Life in frozen veins

Coping with the cold

by
David N. Thomas
(University of Wales, Bangor, UK)
and
Thomas Mock
(Alfred-Wegener Institute, Bremerhaven, Germany)

Every autumn a fundamental transition occurs in the surface waters of polar oceans. Millions of square kilometres of surface waters freeze to form an ice layer that varies from a few centimetres through to several metres thick, and which effectively separates the ocean from the atmosphere above. Ice made from seawater is a porous, semi-solid matrix permeated by a labyrinth of brine channels and pores, and within these a diverse microbial assemblage, including viruses, Archaea, bacteria, flagellates and unicellular algae can thrive. These assemblages can reach such high abundances that the ice becomes a rich coffee colour. The microbial assemblages are in turn a rich food source for grazing protoplankton and zooplankton, especially in winter when food in the water column is scarce.

Frozen seawater

Seawater freezes at approximately −1.8°C, and the salts contained within the water are expelled from the growing ice-crystal matrix. The first visible stage of ice formation is the accumulation of ice crystals that have formed throughout the upper mixed water layers. The 'grease ice' layers form slicks of ice crystals, a few millimetres to a few centimetres thick, on the sea surface. The ice crystals coagulate and, eventually, form a closed ice cover. Once this is established (in a matter of days), the ice becomes thicker, with ice-crystals growing downwards into the water. In undisturbed ice, this can reach depths of a metre or so in just a few months. However, the ice fields are rarely tranquil regions and both wave- and wind-action result in ice floes rafting on top of each other, colliding and/or deforming to produce pressure ridges that can be tens of metres thick. The majority of the ice in the Southern Ocean lasts only less than 1 year, and the average Antarctic sea ice thickness is <1 m. In contrast, in the Arctic Ocean sea ice can last several years and the average thickness is generally >2 m.

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When ice forms from freshwater, the result is a hard brittle solid with the primary inclusions being gas bubbles. In contrast. when seawater freezes the resultant ice is a semi-solid matrix, permeated by a labyrinth of brine-filled channels and pores. The volume of ice occupied by the brine channels is directly proportional to the temperature of the ice, as is the brine concentration within the channels: at −6°C the brine salinity is 100 g/l, at −10°C it is 145 g/l and at −21°C it is 216 g/l. The brines are not static and gravity drainage results in a gradual desalination of sea ice as it ages and brines are expelled into the underlying waters[1].

The temperature at the upper surface of an ice floe is determined by the air temperature (down to −40°C) and the extent of insulating snow cover. In contrast, the temperature at the underside of an ice floe will be at or close to the freezing point of the underlying seawater. This results in gradients of temperature, brine salinity and volume of brine channels and pores throughout an ice floe. During autumn and winter, the ice is generally colder, brine salinities are higher and brine volume is lower in surface ice, compared with underlying ice. Naturally, as ice begins to warm and melt in spring and early summer, these gradients break down.

Sea-ice organisms

Heterotrophic bacteria and unicellular algae represent the two major groups within sea-ice assemblages that have been best studied to date. However, there is a wide diversity of prokaryotes and eukaryotes, and much of the attention of sea-ice studies has focused on identifying the diversity of organisms that are able to survive the transition from the open water to the semi-solid ice matrix[2]. The interest in these organisms is of cause based on a fundamental desire to understand how organisms can both survive and thrive in extremes of temperature, salinity and low light levels[3]. However, we still only have a limited understanding of the biochemical and physiological mechanisms by which these organisms survive, and even less about the molecular controls of these[4,5].

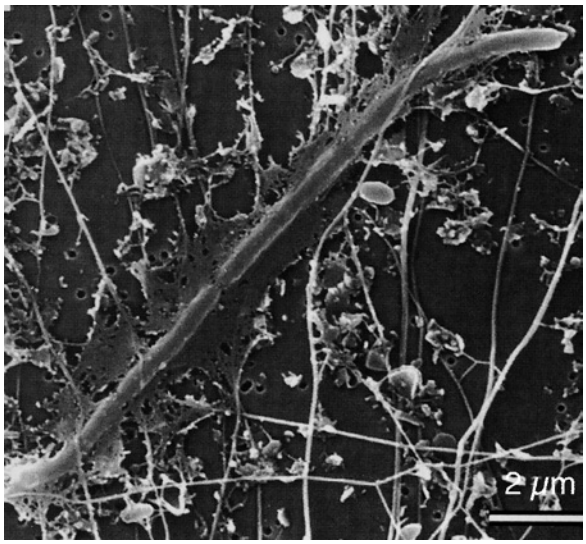
Despite the obvious low temperatures, sea ice is also characterized by highly changeable salinity, pH, dissolved inorganic nutrients, dissolved gas and light conditions[6]. The biology within sea ice is physically constrained to small spaces, in such a way that many of the biological and chemical interactions are probably more akin to those known from aquatic biofilm or sediment/soil studies, especially those exposed to widely changing abiotic conditions such as in intertidal systems[2].

Acclimatization to life in the ice

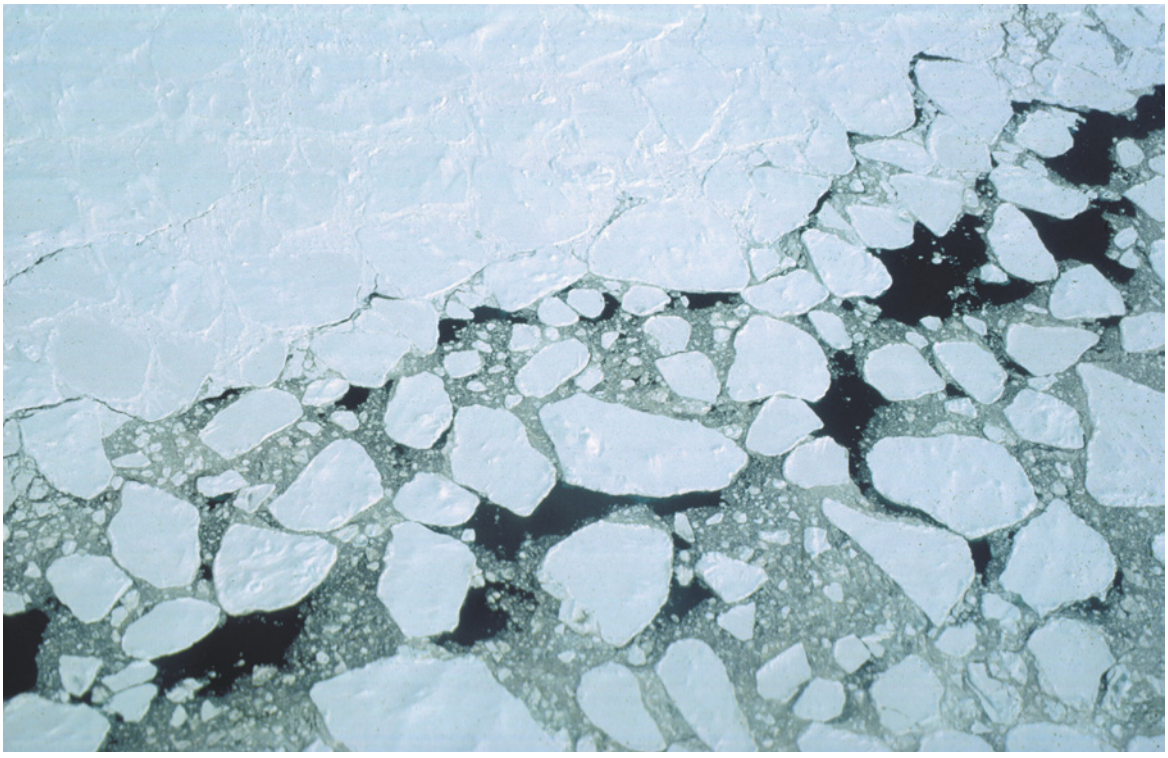
Sea-ice bacteria from Arctic ice have been shown to be active at temperatures down to −20°C, and motile down to −10°C[7]. It is thought that cryoprotectants, such as antifreeze, may be produced to inhibit ice crystal formation[8]. The antifreeze proteins produced by bacteria are possibly part of the large pool of extracellular polymeric substances (EPSs) located on the cell surface. In particular, the CFB (*Cytophaga/Flavobacterium/Bacteroides*) group are known for their abundant slime (EPS) production under decreasing temperatures, and culture experiments with bacteria strains isolated from sea ice produce 30-fold greater yields of EPS at temperatures between −2°C and 10°C, compared with yields at 20°C. Such proteins have been found in other cold adapted bacteria and by unicellular algae isolated from sea ice.

It has been suggested that the ice-active substances (IASs) produced by sea-ice diatoms may be glycoproteins that bind preferentially to ice crystals, causing pitting[9]. This pitting may, in turn, alter the optical properties of sea ice and may also help to maintain fine pore space structure. However, it might be that some interaction of the IASs on the surface of cells increases the ability of cells to stick to ice surfaces, or is involved in protecting cells from freeze–thaw damage. An interesting feature of these IASs is that they seem to be produced mainly while sea ice is growing, and in established sea ice their production is much reduced.

Besides avoiding freezing, the transport of essential nutrients and gases through the cell membranes has to be guaranteed at low temperatures and in high salt concentrations. Hence, increase in membrane



Raster electron micrograph of a chain of bacteria from Baltic Sea ice which produces extracellular polymeric substances for cryoprotection



Ice shelf, sea-ice and open water (Antarctica)

fluidity is one of the most important acclimatization during temperature reduction and is therefore well-documented, particularly for bacteria[10]. It is clear that a decrease in temperature leads to one or a combination of the following changes: an increase in fatty acid unsaturation; a decrease in average fatty-acid chain length; an increase in methyl branching of fatty acids; and an increase in the ratio of anteiso-branching relative to iso-branching. Furthermore, polyunsaturated fatty acids (PUFAs) are not normally detected in temperate bacteria, but ω3 and ω6 fatty acids such as $C_{20:5,\,n-3}$ are often found in significant amounts in lipids of sea-ice bacteria.

Most of the PUFAs in diatoms are concentrated in chloroplast lipid classes where they have essential roles in membrane structure, electron transport and incorporation of protein–pigment complexes. The regulation of membrane lipid composition in sea-ice diatoms has been shown to be essential for efficient electron transport under low temperatures, low irradiances and nitrogen limitation[11,12]. Low temperatures are generally coupled with low irradiance in sea ice. Light also regulates the fluidity of the thylakoid membrane and is indirectly responsible for cold acclimatization of photosynthesis in sea-ice diatoms[12]. At low irradiances, there are increased amounts of PUFAs in monogalactosyldiacylglycerol (MGDG), the main lipid class of thylakoid membranes. Thus, low temperature acclimatization of sea-ice diatoms, in terms of lipid metabolism, is tightly connected with light acclimation, especially for photosystem II processes. PUFAs support the interactions between primary (QA) and secondary (QB) electron acceptors and thus the velocity of electron flow. Beside this influence of lipids on electron transport, they also regulate the structure of thylakoid membranes[11]. During nitrogen limitation, increased production of bilayer-forming digalactosyldiaglycerol (DGDG) and phospatidyglycerol (PG) compensate for the reduction of bilayer-stabilizing proteins and pigments, which would have disrupted entire chloroplast membranes and photosynthetic potential without this compensation[11].

Besides temperature, salinity is probably the factor that has the greatest influence on the organisms living within the brine-channel system, especially in cold ice where the brine salinities can be three or four times higher than in seawater[1]. The ability of diatoms to acclimatization

Sea-ice with layers of micro-algae

to hyperosmotic brine solutions is based on accumulation of free amino acids, such as proline, and other cryoprotectants, such as dimethyl sulphoniopropionate (DMSP)[13]. The intracellular concentrations are dependent on environmental conditions, but also on the physiological potential of each species. Some diatom species are able to produce more of these cryoprotectants and are therefore able to grow at lower temperatures and in higher salinities. In contrast, pelagic diatoms are less prolific producers of DMSP and proline. Studies in the Antarctic have shown that very high concentrations of DMSP can be produced by ice algae assemblages reaching concentrations of over 1500 nM, much higher than open seawater values which typically range from 0 to 50 nM[14].

Light, temperature and nutrient supply, all influence the production of DMSP. In sea ice, however, salinity is the dominant factor influencing DMSP production by the ice algae, with DMSP being synthesized and accumulating in hypersaline conditions. DMSP degrades to DMS (dimethyl sulphide) and acrylic acid when the ambient salinity decreases. Furthermore, DMSP is cleaved to DMS in alkaline conditions, such that the shifts of pH (up to a pH of approximately 10) that have been measured in sea-ice brine may enhance this reaction within the sea-ice habitat. Lastly, DMSP is broken down to DMS and acrylic acid through the action of the enzyme DMSP-lyase and also from grazing by protozoans and metazoans, as well as viral infection.

In remote ocean regions, DMS accounts for most of the non-sea salt sulphate in the atmosphere, and the oxidation of DMS in the atmosphere to aerosol particles and cloud condensation nuclei is part of a complex system of localized and global climate control. The greatest release of DMS from sea-ice regions is associated with melting ice and the corresponding reduction in ambient salinity, when cells containing hypersalinity-induced high concentrations of DMSP are released into the seawater. Periods of ice ablation are also times when elevated grazing activity in ice edge waters will increase the release of DMS into surface waters and therefore into the atmosphere.

The limited data on dissolved gases in sea-ice brines collected to date indicate that when there is high primary production, and accumulation of large algal standing stocks in sea ice, the brines are characterized by substantial reductions in total inorganic carbon, exhaustion of aqueous CO_2, pH values up to 10 and O_2 supersaturation[15]. The ability to sustain photosynthesis at high O_2 and low CO_2 levels in a strongly alkaline environment will be a critical prerequisite for survival of algae within the ice. For example, the ability of algae to actively assimilate HCO_3^- at very low aqueous CO_2 concentrations is considered to be a decisive factor for the success of small diatom species, such as *Chaetoceros* cf. *neogracile*, that are common in established sea-ice algae assemblages.

Low CO_2 conditions can be experienced by phytoplankton in seawater, but hyperoxic conditions are rare in marine systems. It is also possible that toxic photochemical products may accumulate in such environments, especially under conditions of high incident UV radiation associated

with depletions in atmospheric ozone in both the northern and southern hemispheres. Despite ice being an effective barrier to light, UV radiation has been shown to penetrate snow-free ice to a depth of over 1 metre[16]. These products include substances such as hydrogen peroxide and hydroxyl radicals, which can damage nucleic acids, proteins and other cell constituents. Diatoms have been shown to have high activities of antioxidative enzymes, such as catalase, glutathione peroxidase and glutathione reductase, to cope with these potentially damaging conditions, and activities of these enzymes in diatoms isolated from sea ice are very high in the temperature and light ranges experienced within sea ice[17].

Conclusion

The above has been a very brief snap-shot of some of the stresses that prevail within the sea-ice habitats. It is not simply low temperature that governs the ability of sea-ice organisms to survive and grow, but also responses to salinity, pH, dissolved gases and other inorganic nutrients. The sea ice can be a place of both high and low light stress for photosynthetic organisms. While we have identified many of the organisms that are found in the ice (at least groups), the challenge for the future will be to identify the biochemical and genetic adaptations of sea ice organisms, not simply to single stress factors but to the complex interaction of factors that are present within the ice matrix[4,5].

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David Thomas is a Reader and Head of the Biogeochemistry Group in the School of Ocean Sciences, University of Wales, Bangor. Since 1991, he has been active in studying first the biology and more latterly the chemistry of sea ice in the Baltic Sea, the Antarctic and Arctic (www.sos.bangor.ac.uk/staff/php/staffdetails1.php?person=0050)

email: d.thomas@bangor.ac.uk

Thomas Mock is a post-doc at the Alfred Wegener Institute for Polar and Marine Research (AWI) in Bremerhaven, Germany. He obtained his PhD from the University of Bremen in 2003 after graduating from Kiel University with a degree in Biological Oceanography in 1998. He has subsequently done postdoctoral work at the AWI, and is currently working on biochemical and molecular adaptation of polar diatoms.

email: tmock@awi-bremerhaven.de

