FEMS MiniRev

FEMS

FEMS

Article Navigation

NaCl-saturated brines are thermodynamically moderate, rather than extreme, microbial habitats FREE

Callum J D Lee, Phillip E McMullan, Callum J O'Kane, Andrew Stevenson, Inês C Santos, Chayan Roy, Wriddhiman Ghosh, Rocco L Mancinelli, Melanie R Mormile, Geoffrey McMullan, Horia L Banciu, Mario A Fares, Kathleen C Benison, Aharon Oren, Mike L Dyall-Smith, John E Hallsworth ✉

FEMS Microbiology Reviews, Volume 42, Issue 5, 1 September 2018, Pages 672–693, https://doi.org/10.1093/femsre/fuy026

Published: 11 June 2018 **Article history** ▾

Views ▾ PDF Cite Permissions Share ▾

Abstract

NaCl-saturated brines such as saltern crystalliser ponds, inland salt lakes, deep-sea brines and liquids-of-deliquescence on halite are commonly regarded as a paradigm for the limit of life on Earth. There are, however, other habitats that are thermodynamically more extreme. Typically, NaCl-saturated environments contain all domains of life and perform complete biogeochemical cycling. Despite their reduced water activity, ~0.755 at 5 M NaCl, some halophiles belonging to the *Archaea* and *Bacteria* exhibit optimum growth/metabolism in these brines.

Furthermore, the recognised water-activity limit for microbial function, ~0.585 for some strains of fungi, lies far below 0.755. Other biophysical constraints on the microbial biosphere (temperatures of >121°C; pH > 12; and high chaotropicity; e.g. ethanol at >18.9% w/v (24% v/v) and $MgCl_2$ at >3.03 M) can prevent any cellular metabolism or ecosystem function. By contrast, NaCl-saturated environments contain biomass-dense, metabolically diverse, highly active and complex microbial ecosystems; and this underscores their moderate character. Here, we survey the evidence that NaCl-saturated brines are biologically permissive, fertile habitats that are thermodynamically mid-range rather than extreme. Indeed, were NaCl sufficiently soluble, some halophiles might grow at concentrations of up to 8 M. It may be that the finite solubility of NaCl has stabilised the genetic composition of halophile populations and limited the action of natural selection in driving halophile evolution towards greater xerophilicity. Further implications are considered for the origin(s) of life and other aspects of astrobiology.

Keywords: *Dunaliella salina*, extreme halophiles, habitability of Mars, hypersaline brines, limits-of-life on Earth, solar salterns

Topic: biomass, ecosystems, sodium chloride

Issue Section: Review Article

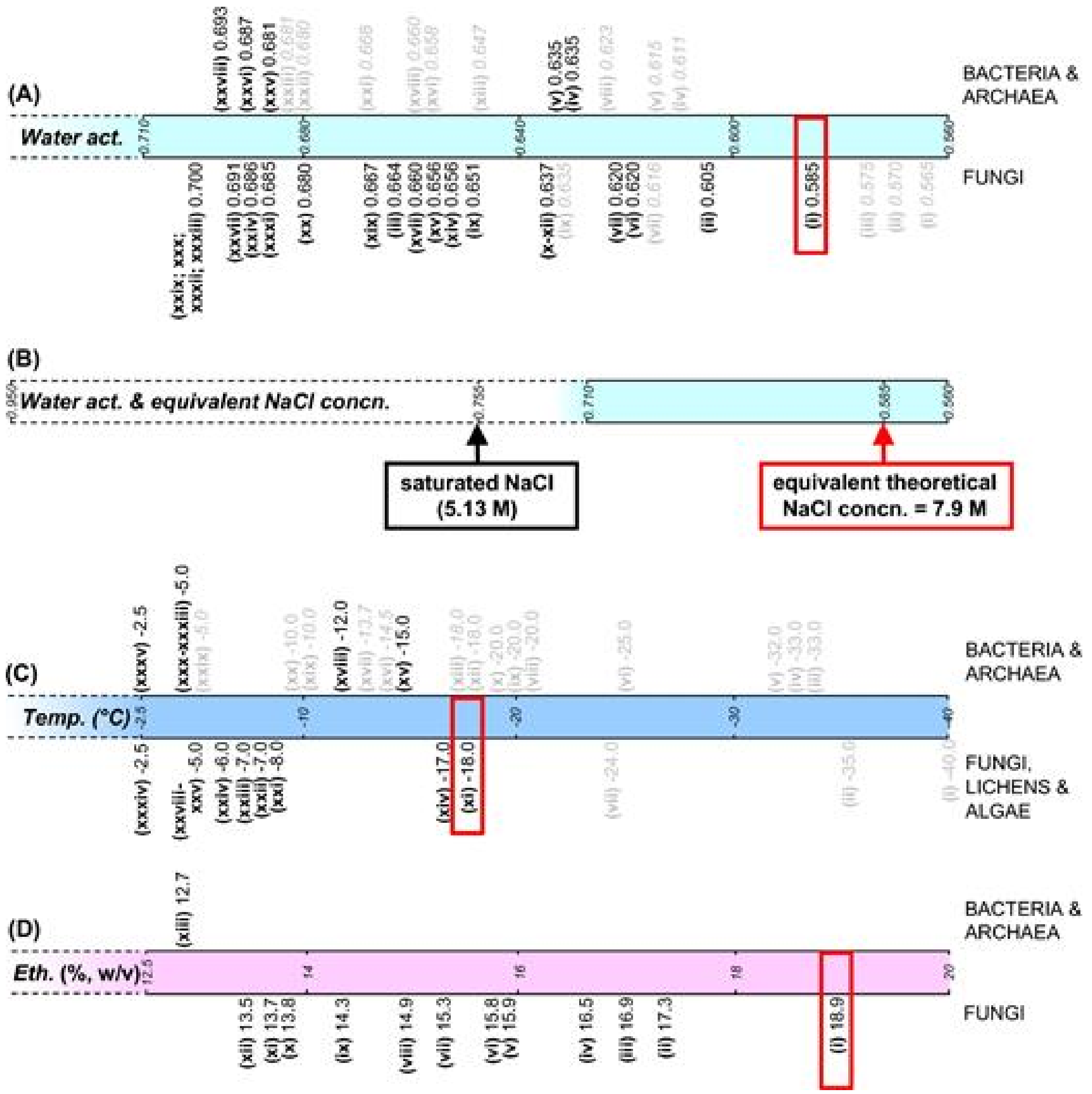
INTRODUCTION

NaCl-saturated environments occur throughout Earth's biosphere, spanning a wide range of scales from solar salterns, inland salt lakes and deep-sea brines to fluid inclusions of NaCl crystals and liquids-of-deliquescence on halite. They also include ephemeral habitats such as those created by the sea spray which lands on rock surfaces or saline aerosols which circulate within the atmosphere. Marine solar salterns, which are of human construction and include NaCl-saturated crystalliser ponds, occur along the coasts of many countries with warm sunny climates, such as Australia, China, Indonesia, Japan, Spain and the USA (Antón *et al.*2000; Zhaxybayeva *et al.*2013; Cui and Qiu 2014; Mori *et al.*2016), while inland solar salterns are often situated where natural saline waters and high evaporation rates occur, such as Australia, India, Spain, Peru and the USA (Maturrano *et al.*2006; Zafrilla *et al.*2010; Jose and Jebakumar 2013). Large-scale inland bodies of water, including the Dead Sea (Rift Valley, Middle East) and the Great Salt Lake (Utah, USA), vary in their solute composition, but their NaCl component is at times saturated or oversaturated. In the Mediterranean Sea there is a cluster of deep-sea basins (e.g. L'Atalante and Bannock), most of which contain NaCl-saturated brines that are known amongst microbiologists as 'deep-sea hypersaline anoxic brine lakes'. NaCl crystals can form in these, as well as other, hypersaline systems. Ancient salt crystals make up evaporite sedimentary rocks that underlie some of Africa, Asia, Australia, Europe, and North and South America (Warren 2016); halite is also present at the surface and has been well studied, for example, in the Atacama Desert in Chile (Crits-Christoph *et al.*2016) and Death Valley in California (Lowenstein and Hardie 1985).

Many of these systems are geochemically complex, containing other types of ions such as Mg^{2+} and SO_4^{2-} (e.g. Yakimov *et al.*2015). Shallow saline lakes in particular are characterised by fluctuating salinity due to cycles of flooding, evapoconcentration and desiccation (Lowenstein and Hardie 1985). For instance, the shallow acid-brine lakes of Western Australia can cycle between ~5% and 35% w/v total salts and so are composed of either saline waters or hypersaline brines (Benison *et al.*2007). Natural brines tend to be enriched in many elements besides Na and Cl; these most commonly include S, Mg, Ca, K and Br, though brines with unusually high or low pH also may have elevated dissolved concentrations of Al, Fe and Si (Bowen and Benison 2009; whereas such elements are often present at trace levels, they can also reach concentrations which are stressful and/or toxic in some brines (see below). All brines are characterised by low water activity, a parameter that can induce potent stresses in microbial cells. Indeed, water activity (i.e. the effective mole fraction of water) acts as a determinant for life processes and habitability of hostile environments (Grant 2004; Rummel *et al.*2014; Stevenson *et al.*2015a,b). This thermodynamic parameter, defined by Raoult's Law, is dependent on temperature and pressure. At the levels of cellular stress biology, and microbial ecology, water activity also interacts with factors such as temperature, pH and chaotropicity to determine rates and windows for metabolic activity and growth of microbial systems (e.g. Williams and Hallsworth 2009; Harrison *et al.*2013; Alves *et al.*2015).

Here, we focus on NaCl-dominated brines that are at or close to saturation with respect to halite, either all or some of the time. These systems are commonly regarded as representing the dry extreme for life—i.e. a limit of the Earth's biosphere—or used as a model for studies of water-constrained ecosystems and habitability of hostile terrestrial environments in the context of astrobiology (e.g. Brown 1990; Grant 2004; Harrison *et al.*2013; Crits-Christoph *et al.*2016). In addition, there has been an assumption that the water-activity limit for the cells and ecosystems of the most extremely halophilic microbes is 0.755, i.e. the value corresponding to water that is saturated with NaCl (Anderson 1954; Brown 1976; Grant 2004; Kminek *et al.*2010; Rummel *et al.*2014). However, recent studies have revealed active cell division of halophilic *Archaea* and *Bacteria* in the range 0.748–0.635 water activity, with theoretically determined water-activity minima as low as 0.611 (Stevenson *et al.*2015b). One study, of *Aspergillus penicillioides* (a xerophilic ascomycete capable of growing at high concentrations of various solutes including NaCl, sugars and glycerol), established a new water-activity limit for microbial differentiation and cell division: 0.585, with a theoretically determined limit of 0.565 (Fig. 1A; Stevenson *et al.*2017a,b,c). Collectively, these studies suggest that some extreme bacterial and archaeal halophiles may grow even when water activity is in the range 0.611–0.565. Furthermore, it is plausible that microbial populations which subsist under extreme conditions may undergo cell divisions over long timescales (Rummel *et al.*2014), yet to be quantified, at even lower water-activity values. Whereas the most-active microbes present in NaCl-saturated environments are known as halophilic extremophiles, the water activity of NaCl-saturated brine falls far short of the limit for extant halophilic life or the theoretical limit to which microbes could plausibly adapt (see below). This article reviews the evidence that microbially diverse, biomass-rich, and highly productive ecosystems occur at 5 M NaCl, and that they are functioning under moderate, rather than thermodynamically extreme, conditions.

Figure 1.



View large   Download slide

The most extreme values thus far reported for microbial cell division based on empirical data (black values); microbial cell metabolism based on empirical data (grey values, normal font); and microbial cell division based on theoretically determined limits (grey values, italics) in relation to: (**A** and **B**) low water-activity (turquoise scale-bars), (**C**) sub-zero temperatures (blue scale-bar), and (**D**) ethanol concentration (pink scale-bar). The scale bars span 0.710 to 0.560 water activity, 0.950 to 0.560 water activity, -2.5 to -40°C, and 12.5 to 20% w/v ethanol, respectively. Red boxes indicate the empirically determined limits for microbial cell division of the most extremely stress-tolerant microbial system in relation to each stress. For (**A**), the microbial systems are: (**i**) *Aspergillus penicillioides* JH06THJ (Stevenson *et al.*2017c), (**ii**) *Xeromyces bisporus* FRR 0025 (basionym *Monascus bisporus*) (Pitt and Christian 1968; extrapolated value from Stevenson *et al.*2017b), (**iii**) *Xerochrysium xerophilium* FRR 0530 (formerly *Chyrsosporium xerophilum*) (Stevenson *et al.*2017b), (**iv**) haloarchaeal strain GN-5 and (**v**) haloarchaeal strain GN-2 Javor 1984; Stevenson *et al.*2015a), (**vi**) *Aspergillus echinulatus* (Snow 1949), (**vii**) *Zygosaccharomyces rouxii* (von Schelhorn 1950; extrapolated value from Stevenson *et al.*2015a), (**viii**) *Halorhodospira halophila* DSM 244^T (Deole *et al.*2013; Stevenson *et al.*2015a), (**ix**) *Eurotium halophilicum* FRR 2471, (**x**) *X. bisporus* FRR 3443, (**xi**) *X. bisporus* FRR 1522 (**xii**) *X. bisporus* FRR 2347 (**ix-**

xii from Stevenson *et al.*2017b), (**xiii**) *Halorhabdus utahensis* DSM 12940^{T} (Wainø *et al.*2000; Stevenson *et al.*2015a), (**xiv**) *Eurotium amstelodami* FRR 2792 and FRR 0475 (Armolik and Dickson 1956), (**xv**) *Eurotium chevalieri* JH06THI (Ayerst 1969), (**xvi**) *Halobacterium* strain 004.1 (Stevenson *et al.*2015a), (**xvii**) *X. xerophilium* CBS 153.67^{T} (Pitt *et al.*2013), (**xviii**) *Actinopolyspora halophila* ATCC 27976^{T} (Yoshida *et al.*1991; Stevenson *et al.*2015a), (**xix**) *Eurotium repens* JH06JPD (Stevenson *et al.*2017b), (**xx**) *A. penicillioides* in complex substrates (Hocking 2003), (**xxi**) *Halanaerobium lacusrosei* DSM 10165^{T} (originally published as *Haloanaerobium lacusroseus*) (Cayol *et al.*1995; Stevenson *et al.*2017b), (**xxii**) *Halorhodospira halochloris* (basonym *Ectothiorhodospira halochloris*) (Deole *et al.*2013; Stevenson *et al.*2015a), (**xxiii**) mixed halophile community (Antón *et al.*2000; Stevenson *et al.*2015a), (**xxiv**) *Bettsia fastidia* FRR 77 (basionym *Chrysosporium fastidium*) (Pitt *et al.*2013), (**xxv**) *Natrinema pallidum* NCIMB 777^{T} (Antón *et al.*2000; Stevenson *et al.*2015a), (**xxvi**) *Halobacterium noricense* DSM 15987^{T} (Stevenson *et al.*2015a), (**xxvii**) *Wallemia sebi* FRR 1473 (Pitt and Hocking 1977), (**xxviii**) *Halcococcus salifodinae* DSM 13046 (Stevenson *et al.*2015a), (**xxix**) *Eurotium rubrum* FRR 0326 (Gock *et al.*2003), (**xxx**) *Polypaecilum pisce* FRR 2733 (Wheeler, Hocking and Pitt 1988), (**xxxi**) *Eurotium repens* FRR 382 (Stevenson *et al.*2017b), (**xxxii**) *Aspergillus conicus* (Pelhate 1968), (**xxxiii**) *Eremascus albus* (Pitt 1975). For (**B**), the limit of NaCl solubility is indicated (at 0.755 water activity) and the theoretical solubility limit of NaCl is indicated at the 0.585 water-activity limit for microbial growth; see (**A**). For (**C**), the microbial systems are: (**i**) *Pleopsidium chlorophanum* (de Vera *et al.*2014), (**ii**) a mixed culture of *Geomyces* and *Leucosporidium* (Panikov and Sizova 2006), (**iii**) *Paenisporosarcina* sp. B5 (Bakermans and Skidmore 2011), (**iv**) *Chryseobacterium* sp. V3519–10 (Bakermans and Skidmore 2011), (**v**) *Nitrosomonas cryotolerans* ATCC 49 181 (Miteva, Sowers and Brenchley 2007), (**vi**) *Planococcus halocryophilus* Or1 (Mykytczuk *et al.*2013), (**vii**) *Cladonia foliacea* (basionym *Cladonia alcicornis*) (Lange 1965), (**viii**) *Colwellia psychrerythraea* 34H (Junge *et al.*2006), (**ix** and **x**) bacterial communities obtained from permafrost (Tuorto *et al.*2014 and Rivkina *et al.*2000, respectively), (**xi**) *Rhodotorula glutinis* FMT157 (Collins and Buick 1989), (**xii**) a mixed culture of *Geomyces* and *Leucosporidium* (Panikov and Sizova 2007), (**xiii**) *Psychrobacter frigidicola* ACAM 304^{T} (Bowman *et al.*1996), (**xiv**) *Umbilicaria aprina* Nyl (Schroeter *et al.*1994), (**xv**) *Planococcus halocryophilus* Or1 (Mykytczuk *et al.*2013), (**xvi**) *Colwellia psychrerythraea* 34H (Huston 2003), (**xvii**) genetically modified *Escherichia coli* (Ferrer *et al.* 2003), (**xviii**) *Psychromonas ingrahamii* GVU73721 (Breezee, Cady and Staley 2004), (**xix**) *Psychrobacter urativorans* ACAM 534 (Bowman *et al.*1996), (**xx**) permafrost Archaea (Rivkina *et al.*2007), (**xxi**) *Nitzschia frigida* (Aletsee and Jahnke 1992), (**xxii**) *Mrakia frigida* (Larkin and Stokes 1968), (**xxiii**) *Mrakia nivalis* (Larkin and Stokes 1968), (**xxiv**) *Thalassosira antarctica* (Aletsee and Jahnke 1992), (**xxv**) *Chlamydomonas* sp. (Eddie, Krembs and Neuer 2008), (**xxvi**) *Thyphula ishikariensis*, (**xxvii**) *Thyphula incarnata*, (**xxviii**) *Thyphula phacorrhiza* (xxvi-xxvii from Snider *et al.*2000), (**xxix**) *Methanogenium frigidum* (Franzmann *et al.*1997), (**xxx**) *Glaciecola punicea* DSM 14233 (Chin *et al.*2010), (**xxxi**) *Psychrobacter glacincola* DSM 12194^{T} (Chin *et al.*2010), (**xxxii**) *P. urativorans* ACAM 534 (Chin *et al.*2010), (**xxxiii**) *Sporosarcina psychrophila* DSM 3^{T} (Chin *et al.*2010), (**xxxiv**) *Humicola marvinii* (Larkin and Stokes 1968), and (**xxxv**) *Methanococcoides burtonii* DSM 6242 (Franzmann *et al.* 1992). For (**D**), the microbial systems are: (**i**) *Saccharomyces cerevisiae* NCYC 1327 (Thomas *et al.*1993), (**ii**) several strains of *S. cerevisiae* (Gray 1941), (**iii**) *S. cerevisiae* shochu strain 1200 (Ohta, Hamada and Nakamura 1993) (**iv**) *S. cerevisiae* IFO 2347 (Hayashida and Ohta 1981), (**v**) *S. cerevisiae* baker's strain (Carlsen, Degn and Lloyd 1991), (**vi**) *S.*

cerevisiae John Labbatt Ltd. 67 (Odumeru *et al.*1992), (**vii**) *S. cerevisiae* PE-2 and CA1185 (Nakamura *et al.*1996), (**viii**) *S. cerevisiae* brewer's strain (Jones, Thomas and Ingledew 1995), (**ix**) *S. cerevisiae* brewer's strain (D'Amore *et al.*1990), (**x**) *S. cerevisiae* brewer's strain (Nagodawitbana and Steinkraus, 1976), (**xi**) *S. cerevisiae* BAW 6 (Omori *et al.*1996), (**xii**) *S. cerevisiae* ale strain (Stewart *et al.*1988), and (**xiii**) *Zymomonas mobilis* (Seo *et al.* 2005; Cray *et al.*2015)

THERE IS NO MICROBIAL ECOLOGY NEAR THE BIOPHYSICAL LIMITS FOR LIFE

In this article, we use the term 'extreme' to refer to conditions which impose near-complete constraints on cellular life and, as such, represent a thermodynamic fringe for microbial life on Earth. For instance, the water-activity window for the microbial biosphere spans from 1 to 0.585, i.e. 0.415 units. Only five or six types of microbe are known to function in the range 0.635 to 0.585; this range represents the most extreme 12% of the known window for microbial functionality (Fig. 1; Stevenson *et al.*2015a, b). Various parameters, either singly or in combination, can prevent microbial cell function; regardless of species. These include extremes of temperature and/or pressure, pH, chaotropicity, ionic strength and low water activity. The biophysical mechanisms by which this occurs operate at the levels of the biomacromolecule, whole cell, ecosystem and, ultimately, the microbial biosphere (Kashefi and Lovley 2003; Hallsworth *et al.*2007; Chin *et al.*2010; Cray *et al.*2015; Santos *et al.*2015; Yakimov *et al.*2015; Stevenson *et al.*2015a,b; Fox-Powell *et al.*2016). For such parameters, the failure points for cell division of the most resilient microbial systems occur close to, for example, 121°C, −18°C, 18.9% w/v (24% v/v) ethanol, and 0.585 water activity (Fig. 1). In relation to water activity, Pitt and Christian (1968) stated that germination of *Xeromyces bisporus* (FRR 0025) aleurospores occurred at 0.605, a finding which has neither been surpassed nor repeated for this species (Fig. 1A; Williams and Hallsworth 2009; Stevenson and Hallsworth 2014; Stevenson *et al.*2015a, b, 2017a,b). At this value, biotic activity was somewhat slow; germination was recorded only after a period of 3 months. The study of *A. penicillioides* strain JH06THJ, which germinated under some conditions at water activities of <0.605, revealed more rapid growth (Fig. 1A; Stevenson *et al.*2017c). Even 0.585 water activity, after 4 days conidia had swollen by >40% on a volume basis and were in a pre-germination phase; by 11.5 days conidia had differentiated to form polarised, tapered germination structures; and between 36 and 57 days fully formed separate germlings had been produced (Stevenson *et al.*2017c).

In relation to low temperatures, there are few reliable reports of microbial growth in the range −5 to −15°C (Fig. 1D; Chin *et al.*2010; Rummel *et al.*2014). Beyond this range, there is a single report of microbial reproduction, for *Rhodotorula glutinis*, at −18°C, although it took 17–18 weeks for cell numbers to double, and the yeast has an optimum temperature for growth between 20°C and 30°C (Fig. 1D; Collins and Buick 1989; Martínez 2006). Ethanol, which is produced in bulk by *Saccharomyces cerevisiae* and other microbes and acts as a potent antimicrobial agent, reduces water activity and acts as a chaotropic stressor for cellular systems (Hallsworth, Nomura and Iwahara 1998; Hallsworth, Heim and Timmis 2003; Cray *et al.*2013a, 2015). There are very few studies reporting microbial tolerance to ethanol at concentrations of >12.5% w/v (15.8% v/v) (Fig. 1C). However, one study demonstrated metabolic activity of, and ethanol production by, *S. cerevisiae* at 18.9% w/v ethanol (Fig. 1C; Thomas *et al.*1993), which corresponds to a water activity of 0.901 and chaotropic activity of 24.31 kJ kg^{-1} (Hallsworth and Nomura 1999; Cray *et al.*2013b). Reduced temperatures and kosmotropic substances, such as the compatible solute proline, can mitigate against and, in some cases, prevent the chaotrope-induced stress associated with ethanol and mechanistically comparable stressors (Hallsworth 1998; Hallsworth *et al.*2007; Bhaganna *et al.*2010; Bell *et al.*2013; Alves *et al.*2015; Cray *et al.*2015). Kosmotropic substances are those which entropically order or stabilise biomacromolecular systems (Collins 1997; Cray *et al.*2013b; Ball and Hallsworth 2015). Recent studies were carried out based on genome analysis, hybridisation and subsequent strain selection to optimise ethanol tolerance and product yield from *S. cerevisiae* strains used for bioethanol fermentations (Fig. 1C; Swinnen *et al.*2012; Pais *et al.*2013). Whereas these were successful, the ethanol concentrations obtained are some way below the 18.9% w/v value reported previously. In relation to the limit for microbial life, each of these extremes will be further examined below.

At or close to such thermodynamic boundaries for life, there is no evidence of any functional microbial ecosystem. Furthermore, there are no reports of microbial metabolism below 0.585 water activity or above 18.9% w/v ethanol (Fig. 1A), and only sporadic reports of limited metabolic activities in a handful of microbial species below −18°C (Fig. 1D; Kminek *et al.*2010; Rummel *et al.*2014). For instance, studies of the lichen *Pleopsidium chlorophanum* that were performed in a Mars Simulation Chamber indicated significant photosynthetic activity at −40°C (Fig. 1D; de Vera *et al.*2014). In natural habitats such as sea ice and permafrost, however, there is no evidence of ecosystem function at temperatures below −18°C; hence, the standard (−20°C) temperature of freezers used for food storage. The data arising from studies of the lower temperature limits for microbial life have been analyzed elsewhere (Chin *et al.*2010; Kminek *et al.*2010; Rummel *et al.*2014) so will not be discussed at length here. There is no evidence of microbial fermentation at ethanol concentrations >18.9% w/v from culture-based studies; similarly, no anthropogenic system or microbial habitat is known to facilitate ecosystem function above such ethanol concentrations according to either culture-dependent or independent studies (Fig. 1C; Cray *et al.*2015; Lievens *et al.*2015). The chaotropicity of $MgCl_2$ also prevents the biotic activity of (all) microbial systems at concentrations ≥ 2.40 M (Hallsworth *et al.*2007), though activity for some halophile systems has been reported in the range 2.50–3.03 M when kosmotropic ions are present (Hallsworth *et al.*2007; Yakimov *et al.*2015). Whereas extrapolations made using data of fungal germination at low water activity suggest theoretical water-activity minima for germination in the range 0.575–0.565 (Fig. 1A; Stevenson *et al.*2017b,c), there is no empirical evidence for metabolic activity or cell division of xerophilic fungi in this range. The few microbes known to be capable of biotic activity at <0.690 water activity inhabit diverse substrates and environments in nature and so are unlikely to occur together or form an active community under these conditions (Fig. 1A; Stevenson *et al.*2015a,b, 2017c). In other words, conditions or habitats of <0.690 might be regarded as 'extreme' and are characterised by low biodiversity and a negligible biomass of active microbes. Each of the findings which demonstrated microbial activity at −18°C, 18.9% w/v ethanol or 0.585 water activity was reported in an individual published study and for a single microbial system (Fig. 1; Collins and Buick 1989; Thomas *et al.*1993; Stevenson *et al.*2017c), and there is no functional ecosystem in any of these contexts. Indeed, chaotropic environments containing high concentrations of $MgCl_2$ or ethanol are functionally sterile (hence the value of such substances as food preservatives and/or biocides) (Hallsworth *et al.*2007; Cray *et al.*2015; Yakimov *et al.*2015).

RICH AND ACTIVE ECOSYSTEMS IN NaCl-SATURATED BRINES

NaCl-saturated systems are highly permissive for microbial life (Table 1). This is also evidenced by the active international community of scientists working on these ecosystems; and the recent and well-attended 13th International Conference on Halophilic Microorganisms of the International Society for Salt Lake Research (2017, in Ulan-Ude, Russia with 122 delegates). There are 2569 papers published on halophiles and their hypersaline habitats, according to the Thomson Reuters Web-of-Science database; a total that was derived (28 May 2018) using the following searches: '(halophil** or halotoler** or microbial community or microbial ecology or microbiology or *Haloquadratum* or *Dunaliella* or *Salinibacter* or *Actinopolyspora* or *Halorhodospira*) and (saltern** or NaCl-crystal** or halite or brine-lake** or deep-sea-lake** or deep-sea-basin** or deep-sea-brine** or fluid-inclus** or crystallizer-pond** or crystalliser-pond** or Dead-Sea or hypersalin** or NaCl-saturat** or salt-saturat** or saturated-NaCl or saturated-salt** or 5-M-NaCl or 5-molar-NaCl)' and 'extreme-halophil** or obligate-halophil**'.

Table 1.

Microbiology of diverse, NaCl-saturated habitats.

Habitat	Microbial biomass	Phylogenetic diversity	Typical members of the community
Crystalliser ponds of marine solar salterns	Compared with most other aquatic microbial habitats, NaCl-saturated solar salterns are biomass rich. Even at ~5 M NaCl, the high cell density of halophilic microbes, and the high intracellular concentrations of their pigments, result in highly coloured turbid brines (Oren 2009). Cell densities are typically in the range 10^6 to 10^8 cells ml^{-1} (Guixa-Boixareu *et al.*1996; Çınar and Mutlu 2016), which is orders of magnitude higher than those observed in a study of coral atolls in the Northern Line Islands (Dinsdale *et al.*2008). Indeed, the cell densities of hypersaline crystalliser ponds are comparable to, or even surpass, those of anthropogenic systems such as aquaculture waters, fishponds and ethanol-producing fermentations (Cocolin, Bisson and Mills 2000; Herrero *et al.*2006; Kalcheva	Marine, NaCl-saturated solar salterns are characterised by an exceptionally high microbial diversity, and contain taxa from each domain of life as well as diverse viruses. One study, which used both culture-based and culture-independent methods (Burns *et al.*2004), revealed that an individual, NaCl-saturated saltern (coast of Australia) contained 57–66 identifiable types of *Archaea* and *Bacteria*. A 16S rRNA gene clone-library study of a Tunisian saltern (S5 pond, 31% total salinity), carried out by Baati	The archaeal genera that are commonly present include *Haloarcula*, *Halobacterium, Halobaculum*, *Halococcus*, *Haloferax*, *Halobacterium, Halorubrum* and *Haloquadratum*, and bacterial genera include *Halomonas*, *Halovibrio*, *Salicola* and *Salinibacter* spp. (Manikandan, Pašić and Kannan 2009; Sabet *et al.*2009; Cray *et al.*2013a). Eukaryotes include fungal genera such as *Aspergillus*, *Cladosporium, Hortaea, Penicillium and Wallemia* spp. (Cantrell, Casillas-Martínez and Molina 2006). The most common algae are *Dunaliella* spp., which are also the most xerophilic of the microalgae (Cifuentes *et al.*2001; Oren 2014;

	*et al.*2008; Taniasuri, Lee and Liu 2016; Wada *et al.*2016).	*et al.* (2008), revealed eight OTUs pertaining to *Bacteria* (*Bacteroidetes* and *Proteobacteria*) and 39 OTUs assigned to *Euryarchaeota* (Halobacteria). For prokaryotes from this saltern, the Shannon Index (Spellerberg and Fedor 2003) was 0.98 for *Bacteria* and 3.04 for *Archaea* (Baati *et al.*2008). Another study retrieved 86 isolates of 26 fungal species from the waters of the Cabo Rojo salterns on the coast of Puerto Rico (Cantrell, Casillas-Martínez and Molina 2006); and a study of a NaCl-saturated crystalliser pond (Spanish coast) revealed the presence of 42 viral genomes (using a fosmid metagenome library) (Garcia-Heredia *et al.*2012).	Stevenson *et al.*2015b). The nanoflagellates *Euplaesiobystra*, *Halocafeteria*, and *Pleurostomum* spp. are the most xerophilic protists known, and have also been found inhabiting NaCl-saturated salterns (Park *et al.*2009; Stevenson *et al.*2015b). Amongst the microbes present in marine solar salterns, there are several species which recurrently dominate halophile communities, most notably *Dunaliella salina*, *Salinibacter ruber* and *Haloquadratum walsbyi* (Cray *et al.*2013a; Oren and Hallsworth 2014).
Liquids-of-deliquescence on halite rock[a]	Halite rock is commonly found in terrestrial surface and subsurface environments, and, under specific conditions, deliquesces to produce hypersaline NaCl brines (Rummel *et al.*2014). These brines, typically exposed to the air, are commonly inhabited by microbes. Cell densities reported for liquids-of-deliquescence on halite rocks of	The phylogenetically diverse communities present include microbes from each domain of life, from photosynthetic bacteria and algae to heterotrophic archaea and bacteria, including both aerobes and the anaerobic	Microbial taxa found in liquids-of-deliquescence include all domains of life but archaeal species are typically the most prevalent (Robinson *et al.*2015). Of the DNA extracted and analysed, 71% of metagenomic reads were assigned to *Archaea* (the majority of which were Halobacteria),

	the Atacama Desert are in the range 2–10 × 10^7 cells g^{-1}, a microbial biomass which is three orders of magnitude greater than that of the surrounding soil (Robinson *et al.*2015).	fermentative bacteria of *Halanaerobiales* (Robinson *et al.*2015; Crits-Christoph *et al.*2016). The microbial diversity of liquids-of-deliquescence can be extraordinarily high, the brine communities on halite of the Atacama Desert have Shannon Indices as high as 6.25, which is in excess of those usually associated with soils (Fierer and Jackson 2006). At least 30 distinct viruses were identified within the cellular biomass found at one sample site; most of these are thought to occur within cells of the Halobacteria (Crits-Christoph *et al.*2016).	27% to *Bacteria* and 1% to eukaryotes (Crits-Christoph *et al.*2016). The most prevalent *Archaea* were, in descending order, *Natronomonas, Halococcus, Haloarcula, Halorhabdus, Salinarchaeum, Halobacterium* and *Halomicrobium,* and the most prevalent bacteria were *Salinibacter*. Cyanobacteria of the genera *Chroococcidiopsis* and *Halothece* carry out the majority of primary production in halite ecosystems (Wierzchos, Ascaso and McKay 2006; Robinson *et al.*2015).
Deep hypersaline anoxic basins	NaCl-saturated, deep-sea brine lakes are characterised by a considerable, highly active biomass (Daffonchio *et al.*2006). The halocline at the interface of the deep-sea Lake Bannock (located beneath the Mediterranean Sea) and the open ocean has a NaCl concentration ranging from that of the overlying sea water (690 mM), where there are 4.32×10^4 cells ml^{-1}, to ~4.5 M which has 4.39×10^5 cells ml^{-1} (Daffonchio *et al.*2006). There is, therefore, an order-of-magnitude increase	Microbial diversity is high in deep-sea basins: the major eukaryotic groups represented include the opisthokonts, plants, cercozoa, alveolates, stramenopiles and discicristates (Edgcomb *et al.*2009); culture-independent studies of Lakes Bannock and Thetis have identified from 40 to	Insights into composition (and function) of microbial assemblages inhabiting the deep-sea brine lakes have been gained mostly by metagenomic analyses. The most-common *Archaea* in the brine layer of the Mediterranean deep-sea hypersaline lakes are MSBL1 and Halophilic Cluster 1 (HC1) candidate divisions (Daffonchio *et al.*2006; La Cono *et al.*2011; Yakimov

	in microbial biomass as NaCl concentration increases, a phenomenon reported within other deep-sea basin systems, such as the NaCl-saturated Lake L'Atalante (Mediterranean Sea) where a cell number of 1.07 × 10^5 ml^{-1} has been recorded (i.e. more than that for the overlying sea water, which has 3.57 × 10^4 ml^{-1}) (Yakimov *et al.*2007).	120 OTUs for protists alone (Edgcomb *et al.*2009; Stock *et al.*2012). Analysis of a Lake Thetis microbial community revealed that 37% of the total number of the OTUs were fungi (Stock *et al.*2012). Additional studies have revealed 71 unique sequences corresponding to *Archaea* and *Bacteria* (in a of more or less 1:1) in Lake Thetis (Ferrer *et al.*2012). Methane oxidisers, methylotrophs, sulphate reducers, sulphur oxidisers and/or microaerophiles are found in Lakes L'Atlante, Bannock and Thetis (Daffonchio *et al.*2006; Yakimov *et al.*2007; La Cono *et al.*2011).	*et al.*2013). The vast majority of the bacteria inhabiting deep-sea brine lakes was assigned to *Alpha-, Delta-*, *Epsilon-* and *Gammaproteobacteria* and KB1 candidate group (Yakimov *et al.*2007; La Cono *et al.*2011; 2013; Pachiadaki *et al.*2014). Bacteria from the *Desulphobulbaceae* and *Sphingobacteria* have been identified in Lake Bannock (Daffonchio *et al.*2006). The most-common protistan genera in Lake Thetis include *Pleuronema, Strombidium* and *Trimyema, w*hile the predominant fungal RNA gene sequences are closely related to those of the genera *Aspergillus, Cladosporium, Malassezia*, and *Rhodotorula* (Stock *et al.*2012; Bernhard *et al.*2014).
Inland salt lakes	Of the large-scale water bodies located inland, it is estimated that 45% are saline (Wetzel 2001), a value which is likely to rise due to global warming (Williams 2002). Lake Kasin, located in Russia, is a shallow, NaCl-saturated water body containing sufficient iron to support specialist, iron-metabolising microbes. Fe(II) ions are weak electron donors, compared with organic substrates for instance, and can limit the efficiency with which	A number of alkaline, NaCl-saturated lakes are located in the Wadi An Natrun Valley in Egypt, including Lakes Rizunia, Gaar, and Zugm, and are characterised by highly diverse communities made up of autotrophic and/or heterotrophic taxa belonging to each domain of life	Both Lakes Chaka and Kasin are dominated by *Archaea* belonging to Halobacteria. Whereas the genera *Haloarcula* and *Halosimplex* prevailed in Lake Chaka, *Halobaculum* spp. seemed to be abundant in Lake Kasin (Jiang *et al.*2006; Emmerich *et al.*2012). The dominant bacterial classes in Lake Kasin were *Bacilli* and *Clostridia*, with *Halothiobacillus* as the

cell-available energy is generated. Living at 5 M NaCl imposes a high energetic cost to the cell. Nevertheless, the NaCl-saturated sediment of Lake Kasin contains ≥ 1×10^4 iron-metabolising microbial cells g^{-1} (and 1.1×10^6 to 6.7×10^7 total microbial cells g^{-1}) (Emmerich *et al.*2012). Lake Chaka, which is located on the Tibetan Plateau at 3200 m above sea level, is 2–3 cm deep and NaCl saturated. Despite the high altitude and relative isolation from other NaCl-rich habitats, the Lake Chaka brine contains 4.8×10^6 cells ml^{-1} and the lake sediment contains 4.2×10^7 cells ml^{-1} (Jiang *et al.*2006). The NaCl-saturated brine of Aran-Bidgol Lake (Iran) has been shown to contain $3–4 \times 10^7$ cells ml^{-1} (Makhdoumi-Kakhki *et al.*2012). Numerous salt lakes with appreciable depths (> 10 m) were reported in Central Romania (Baricz *et al.*2014, 2015; Andrei *et al.*2015, 2017). These permanently stratified bodies of water feature steady physicochemical gradients resulting in sharp niche partitioning and distinctive microbial communities, respectively. As a general characteristic, a less saline (6–20% total salinity) surface layer overlies a NaCl-saturated bottom stratum in such lakes. Estimation of prokaryotic cell densities by qPCR and DAPI indicated values ranging between 10^5 and 10^6 cells ml^{-1} for *Bacteria* and 10^7–10^8 cells ml^{-1} for *Archaea*, with the densest microbial population recorded (Imhoff *et al.*1979; Selim *et al.*2012). A metagenomic study on Lakes Fazda, UmRisha and Hamra, which are all located in the Wadi An Natrun, reported 345 bacterial and 198 archaeal OTUs present in total, and distinct prokaryote communities within, and between, the lakes (Mesbah, Abou-El-Ela and Wiegel 2007). High microbial diversity is also prevalent in Lake Aran-Bidgol, which has been found to contain 37 OTUs corresponding to *Archaea* and *Bacteria*; 63% of these were unrelated to any taxa thus far identified (Makhdoumi-Kakhki *et al.*2012). Andrei *et al.* (2015) reported an increasing prokaryotic diversity along the salinity gradient in two meromictic salt lakes of Central Romania. The highest OTU values were retrieved from the NaCl-saturated layers of Fara Fund (144 archaeal and 330 bacterial OTUs) and Ursu lakes (116 archaeal and 459 bacterial OTUs). Phototrophs most-common genus (Jiang *et al.*2006). Common bacteria in Lake Chaka include *Salinibacter ruber*, and those within the genera *Bacillus* and *Halanaerobium* (Emmerich *et al.*2012). In the Wadi An Natrun Valley, members of the bacterial genus *Halorhodospira* dominated six hypersaline alkaline lakes, and other prevalent microbes within these lakes included the archaeal genus *Halobacterium*, the proteobacterium *Chromatium*, and the cyanobacterial genera *Spirulina* and *Synechcooccus* (Imhoff, Hashwa and Trüper 1978). This has been corroborated by a metagenomic study which also detected members of the bacterial genera *Rhodobaca* and *Roseinatronobacter* and the cyanobacterial genus *Euhalothece* (Mesbah, Abou-El-Ela and Wiegel 2007). The study on permanently stratified salt lakes in Romania pointed to clear niche-based differentiation of microbial communities inhabiting the water column. In the aphotic, anoxic and hypersaline zone of investigated lakes (~35% total salinity), *Bacteria* dominated the microbial communities, being represented by

	in the most-saline lakes (Andrei *et al.*2015, 2017). The concentration and composition of salts within large-scale inland bodies of water including the Dead Sea (Middle East) and the Great Salt Lake (Utah, USA) can vary. The Dead Sea contains relatively high levels of chaotropic $MgCl_2$ (Hallsworth *et al.*2007), but is nevertheless characterised by high levels of microbial biomass during periodic blooming events (Oren 1985; Oren and Gurevich 1995). Biomass-rich microbial communities are also active in the Great Salt Lake, even when NaCl concentrations approach saturation (Post 1980).	(cyanobacteria and *Dunaliella* sp.) were also found in these stratified lakes (Máthé *et al.*2014; Andrei *et al.*2015). One hundred genera of *Archaea* and *Bacteria* were isolated from the NaCl-saturated northern arm of the Great Salt Lake (Weimer *et al.*2009). An analysis of 16S rRNA gene clone libraries recovered from brine samples taken from Lake Kasin identified 290 sequences for bacteria and 231 sequences for Euryarchaeota and other *Archaea* (Emmerich *et al.*2012).	anaerobes classified within the genera *Desulphovermiculus*, *Acetohalobium* and *Halanaerobium*. In such conditions, *Archaea* were less abundant and mostly represented by Halobacteria (*Halorhabdus* sp. and *Halorubrum* sp.). It should be noted that substantial number of reads retrieved in this study related to candidate OP1 and OD1 bacterial clades (Andrei *et al.*2015). The most common eukaryotic species in many inland salt lakes is *Dunaliella salina*, among other *Dunaliella* species (Imhoff, Hashwa and Trüper 1978; Weimer *et al.*2009).
Fluid inclusions within individual NaCl-evaporite crystals[a]	Each year, many 100 millions tons NaCl crystals precipitate from evaporating brines located in both natural halophile habitats and industrial salterns. Halophiles are readily entrapped during crystal precipitation and remain in NaCl-saturated brine inclusions within the crystal (Norton and Grant 1988). The concentrations of cells in these fluid inclusions can be equal to or greater than those found in the evaporate brines from which they came, and can exceed 4 × 10^8 cells g^{-1} (Norton and Grant 1988).	A culture-independent study by Baati *et al.* (2010) identified 21 bacterial and 44 archaeal OTUs in a sample taken from the fluid inclusions of NaCl crystals from the benthic surface of a solar saltern in Sfax, Tunisia (on the Mediterranean coast). Microbes from each domain of life have been found within hypersaline inclusions of NaCl crystals, including *Dunaliella* spp.,	It may be that any of the microbes which inhabit supersaturated saline systems could potentially become entrapped within NaCl crystals. Amongst the taxa thus far identified within fluid inclusions of NaCl crystals are *Dunaliella salina*, *Archaea* such as *Haloarcula*, *Halobacterium*, *Haloferax*, *Halonotius, Halorubrum*, *Haloquadratum* and *Natronococcus*, and the bacteria *Salinibacter* and *Halobacillus* (Norton and Grant 1988; Mormile *et al.*2003; Conner and Benison 2013; Henriet

		Archaea and *Bacteria* (Lowenstein, Schubert and Timofeeff 2011; Conner and Benison 2013).	*et al.*2014).

[a] In some environments, these habitats/ communities may occur simultaneously in the same location.

View Large

Biomass density and richness

In both natural and anthropogenic systems, including industrial salterns, NaCl-saturated brines can have an extraordinarily high microbial biomass. NaCl-saturated solar salterns have 10^6 to 10^8 microbial cells ml^{-1} (Table 1), and this is equal to the yeast-cell densities attained in wine and cider fermentations (Cocolin, Bisson and Mills 2000; Herrero *et al.*2006; Taniasuri, Lee and Liu 2016). Even within the hypersaline fluid inclusions of a single NaCl crystal, cell densities greater than 4×10^8 ml^{-1} have been observed (Table 1) where these communities can remain active; survive within the crystal over long time periods (possibly geological timescales); can be released upon dissolution of crystals or crystal deposits; and collectively constitute enormous microbial ecosystems which extend across considerable regions of the Earth's surface and subsurface (Grant 2004; Schubert *et al.*2009; Sankaranarayanan *et al.*2014). Furthermore, NaCl-saturated systems frequently exhibit a high abundance and diversity of microbial types (Table 1). The Shannon Indices in one marine saltern in Tunisia, for instance, were 0.98 for *Bacteria* and 3.04 for *Archaea* (Table 1; Baati *et al.*2008), values comparable to those for microbial diversity within bioethanol fermentations of corn, i.e. up to 3.23 (Li, Heist and Moe 2016). The salt-saturated bottom water layer (~35% total salinity) of two inland permanently stratified (meromictic) salt lakes situated in Romania revealed unusually high alpha-diversity for both *Bacteria* (SI 3.5 and 5.4; 330 and 459 operational taxonomic units—OTUs$_{0.03}$ – defined as ≥97% 16S rRNA gene sequence similarity clusters) and *Archaea* (SI 4.0 and 3.3; 144 and 116 OTUs$_{0.03}$). In these lakes, the microbial diversity increased along the salinity gradient reaching a maximum at the highest salt concentration (Andrei *et al.*2015). In Mediterranean deep-sea brines, despite the lack of phototrophs, more than 110 OTUs were present, encompassing microbes from each domain of life (the criterion ≥98% was used for 16S rRNA gene sequence similarity) (Table 1; Ferrer *et al.*2012; Stock *et al.*2012).

At specific relative humidities and temperatures, salts absorb water from the vapour phase and liquefy to produce saturated brine (Gough *et al.*2011; Rummel *et al.*2014; Stevenson *et al.*2015b). The liquids-of-deliquescence on halite of the Atacama Desert can contain cell densities of $2–10 \times 10^{7}$ cells g^{-1} (Table 1), and the NaCl-saturated north arm of the Great Salt Lake and saltern crystalliser ponds (in diverse locations) contain $10^{7}–10^{8}$ microbial cells ml^{-1}, a density that gives rise to the opacity and distinctive red colour of these brines (Table 1; Oren 2009). Most other aquatic systems are characterised by microbial cell densities that are significantly lower than this. For example, a study of tropical freshwater reservoirs in Sri Lanka reported up to 4.73×10^{6} bacterial cells ml^{-1} (Peduzzi and Schiemer 2004) and a study of coral atolls reported from 7.2×10^{4} to 8.4×10^{5} microbial cells ml^{-1} seawater, depending on the sampling site (Dinsdale *et al.*2008). A number of studies show that cell number increases as NaCl concentration increases (e.g. Benlloch *et al.*1996; Daffonchio *et al.*2006). There are various reasons which, when combined, help to explain why cell densities are so high in hypersaline environments; these include:

I. salterns, and other NaCl-saturated habitats, can have a plentiful supply of nutrients (including the glycerol leaked by algae; Elevi Bardavid and Oren 2008; Elevi Bardavid, Khristo and Oren 2008; Oren 2017)) that can become further concentrated by evaporative processes,

II. saturated NaCl is not thermodynamically extreme, and daytime temperatures (as well as water activity) are often close to the optimum for haloarchaea and other halophiles (see below, Oren 1994),

III. the most-extreme halophiles are able to fully adapt to hypersaline conditions (Cray *et al.*2013a; Oren and Hallsworth 2014), and so their high growth rates and biomass are a reflection of the high nutrient levels,

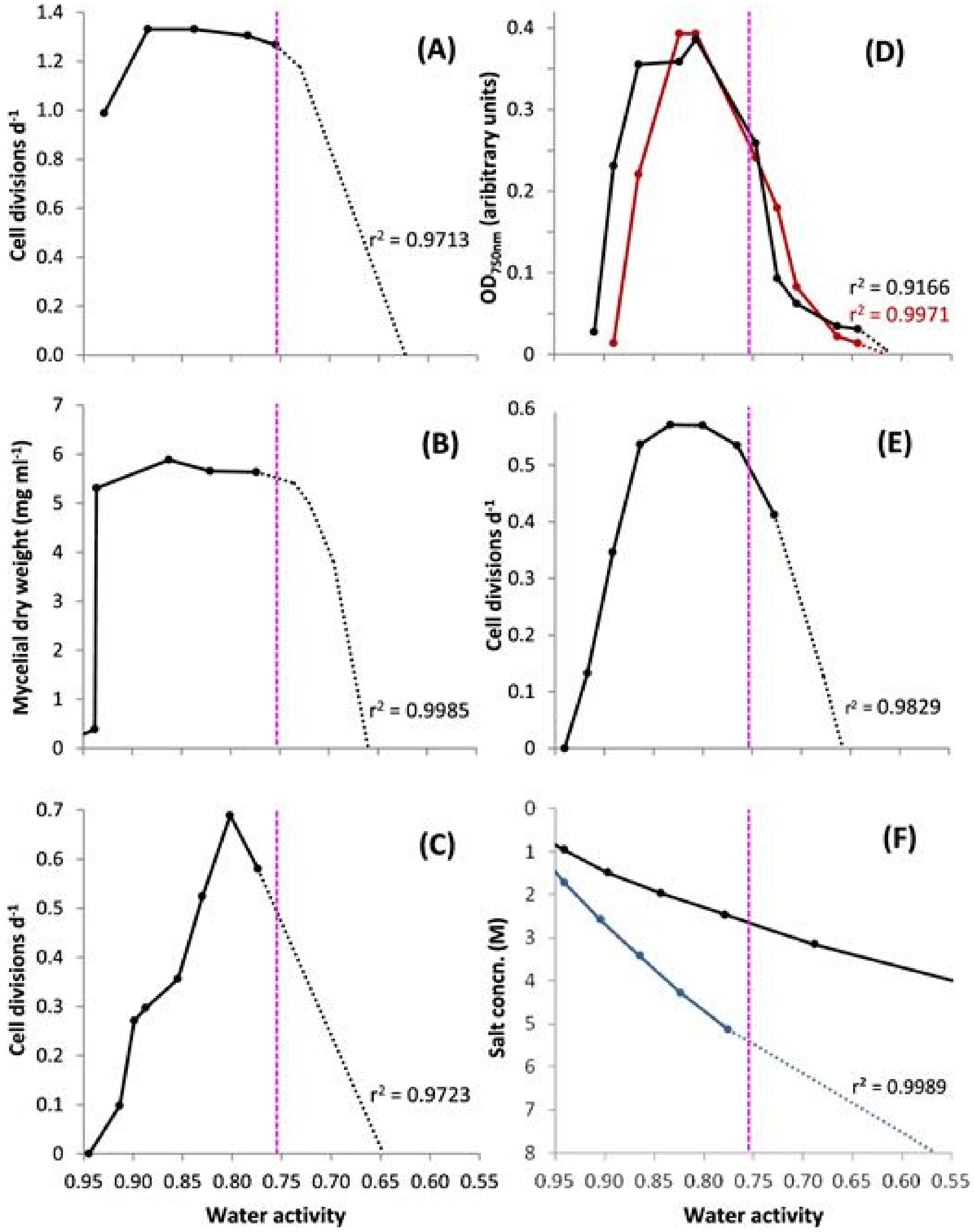
IV. salt concentrations and water activity typically remain more stable than those of saline waters that are not saturated, and

V. the losses due to predation/grazing are relatively low (Guixa-Boixareu *et al.*1996; Pedrós-Alió *et al.*2000; Pedrós-Alió, Calderón-Paz and Gasol 2000).

Halophile function at extremely low water activity

The water activity of NaCl-saturated brines (at ca. 0.755) is hostile to many types of microbe. Hundreds of known microbial species are likely capable of growth in the water-activity range 0.755–0.701, but below this range there is a more restricted number of microbial systems able to function (Fig. 1A; Williams and Hallsworth 2009; Stevenson *et al.*2015a,b, 2017c). Even so, some extremely halophilic prokaryotes—which can be active down to 0.635–0.611—exhibit high or optimum rates of growth at saturated NaCl (Figs 1A and B and 2; Stevenson *et al.*2015a, b). For instance, the extremely halophilic archaeal strains GN-2 and GN-5 (both presumed to be *Halobacterium*), *Halobacterium* strain 004.1, *Haloquadratum walsbyi* (DSM 16790) and *Halorhabdus utahensis* (DSM 12940^T), and the bacteria *Actinopolyspora halophila* (ATCC 27976^T), *Halorhodospira halophila* (DSM 244^T), *Pontibacillus* strain AS2 and *Salinicola* strain LC26 exhibited very high or optimum growth rates at saturated NaCl or at water-activity values equivalent to that of saturated NaCl (Fig. 2; Stevenson *et al.*2015b). Furthermore, their growth windows extend down to ~0.600 water activity, so more than one third of the water-activity window for growth lies beyond the water-activity value for saturated NaCl (Figs 1A, B and 2A–E). Similarly, for *A. penicillioides*, growth rates of germlings are remarkably high at ~0.755 water activity on media supplemented with NaCl and glycerol, and 42% of the water-activity window lies beyond this value; i.e. at water activities lower than (Stevenson *et al.*2017a,b,c). This finding is pertinent to the ecophysiology of halophiles because *A. penicillioides*, along with extreme halophilic fungi such as *Hortaea* and *Wallemia* species and halophilic algae such as *Dunaliella* species (all capable of growth at or close to 5 M NaCl, see Stevenson *et al.*2015b), are exposed to molar concentrations of glycerol which they accumulate in the cytosol at high salt concentrations.

Figure 2.



View large Download slide

In relation to the water activity of saturated NaCl, i.e. 0.755 (pink dashed line), (**A**–**E**) plots are shown of doubling times, dry mass, or optical density measurements—i.e. turbidity—for extreme halophiles and (**F**) salt concentrations for NaCl (blue line) and $MgCl_2$ (black line; Hallsworth *et al.*2007 at 25°C). Growth curves for (**A**) the bacterium *Halorhodospira halophila* (strain DSM 244^T, cultured in a defined medium supplemented with NaCl) (basonym *Ectothiorhodospira halophila*), (**B**) the bacterium *Actinopolyspora halophila* (strain ATCC 27976^T, cultured in a complex medium supplemented with NaCl at 37°C), (**C**) the archaeon *Halorhabdus utahensis* (strain DSM 12940^T in a defined medium supplemented with NaCl at 30°C), (**D**) haloarchaeal strains GN-2 and GN-5 shown in red and black, respectively (cultured in bittern brines supplemented with peptone at 37°C) and (**E**) the archaeon *Halobacterium* strain 004.1 (in a synthetic seawater medium supplemented with NaCl, $MgCl_2$, Na_2SO_4 and KCl) were reproduced from Stevenson *et al.* (2015a); original data were obtained from Javor (1984), Yoshida *et al.* (1991), Wainø, Tindall and Ingvorsen (2000) and Deole *et al.* (2013). Extrapolations of growth curves (dotted lines) were carried out using Excel or Sigmaplot as described by Stevenson *et al.* (2015a). Plots of water activity versus salts concentrations (**F**) were modified from Hallsworth *et al.* (2007). The water activity versus concentration curve for NaCl was extrapolated using polynomial order 2 in Excel (blue dotted line).

Some experimental settings, as well as studies of bittern brines, represent a transition between Mg-rich environments that have no microbial activity, and densely populated NaCl-saturated brine (Javor 1984; Baati *et al.*2011; Yakimov *et al.*2015; Stevenson *et al.*2015b). Biotic activity of halophiles was thereby revealed in culture at <0.755. However, the environmentally relevant salts which reduce water activity to this level are typically chaotropic (see above), and so chaotropicity cuts in as the stress parameter to limit life before water activity can do so (Hallsworth *et al.*2007; Alves *et al.*2015; Stevenson *et al.*2015a,b; Yakimov *et al.*2015). Other substances, such as glycerol, are more permissive such that NaCl+glycerol mixtures enable microbial activity at <0.600 water activity (see above).

Ecosystem activity and complexity

The microbial ecology of biomass-dense, NaCl-saturated habitats is both complex and dynamic (Table 1). Rates of primary production (via photosynthesis) of 120–220 μg carbon l^{-1} d^{-1} have been reported in crystalliser-pond brines (Elevi Bardavid, Khristo and Oren 2008), and the alga *Dunaliella salina* produces (and releases) sufficient amounts of glycerol to be able to sustain communities of heterotrophs (Hart and Gilmour 1991; Elevi Bardavid and Oren 2008; Elevi Bardavid, Khristo and Oren 2008; Oren 2017; Williams *et al.*2017). Heterotrophic microbes are abundant in diverse hypersaline systems, including the haloarchaeal genera *Halobacterium* and *Halococcus* (Table 1), and the bacterium *Salinibacter ruber* which is commonly found in some NaCl-saturated habitats (Antón *et al.*2008; Cray *et al.*2013a). *Salinibacter ruber* can utilise glycerol, converting some to dihydroxyacetone (glycerone) which is excreted, and may be utilised by halophilic *Archaea* such as *Hqr. walsbyi* (Sher *et al.*2004; Elevi Bardavid and Oren 2008; Elevi Bardavid, Khristo and Oren 2008). Halophiles are known to be highly proficient at the symbiotic utilisation of various metabolites excreted by the primary producers, and the necromass produced by microbial cell turnover. This is exemplified both by the utilisation of glycerol leaked from microalgae and some deep-sea NaCl-saturated systems, where communities can be sustained by trophic networks which ultimately rely on the organic matter that migrates from the overlaying seawater (Daffonchio *et al.*2006; Yakimov *et al.*2013). A recent review of the various roles of glycerol in the context of the ecological complexity of hypersaline habitats has been carried out by Oren (2017).

The various halophiles found in hypersaline brines exhibit a wide range of metabolic capabilities. For instance, inland hypersaline alkaline lakes located in Egypt contain microalgae, cyanobacteria, anoxygenic phototrophic bacteria, denitrifying bacteria and (in sediments at least) sulphate reducers (Table 1; Imhoff, Hashwa and Trüper 1978; Oren 2011). Microalgae (such as algae of the *Mamiellaceae* family) and cyanobacteria (*Halothece* spp.), as well as halophilic bacteria and archaea (e.g. *Halorhabdus* spp. and *Halococcus* spp.), inhabit the liquids-of-deliquescence according to studies of halite rock in the Atacama Desert (Robinson *et al.*2015; Crits-Christoph *et al.*2016). Another study of halite rock demonstrated carbon fixation in the absence of light, with ammonia used as an electron donor, further exemplifying the diversity of microbial activities which take place in the communities inhabiting liquids-of-deliquescence (Davila *et al.*2015).

Sulphate-reducing bacteria, such as *Desulphobacter*, *Desulphovi-brio* and *Desulphococcus*, can be active at NaCl saturation in the clay-, silt- and sand sediments of salterns (Kerkar and Loka Bharathi 2007; Mani *et al.*2012); sulphur-oxidising chemolithotrophs of the family *Ectothiorhodospiraceae* are abundant in environments where NaCl concentration ranges from 1.7 to 4.3 M (León *et al.*2014). Together, these findings indicate active bacterial networks for the redox cycling of various sulphur species. Anaerobic iron metabolism has been demonstrated in Lake Kasin (Russia) and Lake Chaka (China) (Jiang *et al.*2006; Emmerich *et al.*2012), while archaeal methanogenesis coupled with active sulphur cycling (by epsilonproteobacterial sulphur oxidisers and deltaproteobacterial sulphate reducers) has been reported to sustain biomass-rich complex microbial communities in deep-sea brine lakes such as those in the Urania basin of the Mediterranean (Borin *et al.*2009) (N.B., in microbiology, the convention has arisen to refer to the bodies of hypersaline water located in deep-sea basins' as brine lakes', whereas geologists use the term' stratified marine brines' when referring to a body of brine which is overlaid by seawater). Active networking between sulphur-oxidising chemolithoautotrophs similar to the endosymbiont of the deep-sea geothermal vent tube worm *Riftia pachyptila*, and sulphate reducers, methanotrophs and nitrogen fixers, has been indicated by metagenomic and metatranscriptomic analyses across the chemoclines of the deep Mediterranean Sea hypersaline anoxic lake Thetis (Ferrer *et al.*2012; Pachiadaki *et al.*2014). Other studies have focused on characterising the geochemical and biological complexity of microbial ecosystems, including biogeochemical cycling in NaCl-saturated deep-sea brines (Table 1; Daffonchio *et al.*2006; Alexander *et al.*2009; Edgcomb *et al.*2009; Danovaro *et al.*2010; Ferrer *et al.*2012).

Many inland alkaline saline lakes result from evaporation of Na-Cl-CO_3-HCO_3-rich waters. These brines precipitate minerals such as halite, calcite, and Na-carbonates such as trona, and halite. Alkaline hypersaline (haloalkaline) waters such as those in Wadi An Natrun (Egypt), Lake Sambhar (Rajasthan, India) or Salton Sea (California, USA) have high NaCl contents but low alkalinity. In this way, their water chemistry differs from that of soda lakes found in arid or semi-arid zones such as North America (e.g. Mono, Big Soda and Soap lakes), Central Asia (e.g. in the SE Siberian steppe and Inner Mongolia) as well as in the East African Rift Valley (e.g. Magadi, Bogoria and Natron lakes) (Sorokin *et al.*2014). These soda lakes are characterised by elevated HCO_3^-/CO_3^{2-} concentrations. The haloalkaline Lake Sambhar is a large, inland, NaCl- and bicarbonate-dominated system where salt concentrations fluctuate depending on season and local rainfall. Total salt content within can reach up to 30% (w/v), with Na^+ at 118.1 g l^{-1}, Cl^- at 147.12 g l^{-1} and HCO_3^- 41.5 g l^{-1}. The remaining ions include other metallic ions Ca^{2+} (0.002 g l^{-1}), Mg^{2+} (0.018 g l^{-1}), K^+ (1.96 g l^{-1}), Fe (0.076 g l^{-1}) and Mn (0.0052 g l^{-1}) and anions NO_3^- (1.07 g l^{-1}) and CO_3^{2-} (33.12 g l^{-1}) (Pathak and Cherekar 2015). The pH varies depending on the salinity, typically ranging from 8.18 to 11 (Yadav, Sarin and Krishnaswami 2007; Pathak and Cherekar 2015). One study revealed 64 morphologically distinct isolates in 26% w/v NaCl samples taken from Lake Sambhar, including the extreme halophile *Natronococcus* that is known to grow up to 32% w/v NaCl (Pathak and Cherekar 2015). It is populated by haloalkaliphilic *Archaea* (Upasani and Desai 1990), *Actinobacteria* (Jose and Jebakumar 2013), several members of the phylum *Firmicutes* (Paul *et al.*2015), *Proteobacteria* (Cherekar and Pathak 2015), a number of halophilic or halotolerant bacteria (Bachani *et al.*2016), as well as phytoplankton that exhibit a range of salt tolerances (Jakher, Bhargava and Sinha 1990).

Despite their multiple stress parameters (salinity; low water activity; pH >9.5), both alkaline hypersaline lakes and soda lakes are among the most productive ecosystems, up to 7 g $C \cdot m^{-2} \cdot day^{-1}$ (Melack and Kilham 1974). Furthermore, they are inhabited by biodiverse communities of microorganisms which drive full carbon-, nitrogen-, phosphorus and sulphur C-, N-, P- and S cycles (Jones *et al.*1998; Sorokin *et al.*2014; Sorokin, Banciu and Muyzer 2015). Soda lakes have, therefore, become a rich source for novel microbes during bioprospecting studies. Some of the microbes isolated from haloalkaline brines are capable of energy generation, e.g. via elemental sulphur disproportionation, acetoclastic and hydrogenotrophic methanogenesis, synthrophic methanogenesis (Sorokin *et al.*2015, 2016a, 2017; Sorokin and Chernyh 2016), and degradation of recalcitrant polymers such as cellulose, chitin and pectin (Zhilina *et al.*2005; Sorokin *et al.*2012a and 2012b, respectively) regardless of the cellular inhibition imposed by the *in-situ* stress parameters. This said, there has been little work done on the water activity of haloalkaline environments. Whereas the NaCl concentration of hypersaline alkaline lakes can reach up to 5 *M* NaCl (e.g., in Wadi An Natrun), it is only 0.5-1 *M* in soda lakes, which contain more carbonates (~2 *M* Na_2CO_3 and $NaHCO_3$ in total). At *in situ* concentrations, sodium carbonate behaves as a weak electrolyte, and organisms inhabiting such environments require less energy for osmotic protection than organisms in NaCl-dominated, pH-neutral environments (Sorokin, Banciu and Muyzer 2015; Sorokin *et al.*2016a). Furthermore, the osmotic pressures of synthetic analogues for waters from soda lakes differ from those of pH-neutral saline lakes at equivalent Na^+ concentrations because carbonate/bicarbonate ions have a less effect than Cl^- ions (Banciu et al. 2004). Collectively, these data suggest that the water activities of soda lakes, and many haloalkaline lakes, are considerably higher than 0.755.

The microbial communities discussed above (see also Table 1) are sufficiently diverse in terms of phylogeny and ecophysiological functions to form sustainable ecosystems. Their primary producers can support heterotrophic, and other trophic, networks without requiring inputs from outside the NaCl-saturated milieu. Furthermore, culture-independent studies indicate the presence of genes that are collectively capable of completing the biogeochemical cycles that can enable the microbial ecosystem to function independently (Allen and Banfield 2005). However, the ecological interactions of halophilic microbes found in NaCl-saturated habitats are neither limited to other microorganisms nor to the immediate locality. For instance, *Dunaliella* and *Asteromonas* and other microbial biomass support high densities of *Artemia* (brine shrimp) in saltmarshes, salterns and brine lakes (Triantaphyllidis, Abatzopoulos and Sorgeloos 1998; Clegg and Trotman 2002) which, in turn, may sustain populations of aquatic insects, and birds such as flamingos, gulls and waders

(Ayadi *et al.*2004; Torrentera and Dodson 2004). Such avian species by nature have a wide home range and/or are migratory, so microbial halophiles can ultimately impact biological systems located far from their hypersaline habitat. This exemplifies how the ecosystems found within many types of NaCl-saturated environment are inherently highly fertile and yet are not self-contained; i.e. that they can provide inputs into other, remote and/or non-saline ecosystems.

NaCl-saturated brines as a hotspot for life

A NaCl-saturated body of water can act as a hotspot for life within in an otherwise low-biomass, low-biodiversity environment. Recent studies of NaCl-dominated lakes in Western Australia, for instance, exemplify this well; these lakes are located in arid, nutrient-poor regions characterised by sandflats and sand dunes. Even those lakes with a low pH (that are hostile due to both extreme acidity and the presence of ions that can be toxic to life) are populated by microbes and are surrounded by plants (including two species of *Eucalyptus* and *Enchylaena tomentosa*, the ruby saltbush), with animal populations also in evidence (Benison 2006). Moreover, the pH-neutral lakes (including those at NaCl saturation) are extremely rich in microbial biomass and diversity, with some also containing dense populations of *Artemia* as well as *Coxiella* gastropods and other invertebrates, and are surrounded by an abundance of plant and animal life including insects and spiders, snakes, emus and other birds, and mammals such as dingoes and kangaroos (Benison 2006).

The NaCl-saturated brines of Death Valley also support a biodiverse ecosystem, including plants of the genus *Salicornia*, some of which can grow while their roots are in NaCl-saturated brine, and gastropods such as the Badwater snail, *Assimnea infirma*. Incredibly, a fish *Cyprinodon salinus* (the pupfish) can also survive at molar concentrations of NaCl (Schoenherr 1992). In addition, many mammal, reptile, bird and insect species live near and interact with the saline waters within the valley (Bryan and Tucker-Bryan 2009). In terms of microbiology and, in many cases flora and fauna, many other types of NaCl-saturated brines act as oases for life in regions which are generally barren (Table 1; Daffonchio *et al.*2006; Crits-Christoph *et al.*2016; Davila *et al.*2015; Robinson *et al.*2015; Çınar and Mutlu 2016).

Species dominance within the open habitats created by NaCl-saturated brines

In some NaCl-rich environments, there is a shift in microbial habitability—a selection for extreme halophiles—at ~3.4 M NaCl. This is evidenced, for instance, by shifts in community structure in solar salterns, as inferred from the relative percentages of the various phylum- or class-level taxa present (Ventosa *et al.*2015, and references therein). In a study of a series of saltern ponds at Santa Pola (Spain), those with lower NaCl concentrations were populated by taxa including *Euryarchaeota* (~46% of the community), *Alphaproteobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Deltaproteobacteria* and *Firmicutes* (Ventosa *et al.*2015). Those with 3.6–5 M NaCl were populated primarily by members of the *Euryarchaeota* (80–90% of the community) and *Bacteroidetes* (9–10%). How the two dominant phyla manage to sustain viable ecosystems (for instrance, via what syntrophic synergies) is a topic worthy of future exploration. Shifts in microbial community composition from moderate- (<3.5 M) to high-salt (>3.5 M) conditions might be governed by a combination of several factors including the availability of light and, perhaps, oxygen, that simultaneously select for dominance of aerobic or microaerophilic haloarchaea and *Bacteroidetes* in hypersaline Santa Pola salterns. Impacts relating to light availability may be due to the albedo effect that can influence how much solar radiation is retained by shallow waters, according the colour of the underlying sediment. If the water is on a white salt, for example, the light is readily reflected by the sediment. For waters that are underlain by darker materials, such as a black mud or dark microbial biomass, more light is absorbed and so is retained within the water body. Abundant chemical precipitation of minerals can also make water turbid. For example, if a salt lake is precipitating clay minerals or iron oxides (which happens in Western Australian acid brine lakes), then the water gets cloudy temporarily, and light would not penetrate the water column as well.

A reverse trend was noted along the salinity gradient in a meromictic hypersaline lake in Romania. *Euryarchaeota* dominated (~90% relative abundance) the communities dwelling in the photic, oxic upper water-layer (20–25% total salinity), whereas the aphotic, anoxic and hypersaline bottom brine (~35% total salinity) was the niche preferred by anaerobic halophilic bacteria (*Deltaproteobacteria*, *Clostridia*, *Spirochaetes*, *Bacteroidetes* and *Actinobacteria*) in an overall abundance of ~75%. Under such conditions, the haloarchaea became secondary (~20%), and were mostly represented by *Halorhabdus* spp. (Andrei *et al.*2015). The structuring of microbial diversity at a global scale, however, may be driven primarily by salinity, and also by habitat type (water versus sediment) and lifestyle (free-living versus host-dependent) (Lozupone and Knight 2007; Auguet, Barberan and Casamayor 2010), and hypersaline sediments are habitats that can exhibit a phylogenetically diversity that is amongst the highest on

Earth (Table 1; Daffonchio *et al.*2006; Lozupone and Knight 2007).

Some types of NaCl-saturated environments, such as salterns, represent open habitats that are characterised by high levels of competition between different microbes; they are resource rich and are available for colonisation, and are habitable, by a large diversity of microbes (Table 1; Hallsworth 1998; Cray *et al.*2013a; Oren and Hallsworth 2014; Lievens *et al.*2015). For a more detailed definition of open habitats, see Cray *et al.* (2013a). In addition, hypersaline systems in which NaCl concentration varies (due to processes such as ingress of freshwater through rivers or streams, precipitation and solar evaporation) are also open habitats for microbes during their transitions between different salt concentrations.

Such transitions can promote competition (Řeháková *et al.*2009) and, under these conditions, some halophiles reach high cell densities and become dominant species within the community; these species are known as ‘microbial weeds’ (Cray *et al.*2013a; Oren and Hallsworth 2014). In solar salterns, weed species include *D. salina*, *S. ruber* and *Hqr. walsbyi* (Cray *et al.*2013a), and can even dominate in low-temperature hypersaline systems such as Deep Lake in the Antarctic (DeMaere *et al.*2013). In this environment, the mean temperature is ~ −20°C and yet haloarchaea, such as *Halohasta litchfieldiae* strain tADL which comprises 44% of the population, are still able to outcompete and outgrow potential competitors (Williams *et al.*2014). Paradoxically, the halophilic archaea isolated from this lake are all mesophiles, with optimum growth temperatures of around 30°C. *Haloferax mediterranei* also has many characteristics of a weed species and, whereas it does not dominate microbial communities in saltern brines (Oren and Hallsworth 2014), it can do so in high NaCl sediments (Youssef, Ashlock-Savage and Elshahed 2012). Microbial weed species, therefore, may simultaneously cause some reduction in species diversity and an increase overall cell density (due to their own abundance). Whereas microbial weeds can in this way act as ‘mavericks’, the ecology of NaCl-saturated systems typically function in both a self-regulating and sustainable manner.

CELLULAR ADAPTATIONS THAT ENABLE HIGH RATES OF BIOTIC ACTIVITY AT 5 M $NaCl$

Strategies to overcome high concentrations of salts

Halophile systems are readily able to function at high concentrations of NaCl because they have many adaptations to enable salt tolerance, and the water activity remains permissive for life. These adaptations are detailed in reviews by Grant (2004), Balashov *et al.* (2005), Oren (2013), Cray *et al.* (2013a) and Lebre, Maayer and Cowan (2017). Adaptations of halophiles to high salinity in the extracellular milieu include several mechanisms to balance the osmotic stress induced by such conditions. Indeed, for some halophilic microbes, NaCl-saturated brines may not significantly stress the cellular system (Stevenson *et al.*2015b; Hallsworth 2018). A number of strategies adopted by halophiles to balance osmotic stress caused by high salt concentrations have been investigated, which allow classifying halophiles into either being 'salt-in', including halophilic archaea of the class *Halobacteria* and a small number of halophilic bacteria, or 'salt-out' represented by halophilic methanogenic archaea, most halotolerant/philic bacteria and all halophilic eukaryotes. Both these strategies involve a number of molecular adaptations that make it possible to adjust to the osmotic conditions of saline environments. Halophiles adopting salt-in strategies balance the low water activity of the extracellular milieu by accumulating compensatory amounts of K^+ sometimes accompanied by minor amounts of Na^+ ions (Oren 2008). In contrast, the salt-out strategy employed by most halophilic bacteria involves mechanisms for excluding salt from the cellular environment or the synthesis of compatible solutes, such as glycine betaine (i.e. N-trimethylated glycine) for bacteria and glycerol for eukaryotes (Roberts 2005; Youssef *et al.*, 2014). Arguably, many extreme halophiles accumulate ions rather than organic compatible solutes for this purpose in order to conserve energy (Oren 1999).

In hypersaline environments, most proteins from non-halophilic microbes are inhibited due to the low water activity and/or high ionic strength, and are typically also denatured and aggregate and/or precipitate (Baldwin 1984; Li, Zhou and Mu 2012; Alves *et al.*2015). However, the extracellular and cytosolic enzyme systems of some halophiles can work optimally at NaCl concentrations as high as 5 M or concentrations of K^+ that are osmotically equivalent, respectively. Intracellular proteins of salt-in species exhibit increased hydrophilicity and charge allowing them to work at very high salt concentrations. Several studies, including those that first sequenced *Halobacterium* sp. NRC-1 (Reistad 1970; Ng *et al.*2000) and those that resolved the structure of halophilic proteins (Dym, Mevarech and Sussman 1995; Kennedy *et al.*2001; DasSarma *et al.*2006), revealed that, for some halophiles, their proteins contain few basic amino acids (relative to their orthologs in non-halophilic proteins), but are enriched in the acidic amino acids, glutamic acid and aspartic acid. They also revealed that halophilic proteins of ‘salt-in’ strategists (Bolhuis, Kwan and Thomas 2008; Capes, DasSarma and DasSarma 2012) have more negative charges on their surfaces than comparable proteins from non-halophiles. Structural studies have also revealed that increasing solvation for halophilic enzymes makes them capable of performing their activities at (or below) 0.755 water activity (Karan, Capes and DasSarma 2012).

Indeed, some enzyme systems remain functional close to a water-activity value of zero (from microbes as well as other organisms; e.g. Dunn and Daniel 2004; Kurkal *et al.*2005; Lopez *et al.*2010). It is the norm in biology that individual components of a cellular or organismal system exhibit their optimal performance (and have maximum and minima for function) at different values of water activity and other biophysical parameters (Hallsworth 2018). The increase of hydrogen bonds between protein-surface acidic amino acids and water molecules, and the reduction of hydrophobic patches, enhance both structure and function of enzymes at high salt concentrations (Dym, Mevarech and Sussman 1995; Britton *et al.*2006; DasSarma and DasSarma 2015). The dynamics of protein folding determined through incoherent neutron spectroscopy of the extreme halophile *Halobacterium salinarum* demonstrated that a large proportion of the proteins in the proteome is strongly dependent on high salt concentrations: cellular vitality becomes compromised at K^+ concentrations below 2.2 M, with many proteins misfolding, precipitating and forming aggregates (Vauclare *et al.*2015).

In general, the acidity of cellular proteins correlates with the salinity of the environment in which halophiles live. For example, the genomes of the extreme halophiles *Acetohalobium arabaticum* and *S. ruber* exhibit extremely acidic proteomes (DasSarma and DasSarma 2015). In general, acidic proteomes are found in salt-in strategists, i.e. the most-extreme halophiles typically have a more acidic proteome. Moderate halophiles and very halotolerant species (e.g. representatives of *Halomonadaceae* family) are typically salt-out strategists with a less acidic proteome (Coquelle *et al.*2010; Schwibbert *et al.*2011; Oren 2013). For salt-in halophiles, the presence of acidic amino acids at protein surfaces correlates with cellular tolerance to salinity. This enables the use of mutations in genes which encode protein-surface amino acids, resulting in the conversion of a halophilic enzyme into a non-halophilic one, demonstrated empirically with the DNA ligase of *Haloferax volcanii* (Tadeo *et al.*2009), *Halobacterium* diphosphate kinase (Ishibashi *et al.*2012), ProtL from *Peptostreptococcus magnus* (Qvist *et al.*2012), and more recently in the protein β-trefoil (Longo *et al.*2015). In conclusion, the ability of halophiles to function in conditions with moderately low water activities (down to ~0.755) is based on the evolution of enzymes that are stable, and work optimally, in these conditions. This hyperstability has evolved through specific modifications of the surface of the enzymes to establish stable hydrogen bonds with water molecules and reduce the exposed hydrophobic patches (Winter *et al.*2009).

At saturation, NaCl is permissive for the activity of extracellular enzyme systems of extreme halophiles, as evidenced by the functional diversity of enzymes observed in hypersaline environments and the well-characterized adaptations of these proteins (Ferrer *et al.*2005, 2012; Borin *et al.*2009; Pachiadaki *et al.*2014). For instance, an extracellular protease from a saltern bacterium was found to be dependent on NaCl for its functionality, and exhibited optimum catalytic activity at 4.5 M NaCl (Vidyasagar *et al.*2009). Another protease, from *Haloferax lucentense*, exhibited a similar NaCl optimum, and also retained high activity at NaCl saturation (Manikandan, Pašić and Kannan 2009). *Halobacterium* strains are also known to produce extracellular proteases which function optimally close to NaCl saturation (Norberg and Hofsten 1968), and halophilie communities are a source of industrially important enzymes which are able to function under various extremes (Ferrer *et al.*2005; Kumar *et al.*2012; Alsafadi and Paradisi 2013; Moreno *et al.*2013).

The genes for these highly adapted proteins do not appear to be confined to individual strains and species. Genome sequencing has revealed that high levels of horizontal gene transfer occur between members of natural consortia of halophilic archaea (DeMaere *et al.*2013), and the transfer of large DNA segments (310–530 kb) between different species of *Haloferax* has been observed in laboratory-based experiments (Naor *et al.*2012). Recently, a membrane vesicle-mediated DNA-transfer mechanism has been discovered in *Halorubrum lacusprofundi* (Erdmann *et al.*2017). This means that genes for halophilic proteins that confer an ecological advantage do not need to evolve by the relatively slow process of mutation and selection, *in situ*, but can spread readily between diverse members of a microbial community.

Other adaptations of halophiles

In addition to osmoadaptive strategy, other survival mechanisms are exhibited by extreme halophiles. For example, sequestration of resources via intracellular storage improves competitive ability and enables tolerance of prolonged periods without carbon substrate, phosphate, etc (Cray *et al.*2013a; Oren and Hallsworth 2014). Some members of the Halobacteria store polyhydroxyalkanoates in the form of intracellular granules (Saponetti *et al.*2011), while *Dunaliella* species can accumulate triacylglycerols as an overflow product of photosynthesis (Chen *et al.*2011). Extreme halophiles can also have adaptations which enhance the generation of cell-available energy, such as light-activated proton pumps, that use light to generate a proton motive force, and have been found in species such as *S. ruber* and *Hqr. walsbyi* (Balashov *et al.*2005; Cray *et al.*2013a). Some species such as *Hqr. walsbyi* and *Hfx. mediterranei* utilise gas vesicles, which facilitate buoyancy and may also maintain the horizontal orientation of the cell thereby optimizing light capture; this may also enhance oxygen acquisition by maintaining the cell's position within the oxygenated regions of stratified brines (Englert, Horne and Pfeifer 1990; Bolhuis *et al.*2006; Cray *et al.*2013a, Oren and Hallsworth 2014). Halophiles can be exposed to high levels of ultraviolet light, and some species such as *D. salina*, *S. ruber* and most haloarchaea synthesise and accumulate high concentrations of carotenoids, thereby protecting their macromolecular systems from ultraviolet radiation (Antón *et al.*2002; Chen and Jiang 2009). It should be noted that some stresses experienced by, and cellular adaptations of, halophiles are not unique to these microbes. Indeed, cellular stress is the norm for all microbial systems and many sources of stress impact the cell via via the same mechanism, regardless of microbial species or habitat type (Hallsworth 2018).

Recently, a number of studies have utilised (culture-independent) analytical techniques to identify metabolites of halophilic microbes, including mass spectrometry coupled with gas or liquid chromatography and/or capillary electrophoresis (e.g. Kido Soule *et al.*2015; Johnson, Kido Soule and Kujawinski 2017). The comprehensive power of these technologies should in future reveal as-yet-unidentified compounds or strategies underlying the cellular phenotype of extreme halophiles.

EVOLUTIONARY BIOLOGY OF HALOPHILES; IMPLICATIONS OF THE FINITE SOLUBILITY OF $NaCl$

For all types of environments (saline or not), habitability by microbes is constrained or prevented by one or more factors; these are usually nutritional, physicochemical and/or biophysical. Environmental parameters—including temperature, water activity, irradiance, ultraviolet radiation, pH, chaotropicity and availability of electron donors or nitrogen substrates—determine biodiversity, abundance, overall biomass and ecosystem function. To put it simply, ecosystems (and the biosphere) are shaped and restricted by available resources and prevailing conditions. And yet, there are few microbial ecosystems in aquatic environments which appear to outperform NaCl-rich microbial habitats. Just as tropical rainforest is considered the pinnacle of biomass and biodiversity when compared with the other plant biomes (Pan *et al.*2013), the halophile communities of solar salterns and deep-sea brines may represent the greatest attainment of aquatic ecosystems in relation to overall metabolism and cell densities. By contrast, in ethanol-producing fermentations, the microbial community is biomass- and diversity limited due to the chaotropicity of ethanol, low pH and low water activity (Cray *et al.*2015). Whereas NaCl-saturated environments clearly select for halophiles, the growth rates, cell densities and microbial abundance of these inhabitants are remarkably high (Table 1, Fig. 2A–E). This is because the NaCl concentration limit is not determined by biology (as discussed above, 5 M NaCl is not the theoretical maximum concentration at which the cellular systems of extreme halophiles can function), but by a quirk of chemistry. The dipoles of water molecules attract ions more than Na and Cl ions attract each other, and these ions (especially Na ions) attract water more than water molecules attract each other, hence the kosmotropicity of NaCl (Cray *et al.*2013b; Alves *et al.*2015). At the saturation point, however, most of the water is interacting with these ions so it becomes more thermodynamically favourable for additional ions to bond with each other; i.e. precipitation of NaCl occurs (Fig. 2f).

The empirically determined and theoretical water-activity limits for fungal cell division and those for growth limits for halophilic prokaryotes (0.585 and 0.565, and 0.635 and 0.611, respectively) are equivalent to a theoretical NaCl concentration of up to 8 M (equivalent to 47%, w/v). This is consistent with the thesis that 5 M NaCl provides mid-range (i.e. moderate) conditions for numerous halophiles (Fig. 2A–E; Stevenson *et al.*2015b). Whereas specific temperatures or ethanol- or $MgCl_2$ concentrations act as barriers to life, there is no threshold value at which NaCl prevents microbial habitation (Figs 1A, B and 2). At NaCl saturation, therefore, there is considerable thermodynamic leeway for extreme halophiles before the theoretical failure of the cellular system is reached.

Whereas the finite solubility of NaCl does not curtail ecosystem development, the biology of some saturated brines may be constrained by the interactive effects of other limiting factors. These include nutrient deficiency (e.g. scarcity of phosphate in hypersaline brines can restrict the growth of phototrophs such as *Dunaliella*; Gasol *et al.*2004; Oren 2017); high pressure and extremes of pH or temperature (Harrison *et al.*2013); high irradiance (Wieland and Kühl 2000); or high concentrations of chaotropic ions (see above) or toxic metals, such as arsenic, though there are some halophile communities that function in arsenic-containing alkaline brines (Oremland and Stolz 2003; Oremland *et al.*2005). Populations of *Bacteria* and *Archaea* can be constrained by the activities of viruses and/or grazers; for instance, bacteriophage-induced lysis and nanoflagellate grazing have been identified as key factors reducing prokaryote abundance in Spanish crystalliser ponds and Korean salterns, respectively (Guixa-Boixareu *et al.*1996; Park *et al.*2003; Cray *et al.*2013a). The ecological importance of viruses within halophile communities was highlighted in a study which successfully assembled several viral genomes from the metagenome of crystalliser pond CR30 of Santa Pola salterns (Garcia-Heredia *et al.*2012). On the basis of shared CRISPR spacer sequences, some of these viruses were found to prey upon *Hqr. walsbyi*. Those salterns with 3.6–5 M NaCl were populated primarily by members of the Euryarchaeota or Nanohaloarchaeota (making up to 80–90% of the community and mostly represented by *Hqr. walsbyi* and *Nanohaloarchaea*) and Bacteroidetes (9–10%; mainly *Salinibacter ruber*) (Garcia-Heredia *et al.*2012), so their phage associations further underscored that viruses are ubiquitous within, and central to the ecologies of, NaCl-saturated habitats.

Novel halophilic methanogenic archaea have been isolated from the anaerobic sediments of alkaline hypersaline lakes, soda lakes and pH-neutral hypersaline lakes (Sorokin *et al.*2017a). The candidate 'Methanohaloarchaea' (HMET) and 'Methanonatronoarchaea' (AMET) strains were capable of methylotrophic methanogenesis at slightly thermophilic (50–60°C) and either saturated NaCl (pH 7) or soda (pH 9.6) conditions, respectively. These strains belong to 'Methanonatronoarchaeia' (former SA1 clade), a distinct class-level deep phylogenetic group within Euryarchaeota possessing the salt-in strategy. Further phylogenomic analyses have suggested that a common halophilic, methanogenic ancestor might have diverged into 'Methanohaloarchaeia' (by gene loss; Spang, Caceres and Ettema 2017; Sorokin *et al.*2017a) and Halobacteria (by massive interdomain gene transfer from aerobic bacteria; Nelson-Sathi *et al.*2012, 2015; Sousa, Nelson-Sathi and Martin 2016). Although the Halobacteria appear to be comprised primarily of aerobic and chemoorganoheterotrophs with a halophilic lifestyle, a few are facultatively anaerobic (Hattori *et al.*2016) or fermenting (Antunes *et al.*2008). However, recent studies allude to greater metabolic capabilities within this group. *Halanaeroarchaeum sulphurireducens*, for example, is a strictly anaerobic, sulphidogenic haloarchaeon that utilises acetate and pyruvate while reducing elemental sulphur (Sorokin *et al.*2016b, c), whereas *Halodesulphurarchaeum formicicum* is the first evidenced lithotrophic

Halobacteria member that uses H_2 or formate as an electron donor and reduced sulphur compounds (thiosulphate, dimethylsufides and S^0) as electron acceptors (Sorokin *et al.*2017b).

The finite solubility of NaCl raises a number of questions in relation to the evolutionary biology of halophiles. In particular, it remains to be investigated how evolvable halophiles are in their natural environments. Because of the strong selective constraints imposed by the requirement for highly acidic proteins, the fixation rate of mutations in halophiles is expected to be low. In fact, the mutation rate of some halophiles, such as *Hfx. volcanii*, is lower than that of microbes that are not halotolerant or halophilic (Mackwan *et al.*2007; Busch and DiRuggiero 2010). Since the fixation rate of mutations is much lower than the mutation rate, it is reasonable to assume a lower fixation rate of mutations for halophiles than for most other microbes. Mutation rates in thermophiles are also reported to be lower than in those for mesophiles (Friedman *et al.*2004; Kissling, Grogan and Drake. 2013), and it has similarly been argued that the functional constraints are much higher for thermophilic than for mesophilic proteins. The greater-than-expected genetic diversity seen in Halobacteria, such as the genera *Halorubrum* and *Haloarcula*, is produced mainly by horizontal gene transfer and homologous recombination (Naor *et al.*2012; DeMaere *et al.*2013; Mohan *et al.*2014). This genome-level diversity could in principle generate a reservoir of genomic variants that can be maintained phenotypically cryptic in the population, but with enormous potential to originate adaptations to other environmental perturbations. In NaCl-saturated environments, selection becomes stronger against many halotolerant (and some halophilic) microbes allowing the persistence of only those genetic variants able to function optimally at 0.755 water activity. However, NaCl is not sufficiently soluble to reduce water-activity values to the 0.755–0.585 range experienced by some extremophiles. Those salts which are sufficiently soluble (e.g. $MgCl_2$) are so chaotropic that the cellular systems of most halophiles are inhibited by their chaotropicity rather than reduced water activity. If genetic variants arose that could grow at water activities below 0.755, even to 0.585, they are not likely to be any fitter than existing species in natural hypersaline environments, and without positive selection are unlikely to maintain or increase their frequency. At the water-activity limit for life, molecular crowding within the cytoplasm and/or inadequate hydration or flexibility of macromolecular systems may act to prevent cellular function (Fields 2001; Zaccai 2004; Miermont *et al.*2013; Parry *et al.*2014). It has yet to be demonstrated that such factors are indeed limiting in NaCl-saturated brines at 0.755 water activity.

IMPLICATIONS FOR ASTROBIOLOGY AND CONCLUDING REMARKS

There are very specific concentrations/proportions of NaCl-$MgCl_2$ or NaCl-$MgCl_2$-glycerol mixtures that permit the proliferation of halophiles below 0.755 water activity (Stevenson *et al.*2015a,b). The highest concentrations of NaCl that can be experienced by the cells of halophiles, 5 M, enables optimum rates of metabolism and growth for some populations, and facilitates the development of biomass dense- and highly active ecosystems. Therefore, we conclude that the environments with NaCl concentrations of ~5 M are not thermodynamically limiting and, thus, this concentration does not represent a biophysical limit for life on Earth. Given the high cell densities and broad functional diversity of many hypersaline brine systems, it is intriguing to ask why their microbial diversity is not even higher. Can this be explained as a product of dominance by a small number of halophile weed species, or are there other factors at play? The solution chemistry of NaCl raises additional questions pertinent to the field of astrobiology:

i. Why are there no truly psychrophilic haloarchaea?

ii. Whereas chaotropic brines may be more readily habitable at subzero temperatures (Ball and Hallsworth 2015), would the evolutionary trajectories of terrestrial halophiles, which are not usually exposed to habitable brines with water activities much below 0.755, make them unable to function in chaotropic brines on Mars or Europa (Hallsworth *et al.*2007; Chin *et al.*2010; Rummel *et al.*2014)?

iii. Would the diverse solution chemistries found on different planetary bodies (e.g. Muñoz-Iglesias, Bonales and Prieto-Ballesteros 2013; Fox-Powell *et al.*2016) result in qualitative differences in the evolution of terrestrial halophiles and their ability to tolerate multiple extremes not found on Earth, and could terrestrial, extreme halophiles tolerate the high ionic strength which characterises some martian brines (Fox-Powell *et al.*2016)?

iv. Diverse NaCl stoichiometries have been reported at high pressures and temperatures (i.e. 200 000 atmospheres and 2000 K), resulting in the formation of stable salts such as $NaCl_3$ (Zhang *et al.*2013). Such salts may be formed during accretion and deep within a planetary or moon body, but only small amounts would be present at or near the surface. Whereas, they may not exist at high concentrations under conditions which are pertinent to (known forms of) life, this finding does raise the question: Do sodium chlorides, that are more soluble than NaCl, reduce water activity to a lower value and exhibit chaotropic activity?

v. Given that saturated NaCl is not stressful for some halophile systems, would these species act as pioneers in the event that NaCl-rich sites on other planetary bodies are contaminated during space exploration missions?

The evolution of life on early Earth is thought to have taken place in extreme aqueous environments such as hydrothermal vents and acid-brine oceans. Hypersaline brines have remained a stable habitat throughout the planet's geological history (Warren 2010), and Buick and Dunlop (1990) describe evaporite deposits from 3.5 Ga Warrawoona Group of Western Australia, confirming the presence of brine surface environments at the time of some of the first life on Earth. Diverse lines of evidence suggest that terrestrial life may have inhabited, and could have even arisen in, brines with a high NaCl, KCl and/or $MgCl_2$ concentration (e.g. Dundas 1998; Stevenson *et al.*2015a; Matveev 2017). Water activity and chaotropicity are likely to have been key determinants for both habitability and the evolution of early cells in relation to early life on Earth (Stevenson *et al.*2015a).

The likelihood that early life was energised by electrochemical Na^+ gradients has been discussed by Price *et al.* (2017), who revealed steep natural gradients (i.e. 4–52 mM Na^+ in the venting liquids versus 468 mM Na^+ in the surrounding sea water) across thin mineral layers, figuratively termed ‘membranes’, within the pores of serpentinite located in shallow sea water. The cell-sized serpentinite pores percolated by the alkaline, reducing (H_2- and CH_4-rich) fluids which contain Na^+ at concentrations one to two orders of magnitude less than in sea water, may have acted as incubators for the development of protocells on the bottom of the Hadean oceans (Sojo *et al.*2016; Price *et al.*2017).

In relation to astrobiology, the occurrence of halophily throughout the phylogenetic tree suggests that it is relatively easy to evolve, and that it evolved more than once. If life evolved in locations beyond Earth, and where salty brines occur, then there would almost certainly be the evolution of halophilic organisms (Mancinelli 2005a,b). There is strong evidence for the past existence of acid brines on Mars (Squyres *et al.*2004; Benison and Bowen 2006). Mars hosts abundant saline minerals, including chlorides (likely halite) and hydrated calcium sulphates (likely gypsum) in lake deposits and lake-derived eolian deposits (Glotch *et al.*2010). The presumed acid- and brine nature of past Mars surface waters have led to speculation that Mars would be inhospitable to life (Tosca, Knoll and McLennan 2008; Rummel *et al.*2014). However, in light of the findings of this study, halophiles may potentially be able to evolve the ability to proliferate at, or below, 0.585 water activity. Therefore, the possibility that such microbes can potentially contaminate saline milieus present on Mars in this water activity range should not be discounted. The findings reported in the current article also expose a need for new experimental and theoretical approaches, which circumvent the apparent limits for life which are imposed merely by solution chemistry, to identify and characterise the thermodynamic constraints of halophilic life.

Acknowledgements

Useful discussion was provided by Josefa Antón (University of Alicante, Spain), Philip Ball (East Dulwich, UK), Catherine Conley (NASA HQ, USA), Terry J. McGenity (University of Essex, UK), Beatriz Sabater-Muñoz (Instituto de Biología Molecular y Celular de Plantas [CSIC-UPV], Spain), Sohini Banerjee (Visvabharati University, India), R. Thane Papke (University of Connecticut, CT, USA), and John D. Rummel (SETI Institute, USA).

FUNDING

This work was supported by Natural Environment Research Council (NERC), United Kingdom (grant number NE/E016804/1).

Conflicts of interest. None declared.

REFERENCES

Aletsee L, Jahnke J. Growth and productivity of the psychrophilic marine diatoms *Thalassiosira antarctica* Comber and *Nitzschia frigida* Grunow in batch cultures at temperatures below the freezing point of sea water. *Polar Biol* 1992;11:643–7.

Google Scholar Crossref

Alexander E, Stock A, Breiner HW et al. Microbial eukaryotes in the hypersaline anoxic L'Atalante deep-sea basin. *Environ Microbiol* 2009;11:360–81.

Google Scholar Crossref PubMed

Allen EE, Banfield JF. Community genomics in microbial ecology and evolution. *Nat Rev Microbiol* 2005;3:489–98.

Google Scholar Crossref PubMed

Alsafadi D, Paradisi F. Effect of organic solvents on the activity and stability of halophilic alcohol dehydrogenase (ADH2) from *Haloferax volcanii*. *Extremophiles* 2013;17:115–22.

Google Scholar Crossref PubMed

Alves FDL, Stevenson A, Baxter E et al. Concomitant osmotic and chaotropicity-induced stresses in *Aspergillus wentii*: compatible solutes determine the biotic window. *Curr Genet* 2015;61:457–77.

Google Scholar Crossref PubMed

Anderson H. The reddening of salted hides and fish. *Appl Microbiol* 1954;2:64–69.

Google Scholar PubMed

Andrei A-Ş, Baricz A, Păuşan M et al. Spatial distribution and molecular diversity of archaeal communities in the extreme hypersaline meromictic Brâncoveanu Lake (Transylvanian Basin, Romania). *Geomicrobiol J* 2017;34:130–8.

Google Scholar Crossref

Andrei A-Ş, Robeson MS, Baricz A et al. Contrasting taxonomic stratification of microbial communities in two hypersaline meromictic lakes. *ISME J* 2015;9:2642–56.

Google Scholar Crossref PubMed

Auguet JC, Barberan A, Casamayor EO. Global ecological patterns in uncultured Archaea. *ISME J* 2010;4:182–90.

Google Scholar Crossref PubMed

Antón J, Oren A, Benlloch S et al. *Salinibacter ruber* gen. nov., sp. nov., a novel, extremely halophilic member of the *Bacteria* from saltern crystallizer ponds. *Int J Syst Evol Microbiol* 2002;52:485–91.

Google Scholar Crossref PubMed

Antón J, Peña A, Santos F et al. Distribution, abundance and diversity of the extremely halophilic bacterium *Salinibacter ruber*. *Saline Syst* 2008;4:15.

Google Scholar Crossref PubMed

Antón J, Roselló-Mora R, Rodríguez-Valera F et al. Extremely halophilic bacteria in crystallizer ponds from solar salterns. *Appl Environ Microbiol* 2000;66:3052–7.

Google Scholar Crossref PubMed

Antunes A, Taborda M, Huber R et al. *Halorhabdus tiamatea* sp. nov., a non-pigmented extremely halophilic archaeon from a deep-sea, hypersaline anoxic basin of the Red Sea, and emended description of the genus *Halorhabdus*. *Int J Syst Evol Microbiol* 2008;58:215–20.

Google Scholar Crossref PubMed

Armolik N, Dickson JG. Minimum humidity requirement for germination of conidia of fungi associated with storage of grain. *Phytopathology* , 1956;46:462–5.

Ayadi H, Abid O, Elloumi J et al. Structure of the phytoplankton communities in two lagoons of different salinity in the Sfax saltern (Tunisia). *J Plankton Res* 2004;26:669–79.

Google Scholar Crossref

Ayerst G. The effects of moisture and temperature on growth and spore germination in some fungi. *J Stored Prod Res* , 1969;5:127–41.

Google Scholar Crossref

Baati H, Guermazi S, Gharsallah N et al. Prokaryotic diversity of a Tunisian multipond solar saltern. *Extremophiles* 2008;12:505–18.

Google Scholar Crossref PubMed

Baati H, Guermazi S, Gharsallah N et al. Microbial community of salt crystals processed from Mediterranean seawater based on 16S rRNA analysis. *Can J Microbiol* 2010;56:44–51.

Google Scholar Crossref PubMed

Baati H, Jarboui R, Ghasallah N et al. Molecular community analysis of magnesium-rich bittern brine recovered from a Tunisian solar saltern. *Can J Microbiol* 2011;57:975–81.

Google Scholar Crossref PubMed

Bachani P, Bhattacharya S, Jain D et al. Bioprospecting of halotolerant bacterial isolates for potassium recovery from K-feldspar. *Chem Eng Technol* 2016;39:1645–52.

Google Scholar Crossref

Bakermans C, Skidmore M. Microbial respiration in ice at subzero temperatures (−4°C to −33°C). *Environ Microbiol Rep* 2011;3:774–82.

Google Scholar Crossref PubMed

Balashov SP, Imasheva ES, Boichenko VA et al. Xanthorhodopsin: a proton pump with a light-harvesting carotenoid antenna. *Science* 2005;309:2061–4.

Google Scholar Crossref PubMed

Baldwin RL. How Hofmeister ion interactions affect protein stability. *Biophys J* 1996;71:2056–63.

Google Scholar Crossref PubMed

Ball JP, Hallsworth JE. Water structure and chaotropicity: their uses, abuses and biological implications. *Phys Chem Chem Phys* 2015;17:8297–305.

Google Scholar Crossref PubMed

Banciu H, Sorokin DY, Galinski EA et al. *Thialkalivibrio halophilus* sp. nov., a novel obligately chemolithoautotrophic, facultatively alkaliphilic, and extremely salt-tolerant, sulfur-oxidizing bacterium from a hypersaline alkaline lake. *Extremophiles* 2004;8:325–34.

Google Scholar Crossref PubMed

Baricz A, Coman C, Andrei A-Ş et al. Spatial and temporal distribution of archaeal diversity in meromictic, hypersaline Ocnei Lake (Transylvanian Basin, Romania). *Extremophiles* 2014;18:399–413.

Google Scholar Crossref PubMed

Baricz A, Cristea A, Muntean V et al. Culturable diversity of aerobic halophilic archaea (Fam. *Halobacteriaceae*) from hypersaline, meromictic Transylvanian lakes. *Extremophiles* 2015;19:525–37.

Google Scholar Crossref PubMed

Bell AN, Magill E, Hallsworth JE et al. Effects of alcohols and compatible solutes on the activity of β-galactosidase. *Appl Biochem Biotech* 2013;169:786–94.

Google Scholar Crossref

Benison KC. Life and death around acid-saline lakes. *Palaios* 2008;23:571–3.

Google Scholar Crossref

Benison KC, Bowen BB. Acid saline lake systems give clues about past environments and the search for life on Mars. *Icarus* 2006;183:225–9.

Google Scholar Crossref

Benison KC, Bowen BB, Oboh-Ikuenobe FE et al. Sedimentology of acid saline lakes in southern Western Australia: newly described processes and products of an extreme environment. *J Sediment Res* 2007;77:366–88.

Google Scholar Crossref

Benlloch S, Acinas SG, Martínez-Murcia AJ et al. Description of prokaryotic biodiversity along the salinity gradient of a multipond solar saltern by direct PCR amplification of 16S rDNA. *Hydrobiologia* 1996;329:19–31.

Google Scholar Crossref

Bernhard JM, Kormas K, Pachiadaki M et al. Benthic protists and fungi of Mediterranean deep hypsersaline anoxic basin redoxcline sediments. *Front Microbiol* 2014;5:605.

Google Scholar Crossref PubMed

Bhaganna P, Volkers RJM, Bell ANW et al. Hydrophobic substances induce water stress in microbial cells. *Microbial Biotechnol* 2010;3:701–16.

Google Scholar Crossref

Bolhuis H, Palm P, Wende A et al. The genome of the square archaeon *Haloquadratum walsbyi*: life at the limits of water activity. *BMC Genomics* 2006;7:169.

Google Scholar Crossref PubMed

Bolhuis A, Kwan D, Thomas JR. Halophilic adaptations of proteins. In Sidiqui KS, Thomas T (eds). *Protein Adaptation in Extremophiles* . New York, USA: Nova Science Publishers Inc., 2008, 70–104.

Borin S, Brusetti L, Mapelli F et al. Sulfur cycling and methanogenesis primarily drive microbial colonization of the highly sulfidic Urania deep hypersaline basin. *Proc Natl Acad Sci USA* 2009;106:9151–6.

Google Scholar Crossref PubMed

Bowen BB, Benison KC. Geochemical characteristics of naturally acid and alkaline saline lakes in southern Western Australia. *Appl Geochem* 2009;24:268–84.

Google Scholar Crossref

Bowman JP, Cavanagh J, Austin JJ et al. Novel *Psychrobacter* species from Antarctic ornithogenic soils. *Int J Syst Evol Microbiol* 1996;46:841–8.

Breezee J, Cady N, Staley JT. Subfreezing growth of the sea ice bacterium “Psychromonas ingrahamii”. *Microbiol Ecol* 2004;47:300–4.

Google Scholar Crossref

Britton KL, Baker PJ, Fisher M et al. Analysis of protein solvent interactions in glucose dehydrogenase from the extreme halophile *Haloferax mediterranei*. *Proc Natl Acad Sci USA* 2006;103:4846–51.

Google Scholar Crossref PubMed

Brown AD. Microbial water stress. *Bacteriol Rev* 1976;40:803–46.

Google Scholar PubMed

Brown AD. *Microbial Water Stress Physiology. Principles and Perspectives* . Chichester, UK: John Wiley & Sons. 1990.

Bryan TS, Tucker-Bryan B. *The Explorer's Guide to Death Valley National Park* , 2nd edn.

Colorado, USA: University Press of Colorado. 2009.

Buick R, Dunlop JSR. Evaporitic sediments of Early Archaean age from the Warrawoona Group, North Pole, Western Australia. *Sedimentology* 1990;37:247–77.

Google Scholar Crossref

Burns DG, Camakaris HM, Janssen PH et al. Combined use of cultivation-dependent and cultivation-independent methods indicates that members of most haloarchaeal groups in an Australian crystallizer pond are cultivable. *Appl Environ Microbiol* 2004;70:5258–65.

Google Scholar Crossref PubMed

Busch CR, DiRuggiero J. MutS and MutL are dispensable for maintenance of the genomic mutation rate in the halophilic Archaeon *Halobacterium salinarum* NRC-1. *PLoS One* 2010;5:e9045.

Google Scholar Crossref PubMed

Cantrell SA, Casillas-Martínez L, Molina M. Characterization of fungi from hypersaline environments of solar salterns using morphological and molecular techniques. *Mycol Res* 2006;110:962–70.

Google Scholar Crossref PubMed

Capes MD, DasSarma P, DasSarma S. The core and unique proteins of haloarchaea. *BMC Genomics* 2012;13:39.

Google Scholar Crossref PubMed

Carlsen HN, Degn H, Lloyd D. Effects of alcohols on the respiration and fermentation of aerated suspensions of baker's yeast. *Microbiology* , 1991;137:2879–83.

Cayol JL, Ollivier B, Patel BKC et al. *Haloanaerobium lacusroseus* sp. nov., an extremely halophilic fermentative bacterium from the sediments of a hypersaline lake. *Int J Syst Evol Microbiol* 1995;45:790–7.

Chen H, Jiang JG. Osmotic responses of *Dunaliella* to the changes of salinity. *J Cell Physiol* 2009;219:251–8.

Google Scholar Crossref PubMed

Chen M, Tang H, Ma H et al. Effect of nutrients on growth and lipid accumulation in the green algae *Dunaliella tertiolecta*. *Bioresource Technol* 2011;102:1649–55.

Google Scholar Crossref

Cherekar MN, Pathak AP. Studies on haloalkaliphilic gammaproteobacteria from hypersaline Sambhar Lake, Rajasthan, India. *Indian J Geo-Mar Sci* 2015;44:1646–53.

Chin JP, Megaw J, Magill CL et al. Solutes determine the temperature windows for microbial survival and growth. *Proc Natl Acad Sci USA* 2010;107:7835–40.

Google Scholar Crossref PubMed

Cifuentes AS, González MA, Inostroza I et al. Reappraisal of physiological attributes of nine strains of *Dunaliella* (*Chlorophyceae*): growth and pigment content across a salinity gradient. *J Phycol* 2001;37:334–44.

Google Scholar Crossref

Çınar S, Mutlu MB. Comparative analysis of prokaryotic diversity in solar salterns in eastern Anatolia (Turkey). *Extremophiles* 2016;20:589–601.

Google Scholar Crossref PubMed

Clegg JS, Trotman C. Physiological and biochemical aspects of *Artemia* ecology. In Abatzopoulos TJ, Beardmore JA, Clegg JS et al. (eds) *Artemia Basic and Applied Biology* . 2002, 129–70.

Cocolin L, Bisson LF, Mills DA. Direct profiling of the yeast dynamics in wine fermentations. *FEMS Microbial Lett* 2000;189:81–87.

Google Scholar Crossref

Collins KD. Charge density-dependent strength of hydration and biological structure. *Biophys J* 1997;72:65–76.

Google Scholar Crossref PubMed

Collins MA, Buick RK. Effect of temperature on the spoilage of stored peas by *Rhodotorula glutinis*. *Food Microbiol* 1989;6:135–41.

Google Scholar Crossref

Conner AJ, Benison KC. Acidophilic halophilic microorganisms in fluid inclusions in halite from Lake Magic, Western Australia. *Astrobiology* 2013;13:850–60.

Google Scholar Crossref PubMed

Coquelle N, Talon R, Juers DH et al. Gradual adaptive changes of a protein facing high salt concentrations. *J Mol Biol* 2010;404:493–505.

Google Scholar Crossref PubMed

Cray JA, Bell AN, Bhaganna P et al. The biology of habitat dominance; can microbes behave as weeds? *Microbiol Biotechnol* 2013a;6:453–92.

Google Scholar Crossref

Cray JA, Russell JT, Timson DJ et al. A universal measure of chaotropicity and kosmotropicity. *Environ Microbiol* 2013b;15:287–96.

Google Scholar Crossref

Cray JA, Stevenson A, Ball P et al. Chaotropicity: a key factor in product tolerance of biofuel-producing microorganisms. *Curr Opin Biotechnol* 2015;33:228–59.

Google Scholar Crossref PubMed

Crits-Christoph A, Gelsinger DR, Ma B et al. Functional interactions of archaea, bacteria and viruses in a hypersaline endolithic community. *Environ Microbiol* 2016;18:2064–77.

Google Scholar Crossref PubMed

Cui HL, Qiu XX. *Salinarubrum litoreum* gen. nov., sp. nov.: a new member of the family *Halobacteriaceae* isolated from Chinese marine solar salterns. *Anton Leeuw* 2014;105:135–41.

Google Scholar Crossref

Daffonchio D, Borin S, Brusa T et al. Stratified prokaryote network in the oxic-anoxic transition of a deep-sea halocline. *Nature* 2006;440:203–7.

Google Scholar Crossref PubMed

Danovaro R, Dell'Anno A, Pusceddu A et al. The first metazoa living in permanently anoxic conditions. *BMC Biol* 2010;8:30.

Google Scholar Crossref PubMed

D'Amore T, Pancbal CJ, Russell I et al. A study of ethanol tolerance in yeast. *Crit Rev Biotechnol* 1990;9:287–304.

Google Scholar Crossref PubMed

DasSarma S, Berquist BR, Coker JA et al. Post-genomics of the model haloarchaeon *Halobacterium* sp. NRC–1. *Saline Syst* 2006;2:3.

Google Scholar Crossref PubMed

DasSarma S, DasSarma P. Halophiles and their enzymes: negativity put to good use. *Curr Opin Microbiol* 2015;25:120–6.

Google Scholar Crossref PubMed

Davila AF, Hawes I, Araya JG et al. *In situ* metabolism in halite endolithic microbial communities of the hyperarid Atacama Desert. *Front Microbiol* 2015;6. DOI:10.3389/fmicb.2015.01035.

Deole R, Challacombe J, Raiford DW et al. An extremely halophilic proteobacterium combines a highly acidic proteome with a low cytoplasmic potassium content. *J Biol Chem* 2013;288:581–8.

Google Scholar Crossref PubMed

DeMaere MZ, Williams TJ, Allen MA et al. High level of intergenera gene exchange shapes the evolution of haloarchaea in an isolated Antarctic lake. *Proc Natl Acad Sci USA* 2013;110:16939–44.

Google Scholar Crossref PubMed

de Vera JP, Schulze-Makuch D, Khan A et al. Adaptation of an Antarctic lichen to Martian niche conditions can occur within 34 days. *Planet Space Sci* 2014;98:182–90.

Google Scholar Crossref

Dinsdale EA, Pantos O, Smriga S et al. Microbial ecology of four coral atolls in the Northern Line Islands. *PLoS One* 2008. DOI: 10.1371/journal.pone.0001584.

Dundas I. Was the environment for primordial life hypersaline?. *Extremophiles*

1998;2:375–7.

Google Scholar Crossref PubMed

Dunn RV, Daniel RM. The use of gas-phase substrates to study enzyme catalysis at low hydration. *Philos T Roy Soc B* 2004;359:1309–20.

Google Scholar Crossref

Dym O, Mevarech M, Sussman JL. Structural features that stabilize halophilic malate dehydrogenase from an archaebacterium. *Science* 1995;267:1344–6.

Google Scholar Crossref PubMed

Eddie B, Krembs C, Neuer S. Characterization and growth response to temperature and salinity of psychrophilic, halotolerant *Chlamydomonas* sp. ARC isolated from Chukchi Sea ice. *Mar Ecol Prog Ser* 2008;354:107–17.

Google Scholar Crossref

Edgcomb V, Orsi W, Leslin C et al. Protistan community patterns within the brine and halocline of deep hypersaline anoxic basins in the eastern Mediterranean Sea. *Extremophiles* 2009;13:151–67.

Google Scholar Crossref PubMed

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

Google Scholar Crossref PubMed

Elevi Bardavid R, Oren A. Dihydroxyacetone metabolism in *Salinibacter ruber* and in *Haloquadratum walsbyi*. *Extremophiles* 2008;12:125–31.

Google Scholar Crossref PubMed

Emmerich M, Bhansali A, Lösekann-Behrens T et al. Abundance, distribution, and activity of Fe(II)-oxidizing and Fe(III)-reducing microorganisms in hypersaline sediments of Lake Kasin, southern Russia. *Appl Environ Microbiol* 2012;78:4386–99.

Google Scholar Crossref PubMed

Englert C, Horne M, Pfeifer F. Expression of the major gas vesicle protein gene in the halophilic archaebacterium *Haloferax mediterranei* is modulated by salt. *Mol Gen Genet* 1990;222:225–32.

Google Scholar Crossref PubMed

Erdmann S, Tschitschko B, Zhong L et al. A plasmid from an Antarctic haloarchaeon uses specialized membrane vesicles to disseminate and infect plasmid-free cells. *Nat Microbiol* 2017;2:1446–55.

Google Scholar Crossref PubMed

Ferrer M, Golyshina OV, Chernikova TN et al. Microbial enzymes mined from the Urania deep-sea hypersaline anoxic basin. *Chem Biol* 2005;12:895–904.

Google Scholar Crossref PubMed

Ferrer M, Werner J, Chernikova TN et al. Unveiling microbial life in the new deep-sea hypersaline Lake Thetis. Part II: a metagenomic study. *Environ Microbiol* 2012;14:268–81.

Google Scholar Crossref PubMed

Fields PA. Review: protein function at thermal extremes: balancing stability and flexibility. *Comp Biochem Phys A* 2001;129:417–31.

Google Scholar Crossref

Fierer N, Jackson RB. The diversity and biogeography of soil bacterial communities. *Proc Natl Acad Sci USA* 2006;103:626–31.

Google Scholar Crossref PubMed

Fox-Powell MG, Hallsworth JE, Cousins CR et al. Ionic strength is a barrier to the habitability of Mars. *Astrobiology* 2016;16:427–42.

Google Scholar Crossref PubMed

Franzmann PD, Liu Y, Balkwill DL et al. *Methanogenium frigidum* sp. nov., a psychrophilic, H_2-using methanogen from Ace Lake, Antarctica. *Int J Syst Evol Microbiol* 1997;47:1068–72.

Friedman R, Drake JW, Hughes AL. Genome-wide patterns of nucleotide substitution reveal stringent functional constraints on the protein sequences of thermophiles. *Genetics* 2004;167:1507–12.

Google Scholar Crossref PubMed

Garcia-Heredia I, Martin-Cuadrado A-B, Mojica FJM et al. Reconstructing viral genomes from the environment using fosmid clones: the case of haloviruses. *PLoS One* 2012;7:e33802.

Google Scholar Crossref PubMed

Gasol JM, Casamayor EO, Joint I et al. Control of heterotrophic prokaryotic abundance and growth rate in hypersaline planktonic environments. *Aquat Microbiol Ecol* 2004;34:193–206.

Google Scholar Crossref

Glotch TD, Bandfield JL, Tornabene LL et al. Distribution and formation of chlorides and phyllosilicates in Terra Sirenum, Mars. *Geophys Res Lett* 2010;37:L16202.

Google Scholar Crossref

Gock MA, Hocking AD, Pitt JI et al. Influence of temperature, water activity and pH on growth of some xerophilic fungi. *Int J Food Microbiol* 2003;81:11–9.

Google Scholar Crossref PubMed

Gough RV, Chevrier VF, Baustian KJ et al. Laboratory studies of perchlorate phase transitions: support for metastable aqueous perchlorate solutions on Mars. *Earth Planet Sci Lett* 2011;312:371–7.

Google Scholar Crossref

Grant WD. Life at low water activity. *Philos T Roy Soc B* 2004;359:1249–67.

Google Scholar Crossref

Gray WD. Studies on the alcohol tolerance of yeasts. *Int J Bacteriol* 1941;42:561.

Guixa-Boixareu N, Calderón-Paz JI, Heldal M et al. Viral lysis and bacterivory as prokaryotic loss factors along a salinity gradient. *Aquat Microbiol Ecol* 1996;11:215–27.

Google Scholar Crossref

Hallsworth JE. Ethanol-induced water stress in yeast. *J Ferment Bioeng* 1998;85:125–37.

Google Scholar Crossref

Hallsworth JE. Stress-free microbes lack vitality. *Fungal Biol* 2018;122:379–85.

Google Scholar Crossref PubMed

Hallsworth JE, Heim S, Timmis KN. Chaotropic solutes cause water stress in *Pseudomonas putida*. *Environ Microbiol* 2003;5:1270–80.

Google Scholar Crossref PubMed

Hallsworth JE, Nomura Y, Iwahara M. Ethanol-induced water stress and fungal growth. *J Ferment Bioeng* 1998;86:451–6.

Google Scholar Crossref

Hallsworth JE, Nomura YA. A simple method to determine the water activity of ethanol-containing samples. *Biotechnol Bioeng* 1999;62:242–5.

Google Scholar Crossref PubMed

Hallsworth JE, Yakimov MM, Golyshin PN et al. Limits of life in $MgCl_2$-containing environments: chaotropicity defines the window. *Environ Microbiol* 2007;9:801–13.

Google Scholar Crossref PubMed

Hamaguchi K, Geiduschek EP. The effect of electrolytes on the stability of the deoxyribonucleate helix. *J Am Chem Soc* 1962;84:1329–38.

Google Scholar Crossref

Hattori T, Shiba H, Ashiki KI et al. Anaerobic growth of haloarchaeon *Haloferax volcanii* by denitrification is controlled by the transcription regulator NarO. *J Bacteriol* 2016;198:1077–86.

Google Scholar Crossref PubMed

Harrison JP, Gheeraert N, Tsigelnitskiy D et al. The limits for life under multiple extremes. *Trends Microbiol* 2013;21:204–12.

Google Scholar Crossref PubMed

Hart BC, Gilmour DJ. A mutant of *Dunaliella parva* CCAP 19/9 leaking large amounts of glycerol into the medium. *J Appl Phycol* 1991;3:367–72.

Google Scholar Crossref

Hayashida S, Ohta K. Formation of high concentrations of alcohol by various yeasts. *J Inst Brew* 1981;87:42–4.

Google Scholar Crossref

Henriet O, Fourmentin J, Delincé B et al. Exploring the diversity of extremely halophilic archaea in food-grade salts. *Int J Food Microbiol* 2014;17:36–44.

Google Scholar Crossref

Herrero M, Quirós C, García LA et al. Use of flow cytometry to follow the physiological states of microorganisms in cider fermentation processes. *Appl Environ Microbiol* 2006;72:6725–33.

Google Scholar Crossref PubMed

Hocking AD. Microbiological facts and fictions in grain storage. In: *Stored Grain in Australia* Wright EJ, Webb MC, Highley E, (eds). Proceedings of the Australian Postharvest Technical Conference. *CSIRO*: Canberra, 2003, 55–8.

Huston AL. Bacterial adaptation to the cold: in situ activities of extracellular enzymes in the North Water polynya and characterization of a cold-active aminopeptidase from Colwellia *psychrerythraea* strain 34H. *PhD thesis* (Univ of Washington, Seattle) 2003.

Imhoff JF, Hashwa F, Trüper HG. Isolation of extremely halophilic phototrophic bacteria from the alkaline Wadi Natrun, Egypt. *Arch Hydrobiol* 1978;84:381–8.

Imhoff JF, Sahl HG, Soliman GS et al. The Wadi Natrun: chemical composition and microbial mass developments in alkaline brines of eutrophic desert lakes. *Geomicrobiol J* 1979;1:219–34.

Google Scholar Crossref

Ishibashi M, Uchino M, Arai S et al. Reduction of salt-requirement of halophilic nucleoside diphosphate kinase by engineering S-S bond. *Arch Biochem Biophys* 2012;525:47–52.

Google Scholar Crossref PubMed

Jakher GR, Bhargava SC, Sinha RK. Comparative limnology of Sambhar and Didwana lakes (Rajasthan, NW India). *Hydrobiologia* 1990;197:245–56.

Google Scholar Crossref

Javor BJ. Growth potential of halophilic bacteria isolated from solar salt environments: carbon sources and salt requirements. *Appl Environ Microbiol* 1984;48:352–60.

Google Scholar PubMed

Jiang H, Dong H, Zhang G et al. Microbial diversity in water and sediment of Lake Chaka, an athalassohaline lake in northwestern China. *Appl Environ Microbiol* 2006;72:3832–45.

Google Scholar Crossref PubMed

Johnson WM, Kido Soule MC, Kujawinski EB. Extraction efficiency and quantification of dissolved metabolites in targeted marine metabolomics. *Limnol Oceanogr-Meth* 2017;15:417–28.

Google Scholar Crossref

Jones AM, Thomas KC, Ingledew WM. Very high gravity fermentation for fuel alcohol production. *Int Sugar Jnl* 1995;97:606–10.

Jones BE, Grant WD, Duckworth AW et al. Microbial diversity of soda lakes. *Extremophiles* 1998;2:191–200.

Google Scholar Crossref PubMed

Jose PA, Jebakumar SR. Phylogenetic appraisal of antagonistic, slow growing actinomycetes isolated from hypersaline inland solar salterns at Sambhar salt Lake, India. *Front Microbiol* 2013;4:190.

Google Scholar PubMed

Junge K, Eicken H, Swanson BD et al. Bacterial incorporation of leucine into protein down to −20°C with evidence for potential activity in sub-eutectic saline ice formations. *Cryobiology* 2006;52:417–29.

Google Scholar Crossref PubMed

Kennedy SP, Ng WV, Salzberg SL et al. Understanding the adaptation of *Halobacterium* species NRC-1 to its extreme environment through computational analysis of its genome sequence. *Genome Res* 2001;11:1641–50.

Google Scholar Crossref PubMed

Kalcheva HV, Nikolova LN, Terziyski DI et al. Relationships between bacterioplankton, zooplankton and environmental factors in fertilized and non-fertilized carp fishponds. *Acta Zool Bulg* 2008;2:175–84.

Karan R, Capes MD, DasSarma S. Function and biotechnology of extremophilic enzymes in low water activity. *Aquat Biosyst* 2012;8:4.

Google Scholar Crossref PubMed

Kashefi K, Lovley DR. Extending the upper temperature limit for life. *Science* 2003;301:934.

Google Scholar Crossref PubMed

Kerkar S, Loka Bharathi PA. Stimulation of sulfate-reducing activity at salt-saturation in the salterns of Ribandar, Goa, India. *Geomicrobiol J* 2007;24:101–10.

Google Scholar Crossref

Kido Soule MC, Longnecker K, Johnson WM et al. Environmental metabolomics: analytical strategies. *Mar Chem* 2015;177:374–87.

Google Scholar Crossref

Kissling GE, Grogan DW, Drake JW. Confounders of mutation-rate estimators: selection and phenotypic lag in *Thermus thermophilus*. *Mutat Res* 2013;749:16–20.

Google Scholar Crossref PubMed

Kminek G, Rummel JD, Cockell CS et al. Report of the COSPAR Mars Special Regions Colloquium. *Adv Space Res* 2010;46:811–29.

Google Scholar Crossref

Kumar S, Karan R, Kapoor S et al. Screening and isolation of halophilic bacteria producing industrially important enzymes. *Braz J Microbiol* 2012;43:1595–603.

Google Scholar Crossref PubMed

Kurkal V, Daniel RM, Finney JL et al. Enzyme activity and flexibility at very low hydration. *Biophys J* 2005;89:1282–7.

Google Scholar Crossref PubMed

La Cono V, Smedile F, Bortoluzzi G et al. Unveiling microbial life in new deep-sea hypersaline Lake *Thetis*. Part I: Prokaryotes and environmental settings. *Environ Microbiol* 2011;13:2250–68.

Google Scholar Crossref PubMed

Lange OL. Der CO_2-Gaswechsel von Flechten bei tiefen Temperaturen. *Planta* 1965;64:1–19.

Google Scholar Crossref

Lebre PH, Maayer PD, Cowan DA. Xerotolerant bacteria: surviving through a dry spell. *Nat Rev Microbiol* 2017;15:285–96.

Google Scholar Crossref PubMed

León MJ, Fernández AB, Ghai R et al. From metagenomics to pure culture: isolation and characterization of the moderately halophilic bacterium *Spiribacter salinus* gen. nov., sp. nov. *Appl Environ Microbiol* 2014;80:3850–7.

Google Scholar Crossref PubMed

Li Q, Heist EP, Moe LA. Bacterial community structure and dynamics during corn-based bioethanol fermentation. *Microbial Ecol* 2016;71:409–21.

Google Scholar Crossref

Li W, Zhou R, Mu Y. Salting effects on protein components in aqueous NaCl and urea solutions: toward understanding of urea-induced protein denaturation. *J Phys Chem* 2012;116:1146–51.

Google Scholar Crossref

Lievens B, Hallsworth JE, Pozo MI et al. Microbiology of sugar-rich environments: diversity, ecology and system constraints. *Environ Microbiol* 2015;17:278–98.

Google Scholar Crossref PubMed

Longo LM, Tenorio CA, Kumru OS et al. A single aromatic core mutation converts a designed “primitive” protein from halophile to mesophile folding. *Protein Sci* 2015;24:27–37.

Google Scholar Crossref PubMed

Lopez M, Kurkal-Siebert V, Dunn RV et al. Activity and dynamics of an enzyme, pig liver esterase, in near-anhydrous conditions. *Biophys J* 2010;99:L62–4.

Google Scholar Crossref PubMed

Lozupone CA, Knight R. Global patterns in bacterial diversity. *Proc Natl Acad Sci USA* 2007;104:11436–40.

Google Scholar Crossref PubMed

Lowenstein TK, Hardie LA. Criteria for the recognition of salt-pan evaporites. *Sedimentology* 1985;32:627–44.

Google Scholar Crossref

Lowenstein TK, Schubert BA, Timofeeff MN. Microbial communities in fluid inclusions and long-term survival in halite. *GSA Today* 2011;21:4–9.

Google Scholar Crossref

Mackwan RR, Carver GT, Drake JW et al. An unusual pattern of spontaneous mutations recovered in the halophilic Archaeon *Haloferax volcanii*. *Genetics* 2007;176:697–702.

Google Scholar Crossref PubMed

Makhdoumi-Kakhki A, Amoozegar MA, Kazemi B et al. Prokaryotic diversity in Aran-Bidgol salt lake, the largest hypersaline playa in Iran. *Microbiol Environ* 2012;27:87–93.

Google Scholar Crossref

Mancinelli RL. Halophiles: A terrestrial analog for life in brines on Mars. In Gunde-Cimerman N, Plemenitaš A, Oren A. (eds). *Adaptation to Life at High Salt Concentrations in Archaea, Bacteria and Eukarya* . Volume 9 in the series on Cellular Origins, Life in Extreme Habitats and Astrobiology (COLE). Dordrecht, the Netherlands: Kluwer Academic Publishers, 2005a, 137–49.

Mancinelli RL. Microbial life in brines, evaporites and saline sediments: The search for life on Mars. In Tokano T (ed.) *Water on Mars and Life* Berlin, Germany: Springer, 2005b, 277–98.

Mani K, Salgaonkar BB, Das D et al. Community solar salt production in Goa, India. *Aquat Biosyst* 2012;8:30.

Google Scholar Crossref PubMed

Manikandan M, Pašić L, Kannan V. Purification and biological characterization of a halophilic thermostable protease from *Haloferax lucentensis* VKMM 007. *World J Microbiol Biot* 2009;25:2247–56.

Google Scholar Crossref

Martínez C. Production of *Rhodotorula glutinis*: a yeast that secretes α-L-arabinofuranosidase. *Electron J Biotechnol* 2006;9:407–13.

Google Scholar Crossref

Máthé I, Borsodi AK, Tóth EM et al. Vertical physico-chemical gradients with distinct microbial communities in the hypersaline and heliothermal Lake Ursu (Sovata, Romania). *Extremophiles* 2014;18:501–14.

Google Scholar Crossref PubMed

Maturrano L, Santos F, Rosselló-Mora R et al. Microbial diversity in Maras salterns, a hypersaline environment in the Peruvian Andes. *Appl Environ Microbiol* 2006;72:3887–95.

Google Scholar Crossref PubMed

Matveev VV. Comparison of fundamental physical properties of the model cells (protocells) and the living cells reveals the need in protophysiology. *Int J Astrobiol* 2017;16:97–104.

Google Scholar Crossref

Melack JM, Kilham P. Photosynthetic rates of phytoplankton in East African alkaline, saline lakes. *Limnol Oceanogr* 1974;19:743–55.

Google Scholar Crossref

Mesbah NM, Abou-El-Ela SH, Wiegel J. Novel and unexpected prokaryotic diversity in water and sediments of the alkaline, hypersaline lakes of the Wadi An Natrun, Egypt. *Microbial Ecol* 2007;54:598–617.

Google Scholar Crossref

Miermont A, Waharte F, Hu S et al. Severe osmotic compression triggers a slowdown of intracellular signaling, which can be explained by molecular crowding. *Proc Natl Acad Sci USA* 2013;110:5725–30.

Google Scholar Crossref PubMed

Miteva V, Sowers T, Brenchley J. Production of N_2O by ammonia oxidizing bacteria at subfreezing temperatures as a model for assessing the N_2O anomalies in the Vostok ice core. *Geomicrobiol J* 2007;24:451–9.

Google Scholar Crossref

Mohan NR, Fullmer MS, Makkay AM et al. Evidence from phylogenetic and genome fingerprinting analyses suggests rapidly changing variation in *Halorubrum* and *Haloarcula* populations. *Front Microbiol* 2014;5:143.

Google Scholar PubMed

Moreno MDL, Pérez D, García MT et al. Halophilic bacteria as a source of novel hydrolytic enzymes. *Life* 2013;3:38–51.

Google Scholar Crossref PubMed

Mori K, Nurcahyanto DA, Kawasaki H et al. *Haloarchaeobius baliensis* sp. nov., isolated from a solar saltern. *Int J Syst Evol Microbiol* 2016;66:38–43.

Google Scholar Crossref PubMed

Mormile MR, Biesen MA, Gutierrez MC et al. Isolation of *Halobacterium salinarum* retrieved directly from halite brine inclusions. *Environ Microbiol* 2003;5:1094–102.

Google Scholar Crossref PubMed

Muñoz-Iglesias V, Bonales LJ, Prieto-Ballesteros O. pH and salinity evolution of europa's brines: Raman spectroscopy study of fractional precipitation at 1 and 300 bar. *Astrobiology* 2013;13:693–702.

Google Scholar Crossref PubMed

Mykytczuk NC, Foote SJ, Omelon CR et al. Bacterial growth at −15°C; molecular insights from the permafrost bacterium *Planococcus halocryophilus* Or1. *ISME J* 2013;7:1211–26.

Google Scholar Crossref PubMed

Nagodawitbana TW, Steinkraus KH. Influence of the rate of ethanol production and accumulation on the viability of *Saccharomyces cerevisiae* in “rapid fermentation”. *Appl Environ Microbial* 1976;31:158–62.

Nakamura T, Ogata Y, Hamada S et al. Ethanol production from Jerusalem artichoke tubers by *Aspergillus niger* and *Saccharomyces cerevisiae*. *J Ferment Bioeng* . 1996;81:564–6.

Google Scholar Crossref

Naor A, Lapierre P, Mevarech M et al. Low species barriers in halophilic *Archaea* and the formation of recombinant hybrids. *Curr Biol* 2012;22:1444–8.

Google Scholar Crossref PubMed

Ng WV, Kennedy SP, Mahairas GG et al. Genome sequence of *Halobacterium* species NRC-1. *Proc Natl Acad Sci USA* 2000;97:12176–81.

Google Scholar Crossref PubMed

Norberg P, Hofsten BV. Proteolytic enzymes from extremely halophilic bacteria. *J Gen Microbiol* 1969;55:251–6.

Google Scholar Crossref PubMed

Odumeru JA, D'Amore T, Russell I et al. Changes in protein composition of *Saccharomyces* brewing strains in response to heat shock and ethanol stress. *J Ind Microbiol Biotechnol* , 1992;9:229–34.

Ohta K, Hamada S, Nakamura T. Production of high concentrations of ethanol from inulin by simultaneous saccharification and fermentation using *Aspergillus* niger and *Saccharomyces cerevisiae*. *Appl Environ Microbiol* 1993;59:729–33.

Google Scholar PubMed

Omori T, Ogawa K, Umemoto Y et al. Enhancement of glycerol production by brewing yeast (*Saccharomyces cerevisiae*) with heat shock treatment. *J Ferment Bioeng* 1996;82:187–90.

Google Scholar Crossref

Oren A. The rise and decline of a bloom of halobacteria in the Dead Sea. *Limnol Oceanogr* 1985;30:911–5.

Google Scholar Crossref

Oren A. The ecology of the extremely halophilic archaea. *FEMS Microbiol Rev* 1994;13:415–39.

Google Scholar Crossref

Oren A. Bioenergetic aspects of halophilism. *Microbiol Mol Biol Rev* 1999;63:334–48.

Google Scholar PubMed

Oren A. Microbial life at high salt concentrations: phylogenetic and metabolic diversity. *Saline Systems* 2008;4:2.

Google Scholar Crossref PubMed

Oren A. Microbial diversity and microbial abundance in salt-saturated brines: why are the waters of hypersaline lakes red? *Nat Resour Env Iss* 2009;15:247–55.

Oren A. Thermodynamic limits to microbial life at high salt concentrations. *Environ Microbiol* 2011;13:1908–23.

Google Scholar Crossref PubMed

Oren A. Life at high salt concentrations, intracellular KCl concentrations, and acidic proteomes. *Front Microbiol* 2013;4:315.

Google Scholar Crossref PubMed

Oren A. The ecology of *Dunaliella* in high-salt environments. *J Biol Res-Thessaloniki* 2014;21:23.

Google Scholar Crossref

Oren A. Glycerol metabolism in hypersaline environments. *Environ Microbiol* 2017;19:851–63.

Google Scholar Crossref PubMed

Oren A, Gurevich P. Dynamics of a bloom of halophilic archaea in the Dead Sea. *Hydrobiologia* 1995;315:149–58.

Google Scholar Crossref

Oren A, Hallsworth JE. Microbial weeds in hypersaline habitats: the enigma of the weed-like *Haloferax mediterranei*. *FEMS Microbiol Lett* 2014;359:134–42.

Google Scholar Crossref PubMed

Oremland RS, Kulp TR, Blum JS et al.   A microbial arsenic cycle in a salt-saturated, extreme environment. *Science* 2005;308:1305–8.

Google Scholar   Crossref   PubMed

Oremland RS, Stolz JF. The ecology of arsenic. *Science* 2003;300:939–44.

Google Scholar   Crossref   PubMed

Nelson-Sathi S, Dagan T, Landan G et al.   Acquisition of 1,000 eubacterial genes physiologically transformed a methanogen at the origin of Haloarchaea. *Proc Natl Acad Sci USA* 2012;109:20537–42.

Google Scholar   Crossref   PubMed

Nelson-Sathi S, Sousa FL, Roettger M et al.   Origins of major archaeal clades correspond to gene acquisitions from bacteria. *Nature* 2015;517:77–80.

Google Scholar   Crossref   PubMed

Norton CF, Grant WD. Survival of halobacteria within fluid inclusions in salt crystals. *Microbiology* 1988;134:1365–73.

Google Scholar   Crossref

Pachiadaki MG, Yakimov MM, La Cono V et al.   Unveiling microbial activities along the halocline of Thetis, a deep-sea hypersaline anoxic basin. *ISME J* 2014;8:2478–89.

Google Scholar   Crossref   PubMed

Pais TM, Foulquié-Moreno MR, Hubmann G et al.   Comparative polygenic analysis of maximal ethanol accumulation capacity and tolerance to high ethanol levels of cell proliferation in yeast. *PLoS Genet* 2013;9:e1003548.

Google Scholar   Crossref   PubMed

Pan Y, Birdsey RA, Phillips OL et al.   The structure, distribution, and biomass of the world's forests. *Annu Rev Ecol Evol S* 2013;44:593–622.

Google Scholar   Crossref

Panikov NS, Sizova MV. Growth kinetics of microorganisms isolated from Alaskan soil and permafrost in solid media frozen down to −35°C. *FEMS Microbiol Ecol* 2006;59:500–12.

Google Scholar Crossref PubMed

Park JS, Kim H, Choi DH et al. Active flagellates grazing on prokaryotes in high salinity waters of a solar saltern. *Aquat Microbiol Ecol* 2003;33:173–9.

Google Scholar Crossref

Park JS, Simpson A, Brown S et al. Ultrastructure and molecular phylogeny of two heterolobosean amoebae, *Euplaesiobystra hypersalinica* gen. et sp. nov. and *Tulamoeba peronaphora* gen. et sp. nov., isolated from an extremely hypersaline habitat. *Protist* 2009;160:265–83.

Google Scholar Crossref PubMed

Paul S, Aggarwal C, Thakur JK et al. Induction of osmoadaptive mechanisms and modulation of cellular physiology help *Bacillus licheniformis* strain SSA 61 adapt to salt stress. *Curr Microbiol* 2015;70:610–7.

Google Scholar Crossref PubMed

Parry BR, Surovtsev IV, Cabeen MT et al. The bacterial cytoplasm has glass-like properties and is fluidized by metabolic activity. *Cell* 2014;156:183–94.

Google Scholar Crossref PubMed

Pathak AP, Cherekar MN. Hydrobiology of hypersaline Sambhar salt Lake a Ramsar site, Rajasthan, India. *Indian J Geo-Mar Sci* 2015;44:1640–5.

Pedrós-Alió C, Calderón-Paz JI, MacLean MH et al. The microbial food web along salinity gradients. *FEMS Microbiol Ecol* 2000;32:143–55.

Google Scholar Crossref PubMed

Pedrós-Alió C, Calderón-Paz JI, Gasol JM. Comparative analysis shows that bacterivory, not viral lysis, controls the abundance of heterotrophic prokaryotic plankton. *FEMS Microbiol Ecol* 2000;32:157–65.

Google Scholar Crossref PubMed

Peduzzi P, Schiemer F. Bacteria and viruses in the water column of tropical freshwater reservoirs. *Environ Microbiol* 2004;6:707–15.

Google Scholar Crossref PubMed

Pelhate J. Recherche des besoins en eau chez quelques moisissures des grains. *Mycopathologia* , 1968;36:117–28.

Pitt JI, Christian JHB. Water relations of xerophilic fungi isolated from prunes. *Appl Microbiol* 1968;16:1853–8.

Google Scholar PubMed

Pitt JI, Lantz H, Pettersson OV et al. *Xerochrysium* gen. nov. and *Bettsia*, genera encompassing xerophilic species of *Chrysosporium*. *IMA Fungus* 2013;4:229–41.

Google Scholar Crossref PubMed

Post FJ. Biology of the North Arm. In: Gwynn JW (ed). *Great Salt Lake: A Scientific, Historical and Economic Overview* . Utah, USA: Utah Department of Natural Resources. pp. 1980;313–23.

Price R, Boyd ES, Hoehler TM et al. Alkaline vents and steep Na^+ gradients from ridge-flank basalts—Implications for the origin and evolution of life. *Geology* 2017;45:1135–8.

Google Scholar Crossref

Qvist J, Ortega G, Tadeo X et al. Hydration dynamics of a halophilic protein in folded and unfolded states. *J Phys Chem B* 2012;116:3436–44.

Google Scholar Crossref PubMed

Řeháková K, Zapomělová E, Prášil O et al. Composition changes of phototrophic microbial communities along the salinity gradient in the solar saltern evaporation ponds of Eilat, Israel. *Hydrobiologia* 2009;636:77–88.

Google Scholar Crossref

Reistad R. On the composition and nature of the bulk protein of extremely halophilic bacteria. *Archiv Mikrobiol* 1970;71:353–60.

Google Scholar Crossref

Rivkina EM, Friedmann EI, McKay CP et al. Metabolic activity of permafrost bacteria below the freezing point. *Appl Environ Microbiol* 2000;66:3230–3.

Google Scholar Crossref PubMed

Rivkina E, Shcherbakova V, Laurinavichius K et al. Biogeochemistry of methane and methanogenic archaea in permafrost. *FEMS Microbiol Ecol* 2007;61:1–15.

Google Scholar Crossref PubMed

Roberts MF. Organic compatible solutes of halotolerant and halophilic microorganisms. *Saline Syst* 2005;1:5.

Google Scholar Crossref PubMed

Robinson CK, Wierzchos J, Black C et al. Microbial diversity and the presence of algae in halite endolithic communities are correlated to atmospheric moisture in the hyper-arid zone of the Atacama Desert. *Environ Microbiol* 2015;17:299–315.

Google Scholar Crossref PubMed

Rummel JD, Beaty DW, Jones MA et al. A new analysis of Mars ‘‘Special Regions’’: findings of the second MEPAG Special Regions Science Analysis Group (SR-SAG2). *Astrobiology* 2014;14:887–968.

Google Scholar Crossref PubMed

Sabet S, Diallo L, Hays L et al. Characterization of halophiles isolated from solar salterns in Baja California, Mexico. *Extremophiles* 2009;13:643–56.

Google Scholar Crossref PubMed

Sankaranarayanan K, Lowenstein TK, Timofeeff MN et al. Characterization of ancient DNA supports long-term survival of haloarchaea. *Astrobiology* 2014;14:553–60.

Google Scholar Crossref PubMed

Santos R, de Carvalho CCR, Stevenson A et al. Extraordinary solute-stress tolerance contributes to the environmental tenacity of mycobacteria. *Environ Microbiol Rep* 2015;7:746–64.

Google Scholar Crossref PubMed

Saponetti MS, Bobba F, Salerno G et al. Morphological and structural aspects of the extremely halophilic archaeon *Haloquadratum walsbyi*. *PLoS One* 2011;6:e18653.

Google Scholar Crossref PubMed

Schoenherr AA. Inland waters. In: Schoenherr AA (ed). *A Natural History of California* . California, USA: University of California Press, 1992, 553–627.

Schroeter B, Green TGA, Kappen L et al. Carbon dioxide exchange at subzero temperatures. Field measurements on *Umbilicaria aprina* in Antarctica. *Cryptogam Bot* 1994;4:233–41.

Schubert BA, Lowenstein TK, Timofeeff MN et al. How do prokaryotes survive in fluid inclusions in halite for 30 k.y.? *Geology* 2009;37:1059–62.

Google Scholar Crossref

Schwibbert K, Marin-Sanguino A, Bagyan I et al. A blueprint of ectoine metabolism from the genome of the industrial producer *Halomonas elongata* DSM 2581T. *Environ Microbiol* 2011;13:1973–94.

Google Scholar Crossref PubMed

Selim SA, El-Alfy SM, Hagagy NI et al. Oil-biodegradation and biosurfactant production by haloalkaliphilic Archaea isolated from Soda Lakes of the Wadi An Natrun, Egypt. *J Pure Appl Microbiol* 2012;6:1011–20.

Sher J, Elevi R, Mana L et al. Glycerol metabolism in the extremely halophilic bacterium *Salinibacter ruber*. *FEMS Microbiol Lett* 2004;232:211–5.

Google Scholar Crossref PubMed

Snow D. The germination of mould spores at controlled humidities. *Ann Applied Biology* 1949;36:1–13.

Google Scholar Crossref

Snider CS, Hsiang T, Zhao G et al. Role of ice nucleation and antifreeze activities in pathogenesis and growth of snow molds. *Phytopathology* 2000;90:354–61.

Google Scholar Crossref PubMed

Sojo V, Herschy B, Whicher A et al. The origin of life in alkaline hydrothermal vents. *Astrobiology* 2016;16:181–97.

Google Scholar Crossref PubMed

Sorokin DY, Abbas B, Geleijnse M et al. Syntrophic associations from hypersaline soda lakes converting organic acids and alcohols to methane at extremely haloalkaline conditions. *Environ Microbiol* 2016a;18:3189–202.

Google Scholar Crossref

Sorokin DY, Abbas B, Merkel AY et al. *Methanosalsum natronophilum* sp. nov., and *Methanocalculus alkaliphilus* sp. nov., haloalkaliphilic methanogens from hypersaline soda lakes. *Int J Syst Evol Microbiol* 2015;65:3739–45.

Google Scholar Crossref PubMed

Sorokin DY, Banciu HL, Muyzer G. Functional microbiology of soda lakes. *Curr Opin Microbiol* 2015;25:88–96.

Google Scholar Crossref PubMed

Sorokin DY, Berben T, Melton ED et al. Microbial diversity and biogeochemical cycling in soda lakes. *Extremophiles* 2014;18:791–809.

Google Scholar Crossref PubMed

Sorokin DY, Chernyh NA. '*Candidatus* Desulfonatronobulbus propionicus': a first haloalkaliphilic member of the order *Syntrophobacterales* from soda lakes. *Extremophiles* 2016;20:895–901.

Google Scholar Crossref PubMed

Sorokin DY, Chernyh NA. *Desulfonatronospira sulfatiphila* sp. nov., and *Desulfitispora elongata* sp. nov., two novel haloalkaliphilic sulfidogenic bacteria from soda lakes. *Int J Syst Evol Microbiol* 2017;67:396–401.

Google Scholar Crossref PubMed

Sorokin DY, Kublanov IV, Gavrilov SN et al. Elemental sulfur and acetate can support life of a novel strictly anaerobic haloarchaeon. *ISME J* 2016b;10:240–52.

Google Scholar Crossref

Sorokin DY, Kublanov IV, Yakimov MM et al. *Halanaeroarchaeum sulfurireducens* gen. nov., sp. nov., the first obligately anaerobic sulfur-respiring haloarchaeon, isolated from a hypersaline lake. *Int J Syst Evol Microbiol* 2016c;66:2377–81.

Google Scholar Crossref

Sorokin DY, Makarova KS, Abbas B et al. Discovery of extremely halophilic, methyl-reducing euryarchaea provides insights into the evolutionary origin of methanogenesis. *Nat Microbiol* 2017a;2:17081.

Google Scholar Crossref

Sorokin DY, Messina E, Smedile F et al. Discovery of anaerobic lithoheterotrophic haloarchaea, ubiquitous in hypersaline habitats. *ISME J* 2017b;11:1245–60.

Google Scholar Crossref

Sorokin DY, Tourova TP, Mordanov AV et al. Bacterial chitin utilisation at extremely haloalkaline conditions. *Extremophiles* 2012a;16:883–94.

Google Scholar Crossref

Sorokin DY, Tourova TP, Panteleeva AN et al. Anaerobic utilization of pectinous substrates at extremely haloalkaline conditions by *Natranaerovirga pectinivora* gen. nov., sp. nov., and *Natranaerovirga hydrolytica* sp. nov., isolated from hypersaline soda lakes. *Extremophiles* 2012b;16:307–15.

Google Scholar Crossref

Sousa FL, Nelson-Sathi S, Martin WF. One step beyond a ribosome: the ancient anaerobic core. *BBA- Bioenergetics* 2016;1857:1027–38.

Google Scholar Crossref PubMed

Spang A, Caceres EF, Ettema TJ. Genomic exploration of the diversity, ecology, and evolution of the archaeal domain of life. *Science* 2017;357:eaaf3883.

Google Scholar Crossref PubMed

Spellerberg IF, Fedor PJ. A tribute to Claude Shannon (1916-2001) and a plea for more rigorous use of species richness, species diversity and the ‘Shannon-Wiener’ Index. *Glob Ecol Biogeogr* 2003;12:177–9.

Google Scholar Crossref

Squyres SW, Grotzinger JP, Arvidson RE et al. In situ evidence for an ancient aqueous environment at Meridiani Planum, Mars. *Science* 2004;306:1709–14.

Google Scholar Crossref PubMed

Stevenson A, Burkhardt J, Cockell CS et al. Multiplication of microbes below 0.690 water activity: implications for terrestrial and extraterrestrial life. *Environ Microbiol* 2015a;17:257–77.

Google Scholar Crossref

Stevenson A, Cray JA, Williams JP et al. Is there a common water-activity limit for the three domains of life? *ISME J* 2015b 9:1333–51.

Google Scholar Crossref

Stevenson A, Hallsworth JE Water and temperature relations of soil Actinobacteria. *Environ Microbiol Rep* 2014;6:744–55.

Google Scholar Crossref PubMed

Stevenson A, Hamill PG, Dijksterhuis J et al. Water-, pH- and temperature relations of germination for the extreme xerophiles *Xeromyces bisporus* (FRR0025), *Aspergillus penicillioides* (JH06THJ) and *Eurotium halophilicum* (FRR 2471). *Microbiol Biotechnol* 2017a;10:330–40.

Google Scholar Crossref

Stevenson A, Hamill PG, Medina A et al. Glycerol enhances fungal germination at the water-activity limit for life. *Environ Microbiol* 2017b;19:947–67.

Google Scholar Crossref

Stevenson A, Hamill PG, O’Kane CJ et al. *Aspergillus penicillioides* differentiation and cell division at 0.585 water activity. *Environ Microbiol* 2017c;19:687–97.

Google Scholar Crossref

Stewart GG, D’Amore T, Panchal CJ et al. Factors that influence the ethanol tolerance of brewer's yeast strains during high gravity wort fermentations. *MBAA Technic Quarterly* , 1988;25:47–53.

Stock A, Breiner HW, Pachiadaki M et al. Microbial eukaryote life in the new hypersaline deep-sea basin Thetis. *Extremophiles* 2012;16:21–34.

Google Scholar Crossref PubMed

Swinnen S, Schaerlaekens K, Pais T et al. Identification of novel causative genes determining the complex trait of high ethanol tolerance in yeast using pooled-segregant whole-genome sequence analysis. *Genome Res* 2012;22:975–84.

Google Scholar Crossref PubMed

Tadeo X, López-Méndez B, Trigueros T et al. Structural basis for the aminoacid composition of proteins from halophilic Archea. *PLoS Biol* 2009;7:e1000257.

Google Scholar Crossref PubMed

Taniasuri F, Lee PR, Liu SQ. Induction of simultaneous and sequential malolactic fermentation in durian wine. *Int J Food Microbiol* 2016;230:1–9.

Google Scholar Crossref PubMed

Thomas KC, Hynes SH, Jones AM et al. Production of fuel alcohol from wheat by VHG technology. *Appl Biochem Biotech* 1993;43:211–26.

Google Scholar Crossref

Torrentera L, Dodson SI. Ecology of the brine shrimp *Artemia* in the Yucatan, Mexico, salterns. *J Plankton Res* 2004;26:617–24.

Google Scholar Crossref

Tosca NJ, Knoll AH, McLennan SM. Water activity and the challenge for life on early Mars. *Science* 2008;353:1204–7.

Google Scholar Crossref

Triantaphyllidis GV, Abatzopoulos TJ, Sorgeloos P. Review of the biogeography of the genus *Artemia* (*Crustacea, Anostraca*). *J Biogeography* 1998;25:213–26.

Google Scholar Crossref

Tuorto SJ, Darias P, McGuinness LR et al. Bacterial genome replication at subzero temperatures in permafrost. *ISME J* 2014;8:139–49.

Google Scholar Crossref PubMed

Upasani V, Desai S. Sambhar Salt Lake. *Arch Microbiol* 1990;154:589–93.

Google Scholar Crossref

Vauclare P, Marty V, Fabiani E et al. Molecular adaptation and salt stress response of *Halobacterium salinarum* cells revealed by neutron spectroscopy. *Extremophiles* 2015;19:1099–107.

Google Scholar Crossref PubMed

Ventosa A, de la Haba RR, Sánchez-Porro C et al. Microbial diversity of hypersaline environments: a metagenomic approach. *Curr Opin Microbiol* 2015;25:80–87.

Google Scholar Crossref PubMed

Vidyasagar M, Prakash S, Mahajan V et al. Purification and characterization of an extreme halothermophilic protease from a halophilic bacterium *Chromohalobacter* sp. TVSP101. *Braz J Microbiol* 2009;40:12–19.

Google Scholar Crossref PubMed

von Schelhorn M. Untersuchungen über den Verderb wasserarmer Lebensmittel durch osmophile Mikroorganismen. II. Grenzkonzentrationen für den osmophilen Schimmelpilz *Aspergillus glaucus* in Abhängigkeit vom pH Wert des Substrates. *Z Lebensm Untersuch Forsch* 1950;91:91338–42.

Wada M, Mori F, Yokouchi K et al. Comparison of planktonic microbial abundance and dissolved oxygen consumption between the aquaculture ponds of mudskippers and shrimps in the Mekong Delta, southern Vietnam. *Fish Sci* 2016;82:787–97.

Google Scholar Crossref

Wainø M, Tindall BJ, Ingvorsen K. *Halorhabdus utahensis* gen. nov., sp. nov., an aerobic, extremely halophilic member of the Archaea from Great Salt Lake, Utah. *Int J Syst Evol Microbiol* 2000;50:183–90.

Google Scholar Crossref PubMed

Warren JK. Evaporites through time: tectonic, climatic and eustatic controls in marine and nonmarine deposits. *Earth Sci Rev* 2010;98:217–68.

Google Scholar Crossref

Warren JK. *Evaporites: A Geological Compendium* , 2nd edn. Berlin, Germany: Springer, 2016, 1813.

Weimer BC, Rompato G, Parnell J et al. Microbial biodiversity of Great Salt Lake, Utah. *Nat Resour Env Iss* 2009;15:15–22.

Wetzel RG. *Limnology: Lake and River Ecosystems* , 3rd edn. Texas, USA: Gulf Professional Publishing, 2001, 4.

Wheeler KA, Hocking AD, Pitt JI. Influence of temperature on the water relations of *Polypaecilum pisce* and *Basipetospora halophila*, two halophilic fungi. *Microbiology* 1988;134:2255–60.

Google Scholar Crossref

Wieland A, Kühl M. Irradiance and temperature regulation of oxygenic photosynthesis and O_2 consumption in a hypersaline cyanobacterial mat (Solar Lake, Egypt). *Mar Biol* 2000;137:71–85.

Google Scholar Crossref

Wierzchos J, Ascaso C, McKay CP. Endolithic cyanobacteria in halite rocks from the hyperarid core of the Atacama Desert. *Astrobiology* 2006;6:415–22.

Google Scholar Crossref PubMed

Williams JP, Hallsworth JE. Limits of life in hostile environments: no barriers to biosphere function? *Environ Microbiol* 2009;11:3292–308.

Google Scholar Crossref PubMed

Williams TJ, Allen MA, DeMaere MZ et al. Microbial ecology of an Antarctic hypersaline lake: genomic assessment of ecophysiology among dominant haloarchaea. *ISME J* 2014;8:1645–58.

Google Scholar Crossref PubMed

Williams TJ, Allen M, Tschitschko B et al. Glycerol metabolism of haloarchaea. *Environ Microbiol* . 2017;19:864–77.

Google Scholar Crossref PubMed

Williams WD. Environmental threats to salt lakes and the likely status of inland saline ecosystems in 2025. *Envir Conserv* 2002;29:154–67.

Google Scholar Crossref

Winter JA, Christofi P, Morroll S et al. The crystal structure of *Haloferax volcanii* proliferating cell nuclear antigen reveals unique surface charge characteristics due to halophilic adaptation. *BMC Struct Biol* 2009;9:55.

Google Scholar Crossref PubMed

Yadav DN, Sarin MM, Krishnaswami S. Hydrogeochemistry of Sambhar Salt Lake, Rajasthan: implication to recycling of salt and annual salt budget. *J-Geol Soc India* 2007;69:139–52.

Yakimov MM, La Cono, Denaro V et al. Primary producing prokaryotic communities of brine, interface and seawater above the halocline of deep anoxic lake L'Atalante, Eastern Mediterranean Sea. *ISME J* 2007;1:743–55.

Google Scholar Crossref PubMed

Yakimov MM, La Cono V, Slepak VZ et al. Microbial life in the Lake *Medee*, the largest deep-sea salt-saturated formation. *Sci Rep* 2013;3. doi: 10.1038/srep03554.

Yakimov MM, La Cono V, Spada GL et al. Microbial community of the deep-sea brine Lake *Kryos* seawater-brine interface is active below the chaotropicity limit of life as revealed by recovery of mRNA. *Environ Microbiol* 2015;17:364–82.

Google Scholar Crossref PubMed

Yoshida M, Matsubara K, Kudo T et al. *Actinopolyspora mortivallis* sp. nov., a moderately halophilic actinomycete. *Int J Syst Bacteriol* 1991;41:15–20.

Google Scholar Crossref

Youssef NH, Ashlock-Savage KN, Elshahed MS. Phylogenetic diversities and community structure of members of the extremely halophilic Archaea (order *Halobacteriales*) in multiple saline sediment habitats. *Appl Environ Microbiol* 2012;78:1332–44.

Google Scholar Crossref PubMed

Youssef NH, Savage-Ashlock KN, McCully AL et al. Trehalose/2-sulfotrehalose biosynthesis and glycine-betaine uptake are widely spread mechanisms for osmoadaptation in the *Halobacteriales*. *ISME J* 2014;8:636–49.

Google Scholar Crossref PubMed

Zaccai G. The effect of water on protein dynamics. *Philos T Roy Soc B* 2004;359:1269–75.

Google Scholar Crossref

Zafrilla B, Martínez-Espinosa RM, Alonso MA et al. Biodiversity of Archaea and flora of two inland saltern ecosystems in the Alto Vinalopo Valley, Spain. *Saline Syst* 2010;6:10.

Google Scholar Crossref PubMed

Zhang W, Oganov AR, Goncharov AF et al. Unexpected stable stoichiometries of sodium chlorides. *Science* 2013;342:1502–5.

Google Scholar Crossref PubMed

Zhaxybayeva O, Stepanauskas R, Mohan NR et al. Cell sorting analysis of geographically separated hypersaline environments. *Extremophiles* 2013;17:265–75.

Google Scholar Crossref PubMed

Zhilina TN, Kevbrin VV, Tourova TP et al. *Clostridium alkalicellum* sp. nov., an obligately alkaliphilic cellulolytic bacterium from a soda lake in the Baikal region. *Microbiology* (Moscow, English Translation) 2005;74:557–66.

Google Scholar Crossref

© FEMS 2018.

This article is published and distributed under the terms of the Oxford University Press, Standard Journals Publication Model (https://academic.oup.com/journals/pages/open_access/funder_policies/chorus/standard_publication_model)

SIX KEY TOPICS IN 2018
Discover leading research into current trends in microbiology
FEMS
FEDERATION OF EUROPEAN MICROBIOLOGICAL SOCIETIES

3 View Metrics

Email alerts

New issue alert

Advance article alerts

Article activity alert

Receive exclusive offers and updates from Oxford Academic

More on this topic

Forest microbiome: diversity, complexity and dynamics

The microbiology of biological phosphorus removal in activated sludge systems

Contaminated environments in the subsurface and bioremediation: organic contaminants

Energetics of syntrophic fatty acid oxidation

Related articles in PubMed

Maximization of CO2 storage for various solvent types in indirect carbonation using paper sludge ash.

Realized niche and spatial pattern of native and exotic halophyte hybrids.

Influence of leaf damage by the horse chestnut leafminer (Cameraria ohridella Deschka & DimiÄ‡) on mycorrhiza of Aesculus hippocastanum L.

Serum Electrolyte Levels and Outcomes in Patients Hospitalized with Hepatic Encephalopathy.

Citing articles via

Google Scholar

CrossRef

Latest | **Most Read** | **Most Cited**

Editorial: Editorial for thematic issue on Archaea

The expanding structural variety among bacteriocins from Gram-positive bacteria

From hairballs to hypotheses–biological insights from microbial networks

Strategies to combat antimicrobial resistance: anti-plasmid and plasmid curing

On the maverick Planctomycetes

Online ISSN 1574-6976
Print ISSN 0168-6445

Copyright © 2018 Federation of European Microbiological Societies

Oxford University Press is a department of the University of Oxford. It furthers the University's objective of excellence in research, scholarship, and education by publishing worldwide

OXFORD
UNIVERSITY PRESS

Copyright © 2018 Oxford University Press