ASTROBIOLOGY
Volume 3, Number 4, 2003

Europa

The Search for Life on Europa: Limiting Environmental Factors, Potential Habitats, and Earth Analogues

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ABSTRACT

The putative ocean of Europa has focused considerable attention on the potential habitats for life on Europa. By generally clement Earth standards, these Europan habitats are likely to be extreme environments. The objectives of this paper were to examine: (1) the limits for biological activity on Earth with respect to temperature, salinity, acidity, desiccation, radiation, pressure, and time; (2) potential habitats for life on Europa; and (3) Earth analogues and their limitations for Europa. Based on empirical evidence, the limits for biological activity on Earth are: (1) the temperature range is from 253 to 394 K; (2) the salinity range is a_{H_2O} = 0.6–1.0; (3) the desiccation range is from 60% to 100% relative humidity; (4) the acidity range is from pH 0 to 13; (5) microbes such as *Deinococcus* are roughly 4,000 times more resistant to ionizing radiation than humans; (6) the range for hydrostatic pressure is from 0 to 1,100 bars; and (7) the maximum time for organisms to survive in the dormant state may be as long as 250 million years. The potential habitats for life on Europa are the ice layer, the brine ocean, and the seafloor environment. The dual stresses of lethal radiation and low temperatures on or near the icy surface of Europa preclude the possibility of biological activity anywhere near the surface. Only at the base of the ice layer could one expect to find the suitable temperatures and liquid water that are necessary for life. An ice layer turnover time of 10 million years is probably rapid enough for preserving in the surface ice layers dormant life forms originating from the ocean. Model simulations demonstrate that hypothetical oceans could exist on Europa that are too cold for biological activity (T < 253 K). These simulations also demonstrate that salinities are high, which would restrict life to extreme halophiles. An acidic ocean (if present) could also potentially limit life. Pressure, *per se*, is unlikely to directly limit life on Europa. But indirectly, pressure plays an important role in controlling the chemical environments for life. Deep ocean basins such as the Mariana Trench are good analogues for the cold, high-pressure ocean of Europa. Many of the best terrestrial analogues for potential Europan habitats are in the Arctic and Antarctica. The six factors likely to be most important in defining the environments for life on Europa and the focus for future work are liquid water, energy, nutrients, low temperatures, salinity, and high pressures. Key Words: Limiting factors for life—Extreme environments—Potential habitats for Europa—Earth analogues. Astrobiology 3, 785–811.

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INTRODUCTION

In the past few decades, our concept of a potentially habitable world has broadened from the narrow range between Venus and Mars to literally cover most of the Solar System. For example, there is evidence that the moon of Pluto, Charon, may be hot enough to produce liquid water (Vogel, 1999). Two primary factors have contributed to broadening our concept of a habitable world. First, there has been an explosion of research on the ability of microorganisms, especially bacteria and archaea, to grow in extreme environments with respect to temperature, salinity, acidity, radiation, and pressure. Environments that would have been considered inhospitable for life a few decades ago have been found thriving with life (e.g., hydrothermal vents). The other factor in broadening our concept of a hospitable world is a better understanding of the environments within which liquid water can exist. Today in the planetary sciences, the first step in the search for life is often the search for liquid water. The current mantra of astrobiology is "Follow the water."

Europa is a cold, ice-covered moon of Jupiter that might at first seem inhospitable for life. But there is abundant evidence for the presence of a subsurface briny ocean (Pappalardo *et al.*, 1999; Kargel *et al.*, 2000; Stevenson, 2000). The putative ocean of Europa has focused considerable attention on the possible habitats for life on Europa (Reynolds *et al.*, 1983; Jakosky and Shock, 1998; Gaidos *et al.*, 1999; McCollom, 1999; Chyba, 2000; Kargel *et al.*, 2000; Chyba and Hand, 2001; Chyba and Phillips, 2001; Navarro-Gonzalez *et al.*, 2002; Pierazzo and Chyba, 2002; Schulze-Makuch and Irwin, 2002). By generally clement Earth standards, the habitats for life on Europa are likely to be extreme environments. On the other hand, extraterrestrial life, if it exists, may be well adapted to its environments and find Earth environments extreme. Nevertheless, we have adapted an Earth-centric perspective and will judge environments by terrestrial life standards ("Life as we know it").

The objectives of this paper were to examine: (1) the limits for biological activity on Earth with respect to temperature, salinity, acidity, desiccation, radiation, pressure, and time; (2) potential habitats for life on Europa; and (3) potential Earth analogues and their limitations for Europa. In this review, we will use a chemical thermodynamic model (FREZCHEM) to simulate how hypothetical Europan chemical compositions are controlled by temperature and pressure. This will enable us to evaluate how environmental factors such as salinity, acidity, desiccation, temperature, and pressure may limit life on Europa.

THE LIMITS FOR LIFE ON EARTH

In this work, we will examine temperature, salinity, acidity, desiccation, radiation, pressure, and time as potential limiting factors for life on Europa. We are implicitly assuming that the supplies of liquid water, energy, and nutrients are, at least, present in some minimal levels to support life on Europa (Reynolds *et al.*, 1983; Jakosky and Shock, 1998; Gaidos *et al.*, 1999; McCollom, 1999; Chyba, 2000; Kargel *et al.*, 2000; Chyba and Hand, 2001; Chyba and Phillips, 2001; Navarro-Gonzalez *et al.*, 2002; Pierazzo and Chyba, 2002; Schulze-Makuch and Irwin, 2002). Other potential limiting factors such as toxic metals and the presence or absence of oxygen or other oxidants will only be peripherally examined in this work. We will first examine the limits for life on Earth. For most of the factors examined (temperature, salinity, acidity, desiccation, radiation, and pressure), we will focus on "normal" biological activity (i.e., respiration, growth, reproduction); for the factor of time, we will examine how much time is required for life to develop on a planet, and how long can organisms survive in the dormant state.

Temperature

There are three environments on Earth where microbes have been identified with temperature tolerances in the range from 100°C to 121°C, namely, submarine hydrothermal vents, the subterranean deep biosphere, and terrestrial hot springs (Table 1). The highest temperature tolerances (110–121°C) are found in microbes from marine hydrothermal vents and the subterranean deep biosphere; high pressures prevent these waters from boiling at 100°C, the normal boiling point of water at 1.01 bar (1 atm) pressure. From terrestrial hot springs, microbes have been isolated that can tolerate temperatures up to 103°C (Table 1).

Hyperthermophiles are invariably either bacteria or archaea. Eukaryotes have an upper temperature range of ~50–60°C (Madigan and Marrs,

TABLE 1. A SUMMARY OF LIMITS FOR BIOLOGICAL ACTIVITY AT HIGH AND LOW TEMPERATURES

Factor	*Type of environment*	*Limits*	*References*
High temperatures	Submarine hydrothermal vents	110–121°C	Pledger and Baross (1991), Segerer *et al.* (1993), Blöchl *et al.* (1997), Huber and Stetter (1998), Stetter (1999, 2002), Madigan and Oren (1999), Kashefi and Lovley (2003)
	Subterranean deep biosphere	110°C	Pedersen (1993), Fyfe (1996), Stetter (1996)
	Terrestrial hot springs	103°C	Kushner (1981), Cometta *et al.* (1982), Smith (1982), Farmer (1998, 2000), Stetter (1999), Rothschild and Mancinelli (2001)
Low temperatures	Ice	−17 to −20°C	Vogel (1999), Rivkina *et al.* (2000), Junge *et al.* (2001, 2004), Rothschild and Mancinelli (2001), Gilichinsky (2002), Junge (2002)
	Dry terrestrial	−17°C	Friedmann and Ocampo (1976), Friedmann and Ocampo-Friedmann (1984), Schroeter and Scheidegger (1995), Gilichinsky (2002)
	Deep sea	~2°C	Yayanos (1995), Sassen *et al.* (1999), Krumgalz *et al.* (1999)

1997; Nealson, 1997; Nealson and Conrad, 1999; Rothschild and Mancinelli, 2001). Until recently, *Pyrolobus fumarii* (an archaea) had the highest temperature tolerance of 113°C (Blöchl *et al.*, 1997); this organism has a minimum temperature for growth of 90°C and an optimum temperature of 106°C and is a strict hyperthermophile (Stetter, 1999). Recently, an archaea was isolated from a hydrothermal vent with a temperature tolerance of 121°C; this organism is closely related to *Pyrodictium occultum* and *Pyrobaculum aerophilum* and doubled in cell number after 24 h at 121°C (Kashefi and Lovley, 2003).

There are three types of environments on Earth where low temperatures are prevalent, namely, ice, cold terrestrial environments, and the deep sea (Table 1). Ice includes snow, glaciers, frozen lakes, sea-ice, and permafrost. Examples of cold terrestrial environments include the Dry Valleys of Antarctica and Arctic polar deserts. Temperatures in the oceanic abysses hover around 2°C at a maximum hydrostatic pressure of 1,100 bars (10,660 m) in the Mariana Trench (Yayanos, 1995).

There are a number of reports in recent years that have demonstrated that some microbes can metabolize, albeit slowly, at temperatures in the range from −17°C to −20°C (Table 1). These organisms include bacteria, lichens (a symbiotic association of algae and fungi), and fungi (yeasts). Many of these ecosystems are in protected environments such as in aqueous pockets in ice (Priscu *et al.*, 1998; Psenner and Sattler, 1998; Thomas and Dieckmann, 2002) and within rocks (cryoendoliths) (Friedmann and Ocampo, 1976; Friedmann and Ocampo-Friedmann, 1984), where climate is more clement than in exposed areas.

Salinity

Salinity affects microbial activity because it controls water availability. The higher the salinity, the more energy an organism must expend to maintain a favorable osmotic balance. Because salinity has been studied by scientists representing many disciplines, measures of salinity vary widely among disciplines. Chemists tend to prefer concentration units of molality [mol/kg (water)], while physiological microbiologists often report salinities as salt % (wt/wt) [g/100 g (soln)], as salt % (wt/vol) (g/100 ml), or as the activity of water (a_{H_2O}). The activity of water is probably the best measure of salinity as it relates directly to the osmotic gradient controlling flows of salts and water into and out of organisms.

This diversity of measures of salinity is especially a problem in cross-disciplinary work, where alternative measures from other disciplines are often unfamiliar. The equations needed to convert from one measure to another are:

$$m\left(\frac{kg(\text{water})}{kg(\text{solution})}\right)\rho = M \quad (1)$$

$$\frac{\Sigma m_i MW_i}{10}\left(\frac{kg(\text{water})}{kg(\text{solution})}\right) = \%salt(\text{wt/wt}) \quad (2)$$

$$\%salt(\text{wt/wt})\rho = \%salt(\text{wt/vol}) \quad (3)$$

$$\frac{kg(\text{water})}{kg(\text{solution})} = \frac{1{,}000}{1{,}000 + \Sigma m_i MW_i} \quad (4)$$

where m is the molality [= mol/kg (water)], M is the molarity [= mol/L], ρ is the density (= kg (solution)/L), and MW is the molecular weight (g/mol). The density of solutions (ρ), which is needed for some of the above conversions, can either be measured experimentally or estimated with a model [e.g., FREZCHEM (G.M. Marion *et al.*, manuscript submitted for publication)].

Figure 1 depicts several measures of salinity for pure NaCl solutions at 25°C and 1.01 bar total pressure calculated using the FREZCHEM model (Marion and Farren, 1999; Marion, 2001, 2002; G.M. Marion *et al.*, manuscript submitted for publication) and Eqs. 1–4. NaCl solutions were used in this example because this salt is often used as a background salt in physiological studies (Madigan and Oren, 1999; Kaye and Baross, 2000). For salts other than NaCl or at temperatures other than 25°C, Eqs. 1–4 must be relied upon to make conversions.

Table 2 outlines the approximate salinity limits for biological activity of bacteria/archaea and fungi. Salinity is one of the few limiting factors for life where a eukaryote (fungi) has a higher tolerance than prokaryotes (bacteria/archaea). To place these limiting a_{H2O} values in perspective, the a_{H2O} for seawater is 0.98. Most prokaryotes and fungi can tolerate much higher salinities than seawater (Table 2).

TABLE 2. TOLERANCES TO SALINITY FOR MICROBIAL ACTIVITY

	Tolerances
Most bacteria/archaea	$a_{H_2O} > 0.9$
Extreme bacteria/archaea	$a_{H_2O} \sim 0.70$
Most fungi	$a_{H_2O} > 0.85$
Extreme fungi	$a_{H_2O} \sim 0.60$

Adapted from Mazur (1980) and Kushner (1981).

This review only provides a brief overview of salinity as a limiting factor for microbial growth. Several books and reviews in recent years provide a much more extensive review of the biology, chemistry, and geology of saline environments (e.g., Eugster and Hardie, 1978; Friedman and Krumbein, 1985; Javor, 1989; Rodriguez-Valera, 1991; Lowe *et al.*, 1993; Ventosa *et al.*, 1998; Kargel *et al.*, 2000). In the section on Earth analogues, we will discuss specific examples of terrestrial saline environments.

Acidity

Acidity is typically quantified using the pH scale:

$$\text{pH} = -\log_{10}(a_{H^+}) \quad (5)$$

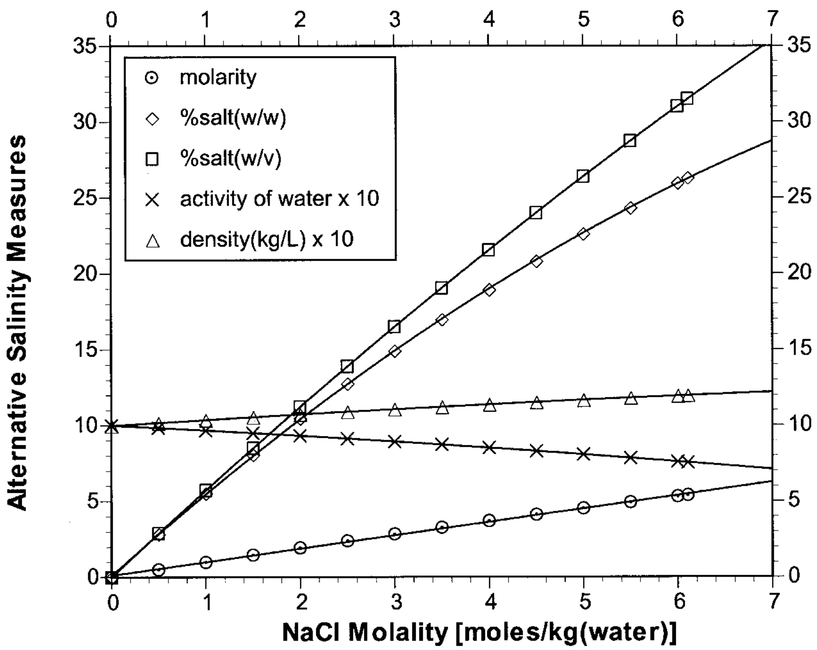

FIG. 1. Alternative measures of salinity for pure NaCl solutions at 25°C and 1.01 bars of pressure.

where a_{H^+} is the hydrogen ion activity. The activity of a single ion cannot be measured unambiguously (Pitzer, 1995); therefore, assumptions must be made in defining pH. One convention is to use a chemical thermodynamic model to estimate the activity coefficient of $H^+(\gamma_H{}^+)$. Then, given an experimental measurement of the molal concentration (*m*), one can calculate the activity as follows: $a = \gamma\, m$. This is how most geochemical models work. Another assumption, most frequently used in calibrating pH standards, is the MacInnis convention (Harvie *et al*., 1984). In this case, the assumption is made that $\gamma_{K^+} = \gamma_{Cl^-}$ in all solutions of the same ionic strength. This allows one, indirectly, to estimate γ_{H^+} and to define a_{H^+}. The reason for raising this issue is that pH values calculated with these two conventions can lead to very different pH values in extreme high acidities (pH <1.0). [See Marion (2002) for a fuller discussion of this issue.]

There are many studies demonstrating that a wide range of organisms can tolerate pH values <1.0. For example, bacteria, archaea, fungi, and algae have all been demonstrated to tolerate pH values ≤1.0 (Table 3). The current record holders are *Picrophilus oshimae* and *Picrophilus torridus* (archaea) that can grow at a pH of −0.06 (Schleper *et al*., 1995). Unfortunately in these acid studies, it is not always clear which of the above two pH conventions was used. For example, the Schleper *et al*. (1995) study, which reported the lowest pH value of −0.06, only indicated that 1 *M* HCl was used as a reference for pH 0, but there is no clear indication which convention was used to calibrate their pH electrode. On the other hand, the Iron Mountain acid studies (e.g., Schrenk *et al*., 1998; Edwards *et al*., 1999; Robbins *et al*., 2000) clearly used the MacInnis convention in calibrating electrodes at low pH (Nordstrom *et al*., 2000).

Invariably, at least on Earth, high acidities are associated with high concentrations of heavy metals because strong acids are highly effective in dissolving primary minerals, releasing heavy metals into the environment (Krishnaswamy and Hanger, 1998; Robbins *et al*., 2000; Lopez-Archilla *et al*., 2001). Therefore organisms that tolerate strong acidity also tolerate high levels of heavy metals.

There are fewer studies of high alkalinities (pH >10) than of high acidities (pH <1.0) probably because high alkalinities are more rare in nature. Nevertheless, there are reports of organisms tolerating pH values >11 (Table 3), and maybe even as high as 12.5–13 (Bachofen, 1986; Duckworth *et al*., 1996).

Desiccation

The desiccating power of the atmosphere is generally measured by relative humidity (RH), which is related to the activity of water (a_{H_2O}) by:

$$a_{H_2O} = RH/100 \qquad (6)$$

Just as $a_{H_2O} = 0.6$ is considered the lower limit for biological activity in saline solutions (Table 2), RH = 60% is considered the lower limit for biological activity under dry atmospheric conditions (Kushner, 1981; Dose *et al*., 2001).

A clear distinction must be made between biological activity and survival under desiccating conditions. Some organisms can survive 99% loss of water with a_{H_2O} ~0 (Mazur, 1980). *Bacillus sphaericus* spores survived 25 million years of desiccation in amber through a process called anhydrobiosis (Fischman, 1995). Bacteria, fungi, plants, and insects have been shown to survive extensive periods of dehydration (Rothschild and Mancinelli, 2001).

Examples of especially dry environments include the Atacama Desert of northern Chile and the Dry Valleys of Antarctica. Dose *et al*. (2001)

TABLE 3. A SUMMARY OF EXTREME pH TOLERANCES

Type of environment	*pH limits*	*Selected references*
Acidic systems Acid mine drainage Volcanic springs	pH = −0.06 to 1.0 Organisms: bacteria, archaea, fungi, algae Record pH = −0.06 [*Picrophilus* (archaea)]	Bachofen (1986), Schleper *et al*. (1995), Johnson (1998), Huber and Stetter (1998), Schrenk *et al*. (1998), Edwards *et al*. (1999), Robbins *et al*. (2000)
Alkaline systems Soda lakes	pH > 11 Organisms: bacteria	Bachofen (1986), Zhilina and Zavarzin (1994), Duckworth *et al*. (1996)

exposed spores, conidia, and cells of several microbes to 15 months of desiccation in the dark at two locations of the Atacama Desert. *Bacillus subtilis* (bacteria) spores (survival ~15%) and *Aspergillus niger* (fungi) conidia (survival ~30%) outlived other species. *Deinococcus radiodurans* (bacteria) did not survive the desert exposure because they were readily inactivated at RH between 40% and 80%, which occurred during desert nights (Dose *et al.*, 2001).

Radiation

Two types of radiation can limit life: ultraviolet (UV) and ionizing radiation. UV radiation is a significant component of sunlight. Sources of ionizing radiation include cosmic rays, x-rays, and radioactive decay.

Resistance to one form of radiation does not necessarily convey protection from other forms. Almost all organisms are prone to UV damage because the macromolecules that propagate genetic information (DNA) absorb UV radiation. For example, the experiments in the Atacama Desert cited in the previous section were done in the dark (shade). Direct exposure to UV radiation in these experiments killed all organisms within hours (Dose *et al.*, 2001).

Table 4 depicts resistance to UV and ionizing radiation for several microbes. *D. radiodurans* is well known to have a high resistance to ionizing radiation (Table 4). This resistance to radiation is thought to have evolved initially as a resistance to desiccation. The mechanism for conveying this resistance is believed due to their ability to quickly repair DNA damage (Kushner, 1981; Smith, 1982; Bachofen, 1986; Jawad *et al.*, 1998; Rothschild and Mancinelli, 2001). Other mechanisms to protect organisms from UV radiation include the development of iron-enriched silica crusts (Phoenix *et al.*, 2001) and self-shading (Smith, 1982). Also, both water and ice are effective in absorbing UV radiation (Baumstark-Khan and Facius, 2002).

Pressure

High pressures can occur in both deep-earth and deep-sea environments, but there are some fundamental differences between these two systems. In the deep sea, hydrostatic pressure on the organism is easily calculated by the depth (m). For example, 1 atm = 1.01325 bars = 0.101325 MPa = 9.816 m (Yayanos, 1995). In the deep earth, the confining pressure could be atmospheric with organisms growing in air pockets or, in contrast, very high as in brine pockets, where the organisms may be subjected to both hydrostatic and lithostatic pressures. Unfortunately, the actual pressures under which these deep-earth microbes grow are poorly documented (Pedersen, 1993). Another fundamental difference is that deep-sea environments decrease in temperature with increasing depth, while deep-earth environments increase in temperature with increasing depth.

Hydrostatic pressure affects physics, chemistry, and biology. For example, organisms with significant gas vacuoles do not handle pressure well (Bachofen, 1986). Pressure squeezes objects into smaller volumes. Chemical reactions that lead to a decrease in volume are favored by pressure. For example, the dissolution of gypsum is as follows:

$$CaSO_4 \cdot 2H_2O\ (V^0 = 74.69) \leftrightarrow Ca^{2+}\ (V^0 = -17.83) + SO_4^{2-}\ (V^0 = 13.98) + 2H_2O\ (V^0 = 18.07) \quad (7)$$

where V^0 is the molal volume (cm^3/mol) at infinite dilution:

TABLE 4. RADIATION DOSE GIVING ~37% SURVIVAL FOR UV AND IONIZING RADIATION

Microorganism	*UV radiation (J/m^2)*	*Ionizing radiation (Gy)*
T1-phage (virus)	—	2,600
E. coli (bacteria)	50	20–30
B. subtilis (bacteria)	—	33
D. radiodurans (bacteria)	600	1,500–6,000
Saccharomyces cerevisiae (yeast)	80	30–150
Chlamydomonas (algae)	—	24
Bodo marina (eukaryote: heterotrophic flagellate)	5,000	—
Humans (eukaryote)	—	1.4

Adapted from Kushner (1981) and Baumstark-Khan and Facius (2002).

$$\Delta V^0_r = V^0_{Ca} + V^0_{SO_4} + 2V^0_{H_2O} - V^0_{CaSO_4 \cdot 2H_2O} = -41.40\ cm^3/mol \quad (8)$$

In this case, ΔV^0_r is negative, which implies that pressure will cause the reaction to shift to the right causing a dissolution of gypsum. Another important reaction with special significance for Europa is the stability of water ice and liquid water at subzero temperatures under pressure:

$$H_2O(I,cr)\ (V^0 = 19.6) \leftrightarrow H_2O(aq)\ (V^0 = 18.0) \quad (9)$$

$$\Delta V^0_r = V^0_{H_2O(aq)} - V^0_{H_2O(I,cr)} = -1.6\ cm^3/mol \quad (10)$$

In this case, pressure will cause a melting of ice (to reduce the volume) with a consequent lowering of the freezing point (Fig. 2). Effects of pressure on the freezing point of aqueous solutions and other chemical equilibria will play an important role in defining boundaries for hypothetical Europan oceans (see the discussion in "Potential Europan Habitats for Life").

Microorganisms have been isolated from the Mariana Trench in the Pacific (10,660 m depth) where pressures reach 1,100 bars (Yayanos, 1995; Kato *et al.*, 1998; Abe *et al.*, 1999). Two bacteria similar to *Moritella* and *Shewanella* are apparently obligately barophilic with optimum pressures for growth occurring at 700 bars and no growth below 500 bars (Kato *et al.*, 1998). These Mariana Trench organisms grow at a temperature of 2°C.

There are some archaea associated with deep-sea hydrothermal vents that can survive at pressures as high as 890 bars (Pledger *et al.*, 1994). The high pressure of hydrothermal vents has a compensatory effect that allows stabilization of molecules, which allows growth at elevated temperatures up to 121°C (Table 1).

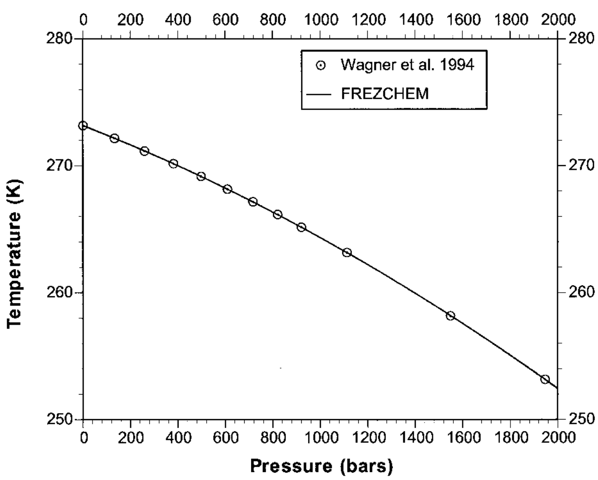

FIG. 2. The freezing point depression of a pure ice/water system as a function of pressure.

Recently, it was demonstrated in a diamond anvil cell that *Shewanella oneidensis* and *Escherichia coli* strains remain physiologically and metabolically active at pressure of 680–16,800 bars for up to 30 h (Sharma *et al.*, 2002). At pressures of 12,000–16,000 bars, living bacteria resided in fluid inclusions in Ice VI crystals and continued to be viable when pressure returned to 1 bar. However, only 1% remained alive; whether this constitutes viability or survival under pressure is contentious (Couzin, 2002). Nevertheless, it demonstrates that pressure may not be much of an impediment for some life forms.

Another phenomenon found under pressure at depth in terrestrial oceans and in permafrost is gas hydrate deposits. Hydrates of natural gases such as methane ($CH_4 \cdot 6H_2O$) and carbon dioxide ($CO_2 \cdot 6H_2O$) form on Earth beneath low permeability strata under high pressure and low temperature (Kvenvolden, 1993; Sloan, 1998; Blunier, 2000) (Fig. 3):

$$CH_4(g) \leftrightarrow CH_4(aq) + 6H_2O(l) \leftrightarrow CH_4 \cdot 6H_2O(cr) \quad (11)$$

The stability of these solid-phase compounds is a function of pressure, temperature, and matrix salt composition. Gas hydrates could be important sources of high-energy carbon (Carney, 1994). On Earth, gas hydrate deposits can sustain complex chemosynthetic communities (Sassen *et al.*, 1999; Fisher *et al.*, 2000). There is speculation that gas hydrates may be present on Europa (Kargel *et al.*, 2000).

Microorganisms have been found growing at depths of 2.8–4.2 km on land (Pedersen, 1993; Fyfe, 1996; Kerr, 1997). Microbes at 4.2 km are growing at a temperature of 110°C, which is probably the most important growth-limiting factor for deep-earth microbes (Pedersen, 1993; Fyfe, 1996). Because of the slow cycling of water, energy, and nutrients at depth, metabolic activity is believed to be extremely slow (Kerr, 1997).

Time

Two aspects of time have a bearing on the possibility of life on Europa: (1) How much time is

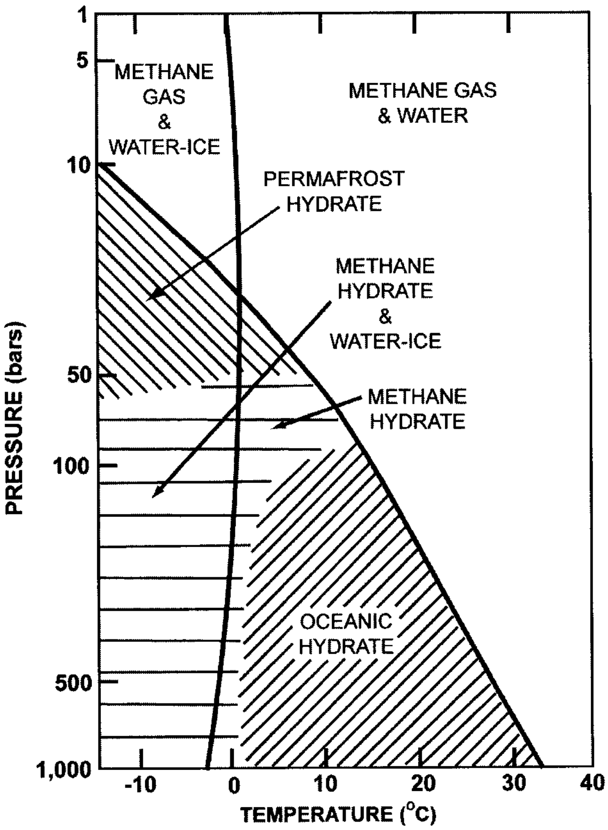

FIG. 3. Stability fields of methane hydrate in pure water (adapted from Kvenvolden, 1993; Pellenbarg and Max, 2000).

required for life to develop on a planet? (2) How long can life survive in the dormant stage in isolation from conditions normally considered vital for life such as cycling of liquid water, energy, and nutrients?

The Earth began forming about 4.6 billion years (Byr) ago. The first 600–800 million years (Myr) of Earth's existence have been erased by the constant early bombardment of asteroids and comets (Arrhenius and Lepland, 2000; Delsemme, 2001; Ehrenfreund and Menten, 2002; Wharton, 2002). The earliest geologic evidence for life on Earth dates to 3.5–3.8 Byr (Schopf and Packer, 1987; Mojzsis *et al.*, 1996; Ehrenfreund and Menten, 2002; Stetter, 2002; Wharton, 2002). Based on this evidence, it has been argued that life on Earth developed rapidly within about 200–300 Myr. During this interval, the Earth evolved from a hot dry rock to a cool wet world. Evidence suggests that the rain of asteroids and comets brought to Earth, water, organic molecules, and gases that are key ingredients for the establishment of life (Delsemme, 2001; Horneck and Baumstark-Khan, 2002). According to present knowledge, the time necessary for life to develop might require hundreds of millions of years.

This model of the evolution of life on Earth does not preclude the possibility that life arrived on Earth fully formed from another body [the Panspermia hypothesis (Horneck and Baumstark-Khan, 2002; Wharton, 2002)]. If this were the case, then only a short-term temporary abode would be necessary for life to become established.

Another, perhaps more important, question is: How long can life survive in the dormant state on a planet even under hostile conditions? A number of reports in recent years have suggested that microbes can survive in the frozen state (in ice or permafrost) for periods ranging from thousands to three million years (Soina *et al.*, 1995; Stone, 1999; Christner *et al.*, 2000; Gilichinsky, 2002). On an even longer time scale (25–40 Myr), viable microbes, similar to *B. sphaericus*, have been isolated from bees encased in amber (Fischman, 1995; Cano and Borucki, 1995). The longest reputed record for survival goes to a *Bacillus* spp. that has been isolated from halite crystals believed to be 250 Myr (Vreeland *et al.*, 2000). In that study, only two of 53 salt crystals had viable bacteria, suggesting that survival is a rare occurrence. However, Hazen and Roedder (2001) have argued that in the absence of primary growth features in the specific halite crystals studied, the age of these crystals and their fluids must remain in doubt. In a reply to these concerns, Powers *et al.* (2001) defended their crystal and fluid inclusion ages.

Life in our Solar System could have started rapidly if the Panspermia hypothersis is correct and life was seeded to Earth (and Europa?) from outside, or it may have taken hundreds of millions of years. At this time, only the crudest boundaries can be placed on the time for life to develop or the survival time for life after environmental conditons become hostile, but it may be on the order of hundreds of millions of years.

POTENTIAL EUROPAN HABITATS FOR LIFE

Figure 4 is a schematic diagram of a cross-section of the Europan surface. Many of the properties and processes are inferred based on indirect evidence. For example, estimates of the thickness

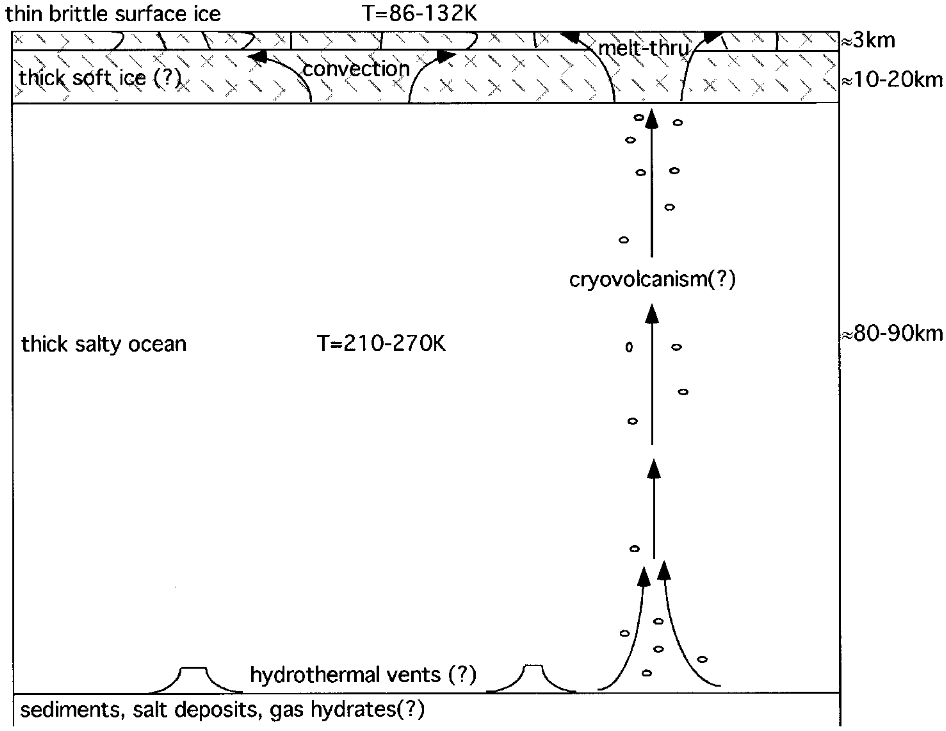

FIG. 4. A schematic diagram of the surface layers of Europa.

of the water/ice layers range from a few kilometers to almost 200 km (McKinnon, 1997; Anderson *et al.*, 1998; Pappalardo *et al.*, 1999; Kargel *et al.*, 2000). The total thickness of this water/ice shell has a direct bearing on pressures that organisms might face in an Europan ocean and the cycling of critical elements for life such as oxidants and nutrients between the ocean and the surface. The surface of Europa is strongly oxidizing because of the heavy radiation load on the surface (Carlson *et al.*, 1999a; Cooper, 2001; Cooper *et al.*, 2002; Greenberg, 2002; Greenberg *et al.*, 2002). The ocean and seafloor sediments are presumably reducing environments. Biology utilizes oxidation–reduction reactions to fuel metabolism (Gaidos *et al.*, 1999). To maintain a long-term viable ecosystem on Europa would require cycling of oxidants and reductants. Presumably a thin crust would lead to quicker cycling between the surface ice and the subsurface ocean. If the crust is thick enough, slow cycling of surface oxidants into the ocean could limit life in the ocean (Barr *et al.*, 2002).

The composition of an Europan ocean will have a major bearing on the suitability of this ocean for life. It has been hypothesized that an Europan ocean could be (1) a neutral Na-Mg-SO_4-H_2O solution, (2) an alkaline Na-SO_4-CO_3 solution, or (3) an acidic Na-H-Mg-SO_4 system (Kargel *et al.*, 2000; Marion, 2001, 2002; Kempe and Kazmierczak, 2002). Simulations of these three alternatives and their bearing on potential life on Europa will be discussed below.

Kargel (2001), Thomson and Delaney (2001), and O'Brien *et al.* (2002) have hypothesized that a Europan ocean could become thermally and compositionally stratified. Disturbance and overturning of this ocean could lead to exsolution of gases and a massive thermal flux to the surface via cryovolcanism (Fig. 4). Such massive fluxes, if frequent enough, could play an important role in cycling oxidants compared with the presumably slower processs of solid-phase convective flow in the soft ice. There may also be hydrothermal vents in an Europan ocean. If massive enough, these vents could play an important role in the cycling of oxidants, nutrients, and heat. Even small hydrothermal vents could serve as refugia for life in an otherwise inhospitable environment.

The variability in the surface temperature (Fig. 4) reflects measured diurnal fluctuations (Spencer *et al.*, 1999). The variability in the ocean temperature reflects uncertainties in the chemical composition, the thicknesses of the solid-ice and liquid brine layers, and internal and external sources of heat. For example, the eutectic temperature (the temperature below which only solid phases are possible) for a pure Na-Mg-SO_4-H_2O system at 1.01 bar is ~238 K (Kargel *et al.*, 2000), while the eutectic temperature for a H-Mg-SO_4-H_2O system at 1.01 bar is 211 K (Kargel *et al.*, 2001; Marion, 2002). So, depending on the chemical composition, the ocean temperature could fall anywhere from the initial freezing point of ice for a given composition (~270–255 K) down to the eutectic temperatures (250–210 K). Also, as we will demonstrate later, pressure may play a role in defining the temperature of an ice-covered ocean.

The potential habitats for life on Europa can be divided into three zones: the ice layer, the liquid ocean, and the seafloor environment.

The ice layer

In the ice layer, important potential limiting environmental factors for biological activity are radiation, temperature, and, perhaps, desiccation. Europa lies deep within the magnetosphere of Jupiter and is continuously bombarded with magnetically trapped, ionizing radiation (Baumstark-Khan and Facius, 2002). For a dose rate of 3–5 Gy/min, *E. coli* and *Bacillus* would be inactivated within 10 min; even *Bacillus* spores would be inactivated within 40 h (Baumstark-Khan and Facius, 2002). However, ice can provide effective protection from radiation. A highly resistant bacterium like *D. radiodurans* (Table 4) could survive the radiation field expected at 1 mm depth on Europa; at 10 cm, most organisms could survive the radiation; and at 20–40 m, the radiation load would be similar to that on Earth (Baumstark-Khan and Facius, 2002).

Not only are life forms adversely affected by the surface radiation, but organics and other potential remnants of deeper life would also be destroyed (Phillips and Chyba, 2001; Carlson *et al.*, 2002; Cooper *et al.*, 2002; Greenberg *et al.*, 2002). How far below the surface one could expect to find evidence of life would depend on processes such as impact gardening, solid-state convective flow, and diurnal fluid movement along stress fractures (Chyba and Phillips, 2001; Phillips and Chyba, 2001; Greenberg, 2002; Greenberg *et al.*, 2002).

Ice is a favorable medium for preserving life forms and organics, including life in the dormant stage (Gilichinsky *et al.*, 1993; Soina *et al.*, 1995; Hoover and Gilichinsky, 2001; Gilichinsky, 2002). The paucity of craters on the surface of Europa suggests a young age, which has been estimated anywhere from 10 to 100 Myr (Zahnle *et al.*, 1998; Pappalardo *et al.*, 1999; Cooper *et al.*, 2002; Greenberg *et al.*, 2002). Bacteria on Earth may have been preserved in the dormant state for periods as long as 250 Myr (Vreeland *et al.*, 2000). Therefore, if life exists (or existed) in the ocean or elsewhere, one would expect to find evidence in the ice layers, although perhaps not at the immediate surface because of the destructive radiation load (Phillips and Chyba, 2001).

Temperatures at the surface (86–132 K, Fig. 4) (Spencer *et al.*, 1999) are well below the limit for microbial growth (~253 K, Table 1). These low surface temperatures are sufficient, *per se*, to preclude active life anywhere near the surface. Only at the base of the ice layer where ice temperatures approach oceanic temperatures could we expect temperatures that could be tolerable, albeit not favorable, for biological activity. These basal ice zones could also contain brine pockets with compositions similar to the ocean that could serve as habitats for life.

Biological activity requires that the desiccating power cannot be less than a_{H_2O} = 0.6 (RH = 60%). Low temperatures are, *per se*, strongly desiccating. For liquid water (containing salts) in equilibrium with ice, a_{H_2O} = 0.6 occurs at 220 K (Marion, 2002). It is impossible that any life form could be active anywhere near the Europan surface because of the radiation load, the low temperatures, and the desiccating power of the environment at these low temperatures.

The brine ocean

Salinity, acidity (if present), temperature, and pressure could be important factors limiting life in the brine ocean. We would like to demonstrate how hypothetical chemical compositions of the ocean coupled with their temperature and pressure dependencies can influence potential Europan environments for life. The selected compositions include: (1) a Na-Mg-Ca-SO_4-Cl-H_2O system (neutral pH) (Kargel *et al.*, 2000), (2) a Na-K-Cl-SO_4-CO_3-H_2O system (alkaline pH) (Marion, 2001), and (3) a Na-H-Mg-SO_4-H_2O system (acidic pH) (Marion, 2002). This discussion uses FREZCHEM model simulations assuming different initial chemical compositions for Europa, and evaluates how these compositions would have evolved as temperature cooled over time. Three of these simulations will be done at an assumed pressure of 1.01 bars; a fourth simulation will examine how the neutral pH simulation (composition 1) would be affected by 1,200 bars of pressure.

The initial chemical composition for the Na-Mg-Ca-SO_4-Cl-H_2O system is believed to be representative of chondritic weathering (Kargel, 1991) with the addition of chlorides in amounts observed by Fanale *et al.* (1998) in their leachates (Kargel *et al.*, 2000). According to model calculations, the starting solution is slightly supersaturated with respect to both gypsum and epsomite at 298 K (Fig. 5). At a temperature of 271 K, $MgSO_4 \cdot 7H_2O$ is replaced by $MgSO_4 \cdot 12H_2O$; ice starts forming at 266 K. The dual effect of the precipitation of a highly hydrated sulfate salt and ice causes the precipitous drop in sulfate and the rapid rise in chloride. The colder the ultimate temperature, the higher the Cl/SO_4 ratio (Fig. 5). The eutectic temperature reached by this composition is 238.65 K at 1.01 bars. If the temperature of the ocean is lower than 238.65 K, liquid water cannot exist for this solution. The salinity can be estimated by a_{H_2O}, which rises from 0.85 (298 K) to 0.93 (266 K), where ice begins to form, and then drops to 0.72 at the eutectic. By the salinity standards of Table 2, a_{H_2O} could be a limiting factor, at times, for biological activity of many microbes, but not for extreme halophiles. Zolotov and Shock (2001), using a similar but more dilute starting salt solution and the FREZCHEM model for calculations, found a eutectic temperature of 237.05 K, which is slightly lower than we found (238.65 K). They also found that the last salt to precipitate was $MgCl_2 \cdot 12H_2O$ at the eutectic; in contrast, we found $NaCl \cdot 2H_2O$ precipitating at the eutectic (Fig. 5). These small discrepancies probably reflect variations in the relative concentrations of the starting solutions.

The initial composition for the hypothetical Europan alkaline system is taken from Alkali Valley, Oregon (Marion, 2001) and represents the type of extreme alkaline system that can lead to precipitation of minerals such as trona ($NaHCO_3 \cdot Na_2CO_3 \cdot 2H_2O$) and natron ($Na_2CO_3 \cdot 10H_2O$) (Fig. 6). Natron has been suggested as a possible salt on the Europan surface (McCord *et al.*, 1999). Extreme alkaline systems are dominated by Na and K; Mg and Ca concentrations are extremely low because of the relative insolubility of Mg and Ca carbonates in high pH, high alkalinity solutions (Marion, 2001). The starting solution is supersaturated with respect to trona (Fig. 6), but this

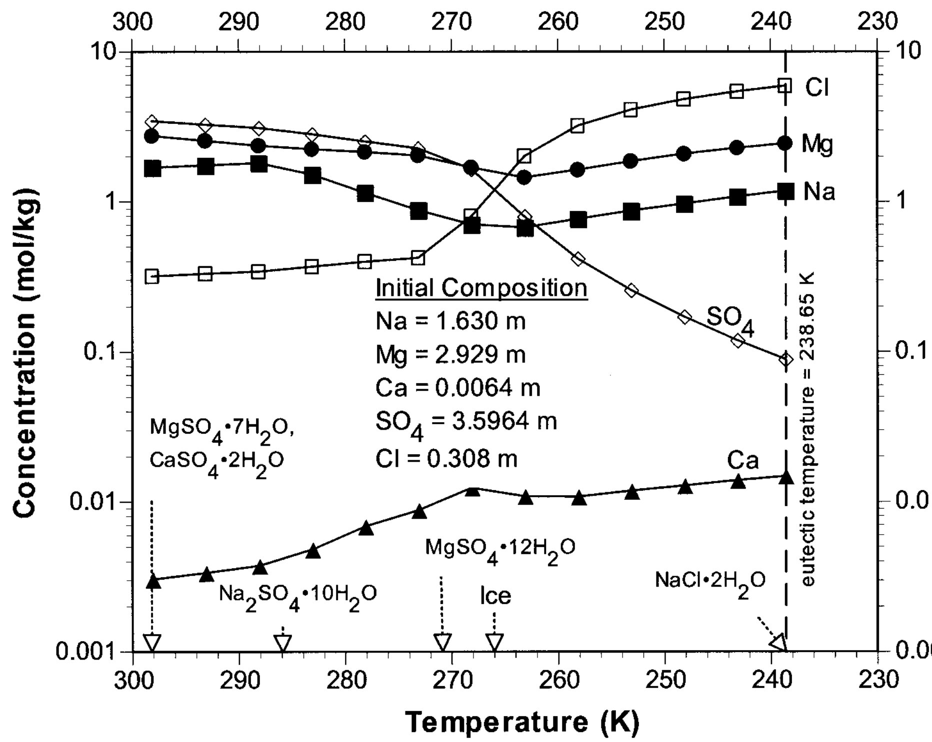

FIG. 5. The evolution of a Na-Mg-Ca-SO_4-Cl brine at 1.01 bars of pressure as temperature decreases to the eutectic.

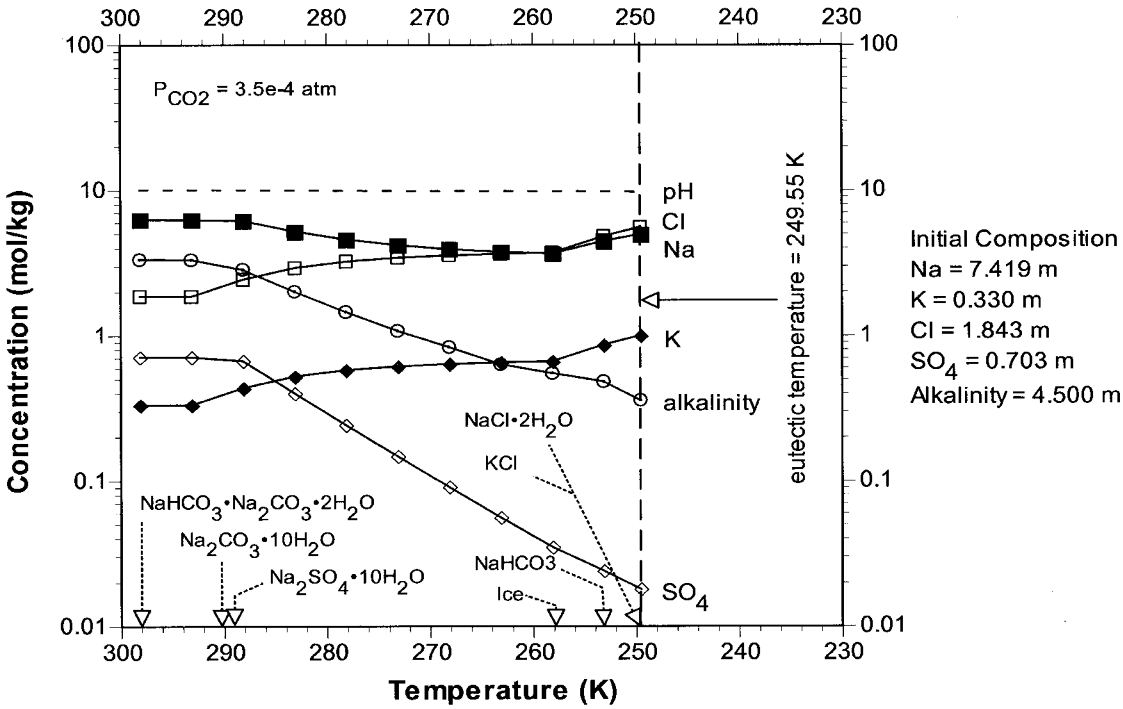

FIG. 6. The evolution of a Na-K-Cl-SO_4-alkalinity brine at 1.01 bars of pressure as temperature decreases to the eutectic.

salt is replaced by natron at 290 K, and eventually by nahcolite ($NaHCO_3$) at 253 K. The high solubility of these alkaline salts keeps ice from forming until the temperature drops to 258 K. The last salts to precipitate are KCl and $NaCl \cdot 2H_2O$ at the eutectic temperature of 249.55 K at 1.01 bars (Fig. 6). Both alkalinity and sulfate drop in concentration with decreasing temperature, leading ultimately to a chloride-dominated system. The calculated pH of this alkaline system ranges from 10.11 at 298 K to 9.73 at the eutectic. But bear in mind that calculated pH is very much a function of the assumed P_{CO_2} (= 3.5e-4 atm). A higher P_{CO_2} would lower pH, and a lower P_{CO_2} would raise the pH. However, it is unlikely that such an alkaline system could rise to levels that would limit life (pH >11, Table 3). The calculated a_{H_2O} values hover between 0.80 and 0.86 across the range of temperatures depicted in Fig. 6. This would limit life forms to extreme halophiles (Table 2).

The initial composition for the acidic system is based on low temperature leaching of chondritic material to produce $MgSO_4$ and high temperature devolatilization and venting of SO_2 into the ocean to produce H_2SO_4 (Kargel *et al.*, 2001; Marion, 2002). The salts that precipitate from this acidic system and the temperatures at which they precipitate (Fig. 7) are very similar to the initially considered Na-Mg-Cl-SO_4-H_2O system (Fig. 5). In this acidic case, the last salt to precipitate is $Na_3H(SO_4)_2$ (an acid salt) at the eutectic temperature of 234.65 K at 1.01 bars (Fig. 7). We also ran this simulation without sodium; in that case, the sink for hydrogen was $H_2SO_4 \cdot 6.5H_2O$, which precipitated at the eutectic temperature of 211 K (Marion, 2002). Because of the high solubility of sulfuric acid, decreasing temperatures and freeze concentration leads to increasing H_2SO_4 concentrations (Fig. 7); this is in marked contrast to the earlier sulfate systems where low temperatures led to the precipitous drop in sulfate concentrations (Figs. 5 and 6). The pH in this acid case dropped below 0.0 (the lower limit for life) at around 263 K (Fig. 7); the minimum pH at −1.09 was reached at the eutectic. The a_{H_2O} rose from 0.85 at 298 K to 0.92 at 265 K, where ice begins to form, and then dropped to 0.69 at the eutectic. Extreme halophiles could survive the salinity, but pH values below 0.0 would place life forms under an additional extreme stress.

The seafloor environment

In addition to salinity and acidity (if present) important potential environmental life-limiting

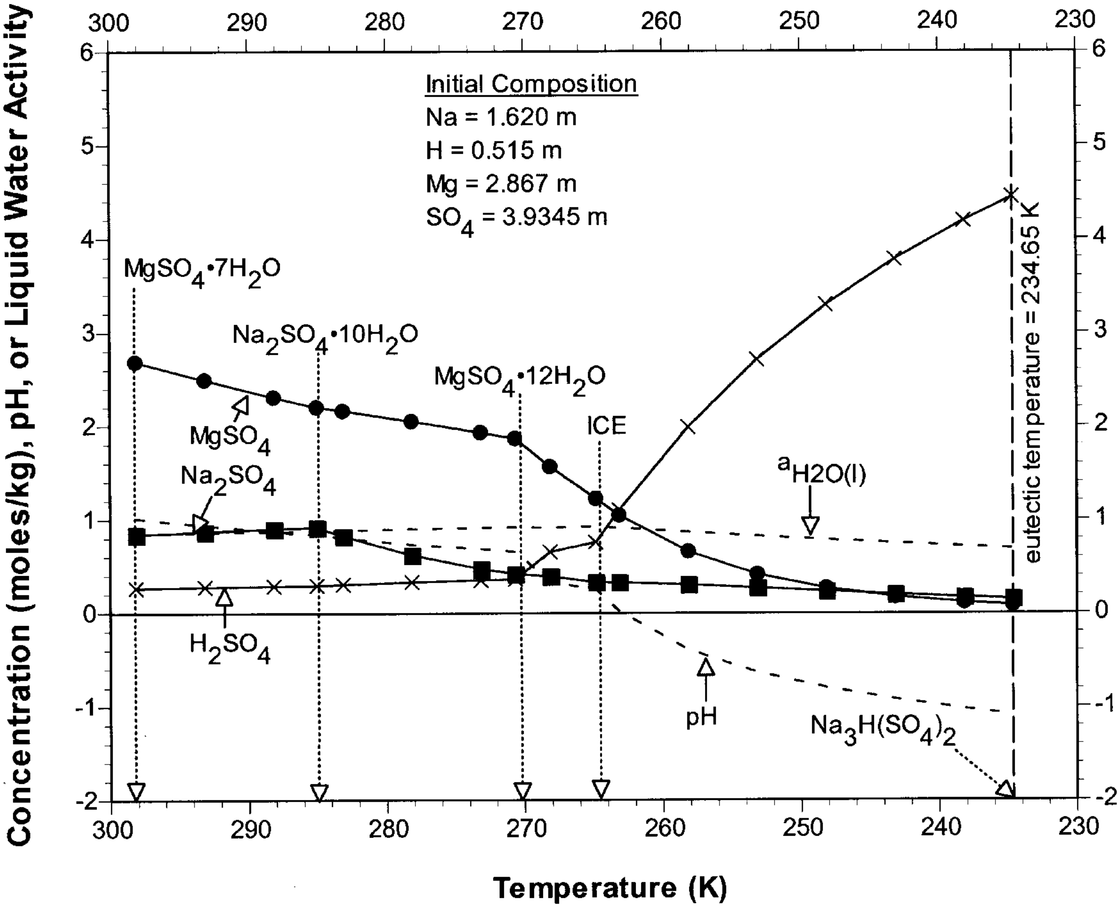

FIG. 7. The evolution of a Na-H-Mg-SO_4 brine at 1.01 bars of pressure as temperature decreases to the eutectic.

factors at the base of the ocean are likely to be pressure and temperature. All of the above simulations (Figs. 5–7) were done at 1.01 bars total pressure. A Europan ocean 100 km deep (Fig. 4) would have a hydrostatic pressure at the seafloor of ~1,200 bars [P (bars) = 12 × depth (km) (Kargel *et al.*, 2000)]. Figure 8 depicts equilibria at 1,200 bars for the system considered in Fig. 5 at 1 bar. In general, the shapes of the curves are similar. What are most strikingly different are the temperatures at which gypsum ($CaSO_4 \cdot 2H_2O$), $MgSO_4 \cdot 12H_2O$, and ice begin to form. At 1 bar, the initial solution is supersaturated with respect to gypsum (Fig. 5); at 1,200 bars, gypsum does not precipitate until the eutectic is reached. Earlier, we used gypsum precipitation as an example on how pressure affects chemical reactions (Eqs. 7 and 8). We showed that pressure should cause the dissolution of gypsum in order to minimize the ΔV^0_r (Eq. 8). In this case, the pressure differential is sufficient to change the solution at 298 K from supersaturated at 1 bar to undersaturated at 1,200 bars. Similarly, in Eqs. 9 and 10 and Fig. 2, we demonstrated that pressure causes the melting of ice (to reduce ΔV^0_r), shifting the freezing point to a lower temperature (cf. Figs. 5 and 8). However, for the reaction:

$$MgSO_4 \cdot 12H_2O \leftrightarrow MgSO_4 \cdot 7H_2O + 5H_2O \quad (12)$$

the calculated ΔV^0_r is +16.55 cm^3/mol. Bringing pressure to bear on this reaction will cause the equilibrium to shift to the left, favoring the precipitation of $MgSO_4 \cdot 12H_2O$ at a higher temperature (cf. Figs. 5 and 8). Another significant difference between 1 and 1,200 bars of hydrostatic pressure is that at 1 bar, chloride precipitates as $NaCl \cdot 2H_2O$ (Fig. 5), while at 1,200 bars, chloride precipitates as $MgCl_2 \cdot 12H_2O$ (Fig. 8). The a_{H_2O} rises from 0.85 (298 K) to 0.95 (257 K), where ice begins to form, then drops to 0.79 at the eutectic (237.55 K). These are generally higher a_{H_2O} values (lower salinities) than was the case at 1 bar of pressure (see previous discussion).

With respect to a Europan ocean, it is the eutectic temperature that governs the lower limit for

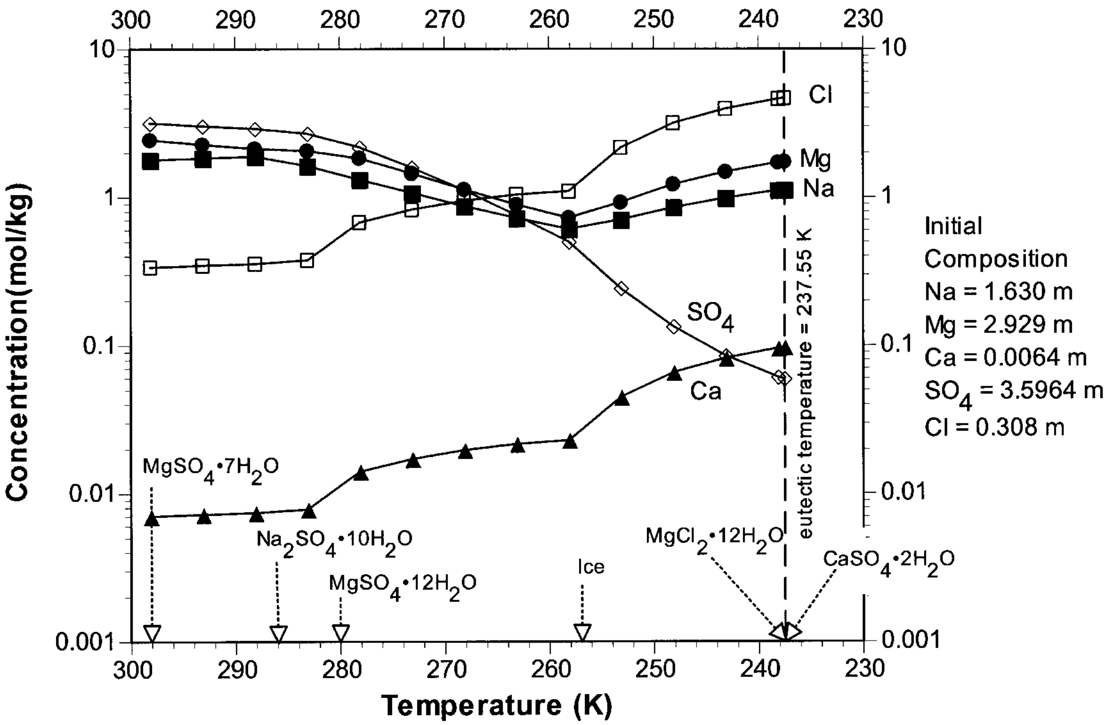

FIG. 8. The evolution of a Na-Mg-Ca-SO_4-Cl brine at 1,200 bars of pressure as temperature decreases to the eutectic.

liquid water at subzero temperatures. In this particular pressure comparison (Figs. 5 and 8), there was only a minor difference in the eutectic temperatures between 1 and 1,200 bars of pressure (1.1 K). There were more noticeable differences in the specific minerals that precipitate and in the temperatures at which individual minerals began precipitating. The effect of pressure on solution chemistry depends on the volumetric properties of specific minerals and their respective solution constituents and concentrations, which makes the outcome of a pressure change highly individualistic.

This pressure simulation assumed an ice/water depth of 100 km (Fig. 4). Estimates of the thickness of this ice/water shell around Europa range up to almost 200 km (McKinnon, 1997). For the later thickness, the seafloor pressure would be 2,400 bars. All of the above simulations assume the presence of Ice Ih. The transition from Ice Ih to the polymorph, Ice III, for a pure ice/liquid water system occurs at 251.165 K at a pressure of 2,099 bars (Wagner *et al.*, 1994). So a 200-km shell would necessitate a consideration of Ice III at great depths and associated high pressures (>2,100 bars). Ice III has a density of 1.165 g cm^{-3} (Petrenko and Whitworth, 1999) and, as a consequence, would sink in a pure water system because the density of water at that temperature and pressure is less than that for Ice III. For example, at 2,000 bars (the maximum pressure for the FREZCHEM model) and 252.46 K, the calculated density of water is 1.090 g cm^{-3} (= kg L^{-1}), and the Ice Ih density is 0.932 g cm^{-3}. However, our estimates of the density of the salt solutions at subzero temperatures at 1,200 bars (Fig. 8) ranged from 1.17 to 1.27 g cm^{-3}; presumably, at a higher pressure these densities would even be greater. Density data for components of a pure $MgSO_4$-H_2O system at high pressures (Fig. 15 in Hogenboom *et al.*, 1995) also fall in the order: salt solution > Ice III > Ice Ih, which is consistent with the FREZCHEM model. Under these conditions, Ice III would float on a dense Europan brine. Or alternatively, if the brine density is significantly less than we have calculated based on the hypothesized compositions, then the liquid ocean could literally be sandwiched between two ice layers, Ice Ih on the top and Ice III on the bottom.

Temperatures in the vicinity of seafloor hydrothermal vents, if they exist, are likely the most favorable environments for life on Europa. Unfortunately, there is no direct evidence that such vents exist on Europa. However, hydrothermal vents have been implicated in the development of melt-through phenomenon such as chaos (Kargel *et al.*, 2000; O'Brien *et al.*, 2002). Whether such chaos areas are due to hydrothermal venting, solid-phase convective flows through soft ice, cryovolcanism, or some combination of these factors (Fig. 4) is still highly speculative.

Kargel (2001) and Thomson and Delaney (2001) have hypothesized that a Europan ocean could be stratified with respect to temperature, composition, and density. Temperatures at the base of the ocean could be quite hot from internal heat flow into the ocean, tidal dissipation, and changes in advective heat losses through the crust (Kargel, 2001). As the bottom waters warm, they could become gravitationally unstable, leading to explosive cryovolcanism (Fig. 4). Any mechanism for increasing temperatures above 273 K would be highly desirable for life. While life can metabolize and grow at temperatures of <273 K (Table 1), this metabolic activity is exceedingly slow (Rivkina *et al.*, 2000).

As mentioned previously, one of the potentially most limiting factors for life in a Europan seafloor environment is the presumed paucity of oxidants at these depths. Rapid communication with the oxidized surface would alleviate this problem, but mechanisms to, at least, theoretically demonstrate that this rapid transport is possible are still under development (Barr *et al.*, 2002).

EARTH ANALOGUES FOR EUROPA

What constitutes a good analogue? Recently Soare *et al.* (2001) proposed a three-rung system for evaluating the meaningfulness of planetary analogues. "Analogues of the first order" are confirmed by direct empirical evidence. We know with scientific certainty that Europa is covered by ice, which makes ice-covered environments on Earth appropriate analogues of the first order, albeit not perfect analogues given the drastically different surface temperatures and radiation loads. "Analogues of the second order" are underpinned by indirect or artifactual, but highly suggestive, evidence awaiting empirical confirmation. The presence of a brine ocean on Europa is an example of an analogue of the second order. There is abundant indirect evidence supporting the presence of a brine ocean (Pappalardo *et al.*, 1999; Kargel *et al.*, 2000). Another example of an analogue of the second order is the composition of the brine ocean. There is abundant evidence that the "non-icy component" of the Europan surface consists of highly hydrated materials (Carlson *et al.*, 1999b; McCord *et al.*, 1999), but whether these are sulfate salts, sulfuric acid, or alkaline minerals such as natron is unclear. "Analogues of the third order" are not sustained by direct or indirect target evidence. A good example of this type of analogue is "life on Europa." There is no direct or indirect evidence that life exists on Europa. Therefore, there may be no appropriate analogues on Earth for life on Europa, if in fact life on Europa does not exist. These three rungs reflect the degree of certainty we can place on the meaningfulness of analogue studies from high (first order) to low (third order).

Does this conclusion imply that Earth analogues for life on Europa are meaningless? Emphatically not! Eicken (2002) recently made the case for why terrestrial analogues for life on Europa are important:

> studies of terrestrial analogs are key insofar as they (1) are an integral component in the development and testing of conceptual models of the potential evolution, presence and detectability of life in Europan environments, (2) help in constraining scenarios of planetary evolution and composition, (3) further our understanding of the fundamental constraints on life at low temperatures and its evolution in extraterrestrial environments, (4) can be coordinated and synthesized from a variety of specific analogs and habitats, and (5) offer the opportunity to collect data necessary to validate and improve numerical models of planetary evolution and life in extreme environments. In the context of future missions to Europa, studies of terrestrial analogs help to (1) define and refine research hypotheses concerning the presence and detectability of life on Europa, (2) develop and test sampling methods for both planetary geology and astrobiological programs, (3) develop and test detection strategies of biomarkers through remote-sensing methods, and (4) identify and delineate the most promising study and sampling areas.

Accepting that this is ample justification for analogue studies, what are the best Earth analogues for evaluating potential habitats for life or preservation of life's signatures on Europa? This justification will be separated into the three zones for potential life on Europa: the ice layer, the brine ocean, and the seafloor environment.

The ice layer

Potentially important limiting environmental factors for biological activity in the ice layer are radiation, temperature, and, perhaps, desiccation. There is no natural analogue on Earth for the extreme low temperatures and high radiation loads associated with the surface of Europa (Fig. 4). Fortunately, the radiation load (primarily ionizing radiation) on Europa is attenuated rapidly with ice depth; by 20–40 m, the background radiation is similar to Earth (Baumstark-Khan and Facius, 2002). An excellent review of the many facets of radiation and their consequences for life on Earth and other Solar System bodies, including Europa, is the chapter by Baumstark-Khan and Facius (2002) on "Life under conditions of ionizing radiation." Background radiation from radionuclides on Earth is generally in the range of 1 mGy/year [Gy = Gray (1 J/kg), which is the SI-unit for physically absorbed radiation]; along certain beaches in Brazil, where monazite sand deposits are found, external radiation may range up to 400 mGy/year (Baumstark-Khan and Facius, 2002). The Earth is largely protected from cosmic radiation by the atmosphere; the dose approximately doubles for every 1,500 m change in elevation. For example, in Germany, the total cosmic radiation dose is 0.3 mGy/year at sea level and 1.2 mGy/year at 3,000 m (Baumstark-Khan and Facius, 2002). Anthropogenic sources of high ionizing radiation include uranium mines and nuclear reactor pools (UNSCEAR, 2000). The actual dose rate at the surface of Europa (3–4 Gy/min) is quickly lethal for all known life forms (Baumstark-Khan and Facius, 2002). The best potential Earth analogues for the high radiation environment near the surface of Europa are probably anthropogenic environments such as uranium mines and nuclear reactor pools (UNSCEAR, 2000). However, given the double stress of lethal radiation and extreme low temperatures at the surface of Europa, future research should focus on preservation of life forms and associated biosignatures in ice in ionizing radiation fields rather than on biological activity, *per se*, because the latter is impossible anywhere near the surface of Europa.

Deeper into the ice layer, the temperature presumably moderates into the range suitable for psychrophiles. Life in ice environments (glaciers, snow, sea ice, lake ice, permafrost) has been the subject of many reviews and studies in recent years (Table 5). There are many physical, chemical, and biological properties of ice that have a bearing on their suitability as Europan analogues; however, not all ices are equivalent for all purposes. For example, the physics of ice dynamics are probably best studied with sea-ice, lake-ice, and deep-ice cores. The relationship between surface chemistry and subsurface brine composition is best studied with ice-covered saline lakes [e.g., perennial ice-covered saline lakes in Antarctica such as Lake Vanda, Lake Bonney, and Lake Vida (Matsumoto, 1993)]. The best ice analogues for microbial adaptations to cold are probably sea-ice and lake-ice. For long-term microbial survival or preservation of biosignatures, deep-ice cores and permafrost are the most suitable ices. Ecosystem structure in ice-isolated environments is best studied in perennial ice-covered lakes such as Lake Vostok, Lake Vanda, Lake Bonney, and Lake Vida.

Desiccation is much less studied than temperature as a growth-limiting factor. But, as pointed out earlier, low temperatures, *per se*, are a strong desiccant. Kennedy (1993) has cited many studies in Antarctic that implicate desiccation as a prime force controlling species distribution. The Dry Valleys of Antarctica are probably the best Earth analogue for cold desiccation (Table 5).

The brine ocean

Salinity, acidity (if present), temperature, and pressure could be important factors limiting life in a Europan ocean. Finding suitable Earth analogues for the hypothetical brine ocean of Europa is difficult because the composition of such an ocean is poorly constrained. Here we are working with an "analogue of the second order" because the only evidence for the ocean composition is indirect and ambiguous (Carlson *et al.*, 1999b; McCord *et al.*, 1999; Kargel *et al.*, 2000). Previously, we examined neutral pH solutions (Figs. 5 and 8), an alkaline solution (Fig. 6), and a strongly acidic solution (Fig. 7) in an attempt to at least examine the range of hypothetical compositions suggested by previous work.

Salinity as a growth-limiting factor has been extensively studied; many books and extensive reviews on the chemistry of saline environments have been published (Table 6). Saline environments include lakes, oceans, sea ice, soils, salterns

TABLE 5. SELECTED REFERENCES OF POTENTIAL EARTH ANALOGUES FOR THE ICE LAYER OF EUROPA

Frozen environments	*Selected references*
Broad-scale reviews	Kennedy (1993), Simmons *et al.* (1993), Ellis-Evans (1996), Vincent and James (1996), Psenner and Sattler (1998), Hoover and Gilichinsky (2001), Fritsen (2002)
Lake Vostok, Antarctica	Ellis-Evans and Wynn-Williams (1996), Kapitsa *et al.* (1996), Jouzel *et al.* (1999), Karl *et al.* (1999), Nadis (1999), Priscu *et al.* (1999), Vincent (1999), Price (2000), Duxbury *et al.* (2001)
Other cold lakes	Matsumoto (1993), Marion (1997), Fritsen and Priscu (1998, 1999), Kepner *et al.* (1998), Priscu *et al.* (1998), Junge *et al.* (2004)
Sea ice	Helmke and Weyland (1995), Arrigo *et al.* (1997), Bowman *et al.* (1997), Brown (1997), Hawes *et al.* (1999), Nadeau and Castenholz (2000), Junge *et al.* (2001), Thomas and Dieckmann (2002)
Permafrost	Gilichinsky *et al.* (1993), Soina *et al.* (1995), Stone (1999), Rivkina *et al.* (2000), Hoover and Gilichinsky (2001), Gilichinsky (2002)
Glaciers	Christner *et al.* (2000)
Dry Valleys, Antarctica	Friedmann and Ocampo (1976), Friedmann and Ocampo-Friedman (1984), Kennedy (1993)

(seawater evaporation basins), and evaporite deposits. The chemical analyses of representative saline waters demonstrate a wide range of properties (Table 7). The Orca Basin is a deep-sea (2,400 m) brine at the bottom of the Gulf of Mexico that formed as the result of dissolution of evaporite salt beds. Because of the high density of the Orca Basin (1.190 kg/L) compared with seawater (1.023 kg/L), these deep-sea brines are stable at the bottom of the ocean. The Great Salt Lake is a predominantly Na-Cl brine, while the Dead Sea is a predominantly Na-Mg-Cl brine. The most dense brine is Don Juan Pond in Antarctica, which is a predominantly Ca-Cl brine. Basque Lake in Canada is a predominantly Mg-SO_4 brine. Mono Lake and Lake Magadi are examples of alkali lakes dominated by Na-Cl-CO_3 brines; note the high pH values (9.9–10.1) and the virtual absence of Mg and Ca from these alkali lakes (Table 7).

We also included in Table 7 the calculated chemical composition of Earth seawater at $-20°C$ (lower temperature limit for biological activity) to demonstrate the powerful influence of freezing on chemical composition. According to model calculations, by the time the temperature has dropped to $-20°C$, ice, calcite, and mirabilite are precipitating; of the original water, 87.6% has pre-

TABLE 6. SELECTED REFERENCES OF POTENTIAL EARTH ANALOGUES FOR THE CHEMICAL COMPOSITION OF A EUROPAN BRINE OCEAN

Chemical factor	*References*
Salinity	
Books and reviews	Jones (1966), Eugster and Hardie (1978), Friedman and Krumbein (1985), Javor (1989), Rodriguez-Valera (1991), Oren (1999), Ventosa *et al.* (1999)
Dead Sea, Israel	Krumgalz (1997), Ventosa *et al.* (1999), Krumgalz *et al.* (2000)
Antarctic lakes	Craig *et al.* (1974), Matsumoto (1993), Smith and Friedman (1993), Marion (1997), Spigel and Priscu (1998)
Others	Rodriguez-Valera *et al.* (1981), de la Pena *et al.* (1982), Del Castillo Arias and Farfan (1997), Kargel *et al.* (2000), Nicolaus *et al.* (2000), Labrenz and Hirsch (2001), Marion (2001)
Acidity	
Iron Mt., California	Edwards *et al.* (1998, 1999, 2000a,b), Schrenk *et al.* (1998), Bond *et al.* (2000a,b), Nordstrom *et al.* (2000), Robbins *et al.* (2000)
Volcanic waters	Africano and Bernard (2000), Delmelle and Bernard (2000)
Others	Babenzien and Babenzien (1990), LeBlanc *et al.* (1996), Krishnaswamy and Hanger (1998), Stapleton *et al.* (1998), Packroff and Woelfl (2000), Lopez-Archilla *et al.* (2001)
Alkalinity	Kempe and Degens (1985), Zhilina and Zavarzin (1994), Marion (2001), Kempe and Kazmierczak (2002)

TABLE 7. CHEMICAL ANALYSES OF SELECTED SALINE WATERS

Property	*Seawater*[a]	*Seawater at −20°C*	*Orca Basin, Gulf of Mexico*[b]	*Great Salt Lake, USA*[c]	*Dead Sea, Israel*[d]	*Don Juan Pond, Antarctica*[e]	*Basque Lake Canada*[c]	*Mono Lake, USA*[c]	*Lake Magadi, Kenya*[f]
Na	0.48610	3.636	5.532	4.860	1.835	0.112	0.797	0.991	6.277
K	0.01058	0.089	0.021	0.139	0.212	0.008	0.054	0.032	0.054
Mg	0.05475	0.460	0.058	0.396	1.944	0.110	2.350	0.001	~0
Ca	0.01065	0.080	0.036	0.008	0.459	5.830	~0	0.0001	~0
Cl	0.56664	4.757	5.633	5.278	6.824	12.192	0.064	0.404	2.476
SO_4	0.02927	0.023	0.051	0.228	0.006	<0.001	2.746	0.081	0.024
Alkalinity	0.00232	0.00073	0.0063	0.0055	0.0060	~0	0.067	0.458	3.899
Salinity (%, wt/wt)	3.51	21.4	25.1	25.2	26.4	40.2	25.8	6.86	32.0
a_{H_2O}[g]	0.981	0.823	0.774	0.776	0.690	0.414	0.919	0.974	0.819
Ionic strength[g]	0.722	5.37	5.88	6.43	9.26	17.75	10.68	1.28	8.28
pH[g]	8.30	8.09	8.36	8.10	7.71	~5.4	8.70	9.90	10.13
Density (kg/L)[g]	1.023	1.180	1.190	1.197	1.223	1.385	1.278	1.047	1.250

Concentration units are mol/kg (water) except for alkalinity, which is given in units of equivalents/kg (water).
[a]Millero and Sohn (1992).
[b]Krumgalz *et al*. (1999).
[c]Eugster and Hardie (1978).
[d]Krumgalz *et al*. (2000).
[e]Marion (1997).
[f]Jones *et al*. (1977).
[g]Calculated with the FREZCHEM model (Marion and Farren, 1999). Model runs assume a temperature of 25°C (except seawater at −20°C), P_{CO_2} of 3.6e-4 atm, and total pressure of 1.01 bars.

cipitated as ice, 11.9% is still present in the aqueous phase, and 0.5% has precipitated as the hydrate mineral, mirabilite. The salinity of this solution ($a_{H_2O} = 0.823$, Table 7) is within the tolerance range of halophiles (Table 2).

Not all measures of "salinity" convey the same degree of salinity. For example, compare Orca Basin, the Great Salt Lake, the Dead Sea, and Basque Lake (Table 7). All four of these waters contain about the same salinity % [25.1–26.4% salt (wt/wt)]. Note, however, that Basque Lake has a much more favorable (for life) a_{H_2O} (0.919) compared with Orca Basin (0.774), Great Salt Lake (0.776), and, especially, the Dead Sea (0.690). Only the most halophilic organisms can live in the Dead Sea (Table 2).

Don Juan Pond is the only selected saline water wherein the reported a_{H_2O} (= 0.414, Table 7) is generally below the experimentally derived lower limit for microbial activity (a_{H_2O} ~0.6, Table 2). There was an early report that suggested that Don Juan Pond had a viable microbial population (Meyer *et al.*, 1962), but later it was found that the isolated bacteria could not grow in the pond water and were likely brought in from outside (Kushner, 1981). The Dead Sea (a_{H_2O} ~0.690, Table 7) was called "Dead" because it was only in 1936 that life forms (e.g., bacteria, algae, yeast) were first isolated from this hypersaline water (Ventosa *et al.*, 1999).

A few studies have examined salinities in cold environments in Antarctica (Marion, 1997; Nicolaus *et al.*, 2000; Labrenz and Hirsch, 2001). Given the strong evidence for sulfates on the Europan surface (Carlson *et al.*, 1999b; McCord *et al.*, 1999; Kargel *et al.*, 2000), focusing more attention on sulfate systems as media for life on Europa would be desirable. Sulfate-dominated systems also maintain highly favorable a_{H_2O} values for life compared with chloride-dominated systems at similar salinities (Table 7).

Lake Vostok in Antarctic is located beneath 3,750 m of ice, and has been isolated from the surface for ~1 Myr (Kapitsa *et al.*, 1996; Jouzel *et al.*, 1999; Nadis, 1999). The thick ice cover and isolation from the surface makes Lake Vostok potentially a good Earth analogue for Europa. However, Lake Vostok is believed to be a freshwater lake located beneath 320 bars of overlying pressure (Kapitsa *et al.*, 1996; Duxbury *et al.*, 2001); such a pressure would lower the freezing point by only −2.5°C (Fig. 2). So it is likely that Lake Vostok would be lower in salts and higher in temperature than a Europan ocean (Figs. 4–8). An ice core has reached 3,623 m, ~120 m above the lake. The coring project is currently (2003) on hold until plans are developed that will allow drilling the final 120 m without contaminating the lake with drilling fluid. From the ice cores, there is evidence of bacterial life to 3,590 m (Priscu *et al.*, 1999; Vincent, 1999).

Extreme pH values in nature arise principally in three environments: acid mine drainage, acidic volcanic springs, and soda lakes (Tables 3 and 6). Acidity associated with acid mine drainage is generally caused by oxidation of sulfide minerals. The following reaction is believed to control acid production at the Iron Mountain, California site (Bond *et al.*, 2000a; Nordstrom *et al.*, 2000):

$$FeS_2 + 14Fe^{3+} + 8H_2O \leftrightarrow 15Fe^{2+} + 2SO_4{}^{2-} + 16H^+ \quad (13)$$

Note that Fe^{3+} is the oxidizing agent in these reactions. The site at Iron Mountain has been extensively studied (Table 6) because of the extreme pH values at this site, which range down to −3.6, reputedly the most extreme pH ever recorded in a natural system (Nordstrom *et al.*, 2000). Many of the cited studies in Table 6 for Iron Mountain deal with microbial activity, which extends down to pH ~0. If the Europan ocean is strongly acidic, Iron Mountain would be a good Earth analogue for life adaptations to cope with strong acidity. Volcanic waters are also frequently associated with high acidities (Table 6).

A characteristic of strongly acidic systems on Earth is that they are almost always associated with extremely high concentrations of sulfate, chloride, iron, aluminum, and heavy metals because strong acids are powerful agents for mineral weathering. Our simulation of a Europan ocean assumed a simpler acid chemistry consisting of sodium and magnesium sulfates and sulfuric acid (Fig. 7); there are not, to our knowledge, any analogous systems on Earth. However, in defense of this simulation, Kargel *et al.* (2000) have argued that there may be kilometers of salt deposits on the seafloor of Europa (Fig. 4), which would isolate an acidic ocean from reactive minerals and the potential toxicity of heavy metals.

Potential Earth analogues for an alkaline ocean on Europa are Mono Lake and Lake Magadi; pH values in these lakes typically range around 10 and are characterized by extremely low magnesium and calcium concentrations (Table 7) because of the insolubility of magnesium and calcium carbonates at these high pH values. Jones (1966) and Eugster and Hardie (1978) have provided many more examples of alkaline lakes. Kempe and Kazmierczak (2002) have argued that an alkaline ocean on Europa would be favorable for biogenesis because of the very low calcium concentrations mandatory for the biochemical function of proteins.

The seafloor environment

Important potential growth-limiting factors at the base of a Europan ocean and in the sediments are likely to be composition, pressure, temperature, and the concentration of oxidants. The question of oxidants in the deep ocean was discussed earlier and depends fundamentally on cycling between the surface ice layer and subsurface ocean and will not be discussed further here.

The hydrostatic pressure at the base of a 100-km Europan ocean is ~1,200 bars. Microorganisms have been found growing in the Mariana Trench, the deepest ocean basin on Earth, where the pressure and temperature are 1,100 bars and 275 K (Yayanos, 1995; Kato *et al.*, 1998; Abe *et al.*, 1999). So, an increase in pressure from 1,100 bars (Earth) to 1,200 bars (Europa) is not likely to be a problem for evolution and adaptation. Furthermore, recent experimental work demonstrated that bacteria can survive pressures up to 16,800 bars (Sharma *et al.*, 2002). Pressures likely to be found in a Europan ocean are not likely to directly limit life, but changing chemistries as a function of pressure could indirectly impact biological activity. For example, the 1,200 bar simulation (Fig. 8) had a generally more favorable salinity than the 1 bar simulation (Fig. 5) (see previous discussion). Also, the freezing point for ice decreased by 9 K between 1 and 1,200 bars of pressure. The Mariana Trench or similar deep, cold ocean waters would be good analogues for a deep, cold ocean on Europa (Fig. 8).

We already mentioned Lake Vostok as a potential Earth analogue for the ice layer and maybe even the ocean (see previous discussions). The depth from the surface of the ice layer to the base of the lake is ~4,300 m, which is equivalent to 444 bars of hydrostatic pressure, which makes Lake Vostok another potentially good analogue for deep cold waters on Europa.

There also exist deep saline basins in the oceans and seas of the Earth. Examples include Orca Basin (2,300 m, T = 4–6°C) in the Gulf of Mexico, and the Tyro and Bannock II Basins (3,400 m, T = 13.5°C) in the Mediterranean (Krumgalz *et al.*, 1999). In addition to high hydrostatic pressures (237–351 bars), these systems have high salinities (a_{H_2O} = 0.742–0.774). Table 7 highlights the chemical characteristics of Orca Basin. Because of the high density of these saline basin waters (e.g., Orca Basin = 1.19 kg L^{-1}) compared with seawater density (1.023 kg L^{-1}) (Table 7), these deep saline basins are stable and do not mix with seawater.

Temperature at the base of a Europan ocean could be relevant for life because hydrothermal vents could provide heat to an otherwise cold ocean, potentially serving as a hotbed of biological activity in an otherwise depauperate ocean, in a similar fashion to what occurs on Earth (Kelley, 2001; Takai *et al.*, 2001). Lake Vanda in Antarctica exhibits a strong thermal and density stratification with temperatures beneath the ice layer (3–5 m) at 0°C and temperatures at the bottom (65–70 m) at 25°C (Spigel and Priscu, 1998). This is the type of density and thermal statification, albeit at a much smaller scale, that Kargel (2001) hypothesized could play a role in cryovolcanic eruptions on Europa (Fig. 4).

SUMMARY

Many studies have examined energy and nutrients as potential limiting factors for life on Europa (e.g., Reynolds *et al.*, 1983; Jakosky and Shock, 1998; Gaidos *et al.*, 1999; McCollom, 1999; Chyba, 2000; Kargel *et al.*, 2000; Chyba and Hand, 2001; Chyba and Phillips, 2001; Navarro-Gonzalez *et al.*, 2002; Pierazzo and Chyba, 2002; Schulze-Makuch and Irwin, 2002). The consensus is that there exist sufficient energy sources and nutrients to at least support a small biomass on Europa. In our paper, we chose to examine other factors such as temperature, salinity, acidity, desiccation, radiation, pressure, and time that could limit life on Europa.

The dual stresses of lethal radiation and cold temperatures on or near the surface of Europa preclude the possibility of biological activity anywhere near the surface. This conclusion is in contrast to Greenberg and Geissler (2002), who argued that a habitable biosphere could exist within centimeters of the surface. Their argument assumes frequent exchange of materials between the surface ice and the ocean through a thin ice layer. While ice is an effective shield for ionizing radiation (Baumstark-Khan and Facius, 2002), ice offers no insulation from the low surface temperatures (86–132 K, Fig. 4) that are well below the level for biological activity (253 K, Table 1) and the eutectic temperatures for hypothetical brines (235–250 K, Figs. 5–8). Liquid water is not stable anywhere near the surface of Europa. Only at the base of the ice layer near the ocean could one expect to fine suitable temperatures and liquid water that are necessary for life. Desiccation could be a problem in the surface ice layers where temperatures are low (<220 K). An ice layer turnover time of 10 Myr is probably rapid enough for preserving in the surface ice layers dormant life forms originating from the ocean.

According to Kargel *et al*. (2000), the physiochemical conditions most likely in Europa's ocean are surmountable based on analogues with Earth micobial adaptations. In contrast, all of our simulations demonstrate that hypothetical oceans could exist on Europa that are too cold for biological activity (T <253 K, Figs. 5–8). Also, all our simulations demonstrate that salinities are high, which would restrict life to extreme halophiles. An acidic ocean could also potentially limit life (pH <0, Fig. 7).

The pressure at the base of a 100 km ice/ocean layer is 1,200 bars, which is similar to the deepest ocean basins on Earth (1,100 bars) where life exists. Pressure, *per se*, is unlikely to directly limit life on Europa. But, indirectly, pressure plays an important role in controlling the chemical environments for life (cf. Figs. 5 and 8).

RECOMMENDATIONS

Based on examining the limits for biological activity on Earth, potential habitats for life on Europa, and Earth analogues for Europa, we recommend that future "Life on Europa" work focus on the following areas:

1. We need to better constrain the thicknesses of the ice layer and the brine ocean. All speculation about life on Europa depends fundamentally on the roles of temperature and pressure as they control physics, chemistry, and biology. First, we must constrain the physical dimensions of the ice/water layers before we can accurately develop temperature and pressure profiles.
2. We need to better constrain heat flow and temperature profiles on Europa. For example, are hydrothermal vents present? Is the temperature of the ocean 240 K or 270 K? Is cryovolcanism an important process on Europa?
3. We need to better constrain the composition of the "non-icy" component (the so-called "Red Stuff") on the surface of Europa. This material may be the only indirect evidence that we have for the composition of a Europan ocean.
4. Future chemical work should focus on sulfate chemistry, sulfate analogues, and life in sulfate systems.
5. Besides the resources of liquid water, energy, and nutrients, low temperature, salinity, and high pressure are likely to be important factors controlling the environments for life on Europa. Future work should focus on these six variables for Europa.
6. The preservation of life forms and associated biosignatures under high radiation in ice needs more work. This improved understanding is important for both remote sensing of the surface and future landed missions on Europa in search for life.

ACKNOWLEDGMENTS

Funding was provided by a NASA Planetary Geology and Geophysics Project, "An Aqueous Geochemical Model for Cold Planets," a NASA EPSCoR Project, "Building Expertise and Collaborative Infrastructure for Successful Astrobiology Research, Technology, and Education in Nevada," and a NASA Space Grant Project, "Earth Analogues for Europan Environments." We thank Annette Risley for assistance in preparing the manuscript. We thank Jeffrey S. Kargel and an anonymous reviewer for providing constructive comments that improved the paper.

ABBREVIATIONS

Byr, billion years; Myr, million years; RH, relative humidity; UV, ultraviolet.

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