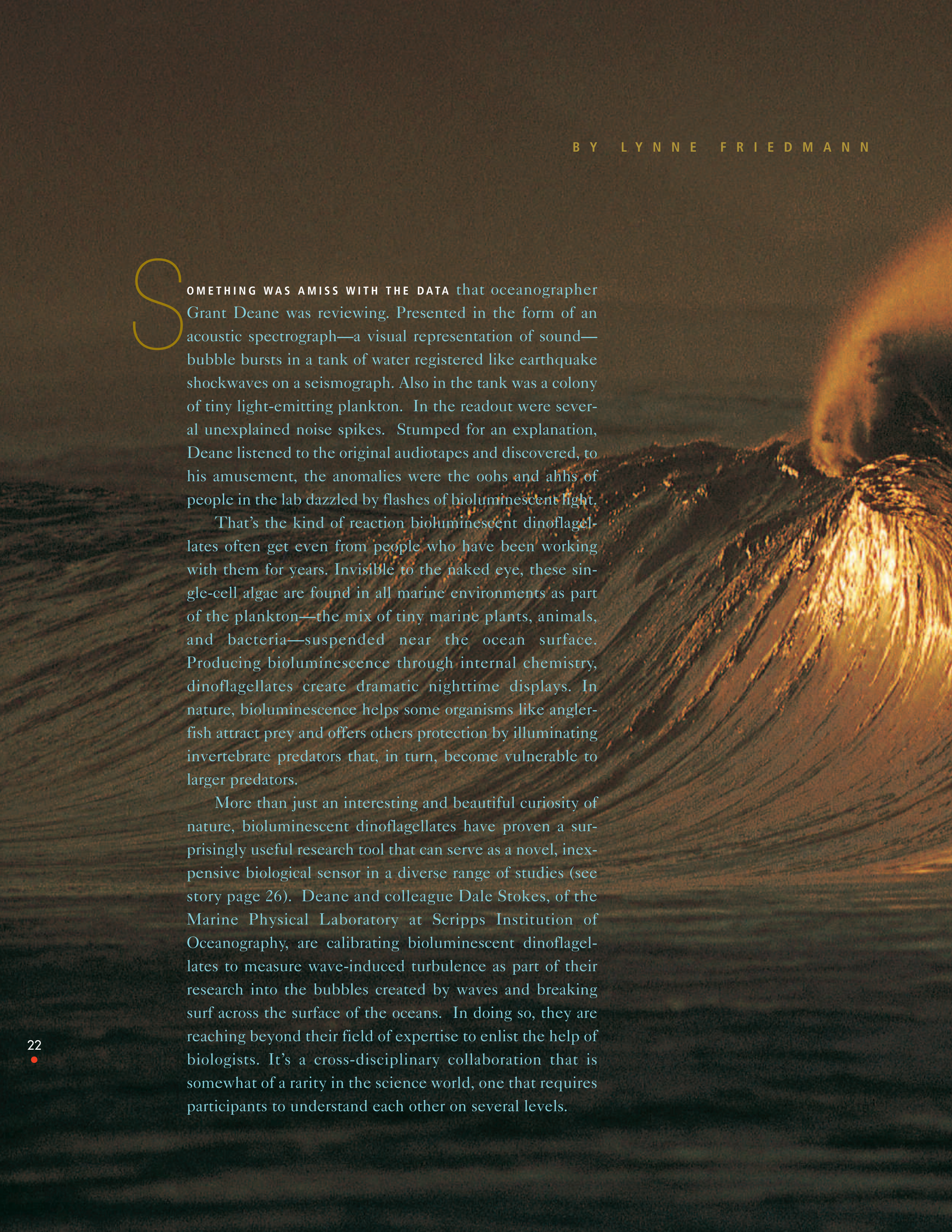


BY LYNNE FRIEDMANN

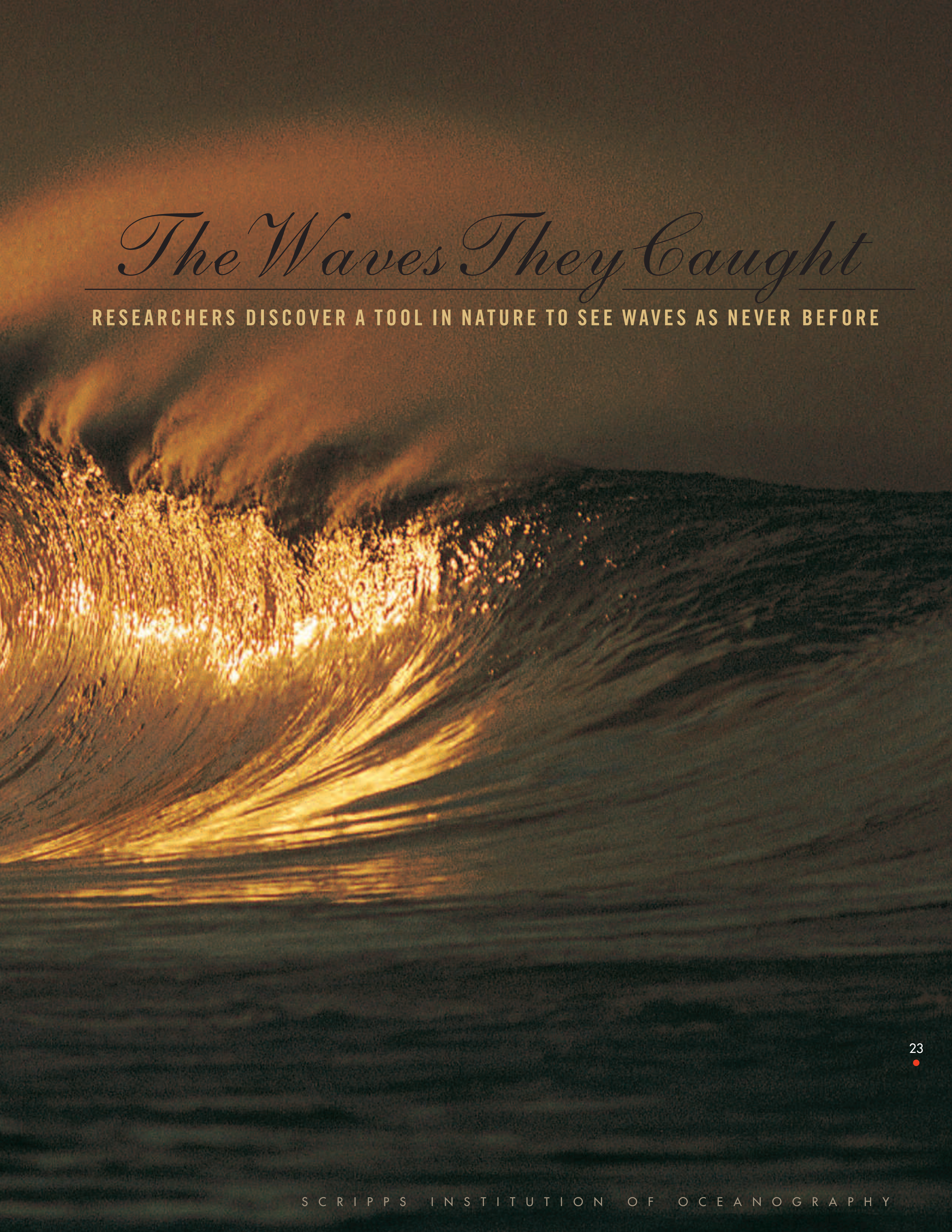
SOMETHING WAS AMISS WITH THE DATA that oceanographer Grant Deane was reviewing. Presented in the form of an acoustic spectrograph—a visual representation of sound—bubble bursts in a tank of water registered like earthquake shockwaves on a seismograph. Also in the tank was a colony of tiny light-emitting plankton. In the readout were several unexplained noise spikes. Stumped for an explanation, Deane listened to the original audiotapes and discovered, to his amusement, the anomalies were the oohs and ahhs of people in the lab dazzled by flashes of bioluminescent light.

That's the kind of reaction bioluminescent dinoflagellates often get even from people who have been working with them for years. Invisible to the naked eye, these single-cell algae are found in all marine environments as part of the plankton—the mix of tiny marine plants, animals, and bacteria—suspended near the ocean surface. Producing bioluminescence through internal chemistry, dinoflagellates create dramatic nighttime displays. In nature, bioluminescence helps some organisms like anglerfish attract prey and offers others protection by illuminating invertebrate predators that, in turn, become vulnerable to larger predators.

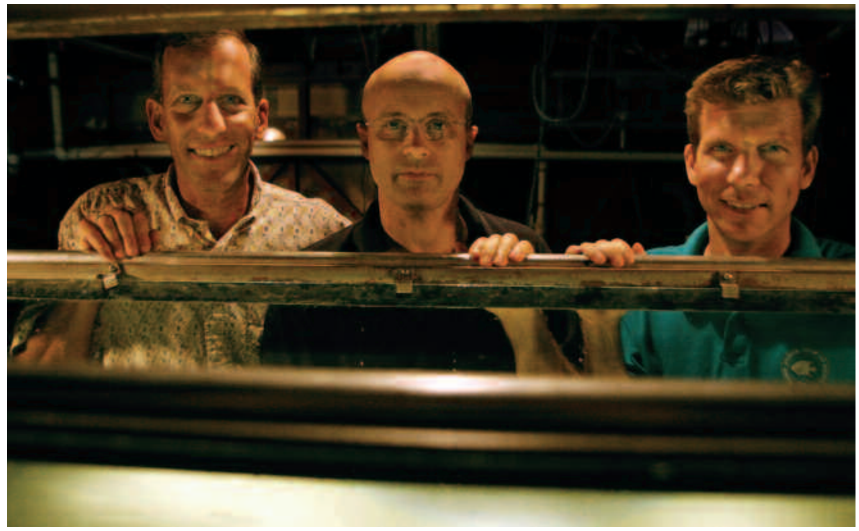
More than just an interesting and beautiful curiosity of nature, bioluminescent dinoflagellates have proven a surprisingly useful research tool that can serve as a novel, inexpensive biological sensor in a diverse range of studies (see story page 26). Deane and colleague Dale Stokes, of the Marine Physical Laboratory at Scripps Institution of Oceanography, are calibrating bioluminescent dinoflagellates to measure wave-induced turbulence as part of their research into the bubbles created by waves and breaking surf across the surface of the oceans. In doing so, they are reaching beyond their field of expertise to enlist the help of biologists. It's a cross-disciplinary collaboration that is somewhat of a rarity in the science world, one that requires participants to understand each other on several levels.

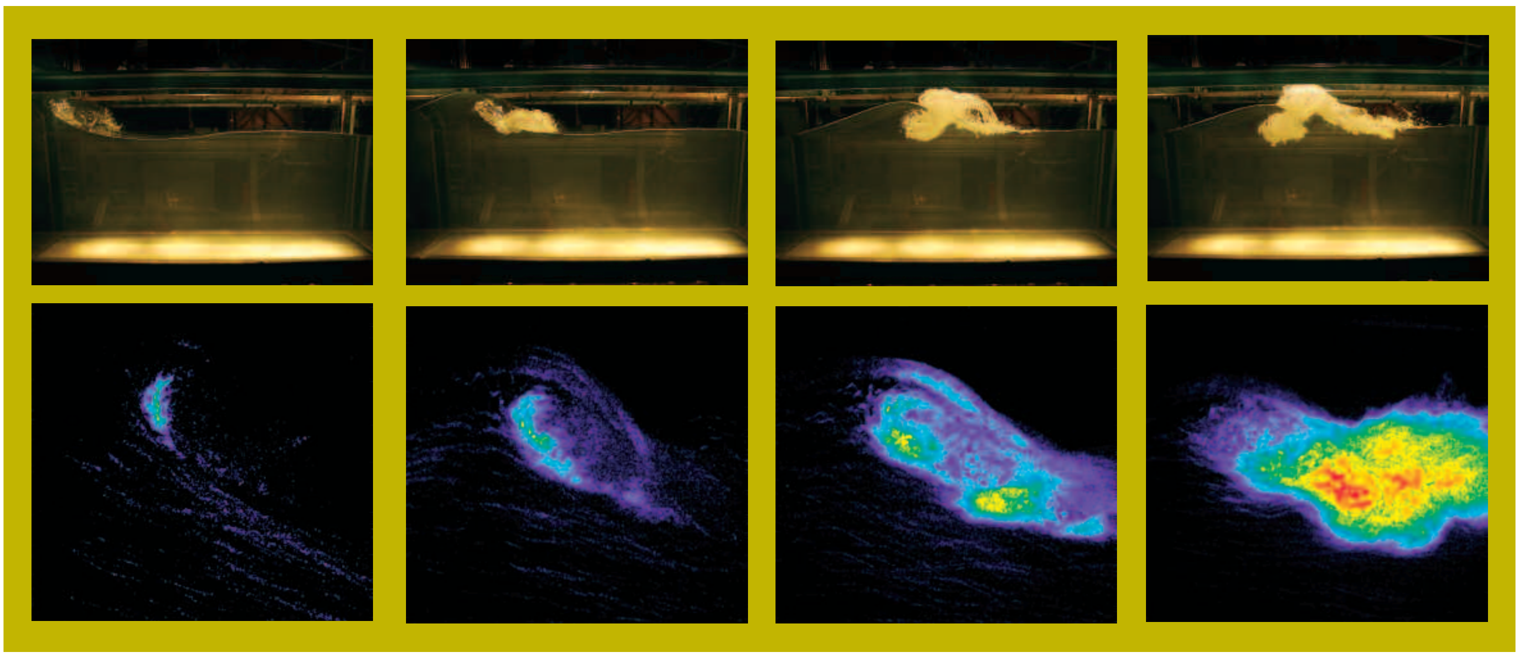
The Waves They Caught

RESEARCHERS DISCOVER A TOOL IN NATURE TO SEE WAVES AS NEVER BEFORE



Right, *Cross-disciplinary collaborators Michael Latz (left), Grant Deane and Dale Stokes.* **Below**, *Two waves, two images: A conventional camera catches an artificially generated breaking-wave sequence at top. The lower false-color image sequence shows dinoflagellate bioluminescence stimulated by the shear stress in another wave, with the red indicating the bright emission and violet the dimmest.* **Opposite**, *Latz with a digital photomultiplier, a light detector that measures bioluminescence.*





"Not only does the science have to match but the people have to fit as well," Deane said.

A CONDUIT FOR CHEMISTRY

Bubbles created by ocean waves play an important role in a variety of ocean and atmospheric processes such as generating ambient noise, producing aerosols, and enhancing air-sea gas exchange. They're a medium through which carbon dioxide enters the oceans, providing a key nutrient to photosynthetic organisms, and a vehicle for a variety of elements that influence the chemistry of sea and air.

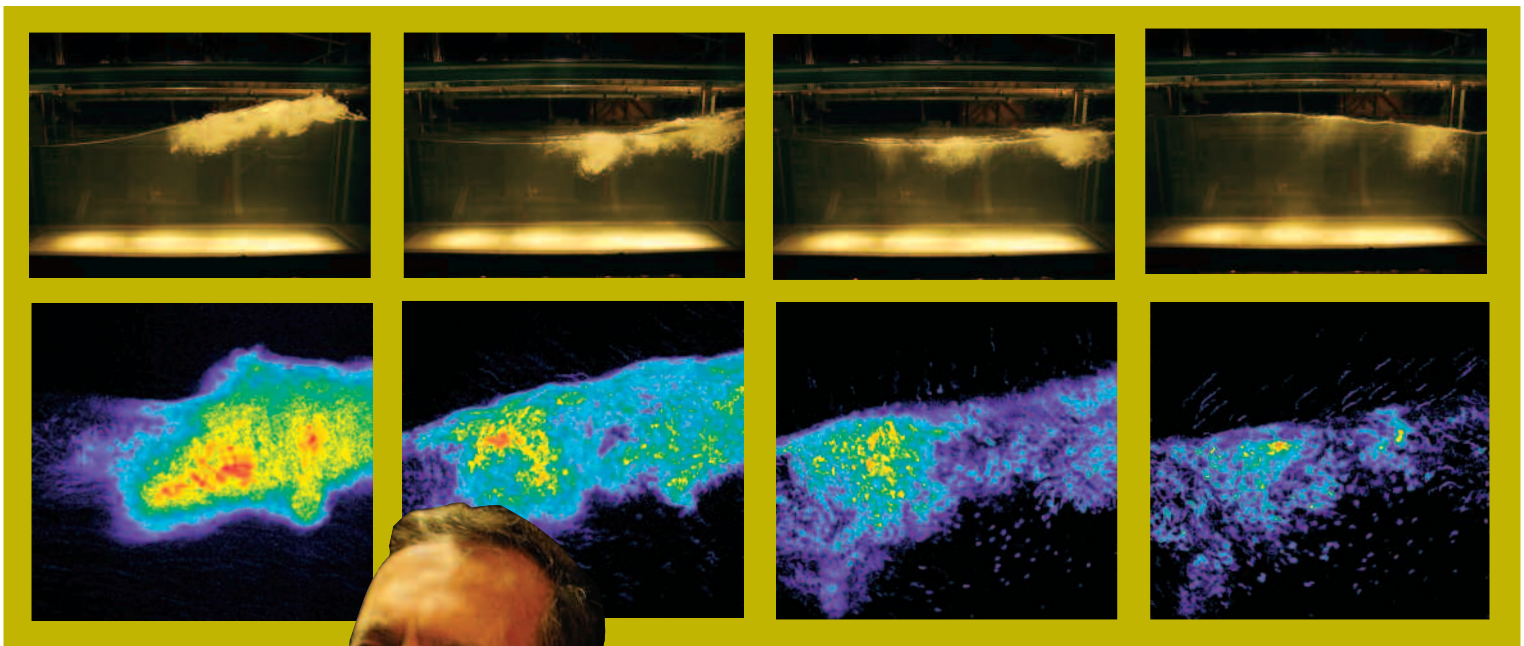
"It's even been speculated that bubbles were responsible for dispersion of biological materials that led to the beginning of life on Earth," said Deane.

As intriguing as bubbles are, their presence has made acoustical and optical measurements of wave turbulence a long-standing problem in physical oceanography.

Combining Deane's interests in acoustics and noise and Stokes's in air-sea gas exchange via bubble formation, they began a line of research in 1996 that initially focused on bubble size distribution in breaking waves. In particular, the researchers wanted to know what takes place in the first seconds after a wave breaks and bubbles are formed.

The pair conducted open-ocean work aboard the Scripps research platform FLIP and wave-tank experiments at the Scripps Hydraulics Laboratory, for which Deane and Stokes developed a unique, high-speed video camera, known as BubbleCam, to track the spectrum of bubbles produced. The work led to the discovery of two distinct mechanisms controlling bubble size and distribution: Bubbles larger than 1 millimeter are formed when a wave curls over onto itself, the "tube" favored by surfers. Smaller bubbles of less than 1 millimeter are created by drop impact as a wave tip splashes.

The observations offered unprecedented insight into the characteristics and dynamics of bubbles inside breaking waves with important implications for the study of air-sea gas exchange—the dynamic interaction that occurs in the thin boundary between the lower surface of the atmosphere and the upper layer of the ocean. As the two environments seek equilibrium, energy transferred from the atmosphere drives ocean circulation. In turn, ocean heat exchange affects atmospheric circulation, weather, and global climate. Moreover because the oceans act as a giant reservoir for dissolved gases such as carbon dioxide,



understanding the method of greenhouse gas transfer between air and sea is crucial to knowledge and prediction of global warming.

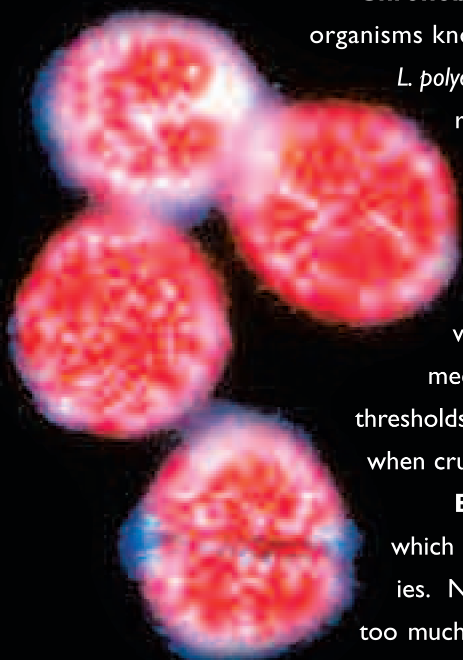
In contemplating the difficult problem of measuring wave turbulence, in order to better understand air-sea exchange, Deane and Stokes turned their attention to dinoflagellates. They were intrigued by the bioluminescence studies of Scripps biologist Michael Latz and Jim Rohr, a physicist at the Space and Naval Warfare Systems Center in San Diego who demonstrated that the amount of light emitted by luminescent dinoflagellates is related to the level of fluid shear stress, the frictional force exerted by the flow of fluid past a surface. In the case of dinoflagellates, this initiates a cascade of intracellular events that culminates in the chemical reaction that produces light.

TWO WORLDS, ONE ROOM

The initial fluid shear work was "basic 101 fluid mechanics experiments to try to tease out some quantitative numbers" related to what triggers these organisms, said Rohr.

Latz and Rohr have applied their findings to imaging studies on the hydrodynamics of dolphins swimming through a concentration of dinoflagellates and flow studies on military watercraft in

GLOWING REVIEWS



For more than 50 years bioluminescent dinoflagellates have proven their worth as an innovative and important research tool used by physicists, biologists, and engineers:

Chronobiology—Examines the cyclic phenomena in living organisms known as biological rhythms. For decades, the dinoflagellate *L. polyedrum* has served as a model organism for the study of circadian rhythms—the roughly 24-hour cycle of physiological processes in plants and animals. The use of dinoflagellates in chronobiology was pioneered at Scripps in 1952.

Military Stealth—Bioluminescence in the nighttime ocean can bring unwanted attention to submarines or other covert craft when a vessel's outline or wake is illuminated by light-emitting plankton. Fluid-mechanic experiments provide the means to quantify the shear-force thresholds that stimulate bioluminescence. Thus, military craft can avoid detection when cruising velocity is below speeds known to excite dinoflagellates.

Bioreactors—Any devices or systems used to grow cultured cells from which important compounds are harvested for medicines or biomedical studies. Nutrients and dissolved gases are supplied through constant mixing, but too much agitation damages or kills cells. With their instantaneous bioluminescence response, dinoflagellates are an effective means to evaluate bioreactor configurations for minimal cell disturbance.

Biomedical Research—The fluid forces in the ocean that stimulate bioluminescence are similar to the forces generated by blood flow. The discovery that dinoflagellates and the endothelial cells that line human blood vessels share the same sensitivity has sparked interest in using bioluminescence as a tool in cardiovascular research to better understanding the thresholds that damage cells or create blood clots.

Toxicology—Dinoflagellate bioluminescence is decreased by contaminants present in coastal areas. A bioassay system developed at the U.S. Navy Space and Warfare Systems Center in San Diego uses dinoflagellate bioluminescence to assess the toxicity of seawater contaminated by metals, organic compounds, and runoff.

Bioluminescent dinoflagellates Pyrocystis fusiformis, *at top showing bioluminescence, and* Lingulodinium polyedrum, *shown at left and above displaying autofluorescence, are valuable tools in a variety of fields, including the marine biology studies of Michael Latz (below).*

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an effort to enable stealth operations when encountering bioluminescence at sea.

"What we needed for our air-sea work was a small, inexpensive sensor," said Stokes.

The answer was plankton.

"Dale and Grant read some of our papers, and being as clever as they are, thought bioluminescence would be a good approach to learn about the flow forces within waves," said Latz.

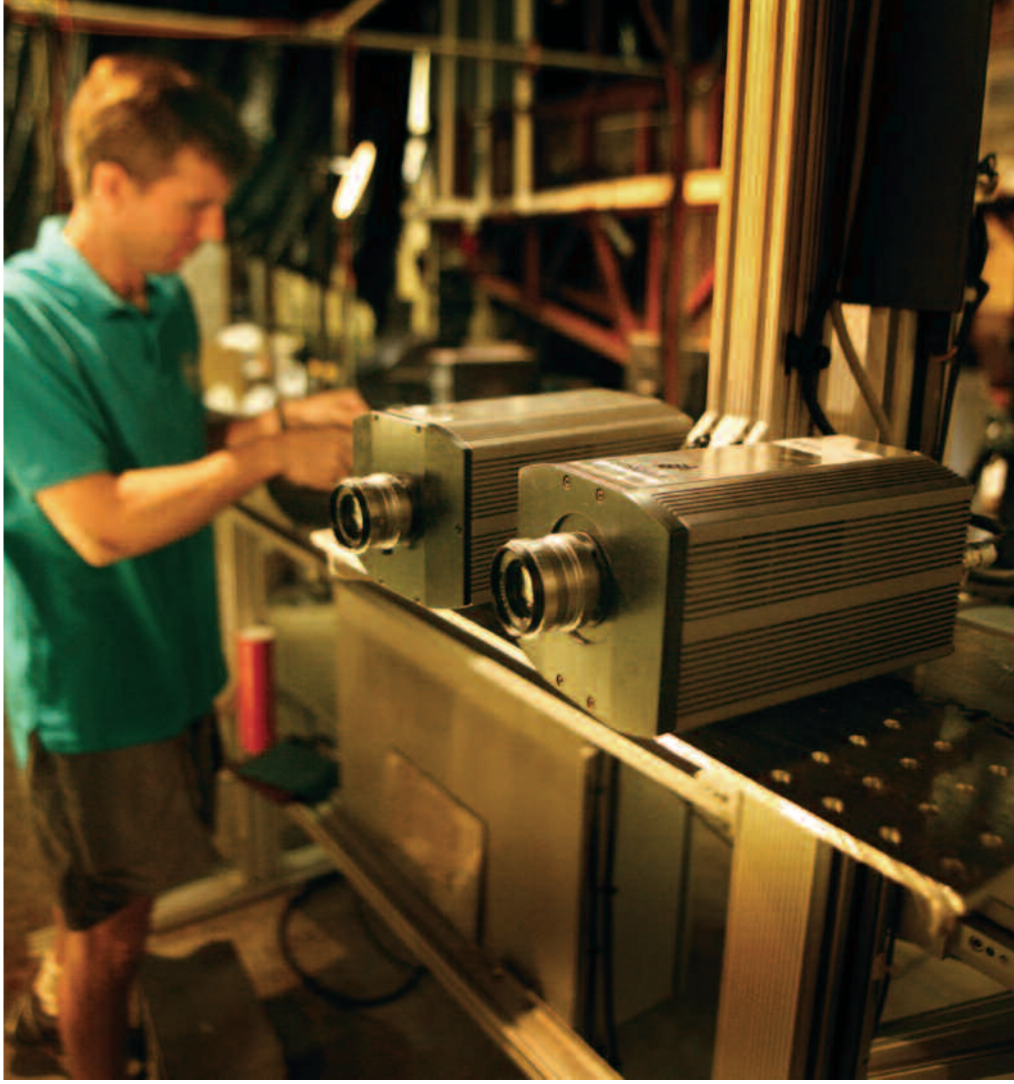
"We came up with the idea of bootlegging on Mike and Jim's work," said Deane. "But what was still missing was a quantitative model."

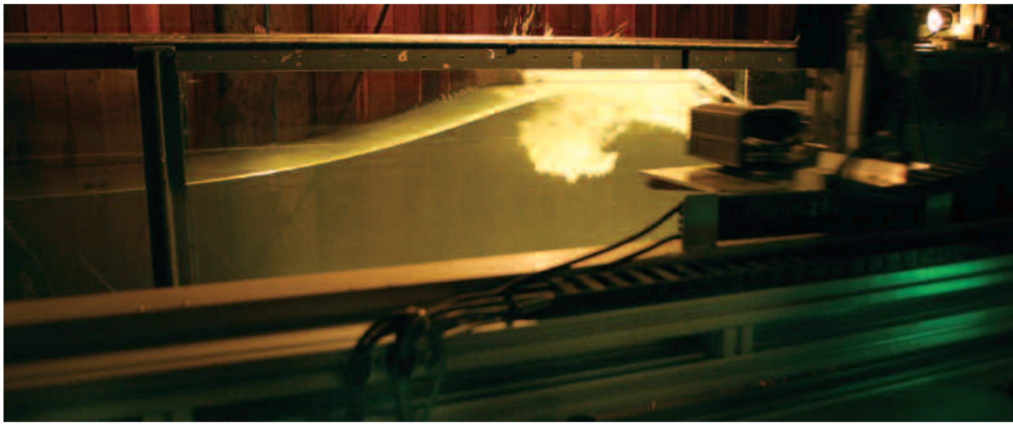
The four researchers developed a statistical model of dinoflagellate cell-stimulation behavior as well as a calibration technique, through bioluminescence imaging, that produces quantitative data of the evolving fluid shear stress field within breaking wave crests. It's a novel approach that also introduces a new concept—"cell anxiety parameter"—that describes the probability of a cell reacting to fluid-shear stress.

"You get Dale, Grant, and Mike in the room and can see their creative juices start flying," said Rohr. "That's what's been so fun for me."

The group's model assumes that over a certain time interval, the probability that a cell will flash is proportional to the level of fluid-shear stress. An additional assumption is that cells produce a detectable flash only once. Testing of the model has produced consistent predicted results.

"What's surprising is how effective the model is," said Stokes.



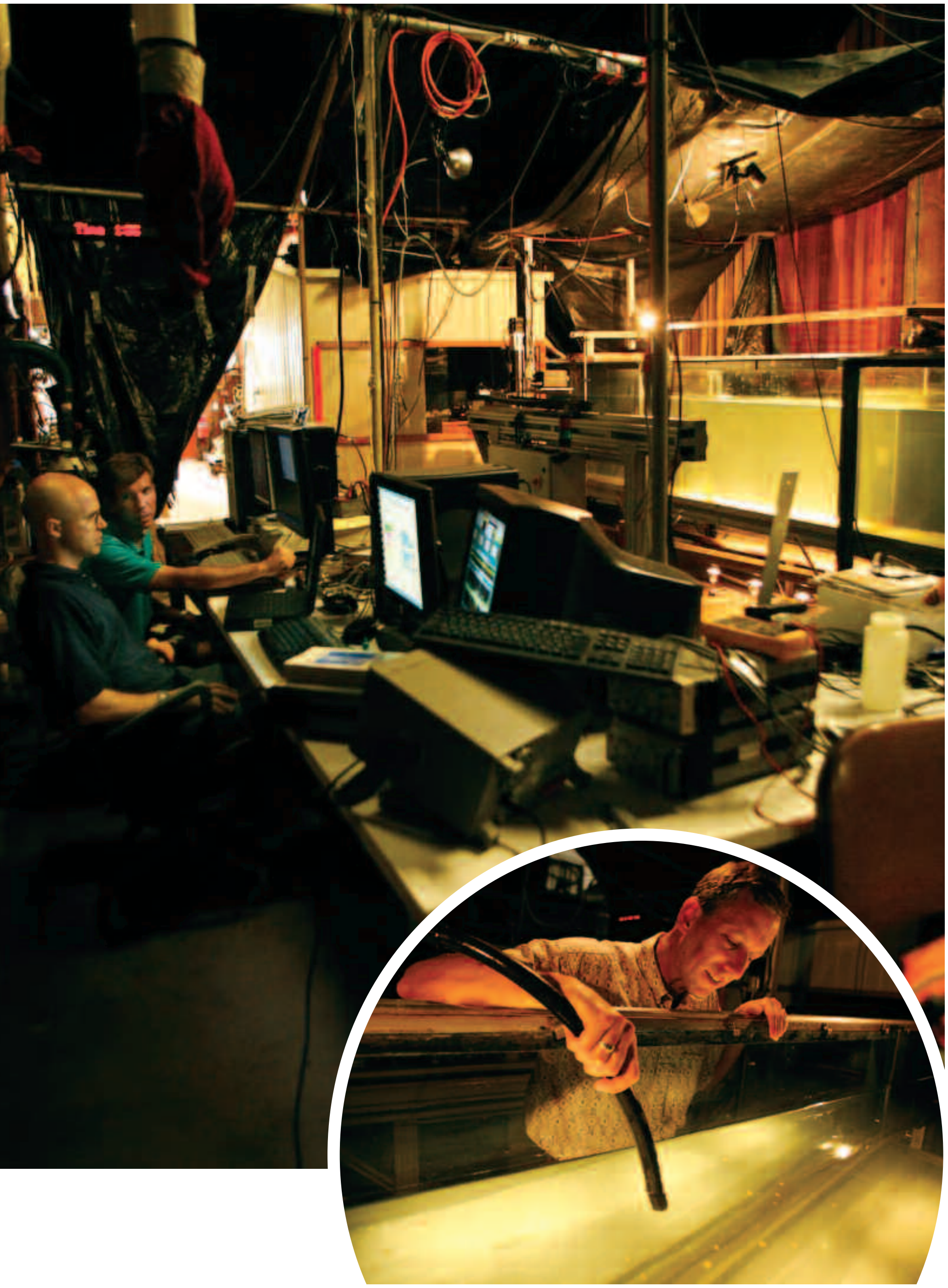


Above, *Specialized high-resolution, low-light cameras track waves across the tank to capture breaking sequences.*

With continued funding from the National Science Foundation, the team embarked this summer on another round of experiments applying the latest computational advances, a new generation of digital camera technology, and the dinoflagellate *Lingulodinium polyedrum*, a local species that is best known for its luminescent red tides.

The experiments are conducted in a glass-walled wave tank that measures 33 meters (108.3 feet) in length by a half meter (19.7 inches) wide. The tank is temperature-controlled and can be filled with fresh or salt water.

"Scripps is the only place for this type of wave-turbulence



Above top, *In a makeshift mission control ringed with blackout curtains, Deane, Stokes, and Latz recorded wave sequences at night for maximum darkness.* **Above**, *Latz collects water samples from the wave tank to quantify cell concentrations.*

research," said Stokes. "Every year more and more capabilities are added."

But before initiating this round of experiments, first came the task of growing the large numbers of dinoflagellates required. Not unlike growing houseplants, phytoplankton need fertilizer in the form of liquid nitrate, phosphate, B vitamins, and trace metals. Light is essential for photosynthesis and to maintain the organism's day/night bioluminescence cycle.

"Growing them is easy," said Latz. "For this experiment we had to figure out how to scale them up in large volumes."

Cultured in 10-liter (2.6-gallon) batches, it takes about two months to arrive at 120 liters (31.7 gallons) of dinoflagellates at a concentration of approximately 3,000 cells per milliliter. This volume is necessary in order to repeat the experiment several times, over the course of a week, using staggered cultures.

After filling the tank to a level of 0.6 meters (24 inches) with filtered seawater, the dinoflagellates are gently added to a tank section under camera observation. Waves of precise height are produced over and over again via a computer-controlled electro-hydraulic wave generator.

Conditions outside the tank are as important as what's going on inside. To maximize bioluminescent light capture, the experiment is performed late at night. In order to eliminate all traces of extraneous light the area surrounding the tank is shrouded in black plastic. Computer monitors are shielded to eliminate light interference.

With the lights turned off, researchers take a few moments to adapt their vision before the first wave is generated. Despite cautions to remain silent for the sake of the acoustic recordings being made, the startling neon flash of blue-green light reliably elicits a few gasps.

Left, *Brainstorming over coffee.* **Below**, *Latz measures cell concentrations from cultures. He grew the dinoflagellates in 10-liter (2.6-gallon) batches over two months to prepare for the test run.*

"I've been seeing it for decades and there's still a 'wow' factor," said Latz. "It's truly incredible because it's light, but produced biologically in living organisms. Just considering everything's that involved, it's amazing."

A robotic camera slides down a track matching the wave-crest speed in order to capture images of stimulated bioluminesence. This is a critical part of data gathering made possible only through recent improvements in low-light digital camera technology. Because each cell produces a detectable flash, the sensitivity of the new cameras allows researchers to relate pixel intensity of the images to the number of cells flashing.

The latest experiment will calibrate the dinoflagellates as "sensors" for future flow-induced bioluminescence studies in breaking waves.

"This is one piece of a complicated problem," said Deane, "but an important one."

Lynne Friedmann is a freelance science writer who resides in Solana Beach, Calif