How extreme is extreme?

Brandi Kiel Reese[1†], Julie A Koester[2†], John Kirkpatrick[3], Talina Konotchick[4], Lisa Zeigler Allen[4], and Claudia Dziallas[5]*
[1]Texas A&M University-Corpus Christi, Department of Life Sciences, Corpus Christi, TX, USA
[2]University of North Carolina Wilmington, Department of Biology and Marine Biology, 601 S. College Road, Wilmington, NC, 28403 USA
[3]University of Rhode Island, Graduate School of Oceanography, 215 South Ferry Road, Narragansett, RI 02882 USA
[4]J. Craig Venter Institute, 4120 Capricorn Lane, La Jolla, CA 92037 USA
[5]University of Copenhagen, Marine Biological Section, Strandpromenaden 5, 3000 Helsingør, Denmark

Abstract

A vast majority of Earth's aquatic ecosystems are considered to be extreme environments by human standards, yet they are inhabited by a wide diversity of organisms. This review explores ranges of temperature, pH, and salinity that are exploited by the three domains of life and viruses in aquatic systems. Four *Eukarya* subgroups are considered: microalgae, fungi, macroalgae, and protozoa. The breadth of environmental ranges decreases with increasing cellular complexity. *Bacteria* and *Archaea* can live in environments that are not physiologically accessible to the macroalgae. Common strategies of adaptation across domains are discussed; for example, organisms in all three domains similarly alter cellular lipid saturation in membranes in response to temperature. Unique adaptations of each group are highlighted. This review challenges the use of the word "extreme" to describe many ecosystems, as the title is applied in relation to human habitability, yet a majority of life on the planet exists outside our habitable zone. Examining traits of the boundary lineages, those that exist at the physiological edge of the entire biome, or within a specific group, will provide a better understanding of life on Earth and provide a starting point to advance future detection of life on Earth and elsewhere.

Section 1. Introduction

Organisms living in extreme environments are known as extremophiles (from the Latin *extremus* meaning "extreme" and Greek *philos* meaning "loving"). Do they thrive in these extreme environments, or are they simply tolerating it temporarily? What defines an extreme environment? The anthropocentric view is simply defined as anything outside what is normal for humans (e.g., oxic, photic, room temperature, neutral pH). However, life in extreme environments is not rare: most of the ocean would be considered an anthropogenic extreme, as it is cold, dark, and saline. Still the term 'extremophile' invokes images of single-celled *Bacteria* and *Archaea*; however all three domains experience conditions that lie outside the human definition of "normal." The environmental conditions that define "extreme" therefore depend on the taxa being studied. Thus, what is extreme for one organism is not extreme for another, making extreme a relative term. Focus should be given to environmental conditions that represent the true boundaries for taxa specifically, or for Earth's biome as a whole. Therefore, as with the change of no longer taxonomically describing organisms as being "higher" or "lower," the description of extreme should be reconsidered as many organisms are capable of growth outside the limited conditions that humans can tolerate. We suggest that those organisms surviving at the edge of a biosphere habitable zone should be considered *boundary organisms* as they occupy a special niche within Earth's biosphere. This would provide a more descriptive term than *extreme* and remove the implied anthropocentric connotation.

This review outlines the boundary conditions for many different branches on the tree of life. Almost everywhere scientists have looked on Earth, life of some kind is present. Life proliferates in habitats that span nearly every physical or chemical variable, with the single limitation that water must be present for some portion of the organism's life history. Many organisms simultaneously experience, and are adapted to,

boundary conditions in more than one environmental parameter. This review is not meant to be exhaustive but rather indicative of observed trends and the range of adaptations to extreme environments that exist across trophic levels (Fig. 1). It is crucial to note that "adaptation" encompasses multiple strategies, which may be short-term (merely persisting until mesophilic conditions return) or long-term (genetic changes which allow organisms to grow and multiply in the midst of extreme conditions). The distinction between persistence and growth can be clearly delineated for organisms being cultured in ideal laboratory settings, but is often elusive in the environment. We try to reflect this distinction to the extent that it is made in the literature for three main environmental parameters: temperature (Fig. 2), pH (Fig. 3), and salinity (Fig. 4). This type of a study, examining boundary conditions rather than arbitrary "extreme" descriptions, will help define the limits of modern life and identify areas for future study, while also increasing our understanding of the potential for life on other planets and the nature of life on early Earth.

Section 2. Viruses

Viruses are ubiquitous in all environments from oceans to the human microbiome and often are the most abundant entity within ecosystems, generally an order of magnitude greater than *Bacteria* or *Archaea*. Viruses 'live' essentially as parasites on their host, which can be of viral (e.g., Mamavirus), bacterial, archaeal, or eukaryotic origin (Hyman and Abedon 2012). These infections can enable transfer of genetic material between and among viruses and hosts; therefore viruses can serve to induce genetic diversity within varied ecosystems. The microbial food web plays a major role in recycling carbon and nutrients and regulates energy transfer to higher trophic levels (Azam 1998; Kirchman 1994). Current knowledge of virus abundance, diversity, and role within extreme environments is rapidly advancing. The onset of new technologies, such as those involved in community sequencing of genomic material and microscopy, enable discovery within new ecosystems at the boundary of the known biosphere. Still, few studies have described the niche differentiation that leads to specific groups being endemic to certain extreme or boundary conditions.

Temperature

High temperature habitats from marine hydrothermal vents to terrestrial hot springs are active sites for viruses. In general, bacteriophages prevail in hydrothermal vent regions and archaeal phages in terrestrial regions (with notable exceptions), likely due to the available host *Epsilonproteobacteria* and *Crenarchaeota* taxa, respectively (Prangishvili and Garrett 2005; Snyder et al. 2003). Viruses have different modes of 'life' in reference to their replication style, and it has been found that lysogenic phages (i.e., those that can incorporate their genome into their hosts and persist for several rounds of host replication) persist in marine high temperature systems (Williamson et al. 2008). Abundances within marine
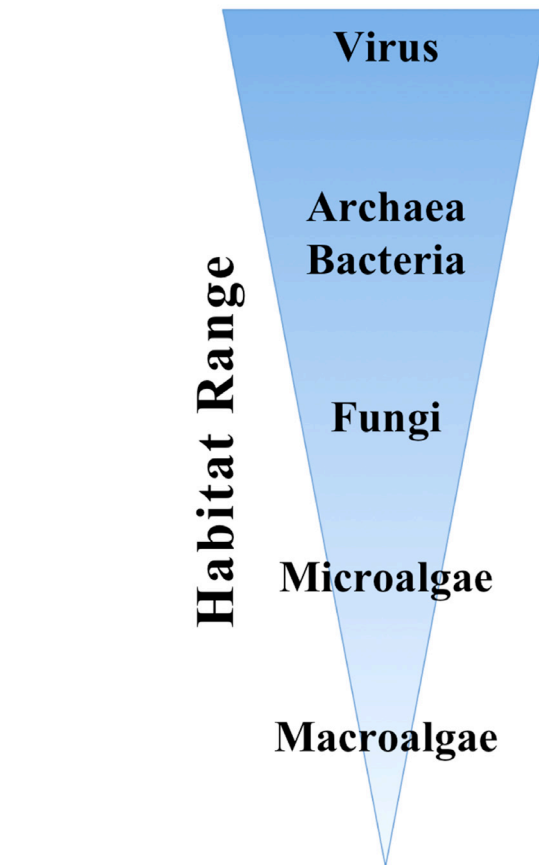

Fig. 1. Hierarchal description of the habitat range of organisms, including viruses, discussed in this review.

vent systems are relatively high (10^5-10^7 mL^{-1}) compared with background seawater (10^4-10^5 mL^{-1}), suggesting active viral production within vent fluids and the subseafloor (Ortmann and Suttle 2005). The highest temperatures in which viruses have been isolated is 100°C from a Yellowstone hot spring (Le Romancer et al. 2007). This field of research is still developing, and since viral abundances appear to track prokaryotic abundances within environments, we suggest that this upper temperature boundary will be extended to the limit for bacterial and archaeal life (discussed below).

High viral abundances have been reported at cold temperatures as well. Polar sea ice contains approximately 10- to 100-fold greater viral counts than the warmer underlying water (Maranger et al. 1994). Viruses persist in temperatures between –2° to –6°C in sea ice where necessary substrates and brine channels vary with temperature causing deviations in virus/host contact rates that ultimately lead to changes in infection dynamics and nutrient availability (Ewert and Deming 2013; Krembs et al. 2000). Culturable virus/host systems from low temperature dependent (psychrophilic) organisms exist for isolates from Arctic sea ice, and like their hosts, these viruses are restricted to reproducing in low temperatures
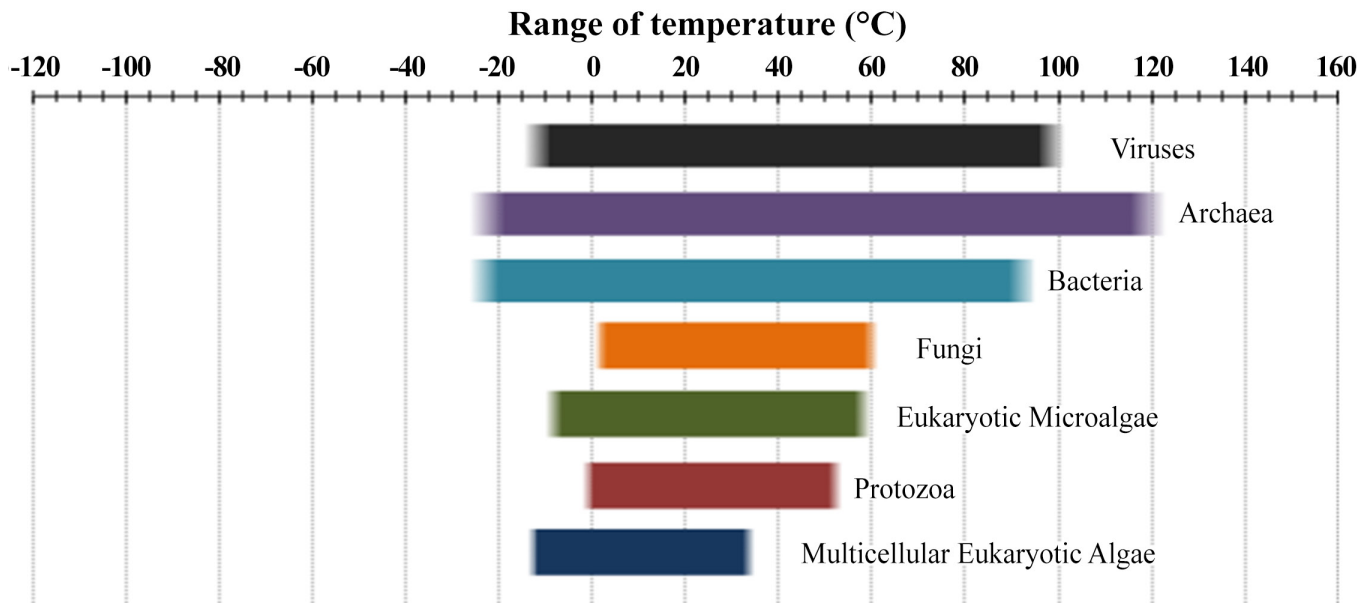
Range of temperature (°C)

Fig. 2. Observed natural temperature ranges of the taxonomic groups outlined in the text, including viruses, based on measurements and observations of activity and growth. References are as follows: high temperature (Tansey and Brock 1972; Knoop and Bate 1990; Huber et al. 1992; Maheshwari et al. 2000; Baumgartner et al. 2002; Ciniglia et al. 2004; Le Romancer et al. 2007; Takai et al. 2008; Toplin et al. 2008), low temperature (Becker 1982; Franzmann et al. 1992; Junge et al. 2001; Krembs et al. 2002; Junge et al. 2004; Magan 2007; Rose and Caron 2007; Yau et al. 2011).
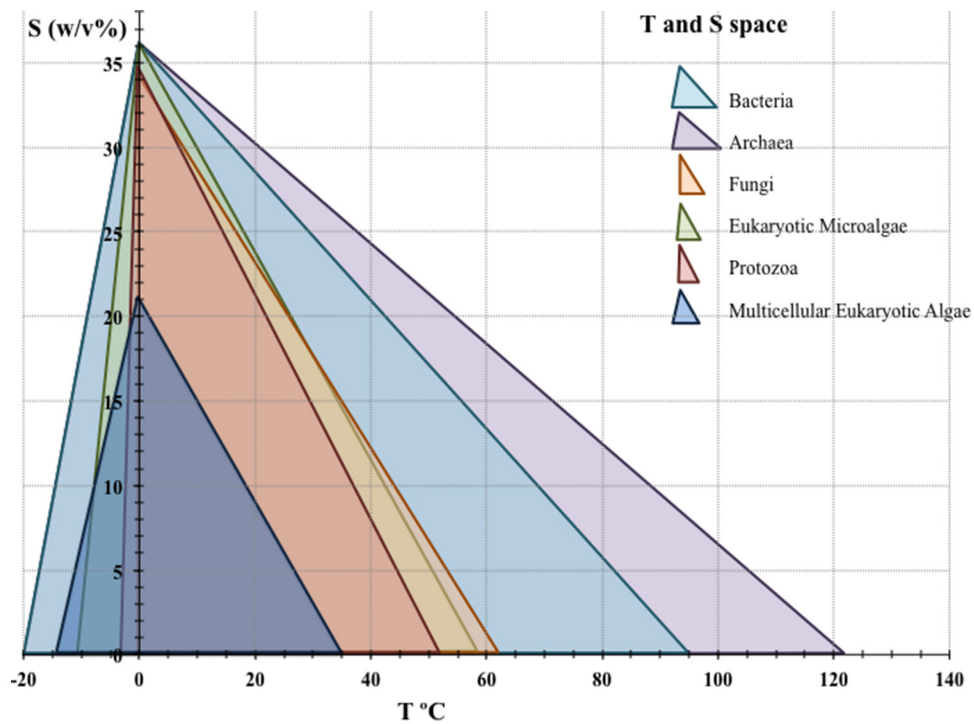

Fig. 3. Ranges of temperature and pH extremes in which various taxonomic groups are found in nature. Where the distinction can be made, the ranges here reflect active metabolism and/or growth, not simple persistence or stasis; for example, Bacteria and Archaea at hydrothermal vents are often periodically exposed to temperatures well in excess of those shown here which represent instead maximum growth temperature. Lines between axes are connectors only and not indicative of data points. References are as follows: high temperature (Tansey and Brock 1972; Knoop and Bate 1990; Huber et al. 1992; Maheshwari et al. 2000; Baumgartner et al. 2002; Ciniglia et al. 2004; Takai et al. 2008; Toplin et al. 2008), low temperature (Becker 1982; Franzmann et al. 1992; Junge et al. 2001, 2004; Krembs et al. 2002; Magan 2007; Rose and Caron 2007), high pH (Skinner 1968; Hecky and Kilham 1973; Maberly 1990; Nagai et al. 1998; Schrenk et al. 2004; Ong'ondo et al. 2013), low pH (Albertano et al. 1981; Schleper et al. 1995; Kelly and Wood 2000; Packroff and Woelfl 2000; Sabater et al. 2003; Baker et al. 2004).
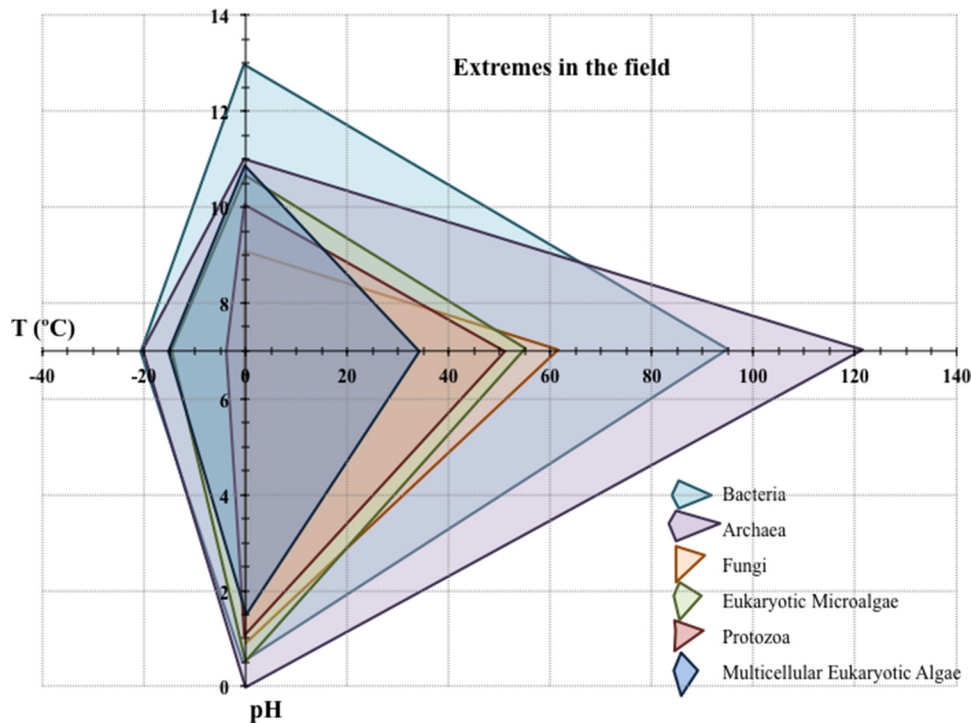
Fig. 4. Ranges of documented high salinity to temperature ranges of taxonomic groups. Salinity values of 35–36% w/v should be regarded as saturated solutions. Extremely low salinity tolerances are an open question, but certainly on a percentage scale are as near to zero as makes little practical difference. Still, absolutely pure water without any ions, gases, or dissolved substrate is, in theory, inhospitable. References are as follows: salinity (Kirby 1934; Tansey and Brock 1972; Henderson 1977; Borowitzka 1981; Knoth and Wiencke 1984; Buchalo et al. 1999; Antón et al. 2000; Oren 2005), high temperature (Knoop and Bate 1990; Huber et al. 1992; Maheshwari et al. 2000; Baumgartner et al. 2002; Ciniglia et al. 2004; Takai et al. 2008; Toplin et al. 2008), low temperature (Becker 1982; Franzmann et al. 1992; Junge et al. 2001, 2004; Krembs et al. 2002; Magan 2007; Rose and Caron 2007).

(Borriss et al. 2003). Hypersaline lakes are particularly harsh environments within polar regions. Phytoplankton viruses have been identified recently in these cold (–14°-15°C), hypersaline (~ 230 g L⁻¹ max salinity) lakes, which require the organisms inhabiting them to be co-adapted to two boundary conditions, and have been a source of phytoplankton viruses in recent studies (Yau et al. 2011).

The viral hot and cold temperature boundary appears to be limited only by the survivability of the host organism. This statement should guide future research and attempts should be made to test these boundaries.

pH

As viruses are found in every system where potential hosts thrive, those that infect alkaliphiles and acidophiles (organisms that persist in high and low pH, respectively) have been identified. Evidence of viruses from alkaline lakes was recently first reported in 2004 from Mono Lake (pH 10) in California (Jiang et al. 2004). At this high pH condition, the estimated abundance was much higher than in other more neutral pH aquatic systems ranging from 10^8-10^9 mL⁻¹ (Jiang et al. 2004).

Conversely, much less data exists for viruses of acidophilic organisms. Archaeal viruses similar to those from the hyperthermophilic environments mentioned above

have been isolated in association with *Sulfolobus*, a common *Crenarchaeota*, from acidic hot springs in Yellowstone National Park at pH 1.5-2 (Bize et al. 2008; Prangishvili et al. 2006; Rice et al. 2001; Zillig et al. 1996). The morphological structures and genome diversity of crenarchaeal viruses is strikingly unique compared with other archaeal groups and bacteriophage, thus providing evidence into evolution and adaptation unrelated to other known viruses (Pietilä et al. 2014). More work in these areas will be needed to determine the boundary for viral persistence within microbial communities.

Salinity

The first haloarchaeal virus was discovered in 1974 (Torsvik et al. 2002), yet to date only about 15 have been described. Recent studies on the activity of haloviruses indicates that these communities are transcriptionally active and that groups with high GC content in their expressed sequences are the most active (Santos et al. 2011). Additionally, in hypersaline marine systems (e.g., the Dead Sea), the reported estimates of viral abundance are high at 10^7 mL⁻¹ (Oren et al. 1997), and vast genomic diversity exists with sizes ranging from 10 to 533 kb (Sandaa et al. 2003). Viruses follow the trend in these environments of being more abundant than their microbial

counterparts and based on estimates of microbial mortality, viruses have been shown to limit the microbial population (Guixa-Boixareu et al. 1996). As salinity increases, the persistence strategies of viruses appear to switch from a prevalence of lytic to lysogenic modes of infection (Bettarel et al. 2011).

Principally, viruses exist wherever potential hosts prevail. Future research should be guided by the knowledge of hosts and their boundary conditions, and attempts should be made to test these boundaries. Their ability to adapt across boundary conditions lies in their genomic and morphological diversity as well as their lysogenic capabilities. Whereas not all viruses are lysogenic, it has been speculated that in some environments, lysogeny is a preferred mode of replication as a means to evade the harsh environments except when absolutely necessary.

Section 3. Archaea

Archaea and *Bacteria* are frequently lumped as the "microbial fraction" in many studies, and indeed they can be indistinguishable morphologically. Ecologically, the two domains of life are distinct and warrant separate discussion here. *Archaea* have been associated with extreme, or boundary, environments since their categorization as a separate domain of life because some of the original representatives came from hot springs and salt lakes. Whereas some *Archaea* are boundary organisms, they are also abundant in environments including terrestrial soils and oxic seawater. The "extremophile" bias has been greatly reduced with increased sampling and culturing efforts. *Archaea* can be difficult to distinguish from *Bacteria* based on size and morphology; therefore, sequence information and/or lipid analyses are often required to make a definitive identification between them. In the environment, both domains co-exist, co-habitate, and even form consortia (e.g., in the case of anaerobic methane oxidation) (Orphan et al. 2000; Orphan et al. 2002; Dekas et al. 2009). Studies of boundary environments, such as sea ice and hydrothermal vents, often assay microbial cells or metabolic activity, thus grouping the two domains because of the difficulty in distinction. Below is a discussion on some of the known environmental boundaries for *Archaea* in which these microorganisms are often incapable of life under "normal" conditions, and therefore the boundary conditions should not be considered "extreme."

Temperature

The current record-holder for high temperature pure culture work is held by *Methanopyrus kandleri* at 122°C (Takai et al. 2008), but it is not the only hyperthermophilic archaeon. There is great interest from the biotechnology industry in high temperature–adapted archaeal lineages. Products including DNA polymerase isolated from *Pyrococcus furiosis* (Pfu) have proven scientifically valuable. Culturing hyperthermophilic *Archaea* is very difficult not just due to the high temperatures, but varying pressures and pH. Once optimal temperature,

pressure, and pH are achieved for archaeal growth, steady state growth is maintainable. In the environment, hot springs and hydrothermal vents can be more thermally variable than lab cultures, which tend to be isothermal. Microbes at vents, for example, can be periodically exposed to fluids in excess of 300°C (Holland and Baross 2003). The extent to which *Archaea* can survive these events is largely unknown, but the upper thermal boundary undoubtedly depends on how the limit is defined. There is a difference between growth and survival, with survivability being temporally dependent on the exposure period to adverse environmental conditions.

The lower temperature boundary for *Archaea* is less well defined. Based on pure cultures and extrapolation from growth curves, a limit of –2.5°C was predicted for *Methanococcoides burtonii* (Nichols and Franzmann 1992). In the environment, aquatic organisms are often frozen into sea ice and can exist in small brine channels of liquid water and extremely cold temperatures (Deming 2002), but whether they are active or not is more difficult to assess. Similarly, *Halorubrum lacusprofundi* was isolated from an extremely cold environment (–20°C), but in lab is grown at room temperature or above (Franzmann et al. 1992). Few cold temperature studies have focused on *Archaea* in situ. Junge et al. (2004) measured microbial activity in sea ice down to –20°C, although this was a mix of *Bacteria* and *Archaea*. Looking specifically at archaeal DNA sequences, Collins et al. (2010) noted the persistence of multiple lineages in sea ice over a winter with in situ temperatures dropping as cold as –26°C. In an artificial setting, laboratory cultures for *Bacteria, Archaea*, and eukaryotic micro-algae are routinely frozen at –80°C or in liquid N_2 (–196°C), and resuscitated for research purposes.

The psychrophilic *Archaea* provide a great example for the need to clearly determine activity versus survivability at biosphere boundaries. Additional research should be done to specifically determine the lower temperature boundary for active metabolic function. A potential additional criterion may be physiological maintenance activity versus growth. Results from such studies will help better define the boundaries of these archaeal lineages and can be applied to other domains.

pH

Archaea are found at the pH boundaries for life on Earth. Many studies of high pH organisms have focused on systems such as hydrothermal vents and hot springs fed by serpentinization reactions, such as the archaeal mats of *Methanosarcinales*, which are found in fluids up to pH 11 (Schrenk et al. 2004). Soda lakes also host notable archaeal phylotypes such as *Natronobacterium* at pH 10 (Tindall et al. 1984). Conversely, *Picrophilus oshimae*, first cultured in the 1990s, has been shown to grow in media as acidic as pH 0 (Schleper et al. 1995). These acidophiles and additional species of *Sulfolobus* and *Acidanus* are typically obligate acidophiles; their adaptations to high hydrogen ion concentrations render them incapable of growth at more neutral pH levels (i.e.,

greater than 4 or 5). As noted earlier, these lineages make good examples for why the term "extreme" is relative and should be reconsidered as it provides a biased description.

Salinity

Many *Archaea* are associated with extreme halophily, and historically were considered dominant lineages within high salt environments. The archaeal class *Halobacteria*, with many cultured representatives, often tolerate completely saturated environments of 36% for standard pressures and temperatures (Henderson 1977). The consequences of high salt concentrations are the same as desiccation (i.e., low water activity). Rather than preventing salt uptake, which could induce lysis, *Halobacteria* cope with extremely high intracellular salt concentrations by using specialized enzymes. These enzymes denature at low salt concentration, and are used to actively pump Na^+ out and K^+ in (Ng et al. 2000). As discussed with *Bacteria*, these strategies and their genetic composition are shared between halophiles such as the archaeon *Halobacteria* and the bacteria *Salinibacter* (Mongodin et al. 2005). Traits that have co-evolved across domains provide general life boundary characteristics that can be used to determine molecular markers. These conserved gene sequences would provide starting points for advanced searches to identify additional halophilic species and potentially in situ quantification of yet to be cultured populations.

Section 4. Bacteria

Phylogenetically, *Bacteria* is a very diverse domain that includes a wide range of metabolic strategies unique only to microbes such as autotrophy, heterotrophy, phototrophy, chemotrophy (using inorganic compounds as energy sources), and fermentation (Oren 2009). Bacteria are ubiquitous; however, only an estimated 0.1% to 1% of bacteria can be cultivated in a laboratory under "normal" conditions. The challenge to microbiologists is to understand the unculturable microorganisms using genetic information. Comparative ribosomal RNA sequencing analysis has lead to the construction of phylogenetic trees that map the natural relationships and quantify the degree of relatedness among all life. Additionally, ribosomal RNA probes identifying specific microorganisms are used to determine the distribution of those organisms throughout the environment. Using molecular techniques, our knowledge of the diversity of bacterial life in extreme environments has rapidly increased in recent decades. Results from these studies have demonstrated that environments previously considered extreme based on our own habitable ranges support highly diverse ecosystems where bacterial and archaeal lineages flourish.

Bacteria survive in a wide and diverse range of environmental parameters that are not limited to the boundary conditions of temperature, pH, and salinity. The environments discussed below are not meant to be an exhaustive list, but rather a starting point using some common environmental parameters where *Bacteria* survive. An interesting example of life at additional boundary environments is that of *Deinococcus radiodurans*, which survives high doses of radiation. This lineage was first discovered in 1956 in a can of ground meat sterilized with radiation. It is able to survive high doses of radiation by using specialized DNA repair enzymes and cell membrane adaptations containing bacterioruberin, a 50 carbon carotenoid pigment that protects against DNA damage caused by UV light (White et al. 1999). Additional boundaries of the biosphere should be explored to identify other boundary lineages, with the goal of understanding diversity from all physiological aspects, and not simply labeling them "extreme" based on our own, human-based environmental limitations.

Temperature

Thomas Brock was a pioneer of much of the work on hyperthermophilic (high temperature loving) bacteria. *Thermotogae* ($t_{max} = 90°C$) and *Aquificae* ($t_{max} = 95°C$) are the only known bacterial phyla that thrive at environmental temperatures above 80°C. The highest recorded marine temperature with active bacterial life was found at a hydrothermal vent (95°C), from which *Aquifex pyrophilus* was isolated (Huber et al. 1992). Specific structural adaptations, which require the optimization of membrane stability and fluidity, have been noted to support life in these conditions. Thermophilic bacteria and cyanobacteria have highly unsaturated fatty acids allowing them to remain stable and retain their shape at high temperatures (Daniel and Cowan 2000; Maslova et al. 2004). Structural protein molecules (e.g., ribosomal proteins, transport proteins) that normally denature at about 60°C, exhibit hyperthermostability through dehydration and slight changes in their primary structure (Kumar et al. 2010). Compounds such as potassium di-inositol-1,17-phosphate (Scholz et al. 1992) or tripotassium cyclic-2,3-diphosphoglycerate (Hensel and König 1988) serve to stabilize protein conformation at high temperatures, and it is suggested that this is also a possible mechanism for the stabilization of nucleic acid secondary structure (Daniel and Cowan 2000). Thermostable protein activity has been measured in excess of 130°C, although theoretical limits are closer to 150°C based on thermodynamic calculations of the protein structure (Hoehler 2007). A clear understanding of how life exists at the upper temperature boundary is hindered by the shortage of data on biomolecule stability above 100°C, which is mostly available for in vitro rather than in vivo conditions.

The lowest temperature limit for active bacterial life is approximately –20°C and motility down to –15°C for bacteria living in sea ice (Junge et al. 2004; Junge et al. 2001). One Arctic marine bacterium, *Psychromonas ingrahamii*, has demonstrated the lowest growth temperature of any organism authenticated by a growth curve (–12 to –15°C) with a generation time of 240 hours (Breezee et al. 2004). This organism is unable to survive at conditions otherwise considered "normal" (25°C), as its maximal growth is 10°C.

Growth optima near 20°C for cultured cyanobacteria isolated from polar environments suggested that these organisms may be cold-tolerant but not cold-adapted (Tang et al. 1997); however, observations of filamentous cyanobacteria in microbial mats found in glacial cryoconite holes and sea-ice melt-water ponds suggests that some species are psychrophiles (De Los Ríos et al. 2004; Gerdel and Drouet 1960).

The ability to protect cells from freezing (and subsequent thawing) relies on the membrane structure itself and cold-temperature adaptation of proteins. Most research of cold-adapted microorganisms at the molecular level focuses on enzymes (e.g., valine dehydrogenase, laminarases, chitobiase, and chitinases) (Deming 2002). However, psychrophiles also regulate the chemical composition of their membranes because the fluidity of membranes decreases with decreasing temperature. The ratio of unsaturated to saturated fatty acids increases to keep them sufficiently fluid to allow for transport processes even at temperatures below freezing (Rothschild and Mancinelli 2001). Regarding proteins, psychrophiles typically use more polar and less hydrophobic residues than proteins from hyperthermophiles. One specific example is the adaptation of the protein M1 aminopeptidase, renamed to cold-active aminopeptidase (ColAP), for which the main function is to assist in proteolytic cleavage (Huston et al. 2004).

Additionally, polar cyanobacteria must survive extended darkness, freeze-thaw cycles and sustained cold temperatures. The structure of the cyanobacterial mats likely aids the survival of the community in which some cyanobacteria fix nitrogen, whereas other members secrete exopolysaccharides (EPS) that provide structure and possibly protection from desiccation and freezing to the mat (De Los Ríos et al. 2004). The ability to recover metabolic processes after desiccation is species specific, and long-term mat survival and propagation may also be attributed stress-resistant spores produced by some species (Hawes et al. 1992). Few research studies address the mechanisms of cold-adaptation in psychrophilic bacteria, instead inferences are made by performing temperature shift experiments on mesophiles (Sato 1995).

Temperature fluctuations are sometimes assumed to affect heterotrophic bacteria more than phototrophic bacteria, and climate change models often assume that primary production will not be directly affected by increases in temperature, but heterotrophy and respiration in soils will be affected (Kirchman 2012). One reason for this is that the light reaction of phototrophy is affected less by temperature changes than heterotrophic respiration reactions. Any differences in how temperature affects heterotrophic organisms versus primary producers or aquatic versus terrestrial organisms would have huge implication for understanding the impact of climate change on the carbon cycle and the rest of the biosphere (Kirchman 2012). The many indirect effects of temperature, such as those on the hydrologic cycle and pH, complicate this understanding even further.

pH

At high pH, cyanobacteria are frequently the dominant primary producers in alkaline lakes and are among the most alkaliphilic organisms known. The cyanobacterium *Plectonema nostocorum* is considered to be the most alkaline tolerant species and can live at a pH of 13 (Skinner 1968). The cyanobacterium *Spirulina platensis* is an obligate alkaliphile, and reaches large population sizes at pH 11 and above (Belkin and Boussiba 1991). The most well-studied alkaliphilic bacteria are within the genus *Bacillus*, which has a broad range for growth (pH 7.5 to 11) and phototrophic purple bacteria (optimum pH 8-10). These organisms are incapable of growth at circumneutral pH conditions.

Alkaliphiles survive by enzyme stability, homeostasis (e.g., pumping ions in and out of the cell to maintain a semi-neutral cytoplasmic pH), and/or reversal of the pH gradient (e.g., coupling Na^+ expulsion to electron transport). For example, *Bacillus firmus* uses a sodium motive force rather than a proton motive force to drive transport reactions and motility (Krulwich 1986). Additionally, cells within a high pH environment are composed of three main components: peptidoglycan, proteins, and polysaccharides or polyol compounds. The peptidoglycans form mechanically rigid three-dimensional net-frame structures outside the membrane, as in the case of a *Bacillus* spp. (Horikoshi 1999). The polyols of the cell wall are also substituted with hexoses or hexosamines in response to high pH surroundings (Krulwich et al. 2007). Within the cell membrane of other alkaliphiles, acidic polymers composed of residues such as galacturonic acid, glutamic acid, aspartic acid, gluconic acid, and phosphoric acid prevent the entry of hydroxide ions yet allow the uptake of hydronium ions (Horikoshi 1999).

The most extreme acidophilic bacteria are *Acidithiobacillus thiooxidans* (pH 0.9–4.5) and *Acidithiobacillus ferrooxidans* (pH 1.5–4), which were first isolated from acid mine drainage (Kelly and Wood 2000). They have since been found in marine environments including the Seto Inland Sea, Japan (Kamimura et al. 2003). Similar to alkaliphiles, many acidophilic microorganisms have the ability to maintain intracellular pH at values that are circumneutral and a proton gradient over their cytoplasmic membranes of several orders of magnitude (Oren 2001; Seckbach 2000), which negates the need for cytoplasmic proteins to evolve acid stability. However, *Acetobacter aceti* has an acidified cytoplasm and nearly all its proteins are acid stable due to an overabundance of acidic residues, which minimizes destabilization at low pH induced by a buildup of positive charge outside the cell (Menzel and Gottschalk 1985).

Each of these adaptations provides the niche specializations required for survival at alkali and acidic boundary conditions while making neutral pH appear "extreme." Evolutionary adaptations allow these lineages to thrive in conditions that are considered extreme, but that view is only from a human perspective. These conditions are boundary environments for the biosphere and should be noted as such. These specific

adaptations should be explored to determine how they provide the needed physiological mechanisms for survival relative to organisms that are adapted to neutral pH. This will help to better understand the range of pH-related adaptations without the bias of considering one extreme and one normal.

Salinity

Most microorganisms in marine systems are naturally halophilic, living in salinities of 3.5% w/v; however, a small subset is uniquely adapted to extremely high salinities. These microorganisms are found in hypersaline lakes and deep-sea brine pools, and can tolerate saturated salt solutions approaching 36% w/v. Extreme halophily has been studied for decades, with several named taxa including *Halobacterium* (Henderson 1977) predating the partitioning in scientific literature between *Archaea* and *Bacteria* (Woese et al. 1983). Subsequently, many of those named taxa were found to be *Archaea*, and extreme halophily was regarded as an archaeal adaptation. This distinction has been overturned in the past decade with the identification of bacteria such as *Salinibacter* (Antón et al. 2000), which grow in saturated salt solutions. Halophiles balance the solute concentration difference by synthesizing or pumping in osmolytes, such as glycerol (Roberts 2005), sucrose (Roberts 2005), glycine betaine (Le Rudulier and Bouillard 1983), or potassium ions (Oren et al. 2002). Similar to the acidophiles discussed above, one strategy for coping with a high salt environment involves the extensive use of acidic amino acid residues to help stabilize protein structures (Oren and Mana 2002).

Paralleling our growing understanding of the significant role that bacteria play in what was thought of as an archaeal world, halophilic *Bacteria* and *Archaea* are thought to use similar adaptations through convergent evolution and gene exchange (Mongodin et al. 2005). The genes associated with the adaptations can be used for molecular studies in higher salt concentrations, allowing for additional targeted studies to determine a true boundary for this environmental condition. These results will help advance our understanding of the limits of Earth's biosphere and assist our search for the life on Mars, as brines have been detected recently on the Martian surface.

Section 5. Fungi

Fungi are ubiquitous in natural environments and have evolved specialized adaptations to a wide variety of environmental, and sometimes boundary, niches. Marine fungi have been reported from hydrothermal vents, deep-sea trenches, cold methane seeps, deep-sea subsurface sediments, and hypersaline, anoxic and suboxic waters. Specialized adaptations have allowed fungi to persist in temperatures, pH, and salinities that approach boundary conditions (Figs. 2-4).

Temperature

Thermophilic fungi, by convention, must grow at 20°C or above and grow optimally between 40 and 50°C. Several species of fungi (e.g., *Thermoascus aurantiacus, Rhizomucor pusilis, Aspergillus candidus*) have been found in natural geothermal systems at temperatures of 60–62°C and enriched at temperatures near 50°C (Maheshwari et al. 2000; Tansey and Brock 1972). Four main hypotheses of fungal thermophily have been investigated: 1) lipid solubilization to maintain fluidity of the cell; 2) resynthesizing essential metabolites rapidly; 3) molecular thermostability of the genetic material; and 4) structural thermostability of the cell (Maheshwari et al. 2000). Most notably, an increase in temperature results in cellular lipids containing more saturated fatty acids, which have a higher melting point than their mesophilic counterparts (Maheshwari et al. 2000). These strategies are similar to the archaeal and bacterial adaptations, suggesting similar, domain-level biological solutions to survival at high temperatures.

At the psychrophilic boundary for fungi, some *Aureobasidium* species have been isolated from Antarctic rocks, which can grow and reproduce at 0–5°C, but are reported to withstand temperatures as low as –80°C (Magan 2007). In sub-Arctic regions, it is important for fungi and other microorganisms to survive harsh winter months to grow actively again during warm months. The conidia (i.e., spores) of *Aspergillus flavus* were found to be resistant to freezing in water at –73°C (Mazur 1956). These fungi have evolved multiple, psychrophilic-associated physiological characteristics, including the capacity to produce antifreeze compounds, dehydrate, high supercooling activity, and chill tolerance (Magan 2007).

pH

Acidophilic fungi are found in volcanic areas, soda lakes, and mine drainages. In the natural environment, filamentous fungi *Acontium pullulans* was isolated at pH 2.5 from an acidic coal waste stream (Belly and Brock 1974). Metabolically active *Dothideomycetes* and *Eurotiomycetes* and additional ascomycete fungi were found in acid mine drainage at pH 0.8 to 1.38 (Baker et al. 2004). Three species of fungi, *Acontium cylatium*, *Cephalosporium* sp., and *Trichosporon cerebriae*, have been grown near pH 0 under laboratory conditions (Schleper et al. 1995). The most acid-tolerant fungi known are *Acontiuni velatum* and fungus D (an unidentified member of the *Dermatiaceae*), originally isolated from strong acid solutions containing 4% $CuSO_4$ in an industrial plant (Sletten and Skinner 1948). These two lineages grew well when submerged in nutrient-enriched sulfuric acid solutions at pH values as low as 0.4. Although these lineages were not isolated from a marine environment, they represent the lower range at which fungi are capable of life and can direct future research. The marine fungi, *Aspergillus awamori*, was isolated from seawater with a pH optimum of 2°C and 30°C (Beena et al. 2010)

The maximum pH of fungal growth at pH 11 was determined using *Penicillium variables* and *Fusarium bullatum* in laboratory experiments; however, both of these species were found to neutralize the growth media to pH 7 (Johnson 1923).

Likewise, Johnson also reported that fungi growing in acidic media increased the pH to neutral. The genera *Acremonium* and *Fusarium* were detected in alkaline caves with a pH 9 and above (Nagai et al. 1998). The ability for fungi to survive in extremely acidic or alkaline systems is similar to that of *Bacteria* and *Archaea* in that they need efficient trans-membrane transport systems and control of protons moving in and out of the cells to meet necessary energy requirements (Kroll 1990). These mechanisms are also linked to the control of the osmotic potential and the ability to survive in hypersaline environments.

Salinity

In general, the diversity of eukaryotic species and their abundances decrease with increasing salinity. The halotolerant (e.g., able to tolerate high salinity) fungal strain, *Hortaea werneckii*, has been isolated from several hypersaline environments, and the maximum salinity it can tolerate is 26% (Gostincar et al. 2010). However, halophilic (e.g., requires high salinity for optimal growth) isolates from the Dead Sea, which has a salinity of 34%, included *Gymnascella marismortui, Ulocladium chlamydosporum*, and *Penicillium westlingii* (Buchalo et al. 1999). Halophilic organisms will progressively replace halotolerant species as salinity increases. Adaptations, such as plasma membrane composition, production of enzymes involved in fatty acid modifications, osmolyte composition, and accumulation of ions allow these species to exist in such extreme environments.

Section 6. Eukaryotic microalgae

The evolutionary history of algae includes two endosymbiotic events involving bacteria that developed into mitochondria and chloroplasts. The ancestors of extant cyanobacteria were the first chlorophyll-based photosynthetic organisms that ultimately lead to the eukaryotic algal lineages and higher plants through endosymbiosis. Primary endosymbiosis, in which a heterotroph engulfed a cyanobacterium, led to green (Chlorophyta) and red algal lineages (Rhodophyta), and subsequent secondary endosymbioses of those lineages evolved into dinoflagellates (Alveolata), diatoms, and the macrophytic brown algae (Heterokontophyta) (Keeling 2004). Some adaptations specific to the boundary conditions of eukaryotic algae are similar to those of *Bacteria* and *Archaea*. In the case of one red thermoacidophile, the adaptive genes were transferred directly from *Bacteria* and *Archaea*, but further work is required to determine if adaptive traits for boundary conditions in the green and brown lineages are from horizontal gene transfer or convergent evolution.

Temperature

Like cyanobacteria, saturation of membrane lipids and increased photosynthetic protein stability are important adaptions in the eukaryotic microalgae. Species within the eukaryotic red algal order *Cyandiales* are thermoacidophiles. Within this order, *Cyanidium caldarium* inhabits the highest

temperatures of any eukaryote, with a maximum temperature of 57°C (Doemel and Brock 1971). These single celled algae are phylogenetically and ecologically diverse, living in and around acid hotsprings at pH 0.5–1.5 and temperatures from 18 to 55°C (Ciniglia et al. 2004; Toplin et al. 2008). In *Cyanidium caldarium,* grown at 55°C, the total lipid fractions containing saturated fatty acids and saturated phospholipids were greater than at 20°C (Kleinschmidt and Mcmahon 1970). The photosynthetic pigment phycocyanin maintains structural integrity at high temperatures due to a high isoelectric point, relative to mesophilic species, suggesting that the charge of the protein at cellular pH allows it to bind tightly to other pigment monomers (Kao et al. 1975). Carbon acquisition in *Galderia* species is aided by the uptake of organic carbon and by mixotrophy, phenotypes encoded by a suite of specialized genes (Barbier et al. 2005).

Toxic chemicals, such as arsenic and heavy metals, that dissolve easily in acid are found in high concentrations in thermoacidic habitats. Arsenic and antimony are enzymatically oxidized by the *Cyandiales* by methyltransferases; these proteins have in vitro temperature optima between 60°C and 70°C (Lehr et al. 2007; Qin et al. 2009). Similarly, the heavy metal chelator produced by *Cyanidioschyzon merolae* has a temperature optimum of 50°C (Osaki et al. 2009). In *Galdieria sulphuraria*, many mechanisms for adaption to thermoacidic environments, such as arsenic removal, are horizontally transferred from *Bacteria* and *Archaea* (Schonknecht et al. 2013).

Viable microalgal life at low temperatures (–2° to –14°C) is accommodated by hypersaline conditions in sea-ice brine channels. Diatoms, chrysophytes, and dinoflagellates were observed with microscopy, both light and epifluorescence, and in situ ^{14}C uptake experiments in sea ice (Krembs et al. 2002). Chrysophytes and dinoflagellates survive the dark winter months by forming dormant cysts (Stoecker et al.1997).

Algal individuals of the sea-ice community are not only physiologically adapted to cold saline conditions, but they physically modify the ice by releasing EPS that also provides energetic substrates for heterotrophic bacteria (reviewed in (Ewert and Deming 2013)). Sea-ice diatoms have several mechanisms of cryoprotection. They secrete ice-binding proteins that interfere with the growth of damaging crystals outside the cells (Janech et al. 2006; Krell et al. 2008). Some diatoms produce dimethylsulphoniopropionate (DMSP) (Levasseur et al. 1994), which functions as a cryoprotectant and as an antioxidant in high salinity (Lyon et al. 2011). Osmotic stress is reduced by increased production of osmolytes such as proline (Krell et al. 2007).

To increase membrane fluidity at low temperatures, fatty acids of chloroplast thylakoid membranes are less saturated in the green ice alga *Chlamydomonas subcaudata* than in the mesophyllic *C. reinhardtii* (Morgan-Kiss et al. 2002). Active photosynthesis extends from the relatively warm (–2°C) seawater-ice interface to greater than 0.5 m above that (Mock and

Kroon 2002). Although ice algae are able to photosynthesize at –11°C, photochemistry is more efficient at higher temperatures with significant increases at –5° and 1.8°C (Ralph et al. 2005).

pH

Diatoms frequently dominate eukaryotic phytoplankton communities living in pH extremes, both acidic and alkaline. The highest pH at which diatoms have been recorded in the field is 10.6 in an East African rift valley lake (Hecky and Kilham 1973). Diatoms, like the cyanobacteria, concentrate carbon using plasma-membrane integrated bicarbonate transporters (Nakajima et al. 2013; Reinfelder 2011). The tiny green alga *Picocystis salinarum* is also important in alkaline lakes. A strain from Dogenoer Soda Lake in Inner Mongolia with natural conditions of pH = 10 and 18.8% salinity was experimentally grown at pH = 12 (Fanjing et al. 2009). In alkaline lakes *Picocystis* mitigates osmotic stress by balancing environmental solute concentrations with matching intracellular concentrations of the osmolytes glycine betaine and DMS*P* (Roesler et al. 2002).

In addition to the thermoacidophiles, some microalgae are simply acidophiles. The green alga *Dunaliella acidophila* is adapted to low pH and survives in nature at pH 0.5–1 (Albertano et al. 1981). Experimentally, *D. acidophila* has tolerated pH 0.2, but its pH optimum for growth was pH 1 (Fuggi et al. 1988). Similar to acidophilic bacteria, *Dunaliella acidophila* maintains a near-neutral internal cellular pH and has a positive electric potential across the plasma membrane, thus decreasing its permeability to hydronium ions, and alleviating the need for internal proteins to have special adaptations (Gimmler et al. 1989). Protein regulation is an important adaptive mechanism; *Chlamydomonas acidophila* increases its basal levels of heat shock proteins, which aid in protein cycling and assembly, relative to the mesophyllic *C. reinhardtii* (Gerloff-Elias et al. 2006).

Salinity

Species within the genus *Dunaliella* tolerate a wide range of salinities, but *D. salina* survives saturating salt conditions (Borowitzka 1981; Oren 2005). The optimal salinity for growth of *D. salina* is ~ 15%, but it is found naturally at higher salt concentrations (Brock 1975). *Dunaliella salina* is valued for the commercial production of β-carotenes, and therefore, the adaptive response to high salinity is well characterized and known to be integrated throughout the cell. Osmotic stress is controlled through the removal of Na^+ by NADPH driven redox reactions and Na^+-ATPases located in the plasma membrane (Katz and Pick 2001). Relative to low salinity treatments, cells grown in high salinity have an increased amount of ion and nutrient transporters, carbonic anydrases that increase the flux of CO_2 into the cell via bicarbonate, signal transduction proteins, and enzymes that reduce oxidative stress (Katz et al. 2007). The increased flux of CO_2 at the plasma membrane is driven by photosynthesis and an increase in Calvin cycle enzymes (Liska

et al. 2004). This fixed carbon is shunted to the production of glycerol, an osmolyte under high salinity (Liska et al. 2004). Although the cellular concentrations of glyercol in *D. parvum* can balance the external concentration of NaCl (Ben-Amotz and Avron 1973), glycine betaine and proline may also be important osmolytes (Mishra et al. 2008). The increased metabolic activity is aided by the fluidity of internal membranes, which are enriched in unsaturated lipids (Azachi et al. 2002).

Oxidative stress increases with increasing salinity, but the nature of the physiological response appears to be salinity dependent (Mishra and Jha 2011). Under low to moderate concentration of NaCl (~3–12%), oxygen radicals are enzymatically controlled, whereas at higher salt concentrations carotenes are the primary anti-oxidants (Borowitzka et al. 1990; Mishra and Jha 2011).

Section 7. Multicellular eukaryotic algae

Multicellular eukaryotic algae range in size over four orders of magnitude, from filamentous algae millimeters in length to the giant kelp *Macrocystis pyrifera*, which can span tens of meters of water column. Multicellularity evolved from unicellular members of the Chlorophyta, Rhodophyta, and Heterokontophyta, each with unique abilities to interact with their environment. Algae also display a wide range of morphologies (e.g., encrusting, calcareous, sheet-like). Macroalgae may exist completely submerged in water and/or exposed to the air and thus experience a range of physical conditions. Factors such as those described below, can vary with tidal cycle and seasons so algae must be able to cope with variations in the conditions as well.

Temperature

Temperature can limit the geographic range of a species as well as its vertical distribution from the subtidal to intertidal zones. Macroalgae from the northern Indian Ocean have a temperature limit of 28°C providing a biogeographic boundary (Schils and Wilson 2006). Tolerance to high temperatures (i.e., up to ~ 30°C) is correlated with vertical position in the intertidal or subtidal zone in Caribbean seaweeds (Pakker et al. 1995), and exposure to air temperatures. The red alga, *Chondrus crispus*, has an upper lethal limit of 34.4°C (Knoop and Bate 1990). At high temperatures trade-offs in energy allocation may occur. For example increased rates of photorespiration can lead to decreased net photosynthesis. At 35°C, the photosynthetic performance of the siphonous green alga *Codium edule* declined over a period of several hours, with thermal denaturation of PSII proteins occurring at 40°C (Lee and Hsu 2009). Chlorophyll degrades above 75°C (Rothschild and Mancinelli 2001), thus setting a theoretical upper temperature limit of chlorophyll-based photosynthesis (Rothschild and Mancinelli 2001).

In salt water, the lower temperature limit of multicellular eukaryotic algae is generally at the freezing point. Filamentous Antarctic algae, *Geminocarpus geminatus* (Ectocarpales:

Phaeophyceae) and *Cladophora repens* (Cladophorales: Cladophorophyceae), have positive growth at temperatures of –1.5°C (Mckamey and Amsler 2006). The red algae *Devaleraea ramentacaea* grew well at –2°C and was able to survive short time periods of freezing at –20°C (Novaczek et al. 1990). *Ascoseira mirabilis*, a brown Antarctic algae, has a temperature optimum near 1°C (Drew 1977). Adaptations for increasing freezing tolerance include the possession of macromolecules that prevent the recrystallization of ice such as in the Antarctic eukaryotic green alga *Prasiola* sp. (Raymond and Fritsen 2001); *Prasiola crispa* was found to be photosynthetically active as low as –15°C (Becker 1982).

pH

The filamentous algae *Klebsormidium flaccidum* and *Mougeotia* sp. survive at pH 1.5 in the Río Tinto River in Spain, which has been acidified by mining, and has high concentration of heavy metals (Sabater et al. 2003). At the high pH boundary, algal species inhabiting tide pools modify the pH in the pool by enzymatically using bicarbonate as a carbon substrate and leaving hydroxide ions (Maberly 1990). *Enteromorpha intestinalis* (now: *Ulva intestinalis*) effectively depletes the inorganic carbon and can raise the external pH to 10.82 (Maberly 1990). Likewise, the intertidal brown alga *Fucus vesiculosus* increased pH to 10.19 in its environment (Middelboe and Hansen 2007).

Salinity

Macroalgae are able to tolerate a range of naturally occurring salinities from freshwater to highly concentrated salinities in evaporative tide pools. Marine macroalgae experience a range of salinities, from brackish to 3.5% (ocean salinity), that varies with tidal cycle, season, and riverine input. In *Fucus distichus*, and upper intertidal alga, the effective quantum yield of photosynthesis was not affected by changes in salinity ranging from 0.5 to 6% (Karsten 2007). *Fucus* species are able to maintain turgor pressure across a range of external salinities via osmotic adjustment of ions Na^+, K^+, and Cl^- and organic compounds such as mannitol (Karsten et al. 1991; Kirst and Bisson 1979; Reed et al. 1985). *Porphyra umbilicalis*, an intertidal red algae, tolerates osmotic conditions up to 6× (~21%) artificial seawater medium (Knoth and Wiencke 1984). Seaweeds alter their transcriptome (i.e., regulate gene expression) as a means to cope with both hypo- and hyper- salinity stress (Teo et al. 2009).

Other extreme conditions

Algae require light for photosynthesis. Low light will limit primary productivity, and excess light can cause damage to the alga. As light is attenuated with depth in the ocean, certain wavelengths disappear faster than others, which can set a depth limit to where algae can live. Algae have evolved a diversity of light harvesting complexes such as the phycobilisome found in cyanobacteria and red algae to exploit a range of wavelengths (Grossman et al. 1995; Grossman et al. 1993).

Polar species experience a period of low light during winter months; kelp gametophytes can survive 18 months of darkness (Tom Dieck 1993). On the other hand, too much light may cause damage to photosystem proteins and generate reactive oxygen species (Ledford and Niyogi 2005). Algae have several photoprotective mechanisms for coping with this type of stress including thermal dissipation of excess energy, alternative electron transport pathways, antioxidant systems, and repair mechanisms (Niyogi 1999). For example, mycosporine-like amino acids (MMAs) protect cells against UV radiation (Oren and Gunde-Cimerman 2007).

Physical characteristics (e.g., light, temperature, nutrients) of the water column differ between the top and bottom, and vary at multiple time scales (e.g., hourly, seasonally, interdecadally), meaning the same individual must cope with different conditions simultaneously. During the summer when the ocean is stratified, the giant kelp may be limited by nutrients at the surface and by light at depth. The ability to transport materials (e.g., amino acids and sugars) through a sieve tube system (only found in some kelps) allows for different physiological processes to occur in different parts of the kelp as determined by experiments testing photophysiology and gene expression (Colombo-Pallotta et al. 2006; Konotchick et al. 2013; Parker and Huber 1965; Sargent and Lantri*p* 1952).

Section 8. Protozoa

Heterotrophic and mixotrophic motile protists were originally classified as their own taxonomic group—the protozoa. This classification is now outdated due to the difficulty in differentiating pure autotrophs from heterotrophs. This section will focus on unicellular, non-autotrophic eukaryotes and their capability to survive and grow under "extreme," boundary conditions.

Temperature

Like the other eukaryotic groups, the temperature boundaries at which protozoa are able to grow are moderate compared with *Archaea* and *Bacteria*. The highest reported temperature for growth of an anaerobic ciliate is 52°C (Baumgartner et al. 2002). At low temperatures, protozoa grow down to –3°C, whereas temperatures below that require highly saline waters for active growth (Rose and Caron 2007). In Antarctic ciliates, reports of posttranslational modifications of tubulin were correlated to survival down to –2°C (Pucciarelli et al. 1997). One strategy to survive cold winter temperatures, below those temperatures where active growth is possible, is to encyst (Simon and Schneller 1973). An interesting aspect of the temperature range of protozoans is that mixotrophy seems to be more common among Antarctic protists than among species from temperate waters (Laybourn-Parry 2002). This suggests a potentially unique and diagnostic target for molecular analysis of psychrophiles that should be explored.

pH

Many protozoans are found in animal guts, therefore they can cope with very low pH in their surroundings, while maintaining a neutral internal pH (Seckbach 2000). The protozoan record holder for life at low pH is an amoeba living at pH 1.1 in a crater lake outflow in Argentina (Packroff and Woelfl 2000). Ciliates from acidic mining drainages were reported living at environmental pH < 3.0 (Packroff and Woelfl 2000). At the other end of the pH range, ciliates are found up to pH 10.1 in Kenyan Rift Valley Lakes (Ong'ondo et al. 2013). Unlike other abiotic parameters, the strategies used by free-living protozoans to adapt to high and low pH have rarely been studied.

Salinity

Protozoa, like other groups discussed can live in a range of salinities from freshwater to the hypersaline, reaching boundaries established for *Archaea* and *Bacteria*. Taxonomically diverse protozoa live at salinities that are nearly saturating (Post et al. 1983), with the ciliate *Rhopalophyra salina* apparently having the greatest tolerance at 34.8% (Kirby 1934). The ciliate *Mesodinium rubrum* (earlier *Myrionecta rubra*), which is often seen as purely autotrophic but is still capable of ingesting prey organisms (Gustafson et al. 2000), was found in Antarctic lakes with a salinity of up to 10% (Perriss and Laybourn-Parry 1997). This example shows that ciliates occurring in large numbers in nonextreme habitats can adapt efficiently to extreme conditions like low temperature and salinity. Ciliates osmoregulate by maintaining intracellular solute concentrations that are hyperosmotic relative to the environment; the excess water is removed by a contractile vacuole (Patterson 1980). For example, the marine ciliate *Miamensis avidis* increases the intracellular concentrations of free amino acids linearly from 0.9 to the asymptote at 5.25% (Kaneshiro et al. 1969).

Other extreme conditions

Other conditions that may be considered as extreme are those of low nutrient and oxygen availability. To cope with low nutrient concentrations, many protozoans live mixotrophically by acquiring nutrients from symbionts or kleptochloroplasts. Anaerobic protozoans often contain methanogenic symbionts, which use the H_2 produced by the protozoan host as an electron donor (Fenchel and Finlay 1992). True anaerobic protozoans (ciliates) survive for hours under anoxic conditions due to their photoautotrophic symbionts, which produce sufficient oxygen for the host's respiratory requirements (Finlay et al. 2006). Anoxic, sulfide-rich conditions are toxic for most organisms including the majority of protozoans. However, there are sulfide-loving ciliate genera, including *Plagiopyla* spp. and *Metopus* spp., that have been found in sulfide concentrations of 1.2 mM (Dyer et al. 1986). Three hydrothermal vent flagellates survived 24 h in anoxic water with 30 mM sulfide (Atkins et al. 2002).

Pressure can limit survival and growth. About 25 years ago,

a flagellate from a depth of 4500 m was isolated and cultured (Turley et al. 1988). In the central Pacific Ocean, ciliates were found at concentrations of 0.3-0.8 cells per liter at a depth of 4000 m but were not detected at depths greater than 5000 m; in contrast, heterotrophic nanoflagellates were found at 5000 m (Sohrin et al. 2010). More research in these areas will need to be completed to firmly define the boundaries for protozoans in situ.

Section 9. Concluding remarks

The term "extremophile" is frequently limited to single-celled *Bacteria* and *Archaea*; however this review documents lineages from all three domains fitting this description, a description that is better stated as "boundary lineages." Lineages living at these boundary conditions are abundant and diverse, with many of them incapable of living at "normal" conditions, highlighting the relative, and less-than-descriptive nature of the term "extreme." Here we have discussed some of the predominant physical boundaries selecting for, or against, aquatic life across several tropic levels. Similar physiological strategies have been noted for all three domains of life to survive and grow at boundary conditions, suggesting convergent evolution or horizontal gene transfer. These strategies can provide direction for future work to develop advanced molecular tools to push these boundaries beyond the current culture-limited restrictions for identification.

Each of the three environmental conditions considered here have boundaries where life has currently been identified. Interestingly, each has one boundary that is more survivable than the other. For temperature, cold is survivable, and is best shown with cryostorage. Growth at cold temperatures appears to be limited to the ability of water to flow or increases salinity as the surrounding water freezes (e.g., brine channels). However, a thermal maximum has been well noted for all three domains, with *Archaea* currently occupying the biome's highest temperature niche for growth. For salinity, conditions approaching near zero salinity are broadly survivable, but there is a saturation point that limits many lineages. These boundary brine conditions approaching 37% (w/v) appear to be limiting to life as we currently know it. For pH, this review notes multiple lineages that grow at pH 0, however no lineage to date can survive pH 14. Alkalinity is more difficult for lineages to survive. So a true "extreme" environment for all life on Earth, based on our current knowledge, would be hot brine with very high pH.

Increasing cellular organization and the proliferation of multicellular life on Earth appears to limit the range of environments that eukaryotic, and especially multicellular organisms, can survive. As environments become "extreme," food webs become truncated and microorganisms predominate. *Bacteria*, *Archaea*, fungi, and microeukaryotes are predominantly asexual and have faster generation times than the multicellular macrophytes, thus potentially providing a greater genetic diversity through mutations that may be

selected for through Earth's history. Evolutionary trajectories in multicellular organisms tend toward a greater diversity in morphologies, whereas single-celled organisms, particularly *Bacteria* and *Archaea*, exhibit metabolic diversity (Poole et al. 2003). There are three possible ways to survive or thrive in a habitat that exceeds the tolerable limits: acclimatize, evolve, or disperse. If none of the above occurs, the final outcome is extinction. The replacement of taxa in response to salinity changes over a 17,000 year period suggest that local extinction and emigration are the dominant processes working in microbial communities; although, variation in phylogenetic DNA markers indicated that microevolution is also occurring (Jiang et al. 2007). Range shifts, and slow adaptation, are most likely to occur for macrophytes that have slower generation times. Extremophiles are in some ways the boundary organisms that demarcate what is currently biologically possible—for a species, a domain, a community, and an ecosystem—from the impossible. In times of change, these organisms can serve as both the warning signs of extinction or the wave of things to come.

Understanding the adaptations that promote life at environmental boundaries is critical. It is important to determine where these boundaries are, how life survives within these niches, and then push forward to test those boundaries. Such studies will help describe life's origins and extent, both on Earth and elsewhere. The list of organisms referenced here with these specific boundary conditions is meant to be a point of comparison and inspiration for additional studies. More boundaries should be examined to reveal additional survival strategies and identify novel lineages.

References

Albertano, P., G. Pinto, S. Santisi, and R. Taddei. 1981. Spermatozopsis acidophila Kalina (Chlorophyta, Volvocales), a little known alga from highly acidic environments. Giornale Botan. Ital. 115:65-76 [doi:10.1080/11263508109427995].

Antón, J., R. Rosselló-Mora, F. Rodríguez-Valera, and R. Amann. 2000. Extremely halophilic bacteria in crystallizer ponds from solar salterns. Appl. Environ. Microbiol. 66:3052-3057 [doi:10.1128/AEM.66.7.3052-3057.2000].

Atkins, M. S., M. A. Hanna, E. A. Kupetsky, M. A. Saito, C. D. Taylor, and C. O. Wirsen. 2002. Tolerance of flagellated protists to high sulfide and metal concentrations potentially encountered at deep-sea hydrothermal vents. Mar. Ecol. Progr. Ser. 226:63-75 [doi:10.3354/meps226063].

Azachi, M., A. Sadka, M. Fisher, P. Goldshlag, I. Gokhman, and A. Zamir. 2002. Salt induction of fatty acid elongase and membrane lipid modifications in the extreme halotolerant alga *Dunaliella salina*. Plant Physiol. 129:1320-1329 [doi:10.1104/pp.001909].

Azam, F. 1998. Microbial control of oceanic carbon flux: The plot thickens. Science 280:694-696 [doi:10.1126/science.280.5364.694].

Baker, B. J., M. A. Lutz, S. C. Dawson, P. L. Bond, and J. F. Banfield. 2004. Metabolically active eukaryotic communities in extremely acidic mine drainage. Appl. Environ. Microbiol. 70:6264-6271 [doi:10.1128/AEM.70.10.6264-6271.2004].

Barbier, G., and others. 2005. Comparative genomics of two closely related unicellular thermo-acidophilic red algae, *Galdieria sulphuraria* and *Cyanidioschyzon merolae*, reveals the molecular basis of the metabolic flexibility of *Galdieria sulphuraria* and significant differences in carbohydrate metabolism of both algae. Plant Physiol. 137:460-474 [doi:10.1104/pp.104.051169].

Baumgartner, M., K. Stetter, and W. Foissner. 2002. Morphological, small subunit rRNA, and physiological characterization of *Trimyema minutum* (Kahl, 1931), an anaerobic ciliate from submarine hydrothermal vents growing from 28 degrees C to 52 degrees C. J. Eukary. Microbiol. 49:227-238 [doi:10.1111/j.1550-7408.2002.tb00527.x].

Becker, E. 1982. Physiological studies on *Antarctic Prasiola* crispa and *Nostoc* commune at low temperatures. Polar Biol. 1:99-104.

Beena, P., K. Elyas, and M. Chandrasekaran. 2010. Acidophilic tannase from marine *Aspergillus awamori* BTMFW032. J. Microbiol. Biotechn. 20:1403-1414 [doi:10.4014/jmb.1004.04038].

Belkin, S., and S. Boussiba. 1991. Resistance of *Spirulina platensis* to ammonia at high pH values. Plant Cell Physiol. 32:953-958.

Belly, R. T., and T. D. Brock. 1974. Widespread occurrence of acidophilic strains of *Bacillus coagulans* in hot springs. J. Appl. Bacteriol. 37:175-177 [doi:10.1111/j.1365-2672.1974.tb00427.x].

Ben-Amotz, A., and M. Avron. 1973. The role of glycerol in the osmotic regulation of the halophilic alga *Dunaliella parva*. Plant Physiol. 51:875-878 [doi:10.1104/pp.51.5.875].

Bettarel, Y., and others. 2011. Ecological traits of planktonic viruses and prokaryotes along a full salinity gradient. FEMS Microbiol. Ecol. 76:360-372 [doi:10.1111/j.1574-6941.2011.01054.x].

Bize, A., and others. 2008. Viruses in acidic geothermal environments of the Kamchatka Peninsula. Res. Microbiol. 159:358-366 [doi:10.1016/j.resmic.2008.04.009].

Borowitzka, L. 1981. The microflora: Adapations of life in extremely saline lakes, p. 33-46. *In* W. D. Williams [ed.], Developements in hydrobiology: Salt lakes. Developments in Hydrobiology. Springer Netherlands [doi:10.1007/978-94-009-8665-7_4].

Borowitzka, M. A., L. J. Borowitzka, and D. Kessly. 1990. Effects of salinity increase on carotenoid accumulation in the green alga *Dunaliella salina*. J. Appl. Phycol. 2:111-119 [doi:10.1007/BF00023372].

Borriss, M., E. Helmke, R. Hanschke, and T. Schweder. 2003. Isolation and characterization of marine psychrophilic phage-host systems from Arctic sea ice. Extremophiles 7:377-384 [doi:10.1007/s00792-003-0334-7].

Breezee, J., N. Cady, and J. T. Staley. 2004. Subfreezing growth of the sea ice bacterium "Psychromonas ingrahamii." Microb. Ecol. 47:300-304 [doi:10.1007/s00248-003-1040-9].

Brock, T. 1975. Salinity and the ecology of Dunaliella from Great Salt Lake. J. General Microbiol. 89:285-292 [doi:10.1099/00221287-89-2-285].

Buchalo, A. S., and others. 1999. Species diversity and biology of fungi isolated from the Dead Sea, pp. 283-300. *In* Wasser, S. P. (ed.), evolutionary theory and processes: modern perspectives. Springer [doi:10.1007/978-94-011-4830-6_17].

Ciniglia, C., H. S. Yoon, A. Pollio, G. Pinto, and D. Bhattacharya. 2004. Hidden biodiversity of the extremophilic Cyanidiales red algae. Mol. Ecol. 13:1827-1838 [doi:10.1111/j.1365-294X.2004.02180.x].

Collins, R. E., G. Rocap, and J. W. Deming. 2010. Persistence of bacterial and archaeal communities in sea ice through an Arctic winter. Environ. Microbiol. 12:1828-1841 [doi:10.1111/j.1462-2920.2010.02179.x].

Colombo-Pallotta, M. F., E. Garcia-Mendoza, and L. B. Ladah. 2006. Photosynthetic performance, light absorption, and pigment composition of *Macrocystis pyrifera* (Laminariales, Phaephyceae) blades from different depths. J. Phycol. 42:1225-1234 [doi:10.1111/j.1529-8817.2006.00287.x].

Daniel, R. M., and D. A. Cowan. 2000. Biomolecular stability and life at high temperatures. Cell. Mol. Life Sci. 57:250-264 [doi:10.1007/PL00000688].

De Los Ríos, A., C. Ascaso, J. Wierzchos, E. Fernández-Valiente, and A. Quesada. 2004. Microstructural characterization of cyanobacterial mats from the McMurdo Ice Shelf, Antarctica. Appl. Environ. Microbiol. 70:569-580 [doi:10.1128/AEM.70.1.569-580.2004].

Dekas, A. E., R. S. Poretsky, and V. J. Orphan. 2009. Deep-sea archaea fix and share nitrogen in methane-consuming microbial consortia. Science 326:422-426 [doi:10.1126/science.1178223].

Deming, J. W. 2002. Psychrophiles and polar regions. Curr. Opin. Microbiol. 5:301-309 [doi:10.1016/S1369-5274(02)00329-6].

Doemel, W. N., and T. D. Brock. 1971. The physiological ecology of *Cyanidium caldarium*. J. Gen. Microbiol. 67:17-32 [doi:10.1099/00221287-67-1-17].

Drew, E. A. 1977. The physiology of photosynthesis and respiration in some Antarctic marine algae. Brit. Antarct. Surv. Bull. 46:59-76.

Dyer, B. D., N. Gaju, P.-A. Carlos, I. Esteve, and R. Guerrero. 1986. Ciliates from a fresh water sulfuretum. BioSystems 19:127-135 [doi:10.1016/0303-2647(86)90025-0].

Ewert, M., and J. W. Deming. 2013. Sea ice microorganisms: Environmental constraints and extracellular responses. Biology 2:603-628 [doi:10.3390/biology2020603].

Fanjing, K., J. Qinxian, E. Jia, and Z. Mianping. 2009. Characterization of a eukaryotic picoplankton alga, strain DGN-Z1, isolated from a soda lake in Inner Mongolia, China. Nat. Res. Environ. Issues Vol. 15, Article 38. <http://digitalcommons.usu.edu/nrei/vol15/iss1/38>

Fenchel, T., and B. Finlay. 1992. Production of methane and hydrogen by anaerobic ciliates containing symbiotic methanogens. Arch. Microbiol. 157:475-480.

Finlay, B.J., S.C. Maberly, and G.F. Esteban. 2006. Spectacular abundance of ciliates in anoxic pond water: contribution of symbiont photosynthesis to host respiratory oxygen requirements. FEMS Microbial Ecol. 20:229-235 [doi:10.1111/j.1574-6941.1996.tb00321.x].

Franzmann, P., N. Springer, W. Ludwig, E. Conway De Macario, and M. Rohde. 1992. A methanogenic archaeon from Ace Lake, Antarctica: *Methanococcoides burtonii* sp. nov. System. Appl. Microbiol. 15:573-581 [doi:10.1016/S0723-2020(11)80117-7].

Fuggi, A., G. Pinto, A. Pollio, and R. Taddei. 1988. Effects of NaCl, Na$_2$SO$_4$, H2SO4, and glucose on growth, photosynthesis, and respiration in the acidophilic alga *Dunaliella acidophila* (Volvocales, Chlorophyta). Phycologia 27:334-339 [doi:10.2216/i0031-8884-27-3-334.1].

Gerdel, R. W., and F. Drouet. 1960. The cryoconite of the Thule area, Greenland. Trans. Am. Microscop. Soc. 79:256-272 [doi:10.2307/3223732].

Gerloff-Elias, A., D. Barua, A. Molich, and E. Spijkerman. 2006. Temperature- and pH-dependent accumulation of heat-shock proteins in the acidophilic green alga *Chlamydomonas acidophila*. FEMS Microbiol. Ecol. 56:345-354 [doi:10.1111/j.1574-6941.2006.00078.x].

Gimmler, H., U. Weis, C. Weis, H. Kugel, and B. Treffny. 1989. *Dunaliella acidophila* (Kalina) Masyuk—an alga with a positive membrane potential. New Phytol. 113:175-184 [doi:10.1111/j.1469-8137.1989.tb04704.x].

Gostincar, C., M. Grube, S. De Hoog, P. Zalar, and N. Gunde-Cimerman. 2010. Extremotolerance in fungi: Evolution on the edge. FEMS Microbiol. Ecol. 71:2-11 [doi:10.1111/j.1574-6941.2009.00794.x].

Grossman, A. R., M. R. Schaefer, G. G. Chiang, and J. L. Collier. 1993. The phycobilisome, a light-harvesting complex responsive to environmental conditions. Microbiol. Rev. 57:725-749.

———, D. Bhaya, K. E. Apt, and D. M. Kehoe. 1995. Light-harvesting complexes in oxygenic photosynthesis: Diversity, control, and evolution. Ann. Rev. Genetics 29:231-288 [doi:10.1146/annurev.ge.29.120195.001311].

Guixa-Boixareu, N., J. I. Calderonpaz, M. Heldal, G. Bratbak, and C. Pedrosalio. 1996. Viral lysis and bacterivory as prokaryotic loss factors along a salinity gradient. Aquat. Microb. Ecol. 11:215-227 [doi:10.3354/ame011215].

Gustafson, D.E. Jr., D.K. Stoecker, M.D. Johnson, W.F. Van Heukelem, and K. Sneider. 2000. Cryptophyte algae are robbed of their organelles by the marine ciliate *Mesodinium rubrum*. Nature 405:1049-1052 [doi:10.1038/35016570].

Hawes, I., C. Howard-Williams, and W. F. Vincent. 1992. Desiccation and recovery of Antarctic cyanobacterial mats. Polar Biol. 12:587-594 [doi:10.1007/BF00236981].

Hecky, R. E., and P. Kilham. 1973. Diatoms in alkaline, saline lakes: ecology and geochemical implications. Limnol. Oceanogr. 18:53-71 [doi:10.4319/lo.1973.18.1.0053].

Henderson, R. 1977. The purple membrane from *Halobacterium Halobium*. Ann. Rev. Biophys. Bioeng. 6:87-109 [doi:10.1146/annurev.bb.06.060177.000511].

Hensel, R., and H. König. 1988. Thermoadaptation of methanogenic bacteria by intracellular ion concentration. FEMS Microbiol. Lett. 49:75-79 [doi:10.1111/j.1574-6968.1988.tb02685.x].

Hoehler, T. M. 2007. An energy balance concept for habitability. Astrobiology 7:824-838 [doi:10.1089/ast.2006.0095].

Holland, M. E., and J. A. Baross. 2003. Limits to life in hydrothermal systems, p. 235–248. *In* P. Halbach, V. Tunnicliffe, and J. R. Hein [eds.], Energy and mass transfer in marine hydrothermal systems. Dahlem Univ. Press.

Horikoshi, K. 1999. Alkaliphiles: Some applications of their products for biotechnology. Microbiol. Mol. Biol. Rev. 63:735-750.

Huber, R., and others. 1992. Aquifex pyrophilus gen. nov., sp. nov. represents a novel group of marine hyperthermophilic hydrogen oxidizing bacteria. System. Appl. Microbiol. 15:340-351 [doi:10.1016/S0723-2020(11)80206-7].

Huston, A. L., B. Methe, and J. W. Deming. 2004. Purification, characterization, and sequencing of an extracellular cold-active aminopeptidase produced by marine psychrophile *Colwellia psychrerythraea* strain 34H. Appl. Environ. Microbiol. 70:3321-3328 [doi:10.1128/AEM.70.6.3321-3328.2004].

Hyman, P., and S. T. Abedon. 2012. Smaller fleas: Viruses of microorganisms. Scientifica 2012 [doi:10.6064/2012/734023].

Janech, M. G., A. Krell, T. Mock, J. S. Kang, and J. A. Raymond. 2006. Ice-binding proteins from sea ice diaotms (bacillariophyceae). J. Phycol. 42:410-416 [doi:10.1111/j.1529-8817.2006.00208.x].

Jiang, H., and others. 2007. Microbial response to salinity change in Lake Chaka, a hypersaline lake on Tibetan plateau. Environ. Microbiol. 9:2603-2621 [doi:10.1111/j.1462-2920.2007.01377.x].

Jiang, S., G. Steward, R. Jellison, W. Chu, and S. Choi. 2004. Abundance, distribution, and diversity of viruses in alkaline, hypersaline Mono Lake, California. Microb. Ecol. 47:9-17 [doi:10.1007/s00248-003-1023-x].

Johnson, H. W. 1923. Relationships between hydrogen ion, hydroxyl ion and salt concentrations and the growth of seven soil molds. Agricultural Experiment Station, Iowa State College of Agriculture and the Mechanic Arts.

Junge, K., C. Krembs, J. Deming, A. Stierle, and H. Eicken. 2001. A microscopic approach to investigate bacteria under in situ conditions in sea-ice samples. Annals Glaciol. 33:304-310.

———, H. Eicken, and J. W. Deming. 2004. Bacterial activity at -2 to -20 degrees C in Arctic wintertime sea ice. Appl. Environ. Microbiol. 70:550-557 [doi:10.1128/AEM.70.1.550-557.2004].

Kamimura, K., E. Higashino, S. Moriya, and T. Sugio. 2003. Marine acidophilic sulfur-oxidizing bacterium requiring salts for the oxidation of reduced inorganic sulfur compounds. Extremophiles 7:95-99.

Kaneshiro, E., G. Holz, and P. Dunham. 1969. Osmoregulation in a marine ciliate, Miamiensis avidus. II. Regulation of intracelluar free amino acids. Biol. Bull. 137:161-169 [doi:10.2307/1539939].

Kao, O. H., M. R. Edwards, and D. S. Berns. 1975. Physical-chemical properties of C-phycocyanin isolated from an acido-thermophilic eukaryote, Cyanidium caldarium. Biochem. J 147:63-70.

Karsten, U. 2007. Research note: salinity tolerance of Arctic kelps from Spitsbergen. Phycol. Res. 55:257-262 [doi:10.1111/j.1440-1835.2007.00468.x].

———, C. Wiencke, and G. Kirst. 1991. The effect of salinity changes upon the physiology of eulittoral green macroalgae from Antarctica and southern Chile II. Intracellular inorganic ions and organic compounds. J. Exper. Bot. 42:1533-1539 [doi:10.1093/jxb/42.12.1533].

Katz, A., and U. Pick. 2001. Plasma membrane electron transport coupled to Na+ extrusion in the halotolerant alga *Dunaliella*. Biochim. Biophys. Acta Bioenerg. 1504:423-431 [doi:10.1016/S0005-2728(01)00157-8].

———, P. Waridel, A. Shevchenko, and U. Pick. 2007. Salt-induced changes in the plasma membrane proteome of the halotolerant alga *Dunaliella salina* as revealed by blue native gel electrophoresis and nano-LC-MS/MS analysis. Mol. Cell. Proteom. 6:1459-1472 [doi:10.1074/mcp.M700002-MCP200].

Keeling, P. J. 2004. Diversity and evolutionary history of plastids and their hosts. Am. J. Botany 91:1481-1493 [doi:10.3732/ajb.91.10.1481].

Kelly, D. P., and A. P. Wood. 2000. Reclassification of some species of *Thiobacillus* to the newly designated genera *Acidithiobacillus* gen. nov., *Halothiobacillus* gen. nov and *Thermithiobacillus* gen. nov. Int. J. System. Evol. Microbiol. 50:511-516 [doi:10.1099/00207713-50-2-511].

Kirby, H. 1934. Some ciliates from salt marshes in California. Arch. Protistenkd. 82:114-133.

Kirchman, D. L. 1994. The uptake of inorganic nutrients by heterotrophic bacteria. Microb. Ecol. 28:255-271 [doi:10.1007/BF00166816].

———. 2012. Processes in microbial ecology. Oxford Univ. Press.

Kirst, G., and M. Bisson. 1979. Regulation of turgor pressure in marine algae: ions and low-molecular-weight organic compounds. Funct. Plant Biol. 6:539-556.

Kleinschmidt, M. G., and V. A. Mcmahon. 1970. Effect of growth temperature on the lipid composition of *Cyanidium caldarium*. I. Class separation of lipids. Plant Physiol. 46:286-289 [doi:10.1104/pp.46.2.286].

Knoop, W., and G. Bate. 1990. A model for the

description of photosynthesis-temperature responses by subtidal Rhodophyta. Botan. Mar. 33:165-172 [doi:10.1515/botm.1990.33.2.165].

Knoth, A., and C. Wiencke. 1984. Dynamic changes of protoplasmic volume and of fine structure during osmotic adaptation in the intertidal red alga Porphyra umbilicalis. Plant Cell Environ. 7:113-119 [doi:10.1111/j.1365-3040.1984.tb01564.x].

Konotchick, T., C. Dupont, R. Valas, J. Badger, and A. Allen. 2013. Transcriptomic analysis of metabolic function in the giant kelp, Macrocystis pyrifera, across depth and season. New Phytol. 198:398-407 [doi:10.1111/nph.12160].

Krell, A., D. Funck, I. Plettner, U. John, and G. Dieckmann. 2007. Regulation of proline metabolism under salt stress in the psychrophilic diatom *Fragilariopsis cylindrus* (Bacillariophyceae). J. Phycol. 43:753-762 [doi:10.1111/j.1529-8817.2007.00366.x].

———, B. Beszteri, G. Dieckmann, G. Glockner, K. Valentin, and T. Mock. 2008. A new class of ice-binding proteins discovered in a salt-stress-induced cDNA library of the psychrophilic diatom *Fragilariopsis cylindrus* (Bacillariophyceae). Eur. J. Phycol. 43:423-433 [doi:10.1080/09670260802348615].

Krembs, C., R. Gradinger, and M. Spindler. 2000. Implications of brine channel geometry and surface area for the interaction of sympagic organisms in Arctic sea ice. J. Exp. Mar. Biol. Ecol. 243:55-80 [doi:10.1016/S0022-0981(99)00111-2].

Krembs, C. E., H. Eicken, K. Junge, and J. Deming. 2002. High concentrations of exopolymeric substances in Arctic winter sea ice: implications for the polar ocean carbon cycle and cryoprotection of diatoms. Deep Sea Res. I 49:2163-2181 [doi:10.1016/S0967-0637(02)00122-X].

Kroll, R. G. 1990. Alkalophiles, p. 55-92. *In* C. Edwards [ed.], Microbiology of extreme environments. Open Univ. Press.

Krulwich, T., D. B. Hicks, T. Swartz, and M. Ito. 2007. Bioenergetic adaptations that support alkaliphily, pp. 311-329. *In* C. Gerday and N. Glansdorff, (eds.), Physiology and biochemistry of extremophiles. ASM Press [doi:10.1128/9781555815813.ch24].

Krulwich, T. A. 1986. Bioenergetics of alkalophilic bacteria. J. Membrane Biol. 89:113-125 [doi:10.1007/BF01869707].

Kumar, S., S. K. Singh, and M. M. Gromiha. 2010. Temperature-dependent molecular adaptations, microbial proteins. Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology [doi:10.1002/9780470054581.eib516].

Laybourn-Parry, J. 2002. Survival mechanisms in Antarctic lakes. Phil. Trans. R. Soc. B 357:863-869 [doi:10.1098/rstb.2002.1075].

Le Romancer, M., M. L. Gaillard, C. Geslin, and D. Prieur. 2007. Viruses in extreme environments, p. 99-113. *In* Life in extreme environments. Springer [doi:10.1007/978-1-4020-6285-8_6].

Le Rudulier, D., and L. Bouillard. 1983. Glycine betaine, an osmotic effector in *Klebsiella pneumoniae* and other members of the *Enterobacteriaceae*. Appl. Environ. Microbiol. 46:152-159.

Ledford, H. K., and K. K. Niyogi. 2005. Singlet oxygen and photo-oxidative stress management in plants and algae. Plant Cell Environ. 28:1037-1045 [doi:10.1111/j.1365-3040.2005.01374.x].

Lee, T.-C., and B.-D. Hsu. 2009. Disintegration of the cells of the siphonous green alga *Codium edule* (Bryopsidales, Chlorophyta) under mild heat stress. J. Phycol. 45:348-356 [doi:10.1111/j.1529-8817.2009.00656.x].

Lehr, C. R., D. R. Kashyap, and T. R. Mcdermott. 2007. New insights into microbial oxidation of antimony and arsenic. Appl. Environ. Microbiol. 73:2386-2389 [doi:10.1128/AEM.02789-06].

Levasseur, M., M. Gosselin, and S. Michaud. 1994. A new source of dimethylsulfide (DMS) for the Arctic atmosphere: Ice diatoms. Mar. Biol. 121:381-387 [doi:10.1007/BF00346748].

Middelboe, A. L., and P. Juel Hansen. 2007. Direct effects of pH and inorganic carbon on macroalgal photosynthesis and growth. Mar. Biol. Res. 3:134-144 [doi:10.1080/17451000701320556].

Liska, A. J., A. Shevchenko, U. Pick, and A. Katz. 2004. Enhanced photosynthesis and redox energy production contribute to salinity tolerance in *Dunaliella* as revealed by homology-based proteomics. Plant Physiol. 136:2806-2817 [doi:10.1104/pp.104.039438].

Lyon, B. R., P. A. Lee, J. M. Bennett, G. R. Ditullio, and M. G. Janech. 2011. Proteomic analysis of a sea-ice diatom: salinity acclimation provides new insight into the dimethylsulfoniopropionate production pathway. Plant Physiol. 157:1926-1941 [doi:10.1104/pp.111.185025].

Maberly, S. C. 1990. Exogenous sources of inorganic carbon for photosynthesis by marine macroalgae. J. Phycol. 26:439-449 [doi:10.1111/j.0022-3646.1990.00439.x].

Magan, N. 2007. Fungi in extreme environments, pp. 85-103. *In* C. Kubicek and I. Druzhinina (eds.), Mycota: Environmental and microbial relationships. Springer Berlin Heidelberg [doi:10.1007/978-3-540-71840-6_6].

Maheshwari, R., G. Bharadwaj, and M. K. Bhat. 2000. Thermophilic fungi: Their physiology and enzymes. Microbiol. Mol. Biol. Rev. 64:461-488 [doi:10.1128/MMBR.64.3.461-488.2000].

Maranger, R., D. Bird, and S. Juniper. 1994. Viral and bacterial dynamics in Arctic sea ice during the spring algal bloom near Resolute, N. W. T., Canada. Mar. Ecol. Progr. Ser. 111:121-127 [doi:10.3354/meps111121].

Maslova, I., E. Mouradyan, S. Lapina, G. Klyachko-Gurvich, and D. Los. 2004. Lipid fatty acid composition and thermophilicity of cyanobacteria. Russ. J. Plant Physiol. 51:353-360 [doi:10.1023/B:RUPP.0000028681.40671.8d].

Mazur, P. 1956. Studies on the effects of subzero temperatures on the viability of spores of *Aspergillus flavus*. J. Gen.

Physiol. 39:869-888 [doi:10.1085/jgp.39.6.869].

Mckamey, K. A., and C. D. Amsler. 2006. Effects of temperature and light on growth of the Antarctic algae *Geminocarpus geminatus* (Ectocarpales: Phaeophyceae) and *Cladophora repens* (Cladophorales: Cladophorophyceae) in culture. Phycologia 45:225-232 [doi:10.2216/04-75.1].

Menzel, U., and G. Gottschalk. 1985. The internal pH of *Acetobacterium wieringae* and *Acetobacter aceti* during growth and production of acetic acid Archives of Microbiology 143:47-51 [doi:10.1007/BF00414767].

Mishra, A., A. Mandoli, and B. Jha. 2008. Physiological characterization and stress-induced metabolic responses of *Dunaliella salina* isolated from salt pan. J. Industr. Microbiol. Biotechnol. 35:1093-1101 [doi:10.1007/s10295-008-0387-9].

———, and B. Jha. 2011. Antioxidant response of the microalga *Dunaliella salina* under salt stress. Botan. Mar. 54:195-199 [doi:10.1515/bot.2011.012].

Mock, T., and B. M. A. Kroon. 2002. Photosynthetic energy conversion under extreme conditions - II: the significance of lipids under light limited growth in Antarctic sea ice diatoms. Phytochemistry 61:53-60 [doi:10.1016/S0031-9422(02)00215-7].

Mongodin, E. F., and others. 2005. The genome of *Salinibacter ruber*: Convergence and gene exchange among hyperhalophilic bacteria and archaea. Proc. Nat. Acad. Sci. U.S.A. 102:18147-18152 [doi:10.1073/pnas.0509073102].

Morgan-Kiss, R., A. G. Ivanov, J. Williams, K. Mobashsher, and N. P. A. Huner. 2002. Differential thermal effects on the energy distribution between photosystem II and photosystem I in thylakoid membranes of a psychrophilic and a mesophilic alga. Biochim. Biophys. Acta 1561:251-265 [doi:10.1016/S0005-2736(02)00352-8].

Nagai, K., K. Suzuki, and G. Okada. 1998. Studies on the distribution of alkalophilic and alkali-tolerant soil fungi. II. Fungal flora in two limestone caves in Japan. Mycoscience 39:293-298 [doi:10.1007/BF02464011].

Nakajima, K., A. Tanaka, and Y. Matsuda. 2013. SLC4 family transporters in a marine diatom directly pump bicarbonate from seawater. Proc. Nat. Acad. Sci. 110:1767-1772 [doi:10.1073/pnas.1216234110].

Ng, W. V., and others. 2000. Genome sequence of Halobacterium species NRC-1. Proc. Nat. Acad. Sci. 97:12176-12181 [doi:10.1073/pnas.190337797].

Nichols, P. D., and P. D. Franzmann. 1992. Unsaturated diether phospholipids in the Antarctic methanogen methanococcoides-burtonii. FEMS Microbiol. Lett. 98:205-208 [doi:10.1111/j.1574-6968.1992.tb05515.x].

Niyogi, K. K. 1999. Photoprotection revisited: genetic and molecular approaches. Ann. Rev. Plant Biol. 50:333-359 [doi:10.1146/annurev.arplant.50.1.333].

Novaczek, I., G. Lubbers, and A. Breeman. 1990. Thermal ecotypes of amphi-Atlantic algae. I. Algae of Arctic to cold-temperate distribution (*Chaetomorpha melagonium*,

Devaleraea ramentacea, and *Phycodrys rubens*). Helgolander Meeresuntersuchungen 44:459-474 [doi:10.1007/BF02365480].

Ong'ondo, G. O., and others. 2013. Ecology and community structure of ciliated protists in two alkaline, Äìsaline Rift Valley lakes in Kenya with special emphasis on Frontonia. J. Plankton Res. 35:759-771 [doi:10.1093/plankt/fbt044].

Oren, A. 2001. Acidophiles. eLS. Wiley.

———. 2005. A hundred years of Dunaliella research: 1905-2005. Saline Syst. 1:1-14 [doi:10.1186/1746-1448-1-1].

———. 2009. Microbial diversity. eLS. Wiley.

———, G. Bratbak, and M. Heldal. 1997. Occurrence of virus-like particles in the Dead Sea. Extremophiles 1:143-149 [doi:10.1007/s007920050027].

———, and L. Mana. 2002. Amino acid composition of bulk protein and salt relationships of selected enzymes of Salinibacter ruber, an extremely halophilic bacterium. Extremophiles 6:217-223 [doi:10.1007/s007920100241].

———, M. Heldal, S. Norland, and E. A. Galinski. 2002. Intracellular ion and organic solute concentrations of the extremely halophilic bacterium Salinibacter ruber. Extremophiles 6:491-498 [doi:10.1007/s00792-002-0286-3].

———, and N. Gunde-Cimerman. 2007. Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? FEMS Microbiol. Lett. 269:1-10 [doi:10.1111/j.1574-6968.2007.00650.x].

Orphan, V. J., L. T. Taylor, D. Hafenbradl, and E. F. Delong. 2000. Culture-dependent and culture-independent characterization of microbial assemblages associated with high-temperature petroleum reservoirs. Appl. Environ. Microbiol. 66:700-1248 [doi:10.1128/AEM.66.2.700-711.2000].

———, C. H. House, K.-U. Hinrichs, K. D. Mckeegan, and E. F. Delong. 2002. Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. Proc. Nat. Acad. Sci. 99:7663-7668 [doi:10.1073/pnas.072210299].

Ortmann, A. C., and C. A. Suttle. 2005. High abundances of viruses in a deep-sea hydrothermal vent system indicates viral mediated microbial mortality. Deep Sea Res. I 52:1515-1527 [doi:10.1016/j.dsr.2005.04.002].

Osaki, Y., T. Shirabe, H. Nakanishi, T. Wakagi, and E. Yoshimura. 2009. Characterization of phytochelatin synthase produced by the primitive red alga *Cyanidioschyzon merolae*. Metallomics 1:353-358 [doi:10.1039/b823013g].

Packroff, G., and S. Woelfl. 2000. A review on the occurrence and taxonomy of heterotrophic protists in extreme acidic environments of pH values < 3. Hydrobiologia 433:153-156 [doi:10.1023/A:1004039309694].

Pakker, H., A. M. Breeman, W. F. Prud'homme Van Reine, and C. Hock. 1995. A comparative study of temperature responses of Caribbean seaweeds from different biogeographic regions. J. Phycol. 31:499-507 [doi:10.1111/j.1529-8817.1995.tb02543.x].

Parker, B. C., and J. Huber. 1965. Translocation in Macrocystis. II. Fine structure of the sieve tubes. J. Phycol. 1:172-179

[doi:10.1111/j.1529-8817.1965.tb04579.x].

Patterson, D. 1980. Contractile vacuoles and associated structures: their organization and function. Biol. Rev. 55:1-46 [doi:10.1111/j.1469-185X.1980.tb00686.x].

Perriss, S.J., and J. Laybourn-Parry. 1997. Microbial communities in saline lakes of the Vestfold Hills (eastern Antarctica). Polar Biol. 18:135-144 [doi:10.1007/s003000050168].

Pietilä, M. K., T. A. Demina, N. S. Atanasova, H. M. Oksanen, and D. H. Bamford. 2014. Archaeal viruses and bacteriophages: Comparisons and contrasts. Trends Microbiol [doi:10.1016/j.tim.2014.02.007].

Poole, A. M., M. J. Phillips, and D. Penny. 2003. Prokaryote and eukaryote evolvability. BioSystems 69:163-185 [doi:10.1016/S0303-2647(02)00131-4].

Post, F., L. Borowitzka, M. Borowitzka, B. Mackay, and T. Moulton. 1983. The protozoa of a Western Australian hypersaline lagoon. Hydrobiologia 105:95-113 [doi:10.1007/BF00025180].

Prangishvili, D., and R. A. Garrett. 2005. Viruses of hyperthermophilic Crenarchaea. Trends Microbiol. 13:535-542 [doi:10.1016/j.tim.2005.08.013].

———, R. A. Garrett, and E. V. Koonin. 2006. Evolutionary genomics of archaeal viruses: Unique viral genomes in the third domain of life. Virus Res. 117:52-67 [doi:10.1016/j.virusres.2006.01.007].

Pucciarelli, S., P. Ballarini, and C. Miceli. 1997. Cold-adapted microtubules: Characterization of tubulin posttranslational modifications in the Antarctic ciliate Euplotes focardii. Cell Motility Cytoskeleton 38:329-340 [doi:10.1002/(SICI)1097-0169(1997)38:4<329::AID-CM3>3.0.CO;2-Z].

Qin, J., C. R. Lehr, C. Yuan, X. C. Le, T. R. Mcdermott, and B. P. Rosen. 2009. Biotransformation of arsenic by a Yellowstone thermoacidophilic eukaryotic alga. Proc. Nat. Acad. Sci. 106:5213-5217 [doi:10.1073/pnas.0900238106].

Ralph, P. J., A. McMinn, K. G. Ryan, and C. Ashworth. 2005. Short-term effect of temperature on the photokinetics of microalgae from the surface layers of antarctic pack ice1. J. Phycol. 41:763-769 [doi:10.1111/j.1529-8817.2005.00106.x].

Raymond, J. A., and C. H. Fritsen. 2001. Semipurification and ice recrystallization inhibition activity of ice-active substances associated with Antarctic photosynthetic organisms. Cryobiology 43:63-70 [doi:10.1006/cryo.2001.2341].

Reed, R., I. Davison, J. Chudek, and R. Foster. 1985. The osmotic role of mannitol in the Phaeophyta: an appraisal. Phycologia 24:35-47 [doi:10.2216/i0031-8884-24-1-35.1].

Reinfelder, J. R. 2011. Carbon concentrating mechanisms in eukaryotic marine phytoplankton. Mar. Sci. 3 [doi:10.1146/annurev-marine-120709-142720].

Rice, G., and others. 2001. Viruses from extreme thermal environments. Proc. Nat. Acad. Sci. 98:13341-13345 [doi:10.1073/pnas.231170198].

Roberts, M. F. 2005. Organic compatible solutes of halotolerant and halophilic microorganisms. Saline Syst. 1:1-30 [doi:10.1186/1746-1448-1-5].

Roesler, C. S., and others. 2002. Distribution, production, and ecophysiology of Picocystis strain ML in Mono Lake, California. Limnol. Oceanogr. 47:440-452 [doi:10.4319/lo.2002.47.2.0440].

Rose, J. M., and D. A. Caron. 2007. Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implications for algal blooms in cold waters. Limnol. Oceanogr. 52:886-895 [doi:10.4319/lo.2007.52.2.0886].

Rothschild, L. J., and R. L. Mancinelli. 2001. Life in extreme environments. Nature 409:1092-1101 [doi:10.1038/35059215].

Sabater, S. and others. 2003. Structure and function ofbenthic algal communities in an extremely acid river. J. Phycol. 39:481-489 [doi:10.1046/j.1529-8817.2003.02104.x].

Sandaa, R.-A., E. F. Skjoldal, and G. Bratbak. 2003. Virioplankton community structure along a salinity gradient in a solar saltern. Extremophiles 7:347-351 [doi:10.1007/s00792-003-0328-5].

Santos, F., and others. 2011. Metatranscriptomic analysis of extremely halophilic viral communities. ISME J. 5:1621-1633 [doi:10.1038/ismej.2011.34].

Sargent, M. C., and L. W. Lantrip. 1952. Photosynthesis, growth and translocation in giant kelp. Am. J. Botany 39:99-107 [doi:10.2307/2438175].

Sato, N. 1995. A family of cold-regulated RNA-binding protein genes in the cyanobacterium Anabaena variabilis M3. Nucleic Acids Res. 23:2161-2167 [doi:10.1093/nar/23.12.2161].

Schils, T., and S. C. Wilson. 2006. Temperature threshold as a biogeographic barrier in Northern Indian ocean macroalgae. J. Phycol. 42:749-756 [doi:10.1111/j.1529-8817.2006.00242.x].

Schleper, C., G. Puhler, B. Kuhlmorgen, and W. Zillig. 1995. Life at extremely low pH. Nature 375:741-742 [doi:10.1038/375741b0].

Scholz, S., J. Sonnenbichler, W. Schäfer, and R. Hensel. 1992. Di-myo-inositol-1, 1'-phosphate: A new inositol phosphate isolated from Pyrococcus woesei. FEBS Lett. 306:239-242 [doi:10.1016/0014-5793(92)81008-A].

Schonknecht, G., and others. 2013. Gene transfer from bacteria and archaea facilitated evolution of an extremophilic eukaryote. Science 339:1207-1210 [doi:10.1126/science.1231707].

Schrenk, M. O., D. S. Kelley, S. A. Bolton, and J. A. Baross. 2004. Low archaeal diversity linked to subseafloor geochemical processes at the Lost City Hydrothermal Field, Mid-Atlantic Ridge. Environ. Microbiol. 6:1086-1095 [doi:10.1111/j.1462-2920.2004.00650.x].

Seckbach, J. 2000. Acidophilic microorganisms, p. 107-116. *In* J. Seckbach [ed.], Journey to diverse microbial worlds. Kluwer Academic Publishers.

Simon, E. M., and M. V. Schneller. 1973. The preservation of ciliated protozoa at low temperature. Cryobiology 10:421-426 [doi:10.1016/0011-2240(73)90069-2].

Skinner, F. 1968. The limits of microbial existence. Proc. R. Soc. London. B 171:77-89.

Sletten, O., and C. E. Skinner. 1948. Fungi capable of growing in strongly acid media and in concentrated copper sulfate solutions. J. Bacteriol. 56:679-681.

Snyder, J. C., K. Stedman, G. Rice, B. Wiedenheft, J. Spuhler, and M. J. Young. 2003. Viruses of hyperthermophilic Archaea. Res. Microbiol. 154:474-482 [doi:10.1016/S0923-2508(03)00127-X].

Sohrin, R., M. Imazawa, H. Fukuda, and Y. Suzuki. 2010. Full-depth profiles of prokaryotes, heterotrophic nanoflagel-lates, and ciliates along a transect from the equatorial to the subarctic central Pacific Ocean. Deep Sea Res. II 57:1537-1550 [doi:10.1016/j.dsr2.2010.02.020].

Stoecker, D. K., D. E. Gustafson, J. R. Merrell, M. Black, and C. T. Baier. 1997. Excystment and growth of chrys-ophytes and dinoflagellates at low temperatures and high salinities in antarctic sea-ice1. J. Phycol. 33:585-595 [doi:10.1111/j.0022-3646.1997.00585.x].

Takai, K., and others. 2008. Cell proliferation at 122 degrees C and isotopically heavy CH4 production by a hyperther-mophilic methanogen under high-pressure cultivation. Proc. Nat. Acad. Sci. U.S.A. 105:10949-10954 [doi:10.1073/pnas.0712334105].

Tang, E. P., R. Tremblay, and W. F. Vincent. 1997. Cyanobacterial domnance of polar freshwater ecosystems: Are high-lati-tude mat-formers adapted to low temperature? J. Phycol. 33:171-181 [doi:10.1111/j.0022-3646.1997.00171.x].

Tansey, M. R., and T. D. Brock. 1972. Upper temperature limit for eukaryotic organisms. Proc. Nat. Acad. Sci. U.S.A. 69:2426-2428 [doi:10.1073/pnas.69.9.2426].

Teo, S. S., C. L. Ho, S. Teoh, R. Abdul Rahim, and S. M. Phang. 2009. Transcriptomic analysis of *Gracilaria changii* (Rhodophyta) in response to hyper-and hypo-osmotic stresses. J. Phycol. 45:1093-1099 [doi:10.1111/j.1529-8817.2009.00724.x].

Tindall, B., H. Ross, and W. Grant. 1984. *Natronobacterium* gen. nov. and *Natronococcus* gen. nov., Two new genera of haloalkaliphilic Archaebacteria. System. Appl. Microbiol. 5:41-57 [doi:10.1016/S0723-2020(84)80050-8].

Tom Dieck, I. 1993. Temperature tolerance and survival in darkness of kelp gametophytes (Laminariales, Phaeophyta): ecological and biogeographical implications. Mar. Ecol. Progr. Ser. 100:253-253 [doi:10.3354/meps100253].

Toplin, J. A., T. B. Norris, C. R. Lehr, T. R. Mcdermott, and R. W. Castenholz. 2008. Biogeographic and phylogenetic diversity of thermoacidophilic cyanidiales in Yellowstone National Park, Japan, and New Zealand. Appl. Environ. Microbiol. 74:2822-2833 [doi:10.1128/AEM.02741-07].

Torsvik, V., L. Ovreas, and T. F. Thingstad. 2002. Prokaryotic diversity—magnitude, dynamics, and controlling factors. Sci. Signal. 296:1064-1066.

Turley, C., K. Lochte, and D. Patterson. 1988. A barophilic flagel-late isolated from 4500 m in the mid-North Atlantic. Deep Sea Res. A 35:1079-1092 [doi:10.1016/0198-0149(88)90001-5].

White, O., and others. 1999. Genome sequence of the radio-resistant bacterium *Deinococcus radiodurans* R1. Science 286:1571-1577 [doi:10.1126/science.286.5444.1571].

Williamson, S. J., and others. 2008. Lysogenic virus-host inter-actions predominate at deep-sea diffuse-flow hydrothermal vents. ISME J. 2:1112-1121 [doi:10.1038/ismej.2008.73].

Woese, C. R., R. Gutell, R. Gupta, and H. F. Noller. 1983. Detailed analysis of the higher-order structure of the 16S-like ribosomal ribonucleic acids. Microbiol. Rev. 47:621-669.

Yau, S., and others. 2011. Virophage control of antarctic algal host-virus dynamics. Proc. Nat. Acad. Sci. U.S.A. 108:6163-6168 [doi:10.1073/pnas.1018221108].

Zillig, W., and others. 1996. Viruses, plasmids and other genetic elements of thermophilic and hyperthermo-philic Archaea. FEMS Microbiol. Rev. 18:225-236 [doi:10.1111/j.1574-6976.1996.tb00239.x].