

A Green Thumb for *Synechococcus*

GROWING MARINE BACTERIA IN THE LABORATORY IS A CHALLENGE by Kristen Hunter-Cevera

Anyone who has tried to grow orchids or keep a bonsai tree alive will tell you that cultivating plants is not always simple. My thesis research absolutely depended on cultivating certain types of “plants” and keeping them alive, so that I could investigate the factors that boost or thwart their ability to grow.

My specimens were among the most ubiquitous organisms on Earth and critical to supporting life on our planet. Yet we know surprisingly little about them. That’s because it’s so difficult to observe them in their natural habitat—the ocean.

To make some headway into learning what allows them to grow, reproduce, and survive in the ocean, ironically we needed to be able to grow them in the laboratory. And that’s just a very different environment from what they encounter in nature.

My specimens are remarkably abundant and are found across the world’s oceans. Yet no one even knew they existed until they were discovered in 1979 by scientists at Woods Hole Oceanographic Institution (WHOI): Stanley Watson, John Waterbury, and Freddy Valois.

They are called *Synechococcus*, and they aren’t plants, but rather single-cell bacteria. Though they are leafless, rootless, and adrift in the sea, they use the same machinery as terrestrial plants—photosynthesis—for the same end: to convert carbon dioxide into organic building blocks. Collectively they move mammoth amounts of carbon around the planet. They take about 20 percent of the carbon dioxide drawn from the air into coastal waters and transform it into biomass—creating a critical source of food at the base of the marine food chain. In the process, they also release a significant portion of the oxygen we breathe.

Unexplored population dynamics

The ocean is filled with photosynthetic plankton more numerous and varied than any fields or forests of terrestrial plants. But these vital ecosystems remain largely unexplored because of limited access and observational tools.

In 2003, my Ph.D. co-advisor, WHOI biologist Heidi Sosik, began using a new automated underwater instrument that she co-invented with WHOI scientist Rob Olson. The instrument, called FlowCytobot, detects and records small phytoplankton. Plugged into a seafloor node, it receives power via an undersea cable from WHOI’s Martha’s Vineyard Coastal Observatory. It transmits back to shore hourly observations of microscopic plankton off the coast of Martha’s Vineyard.

Over the past twelve years, FlowCytobot has revealed complex patterns of *Synechococcus* abundance. To understand some of these patterns, we needed information about the different types of *Synechococcus* that could make up the population. While FlowCytobot can detect *Synechococcus*, it cannot distinguish among different types. So we analyzed genetic material in seawater samples and identified five main types of *Synechococcus* off Martha’s Vineyard. Interestingly, these different types were more or less abundant at different times of year.

Obvious questions arose: Why are certain *Synechococcus* more abundant during some seasons than at other times? Even though they are all in the same genus, do different types of *Synechococcus* have different responses to different water temperatures, light, nutrients, or other environmental factors?

To answer these questions, we needed to conduct experiments on the different types of *Synechococcus*. To do that, we needed to maintain populations of them in the lab, which scientists refer to as “cultures.” My challenge was to find the right laboratory conditions that would enable the growth of the five main types of *Synechococcus*. As I found out, just as each type of plant has specific needs—for sun, soil, moisture, and fertilizer, for example—so do bacteria.

Master gardeners

Figuring out the exact requirements or conditions that allow bacteria to thrive in the lab can be a less-than-straightforward process. Being new to culturing *Synechococcus*, I was fortunate



enough to be able to seek guidance on how to isolate different types of *Synechococcus* into culture from the pioneers, John Waterbury and Freddy Valois, still working here at WHOI.

When they first placed natural samples of seawater containing *Synechococcus* into standard lab culture media in the late 1970s, the cells quickly died. “It took over a year to figure it out,” Waterbury said. At the time, media for bacteria contained a little bit of copper. It turned out that *Synechococcus* were quite sensitive to the metal. It wasn’t failing to add something that the bacteria needed, but adding something inadvertently that killed them.

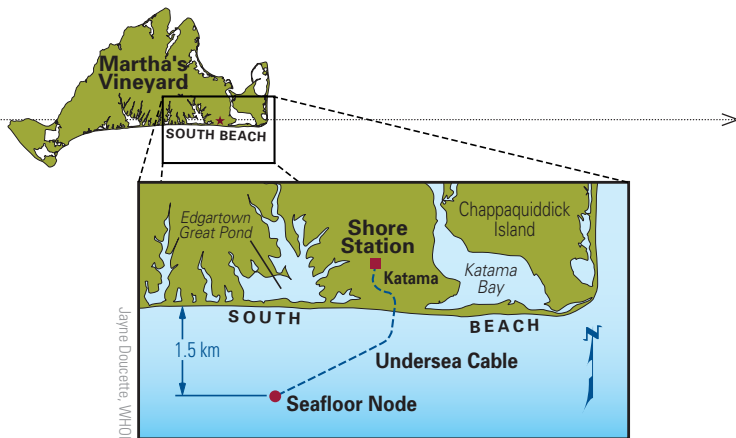
Valois said that a lot of the process is trial and error. Many attempts will fail, but the one that works makes it all worthwhile. “You build on what has been done and then try new things—many new things.”

Smaller details matter when trying to coax an organism to grow in the lab—everything from sterilization procedures for equipment to the types of containers the cultures are kept in can affect growth. Many microbes that live in the open ocean are sensitive to glass, for example, whereas others, such as coastal *Synechococcus*, seem to prefer it, even though it isn’t part of their natural environment.

Some recipes for media are unexpected and seem illogical. A great example is that the most successful medium used to keep *Synechococcus* in long-term culture is made of 75 percent seawater and 25 percent fresh water. When asked why this works better than 100 percent seawater, Waterbury had no exact explanation: “They just like it better.”

Most researchers have a few tales of cultures either growing happily or dying for no apparent reason. Another member of my Ph.D. thesis committee, MIT microbiologist Penny Chisholm, tells the story of a photosynthetic bacterium that she discovered called *Prochlorococcus*, which is a sister genus to *Synechococcus*. She had been culturing them in her lab, but when their building was being renovated and the lab had to move across the street for a year, *Prochlorococcus* was apparently not pleased with the location change.

“We had these beautiful new incubators that the cells simply would not grow well in,” Chisholm said. Everything else, from the containers to the media recipe, had remained the same. When



the lab got to move back to the original building, the *Prochlorococcus* cultures resumed their previous strong growth.

Step 1: Spike your seawater

Armed with some well-tested methods of getting *Synechococcus* to grow in the lab, I set out to try to culture as many types as I could from seawater samples taken at the Martha’s Vineyard Coastal Observatory. The process starts with spiking the seawater with a small amount of media that supports *Synechococcus* growth. You hope that the extra nutrients in the media will encourage the cell growth you want.

However, the sample probably contains other organisms that find the media tasty, and they may start to grow as well. This can be a problem: If these organisms grow faster than *Synechococcus* in culture, they will outcompete the bacteria. In addition, different types of *Synechococcus* may also be in competition with one another, with the result that one type may end up dominating the culture. This was a special concern for me because I was trying to isolate as many different types of *Synechococcus* as I could.

To preclude competition, I used techniques to try to separate different types of *Synechococcus*. One technique is to spread out the seawater samples onto media made with agar, a gel-like substance. Oddly enough, *Synechococcus* is amenable to growing on a semi-solid surface, even though it ordinarily spends its entire life in seawater.

Spread out on the agar surface, cells have an opportunity to grow away from other cells. Once the cells have divided enough to be seen with a microscope, I could pick cells off the surface—trying to get only *Synechococcus*—and put them back into liquid media. This process can be repeated multiple times to ensure that only *Synechococcus* remains in the cultures.

Different strokes for different bacteria

Using the above methods, combined with a lot of patience, I cultured not five but 13 different types of *Synechococcus* from the Martha’s Vineyard seawater samples. Many of these matched the main types of *Synechococcus* that I identified from the genetic analyses. These additional types of *Synechococcus* were in the seawater, but likely in much less abundant populations.

Kristen Hunter-Cevera’s Ph.D. thesis focused on exploring and understanding the population dynamics and diversity of photosynthetic bacteria called *Synechococcus* observed at the Martha’s Vineyard Coastal Observatory (top). But getting them to grow in the lab, so she could study them in detail, proved challenging.



Debraan Fournie, WHOI

With cultures of these different types of *Synechococcus* in hand, we are now poised to investigate some of the differences among them. We can further explore the various physiological factors and environmental conditions that control when and where each type grows, reproduces, and survives.

We have indications that certain *Synechococcus* can't use nitrate for growth, while others can use different sources of nitrogen, such as urea and amino acids, as nutrients. Some types have different photosynthetic pigments, indicating that they can harvest different wavelengths of light and thus have advantages in clear or turbid waters. Different types exhibit different tolerances for water temperature or salt content. Others may be more or less palatable to various creatures that graze on them, or more or less vulnerable to viruses that can kill them.

All these differences favor different types of *Synechococcus* at different times and in different locations, allowing them to occupy distinct ecological niches as environmental conditions shift. Ultimately, the more we learn about how individual *Synechococcus* types behave, the more we can understand the complex population dynamics of this essential marine organism.

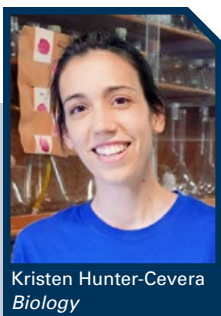
Cultivating cultural expertise

Synechococcus cultivation has been a rewarding part of my Ph.D. thesis and has suggested new research ideas for the future. As a caretaker of *Synechococcus*, I gained perspective on the organism that I know I would not have had if I had not spent time growing them. I came to appreciate that, just as humans have different likes and dislikes, so do these amazing and highly productive marine bacteria.

I also developed a deep appreciation for the value of culture collections and the hard work of their curators. Culturing microorganisms involves tenacity, ingenuity, and patience, but even with these attributes, luck and serendipity can also be an important part of the recipe for success.

Every Ph.D. student's thesis includes a section gratefully acknowledging those who helped them through their graduate studies. Mine, of course, thanked my advisors, other scientists, family, and friends for all their support. But it also included another line: "To my cultures, for growing away from the ocean." ▲

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Biology

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After a trip to Woods Hole when she was eight years old, Kristen Hunter-Cevera declared to her parents that she wanted to become a marine biologist. The California native eventually returned to Woods Hole and kept her word. On the way, she graduated from West Virginia University with degrees in biology and mathematics. During a slight detour, she also received a master's of business administration degree from WVU. She graduated from



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Tom Kleindinst, WHOI

Top: WHOI scientists Rob Olson and Heidi Sosik created Imaging FlowCytobot, an automated underwater microscope that captures high-resolution images of phytoplankton.

Middle: Graduate student Bennett Spencer Lambert helps install an Imaging FlowCytobot at the Martha's Vineyard Coastal Observatory.

Bottom: WHOI scientists John Waterbury and Frederica Valois, and the late Stanley Watson, first discovered *Synechococcus* in 1979. They have mastered the subtle tricks of culturing the bacteria.

the MIT-WHOI Joint Program in 2014 with a Ph.D. in biological oceanography. When not in the lab transferring her *Synechococcus* cultures, Hunter-Cevera can often be found running on the beach, tending to her multiple house plants and betta fish, enjoying almost any arts and craft project, or undertaking occasional moderate lab or office redecoration. Her mentor for this article was David Levin, a freelance science and technology journalist.