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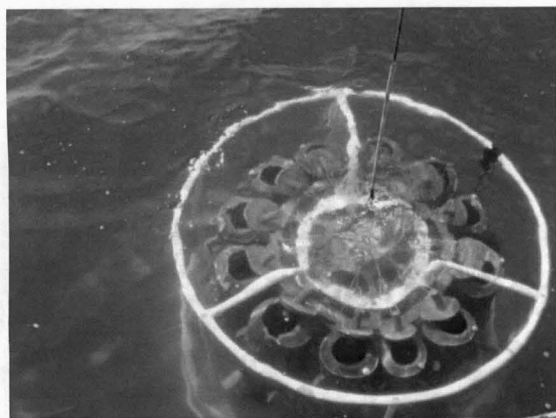
Nutrients that feed red tide 'under the microscope' in major study

Date: November 6, 2014

Source: Bigelow Laboratory for Ocean Sciences

Summary: The 'food' sources that support Florida red tides are more diverse and complex than previously realized, according to five years' worth of research on red tide and nutrients. The microbiology, physiology, ecology and physical oceanography factors affecting red tides were documented in new detail and suggestions for resource managers addressing red tide in the coastal waters of southwest Florida were offered.

FULL STORY



The rosette of Niskin bottles is submerged to collect water samples.

Credit: Mote Marine Laboratory

The "food" sources that support Florida red tides are more diverse and complex than previously realized, according to five years' worth of research on red tide and nutrients published recently as an entire special edition of the scientific journal *Harmful Algae*.

The multi-partner project was funded by the National Oceanic and Atmospheric Administration's ECOHAB program and included 14 research papers from seven institutions.

The research team studied four red tide blooms caused by the harmful algae species *Karenia brevis* in 2001, '07, '08 and '09, plus the non-bloom year 2010. Their goal was to understand which nutrients supported these red tides and the extent to which coastal pollution might contribute, helping reveal what drives red tide in southwest Florida.

Study partners documented 12 sources of nutrients in southwest Florida waters -- including some never before associated with *K. brevis*. Results supported the consensus that blooms start 10-40 miles offshore, away from the direct influence of land-based nutrient pollution, but once moved inshore blooms can use both human-contributed and natural nutrients for growth.

The project documented the microbiology, physiology, ecology and physical oceanography factors affecting red tides in new detail, provided a synthesis of results and offered suggestions for resource managers addressing red tide in the coastal waters of southwest Florida.

Florida red tide blooms -- which occur naturally in the Gulf of Mexico and most frequently off southwest Florida -- are higher-than-normal concentrations of the microscopic algae species *K. brevis*, a plant-like organism whose toxins can kill fish and other marine species, make shellfish toxic to eat and cause respiratory irritation in humans. These blooms occurred centuries before the mid-to-late twentieth century population boom along Florida's coast. Now, with large numbers of coastal residents and visitors in Florida, blooms can significantly affect public health and the economy.

Public information and short-term forecasts help mitigate red tide impacts, but ongoing research is critical to inform resource managers working to understand and potentially reduce nutrients available to blooms.

"Data go a long way toward increasing our understanding," said Dr. Cynthia Heil, Senior Research Scientist at Bigelow Laboratory for Ocean Sciences in Maine, who co-edited the special issue of *Harmful Algae* and was formerly with FWC's Fish and Wildlife Research Institute. "This report, which includes data from four different red tides and numerous laboratory studies and modeling efforts by biological, chemical and physical oceanographers, shows the collaborative efforts needed to understand why Florida red tides are so frequent and harmful in this region."

Co-editor Dr. Judith O'Neil, Research Associate Professor at the University of Maryland Center for Environmental Science, added, "We learned that *K. brevis* is an adaptable and flexible organism. We identified 12 different sources of nutrients that it can take up and use. One of the most interesting things that hadn't previously been taken into account is this organism's ability to not just use sunlight, like plants, but to also consume other single-celled organisms as a nutrient source. Additionally, its migratory behavior and directed swimming allows *K. brevis* access to nutrient sources everywhere it finds them -- at the surface, bottom and throughout the water column."

According to the study, *K. brevis* can get the nutrients nitrogen and/or phosphorus from the following sources (bold sources were newly linked to *K. brevis* blooms through the ECOHAB project):

- Undersea sediments
- Decaying fish
- Water flowing out of estuaries
- Deposits from the atmosphere
- Nitrogen from the air transformed, or "fixed," into a more useable form by the naturally occurring bacteria *Trichodesmium*. (They are a type of cyanobacteria, which use energy from sun to make food, like plants. They can multiply and form blooms.)
- Waste from zooplankton -- small aquatic animals visible to the naked eye
- The "grazing" of smaller zooplankton, dubbed "microzooplankton" because they can only be seen under a microscope. (Grazing includes their "sloppy eating" of other tiny life forms, along with the their waste.)

- Picoplankton -- tiny life forms that *K. brevis* consumes
- Bacteria transforming nitrogen in the water into more useful forms
- Light creating available nutrients from natural, dissolved compounds like tannins in the water
- Decay of Trichodesmium blooms (newly documented as a long-term nutrient source for *K. brevis* blooms)
- Nitrogen from the air "fixed" by other cyanobacteria that are NOT Trichodesmium

The researchers concluded that many of these nutrient sources are individually more than enough to support observed blooms, but no single nutrient source is solely responsible.

Naturally occurring Trichodesmium (defined above) provided the most nitrogen, but not all, for *K. brevis* blooms developing offshore. Nearer to shore and within estuaries, major nitrogen sources believed to support blooms included estuary water carrying land-based nutrients to sea, underwater sediments and dead fish decomposing, in addition to other sources.

A few coastal sources -- estuary water, deposits from the atmosphere and underwater sediments -- are known to carry natural nutrients as well as some enhanced levels due to human activity. With other nutrient sources -- such as microscopic life forms -- connections with human activities are less direct, so it is harder to predict how they might be influencing red tides.

"Nature is messy, but this project has put several new pieces in place," said Dr. Kellie Dixon, Senior Scientist at Mote Marine Laboratory and Co-Principal Investigator for the ECOHAB project. "Until now we had not looked at this many of the 12 sources and their specific quantities simultaneously. Some of the sources, like nutrients released from the sediments, had never been measured in southwest Florida's coastal waters until we studied them for ECOHAB."

The project blended nutrient studies with physical oceanography, shedding new light on how blooms are brought to shore.

"Until now, effective management of harmful algal blooms caused by *K. brevis* was complicated because we didn't know enough about how different nutrient sources and forms taken up by *K. brevis* interacted with the physical environment," said Matt Garrett of the Fish and Wildlife Research Institute, who managed the ECOHAB project. "This project provides data that can help inform management recommendations on how to control nutrient sources and possibly improve forecasting models."

The special issue of *Harmful Algae* includes the following management recommendations:

- Maximize efforts to reduce potentially controllable nutrient inputs and sources that contribute to *K. brevis* blooms.
- Monitor for known physical conditions that favor/disfavor the initiation, transport and export of *K. brevis* blooms in the southwest Florida region.
- Identify and provide necessary funding at state and federal levels to maintain the southwest Florida coastal observing system infrastructure on an operational basis.

See abstracts for all the papers at: <http://www.sciencedirect.com/science/journal/15689883/38>

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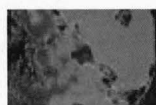
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Do you have questions about Florida's red tide? Find the answers here.

What?

What is a Florida red tide?

A red tide, or harmful algal bloom, is a higher-than-normal concentration of a microscopic alga (plantlike organism). In Florida and the Gulf of Mexico, the species that causes most red tides is *Karenia brevis*, often abbreviated as *K. brevis*. To distinguish *K. brevis* blooms from red tides caused by other species of algae, researchers in Florida call the former the "Florida red tide."

Are red tides red?

At high enough concentrations, Florida red tide can discolor water a red or brown hue. Blooms caused by other algal species can appear red, brown, green or even purple. The water can also remain its normal color during a bloom.

When?

Is red tide a new phenomenon?

No, red tides were documented in the southern Gulf of Mexico as far back as the 1700s and along Florida's Gulf coast in the 1840s. Fish kills near Tampa Bay were even mentioned in the records of Spanish explorers.

How long do Florida red tides last?

Red tides can last as little as a few weeks or longer than a year. They can even subside and then reoccur. The duration of a bloom in nearshore Florida waters depends on physical and biological conditions that influence its growth and persistence, including sunlight, nutrients and salinity, as well as the speed and direction of wind and water currents.

Where?

Do red tides occur anywhere else?

Yes, many algae species cause red tides all over the world. Yet, the organism that causes Florida's red tide, *K. brevis*, is found almost exclusively in the Gulf of Mexico from Mexico to Florida. Florida red tides can be transported around the Gulf of Mexico as coastal waters move with winds and currents. Some red tides have even been carried by the Gulf Stream current into the Atlantic Ocean as far north as Delaware.

Is the Florida red tide found in estuaries, bays or freshwater systems?

The Florida red tide can be found in bays and estuaries but not in freshwater systems such as lakes and rivers. Because *K. brevis* cannot tolerate low-salinity waters for very long, blooms usually remain in salty coastal waters and do not penetrate upper reaches of estuaries. However, other harmful algae, including cyanobacteria (blue-green algae), typically bloom in freshwater lakes and rivers.

Can we predict where a red tide will occur?

Although the occurrence of a red tide cannot be predicted, scientists can forecast its movement using wind and water current data once a bloom is located. Scientists also monitor the concentration of the red tide organism by collecting water samples routinely and in response to blooms. Red tide movement and concentration are important because the effects of a red tide, such as dead fish and human respiratory irritation, depend on these factors. The information provided by forecasting and monitoring allows people to make informed decisions regarding their beach-going activities.

Why?

Why are red tides harmful?

Many red tides produce toxic chemicals that can affect both marine organisms and humans. The Florida red tide organism, *K. brevis*, produces brevetoxins that can affect the central nervous system of fish and other vertebrates, causing these animals to die. Wave action can break open *K. brevis* cells and release these toxins into the air, leading to respiratory irritation. For people with severe or chronic respiratory conditions, such as emphysema or asthma, red tide can cause serious illness. The red tide toxins can also accumulate in molluscan filter-feeders such as oysters and clams, which can lead to Neurotoxic Shellfish Poisoning in people who consume contaminated shellfish.

Has coastal (nutrient) pollution caused the Florida red tide?

Florida red tides develop 10-40 miles offshore, away from man-made nutrient sources. In contrast to the many red tide species that are fueled by nutrient pollution associated with urban or agricultural runoff, there is no direct link between nutrient pollution and the frequency or initiation of red tides caused by *K. brevis*. Red tides occurred in Florida long before human settlement, and severe red tides were observed in the mid-1900s before the state's coastlines were heavily developed. However, once red tides are transported inshore, they are capable of using man-made nutrients for their growth.

How?**How can we control Florida's red tides?**

Control of Florida red tides is not a simple issue. The harmful effects of a red tide are caused by toxins released when the organism dies. Potential controls must not only kill the red tide organism but also eliminate the toxins from the water. To date, this has not been possible; however, researchers are identifying ways to reduce shellfish toxicity. In addition, any control strategy must not harm the environment. In the 1950s, U.S. Fish and Wildlife Service and State of Florida scientists used copper sulfate to attempt to eliminate a red tide in coastal Florida waters. Although the copper sulfate killed some of the red tide cells, it led to the release of toxins that, along with the copper sulfate, had negative effects on other marine organisms. Controls must also be practical. Red tides vary greatly in size – expanding as far as 10,000 square miles – and can be present from the surface of the water to the seafloor. Presently, there is no practical and acceptable way to control or kill red tide blooms.

Health and Safety**Will I experience respiratory irritation during a Florida red tide?**

Some people experience respiratory irritation (coughing, sneezing, tearing and an itchy throat) when the Florida red tide organism, *K. brevis*, is present and winds blow onshore. Offshore winds usually keep respiratory effects experienced by those on the shore to a minimum. The Florida Department of Health advises people with severe or chronic respiratory conditions, such as emphysema or asthma, to avoid red tide areas.

Is it safe to swim during a Florida red tide?

Swimming is safe for most people. However, the Florida red tide can cause some people to suffer skin irritation and burning eyes. People with respiratory illness may also experience respiratory irritation in the water. Use common sense. If you are particularly susceptible to irritation from plant products, avoid an area with a red tide bloom. If you experience irritation, get out of the water and thoroughly wash off. Do not swim among dead fish because they can be associated with harmful bacteria.

Is it OK to eat shellfish at a restaurant or purchase shellfish from a seafood market during a red tide?

Store-bought and restaurant-served shellfish are safe to eat during a bloom because the shellfish are monitored by the government for safety. Commercially available shellfish are often not locally harvested and, if harvested locally, are tested for red tide toxins before they are sold.

Is it OK to eat recreationally harvested shellfish during a red tide?

Recreational harvesting of bivalve molluscs such as hard clams, oysters and mussels from conditionally approved or approved shellfish harvesting areas is banned during red tide closures; these organisms may not legally be harvested and, therefore, should not be eaten during any closure of a shellfish harvesting area. To determine whether or not harvesting of shellfish is permitted in an area, visit the Florida Department of Agriculture and Consumer Services, Division of Aquaculture website. Edible parts of other animals commonly referred to as shellfish (crabs, shrimp and lobsters) are not affected by the red tide organism and can be eaten. Do not eat the tomalley (green stuff, hepatopancreas). During scallop season, locally harvested scallops from open scallop harvesting areas are also safe to eat as long as you eat only the muscle of the scallop and not the whole animal.

In addition, illegally harvested and unregulated shellfish are particularly dangerous and should never be consumed. For example, coquina clams and molluscan predators, such as whelks that feed on toxic bivalves, readily accumulate toxins in their tissues. An illegal harvest is a dangerous harvest.

Is it OK to eat local finfish during a red tide?

Yes, it is safe to eat local finfish as long as the fish are filleted before eaten. Although toxins may accumulate in the guts of fish, these areas are disposed of when the fish are filleted. However, it is never a good idea to eat dead or distressed animals, especially in a red tide area, because the reason for the animal's strange behavior or death cannot be absolutely known.

Does cooking or freezing destroy the Florida red tide toxin?

No, cooking or freezing does not destroy the red tide toxin. Furthermore, the toxin cannot be seen or tasted.

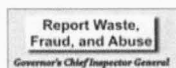
How can the Florida red tide affect my pets?

Just like people, pets may be affected by the Florida red tide. If you live close to the beach, consider bringing outdoor pets inside during a bloom to prevent respiratory irritation. If you are at the beach with your pets, do not allow them to play with dead fish or foam that may accumulate on the beach during or after a red tide. If your pet eats dead fish, it may get sick. If your pet swims in the red tide, wash it as soon as possible. Most dogs lick themselves after swimming and will consume any toxins on their fur.

Where can I get more health and safety information on harmful algae?Centers for Disease Control and Prevention Florida Department of Health Florida Department of Agriculture and Consumer Services **Download these factsheets to learn more about different algae species.***Karenia brevis* factsheet ( 490 KB)*Pseudo-nitzschia* factsheet ( 563 KB)*Pyrodinium bahamense* factsheet ( 496 KB)**FWC Facts:****Gutters and storm drains can transport excess lawn chemicals to coastal waters and damage seagrass beds.****Learn More at AskFWC**Get FWC News Feeds 

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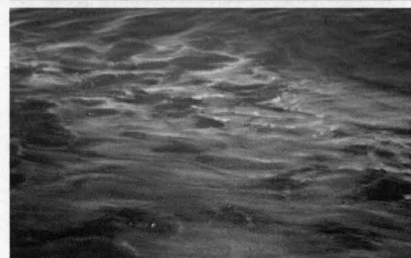
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Find out how dust from the Sahara Desert fuels annual Trichodesmium blooms in the Gulf of Mexico.

Trichodesmium, a marine cyanobacterium (blue-green algae) found worldwide in tropical and subtropical waters, blooms every year in the Gulf of Mexico. In southwest Florida, blooms can extend for miles and are visible from space. Occasionally, wind and currents can transport *Trichodesmium* blooms to the east coast of Florida. In general, *Trichodesmium* is not a good food source for other organisms. Only a few specialized animals actively feed on it. Some strains of *Trichodesmium* do produce toxins, but researchers have not documented any negative effects of *Trichodesmium* on marine life or people in Florida.

Reports of *Trichodesmium* blooms date back to the 1700s, when Captain James Cook of the British Royal Navy wrote about large, brown blooms that resembled sandbars. Sailors sometimes refer to *Trichodesmium* as "sea sawdust" because it forms colonies that can be quite large (up to 1 centimeter) and visible to the naked eye. Small blooms look like sawdust floating on the water surface, whereas larger blooms can look like oil slicks or foamy pollution. The amount of *Trichodesmium* on the surface may vary with time of day, as this species is capable of moving up and down in the water column. *Trichodesmium* blooms can appear brown when the bloom is healthy; green when the bloom is starting to decay; pink or red when certain pigments leak out of the cells; or white after pigments decay. *Trichodesmium* blooms have a sweet smell similar to freshly cut hay when they decay.

In the ocean, the growth of most algae is limited by the amount of nitrogen dissolved in the water. *Trichodesmium* is unique because it gets most of its nitrogen from the atmosphere. It has specialized structures that allow it to convert nitrogen gas into a usable form of nitrogen, ammonia. This process is called nitrogen-fixation. Because nitrogen fixation is limited by iron availability, new iron introduced into the water will stimulate *Trichodesmium* blooms. Typically between May and September, iron-rich dust blows from the Sahara Desert in Africa across the Atlantic and settles in the Gulf of Mexico. Blooms usually occur during those months, as *Trichodesmium* uses this iron to fix nitrogen and grow. Other algae, like the Florida red tide organism, *Karenia brevis*, can also use the new nitrogen fixed by *Trichodesmium*.



Dense and thin *Trichodesmium* blooms look very different. Dense blooms are thick and brown (top), whereas thin blooms look like sawdust (bottom). The dark green patches in the top image are seagrass beds.

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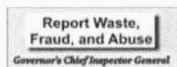
Sea turtles range in size from the 75- to 100-pound Kemp's ridley to the 1,300-pound, 8-foot-long leatherback.

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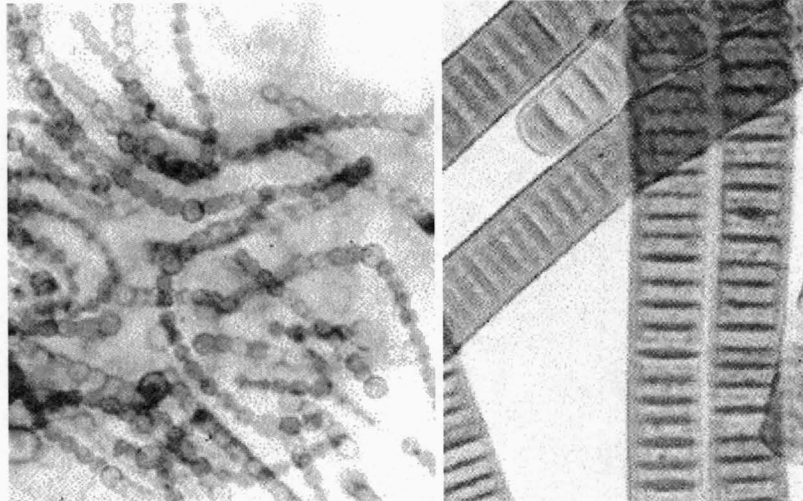
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Introduction to the Cyanobacteria

Architects of earth's atmosphere



Cyanobacteria are aquatic and photosynthetic, that is, they live in the water, and can manufacture their own food. Because they are bacteria, they are quite small and usually unicellular, though they often grow in colonies large enough to see. They have the distinction of being the oldest known fossils, more than 3.5 billion years old, in fact! It may surprise you then to know that the cyanobacteria are still around; they are one of the largest and most important groups of bacteria on earth.

Many Proterozoic oil deposits are attributed to the activity of cyanobacteria. They are also important providers of nitrogen fertilizer in the cultivation of rice and beans. The cyanobacteria have also been tremendously important in shaping the course of evolution and ecological change throughout earth's history. The oxygen atmosphere that we depend on was generated by numerous cyanobacteria during the Archaean and Proterozoic Eras. Before that time, the atmosphere had a very different chemistry, unsuitable for life as we know it today.

The other great contribution of the cyanobacteria is the origin of plants. The chloroplast with which plants make food for themselves is actually a cyanobacterium living within the plant's cells. Sometime in the late Proterozoic, or in the early Cambrian, cyanobacteria began to take up residence within certain eukaryote cells, making food for the eukaryote host in return for a home. This event is known as **endosymbiosis**, and is also the origin of the eukaryotic mitochondrion.

Because they are photosynthetic and aquatic, cyanobacteria are often called "blue-green algae". This name is convenient for talking about organisms in the water that make their own food, but does not reflect any relationship between the cyanobacteria and other organisms called algae. Cyanobacteria are relatives of the bacteria, not eukaryotes, and it is only the *chloroplast* in eukaryotic algae to which the cyanobacteria are related.

Click on the buttons below to find out more about the Cyanobacteria.

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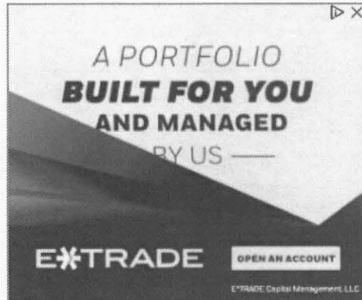
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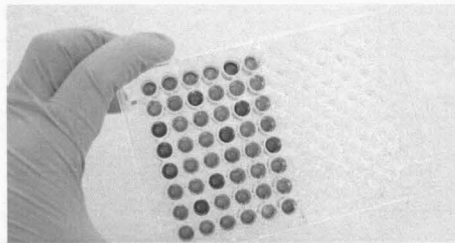
Zooplankton

BY EDITORS

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Zooplankton Definition

Zooplankton (pictured below) are a type of heterotrophic plankton that range from microscopic organisms to large species, such as jellyfish. Zooplankton are found within large bodies of water including oceans and freshwater systems. Zooplankton are drifting ecologically important organisms that are an integral component of the food chain.

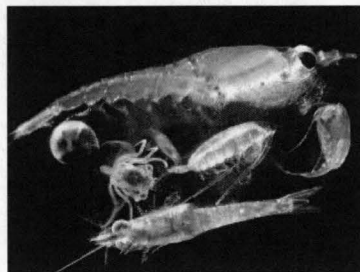


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Types of Zooplankton

The most important types of zooplankton include the radiolarians, foraminiferans, and dinoflagellates, cnidarians, crustaceans, chordates, and molluscs.

Radiolarians

Radiolarians are small protozoan species that are characterized by the production of mineral skeletons made of silica. The remains of these organisms can be found at the bottom of oceans comprising a large part of the sediment.

Foraminiferans

Foraminiferans are a type of amoeboid protist that exhibit an external shell and ectoplasm to obtain food. While the shell is typically comprised of calcium carbonate, the shells of some

species contain other minerals. These zooplankton can be found in the sediment or drifting the upper surface waters.

Dinoflagellates

Dinoflagellates are considered a mixotrophic species, meaning that they can be both photosynthetic or ingest other species. This type of zooplankton is extremely small and represents a significant portion of marine eukaryotes and are important for the health of coral reefs.

Cnidarians

Cnidarians are marine species that are characterized by specialized cells called "cnidocytes" which are used to capture their prey. They have bodies consisting of a jelly-like substance called mesoglea, a mouth, and tentacles that contain the cnidocytes (e.g., jellyfish).

Crustaceans

Crustaceans are a type of arthropod that consists of crabs, krill, shrimp, and barnacles. Crustaceans range in terms of size, and comprise a significant part of the food chain. Krill and copepods in particular, are important zooplankton species.

Chordates

Chordates are animals that possess a notochord, dorsal nerve chord, endostyle, post-anal transverse pharyngeal slits. This is a highly diverse family that includes sea stars, scalps, and many other species.

Molluscs

Molluscs are a highly diverse group of organisms, which include squid species as well as sea and sea snails. Molluscs comprise a large component of all marine life.

What do Zooplankton Eat?

Zooplankton consume a variety of bacterioplankton, phytoplankton, and even other zooplankton species. Since such organisms reside at the surface of bodies of water, zooplankton are also typically found in the upper waters.

Zooplankton Examples

Krill

Krill (shown below) are a type of crustacean found populating oceans throughout the world. They consume phytoplankton and other zooplankton species. Krill are consumed by larger marine animals, thus making them a significant contributor to the lower food chain in marine environments. As such, krill are extremely abundant and provide a primary dietary component for several large marine species, such as whales and seals. Krill reside at the surface at night and move down into deeper waters during the day.

Jellyfish

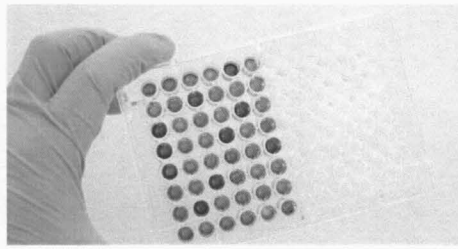
Jellyfish (shown below) are a type of cnidarian as described above, and are found residing throughout marine environments; some reside near the surface, while some species can be found in deeper waters. Jellyfish are most commonly found near coastal regions throughout the world.

Conger Eel

Conger eels (pictured below) are large organisms found in both European and North American coastal waters. They typically reside close to the sediment and prey on crustaceans and small fish.

Segmented worm

Segmented worms include leeches (shown below) and other forms of ringed worms. Marine species are most frequently found in coral reefs and tidal zones, borrowing into the sediment. This type of zooplankton is important as its borrowing ability oxygenates the sediment, thereby facilitating the growth of aerobic bacterial species and other animals.

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Quiz

1. Which of the following is NOT an example of a type of zooplankton?

- A. Krill
- B. Jellyfish
- C. Leech
- D. Squid
- E. All of the above

> Answer to Question #1

2. Which of the following is NOT food for zooplankton?

- A. Other zooplankton species.
- B. Phytoplankton
- C. Aquatic mammals
- D. None of the above. Zooplankton are photosynthesizing species and do not require addit food.

> Answer to Question #2

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Picoplankton

Picoplankton is the fraction of plankton composed by cells between 0.2 and 2 μm that can be either prokaryotic and eukaryotic phototrophs and heterotrophs:

- photosynthetic
- heterotrophic

They are prevalent amongst microbial plankton communities of both freshwater and marine ecosystems. They have an important role in making up a significant portion of the total biomass of phytoplankton communities

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Classification

In general, plankton can be categorized on the basis of physiological, taxonomic, or dimensional characteristics. Subsequently, a generic classification of a plankton includes:

- Bacterioplankton
- Phytoplankton
- Zooplankton

However, there is a simpler scheme that categorizes plankton based on a logarithmic size scale:

- Macroplankton (200-2000 μm)
- Micro-plankton (20-200 μm)
- Nanoplankton (2-20 μm)

This was even further expanded to include picoplankton (0.2-2 μm) and fem-toplankton (0.02-0.2 μm), as well as net plankton, ultraplankton. Now that picoplankton have been characterized, they have their own further subdivisions such as prokaryotic and eukaryotic phototrophs and heterotrophs that are spread throughout the world in various types of lakes and tropic states. In order to differentiate between autotrophic picoplankton and heterotrophic picoplankton, the autotrophs could have photosynthetic pigments and the ability to show autofluorescence, which would allow for their enumeration under epifluorescence microscopy. This is how minute eukaryotes first became known.^[1] Overall, picoplankton play an essential role in oligotrophic dimicite

lakes because they are able to produce and then accordingly recycle dissolved organic matter (DOM) in a very efficient manner under circumstance when competition of other phytoplankters is disturbed by factors such as limiting nutrients and predators. Picoplankton are responsible for the most primary productivity in oligotrophic gyres, and are distinguished from nanoplankton and microplankton.^[2] Because they are small, they have a greater surface to volume ratio, enabling them to obtain the scarce nutrients in these ecosystems. Furthermore, some species can also be mixotrophic. The smallest of cells (200 nm) are on the order of nanometers, not picometers. The SI prefix pico- is used quite loosely here, as nanoplankton and microplankton are only 10 and 100 times larger, respectively, although it is somewhat more accurate when considering the volume rather than the length.

Role in ecosystems

Picoplankton contribute greatly to the biomass and primary production in both marine and freshwater lake ecosystems. In the ocean, the concentration of picoplankton is 10^5 – 10^7 cells per millilitre of ocean water.^[3] Algal picoplankton is responsible for up to 90 percent of the total carbon production daily and annually in oligotrophic marine ecosystems.^[4] The amount of total carbon production by picoplankton in oligotrophic freshwater systems is also high, making up 70 percent of total annual carbon production.^[4] Marine picoplankton make up a higher percentage of biomass and carbon production in zones that are oligotrophic, like the open ocean, versus regions near the shore that are more nutrient rich.^{[4][5]} Their biomass and carbon production percentage also increases as the depth into the euphotic zone increases. This is due to their use of photopigments and efficiency at using blue-green light at these depths.^[4] Picoplankton population densities do not fluctuate throughout the year except in a few cases of smaller lakes where their biomass increases as the temperature of the lake water increases.^[5]

Picoplankton also play an important role in the microbial loop of these systems by aiding in providing energy to higher trophic levels.^[4] They are grazed by a various number of organisms such as flagellates, ciliates, rotifers and copepods. Flagellates are their main predator due to their ability to swim towards picoplankton in order to consume them.^[5]

Oceanic picoplankton

Picoplankton are important in nutrient cycling in all major oceans, where they exist in their highest abundances. They have many features that allow them to survive in these oligotrophic (low-nutrient) and low-light regions, such as the use several nitrogen sources, including nitrate, ammonium, and urea.^[6] Their small size and large surface area allows for efficient nutrient acquisition, incident light absorption, and organism growth.^[7] A small size also allows for minimal metabolic maintenance.^[8]

Picoplankton, specifically phototrophic picoplankton, play a significant role in the carbon production of open oceanic environments, which largely contributes to the global carbon production.^[6] Their carbon production contributes to at least 10% of global aquatic net primary productivity.^[7] High primary productivity contributions are made in both oligotrophic and deep zones in oceans.^[6] Picoplankton are dominant in biomass in open ocean regions.^[9]

Picoplankton also form the base of aquatic microbial food webs and are an energy source in the microbial loop. All trophic levels in a marine food web are affected by picoplankton carbon production and the gain or loss of picoplankton in the environment, especially in oligotrophic conditions.^[8] Marine predators of picoplankton include heterotrophic flagellates and ciliates.^[6] Protozoa are a dominant predator of picoplankton.^[8] Picoplankton are often lost through processes such as grazing, parasitism, and viral lysis.^[8]

Measurement

Marine scientists have slowly begun to understand in the last 10 or 15 years the importance of even the smallest subdivisions of plankton and their role in aquatic food webs and in organic and inorganic nutrient recycling. Therefore, being able to accurately measure the biomass and size distribution of picoplankton communities has now become rather essential. Two of the prevalent methods used to identify and enumerate picoplankton are fluorescence microscopy and visual counting. However, both methods are outdated because of their time-consuming and inaccurate nature. As a result, newer, faster, and more accurate methods have emerged lately, including flow cytometry and image-analyzed fluorescence microscopy. Both techniques are efficient in measuring nano plankton and auto-fluorescing phototrophic picoplankton. However, measuring very minute size ranges of picoplankton has often proven to be difficult to measure, which is why Charge-coupled devices (CCD) and video cameras are now being used to measure small picoplankton, although a slow-scan CCD-based camera is more effective at detecting and sizing tiny particles such as bacteria that is fluorochrome-stained. [2] (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC195288/>)

See also

- Plankton § Size groups

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Review of Florida Red Tide and Human Health Effects

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Abstract

This paper reviews the literature describing research performed over the past decade on the known and possible exposures and human health effects associated with Florida red tides. These harmful algal blooms are caused by the dinoflagellate, *Karenia brevis*, and similar organisms, all of which produce a suite of natural toxins known as brevetoxins. Florida red tide research has benefited

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from a consistently funded, long term research program, that has allowed an interdisciplinary team of researchers to focus their attention on this specific environmental issue—one that is critically important to Gulf of Mexico and other coastal communities. This long-term interdisciplinary approach has allowed the team to engage the local community, identify measures to protect public health, take emerging technologies into the field, forge advances in natural products chemistry, and develop a valuable pharmaceutical product. The Review includes a brief discussion of the Florida red tide organisms and their toxins, and then focuses on the effects of these toxins on animals and humans, including how these effects predict what we might expect to see in exposed people.

Keywords

Florida red tide; red tide; neurotoxic shellfish poisoning; NSP; brevetoxins; harmful algal bloom; HAB; *Karenia brevis*; shellfish poisoning; respiratory irritation; marine toxin diseases; neurotoxic fish poisoning

1.0 Introduction

Florida red tides are predominantly associated with the blooms of the toxic dinoflagellate, *Karenia brevis* (*K. brevis*), formerly known as *Gymnodinium breve* and *Ptychodiscus brevis*. *K. brevis* produces a group of potent natural neurotoxins, the brevetoxins (i.e. PbTx or Ptychodiscus toxins), which can cause illness and mortalities in fish, seabirds, and marine mammals. Humans are susceptible to the effects of brevetoxin exposure, and public health surveillance activities have documented cases of intoxications from eating contaminated seafood and many respiratory complaints from inhaling contaminated aerosols (Kirkpatrick et al., 2004a).

Florida red tide blooms have been documented on the Florida west coast since the 1800s. More recently, Florida red tides have spread as far as the eastern coast of Mexico and have been entrained in the Gulf Loop, the current that brings Gulf waters to the shores of North Carolina. Other brevetoxin-producing dinoflagellate blooms have been identified in diverse geographic locations worldwide, including New Zealand, Australia and Scotland (Baden and Fleming 2007; Hernandez Becerril et al., 2007; Haywood et al., 2004; Kirkpatrick et al., 2004a; Nozawa et al., 2003; Steidinger et al., 1983).

In the 1980s and 1990s, there was increased interest in and research activity on harmful algal blooms (HABs). Much of this interest was driven by media attention on the discovery of several new HAB organisms purportedly associated with animal and human exposures and health impacts (e.g. *Pseudo-nitzschia*, *Pfiesteria*, and the phytoplankton producing the newly discovered toxins, the Azaspiracids) (Abraham and Baden 2006; Backer et al., 2003a; Backer et al., 2005a; Backer and Fleming 2008; Fleming et al., 2001; Fleming et al., 2004; Okamoto and Fleming 2005; Twiner et al., 2008; Zaias et al., 2010). There were also concerted efforts by the HAB research and response community to increase national and international attention on the apparent increase in HABs and the resulting increased risk for human exposure and subsequent adverse health effects (e.g. National HAB plan at <http://www.esa.org/HARRNESS/>). These activities lead to increased funding and interest in HAB research (e.g. the ecology and oceanography of harmful algal blooms [ECOHAB] <http://www.whoi.edu/science/B/redtide/nationplan/ECOHAB/ECOHABhtml.html>).

Over the past decade, there has been an intensive interdisciplinary and inter-agency research program focused on the possible exposures and health effects in humans and other animals from the Florida red tide toxins, particularly the aerosolized toxins (Abraham and Baden

2006; Backer et al., 2003a; Backer et al., 2005a; Fleming et al., 2004; Fleming et al., 2005a; Red tide research group 2002; Zaias et al., 2010). The findings of this particular research program are summarized below, with particular emphasis on the implications for human health.

2.0 Organisms

In the past decade, through the use of new technologies, it has become clear that *K. brevis* is only one of several different species of the Genus, *Karenia*, found throughout the world's oceans. Blooms in the Gulf of Mexico may contain both *K. brevis* and *K. mikimotoi* (another brevetoxin-producing *Karenia* species) (Haywood et al., 2004). Other research has demonstrated varying brevetoxin production among the *Karenia* species, and even among individual *K. brevis* organisms. There have also been major advances in understanding the genomics of these dinoflagellates, such as the identification of the toxin-producing PKS genes, exploration of the impact of environmental change (e.g. temperature, light/dark cycles, etc) on gene expression, and the appreciation of the apparently unique complexity of the *Karenia* genome (Lidie et al., 2005; Monroe et al., 2008; Rein and Snyder 2006).

Despite what has been learned in the past few decades, there remains ongoing controversy concerning the sources and factors contributing to the bloom behavior of dinoflagellates, including *K. brevis*. The actual life cycle of *K. brevis* is still undefined, especially the location or existence of resting cysts. However, the major controversy has centered on the ability of anthropogenic change to influence *K. brevis* bloom dynamics. In particular, the relative importance of the role of nutrients (e.g. nitrates, phosphorus, silica, and iron) from coastal rivers, non-point coastal sources, or atmospheric deposition in initiating and/or sustaining *K. brevis* blooms is currently an important research and environmental policy topic (Brand and Compton 2007; Olascoaga et al., 2006; Walsh J et al., 2006).

3.0 Toxins

There has been an explosion of research on brevetoxins research over the past decade due to increased scientific and public health interest, and the potential to apply a range of new technologies. The brevetoxins (M.W. ~900) are lipid soluble, cyclic polyethers. In biological systems, they act to open voltage gated sodium (Na⁺) ion channels in cell membranes, leading to Na⁺ influx into the cell (Baden and Fleming 2007; LePage et al., 2003; Mattei et al., 2008; Twiner et al., 2007). There are over 10 different brevetoxins isolated in sea water blooms and *K. brevis* cultures in the laboratory, as well as multiple analogs and derivatives from the metabolism of shellfish and other organisms (Baden et al., 2005; Baden and Fleming 2007; Campbell et al., 2004; Michelliza et al., 2004 and 2007; Satake et al., 2008 and 2009). Recently, several laboratories have successfully synthesized brevetoxins *de novo* (Crimmins et al., 2009; Fuwa et al., 2006; Kuranaga et al., 2009). During Florida red tide blooms, the major brevetoxin produced is PbTx-2, along with lesser amounts of PbTx-1, PbTx-3, and other brevetoxin analogs (Cheng et al., 2004; Cheng et al., 2005a; Cheng et al., 2005b; Hardman et al., 2004; Lamberto et al., 2004; Pierce et al., 2003; 2005; 2008).

One of the most exciting discoveries of the last decade has been the identification of brevenal, a brevetoxin antagonist, in both *K. brevis* laboratory culture and in the environment during *K. brevis* blooms. This is apparently the first documented case of a toxin-producing organism also producing its own antagonist. Brevenal is produced by *K. brevis* in significant amounts, particularly during bloom senescence, and it acts at a different receptor site on nerve cells than the brevetoxins. Other brevetoxin analogs with varying degrees of antagonism or brevetoxin-like characteristics have also been identified (Abraham et al., 2003; Abraham et al., 2005a and 2005b; Bourdelais et al., 2002; Bourdelais et al., 2004a and 2004b; Bourdelais et al., 2005; Potera 2007).

From the point of view of human exposure and health, the brevetoxins are tasteless, odorless, and heat and acid stable. Thus, these toxins cannot be easily detected, nor can they be removed by food preparation procedures (Backer et al., 2003a; Backer et al., 2005a; Backer and Fleming 2008; Baden and Fleming 2007). Thus, the normal warning mechanisms (e.g. bad taste) or other protections (e.g. cooking contaminated seafood) are useless, and public health protection must focus on preventing human exposure (i.e. primary prevention).

Over the past decade, again thanks to the application of new technologies, major advances have been made in the detection of brevetoxins in a range of substrates, including seawater, air, seafood, and various animal and human clinical specimens (Dechraoui 2005; Dickey et al., 2004; Flewelling et al., 2005; Naar et al., 2007; Plakas et al., 2008; Poli et al., 2007; Weidner et al., 2004; Woofter et al 2003, 2005a and b, 2007). In particular, the creation, development and application of a new brevetoxin ELISA to all of these substrates, coupled with significant improvements in the detection limits of more traditional toxicologic analyses (e.g. Liquid Chromatography Mass Spectrometry (LCMS)), have allowed researchers and regulators to identify brevetoxins at very low levels in multiple environments and in a range of substrates (Naar et al., 2002 and 2004). This improved detection ability has been particularly important in advancing research to document exposures to brevetoxin-contaminated aerosols generated during Florida red tides and to identify the associated health effects in animals and humans. Specifically, using this highly sensitive ELISA, brevetoxins (particularly PbTx2 and 3, as well as brevenal) have been found in seawater and aerosols during active *K. brevis* blooms, as well as during non bloom periods (although at much lower levels).

In addition to establishing the concentrations of brevetoxins seen during a Florida red tide bloom (ranging from 15–90 mg/m³), the particle size of the brevetoxin aerosol has been characterized. The particles have a geometric mean of approximately 8–9 μ . This is important information in terms of the potential for respiratory effects of brevetoxin aerosols in humans. Particle size needs to be less than 5 μ to enter the lower airway; therefore, with a geometric mean of 8–9 μ , only 10–20% of these particles are small enough to enter the human lung (Cheng et al., 2004; Cheng et al., 2005a; Cheng et al., 2005b; Pierce et al., 2003; 2005; 2008). Using the same air sampling technologies, brevetoxin aerosols have been demonstrated to travel as much as a mile inland from coastal areas during an active Florida red tide, particularly when there are strong onshore winds (Kirkpatrick et al., 2010).

In addition to its application for aerosol analysis, improvements in brevetoxin detection and measurement have lead to the discovery of measurable levels of toxin in fish, both in the internal organs and in the filets that might be eaten by people. This creates the new possibility that there is an additional disease, “brevetoxin fish poisoning” (not just shellfish poisoning) that could effect marine mammals and people as discussed below (Flewelling et al., 2005; Kirkpatrick et al., 2009a; Naar et al., 2007).

4.0 Cellular processes

Cellular effects associated with both natural and experimental exposure to brevetoxins have been observed in the immune system of many species, although the mechanisms of action of brevetoxin exposure on immune cells and immune competence are not well understood. The number and variety of mediators, critical checkpoints, and key regulators in the immune system are vast, and brevetoxin may impact any one of these pathways individually or in combination. Full characterization of cellular consequences of brevetoxin exposure is critical to fully understand the impact of recurrent red tide events on human health.

Several potential mechanisms for brevetoxin immunotoxicity have been suggested, including the inhibition of cathepsin active sites (Katunuma et al., 2003; Sudarsanam et al., 1992); apoptosis (Bossart et al., 1998; Sayer et al., 2005; Walsh et al., 2008; Murrell and Gibson, 2009; 2010); the release of inflammatory mediators (Bossart et al., 1998; Murrell and Gibson 2010); effects on cell cycle (Han et al., 2003; Murrell and Gibson, 2009; Sayer et al., 2006; Walsh et al., 2005; 2008); and oxidative stress (Radwan and Ramsdell, 2006; Walsh et al., 2009). Brevetoxin exposure has been shown to have the potential to impair the immune system of many species, including manatee (Bossart et al., 1998; Walsh et al., 2005), cormorant (Kreuder et al., 2002), rat (Benson et al., 1999; 2004a and b; 2005) and loggerhead sea turtle (Walsh et al., 2010). Demonstrated effects resulting from brevetoxin exposure include: reduced phagocytosis (Benson et al., 1999); decreased plaque-forming ability (Benson et al., 1999; 2004a and b); and decreased lymphocyte proliferation (Walsh et al., 2005). Levels of lysozyme were found to be elevated in rescued loggerhead sea turtles (Walsh et al., 2010). *In vitro* experiments have demonstrated possible DNA damage (Murrell and Gibson, 2010; Sayer et al., 2005); chromosomal aberrations (Sayer et al., 2006); and effects on cellular growth (Han et al., 2003; Murrell and Gibson, 2009; Sayer et al., 2006; Walsh et al., 2008). Other immune system effects include mast cell degranulation (Hilderbrand et al., 2010) and histamine release (Abraham et al., 2005), cellular effects which may contribute to observed airway responses following the inhalation of aerosolized brevetoxins. Production of the pro-inflammatory cytokine, IL-6, was increased at both the protein (Hilderbrand et al., 2010) and gene (Murrell and Gibson, 2010) level in response to brevetoxin exposure. Several other cytokine genes with roles in pathogenesis of respiratory diseases were also shown to be increased in Jurkat E6-1 cells in response to *in vitro* brevetoxin exposure (Murrell and Gibson, 2010).

Apoptosis as potential mechanism of brevetoxin immunotoxicity was suggested based on the presence of interleukin-1 converting enzyme in lymphocytes and macrophages in manatee tissues collected during an epizootic (Bossart et al., 1998). DNA damage in human lymphocytes treated with brevetoxins *in vitro* (Sayer et al., 2005) supports apoptotic effects. Apoptosis, as measured by activity of caspase-3, was reported in a cell line (Jurkat E6-1) exposed to PbTx-2 and PbTx-6, but not when exposed to PbTx-3 (Walsh et al., 2008). Murrell and Gibson (2009) also demonstrated apoptosis occurring in brevetoxin-treated Jurkat cells through an increase in caspase 3/7 activity and activation of poly (ADP-ribose) polymerase (PARP), processes which were toxin-congener dependent, again with PbTx-3 failing to induce apoptosis. Several genes involved in apoptotic processes were affected by *in vitro* brevetoxin exposure in Jurkat cells (Murrell and Gibson, 2010).

Several studies have indicated that oxidative stress may play a role in the cellular response to brevetoxins. Glutathione depletion, an indication of oxidative stress, resulted in a U-937 human monocyte cell line treated with PbTx-2 (Walsh et al., 2009). Observations of DNA strand breaks (Sayer et al., 2005) and chromosomal aberrations (Sayer et al., 2006) are also consistent with oxidative stress. Brevetoxins have been shown to proceed through cytochrome P450 metabolic pathways, which may lead to oxidative damage. Evidence for cytochrome P450 involvement includes the metabolism of PbTx-2 by rat hepatocytes (Radwan and Ramsdell, 2006) and the U-937 human monocyte cell line (Walsh et al., 2009), and following treatment with cDNA-expressed rat cytochrome P450 enzymes (Radwan et al., 2005). The systemic administration of PbTx-2 to rats (Radwan et al., 2005) also demonstrated brevetoxin metabolism through cytochrome P450 pathways. Such metabolic processes can generate nucleophilic intermediates with the potential to bind DNA, and may have led to the brevetoxin-nucleic acid adducts detected in rat lung cells following both *in vitro* and *in vivo* exposure (Radwan and Ramsdell, 2008). Leighfield et al. (2009), however, reported that neither PbTx-2 nor the epoxide (PbTx-6) showed mutagenic potential. Some

genes related to DNA damage, however, were increased in expression in Jurkat cells exposed to brevetoxin (PbTx-2) (Murrell and Gibson, 2010).

5.0 Animals

Significant die-offs of marine mammals, seabirds, and other animals throughout the 1990s and early 2000s enhanced awareness of the impacts of Florida red tides, and led to substantial increases in the resources available to support relevant interdisciplinary research (Kirkpatrick et al., 2004; Kreuder et al., 2002; Van Dolah et al., 2003; Zaias et al., 2010). In particular, the deaths of a significant population of the highly endangered Florida manatee during the prolonged 1996 Florida red tide focused attention on the potential health impacts for both animals and humans, particularly those associated with inhaling aerosolized toxins (Bossart et al., 2002; 2003a; 2003b). Another important finding demonstrating the impacts of brevetoxins on animals involved a major dolphin die-off in the early 2000s. Although not temporally associated with an active Florida red tide bloom, the cause of death was exposure to brevetoxins via the food web. Fish found in the dolphins' stomachs tested positive for brevetoxins, particularly in the organs but also in the muscle. This episode raised the possibility of "brevetoxin fish poisoning" in humans and other animals who consumed whole fish contaminated with brevetoxins (Flewelling et al., 2005; Kirkpatrick et al., 2009a; Naar et al., 2007).

Recent laboratory studies in animals have been particularly important in exploring exposure and toxicity mechanisms, validating brevetoxin exposure, and demonstrating biological plausibility and possible mechanisms of action for the health effects reported in human studies (described below). Short and long term exposures of rodents (rats and mice) to aerosols containing brevetoxin have not demonstrated the same level of toxicity as seen in humans and other animal models. However, rodent studies have shown that aerosolized exposure to brevetoxins can lead to rapid systemic distribution, particularly to the neurologic system, implying potential adverse neurologic health impacts with respiratory exposure to aerosols (Benson et al., 2004a and 2004b; Benson et al., 2005a; Tibbetts et al., 2006). For example, exposing mice to aerosols containing brevetoxins (and exposing fish to water containing brevetoxins) caused changes in vestibular and auditory nerve function (Benson et al., 2005b; Lu and Tomchik 2002). Long term exposures of rodents to aerosolized brevetoxins have demonstrated immune dysfunction, including delay of viral clearance and possible enhancement of the pathogenicity of influenza A (J Benson, Lovelace Respiratory Research Institute, personal communication). Rodent studies have also been important in demonstrating that brevetoxins delivered in aerosols are not teratogenic in multi-generational exposure studies (Benson et al., 2006).

The sheep model of asthma has served as an important tool for the exploration of possible health effects from aerosolized brevetoxin exposures. The highlight of this model is that the sheep respond to brevetoxin exposures at levels similar to those experienced by humans at the beach during a Florida red tide bloom (Abraham and Baden 2006; Abraham et al., 2003; Abraham et al., 2004; Abraham et al., 2005a and b; Abraham et al., 2009; Zaias et al., in press). This model has demonstrated that both asthmatic and non asthmatic sheep react with significantly decreased respiratory function and experience dose-dependent airway hypersensitivity after exposure to very small concentrations of aerosolized brevetoxins (~10 pg/ml of PbTx-2 or PbTx-3, the two main toxins found in the air during a *K. brevis* bloom). In the asthmatic sheep, these effects are larger and last longer, particularly when there has been exacerbation of the asthma prior to the brevetoxin exposure. Chronic exposures in sheep demonstrate reduced function of alveolar macrophages, suggesting immune dysfunction (Zaias et al in press). This same model system has been important in the exploration of the pathogenesis and binding of many different types of brevetoxins and their

analogs, including the new antagonist brevenal, since it allows for precise exposure delivery and effect measurement.

6.0 Humans

Humans can be exposed to brevetoxins through food, water, and air (Backer et al., 2003a; Backer et al., 2005a; Backer and Fleming 2008; Fleming et al., 2001; Fleming et al., 2002; Okamoto and Fleming 2005). Until recently, the health effects associated with exposure to Florida red tide have been driven primarily by anecdote and case report, as well as the evidence described above from wild marine mammal illnesses and deaths. It is only in the past decade that interdisciplinary epidemiologic research has been applied to the exposures and health effects of Florida red tide and its toxins.

6.1. Consumption of contaminated seafood

The traditional illness associated with exposure to Florida red tide and its toxins through the consumption of contaminated shellfish is neurotoxic shellfish poisoning (NSP). The assumption has been that this is a relatively rare disease due to the stringent monitoring and timely closure of toxin-contaminated shellfish beds in the Gulf of Mexico. However, a recent comprehensive Review by Watkins et al., (2009) found that, this illness is likely to be misdiagnosed, and is probably more common than previously thought, particularly among visitors and subpopulations not informed of shellfish bed closures or shellfish harvesting bans. Based on a review of emergency room cases in Florida, it is clear that NSP can be a severe acute disease with emergency room and intensive care required during the first hours, and, in severe cases days, to prevent respiratory failure (Abraham A et al., 2008; Watkins et al., 2009). Even with a severe acute illness, victims are usually discharged from the hospital within days; there is almost nothing known about the subchronic or chronic sequelae of an acute NSP episode. Furthermore, nothing is known about the possible health effects of long term very low level exposures from eating shellfish with low levels of contamination over a long period of time.

With regards to the possible new illness of “brevetoxin fish poisoning,” it is not known if there are human cases of illness associated with eating brevetoxin-contaminated finfish. Nevertheless, evaluation of emergency room admissions for gastrointestinal illnesses during an active Florida red tide and again when there was not an active bloom demonstrated significantly increased gastrointestinal illness emergency room admissions during the active Florida red tide period (Flewelling et al., 2005; Kirkpatrick et al., 2009a; Naar et al., 2007; Perez Linares et al., 2009).

6.2 Inhalation exposure

The major advances in the past decade in defining the nature and extent of human health effects from exposure to Florida red tide and its toxins have been in the area of aerosol exposures (Backer et al., 2003b; Backer et al., 2005b; CDC 2008; Fleming et al., 2005a and b; Fleming et al., 2009; Kirkpatrick et al., 2002; Kirkpatrick et al., 2004a; Kirkpatrick et al., 2006; Kirkpatrick et al., 2009a; Kirkpatrick et al., 2009b; Kirkpatrick et al., 2010; Milian et al., 2007; Quirino et al., 2004; Steensma 2007). An early study demonstrated that (similar to the studies in the sheep model) all persons, regardless of their underlying respiratory function, can be affected by exposure to aerosols generated during Florida red tides. Complaints included respiratory symptoms which appeared to move from the upper to the lower respiratory track with increasing exposure levels of the aerosolized brevetoxins (Backer et al., 2003b). Subsequent studies in a group of very healthy non-asthmatic lifeguards found significantly increased self-reports of respiratory symptoms after completing an 8 hour work shift during an active Florida red tide confirmed by finding

brevetoxin in the water and in aerosols. There was no increase in symptoms after completing a work shift in the absence of a Florida red tide. Measurements in these lifeguards did not demonstrate a significant decrease in their pulmonary function associated with exposure to aerosolized brevetoxins during an active Florida red tide nor during a non exposure period (Backer et al., 2005b).

While the results in the lifeguards suggested that the effects from aerosolized Florida red tide were acute and of minor significance in a very healthy cohort, there are other groups of people for whom these aerosols might pose an important health risk. Asthmatics aged 12 and older had significant increases in self-reported respiratory symptoms and significant decreases in respiratory function measured by spirometry after only 1 hour of acute exposure to aerosolized brevetoxins during an active Florida red tide. The changes in symptoms and lung function were not present when the participants went to the beach when there was no active Florida red tide (Cheng et al., 2004; Cheng et al., 2005a; Cheng et al., 2005b; Fleming et al., 2005a; Fleming et al., 2007b; Milian et al., 2007; Pierce et al., 2003; 2005; 2008;). In addition, there was a dose response relationship to the symptoms (but not the pulmonary function changes) during the 1 hour of brevetoxin exposure (Fleming 2009). The subgroups that appeared to react most significantly in terms of both symptoms and pulmonary function changes to the 1 hour brevetoxin exposure were those reporting recent use of asthma medications (suggestive of less controlled asthma), and those who lived over 1 mile inland from the coastal regions (possibly relatively unexposed prior to the study beach exposure) (Fleming et al., 2005b; Fleming et al., 2007b; Fleming et al., 2009; Kirkpatrick et al., 2010; Milian et al., 2007).

As seen in the sheep asthma model, when the asthmatic study participants were followed up over a 5 day time period, their symptoms persisted for 3–4 days after the acute 1 hour beach exposure (Kirkpatrick et al., 2009b). Even more importantly, their pulmonary function decreased even further the day after the 1 hour beach exposure and did not recover for the subsequent 4 more days of follow up. Again, the less controlled asthmatics and those who live inland seemed to experience the greatest changes in symptoms and lung function.

Another way to assess the public health impact from an environmental exposure is to examine contacts with the local health care systems. Kirkpatrick et al. (2006) evaluated emergency room admissions during an active Florida red tide period and compared them with admissions during a non active period. The authors found an increase in emergency room admissions for acute and subchronic respiratory health effects (e.g. asthma, bronchitis, and pneumonia) during active Florida red tides, particularly for coastal residents. Another study compared calls to the Poison Information Center with and without reported Florida red tide exposure. The results suggested that callers experienced prolonged respiratory symptoms, and increased use of medication and doctor visits, among those who were exposed to the Florida red tide aerosols (Quirino et al., 2004).

Taken together, these data suggest that at least in asthmatics (and possibly in others with underlying respiratory illness), exposure to aerosolized Florida red tide toxins leads to acute, subacute, and possibly more chronic illness (Kirkpatrick et al., 2006; Kirkpatrick et al., 2009b; Zaias et al., 2010). However, initial evaluation of longitudinal data in the cohort of asthmatic residents who were exposed almost annually to *K. brevis* blooms did not identify any particular subgroup of asthmatics at increased risk, nor does there appear to be any longitudinal health effect over years of intermittent exposure. Despite potential intermittent brevetoxin environmental exposures, continued intermittent exposure to aerosolized Florida red tide toxins over several years follow up did not appear to cause major respiratory changes in the asthmatic study group; however, these results need to be explored further to be confirmed (J. Bean, the Cincinnati Children's Hospital, personal communication).

7.0 Treatment and Prevention

The sheep model of asthma discussed above has demonstrated that commonly used asthma medications (e.g. beta agonists, cromolyn, and steroids), as well as antihistamines and brevenal, can prevent the respiratory effects of subsequent aerosolized brevetoxin exposure. In addition, the beta agonists and brevenal can reverse or treat these effects if given after the brevetoxin exposure (Abraham and Baden 2006; Abraham et al., 2003; Abraham et al., 2004; Abraham et al., 2005a and b; Abraham et al., 2009; Zaias et al., in press). The sheep model is now being used to explore the mechanisms of the cellular response to brevetoxin exposure in the lung, including the possibility that there are additional brevetoxin receptors in addition to those associated with sodium channels. Finally, aside from the effects of Florida red tide aerosols, experiments with brevenal in the sheep model have identified a potentially powerful new pharmacokinetic activity. Specifically, administration of brevenal substantially increased the mucociliary clearance in the lung. Furthermore, the similarities between the response to brevenal and sodium channel blockers in mucus clearance, suggest that brevenal might be a possible candidate to treat other diseases with impaired mucus clearance (such as chronic obstructive lung disease (COPD) or cystic fibrosis) (Clarke et al., 2008; Sabeter et al., 2008; Sabeter et al., 2009; Potera 2007; Raloff 2005).

While there is still much more to learn, we can develop some preliminary public health messages about exposure to Florida red tides. For example, during an active Florida red tide, particularly if the bloom is close to the coast and with a strong onshore wind, asthmatics and others with underlying respiratory diseases should consider staying inland (at least 1–2 miles from the coast) or indoors with the windows shut and air conditioning on (Fleming et al., 2005a; Kirkpatrick et al., 2010). Other research by Cheng et al., (Y-S Cheng, Lovelace Respiratory Research Institute, personal communication) has demonstrated that inhalation of brevetoxin-contaminated particles from Florida red tide aerosols can be decreased by up to 45% by using a paper surgical mask. However, it is not known for how long this protective effect lasts, nor is it known if this is a sufficient decrease in exposure to the aerosolized brevetoxins to protect asthmatics and other susceptible persons with compromised lung function.

8.0 Economics

There has been considerable interest in quantifying the economic impacts of HABs in general in the US since the work of Hoagland and Anderson which estimated these impacts based on public health, commercial fisheries, recreation and tourism, and monitoring and management effects (Anderson et al., 2000; Hoagland et al, 2002). Since then, as part of a cost-of-illness study, Hoagland et al., (2009) created a model to explore the relationship between *K. brevis* blooms and respiratory illness visits to hospital emergency rooms while controlling for environmental factors, disease, and other factors. The model included data on emergency room visits for respiratory complaints, *K. brevis* cell densities, and measures of pollen, pollutants, community illness (influenza), and intra-annual population changes. *K. brevis* cell counts lagged by 1 week from measurement (as well as low air temperatures, influenza outbreaks, high pollen counts, and tourist visits) were predictive of the number of respiratory-specific emergency room diagnoses. The authors estimated that capitalized estimated marginal costs-of-illness for emergency room respiratory illnesses associated with *K. brevis* blooms in Sarasota County (FL) ranged from \$0.5 to \$4 million, depending upon bloom severity. Of note, this study did not capture the entire cost of interacting with other components of the medical care system (such as visits to the pharmacy, primary care physicians or specialists, or hospitalizations).

Nierenberg et al., (2010) evaluated lifeguard attendance data during an active Florida red tide bloom. Absenteeism results in significant direct and indirect increased costs to organizations; absenteeism associated with the lifeguards and Florida red tide was significantly increased during an active bloom with costs estimated of \$3,000/bloom. The capitalized costs of lifeguard absenteeism were estimated to be up to \$100,000 at Sarasota County (Florida) beaches alone. The costs could not be estimated for presenteeism, that is, the cost of people coming to work when they are ill. However, lifeguards reported not only that they experienced adverse health effects from exposure to Florida red tide, but also that they believed their attentiveness and abilities to take preventative actions decreased when they worked during a bloom. The authors noted that this presenteeism could affect beach safety by hampering the abilities of lifeguards to identify and respond to emergency situations.

9.0 Monitoring, surveillance, outreach & education

Over the decade, there has been an explosion of outreach and education on Florida red tide, using diverse mechanisms and targeted at a range of populations. Kuhar et al., (2009) investigated the public perception and consequent reactions to Florida red tides among persons living and visiting coastal areas regularly affected by the HABs. Using questionnaire surveys and semi-structured interviews, the researchers explored various perceptions of the risk surrounding red tides along two beaches on the west coast of Florida. Among other findings, the results indicated that coastal residents and tourists may not have efficiently delivered and up-to-date information about Florida red tides and their impacts because of inconsistent public outreach. Nierenberg et al., (in press) further pursued these issues assessing the public knowledge about Florida red tide. Again gaps in public knowledge regarding Florida red tides were identified, even among coastal residents; and preferred information sources for obtaining information on Florida red tide were also identified. Specifically, the biology of Florida red tide blooms, what is and is not safe to ingest (commercial versus recreationally caught), swimming in, and Florida red tide associated human mortalities were found to be inconsistently and incorrectly responded to by both tourists and residents of coastal Florida counties. The informational sources preferred by 80% of tourists and by 53% of residents was the Internet; these participants also looked to Mote Marine Laboratory, the local science organization, the majority of the time (66%), while toll free numbers were rarely (14%) used by tourists.

The Beach Conditions Reporting System (<http://coolgate.mote.org/beachconditions/>; tel: 1-941-BEACHES) has been set up to collect and report real time data from life guards and beach managers on a range of beach-related issues (including respiratory irritation and dead fish) to the public. The information is accessible through phone or internet. Initiated in Sarasota County (FL), the Beach Conditions Reporting System has now spread throughout the western coast of Florida, with plans to extend this reporting system throughout the Gulf of Mexico (Kirkpatrick et al., 2004b; Kirkpatrick et al., 2008; Nierenberg et al., 2009). The Florida Poison Information Center has created and formally evaluated a 24/7 toll free number (tel: 888 232 8635) on aquatic toxins which allows the caller to access information on a range of aquatic toxin issues including Florida red tide in English and Spanish, as well as speak directly with a trained Florida Poison Information Specialist (Fleming et al., 2007b).

Monitoring information for the Florida red tide organism has become more available through the Florida Fish and Wildlife Commission (http://research.myfwc.com/features/view_article.asp?id=9670), and through the NOAA Gulf of Mexico HAB Bulletin (<http://tidesandcurrents.noaa.gov/hab/bulletins.html>). The FWC monitoring data are collected by a volunteer network, and report *K. brevis* cell counts

throughout Florida marine waters biweekly. The NOAA HAB Bulletin uses these monitoring data, the Beach Conditions Reporting Data, and satellite remote sensing data incorporated into a wind and current model to identify and predict the location of Florida red tide blooms throughout the Florida coast. Of note, ongoing work by other researchers is also leading to increasingly accurate detection and predictions of Florida red tide blooms. These efforts include creating and making operational an optical phytoplankton discriminator (OPD) also known as the “BreveBuster.” The BreveBuster has been installed on a variety of different platforms including fixed buoys and in the payload of autonomous underwater vehicles (AUVs). The Slocum glider (Web Research, Falmouth, MA) AUVs are buoyancy-driven through the water column, and use the OPD to identify blooms. The AUVs surface periodically to send back real-time data via satellite). Other efforts include detecting of *K. brevis* by off-shore portable buoys (again with transmission of real time data by satellite), and improvements in the algorithms used to detect *K. brevis* blooms using remote sensing data (Carvahlo et al., 2010; G Kirkpatrick et al., 2000; Stumpf et al., 2009; Wynne et al., 2005; J Walsh 2006).

The Centers for Disease Control and Prevention (CDC), in collaboration with a range of State Departments of Public Health, has created a unique surveillance system that captures health effects and exposure information for humans and animals, as well as information characterizing the blooms themselves; the system is called the Harmful Algal Bloom-related Illness Surveillance System (HABISS) (<http://www.cdc.gov/hab/surveillance.htm>). This system incorporates data from the National Poison Data System, public health reports, and media reports, as well as environmental monitoring data. Major objectives of this system are to establish baselines for the incidence of the harmful algal bloom-related illnesses (including for Florida red tide), and to identify new occurrences of HABs that cause human and animal exposures and health effects.

Finally, community groups (e.g. Solutions to Avoid Red Tide [START] <http://www.start1.com/>), health educators, public health managers, and researchers have developed targeted materials to educate various groups about exposure to and health effects from Florida red tide toxins, including: coastal residents and tourists, healthcare providers, and beach managers. The Florida Department of Health has established a fulltime position called the Aquatic Toxins Coordinator. In addition to coordinating surveillance and outreach about HABs, including Florida red tide, the Coordinator has established a model HAB response plan that can be adapted and adopted for all of the Florida County Departments of Health (<http://www.myfloridaeh.com/medicine/aquatic/index.html>). The Florida Department of Health in conjunction with START, Mote Marine Laboratory, the University of Miami Oceans and Human Health Center, and the Florida Poison Information Center have also created a range of educational materials including: annotated Power Points® presentations and video for healthcare providers and beach managers, (<http://www.med.miami.edu/poisoncontrol/x57.xml> and <http://www.mote.org/niehsredtidestudy/>) for tourists and residents. In addition, they have created the START Florida Red Tide Card and traveling Florida Red Tide Exhibit, and Florida red tide beach signage (Figure 1).

10.0 Conclusions

This Review of recent developments in Florida red tide research has focused on the potential exposures and subsequent health effects in humans and animals. It has necessarily left out additional exciting basic science and ecology research which is also important and relevant. Nevertheless, the lessons to be learned from this Review include not only the establishment of new and potential exposures and health effects from Florida red tides and their toxins, but also a clear message that the application of new technologies, additional resources, and the

focus of truly interdisciplinary research efforts can lead to remarkable new discoveries and insights in harmful algal bloom research, as well as expanded resources for effected communities in knowledge, outreach and education. Without these interdisciplinary efforts and resources, brevetoxin would not have been discovered, and a new and important natural product which could treat chronic obstructive pulmonary disease (COPD) and cystic fibrosis, deadly human illnesses, would never have been developed (Fleming et al., 2005a). Furthermore, the issue of acute and subacute respiratory effects after exposure to aerosolized Florida red tide toxin exposure was considered to be anecdotal until formal interdisciplinary epidemiologic research in collaboration with effected coastal residents demonstrated measurable respiratory effects, particularly among asthmatics (Fleming et al., 2007a). One can only imagine the new and important discoveries that can be made by the HAB research community with the application of resources and interdisciplinary research activities in the future.

Abbreviations

PbTx	Brevetoxin
cm	Centimeter
COPD	chronic obstructive pulmonary disease
ELISA	Enzyme linked immunosorbent assay
HAB	Harmful algal bloom
HABISS	Harmful Algal Bloom-related Illness Surveillance System
<i>K. brevis</i>	<i>Karenia brevis</i>
km	Kilometer
LCMS	Liquid chromatography mass spectroscopy
mg/m³	milligram per meter cubed
ug/L	Microgram per liter
ng/m³	nanogram per meter cubed
NSP	Neurotoxic shellfish poisoning
OPD	Optical phytoplankton discriminator

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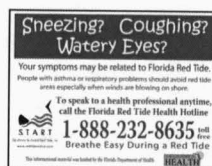


Figure 1.
Florida Red Tide Beach Signage (Florida Dept of Health, START, Mote Marine, University of Miami Oceans & Human Health Center, Florida Poison Information Center, and Ms Wendy Stephan MPH)

Toxic Algae, Nutrients, Stormwater, Science, and Public Policy Consequences ... Connecting the Dots

The profound economic and ecologic impacts as well as those to the quality of life of Florida's businesses, residents and visitors caused by the recent prolonged and intense red tide and blue-green toxic algae blooms can no longer be ignored by the State of Florida policy makers. While there may be much confusion and disinformation regarding these toxic algae, there are several things that we do know:

The fuel for these toxic algae are nitrogen and phosphorus nutrients.

Stormwater runoff washes nutrients from the landscape to streams, rivers, estuaries, and ultimately the Gulf of Mexico.

In 1982 and 1990, the State of Florida established regulations requiring new development to implement stormwater treatment systems to reduce 80% to 95% of pollutant loads associated with "Total Suspended Solids" and "pollutants that cause or contribute" to the impairment of water quality in receiving streams, rivers, estuaries, and Gulf of Mexico, respectively. These stormwater regulations, based upon science available in the 1970's and 1980's, established presumptive criteria – if this design criteria is followed, it is *presumed* that reduction targets are met. **The most common treatment systems installed and presumed to reduce nutrient pollutant loads are stormwater ponds.**

However over a decade ago, the State of Florida noted that nutrient related levels were increasing, bringing into question the effectiveness of the presumptive criteria and a critical need for better means to address nutrient pollutant loads from stormwater. Of particular significance, the State determined that stormwater ponds were only capable of reducing nitrogen loads by 40% to 50%, at most. It was also quantified by the State that there were 200,000 metric tons of legacy phosphorus in the upper soil horizon of the Lake Okeechobee watershed. At 500 metric tons per year it would take 400 years for this legacy phosphorus to wash out and drain to Lake Okeechobee. This is independent of and in addition to average annual TP pollutant loads which has been estimated at approximately 1000 metric tons per year. The assimilation capacity determined by the State for Lake Okeechobee is at most about 140 metric tons per year, including an estimated 35 metric tons associated with atmospheric deposition.

With these revelations the State of Florida, consisting of the Florida Department of Environmental Regulation and all 5 Water Management Districts, proceeded to update and revise regulations with the specific goal of addressing nitrogen and phosphorus nutrient pollutant loads in stormwater. This multi-year effort incorporated a solid body of research from Florida's Academic Institutions including the UCF Stormwater Academy, engaged a multi-stakeholder Technical Advisory Committee that convened on a regular basis to vet the proposed new Rule known as the Statewide Stormwater Treatment Rule, and culminated in proposed Rule - Chapter 62-347 Florida Administrative Code in 2010. **However, the public vetting and adoption of the drafted regulations to address Florida's recognized stormwater nutrient**

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CHAPTER 62-347
STORMWATER QUALITY

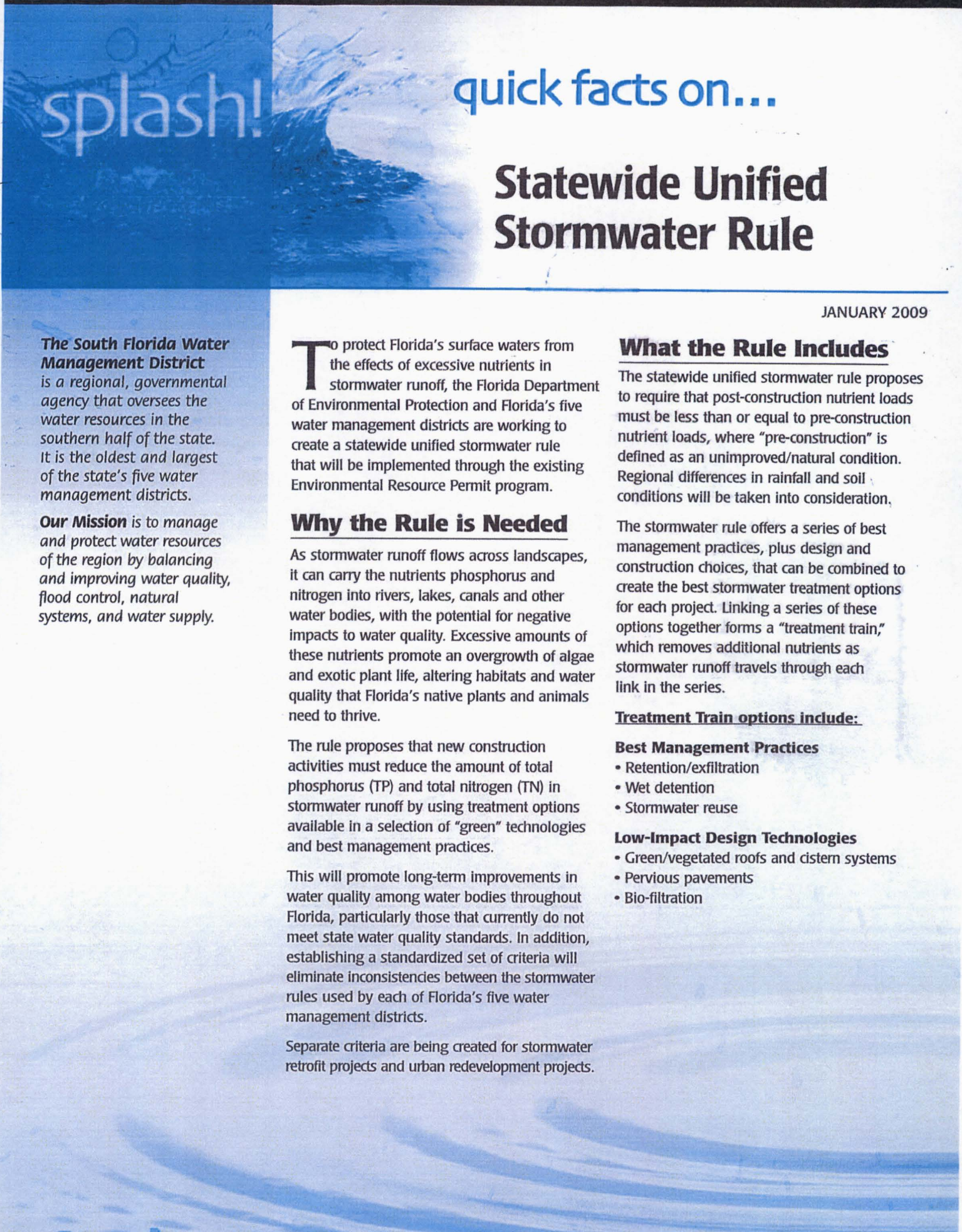
62-347.010 Performance Standards.

(1) Except as provided in Rule 62-347.051, F.A.C., this chapter provides the minimum level of stormwater treatment and design criteria (hereinafter stormwater quality treatment requirements) that must be incorporated into the design, construction, alteration, modification, operation, and maintenance or repair (excluding routine custodial maintenance), removal, or abandonment of a stormwater management system that requires a permit, other than a noticed general permit, under Part IV of Chapter 373, F.S., from the Department of Environmental Protection, a water management district under Section 373.069, F.S., or a local government that has received a delegation from the Department or water management district under Section 373.441, F.S. (hereinafter for purposes of this chapter, all of these entities are referred to as "Agency").

(2) The specific stormwater quality treatment requirements that must be met under this chapter are contained in the "Department of Environmental Protection and Water Management Districts Environmental Resource Permit Stormwater Quality Applicant's Handbook: Design Requirements for Stormwater Treatment Systems in Florida" (hereinafter referred to as the "Applicant's Handbook: Stormwater Quality"), effective [effective date], which is herein adopted and incorporated by reference. An electronic copy of this handbook may be obtained from the Department's Internet set at <http://www.dep.state.fl.us/water/wetlands/erp/rules/guide.htm>. The requirements of the Applicant's Handbook: Stormwater Quality shall supersede the requirements contained in the following rules of the water management districts:

(a) Within the Northwest Florida Water Management District:

Sections 2.7 through 2.7.2 and Parts IV through V of Department of Environmental Protection and Northwest Florida Water Management District Environmental Resource Permit Applicant's Handbook — Volume II (Design Requirements for Stormwater Management Systems


 splash!

quick facts on...

Statewide Unified Stormwater Rule

JANUARY 2009

The South Florida Water Management District

is a regional, governmental agency that oversees the water resources in the southern half of the state. It is the oldest and largest of the state's five water management districts.

Our Mission is to manage and protect water resources of the region by balancing and improving water quality, flood control, natural systems, and water supply.

To protect Florida's surface waters from the effects of excessive nutrients in stormwater runoff, the Florida Department of Environmental Protection and Florida's five water management districts are working to create a statewide unified stormwater rule that will be implemented through the existing Environmental Resource Permit program.

Why the Rule is Needed

As stormwater runoff flows across landscapes, it can carry the nutrients phosphorus and nitrogen into rivers, lakes, canals and other water bodies, with the potential for negative impacts to water quality. Excessive amounts of these nutrients promote an overgrowth of algae and exotic plant life, altering habitats and water quality that Florida's native plants and animals need to thrive.

The rule proposes that new construction activities must reduce the amount of total phosphorus (TP) and total nitrogen (TN) in stormwater runoff by using treatment options available in a selection of "green" technologies and best management practices.

This will promote long-term improvements in water quality among water bodies throughout Florida, particularly those that currently do not meet state water quality standards. In addition, establishing a standardized set of criteria will eliminate inconsistencies between the stormwater rules used by each of Florida's five water management districts.

Separate criteria are being created for stormwater retrofit projects and urban redevelopment projects.

What the Rule Includes

The statewide unified stormwater rule proposes to require that post-construction nutrient loads must be less than or equal to pre-construction nutrient loads, where "pre-construction" is defined as an unimproved/natural condition. Regional differences in rainfall and soil conditions will be taken into consideration.

The stormwater rule offers a series of best management practices, plus design and construction choices, that can be combined to create the best stormwater treatment options for each project. Linking a series of these options together forms a "treatment train," which removes additional nutrients as stormwater runoff travels through each link in the series.

Treatment Train options include:

Best Management Practices

- Retention/exfiltration
- Wet detention
- Stormwater reuse

Low-Impact Design Technologies

- Green/vegetated roofs and cistern systems
- Pervious pavements
- Bio-filtration

Other Benefits

Effective use of the "treatment train" in project design may reduce or eliminate the need to enlarge water detention areas on sites. It also may reduce the volume of runoff discharged from the site, recharging groundwater and reducing downstream flood impacts.

Complying with the statewide rule criteria should help address the requirements for new construction associated with Florida's Total Maximum Daily Load Basin Management Action Plans. These plans are designed to help restore Florida's impaired waterways.

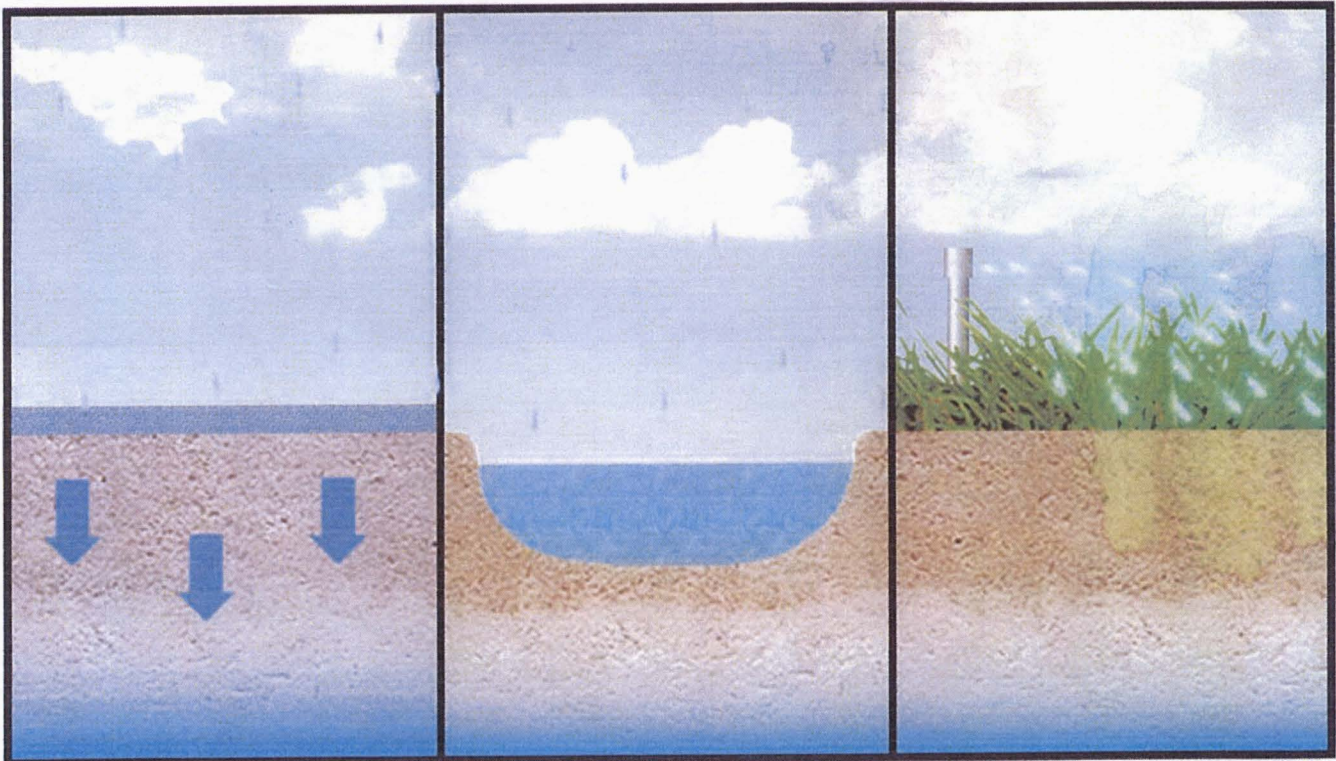
Treatment Train

A series of different best management practices linked together forms a stormwater runoff treatment train. Additional nutrients are removed as stormwater passes through each section. The treatment train may also reduce the volume of runoff discharged from a site, improving groundwater recharge and reducing downstream flood impacts.

Additionally, local governments can implement more stringent requirements for new development in accordance with their authority.

When the Rule Will Go Into Effect

A Technical Advisory Committee has been meeting since March 2008 to provide input on the proposed rule. Technical review will continue in 2009. Public workshops will be scheduled after the advisory committee finishes its work. The rule is anticipated to be adopted in mid to late 2010.



Rainfall passes through pervious pavement and is absorbed by the ground.

At the same time, rainfall and runoff collect in a stormwater treatment pond that reduces nutrients and recharges groundwater.

Water from the stormwater treatment pond is then recycled through irrigation.



JR01/29/09

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 Broward
 954-713-3200
 Florida Keys (Plantation Key)
 305-853-3219 or 800-464-5067
 Lower West Coast
 239-338-2929 or 800-248-1201

Martin/St. Lucie
 772-223-2600 or 800-250-4100
 Miami-Dade
 305-377-7274 or 800-250-4300
 Okeechobee
 863-462-5260 or 800-250-4200
 Orlando
 407-858-6100 or 800-250-4250
 Palm Beach County
 561-682-6000 or 800-432-2045

MARCH 2010 DRAFT

**DEPARTMENT OF ENVIRONMENTAL PROTECTION AND
WATER MANAGEMENT DISTRICTS**

**ENVIRONMENTAL RESOURCE PERMIT
STORMWATER QUALITY
APPLICANT'S HANDBOOK**

**DESIGN REQUIREMENTS FOR
STORMWATER TREATMENT SYSTEMS IN FLORIDA**

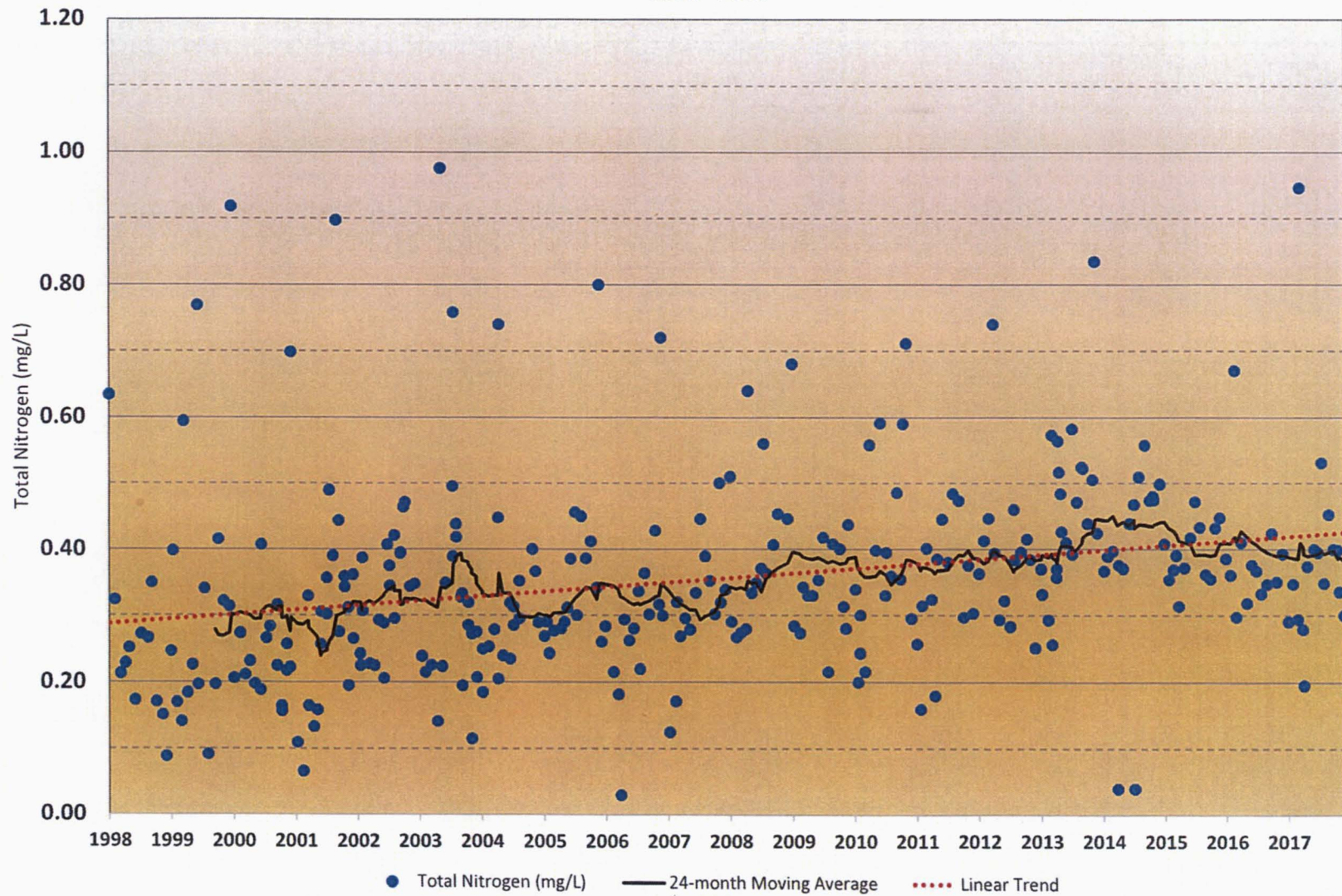
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**Southwest Florida
Water Management District**



Sarasota Bay
Average Total Nitrogen (All Stations)
1998 - 2017



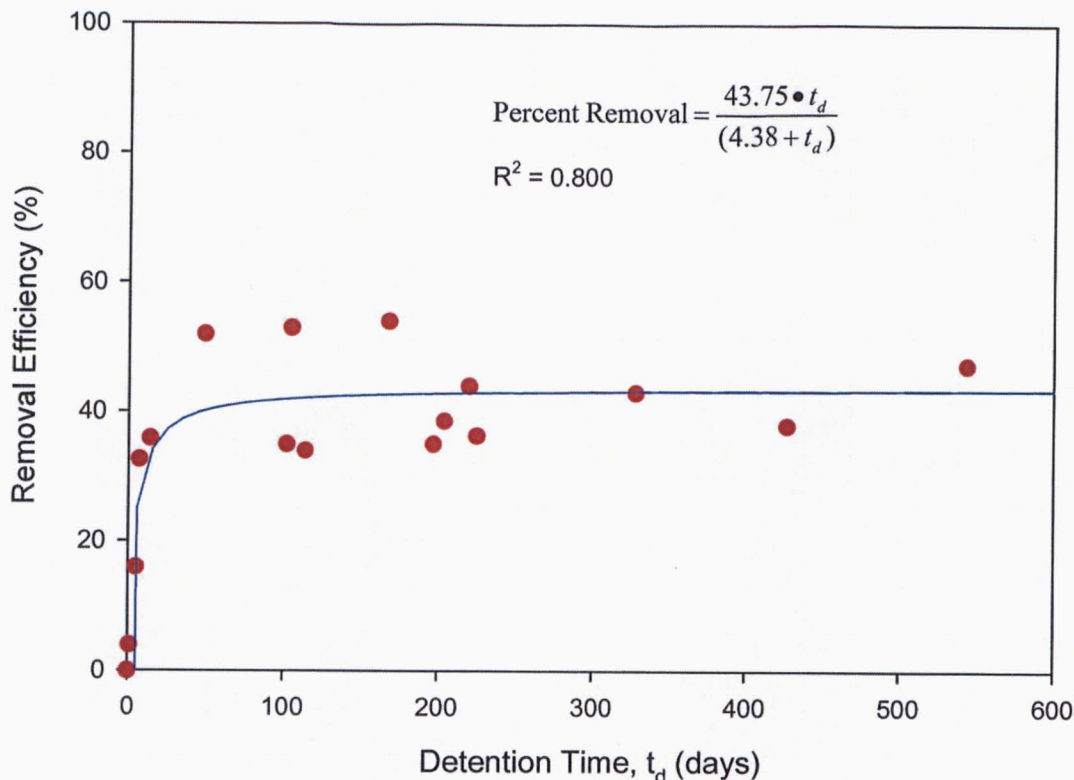


Figure 13.3 Removal Efficiency of Total Nitrogen in Wet Detention Ponds as a Function of Residence Time

- (c) **Pond Depth** – The maximum depth to be used in calculating the water quality permanent pool volume shall be no greater than 12 feet, unless the applicant demonstrates, based on Equation 13-1 and as described in Section 23.3 of this Handbook, that an alternative depth is appropriate for the specific site conditions. The maximum allowable permanent pool depth as it relates to the aerobic zone is directly related to the anticipated algal productivity within the pond. The maximum depth of the pond may be deeper, provided the applicant demonstrates that permanent pool credit for deeper pond depths only includes volumes that are based on the depth below the control elevation that remains aerobic throughout the year (based on a monthly analysis). The general relationship for determining the depth to anoxic conditions is expressed as the following equation:

Equation 13-1:

$$\text{Depth of DO} < 1 = 3.035 * \text{Secchi} + 0.02164 * (\text{chl-a}) - 0.004979 * \text{Total P}$$

Where: DO = dissolved oxygen (mg/L)

Secchi = estimated Secchi depth (meters)

Chl a = estimated chlorophyll a (mg/m³)

Total P = estimated total phosphorus (ug/L)

The above calculation must be performed on a monthly basis in order to determine the most limiting time of year (month with shallowest depth to the anoxic zone). Alternatively, the