295:641–644. doi:10.1126/science.1063391

Thomas DN, Fogg GE, Convey P et al (2008) Biology of polar regions. Oxford University Press, Oxford

Tomczak M, Godfrey JS (2003) Regional oceanography: an introduction, 2nd edn. Elsevier Science, Tarrytown, NY

Toseland A, Daines SJ, Clark JR et al (2013) The impact of temperature on marine phytoplankton resource allocation and metabolism. Nat Clim Chang 3:979–984. doi:10.1038/nclimate1989

Uhlig C, Kilpert F, Frickenhaus S et al (2015) In situ expression of eukaryotic ice-binding proteins in microbial communities of Arctic and Antarctic sea ice. ISME J 9:2537–2540. doi:10.1038/ismej.2015.43

Underwood GJC, Fietz S, Papadimitriou S et al (2010) Distribution and composition of dissolved extracellular polymeric substances (EPS) in Antarctic sea ice. Mar Ecol Prog Ser 404:1–19. doi:10.3354/meps08557

Underwood GJC, Aslam SN, Michel C et al (2013) Broad-scale predictability of carbohydrates and exopolymers in Antarctic and Arctic sea ice. Proc Natl Acad Sci U S A 110:15734–15739. doi:10.1073/pnas.1302870110

Van Oijen T, van Leeuwe M, Gieskes WWC (2003) Variation of particulate carbohydrate pools over time and depth in a diatom-dominated plankton community at the Antarctic Polar Front. Polar Biol 26:195–201. doi:10.1007/s00300-002-0456-x

von Quillfeldt C (2004) The diatom *Fragilariopsis cylindrus* and its potential as an indicator species for cold water rather than for sea ice. Vie Milieu 54:137–143

Vincent WF, Ja G, Pienitz R et al (2000) Ice shelf microbial ecosystems in the high arctic and implications for life on snowball earth. Naturwissenschaften 87:137–141. doi:10.1007/s001140050692

Wang DS, Xu D, Wang YT et al (2015) Adaptation involved in nitrogen metabolism in sea ice alga Chlamydomonas sp. ICE-L to Antarctic extreme environments. J Appl Phycol 27:787–796. doi:10.1007/s10811-014-0372-9

Wang Q, Lu Y, Xin Y et al (2016) Genome editing of model oleaginous microalgae *Nannochloropsis* spp. by CRISPR/Cas9. Plant J 88:1071–1081. doi:10.1111/tpj.13307

Weissenberger J, Dieckmann G, Gradinger R, Spindler M (1992) Sea ice: a cast technique to examine and analyze brine pockets and channel structure. Limnol Oceanogr 37:179–183. doi:10.4319/lo.1992.37.1.0179

Werner I (2006) Seasonal dynamics, cryo-pelagic interactions and metabolic rates of arctic pack-ice and under-ice fauna – a review. Polarforschung 75:1–19

Wheeler P, Watkins JM, Hansing RL (1997) Nutrients, organic carbon and organic nitrogen in the upper water column of the Arctic Ocean: implications for the sources of dissolved organic carbon. Deep Sea Res Part II Top Stud Oceanogr 44:1571–1592. doi:10.1016/S0967-0645(97)00051-9

Willem S, Srahna M, Devos N et al (1999) Protein adaptation to low temperatures: a comparative study of α-tubulin sequences in mesophilic and psychrophilic algae. Extremophiles 3:221–226. doi:10.1007/s007920050119

Williams WE, Gorton HL, Vogelmann TC (2003) Surface gas-exchange processes of snow algae. Proc Natl Acad Sci U S A 100:562–566. doi:10.1073/pnas.0235560100

Xu D, Wang Y, Fan X et al (2014) Long-term experiment on physiological responses to synergetic. Environ Sci Technol 48:7738–7746

Zhang P, Liu S, Cong B et al (2011) A novel omega-3 fatty acid desaturase involved in acclimation processes of polar condition from Antarctic ice algae *Chlamydomonas* sp. ICE-L. Mar Biotechnol 13:393–401. doi:10.1007/s10126-010-9309-8