TESTING LIFE'S LIMITS ON THE SM

Robert M. Hazen

Traditionally, we think of life in the universe like the story of Goldilocks and the three bears, with life searching for the perfect "bed" in which to host its existence. Moving through our solar system, we know some planets are too cold, some are too hot, and in general, all but one, are not the right place for life to make a go of it.

Earth is just right for living. And because we live at the surface of a temperate, sunny and water-rich planet, planetary scientists have traditionally defined the "habitable zone" as the narrow region around a star where temperatures might permit liquid water to exist at or near a planetary surface. Given this viewpoint, living worlds might be correspondingly few and far between. But our view of what is normal and habitable is changing.

A flood of discoveries over the past half century have radically altered our views of the limits of life, and consequently have expanded our vision of habitable zones in the universe. These discoveries in the field and the lab have not only given us new places to look for life off Earth, but also have shaped new paths of thought about the interactions between life and various environmental conditions on Earth. The bottom line: Microbial life survives at astonishing environmental extremes of temperature, pressure, acidity, radiation and more.

HOT AND COLD

The idea that life might readily adapt to conditions inimical to multicellular animals (or people) came into sharp focus in the 1960s with studies of hot springs, such as the boiling pools of Yellowstone National Park. Conventional wisdom suggested that life could not exist much above 70 degrees Celsius (the upper limit for photosynthetic organisms), but the discovery by Thomas Brock and University of Wisconsin colleagues of *Thermus aquaticus* and other microbes thriving at temperatures close to water's boiling point changed that perception.

Such "hyperthermophiles" proved to be more than mere curiosities. Biologists seized upon the technological potential of unusually stable microbial proteins.

This microbial "biofilm" community grows on acid mine drainage in the Richmond Mine, Calif. This tiny life survives at battery acid-like conditions and also contribute to processes that keep the mine water's acidity high.

Rudy Carver and Jill Banfield

MALL SCALE

Thermus aquaticus yielded the thermally robust DNA "polymerase enzyme" that is at the heart of the polymerase chain reaction, or PCR, a technique by which a single segment of DNA can be copied over and over again to speed medical diagnoses and forensic analyses. Given the success of that procedure, scientists began paying special attention to life at the extremes.

The study of hyperthermophiles has not been limited to hot springs, however. Water boils at 100 degrees Celsius at sea level, but in the deep ocean, the liquid state is possible at higher temperatures because of higher pressures.

The February 1977 discovery by Jack Corliss and his Oregon State University colleagues of diverse ecosystems at submarine hydrothermal vents, known as "black smokers," revolutionized our concepts of extreme life. Deep-vent ecosystems, some more than 5 kilometers deep, teem with microbes at crushing pressures and boiling temperatures in total darkness.

One such superbug, *Pyrolobus fumarii*, continues to reproduce at 113 degrees Celsius, while it stops growing at 90 degrees Celsius, which is too cold for its specialized metabolism to operate. Researchers have recovered submarine vent microbes from the Mid-Atlantic Ridge and the East Pacific Rise at temperatures close to 120 degrees Celsius — a record that seems likely to fall. Indeed, extremophile expert John Baross of the University of Washington in Seattle thinks life's upper limit lies closer to 150 degrees Celsius. Life at such extreme temperatures dramatically expands the range of planets and moons that might harbor life.

What about cold? It's hard to imagine life coping with temperatures below water's freezing point, but recent polar studies reveal robust communities of so-called psychrophiles — microbes that spend their lives near 0 degrees Celsius.

Current record low temperatures for replicating cells is about minus 10 degrees Celsius in ice-trapped brines, but Bruce Jakosky, an astrobiologist at the University of Colorado in Boulder, has suggested that life-supporting thin films of liquid water could persist to below minus 20 degrees Celsius on some mineral surfaces, including those found on Mars. If life can persist over the vast range from minus 20 to more than 150 degrees Celsius, then we have to rethink our view of habitable zones on Earth, in the solar system and throughout the galaxy. Currently planned space missions work off just such a premise: that life may lurk in extreme environments.

LIFE UNDER PRESSURE

Life may have adapted to a surprising range of temperatures, but surely pressure is a different story. The crush of a few hundred times atmospheric pressure, equal to hundreds of kilograms per square centimeter, seems beyond the endurance of even the hardiest living cell. Yet researchers have recovered viable microbes from the deepest trenches of the oceans and from drill cores several kilometers deep — places where pressures exceed 1,000 atmospheres. Indeed, microbes are found in drill cores from every conceivable lithology: granite and basalt, gneiss and schist, sandstone and limestone, salt domes and polar ice.

The ubiquity of deep life led the late Cornell University astrophysicist Thomas Gold to propose that half of Earth's biomass is hidden from view beneath our feet. And indeed, deep drilling reveals that life can thrive at pressures above 1,000 atmospheres. But what's the limit?

In recent experiments at the Carnegie Institution in Washington D.C., a team led by Anurag Sharma and James Scott (and that included myself) subjected a strain of the familiar intestinal bacterium *Escherichia coli* to the ridiculous pressure of 16,000 atmospheres — a value obtained accidentally by overzealous tightening of a diamond anvil pressure cell. (Our diamond anvil cell — used for a variety of research studies — generates high pressures by squeezing a gasketed liquid sample between a pair of gem-quality diamonds.)

Much to our surprise, a small fraction of the microbes survived this hyperbaric insult. Analysis revealed that temperatures below 150 degrees Celsius — currently thought to be the maximum temperature for sustaining life — correspond to pressures no greater than about 5,000 atmospheres. Therefore, based on our results at 16,000 atmospheres, at no known place on Earth (or on any other promising terrestrial planet or moon, for that matter) could high pressure limit life.

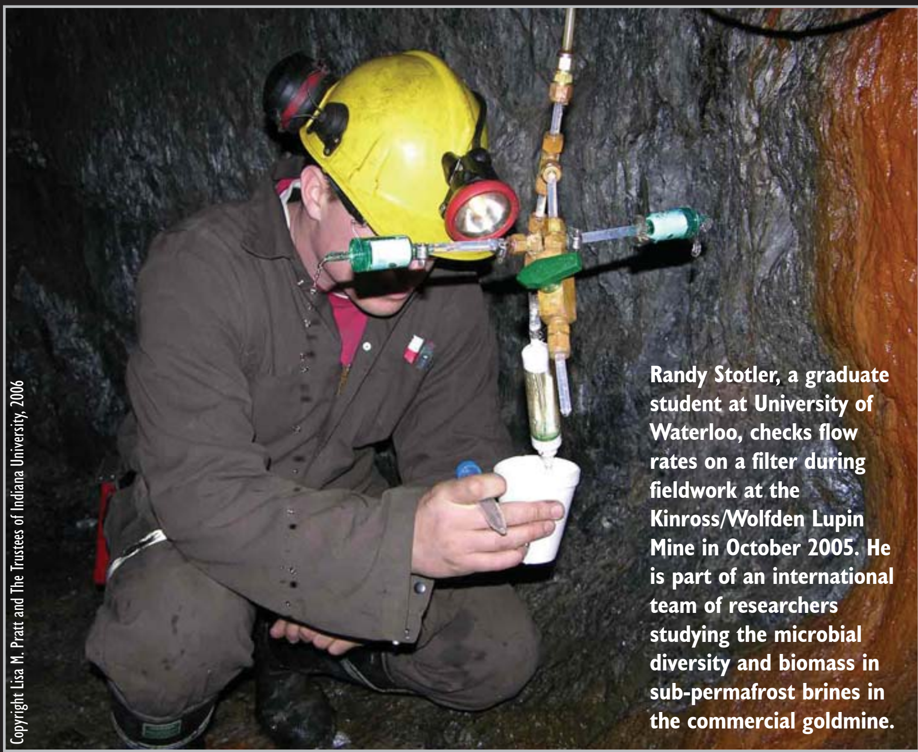
At the opposite extreme, in a vacuum — conditions devoid of almost all pressure — many microbial species appear to be equally resilient. These cells simply dehydrate and wait for the next cool drink. Such cells cannot metabolize or replicate without pressure, to be sure, but many microbes are able to hunker down in a dormant, desicated state until conditions return to normal. This ability to hibernate has led a number of astrobiologists to speculate on the probability of interplanetary transfers of microbes.

Jay Melosh at the University of Arizona in Tuscon and colleagues at Caltech have shown that, while large impacts vaporize vast volumes of rock, peripheral stony chunks may be hurled into space with only mild shock and heating. If such a far-flung rock contains microbes, the cells might survive a journey through the vacuum of space to another world. Given the additional insight that Mars appears to have been a warm, wet planet hundreds of millions of years earlier than Earth, the idea of life originating on Mars, hibernating in rocks and then being transferred to Earth is very much in play. Perhaps we are all martians!

CHEMICAL EXTREMES

Most life is mostly water, a fact that

TESTING LIFE'S LIMITS ON THE SMALL SCALE



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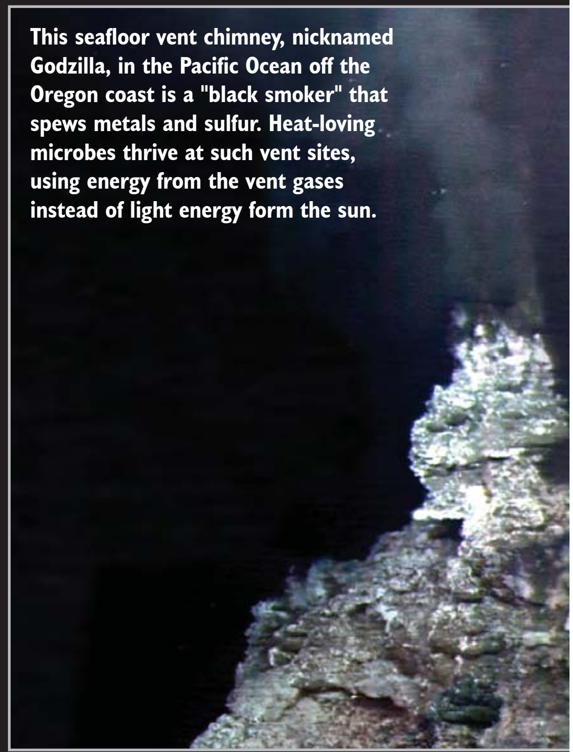
Randy Stotler, a graduate student at University of Waterloo, checks flow rates on a filter during fieldwork at the Kinross/Wolfden Lupin Mine in October 2005. He is part of an international team of researchers studying the microbial diversity and biomass in sub-permafrost brines in the commercial goldmine.



This seafloor vent chimney, nicknamed Godzilla, in the Pacific Ocean off the Oregon coast is a "black smoker" that spews metals and sulfur. Heat-loving microbes thrive at such vent sites, using energy from the vent gases instead of light energy form the sun.

prompted British biologist John Haldane to quip, "Even the Pope is 70 percent water." This need for a wet cellular environment is at the heart of the most ancient processes to protect food from hungry microbes. Simply adding salt or sugar — ingredients that draw water by osmosis from inside cells — can drastically reduce water's "activity", which is water's chemical concentration, from the normal cellular value of about 0.99.

Most bacterial growth stops below an activity of about 0.91, though some molds can survive conditions as low as 0.80. But in spite of life's dependence on water, these extremes in the activity of water are not the limit to life. Some salt-loving halophiles from icy brines, such as the Arctic microbe *Psychrobacter arcticum*, cope with activities of water closer to 0.3. That's much drier than the driest desert (where microbes also manage to eke out a living).

Versatile microbes have evolved biochemical pathways to deal with a host of other seemingly lethal chemical environments. Some hardy cells thrive at battery acid-like conditions of a pH less than 1 in some mine drainage systems. Biogeologist Jill Banfield and co-workers at the University of California in Berkeley study *Ferroplasma acidiphilum*, a microbe from the Iron Mountain district of California that can reproduce at pH near 0. Such resilient mine water cells, which often have to contend with extremely high metal contents of iron, copper and zinc, survive by oxidizing iron and releasing hydrogen as a waste product. Consequently, the microbes not only survive the harsh conditions, but they turn out to be the principal culprit in the mine water's high acidity.

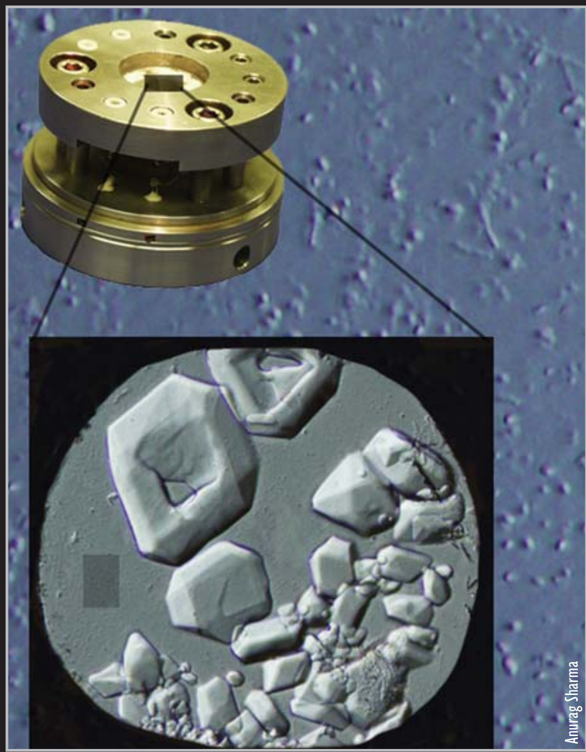
Other populations of bacteria, such as Bacillus alcalophilus, live in the extreme alkaline environments of "soda lakes," some of which have a pH greater than 11. Like "acidophiles," these "alkaliphiles" have complex biochemical mechanisms that maintain the cell's internal pH at close to neutral despite the harsh external environments. The rule of thumb seems to be that no matter how extreme the chemical environment, if a source of metabolic redox energy is available, life will find a way to survive.

Perhaps most remarkable, however, are cells that survive seemingly lethal dosages of radiation. The unrivaled superbug of radiation studies is the euphonious *Deinococcus radiodurans*, discovered in 1956 by Arthur W. Anderson at the Oregon Agricultural Experiment Station in Corvallis. Anderson noticed reddish colonies of microbes happily munching away in supposedly sterilized cans of gamma-irradiated meat. He found that these hardy microbes can withstand 10 times the dosage of gamma radiation that kills almost any other single-celled organism (and 3,000 times the lethal dose for humans).

That unrivaled resistance to radiation has led geneticists to engineer new *D. radiodurans* variants that can digest toxic chemicals in nuclear waste. The secret to its success appears to lie in an unusually high number of duplicate chromosomes — four to 10 copies in each cell — coupled with as yet poorly understood DNA repair mechanisms. Whatever *D. radiodurans'* trick, it adds fuel to speculations that microbes might be able to survive an extended journey in space.

TIME TO SPARE

Many microbes seem to live at a frenetic pace, growing and dividing in hours or even minutes. Yet some microbial populations now appear able to all but stop their



Under pressure: experiment with a diamond anvil cell at the Carnegie Institution, researchers observed the bacteria ***Escherichia coli*** under high pressure. At 13 kilobars, nutrient rich fluid with ***E. coli*** is clearly visible as rod-shaped bacteria sticking on the diamond and icy surface in the background image (magnified from the grey rectangle within the inset). These bacteria show continued motility that increases with lowering of pressure. These pressures are significantly higher than the upper limits traditionally considered for life to be able to exist.

metabolism and survive over immense spans of geological time.

Tullis Onstott and colleagues at Princeton University study subterranean microbial communities from the deepest South African gold mines. These cells, which live more than a kilometer below the surface in solid rock, are occasionally released when a miner's blast uncorks a reservoir of pressurized water. Onstott's crew quickly recovers bottles of the pristine water and its precious hoard of microbes. They find that these sparse populations live at the most leisurely pace; some cells appear to take more than a thousand years on average to reproduce, while some communities are estimated to have been isolated from the surface for millions of years.

Much older cell populations have been teased out of ancient amber-entombed insects. In 1997, California microbiologist Raul Cano made headlines when he claimed that he had recovered viable cells of *Bacillus sphaericus* from a 25- to 30-million-year-old fossil bee. Though the results were first greeted with skepticism, the scientific community now appears convinced, and Cano and his colleague Monica Borucki have even been granted a patent for "An isolated, viable culture of a microorganism obtained from within a naturally occurring resin" (although it's not yet obvious what commercial use might be made of that microbial culture).

But these ancient cells may be mere toddlers compared to a salt-bound *B. sphaericus* strain recovered from 250-million-year-old Permian salt domes, by West Chester University environmental microbiologist Russell Vreeland and his colleagues in 2000. Although the unexpected results are still a matter of debate, Vreeland's group claims that these salt-loving microbes began to reproduce after a quarter of a billion years of dormancy. If they're right, then the time required for interplanetary transfer of microbes does not represent a limit to life.

LOOKING FOR LIFE

We live in a universe of a hundred billion galaxies, many of which boast hundreds of billions of stars. Given this cosmic extravagance, conservative estimates place the number of terrestrial planets at greater than 1,020, with many more potentially habitable moons. Of those myriad worlds, a relatively small fraction is likely to be similar to Earth, with globe-spanning oceans and a temperate, sunny climate.

But with our new understanding of life at extreme limits of temperature, pressure, chemical environment, radiation and more, we must expand our search to planets and moons of many sorts. Planets like Mars may be dry and blasted at their surfaces, but still harbor benign environments a few kilometers down. Similar environs may occur deep within Europa, Callisto, Titan and other moons of giant gas planets, which may be far from the sun and tortured by radiation, yet remain wet and warm inside from tidal heating. Thus, the current work on Earth to understand extreme life will prove invaluable as humankind continues to explore the solar system and beyond.

We have much to learn about life at the limits, but one thing is sure: Each year, as our understanding of life's resilience expands, so too does our view of habitable zones in space.

Hazen is Staff Scientist at the Carnegie Institution's Geophysical Laboratory and Clarence Robinson Professor of Earth Science at George Mason University. E-mail: r.hazen@gl.ciw.edu.