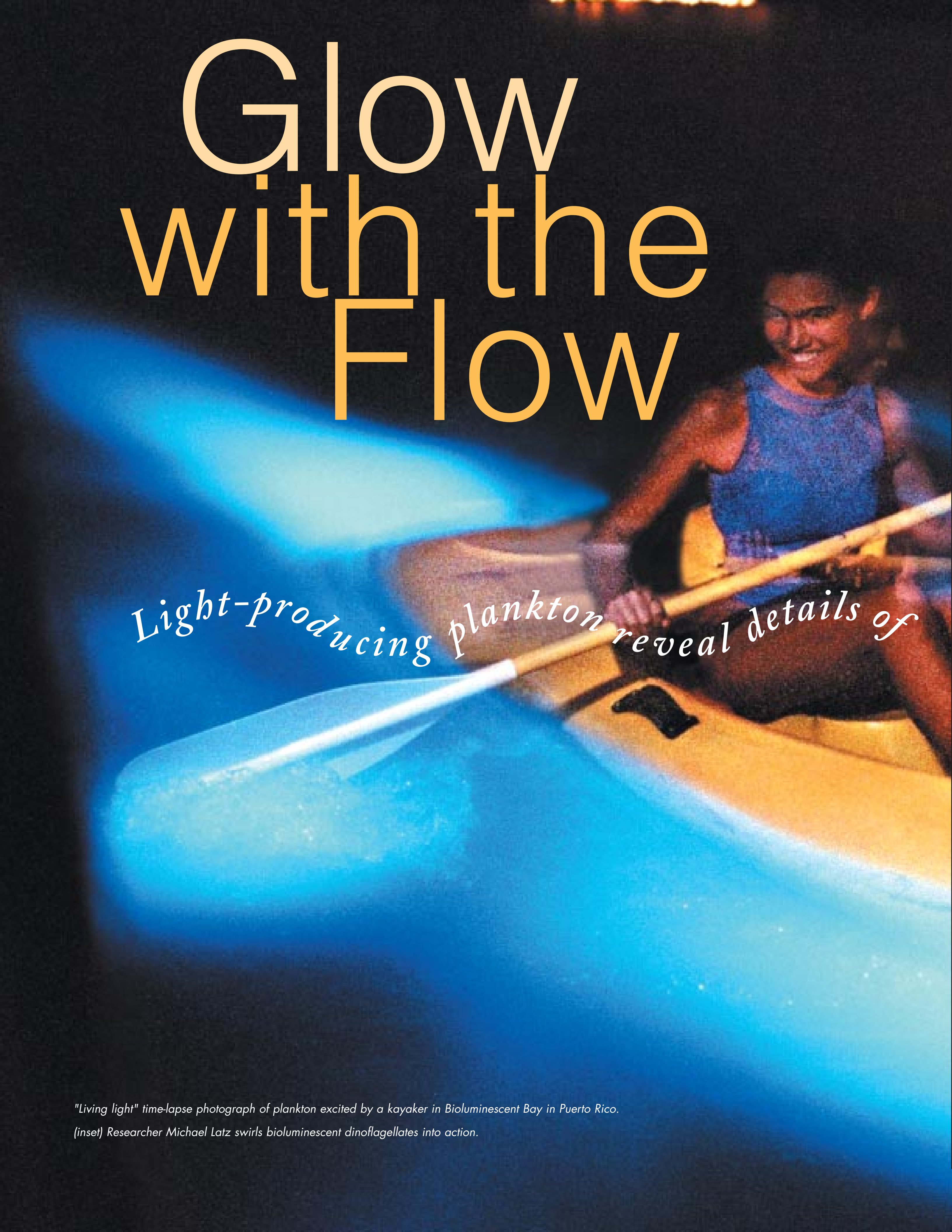
Glow with the Flow

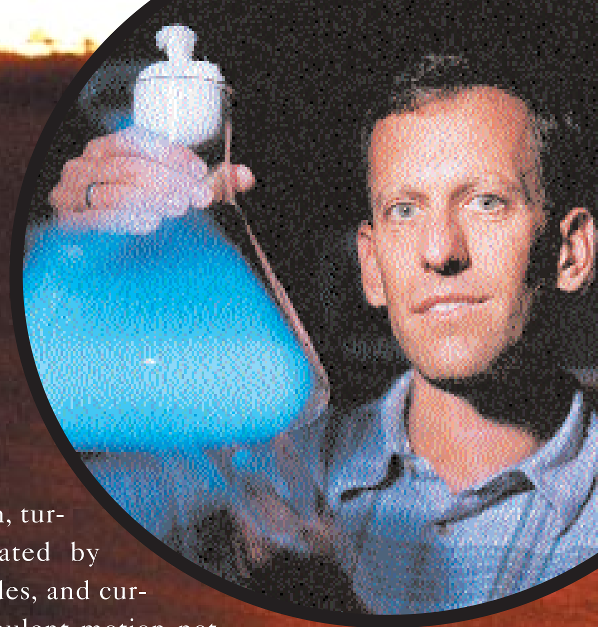
Light-producing plankton reveal details of



"Living light" time-lapse photograph of plankton excited by a kayaker in Bioluminescent Bay in Puerto Rico.
(inset) Researcher Michael Latz swirls bioluminescent dinoflagellates into action.

cellular dynamics

BY PAIGE JENNINGS



Many people have experienced the roll of a boat on a rough body of water—along with a queasy stomach and uneasy legs. The pitch of the boat and the distasteful physiological effects can be blamed on fluid motion. Luckily, people can escape an uncomfortable boat ride by eventually returning to port. But for organisms that live in the ocean there is no escape. They exist continuously in a dynamic fluid environment, which scientists do not yet fully understand.

Scripps marine biologist Michael Latz and his graduate students are studying the effects of fluid motion on single-celled algae known as dinoflagellates.

In the ocean, turbulence is created by wind, waves, tides, and currents. This turbulent motion not only carries around plankton, including dinoflagellates, but also directly affects their biology.

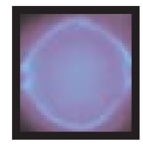
"We know essentially nothing about the physiological effects of flow on marine cells," explains Latz. "But we do know that dinoflagellates are among the most flow-sensitive cells, far more so than mammalian, plant, or insect cells."

Dinoflagellates commonly occur throughout the world's oceans and have several interesting characteristics.

Certain species form red tides, which occur when populations congregate and reproduce so densely that they discolor the water red or brown. Some dinoflagellate "blooms" can degrade water quality and some produce toxins that are harmful to other marine organisms, such as seals or whales. The same toxins also affect humans through paralytic shellfish poisoning.

Many dinoflagellate species are bioluminescent, emitting bright flashes of light at night in response to agitation. In large accumulations, they produce "phosphorescent seas" in which crests of waves, surf, and waters around boats and swimming organisms glow electric blue. In San Diego, a common dinoflagellate named *Lingulodinium polyedrum* is responsible for these light displays, which are most prominent during red tides.

Photo courtesy Frank Borges Llosa.



Clockwise: *Graduate student Andrew Juhl monitors the health of natural populations of dinoflagellates from Scripps Pier. Research vessel monitoring red tide in San Sebastian, Spain. Red tide on Tanabe Bay, Japan.*

Although luminescent dinoflagellates have been studied at Scripps by Latz for several years and by others since the 1950s, some of the cellular and environmental factors affecting the bioluminescent response are still not understood.

STIMULATING A RESPONSE

There are theories, but no concrete explanations as to how dinoflagellates sense their fluid environment, such as the turbulence caused by wind and breaking waves. Scientists also have not identified the internal pathways or mechanisms in the organisms that trigger physiological responses to flow, such as bioluminescence and changes in growth rate, nutrient uptake, and structure.

Flows capable of stimulating a bioluminescent response in dinoflagellates must be quite strong. Beneath the ocean surface on a windy day the turbulence usually isn't strong enough to stimulate bioluminescence, but it does affect the cells in other ways, causing them to reproduce more slowly and even to change shape.

Dinoflagellates and other microscopic plankton experience their environment much differently than do larger animals. Because of their small size, dinoflagellates feel turbulence as laminar shear, a difference in flow velocity across the cell diameter.

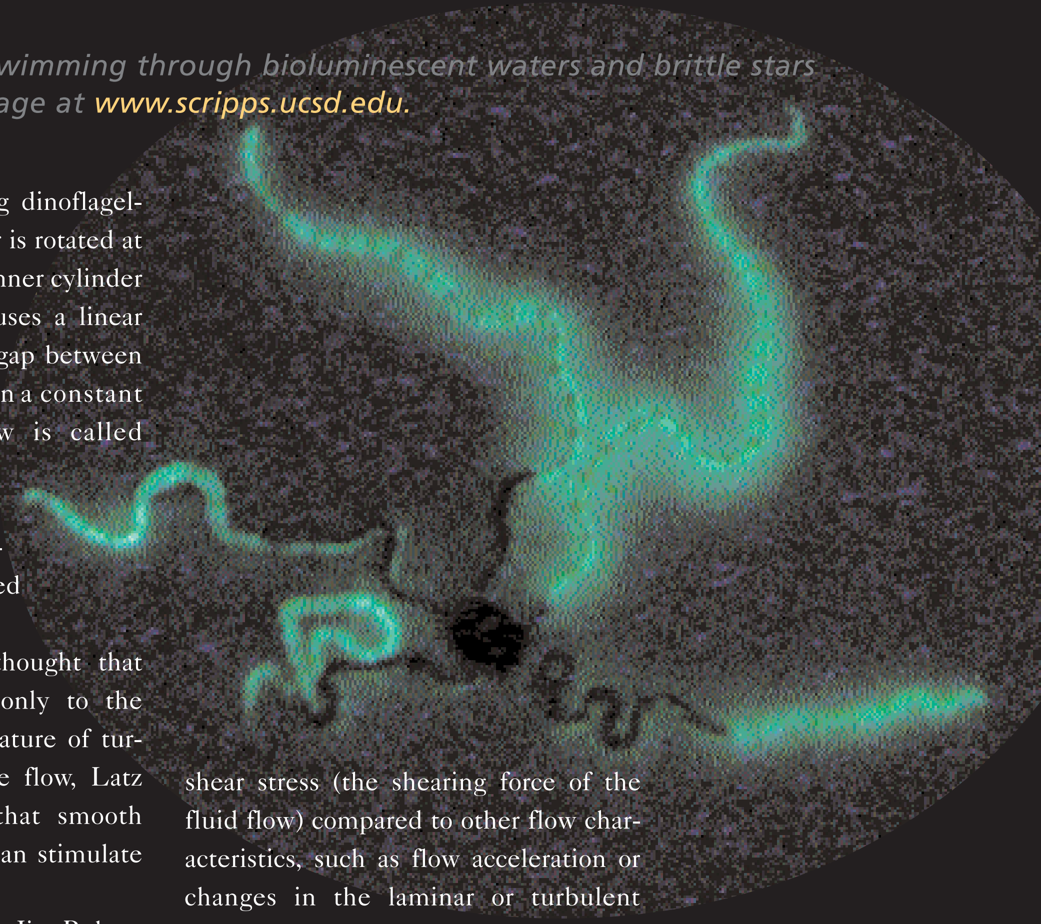
"If you are on a ship in windy conditions, you feel lots of acceleration," explained Latz. "For plankton, acceleration isn't as important; they are so small that they live in a viscous world dominated by shear."

According to Latz, although the velocity difference across a dinoflagellate is extremely small, there is sufficient shear for them to sense and respond to.

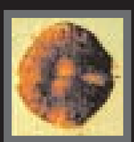
Latz is using dinoflagellate bioluminescence as a way of reporting how cells are affected by flow. Agitating water containing dinoflagellates results in flashes of light from the cells. The flashes are bright and nearly instantaneous, allowing Latz to observe exactly where, when, and to what types of flow the dinoflagellates respond.

In the lab, Latz and graduate students Andrew Juhl and Peter von Dassow create carefully defined experimental flow conditions to test dinoflagellate flow sensitivity. Simple fluid shear is created using special flow chambers consisting of two clear, concentric cylinders. The space between the two cylinders-

ON THE WEB - See video of dolphins swimming through bioluminescent waters and brittle stars lighting up for science at SIO's homepage at www.scripps.ucsd.edu.



filled with water containing dinoflagellates, and the outer cylinder is rotated at different speeds while the inner cylinder is held stationary. This causes a linear gradient of velocity in the gap between the two cylinders, resulting in a constant shear. This type of flow is called Couette flow and is used by the scientists to test the response of dinoflagellates to an exactly defined shear.



At one time it was thought that dinoflagellates responded only to the rapidly changing, chaotic nature of turbulent flow. Using Couette flow, Latz has been able to show that smooth unchanging laminar flows can stimulate bioluminescence too.

Latz and his collaborator Jim Rohr, a physicist at the Space and Naval Warfare Systems Center in San Diego, have conducted other studies combining experimental fluid mechanics with more complex flows. For example, in some studies they send water filled with dinoflagellates through a clear pipe. By controlling the flow rate and thus the characteristics of the flow, they test how the bioluminescent response is affected by the shear stress (the shearing force of the fluid flow) compared to other flow characteristics, such as flow acceleration or changes in the laminar or turbulent nature of the flow.

IMPLICATIONS FOR ALL CELLS

The results of these studies have powerful implications for understanding how all cells might be affected by fluid flows. But scientists still know very little about the internal pathways by which dinoflagellates detect their fluid environment. How do flows trigger physiological responses such as bioluminescence in organisms, or changes in growth rate, nutrient uptake, and cell structure?

"Not all cells are bioluminescent," explains von Dassow, a third-year graduate student with Latz. "But probably the trigger that controls that response in dinoflagellates has general similarities to

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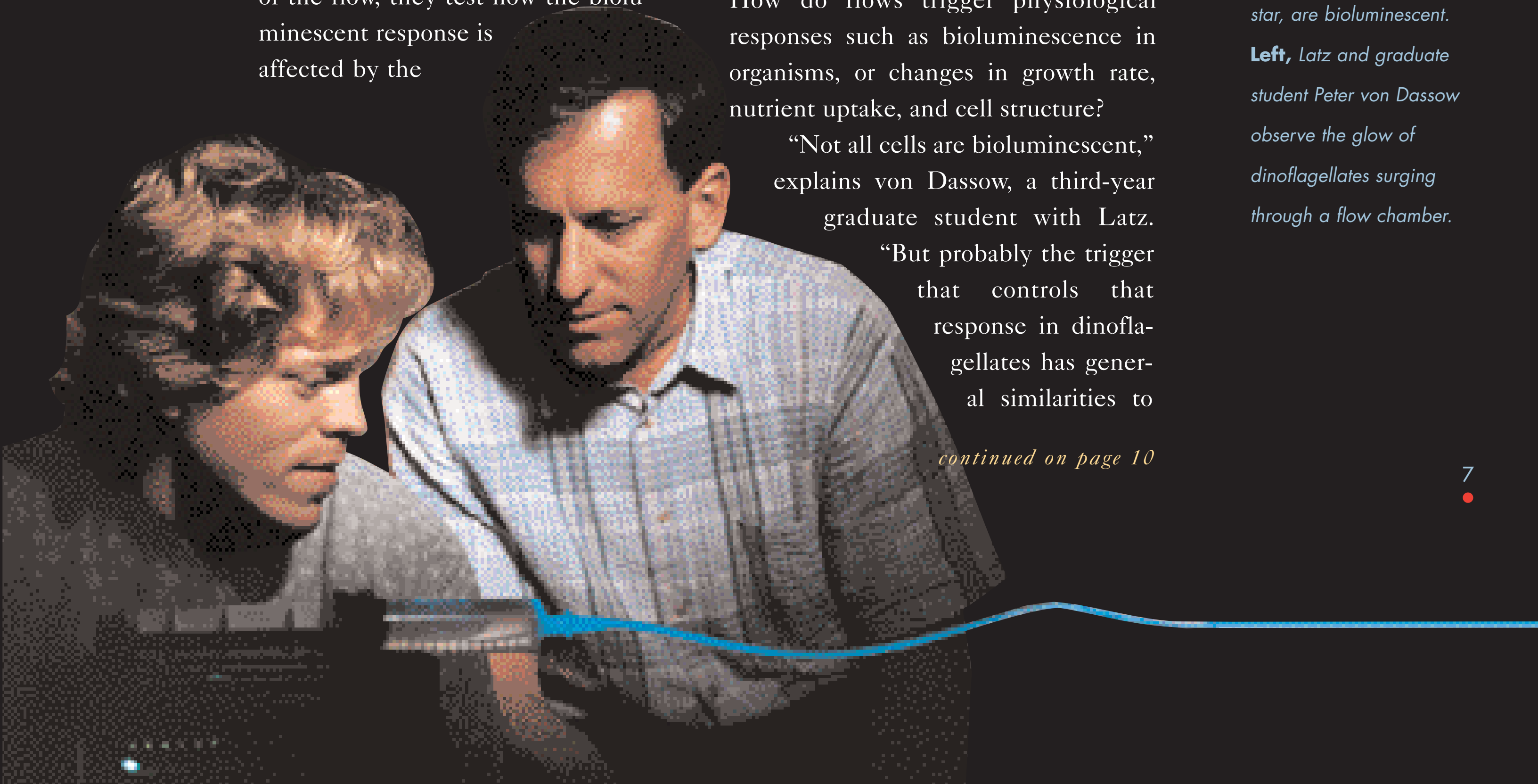

Above, *Many marine organisms, such as this large species of brittle star, are bioluminescent.* **Left,** *Latz and graduate student Peter von Dassow observe the glow of dinoflagellates surging through a flow chamber.*

Indicators of Pollution

STUDIES IN SAN DIEGO BAY

Ocean pollution is a serious environmental concern, especially in developed coastal areas where shoreline and bay sediments may become reservoirs of urban and industrial pollutants, including heavy metals. As a consequence, organisms living in or on the sediment, or those that prey upon bottom-living organisms, face heavy-metal exposure.

Marine organisms living in direct contact with contaminated coastal sediments may serve as biological indicators to help scientists determine the locations and severity of pollution. Dimitri Deheyn, a postdoctoral fellow from Belgium, is working in Michael Latz's lab at Scripps to develop new ways of using common bioluminescent brittle stars for this purpose.

"Brittle stars are cousins of starfish, and their name refers to their tendency to release an arm when stressed," explains Deheyn. "They live in contact with the sediment on which they feed and are sensitive to environmental quality, being easily killed by exposure to high enough levels of certain heavy metals."

Commonly, bioluminescent bacteria are used to determine sublethal effects of toxicity, but Deheyn thinks they are not accurate indicators of how heavy-metal toxicity will affect larger multicellular organisms with nervous systems.

Deheyn explains, "Bacteria lack the complex organization of tissues and organs found in higher organisms, including humans. The human nervous system can be very sensitive to pollutants, while other tissues in our bodies are less sensitive or even help remove pollutants. "This appears to be the case for brittle stars.

In luminescent brittle stars, light production is under the control of the nervous system and originates in photocells in the five arms. By comparing bioluminescence from individual photocells to that from the arms, Deheyn's experiments at Scripps will determine whether the effect of heavy metals on bioluminescence is due to nervous system toxicity or a more general physiological impairment.

One of Deheyn's main field projects is taking place this summer in San Diego Bay at six sites exposed to varying levels of heavy metals. He will transplant brittle stars collected from uncontaminated areas outside of the bay into mesh cages buried in the sediment at each site. The locations range from the relatively clean waters at the mouth of the bay, to the back of the bay, where the sediments support high levels of contamination due to heavy industry and a slow rate of water recirculation.

During the course of one month, Deheyn will remove samples from each site for evaluation in the lab. He will determine the types and quantities of metals in the tissues and will measure the light production of animals from each site. He will then compare the physiological toxicity to the amount of heavy metals in the tissue as measured by mass spectrometry.

"If light production decreases, pollution might limit the fitness of any individual brittle star. The bay brittle star can be considered a model for other organisms in which such a causal link between anthropogenic pollutants and individual fitness may not be so obvious," says Deheyn.

Deheyn's field work is being conducted in collaboration with the Marine Environmental Quality Branch of the Space and Naval Warfare Systems Center in San Diego. He also receives research support from NATO, the Belgian-American Educational Foundation, and the UC Toxic Substances Research and Training Program.

Top left and middle, *In the backwater of San Diego Bay, Dimitri Deheyn prepares to dive to one of his research sites.* **Above,** *Collecting during the dive.* **Next page,** *With a keen eye and gentle grasp, Deheyn searches for tiny brittle stars living within a locally collected kelp holdfast.*

“My goal,” explains Deheyn, “is to use the light production as a physiological indicator of pollution, with the understanding that the amount of light produced will vary with the amount of heavy metals that an animal encounters and absorbs into its tissues.”

Left, *Deheyn's research requires a large, healthy population of brittle stars. In Scripps's experimental aquarium he enlists the help of Latz and UCSD undergraduate Laura Brams to tend the specimens maintained for his experiments.* **Facing page,** *UCSD undergraduate Lisa Schile handles sterile cultures of luminescent dinoflagellates.*

those controlling other responses in other cells. A big lesson of the last few decades of cell biology is that all cells use the same basic building blocks for a large number of processes. We just need to figure out which fundamental elements are being used by the bioluminescent cells, in order to know how our studies of dinoflagellate bioluminescence might be relevant to other cells in flowing fluid."

TRACING BIOCHEMICAL PATHWAYS

In a project funded by the National Science Foundation, von Dassow, Latz, and John Frangos in the UCSD Department of Bioengineering are studying how the fluid forces acting on the cell are translated into a biochemical signal that tells the cell to produce light. Von Dassow is conducting tests to determine if shear causes calcium ions from seawater to enter the dinoflagellate cell, triggering bioluminescence. Shear is known to result in calcium entry into mammalian endothelial cells (cells that form the lining of blood vessels). If his hypothesis is correct, it would indicate that shear affects bioluminescent dinoflagellates—which, unlike endothelial cells, are not attached to anything and must move with the fluid—in a similar way. In the future, Latz plans to test whether other biochemical events in the cells are triggered by fluid motion.

The relatively large shear forces that stimulate dinoflagellate bioluminescence are higher than typical levels of oceanic turbulence. But dinoflagellates are also affected by lower levels of fluid motion, such as those present near the surface on a windy day.

"Because dinoflagellates swim to surface layers during the day," explains Latz, "they are exposed to stronger levels of turbulence than exist in deeper layers."

Generally, dinoflagellate red tides occur during calm conditions. In contrast, other planktonic algae, such as diatoms, thrive in more turbulent conditions, which stir up nutrients from deeper layers. Juhl, who is just completing his dissertation, has been studying whether the dinoflagellates' preference for calm conditions results from their extraordinary flow sensitivity. It is possible that red tides don't occur during turbulent conditions because the turbulence prevents dinoflagellate populations from growing.

"The idea that flow affects cell physiology is well developed in other fields of biology, but it is a novel idea for oceanography," says Juhl. "Typically people only think of oceanic flow in terms of its ability to move things from here to there, not in terms of what it is actually doing directly to the cell. I'm looking at whether the growth of dinoflagellates is sensitive to oceanic levels of flow."

IN THE LABORATORY

Juhl has designed special Couette flow chambers that allow him to grow dinoflagellates in the gap between the two cylinders. He studies and records this growth by periodically removing a sample of water and counting the cells it contains.

He exposes dinoflagellates to very low levels of shear for an hour or more each day. Other Couette flow chambers remain stationary to serve as experimental controls. Juhl finds that the population growth rate, the rate at which the dinoflagellates are increasing in number, is much reduced in the sheared chambers

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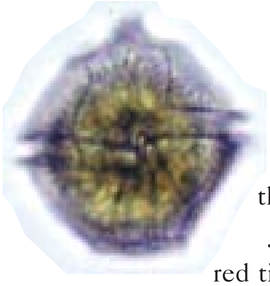


compared with those that remain still.

Juhl has studied the local red tide dinoflagellate species *Lingulodinium polyedrum* extensively and is now working with *Alexandrium fundyense*, a toxic dinoflagellate that causes paralytic shellfish poisoning in other areas of the world, including the East Coast and the Pacific Northwest.

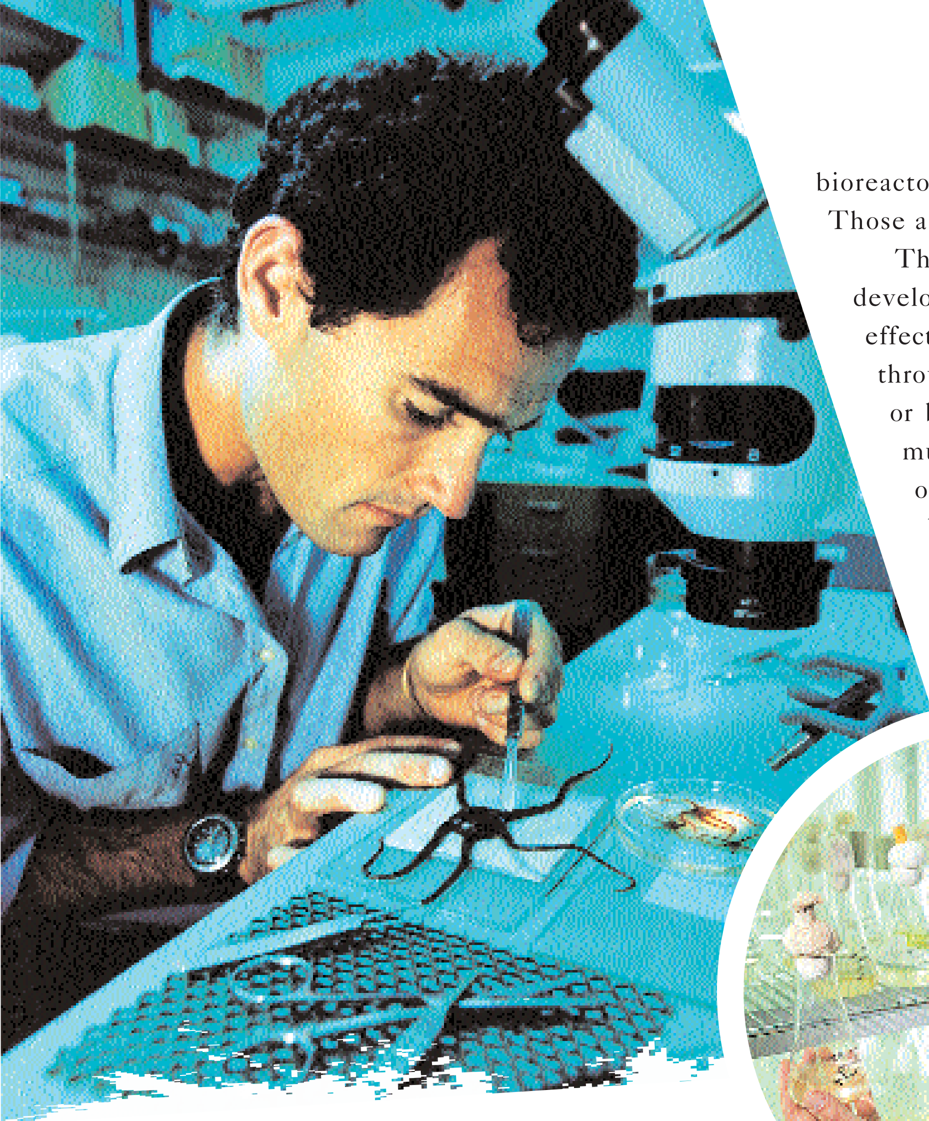
"This species causes huge economic losses to fisheries and can make people sick," Juhl explains. "When you expose the dinoflagellates to shear, their toxin levels go way up. So there might be fewer cells because of their decreased growth rate, but they might be more toxic." This may be important because cell counts of toxic species might not represent the potential toxicity of the seawater and shellfish during turbulent conditions.

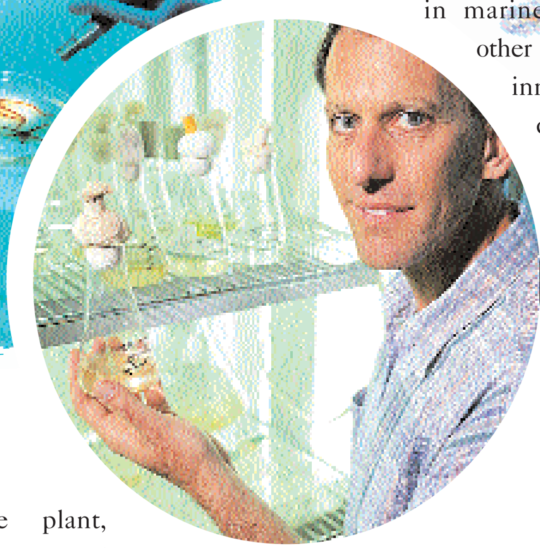
EXPLORING PRACTICAL APPLICATIONS

In addition to using dinoflagellates as models for understanding how cells are affected by flows, Latz also has been exploring practical applications for dinoflagellate bioluminescence. Fluid physicists cannot yet measure flows directly at the very small scales of individual plankton. Latz's studies of the relationship between fluid shear and bioluminescence suggest that dinoflagellates can be used as microscopic flow sensors to study the complexities of fluid flow.

In a dramatic example of this possibility, Latz and Rohr observed bioluminescence generated around dolphins as they swam through

At night, when they can't be seen by predatory fish, many zooplankton swim to the upper layers of the ocean to feed on algae, including dinoflagellates. However, when a zooplankton attacks bioluminescent dinoflagellates, the dinoflagellates are stimulated to flash, making the zooplankton vulnerable to being eaten by a nearby fish alerted by the light. According to Michael Latz, the bioluminescence acts as an alarm when the dinoflagellate is being attacked by a zooplankton and results in fewer dinoflagellates being eaten. So, if dinoflagellates use bioluminescence for protection, why is the light response also triggered by waves or the flow around swimming organisms? Just as a car alarm can be inadvertently triggered by an accidental bump or a heavy rain storm, dinoflagellate bioluminescence is stimulated by flow conditions that have sufficient force to set off the "alarm."





water containing dinoflagellates. By comparing their results to mathematical solutions and to flows created around objects in the laboratory, they identified different regions of flow around the dolphins' streamlined bodies.

The success with swimming dolphins demonstrates the potential usefulness of the approach. According to Latz, "We are now interested in applying this knowledge to other flow conditions, such as in bioreactors, the laboratory chambers used to grow cultured cells from which important compounds can be harvested for medicines or biomedical research."

Cells grown in this manner include plant, yeast, insect, and even mammalian cells. These cells cannot swim, so when grown in bioreactors, they must be supplied with nutrients and dissolved gases through constant mixing. This requirement can cause more harm than good—mixing too vigorously will damage or kill the cells.

"The ideal bioreactor mixes well but with low shear. Using bioluminescence is a way to verify the claims of a bioreactor manufacturer that their product is more gentle than the competition," Latz explains. "You can put the dinoflagellates in a bioreactor and observe the areas that light up. Those are areas of high shear."

This same technique might also be used to develop artificial hearts that are safer and more effective. The shear caused by blood pumping through an artificial heart must not be too high or blood cells will be damaged, but the shear must also not be too low or clots might develop. Latz believes it might be possible to use bioluminescent dinoflagellates to determine whether the shear is too high, too low, or just right.

As work in the lab continues, Latz plans to pursue new collaborations within marine biology and in other fields where his innovative techniques can help physicists and engineers learn more about intricacies of fluid flow.

Visit the Latz laboratory website at http://siobiolum.ucsd.edu

Above, *Deheyn removes the arms of a large brittle star for use in his experiments. Brittle stars can detach their arms for self-defense and then regenerate them.* ***Middle,*** *Michael Latz cultures dinoflagellates in a lab incubator.*