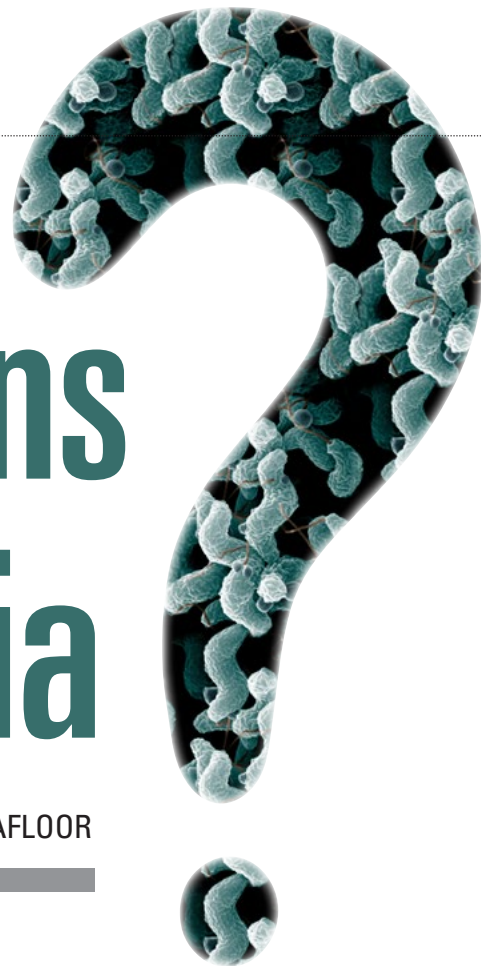


# Big Questions About Tiny Bacteria

NOVEL EXPERIMENTS PROBE HOW LIFE THRIVES AT THE SEAFLOOR

by David Levin



It's 3 a.m., and Jesse McNichol is struggling to stay awake. Since midafternoon he's been in his lab, tending to a jumble of glassware, plastic tubing, and metal cylinders filled with microbes. He sighs, rubs his bloodshot eyes, and heads back to work.

As a Ph.D. student in the MIT-WHOI Joint Program, McNichol says he's used to keeping odd hours like these—but normally, he's able to retreat to his apartment around the corner when he needs to recharge. For the entire month of January 2014, however, that quick trip home wasn't an option. His lab and his late nights were more than 1,000 miles off the coast of Panama, in the east Pacific Ocean.

McNichol, a biological oceanographer, joined an international team of researchers aboard WHOI's research vessel *Atlantis* to study microbes living at some of the most extreme environments on Earth—hydrothermal vents. These vents are like undersea geysers that form in volcanic areas on the ocean floor. Some spew liquid that can reach more than 750° F (400° C); others are lukewarm jets, formed as cold seawater mixes with hot vent fluid under the seafloor. All are situated a mile and a half below the surface, where they experience enormous pressure—more than 3,500 pounds per square inch. Yet despite these seemingly harsh conditions, the areas near these vents support lush communities of life, which can range from six-foot-tall tubeworms to microscopic single-cell organisms.

McNichol is specifically interested in a class of microbes called *Epsilonproteobacteria*, that dominates the vents. On

Earth's surface, he said, these bacteria are found only in small numbers, in environments ranging from mudflats and swamps to cows' stomachs. But at vent sites, they thrive.

For McNichol, what makes them interesting isn't *where* they live, though; it's *how* they live. Unlike plants and animals at the surface, organisms living at deep-sea vent sites exist in total darkness and can't take advantage of energy coming from the sun. Rather than photosynthesis, these *Epsilonproteobacteria* survive using chemosynthesis: To build their bodies, they extract energy from inorganic chemicals that leach out of rocks below the seafloor and into the vent fluid. The bacteria also flourish in places with little or no oxygen. It's an unusual method of making a living—one that might offer clues to the way early organisms first carved out an existence on a highly volcanic early Earth, before oxygen appeared in its atmosphere.

"In many ways, these bacteria could be similar to some of the earliest life on the planet," McNichol said.

Exactly what they do to live and grow is something he wants to find out.

## Early life on Earth

The origins of life—and the many forms it has evolved into over billions of years—is a topic that has always fascinated McNichol. Growing up in Canada, he spent hours exploring in the woods, and identifying edible plants and mushrooms. He read books about natural history and reveled in the outdoors. But it wasn't until he was an undergraduate at Mount Allison

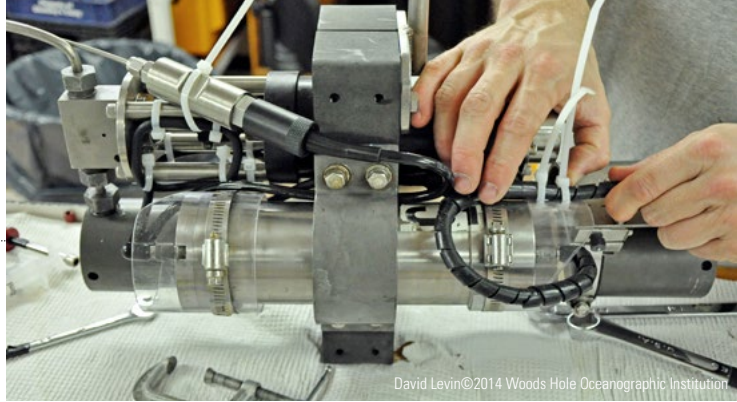
University in New Brunswick, Canada, that he realized just how little humans actually know about the first organisms on our planet.

“When I was young, I was of course interested in dinosaurs. But when I got to university, I realized there was three billion years of history before that, with many big, unanswered questions,” he said. “I mean, there’s very little known about dinosaurs, but there’s even less known about bacteria from three billion years ago. For me, it all comes down to that mystery about the history of life on Earth.”

In his undergraduate research, McNichol strove to chip away at those big questions. He moved through several fields searching for answers, studying botany, the biology of algae, and the symbiotic relationships between plants and fungi. As it turned out, that interdisciplinary background comes in quite handy when studying chemosynthetic bacteria at hydrothermal vents, said WHOI microbiologist Stefan Sievert, McNichol’s Ph.D. advisor at WHOI.

“He has enough practical knowledge to approach topics in both microbiology and biochemistry,” said WHOI geochemist Jeff Seewald, with whom McNichol also works. “That’s quite an accomplishment, especially for someone just starting out in the field.”

McNichol is hoping his work will help explain which chemicals these *Epsilonproteobacteria* use to survive, and how they metabolize them. “That’s something we don’t have much



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In the deep sea, pressure can reach 3,500 pounds per square inch. To sample fluids (and microbes in them) at these depths, WHOI scientists and engineers invented a device called an Isobaric Gas-Tight sampler. Made of a titanium cylinder, it can suck in bacteria and fluids and maintain them at deep-sea pressures, even when the sampler comes back to the surface.

knowledge about,” Sievert said, “yet it’s critical if we’re going to assess the role these ecosystems play in the deep ocean.”

In the past, expeditions have catalogued the basic species that live in and around vent systems on the East Pacific Rise, a vast volcanic mountain chain on the seafloor beneath the Pacific. Other research has shown that the microbes survive by using chemicals in the vent fluids as raw material. They use a series of chemical reactions called reduction-oxidation, or “redox” reactions, which transfer electrons among molecules and release energy in the process. Yet even with these existing studies, Sievert said, it’s still unclear exactly which combinations of vent chemicals different microbial species prefer to use, which biochemical reactions they employ to make energy, and which of their genes allow these processes to happen.

### High-pressure experiments at sea

To truly understand how vent bacteria behave, McNichol said, it’s necessary to study them in their natural habitat. If

The manipulator arm of the remotely operated vehicle *Jason* positions an Isobaric Gas-Tight sampler to collect samples near a hydrothermal vent site on the bottom of the Pacific Ocean. The vents have a thriving community of tubeworms, clams, and microbes such as *Epsilonproteobacteria*, the target of graduate student Jesse McNichol’s research.

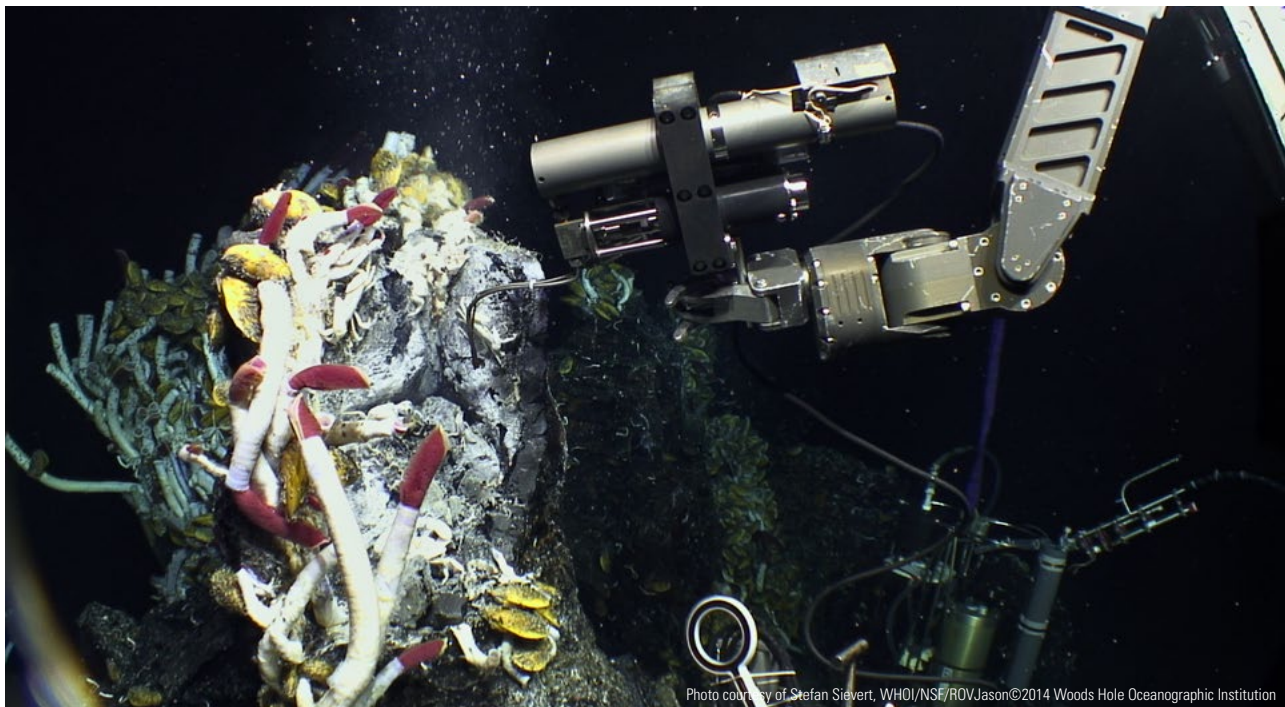


Photo courtesy of Stefan Sievert, WHOI/NSF/ROVJason©2014 Woods Hole Oceanographic Institution

the team of researchers simply brought samples back to the surface in an unsealed container, the change in pressure would cause gaseous chemicals dissolved in the fluids to bubble out, changing the fluid's chemistry, and potentially altering the microbes' activity.

To get around this problem, the researchers used the remotely operated deep-sea vehicle *Jason* to send down devices called Isobaric Gas-Tight samplers (IGTs) to the vents. These samplers, made of a titanium cylinder, can suck in bacteria and fluids, and then maintain the intense pressures of the deep sea inside the cylinder as it journeys all the way back to the surface.

Once the cylinders are aboard *Atlantis*, McNichol then incubated them, allowing the bacteria inside to grow as they normally would on the seafloor. Over 24 hours, he removed small amounts of fluid from the device and analyzed its chemistry. Over time, a drop-off of certain chemical compounds or a dramatic increase in others, he reasoned, would offer clues to what the vent microbes inside were using to generate energy.

Doing these kinds of experiments while aboard a ship wasn't easy, however. The constant motion of the deck made simple procedures—like weighing chemical solutions—impossible, and if a piece of equipment failed, running out for a spare part was out of the question.

"Most people use cruises just to collect samples, bring them back to the lab, and analyze them," Sievert said. "Conducting microbiological experiments aboard a ship is pretty unique. Very few researchers have tried to do this while at sea."

In fact, it's almost unheard of. According to McNichol, he and Sievert are among the first to conduct high-pressure experiments on deep-sea bacteria while aboard a research ship—yet he offers that detail with trademark humility.

"I really don't like to exaggerate my work. Sometimes maybe I tend to go to the other extreme, and make it sound like it's not that novel or new. But it really is. And I guess it's novel for the exact reasons you would expect—who else has access to the kinds of equipment we have here at Woods Hole? If it wasn't for Jeff Seewald's IGT samplers, there'd be no way we could do it. In this little unassuming village, we have access to unbelievable technology, which is really cool."

During the 2014 cruise, McNichol and his colleague, François Thomas, used Seewald's samplers to run more than 18 individual experiments, each lasting 24 hours. In addition to testing the changing chemistry inside the IGTs, the pair added other chemicals, including hydrogen gas, nitrate, and oxygen, to see which ones the vent microbes might use as "fuel" to survive and grow.

"We were able to get pretty interesting results from doing that," McNichol said. "We

found that the microbes were able to not only tolerate high levels of oxygen—more than 50 times the level that would normally be available in a vent ecosystem—but they could actually *use* it all as well, which is really striking and surprising. We also found that the microbes were able to use chemicals like hydrogen as an energy source, which was already predicted by other researchers—so our experiments are starting to confirm those ideas."

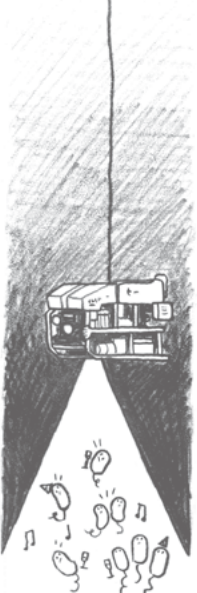
### Meanwhile, back on shore

The data McNichol collected at sea are only part of the puzzle, however. Back on shore, he's working to sequence the DNA of the microbes from each sample, which will help him identify which species were present in the IGTs. Working with collaborator Niculina Musat in Leipzig, Germany, he is also using a new technique called NanoSIMS (for Nanoscale Secondary Ion Mass Spectrometry), which can analyze cells of individual bacteria to tell him which species created the most biomass (the organic material that makes up a microbe's "body") during his experiments. With this information, he'll be able to calculate which microbes experienced the most growth when exposed to specific chemicals, providing clues to the biochemical reactions the bacteria use to survive. The research will also help pinpoint which microbes are the most active in the vent ecosystem.

The January 2015 cruise gave McNichol a wealth of new data, but he's still working on fleshing out his research. In November 2015, he returned to the East Pacific Rise to continue his experiments. Although he braced himself for another month of sleepless nights in the lab, he said, he was motivated by the fact that there's still much to be learned about these vent bacteria—and that every new discovery could lead to new questions.

"I recently read naturalist E.O. Wilson's *Letters to a Young Scientist*, and his advice is, 'Find a field where there are as few people as possible, and many open questions.' That's exactly how I feel about oceanography," McNichol said. "In the grand scheme of things, we know very little about these organisms—so any work I do, as long as I don't totally screw it up, is going to provide useful information. That's really exciting and empowering." ▲

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Jesse McNichol  
Biology

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Jesse McNichol has always been fascinated by stories about the Earth and how life has evolved. Books about dinosaurs and outer space as a child gave way to university courses on astronomy, botany, microbiology, and earth systems science at Mount Allison University in Canada, a two-year stint at the National Research Council of Canada,

and the MIT-WHOI Joint Program. Despite getting seasick easily, McNichol has managed to complete three research cruises while at WHOI, getting plenty of interesting data about the deep sea. He has also been fortunate to have the opportunity to travel internationally to work with collaborators in Germany and China. In his spare time, he enjoys foraging, fermenting things, cooking, and listening to music.