Listening in as bacteria 'talk' to each other

A graduate student investigates the microbial mysteries of quorum sensing

The 27th of January, at the entrance of the vast Bay of Bengal ... about seven o'clock in the evening, the Nautilus ... was sailing in a sea of milk. ... Was it the effect of the lunar rays? No: for the moon ... was still lying hidden under the horizon. ... The whole sky, though lit by the sidereal rays, seemed black by contrast with the whiteness of the waters. "It is called a milk sea," I explained.

—20,000 Leagues Under the Sea, *Jules Verne, 1870*

By Laura Hmelo

Throughout the vast oceans, silent conversations are regularly taking place among the Earth's most primordial life forms. The multitudes of single-celled bacteria that inhabit the oceans have evolved a way to communicate with one another, come together, and coordinate their behavior. Bacteria talk, and in our lab at Woods Hole Oceanographic Institution (WHOI), we are trying to eavesdrop on their chatter.

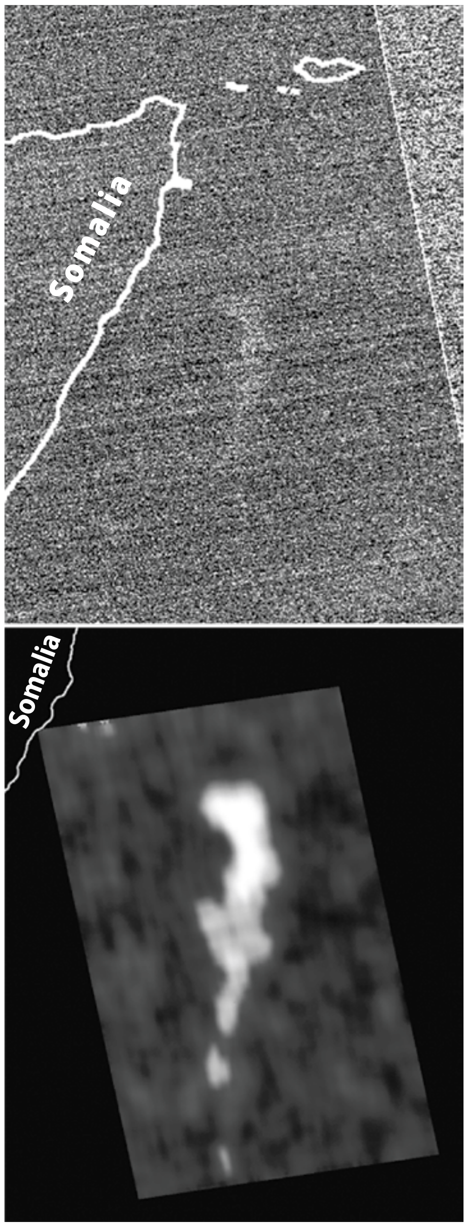
The process bacteria use has been dubbed "quorum sensing." You can think about it this way: In Congress or a corporate boardroom, you need to muster a minimum number of individuals—a quorum—to conduct business or accomplish something together; the same is true in the microscopic realms of the ocean.

One example is collective defense: A bacterium, alone and floating in the ocean, is an easy target for a marauding single-celled protist. But itinerant bacteria have evolved ways to aggregate into tightly packed, highly organized, and usually slimy communities called "biofilms," which attach to hard surfaces. Like walled cities, these biofilms protect individuals living within them from assaults.

The good, the bad, and the slimy

Biofilms are everywhere, and they have wide-reaching impacts. If you have ever felt the plaque on your teeth, slipped on a slimy rock at the beach, or been frustrated by ugly green slime on the hull of your boat, you have had a close encounter with a biofilm.

In the human body, bacteria that cause dental cavities, ear infections, and fatal lung diseases in cystic fibrosis patients, for example, all forge biofilms. These act as a fortress to protect the bacteria from the body's immune response or antibiotic treatments while they build their ranks and prepare to attack their host.



Satellite images of the Indian Ocean off Somalia from the night of Jan. 25, 1995 (unfiltered at top, filtered in bottom) show where bioluminescent marine bacteria aggregated to illuminate the ocean over a 5,946-square-mile area—a bit larger than the state of Connecticut.

"Milky Sea" images courtesy of Steven D. Miller, Naval Research Laboratory (NRL); Steven H. D. Haddock, Monterey Bay Aquarium Research Institute; Christopher D. Elvidge, National Geophysical Data Center; and Thomas F. Lee, NRL

Similarly, biofilms serve as refuges for disease-causing bacteria in the ocean. Biofilms transported on ships, the shells of marine animals such as lobsters, or on microscopic copepods can help spread and transmit pathogens such as cholera.

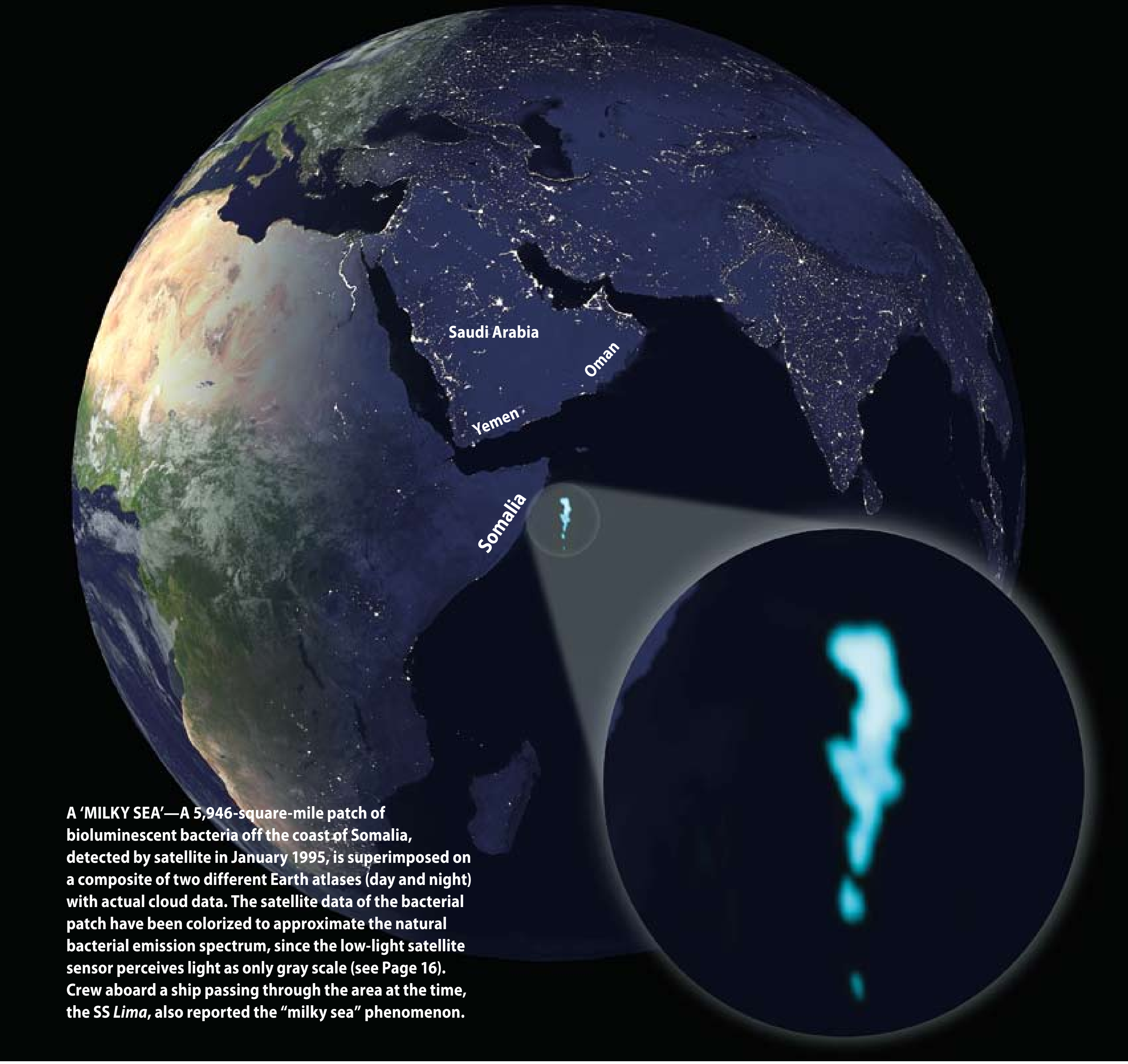
Biofilms are detrimental in other ways. They foul and clog water pipes. They form the substrate on which nuisance organisms such as barnacles settle on ship hulls. The U.S. Navy spends more than $100 million every year on fuel to overcome biofilm-induced drag on its vessels. In fact, much of the information we have about the structure and function of biofilms has been discovered by scientists interested in controlling and eradicating them. This research includes searching for ways to prevent certain infectious bacteria from building biofilm fortresses, thereby making them vulnerable to more traditional antibiotic approaches.

But biofilms also play essential, positive roles in a variety of critical natural processes and provide innumerable crucial ecological services. They provide habitats for beneficial bacteria, algae, and higher organisms. Swarms of bacteria detoxify pollutants in lakes, rivers, and oceans. By decomposing and recycling organic material, they help keep nutrients circulating in the food chain like money in financial markets.

Via behaviors regulated by quorum sensing, bacteria play a significant role in determining what happens to carbon in the ocean: whether it sinks to the depths and is buried as detritus on the seafloor or converted into the greenhouse gas carbon dioxide and released back into the atmosphere.

How bacteria talk the talk

Bacteria communicate via "chemical" conversations. The "words" they use are small molecules. Among the most common chemical "words" in the marine environment are modified amino acids called acylated homoserine lactones, or AHLs.



A 'MILKY SEA'—A 5,946-square-mile patch of bioluminescent bacteria off the coast of Somalia, detected by satellite in January 1995, is superimposed on a composite of two different Earth atlases (day and night) with actual cloud data. The satellite data of the bacterial patch have been colorized to approximate the natural bacterial emission spectrum, since the low-light satellite sensor perceives light as only gray scale (see Page 16). Crew aboard a ship passing through the area at the time, the SS *Lima*, also reported the "milky sea" phenomenon.

The basic process of quorum sensing is pretty simple. Bacteria are constantly producing a few AHLs, which diffuse through bacterial cell membranes into the environment. If no other AHL-producing bacteria are out there, the AHLs will diffuse away and quickly degrade, and bacterial "silence" will prevail.

But if enough AHL-producing bacteria are in the vicinity, the concentration of AHLs outside the bacteria will eventually rise. That's the chemical signal to each bacterium that it's got a lot of buddies in the area. In this way, the bacteria sense that they have sufficient density—that they have achieved a quorum.

The communal buildup of AHLs triggers the production of more AHLs by individual bacteria, which keeps the process going—something called an autoinduction response. The AHLs bind to protein receptors, which interact with bacterial DNA and help to turn on genes inside all the bacteria throughout the quorum. The genes activate behaviors that would not have been worthwhile for an individual bacterium but are now collectively advantageous: They produce enzymes, secrete toxins, or produce the biochemical building blocks that will fortify the biofilm.

Ready, set, glow

In some bacteria, quorum sensing triggers the production of enzymes that catalyze light-producing reactions. The bacteria appear to glow! Swarms of bioluminescent bacteria are the presumed source of the "milky seas" that many sailors have periodically observed over the centuries and that Jules Verne described in *20,000 Leagues Under the Sea*.

In fact, the phenomenon of bioluminescence sparked the discovery of quorum sensing 40 years ago, when researchers were studying how Hawaiian bobtail squids glow. They found that bacteria called *Vibrio fischeri* aggregate in the squids' light organs, forging a symbiotic relationship. In exchange for a protected, nutrient-rich environment to live in, the bacteria do something as a community that they wouldn't do as individuals: They glow.

The benefit that the bacteria get from bioluminescing is still in debate, but we do know what's in it for the squids. The glowing counterilluminates and camouflages squids' shadows when they are active on moonlit nights, so they aren't as easily detectable by predators.

Quorum sensing research at WHOI

As it turns out, quorum sensing is a ubiquitous process in the types of bacteria that dominate marine microbial communities. With my research advisor, WHOI biogeochemist Ben Van Mooy, I have been using novel analytical approaches to try to home in on the fleeting chemical "words" used by marine bacteria.

I sample biofilms from all manner of marine environments in hopes of catching these conversations in action. I scrape slime from rocks I find on Vineyard Sound beaches, from ship hulls in local waters, and from sediments in the Chesapeake Bay. I isolate bacteria from seawater in the Indian Ocean and from the backs of marine organisms.

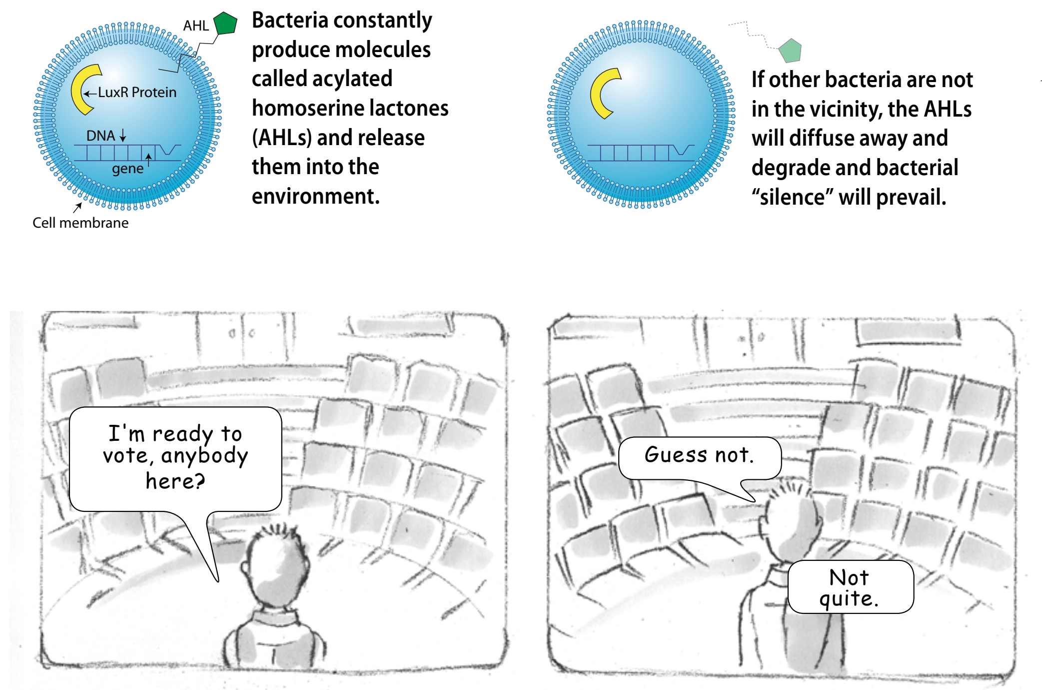
I bring these natural samples back to the lab, where we extract any AHLs present in the samples using organic solvents, just as you might make a cup of tea from tea leaves with hot water. We subject the extract to liquid chromatography, a process that teases apart the different molecules in the extract.

We then use a mass spectrometer to bombard the molecules with high energy, which smashes them into smaller pieces (think about what happens if you drop a ceramic dish onto the floor). By examining the size of each of these pieces, we can put them back together like a puzzle and identify the exact chemicals that were in the original extract.

Quorum-sensing bacteria are very sensitive to AHLs, and so the molecules are usually present in very low concentrations. In the lab, scientists can cultivate very large batches of bacteria and isolate very large quantities of AHLs. However, in the environment, bacterial populations are much smaller and dispersed over much wider areas, making it much more challenging to obtain detectable quantities of natural AHL.

But these aren't the only obstacles that have limited scientists' ability to eavesdrop on natural bacterial communities. AHLs

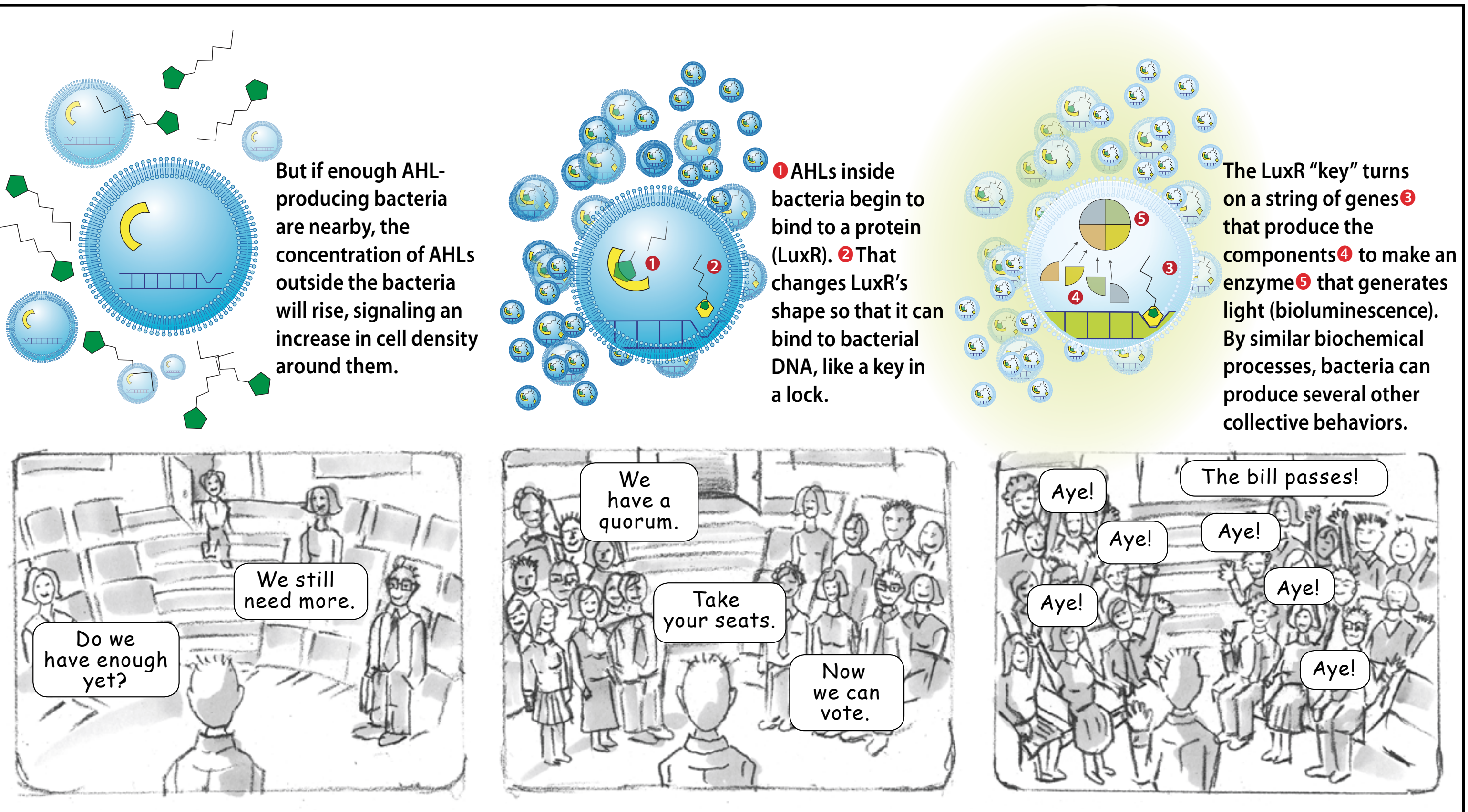
E pluribus unum, politically and biochemically:
How bacteria achieve a "quorum" to coordinate collective behavior




Tom Kleindinst, WHOI


Jim Canavan, WHOI

Bacteria floating in the ocean use quorum sensing to aggregate into tightly packed, highly organized, and usually slimy communities called biofilms, which attach to hard surfaces such as ship hulls (right). On the other hand, biofilms also play essential beneficial roles in ecosystems, providing habitats for creatures in tidal pools, for example (left).



E. Paul Oberlander and Katherine Joyce, WHOI

are very sensitive to the acidity or alkalinity of seawater, and they degrade quickly in the ocean. In our lab, we have been measuring how quickly they degrade; it turns out that, on average, each AHL molecule self-destructs in just a few hours. So we have to make our measurements soon after we collect our samples from the ocean.

'It is called a milk sea'

Our pot of gold would be to find a large-scale bacterial quorum in the ocean. Indeed, a recent and tantalizing observation suggests that they do exist. In 2005, scientists at the Naval Research Laboratory, Monterey Bay Research Institute, and the National Geophysical Data Center reported a "milky sea" that had occurred a decade earlier. It covered an area of 5,946 square miles in the Indian Ocean.

The Indian Ocean bioluminescence had all the characteristics that it had been produced by bacteria. A huge bloom of phytoplankton had blanketed the ocean surface. Hordes of bacteria in the area massed onto the phytoplankton like swimmers on a fleet of rafts. When they sensed they had achieved a quorum, they signaled one another to glow, becoming collectively bright enough to be seen by satellite.

To produce that much light, more than a billion trillion (10^{22}, or 1 with 22 zeroes after it) cells would have had to be present in that 5,946 square miles. That's quite a large quorum.

It is just a matter of time before we catch a conspicuous bacterial quorum in action in the ocean. We hold out hope that we may be at the right place at the right time during research cruises we have planned over the next two years.

Meanwhile, our lab continues to make progress in deciphering the chemical conversations of bacteria within less spectacular, though no less significant, biofilms on marine particles and phytoplankton. Ultimately, our work may reveal how decisions made in tiny bacterial boardrooms have impacts felt throughout the vast oceans and atmosphere.

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Laura Hmelo was raised by a family of scientists in Nashville, Tenn. She earned a degree in chemistry in 2002 at Carleton College in Northfield, Minn., where she was active in environmental politics. In 2001, as a summer fellow at WHOI, she conducted paleoclimate research in a lab where she continued to work for two years after college. But she decided she preferred to study modern biogeochemical processes, focusing on bacterial quorum sensing and biofilms. That has led her to become—in addition to chemist and oceanographer—a microbiologist. While taking breaks from conversing with bacteria, she finds time to run, take photos, and dangle things in front of her cat.