15 Life in ice

Jody W. Deming
University of Washington, Seattle
Hajo Eicken
University of Alaska, Fairbanks

White men think of ice as frozen water, but Inuit think of water as melted ice. To us, ice is the natural state.

Nuka pinguaq, nineteenth-century native Arctic hunter (Kobalenko, 2002)

15.1 Introduction

Ice is the “natural state” or predominant form of water in our Solar System. The surfaces of most planets and moons are currently at temperatures well below the freezing point of pure water, including two of the more promising sites in the search for extraterrestrial life, Mars and Europa. The amount of water-ice on Europa exceeds the volume of liquid water on Earth. Comets, considered potential vectors for precursors or early stages of life, are also icy bodies (Chapter 3). Even Earth may have undergone a series of complete (or near-complete) glaciations in its recent history, earning the title “Snowball Earth” (Section 4.2.4).

The presence of the liquid phase of water, however, is essential to the prospering of life as we know it. In order to study where liquid water can occur, we must understand scales ranging from the structure of the water molecule to the temperatures possible on a planetary surface or subsurface. In our Solar System, only Earth allows for a planetary surface with abundant liquid water (Chapter 4). Mars, however, may well have some liquid water in a permafrost (perennially frozen soil) beneath its surface today (Section 18.4.1), and the evidence is strong that Europa and perhaps other moons of Jupiter have water oceans below their icy crusts (Chapter 19).

Dismissing the surfaces of other Solar System bodies as currently lifeless for lack of a significant body of liquid H₂O might seem reasonable if only planetary-scale parameters are considered. However, on the scale of micrometers relevant to microbial life, the “natural state of water” – ice – does not in fact automatically exclude the simultaneous presence of the liquid phase. Consider that natural ice is rarely if ever formed from pure water. The impurities of ice on Earth – for example, the salts in ice derived from seawater or the impurities in permafrost – allow for the presence of liquid water as a significant fraction of the ice volume (Table 15.1) even though, on a larger bulk scale, the ice appears to be solid. Like virtually all natural waters on Earth, this remaining liquid within the ice is inhabited by microorganisms. The impurity effect allowing for liquid water holds true even at temperatures approaching the average surface temperature of Mars today (−55°C). Indeed, recent evidence suggests the potential existence of unfrozen water in subsurface permafrost on Mars (Sec. 18.4.1).

Water is bound in the solid phase on Earth in five major types of so-called ice formations (Table 15.1). Listed by global volume, they are the terrestrial ice sheets (mainly those covering Antarctica and Greenland), mountain glaciers and polar marine ice shelves, sea ice (encircling Antarctica and covering the Arctic Ocean and surrounding seas), and permafrost (as in Siberia and Alaska). Although seasonal snow covers the largest fraction of Earth’s surface area compared to the other major ice formations (9% versus 0.1–5%), snow represents only a minor form of frozen water volumetrically (< 0.007%). The most prevalent type of ice on Earth is the terrestrial ice sheet (“terrestrial” meaning “on land”, as opposed to “marine”), which is formed from water vapor deposited onto snow crystals with limited impurities; such ice contains the lowest fraction by volume of liquid water (0.0001, a generous upper limit). Underlining the importance of salt impurities for the presence of a significant liquid phase, the average fraction of liquid present in sea ice (0.08) greatly exceeds that of all other ice forms on Earth (Table 15.1). For perspective, the total amount

of liquid water present at sub-freezing temperatures, as a result of salt and other impurities, within all ice formations on Earth exceeds the volume of freshwater flowing in all rivers. From the microbial perspective, even a fraction of a microliter of water contained within a block of ice represents a luxurious water world. Critical to that water being supportive of ongoing metabolism and growth, however, is the presence of connections between numerous liquid niches: an open system allows for the essential exchange of nutrients and waste products by diffusion or advection (carrying of materials or molecules via (small-scale) currents). At the very lowest temperatures of ice on Earth, and thus for our best analogues to the frozen environments on Mars and Europa, salt or organic impurities are essential to the presence of any liquid water within the ice. Thermal gradients across an ice formation can even allow fluids to flow within the ice on a scale relevant to microorganisms.

In this chapter, we present the underlying physical and chemical reasons for the occurrence and characteristics of liquid water in ice. We then discuss emerging relationships between the amount of water available in various ice formations and the abundance and activity of microbial life forms found therein. Figure 15.1 presents the temperature ranges for the occurrence of liquid water on Earth and for laboratory-confirmed microbial growth and enzyme activity. We focus on microbial life (single-celled organisms, typical dimension of 1 μm or less) in the domains of Bacteria and Archaea (Chapter 10), here generically called bacteria (with lower case b). Bacteria, themselves composed of 70–90% water, must conduct all of their basic metabolic functions within an aqueous medium.

Note:
“excluding sub-glacial permafrost in Antarctica and Greenland and subseabed permafrost under the Arctic Ocean (since extent is not well known) (from Zhang et al., 2000)

1 A niche is a habitat providing the factors necessary for growth of a particular organism or species.
as must all of the enzymes that enable life (Madigan et al., 1997). More complex organisms (Eukarya), whether single-celled or multicellular (metazoa), also live in some types of ice — most notably the photosynthesizing algae that flourish along with their metazoan grazers during sunlit seasons in sufficiently clear sea ice (and sometimes also on the surface of snow formations and in rock crevices). Indeed, the first organisms living in a subzero environment to be recorded were later identified as photosynthetic Eukarya or “red algae” — Aristotle noted that “living animals are produced ... in long-lying snow” that “gets reddish in color” (Barnes, 1984). Here we pay more attention to heterotrophic bacteria — those capable of deriving nutrition from pre-formed organic compounds and thus living independently of solar radiation.

Heterotrophy is an obvious focus, given that the dissolved impurities in the liquid contained in ice formations on Earth invariably include not only mineral salts but also organic compounds. Metabolisms independent of light are also highly relevant to an astrobiological consideration of life in ice: on Earth, many of the liquid niches in ice are not sunlit; on Mars and Europa, a persistent liquid phase of water most likely exists only at subsurface depths in the dark. We also focus on heterotrophic bacteria because of their long history on Earth, their dominance globally in terms of abundance (Deming and Baross, 1993), their profound effects on geochemistry, and their occurrence in all natural ice formations examined to date.2

Finally, in the course of considering the physics, chemistry, and microbiology of Earth ice and its fluid inclusions, we have built a rationale for the search for microbial life in ice formations elsewhere in the Solar System. Of interest is the detection of accidental tourists in extraterrestrial ice — those microorganisms not native to their icy environments but preserved upon arrival by the extreme cold and possibly recoverable upon return to conditions permissive to their growth (see also Chapter 25). Of even greater interest is the detection of cold-adapted microbial life actively metabolizing or possibly growing within an ice formation. On Earth, the bacteria known to be cold-adapted, whether requiring the cold for growth (psychrophilic) or simply tolerating it as they grow (psychrotolerant), were late arrivals in the broad evolutionary scheme of things, according to molecular-phylogenetic analyses (Fig. 15.2). Psychrophilic bacteria are generally understood to have evolved from mesophilic bacteria, organisms that grow at room temperature and warmer (to about 40–50°C) but are inactive below about 10°C. Permanently cold habitats that would favor the evolution of obligate psychrophiles (bacteria that must have cold to reproduce, growing optimally below 10°C) were not available on Earth until about half way through its history (~2.3 Ga), when the first glaciation events appear in the geological record (Section 4.2.4). Had persistent ice formations appeared sooner, cold-adapted bacteria may well have evolved sooner: the genetic steps from mesophily to psychrophyly do not appear to be particularly daunting. Detection of metabolically active microorganisms (or their traces) in the ice formations of Mars or Europa could thus imply either a pioneer community struggling to colonize the ice or the presence of a well-established, resident

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2 Global occurrence in ice is only known now with respect to the domain of Bacteria, since study of the distribution of Archaea in ice has only just begun.
microbial world in milder regions (or eras) of the planet or satellite from which evolved successful ice inhabitants. Regardless of the possible types of organisms envisioned – tourists, pioneers, or residents – and regardless of the thermal history of a given planet or moon, the extraterrestrial surfaces that we can expect to examine in the foreseeable future (on Mars and possibly Europa) are frozen environments. Their successful exploration will benefit from what we can learn on Earth about life in ice.

15.2 Physics and chemistry of ice

15.2.1 Liquid water at subzero temperatures

To approach the question of how microbial life may persist and even be active in frozen environments, the basic physics and phase chemistry of liquid water and ice must first be appreciated. Understanding how impurities (salt and other freezing point depressants) in natural ice formations, as well as kinetic and other constraints, enhance the occurrence of the liquid phase even at very low temperatures is also essential. Together, the specific physical and chemical aspects of a frozen system yield a three-dimensional microstructure that defines the inhabitable liquid space for microbial life. The amount of liquid and degree of connectivity of those spaces determine in large part whether bacteria simply persist in ice or actually benefit from diffusive or advective exchange of chemical compounds (of nutritional or other value) and thus have the opportunity to evolve through collaboration or competition at very cold temperatures.

15.2.1.1 Phase diagram of liquid and solid water

The structure of the water molecule and the nature of its hydrogen bonds play key roles in the physicochemical and biochemical processes that underlie the origin and evolution of life on Earth. They also account for a remarkable range of different ice phases, each of which are stable under particular conditions that can be encountered at the surface or in the interior of the various moons and planets in the Solar System. To date, thirteen different stable crystalline phases of ice have been described (Petrenko and Whitworth, 1999; Fig. 15.3). In the terrestrial and marine environments of Earth, the only important form of ice is the hexagonal modification of ice I, termed ice Ih (throughout this chapter, the term “ice,” if used without a qualifier, always refers to ice Ih). This form is stable under conditions encountered throughout the Earth’s atmosphere and hydrosphere.

A key property of ice Ih is apparent from the negative slope of the phase boundary (called the liquidus) that distinguishes the field of stability of ice Ih from that of liquid water (Fig. 15.3). Owing to the lower density of this ice (0.917 g cm$^{-3}$ at standard conditions of 0°C and 1 bar = 0.1 MPa) compared to water, the freezing/melting point of water/ice Ih decreases with increasing pressure. At the base of the Antarctic ice sheet, where sub-glacial Lake Vostok is located, the pressure of more than 3 km of ice pushes the freezing/melting point (and hence water temperature in the lake) to just below −3°C. The other phases of ice in equilibrium with liquid water do not show this anomalous behavior; instead, the freezing/melting point increases with increasing pressure. Consequently, the lowest temperature at which liquid

\[\text{FIGURE 15.3 Phase diagram of pure water at high pressures, showing the fields of stability of liquid water (L) and of ices I through IX. Ice I is the dominant form of ice on Earth. Atmospheric pressure on Earth corresponds to 10^{-1}\text{ kbar} and the bottom of a 1-km thick glacier to ~0.1\text{ kbar. (From Hobbs, 1974)}}\]
water can be present (in the absence of chemical freezing-point depressants such as salts or various organic compounds) is the point where the phase boundaries between ice Ih, liquid water and ice III meet. This triple point lies at a temperature of $-22.3^\circ$C and a pressure of 2.1 kbar (Petrenko and Whitworth, 1999), a combination of conditions not likely to occur on Earth.4

At considerably higher pressures (100 kbar), ice can be stable at temperatures approaching 400°C. While high-pressure phases of ice may be stable in the interior of some icy planets or moons, the ice crust overlying the putative ocean on Europa (Pappalardo et al., 1999) only barely, if at all, experiences pressures high enough (a few kbar) for ice II or III to appear (Lupo and Lewis, 1979). Nevertheless, the possible occurrence of modified forms of ice under high pressure may be important to consider, since the presence of any form of ice in hydrothermal environments would have substantial consequences for processes related to the possible origin and evolution of life in such settings. Furthermore, in the surface environments of some planets and moons, very low temperatures (and the resulting slowness of chemical changes) may create conditions such that a normally unstable state of ice is instead metastable, meaning that in effect it does not undergo transformations over time periods of interest.

15.2.1.2 Freezing point depression in brines

The importance of water as a prerequisite for life on Earth derives in large part from the polar nature of the water molecule and its role as a solvent for ionic and other compounds (see also Chapter 14). The same molecular-level properties and electrostatic forces governing the interaction between water, ionic compounds, and many types of organic compounds – hydrogen bonding and van der Waals interaction – are also critical for freezing-point depression that allows the survival and activity of microorganisms at subzero temperatures. The distribution of electrons within the water molecule, with a net negative charge associated with the oxygen atom and a net positive charge associated with the two hydrogen atoms, imparts a permanent dipole moment to the molecule as a whole (Petrenko and Whitworth, 1999). This inherent feature results in the hydration of ions (or other polar molecules), meaning that water dipoles orient themselves such that a layer (called a hydrate shell) surrounds individual anions or cations. The change in potential energy (Gibbs free energy) associated with such hydration, which depends on the radius and electronegativity of a specific ion, governs the solubility of any compound in water.

Hydration, however, also strongly affects phase changes, including the freezing of water, since the electrostatic interaction between ions and water molecules can substantially lower the temperature at which water undergoes a phase change from liquid to solid. In addition, the crystal lattice of ice does not allow for incorporation into the solid phase of most salt ions (F$^-$ and NH$_4^+$ being notable and biologically relevant exceptions; Hobbs, 1974), causing ions to be concentrated in any liquid that remains upon ice formation. Thus, for a saturated solution of NaCl in water (23% salt), ice only forms when the temperature is dropped to $-21.2^\circ$C (Fig. 15.4); until that point water molecules in the liquid phase survive in hydrate shells.5 Ionic salts, as well as polar molecules such as methanol or ethylene glycol (anti-freeze), hence depress the freezing point of water well below that of pure water.

The impurity effect on the availability of liquid water in frozen systems can be illustrated by a phase diagram for the two-component system NaCl-H$_2$O (Fig. 15.4). Although brines usually include several salts other than NaCl, this diagram serves as a reasonable proxy for natural systems such as permafrost soils or seawater, where the composition of the liquid brine fraction is typically more than 80% NaCl (Weeks and Ackley, 1986). The liquidus curves in the phase diagram indicate the first appearance of solid ice in a solution of given NaCl composition as the solution is cooled. Upon further cooling, more and more of the water turns to ice, with the salt becoming increasingly enriched in the remaining liquid. The amount of ice freezing out of solution and the concentration of salt in the remaining liquid is dictated by the freezing-point depression specific to each ionic compound. At the so-called eutectic point, the remaining liquid water simultaneously freezes with the salt (forming hydrohalite, NaCl$\cdot$2H$_2$O), leaving a mixture of pure ice and hydrohalite (bottom of Fig. 15.4).

Although such liquid-free mixtures of ice and salt precipitates are believed to occur only rarely in natural ice formations on Earth, the eutectic point is

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4 Pressure and temperature at the bottom of the deepest ocean (~11 km) are ~1.1 kbar and 2°C.

5 For standard seawater (3.5% NaCl), the freezing temperature is depressed to $-1.9^\circ$C.
15.2 Physics and chemistry of ice

approached when severe conditions sufficiently chill an ice formation (e.g., in South Pole snow, Arctic winter sea ice, or Siberian permafrost). Presumably, as the fraction of liquid water becomes vanishingly low, bacteria also present as “impurities” in the ice would cease to function (though might well be preserved in an inactive but recoverable state) or become damaged (perhaps irreversibly). Such effects could happen as a result of (a) low water activity (see below) that prevents the maintenance of essential cellular components, or (b) physical damage from ice crystals forming either within or adjacent to the cells. The precise physical and chemical conditions leading to ice formation are of critical importance to bacterial survival. As we shall see, however, bacteria themselves, as living organic entities, have their own strategies for surviving extreme conditions and even altering to their advantage both the freezing and eutectic points of the fluid niches they inhabit.

In a simple (life-free) water system, the magnitude of the freezing-point depression can be found by solving for $T_f$ in a derivation of the Clausius–Clapeyron equation (e.g., Fletcher, 1993):

$$\ln(a_i) = \frac{\Delta H_f}{R} \left( \frac{1}{T_f} - \frac{1}{T_i} \right),$$

with $\Delta H_f$ the enthalpy of fusion (latent heat of fusion) of water, $R$ the universal gas constant, $T_f$ the freezing temperatures of the solution and the pure compound (superscript $\bullet$), and $a_i$ the activity of the water in the liquid phase fraction. The activity corresponds to the product of an activity coefficient and the molar fraction of the relevant component; it represents a measure of the extent to which water molecules are available to participate in chemical reactions or phase changes. The fact that the magnitude of freezing-point depression depends on the number rather than the mass of ions of the component per unit volume is an important aspect of a system (often referred to as a colligative property). The activity of water decreases substantially with increasing salt concentration or ionic strength of the remaining solution (Fig. 15.5). Although theoretical modeling of low-temperature and sub-freezing processes is progressing (e.g., Marion and Farren, 1999), semi-empirical approaches are still common (Eicken, 2003).

What and where are the lowest temperatures at which we can expect to find liquid water on Earth? They will not be in ice formations derived from source waters (or vapors) with limited impurities, but rather in those derived from salty solutions. Liquid water is found routinely at fractions of several parts per thousand even in the coldest types of saline ice; for example, in Arctic winter sea ice at temperatures from $-20^\circ C$ to below $-30^\circ C$ (Eicken, 2003). Consider, for example, a volume of seawater with “standard” composition (six major salt components) and a total salt concentration of 35g solute per kg of solution (35%). Modeling by Marion and Farren (1999) shows that as the fraction of liquid water decreases with dropping temperature, the concentration (expressed as the ionic strength) of inorganic ions in solution
Life in ice

increases (Fig. 15.5). The liquid fraction becomes supersaturated with respect to several salts at specific temperatures begin to precipitate. The first of these precipitates (forming at about \(-6.3^\circ\text{C}\)) is mirabilite (Na\(\text{SO}_4\cdot10\text{H}_2\text{O}\)), accounting for roughly 15% of the total salt fraction in standard seawater. At \(-22.9^\circ\text{C}\), with the volumetric fraction of liquid water still above 10%, hydrohalite (NaCl\(\cdot2\text{H}_2\text{O}\)) begins to precipitate. Only at a temperature of \(-54^\circ\text{C}\), the eutectic point for seawater, does the model indicate that the liquid fraction drops to zero (upon precipitation of Antarcticite, CaCl\(\cdot6\text{H}_2\text{O}\)). Another pathway, involving precipitation of gypsum (Ca\(\text{SO}_4\cdot2\text{H}_2\text{O}\)), results in the liquid phase vanishing at a higher temperature of \(-36^\circ\text{C}\). The model assumes thermodynamic equilibrium and the absence of any other compounds in the system that could affect ice formation or salt precipitation. That the latter assumption will not always pertain to highly concentrated solutions at very low temperatures in natural systems (see below) points the way for future research.

While freezing point depressants can significantly extend the presence of liquid water into the subzero realm – and by implication the presence of living microorganisms – the benefits of acquiring a fluid niche in ice come at a cost to the organism in terms of maintaining its metabolism. The high ionic strengths and low activities of the liquid water may represent more severe challenges to microbial life than the usually cited reduced reaction rates and sluggish kinetics associated with a drop in temperature (Chapter 14). A bacterium living successfully in a salty solution (whether cold or not) is generally understood to keep its interior water activity at a sufficient level by raising interior concentrations of certain components high enough to cause \(\text{H}_2\text{O}\) (alone) to diffuse into the cell. Accumulating these so-called “compatible solutes” (examples are the sugars – sorbitol, trehalose, and glycerol) thus constitutes a primary strategy to avoid osmotic shock\(^6\) and cell lysis (rupture and loss of contents) (Madigan et al., 1997). Expending energy to pump salt ions out of the cell is another approach. Even with these identified strategies, however, no cultured bacterium is known to grow at water activities below 0.75 and even the hardiest of all xerophilic (dry-loving) organisms – some yeast and fungi – fail to grow at water activities below 0.61 (Madigan et al., 1997).

These perceived limits, however, are not based on studies of bacteria uniquely adapted to life in saline ice (e.g., the best-known users of compatible solutes are found in canned food, while the xerophiles mentioned above are mesophilic Eukarya). Appreciating the relative or combined impacts of temperature and ionic strength or water activity on life in ice is hampered by an absence of suitable model organisms. Although extreme halophiles (salt-lovers that grow best in solutions of 15–30% salt) and obligate psychrophiles have been known for decades, the former come from warm habitats and cannot grow in the cold, while the latter cannot grow in salt concentrations above 10%. If organisms that combine the growth traits\(^7\) of extreme halophily and obligate psychrophily exist today, they continue to evade cultivation. Instead, progress in understanding microbial life in ice is coming from (a) the study of organisms that experience some degree of thermal and/or saline stress in ice (e.g., psychrophiles that are moderately halophilic, requiring seawater concentrations of salt [3.5%] for best growth), and (b) the direct study of natural ice formations as non-invasively as possible (Section 15.3.3).

Complicating the problem, however, is the close coupling between the temperature of any ice formation and the composition and ionic strength of its liquid fraction: each of these factors becomes more extreme simultaneously (Eicken, 2003). Effectively separating one factor from others for an experimental evaluation (as often attempted in microbiological studies) may not be practical or desirable. In the coldest ice formations, organisms must overcome the stress factors in combination in order to survive and grow. Recent studies of Arctic-winter sea ice suggest that bacteria can indeed succeed in this struggle (even if we do not yet have them in culture or understand their strategies for success). Significant numbers of active bacteria (Junge et al., 2004) and high concentrations of previously unrecognized organic cryoprotectants (compounds that prevent freeze damage to cells; Krembs et al., 2002) have been detected, even at the lowest temperature examined, \(-20^\circ\text{C}\), and with a concomitant salinity of \(\sim21\%\) (see also Section 15.3.3).

\(^6\) Osmotic shock is a sudden change in the osmotic state of a cell, which can lead to its rupture and loss of contents (lysis). The osmotic state refers to the balance in solutes (largely controlled by diffusion) between the interior and exterior of a cell.

\(^7\) Standard categorizations are based on parameters of growth, since reproductive success is the traditional (Eukaryotic) measure of “survival of the fittest”; but tolerance and survival traits in the microbial world (Bacterial and Archaeal), even in the absence of growth, deserve more consideration and study than they receive.
15.2 Physics and chemistry of ice

15.2.1.3 Other mechanisms enabling liquid water in ice

In contrast to freezing-point depression proportional to the concentration of inorganic salts, as discussed above, other processes may help to maintain liquid water in ice at even lower temperatures than the eutectic point of seawater. They involve properties of organic compounds produced by living organisms, as well as the molecular interactions that occur at interfaces between ice and liquids, gels (organic polymers), and solids such as entrained mineral grains or ice-crystal boundaries.

These processes may be particularly important to evaluate when considering the potential for active or dormant (recoverable) microorganisms in environments on Mars and Europa that are colder than anywhere on Earth.

Compared to the role of inorganic solutes in lowering the freezing point, organic-solute colligative properties (having to do with levels of concentration and how solute molecules bind) have received much less attention. Only recently has the widespread occurrence of high levels of dissolved organic matter in such frozen environments as sea ice been well established (Thomas and Dieckmann, 2002). From the biochemical literature (often in medical and food sciences), however, certain classes of organic compounds, particularly glycoproteins, are well known to repress ice formation at temperatures well below those predicted on the basis of equilibrium processes. This phenomenon is particularly well documented for Antarctic bottom-dwelling fish that combat ice formation in their blood stream (Deluca et al., 1998). Rather than increase the concentration of freezing-point depressants, an energetically costly strategy that would affect other metabolic processes, some fish produce compounds that even at low concentrations kinetically inhibit ice formation and growth. These “surface-active” freeze protectants readily adsorb in a mono-molecular layer onto free ice surfaces in a solution. This organic film then prevents or greatly reduces the rate of further ice accretion onto those surfaces at temperatures well below the equilibrium freezing point. For example, a solution containing 50 mg ml⁻¹ of a fish-derived anti-freeze glycoprotein can be supercooled by more than 3°C below the freezing point without any ice formation (Woehrmann, 1993).

Such “ice-active” compounds are also known in the microbial world, especially among bacteria studied as contaminants of frozen foods. Among natural ice formations, Antarctic summer sea ice has yielded at least one type of micro-algal community that releases protein-rich substances that are “ice-active.” In addition to inhibiting ice formation (Raymond and Fritsen, 2001), some of these compounds roughen the surface of existing ice crystals on a micro-scale, which alters fluid flow and primes the surfaces for more effective bacterial colonization. In general, more attention should be paid to the ecological and geophysical consequences of bacterial processes that suppress ice formation and its ensuing damage to cells, or that physically alter the ice itself for greater habitability.

Recent studies of Arctic sea ice in winter suggest that gelatinous organic (sugar-based) polymers, known to be released by several types of microorganisms in sea ice, may prevent or reduce ice formation and hence act as cryoprotectants (Krembs et al., 2002). The precise mechanism of action of these large molecular weight exopolymers has not yet been determined, but possibilities include the gamut of known mechanisms for other organic compounds and some new angles. In gel form, the exopolymers can fill space within ice and help to retain dissolved impurities during ice growth, thus altering the properties of the ice. These changed properties include its habitability, since gels (as “impurities” in the liquid fraction) represent occupiable space that the solid phase of ice has not encroached upon. A cell fully encased by ice is subject to direct physical damage from ice crystals; a cell first encased by gelatinous material, however, should have a physically and chemically protective advantage. Exopolymeric gels can also scavenge water directly within their molecular structures. As thick coatings around cells living in very cold brines, such gels have the potential to act as a buffer between organism and brine (or organism and ice), protecting against osmotic shock or physical ice damage by elevating the effective water activity in the immediate vicinity of the cell. Given that a variety of organic polymers can occur in significant concentrations in space environments such as comets and interstellar gas, studies of the habitability of natural ice formations on Earth need to consider in more detail these and other organic chemical processes.

The abiotic formation of organic polymers in deeply frozen ice has several interesting implications for astrobiology. Just as the freezing process can concentrate dissolved salts to the point of precipitation, organic compounds can be brought to concentrations higher.

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8 For example, how does the freezing rate of an organic-rich fluid influence its final properties and the survival of entrapped bacteria (Dumont et al., 2004)?
than usually seen elsewhere in nature. Recent work capitalizing on this freeze-concentration effect has included detection of polymerization reactions without the enzymes usually required under milder conditions (Kanavarioti et al., 2001), as well as enhanced rates or yields of other biomolecular reactions and improved amplification of L-enantiomers (stereoselection; see Vajda and Hollosi, 2001). Such organic reactions and products may enhance, now and in the past, the availability of liquid water at very low temperatures, and thus the persistence and evolution of life in frozen environments. Further, we perhaps should add a selection of “sorbets” to the warm or hot “primordial soup” on the menu of options for providing the biochemical precursors to life.

Also independent of biology but of potential importance to life in ice is another phenomenon known as premelting. Recent studies have shown that liquid or quasi-liquid water films persist at sub-freezing temperatures on interfaces such as those between an ice matrix and enclosed mineral particles, or even along ice grain boundaries. This premelting phenomenon is due to thermomolecular forces, which are interactions between molecules in the liquid and the solid that affect the free energy of the system and hence the thermodynamics of ice formation. Wettlaufer (1999) has shown that the presence of ionic impurities can increase this film thickness substantially, for example to more than 0.1 μm at a supercooling of 0.1°C. (The effects of organic impurities on film thickness await study.) Such liquid films may have important implications for the survival of individual bacteria embedded within an ice matrix and for the advection of nutrients and other materials driven by thermomolecular forces. Advection of critical compounds to and from a bacterium can enable metabolic activities beyond those required simply to persist. Over 99% of all actively respiring bacteria in Arctic sea ice, when examined at a winter temperature of −20°C (and corresponding brine salinity of 21%), have been found to be associated with surfaces such as mineral grains and organic particles encased in the ice, as well as the walls of ice crystals framing a brine pore (Junge et al., 2004) (Fig. 15.6). Further investigations of the life-facilitating aspects of these microscopic surfaces and interfaces promise to be important in considering life on Earth or elsewhere.

A final aspect of the liquid phase of frozen environments to consider is viscosity. Given the impurity-concentration effect inherent to the freezing process, the resulting salty and organic-rich liquids are more viscous than the source waters. Relationships between viscosity and the freezing characteristics of ice are complex and poorly known. Highly viscous fluids (imagine molasses) might be expected to pose problems to organisms that need to move or, if attached to a surface, that rely on diffusion to deliver nutrients or remove wastes. Recent work, however, shows that an obligately psychrophilic bacterium can swim easily and rapidly through a highly viscous sugar (glycerol) solution at temperatures as low as −10°C, though not in an equally cold brine solution (Junge et al., 2003b). Viscosity itself (just as temperature alone) may therefore not present too serious a limit on microbial activity in ice, returning us to high salt and low water activity as the primary challenge to life within the inhabitable niches of ice formations.

15.2.2 Structural constraints on life in ice
Summarizing the discussion of the previous sections, Fig. 15.7 provides a qualitative overview of the different types of frozen environments on Earth, all harboring microbial life in either active or dormant states (next section). The environments are located on the
diagram according to the fraction of their volume occupied by solid ice, liquid brine, and salt precipitates, the primary determinants for the presence of liquid water in frozen environments and hence for their habitability. Although other variables can contribute to available water in important ways (for example, the previously discussed “ice-active” organic compounds and particles known to promote premelting), they are not depicted because they may be present in any frozen environment. In this scheme, the cleanest of freshwater ice (glacier and lake) occupies one apex, while purely liquid brines (salt lakes) and salt crusts at similarly extreme temperatures occupy the others. Terrestrial permafrost, the frozen soil environment, may locate in various positions on the diagram, depending on the salinity of the initial liquid water and subsequent complex processes that can occur during seasonal temperature variations. The bulk of the diagram is occupied by the various seasonal and perennial forms of sea ice, as well as the saline slush that leads to its formation at the start of winter.

Until recently, the frozen Earth environments receiving the most widespread attention from an astrobiological perspective have been freshwater ice and permafrost; for example, deep glacial ice overlying Lake Vostok in Antarctica and permafrost in Siberia (Priscu and Christner, 2004; Rivkina et al., 2000). As we have seen, glacial and lake-ice formations cluster in a small section of the entire suite of habitats (near the ice apex in Fig. 15.7), while permafrost can occur anywhere within this spectrum of possibilities for liquid water. Other potentially habitable worlds in the Solar System, such as Mars and Europa, are known to have salts on their surfaces, and may now or in the past have had briny environments (Chapters 18 and 19). Sea ice can thus serve as an instructive and contrasting habitat to freshwater ice and permafrost. Remember that it contains the largest volumetric fraction of liquid water of the various ice formations on Earth – and at the coldest temperatures (Table 15.1). What remains to be considered from a physical perspective is the comparative microstructure of these ice formations, in particular the three-dimensional shape of the interior (liquid-filled) spaces within the ice matrix and their degree of connectivity.

In freshwater ice with no thermal or chemical gradients, the distribution of the liquid phase can be limited to isolated single inclusions (closed pores) that remain disconnected from each other on the scale of thousands of years or more (in some glacial ices). In contrast, the microstructure of sea ice, when examined at even much lower temperatures than glacial ice, includes a connected network of liquid water that enables diffusion between pores and favors advective processes conducive to microbial activity. The distribution and activity of microorganisms in the various frozen environments of Earth (next section) supports the idea that frozen low-salt environments are more relevant to preserved (dormant) life and opportunities to detect and possibly recover ancient organisms from extraterrestrial environments. On the other hand, knowledge of frozen habitats of higher salt content may better instruct on the possibility of finding active life elsewhere.

The microstructure of natural sea ice depends on the growth environment, on boundary conditions at the advancing ice–water interface, and on the temperature and chemical composition of the ice. Among the effects of growth and boundary conditions, many of which are peculiar to Earth ice and the three-dimensional forms it takes (see Eicken, 2003), the rate of ice formation may be of particular astrobiological relevance. In general, the more rapid the freezing rate of sea ice on Earth, the greater the retention of salts and other impurities, with consequent effects on the distribution of phases within the ice (solid ice, liquid brine, salt precipitates, gas inclusions). Indeed, some of the highest concentrations of bacteria (“impurities” in this context) in sea ice have been observed just after the period of rapid freezing in late autumn (Grossmann and Dieckmann, 1994; Delille et al., 1995). If salty ocean water on Europa were to rise through tidal cracks towards a deeply
frozen surface (mean temperature of −160°C), as predicted by analyses of cycloidal features (Hoppa et al., 1999; Section 19.7), the formation rate of new ice from that liquid would be very rapid. Any liquid water flowing to the very cold surface of Mars (−55°C) would also experience rapid freezing. By analogy with Earth ice, salt – and any microscopic life forms also present as “impurities” – would tend to be retained in this new ice, making such formations ideal targets for exploration.

The temperature and chemical composition of the ice is also of prime importance to the morphology and connectivity of inhabitable pore spaces within an ice formation. Recent advances in appreciating these small-scale microstructural features have been made by the direct application of magnetic resonance imaging (MRI) to sea-ice samples. The specific example in Fig. 15.8 tracks the pore morphology in Arctic winter sea ice as the sample was warmed from its \textit{in situ} temperature of −21°C to −6°C (Eicken et al., 2000). As predicted by the phase relations in thermodynamic equilibrium, the brine fraction in the ice was observed to increase with warming. At the same time, however, the size, morphology, and connectivity of the pores (whose original spacing and orientation was determined by ice-growth conditions) clearly evolved, with pores observed to link up at warmer temperatures. The MRI images suggest that these connections exist at −10°C, but recent microscopy with an effective magnification of more than 3200 has confirmed the interconnectedness of brine-filled pores on the \textmu m scale at even colder temperatures of −15°C (Fig. 15.6; Stierle and Eicken, 2002) and −20°C (Junge et al., 2001, 2004). With an ability to exchange fluids and chemical compounds between even the smallest of brine pores in salty ice formations, bacteria are not confined to single closed pores (as they are in cold glacial ice), and thus have a better chance to metabolize and evolve.

15.3 Microbiology of ice

15.3.1 Bacterial abundance

Bacteria are known to be present in significant numbers in all types of natural ice formations on Earth; a “sterile” or bacteria-free sample of any type of natural ice formation remains to be documented. Rare reports of sterile ice in the past (reviewed by Baross and Morita, 1978) were based on highly selective culturing techniques that would have missed the vast majority of bacteria likely to be present. The known densities of bacteria in ice today, based on microscopic counts of all DNA-staining forms present, range over six orders of magnitude, from 200 ml\(^{-1}\) in South Pole snow to \(>6 \times 10^7\) ml\(^{-1}\) in sediment-laden glacial ice to \(>10^8\) g\(^{-1}\) of soil in permafrost (Table 15.2). Note that the highest concentrations of bacteria, across all types of natural ice formations, have been observed in ice that was also enriched with other organic particles or with \textit{lithogenic} (originating in rocks) sediments (Table 15.2).

Also note that for all ice formations but permafrost, bacterial densities are reported routinely for a given volume of \textit{melted ice}. This approach reflects the fact that, until very recently, all ice samples (including permafrost) were first allowed to melt before microscopically examining their contents to count the number of bacteria present. Melting natural ice samples provides for ready examination of their contents by established methods for water samples, but presents an osmotic shock to organisms that may have acclimated to the higher ionic strength (and organic content) of their former liquid water home within the ice. This problem pertains especially to sea ice, but also to other frozen systems, since the “impurity” concentration mechanism inherent to the freezing process leads to liquid inclusions of higher ionic strength even in freshwater ice. The loss of bacteria during melting has been investigated by comparing ice samples melted...
directly, melted into source waters (first made bacteria-
free by filtration), and melted into a brine solution that
provides for a final melt salinity equivalent to the liquid
phase in the ice before melting. The latter approaches
yield the higher counts of intact bacteria, up to twice as
many.

Scaling the number of bacteria to the total volume
of the original ice sample, even when melted in a way
to minimize osmotic shock, masks another reality. As
revealed by new microscopic techniques for examining
intact (unmelted) ice, bacteria do not inhabit the ice
uniformly in space (Junge et al., 2001, 2004). Instead,
most of the organisms reside within the liquid phase
of the ice (Fig. 15.6), where they become increasingly
concentrated during the freezing process along with
other “impurities.” If the number of bacteria is scaled
to the inhabitable space within an ice formation – the
volume of water in the liquid phase (the brine), then in
situ bacterial densities are of course much greater. For
example, in Arctic winter sea ice, the range for bacterial
abundance in “standard” terms is 0.5–3 × 10^6 ml^-1
melted ice (Table 15.2); but when scaled to brine volume,
the densities are ~100 times higher. When considered
in terms of the volume of melted ice, relationships
between heterotrophic bacteria and their food (dissolved
organic matter) in the ice have been ambiguous or elusive;
when scaled to brine volume, the puzzle resolves, with
bacterial density correlating strongly with dissolved organic matter (Junge et al., 2004).

Few researchers have considered this fluid-phase
scaling approach for ice samples, but it is important
to conceptualizing and understanding how bacteria
colonize, survive, and metabolize within ice, especially
the colder forms of liquid-filled ice on Earth – winter
sea ice and permafrost. Note that in general bacteria
more homogeneously distributed in a given volume
e.g., microbial life in water) interact minimally
compared to those in closer contact with each other
within a biofilm or connected network. The latter
scenario best describes life in the liquid phase of natural
ices. The extreme conditions of Earth’s coldest ice
formations would seem to severely limit if not preclude
microbial activity, but on the other hand the freeze-
concentration aspects of ice formation should make
ice a compelling setting for intensive cell-to-cell com-
 munications, including genetic exchange.

Which factors account for the universal presence
of bacteria in natural ice formations on Earth? At
the most basic level, the answer lies in the fact that all
natural source waters (and sediments) for ice on the
planet contain bacteria. Knowledge of the bacterial
contents of the source water and of the concentration
process inherent to freezing might thus seem sufficient
to account for bacterial numbers in any final ice for-

mation. However, the bacterial content of an ice sam-
ple (even when melted to minimize osmotic shock)
rarely matches the bacterial density of the source
water: bacteria are not passive particles, nor are the
physics and chemistry of natural ice formation simple
linear processes. Even if bacteria were to behave as
relatively passive particles (expending minimal energy),
their lipid- and polysaccharide-rich outer coats can
result in attachment to other particles and surfaces.

### TABLE 15.2 Bacterial abundance in ice

<table>
<thead>
<tr>
<th>Type of ice formation</th>
<th>Sampling location</th>
<th>Sample T (°C)</th>
<th>Particle-poor ice</th>
<th>Particle-rich ice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snow</td>
<td>South Pole</td>
<td>−15</td>
<td>0.2–5 × 10^3</td>
<td></td>
</tr>
<tr>
<td>Ice sheet</td>
<td>Over Lake Vostok (2–4 km)</td>
<td>−3</td>
<td>0.2–8 × 10^3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Greenland (bottom of sheet)</td>
<td>−9</td>
<td></td>
<td>&gt;6 × 10^7</td>
</tr>
<tr>
<td>Lake ice</td>
<td>Lake Bonney, Antarctica</td>
<td>&lt;−5?</td>
<td>5 × 10^3</td>
<td>0.1–4 × 10^5</td>
</tr>
<tr>
<td></td>
<td>Imikpuk Lake, Alaska</td>
<td>−5</td>
<td>7 × 10^4</td>
<td>7 × 10^5</td>
</tr>
<tr>
<td>Sea ice</td>
<td>Southern Ocean, summer</td>
<td>−2</td>
<td>0.01–3 × 10^6</td>
<td>0.02–2 × 10^6</td>
</tr>
<tr>
<td></td>
<td>Southern Ocean, winter</td>
<td>−2</td>
<td>0.2–2 × 10^6</td>
<td>1 × 10^7</td>
</tr>
<tr>
<td></td>
<td>Arctic Ocean, summer</td>
<td>−2</td>
<td>0.4–2 × 10^6</td>
<td>0.05–1 × 10^7</td>
</tr>
<tr>
<td></td>
<td>Arctic Ocean, winter</td>
<td>−2 to ~20</td>
<td>0.2–1 × 10^5</td>
<td>0.5–3 × 10^6</td>
</tr>
<tr>
<td>Permafrost</td>
<td>Northeast Siberia</td>
<td>−10</td>
<td></td>
<td>&gt;1 × 10^6</td>
</tr>
</tbody>
</table>

**Note:**

“Number ml^-1 melted ice or g^-1 soil for permafrost; data compiled from Carpenter et al. (2000), Delille et al. (1995), Gradinger and Zhang (1997); Grossmann and Dieckmann (1994); Helmke and Weyland (1995); Junge et al. (2001, 2003a, 2004), Karl et al. (1999), Priscu and Christner (2004), Rivkina et al. (2000), and Sheridan et al. (2003)."
The presence of particulate matter in the source water can thus influence in non-linear ways the number of bacteria ending up in the fluid phase of the ice. As discussed in the previous section, the rate of freezing also influences this process, with more rapid freezing leading to higher concentrations of impurities. Airborne and snow delivery of particles to ice forming at an atmosphere–water interface also contributes to the overall bacterial count.

The sum of these physical inputs of bacteria can be nullified, however, by physical losses from the system during ice formation. Even as impurities (bacteria) become concentrated in the liquid fraction of ice forming over a body of water, a significant portion of those fluids (and bacteria) will be expelled from the ice back into the source water. The classic case of fluid loss from an ice formation is brine rejection from young sea ice (the expulsion of liquid from within the ice pore network into the underlying ocean), creating dense water that sinks below the ocean surface and helps to drive global thermohaline circulation.

The only physical mechanism to account for very high bacterial numbers in an ice formation is the entrainment of high concentrations of lithogenic particles (and their attached bacteria). Such entrainment occurs in sea ice as a result of benthic storms (Stierle and Eicken, 2002) and in terrestrial ice sheets at the ice–soil interface (Table 15.2). Note that soils and sediments contain many billions of bacteria per gram of particulate matter, compared to source waters for ice formation that typically contain only millions per milliliter (Deming and Baross, 1993).

An accounting of bacterial numbers in ice via physical inputs and losses will inevitably fall short, since bacteria rarely behave as passive particles. The full range of metabolic states, from dormancy to ongoing cell division, can be expected among the bacteria present in source waters (and sediments) prior to ice formation. Many bacteria will move to position themselves advantageously within the physico-chemical gradients of their aquatic environments, a process known as chemotaxis. If chemotaxis is possible within an ice–brine network (Junge et al., 2003b, and citations therein), then altered densities at favorable locations may result. Aquatic bacteria in general are known to attach to surfaces and particulate matter, either to remain in position against fluid flow (receiving new nutrients, while waste moves downstream) or to travel more rapidly as a passenger to a new position. Attachment is a common mode of existence for bacteria within permafrost (Rivkina et al., 2000) and sea ice (Junge et al., 2004), where the number of attachment sites can be very high due to the presence of sediments (Stierle and Eicken, 2002). Even when sediment particles are rarities in ice, they can serve as “hot spots” of bacterial abundance and activity (Priscu and Christner, 2004). When conditions are particularly favorable in the ice for a given bacterium, it will also grow (in size) and divide (as in Fig. 15.6), increasing population densities even under what we perceive as extreme conditions.

Once a distinct ice habitat has formed from source waters, some of the bacteria that were best or uniquely adapted to the prior aquatic conditions can be expected to succumb to their new circumstances. Complicating analysis of this loss term is that both the dissolved and particulate debris from such bacteria represent fuel for the growth of neighbors better adapted to life in ice. In a relatively closed ice system, losses might be expected to continue as the ice ages and bacteria spend more time under unfavorable conditions without replenishment of nutrients; the ice sheet overlying Lake Vostok, for example, contains only very low concentrations of bacteria (Table 15.2). Arguing against significant losses due only to the age of the ice, however, are the well-known preserving effects of freezing temperatures (and of brines) on a wide variety of bacteria from various milder habitats (Madigan et al., 1997; Dumont et al., 2004). Low bacterial abundance in ancient glacial ice is more likely due to low bacterial content of its source waters and the ice formation process than to massive cell lysis over time. If the latter were a common phenomenon, the base of the Greenland ice sheet (where sediments were entrained 0.1 to 2 Ma) would not still contain the observed high concentrations of bacteria (Table 15.2).

The permafrost environment, which includes some of the oldest (millions of years) and coldest frozen habitats on Earth, contains the highest numbers of intact bacteria among natural ice formations. These high densities are readily explained by the equally high abundances that characterize source (unfrozen) soils and the long-term preserving effects of sub zero conditions once the soils are frozen. The extent to which high numbers may also indicate a dynamic population of bacteria, metabolizing and growing in situ, depends, however, upon the availability of a liquid phase. The search for dynamic bacterial populations
in frozen habitats has thus been most rewarding in the most liquid-rich of ice formations – sea ice (Section 15.3.3).

In very cold ice formations and ice formed from source waters of limited impurities, the inhabitable (liquid-filled) space is so restrictive in its morphology that the most common form of bacterial loss – grazing by larger and more complex organisms (eukaryotic protists) – ceases to be a factor. Such grazers are excluded from these ice habitats by their size (Krembs et al., 2000), if not their greater fragility under extreme conditions. Bacteria cannot entirely escape predation through these spatial refugia, however, since they have other attackers in the form of nm-scale bacteriophage (bacteria-specific viruses). In frozen environments, such viral particles appear to become concentrated in the liquid phase along with salts, organic polymers and colloids (in the same nm-size class as viruses), and their μm-sized bacterial targets. Some of the highest viral concentrations in aquatic environments have been found in sea ice (>1 × 10^8 viral particles ml^-1 melted volume of sea ice; Maranger et al., 1994). Yet, the well-known role that viruses play in bacterial mortality in milder aquatic habitats has not been studied in frozen systems. The profound role that viruses can play in the evolutionary process, by mediating gene exchange between organisms for improved survival, constitutes an essential astrobiological question, but one that has not yet been addressed for life in ice.

15.3.2 Bacterial diversity

The advent of modern molecular techniques for assessing genetic diversity within and between natural microbial assemblages, without having to bring the organisms into culture, has greatly enhanced our general understanding of bacterial diversity. Some extreme environments, especially hydrothermal vents (Chapter 14), have received considerable attention from this perspective, but by comparison the diversity of bacteria in frozen environments is poorly known.

The handful of studies available on direct phylogenetic analyses of ice well fit the history and physical-chemical nature of the various types of ice formations that have been examined. For example, in glacial ice sheets and Antarctic lake ice, where bacterial abundance is usually very low (Table 15.2), bacterial diversity reflects a cosmopolitan input of Bacterial tourists. Notably absent from these DNA libraries to date (likely reflecting low numbers not detectable by the methods in use) are any Archaea, even though cold-adapted methanogens (methane-producing members of the Archaea) have been cultured from the source waters (Fig. 15.2). Many of the Bacterial organisms detected, typically dominated by gram-positive bacteria (known for their thick cell walls) and several subdivisions of the common Proteobacteria, are recognizable as delivered aerially or via entrained soils. They are either associated with atmospheric indicators, adapted to dessication (thick-walled spore formers), or of known soil origin (Priscu and Christner, 2004; Sheridan et al., 2003). The detection of thermophilic bacteria in glacial ice (!) is unambiguous evidence of the preserved state of many of these airborne tourists (Priscu and Christner, 2004; Sheridan et al., 2003), since thermophiles cannot function at temperatures below about +30°C, even if all other requirements for activity are met in glacial ice. The latter is unlikely for any type of microorganism, given the very limited fraction of liquid and rare chemical impurities that could serve as a source of energy or nutrition. The presence of photosynthetic bacteria, however, in ice overlying some permanent, surface Antarctic lakes (Priscu and Christner, 2004) does point to potential activity in situ when sunlight becomes available as a source of energy.

The detection in glacial ice of other bacteria (and viruses) normally associated with the human body has fueled speculation that ancient ice may provide a repository of preserved organisms that reflect human health conditions of ages past. Such speculation is tempered by the issue of contamination (and methodological artifacts) when applying DNA amplification techniques to natural samples. Considerable effort has thus been expended on contaminant-free collection of ancient ice samples, so that confidence is increasing in techniques that distinguish sample-derived organisms from contaminants (Sheridan et al., 2003).

Another form of freshwater ice – Antarctic snow, with very low bacterial abundance (Table 15.2) and a vanishingly low content of liquid water (Table 15.1; Warren and Hudson, 2003) – has also been examined by direct amplification of DNA. Carpenter et al. (2000), who targeted only Bacteria, recovered DNA sequences indicative of organisms known for their ability to survive stresses related to dryness or low water activity, in particular various members of the genus Deinococcus. Other bacteria related to known (cultured) marine psychrophiles also appeared in this DNA library of snow from the South Pole, suggesting that aerially delivered organisms came from other cold environments.
The ice formation expected to support the highest bacterial diversity, for both Bacteria and Archaea, is permafrost. Temperate soils (that may experience only brief periods of freezing in winter) are well known for their high microbial diversity, often one or two orders of magnitude higher than in associated bodies of water (or ice). This difference is due to the wide range of niches, including anoxic ones, present in soils and sediments. Such structural complexity and niche diversity also pertains to permafrost, even if deeply frozen by atmospheric conditions: upon warming, permafrost samples have yielded a wide variety of culturable Bacteria, as well as methanogens. The full extent of microbial diversity in deeply frozen permafrost, however, has yet to be examined using modern phylogenetic or DNA amplifying techniques; the habitat is wide open for discovery. At issue is the degree to which the severity of conditions in deeply frozen ice may alter the initially high microbial diversity prior to freezing. A further unanswered question (which can also pertain to sea ice) is the degree to which freeze–thaw cycles influence microbial diversity.

The ice formation perhaps most intriguing with regards to bacterial diversity is sea ice. Spring and summer sea ice – sunlit, near the melting point of water, and with nutrient-rich seawater infiltrating from below – is the best studied and most biologically active of all ice formations, even trumping the underlying ocean in terms of biological productivity on a per-volume (of bulk ice) basis (Arrigo et al., 1997). The total biomass of microscopic organisms, especially the pigmented photosynthetic algae that bloom in the ice during the sunlit seasons, often becomes visible to the naked eye (Fig. 15.9). Given such high levels of biological growth and production, warm-season sea ice (called “summer” sea ice, as opposed to the much colder winter sea ice) should be populated primarily by residents (as opposed to tourists) that are well adapted to conditions in the ice; and indeed it is, for every class of organism and by every type of analysis (Krembs et al., 2000; Thomas and Dieckmann, 2002). From the bacterial perspective, sea ice is a global seed bed for psychrophilic marine bacteria, providing a highly selective habitat for their competitive survival through the winter season, then releasing them to the ocean (and atmosphere) during melt seasons (Helmke and Weyland, 1995). Unlike glacial ice formations, where thermophilic tourists can be preserved in a dormant state, no thermophiles and rarely even mesophiles have been cultured from sea ice. Their absence further supports the cold-adapted and dynamic state of sea-ice bacteria: tourists and even pioneers from other climes are poor competitors with residents for local resources.

Although resident sea-ice bacterial communities may be predominantly psychrophilic, how diverse are they phylogenetically? The structural complexity of the ice, as well as seasonal opportunities to associate with a wide range of higher organisms (the algae and their metazoan grazers) not found in other ice formations, should favor an abundance of niches, including anoxic ones, and thus relatively high diversity. The stress factors of low temperature and high salt, however, might be expected to place limits on diversity, especially during winter. Recent applications of DNA amplification techniques to summer sea ice do suggest a gradient in Bacterial diversity, with higher diversity in algal bands near the seawater (Fig. 15.9) and lower diversity in other portions of the ice. A study of winter sea ice suggests a further reduction in bacterial diversity as the severity of conditions increase (Junge et al., 2004). An important exception to this trend is that winter sea ice has also yielded the first evidence for Archaea in any ice formation other than frozen soil (which is a repository of Archaeal methanogens even before freezing). If these results hold, then one of the coldest of ice formations on Earth – Arctic winter sea ice – supports diversity at the Domain level unseen in other (particle-poor) frozen environments. Because the detection method for these Archaea also implied an actively metabolizing state (Junge et al., 2004), the
implications for adaptation of life to subzero temperatures are potentially important. Future work on winter sea ice (and possibly permafrost) may yield organisms that define a deeper root for cold-adapted Archaea on the Tree of Life (Fig. 15.2), changing the way we think about temperature as a driving force in the origin and evolution of life on Earth.

### 15.3.3 Bacterial activity

The heterotrophic bacteria emphasized in this chapter, being free of any direct requirement for light, need only an adequate supply of dissolved organic and inorganic nutrients for their life activities. Many if not most are facultative anaerobes, able to live independently of free oxygen as well, using other oxidized compounds (e.g., dissolved nitrate, sulfate, metal oxides, or organic compounds) as electron acceptors during the metabolism that generates energy for the cell. The requirement for “dissolved” nutrients implies the presence of a liquid phase within the ice matrix. Liquid allows for diffusion of compounds to and from the organism, i.e., delivery of nutrients and removal of waste products (required for continued activity and growth). The minimal liquid fractions and spatially restricted and disconnected inhabitable spaces within freshwater ice formations do not favor bacterial growth; the “dryness” of some may preclude even cellular maintenance activities, leaving saline ice formations as the better candidates to house dynamic populations of bacteria. In fact, the natural ice formation on Earth most supportive of a flourishing and diverse microbial (and Eukaryotic) community (and most flushed with liquid water) is spring–summer sea ice.

In sea ice (or any ice formation on Earth), the organic nutrient supply for heterotrophic bacteria traces back either to the byproducts of algae that also inhabit the ice or to the dissolved organic materials (also biologically produced) in the source seawater. Although abiotic sources of organic nutrients may well pertain to more deeply frozen systems on Earth and elsewhere (Section 15.2.1), the dominant role of photosynthesizing organisms in Earth’s contemporary, sunlit global ecosystem makes their influence on other life forms virtually inescapable. Levels of both bacterial abundance and activity in sample cores of sea ice invariably reach their maxima in association with a band of springtime ice algae (Fig. 15.9) and are best understood for that reason (Thomas and Dieckmann, 2002). The levels achieved rival those known in milder environments on Earth.

In the dark polar winter, however, Earth’s photosynthetic organisms are the least influential in a given habitat; heterotrophic organisms reign. As we have seen, bacteria are present in significant numbers in every cubic centimeter of ice that covers the polar seas (or land; Table 15.2), regardless of the season, age, or other conditions of the ice, but during the winter season they account overwhelmingly for the total biomass of all organisms present in sea ice (Delille et al., 1995; Krembs et al., 2002). A relevant astrobiological question is whether or not these bacteria continue to be metabolically active or even reproductive under the most extreme of winter conditions (below −20°C and in brine solutions of >21% salt). If the most liquid-filled niches of Earth’s coldest ice formations – winter sea ice in the high Arctic – do allow for continued activity, what factors may ultimately impose a limit on that activity? Could other types of extremely cold ice formations akin to sea ice, whether on Earth or elsewhere, also support dynamic bacterial populations? To what extent is microbial life itself in control of its own environment and threshold settings for continued activity?

Empirical research to address some of these astrobiological questions has only recently begun. There have been few direct attempts to measure bacterial activity in natural (or simulated) ice formations under extreme conditions, e.g., at temperatures near or below −15°C. This temperature has sometimes been considered a lower limit for growth of bacteria in culture, based on extrapolations from measured growth (production of new cells) at warmer temperatures where it can be readily detected by conventional methods. Still speculative are much lower temperature limits for metabolic activities that, while not supporting growth, would ensure cell maintenance and genetic survival (undamaged DNA). Table 15.3 summarizes recent results, but differences among the available studies in both methods and motivations make comparisons difficult. Furthermore, deeply frozen samples must be manipulated or altered (e.g., warmed, melted, amended, incubated), usually to a greater degree than other types of environmental samples, in order to make an activity measurement, sometimes calling into question the relevance of results to the in situ frozen environment. A microscopic approach for working with natural ice samples that are neither warmed nor melted does exist (Junge et al., 2001) and cross-disciplinary

11 Some inhabitants of the deep subsurface biosphere are important and exciting exceptions (Chapter 14).
interaction, one of the hallmarks of astrobiology (Chapter 28), is further advancing the issue of how to measure or deduce metabolic activity in unaltered ice (Carpenter et al., 2000; Warren and Hudson, 2003; Junge et al., 2004; Price and Sowers, 2004).

In spite of the limited experimental work to date and the various caveats inherent to it, activity measurements made on the coldest of ice formations (Arctic winter sea ice and Siberian permafrost) indicate that the lowest temperature yet tested, −20°C, still permits significant bacterial activity. The measured activities include those required for cellular maintenance, not necessarily growth: oxygen respiration to generate chemical energy for the cell; lipid production to keep membranes well structured and functional; and protein production to ensure critical biochemical functions. Where rates have been determined for a range of temperatures (in permafrost samples), activities are lower than under warmer conditions, as thermodynamics would predict. One theoretical treatment of data available in the literature, including a deduction of microbial activity at −40°C from chemical anomalies in glacial ice, suggests that an absolute temperature minimum for cellular activity may not exist (Price and Sowers, 2004). If one does exist, it may simply define the point at which critical (DNA) repair mechanisms in the cell fail to keep pace with any degradative processes, however slow they may be in deeply frozen systems. In any case, a temperature minimum would not be the same for all frozen systems, but instead would vary according to the specific physical-chemical features of a given ice formation. Since attempts to measure DNA replication or bacterial growth in natural ice formations have not yet been reported at −20°C (only to −15°C; Table 15.3 and Fig. 15.6), nor have empirical tests for any type of activity been reported at temperatures below −20°C, surprises may lie in store. Determining the physical-chemical ice features and/or biological mechanisms that enable continued bacterial activity in frozen environments at temperatures of −20°C and possibly lower represents a field of inquiry of critical importance.

### TABLE 15.3 Lowest known temperatures for bacterial activity in ice

<table>
<thead>
<tr>
<th>Type of ice formation</th>
<th>Samples examined</th>
<th>Lowest T (°C) examined</th>
<th>Type of activity detected</th>
<th>Sample manipulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial ice VI</td>
<td>Liquid cultures</td>
<td>+25 (at 1.25 GPa)</td>
<td>Anaerobic formate oxidation (slow rate)</td>
<td>Amended with formate (no warming), pressurized, incubated 300 h at test T</td>
</tr>
<tr>
<td>Artificial glacial ice</td>
<td>Liquid cultures</td>
<td>−15</td>
<td>DNA and protein production (slow rate)</td>
<td>Pre-frozen to −70°C, amended with liquid tracer (warmed), incubated 280 d at test T</td>
</tr>
<tr>
<td>Antarctic snow</td>
<td>Snow samples (at −28°C <em>in situ</em>)</td>
<td>−12 to −17</td>
<td>DNA and protein production (slow rate)</td>
<td>Amended with liquid tracer (warmed), incubated 24 h at test T</td>
</tr>
<tr>
<td>Siberian permafrost</td>
<td>Soil samples (at −10°C <em>in situ</em>)</td>
<td>−20</td>
<td>Lipid production (near detection limit)</td>
<td>Amended with liquid tracer (warmed and mixed), incubated 550 d at test T</td>
</tr>
<tr>
<td>Arctic winter sea ice</td>
<td>Sea-ice sections (at −20°C <em>in situ</em>)</td>
<td>−20</td>
<td>Oxygen respiration (1–4% of cells)</td>
<td>Melted in −20°C brine, amended with respiratory stain (no warming), incubated 24 h at test T</td>
</tr>
<tr>
<td>Arctic winter sea ice</td>
<td>Sea-ice sections (at −20°C <em>in situ</em>)</td>
<td>−20</td>
<td>Possible protein production (70% of cells)</td>
<td>Fixed (no warming or incubation), examined microscopically using fluorescent 16S rRNA probes</td>
</tr>
</tbody>
</table>

*Note:* data compiled from Carpenter et al. (2000), Rivkina et al. (2000), Christner (2002), Sharma et al. (2002), and Junge et al. (2004)
to astrobiology. Pursuing even colder temperatures will not be the only direction to take. Recall that under extremely high pressures, ice VI can form over the temperature range of 0 to +80°C. Novel experiments on bacterial activity under such unusual conditions (not found on Earth) have yielded intriguing results (Sharma et al., 2002; Table 15.3) that include apparent bacterial motility, pointing to the importance of an interconnected network of liquid water within an ice formation. We again emphasize that the basic biology of the resident organisms themselves is only one part of the story. The other part comprises the combined physical and chemical features of the frozen environment – its microstructure, the availability of interconnected fluids as well as attachment surfaces, the hydrated state of organic gels in which cells may be imbedded, the initial freezing rate of the source fluids, etc.

15.4 Summary and prospectus

In this chapter, we have emphasized the range of frozen environments on Earth and thus the known range of specific physical and chemical conditions encountered by microorganisms either thriving or simply managing to survive and persist within those ice formations. Perfect analogues for potential habitats elsewhere in the frozen realm of the Solar System may not exist on Earth, but examining and comparing the possibilities on this planet provides an excellent basis for developing predictions – and often novel experimental approaches to test them – of where explorers might find microbial life, and in what stage of development or survival. A synthesis approach, integrating the available information across many different types of ice, reveals new avenues for research and discovery in well-studied ice formations, as well as gaps in the astrobiological exploration of ice on Earth. In the latter case, for example, the surfaces of remote high-salt lakes in Antarctica often experience atmospheric conditions colder than those of high Arctic sea ice or permafrost, yet the microbiology of saline ice that forms on some of them during the coldest winter months awaits study. Although terrestrial permafrost formations are well studied from some perspectives, modern molecular techniques for evaluating basic microbial diversity have not yet been applied to these environments that are often presented as analogues for frozen martian soils.

Regardless of where on Earth we search for life in ice, the issue of paramount importance to microbial and enzymatic processes in an ice formation is the availability of liquid water within the ice matrix. Although a threshold fraction or absolute amount of liquid water required for microbial activity in ice is not known, connectivity of the liquid on a scale relevant to bacteria, the presence of nutrients, and materials they exude all favor activity. We know that the most liquid-rich ice formations on Earth (sea ice) support very dynamic microbial populations dominated by heterotrophic bacteria adapted to the cold. In contrast, liquid-poor ice formations (e.g., glacial ice) appear to support only the limited maintenance activities that may be required for long-term persistence and survival of those microorganisms, usually in very low numbers and sometimes of diverse origins (aerially delivered thermophiles as well as psychrophiles). As we have seen from physical-chemical observations and theory, however, the amount of liquid water in ice is not a simple function of temperature considered in isolation from other factors, just as a lower temperature threshold for bacterial activity or survival may not exist. Salt and other impurities are essential to the existence of the liquid phase at temperatures below the freezing point of water. On other bodies such as Europa, high pressure may be another key factor.

Let us push the physical-chemical boundaries of ice that can support microbial life to limits that are relevant off Earth, namely −55°C in the soils of Mars, and even colder in the saline ice covering Europa. The frozen systems of choice for the question of bacterial survival or preservation under severe extraterrestrial conditions may not be natural ice formations on Earth but rather artificial constructs in the laboratory. Under controlled conditions, study of the deep-freezing process (e.g., to −80°C or lower) known as vitrification, whereby water freezes to a glassy state without the formation of cell-damaging ice crystals or the retention of detectable liquid water (not discussed in this chapter; see Dumont et al., 2004), may be particularly illuminating. To move beyond microbial survival, however, and consider dynamic life processes, ice formations that retain some liquid should be best. Among the relatively unexplored ice formations on Earth that still retain an unfrozen fraction under the most extreme of natural conditions – lowest temperatures and highest concentrations of salt impurities (with the accompanying benefits of organic compounds and lithogenic particles, as we have discussed) – the various sea-ice formations over the Arctic Ocean

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12 Extensive permafrost formations that lie frozen beneath the Arctic Ocean have not at all been explored for their microbial content.
during wintertime offer great promise, with Arctic winter permafrost a close second.

In addition to being the ice formation on Earth that contains the highest fraction of liquid water, retaining liquid even at its coldest *in situ* temperatures (due to salt and other impurities), natural sea ice, especially during winter, presents within a single study area the advantage of access to a wide range of ice features and conditions (Fig. 15.10). The winter ice is wedged between severe atmospheric conditions above, which can approach the mean martian surface temperature of \(-55^\circ C\), and the warmer ocean below, at \(-1.9^\circ C\). Level pans of undeformed ice grow through the winter to a thickness of 2–3 meters as seawater continues to freeze to the bottom of an ice floe. Analysis of winter ice cores from these pans, with temperature gradients from about \(-20^\circ C\) (warmer than the atmosphere due to an insulating snow cover) to \(-2^\circ C\), has elucidated the roles of concentrated organic polymers and provided insight into bacterial abundance, diversity, and activity under extreme conditions, as discussed throughout this chapter. Cracks or leads that open and then freeze over again in winter can provide samples of rapidly frozen ice to test hypotheses on the effects of freezing rate on bacteria. The very coldest ice (well below \(-20^\circ C\) in winter) occurs at the surface of windblown ridges formed by compressive forces, windblown ice in rubble fields formed by shear forces, and windblown (thus nearly free of insulating snow) level ice (Fig. 15.10). The microbiology of these rapidly and/or deeply frozen environments awaits interdisciplinary study.

Although formed by large-scale planetary processes, the ability of these Arctic winter sea-ice formations – or any frozen environment – to support an active microbial population depends upon features evaluated on the scale of micrometers and less. As reinforced in this chapter, evaluating information on the dimensional scale directly relevant to an individual microorganism can reveal much about the inherent habitability of a given environment and thus whether to expect long-term residents, pioneers, or accidental tourists. Without such information from Earth, assessments of extraterrestrial habitability will be limited if not inaccurate. Nevertheless, the larger scale features of an extreme environment on Earth can be instructive and compelling; those observed in aerial images of the ice-covered Arctic Ocean (Fig. 15.10) in particular have captured the attention of planetary scientists and astrobiologists focused on the icy moons of Jupiter. Even though occurring on still larger scales and resulting from deformation mechanisms that differ in some ways from those on Earth, the frozen and salty surface of Europa also clearly exhibits ridged ice, refrozen cracks, and level rafts of ice of variable thickness (Pappalardo *et al.*, 1999; Chapter 19). The possible relevance to Europa of microbial life in saline ice formations on Earth provides a powerful motivation for future study.

Studies of frozen environments on Earth can significantly help in achieving key objectives of the emerging science of astrobiology. Specifically, they further our understanding of fundamental constraints on the evolution of life at low temperatures and aid in testing conceptual models of the potential presence and detectability of life in extraterrestrial environments such as Mars or Europa. They also help to constrain scenarios of planetary development and composition that may include microbial colonization and evolution. Moreover, experience has shown that closer looks at Earth’s frozen environments have almost invariably yielded remarkable and sometimes unexpected evidence for the sustainability of microbial life under ever more extreme conditions than previously imagined.

Ultimately, the interdisciplinary study of life within the full range of frozen environments on Earth may lead us towards more meaningful predictions of life – or its absence – in ice elsewhere. The liquid water boundaries on Earth have not yet been adequately explored from the microbial, enzymatic, or physical-chemical perspectives. The phase fractions of solid ice, liquid brine, and salt precipitates need to be considered in parallel with microbiological parameters.
In this chapter we have only just begun such an analysis from the existing literature – the field is wide open for discovery of microbial strategies for surviving and even thriving in ice, and the critical physics and chemistry underlying them. And beyond water ice, the natural state of H$_2$O in our Solar System, the possibilities for life in more exotic frozen environments, such as methane or hydrocarbon ice on Titan (Chapters 20 and 27), further beckon.

References


References


**Further reading and surfing**

