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## **Multiplication of microbes below 0.690 water activity: implications for terrestrial and extraterrestrial life**

Andrew Stevenson, Jürgen Burkhardt, Charles S. Cockell, Jonathan A. Cray,  
Jan Dijksterhuis, Mark Fox-Powell, Terence P. Kee, Gerhard Kminek, Terry J.  
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1 **Multiplication of microbes below 0.690 water activity: implications for terrestrial and**  
2 **extraterrestrial life**

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5 Andrew Stevenson<sup>1</sup>, Jürgen Burkhardt<sup>2</sup>, Charles S. Cockell<sup>3</sup>, Jonathan A. Cray<sup>1</sup>, Jan  
6 Dijksterhuis<sup>4</sup>, Mark Fox-Powell<sup>3</sup>, Terence P. Kee<sup>5</sup>, Gerhard Kminek<sup>6</sup>, Terry J. McGenity<sup>7</sup>,  
7 Kenneth N. Timmis<sup>8</sup>, David J. Timson<sup>1</sup>, Mary A. Voytek<sup>9</sup>, Frances Westall<sup>10</sup>, Michail  
8 M.Yakimov<sup>11</sup>, John E. Hallsworth<sup>1</sup>

9  
10 <sup>1</sup>Institute for Global Food Security, School of Biological Sciences, MBC, Queen's University  
11 Belfast, Belfast, BT9 7BL, Northern Ireland. <sup>2</sup>Plant Nutrition Group, Institute of Crop Science  
12 and Resource Conservation, University of Bonn, Karlrobert-Kreiten-Str. 13, D-53115 Bonn,  
13 Germany. <sup>3</sup>UK Centre for Astrobiology, School of Physics and Astronomy, University of  
14 Edinburgh, Edinburgh EH9 3JZ, UK. <sup>4</sup>CBS Fungal Biodiversity Centre, Uppsalalaan 8, NL-  
15 3584, CT Utrecht, Netherlands. <sup>5</sup>University of Leeds, School of Chemistry, Leeds, LS2 9JT,  
16 West Yorkshire, UK. <sup>6</sup>ESA-ESTEC, Keplerlaan 1, 2200 Noordwijk, The Netherlands.  
17 <sup>7</sup>University of Essex, School of Biological Sciences, Colchester, CO4 3SQ, Essex, UK.  
18 <sup>8</sup>Institute of Microbiology, Technical University Braunschweig, Spielmannstrasse 7, D-38106  
19 Braunschweig, Germany. <sup>9</sup>NASA Headquarters, Washington, DC 20546-0001, USA.  
20 <sup>10</sup>Centre de Biophysique Moléculaire, CNRS, Rue Charles Sadron, and Centre de  
21 Recherches sur les Matériaux à Haute Température, 1D, avenue de la recherche  
22 scientifique, 45071 Orléans Cedex 2, France. <sup>11</sup>Istituto per l'Ambiente Marino Costiero, CNR,  
23 98122 Messina, Italy.

24  
25 \*For correspondence. E-mail [j.hallsworth@qub.ac.uk](mailto:j.hallsworth@qub.ac.uk); Tel: (+44) 289097 2314; Fax: (+44)  
26 289097 5877.

27  
28 Running title: Multiplication of microbes at low water-activity

29  
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31 xerophilic fungi, water activity

## 33 **Summary**

34  
35 Since a key requirement of known life-forms is available water (water-activity;  $a_w$ ), searches  
36 for signatures of past life in terrestrial and extraterrestrial environments have recently  
37 targeted places known to have contained significant quantities of biologically available water.  
38 The lower limit of water activity that enables cell division is  $\sim 0.605$  which, until now, was only  
39 known to be exhibited by a single eukaryote; the sugar-tolerant, fungal xerophile *Xeromyces*  
40 *bisporus*. The first forms of life on Earth were, however, prokaryotic. Furthermore, early life  
41 on Earth inhabited high-salt environments, suggesting an ability to withstand low water-  
42 activity. Recent evidence indicates that some halophilic Archaea and Bacteria have water-  
43 activity limits more or less equal to those of *X. bisporus*. Regardless of species, cellular  
44 systems are sensitive to minute differences in water activity (of  $< 0.005$   $a_w$ -units) so there is a  
45 need to determine water-activity values to three decimal places. We discuss water activity in  
46 relation to the limits of Earth's present-day biosphere; the possibility of microbial  
47 multiplication by utilizing water from thin, aqueous films or non-liquid sources; whether  
48 prokaryotes were the first organisms able to multiply at the  $0.605$ - $a_w$  limit; and whether  
49 extraterrestrial aqueous milieu of  $\geq 0.605$   $a_w$  can resemble fertile microbial habitats found on  
50 Earth.

51

52

53

## 54 **Introduction**

55 Given the fact that water is one of the principal ingredients of cellular life (Daniel et al.,  
56 2004), insights into the minimum water requirements of cells are imperative to understanding  
57 the functionality of living-systems at every level (from biomacromolecule to biosphere), as  
58 well as the origins of life, in an environmental context. The generally held opinion is that life  
59 appeared independently on Earth and, possibly, elsewhere in the Solar System (Clancy et al.  
60 et al., 2005); though one other explanation for the presence of life on Earth is that it appeared  
61 on another planet and was transported here in the form of prokaryotes or their ancestors (an  
62 idea known as panspermia; Thomson, 1871). Until recently, eukaryotic microbes have held  
63 the record for life under water-constrained conditions, as some species are capable of cell  
64 division down to a water activity ( $a_w$ )<sup>1</sup> of  $0.605$  at high sugar concentrations (Pitt and  
65 Christian, 1968; Williams and Hallsworth, 2009). Whereas such data have formed the basis  
66 of international policy for planetary protection in relation to space-exploration missions (see

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<sup>1</sup> Water activity, the mole fraction of water, is defined by an equation (water activity = vapour pressure of the solution/vapour pressure of the water) which is derived from Raoult's Law; this parameter and its derivation are discussed in detail by Brown (1990) and Grant (2004).

67 below), sugar-rich substrates have very limited applicability to those extraterrestrial habitats  
68 with which we are familiar. Historically, the accepted limit for cell division of prokaryotic  
69 microbes has been 0.755  $a_w$ ; this applied to a small fraction of halophilic species at high salt  
70 concentrations (for references, see Grant, 2004). However, both culture-based and culture-  
71 independent studies provide evidence for multiplication and metabolic activity of halophilic  
72 Archaea and Bacteria in the range 0.680 to 0.605  $a_w$ , both in their natural habitats in situ,  
73 and in vitro (Javor, 1984; Yakimov et al., 2014; A. Stevenson et al., submitted). Other studies  
74 have shown that, whereas the vast majority of yeasts and fungi are active somewhere within  
75 the range 1 to 0.720  $a_w$  (Pitt, 1975; Brown, 1976), only ~12 species have been observed to  
76 grow and/or germinate at < 0.700  $a_w$  (Williams and Hallsworth, 2009; A. Stevenson et al.,  
77 submitted). Here, we discuss the evidence for microbial activity below at or below 0.690,  
78 which represents the very edge of the functional biosphere on Earth. Low water activity is  
79 also discussed in relation to early life on Earth, the plausibility of cell division in habitable  
80 extraterrestrial environments in which biologically available water is present, and a series of  
81 unanswered scientific questions.

## 82 83 Water-activity at the outer edges of the microbial biosphere

84  
85 The primary physical determinants of the habitable space on Earth are temperature and  
86 water activity; these parameters are also used to designate the 'Special Regions' of Mars in  
87 which microbial cell-division might feasibly take place (Beaty et al., 2006; Kminek et al.,  
88 2010; J. D. Rummel et al., unpublished)<sup>2</sup>. The temperature window over which microbes are,  
89 collectively, capable of cell division (i.e. from -18 to +122°C; Takai et al., 2008; Chin et al.,  
90 2010) spans  $\leq 40\%$  of the entire range of temperatures to which life-systems on Earth can  
91 be exposed; i.e. from approximately -90°C to  $\geq 250^\circ\text{C}$  (for some hydrothermal vents; Fig.  
92 1a). By contrast, environmental water-activity values range from 1 to 0 and most cellular  
93 systems of known life-forms on Earth are only active in the range, or a segment of the range,  
94 1 to 0.900  $a_w$  (Fig. 1b; Brown, 1976; Grant, 2004). For example, there is a drop-off in  
95 measurable metabolic activity in many soils at  $\leq 0.890 a_w$  (Moyano et al., 2012; 2013;  
96 Stevenson and Hallsworth, 2014). However, metabolic activity and cell-division has been

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<sup>2</sup> Planetary protection in relation to space missions aims to protect those planets where spacecraft are landed, as well as Earth, from accidental contamination with non-native life-forms (Kminek et al., 2010; 2014). Mars Special Regions have been defined according to the activities of the NASA Mars Exploration Program Analysis Group (MEPAG), Special Regions-Scientific Analysis Group 1 (SR-SAG1) and the Committee on Space Research (COSPAR), which is part of the International Council for Science. Both these committees conservatively recommended 0.500  $a_w$  as the limit beyond which no known terrestrial microorganism is capable of multiplication; implying that any environment of Mars with a water activity of > 0.500 may potentially enable proliferation of xerophilic microbes if they happened to arrive as accidental passengers on spacecraft sent from Earth (Fig. 1; Beaty et al., 2006; Kminek et al., 2010). A revised analysis of Mars Special Regions is currently underway by the MEPAG SR-SAG2 (J. D. Rummel et al., unpublished).

97 reported below 0.900  $a_w$  for a great number of xerotolerant/philic and halotolerant/philic  
98 microbes (Brown, 1976; Grant, 2004), and even below 0.755  $a_w$  for both eukaryotic and  
99 prokaryotic species (Javor, 1984; Williams and Hallsworth, 2009; Yakimov et al., 2014; A.  
100 Stevenson et al., submitted). Of the microbes known to multiply below 0.720, the majority  
101 (unlike *X. bisporus*) are not obligate osmophiles that must inhabit sugar-rich substrates;  
102 these include halophilic prokaryotes and xerophilic fungi such as *Aspergillus penicillioides*  
103 and *Eurotium herbariorum* (Samson and Lustgraaf, 1978; Williams and Hallsworth, 2009;  
104 Yakimov et al., 2014; A. Stevenson et al., submitted)<sup>3</sup>. Even for the most xerophilic microbes  
105 thus far characterized (see Pitt, 1975; Javor, 1984; Williams and Hallsworth, 2009; A.  
106 Stevenson et al., submitted), rates of cell division typically decrease by an order of  
107 magnitude between 0.870 and 0.770  $a_w$ , and by a further order of magnitude between 0.770  
108 and 0.670  $a_w$  (Stevenson and Hallsworth, 2014; A. Stevenson et al., submitted). There are  
109 only reports of cell division for between 20 and 30 microbial species or communities at  $\leq$   
110 0.690  $a_w$  (see Javor, 1984; Yakimov et al., 2014; A. Stevenson et al., submitted). Whereas  
111 all of these species are extreme, obligately xerophilic eukaryotes or extreme, obligately  
112 halophilic prokaryotes which have low rates of cell division - or are incapable of growth -  
113 close to 1  $a_w$ , the ultimate limit for multiplication of even the most resilient strains appears to  
114 be  $\sim 0.61 a_w$  (Pitt and Christian, 1968; A. Stevenson et al., submitted). For microbes on  
115 Earth, therefore, biotic activity spans approximately 40% of the available water-activity  
116 range, thus emphasizing the potency of water as a determinant of the functional biosphere.  
117 The overwhelming majority of microbial systems are metabolically active somewhere within  
118 the ranges 5 to 40°C, and 1 to 0.900  $a_w$ , which represent even smaller portions of the  
119 environmentally pertinent temperature and water-activity ranges; i.e. only 10% in each case  
120 (Fig. 1). Of the microbial systems characterized thus far, the 20 to 30 known to be active at  $\leq$   
121 0.690  $a_w$  (Fig. 1; Javor, 1984; Yakimov et al., 2014; A. Stevenson et al., submitted) represent  
122 the most extreme forms of life to have penetrated these kinds of hostile environment<sup>4</sup>.

123 Some reports have alluded to the possibility of microbial growth and metabolism at  
124 the otherwise unprecedented water-activity values of 0.382 (for deep-sea halophiles in  
125  $MgCl_2$ -saturated brine; van der Wielen et al., 2005),  $< 0.450$  (for halophiles in the  $CaCl_2$ -rich,  
126 Antarctic Don Juan Pond; Siegel, 1979), 0.500 (Actinobacteria isolated from algal mats and  
127 cultured in soil-based substrates; Doroshenko et al., 2005; 2006; Zvyagintsev et al., 2009;

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<sup>3</sup> This has implications for preventing contamination of other planetary bodies (see above) which, as far as we know, lack sugar-rich environments, during space exploration missions.

<sup>4</sup> Habitats which have sufficiently low water-activity to exclude almost all forms of life on Earth and, therefore, have a characteristically low biodiversity (especially those of  $< 0.690 a_w$ ) are fertile habitats for those extremophiles which thrive there due to minimal competition and, frequently, a lack of grazers and predators (for references, see Cray et al., 2013b). Such low-water activity habitats are, however, typically too biologically hostile and insufficiently biodiverse to act as open habitats for microorganisms (Cray et al., 2013b; Lievens et al., 2014; Oren and Hallsworth, 2014).

128 2012), 0.570 (for halophiles in acidic saline lakes; Mormile et al., 2009), 0.600 (for  
129 germination of *Wallemia sebi* [a xerophilic basidiomycete] on high-sugar substrates; Frank  
130 and Hess, 1941) and 0.600 (reported value for optimum growth of halophiles (Jaenicke and  
131 Bohm, 1998), and biotic activity in salt lakes; Cobucci-Ponzano et al., 2006). Some of these  
132 values were hypothetical (see below), and the other claims have not been accepted or have  
133 been refuted by authors of a number of subsequent studies (Pitt and Christian, 1968; Wynn-  
134 Williams, 1996; Beaty et al., 2006; Hallsworth et al., 2007; Kminek et al., 2010; Oren, 2011;  
135 Stevenson and Hallsworth, 2014; A. Stevenson et al., submitted; J. D. Rummel et al.,  
136 unpublished). The Don Juan Pond (located within the McMurdo Dry Valleys, Antarctica) is a  
137  $\text{CaCl}_2$ -saturated brine-pool situated in a closed basin and fed by seasonal melt-water  
138 streams and deliquescent seepages, both of which are thought to deliver  $\text{CaCl}_2$  to the lake  
139 (Dickson, 2013). Its volume fluctuates but is typically  $\sim 3000 \text{ m}^3$  (slightly larger than an  
140 Olympic swimming pool), and it is amongst the most saline large-scale bodies of water  
141 known on Earth. This pond rarely, if ever, freezes despite winter temperatures of  $\leq -51^\circ\text{C}$   
142 (Siegel 1979; Marion 1997; Grant, 2004). While annual temperatures of the pond's water and  
143 the surrounding sediments are occasionally above  $0^\circ\text{C}$ , they remain below  $-20^\circ\text{C}$  for the  
144 majority of the year (Samarkin et al., 2010) so it is highly unlikely that microbial life could  
145 multiply there (for references, see Chin et al., 2010; Kminek et al., 2010; J. D. Rummel et al.,  
146 unpublished). Saturated solutions of  $\text{CaCl}_2$ , as found in the Don Juan Pond, are highly  
147 chaotropic and are therefore likely to prevent microbial growth (and may even be sterile  
148 environments; Duda et al., 2004; 2005; Hallsworth et al., 2007; Cray et al., 2013a; 2013b;  
149 Oren, 2013; Yakimov et al., 2014). Nitrous oxide emissions recorded from the surrounding  
150 sediments, frequently attributed to the biological transformation of nitrogenous compounds,  
151 are apparently the result of abiotic reactions between brine nitrates and  $\text{Fe}^{\text{II}}$ -bearing minerals  
152 (Samarkin et al., 2010). The water activity of the  $\text{MgCl}_2$ -dominated, deep-sea hypersaline  
153 brine studied by van der Wielen et al. (2005) is  $\sim 0.382$  at the in situ temperature of  $14.5^\circ\text{C}$   
154 (Winston and Bates, 1960; Hallsworth et al., 2007). Culture-dependent and culture-  
155 independent studies of this brine, and investigations into the biophysics of macromolecular  
156 interactions, indicate that both its potent chaotropicity (even at water-activity values which  
157 would otherwise be permissive for cell division) and exceptionally low water-activity prohibit  
158 life processes (Hallsworth et al., 2007; Yakimov et al., 2014), as these parameters do for  
159 solutions of comparable salts (Winston and Bates, 1960; Duda et al., 2004; Hallsworth et al.,  
160 2003a; Kminek et al., 2010; Oren, 2011; Cray et al., 2013a; 2013b). Speculations that  
161 microbial metabolism and cell division occur at  $\sim 5 \text{ M MgCl}_2$  are inconsistent with the virtual  
162 sterility of the Dead Sea when  $\text{MgCl}_2$  concentrations become elevated (Oren, 1999; 2010;  
163 Oren, 2013) or the  $\text{CaCl}_2$ -dominated Don Juan Pond (Siegel et al., 1983; Samarkin et al.,

164 2010; Oren, 2013) where concentrations of divalent chloride salts reach critical  
165 concentrations which are prohibitive for all life processes (Hallsworth et al., 2007; Cray et al.,  
166 2013a; Oren, 2013; Yakimov et al., 2014). Although there is a theoretical possibility that  
167 some microbes have evolved specialised structures which isolate cells from such hostile  
168 habitats whilst permitting biotic activity, to our knowledge no such structures have yet been  
169 reported for any microbial species in any type of extremely chaotropic (e.g. Hallsworth et al.,  
170 2007; Yakimov et al., 2014) or low water-activity ( $\leq 0.600$ ) environment.

171 Reports of germination and subsequent cell division during germ-tube formation of  
172 several Actinobacteria (i.e. *Streptomyces albidoflavus* [syn. *Streptomyces odorifer*],  
173 *Streptomyces rectiviolaceus*, and a *Micromonospora* strain) at 0.500  $a_w$  (which were carried  
174 out by one group: Doroshenko et al., 2005; 2006; Zvyagintsev et al., 2009; 2012) are  
175 apparently erroneous (see Stevenson and Hallsworth, 2014). Independent studies have  
176 demonstrated that none of these species was capable of growth below 0.895  $a_w$ , and the  
177 theoretical water-activity minimum for the most xerotolerant (a strain of *Streptomyces*  
178 *albidoflavus*) was  $\sim 0.870$  (Stevenson and Hallsworth, 2014). Proposed limits of 0.570 or  
179 0.600  $a_w$  for biotic activity of halophiles were speculative (i.e. not derived from  
180 determinations of water-activity; Jaenicke and Bohm, 1998; Mormile et al., 2009; Cobucci-  
181 Ponzano et al., 2006), and sources of experimental error in studies of *W. sebi* germination  
182 have been discussed previously (Pitt and Christian, 1968). Furthermore, apparent microbial  
183 growth within terrestrial brine lakes which can reach values of  $\leq 0.600 a_w$  may have actually  
184 occurred at higher water-activity values given the seasonal and other temporal fluctuations of  
185 the in situ salt concentrations (Oren, 1988; 1993; Cobucci-Ponzano et al., 2006; Mormile et  
186 al., 2009).

187 Although the established temperature minima for multiplication of the most  
188 psychrophilic microbes are in the region of  $-15$  to  $-18^\circ\text{C}$  (for references, see Chin et al.,  
189 2010; Kminek et al., 2010), there are numerous sources of evidence for metabolic activity  
190 considerably below this range (Kminek et al., 2010; J. D. Rummel et al., unpublished). By  
191 contrast, there is a paucity of data to demonstrate metabolic activity below the accepted  
192 water-activity minimum for microbial cell division (i.e. 0.605; Kminek et al., 2010; Yakimov et  
193 al., 2014; A. Stevenson et al., unpublished; J. D. Rummel et al., unpublished). In relation to  
194 the water-activity limit for life, it is noteworthy that trehalose, a hygroscopic substance which  
195 accumulates in desiccated microbial cells and may facilitate the acquisition and retention of  
196 water, cannot efficiently absorb water from the vapour phase at equilibrium relative  
197 humidities of less than  $\sim 50\%$ , equivalent to 0.500  $a_w$  (Fakes et al., 2000). Whereas some  
198 enzymes can remain catalytic at water activities of  $< 0.500$  (Kurkal et al., 2005; Lopez et al.,  
199 2010), there is evidence that DNA becomes disordered, and is therefore no longer



200 transcribable, below a water activity of 0.550 (Falk et al., 1963). Furthermore, strand breaks  
201 have been recorded at 0.530  $a_w$  in bacterial cells (Asada et al., 1979). It has, therefore, long-  
202 been considered unlikely that cellular systems could function at water activities substantially  
203 lower than 0.600 (e.g. Pitt, 1975; Brown, 1976; 1990; Sutton and Hildebrand, 1985; J. D.  
204 Rummel et al., unpublished). However, interactions between the various factors which  
205 determine the biophysical limits for cellular integrity and biotic activity at low water-activity  
206 are complex and have yet to be fully elucidated. Macromolecular integrity and functionality  
207 can depend on the net effect of prevailing conditions such as temperature, chaos-  
208 /kosmotropicity, pressure and water activity (Hallsworth, 1998; Hallsworth et al., 2007;  
209 Williams and Hallsworth, 2009; Bhaganna et al., 2010; Chin et al., 2010; Yakimov et al.,  
210 2014) and it may be possible that, in some as-yet-undiscovered environments, cells are  
211 capable of metabolism at  $< 0.600 a_w$ .

212

213 Microbial cell division via utilization of water which is not in the bulk liquid-phase

214

215 Water is more or less ubiquitous on Earth and in other parts of the Solar System (Bradley et  
216 al., 2014; Küppers et al., 2014); it may be present within the atmospheres, subsurface, rocks  
217 and regolith, polar ice-sheets, glaciers, and/or subsurface oceans of planetary bodies, in  
218 vapour plumes extruded into space, and – indeed – within space itself<sup>5</sup>. Whereas here on  
219 Earth, we tend to be most familiar with water in its bulk-liquid phase, in both terrestrial and  
220 extraterrestrial environments, it can also be present in a variety of forms. In addition to ice  
221 and vapour, these include thin aqueous films on/at various types of surfaces and interfaces,  
222 or as molecules hydrating mineral, organic, and other substances (Kminek et al., 2010;  
223 Toner et al., 2014; J. D. Rummel et al., unpublished). Liquidity of water is determined by  
224 temperature, pressure, the presence of solutes and/or gases, and molecular interactions  
225 between other materials or substances and water molecules - as well as processes such as  
226 salt deliquescence, sublimation of ice, frost formation, condensation or dew-formation on  
227 surfaces or within the gaseous phase, aerosol formation, and precipitation (Watanabe and  
228 Mizoguchi, 2002; Jepsen et al., 2007; Möhlmann; 2008; 2009; 2012; Argyris et al., 2008;  
229 Chin et al., 2010; Pavlov et al., 2010; Bing and Ma, 2011).

230 Thin aqueous films can exist on various surfaces including those of ice and biological  
231 and mineral structures, and the water within these films can remain in the liquid phase under  
232 a wide range of conditions (Pearson and Derbyshire, 1974; Raviv et al., 2001; Wolfe et al.,  
233 2002; Jepsen et al., 2007; Möhlmann; 2004; 2008; 2009; 2011; 2012; J. D. Rummel et al.,

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<sup>5</sup> See Waite et al. (2006); Nimmo et al. (2007); Tosca et al. (2008); Campins et al. (2010); Sohl et al. (2010);  
Carter et al. (2013); Martínez and Renno (2013); and Bradley et al. (2014).

234 unpublished). The depth of thin films can range from > 1 mm to a monolayer of water  
235 molecules (~0.3 nm; Möhlmann, 2004; 2005), and they can be stable (Möhlmann, 2012) or  
236 highly ephemeral (Burkhardt and Hunsche, 2013). At the temperatures and pressures which  
237 typically prevail in Earth's biosphere, aqueous films of ~1 mm are primarily made up of water  
238 which is biologically available (e.g. Qvit-Raz et al., 2008, Burch et al., 2013). Whereas we  
239 speculate that single-monolayer films do not provide water that can be accessed by cellular  
240 systems. It has, however, been suggested that microbes can utilize fluid films with a mean  
241 thickness equivalent to that of three water molecules (Harris, 1981; Beaty et al., 2006); a  
242 hypothesis that may be inconsistent with the lack of solute diffusion in very thin films  
243 (Derjaguin and Churaev, 1986; Hu and Wang, 2003) which indicate that the water in films as  
244 thin as this is not in the liquid phase<sup>6</sup>. Despite the circumstantial evidence (see also Rivkina  
245 et al., 2000), there is a paucity of data thus far available which convincingly demonstrate that  
246 water in thin films that are equivalent to between one and three water molecules in depth is  
247 biologically available.

248 There are three possible sources of liquid water in otherwise desiccated and cold  
249 areas such as those which are characteristic of Mars: (1) interfacial water as a thin film  
250 (several water-molecular in depth) forming on mineral surfaces by adsorption or, on ice, as  
251 pre-melted ice (Dash et al. 2006, Möhlmann, 2011); (2) brines forming on salt crystals via  
252 deliquescence; and (3) subsurface melt-water below an ice covering due to a solid-state  
253 'greenhouse' effect (Möhlmann, 2011). Process (2) is a particularly effective mechanism by  
254 which liquid water can be generated on Earth and, almost certainly, in extraterrestrial  
255 locations (Möhlmann, 2011). The condensing water vapour can potentially reach the dry  
256 weight of the deliquescent salt, and will exceed it if the humidity exceeds the deliquescence  
257 relative humidity. Deliquescence of NaCl, as equilibrium relative humidity increases from 65  
258 to 80%, can be observed in Movie S1. Most salts (and, indeed, many organic substances)  
259 are hygroscopic and will attract water to their surface at equilibrium relative humidities of ≤  
260 100%. Each salt becomes deliquescent at a specific relative humidity, thereby dissolving as  
261 the water vapour condenses. The deliquescence relative humidity for a given salt and its  
262 (usually slight) temperature-dependence quantitatively correspond to both the water activity  
263 values of, and equilibrium relative humidity values for, saturated solutions of a given salt  
264 (Winston and Bates, 1960). If the equilibrium relative humidity is higher than a salt's  
265 deliquescence relative humidity, the water activity of the salt solution will equilibrate with the  
266 relative humidity of the atmosphere, so the salt solution will become more dilute. Mixtures of  
267 substances (e.g. mixtures of different salts or salts plus sugars) will have a deliquescence

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<sup>6</sup> This inconsistency also raises the possibility that the high water-activity values associated with very thin films (Harris, 1981; Papendick and Campbell, 1981) could be a consequence of methodological error.

268 relative humidity below that of each individual component (Mauer and Taylor, 2010). In  
269 addition to the reduced water activity, salts also reduce the freezing point, and cryobrines  
270 may be stable far below the melting point of water, e.g. under Martian conditions (Möhlmann,  
271 2011, Martínez and Renno, 2013).

272 Within the Earth's biosphere, brine formation may play a role for diverse microbial  
273 species – especially those that are halotolerant or halophilic – which are located within  
274 bioaerosols, or on mineral or biological surfaces (e.g. leaf surfaces) and are exposed to  
275 humid air (Potts, 1994). For example, adapted species can reproduce within the  
276 phyllosphere of salt-exuding desert plants (Qvit-Raz et al., 2008, Burch et al., 2013) and, at  
277 subzero temperatures, in supercooled water in the atmosphere (Sattler et al., 2001).  
278 *Pseudomonas syringae*, which is not halophilic, is a species widely transported within  
279 bioaerosols and its cells are highly effective as ice nuclei because they have protein coatings  
280 that cause water to freeze at relatively warm temperatures (Christner et al., 2008; Morris et  
281 al., 2014). Being surrounded by ice, they may benefit from the solid-state greenhouse effect  
282 which involves the internal formation of thin films due to the penetration and retention of  
283 shortwave radiation within the ice.

284 Microbes can obtain water from the vapour-phase, a process which has been  
285 observed in lichens (Lange et al., 2006; Pintado and Sancho, 2002) as well as the  
286 propagules of various species (Waldham and Halvorson, 1954; Pasanen et al., 1991;  
287 Reponen et al., 1996). Other studies have demonstrated that microbial cells also generate  
288 considerable quantities of water via their metabolic activity (Oriol et al., 1988; Nagel et al.,  
289 2001; Marcano et al., 2002; Kreuzer-Martin et al., 2005; 2006; de Goffau et al., 2011), up to  
290 70% of the cell's water according to radio-labelled gas uptake experiments (Kreuzer-Martin  
291 et al. 2005; 2006). Spore germination of powdery mildews, such as by the *Erysiphe* and  
292 *Uncinula* species, has been observed at low equilibrium relative humidities (0 to 10%)  
293 without a visible extracellular source of liquid water (Brodie and Neufield, 1942; Manners and  
294 Hossain, 1963; Carroll and Wilcox, 2003); although it is not clear whether condensation  
295 processes and/or thin films might act to shuttle water to the cell. Desiccated lichens are able  
296 to absorb water at an equilibrium relative humidity of  $\geq 82\%$  and thereby commence  
297 photosynthesis (Pintado and Sancho, 2002; Lange et al. 2006). Various lines of evidence  
298 suggest that microorganisms may be capable of cell division without an extracellular supply  
299 of liquid water (see also Miller and Chibnall, 1932; Yarwood, 1950; Peterson and Cowling,  
300 1972; Lange et al., 1986; 1994). However, there is a paucity of convincing data to irrefutably  
301 affirm this hypothesis. Furthermore, systematic studies of water-activity limits for cell division  
302 of phylogenetically diverse extremotolerant and extremophilic microbes suggest that cell  
303 division would be implausible at values much below 0.600  $a_w$  (i.e. 60% equilibrium relative

304 humidity) (Pitt and Christian, 1968; Brown, 1976; Williams and Hallsworth, 2009; A.  
305 Stevenson et al., unpublished). This question is equally pertinent to life on Earth, and the  
306 aqueous milieu found elsewhere in the Solar System (not least in relation to planetary  
307 protection; see above).

308  
309 Implications for the evolution of microbial life on Earth

310  
311 The most solute-tolerant Bacteria and Archaea (i.e. extreme, obligate halophiles) are only  
312 able to grow at their water-activity minima under hypersaline conditions. Some of these  
313 organisms thrive under conditions which resemble those that would have been available on  
314 the early Earth; indeed, many of the extreme halophiles thus far studied exhibit their water-  
315 activity minimum for cell division at elevated temperatures (Robinson et al., 2005). There is  
316 some debate regarding the temperature of the early seas; earlier estimates of 70-80°C  
317 (Knauth and Lowe, 2003) are now considered to be too high (the  $\delta^{18}\text{O}$  values on which the  
318 calculations were based were skewed due to increased seawater temperatures which  
319 resulted from inputs of hydrothermal fluids from the crust). More recent estimates based on  
320 analysis of oxygen and hydrogen isotopes (i.e.  $\delta^{18}\text{O}$  and  $\delta\text{D}$ , respectively) are about 40°C  
321 (Blake et al., 2010). However, the high mantle heat flow on the early Earth drove a highly  
322 active hydrothermal circulatory system that contributed hot, salty (de Ronde et al., 1997),  
323 silica-rich fluids to the local environment (Westall, 2012). It has been proposed that  
324 primordial life may have first occurred within saline environments on early Earth (Dundas,  
325 1998), and recent evidence suggests that the abiotic formation of primitive proteins can  
326 indeed occur under saline conditions (Longo et al., 2013; Longo and Blaber, 2014).  
327 Understanding the way in which water-condensing chemical reactions could have led to the  
328 emergence of key biomolecules (eg: peptides and nucleic acids) is essential to  
329 understanding the origins of life (da Silva and Holm 2014 and references therein).  
330 Prokaryote life (anaerobic) was relatively abundant in these early environments and left  
331 behind numerous signatures of its presence (Westall, 2012). There are stratified salt  
332 deposits of various ages across large regions of the Earth, indicating that concentrated salt-  
333 waters/brines have existed across the planet's geologic history (Warren, 2010). Direct  
334 association of an early photosynthetic microbial community with evaporitic conditions is  
335 documented in 3.33 billion-year-old volcanic sands from the Barberton greenstone belt,  
336 South Africa (Figure 2; Westall et al., 2006, 2011). The uppermost layers of a desiccated  
337 biofilm, formed on sediments deposited in shallow waters that were partially exposed to air,  
338 are interlayered with tiny evaporate crystals (microns in size and including aragonite,  
339 gypsum, halite and magnesium calcite; Figure 2). Evaporitic precipitates have been

340 described from other formations on the early Earth, including the 3.42 billion-year-old Buck  
341 Reef Chert in Barberton (Lowe and Fisher-Worrell, 1999) and the 3.43 billion-year-old  
342 Strelley Pool Chert of the Pilbara in Australia (Allwood et al., 2007). The early terrestrial  
343 phototrophs were quite advanced on the evolutionary scale compared to chemotrophs.  
344 Although, to date, no direct association of chemotrophic biosignatures with the early  
345 evaporitic deposits has been identified, these more primitive organisms were nevertheless  
346 also common (Westall, 2012; Westall et al., 2013). If primitive cells did reach the early Earth  
347 through panspermia, experiments simulating the entry of meteorites containing  
348 microorganisms into the Earth's atmosphere have shown (1) that phototrophs could not have  
349 been transported to Earth by these means (Cockell et al., 2007) and (2) that, if resilient forms  
350 of life were hidden in meteorites, they would need to be buried at depths of at least 5 cm in  
351 cracks within the meteorite in order to withstand the heat of entry (Foucher et al., 2010).

352         Regardless of how (and where) life originated, it seems most likely that it was  
353 prokaryotes (known to have preceded eukaryotes by ~2 billions years), in saline  
354 environments, which first reached the 0.605- $a_w$  limit. Some of the oldest known fossils are  
355 those of prokaryotic cells (dating from ~3.5 billion years ago [Frances, is this the same  
356 environment as the 3.33 billion above? And if so, do we need to use the same  
357 number?...also is there any repetition between these sentences concerning the Barberton  
358 work here and those in the paragraph above? If not then that's fine.]) which apparently lived  
359 in salt-rich environments, as evaporite minerals such as magnesium calcite and halite were  
360 found embedded in the biofilm of an extant [Frances, was this mat fossilized or alive?]  
361 microbial mat, discovered in the Barberton greenstone belt, South Africa [Frances, given that  
362 the Barberton greenstone belt and it's location were mentioned above does this need  
363 repeating here?] (Westall et al., 2001; 2006), and similarly within ancient stromatolite  
364 columns from the Pilbara Craton, Western Australia (Allwood et al., 2007). Intriguingly,  
365 molecular analysis of modern stromatolite communities revealed that 74% of archaeal clones  
366 were closely related to the Halobacteria (Burns et al., 2004), which frequently dominate  
367 hypersaline environments (Oren, 2002). These prokaryotic halophiles were exposed to, and  
368 presumably inhabited [Frances should there be a comma here...] evaporitic environments  
369 containing [...or a comma here?] elevated concentrations of magnesium and characterized  
370 by water activities of considerably less than 0.755 (and can, indeed, be considerably below  
371 0.600  $a_w$ , depending on salt concentrations; Winston and Bates, 1960; Hallsworth et al.,  
372 2007; Yakimov et al., 2014; A. Stevenson et al., submitted). Indeed, the signatures of past  
373 life forms, including stromatolites, can be common in evaporitic deposits (Rothschild and  
374 Mancinelli, 2001).

375 Much later, and presumably in terrestrial locations, the Eukarya must have developed  
376 a similar resilience during growth at high concentrations of solutes which are produced via  
377 biogenic activity; namely sugars and polyols. Indeed, extremophilic Eukarya are considerably  
378 less salt-tolerant than their bacterial and archaeal counterparts, and it may be that the  
379 prokaryotes are yet to evolve an ability to grow at low water-activity in non-saline substrates  
380 (their current record is in the range 0.850 to 0.800; Lievens et al., 2014; R. Santos et al.,  
381 submitted; A. Stevenson et al., submitted). Microbial, and indeed all biological, cells are not  
382 pure-water reactors with water activity of 1 (Trevors and Pollack, 2005), but consist of gels  
383 within which modulation of water activity along with speciation as a result of the solute-  
384 exclusion principle are central to effective cellular function. Indeed, a metabolic ability to  
385 maintain the cellular system at this level is one of the fundamental, defining characteristics of  
386 life itself.

387

388 Extraterrestrial, aqueous milieu which resemble fertile habitats on Earth

389

390 Liquid water was, and may still be, present in numerous locations in the Solar System. On  
391 Mars, for example, there is abundant geomorphological evidence for the presence of liquid  
392 water on the planet in the past (Carr, 2006) and possibly even, ephemerally, in the present  
393 (Möhlmann, 2011; McEwan et al., 2014; J. D. Rummel et al., unpublished). Such evidence  
394 includes the formation of secondary minerals through the aqueous alteration of the basaltic  
395 rocks that cover the surface of the planet (e.g. Carter et al., 2013; Martínez and Renno,  
396 2013). It has been calculated that the water activities of evaporite deposits and bodies of  
397 saline water on early Mars were as high as 0.780 to 0.860 (Tosca et al., 2008), which is well  
398 within the ranges for microbial species from each Domain-of-life (Javor, 1984; Grant, 2004;  
399 Williams and Hallsworth, 2009; Stevenson et al., submitted).

400 The various brines on Jupiter's moon Europa are composed primarily of water and  
401 salts such as  $\text{MgSO}_4$ ,  $\text{Na}_2\text{SO}_4$ , and/or  $\text{Na}_2\text{CO}_3$  (and, in some cases also contain sulfuric  
402 acid; Muñoz-Iglesias et al., 2013). Saturated solutions of these salts have water-activity  
403 values of 0.900, 0.930 and 0.920 respectively (at 20°C, 1 atm; Winston and Bates, 1960),  
404 although it is currently unclear what the values would be under the prevailing conditions on  
405 Europa. At the lower temperatures, and the in situ pressures, on Europa the solubility of ions  
406 and, conversely, the precipitation of salts can also vary leading to increases in water activity  
407 (Marion et al., 2003; 2005), the water activity of a saturated  $\text{Na}_2\text{CO}_3$  solution at 10°C, for  
408 example, is 0.990 (Winston and Bates, 1960). Whereas water-activity values for individual  
409 brines will vary according to their ionic composition (and pH, which also influences  
410 solubilities of some salts), it seems likely that the in-situ water activities are sufficiently high

411 to span the entire range for known life (Javor, 1984; Williams and Hallsworth, 2009;  
412 Stevenson et al., submitted).

413 Water has also been identified in asteroidal materials, for example the Monahans  
414 (1998) H5 chondrite which contained hypersaline fluid inclusions composed predominantly of  
415 saturated NaCl (Zolensky et al., 1999) having a water activity of 0.760 at 20°C and 0.750 at  
416 2°C at 1 atm (Winston and Bates, 1960), although these values will vary with pressure. Fluid  
417 inclusions have been identified in an increasing number of asteroidal specimens including  
418 the Zag (1998) meteorite (Rubin et al., 2002). Furthermore, organic molecules have been  
419 detected in the fluid inclusions of some of these asteroidal bodies (e.g. Fries et al., 2012), so  
420 the composition of these asteroidal fluids can be close to those of the media and substrates  
421 in which halophiles occur. For instance, halophiles in hypersaline fluid inclusions of salt  
422 crystals from evaporite deposits contain Archaea, Bacteria, and algae (*Dunaliella* species)<sup>7</sup>.  
423 Many NaCl-saturated habitats contain a remarkably high microbial biomass and are  
424 characterised by intense competition (Antón et al., 2002; Daffonchio et al., 2006; Baati et al.,  
425 2008; Elevi Bardavid et al., 2008; Khemakhem et al., 2010) during which some species -  
426 which are known as “microbial weeds” (Cray et al., 2013b; Oren and Hallsworth, 2014) -  
427 achieve dominance of the communities including Archaea, Bacteria and Eukarya (e.g.  
428 *Haloquadratum walsbyi*, *Salinibacter ruber*, and *Dunaliella salina*; for references see Cray et al.  
429 et al., 2013b; Oren and Hallsworth, 2014). The microbes that dominate and/or are most  
430 frequently isolated from the fluid inclusions of salt crystals found in evaporite deposits  
431 include a number of species known to be capable of cell division in the range 0.710 to 0.605  
432 (or their close relations, such as *Dunaliella*, *Halocarcula*, *Halobacterium*, *Halococcus*,  
433 *Halorubrum* and *Natrinema* spp.: Stan-Lotter et al., 2000; Schubert et al., 2009b; Lowenstein  
434 et al., 2011; Gramain et al., 2011; A. Stevenson et al., submitted). In relation to water  
435 activity, the biotic activity of microorganisms - including halophiles – is plausible for some of  
436 the aqueous milieu found in extraterrestrial environments. Indeed, some of these locations  
437 resemble highly fertile habitats for known halophiles (see also A. Stevenson et al.,  
438 submitted).

439 Planets which are neither too close to nor too far from a star and could, theoretically  
440 at least, accommodate active biological systems are said to be in the Circumstellar Habitable  
441 Zone or Goldilocks Zone of their respective solar system (Strughold, 1953). This designation  
442 is based on criteria, such as size of the planet and its absolute distance from the star it  
443 orbits, whether illuminosity could permit photosynthesis, having surface temperatures which

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<sup>7</sup> See McGenity et al. (2000); D'Hondt et al. (2002); Schubert et al. (2009a); Gramain et al. (2011); Lowenstein et al. (2011); Lomstein et al. (2012); Valentine (2013). Cyanobacteria are known to be metabolically active in evaporite deposits (the in situ water-activity limit for this activity has yet to be determined; Rothschild et al., 1994).

444 are biologically permissive for at least some of the time (variously defined as 0 to 100°C, or -  
445 25 to +122°C; Franck et al. 2007; Takai et al., 2008; Kminek et al., 2010; Harrison et al.,  
446 2013), and/or whether they have liquid water (Rampino and Caldeira, 1994; von Bloh et al.,  
447 2011). However, these criteria (and indeed the habitable-zone concept) have limited  
448 applicability or validity for a variety of reasons. Ecosystems exist on Earth which do not  
449 depend on photosynthetic activity (Chivian et al., 2008; Teixeira et al., 2013) and, indeed, the  
450 earliest forms of life were not photosynthetic (Westall, 2012); furthermore, there is  
451 circumstantial evidence that an extracellular source of liquid water is not obligatory for  
452 microbial life (see above). What is more, biologically permissive conditions may prevail in  
453 specific environments or substrates on otherwise hostile planetary bodies (for examples in  
454 relation to moons of Saturn, see Raulin, 2006; Nimmo et al., 2007; Parkinson et al., 2008).  
455 And finally, various activities of solutes can both prevent freezing of water and expand biotic  
456 windows of microbes and may do so to a degree yet to be determined (see below; Chin et  
457 al., 2010; J. D. Rummel et al., unpublished).

458 Water can remain liquid at temperatures far lower than those known to permit  
459 microbial cell-division (i.e. approximately -18°C; see references in Chin et al., 2010). Liquid  
460 water (in various forms, from thin films to underground oceans) may be found in many  
461 environments on Mars as well as planetary moons (Europa, Ganymede, Enceladus, etc).  
462 Diverse lines of evidence suggest that both photosynthetic and non-photosynthetic microbes  
463 may be capable of metabolism and cell division by hygroscopic absorption of water vapour  
464 and/or acquiring water from their substratum (as a sole extracellular source of water) both in  
465 vitro and in their natural habitats on Earth<sup>8</sup>, and utilize a variety of mechanisms for the  
466 acquisition and retention of water (e.g. production and accumulation of trehalose and other  
467 hygroscopic substances which optimize the acquisition and retention of water, morphological  
468 changes which minimize water loss, hydrotactic responses, inhabiting high-humidity niches,  
469 and construction of soil features to enhance water capture and retention; Garcia-Pichel and  
470 Pringault, 2001; Garvie et al., 2008; de Goffau et al. 2011; Williams et al., 2012; Rajeev et  
471 al., 2013; Zakharova et al., 2013). Furthermore, as noted above, some microbial cells can  
472 generate vast quantities of water via their metabolic activities (Miller, 1932; Peterson and  
473 Cowling, 1973; Oriol et al., 1988; Nagel et al., 2001; Marcano et al., 2002; Hocking, 2003;  
474 Kreuzer-Martin et al., 2005; 2006). Indeed, studies of bacterial cells demonstrate that up to  
475 70% of intracellular water can be derived in this way (Kreuzer-Martin et al., 2005; 2006) and  
476 other studies demonstrate that cells can maintain higher intracellular water-activity than that  
477 of the environment; de Goffau et al. (2011).

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<sup>8</sup> E.g. fungi, lichens and cyanobacteria (Snow, 1949; Armolick and Dickson, 1956; Pitt and Christian, 1968; Ayerst, 1969; Bootsma et al., 1973; Drewello and Weissmann, 1997; Shomari and Kennedy, 1999; Lange et al., 2006; Wierzchos et al., 2011; Zakharova et al., 2013).



478 The atmosphere of Saturn's moon Enceladus can contain  $\geq 90\%$  water vapour (Waite  
479 et al., 2006) and, whereas its terrestrial surface is approximately  $-200^{\circ}\text{C}$  (Brown et al., 2006),  
480 plumes of water vapour and ice which are released into space are thought to originate in  
481 subsurface oceans that have temperatures in the range  $-23$  to  $-3^{\circ}\text{C}$  (Nimmo et al., 2007;  
482 Parkinson et al., 2008); i.e. temperatures which are permissive for the metabolic activity of  
483 psychrotolerant and psychrophilic microbes (Collins and Buick, 1989; Chin et al., 2010,  
484 Kminek et al., 2010, Mykytczuk et al., 2013). Various salts, nitrogenous compounds, and  
485 organic substances have been identified in the atmosphere of Enceladus and E-ring ice  
486 grains of Saturn (which may originate from Enceladus) including NaCl,  $\text{NaHCO}_3$ ,  $\text{NaCO}_3$ ,  $\text{N}_2$ ,  
487 ammonia, hydrogen cyanide, CO and  $\text{CO}_2$ , methane, acetylene, and propane (Matson et al.,  
488 2007; Postberg et al., 2009; 2011). Under conditions prevalent on Earth, bioaerosols can be  
489 fertile habitats characterized by high levels of microbial diversity, biomass, and metabolic  
490 activity (Fahlgren et al., 2010; Womack et al., 2010; 2012). In relation to the atmosphere of  
491 Enceladus and/or the watery plumes which it emits into space, it is intriguing to speculate  
492 what the water activity of liquid droplets in, or the humidity of, the gaseous phase might be  
493 (presumably close to 100%) and whether the temperatures within these plumes can ever be  
494 considerably higher than  $-200^{\circ}\text{C}$ . It should be noted that, whereas definitive evidence from  
495 culture-based studies of microbial systems on Earth indicate limits for cell division of  
496 approximately  $+122^{\circ}\text{C}$  or  $-18^{\circ}\text{C}$  (Collins and Buick, 1989; Takai et al., 2008; Chin et al.,  
497 2010; Harrison et al., 2013), circumstantial evidence from other biochemical or geochemical  
498 data suggest biotic activity under more extreme conditions (down to about  $-40^{\circ}\text{C}$ , and up to  
499 approximately  $+140^{\circ}\text{C}$ ; Parkes et al., 2000; Kminek et al., 2010; J. D. Rummel et al.,  
500 unpublished).

501 Although the Earth is located within the region allocated as the Goldilocks Zone of our  
502 own Solar System, it hosts many environments which do not permit life process and are  
503 therefore essentially sterile due to, for example, low water activity, high chaotropicity,  
504 excessively high or low temperatures, pH of  $> 12$ , plus combinations of conditions such as  
505 high salt and low pH or high temperature and high pH (e.g. Brown, 1990; Hallsworth, 1998;  
506 Parkes et al., 2000; Grant, 2004; Hallsworth et al., 2007; Harrison et al., 2013; Yakimov et  
507 al., 2014). Under all these conditions cells also need adequate energy sources and nutrients  
508 for maintenance and growth which may require electron donors and acceptors for  
509 respiration etc. Some combinations of conditions can slightly extend extremes for growth,  
510 such as high pressure and temperatures; furthermore survival can occur under conditions

511 where growth cannot<sup>9</sup>. Conversely, planetary bodies which are basically hostile to life may  
512 nevertheless harbour small-scale, biologically permissive domains (Kminek et al., 2010; J. D.  
513 Rummel et al., unpublished). Solute activities represent one of the determinants for potential  
514 habitability on Earth; for example, chaotropicity can enable cellular function at low  
515 temperatures and kosmotropicity may enable cellular function in high-temperature  
516 environments or those dominated by chaotropic substances<sup>10</sup>. The ways in which water  
517 activity and other solute activities can interact to determine the physicochemical limits for life  
518 (e.g. Williams and Hallsworth, 2009; Chin et al., 2010) have yet to be fully characterized.  
519 Furthermore, there is little information on the way in which availability of nutrients and other  
520 resources can determine tolerance limits to physicochemical stress parameters (e.g.  
521 Daffonchio et al., 2006; J. P. Harrison et al., submitted). Once the interactions between such  
522 factors are better understood, the currently accepted criteria for habitability will require  
523 revision (Beaty et al., 2006; Marion et al., 2003; Marion and Kargel, 2008; Tosca et al., 2008;  
524 Kminek et al., 2010; Harrison et al., 2013; J. D. Rummel et al., unpublished).

525

526 How sensitive are cells to minute changes in water activity? And other unanswered  
527 questions

528

529 In their environmental context, microbes are exposed to complexity at multiple levels; in  
530 relation to (i) the dynamics of physical and chemical parameters, (ii) the antimicrobials and  
531 other substances produced by other cells in the vicinity, (iii) varying availability of resources,  
532 and countless other factors. Water activity, in particular, can oscillate (Cray et al., 2013b;  
533 Lievens et al., 2014), and may do so across a range of timescales from a fraction of a  
534 second, for example to days or longer. The majority of stress-biology studies which quantify  
535 water activity do so to either one or two decimal places. We propose here that water activity  
536 ought to be determined to an accuracy of three decimal places (Winston and Bates, 1960;  
537 Williams and Hallsworth, 2009; A. Stevenson et al., submitted) as this is more closely  
538 aligned with the sensitivity of cellular systems. All technologies used to quantify the water  
539 activity of undefined substrates are associated with some degree of error (see Winston and  
540 Bates, 1960, Greenspan, 1977, Hallsworth and Nomura, 1999, Yu et al., 2009).  
541 Commercially available apparatus for water-activity determination are associated with a net

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<sup>9</sup> The propagules/cells of many microbes are highly resilient to exposure to extremes of temperature, uv, pH, chaotropicity, desiccation and other stresses (e.g. Wyatt et al., 2014; R. Santos et al., submitted), even over long timescales, and so are capable of surviving conditions found in extraterrestrial locations (see above).

<sup>10</sup> See Hallsworth (1998a); Hallsworth et al. (1998b; 2003a; 2003b; 2007); Williams and Hallsworth (2009) Bhaganna et al. (2010); Chin et al. (2010); McCammick et al. (2010); Bell et al. (2013); Cray et al. (2013a; 2013b); Lievens et al. (2014); Yakimov et al. (2014). Whereas chaotropic substances are typically less polar than water and disorder biomacromolecules, kosmotropic substances are usually more polar than water and thereby structure or rigidify macromolecular systems (see Cray et al., 2013a, and references therein).

542 variation (accounting for both accuracy and repeatability) of  $\pm 0.010$  to  $0.020$  water-activity  
543 units (A. Stevenson et al., submitted). At  $0.600$  water activity, this is equivalent to variations  
544 of water potential between  $\pm -2.3$  and  $-4.5$  MPa respectively). For the purposes of biological  
545 and food-related research it has been suggested, that levels of accuracy of  $\pm 0.010$  (Labuza  
546 et al., 1976; Roa and Tapia, 1998),  $\pm 0.020$  (Troller and Christian, 1978; Sereno et al., 2001),  
547  $\pm 0.005$  (Ferro Fontán and Chirife, 1981; Hallsworth and Nomura, 1999), or  $\pm 0.001 a_w$  are  
548 appropriate (Winston and Bates, 1960). Our earlier studies (Williams and Hallsworth, 2009;  
549 A. Stevenson et al., submitted) suggest that microbial cells can be sensitive to  
550 differences/changes of  $< 0.010$  water activity. For example, water-activity differences of  $<$   
551  $0.005$  units have impacted growth rates for diverse strains of xerophilic fungi by between 40  
552 and 80% (A. Stevenson et al., submitted) which, in turn, implies fundamental differences at  
553 every level of the cellular system, from gene expression to physiological and developmental  
554 processes. On glycerol-supplemented media at water activities of  $0.799$  and  $0.795$  growth-  
555 rates for *A. penicillioides* varied between  $1.13$  and  $0.642$  mm d<sup>-1</sup> for strain JH06THH and  
556 between  $1.20$  and  $0.732$  mm d<sup>-1</sup> for strain JH06THJ; and on MgCl<sub>2</sub>-supplemented media at  
557 water activities of  $0.915$  and  $0.907$  rates for *X. bisporus* varied between  $3.96$  and  $1.43$  mm d<sup>-</sup>  
558 <sup>1</sup> for strain FRR 0025,  $2.55$  and  $0.533$  mm d<sup>-1</sup> for strain FRR 2347, and  $2.13$  and  $0.800$  mm  
559 d<sup>-1</sup> for strain FRR 3443 (A. Stevenson et al., submitted). These data raise the tantalizing  
560 question of whether microbial cells are sensitive to water-activity differences down to the  
561 fourth, or even fifth, decimal place<sup>11</sup>. It is noteworthy that, for a hypothetical microbial species  
562 which has a temperature window for cell division spanning from  $5$  to  $40^\circ\text{C}$  (i.e. a  $35^\circ\text{C}$   
563 range), a temperature change of  $10$ ,  $1$  or  $0.1^\circ\text{C}$  would represent a  $1/3.5$ ,  $1/35$  and  $1/350$   
564 fraction of this window, respectively. If the water-activity window for this microbe spanned  
565 from  $1$  to  $0.900 a_w$  (i.e.  $0.100 a_w$ -units in total),  $1/3.5$ ,  $1/35$ - and  $1/350$ -portions of this window  
566 would correspond to  $0.02857$ ,  $0.00286$  and  $0.00029 a_w$  units, respectively. This underlines  
567 the fact that water-activity determinations to one decimal place (equivalent, in this example,  
568 to  $\sim 29^\circ\text{C}$ ) can lack biological meaning, and those made to two decimal places (equivalent to  
569 an accuracy level of up to  $2.9^\circ\text{C}$ ) are far less accurate than we would accept for biological  
570 studies of temperature or other environmental parameters. In relation to microbial  
571 multiplication on Earth, the water-activity and temperature windows for life span  $0.400 a_w$ -  
572 units and  $140^\circ\text{C}$ , respectively (Fig. 1). In the context of stress biology, and at the scale of the

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<sup>11</sup> Based on the use of Novasina technology (Axair Ltd., Pfäffikon, Switzerland) and a protocol incorporating a range of precautionary measures we achieve an accuracy of  $\pm 0.001$  water-activity units (A. Stevenson et al., submitted). Whereas calculations can be carried out to enable the expression of water-activity values to the fourth decimal place, these have been based on a number of assumptions which, collectively, result in unacceptable levels of uncertainty (Greenspan, 1977; Yu et al., 2009). Such a level of accuracy would be highly desirable in many spheres of biological research but empirical determinations of water activity to the fourth decimal place are currently unattainable.

573 biosphere, the expression of water activity to decimal place leads to an unacceptable level of  
574 accuracy, as 0.100  $a_w$  units equates to a temperature of 35°C. Even water-activity  
575 determinations to three decimal places (equivalent to an accuracy level of ~0.3°C) are  
576 imposed by technological limitations rather than being dictated by the sensitivity level of the  
577 cell.

578 It remains unclear whether microorganisms are capable of subsistence without an  
579 extracellular supply of liquid water, and the biological availability of water in various types of  
580 aqueous film has also yet to be quantified. Cells may be able to acquire and retain water (de  
581 Goffau et al., 2011) which can be utilized when water activity falls below biologically  
582 permissive levels (for instance, see the studies of powdery mildew cited above) but there is  
583 no definitive evidence that this does indeed occur (and, if so, what mechanisms are involved)  
584 at present (J. D. Rummel et al., unpublished). Culture-independent studies are needed for  
585 high-solute, and other low-water activity, habitats to establish whether metabolic activity  
586 below the threshold for cell division (0.605  $a_w$ ) is commonplace at different locations within  
587 the microbial biosphere. In contrast with the increasing understanding of molecular-level  
588 adaptations in many other forms of extremophile, there is a paucity of information in relation  
589 to physiological, biochemical, and genetic mechanisms which facilitate halophile/xerophile  
590 function at  $< 0.690 a_w$ <sup>12</sup>. Further work is also needed to elucidate the roles that low water-  
591 activity substrates have played, and continue to play, in the evolution of both prokaryotic and  
592 eukaryotic systems. In the context of habitability, work is also needed to elucidate the  
593 interactions between type and concentration of ions, chao-/kosmotropicity, and water activity  
594 in relation to complex brines such as current those found in various locations on Earth  
595 (Siegel et al., 1983; Oren, 1988; Hallsworth et al., 2007; Yakimov et al., 2014) and those  
596 likely to have existed on early Earth or ancient Mars (Tosca et al., 2008). For ecosystems  
597 located in extremely hostile habitats, some reports hint that microbial life can be  
598 discontinuous and fragmented (Hopkins et al., 2005). In some low water-activity habitats, it  
599 may be that active cells can be located in otherwise biologically non-permissive zones, and  
600 pockets of sterility exist within otherwise inhabited zones. Furthermore, in some locations  
601 microbes may be inactive for most of the time and yet functional for short periods. It has yet  
602 to be determined, for example, whether slow cell divisions (over 100s or 1000s years) can  
603 occur in microbial communities which may subsist in nature at water activities below the  
604 known 0.605  $a_w$  limit. In relation the water-activity limits for microbial life, we know much  
605 about the outer edges of Earth's biosphere yet, in the relation to the in situ conditions of  
606 microbial habitats, we still know relatively little.

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<sup>12</sup> This also acts as a barrier to the biotechnological exploitation of these extremophiles and the macromolecular systems derived from them.

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628 **References**

629

630 Allwood, A.C., Walter, M.R., Burch, I.W., and Kamber, B.S. (2007) 3.43 billion-year-old  
631 stromatolite reef from the Pilbara Craton of Western Australia: ecosystem-scale insights to  
632 early life on Earth. *Precambrian Res* **158**: 198–227.

633

634 Antón, J., Oren, A., Benloch, S., Rodríguez-Valera, F., Amann, R., and Rosselló-Mora, R.  
635 (2002) *Salinibacter ruber* gen. nov., sp. nov., a novel, extremely halophilic member of the  
636 Bacteria from saltern crystallizer ponds. *Int J Syst Evol Microbiol* **52**: 485–491.

637

638 Argyris, D., Tummala, N.R., Striolo, A., and Cole, D.R. (2008) Molecular structure and  
639 dynamics in thin water films at the silica and graphite surfaces *J Phys Chem C* **112**: 13587–  
640 13599.

641

642 Armolick, N., and Dickson, J.G. (1956) Minimum humidity requirement for germination of  
643 conidia of fungi associated with storage of grains. *Phytopathology* **46**: 462–465.

644

645 Asada, S., Takano, M., and Shibasaki, I. (1979) Deoxyribonucleic acid strand breaks during  
646 drying of *Escherchia coli* on a hydrophobic filter membrane. *Appl Environ Microbiol* **37**: 266–  
647 273.

648

649 Ayerst, G. (1969) The effects of moisture and temperature on growth and spore germination  
650 in some fungi. *J Stored Prod Res* **5**: 127–141.

651

652 Baati, H., Guerhazi, S., Amdouni, R., Gharsallah, N., Sghir, A., and Ammar, E. (2008)  
653 Prokaryotic diversity of a Tunisian multipond solar saltern. *Extremophiles* **12**: 505–518.

654

655 Beaty, D.W., Buxbaum, K.L., Meyer, M.A., Barlow, N.G., Boynton, W.V., Clark, B.C., et al.  
656 (2006) Findings of the special regions science analysis group. *Astrobiology* **6**: 677–732.

657

658 Bell, A.N.W., Magill, E., Hallsworth, J.E., and Timson, D.T. (2013) Effects of Alcohols and  
659 Compatible Solutes on the Activity of beta-Galactosidase. *Appl Biochem Biotech* **169**: 786–  
660 796.

661

662 Bhaganna, P., Volkers, R.J.M., Bell, A.N.W., Kluge, K. Timson, D.J., McGrath, J.W., et al.  
663 (2010) Hydrophobic substances induce water stress in microbial cells. *Microb Biotechnol* **3**:  
664 701–716.

665

666 Bing, H., and Ma, W. (2011) Laboratory investigation of the freezing point of saline soil. *Cold*  
667 *Reg Sci Technol* **67**: 79–88.

668

669 Blake, R.E., Chang, S.J. and Lepland, A. (2010) Phosphate oxygen isotope evidence for a  
670 temperate and biologically active Archean ocean. *Nature* **464**: 1029–1033.

671

672 Bootsma, A., Gillespie, T.J., and Sutton, J.C. (1973) Germination of *Phyllosticta maydis*  
673 conidia in an incubation chamber with control of high relative humidities. *Phytopathology* **63**:  
674 1157–1161.

675  
676 Bradley, J.P., Ishii, H.A., Gillis-Davis, J.J, Ciston, J., Nielsen, M.H., Bechtel, H.A., et al.  
677 (2014) Detection of solar wind-produced water in irradiated rims on silicate minerals. Proc  
678 Natl Acad Sci doi: 10.1073/pnas.1320115111  
679  
680 Brodie, H.J., and Neufeld, C.C. (1942) The development and structure of the conidia of  
681 *Erysiphe polygoni* DC and their germination at low humidity. Can J Res **20**: 41–61.  
682  
683 Brown, A.D. (1976) Microbial water stress. Bacteriol Rev **40**: 803–846.  
684  
685 Brown, A.D. (1990) Microbial Water Stress Physiology. Principles and Perspectives. John  
686 Wiley and Sons, Chichester, United Kingdom.  
687  
688 Brown, R.H., Clark, R.N., Buratti, B.J., Cruickshank, D.P., Barnes, J.W., Mastrapa, R.M.E.,  
689 et al. (2006) Composition and physical properties of Enceladus' surface. Science **10**: 1425–  
690 1428.  
691  
692 Burch, A.Y., Finkel, O.M., Cho, J.K., Belkin, S. and Lindow, S.E. (2013) Diverse  
693 microhabitats experienced by *Halomonas variabilis* on salt-secreting leaves. Appl Environ  
694 Microbiol **79**: 845–852.  
695  
696 Burkhardt J. and Hunsche M. (2013) “Breath figures” on leaf surfaces – formation and effects  
697 of microscopic leaf wetness. Front Plant Sci. **4**: 1–9.  
698  
699 Burns, B.P., Goh, F., Allen, M., and Neilan, B.A. (2004) Microbial diversity of extant  
700 stromatolites in the hypersaline marine environment of Shark Bay, Australia. Environ  
701 Microbiol **6**: 1096–1101.  
702  
703 Campins, H., Hargrove, K., Pinilla-Alonso, N., Howell, E.S., Kelley, M.S., Licandro, J., et al.  
704 (2010) Water ice and organics on the surface of the asteroid 24 Themis. Nature **464**: 1320–  
705 1321.  
706  
707 Carr, M. (2006) The surface of Mars. Cambridge University Press.  
708  
709 Carroll, J.E., and Wilcox, W.F. (2003) Effects of humidity on the development of grapevine  
710 powdery mildew. Phytopathology **93**: 1137–1144.  
711  
712 Carter, J., Poulet, F., Bibring, J.P., Mangold, N., and Murchie, S. (2013) Hydrous minerals on  
713 Mars as seen by the CRISM and OMEGA imaged spectrometers: updated global view. J  
714 Geophys Res-Planet **118**: 831–858.  
715  
716 Chin, J.P., Megaw, J., Magill, C.L., Nowotarski, K., Williams, J.P., Bhaganna, P., et al. (2010)  
717 Solutes determine the temperature windows for microbial survival and growth. Proc Natl  
718 Acad Sci USA **107**: 7835–7840.  
719

720 Chivian, D., Brodie, E.L., Alm, E.J., Culley, D.E., Dehal, P.S., DeSantis, T.Z., et al. (2008)  
721 Environmental genomics reveals a single-species ecosystem deep within Earth. *Science*  
722 **322**: 275–278.

723

724 Christner, B.C., Morris, C.E., Foreman, C.M., Cai, R., and Sands, D.C. (2008) Ubiquity of  
725 Biological Ice Nucleators in Snowfall. *Science* **319**: 1214-1214.

726

727 Clancy, P., Brack, A., Horneck, G. (2005) *Looking for life: Searching the Solar System*.  
728 Cambridge University Press, Cambridge.

729

730 Cobucci-Ponzano, B., Rossi, M., and Moracci, M. (2006) Interrupted genes in extremophilic  
731 Archaea: mechanisms of gene expression in early organisms. *Orig Life Evol Biosph* **36**:  
732 487–492.

733

734 Cockell, C.S., Brack, A., Wynn-Williams, D.D., Baglioni, P., Brandstätter, F., Demets, R., et  
735 al. (2007) Interplanetary transfer of photosynthesis: an experimental demonstration of a  
736 selective dispersal filter in planetary island biogeography. *Astrobiology* **7**: 1–9.

737

738 Collins, M.A., Buick, and R.K. (1989) Effect of temperature on the spoilage of stored peas by  
739 *Rhodotorula glutinis*. *Food Microbiol* **6**: 135–142

740

741 Cray, J.A., Bell, A.N.W., Bhaganna, P., Mswaka, A.Y., Timson, D.J., and Hallsworth,  
742 J.E. (2013a) The biology of habitat dominance; can microbes behave as weeds? *Microb*  
743 *Biotechnol* **6**: 453–492.

744

745 Cray, J.A., Russell, J.T., Timson, D.J., Singhal, R.S., and Hallsworth, J.E. (2013b) A  
746 universal measure of chaotropy and kosmotropy. *Environ Microbiol* **15**: 287–296.

747

748 Da Silva, J.A.L. and Holm, N.G. (2014) Borophosphates and silicophosphates as plausible  
749 contributors to the emergence of life. *J Coll Interface Sci*. DOI: 10.1016/j.jcis.2014.02.034.

750

751 Daffonchio, D., Borin, S., Brusa, T., Brusetti, L., van der Wielen, P.W.J.J., Bolhuis, H., et al.  
752 (2006) Stratified prokaryote network in the oxic–anoxic transition of a deep sea halocline.  
753 *Nature* **440**: 203–207.

754

755 Daniel, R.M., Finney, J.L., and Stoneham, M. (2004) The molecular basis of life: is life  
756 possible without water? A discussion meeting held at the Royal Society, London, UK, 3-4  
757 December 2003. *Philos Trans R Soc Lond B Biol Sci* **359**: 1141–1328.

758

759 Dash, J.G., Rempel, A.W., and Wettlaufer, J.S. (2006) The physics of premelted ice and its  
760 geophysical consequences. *Rev Mod Phys* **78**: 698–741.

761

762 de Goffau, M.C., van Dijk, J.M., and Harmsen, H.J.M. (2011) Microbial growth on the edge of  
763 desiccation. *Environ Microbiol* **13**: 2328–2335.

764



765 de Ronde, C.E.J., Channer, D.M.DeR., Faure, K., Bray, C.J., and Spooner, E.T.C. (1997)  
766 Fluid chemistry of Archean seafloor hydrothermal vents; implications for the composition of  
767 circa 3.2 Ga seawater. *Geochim Cosmochim Acta* **61**: 4025–4042.

768  
769 Derjaguin, B.V., and Churaev, N.V. (1986) Properties of water layers adjacent to interfaces.  
770 In: *Fluid Interfacial Phenomena*, Croxton C.A. (Ed.). John Wiley & Sons, New York, pp. 663–  
771 738.

772  
773 D’Hondt, S., Rutherford, S., and Spivack, A.J. (2002) Metabolic activity of subsurface life in  
774 deep-sea sediments. *Science* **295**: 2067–2070.

775  
776 Dickson, J.L., Head, J.W., Levy, J.S., and Marchant, D.R. (2013) Don Juan Pond, Antarctica:  
777 near-surface CaCl<sub>2</sub>-brine feeding Earth’s most saline lake and implications for Mars. *Sci Rep*  
778 **3**: 1166.

779  
780 Doroshenko, E.A., Zenova, G.M., Zvyagintsev, D.G., and Sudnitsyn, I.I. (2005) Spore  
781 germination and mycelial growth of streptomycetes at different humidity levels. *Mikrobiologiya*  
782 **74**: 690–694.

783  
784 Doroshenko, E.A., Zenova, G.M., Sudnicin, I.I., and Zvyagintsev, D.G. (2006) Influence of  
785 humidity on soil mycelial bacteria. *Vestn Mosk U Poch* 1: 45–48.

786  
787 Drewello R., and Weissmann, R. (1997) Microbially influenced corrosion of glass. *Appl*  
788 *Microbiol Biotechnol* **47**: 337–346

789  
790 Duda, V.I., Danilevich, V.N., Suzina, N.E., Shorokhova, A.P., Dmitriev, V.V., Mokhova, O.N.,  
791 et al. (2004) Changes in the fine structure of microbial cells induced by chaotropic salts.  
792 *Mikrobiologiya* **73**: 341–349.

793  
794 Duda, V.I., Danilevich, V.N., Akimov, V.N., Suzina, N.E., Dmitriev, V.V., and Shorokhova,  
795 A.P. (2005) Fluorescence microscopic study of microorganisms treated with chaotropic  
796 agents. *Mikrobiologiya* **74**: 434–439.

797  
798 Dundas, I. (1998) Was the environment for primordial life hypersaline? *Extremophiles* **2**:  
799 375–377.

800  
801 Elevi Bardavid, R., Khristo, P., and Oren, A. (2008) Interrelationships between *Dunaliella* and  
802 halophilic prokaryotes in saltern crystallizer ponds. *Extremophiles* **12**: 5–14.

803  
804 Fahlgren, C., Hagström, A., Nilsson, D., and Zweifel, U.L. (2010) Annual variations in the  
805 diversity, viability, and origin of airborne bacteria. *Appl Environ Microbiol* **76**: 3015–3025.

806  
807 Fakes, M.G., Dali, M.V., Haby, T.A., Morris, K.R., Varia, S.A., and Serajuddin, A.T.M. (2000)  
808 Moisture sorption behavior of selected bulking agents used in lyophilized products. *PDA J*  
809 *Pharm Sci Technol* **54**: 144–149.

810

- 811 Falk, M., Hartman, K.A., and Lord, R.C. (1963) Hydration of deoxyribonucleic acid. II. An  
812 infrared study. *J Am Chem Soc* **85**: 387–391.
- 813
- 814 Ferro Fontán, C., and Chirife, J. (1981) The evaluation of water activity in aqueous-solutions  
815 from freezing-point depression. *J Food Technol* **16**: 21–30.
- 816
- 817 Foucher, F., Westall, F., Brandstatter, F., Demets, R., Parnell, J., Cockell, C.S., et al. (2010)  
818 Testing the survival of microfossils in artificial martian sedimentary meteorites during entry  
819 into Earth's atmosphere: the STONE 6 experiment. *Icarus* **207**: 616–630.
- 820
- 821 Franck, S., von Bloh, W., and Bounama, C. (2007) Maximum number of habitable planets at  
822 the time of Earth's origin: new hints for panspermia and the mediocrity principle. *Int J*  
823 *Astrobiol* **6**: 153–157.
- 824
- 825 Frank, M., and Hess, E. (1941) Studies on salt fish: V. Studies on *Sporendonema epizoum*  
826 from "Dun" salt fish. *Can J Fish Aquat Sci* **5b**: 276–286.
- 827
- 828 Fries, M.D., Steele, A., and Zolensky, M. (2012) Halogen-Substituted Methane in Monahans  
829 Halite. *Meteorit Planet Sci Supplement* **75**: 5381.
- 830
- 831 Garcia-Pichel, F., and Pringault, O. (2001) Cyanobacteria track water in desert soil.  
832 *Nature* **413**: 380–381.
- 833
- 834 Garvie, L.A., Knauth, L.P., Bungartz, F., Klonowski, S., and Nash, T.H. 3<sup>rd</sup>. (2008) Life in  
835 extreme environments: survival strategy of the endolithic desert lichen *Verrucaria*  
836 *rubrocincta*. *Naturwissenschaften* **95**: 705–712.
- 837
- 838 Gramain, A., Díaz, G.C., Demergasso, C., Lowenstein, T.K., and McGenity, T.J. (2011)  
839 Achaean diversity along a subterranean salt core from the Salar Grande (Chile). *Environ*  
840 *Microbiol* **13**: 2105–2121.
- 841
- 842 Grant, W.D. (2004) Life at low water activity. *Philos Trans R Soc Lond B Biol Sci* **359**: 1249–  
843 1266.
- 844
- 845 Greenspan L. (1977) Humidity fixed points of binary saturated aqueous solutions. *J Res Nat*  
846 *Bur Stand-A Phys Chem* **81A**: 89–96.
- 847
- 848 Hallsworth, J.E. (1998) Ethanol-induced water stress in yeast. *J Ferment Bioeng* **85**: 125–  
849 137.
- 850
- 851 Hallsworth, J.E., Nomura, Y., and Iwahara, M. (1998) Ethanol-induced water stress and  
852 fungal growth. *J Ferment Bioeng* **86**: 451–456.
- 853
- 854 Hallsworth, J.E., and Nomura, Y. (1999) A simple method to determine the water activity of  
855 ethanol-containing samples. *Biotechnol Bioeng* **62**: 242–245.

856 Hallsworth, J.E., Heim, S. and Timmis, K.N. (2003a) Chaotropic solutes cause water stress  
857 in *Pseudomonas putida*. *Environ Microbiol* **5**: 1270–1280.

858

859 Hallsworth, J.E., Prior, B.A., Nomura, Y., Iwahara, M., and Timmis, K.N. (2003b) Compatible  
860 solutes protect against chaotrope (ethanol)-induced, nonosmotic water stress. *Appl Environ*  
861 *Microbiol* **69**: 7032–7034.

862

863 Hallsworth, J.E., Yakimov, M.M., Golyshin, P.N., Gillion, J.L.M., D'Auria, G., Alves, F.L., et al.  
864 (2007) Limits of life in MgCl<sub>2</sub>-containing environments: chaotropicity defines the window.  
865 *Environ Microbiol* **9**: 803–813.

866

867 Harris, R.F. (1981). The effect of water potential on microbial growth and activity. *Water*  
868 *Potential Relations in Soil Microbiology*, J.F. Parr et al., (Eds.). Soil Science Society of  
869 America, Madison, WI, pp. 23-95.

870

871 Harrison, J.P., Gheeraert, N., Tsigelnitskiy, D., and Cockell, C.S. (2013) The limits for life  
872 under multiple extremes. *Trends Microbiol* **21**: 204–212.

873

874 Harrison, J. P., Hallsworth, J. E. and C. S. Cockell (2014) Resource limitation redefines  
875 the limits for life on Earth. Under review for *Environmental Microbiology Special Issue*.

876

877 Hocking, A.D. (2003) Microbiological facts and fictions in grain storage. In *Stored Grain in*  
878 *Australia*. Wright, E.J., Webb, M.C., and Highley, E., (Eds.). Proceedings of the Australian  
879 Postharvest Technical Conference, Canberra. CSIRO: 55-58.

880

881 Hopkins, B., Elberling, B., Greenfield, L.G., Gregorich, E.G., Novis, P., O'Donnell, A.G., et al.  
882 (2005) Soil microorganisms in Antarctic dry valleys: resource supply and utilization. In *Micro-*  
883 *organisms and Earth Systems-Advances in Geomicrobiology*, Gadd, G., Semple, K., and  
884 Lappin-Scott, H. (Eds.). Cambridge University Press, Cambridge, UK, pp. 71–84.

885

886 Hu, Q., and Wang, J.S.Y. (2003) Aqueous-phase diffusion in unsaturated geologic media: a  
887 review. *Crit Rev Environ Sci Technol* **33**: 275–297.

888

889 Jaenicke, R., and Bohm, G. (1998) The stability of proteins in extreme environments. *Curr*  
890 *Opin Struct Biol* **8**: 738–748.

891

892 Javor, B.J. (1984) Growth potential of halophilic bacteria isolated from solar salt  
893 environments: carbon sources and salt requirements. *Appl Environ Microbiol* **48**: 352–360.

894

895 Jepsen, S.M., Priscu, J.C., Grimm, R.E., and Bullock, M.A. (2007) The potential for  
896 lithoautotrophic life on Mars: application to shallow interfacial water  
897 environments. *Astrobiology* **7**: 342–354.

898

899 Khemakhem, H., Elloumi, J., Moussa, M., Aleya, L., and Ayadi, H. (2010) The concept of  
900 ecological succession applied to phytoplankton over four consecutive years in five ponds  
901 featuring a salinity gradient. *Estuar Coast Shelf Sci* **88**: 33–44.

902

- 903 Kminek, G., Rummel, J. D., Cockell, C. S., Atlas, R., Barlow, N., Beaty, D., et al. (2010)  
904 Report of the COSPAR Mars special regions colloquium. *Adv Space Res* **46**: 811–829.  
905
- 906 Kminek, G., Conley, C., Allen, C.C., Bartlett, D.H., Beaty, D. W., Benning, D.G., et al. (2014)  
907 Report of the workshop for life detection on samples from Mars. *Life Sci Space Res* **2**: 1–5.  
908
- 909 Knauth, L.P., and Lowe, D.R. (2003) High Archean climatic temperature inferred from  
910 oxygen isotope geochemistry of cherts in the 3.5 Ga Swaziland Supergroup, South Africa.  
911 *Geol Soc Am Bull* **115**: 566–580.
- 912 Kreuzer-Martin, H.W., Ehrlinger, J.R., and Hegg, E.L. (2005) Oxygen isotopes indicate most  
913 intracellular water in log-phase *Escherichia coli* is derived from metabolism. *Proc Natl Acad*  
914 *Sci* **102**: 17337–17341.
- 915 Kreuzer-Martin, H.W., Lott, M.J., Ehrlinger, J.R., and Hegg, E.L. (2006) Metabolic  
916 processes account for the majority of the intracellular water in log-phase *Escherichia coli*  
917 cells as revealed by hydrogen isotopes. *Biochemistry* **45**: 13622–13630.
- 918 Kurkal, V., Daniel, R.M., Finney, J.L., Tehei, M., Dunn, R.V., and Smith, J.C. (2005) Enzyme  
919 activity and flexibility at very low hydration. *Biophys J* **89**: 1282–1287.
- 920 Küppers, M., O'Rourke, L., Bockelée-Morvan, D., Zakharov, V., Lee, S., von Allmen, P., et  
921 al. (2014) Localized sources of water vapour on the dwarf planet (1) Ceres. *Nature* **505**:  
922 525–527.  
923
- 924 Labuza, T.P., Acott, K., Tatini, S.R., Lee, R.Y., Flink, J., and McCall, W. (1976) Water activity  
925 determination - collaborative study of different methods. *J Food Sci* **41**: 910–917.  
926
- 927 Lange, O.L., Kilian, E., and Ziegler, H. (1986) Water vapour uptake and photosynthesis of  
928 lichens: performance differences in species with green and blue-green algae as  
929 phycobionts. *Oecologia* **71**: 104–110.  
930
- 931 Lange, O.L., Meyer, A., Zellner, H., and Heber, U. (1994) Photosynthesis and water relations  
932 of lichen soil crusts: field measurements in the coastal fog zone of the Namib Desert. *Funct*  
933 *Ecol* **8**: 253–264.  
934
- 935 Lange, O.L., Allan Greene, T.G., Melzer, B., Meyer, A., and Zellner, H. (2006) Water  
936 relations and CO<sub>2</sub> exchange of the terrestrial lichen *Teloschistes capensis* in the Namib fog  
937 desert: measurements during two seasons in the field and under controlled conditions. *Flora*  
938 **201**: 268–280.  
939
- 940 Lievens, B., Hallsworth J.E., Belgacem, Z.B., Pozo, M.I., Stevenson, A., Willems, K.A., et al.  
941 (2014) Microbiology of sugar-rich environments: diversity, ecology, and system constraints.  
942 *Environ Microbiol*. DOI: 10.1111/1462-2920.12570  
943

944 Lomstein, B.A., Langerhuus, A.T., D'Hondt, S., Jorgensen, B.B., and Spivack, A.J (2012)  
945 Endospore abundance, microbial growth and necromass turnover in deep sub-seafloor  
946 sediment. *Nature* **484**: 101–104.

947

948 Longo, L.M., Lee, J., and Blaber, M. (2013) Simplified protein design biased for prebiotic  
949 amino acids yields a foldable, halophilic protein. *Proc Natl Acad Sci USA* **110**: 2135–2139.

950

951 Longo, L.M., and Blaber, M. (2014) Prebiotic protein design supports a halophile origin of  
952 foldable proteins. *Front Microbiol* **4**: 418.

953

954 Lopez, M., Kurkal-Siebert, V., Dunn, R.V., Tehei, M., Finney, J.L., Smith, J.C. et al. Activity  
955 and dynamics of an enzyme, pig liver esterase, in near-anhydrous conditions. *Biophys*  
956 *J* **99**: L62–64.

957

958 Lowe, D.R., and Fisher-Worrell, G., (1999) Sedimentology, mineralogy, and implications of  
959 silicified evaporites in the Kromberg Formation, Barberton greenstone belt, South Africa. In  
960 *Geologic evolution of the Barberton greenstone belt, South Africa*, Lowe, D.R., and Byerly,  
961 G.R., (Eds.). *Geol Soc Am Spec Pap* **329**: 167–188.

962

963 Lowenstein, T.K., Schubert, B.A., and Timofeeff, M.N. (2011) Microbial communities in fluid  
964 inclusions and long-term survival in halite. *GSA Today* **21**: 4–9.

965 Marcano, V., Benitez, P., and Palacios-Prü, E. (2002) Growth of a lower eukaryote in non-  
966 aromatic hydrocarbon media C<sub>12</sub> and its exobiological significance. *Planet Space Sci* **50**:  
967 693–709.

968

969 Manners, J.G., and Hossain, S.M.M. (1963) Effects of temperature and humidity on conidial  
970 germination in *Erysiphe graminis*. *T Brit Mycol Soc* **46**: 225–234.

971

972 Marion, G.M. (1997) A theoretical evaluation of mineral stability in Don Juan Pond, Wright  
973 Valley, Victoria Land. *Antarct Sci* **9**: 92–99.

974

975 Marion, G.M., Fritsen, C.H., Eicken, H., and Payne, M.C. (2003) The search for life on  
976 Europa: limiting environmental factors, potential habitats, and Earth analogues. *Astrobiology*  
977 **3**: 785–811.

978

979 Marion, G.M., Kargen, J.S., Catling, D.C., and Jakubowski, S.D. (2005) Effects of pressure  
980 on aqueous chemical equilibria at subzero temperatures with applications to Europa.  
981 *Geochim Cosmochim Acta* **69**: 259–274.

982

983 Marion, G.M., and Kargel, J.S. (2008) *Cold Aqueous Planetary Geochemistry with*  
984 *FREZCHEM: From Modeling to the Search for Life at the Limits*. Springer-Verlag, Berlin,  
985 Germany.

986

987 Martínez, G.M., and Renno, N.O. (2013) Water and brines on Mars: current evidence and  
988 implications for MSL. *Space Sci Rev* **175**: 29–51.

989

990 Matson D.L, Castillo, J.C., and Lunine, J. (2007) Enceladus' plume: compositional evidence  
991 for a hot interior. *Icarus* **187**: 569–573.  
992  
993 Mauer, L.J., and Taylor, L.S. (2010) Water-Solids Interactions: deliquescence. *Annu Rev*  
994 *Food Sci Tech* **1**: 41–63.  
995  
996 McCammick, E.M., Gomase, V.S., Timson, D.J., McGenity, T.J., and Hallsworth, J.E. (2009)  
997 Water-hydrophobic compound interactions with the microbial cell. In *Handbook of*  
998 *Hydrocarbon and Lipid Microbiology – Hydrocarbons, Oils and Lipids: Diversity, Properties*  
999 *and Formation*, Timmis, K.N. (Ed.). Springer, New York, USA, Vol. **2**, pp. 1451– 1466.  
1000  
1001 McEwen, A.S., Dundas, C.M., Mattson, S.S., Toigo, A.D., Ojha, L., Wray, J.J., et al.. (2014)  
1002 Recurring slope lineae in equatorial regions of Mars. *Nature Geoscience* **7**: 53–58.  
1003  
1004 McGenity, T.J., Gemmell, R.T., Grant, W.D., and Stan-Lotter, H. (2000) Origins of halophilic  
1005 microorganisms in ancient salt deposits. *Environ Microbiol* **2**: 243–250.  
1006  
1007 Miller, E.J., and Chibnall, A. C. (1932) The proteins of grasses. *Biochem J* **26**: 392–402.  
1008  
1009 Möhlmann, D.T.F. (2004) Water in the upper Martian surface at mid- and low-latitudes:  
1010 presence, state, and consequences. *Icarus* **168**: 318–323.  
1011  
1012 Möhlmann, D. (2005) Adsorption water-related potential chemical and biological processes  
1013 in the upper Martian surface. *Astrobiology* **5**: 770–777.  
1014  
1015 Möhlmann, D.T.F. (2008) The influence of van der Waals forces on the state of water in the  
1016 shallow subsurface of Mars. *Icarus* **195**: 131–139.  
1017  
1018 Möhlmann, D. (2009) Are nanometric films of liquid undercooled interfacial water bio-  
1019 relevant? *Cryobiology* **58**: 256–261.  
1020  
1021 Möhlmann, D. (2011) Three types of liquid water in icy surfaces of celestial bodies. *Planet*  
1022 *Space Sci* **59**: 1082–1086.  
1023  
1024 Möhlmann, D. (2012) Widen the Belt of Habitability! *Orig Life Evol Biosph* **42**: 93–100.  
1025  
1026 Mormile, M.R., Hong, B.Y., and Benison, K.C. (2009) Molecular analysis of the microbial  
1027 communities of Mars analog lakes in Western Australia. *Astrobiology* **9**: 919–930.  
1028  
1029 Morris, C.E., Conen, F., Alex Huffman, J. Phillips, V., Pöschl, U., and Sands, D.C. (2014)  
1030 Bioprecipitation: a feedback cycle linking Earth history, ecosystem dynamics and land use  
1031 through biological ice nucleators in the atmosphere. *Glob Change Biol* **20**: 341–351.  
1032  
1033 Moyano, F.E., Vasilyeva, N., Bouckaert, L., Cook, F., Craine, J., Curiel Yuste, J., et al.  
1034 (2012) The moisture response of soil heterotrophic respiration: interaction with soil  
1035 properties. *Biogeosciences* **9**: 1173–1182.  
1036

1037 Moyano, F.E., Manzoni, S., and Chenu, C. (2013) Responses of soil heterotrophic  
1038 respiration to moisture availability: An exploration of processes and models. *Soil Biol*  
1039 *Biochem* **59**: 72–85.  
1040  
1041 Muñoz-Iglesias, V., Bonales, L.J., and Prieto-Ballesteros, O. (2013) pH and salinity evolution  
1042 of Eurpa’s brines: raman spectroscopy study of fractional precipitation at 1 and 300 bar.  
1043 *Astrobiology* **13**: 693–702.  
1044  
1045 Mykytczuk, N.C.S., Foote, S.J., Omelon, C.R., Southam, G., Greer, C.W., and Whyte, L.G.  
1046 (2013). Bacterial growth at -15C; molecular insights from the permafrost bacterium  
1047 *Planococcus halocryophilus* Or1. *ISME J* **7**: 1211–1226.  
1048  
1049 Nagel, F.J.J.I., Tramper, J., Bakker, M.S.N., and Rinzema, A. (2001) Model for on-line  
1050 moisture-content control during solid-state fermentation. *Biotechnol Bioeng* **76**: 291–302.  
1051  
1052 Nimmo, F., Spencer, J.R., Pappalardo, R.T., and Mullen, M.E. (2007) Shear heating as the  
1053 origin of the plumes and heat flux on Enceladus. *Nature* **447**: 289–291.  
1054  
1055 Oren, A. (1988) The microbial ecology of the Dead Sea. In *Advances in microbial ecology*,  
1056 Marshall, K.C. (Ed.). Plenum Press, New York, pp. 193–229.  
1057  
1058 Oren, A. (1993) The Dead Sea—alive again. *Experientia* **49**: 518–522.  
1059  
1060 Oren, A. (1999) Bioenergetic aspects of halophilism. *Microbiol Mol Biol Rev* **63**: 334–348.  
1061  
1062 Oren, A. (2002) Diversity of halophilic microorganisms: environments, phylogeny,  
1063 physiology, and applications. *J Ind Microbiol Biotechnol* **28**: 56–63.  
1064  
1065 Oren, A. (2010) The dying Dead Sea: the microbiology of an increasingly extreme  
1066 environment. *Lakes Reser: Res Manag* **15**: 215–222.  
1067  
1068 Oren, A. (2011) Thermodynamic limits to microbial life at high salt concentrations. *Environ*  
1069 *Microbiol* **13**: 1908–1923.  
1070  
1071 Oren, A. (2013) Life in magnesium- and calcium-rich hypersaline environments: salt stress  
1072 by chaotropic ions. In *Polyextremophiles: Life Under Multiple Forms of Stress. Cellular*  
1073 *Origin, Life in Extreme Habitats and Astrobiology*. Seckbach, J., Oren, A., and Stan-Lotter,  
1074 H. (Eds.) Springer Science and Business Media, Dordrecht, Netherlands.  
1075  
1076 Oren, A. and Hallsworth, J.E. (2014) Microbial weeds in hypersaline habitats: the enigma of  
1077 the weed-like *Haloferax mediterranei*. Revised for *FEMS Microbiol Lett*.  
1078  
1079 Oriol, E., Raimbault, M., Roussos, S., and Viniegra-Gonzales, G. (1988) Water and water  
1080 activity in the solid state fermentation of cassava starch by *Aspergillus niger*. *Appl Microbiol*  
1081 *Biotechnol* **27**: 498–503.  
1082

- 1083 Papendick, R.I. and Campbell, G.S. (1981) Theory and measurement of water potential.  
1084 In *Water Potential Relationships in Soil Microbiology*, Parr, J.F., et al. (Eds.). Soil Science  
1085 Society of America Publications, Madison, WI, pp. 1–22.
- 1086
- 1087 Parkes, R.J., Cragg, B.A., and Wellsbury, P. (2000) Recent studies on bacterial populations  
1088 and processes in subseafloor sediments: a review. *Hydrogeol J* **8**:11–28.
- 1089
- 1090 Parkinson, C.D., Liang, M.-C., Yung, Y.L., and Kirshcivnk, J.L. (2008) Habitability of  
1091 Enceladus: planetary conditions for life. *Orig Life Evol Biosph* **38**: 355–369.
- 1092
- 1093 Pasanen, A.L., Pasanen, P., Jantunen, M.J., and Kalliokoski, P. (1991) Significance of air  
1094 humidity and air velocity for fungal spore release into the air. *Atmos Environ* **25**: 459–462.
- 1095
- 1096 Pavlov, A.K., Shelegedina, V.N., Vdovina, M.A., and Pavlov, A.A. (2010) Growth of  
1097 microorganisms in Martian-like shallow subsurface conditions: laboratory modeling. *Int J*  
1098 *Astrobiol* **9**: 51–58.
- 1099
- 1100 Pearson, R.T., and Derbyshire, W. (1974) NMR studies of water adsorbed on a number of  
1101 silica surfaces. *J Colloid Interf Sci* **46**: 232–248.
- 1102
- 1103 Peterson, C.A., and Cowling, E.B. (1973) Influence of various initial moisture contents on  
1104 decay of sitka spruce and sweetgum sapwood by *Polyporus versicolor* in the soil-block test.  
1105 *Phytopathology* **63**: 235–237.
- 1106
- 1107 Pintado, A., and Sancho, L.G. (2002) Ecological significance of net photosynthesis activation  
1108 by water vapour uptake in *Ramalina capitata* from rain-protected habitats in central  
1109 Spain. *Lichenologist* **34**: 403–413.
- 1110
- 1111 Pitt, J.I., and Christian, J.H.B. (1968) Water relations of xerophilic fungi isolated from prunes.  
1112 *Appl Environ Microbiol* **16**: 1853–1858.
- 1113
- 1114 Pitt, J.I. (1975) Xerophilic fungi and the spoilage of foods of plant origin. In *Water Relations*  
1115 *of Foods*, Duckworth, R.B. (Ed.). London, United Kingdom: Academic Press, pp. 273–307.
- 1116
- 1117 Postberg, F., Kempf, S., Schmidt, J., Brilliantov, N., Beinsen, A., Abel, et al. (2009) Sodium  
1118 salts in E-ring ice grains from an ocean below the surface of Enceladus. *Nature* **459**: 1098–  
1119 1101.
- 1120
- 1121 Postberg, F., Schmidt, J. hillier, J., Kempf, S., and Srama, R. (2011) A salt-water reservoir as  
1122 the source compositionally stratified plume on Enceladus. *Nature* **474**: 620–622.
- 1123
- 1124 Potts, M. (1994) Desiccation tolerance of prokaryotes. *Microbiol Rev* **58**: 755–805.
- 1125
- 1126 Qvit-Raz, N., Jurkevitch, E., and Belkin, S. (2008) Drop-size soda lakes: transient microbial  
1127 habitats on a salt-secreting desert tree. *Genetics* **178**: 1615–1622.
- 1128
- 1129 Rajeev, L., Da Rocha, U.N., Klitgord, N., Luning, E.G., Fortney, J., Axen, S.D., et



1130 al. (2013). Dynamic cyanobacterial response to hydration and dehydration in a desert  
1131 biological soil crust. *ISME J* **7**: 2178–2191.

1132

1133 Rampino, M.R., and Caldeira, K. (1994) The goldilocks problem: climate evolution and long-  
1134 term habitability of terrestrial planets. *Annu Rev Astron Astrophys* **32**: 83-114.

1135

1136 Raulin, F. (2008) Astrobiology and habitability of Titan. *Space Sci Rev* **135**: 37–48.

1137

1138 Raviv, U., Laurat, P., and Klein, J. (2001) Fluidity of water confined to subnanometre films.  
1139 *Nature* **413**: 51–54.

1140

1141 Rivkina, E., Friedmann, E., McKay, C., and Gilichinsky, D. (2000) Metabolic activity of  
1142 permafrost bacteria below the freezing point. *Appl Environ Microbiol* **66**: 3230–3233.

1143

1144 Roa, V., and Tapia, M.S. (1998) Estimating water activity in systems containing multiples  
1145 solutes based on solute properties. *J Food Sci* **63**: 559–564.

1146

1147 Robinson, J.L., Pyzyna, B., Atrasz, R.G., Henderson, C.A., Morrill, K.L., Burd, A.M., et al.  
1148 (2005) Growth kinetics of extremely halophilic Archaea (family Halobacteriaceae) as  
1149 revealed by Arrhenius plots. *J Bacteriol* **187**: 923–929.

1150

1151 Rothschild, L. J. and Mancinelli, R. L. (2001) Life in Extreme Environments. *Nature* **409**:  
1152 1092–1101.

1153

1154 Rubin, A.E, Zolensky, M.E., and Bodnar, R.J. (2002) The halite-bearing Zag and Monahans  
1155 (1998) meteorite breccias: Shock metamorphism, thermal metamorphism and aqueous  
1156 alteration on the H-chondrite parent body. *Meteorit Planet Sci* **37**: 125–141.

1157

1158 Samarkin, V.A., Madigan, M.T., Bowles, M.W., Casciotti, K.L., Priscu, J.C., McKay, C.P., et  
1159 el. (2010) Abiotic nitrous oxide emissions from the hypersaline Don Juan Pond in Antarctica.  
1160 *Nature* **3**: 341–344.

1161

1162 Samson, R.A., van der Lustgraaf, B. (1978) *Aspergillus penicilloides* and *Eurotium*  
1163 *halophilicum* in association with house-dust mites. *Mycopathologia* **64**: 13–16.

1164

1165 Sattler, B., Puxbaum, H., and Psenner, R., (2001) Bacterial growth in supercooled cloud  
1166 droplets. *Geophys Res Lett* **28**: 239-242.

1167

1168 Schubert, B.A., Lowenstein, T.K., Timofeeff, M.N., and Parker, M.A. (2009a) How do  
1169 prokaryotes survive in fluid inclusions in halite for 30,000 years? *Geology* **37**: 1059–1062.

1170

1171 Schubert, B.A., Lowenstein, T.K., and Timofeeff, M.N. (2009b), Microscopic identification of  
1172 prokaryotes in modern and ancient halite, Saline Valley and Death Valley, California:  
1173 *Astrobiology* **9**: 467–482.

1174

1175 Sereno, A.M., Hubinger, M.D., Comesaña, J.F., and Correa, A. (2001) Prediction of water  
1176 activity of osmotic solutions. *J Food Eng* **49**: 103–114.

1177  
1178 Shomari, S.H., and Kennedy, R. (1999) Survival of *Oidium anacardii* on cashew  
1179 (*Anacardium occidentale*) in southern Tanzania. *Plant Path* **48**: 505–513.  
1180  
1181 Siegel, B.Z., McMurty, G., Siegel, S.M., Chen, J., and LaRock, P. (1979) Life in the calcium  
1182 chloride environment of Don Juan Pond, Antarctica. *Nature* **280**: 828–829.  
1183  
1184 Siegel, B.Z., Siegel, S.M., Chen, J., and LaRock, P. (1983) An extraterrestrial habitat on  
1185 Earth: the algal mat of Don Juan Pond. *Adv Space Res* **3**: 39–42  
1186  
1187 Snow, D. (1949) The germination of mould spores at controlled humidities. *Ann Appl Biol* **36**:  
1188 1–13.  
1189  
1190 Sohl, F., Choukroun, M., Kargel, J., Kimura, J., Pappalardo, R., Vance, S., et al.  
1191 (2010) Subsurface water oceans on icy satellites: Chemical composition and exchange  
1192 processes, *Space Sci Rev* **153**: 485–510.  
1193  
1194 Stan-Lotter, H., Radax, C., Gruber, C., McGenity, T.J., Legat, A., Wanner, G., et al. (in  
1195 press) The distribution of viable micro-organisms in Permo-Triassic rock salt. In 8<sup>th</sup> World  
1196 Salt Symposium, 2000, Geertman, R.M. (Ed.). Elsevier Science, Amsterdam.  
1197  
1198 Stevenson, A., and Hallsworth, J.E. (2014) Water and temperature relations of soil  
1199 Actinobacteria. Revised for *Environ Microbiol Reports*.  
1200  
1201 Strughold, H. (1953) *The Green and the Red Planet: a Physiological Study of the Possibility*  
1202 *of Life on Mars*. University of New Mexico Press, Albuquerque.  
1203  
1204 Sutton, J.C., and Hildebrand, P.D. (1985) Environmental water in relation to *Peronospora*  
1205 *destructor* and related pathogens. *Can J Plant Pathol* **7**: 323–330.  
1206  
1207 Takai, K., Nakamura, K., Toki., T., Urumu, T., Miyazaki, M., Miyazaki, J., et al. (2008) Cell  
1208 proliferation at 122°C and isotopically heavy CH<sub>4</sub> production by a hyperthermophilic  
1209 methanogen under high-pressure cultivation. *Proc Natl Acad Sci* **105**: 10949–10954.  
1210  
1211 Teixeira, S., Olu K., Decker, C., Cunha, R.L, Fuchs, S., Hourdez, S., et al. (2013) High  
1212 connectivity across the fragmented chemosynthetic ecosystems of the deep Atlantic  
1213 Equatorial Belt: efficient dispersal mechanisms or questionable endemism? *Mol Ecol* **22**:  
1214 4663–4680.  
1215  
1216 Thomson, W. (1871) Presidential Address to the British Association for the Advancement of  
1217 Science. *Nature* **4**: 262.  
1218  
1219 Toner, J.D., Catling, D.C., and Light, B. (2014) Soluble salts at the Phoenix Lander site,  
1220 Mars: a reanalysis of the wet chemistry laboratory data. *Geochim Cosmochim Ac* **136**: 142–  
1221 168.  
1222

1223 Tosca, N.J., Knoll, A.H., and McLennan, S.M. (2008) Water activity and the challenge for life  
1224 on early Mars. *Science* **320**: 1204–1207.

1225

1226 Trevors, J.T. and Pollack, G.H. (2005) Hypothesis: the origin of life in a hydrogel  
1227 environment. *Prog Biophys Mol Biol* **89**: 1–8.

1228

1229 Troller, J.A., and Christian, J.H.B. (1978) *Water Activity and Food*. Academic Press, New  
1230 York, USA.

1231

1232 Valentine, D.L. (2013) Microbiology: intraterrestrial lifestyles. *Nature* **496**: 176–177.

1233

1234 van der Wielen, P.W.J.J., Bolhuis, H., Borin, S., Daffonchio, D., Corselli, C., Giuliano, L., et  
1235 al. (2005) The enigma of prokaryotic life in deep hypersaline anoxic basins. *Science* **307**:  
1236 121–123.

1237

1238 von Bloh, W., Cuntz, M., Franck, S., and Bounama, C. (2011) Habitability of the Goldilocks  
1239 Planet Gliese 581g: results from Geodynamic Models. *Astron Astrophys* **528**: A133.

1240

1241 Waite, J.H., Combi, M.R., Ip, W.-H., Cravens, T.E., McNutt, R.L., Kasprzak, W., et al. (2006)  
1242 Cassini ion and neutral mass spectrometer: Enceladus plume composition and structure.  
1243 *Science* **311**: 1419–1422.

1244

1245 Waldham, D.C., and Halvorson, H.O. (1954) Studies on the relationship between equilibrium  
1246 vapor pressure and moisture content of bacterial endospores. *Appl Microbiol* **2**: 333–338

1247

1248 Warren, J.K. (2010) Evaporites through time: tectonic, climatic and eustatic controls in  
1249 marine and nonmarine deposits. *Earth Sci Rev* **98**: 217–268.

1250

1251 Watanabe, K., and Mizoguchi, M. (2002) Amount of unfrozen water in frozen porous media  
1252 saturated with solution. *Cold Regions Sci Technol* **34**: 103–110.

1253

1254 Westall, F., de Wit, M.J, Dann, J., van der Gaast, S., de Ronde C.E.J, and Gerneke, D.  
1255 (2001) Early Archean fossil bacteria and biofilms in hydrothermally-influenced sediments of  
1256 the Barberton greenstone belt, South Africa. *Precambrian Res* **106**: 93–116.

1257

1258 Westall, F., de Ronde, C.E.J., Southam, G., Grassineau, N., Colas, M., Cockell, C.S., et al.  
1259 (2006) Implications of a 3.472–3.333 Gyr-old subaerial microbial mat from the Barberton  
1260 greenstone belt, South Africa for the UV environmental conditions on the early Earth. *Phil  
1261 Trans R Soc* **361**: 1857–1875.

1262

1263 Westall, F., Cavalazzi, B., Lemelle, L., Marrocchi, Y., Rouzaud, J.-N., Simionovici, A., et  
1264 al. (2011) Implications of in situ calcification for photosynthesis in a ~ 3.3 Ga-old microbial  
1265 biofilm from the Barberton greenstone belt, South Africa. *Earth Planet Sci Lett* **310**, 468–479.

1266

1267 Westall, F. (2012) Early Earth. In *Astrobiology*, Lunine, J., et al. (Eds.). Cambridge University  
1268 Press, pp. 89–114.

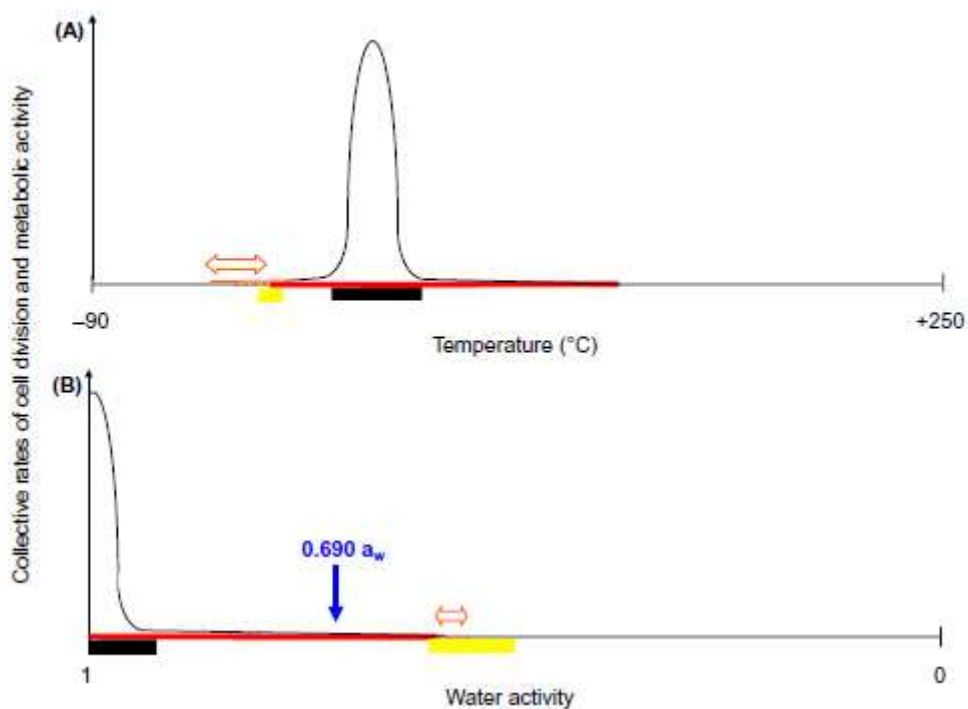
1269

1270 Westall, F., Loiseau, D., Foucher, F., Bost, N., Bertrand, M., Vago, J., et al. (2013)  
1271 Habitability on Mars from a Microbial point of view. *Astrobiology* **13**: 887–897.  
1272  
1273 Wierzchos, J., Cámara, B., de los Rios, A., Davila, A.F., Sánchez Almazo, I.M., Arteida, O.,  
1274 et al. (2011) Microbial colonization of Ca-sulfate crusts in the hyperarid core of the Atacama  
1275 Desert: implications for the search for life on Mars. *Geobiology* **9**: 44–60.  
1276  
1277 Williams, J.P., and Hallsworth, J.E. (2009) Limits of life in hostile environments; no limits to  
1278 biosphere function? *Environ Microbiol* **11**: 3292–3308.  
1279  
1280 Williams, A.J., Buck, B.J., and Beyene, M.A. (2012) Biological soil crusts in the Mojave  
1281 Desert, USA: micromorphology and pedogenesis. *Soil Sci Am J* **76**: 1685–1695.  
1282  
1283 Winston, P.W. and Bates, P.S. (1960) Saturated salt solutions for the control of humidity in  
1284 biological research. *Ecology* **41**: 232–237.  
1285  
1286 Wolfe, J., Bryant, G., and Koster, K.L. (2002) What is “unfreezable water”, how unfreezable  
1287 is it and how much is there? *CryoLetters* **23**: 157–166.  
1288  
1289 Womack, A.M., Bohannan, B.J.M., and Green, J.L. (2010) Biodiversity and biogeography of  
1290 the atmosphere. *Phil Trans R Soc B* **365**: 3645–3653.  
1291  
1292 Womack, A.M., Artaxo, P.E., Ishida, F., Jardine, K.J., Saleska, S.R., Wiedemann, K.T., et al.  
1293 (2012) Microbial community composition and gene expression in the atmosphere over the  
1294 Brazilian Amazon. ASM, 112th General Meeting American Society for Microbiology, San  
1295 Francisco, California.  
1296  
1297 Wyatt, T.T., Golovina, E.A., van Leeuwen, M.R., Hallsworth, J. E., Wösten, H.A.B. and  
1298 Dijksterhuis, J. (2014) Decreases in bulk water and mannitol and accumulation of trehalose  
1299 and trehalose-based oligosaccharides define a two-stage maturation process towards  
1300 extreme stress-resistance in ascospores of *Neosartorya fischeri* (*Aspergillus fischeri*).  
1301 *Environ Microbiol* In press. DOI: 10.1111/1462-2920.12557.  
1302  
1303 Wynn-Williams, D.D. (1996) Antarctic microbial diversity: the basis of polar ecosystem  
1304 processes. *Biodivers Conserv* **5**: 1271–1293.  
1305  
1306 Yakimov, M.M., Lo Cono, V., La Spada, G., Bortoluzzi, G., Messina, E., Smedile, F., et al.  
1307 (2014) Microbial community of seawater-brine interface of the deep-sea brine Lake Kryos as  
1308 revealed by recovery of mRNA are active below the chaotropicity limit of life. Revised for  
1309 *Environ Microbiol*  
1310  
1311 Yarwood (1950) Water content of fungus spores. *Am J Bot* **37**: 636–639.  
1312  
1313 Yu, X., Schmidt, A.R., and Schmidt, S.J. (2009) Uncertainty analysis of hygrometer-obtained  
1314 water activity measurements of saturated salt slurries and food materials. *Food Chem* **115**:  
1315 214–226.  
1316

- 1317 Zakharova, K., Tesei, D., Marzban, G., Dijksterhuis, J., Wyatt, T., and Sterflinger, K. (2013)  
1318 Microcolonial fungi on rocks: A life in constant drought? *Mycopathologia* **175**: 537–547.  
1319
- 1320 Zolensky, M.E. Bodnar, R.J., Gibsion, E.K., Nyquist, L.E., Reese, Y., Shih, C.–Y., et al.  
1321 (1998) Asteroidal water within fluid inclusion-bearing halite in an H5 chondrite, Monahans  
1322 (1998). *Science* **285**: 1377–1379.  
1323
- 1324 Zvyagintsev, D.G., Zenova, G.M., Sudnitsyn, I.I., Gracheva, T.A., Napol'skaya, K.R., and  
1325 Belousova, M.A. (2009) Dynamics of spore germination and mycelial growth of  
1326 streptomycetes under low humidity conditions. *Microbiology* **78**: 440–444.  
1327
- 1328 Zvyagintsev, D.G., Zenova, G.M., Sudnitsyn, I.I., Gracheva, T.A., Lapygina, E.E.,  
1329 Napol'skaya, K.R., et al. (2012) Development of actinomycetes in brown semidesert soil  
1330 under low water pressure. *Eurasian Soil Sci* **45**: 717–725.  
1331

1332 **Figure legends**

1333

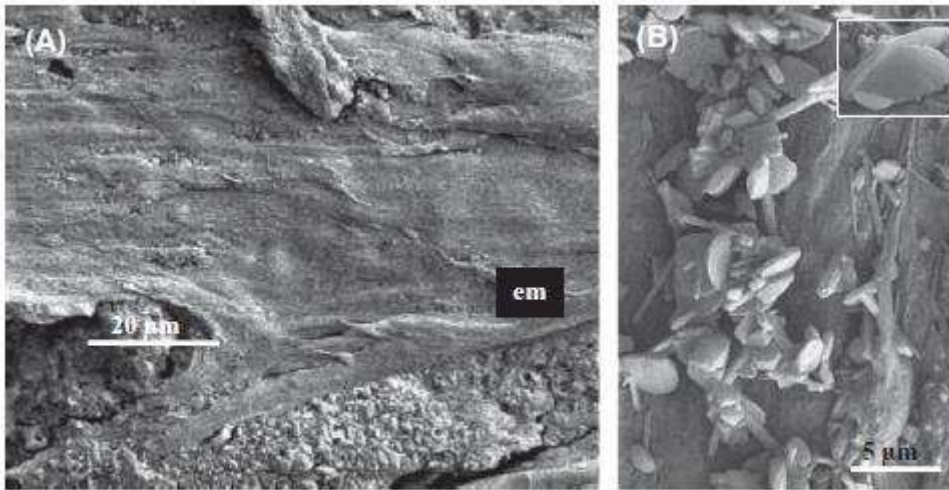


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1335 **Figure 1.** Diagrammatic representation of collective biological activity (compound rates cell  
 1336 division and metabolic activity) for microbes on Earth in relation to prevailing environmental  
 1337 (a) temperatures and (b) water activities. Red bars indicate the known range for cell division  
 1338 of microbes (-18 to +122°C, and 1 to 0.605 a<sub>w</sub>), and orange dotted lines indicate for (a) the  
 1339 established limit for cellular metabolism (33°C), and (b) the known limit for physiological  
 1340 function of DNA (down to 0.530 a<sub>w</sub>). Black bars indicate the range in which the overwhelming  
 1341 majority of microbial activity takes place, and curves represent collective biotic activity of  
 1342 microbes on Earth. Yellow bars indicate safety margins used for the designation of ‘Special  
 1343 Regions’ on Mars (down to -25°C and 0.500 a<sub>w</sub>; Kminek et al., 2010) in relation to  
 1344 international policy on planetary protection. Horizontal orange arrows indicate zones in which  
 1345 cell division may take place over extended timescales (10s to 1000s years) though there is a  
 1346 paucity of data on this topic; this zone for temperature extends considerably below -33°C  
 1347 because of the possibility that chaotropic substances may enhance flexibility of  
 1348 macromolecular systems and thereby reduce the temperature minima for microbial activity  
 1349 by a further 10 to 20°C (Chin et al., 2010).

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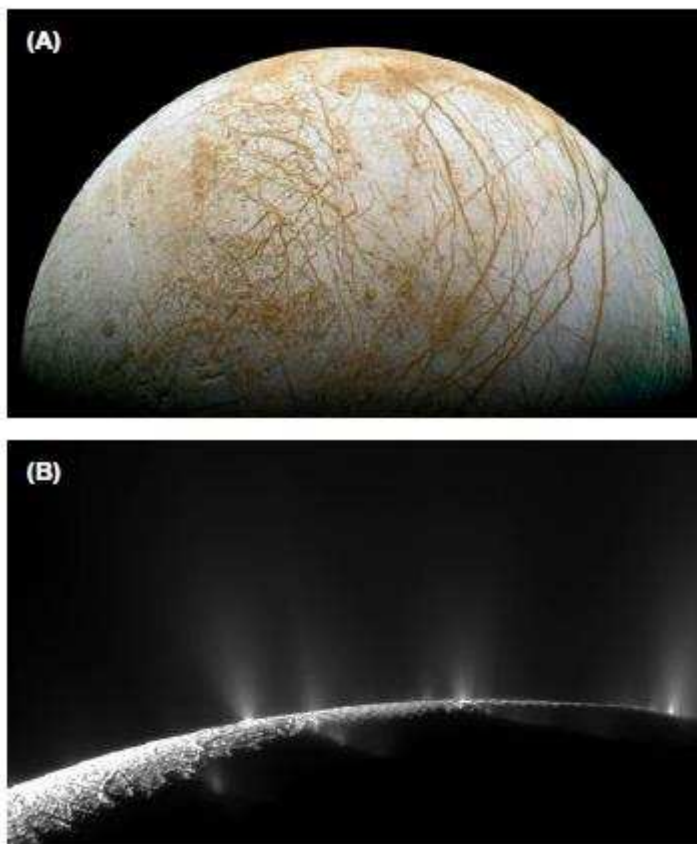
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1353 **Figure 2.** Early Archaean microbes and evaporites; example from the 3.33 billion-year-old  
 1354 Josefsdal Chert, Barberton Greenstone Belt: (a) layer of evaporite minerals interbedded with  
 1355 layers of a photosynthetic microbial biofilm, (em) evaporite minerals, and (b) details of the  
 1356 diversity of minerals encrusted on the surface of the biofilm. They include here  
 1357 pseudomorphs (silica replaced) of acicular aragonite and losenge-shaped gypsum.  
 1358 Reproduced from Westall et al. (2006) with permission from The Royal Society Press.

1359



1360

1361 **Figure 3.** Views of two planetary moons which are known to have an abundance of water,  
 1362 some of which may be present as subsurface oceans: (a) the icy surface of Europa, and (b)

1363 jets composed of water vapour, ice particles and organic compounds released from beneath  
1364 the surface of Enceladus. Courtesy NASA/JPL-Caltech.

1365

1366 **Supplemental information**

1367

1368 **Movie S1.** Deliquescence of NaCl crystals on the surface of a pine needle (*Pinus sylvestris*)  
1369 as humidity rises from approximately 65 to 80% equilibrium relative humidity. The  
1370 deliquescence point of NaCl is approximately 75.0% equilibrium relative humidity at 2°C. An  
1371 epistomatal chamber is visible but the guard cells are located below this section and cannot,  
1372 therefore, be seen. The recording was made using an environmental scanning electron  
1373 microscope and equilibrium relative humidity was controlled experimentally within a chamber  
1374 (see Burkhardt and Hunsche, 2013).