# It's a *Breve* New World for Nitrogen Research at VIMS

*By David Malmquist*

In recent years, blooms of harmful algae have killed thousands of fish and raised public concern about the increasing number of such episodes along coastlines worldwide.

VIMS scientist Dr. Debbie Bronk and colleagues are now working to better understand harmful algal blooms by focusing on one of their key ingredients—the dissolved nitrogen that algae require for rampant growth.

Algal blooms are formed by aggregations of tiny marine plants that require nitrogen and other nutrients. Many scientists attribute the recent escalation in harmful algal blooms at least in part to nutrient enrichment of coastal waters due to human activities such as the application of nitrogen fertilizers to fields and lawns. Rivers and the wind can transport nitrogen from these sources into shallow waters offshore.

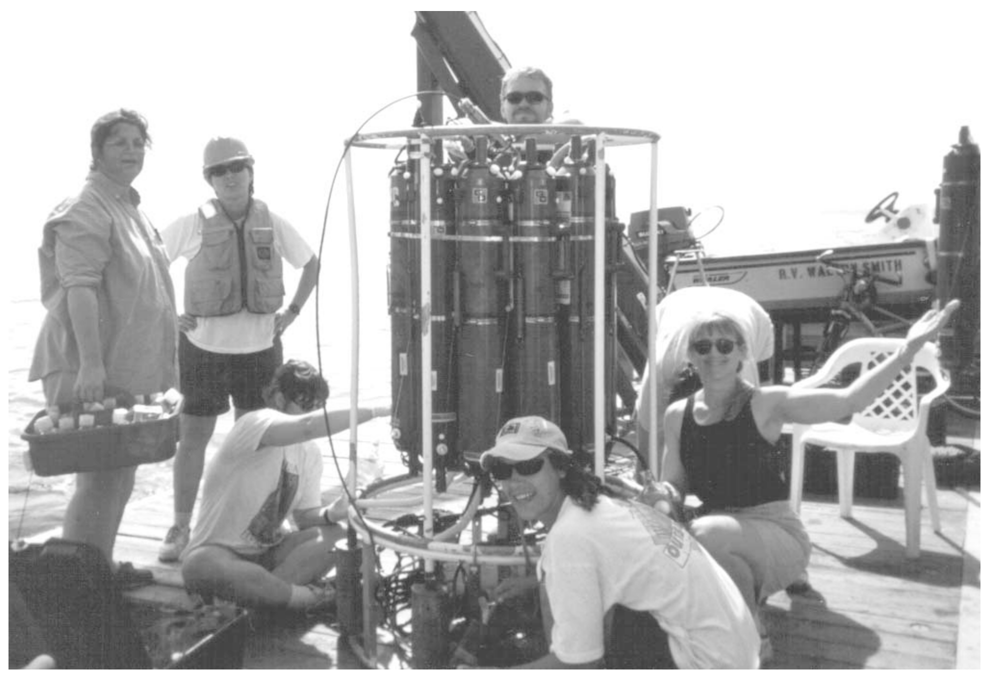
But such thinking fails to fully explain the algal blooms that typically occur during fall in the eastern Gulf of Mexico. The waters there are nitrogen-poor, yet they frequently nurture vast blooms of the toxic algae *Gymnodinium breve*, or *G. breve* as researchers call it.

These "red tides" threaten fishing and tourism along Florida's west coast. The algae release a neurotoxin that can kill fish, which then often float ashore and foul nearby beaches. Build-up of the toxin in shellfish such as oysters precludes their consumption by humans. There is also anecdotal evidence that the toxin can affect humans directly. Swimmers exposed to the toxin have complained of itchy eyes and scratchy throats, as have boaters, who could be exposed to the toxin if it enters the air when waves break.

The luxuriant growth of nitrogen-loving algae in nitrogen-poor waters poses a fundamental question. Asks Bronk, "What's fueling these guys?" The situation also provides a natural laboratory for studying the role that nitrogen plays in marine food webs elsewhere, including Chesapeake Bay.

Along with colleagues from the University of South Florida and Old Dominion University, Bronk is testing the idea that the nitrogen required to fuel *G. breve* in the Gulf of Mexico is supplied naturally by a colonial, nitrogen-fixing marine bacterium called *Trichodesmium*.

On land, bacteria within root nodules on plants like peas and beans help the plants extract or "fix" nitrogen directly from the atmosphere. These plants play a key role in terrestrial ecosystems by allowing vegetative growth in areas with nitrogen-poor soils. Farmers take advantage of this ability when they alternate plantings of corn, a heavy nitrogen feeder, with soybeans, a nitrogen-fixer that returns nitrogen to the soil.



Dr. Deborah Bronk (far right) collecting water samples aboard the *R/V Walton Smith* during a research cruise studying the fate of recently fixed nitrogen by *Trichodesmium* in the eastern Gulf of Mexico.

*Trichodesmium* is thought to play a similar role in nitrogen-poor tropical seas. Scientists suspect that specialized cells within a *Trichodesmium* colony extract atmospheric nitrogen dissolved in seawater for their own growth and development. Then, scientists think, these cells release nitrogen-bearing compounds such as amino acids into the surrounding water, to nurture other cells in the colony. It is this dissolved organic nitrogen, Bronk and her colleagues believe, that leaks into the surrounding water and later stimulates the growth of *G. breve*.

The team's hypothesis is based partly on the process of elimination, as measurements show that inputs of nitrogen from rivers and the air into local waters fail to supply the quantities needed to support blooms of the extent observed. The team also points to the timing of *G. breve* blooms along the west Florida coast. These occur in early fall, shortly after *Trichodesmium* undergoes its own annual summertime bloom.

Bronk suggests that previous research has overlooked the nitrogen derived from *Trichodesmium* because it occurs in a dissolved organic form, within compounds such as urea and amino acids. To date, researchers have mostly looked for forms of dissolved inorganic nitrogen, such as ammonia and nitrate, when trying to balance nitrogen budgets in the ocean.

To test their hypothesis, Bronk and her colleagues have received a grant from the National Science Foundation to conduct a pair of research cruises off Tampa Bay, Florida during each of the next three years. The project is part of the national ECOHAB (Ecology and Oceanography of Harmful Algal Blooms) program, which was established by NSF and NOAA in response to the upward trend in harmful algal blooms along America's coasts. A July cruise is timed to collect samples of *Trichodesmium* during its summer bloom. A September cruise is designed to sample *G. breve*.

This year the team took their first pair of cruises. During the July cruise, Bronk and her laboratory manager Marta Sanderson began experiments to test whether *Trichodesmium* colonies fix nitrogen according to accepted thinking. They did so by adding a dose of heavy nitrogen isotopes to a sample of nitrogen gas, and then adding this "labeled" nitrogen gas to seawater containing colonies of *Trichodesmium*. If specialized *Trichodesmium* cells do indeed fix nitrogen and share it with other cells, labeled nitrogen should appear throughout the colony.

After sampling an unusually rich bloom of *G. breve* during their September cruise, Bronk and Sanderson began investigating the relationship between *G. breve* and *Trichodesmium*. They added labeled nitrogen to a vial of seawater containing a population of *Trichodesmium* separated from a surrounding population of *G. breve* by a semi-permeable membrane. The openings in this membrane are large enough to allow passage of dissolved organic nitrogen, but too small for either organism. Thus any labeled nitrogen they detect in the tissues of *G. breve* will provide evidence that this organism does indeed take up organic nitrogen released by *Trichodesmium*. Early results from these tests confirm their hypothesis.

Bronk is also working with Craig Tobias, a current post-doctoral student at the Woods Hole Oceanographic Institution and VIMS graduate. Their goal is to further test the idea that dissolved organic nitrogen produced by the summertime *Trichodesmium* bloom persists and is used by *G. breve* in the fall.

The laboratory technique they use to test this link was developed by Bronk. Applying this technique to measure the natural abundance of organic nitrogen dissolved in seawater has never been done before. It relies on measuring the tell-tale ratio between two naturally occurring nitrogen isotopes that results when *Trichodesmium* fixes nitrogen. Detecting nitrogen compounds within *G. breve* containing a similar ratio would provide strong evidence of a link between the two organisms.

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The project has been an overall success. CBNERRVA plans to continue the field trips to YRSP this fall and throughout the next year. For those who are visually impaired, an information audio tape of the trail immediately surrounding the YRSP Visitor's Center is being produced and will be released in September. An introductory guide to developing environmental education programs for the visually and hearing impaired is being completed to be distributed to the other National Estuarine Research Reserves (NERRS) around the country.