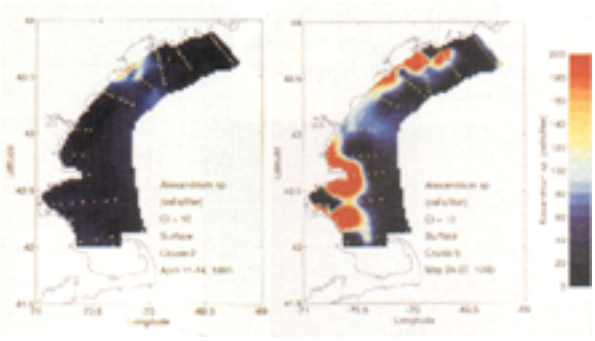
TRACING A TOXIC TIDE

Toxic red tides were virtually unknown in southern New England until 1972, when Hurricane Carrie may have swept a population of the dinoflagellate *Alexandrium tamarense* south from the Bay of Fundy and down-east Maine into the region's waters. Since then, HABs have materialized every spring in a north-to-south progression from southern Maine to Cape Cod Bay. The toxic algae are eaten by shellfish in Massachusetts, New Hampshire, Maine, and even far offshore on Georges Bank. When these shellfish are consumed in turn by humans, the neurotoxin from *Alexandrium* can cause paralytic shellfish poisoning (PSP)—numbness, dizziness, vomiting, and, sometimes, respiratory paralysis and death. Although PSP toxins regularly taint coastal shellfish beds, there has been no good explanation for how the blooms recur each year. But after fifteen years of work, Don Anderson and his students have found the answer.



The north-south movement of toxic *Alexandrium*, determined from cruises in the southwestern Gulf of Maine. Red areas are highest concentrations.

Left: The bloom begins near Casco Bay.

Right: Winds and currents have spread the bloom along hundreds of kilometers of coastline. Note the localized bloom in Casco Bay, where the red tide "reseeds" itself. Presumably cysts deposited from that northern bloom initiate the next spring's event.

In 1977, as a doctoral student in Civil Engineering at MIT, Anderson thought heavy metals played a role in the PSP outbreaks. When Anderson treated *Alexandrium* cultures with copper, they made dormant cysts that could revive if favorable conditions returned. With the help of then-WHOI researcher David Wall, one of the world's experts on dinoflagellate cysts, Anderson described the first true *Alexandrium* cysts— isolated from Cape Cod mud and germinated to yield swimming cells.

Anderson came to WHOI the next year as a post-doctoral investigator, intent on exploring two paradoxes: Why are some years free of red tides? And since *Alexandrium* are plants, shouldn't they bloom first in New England's warmer, southern waters instead of along the colder, northern coast?

Anderson obtained NOM Sea Grant funds to study the region's currents, temperatures, winds, and estuaries. He found that dormant cysts near Maine's Andrescoggin and Kennebeck Rivers awaken and germinate in the spring, then ride south on a buoyant plume of river outflow. When the summer winds blow consistently from the northeast, the plume is trapped against the coast, and shellfish become toxic progressively in a north-to-south direction. When the wind blows to the northeast, the plumes and cells move offshore, decreasing the toxicity of coastal waters. Anderson is now exploring how *Alexandrium*

populations near the Maine estuaries re-seed that area each year for the one-way trip to the south.

The toxins enter the food chain when higher organisms such as clams, mussels, oysters, and scallops ingest the algae and retain the toxins in their tissues. "Typically, the shellfish themselves are only marginally affected, but a single clam can sometimes accumulate enough toxin to kill a human being," Anderson says.

Shellfish accumulate four types of toxins that play havoc with the human body's chemistry. They alter the ion balance within individual cells, disrupt electrical conduction through the nervous system, and bring essential physiological processes to a halt.

The result is paralytic, diarrhetic, neurotoxic, or amnesic shellfish poisoning, shortened to PSP, DSP, NSP, and ASP. DSP causes diarrhea, nausea and vomiting. PSP prompts tingling and numbness of the mouth, lips, and fingers, muscle weakness, and, in acute cases, respiratory paralysis and death. NSP triggers stomach pain, muscle aches, dizziness, anxiety, and tingling in the limbs. ASP causes disorientation, stomach cramps and temporary or permanent loss of short term memory—ASP victims can remember their names and addresses, for example, but not what they had for lunch.

Illnesses and deaths from shellfish poisonings are rare in developed countries where monitoring programs detect contaminated shellfish before they reach the market. In developing countries, however, shellfish toxins can poison thousands and kill scores in unexpected outbreaks, while a related toxin, ciguatera fish poisoning, affects tens of thousands annually (see "Danger On The Reefs" which follows).

In developed and developing countries alike, HABs are taking a rising economic toll. Fish and shellfish losses total tens of millions of dollars every year. And as the world's fishing grounds are picked clean and nations turn to aquaculture to meet consumer seafood demand, HABs are devastating finfish bred in coastal pens. While wild fish can swim away from algal blooms that might burst their blood cells or destroy their gills, penned fish are trapped. Within the last ten years, massive HAB-induced kills at fish farms have become routine.

"We're spending much more time dealing with new toxins in new parts of the world," Anderson says. "It's evidence of the global expansion that I feel and see personally every day."

As yet there is no definite explanation for the rise in reported red tides. Some observers say scientists are merely finding toxins and HABs that have always been with us. But other HABs are clearly the result of human activity. Freighters that use water for ballast suck up dormant cysts in one harbor and discharge them in another halfway across the globe. Coastal dredging disperses buried cysts into currents that carry them long distances. And shellfish spat,

shipped from place to place to seed aquaculture beds, carry hitchhiking dinoflagellates that colonize their new surroundings.

More worrisome is the evidence that the discharges of nitrogen-laden effluent from the world's increasingly developed coastlines stimulate dinoflagellate growth. Studies in the North Sea, Hong Kong, the Netherlands, and Australia link HABs to coastal pollution. "We have abused our coastal waters, and one sign of that abuse is increased red tides," says Anderson.

This l995 non-toxic bloom of *Noctiluca* stretched along the coast of southern California from Santa Barbara to the Mexican border.

It will take decades of research to fully clarify the causes of HABs. In the meantime, Anderson and his associates are running full-tilt to meet requests for help from around the world.

"There are countries struggling with issues that we take for granted. I will eat shellfish here because I know our monitoring programs are effective, but that's not true in a lot of countries." Anderson helps governments design monitoring and research programs, and visiting scientists are always present in his lab, learning to grow cultures from cysts, to isolate dinoflagellates from water samples, and to extract and analyze toxins.

He recently completed a project with the government of China, where salt marshes, mangrove swamps, and wetlands have been uprooted for fish, and shellfish farms, and red tides plague the coastline. And here at home, Anderson and colleagues from U.S. government agencies and universities have just put the finishing touches on a national plan to coordinate research, monitoring, and regulatory policies.

As his field has matured, Anderson's schedule has fragmented. He spends more and more time as a laboratory administrator, research fund raiser, and policy advisor and less with the hands-on research he loves. He's increasingly on the road, away from his wife Kay, his young sons Brian and Eric, and his daughter Lauren. Since June, his itinerary has included conferences in Japan, policy meetings in France and Chile, and a stint at a Swedish marine station where he was able to devote an uninterrupted week to pure research.

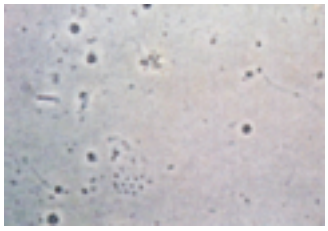
Yet if he sometime laments the demands on his time, his sense of duty keeps him going. "Almost half of my working hours are spent doing things you can't call research, but which are helping my field progress in ways that are helping society," he says. "I have a great job and am blessed with the opportunity to do exciting research. I owe it to my field to provide guidance and advice to those who are asking for it."

LIGHT UP AND BE COUNTED

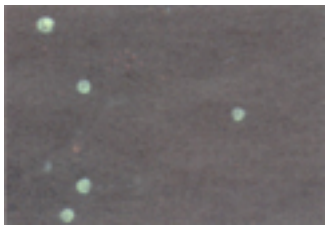
Most red tide studies begin with a seemingly simple task—counting the cells in scrutinizes a sample on a microscope slide and taps a counter for every cell of the desired species.

This chore is time consuming: a slide may be crowded with thousands of cells of dozens of phytoplankton species, and toxic and nontoxic strains of the same species can look deceptively alike.

Anderson is developing new counting methods, adapting techniques used in medicine. One method involves antibodies, large proteins manufactured by the immune systems of living organisms; when a foreign cell invades the organism's blood stream the antibodies bind to the invading cell and mark it for destruction by white blood cells. When these antibodies are purified and introduced into a laboratory sample that contains the target organism, they bind to that target just as they did within the host.



1. A field sample from Great South Bay, Long Island, magnified over 1,000 times. Even skilled scientists are unable to identify the small, non-descript organisms in this type of sample.



2. The same sample after treatment with an antibody specific for *Aureococcus onorexefferens*, a brown tide algae. Five cells are now identifiable, thanks to the fluorescent halo indicating where the antibody bound to the cell surface.

In one technique, called immunofluorescent detection, antibodies specific for a particular red tide species are obtained from laboratory animals that were inoculated with preserved algal cells. A serum containing these antibodies is then introduced into plankton samples. The antibodies, called primary antibodies, bind to the outside of the red tide cells. Next, a secondary antibody is added that binds to the primary antibody. These secondary antibodies glow when struck by light of a particular wavelength.

The sample is then passed through a flow cytometer, which dribbles the sample through an orifice barely larger than a single cell. As lasers bombard each falling cell, the secondary antibodies on target cells glow, and the glow is measured by the cytometer. The instrument can count thousands of cells per second and can distinguish glowing cells, not only of separate species, but of separate genetic strains.

Another technique physically isolates the target species. Instead of introducing a secondary antibody by itself to the sample, Anderson adds the secondary antibody coupled to microscopic magnetic beads. This complex binds to the primary antibody on the target cell. When a magnet is placed near the sample, the beaded cells zip to the magnet like iron filings. They can then be counted or analyzed for toxins or other physiological parameters.