Water-Activity Limit for Life As We Know It. J. E. Hallsworth[1]. [1]Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Northern Ireland, UK. E-mail: johnhallsworth@yahoo.com

Introduction: Life as we know it requires sufficient water to enable cellular hydration and function. This is dependent on the effective water concentration; the thermodynamic parameter 'water activity' which is based on Raoult's Law allocated a scale from 0 to 1 (and is dependent on temperature and pressure). Each individual cell, enzyme, biological process, etc has its own water-activity window for functionality and its own water-activity optimum. These values can be plastic to some degree because they depend on factors such as physiological condition and temperature. However, water activity imposes potent limits on active life even under conditions that are otherwise optimal.

Some terrestrial microbes – termed xerophiles – grow optimally at reduced water activity, and some of these – termed halophiles – are adapted to life in brines. Xerophiles have been described as those microbes able to grow below a water activity of 0.850 (under at least two sets of environmental conditions) and must also grow optimally below 0.950 [1]. Remarkably, species such as the ascomycete *Aspergillus penicillioides* are able to flourish at low water-activity in brines, at low relative humidity, <u>or</u> at high concentrations of other solutes including sugars. For such organisms, high-glycerol milieux are the most-permissive for growth and metabolism at low water-activity [2,3], whereas brines are more challenging [4].

Terrestrial brines are typically complex in their chemical composition and can fluctuate in their temperature, concentration, composition, water activity, pH, etc on timescales from seconds or minutes up to 1000s years or more. Furthermore, brines can exhibit numerous parameters that can cause cellular stress: low water activity, high osmolarity, chaotropicity, extremes of temperature or pH, exposure to ultra-violet radiation, lack of nutrients, etc [5]. Indeed, individual salts can impose multiple parameters that cause concomitant mechanistically diverse stresses to the cell [6]. Nevertheless, even within this complexity water activity remains a major determinant for terrestrial biology; and for the limitations of terrestrial biology.

Water Activity Limits for Active Life on Earth: Water-activity values, expressed as a fraction of 1, can seem small and insignificant yet cells are sensitive to changes of about ±0.001 [7]. The past 100 years or so of culture-based growth studies has not yielded any verifiable evidence of microbial division below 0.585 water activity (= 58.5% relative humidity) [2,3,8-10]. Furthermore, differentiation and cell division have only been observed at 0.585 water activity for one species, *A. penicillioides*, and only at 24°C (297 K) [3]. According to reliable studies which yield empirical data for microbial proliferation, the lowest water activity at which growth has been observed in brines is 0.635 (for halophilic archaea), at 37°C (310 K) [9]. No data indicate that any life-form can function at ≤0.585 water activity at temperatures far from 24°C (e.g., subzero, or temperatures over 50°C) or at other extremes (e.g., below pH 4 or above 9). Furthermore, there are no empirical data which show cellular metabolism at any water-activity values below 0.585, for any type of xerophile and regardless of the domain of life.

Bosch et al. [11] recently reassessed the metabolism of microbes during entry into and out of a desiccated (anhydrobiotic) state, including taxa living in high-salt desert soil crusts. The authors (of which I was one) present circumstantial evidence that DNA repair and other enzyme-mediated processes occur during anhydrobiosis, when the cytosol is <0.250 water activity. It is already known that some enzymes can function well below the 0.585 water-activity limit for cell division [10]. The question is whether we regard these processes as cellular metabolism *per se*; for a discussion of this distinction, see Section 3.1 of Rummel et al. [12]. We know that some halophiles can survive desiccation–rehydration cycles; their long-term survival in an anhydrobiotic state can enable the colonisation of liquids-of-salt deliquescence here on Earth and, potentially, on Mars and other planetary bodies [13-15].

Saturated NaCl is Not so Extreme: The most-abundant salt in Earth's biosphere is NaCl, so this salt has in large part governed the evolutionary trajectory of terrestrial halophiles [16]. In relation to the scale of water activity over which some microbial cells can survive (0 to 1) and the window over which we know they can grow (0.585 to 1), the water activity of NaCl-saturated solutions is moderate [16]. For a temperature range of 0 to 50°C (1 atm), for example, a pure NaCl-saturated brine has water-activity values ranging from a modest 0.745 to 0.765 [17]. Brines with water-activity values lower than this (see below) are typically so chaotropic or acidic (and/or exhibit other extremes) that their water activity is not the life-limiting parameter; with very few exceptions [9]. As discussed by Lee et al. [16], natural selection to favour the evolution of halophiles that are more-xerophilic than those we currently know [9] has therefore been limited.

Testament to the thermodynamically moderate nature of NaCl-saturated brines, they host biodiverse and biomass-rich ecosystems consisting of all domains of life (see Table 1 of [16]). These ecosystems are capable of complete biogeochemical cycling, with complex ecologies which include predatory amoebae [16] and vampire bacteria [18, 19], as well as symbiotic nanohaloarchaea [20] and halophilic viruses. Furthermore, there are higher organisms that can inhabit NaCl brines including nematodes [21], brine shrimps, and even fish (the Death Valley pupfish) [16]. By contrast, other types of terrestrial brines (as well as those elsewhere in the Solar System) are typically more-complex and more-hostile to life. These include low water-activity brines that are chaotropic or acidic.

Water Activity of Chaotropic Brines and Acidic Brines: Saturated solutions of salts such as $MgCl_2$, $CaCl_2$, and LiCl reduce water activity to levels way below that for pure NaCl. For example, at 5°C (278 K) saturated solutions of these salts have values of 0.345, 0.400, and 0.140, and at 25°C (298 K) 0.325, 0.295, and 0.012, respectively [17]. Unsurprisingly, there are no biological data to demonstrate active life in such brines either *in vitro* or in nature. Studies of mRNA in deep-sea, $MgCl_2$-dominated brines in the sub-saturated concentration range revealed that active microbial life is prevented at water-activity values above the limit for halophile activity in NaCl brines [22-24]. Furthermore, these and other studies [e.g., ref. 6] demonstrate conclusively that the limiting parameter is chaotropicity; the entropic disordering of cellular macromolecules [25, 26]. Addition of NaCl to these $MgCl_2$-rich brines can enable active life to resume because (even though the water activity is further reduced), the kosmotropicity of NaCl mitigates the chaotropicity of $MgCl_2$ [22]. $MgCl_2$ also reduces water activity, causes osmotic stress, and reduces pH but its chaotropicity is the most-potent cellular stress parameter, regardless of the domain of life [6, 9, 22]. Chaotropic substances (including salts) enable the flexibility of cellular macromolecules and thereby enhance biological activity at low temperatures [27, 28], as confirmed by recent studies of enzyme activity at 5°C in the presence of perchlorate salts [29].

Alkaline brines are not known to exhibit water-activity values lower than those for saturated NaCl. By contrast, the values for some acid brines can be extremely low. For example, the brine of Gneiss Lake (Western Australia) was measured to be 0.714 water activity (at the *in-situ* temperature of 34°C [307 K] and pH 1.4) [30]. This water-activity value is within the known window for cellular function of some halophiles; see above. However, we have no data to suggest that any microbe is able to function at this combination of extreme pH and low water-activity [31]. Whereas Gneiss Lake contains DNA and 16S rRNA of microbial communities, these are likely active only when the brine is diluted by rainwater; i.e., when the water activity is higher than 0.714. The most-acidophilic microorganism known is the archaeon *Picrophilus torridus*, which grows down to a pH of -0.06 (at 60°C, 333 K) [32]. However, this species is not halophilic. Furthermore, a recent analysis suggests that its limited xerotolerance (there is no evidence that it can grow below 0.900 water activity), rather than its tolerance to acidity *per se*, is what constrains its ability to grow at even higher concentrations of acid [31].

Conclusions: Water activity, a cellular stress parameter independent of turgor [6] limits life in different types of habitat, not only brines [7-10, 31, 33, 34]. Whereas terrestrial life requires salts, it also requires sufficient water [7, 35] and whilst supra- or sub-optimal water activity can be highly stressful for cellular systems, stress is an inextricable and continuous aspect of life [36]. Terrestrial brine systems do not always emulate those found elsewhere in the Solar System, but do help us to understand the biophysical limits of microbial life. What may be less-well appreciated is that astrobiological approaches to scientific enquiry also help us understand the biology of present-day life here, on Earth [37].

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