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The limits for life under multiple extremes

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Life on Earth is limited by physical and chemical extremes that define the 'habitable space' within which it operates. Aside from its requirement for liquid water, no definite limits have been established for life under any extreme. Here, we employ growth data published for 67 prokaryotic strains to explore the limitations for microbial life under combined extremes of temperature, pH, salt (NaCl) concentrations, and pressure. Our review reveals a fundamental lack of information on the tolerance of microorganisms to multiple extremes that impedes several areas of science, ranging from environmental and industrial microbiology to the search for extraterrestrial life.

The terrestrial biosphere and extremophiles

On a planetary scale, the Earth's biosphere (see Glossary) resembles a thin film in contact with the lithosphere and the atmosphere [1,2]. The parameter space within which life persists is determined by physical, geochemical, and biological factors that act to limit the growth and function of organisms [3,4]. These factors include the availability of liquid water, energy (via photons and/or chemical sources), nutrients, and trace elements. Additionally, conditions including ambient temperature, pH, salinity, and pressure interact with biological systems to define the 'habitable space' for life [5]. Within this space, essential biological processes including cell growth and metabolism are maintained.

Over the past three decades, remarkable advances have been made in charting the boundaries of the habitable space on Earth via research into microbial extremophiles, organisms which either tolerate environmental extremes or require them for growth [3,5–8]. Although extremophiles are represented by all domains of life, extreme environments are typically inhabited by prokaryotes (bacteria and archaea) [7]. Defining the tolerance windows of these organisms in response to environmental stressors is essential to advance our knowledge of the evolution, diversification, and extent of life over time, and to elucidate the habitability of other planets within and beyond our Solar System [2,4,7–10]. Understanding the limits for life is of additional biotechnological interest due to the applications of extremophiles and their biomolecules in industrial processes [3,10].

Both laboratory-based and theoretical investigations have led to significant advances in establishing limits for microbial growth under extremes. These range from high and low temperatures to extremes of salinity (NaCl), hydrostatic pressure, and several other stressors (e.g., ionizing radiation and elevated concentrations of heavy metals) [3,4,6,11,12]. Although the impacts of individual extremes on microorganisms have been widely researched [3], attempts to define their collective influences on life are scarce. This is highly surprising because most extreme environments on Earth are characterized by several coexisting stressors. Moreover, an increasing number of microbial strains isolated from these environments have been found to tolerate multiple extremes [13–20]. Organisms tolerating more than two extremes are known as polyextremophiles [19,21,22] (see [3] for details on the classification of extremophiles).

In addition to the increasing number of isolated polyextremophiles, culture-independent studies have discovered life in environments that were previously considered uninhabitable, including deep hypersaline anoxic brines [18,20,23]. Moreover, we are only beginning to understand the mechanisms by which multiple stressors limit microbial growth and survival [6,15,24–26]. Therefore, the true shape of the terrestrial biosphere remains undefined.

In this review, we systematically explore the boundaries for microbial life under combinations of multiple extreme conditions. Following the construction of visual maps of the terrestrial habitable space, we discuss our understanding of the mechanisms underlying adaptation to multiple extremes, and how this information relates to the search

Glossary

Biofilm: microbial aggregates adhering to a surface and encased within a matrix of extracellular polymeric substances.
Biosphere: the global space occupied by life and biological processes on Earth.
Chaotropicity: the tendency of a compound to disrupt cellular macromolecules.
Habitable space: the physical and chemical parameter space in which cell growth is maintained.
Interspecific interaction: an ecological interaction between members of different taxa within a community.
Ionizing radiation: radiation capable of producing ions by removing electrons from atoms.
Lithosphere: the outer layer of a planet consisting of rock and/or soil.
Polyextremophile: an organism tolerating or requiring more than two extremes for growth.
Prokaryotes: unicellular organisms lacking membrane-bound nuclei and comprising two out of three domains of life (*Bacteria* and *Archaea*).
Water activity: a thermodynamic measure of the biological availability of water in a solution.

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for life in the universe. These data are used to generate recommendations for research, with the aim of advancing future investigation of microbial extremophiles, the limits for life on Earth, and assessing the habitability of extraterrestrial environments.

Maps of habitability under synchronous extremes

Representations of terrestrial habitability may be created by visualizing the growth minima, optima, and maxima of extremophiles in response to environmental constraints. This approach has previously been employed to create bivariate approximations of habitability based on temperature, salinity, pH, and radiation [6]. Additionally, phase diagrams based on temperature and pressure have been used to characterize the habitability of liquid water on Earth [27]. Few multifactorial investigations of microbial responses to different stressors exist and they have focused on a limited selection of strains and environments, as opposed to analyses of the entire biosphere [15,28–31]. For example, the growth optima of several polyextremophilic and extremely halophilic taxa have been explored as a function of salinity, pH, and temperature [31]. To our knowledge, only a single and now dated 3D map of the Earth's biotic window has been constructed approximately two decades ago as a function of temperature, pH, and salinity (NaCl) (see Figure 4 in [4]).

We constructed 3D representations of the habitable volumes for microbial life under synchronous extremes, using cardinal growth data available for extremophilic prokaryotic strains (Box 1). For the purposes of this review, a 'habitable' environment was defined as one in which microbial growth is sustained. Data pertaining to prokaryotic growth under high pressures were considered in addition to temperature, pH, and NaCl concentrations to create maps of the habitable window for prokaryotic life.

Box 1. Data retrieval and visualization

In order to construct visual maps of the boundary space for life on Earth, previously published data were collected for a total of 67 prokaryotic extremophilic isolates (Table S1 in the supplementary material online). The data utilized consisted of measurements of minimal, optimal, and maximal conditions supporting extremophile growth across a range of temperatures (°C), pH values, NaCl concentrations (% w/v), and pressures (MPa). Optimal conditions were defined as those supporting a minimal population doubling time. Limiting values for growth under a given environmental factor were typically based on measurements obtained at otherwise optimal conditions. In the case of certain piezophilic taxa, data were available for different temperature–pressure regimes and were accounted for in order to define the boundary space for growth (Table S1 in the supplementary material online).

3D approximations of the biotic window were constructed by visualizing the 'habitable volumes' of individual strains, using a single limiting growth condition and two optimal conditions for each data point (Table S1 in the supplementary material online). Where measurements of optimal growth conditions were unavailable, the mean of the minimal and maximal conditions for growth was utilized. In the additional absence of minimal and maximal growth data, optimal conditions for growth were estimated using the median of the collective set of measurements corresponding to a given environmental factor and type of extremophile (defined in [3]). Missing data with reference to optimal pressures for growth were assigned a value corresponding to atmospheric pressure (0.1 MPa). To facilitate visualization of the maps, optimal conditions for growth represented by single values were substituted by a range of values based on the original measurement [±2 °C for temperature, ±0.5% (w/v) for salinity, ±0.2 for pH, and ±2 MPa for above-atmospheric pressure].

Temperature, salinity, and pH

Approximating the shape of the biosphere as a function of temperature, NaCl concentrations, and pH illustrated how the limits of growth established for prokaryotic isolates have considerably broadened over the previous two decades (Figure 1) (see original temperature–salinity–pH map in [4]). The minimal and maximal habitable temperatures measured in the absence of corresponding pressure data have decreased from 0 °C to −12 °C and increased from 108 °C to 121 °C, respectively (strains listed in Table S1 in the supplementary material online). However, a higher temperature limit for archaeal growth has been measured under elevated pressures (discussed in 'Temperature, salinity, and pressure'). The lowest reported habitable pH value for prokaryotic isolates has decreased from 2 to 0 (Table S1). Figure 1 also demonstrates that our understanding of the biosphere now covers life within environments characterized by a combination of high NaCl concentrations and low pH values. For example, the bacterial strain Brown 1 is able to grow at a pH value of 3 and within NaCl-saturated media (determined separately) [32].

Figure 1 indicates an absence of hyperthermophilic alkaliphiles and data from habitats characterized by a combination of extreme acidity and sub-zero temperatures, potentially due to insufficient sampling and/or the rarity of such environments on Earth [4–6,31]. There is also a paucity of strains occupying the region of Figure 1 characterized by extremely high temperatures and salinities [6]. Indeed, no strains have been discovered to grow under the joint extremes of high temperature, salinity, and pH, raising the question of whether life is possible under a combination of these factors [31]. Synergistic impacts of these variables on microbial growth have been demonstrated by growth experiments using individual taxa [28–30] and it is possible that similar interactions influence the shape of the entire biosphere.

Temperature, salinity, and pressure

Mapping the limits for life based on temperature, NaCl concentrations, and pressure resulted in a strongly constrained assemblage of livable spaces under the combined influences of high salinity (>15% w/v) and pressure (>20 MPa) (Figure 2). This pattern is probably due to a lack of growth data from environments characterized by these extremes, as opposed to biological adaptations (discussed in 'Microbial adaptations to multiple stressors') [15,33]. In fact, most known piezophilic strains have been isolated from habitats with relatively low NaCl concentrations, whereas halophiles frequently originate from environments characterized by atmospheric or near-atmospheric pressure (Table S1). Even so, viable microorganisms (e.g., *Bacillus* spp.) have been recovered from deep hypersaline sediments in the Mediterranean Sea [34], emphasizing the need to further assess the potential for life under extreme salinity and pressure.

Figure 2 also illustrates that piezophilic life histories are possible at both extremely high and low temperatures