

Scripps Oceanography microbiologist Brian Palenik and second-year graduate student Vera Tai prepare to scoop up a microbial metagenome in a bucket off the Scripps Pier. With each sample, Palenik hopes to reveal some of the secrets of entire ocean ecosystems.

the **Black Box**

BY ROBERT MOUROE

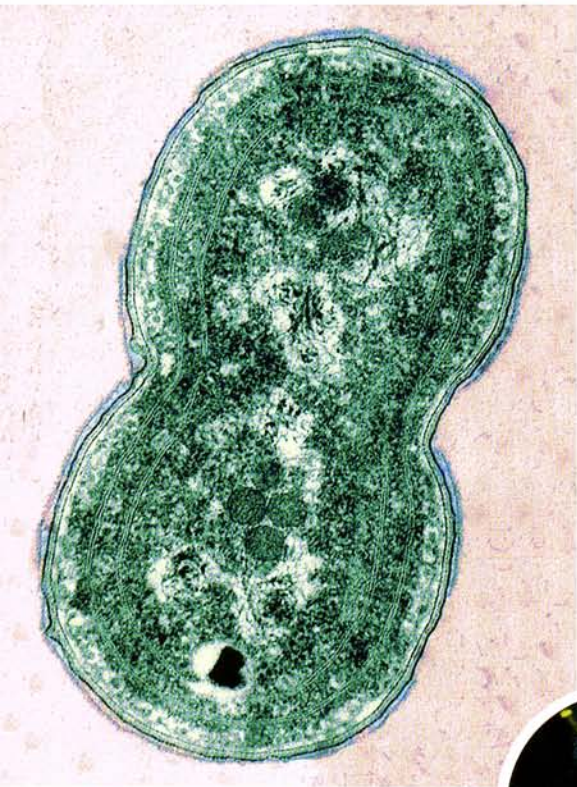
Scripps microbiologists go deeper into the cell to understand entire ecosystems

BRIAN PALENIK ADMITS A BIAS, but the microbial researcher makes a strong case for cyanobacteria being the world's most important group of organisms.

Consider some of the microbes Palenik studies like *Synechococcus* and *Prochlorococcus*. The two phytoplankton species are sometimes called the photosynthetic "engine" of the oceans. They are the core of the ocean's food web. In their ubiquity the world over, they convert as much as a quarter of Earth's yearly carbon dioxide budget into organic compounds. On our home planet, they are a force for habitability. They were probably the planet's first life-forms and their activities are believed to be responsible for filling Earth's early atmosphere with oxygen. Today, cyanobacteria provide the oxygen for nearly one in every four of our inhalations.

So it's only appropriate that Scripps Institution of Oceanography's road to genomics came through Palenik's lab.

It's been five years since he and fellow microbiologist Bianca Brahmsha led the first of 15 genome-sequencing projects that have either originated at Scripps or involved the institution's significant participation. With the completed genome of



Synechococcus in their hands for the past two years, they have made key discoveries about how the microbe equips itself to deal with its surroundings.

They are getting closer to understanding how the state of *Synechococcus* and cyanobacteria like it equates to the health of the ocean as a whole.

"It's kind of counterintuitive. You're going deeper and deeper into the mysteries of a cell to understand this giant ecosystem," Palenik said.

Ditching preconceptions has been a necessity for Palenik's team when dealing with these microbes. For example, *Synechococcus* can swim about 25 body lengths a second, an especially impressive feat considering the creature has no tail, fin, or other apparent mechanism for movement. It is one of the many survival tools Palenik and Brahmsha are investigating.

Indeed the unusual motility is part of a survival strategy suite laid bare for inspection by its genome sequence. Palenik has found, for instance, that strains of *Synechococcus* have genetic instruction packages tailored to different environments. Strain WH8102, found in the open ocean, has a smaller genome than its coastal cousin, strain CC9311. It has 200,000 fewer base pairs, a reflection of its relatively stable, homogeneous surroundings.

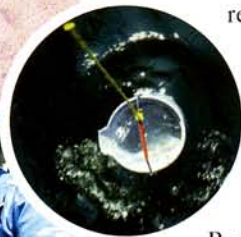
On the other hand, evolution has equipped CC9311 with more "bells and whistles," as

Brahmsha put it, meant to deal with the changing light levels, strange chemicals, and general bustle associated with nearshore environments. CC9311 devotes twice as many genes to regulating its metabolism and sensory response as the open ocean

strain does. Some of them allow it to store or discard toxic metals, a diagnostic clue to the chemical composition of the potentially fouled waters of ports and harbors.

Researchers hope having that kind of knowledge can lead to a host of applications. Perhaps CC9311's ability to sequester metals will make it a suitable tool for bioremediation, the use of biological agents to clean polluted sites in the ocean or on land. In a recent study, Palenik's student Aubrey Davis followed the activity of diatom genes in the presence of copper. The metal is a pollutant commonly found in ports teeming with shipping activity. One way to assess the hazard it poses is to simply measure copper concentrations in the water, but that only tells part of the story. In Davis's study, she determined which genes the microorganisms become active in marine environments with high concentrations of copper. Now identified, the genes with that sensitivity can be used as biomarkers indicating the more important factor of how much of that copper can be taken up and rendered environmentally safe by the port's microbial community.

Similarly, studies of open-ocean cyanobacteria can gauge the adaptive success of organisms dealing with changes in the abundance of essential elements such as iron, fluctuations of which reverberate through the entire ocean food web.



Left, Weekly trips to the pier yield varying amounts of *Synechococcus* (second from bottom), one of the world's most ubiquitous and important organisms.

KEY TERM

BIOREMEDIATION: Use of organisms such as bacteria to chemically alter hazardous substances and reduce their toxicity.



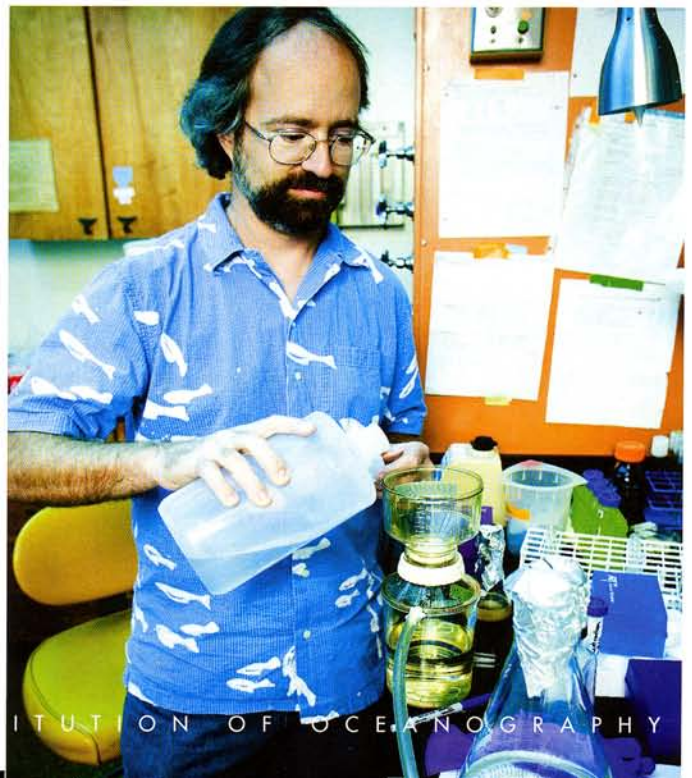
“There’s always a translation between a chemical measurement and a biological effect,” Palenik said. “What we’re trying to get to is a measurement of the biological effect.”

The researchers had been able to get an idea of ecological differences like those between CC9311 and WH8102 before genomics came along, but only through comparison of entire genomes have some been detectable. For instance, *Synechococcus*’s swimming mechanism might come from a large protein it produces. Before the microbe’s genome was sequenced, researchers laboriously sifted through small strings of genes to figure out what function each was responsible for. Brahamsha had been finding what appeared to be an oft-repeated instruction to make a certain protein. Only with the wider perspective of the entire genome could she see that one large gene was actually coding for a single large protein.

“If you look at the entire blueprint for an organism, you’re looking at everything it has,” Brahamsha said. “If we hadn’t sequenced the genome, it would have taken us a lot longer and been much more difficult to find the protein.”

Traditional techniques still complement genomics in Palenik’s research. Every week for the past seven years, sometimes with the help of his now 11-year-old daughter, the researcher has walked to the end of Scripps Pier, lowered a bucket into the ocean and brought the collected seawater back to his lab for analysis. Using an instrument called a flow cytometer, he takes a census of the microorganisms present in each drop. The results contribute to a time study

Above, Some samples are stored in freezers, while others are fed into a flow cytometer that identifies quantities of various microbes in a seawater sample. **Below,** Palenik first filters the seawater to isolate the DNA within the metagenome.



KEY TERM

METAGENOME. The genetic material collected from many species in a microbial community and analyzed for ecological information.

that enables him to track population trends through seasons and changes in water temperature and nutrient availability.

“What we’re trying to do is understand how many different kinds of cyanobacteria there are and why. What are the kinds of forces driving that diversity?” Palenik said.

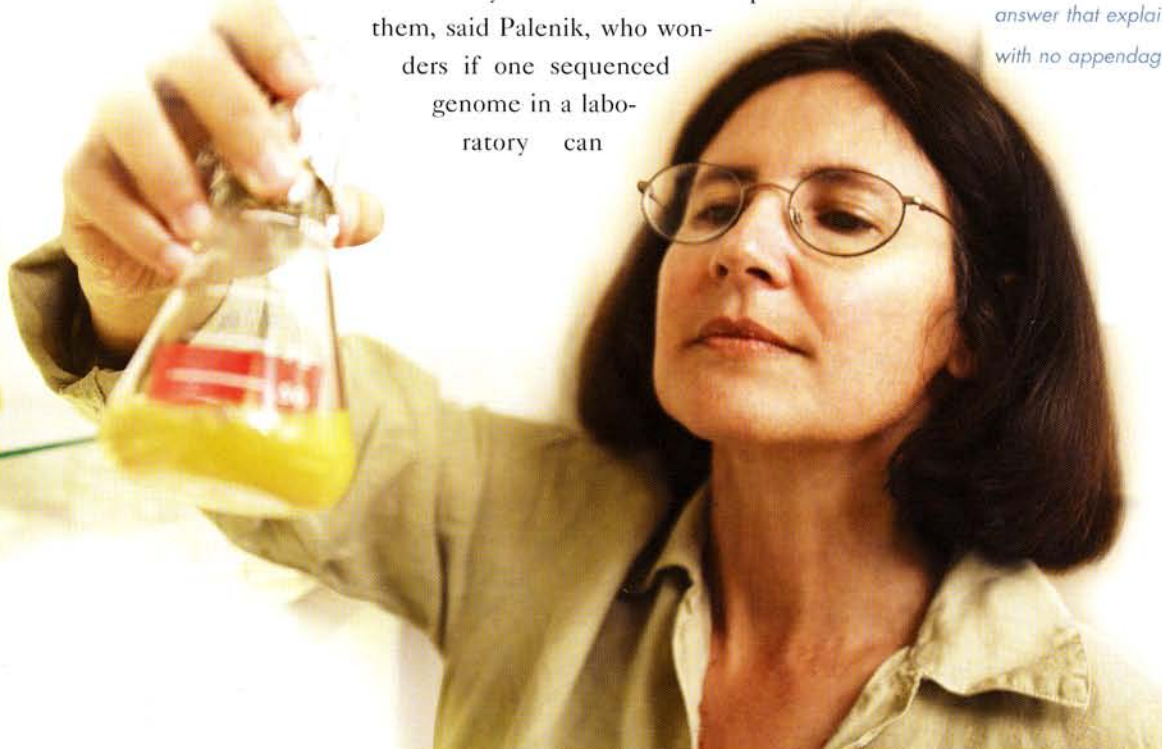
Since the sequencing of *Synechococcus*, Palenik has expanded his study to include *Ostreococcus*. At less than a micron in diameter, the microbe bears the distinction of being the world’s smallest eukaryote (an organism with a nucleus in its cell or cells). At nearly the same size, it is also found nearly everywhere *Synechococcus* is, including in Palenik’s seawater samples. After a few years of noting its constant presence, Palenik became interested in how the two organisms interact and compete. Genomics laboratories are currently completing the sequences of three *Ostreococcus* strains, including a local variety and another found in the Mediterranean Sea.

Despite the promise of genomics, Palenik is skeptical of some of the hype surrounding the young field. The use of genomics as a tool for ocean ecology is still in its infancy because of the expense of sequencing genomes and researchers’ currently

limited ability to know how to interpret them, said Palenik, who wonders if one sequenced genome in a laboratory can



Top, An array of agar plates houses cyanobacterial clones grown for experiments. **Middle and below,** Bianca Brahmsha searches the proteins made by *Synechococcus* for the answer that explains how a microbe with no appendages can swim.





*Microbiologists of the future will need to be able to negotiate genome maps, like the one color-coding every gene possessed by *Synechococcus* 8102 (below), but Palenik sees genomics working in tandem with traditional laboratory techniques for years to come.*



really be an adequate representative for a whole group of organisms.

But to this day, Palenik and Brahamsha share the excitement microbiologists everywhere feel when they think about the magnitude of genomics' potential. They still see the explosion of genetic knowledge continuing unchecked. They see a day when instruction in bioinformatics, the computational systems that process vast quantities of genomic data, is part of every microbiology student's curriculum. It'll all happen in good time.

"There's an idea in oceanography that we want to be a predictive science, not a descriptive science," Palenik said. "So what we're hoping is that by understanding organisms at the genomic level, we'll be able to predict how organisms will respond to different stresses. We certainly have a lot more information now to try to do that." 🌐

