Biological constraints on habitability

Lewis Dartnell discusses how extremophiles have pushed the survival envelope of terrestrial life – and what this means for the possibility of extraterrestrial life.

So far, the search for life beyond Earth has largely focused on the search for bodies of liquid water, ancient or current. While all life on Earth fundamentally relies on liquid water as the biosolvent, finding wet extraterrestrial environments, or at least locales exhibiting signs of ancient water, is only the first step. Liquid water is a necessary but not sufficient prerequisite for life. Multiple physical and chemical parameters further limit the suitability of a wet environment to support life. It is these factors that define the ultimate habitability of an environment, the volume of physicochemical parameter space that can be tolerated by living organisms. So the study of terrestrial organisms that can survive on the extreme boundary of these conditions, the so-called extremophiles, greatly informs astrobiology and the search for life beyond Earth.

Given there is no "normal" or average environment on Earth, and we don't yet know the physical and chemical conditions under which life first arose, it is difficult to set a baseline from which to define an "extreme" environmental condition. Out of necessity, extreme conditions are taken to be those that deviate from what our *mesophile* cells could tolerate, chauvinistic though this may seem! "Extreme" is in the eye of the beholder, and for bacterial cells thriving in the hot anaerobic deep environment of marine crust, it is our kinds of life, lumbering around in the cold, low-pressure, oxygen- and radiation-bathed environment of the planetary surface, that are the evolutionary freaks.

This article presents a short overview of the main physical and chemical parameters that affect an environment, and the extremophilic organisms found flourishing in the greatest extremes of each. Taken together, these maximum ranges for biology on our planet describe the survival envelope of terrestrial life, and they inform the science of astrobiology. What are the limits of terrestrial habitability and what do they mean for the possibility of life beyond Earth?

Desiccation

While liquid water is absolutely essential for the growth and reproduction of all terrestrial life, certain organisms can tolerate periods of extreme desiccation: the xerophiles. They survive by entering a state of anhydrobiosis, in which minimal water remains and cells' metabolic activity enters dormancy. Cases of anhydrobiosis have been discovered within bacteria, yeast, fungi, plants and animals. When environmental conditions deteriorate, tardigrades, or "water bears", enter a desiccated, dormant tun stage, retracting their legs into their bodies. In this anhydrobiotic state they can tolerate very high levels of radiation, vacuum exposure, and temperatures between –253 °C and 151 °C.

Temperature

Heat-loving organisms are termed thermophiles, with the most extreme cases named hyperthermophiles (optimum growth at temperatures above 80 °C). High temperatures denature proteins and nucleic acids. As the temperature rises, the thermal vibration and flexing of these polymers increases until the non-covalent chemical bonds are overcome and the biomolecule begins to lose its precise functional structure. For soluble proteins, water molecules gain access to the hydrophobic core and the macromolecule precipitates out of solution. Membrane fluidity also increases with temperature, until it loses its barrier function and the cell can no longer regulate its own internal composition. Chlorophyll is only stable up to 75 °C, so photosynthesis does not take place in hyperthermophile environments. The most hyperthermophilic organisms are members of the archaea, with one strain growing at up to 121 °C. Bacteria manage up to around 90 °C, while eukaryotic cells cannot cope much beyond 60 °C. Adaption to hot environments involves regulating membrane fluidity and incorporating more amino acids that are able to form non-covalent bonds in order to reinforce the conformational structure of the proteins.

Psychrophile organisms (optimum growth at less than 15 °C) in very cold environments face the opposite problem. At low temperatures, thermal activity is so diminished that proteins become too rigid and inflexible and they cannot function as effective catalysts. Such environments are encountered in the deep sea, Antarctic and Arctic marine environments (including surface waters and sea ice), and glaciers worldwide. Veins of water within marine ice remain liquid down to –35 °C because of the high concentration of dissolved salts as the surrounding seawater freezes, and thin films of water remain fluid around mineral grain boundaries to even lower temperatures. Freezing water further endangers organisms through ice crystal growth and cellular rupture. Solubility of gases in water at low temperature can also be a limiting factor, particularly for organisms requiring oxygen or carbon dioxide for growth. Claims have been made for microorganism metabolism and growth at temperatures of –20 °C or lower, but such cold environments result in exceedingly slow reaction kinetics; even with cold-adapted enzymes, cellular metabolism proceeds at a glacial place. This has frustrated efforts to reliably detect evidence of growth at such low temperatures.

Neutral, acid or alkali

The pH scale measures the concentration of hydrogen ions (H^+, protons) in solution. A pH of 7 denotes neutrality, solutions with a pH value less than 7 are acidic and those greater than 7 are alkaline.

As with high temperatures, very acidic conditions act to denature proteins and hydrolyse cellular components and so are extremely hazardous to life. Acidic environments include geothermal pools with a low pH from dissolved volcanic gases, such as Grand Prismatic Spring, USA (figure 1), and the run-off streams from mines such as Rio Tinto in Spain. Organisms able to tolerate such conditions are termed acidophiles, and members of all three domains of life, archaea, bacteria and eukarya, have been discovered to tolerate conditions down to pH 0. The acidophilic champions are currently archaea in the genus *Picrophilus*, which can tolerate a pH of –0.2. Certain acidic environments, such as acid mine drainages, are also rich in dissolved metal ions (due to the high solubility of metals in acidic water), and so cells must also contend with the toxicity of high concentrations of copper, arsenic, cadmium and zinc.

Organisms living in the opposite extreme, at very high pH, are termed alkaliphiles and are found in environments such as the soda lakes of East Africa or North America. Figure 2 shows

Lake Magadi, a soda lake in Kenya. Extreme alkalinity presents a survival challenge because of the paucity of hydrogen ions in the solution. Cellular metabolism transduces energy by transporting hydrogen ions across the cell membrane to create an electrochemical gradient. These protons are then allowed to flow back in again through the enzyme ATP synthase, which generates molecules of ATP as a chemical energy store for the cell. In extremely alkaline environments, however, the low availability of hydrogen ions for this crucial bioenergetic process becomes limiting. The most alkaliphilic organisms are cyanobacteria able to grow at pH 12–13.

Both acidophiles and alkaliphiles survive by regulating their internal pH to that of mesophilic relatives – near neutral – and so do not require molecular adaptations to pH extremes in their internal cellular components. This is achieved by both active pumping of protons across the cell membrane (outwards for acidophiles, inwards for alkaliphiles), and passive mechanisms including negative surface charges on alkaliphiles (to enhance scavenging of H^+) and positive charges on acidophiles.

Salinity

High salinity reduces the water activity of a solution and disrupts the distribution of charges around macromolecules such as proteins and DNA, forcing them to denature and fall out of solution. Organisms able to flourish in high-salinity environments, such as evaporitic lakes, are termed halophiles and representatives are found in each of the three domains of life. Halophiles exhibit one of two survival strategies to cope with their environment. The first, the "salt-in" approach, maintains intracellular salt concentrations comparable to the external solution (and thus controlling the loss of water from the cell by osmosis), but requires all cellular systems to be adapted to high salinity. The second strategy, "compatible solute", relies on excluding ions such as Na^+ from the cytoplasm by active transport across the membrane, and intracellular accumulation of organic osmolytes such as glutamate, glycine or trehalose, which preserve the osmotic balance with the environment but are not themselves toxic. This strategy is more energetically expensive, requiring constant active maintenance of intracellular conditions, but does not necessitate extensive adaptation of all intracellular machinery to high salinity.

Environments exposing inhabitants to extremes of salinity are often desiccating (as evaporation and water loss is often the mechanism for concentrating dissolved salts) and so organisms must exhibit high tolerance to both stresses.

Pressure

High pressure poses several challenges to survival for piezophilic (also called barophilic) organisms. High pressure compresses the packing of lipids in cellular membranes, and so restricts membrane fluidity, giving a similar outcome to low temperatures. Many organisms respond to this by increasing the proportion of unsaturated fatty acids in the composition of their membranes. Secondly, by Le Châtelier's principle, high pressure causes a shift in the equilibrium of chemical reactions that involve a change in volume; notably in the consumption or production of gases. Thus, biochemical reactions that produce an increase in volume are inhibited by high-pressure environments and piezophilic organisms must adapt to this.

Natural high-pressure environments on the Earth include deep lakes and seas, or the subsurface. Pressure increases at a rate of 10.5 kPa per metre depth in water (hydrostatic pressure), while lithostatic pressure increases at over twice the rate, 22.6 kPa per metre beneath the Earth's surface. The greatest pressure in the Earth's oceans is at the bottom of the Mariana Trench, at just over 11 km depth, corresponding to ~110 MPa water pressure (over a thousand times that at sealevel). Organisms were found even at this crushing depth, many of which were obligate piezophiles only able to grow at pressures above 50 MPa, but many isolates were found to grow at standard temperature and pressure. Microbiological sampling has been performed from a borehole 5.3 km deep in granitic rock in Sweden, discovering a population of anaerobic thermophilic bacteria. Lithostatic pressure at this depth would be around 55 MPa, far less than the Mariana Trench. The ambient temperature at 5.3 km depth was already around 70 °C (and the thermal gradient in oceanic crust is even greater than that in old continental crust), so the limit for life at depth in the Earth's crust is largely defined not by pressure but by temperature – the hardiest piezophiles are likely to reside in the deep cold oceans rather than the planetary crust.

On the other hand, atmospheric pressure diminishes with increasing altitude above the Earth's surface. The limiting factor for growth at high altitude, however, is not reduced pressure but the freezing temperatures atop mountains. As pressures drop very low, towards vacuum, water sublimes and organisms become desiccated. Different exposure experiments on space missions have found that organisms, particularly those in a dormant or spore state, are able to survive the vacuum and consequent desiccation of the space environment, provided they receive adequate shielding from solar ultraviolet radiation. Indeed, freeze-drying or lyopilization, is a standard laboratory procedure for preserving microbial samples for storage.

Radiation

Radiation that affects the survival of organisms comes in a variety of different forms, from short-wavelength electromagnetic waves, such as ultraviolet, X-ray and gamma-ray, to energetic subatomic particles such as the alpha and beta emissions of radioactive decay (helium nuclei and electrons, respectively) or the accelerated protons and heavier atomic nuclei that constitute cosmic rays. Ultraviolet radiation is absorbed by proteins and DNA to drive photochemistry, leading to photolysis or base dimerization and subsequent mutations. The destructive effects of the other radiation types are primarily through ionization: liberating electrons and breaking covalent bonds, and so damaging biomolecules through both direct radiolysis and free radical chemistry.

Protection against ultraviolet radiation is afforded by UV-screening pigments such as carotenoids, or by living endolithically (within cracks or between mineral grains of surface rocks), as do microbial communities in the Antarctic Dry Valleys. Organisms cannot shield themselves against the flux of ionizing radia-

ACIDIC AND ALKALINE ENVIRONMENTS

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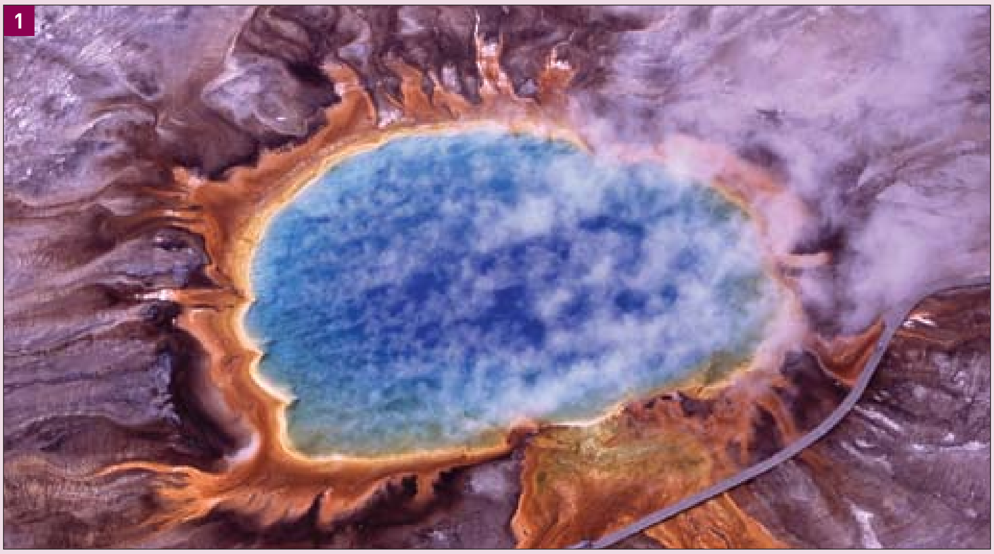

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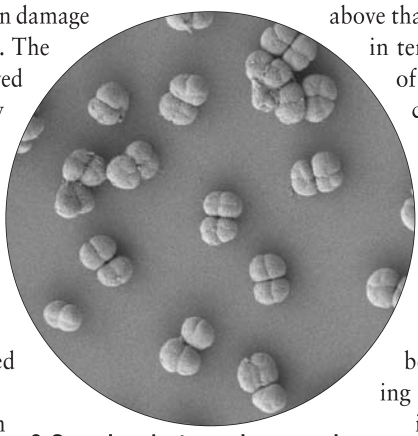
1: Grand Prismatic Spring, a geothermal pool in Yellowstone Park, USA, populated by acidophile thermophiles. 2: Lake Magadi, a highly saline soda lake in Kenya inhabited by halophilic alkaliphilic organisms. (Courtesy of Lottie Davis)

tion, they can only repair cellular damage once it has been inflicted. *Deinococcus radiodurans*, shown in figure 3, is able to survive gamma-ray doses of up to 5000 Grays without measurable loss of viability. Such doses are far higher than ever experienced in the natural environment, and radiation resistance is in fact thought to be a spin-off of desiccation resistance: intracellular drying-out produces similar DNA fragmentation and protein oxidation damage as does ionizing radiation. The survival mechanism employed by *D. radiodurans* is only recently being elucidated as the protection of cellular proteins from radiation-induced oxidation; safeguarding the proteome so that repair enzymes can subsequently reconstitute the fragmented DNA (Daly 2009).



3: Scanning electron microscope image of tetrad clusters of the bacterium *Deinococcus radiodurans*, the most radiation-resistant organism on Earth.

The strict definition of an extremophile is an organism that *requires* a particular environmental extreme in order to grow; a thermophile with thermostable adapted enzymes that can only grow above 70 °C, or a halophile that needs 1.5 M NaCl to maintain their structural integrity, for example. In this sense, xerophiles are not true extremophiles in that they do not require near-complete desiccation for growth; they are merely able to tolerate it by entering a metabolically dormant state. Similarly, there are no known examples of organisms that actually require high dose rates of radiation in order to grow, just those, like *D. radiodurans*, that are able to repair the molecular devastation wrought by irradiation. However, there have been some, as yet unconfirmed, reports of cyanobacteria, algae and fungi exhibiting faster growth rates during irradiation and thus purportedly able to harvest energy delivered by ionizing radiation (see the further reading section below for references).

Chaotropicity

A further factor limiting environmental habitability has come to light recently, in addition to the physicochemical parameters listed above that are most often discussed in terms of the survival limits of terrestrial life. High salt concentrations reduce the water activity of a solution and desiccate and stress a cell in the ways described here under the section on salinity. But the growth-limiting effect of certain salts seems to extend beyond their role in reducing the water activity. $MgCl_2$ is additionally chaotropic in nature, meaning that it exhibits a tendency to destabilize biological macromolecules (Hallsworth *et al.* 2007). $MgCl_2$ is even more destabilizing than ethanol and urea that are commonly used in laboratories to disrupt molecular structures or prevent biological activity.

Natural environments where this effect becomes significant include the briny water of the Dead Sea, which is dominated by $MgCl_2$ rather than NaCl or KCl salts, and even more extreme cases are provided by the deep anoxic basins at the bottom of the Mediterranean Sea, where $MgCl_2$ concentrations can approach saturation. While inhabited by microorganisms, actual microbial reproduction in Dead Sea brine has not been demonstrated, and no microbial activity at all could be detected at the bottom of the $MgCl_2$ chemocline in the Discovery basin of the Mediterranean. It is the chaotropicity of these environments that limits their habitability, more so than their salinity.

Polyextremophiles

While each of these environmental factors limits biology in different ways, it is important to stress that rarely are physicochemical extremes encountered in isolation. In their natural habitat, extremophiles must often tolerate several extremes in combination. For example, organisms living in Grand Prismatic Spring in Yellowstone Park, USA (figure 1), must tolerate both high water temperatures from geothermal heating and low pH from dissolved volcanic gases; inhabitants here are both thermophilic and acidophilic. Similarly, an environment such as the surface mineral soils of the Antarctic Dry Valleys requires tolerance to a combination of low temperatures (and indeed often great swings in temperature due to variability in winds and insolation), desiccation, ultraviolet radiation, and often high salinity. Thus, many extremophiles are in fact better termed "polyextremophilic" to describe their resilience to several stressors simultaneously.

Survival envelope of terrestrial life

A habitable environment is, by definition, one that can support biology. The limits of habitability are thus delineated by the maximum ranges of conditions that life can tolerate: the domain of the extremophiles. In addition to the seven listed environmental parameters that life must contend with, there are many other physicochemical factors that can be limiting such as concentrations of different chemicals including free oxygen or heavy metal ions.

The three most crucial environmental factors that limit biology are commonly taken to be temperature, pH and salinity. Figure 4 displays a plot of the ranges of conditions that known extremophiles can tolerate. The green boundary is the survival envelope of life on Earth (in temperature–pH–salinity space), and at any point within the green volume an organism has been discovered flourishing in that particular combination of hostile conditions. The domain of the mesophiles – non-extremophilic organisms such as *H. sapiens* – lies at the base of this survival envelope: conditions of low–moderate temperature and salinity, and pH neutrality. Polyextremophiles are situated at the very corners of this survival envelope, such as the archaeon *Sulfolobus acidocaldarius* thriving at 80 °C, pH 3 and low salinity. In fact, this figure is slightly out of date and the currently known survival envelope has expanded a little beyond the limits shown here – for instance, an archaeal strain has since been reported to grow at up to 121 °C, slightly extending the "toe" of this boot-shaped envelope.

The extent of this survival envelope of terrestrial life is staggering, protruding into hostile environments that had previously been thought to be completely sterile. These discoveries in terrestrial extremophile research have really encouraged optimism in the possibility of similarly hardy life in extraterrestrial environments.

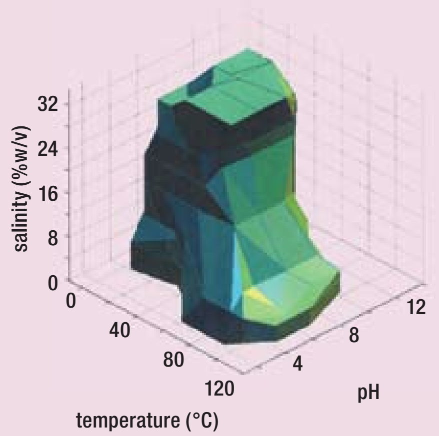
But the question remains: why doesn't life on Earth bulge even further within this parameter space? Why are certain regions of this physicochemical parameter space not populated by terrestrial organisms? Why has biology not colonized these apparent gaps?

Limits on habitability

The furthest extent of environmental parameter space that is theoretically habitable is delineated by physical conditions that permit flowing water and stability of organic macromolecules. Pure water is liquid between 0 °C and 100 °C under standard conditions, but the freezing point can be depressed by dissolved salts and the boiling point delayed by higher pressures. Hydrothermal vents on the ocean floor pump out water at up to around 400 °C, prevented from boiling by the overbearing pressure of the water column, but above about 150 °C organic molecules start to fall apart. So the actual upper survival limit for temperature is defined by chemical constraints and the molecular stability of the components of life.

A meaningful exercise is to consider diverse extremophiles on the outer fringes of the survival envelope of terrestrial life; to identify regions of this parameter space where life appears to be right up against the theoretical maximum limit, and other parameter combinations where terrestrial life could perhaps be improved upon. For example, the hottest limit recorded for growth is 121 °C, with survival for short periods reported even at 130 °C, which isn't far off the theoretical maximum prescribed by organic molecule stability. On the other hand, no examples of terrestrial acidophilic psychrophiolic organisms are known, yet there is no major physical or chemical constraint on why this may be. The reason may simply be a consequence of the lack of such environments on Earth, and so biology has had no opportunity to adapt to such a combination. Perhaps, provided with the appropriate environmental conditions, extraterrestrial life would encounter no barrier to extending its habitability range into the region of low temperature and low pH.

The most notable gap in the extent of terrestrial life shown in figure 4 is the wide space above the "toe" of the boot-shaped survival envelope. This region of the parameter space corresponds to conditions of high temperature and high salinity. Why does biology appear not to tolerate such a combination? Is it that very hot, salty water presents some form of insurmountable challenge or stress to survival, perhaps by compromising membrane integrity or destabilizing DNA? Or alternatively, does terrestrial life not occupy that region of parameter space simply because such a niche is not available in the natural environment? Has terrestrial life simply lacked the opportunity to adapt to such a combination? Cold salty environments exist, such as in Antarctic lakes or the briny veins within sea ice; indeed, it is the high salinity that allows the water to remain liquid, and thus able to sustain active life, at low temperatures. But where in the natural environment would hypersaline solutions become superheated?



4: A slightly dated figure depicting the survival envelope of terrestrial life. Three environmental parameters are considered here – pH, temperature and salinity – and the tolerance ranges of known extremophiles are plotted to produce this boot-shaped volume. (Courtesy of Julian Wimpenny)

So a crucial topic in extremophile research is what defines the boundaries of the survival envelope of terrestrial life. Are the limits determined by genuine biological restrictions (such as the degradation of organic molecules), which may be shared with extraterrestrial organisms, or are they more a consequence of the available range of environments on Earth to which life has had the opportunity to adapt, and so perhaps idiosyncratic to terrestrial life?

Origin of life

There is a final key point on extremophiles and what they reveal about the ranges of habitability and possibility of life beyond Earth. Extremophiles demonstrate the incredible adaptability of life once it has arisen, but make no statement about the likelihood of life arising in the first place. It is almost certain that the physicochemical conditions able to nurture emerging self-organizing networks of prebiotic chemistry are much more tightly constrained than the conditions for survival of cells, protected within a membrane barrier and able to regulate internal conditions.

We can derive hints of the environmental conditions in which the universal common ancestor of terrestrial life lived (which presumably are similar to the conditions for prebiotic chemistry leading to the first cells). Organisms that lie at the root of the three-domained phylogenetic tree of life on Earth, the organisms alive today that appear most closely related to all others, are all thermophiles, suggesting that the habitat of the earliest life was hot and not very salty (which fits with expectations of the environment of the primordial Earth). Additionally, the fact that both acidophiles and alkaliphiles actively maintain their cytoplasm near neutrality suggests that the universal ancestor lived in waters neither very acidic nor alkaline. So it seems likely that the first life on Earth occupied an environmental niche somewhere within the toe of the boot-shaped envelope in figure 4 – hot, neutral and not particularly salty – and has since diversified and expanded outwards from this point to fill the whole survival envelope of modern life.

But if the environment of another world, such as Mars or Europa that are championed as potential habitats for extraterrestrial life, cannot provide the right combination of physicochemical conditions for the emergence of life in the first place, then the astounding extent of habitability revealed by the extremophiles is largely irrelevant.

There are, therefore, two major challenges that remain in fully defining the biological constraints on habitability, and thus which extraterrestrial locales offer the best hopes for life. Firstly, it is key to characterize the theoretical limits for supporting biological processes as distinct from the limits exhibited by terrestrial extremophiles, which may be idiosyncratic to the Earth and its repertoire of habitats available for life to adapt to.

Secondly, researchers in the field of prebiotic chemistry can provide insights into the necessary physical and chemical conditions for the original synthesis of life, from which starting point organisms can adapt to the full survival range of terrestrial extremophiles. ●

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Further reading

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