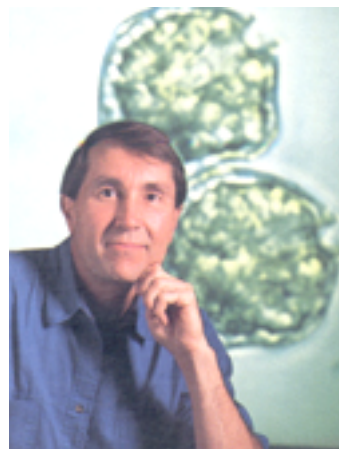


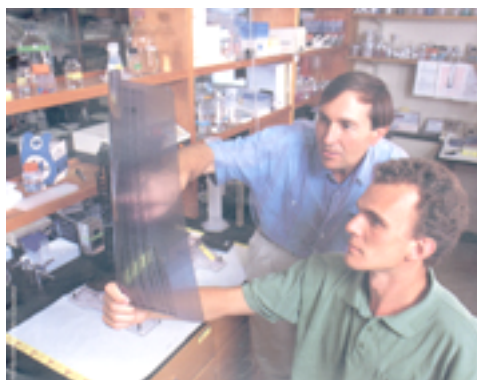
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TOXIC RED TIDES ARE ON THE INCREASE, AND BIOLOGIST DON ANDERSON IS WORKING TO FIND OUT WHY.



The twisting warren of rooms in Don Anderson's lab have the beehive pace of a factory. In one nook a technician downloads data originating from a satellite high in space, while two other workers sit with their eyes glued to microscopes, tap-tapping counters, tallying thousands of cells. Down the hall, a visiting scientist from Spain experiments with antibodies, while in another room a graduate student works with DNA. The focus of their efforts are stored nearby in a bank of humming refrigerators, in jars of mud and in hundreds of test tubes holding two ounces of seawater and a floating wisp of color. These are living cultures from around the world, an archive of tiny killers that can produce poisons one thousand times more lethal than cyanide. The killers are toxic dinoflagellates, members of a class of nearly two thousand single-celled organisms found in the sea, lakes, and polar ice. The great majority of these algae are beneficial: One of the lowest links in the aquatic food chain, dinoflagellates are prey for zooplankton, and ultimately for fish, marine mammals, and human beings. But when conditions are right, dinoflagellate populations skyrocket, forming dense, colored blooms that vividly stain the water they swim in and turn it opaque as paint. Most of these "red tides" are harmless, but they can turn catastrophic—when they stop growing and decompose they rob sea life of oxygen; when alive, some produce toxins that accumulate in the tissues of animals that consume the algae as food, killing fish, birds, and marine mammals, and robbing humans, of their memories, the use of their limbs, and their lives.

Marine biologist Don Anderson and Joint Program student Gaspar Taroncher- Oldenburg examine an autoradiograph of the RNA of a dinoflagellate, a single-celled organism responsible for many toxic red tides. The differential display method they are using allows them to compare the genetics of toxic and non-toxic species, in search of the elusive genes that make the toxin.



Although one of the first recorded red tide blooms appears as a plague in the Bible ("...all the waters that were in the river were turned to blood. And the fish that was in the river died; and the river stank and the Egyptians could not drink of the water of the river." Exodus 7:20,21), red tide blooms still occur worldwide and at an increasing rate.

"There has been a tremendous expansion over the last twenty years and it shows no signs of abating," says Anderson, a tall, soft-spoken biologist who has focused on red tides, or harmful algal blooms (HABs), since the early 1970s.

Consider this: Reports of certain types of red tide, which formerly involved only the waters of Europe, North America, and Japan, now are reported in South Africa, Australia, India, Southeast Asia, and South America. In 1985, *Allreococcus anorexeferens* turned Long Island waters into a brown soup and all but destroyed the area's multi-million dollar scallop industry. In 1987 more than one hundred people fell victim to an unprecedented outbreak of shellfish poisoning in Canada, with three dead and more than a score suffering memory loss. Scientists blame toxic *Alexarldrium tamarens* for the death of fourteen humpback whales off New England that same year; simultaneously a huge bloom of *Ptychodiscus brevis* drifted from Florida to North Carolina, closing shellfish beds and sickening scores of residents and tourists.

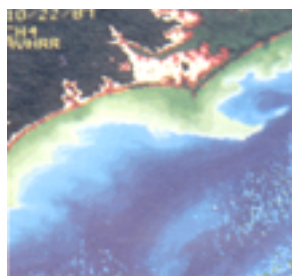
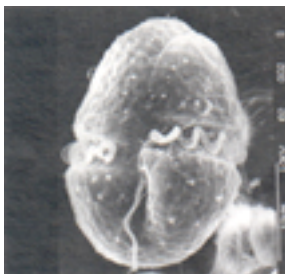


A red tide on an Italian freshwater lake. This recurring harmless bloom became a popular tourist attraction.

Anderson is spearheading an assault on the red tide epidemic, and his work has established WHOI as one of the world's leading HAB research centers. His painstaking experiments and seemingly limitless energy have made his lab in the Redfield building a Mecca to scientists and students from around the world.

"We take cruises into the coastal waters to find how the water's moving, and the way these algae are growing and getting eaten," Anderson says in his crowded office, where books and papers are piled high and e-mail arrivals regularly flash on his computer. "We grow them in the lab and look at how the nutrients we give them change the amount of toxins they produce. We're trying to find the genes that code for the toxin, and we're trying to make tests and kits to detect the toxins and the cells."

Gyrodinium uncatenum, a non-toxic dinoflagellate. The flagellum around its middle is for propulsion. The flagellum at the bottom is for steering.



In 1987, a toxic red tide developed in the Gulf of Mexico, rounded the Florida peninsula and then moved north on the Gulf Stream. Off the Carolina coast, filaments of warm water broke away from the Gulf Stream and carried the toxic cells to shore, causing a 520 million loss to tourist and shellfish industries. This remote sensing image shows the warmer water (indicated by blue) approaching North Carolina's Outer Banks.

A single red tide bloom of *Cochlodinium* killed over \$7 million worth of farmed yellowtail in Japan in 1985.



"Don has shown stunning scientific leadership," says Ted Smayda, Professor of Oceanography at the University of Rhode Island. "He has put himself amongst the avant-garde in the discipline by his eagerness to acquire and test new techniques. A lot of people play it safe, but Don keeps reaching."

Part of his success, some colleagues say, comes from an uncanny ability to detect cause and effect, to see all the doors that open with each discovery. Others credit his doggedness, his unwillingness to rest until he has disarmed every potential challenge to a new solution. And more often than not, his persistence is warranted: "These cells," he says, "are always fooling us."

Despite their tiny size, dinoflagellates are enormously puzzling. Tinted with chlorophyll and other pigments, many can photosynthesize and are thus

considered plants. However, they exhibit behavior normally associated with animals—they use their long, undulating tails and a waving belt about their middle to swim (dinoflagellate comes from the Greek *dinos*—to spin, and Latin *flagellum*—whip); some even eat their neighbors, and still others perform what seems to be a mating dance.

Like most single-celled algae, dinoflagellates reproduce by mitosis: Cells divide in two, the two into four, and so on. With sufficient light, nutrients, calm waters, and few zooplankton or larger animals to graze upon them, their numbers can quickly increase to millions of cells in a liter of water.

When nutrients are scarce, however, some species turn to sexual reproduction—cells split into male and female forms, which then fuse into one-celled cysts that settle on the seafloor and can survive for years. When favorable conditions return, the cysts germinate and reinoculate the water with swimming cells that can then bloom. Dinoflagellates can swim as much as ten meters per day, a key advantage when the warm sun or freshwater runoff creates a buoyant surface layer above colder, denser, nutrient-rich waters. They reside on the surface during the day, "harvesting sunlight like sunbathers," Anderson says. "They then swim down to take up nutrients by night." The result is a sudden bloom in water that seems inhospitable to growth.

Researchers are unsure why dinoflagellates produce toxins. They may incapacitate predators or cause the dinoflagellates to taste bad, or may perform an essential biochemical function and are only coincidentally harmful to other species. Scientists do know that a species' toxicity may change with differing light levels, temperature, nutrients or water agitation.

A non-toxic dinoflagellate bloom covered this Chilean beach with tremendous amounts of thick foam that eventually decayed and smelled.



TRACING A TOXIC TIDE

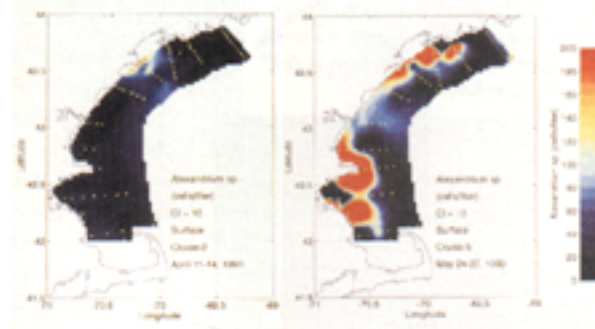
Toxic red tides were virtually unknown in southern New England until 1972, when Hurricane Carrie may have swept a population of the dinoflagellate *Alexandrium tamarense* south from the Bay of Fundy and down-east Maine into the region's waters. Since then, HABs have materialized every spring in a north-to-south 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 Androscoggin and Kennebec 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 1995 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."

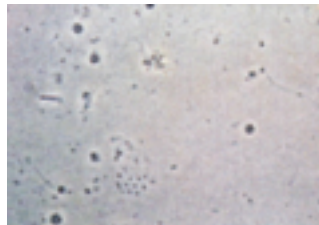
LIGHT UP AND BE COUNTED

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.

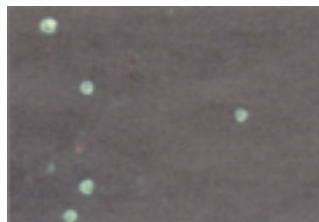
This chore is time consuming: a slide may be crowded with thousands of cells of dozens of phytoplankton species, and toxic and nontoxic strains of the same species can look deceptively alike.

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 anophagefferens*, 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.