

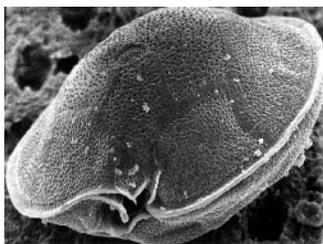
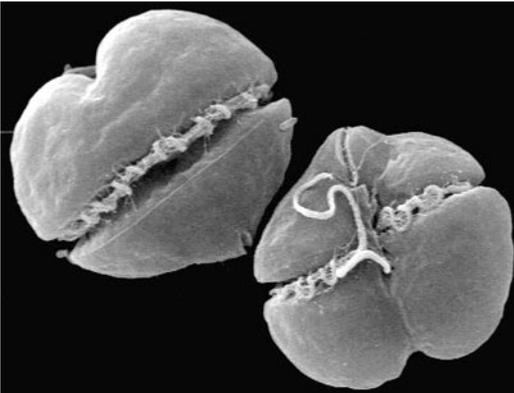


*submitted to*  
**Florida's  
Harmful Algal Bloom  
Task Force**



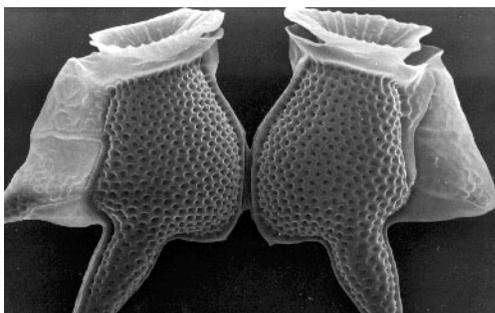
*by the*  
**Harmful Algal Bloom Task Force  
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# **Harmful ALGAL BLOOMS in Florida**



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**March 8  
1999**

## FOREWORD

The Florida Harmful Algal Bloom Task Force was established in 1997 by Virginia Wetherell, then Secretary of the Florida Department of Environmental Protection. It was formed to develop recommendations on how to handle recurring harmful algal bloom (HAB) problems caused by red tides and their impacts on public health and natural resources as well as new emerging issues such as *Pfiesteria*, which was causing resource and possible human health problems along the eastern seaboard.

The first Task Force report, *Harmful Algal Blooms in Florida*, dated March 8, 1999, was prepared by the Technical Advisory Group and made recommendations on priority research. The recommendations were reached by consensus of the Task Force. Some of the priority research was funded by money made available through the Legislature to the Department of Environmental Protection and later to the Florida Fish and Wildlife Conservation Commission.

In 1999, the Florida Legislature created the Harmful Algal Bloom Task Force in Chapter 370 F.S. “The Harmful-Algal-Bloom Task Force shall: (a) Review the status and adequacy of information for monitoring physical, chemical, biological, economic, and public health factors affecting harmful algal blooms in Florida; (b) Develop research and monitoring priorities for harmful algal blooms in Florida, including detection, prediction, mitigation, and control; (c) Develop recommendations that can be implemented by state and local governments to develop a response plan and to predict, mitigate, and control the effects of harmful algal blooms; and (d) Make recommendations to the Florida Marine Research Institute by October 1, 1999, for research, detection monitoring, prediction, mitigation, and control of harmful algal blooms in Florida.” This same legislation created a financial disbursement program within the Florida Marine Research Institute to implement provisions of the act.

New and sustained impacts from HAB events continue to plague Florida’s waters. Since early 2002, more than 28 cases of saxitoxin food poisoning were caused by the consumption of toxic puffer fish harvested from the Indian River Lagoon on Florida’s east coast. These saxitoxins were subsequently confirmed in the dinoflagellate *Pyrodinium bahamense* for the first time in the United States. All major groups of HABs with a potential to affect public health, cause economic losses, and impact ecological resources have now been documented to occur in Florida waters. Most recently, the year-long 2005 red tide event on Florida’s west coast caused significant ecological and economic impacts.

Management plans need to be regularly re-appraised to address the changing scope and impacts associated with HABs in Florida’s waters. Networking and surveillance activities established through the Florida HAB Task Force have ensured in-state investigations, research collaborations, and continued monitoring of HAB events and their significant effects.

Since the first report in March 1999, there have been significant HAB events and new issues; however, there have also been substantial advances in HAB research that need to be included in an updated report. The report will be revised and updated in Fiscal Year 2006–2007 to accomplish this task.

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## EXECUTIVE SUMMARY

Florida's Harmful Algal Bloom Task Force was created in October 1997 by Virginia Wetherell, then Secretary of the Florida Department of Environmental Protection. Ms. Wetherell was the chair and Dr. James Howell, Florida Department of Health, was the co-chair. The Task Force was charged with investigating harmful algal blooms (HABs) and assessing their potential effects on marine resources and public health. This charge included investigating historical HAB events such as Florida red tides, and new emerging issues such as the potential for *Pfiesteria piscicida* becoming established in Florida. Along with these responsibilities, the Task Force was also charged with evaluating critical HAB issues and making recommendations for action. A Technical Advisory Group (TAG) was also appointed in order to serve the Task Force. The TAG was assigned the responsibility of developing a resource document for the Task Force to review and approve. This document "Harmful Algal Blooms in Florida" is the result of that assignment.

Six major Florida HABs are reviewed from the aspect of an historical perspective, natural resource and public health risks, and economic losses. Results can best be summarized by *Findings* and *Recommendations*.

## MAJOR FINDINGS

HABs occur world-wide and represent an increase in nonvascular aquatic plants that cause negative impacts. They can occur in open coastal waters and estuaries.

HABs have become a common feature of Florida's coastal environment. Their negative impacts affect natural resources, human health, and the coastal economy. Impacts can be minimized by applied research and public outreach.

Specifically, HABs in Florida are associated with fish, bird, manatee, and other wildlife kills, human illness caused by contaminated seafood, human respiratory problems and other potential natural resource and human health risks.

The ever-expanding influence of human activities in our popular coastal waters may allow HABs to extend their range and time of residency.

Rapid detection and prediction of HABs through testing and modeling are essential for minimizing the negative effects of HABs.

Methods must be explored and developed to help manage the negative impacts of the HAB events including innovative engineering procedures to rid the shore of dead fish, decrease the size of blooms, eliminate the occurrence of blooms, or neutralize the toxins released into the water.

Although Florida has more than 60 toxic or potentially toxic microorganisms in its freshwater, estuarine, and marine waters, the effects of only a few are an obvious threat, such as

- HABs that cause red tides and Neurotoxic Shellfish Poisoning (NSP),
- HABs that cause tropical fish poisoning (Ciguatera),
- *Pfiesteria*-like organisms that pose a potential threat to resources and humans,
- HABs that likely cause tumors in marine animals, and
- blue-green algae or cyanobacteria in freshwater that threaten drinking water supplies and natural resources.

NSP and ciguatera are long-standing HAB events in Florida, whereas *Pfiesteria*-like events (e.g., possible fish lesions) and toxic blue-green algae blooms (freshwater) are emerging issues.

In addition, macroalgae can cause nuisance blooms.

The highest priority recommendation is to monitor and survey inshore and offshore waters to develop early warning systems so that contingency plans can be put in place. Contingency plans can involve management and mitigation of HABs.

## RECOMMENDATIONS

### RED TIDE HABs

Predict the onset and movement of red tides in shelf waters

Develop rapid chemical assays to replace the mouse bioassay

Develop economic impact studies to properly evaluate losses by locale and by industry

Develop epidemiological studies to determine what public health risks are involved

Investigate existing technologies for effective dead fish cleanup

Investigate the applicability and efficacy of control and mitigation methods

Determine the fate and effects of toxins in the marine environment, including water, sediment, air, and food webs

Continue and enhance public information and outreach programs

### PFIESTERIA-LIKE SPECIES HABs

Determine occurrence and distribution of PLS in Florida

Develop molecular probes for identification and differentiation of PLS species in Florida

Develop molecular probes for the detection of PLS toxins or bioactive compounds in natural waters

Determine whether isolates are toxic or produce bioactive compounds that affect fish

Determine whether—and if so, how—domestic animal and/or human wastes influence distribution and abundance of PLS

Determine how environmental variables influence toxic events, e.g., freshwater flow and nutrient enrichment

Determine the distribution of PLS in relation to fish disease or fish kill “hot spots” and its relationship to other pertinent environmental factors such as nutrients

Conduct short- and long-term studies of exposure of fish to PLS

Determine the fate and effects of toxins or bioactive compounds in the food web

### CIGUATERA HABs

Develop accurate and rapid tests to detect ciguatera toxins in fish

Expand the testing network (commercial and recreational) to document the occurrence and extent of ciguatoxic fish

Develop a better monitoring system for recording and documenting confirmed incidences of ciguatera poisoning

Increase awareness and training in the medical community for recognizing, documenting, and treating the symptoms

Determine the economic impact and loss factors, including loss of revenue and productivity due to illness and treatment

Survey Florida waters for ciguateric dinoflagellate species and hot spots

Determine the transfer of toxins in the food chain and what animals at what size and weight are a public health risk

Ciguatera as well as other neurotoxins should be evaluated for potential chronic, immunologic, neurologic, and pulmonary effects

Support and encourage the reporting network (hotline) now operating in the Miami area

Produce public outreach literature to increase public awareness and improve reporting

### **TOXIC BLUE–GREEN ALGAE OR CYANOBACTERIA HABS**

Determine distribution of toxic and nontoxic strains in Florida waters

Develop epidemiological studies to determine what public health risks are involved

Develop economic impact studies to properly evaluate losses by locale or industry

Determine the roles of nutrient enrichment and managed freshwater flow in bloom development

Determine the fate and effect of toxins in the food web

Investigate the applicability and efficacy of control and mitigation methods

### **HABS AS TUMOR PROMOTERS**

The potential role of biotoxins such as okadaic acid (OA) in tumor development in sea turtles should be further explored, either for direct tumorigenic effects, as co-factors, or as sublethal immunosuppressive factors that render animals susceptible to oncogenic viruses or other pathogens

Determine the fate and effects of toxins in the food web

Conduct animal exposure studies to determine effects of specific tumor-promoting compounds

Isolate and maintain potentially toxic species for toxin or bioactive compound identification

Include potential species of concern in any state-wide survey

### **MACROALGAE HABS**

Survey *Codium* populations out to 300 feet deep in the area of known occurrence for spatial and temporal variability and determine sources of drift algae

Survey invertebrate herbivore populations and determine food sources and feeding rates

Verify environmental regulators of growth, e.g., temperature, light, nutrients

Determine sources of nutrients and whether there is a lag in growth influenced by source of nutrients or storage of nutrients

Determine submarine groundwater discharge points in relation to *Codium* distribution and develop a groundwater flow model

Monitor nutrient levels in the areas of historical *Codium* buildup

Identify markers to distinguish between sewage-related nutrients and upwelled nutrients

Determine the economic impact of such blooms on local economies

# HARMFUL ALGAL BLOOMS IN FLORIDA

## INTRODUCTION

Harmful Algal Blooms (HABs) are the proliferation of harmful or nuisance algae that adversely affect natural resources or humans and occur worldwide. The algae can be either microscopic plant-like cells in seawater, brackish water, and fresh water or larger aquatic plants like “sea lettuce” (*Ulva*) that can be seen with the unaided eye. Although the term “algae” suggests plants that have chlorophyll, like land plants, not all HAB species have their own chloroplasts and produce their own chlorophyll. Microalgae, both those with and without chloroplasts, are often known as Protista. The term “bloom” indicates an increase in abundance above normal background numbers of the species in a specific geographic area. The increase can be in the water column or on sea-bottom substrates—like blades of seagrass or pieces of seaweed—or in the sediment. HABs are really defined by their harmful effects, which may be visible, such as floating or beached dead fish, or hidden, such as the alteration of a food chain or the gradual loss of benthic vegetation that provides habitat for many organisms, including fishes. HABs may also affect public health; people can become ill when they consume shellfish that have been exposed to toxins from a bloom.

Fish kills, instances of disease in animals, and other deleterious aquatic phenomena are not unusual along the Gulf of Mexico and the Atlantic coasts of Florida. HABs can cause massive animal mortalities, neurotoxic shellfish poisoning, and respiratory irritation in humans (Connell and Cross, 1950; Rounsefell and Nelson, 1966; Steidinger et al., 1973, 1998a; Steidinger, 1993; Pierce, 1986, 1987; Pierce et al., 1990; Landsberg and Steidinger, 1998). However, poor water quality, anoxic or hypoxic water, chemical contaminants, and infectious pathogens also have caused such events. All these factors need to be considered in the investigation of any fish mortality or disease event (American Fisheries Society, 1992). About 40 species of toxic marine microalgae, including 11 ichthyotoxic (fish-killing) species, have been identified from Florida marine waters and all may have various effects on natural resources and public health (Steidinger, 1993; Landsberg, 1995; Steidinger et al., 1998a, 1998b; Tomas, 1998). In addition to the marine species, about 20 freshwater and freshwater-estuarine blue-green algae species that are toxic or potentially toxic occur in Florida waters (see Appendix I for a list of toxic or potentially toxic species in Florida).

Although there are more than 60 toxic microalgae, we are fortunate that not all the potential deleterious effects, particularly those involving public health, occur. For example, although we have toxic *Pseudo-nitzschia* species (diatoms that produce domoic acid) in Florida waters, we do not have any recorded cases of amnesic shellfish poisoning. The same is true for toxic *Prorocentrum* and *Dinophysis* species (dinoflagellates that produce okadaic acid); there have been no documented cases of diarrhetic shellfish poisoning in Florida. This paper concentrates on the HAB species most that affect Florida’s resources, residents and visitors, and economy. The HAB Task Force based on several factors, including frequency of occurrence, geographic extent, public health risks, natural resource impacts and economic losses has ranked Florida’s HABs in order of concern. The ranking of species or taxonomic group is as follows:

***Gymnodinium breve***, the red tide organism that causes neurotoxic shellfish poisoning (NSP), human respiratory irritation, and animal mortalities;

***Pfiesteria*-like organisms** that potentially pose a threat to natural resources and human health; ***Gambierdiscus toxicus***, species of ***Prorocentrum*** and ***Ostreopsis***, and other benthic dinoflagellates that are associated with the tropical fish poisoning known as ***ciguatera***;

**Blue-green algae** that can contaminate drinking water and kill domestic and wild animals;

**Dinoflagellates and blue-green algae** that are associated with tumor promotion in experimental animals and may be associated with tumors in marine fish, turtles, and mammals; and

**Macroalgae** that cause nuisance blooms which can displace fish and smother bottom communities.

The key to understanding any HAB lies in knowing how one algal species has adapted and can dominate the plankton or epiflora in its particular ecosystem. HAB species exploit their physical and biogeochemical environment, but what physiological and behavioral adaptations represent successful survival and dispersal strategies in a fluctuating environment? Have red tides increased in the Gulf of Mexico over the last 400 years as part of a global epidemic (Smayda, 1990), or has our observational network grown instead?

Of the causal factors leading to HABs, excess nutrients often dominate the discussion. Consequently, there is an active debate among environmental scientists as to the role of nutrients in stimulating and spreading harmful algal blooms. One component argues that the increase in man's activity over the past decades has altered conditions, resulting in elevated nutrient levels in coastal waters. These activities include nutrient runoff from farm crops and animal pens, phosphate detergents that find their way into coastal waters, human waste from malfunctioning or nonfunctioning septic systems, organic compounds from food processing and tanning industries as well as toxic metal pollution from other industries, and modification of estuarine circulation. A commonly cited example is the increase in red tides in Tolo Harbor, Hong Kong (Lam and Ho, 1989) that has accompanied a decade of rapid increases in populations in the crowded coastal areas. Similarly, the change in the nitrogen, phosphorus, and silica ratios in the Rhine River discharge during the past 50 years has stimulated flagellate blooms in the North Sea. Over the years, the addition of phosphorus (detergents) and nitrogen (fertilizers) was not matched by the natural silica required for non-red tide species, and the ratio slowly favored harmful species in place of the benign and necessary "normal" phytoplankton (Smayda, 1989, 1990). Excess nutrients often result in eutrophication, which means that there is an excessive algal biomass. These blooms discolor the water by their sheer abundance, but for the most part, are harmless unless decay leads to excess oxygen demand and hypoxia or anoxia results. During the 1970s, the Japanese were reporting more than 300 red tides per year in the Seto Inland Sea of Japan (Okaichi, 1989), where heavy use for aquaculture was loading bays with organic substances as products of animal and plant cultivation. Of these 300 blooms reported, most of them were of harmless species, which simply colored the water, and only a small percentage (<2%) consisted of truly toxic species.

When it comes to species that produce actual toxins, the connection is less clear. Toxic blooms can begin in open water miles away from shore or the immediate influence of human activities. This is the case for the Florida red tide organism (*Gymnodinium breve*) as well as those organisms that poison clams in the northeastern and northwestern U.S. (*Alexandrium tamarense*, *A. catenella*). Elevated nutrients in inshore areas did not start the bloom but, in some instances, can allow it to persist in the nutrient-rich environment for a slightly longer period than normal.

However, in the same areas, the harmful bloom species can also be out competed by non-harmful species, and, thus, the elevated nutrients can work against persistence of the HABs. The oldest recorded food poisoning, ciguatera fish poisoning, occurs in tropical waters where nutrient levels are usually so low they are difficult to detect. This poisoning is caused by bottom-dwelling microalgae such as *Gambierdiscus toxicus* that grows on seaweeds and is inadvertently eaten by small fish and crustaceans which, in turn, are eaten by larger ones. Again, all this can occur in areas where nutrients are hardly detected. The invasion of *Gambierdiscus* has been connected to man's disturbance of reefs by dredging, anchoring, and construction (De Sylva, 1994). The role of nutrients and blooms of *Pfiesteria* and *Pfiesteria*-like species is yet more complicated. Nutrients may stimulate *Pfiesteria* indirectly by supporting small autotrophic prey species and bacteria, which *Pfiesteria* uses as food, or they may support *Pfiesteria* and *Pfiesteria*-like species stages directly. The relationship of *Pfiesteria* and nutrients needs to be better characterized and clarified.

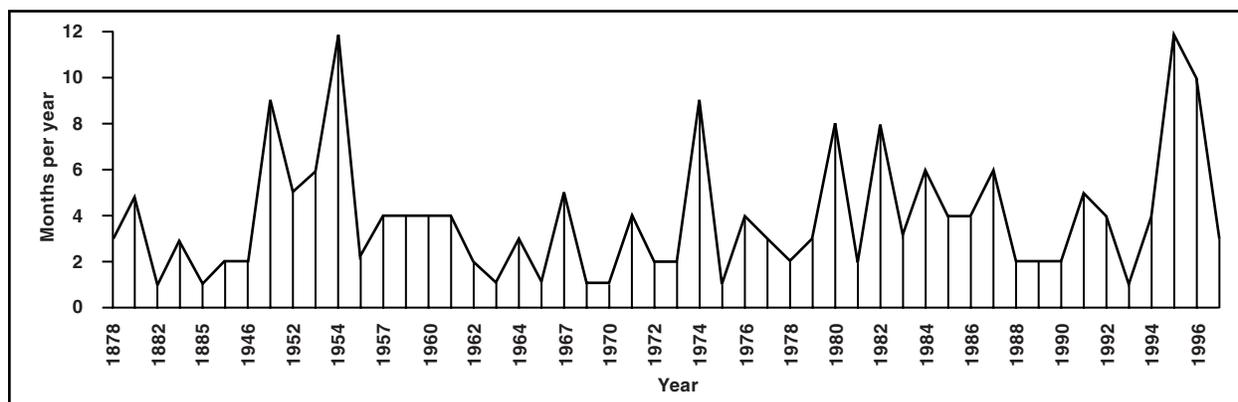
Thus, there is no simple answer for nutrient enrichment in coastal waters. Elevated nutrients do not automatically result in a higher incidence of HABs. HABs can bloom in nutrient-poor waters away from shore with no direct influence by humans. HABs may bloom in such small numbers that it does not take elevated nutrients to cause the appearance of their toxins in shellfish. HABs may start in eutrophic waters and terminate in the same waters that still have excess nutrients. In other instances, however, there is direct evidence of increased manmade influences with increased HABs. Each incidence has to be examined and evaluated separately as to the role nutrients may play.

By far, *G. breve* HABs are the most serious in Florida because they occur almost every year; cause NSP and human respiratory irritation; cause fish, bird, and marine mammal mortalities; can extend from Pensacola to Jacksonville at different times during a red tide event; can be transported to other states, as they were in 1987 and 1997; and can cause a minimum of \$15-25 million in economic losses each year. We do not have sufficient information about many of the other HABs to determine the geographical extent of a potential problem, but large-scale visible resource or public health effects have not been noted. This review presents background information on the Florida HABs of special concern that affect living resources, public health, and the economy. Following the background information are recommendations for action that require further research, development of methods, evaluation of methods, or surveys to determine the extent of the problem. The recommended actions that are highlighted in **bold** are being funded with a special appropriation from the Florida legislature to the Florida Department of Environmental Protection (FDEP). In addition to this special State appropriation, the National Oceanic and Atmospheric Administration, the U.S. Environmental Protection Agency, and the Navy, among others, are funding studies to provide data for developing models to predict the occurrence and magnitude of several HABs in Florida (e.g., *G. breve* red tides and *Pfiesteria*-like events).

## RED TIDE

### Background

Red tides causing massive fish kills in the Gulf of Mexico have been reported anecdotally since the 1500s, but written records exist only since 1844. For example, during the 1800s, red water or



**Figure 1**

Florida west coast red tide data, 1871–present. Number of red tide months per year.

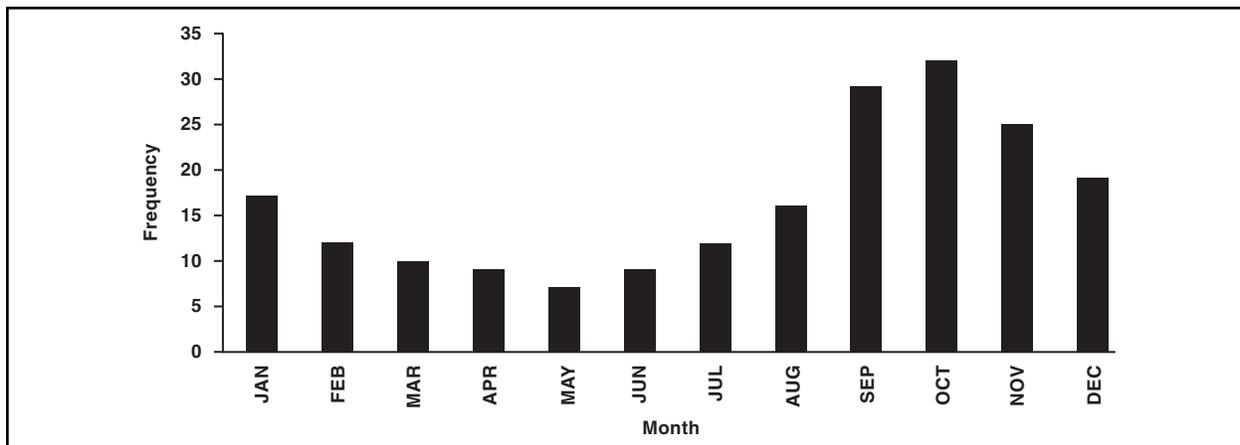
“poisoned water” off Florida’s coast was associated with fish, invertebrate, and bird kills; toxic shellfish; and a human respiratory irritant (Rounsefell and Nelson, 1966). By 1996, all states in the Gulf of Mexico had had *G. breve* blooms that affected natural resources and public health. Although Texas had recorded *G. breve* red tides in 1935, 1955, 1974, 1986, and 1996 (Buskey, 1996), 1996 was the first record for Alabama, Mississippi, and Louisiana.

Most HAB events are recognized and documented by their effects, which can depend on cell concentration. For example, at  $>5 \times 10^3$  cells  $l^{-1}$ , *G. breve* can cause closure of shellfish beds because of the potential for causing NSP. At cell concentrations  $>1 \times 10^5$   $l^{-1}$ , it can cause fish kills and manatee mortalities. At cell concentrations of  $1 \times 10^5$   $l^{-1}$  in surface waters, chlorophyll can be detected by satellite sensors, but only when the level reaches  $1 \times 10^6$  cells  $l^{-1}$  can the human eye detect discolored surface water (Tester et al., 1998). Cell concentrations have been recorded as high as  $1 \times 10^8$   $l^{-1}$  in Texas waters (Buskey, 1996).

Over the last century, the duration of red tides off west Florida has varied, the maximum being 20 months, and 70% of the blooms have occurred in late summer-fall (Steidinger et al., 1977) (Fig. 1). Red tides have been observed in 22 of the last 23 years (Fig. 2) within the region between Tampa Bay and Charlotte Harbor, compared to 5 to 10 outbreaks north and south of that region. This region is thus defined as the epicenter of *G. breve* abundance along the west Florida coast and constitutes a focused study area for ECOHAB:Florida, a federally funded program to predict red tides. However, red tides may initiate in and be confined to areas north and south of the epicenter.

*Gymnodinium breve* is common in the Gulf of Mexico all year long at cell concentrations of  $<1 \times 10^3$   $l^{-1}$  (Geesey and Tester, 1993), which is considered to be the background level. However, blooms of toxic *G. breve* originate 18 to 74 km offshore of central Florida at depths of 12 to 40 m (Steidinger and Haddad, 1981). These blooms may yield surface stocks of  $>90$   $\mu g$  chlorophyll  $a$   $l^{-1}$  (Carder and Steward, 1985) and carbon fixation rates of  $1.9$   $g$   $C$   $m^{-2}$   $day^{-1}$  (Vargo et al., 1987). Also, these blooms are apparently not subjected to much grazing pressure, are not found in salinities  $<24$  psu (at least in Florida).

From its source waters in the Gulf of Mexico, *G. breve* is moved throughout its oceanic range by major currents and eddy systems, and bloom initiation is closely coupled to physical



**Figure 2**

Florida west coast red tide data, 1878–1997. Frequency by month. 52% of blooms occur September–December. 46% of the blooms are initiated in September; 21% are initiated in October and November.

processes (Tester and Steidinger, 1997). Red tide blooms are most frequent along the west Florida shelf, especially from Clearwater to Sanibel Island, areas that experience either persistent, intermittent, or event-related slope/shelf upwelling. Blooms first appear offshore and have been associated with oceanic fronts caused by the onshore-offshore meanders of the Loop Current water along the outer southwest Florida shelf. Fronts represent a dynamic area of nutrient regimes and light conditions that can favor accumulation and growth of cells. *G. breve* has a high photosynthetic capacity at low light levels, is able to use organic as well as inorganic nutrients, and may competitively exclude other dinoflagellate species so that a nearly monospecific dinoflagellate bloom results.

Once a bloom has developed offshore in typically oligotrophic waters, cell concentrations at the  $10^5$  level can be maintained for months. These coastal blooms are thought to be sustained by nutrients upwelled from deep waters offshore or nutrients generated from preceding phytoplankton blooms. For example, at intermediate red tide levels of  $1 \times 10^6$  cells  $l^{-1}$ , or  $\sim 13 \mu g$  Chl *a*  $l^{-1}$ , initial nutrient stocks of  $8.0 \mu g$ -at  $NO_3$   $l^{-1}$  and  $0.5 \mu g$ -at  $PO_4$   $l^{-1}$  would be required (Wilson, 1966; Vargo and Shambloott, 1990) to sustain this population level. However, these concentrations of both inorganic N and P are not found even within 2–4 km of the Florida coast ( $<0.2 g$ -at  $l^{-1}$ ; Dragovich et al., 1961, 1963; Vargo and Shanley, 1985). Furthermore, the atomic ratios of dissolved inorganic nitrogen ( $NO_3 + NH_4 + NO_2$ ) and phosphate in the Peace River, entering Charlotte Harbor (Fraser and Wilcox, 1981, McPherson et al., 1990), and in the Alafia River, entering Tampa Bay, are usually  $<2$ . Remember that they both drain the phosphate-rich Hawthorne formation of central West Florida (Dragovich et al., 1968). Once growth occurs offshore, it takes 2 to 8 weeks to develop into a bloom of fish-killing proportions, depending on physical, chemical, and biological conditions.

A bloom in open water may cover a surface area up to  $1.4$  to  $3.0 \times 10^4$   $km^2$ , and although biomass concentration is patchy, chlorophyll *a* values make the resultant discolored surface water detectable by color sensors onboard satellites. In the case of *G. breve*, the CZCS sensor detected chlorophyll *a* from cells at densities at least two orders of magnitude less ( $10^4$ ) than are

present when discolored water is detectable by human eyesight ( $>10^6$ ) (Tester et al., 1998). There is evidence that some blooms can be maintained within the midshelf zone by means of seasonal wind reversals (northeast–southwest flow), which results in zero mean flows both in the along-shore and across-shore directions over periods of months. These offshore populations can continually inoculate the nearshore waters. Recent research on the west Florida shelf circulation describes two basic patterns, a summer pattern (April–September) and a winter pattern (October–March) characterized by a semi-permanent, anticyclonic eddy on the northwestern Florida shelf in the Apalachee Bay–Middle Grounds area (Weisberg et al., 1996; Yang et al., 1998). This feature dominates the northeastern shelf onshore of the 50-m isobath and may be responsible for the entrainment and transport of cells northward to the Florida panhandle. A recent question asks, “Is there a resident population of *G. breve* at high concentrations somewhere on the west Florida shelf all year long that may or may not inoculate inshore waters?”

From Tampa Bay south, small-scale eddies or filaments may also play a role in the translocation of offshore blooms. Frontal eddies (Loop Current water) and onshore–offshore meanders of the Loop Current move southward along the outer southwest Florida shelf every 2 to 14 days, and there is evidence for a southward mean flow over the shelf when the Loop Current is at the shelf edge. The annual cycle of wind stress, northward during the summer and southward in the fall, is responsible for the persistent upwelling (summer) or downwelling (fall) found over the west Florida shelf and may concentrate or disperse blooms depending on the site and timing of the bloom (Tester and Steidinger, 1997). Another recently described eddy system operative between the Tortugas and the upper Keys is dependent on a well-developed Loop Current and the consequent offshore position of the Florida Current (Lee et al., 1994). The Tortugas Gyre is a cyclonic recirculating feature (100 to 180 km) that has a duration of 40 to 108 days that has a strong influence on the transport and retention of zooplankton and larval fish in the lower Florida Keys. In the intervals between gyre recirculation periods, there are 20- to 30-day episodes of intense eastward flow. Dissipation or termination of blooms is thought to occur when the offshore bloom component is entrained and transported out of the area or when mixing weakens the integrity of the water mass.

### Resource Impacts

*Gymnodinium breve* blooms, and the subsequent toxins produced, can cause animal mortalities and affect human health. Organisms are exposed to brevetoxins through ingestion of *G. breve* cells (by filter feeders), by consumption of toxic prey (e.g., by birds, humans [NSP]), by aerosol transport (causing respiratory irritation in humans and potentially in manatees, turtles, and birds), by water-borne toxin after cells dissolve (affecting fish), from sediment sinks (affecting benthic organisms), and possibly through consumption of toxic benthic stages (Steidinger et al., 1973; Hemmert, 1975; Quick and Henderson, 1975; Forrester et al., 1977; Roberts et al., 1979; Baden et al., 1982; Fowler and Tester, 1989; Geraci, 1989; Pierce, 1986; Pierce et al., 1990; Summerson and Peterson, 1990; O’Shea et al., 1991; Landsberg and Steidinger, 1998).

Although the majority of red tide blooms are usually associated with invertebrate, fish, and bird mortalities, there have also been numerous accounts of other marine animal mortalities, especially dolphins and sea turtles (Rounsefell and Nelson, 1966). The role of biotoxins in marine mammal mortalities has been controversial for many years (Geraci, 1989; Geraci et al., 1989; Anderson and White, 1992). Yet *G. breve* has been implicated in mortalities of the endangered Florida manatee, *Trichechus manatus latirostris*, in 1963 (Layne, 1965), 1982 (O’Shea et al.,

1991), and 1996 (Bossart et al., 1998; Wright et al., in prep.), when 7, 39, and 149 animals, respectively, died in southwest Florida during the winter/spring (February to April) period.

*Gymnodinium breve* most frequently comes inshore during the fall–winter months and then dissipates. Red tides do not usually appear inshore during the winter–spring months (Tester and Steidinger, 1997) when manatees are congregated upstream in freshwater or low-salinity areas, warmer waters of coastal power plants, warm-water spring refugia, or residential canals (Reynolds and Wilcox, 1986). As the water temperature warms, manatees disperse into the inshore bays. If a red tide has come inshore during this period, then certain high-salinity areas (above 28‰) may maintain persistently high concentrations of *G. breve* cells ( $> 1 \times 10^5$  per liter), so the likelihood of manatees being exposed to red tide during their post-winter movements is fairly high (Landsberg and Steidinger, 1998). In 1996, several factors contributed to an unusually persistent red tide bloom that encroached inside the barrier islands in southwest Florida. These include the potential for an offshore population of *G. breve* to move inshore; a warm-water shelf intrusion during February (R. Stumpf, USGS, pers. comm.); high west-to-southwest winds during several cold fronts; high salinities above 28‰; and low rainfall. All of these factors contributed to the movement and persistence of the bloom once it reached inshore areas. Manatees that began to disperse encountered a red tide when entering the estuary. However, some safe zones were present in areas where lower salinities persisted. Therefore, not all manatees in the southwest region would necessarily be exposed to red tide if their movement patterns kept them in low-salinity areas. This scenario would partially explain why there were many manatees in southwest Florida that survived the red tide event.

During 1946–1947 and 1953–1955, two of the largest red tide events on record, with respect to both geographical distribution and longevity, occurred in central and southwest Florida (Gunter et al., 1948; Rounsefell and Nelson, 1966). Catastrophic mortalities of marine animals were recorded from Tarpon Springs to Key West (some 150 miles of coastline), far exceeding the geographical extent of the manatee epizootics described above. During these events, however, there were no reports of dead manatees, only bottlenose dolphins (*Tursiops truncatus*), sea turtles, and numerous fish species (Gunter et al., 1948; Rounsefell and Nelson, 1966). Landsberg and Steidinger (1998) suggested that the 1946–1947 and 1953–1955 red tide events differed from the 1963, 1982, and 1996 events because manatee movement from inland warm-water refugia to estuarine waters did not coincide with the presence of the red tide in the estuary. In 1946–1947, red tide was reported from November 1946 through January 1947, throughout April 1947, and again from the end of June through August 1947. During April 1947, reports of dead fish associated with the presence of red tide were recorded only from the most southwestern region of Florida, near Cape Sable and Key West (Gunter et al., 1948). In 1953–1955, red tide was present in the Pine Island Sound area from November 1952 through January 1953 and again from July 1954 through February 1955 (Rounsefell and Nelson, 1966). Red tide was apparently not present in the Pine Island Sound and Marco Island areas when manatees dispersed there from late February through the end of April.

In 1963, 7 dead manatees were reported in the Caloosahatchee River area between March 26 and April 9 (Layne, 1965). Red tide counts from the Sarasota area at the end of March were as much as  $6.0 \times 10^6$  cells/liter and up to  $8.2 \times 10^5$  cells/liter half a mile offshore of Pine Island Sound (B. Roberts and K. Steidinger, FWC). In 1982, sick and dying manatees were observed throughout the red tide event (O’Shea et al., 1991), but there was a lag between the time that the

red tide cell counts had decreased to below  $1 \times 10^5$  cells/liter (March 22; B. Roberts and K. Steidinger, FWC) and the time that the last manatee died (April 16) (O'Shea et al., 1991). Tunicates presumed to have accumulated toxins were found in manatee stomachs and intestines. Filter-feeding organisms can retain brevetoxins for weeks or even months after a red tide has dissipated (K. Steidinger, unpubl. data) and tunicates are known to accumulate brevetoxins (R. Pierce, Mote Marine Laboratory, FL, pers. comm.). Tunicates collected from seagrass beds in the same area contained toxins, but these were not confirmed as brevetoxins (D. Baden, unpublished data).

In 1996, there was no significant lag time between the dissipation of the red tide and the last manatee mortality, if April 27 is accepted as the end date of the epizootic. Unlike 1982, distressed manatees were not observed in 1996 until towards the end of the red tide event. Necropsies indicated that the stomach contents of only three manatees contained tunicates (Wright et al., in prep.), the organisms suspected of transmitting toxins during the 1982 event (O'Shea et al., 1991). Also in contrast to 1982, manatees were reported to be healthy animals that were dying rapidly in hours or days (Wright et al., in prep.). In 1996, the duration of the manatee mortality was coincident with the duration of the red tide bloom. Manatee stomachs contained enough brevetoxin to kill manatees (Baden et al., in prep.), but the sources of the ingested toxin are still unknown. The coincidence of the mortality with the red tide bloom would also suggest exposure through inhalation. Residual toxin bound up in the food web, water, or substrate would persist after the bloom had dissipated, but aerosolized brevetoxin that manatees could inhale would disperse relatively quickly. Presence of brevetoxins in nasal and lung tissue also implicate the aerosol (Bossart et al., 1998; Baden et al., in prep.). Therefore, there are three potential routes of toxicosis in manatees: (1) toxic aerosol inhalation, (2) toxic food ingestion, and (3) toxic seawater intake. There is also a potential for immunosuppressive effects associated with persistent chronic exposure to brevetoxins that may influence manatee health in the long term (Bossart et al., 1998).

Although acute exposure to lethal doses of brevetoxin results in massive animal mortalities, effects from exposure to low-level brevetoxins are unknown. The stability of brevetoxins in the environment is also unknown. Other biotoxins can be transferred through the food web and cause mortality of animals, including fish and birds (e.g., White et al., 1989; Work et al., 1993). Numerous unexplained fish kills reported by Williams and Bunkley-Williams (1990) may have been attributable to biotoxin transfer through dietary exposure (Landsberg, 1995). Also, the worldwide distribution of two major types of cancer in shellfish has recently been hypothesized to be related to chronic exposure of bivalves to biotoxins (Landsberg, 1996). Chronic dietary exposure to brevetoxins could exert lethal or sublethal effects at all trophic levels, leading to impaired feeding, avoidance behavior, physiological dysfunction, impaired immune function, reduced growth and reproduction, pathological effects, or mortality.

### Public Health Impacts

*Gymnodinium breve* produces hemolysins and neurotoxins that can affect humans through several routes. Poisonings from edible bivalves that have accumulated brevetoxins can cause temporary illness, e.g., gastrointestinal and neurological distress known as Neurotoxic Shellfish Poisoning (NSP) in humans. Symptoms include hot/cold reversals, tingling sensations in the extremities, nausea, diarrhea, vertigo, and pupil dilation and full recovery typically takes two to three days. Of the >60 cases of NSP in the southeast, there have been no mortalities. However,

there are two cases where patients (one less than 5 years old and the other a teenager) were put on ventilators.

To protect public health during red tide events, shellfish areas are closed to harvesting when *G. breve* levels are above background concentrations and are opened when mouse bioassay test results are less than 20 mouse units (MU) per 100 g of shellfish meats and cell counts are at background levels. This protocol is in compliance with Florida's Biotxin Control Plan established in 1984. At 20 MU per 100 g or less, shellfish are safe for human consumption. However, the mouse bioassay is a slow and labor-intensive method that delays final results for 3–5 days, and alternate chemical analyses are being sought. Typically, when red tide has dissipated from the area, shellfish can cleanse themselves of toxin within 2 to 6 weeks. There is one incident where an edible clam, *Chione cancellata*, retained toxicity for 1 year (Steidinger et al., 1998).

In addition to shellfish poisoning, brevetoxins from *G. breve* (and possibly flagellates such as *Chattonella*) can cause respiratory irritation when the toxin in seawater becomes airborne as an aerosol due to bubble-mediated transport (Pierce et al., 1990). Humans can experience eye irritation, nonproductive cough, and bronchoconstriction (Baden et al., 1995). This route of intoxication is considered to be reversible with the removal of the irritant source; however, there are no comprehensive epidemiological studies for chronic exposures during a red tide event. These symptoms are reversible once the person is no longer exposed. The Florida Department of Health, in collaboration with the University of Miami, Centers for Disease Control and Prevention (CDC), Mote Marine Laboratory, and other partners, is conducting preliminary investigations on human risk involved with exposure to the aerosol.

Other public health effects involve rare cases of contact dermatitis from exposure to brevetoxins in seawater and cases of puncture wounds from spines of beached dead fish.

### **Economic Impacts**

Two 3- to 5-month red tides off Florida caused \$15–\$20 million in losses to local communities in the 1970s (Habas and Gilbert, 1974, 1975). In 1987, when a Florida red tide was transported to North Carolina waters by the Gulf Stream, closures of shellfish-harvesting areas alone caused a loss of \$25 million (Tester and Fowler, 1990). Those who live and work on the beach will tell you that businesses, tourism, and community recreational activities are adversely affected by red tides, e.g., Longboat Key, 1994–1996 (Boesch et al., 1997), but there are no current data documenting actual losses that would justify expenditures to reduce or eliminate the perceived effects. Protocols for collecting such data are being evaluated by economists at the University of Florida (C. Adams, pers. comm.).

### **Recommendations for Action**

Predict the onset and movement of red tides in shelf waters (federal and state initiatives)

**Develop rapid chemical assays to replace the mouse bioassay (special state appropriation)**

**Develop economic impact studies to properly evaluate losses by locale and by industry (special state appropriation)**

**Develop epidemiological studies to determine what public health risks are involved (special state appropriation)**

**Investigate existing technologies for effective dead fish cleanup (special state appropriation)**

### Investigate the applicability and efficacy of control and mitigation methods (special state appropriation)

Determine the fate and effects of toxins in the marine environment, including water, sediment, air, and food webs (federal and state initiatives)

Continue and enhance public-information and -outreach programs (state)

## **PFIESTERIA-LIKE SPECIES IN FLORIDA'S ESTUARINE WATERS**

### **Background**

From 1980–1989, at least 50% and 69% of fish kills in the Gulf of Mexico and South Atlantic, respectively, were attributed to low levels of dissolved oxygen (Lowe et al., 1991). It is possible that some of these kills were associated with harmful algal blooms caused by small, ephemeral dinoflagellates that were not, until recently, recognized as being ichthyotoxic. Such fish kills would have been attributed to low levels of dissolved oxygen associated with the bloom rather than direct toxicity. Many kills are now known to be caused by blooms of small dinoflagellates such as *Heterocapsa*, *Gymnodinium*, *Gyrodinium*, or *Pfiesteria* (Steidinger et al., 1996a, 1998a). Recently, a series of fish kills, ulcerated fish disease events, and public health threats have highlighted the life strategies of small, heterotrophic, lightly armored dinoflagellates along the eastern seaboard. *Pfiesteria piscicida* was first thought to be a *Gymnodinium* sp. killing tilapia in a North Carolina aquarium (Smith et al., 1988). Although massive fish kills (mostly menhaden) were particularly prevalent in North Carolina's estuaries for many years, it was not until 1991 that *P. piscicida* was implicated in these mortalities (Burkholder et al., 1992; Noga et al., 1993, 1996). *Pfiesteria piscicida* was described in 1996 (Steidinger et al., 1995, 1996a) and has been documented in North Carolina, Maryland, Delaware, and South Carolina (Burkholder et al., 1992, 1995a, 1995b; Lewitus et al., 1995; Steidinger et al., unpubl. data). Fish exposed directly to *P. piscicida* populations in tanks or water filtrate (and presumptive toxin) that contained *P. piscicida* and other microbes showed behavioral neurological signs and developed severe dermatological lesions (Noga et al., 1996). About this time, *Pfiesteria piscicida* and a *Pfiesteria*-like species (PLS) were reported from the St. Johns River (Burkholder et al., 1995a). This record was later modified to two PLS (Burkholder and Glasgow 1997a, 1997b). Several new genera of PLS species have been identified from the St. Johns and St. Lucie rivers, one of which is referred to as a cryptoperidiniopsoid (Steidinger et al., submitted); other PLS have also been documented in Florida. We have not yet confirmed whether our species are the same as the species reported by Burkholder and Glasgow (1997a, 1997b). Neither *P. piscicida* nor the second known *Pfiesteria* species have yet been confirmed in the wild in Florida (Landsberg et al., 1995; Steidinger et al., unpubl. data). The as yet undescribed *Pfiesteria* species was characterized from a Florida aquarium (Landsberg et al., 1995) and is suspected of killing fish in tropical fish tanks. However, it co-occurred with a parasitic dinoflagellate (*Amyloodinium ocellatum*) that is known to kill fish (Landsberg et al., 1994), so its role in the fish kills is uncertain. Also, a second, less toxic, *Pfiesteria*-like species or *Pfiesteria*-complex organism is being described as new by J. Burkholder and H. Glasgow (pers. comm.).

The term “*Pfiesteria*-like species” or PLS, in the context of this review, refers to small heterotrophic dinoflagellates that morphologically resemble *Pfiesteria piscicida*. They are also called *Pfiesteria*-like organisms or PLOs. Some of them may produce toxins or bioactive compounds and some may not, although many of them are no doubt very closely related because of

the habitat and niches they occupy. D. Oldach (pers. comm.) has demonstrated that *P. piscicida*, an undescribed ichthyotoxic species, and a cryptoperidiniopsoid that is not ichthyotoxic are all in the same clade and are of monophyletic origin. One of the pressing needs in PLS research is to determine which species are harmful and what compounds they produce and release.

A critical aspect of the ecology of *Pfiesteria* and PLS is whether the increased frequency of these species is related to anthropogenic inputs and activities. In all probability, these species have been present for many years and misidentified as gymnodinioids. The selection toward increased abundances of flagellated prey, bacteria, and other organisms important to *Pfiesteria* and PLS may be fundamental in regulating the appearance of these HAB species in continually eutrophied coastal waters. Several anthropogenic factors of particular concern that may lead to PLS blooms are nutrient enrichment (inorganic or organic) and bacterial loading from point or non-point discharges from urban runoff, agriculture, and wastewater treatment plants. There is some indication that *P. piscicida* responds directly to increased nutrient loading and in particular to phosphorus (Burkholder and Glasgow, 1996, 1997a; Lewitus et al., in press). About 75% of toxic *Pfiesteria* outbreaks were in nutrient-enriched waters (Burkholder et al., 1995a). In studies comparing *P. piscicida* abundance near wastewater treatment facilities with that in control regions, there was a statistically significant correlation between *P. piscicida* abundance and proximity to treatment sites (Burkholder and Glasgow, 1997a). Whether this is a direct or an indirect response by *P. piscicida* is still being evaluated. Increased biological oxygen demand (BOD) resulting from sewage, wastewater, and animal fertilizer is indicative of increased bacterial densities that provide a food source for protists that are then consumed by *P. piscicida* amoebae or other stages. Elevated N and P concentrations as well as coliform counts either from animal or human sources may be conducive to *Pfiesteria* and PLS HABs. Elevated nutrients that lead to increased algal blooms in turn provide ideal feeding grounds for planktivorous schooling fish, such as menhaden, that are attracted to these areas (Rob Magnien, Maryland DNR report 1998). High concentrations of fish provide optimum conditions for blooms of toxic *P. piscicida* (Burkholder et al., 1995a). Unknown substances produced by fish stimulate *P. piscicida* zoospores to emerge from benthic stages, but if fish are removed within 48 hours, the zoospores become non-toxic (Burkholder and Glasgow, 1997a).

Many fish diseases attributed to *Pfiesteria* and PLS events may actually be attributable to other etiologies. Several species of known or suspected toxic dinoflagellates are being found at the sites of the current fish kill and disease events and may pose risks to natural resources and public health (Steidinger et al., unpubl. data). Strong circumstantial evidence suggests that some of these species may be involved in these events and that not all are *P. piscicida*. It is critical that an overall multi-institutional, multi-disciplinary approach be developed to address all etiological factors in fish kill and disease events and the evaluation of potential human health risks. A priority for advancement in this field is to provide accurate diagnoses and descriptions of dinoflagellates through scanning electron microscopy and more rapid techniques using DNA sequencing. Molecular probes are being developed and field tested to identify *P. piscicida* and associated toxins (P. Rublee, pers. comm.; D. Oldach, pers. comm.; J. Ramsdell, pers. comm.).

### **Resource Impacts**

Since the late 1940s, recurrent fish kills, some of which might have been associated with PLS HABs (Burkholder et al., 1995a; Burkholder & Glasgow, 1997a), have been documented along the eastern USA. In the Gulf of Mexico, reports of fish kills or fish with lesions have been docu-

mented (Plumb et al., 1974; Ahrenholz et al., 1987; Osborne et al., 1989; Noga, 1993; Paulic and Hand, 1994; Steidinger et al., 1998a) but thus far it has not been determined whether PLS are involved. Recently, preliminary studies have been conducted to investigate the potential role of PLS in fish kill and disease events in Florida (Steidinger et al., unpublished) and a survey for *Pfiesteria* and PLS is being planned for the spring of 1999 using established and new techniques (molecular probes).

Ulcerative mycosis (UM) in estuarine fish, predominantly menhaden and mullet, has been known since at least the early 1970s. The distribution of fish with chronic, ulcerative granulomatous lesions ranges from Florida to New York, with a few reports from the Gulf of Mexico (Hargis, 1985; Dykstra et al., 1986, 1989; Noga and Dykstra, 1986; Ahrenholz et al., 1987; Grier and Quintero, 1987; Te Strake and Lim, 1987; Lim and Te Strake, 1988; Noga et al., 1988, 1989; Sindermann, 1988; Levine et al., 1990, 1991; Noga, 1993; FDEP, unpubl. data; Blazer et al., 1998). The basic pathology of UM appears to be similar in all areas surveyed: UM is characterized by deep, penetrating ulcers, chronic inflammation, and the presence of a fungus, usually *Aphanomyces* spp. (Dykstra et al., 1986; Noga and Dykstra, 1986; McGarey et al., 1990; Noga, 1993; Blazer et al., 1998).

Only recently has an association between UM and *P. piscicida* been implicated (Noga et al., 1996). When fish were experimentally exposed to water containing *P. piscicida*, hemorrhaging and sloughing of the skin epithelium were associated with presumptive toxin exposure (Noga et al., 1996). Invasion of damaged fish skin further resulted in deep ulcerations becoming secondarily invaded by opportunistic bacteria and fungi. UM is characterized by shallow to deep ulcers with >50% of the ulcers appearing near the anal area (Noga, 1993), especially in menhaden (Levine et al., 1990). Fish affected by UM have a high mortality rate, and the disease can be seasonal or cyclical (Noga, 1993). UM is common in low to moderate salinities, but not all fish species occurring in these salinities are affected (Noga et al., 1991). Degrading water quality conditions in certain estuarine waters (< 25ppt, [Noga and Dykstra, 1986]) are thought to be associated with disease outbreaks, but no definitive cause-effect relationship has been proven. The potential role of fungi, particularly *Aphanomyces* spp., as primary pathogens in the development of fish lesions is a strong factor in UM (Blazer et al., 1998). In the Far East, ulcerative disease has been documented for the last 25 years in numerous fish species and is associated primarily with *Aphanomyces*; this fungus appears to be the primary pathogen (Lilley & Roberts 1997). This same pathogen has recently been implicated in ulcerative disease in Australia (Callinan et al., 1995).

In March 1998, a severe fish-lesion event occurred in the St. Lucie River and estuary. Strong circumstantial evidence suggests an association between excess water discharges from the St. Lucie canal into the river and the outbreak of fish lesions; this has also been shown for lesion outbreaks in the St. Lucie area in 1979–1980, 1982, and 1995 (FDEP, USGS, SFWMD, unpubl. data). The connection between freshwater input and the appearance of disease in fish can involve a whole suite of environmental factors. In certain geographical locations along the east coast, the occurrence of *P. piscicida* has coincided with the occurrence of active fish kills and fish exhibiting lesions. This has led to the inference that *P. piscicida* is also responsible for the lesions. The spatial and temporal distribution of *P. piscicida* along the eastern seaboard does not always support this conclusion. In some situations, *P. piscicida* may initiate lesions, but this may not always be the case. In Florida and South Carolina, for example, fish with lesions can occur in the absence of active fish kills or *P. piscicida* (Landsberg et al., 1998). The

association of UM and *P. piscicida* or PLS needs further investigation.

There are many fish diseases, including lesions and ulcers, that do not appear to be related to *P. piscicida*, although they may be attributed to *P. piscicida*. In some cases, fish with ulcers are being reported from ongoing kill sites apparently within minutes after exposure to *Pfiesteria*. In most cases, such exposure would lead only to acute hemorrhaging directly associated with exposure to *P. piscicida* toxins (Noga et al., 1996; Burkholder and Glasgow, 1997a) and not the deep ulcers associated with chronic exposure (Burkholder and Glasgow, 1997a), which involves invasion by secondary bacterial or fungal pathogens (Noga, 1993). In experimental exposure of fish to *P. piscicida*, epithelial damage was first apparent in striped bass after 8 hours, and bacterial colonization began only after 48 hours (Noga et al., 1996). In this sense, an area with an ongoing chronic disease syndrome may be confused with a sudden active fish kill event. This does not necessarily imply that *P. piscicida* could not previously have been involved as an initiator of fish lesions.

*Pfiesteria piscicida* has been reported from North Carolina, South Carolina, Maryland, and Delaware, but it has been implicated in fish kills only in North Carolina, Maryland, and Delaware. *Pfiesteria piscicida* has not yet been reported from Virginia, Georgia, or Florida. Although there is some overlap between UM and *P. piscicida*, this does not always imply a causal relationship. Environmental stressors, including *P. piscicida*, may initiate lesions, but *P. piscicida* is not necessarily always the cause of “*Pfiesteria*-type” deep lesions (Landsberg et al., 1998).

A new PLS cryptoperidiniopsoid, which is morphologically and genetically similar to *Pfiesteria*, is associated with sites in the Chesapeake Bay, North Carolina, and Florida where ulcerative lesions have been observed. Additionally, we have documented another new PLS species from southeast Florida. The fact that we have not yet confirmed the presence of *P. piscicida* in Florida but have consistently isolated other PLS (Steidinger et al., in prep) indicates that these species may also have a role in the initiation of fish lesions. However, the relationship of these species to fish lesions is unknown; they too may produce bioactive compounds that are involved in lesion initiation. Small heterotrophic dinoflagellates may produce and release bioactive compounds capable of causing hemolysis or necrosis of fish epithelial tissue without having to be stimulated by the presence of fish to produce such compounds.

In freshwater and brackish habitats in the southeastern USA, red sore disease (RSD) was found in several fish species (Rogers, 1972; Esch et al., 1976; Overstreet and Howse, 1977; Osborne et al., 1989). RSD has characteristics similar to UM. Lesions are associated with *Aeromonas hydrophila* and the parasitic ciliate *Epistylis*, which produce a characteristic hemorrhagic hyperplastic condition beneath the scales that defines the red sore (Rogers, 1972; Esch et al., 1976). RSD is typically found in habitats that contain high levels of organic matter—a condition that is ideal for PLS; but environmental factors associated with nutrients, high BOD, bacteria, fish stress, and infectious agents may all lead to lesions in fish, in the absence of *P. piscicida* or other PLS. Given appropriate environmental stressors, other ubiquitous or pathogenic bacteria, fungi, and protists are also directly responsible for fish disease and the formation of ulcers.

### Public Health Impacts

*Pfiesteria piscicida* has been reported to produce a neurotoxic, water-soluble compound (Burkholder and Glasgow, 1997b) that causes human illness. When fish were exposed to dense, aer-

ated cultures of *P. piscicida* in a poorly vented room, the production of high concentrations of toxins was reported to cause human health-related problems (Glasgow et al., 1995), including memory loss and respiratory stress. Also, significant learning impairment was detected in rats exposed to aquarium water in which *P. piscicida* had been killing fish (Levin et al., 1997). Following a fish-killing episode involving *P. piscicida* in Maryland, 13 people that were exposed to the local waterway demonstrated difficulties with learning and higher cognitive functions. These symptoms were reversible 3–6 months after exposure ceased (Grattan et al., 1998). Other PLS may also produce toxins or bioactive compounds that could be a risk to both natural resources and public health, or they may produce bioactive substances that are a risk only to natural resources. Accurate identification of these small dinoflagellates is therefore paramount for developing risk-assessment strategies and examining the environmental triggers and circumstances that allow these species to bloom. The presence of fish with presumptive *P. piscicida*-induced lesions is being used as a biomarker and early warning signal for potential human health threats. Departments of health are developing criteria for human epidemiological studies based on the prevalence of fish lesions that are suspected to be associated with *P. piscicida* exposure (Centers

**Table 1**  
Possible scenarios for natural resources and public health caused by PLS  
(based on current information, Steidinger and Landsberg, unpubl.).

| Species                       | Fish disease      | Fish kills       | Public health*                   |
|-------------------------------|-------------------|------------------|----------------------------------|
| <i>Pfiesteria piscicida</i>   | +++               | +++              | +++ <sup>1</sup> (SL, N, RI, GI) |
| <i>Pfiesteria</i> sp.         | +++ <sup>2</sup>  | ?                |                                  |
| Cryptoperidiniopsoid          | ?+++ <sup>3</sup> | ?+++             | ? <sup>8</sup>                   |
| <i>Pfiesteria</i> -like sp. 1 |                   | +++ <sup>4</sup> | ? <sup>8</sup>                   |
| <i>Gyrodinium galatheanum</i> | +++ <sup>5</sup>  | +++ <sup>5</sup> |                                  |
| <i>Gymnodinium pulchellum</i> |                   | +++              | +++ <sup>6</sup> (RI, SI)        |
| <i>Pfiesteria</i> -like sp. 2 |                   | ? <sup>7</sup>   |                                  |

\*SL = skin lesions, N = neurocognitive, GI = gastrointestinal, RI = respiratory irritation, SI = skin irritation.

<sup>1</sup> In laboratory exposures and strongly circumstantial in MD field studies (Burkholder et al., 1997a; Levin, 1997; Grattan et al., 1998)

<sup>2</sup> Fish mortalities in home aquarium and strongly circumstantial—toxicity not yet verified. Co-occurred with a known fish pathogen, the dinoflagellate *Amyloodinium ocellatum* (Landsberg et al., 1995).

<sup>3</sup> Found in St. Johns River, FL; Neuse and Pamlico rivers, NC; and Kings Creek and Chicamacomico and Pocomoke rivers, MD (Steidinger et al., in prep.). All sites known for fish lesions and fish kills.

<sup>4</sup> Killed fish in bioassay in 1993 before experiments were stopped because of potential health concerns (Steidinger et al., unpubl. data).

<sup>5</sup> Species has been isolated from sites of ongoing fish kills in TX, SC, and MD and from known sites of historic fish kills in FL. It is reportedly toxic to fish (Nielsen, 1993) and has caused pathological effects in fish gills but no obvious ulcerated skin lesions.

<sup>6</sup> Reports of respiratory irritation experienced by field workers and commercial fishermen during a fish-killing episode in the Indian River, FL, as well as reports of burning-skin sensation on contact with water (Steidinger et al., 1998a).

<sup>7</sup> Co-occurred with cryptoperidiniopsoid in the Pocomoke River, MD, in May samples prior to fish kills; implicated in other fish kills.

<sup>8</sup> Anecdotal accounts of skin lesions in humans. Human health has not been studied in an epidemiological survey in FL (but is being planned now by FL Dept. of Health). At this stage, we do not know which dinoflagellate species, if any, are associated with presence of skin lesions in fish and therefore can only speculate that there may be a connection between a biomarker of inferred PLS presence (i.e., fish lesions) and anecdotal reports of lesions in humans.

for Disease Control and Prevention, 1998). Because many fish mortality and disease events can be attributed to several etiologies, it is absolutely critical that we determine whether PLS are involved in these events and, if they are, whether they are toxic only to natural resources or to the public as well. The potentially harmful effects of these PLS are suspected but are currently unidentified. Scenarios of risk potential are outlined in Table 1.

### **Economic Impacts**

Detailed analysis of the economic impacts caused by this group of organisms is not currently available for Florida. We are aware that an economic evaluation of the 1998 St. Lucie event has been commissioned by the South Florida Water Management District.

### **Recommendations for Action**

Determine occurrence and distribution of PLS in Florida (federal initiative)

#### **Develop molecular probes for identification and differentiation of PLS species in Florida (special state appropriation)**

Develop molecular probes for the detection of PLS toxins or bioactive compounds in natural waters

#### **Determine whether isolates are toxic or produce bioactive compounds that affect fish (special state appropriation)**

Determine whether—and if so, how—domestic animal and/or human wastes influence distribution and abundance of PLS

Determine how environmental variables influence toxic events, e.g., freshwater flow and nutrient enrichment (federal initiative)

Determine the distribution of PLS in relation to fish disease or fish kill “hot spots” and its relationship to other pertinent environmental factors such as nutrients (federal initiative)

Conduct short- and long-term studies of exposure of fish to PLS

Determine the fate and effects of toxins or bioactive compounds in the food web

## **CIGUATERA**

### **Background**

Human intoxication from the consumption of finfish and shellfish, including crustaceans, is not new for the Western Hemisphere. Perhaps the oldest report of seafood intoxication in the Americas was that by the historian Peter Martyr (1457–1526) in the West Indies, where an illness now known as ciguatera poisoning was first noted (Gruder, 1930). In 1606, the first reported cases of ciguatera from the New Hebrides in the Pacific were described. This illness was also reported from the Spanish Antilles (Aruba and Bonaire) during the 18th century, when Spanish explorers ate the “Acigua” or turban shell, *Cittarium pica* (*Livona turbo*) (Withers, 1982). In the early 1800s, Cuban ichthyologist F. Poey y Aloy described an ordinance prohibiting the sale of ciguatera-contaminated fish weighing more than three pounds and noted that, in the Caribbean, toxic fish were unevenly distributed: toxic fish could be found on one side of the island while those on the other side were nontoxic (Gordon, 1977). The origin or causative agent was unknown at the time, and symptoms were attributed to a rabies-like condition.

Since these early reports, ciguatera intoxication has continued to occur, particularly in the Caribbean region. It was not until the 1940s to late 1950s, when more accurate survival manuals for soldiers and explorers in the tropics were required, that a resurgent interest in ciguatera led

Randall (1958) to speculate that the causative agent was a toxin-producing cyanobacterium associated with reef biota. Randall correctly suggested that the toxin is accumulated through the food chain by herbivorous, nonpiscivorous, and detritus-feeding fish and that larger piscivores thus acquired enough toxin to cause symptoms in humans that eat them. Piscivores include large reef-dwelling barracuda, grouper, jacks, snapper, bonefish, eels, toadfish, mackerel, dolphin fish, and hogfish. The major causative organism of ciguatera was finally identified (Yasumoto et al., 1977; Adachi and Fukuyo, 1979) as *Gambierdiscus toxicus*, a toxic dinoflagellate that normally inhabits reefs and seaweeds in subtropical and tropical regions. Since then, several new *Gambierdiscus* species have been found (Faust, Smithsonian Institution, pers. comm.).

Ciguatera outbreaks are sometimes associated with disturbances to reefs from hurricanes (e.g., Andrew and Gilbert) (Bohnsack et al., 1994; Perrotta, see appendix), coral bleaching (Kohler and Kohler, 1992), dredging (Bagnis et al., 1990; Tebana, 1992), commercial harvesting of fish or corals by destructive methods such as rotenone and dynamite, and other anthropogenic alterations. Existing populations of *G. toxicus* increased in boat channels that were being blasted from coral (Kaly and Jones, 1994), but the species did not appear in newly exposed areas where it had not previously been observed. Newly exposed surfaces rather than the addition of nutrients to a system seem to stimulate colonization by *G. toxicus*. Both in Australia and in the Florida Keys, increased abundance of macroalgae, particularly filamentous red algae, was accompanied by increases in *G. toxicus*. Gillespie et al. (1985) also noted *G. toxicus*'s preference for red macroalgae. Other ciguateric dinoflagellates have been associated with dead corals on the inner slope of barrier reefs or in lagoonal patch reefs but not on the barrier reef's outer slope or in the back bay (Grzebyk et al., 1994).

The cultivation of *G. toxicus* has provided some insights into its biology. Laboratory cultures are less toxic than wild strains (Morton and Norris, 1989), which may reflect the laboratory environment's suboptimal conditions rather than real changes in toxin production. Some apparently nontoxic strains of *G. toxicus* have also been isolated and identified. However, the presence or absence of their genetic potential for producing toxins has not been demonstrated. Laboratory studies with *G. toxicus* showed a preference for high temperatures (27°C), elevated salinities (>36‰), and low light intensities (Bomber et al., 1985, 1988a), where it grew slowly (0.3 div/day) but had maximal toxin content. These conditions are consistent with those in its natural environment as an epiphyte on algae or reefs.

Only within the past two decades have there been major advancements and intensive chemical studies of the organisms and toxins causing this illness. In addition to *G. toxicus*, other dinoflagellate species have been implicated in ciguatera poisoning. These include *Prorocentrum lima*, *P. mexicanum*, and *P. concavum* (Besada et al., 1982; Steidinger and Baden, 1983), each of which has slightly different toxins. *Gambierdiscus toxicus*, *P. lima*, *P. mexicanum*, *P. concavum*, *P. hoffmannianum*, *Ostreopsis siamensis*, *Ostreopsis* sp., and undescribed species (Steidinger, unpubl. data) are found along the south Florida coast and in the Keys (Bomber et al., 1988b; Steidinger, unpubl. data). These dinoflagellates colonize macroalgae, algal turf, coral, sponges, detritus, sand, sediment, and seagrasses such as *Thalassia testudinum* (Anderson and Lobel, 1987; Bomber et al., 1989). *Prorocentrum* spp. have been found on seagrasses being screened for disease (Landsberg, unpubl. data) and are also being found in water samples (Steidinger, unpubl. data).

These dinoflagellates produce a number of toxins such as maitotoxin, gambiertoxin, gam-

bieric acid, and ciguatoxin (*G. toxicus*); prorocontrolide, dinophysistoxin, and okadaic acid (*Prorocentrum* spp.); ostreotoxin (*Ostreopsis* spp.); other fast-acting toxins (*Prorocentrum* spp.); and other undescribed toxins that are involved in the human poisonings known as ciguatera or diarrhetic shellfish poisoning. Ciguatera poisoning may thus be a complex of toxins, each contributing to the observed symptoms. Some other dinoflagellates are also thought to contain ciguatoxin analogs (Besada et al., 1982; Norris et al., 1985; Tindall et al., 1989), and the cyanobacterium *Trichodesmium erythraeum* has been suggested to harbor a ciguatoxin-like compound causing death in mice (Hahn and Capra, 1992; Endean et al., 1993). Okadaic acid, which is produced by *Prorocentrum lima* (Murakami et al., 1982) and *P. concavum* (Dickey et al., 1990) and is associated with diarrhetic shellfish poisoning in humans (Yasumoto et al., 1984), has been detected in shellfish and phytoplankton from the Gulf of Mexico (Dickey et al., 1992). Experimentally, okadaic acid has been shown to induce skin tumors (papillomas) in mice (Suganuma et al., 1988; Fujiki et al., 1989).

### Resource Impacts

Ciguatera toxins have been reported to have no effect on fish in the wild (Banner et al., 1966; Swift and Swift, 1993). It was assumed that herbivorous fish would remain behaviorally asymptomatic and would carry the toxin without any adverse effect (Helfrich and Banner, 1963). However, recent experiments have shown that ciguateric toxins can be lethal to fish (Capra et al., 1988) and can induce pathological changes (Capra et al., 1988; Gonzalez et al., 1994) and abnormal behavior (Durand et al. 1985; Davin et al., 1986, 1988; Durand-Clement et al., 1987; Kohler et al., 1989; Kelly et al., 1992; Lewis, 1992; Magnelia et al., 1992; Gonzalez et al., 1994; Goodlett et al., 1994). Herbivorous fish exposed directly to *Gambierdiscus toxicus* toxins or fish that are force-fed ciguatoxic fish flesh may show behavioral changes or pathological effects. It was recently postulated that tropical reef-fish disease and mortalities in southeast Florida and the Florida Keys were triggered by fish immunosuppression associated with the consumption of biotoxins (Landsberg, 1995). The species of fish implicated in the ciguatera food chain in Florida (de Sylva, 1994) are similar to those species that were affected during the 1980 and 1993–1994 reef mortalities and disease outbreaks in Florida (Landsberg, 1995). In addition, snapper, which can be highly ciguateric (Banner et al., 1966), are also susceptible to tumors (Lucké, 1942; Overstreet, 1988).

Primary stressors, such as sublethal or cumulatively lethal dietary biotoxins that may have led to disease and subsequent mortalities, were postulated (Landsberg, 1995). If there are cyclical changes in the distribution or potency of biotoxic organisms and their subsequent effect on aquatic organisms, then there may be a connection between the food preferences of the species affected, the level or type of toxin found, and the cyclical incidence of mortality and associated disease outbreaks in aquatic populations. The fact that there are numerous cases of tumors in aquatic organisms in south Florida merits an intensive study as to potential environmental triggers.

It might be expected that ciguatera outbreaks would increase if there are increased levels of ciguatoxins in the environment. Although this does not appear to be the case, it may be masked by the following factors: (1) sale of barracuda is prohibited in southeast Florida, (2) many ciguatera cases are unreported, and (3) it is difficult to determine the source of ciguateric fish (de Sylva, 1994). Also, we need to understand how ciguatoxins affect organisms at lower trophic levels.

### Public Health Impacts

Ciguatoxin is a lipid-soluble molecule that is produced mainly by *Gambierdiscus toxicus* and

that accumulates in the flesh of fish that consume it. Scaritoxin and maitotoxin were identified after ciguatera organisms were isolated. These two toxins were named for the organisms from which they were isolated, parrotfish (*Scarus viridis*) and surgeonfish (*Acanthurus* sp.), respectively (Yasumoto et al., 1977). Scaritoxin is lipid-soluble, whereas maitotoxin is water-soluble. These toxins are absorbed directly in the viscera and accumulated in muscle tissue or are modified prior to bioaccumulation. The toxins are not affected by heat or freezing and cannot be destroyed by cooking. They are also colorless and odorless and therefore are not easily detected. Recently another toxin, palytoxin, has been implicated in ciguatera-like symptoms in humans that ate smoked mackerel (*Decapterus macrosoma*) (Kodama et al., 1989). Both lipid- and water-soluble components interfere with neural conduction by acting either on the sodium or the calcium channel (Baden et al., 1990). One method of detection exploits this feature by using brain cell membranes and their receptor sites to bind toxin in samples.

Ciguatera is limited to subtropical and tropical regions and is common in the Indo-Pacific, the Hawaiian Islands, and the Caribbean Sea. The only places in the continental United States where indigenous ciguatera phytoplankton species are found, either on algal substrates or on the reefs, and where verified toxicities have been recorded from confirmed local ciguatera fish are the Florida Keys, Florida Bay, the Dry Tortugas including the nearby Tortugas Banks, and in Texas near Matagorda (Perrotta, Texas Dept. Health; Bomber et al., 1988; Tomas, unpubl.). The confirmed ciguatera cases in Texas were associated with fish captured near an oil platform 30 miles from Matagorda. These fish presumably were feeding in the Texas reef area known as the "Flower Garden Banks," where ciguatera species could grow. The threat of ciguatera in the late 1970s caused the prohibition of barracuda sales in Florida (Lawrence et al., 1980). There are at least 900 cases of ciguatera poisoning per year in Dade County alone. Ciguatera is a larger public health problem than NSP in Florida.

Evidence suggests that reef fish that acquire the toxin remain toxic permanently. Because these fish do not migrate, they remain exposed to the toxin sources (Banner et al., 1966). Contradictory evidence, however, suggests a seasonality to the ciguatera barracuda in Puerto Rico; the fish may become less toxic, or there may be a greater exchange of fish between reefs (Tosteson et al., 1988). Ciguatera barracuda have been found off Florida's west coast (Morton and Burklew, 1970; Tomas, 1996) and Florida Keys including the Dry Tortugas (Tomas and Baden, unpubl. data). During the late 1980s, ciguatera intoxications on the east and west coasts of Florida caused further alarm. Since then, confirmed cases have been documented by Florida health officials. The frequency and extent of these intoxications and the species of ciguatera fish involved were documented by de Sylva (1994). Worldwide, an estimated 50,000 victims suffer from ciguatera poisoning each year, and over 1,300 cases per annum occur in Florida alone (de Sylva, 1994). This incidence is relatively unpublicized even among medical professionals (Regalis, 1982; Freudenthal, 1990).

The presence of multiple toxins may explain why there is such a variety of symptoms of ciguatera toxicity in humans (>175 symptoms have been identified) (Swift and Swift, 1993), even though only a few of the toxins have been identified or characterized from toxic fish. Human consumption of toxic herbivorous fish is usually associated with gastrointestinal illness or neurological symptoms, whereas consumption of toxic carnivorous fish is more often associated with cardiovascular and neurological disorders (Bagnis, 1968). The human symptomology begins 1–12 hours after ingestion of contaminated fish and includes nausea, vomiting, diarrhea,

hot–cold reversals, tremors, numbness and weakness of limbs, aching joints, itching, and swelling of the tongue, mouth, and throat (Kodama and Hokama, 1989). Extreme cases, which are rare, result in paralysis, loss of consciousness, and death. In sublethal cases, the symptoms may continue for weeks to months until the acute phases abate, but in some instances, symptoms have continued or recurred years later. Ingestion of fish or alcohol may cause symptoms to recur a long time after the original intoxication. Intravenously administering mannitol to intoxicated patients has helped to reduce brain swelling and diminish the most irritating symptoms (Palafox et al., 1988; Blythe et al., 1992). A permanent cure is not available at this time.

### **Economic Impacts**

The economic impacts from ciguatera poisoning in the Caribbean are estimated to be over \$10 million. In the US and Canada, annual costs for time off work and hospitalization are estimated at \$20 million (de Sylva, 1994). Because we lack an effective screening procedure, the only reliable method of preventing intoxication is to prohibit sales of fish from known ciguatoxic areas. This action causes significant economic losses to the fishing industry in these regions (de Sylva and Higman, 1980). Liability for damages due to exposure to toxic fish falls to the seafood sellers who are considered to be responsible for ciguatera transmission even if all care has been exercised in the preparation and sale of the product (Sturm, 1991). Victims have successfully sued restaurant operators to recover ciguatera-related damages (Nellis and Barnard, 1986).

Because ciguatera cases are underreported by up to 90%, it is difficult to have accurate estimates of the economic impacts on Florida's economy. These impacts include not only lost tourism revenue, restaurant liabilities, hospitalizations, and treatment of patients but also the loss of work and medical costs incurred by recurrent symptoms months or years after the initial inflection.

### **Recommendations for Action**

- Develop accurate and rapid tests to detect ciguatera toxins in fish
- Expand the testing network (commercial and recreational) to document the occurrence and extent of ciguatoxic fish
- Develop a better monitoring system for recording and documenting confirmed incidences of ciguatera poisoning
- Increase awareness and training in the medical community for recognizing, documenting, and treating the symptoms
- Determine the economic impact and loss factors, including loss of revenue and productivity due to illness and treatment
- Survey Florida waters for ciguateric dinoflagellate species and hot spots
- Determine the transfer of toxins in the food chain and what animals at what size and weight are a public health risk
- Support and encourage the reporting network (hotline) now operating in the Miami area
- Produce public outreach literature to increase public awareness and improve reporting
- Ciguatoxin as well as other neurotoxins should be evaluated for potential chronic, immunologic, neurologic, and pulmonary effects

## TOXIC BLUE-GREEN ALGAE (CYANOBACTERIA) IN FRESH/ESTUARINE WATERS

### Background

Many of the preeminent problems associated with algal blooms in Florida's freshwater and estuarine environments involve cyanobacteria (blue-green algae). Given Florida's subtropical climate and the eutrophic nature of many of its lakes, rivers, and estuaries, it is not surprising that cyanobacteria are a major feature of aquatic ecosystems. Although there is little doubt that the phenomenon of cyanobacterial blooms predates human development in Florida, the recent boom in human population (~1,000 people/day; Smith, 1996) and land use has increased the frequency, duration, and intensity of these blooms and precipitated grave concern over their possible harmful effects.

Cyanobacteria blooms in Florida represent a major threat to water quality, ecosystem stability, surface drinking water supplies, and public health. Many of Florida's largest and most important lakes, rivers, and estuaries have been plagued by massive cyanobacteria blooms over the past several decades, including Lake Okeechobee (Havens et al., 1996), Lake Apopka, and the St. Johns River estuary (Burns et al., 1997). In addition, phytoplankton composition in many of Florida's aquatic systems has undergone rapid ecological changes that include replacing dominant types of phytoplankton taxa with potentially toxic cyanobacteria (Chapman and Schelske, 1997).

There is a serious need to define the specific relationships among freshwater cyanobacteria blooms, the production of secondary blooms in estuaries and marine coastal systems, and the harmful effects associated with them. However, because we lack research and sufficient biomonitoring programs in Florida, the relationship between cyanobacteria blooms and these associated environmental problems remains largely in the realm of incidental observation and speculation.

Although the environmental conditions that elicit cyanobacteria blooms in freshwater lakes and reservoirs have received some long-deserved attention around the world, our understanding of the causes and consequences of these blooms in Florida lags far behind. In many coastal areas, cyanobacteria blooms produced within the freshwater-riverine and lacustrine systems concentrate downstream near the more urbanized coastal areas where population centers are typically located. With the death and decay of freshwater phytoplankton downstream in the more saline reaches, there is an increased probability of the release of endotoxins, reduction of light availability in the water column, and the alteration and disruption of food webs. The increase of bioavailable nutrients from decaying freshwater phytoplankton has been observed to coincide with secondary algal blooms that are adapted to more saline environments (Christianson et al., 1988; Paerl, 1988).

The type of toxins (secondary metabolites) produced are neurotoxins, hepatotoxins, and dermatotoxins, and their production can be affected by environmental variables. Some blue-green species isolates may not be toxic, and because there are toxic and nontoxic strains, it is important to have the tools, such as molecular probes, to differentiate the two. Although species known to produce toxins elsewhere in the world have been found in Florida, not all have been associated with toxic events or positive laboratory findings.

There are about 20 species or groups of freshwater or freshwater-estuarine blue-green algae that are toxic or potentially toxic occurring in Florida waters. At risk are recreational and other

uses of eutrophic freshwater lakes and surface-water systems such as the reservoirs used as sources of drinking water. Blue-green algae blooms can taint public water supplies, even killing animals such as cattle, birds, or dogs that drink the contaminated water. Vital questions that need to be addressed include: (1) what environmental or genetic factors stimulate toxicity, (2) whether toxins accumulate in the food chain, (3) whether aquatic mammals are at risk if exposed, (4) whether humans are at risk if exposed, (5) whether toxins are delivered in drinking water, and (6) whether there are protracted effects in aquatic habitats or their components.

### Resource Impacts

Over the past decade, human-induced eutrophication of Florida estuaries has been associated with ulcerated fish, fish kills, declining fisheries resources, the decline of submerged vegetated habitats, and extensive algal blooms. Filamentous green and blue-green algae (e.g., *Cladophora* sp., *Enteromorpha* sp., *Lyngbya wollei*, *Oedogonium* sp., and *Rhizoclonium* sp.) in the St. Johns River estuary are believed to reduce ambient light levels below those required for submerged aquatic vegetation (SAV) to survive (Burns et al., 1997). Blue-green algae (e.g., *Microcystis aeruginosa*, *Anabaena circinalis*) that form surface scum have been associated with low dissolved oxygen levels (<0.5 ppm), a condition that is lethal to many fish and invertebrate species (Burns and Chapman, unpubl. data) (Fig.1). Some of the potentially toxic cyanobacteria that are known to bloom frequently in Florida waters are *Microcystis aeruginosa*, *Anabaena circinalis*, *A. flos-aquae*, *Aphanizomenon flos-aquae*, *Cylindrospermopsis raciborskii*, and *Lyngbya wollei*.

Cyanotoxins, which have been studied intensively, belong to one of two groups (hepatotoxins or neurotoxins) that are defined by the symptoms they produce in animals. Neurotoxins interfere with the functioning of the nervous system and cause paralysis of the respiratory muscles. Hepatotoxins damage liver tissues and cause pooling of blood that can lead to fatal circulatory shock or liver failure. Neurotoxins that have been described in detail include anatoxin, anatoxin-a(s), saxitoxin, and neosaxitoxin. Saxitoxin and neosaxitoxin are also common marine algal toxins that are produced by freshwater cyanobacteria as well as marine dinoflagellates. Anatoxin-a(s) is considered a naturally occurring organic phosphate that is similar in function to synthetic organophosphate insecticides, such as parathion and malathion. Hepatotoxins have been implicated in toxic events in virtually every corner of the world (Carmichael, 1997). It is now known that hepatotoxins are represented by many related forms consisting of cyclic or ringed peptides. Those consisting of seven amino acids are called microcystins, and those consisting of five amino acids are labeled nodularins. An additional hepatotoxin isolated from *Cylindrospermopsis raciborskii* has been labeled cylindrospermopsin. The World Health Organization-recommended guideline for maximum allowable concentrations of microcystins is 1 mg/L for drinking water, but no guidelines for anatoxins, saxitoxins, or cylindrospermopsin have been recommended because existing data are limited (World Health Organization, 1998).

There are few existing reports of toxic cyanobacteria events in Florida waters. Screening of events in the United States, Europe, Asia, and Australia has provided information that a significant percentage of samples analyzed were positive for cyanotoxins. In the United States, 65% of approximately 3,000 analyses (909 sample stations selected within 9 USEPA regions and 4 utilities in Canada) were positive for microcystins, and 22% exceeded World Health Organization guidelines of 1 mg/L of total microcystin (Carmichael, 1998). Of 86 sites containing cyanobacteria in Wisconsin, 25 were found to be toxic; one sample produced a neurotoxic response in a mouse bioassay (Sonzogni et al., 1988). A three-year screening project in Germany (1995–1997)

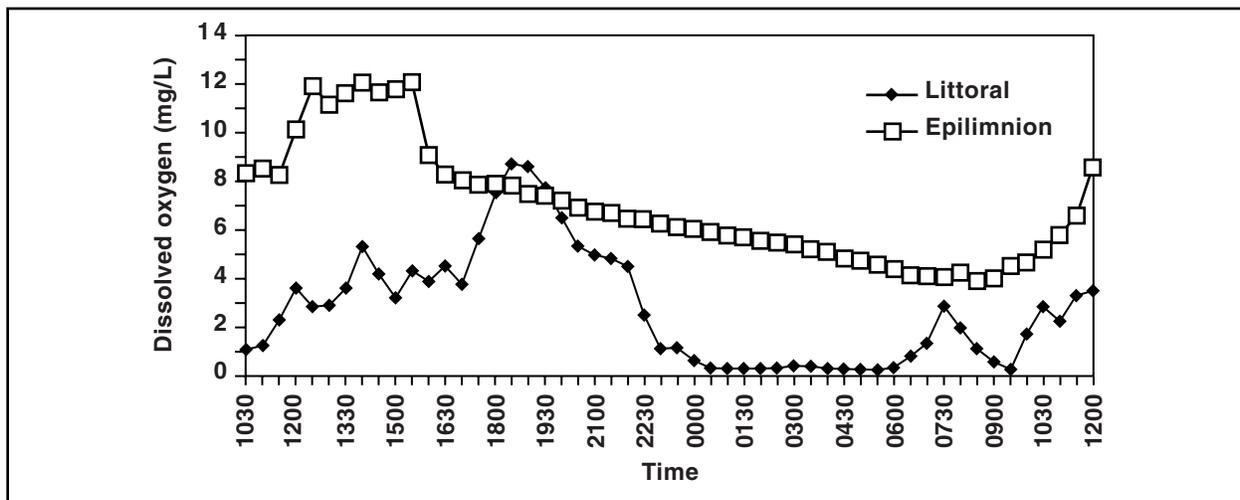
found that almost all samples containing species of *Microcystis* or *Planktothrix* (syn. *Oscillatoria*) contained microcystins and that 22% of the samples analyzed contained anatoxin-a (Chorus, 1998). Microcystins were also detected in 50 of 66 cyanobacteria-bloom samples taken from 17 lakes in Korea between 1992 and 1996 (Ho Dong Park et al., 1998). However, many cyanobacteria blooms in Florida are not reported, and no toxin-monitoring program exists for natural surface waters or potable supplies.

*Anabaena circinalis* and *Microcystis aeruginosa* are two of the most widely distributed freshwater cyanobacteria species in Florida. They are planktonic and often form extensive surface blooms and scums in eutrophic waters during calm wind and warm weather. Toxic events associated with these species have been confirmed in lakes Okeechobee and Istokpoga (Carmichael, 1992). Outside of Florida, *A. circinalis* strains have been reported to produce neurotoxin (anatoxin-a), hepatotoxin (microcystin), and paralytic shellfish poisons [PSPs; e.g., saxitoxin, neosaxitoxin], whereas *Microcystis aeruginosa* strains are known to produce all of the forms of microcystin. Acute hepatotoxicosis involving hepatotoxins is the most commonly encountered toxicosis involving cyanobacteria. Reported hazards to human health include gastroenteritis, asthma, eye irritation, blistering around the mouth and nose, and toxic injury to the liver. Microcystin is also known to be a tumor-promoter in laboratory animals (see section 5), and toxicity can be species-specific in fish and aquatic invertebrates.

*Aphanizomenon flos-aquae* is another freshwater planktonic species that is common throughout Florida. Anatoxin-a (neurotoxin) and two neurotoxic alkaloids resembling saxitoxin and neosaxitoxin (PSPs) have been isolated from *A. flos-aquae*. Anatoxin-a mimics acetylcholine and can affect respiratory muscles, causing paralysis, convulsions, and suffocation (Carmichael et al., 1979). *Anabaena flos-aquae* has been reported to produce anatoxin-a, anatoxin-a(s), and microcystin, and the toxicity of colony and clonal isolates varies, but no toxic blooms of *A. flos-aquae* have been reported in Florida.

*Cylindrospermopsis raciborskii* is a freshwater planktonic species in Florida that is known to produce cylindrospermopsin (hepatotoxin). Toxic effects in mice include problems with kidneys, adrenal glands, lungs, intestine, and liver. This species is found primarily in tropical and subtropical regions and appears to be increasing its distribution in Florida (Chapman and Schelske, 1997); it forms subsurface blooms and has been reported in the St. Johns River, Wekiva River, Newnans Lake, Lake Dora, Lake Eustis, Lake Griffin, Lake George, Lake Okeechobee, Lake Wauberg, and Lake Disston. *C. raciborskii* has been implicated as a possible cause of recent mass mortality and reproductive failure in American alligators (*Alligator mississippiensis*) on Lake Griffin in the Oklawaha River system in central Florida. Preliminary investigations of unusual numbers of dead and moribund alligators during 1998 suggest a temporal association with heavy blooms of *C. raciborskii*, which has become the dominant blooming algae in Lake Griffin. Necropsy examination of four alligators was inconclusive, but analysis of tissues by ELISA immune assay revealed the presence of small quantities of microcystin toxin.

The mat-forming filamentous alga *Lyngbya wollei* can grow to bloom proportions in freshwater littoral zones in Florida, degrading nearshore areas and preventing light from reaching submerged aquatic vegetation (Burns and Chapman, unpubl. data). This filamentous cyanobacterium has also been reported to produce a potent, acutely lethal neurotoxin related to known PSPs (decarbamoyl gonyautoxins 2 and 3) (Carmichael, 1997).



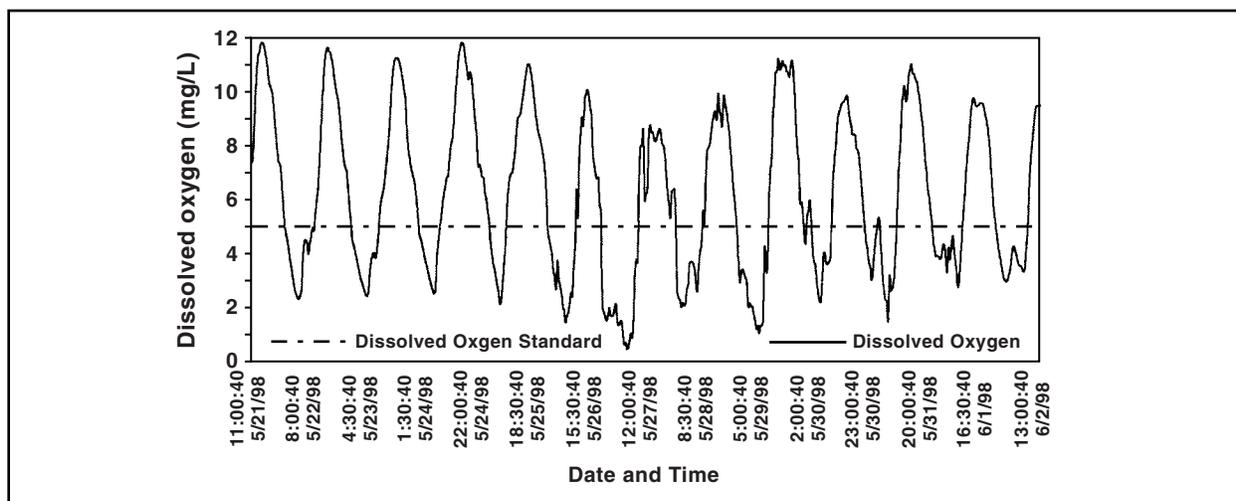
**Figure 3**

Diel dissolved oxygen levels during a cyanobacteria bloom (chlorophyll *a*: 115  $\mu\text{g/L}$ ) in Doctors Lake, tributary of the lower St. Johns River, Florida, on July 17, 1997.

Human-induced eutrophication and the decline of submerged aquatic vegetation (SAV) habitats caused by the production of algal blooms and the subsequent loss of available underwater irradiance have been documented in many coastal estuaries (Batiuk et al., 1992; McComb, 1995). Impacts to SAV habitats caused by filamentous algal mats and phytoplankton surface scums that are concentrated near the shoreline by winds are rarely quantified. These algal mats and surface scums are typically attached to and may be held in place by SAV. SAV provides habitat vital for both freshwater and estuarine species, so these areas are often identified as locations where algal death and decay have the greatest effect on food web dynamics, dissolved oxygen concentrations, aesthetics, and recreation.

Although the production of toxins and the deaths of large vertebrates may be one of the most dramatic consequences of algal blooms, the creation of anoxic conditions may be one of the most common ecological problems. Large amounts of algal biomass in nearshore or flow-restricted regions of aquatic ecosystems can cause highly elevated levels of biological oxygen demand (Figs. 3 and 4). When the oxygen supply is depleted (anoxia), organisms that rely on oxygen die. Prolonged periods of anoxia can also lead to other environmental problems, including foul odor and the proliferation of undesirable microorganisms. During cyanobacteria blooms in the lower St. Johns River, diurnal supersaturation and anoxia are common in nearshore areas (Figs. 3 and 4). Under such conditions, levels of dissolved oxygen can fail to reach the Florida minimum standard (5 ppm) during the night (Figs. 3 and 4), when plants use more oxygen than they produce. Biota that are unable to escape to offshore areas, where dissolved oxygen concentrations remain adequate, may not survive.

It is well known that phytoplankton can be a major absorber of light in the water column (Kirk, 1994), and independent of toxic effects, the influence of cyanobacteria blooms on light transmission can disrupt aquatic ecosystems (Paerl, 1988). In shallow aquatic ecosystems, surface blooms of algae can limit the amount of light that reaches benthic algae and macrophytes. The worldwide loss and decline of SAV and associated communities has been noted following a



**Figure 4**

Diel dissolved oxygen levels in the littoral zone during a cyanobacteria bloom (chlorophyll *a*: 48–64  $\mu\text{g/L}$ ) in Doctors Lake, tributary of the lower St. Johns River, Florida.

decline in underwater irradiance. The loss of underwater light availability has been attributed primarily to eutrophication processes that result in an over-abundance of algae. Light attenuation by cyanobacteria surface scums can reduce surface irradiance to less than 10% at a depth of 0.65 m in the lower St. Johns River. An increase in light attenuation by surface cyanobacteria scums and filamentous mats is in part responsible for the decline of submerged aquatic vegetation in some areas of the river.

The effect of cyanobacteria on grazing activities and the structure of zooplankton communities is another ecological consequence associated with cyanobacteria that has been documented for a number of freshwater ecosystems (e.g., Snell, 1980; Starkweather, 1981; Ostrofsky et al., 1983; Starkweather and Kellar, 1983; Nizan et al., 1986; Fulton and Paerl, 1987; Lampert, 1987; Gilbert, 1990, 1994; Gilbert and Durand, 1990; Penaloza et al., 1990; DeMott and Moxter, 1991; Forsyth et al., 1992; Kirk and Gilbert, 1992). Typically large cladocerans are severely impacted by planktonic cyanobacteria (Gilbert, 1990; Reinikainen, 1997), whereas copepods, rotifers, and small cladocerans are relatively resistant to large blooms of cyanobacteria (Fulton and Paerl, 1987). An additional group of organisms subject to the effects of cyanobacterial blooms are benthic and epifaunal invertebrates. Surface blooms of cyanobacteria in the St. Johns River frequently accumulate and concentrate along the shoreline. This pattern potentially exposes benthic and epifaunal invertebrates to cyanobacterial toxins and low dissolved oxygen concentrations.

### Public Health Impacts

Humans can suffer physically from exposure to cyanobacteria in freshwater or estuaries. Documented cases have involved severe respiratory distress, kidney and liver disease, allergic asthma, neurointoxication, skin rashes or necrosis, and death (Falconer, 1993, 1996, 1997).

As in the case of other HAB species, the majority of studies on the toxicity of cyanobacteria have been animal experiments on acute toxicity or accidental animal or human poisonings (Carmichael and Falconer, 1993; Falconer and Humpage, 1996).

### **Economic Impacts**

Little is known about economic losses resulting from cyanobacteria blooms in Florida's freshwater and estuarine waters. Certainly, there are costs associated with treatment of reservoirs that store drinking water, and there must be accountable losses from reduced recreational activity on waterways during extensive blooms, but to our knowledge this has not been quantified.

### **Recommendations for Action**

Determine distribution of toxic and nontoxic strains in Florida waters (special state appropriation)

Develop epidemiological studies to determine what public health risks are involved

Develop economic impact studies to properly evaluate losses by locale or industry

Determine the roles of nutrient enrichment and managed freshwater flow in bloom development

Determine the fate and effect of toxins in the food web

Determine the relationship between blue-green toxins and alligator deaths

Investigate the applicability and efficacy of control and mitigation methods

## **HARMFUL MICROALGAE AS TUMOR PROMOTERS**

### **Background**

The presence of toxic plants, microbes, and animals in marine systems is well documented (Halstead, 1967; Paul and Fenical, 1986; Paul, 1987). Of interest in recent years are the number and types of plant and animal metabolic byproducts that may be both beneficial and detrimental to humans or other aquatic organisms. A range of organisms with antineoplastic (anticancerous) compounds as well as neoplastic (cancerous) compounds has been documented (Burkholder, 1968). In many cases, the effect that cancer-inducing compounds has on marine systems is completely unknown, and their potential adverse effects on aquatic organisms are not usually considered.

The role of harmful algal blooms both in shellfish poisonings of humans and in mass mortalities of aquatic organisms is well documented. Because many marine harmful algal blooms are planktonic, acute in nature, and may lead to fast-acting shellfish poisoning events or mass mortalities of aquatic organisms, many of the potential longer-term chronic effects associated with these biotoxins are unknown. Filter-feeding bivalves accumulate microalgal biotoxins, which in turn become available to consumers, both animal and human, through the food chain.

In some animal species, the presence of harmful algal blooms has been demonstrated to lead to acute behavioral, physiological, or pathological responses, and in some cases, mortalities. As is true for other contaminants or toxicants, potential long-term effects of biotoxins on aquatic animals or on public health may be expressed in terms of susceptibility to disease, immunosuppression, reduced growth, effects on reproduction, or the development of tumors. There is little information concerning chronic, lethal, or sublethal effects on animals caused by bioaccumulated or biomagnified algal toxins, nor do we know whether such effects render animals susceptible to disease. The potential for some biotoxins to act as immunomodulators has not yet been explored.

Some of these natural toxic or carcinogenic compounds could have increased in the past 20 years because of anthropogenic activities that may have led to an increase in the distribution of toxic algae. More frequent occurrences of harmful algal blooms and incidents of shellfish toxic-

ity affecting humans have been recorded in the past 20 years in certain parts of the world (Hallegraeff, 1993). In many coral reef areas, there have been increased nutrient inputs and associated increases in macroalgal cover on the reefs (Lapointe, 1989). Increased macroalgal or macrophyte cover that is used as substrate by numerous toxic microalgae can lead to an increased abundance of these species. In Florida Bay, declines or shifts in seagrass (*Thalassia testudinum*) abundance (Robblee et al., 1991) and/or concomitant expansion in macroalgal cover may also be increasing the distribution of toxic microalgal species that are inadvertently available to herbivores through diet. The dominance of the planktonic blue-green alga, *Synechococcus elongatus* in Florida Bay has been well documented, but the distribution and abundance of toxic benthic microalgae is not currently well known (Steidinger, unpubl. data).

### Resource Impacts

Microalgal toxins can be ingested by shellfish, herbivorous or omnivorous fish, or turtles when filter feeding and when browsing on plankton, macroalgae, algal turf, coral, sponges, seagrasses, detritus, sand, or sediment. Toxic dinoflagellates (see *Ciguatera*) that are attached to seagrasses could inadvertently be consumed by green turtles (*Chelonia mydas*) feeding on *Thalassia* (Bjorndal, 1980). Loggerhead turtles (*Caretta caretta*) could accumulate toxins by preying on toxic molluscs or crustaceans. The fact that some mollusks filter-feed and retain toxins associated with dinoflagellates is unequivocal. At least four types of human poisonings are attributed to consumption of toxic shellfish (Steidinger, 1993; Baden et al., 1995). A wide range of mollusk species are present in Florida Bay, and even though no human poisonings associated with consumption of toxic shellfish are known from the area, biotoxins that could affect aquatic organisms may still be present in these species. Feeding habits and diet of tumored turtles and fish in this area should be investigated.

Most etiological studies concerned with the development of tumors in aquatic organisms such as shellfish and fish consider several factors that may be involved either alone or together. These factors include oncogenic viruses (Kimura et al., 1981; Oprandy et al., 1981; Cooper and Chang, 1982; Oprandy and Chang, 1983), genetic predisposition (Anders, 1967; Vielkind and Vielkind, 1982; Vielkind and Dippel, 1984), chemical contaminants (Kimura et al., 1984; Vogelbein et al., 1990), ultraviolet radiation from sunlight, or other environmental factors (Herbst, 1994). Although the influence of such factors has been postulated, there still remain many cases where cause-effect relationships of tumor induction have not been proven.

Rarely are biotoxins considered to be responsible for tumor induction. This is surprising given their widespread geographical distribution and the resultant probability that many organisms are exposed to them. Biotoxins have a clearly defined role in (1) human poisonings associated with consumption of shellfish and fish; (2) aquatic mortalities, disease, or immuno-suppression; (3) tumor induction in mammals; and (4) measured effects on vertebrates (Suganuma et al., 1988; Fujiki et al., 1989; Steidinger, 1993; Landsberg, 1995). Recently, the potential role of biotoxins in tumor development in marine organisms has been explored (Landsberg, 1995, 1996; Landsberg et al., in review).

In Florida, there are several locations where the incidence of tumors in aquatic animals is significantly higher than elsewhere in the state. The influence of environmental factors on the development of tumors has in some cases been postulated but not demonstrated. For example, since the mid 1980s, gonadal tumors have been reported in the hard clams, *Mercenaria mercenaria* and *M. campechiensis* and in their hybrids in the Indian River Lagoon (Hesselman et al., 1988; Bert et al.,

1993). Studies (FDEP, unpubl. data) in the Indian River have not revealed any significant contaminant levels at sites where these tumors have been prevalent (Bert et al., 1993). The role of biotoxins as potential promoters of shellfish tumors needs to be investigated.

Sea turtle fibropapillomatosis (FP) is a neoplastic disease that has been documented from the Atlantic Ocean (Florida, Bahamas, Brazil), the Indo-Pacific region (Hawaiian Islands, Australia, Sri Lanka, Sarawak, Malaya, Japan), and the Caribbean Sea (Cayman Islands, Puerto Rico, Dominican Republic, Virgin Islands, Barbados, Antigua and Barbuda, Central America) (Balazs, 1991; Herbst, 1994; Williams et al., 1994). Fibropapillomas are commonly found in green turtles, *Chelonia mydas*, although loggerhead turtles, *Caretta caretta*, and olive ridley turtles, *Lepidochelys olivacea*, are also known to be affected (Jacobson et al., 1991; Herbst, 1994). The tumors are benign, but growths of fibropapillomas can adversely affect locomotion, vision, swallowing, and breathing. Visceral fibromas can disrupt normal organ function to the extent that death ensues (Herbst, 1994). Fibropapillomatosis was first reported when an affected turtle was captured in 1938, and 1.5% (3/200) of free-ranging green turtles in Key West, Florida, were found to be affected (Smith and Coates, 1938). In the last 20 years, FP has increased dramatically in green turtles in the Hawaiian Islands, Florida, and the Caribbean Sea. In the Hawaiian Islands, there is a clearly defined distributional pattern of FP, with up to 92% of turtles in Oahu affected (Balazs, 1991). In Florida Bay in 1995, FP was reported in up to 60% of green turtles and 11% of loggerheads (B. Schroeder, National Marine Fisheries Service, pers. comm.). FP has also increased significantly in the Indian River (Ehrhart, 1991). In the Caribbean, increases in FP have also been noted since the mid-1980s, particularly in Puerto Rico and Colombia (Williams et al., 1994).

Possible causes of FP include infectious agents such as oncogenic viruses (Jacobson et al., 1991; Herbst et al., 1995; Casey et al., 1997) and parasites (Dailey and Morris, 1995; Aguirre et al., in press), genetic predisposition (Herbst, 1994), toxicants (Aguirre et al., 1994), ultraviolet radiation (Smith and Coates, 1938), and other as yet undefined synergistic environmental factors (Herbst, 1994). Herpesvirus was detected in fibropapillomas from Florida green turtles by the use of electron microscopy (Jacobson et al., 1991), and experimental transmission of fibropapillomas was achieved by inoculating disease-free green turtles with cell-free tumor homogenate (Herbst et al., 1995). The presence of retrovirus in both tumored and nontumored green turtles, with a higher incidence of virus in the former, has recently been demonstrated (Casey et al., 1997). Attempts to culture virus have not yet been successful, nor have Koch's postulates been fulfilled. The fact that tumors have been experimentally induced in animals using oncogenic viruses or cell-free filtrates in laboratory studies does not necessarily infer a single cause-effect relationship in what may be a multifactored or multistep neoplastic process occurring in the wild.

Harmful microalgae produce some of the most potent toxins known. Although marine biotoxins cause acute human and animal poisonings (Steidinger, 1993; Baden et al., 1995), act as tumor promoters in mammals (Fujiki and Suganuma, 1993; Falconer and Humpage, 1996), and are suspected for chronic effects or tumor induction in aquatic animals (Landsberg, 1995, 1996), their potential role as tumor promoters in FP has not been studied (Landsberg, 1995). Because toxic algal blooms tend to be acute in nature and lead to fast-acting shellfish poisoning events or mass mortalities of aquatic organisms, many of the potential chronic effects of biotoxins produced by either planktonic or benthic microalgae are unclear.

*Prorocentrum lima*, *P. concavum*, and *P. hoffmannianum* are benthic dinoflagellates produc-

ing the tumor-promoter okadaic acid (OA) (Murakami et al., 1982; Dickey et al., 1990; Aikman et al., 1993), which significantly affects cellular processes (Bialojan and Takai, 1988; Fujiki et al., 1989; Haystead et al., 1989; Herschman et al., 1989; Yamashita et al., 1990; Sakai and Fujiki, 1991; Fujiki and Suganuma, 1993; Schönthal and Feramisco, 1993). OA has been experimentally shown to induce skin papillomas and carcinomas in mice and adenomatous hyperplasia and adenocarcinomas in the stomachs of rats in two-stage carcinogenesis experiments (Suganuma et al., 1990; Fujiki and Suganuma, 1993). Nothing is known about the potential tumor-promoting effects of OA on aquatic animals.

Green turtles show high fidelity toward their feeding sites, where they graze on specific macroalgal and seagrass substrates (Balazs et al., 1994a, 1994b, in press a, in press b; Russell and Balazs, 1994). Benthic *Prorocentrum* have a worldwide distribution (Steidinger, 1997), are common in tropical ecosystems, and are epiphytic on macroalgae and seagrasses (Fukuyo, 1981; Anderson and Lobel, 1987; Bomber et al., 1989) that are normal components of green turtle diets (Mortimer, 1981; Garnett et al., 1985; Russell and Balazs, 1994). Because *Prorocentrum* species are probably consumed by turtles grazing on macroalgae or seagrass, green turtles are potentially exposed to OA. Landsberg (1998a) and Landsberg et al. (in review) document the distributions of *Prorocentrum* species known to produce OA and the presence of FP in turtles in Hawaii and present preliminary evidence that indicates that there is a link between the distribution of FP in the Hawaiian Islands and the distributions of benthic *Prorocentrum* species known to produce OA. They demonstrated that in high-risk FP areas, the turtle's preferred diet of macroalgae and seagrass has a high incidence of toxic *Prorocentrum* species, so turtles are presumably exposed to OA.

More information is known about the role of cyanobacterial toxins in the development of tumors or chronic health effects on animals than is available for dinoflagellates or other microalgal species. A few studies have investigated the subchronic or chronic effects of toxic cyanobacteria; most concentrate on the hepatotoxic effects of *Microcystis*. When mice were exposed to drinking water containing dilutions of toxic *Microcystis* extract during the course of one year, 5.6% of the mice receiving higher concentrations developed tumors, whereas only 0.89% of those exposed to lower concentrations or no toxin did.

In standard two-stage carcinogenicity tests using DMBA as an initiator, a significantly greater weight of skin papillomas developed in mice receiving drinking water with *Microcystis* extract. A parallel experiment using *Anabaena* showed no evidence of tumorigenicity. In order to investigate the potential effects of cyanobacteria toxins on human health, three different areas of investigation have been pursued at the molecular, cellular, and population levels (Falconer and Humpage, 1996).

Like okadaic acid, nodularin and microcystin are phosphatase inhibitors. Protein phosphatases are integral components of cellular processes, and inhibition leads to numerous cellular and organismal effects and to the induction of tumors. The organ specificity of microcystin is different from that of okadaic acid, due to the active concentration of microcystins in the liver and the apparent lack of easy penetration of microcystin into cells without a bile-acid system (Falconer, 1993). Because microcystins are preferentially taken up by hepatocytes (liver cells), it is expected that microcystins would be most likely to promote liver tumors (Carmichael and Falconer, 1993). When mice were exposed to high doses of *Microcystis* extracts in drinking water (89 mg/day), their body weight increased 16% less than controls did. They also showed a steeper

mortality curve, although statistically this was not significantly different from the low-dose group. In all exposed mice, tumors of the duodenum and lymphoid tumors of liver, thymus, and spleen were demonstrated (Falconer and Humpage, 1996).

In addition to the potential for tumor induction in shellfish, toxic microalgal blooms may also precede or coincide with some unexplained mass mortalities or disease phenomena. Conversely, diseased or parasitized shellfish may be more susceptible to, and further weakened by, harmful algal bloom exposure. Bacterial pathogens such as *Vibrio* spp. are often associated with harmful algal blooms. Such bacteria are well known as pathogens in shellfish that are not exposed to harmful algal blooms, but it is not known whether bloom-associated bacteria cause disease in shellfish that are exposed to these blooms. The links between chemical contaminants and tumors or disease susceptibility in shellfish have been relatively well researched. However, studies of the epizootiology of disease and tumors in shellfish should also take into account environmental factors, particularly the distribution of harmful algal blooms, and a potential correlation with accumulated biotoxins.

### Public Health Impacts

Because multiple toxins are associated with many benthic dinoflagellate species, aquatic organisms may be exposed through their diets to various combinations of these numerous toxins during their lifetimes. How these toxins are accumulated, sequestered, excreted, or biochemically transformed by these aquatic consumers is currently unknown. It is unclear what potential risk this may pose to humans who consume these aquatic organisms and are exposed to low toxin levels for a long period of time.

The marine cyanobacterium *Lyngbya majuscula* produces known tumor promoters, lyngbyatoxin a and aplysiatoxins. Although these have been tested by skin application in mice, nothing is known of their oral toxicity or risk to humans.

The potential chronic effects of cyanobacterial toxins on the health of Florida's citizens is currently unknown. The strongest current evidence for a link between human cancer and cyanobacteria in water supplies comes from research in the Department of Epidemiology at Shanghai Medical University (Falconer and Humpage, 1996). These studies were motivated by a very high incidence of primary hepatocellular carcinoma (liver cancer) in particular regions of southeastern China coupled with an overall higher incidence of the disease than in the rest of the world. In hyperendemic regions of southeastern China, incidences of over 60 cases per 100,000 people have been reported (Yu, 1994). Although other compounding factors may contribute to liver cancer in this area, such as the high incidence of the Hepatitis B virus and the presence of aflatoxins (also known carcinogens) in food such as corn, there is still circumstantial evidence for a possible role of cyanobacterial toxins. Surface water samples in areas with high incidences of hepatocellular carcinoma contained an average of 6.5 mg/l microcystin compared to undetectable concentrations in nearly all well-water samples. The drinking water of 20 patients with hepatocellular carcinoma contained microcystin levels that averaged  $0.6 \pm 0.28$  mg/l, whereas the drinking water of 19 control patients averaged  $0.36 \pm 0.22$  mg/l (Yu, 1994).

### Economic Impacts

The chronic presence of natural toxins in the food chain is likely to affect endangered species and commercial and recreational fisheries, causing economic losses and natural resource pressures; it also has important societal and public health implications. It is critical that the role of

natural dietary toxins in marine ecosystems and how their distribution may be potentially affected by anthropogenic factors in stressed ecosystems be understood.

### Recommendations for Action

The potential role of biotoxins such as OA in tumor development in sea turtles should be further explored, either for direct tumorigenic effects, as co-factors, or as sublethal immunosuppressive factors that render animals susceptible to oncogenic viruses or other pathogens

Determine the fate and effects of toxins in the food web

Conduct animal exposure studies to determine effects of specific tumor-promoting compounds

Isolate and maintain potentially toxic species for toxin or bioactive compound identification

Include potential species of concern in any state-wide survey

## MACROALGAE

### Background

Macroalgal blooms in Florida waters can adversely affect natural resources, fisheries, tourism, and local economies. Although these blooms are not as frequent as microalgal blooms, they are dramatic because of the sheer biomass that is produced. For example, photographs show people standing on 6-foot-deep beached algal mats (Tampa Bay, Pinellas County Health Department, pers. comm.) and waist-deep in a shallow south Florida embayment with macroalgae as far as one can see (ECOHAB, 1996). Divers have taken film and video of specific events that show drift macroalgae in such dense aggregations that visibility is substantially reduced (Jim Green, *Codium* bloom, southeast Florida, 1990, pers. comm.; Ray Odor, June grass, west Florida coast, 1997, pers. comm.).

In temperate waters, most of the macroalgal blooms are caused by filamentous green algae such as *Cladophora* (e.g., see Valiela et al., 1997). On the other hand, Florida has temperate, subtropical, and tropical regions from Jacksonville to the Dry Tortugas, and consequently, Florida waters support a wide variety of green, brown, and red algae that can bloom. Although increased biomass of macroalgae can be in the form of attached or drift (dislodged or fragmented) plants, many of the problem events are from drift algae that accumulate because of tidal or other current action. These blooms can displace seagrasses, other macroalgae, corals, and other faunal communities.

In the northern Gulf of Mexico and off north and central Florida, there are June grass events that typically involve drifting filamentous green algae that finally strand on the beaches. Sometimes such events are associated with hypoxia or anoxia that affect benthic communities. In south Florida, algal clumps of *Laurencia* drift in a movement that approximates tumbleweeds. In this case, the drift algae is beneficial, providing habitat for young lobsters (Holmquist, 1994). However, when the algae decays it creates biological oxygen demands that can affect benthic communities. In Tampa Bay in the 1960s and 1970s, drift mats of intermixed *Gracilaria*, *Hypnea*, *Ulva*, and other algae were seasonally dominant. These noxious algal events in Tampa Bay, more specifically Hillsborough Bay, have dramatically declined, principally because of reduction in nitrogen loading from industrial and wastewater inputs (Johansson, pers. comm.).

Nutrients play a role in stimulating macroalgal blooms, as do physical and biological forcing mechanisms such as currents and herbivory by reef or bay-bottom fauna. According to Valiela et

al. (1997), herbivory as a control mechanism is significant only when nitrogen (N) levels are low and the algal species is N-limited. What contributes to the increase in macroalgal biomass—eutrophication, lack of predation, or physical concentration of drift plants? It appears that all three have a role.

Another case in southeast Florida involves *Codium*, a tubular, branching green alga that can smother reefs or have other deleterious effects. *Codium isthmocladum* blooms in southeast Florida between Ft. Lauderdale and Jupiter. Although this is an example of a macroalgal bloom occurring in one specific area, the variables leading to it are similar to those involved in other macroalgal blooms, so *Codium* can be used as a case study. Variables that can limit the occurrence and growth of macroalgae are interwoven in importance. Temperature can limit species occurrence, distribution, seasonality, and growth. Light penetration and availability can influence growth and seasonality. Removal of grazers, i.e., the herbivores, can favor overgrowth. Nutrients, particularly those that are limiting, can provide competitive advantages for certain species. All of these factors are interactive. For example, Valiela et al. (1997) point out that extremely high loading of N in a water system can lead to hypoxia and reduce the populations of invertebrate grazers. According to LaPointe (1997), grazing pressure and low N levels on coral reefs in the Caribbean and south Florida keep macroalgae in check. LaPointe (1997) schematically demonstrated the effects of grazing and nutrients on tropical and subtropical coral reefs. Low levels of nutrients and high levels of grazing resulted in high coral cover, whereas low levels of nutrients and levels of grazing resulted in dominance of turf algae. High levels of nutrients and high levels of grazing resulted in a prevalence of coralline algae, and high levels of nutrients and low levels of grazing resulted in dominance of macroalgae. In some areas, scientists disagree as to whether grazing or increased nutrients is the more significant controlling factor in macroalgal blooms.

High biomass of macroalgae in shallow embayments or on coral reefs does require increased nutrients, but the type of nutrient and the pulsing of that nutrient can determine what species are favored. Some species may respond to increased N but not to increased phosphorus (P), whereas another species may have the opposite requirements or be able to use organic sources. *Codium* appears to be N-limited. According to LaPointe (1997), levels of dissolved inorganic nitrogen (DIN) and soluble reactive phosphate (SRP) in southeast Florida are above the threshold levels for macroalgal blooms. The question then becomes, what is the source of the DIN in southeast Florida? LaPointe et al. (1990), LaPointe (1997), and Shinn (pers. comm.) point out that submarine groundwater reaches reefs off Palm Beach County and in the Florida Keys. It is associated with lower salinity and high Radium-226 in bottom waters (e.g., off Jupiter Inlet) on the reef. Septic tanks influence groundwater discharge off the Keys, and it is thought that rainfall and shallow aquifer discharge off Palm Beach County, Class 1 injection-well seepage, ocean-outfall sewage discharge, ocean upwelling, and even atmospheric deposition could contribute DIN and raise it above the threshold level. Factors in addition to nutrients—e.g., predation, light, temperature, and other factors—shape population increases. Chemical, biological, physical, and meteorological variables all interact to determine whether there will be a *Codium* buildup, what areas will be affected, and how the bloom will dissipate.

The *Codium* event off Palm Beach County was first documented in 1989–1990. It has typically occurred between spring and fall, peaking in summer and dying back in winter. Carlson and Roberts (1991) summarized the results of a *Codium* workshop that detailed the development

of the blooms and identified data gaps needed to assess environmental cause-and-effect relationships. Much of the information indicates that *C. isthmocladum* is a deep-water species, occurring in depths up to 300 feet. This alga can break loose or fragment and can be transported across the shelf into the reef areas, where it can accumulate on the lee side of depressions and create mats greater than four feet thick. It can also reattach to a substrate after being adrift. The sources of nutrients at different depths are still being debated, but the influence of physical parameters and events can be obvious. In August 1992, *Codium* was physically dispersed by Hurricane Andrew. Although continuously present in offshore waters, *C. isthmocladum*, patchy and attached, reappeared in 1996 and was densest in depths between 100 and 130 feet. According to Bill Parks (pers. comm.), this is the area where drift populations have originated historically. This information was presented at a second meeting on *Codium* on February 27, 1997, where discussion continued regarding the respective influences of groundwater discharge and of upwelling as factors that supply additional nutrients, particularly dissolved inorganic nitrogen.

### Resource Impacts

The most obvious natural resource impact is that marine macroalgal blooms smother the sea bottom, whether coral reef or sand. This often kills the bottom community. Blooms can also cause hypoxia and anoxia, with the same result. Whenever there is an anoxic event, there can be a hydrogen sulfide buildup, which leads to associated community shifts. Finfish may avoid affected areas and become displaced or, in some cases, become diseased (Landsberg, 1995). *Codium* blooms appear to have occurred during dry years and were not recorded in two wet years, 1994 and 1995. Wet years, associated with high runoff and high turbidity, may limit light and therefore growth, although causal relationships still need to be determined.

Macroalgal blooms (e.g., *Codium*, *Caulerpa*, *Laurencia*, or other types) can foul lobster and crab traps, aquaculture in-water structures such as ropes, trays, and cages, and even the sea floor. Fouling requires routine monitoring and cleaning of equipment, structures, or leased bottom. In some cases, drift algae that accumulate and decay are a source of hypoxia, and control measures (e.g., nets) may have to be implemented to keep the algae from reaching cultured stock.

Macroalgal blooms are not limited to marine environments in Florida. They can also occur in freshwater habitats. For example, in 1997, blooms of filamentous green algae formed extensive littoral mats in the lower St. Johns River in late winter–early spring. These blooms have raised questions on potential negative effects to submerged aquatic vegetation and the role of nutrients, particularly nitrogen, in the support of such biomass. The most problematic species belong to *Oedogonium*, *Rhizoclonium*, *Enteromorpha*, *Cladophora*, and *Stigeodinium* (Burns and Chapman, unpubl.).

### Public Health Impacts

There are no known public health impacts from macroalgal blooms in Florida.

### Economic Impacts

Because of their visibility and impacts to reef or bay faunal and floral communities, these macroalgal blooms can adversely affect local industries associated with recreational use of waters, such as diving, fishing, and tropical fish collection. The displacement of fish or actual loss of fish (kills or disease) and the loss of corals and other benthic communities have not been quantified, nor has the loss of revenue to local communities.

**Recommendations for Action**

Survey *Codium* populations out to 300 feet deep in the area of known occurrence for spatial and temporal variability and determine sources of drift algae

Survey invertebrate herbivore populations and determine food sources and feeding rates

Verify environmental regulators of growth, e.g., temperature, light, nutrients

Determine sources of nutrients and whether there is a lag in growth influenced by source of nutrients or storage of nutrients

Determine submarine groundwater discharge points in relation to *Codium* distribution and develop a groundwater flow model

Monitor nutrient levels in the areas of historical *Codium* buildup

Identify markers to distinguish between sewage-related nutrients and upwelled nutrients

Determine the economic impact of such blooms on local economies

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**APPENDIX I**  
**HAB SPECIES AND TOXINS**

## Known or potentially toxic or harmful algal species in the Gulf of Mexico and on the Atlantic coast of Florida

| Toxin   | Impact   | Species  | Habitat                              | Distribution                 | Comments   |
|---|--|--|--------------------------------------|------------------------------|--|
| <b>Brevetoxins (acute toxicity [LD<sub>50</sub> i.p. mouse] is 0.2 mg/kg)</b>                             |  |  |                                      |                              |  |
| Brevetoxins   | Shellfish poisoning, fish and bird kills, implicated in manatee and turtle mortality, respiratory irritation in humans | <i>Gymnodinium breve</i> (dinoflagellate)          | Oceanic/coastal/estuarine planktonic | Gulf-wide, Atlantic coast FL | Documented ~60 times in the last 150 years; persistent in FL coastal waters over the last 45 years; from 1994-1996 it ranged in FL from Dry Tortugas to Pensacola and co-occurred with <i>A. monilatum</i> in northwest FL and <i>G. mikimotoi</i> in southwest FL |
| Brevetoxins?  | Fish kills, low dissolved oxygen   | <i>Gymnodinium pulchellum</i> (dinoflagellate)     | Estuarine/planktonic                 | Southeast FL, Indian River   | Implicated in a 1996 fish kill   |
| Brevetoxins   | NSP? Fish kills?   | <i>Chattonella antigua</i> (raphidophyte)          | Coastal/planktonic                   | FL, LA, TX                   |  |
| Brevetoxins   | NSP? Fish kills?   | <i>Chattonella subsalsa</i> (raphidophyte)         | Coastal/planktonic                   | FL, LA, TX                   | FL isolates produce toxins   |
| Brevetoxins   | NSP? Fish kills?   | <i>Fibrocapsa japonica</i> (raphidophyte)          | Coastal/planktonic                   | FL, TX                       | Toxic in lab studies   |
| <b>Pfiesteria-like</b>  |  |  |                                      |                              |  |
| Unknown   | Fish lesions?  | Cryptoperidiniopsis and "Lucy" (dinoflagellates)   | Estuarine/planktonic/benthic         | FL                           | Occurs at northeast FL fish lesion event areas but not proven to produce bioactive compounds   |
| <b>Ciguatera (acute toxicity of ciguatoxin [LD<sub>50</sub> i.p. mouse] is 0.45 µg/kg)</b>                |  |  |                                      |                              |  |
| Ciguatoxin plus others  | Ciguatera, human illness, fish kills, fish disease?  | <i>Gambierdiscus toxicus</i> (dinoflagellate)      | Benthic                              | South FL and TX              | Under-reported illnesses; FL strains produce toxins  |
| Okadaic acid plus others  | DSP?/Ciguatera?  | <i>Prorocentrum belizeanum</i> (dinoflagellate)    | Benthic                              | South FL                     | Toxic in lab studies   |
| Okadaic acid plus others  | DSP?/Ciguatera?  | <i>Prorocentrum hoffmannianum</i> (dinoflagellate) | Benthic                              | North and southwest FL       | Toxic in lab studies; FL strains produce toxins  |
| Okadaic acid plus others  | DSP?/Ciguatera?  | <i>Prorocentrum lima</i> (dinoflagellate)          | Benthic                              | Gulf-wide, Atlantic coast FL | Toxic in lab studies; FL strains produce toxins  |
| Okadaic acid plus others  | DSP?/Ciguatera?  | <i>Prorocentrum marinum</i> (dinoflagellate)       | Benthic                              | Gulf-wide, Atlantic coast FL | Toxic in lab studies   |
| ?   | DSP?/Ciguatera   | <i>Prorocentrum mexicanum</i> (dinoflagellate)     | Benthic                              | Gulf-wide                    | Toxic in lab studies; FL strains produce toxins  |
| Ostreotoxin   | ?  | <i>Ostreopsis lenticularis</i> (dinoflagellate)    | Benthic                              | South FL                     | Co-occurs with ciguatera species   |
| Ostreotoxin   | ?  | <i>Ostreopsis siamensis</i> (dinoflagellate)       | Benthic                              | FL                           | Co-occurs with ciguatera species   |
| <b>Tumor Promoters and DSP (acute toxicity of okadaic acid [LD<sub>50</sub> i.p. mouse] is 210 µg/kg)</b> |  |  |                                      |                              |  |
| Okadaic acid plus others  | DSP?/Ciguatera?  | <i>Prorocentrum belizeanum</i> (dinoflagellate)    | Benthic                              | South FL                     | Toxic in lab studies   |

|                          |   |  |                              |                              |   |
|--------------------------|---|--|------------------------------|------------------------------|---|
| Okadaic acid plus others | DSP/Ciguatera?                              | <i>Prorocentrum hoffmannianum</i> (dinoflagellate)                                 | Benthic                      | North and south FL           | Toxic in lab studies. FL strains produce toxins   |
| Okadaic acid plus others | DSP/Ciguatera?                              | <i>Prorocentrum lima</i> (dinoflagellate)  | Benthic                      | Gulf-wide, Atlantic coast FL | Toxic in lab studies; FL strains produce toxins   |
| Okadaic acid plus others | DSP/Ciguatera?                              | <i>Prorocentrum marinum</i> (dinoflagellate)                                       | Benthic                      | Gulf-wide, Atlantic coast FL | Toxic in lab studies  |
| ?                        | DSP/Ciguatera?                              | <i>Prorocentrum mexicanum</i> (dinoflagellate)                                     | Benthic                      | Gulf-wide                    | Toxic in lab studies; FL strains produce toxins   |
| Unknown                  | Fish kills?                                 | <i>Prorocentrum minimum</i> (dinoflagellate)                                       | Coastal/estuarine/planktonic | Gulf-wide, Atlantic coast FL | Toxic in lab studies  |
| Okadaic acid plus others | DSP   | <i>Dinophysis acuminata</i> (dinoflagellate)                                       | Coastal planktonic           | Gulf-wide                    | Toxic in lab studies  |
| Okadaic acid plus others | DSP?  | <i>Dinophysis caudata</i> (dinoflagellate)   | Estuarine/coastal planktonic | Gulf-wide, Atlantic coast FL | Toxic in lab studies  |
| Okadaic acid plus others | DSP   | <i>Dinophysis fortii</i> (dinoflagellate)  | Coastal planktonic           | Gulf-wide                    | Toxic in lab studies  |
| Okadaic acid plus others | DSP?  | <i>Dinophysis tripos</i> (dinoflagellate)  | Coastal planktonic           | Gulf-wide                    | Toxic in lab studies  |
| Okadaic acid plus others | DSP?  | <i>Phalacrocoma mitra</i> (dinoflagellate)   | Coastal planktonic           | Gulf-wide                    | Toxic in lab studies  |
| Okadaic acid plus others | DSP?  | <i>Phalacrocoma rotundatum</i> (dinoflagellate)                                    | Estuarine/coastal planktonic | Gulf-wide                    | Toxic in lab studies  |
| <b>Others</b>            |   |  |                              |                              |   |
| Hemolysin                | Fish and invertebrate kills                 | <i>Alexandrium monilatum</i> (dinoflagellate)                                      | Estuarine/coastal planktonic | Gulf-wide, east FL coast     | Seasonal in estuaries and coastal waters.; appears sporadically; caused marine animal mortality in FL, MS, and TX; not implicated in shellfish poisoning (e.g., PSP); simultaneous bloom off FL, MS, LA, and TX in September 1995 |
| Yessotoxin               | ?   | <i>Gonyaulax grindleyi</i> (= <i>Protocecratium reticulatum</i> ) (dinoflagellate) | Estuarine/coastal/planktonic | Gulf-wide                    | Common species; no known toxic events in Gulf or Florida waters   |
| Cytolytic toxin, unknown | ?   | <i>Coolia monotis</i> (dinoflagellate)   | Benthic                      | Gulf-wide, Atlantic coast FL | FL strains produce toxins   |
| Gymnodiamine?            | ?   | <i>Gymnodinium mikimotoi</i> (dinoflagellate)                                      | Coastal/planktonic           | FL/TX?                       | Known to be toxic in other areas  |
| ?                        | Fish kills, low dissolved oxygen            | <i>Gymnodinium sanguineum</i> (dinoflagellate)                                     | Estuarine/coastal/planktonic | Gulf-wide, Atlantic coast FL | One lab study indicates toxicity  |
| ?                        | Fish kills in nature and in fish farm ponds | <i>Gyrodinium galatheanum</i> (dinoflagellate)                                     | Estuarine/planktonic         | South FL and TX              | Occurs in Florida Bay; known fish killer at very high cell concentrations   |
| Neurotoxins/ Unknowns    | Fish kills in aquariums                     | <i>Pfiesteria</i> sp. (dinoflagellate)   | Benthic/planktonic           | ?                            | May be responsible for fish kills in tropical saltwater aquaria   |
| Domoic acid              | ASP?  | <i>Pseudo-nitzschia multiseries</i> (diatom)                                       | Benthic/tychoplanktonic      | Gulf-wide                    | No human shellfish toxicity cases in this area, but species have tested positive for domoic acid  |
| Domoic acid              | ASP?  | <i>Pseudo-nitzschia pseudodelicatissima</i> (diatom)                               | Benthic/tychoplanktonic      | Gulf-wide                    | No human shellfish toxicity cases in this area, but species have tested positive for domoic acid  |
| Domoic acid              | ASP?  | <i>Pseudo-nitzschia delicatissima</i> (diatom)                                     | Benthic/tychoplanktonic      | Gulf-wide                    | No human shellfish toxicity cases in this area, but species have tested positive for domoic acid  |
| ?                        | Fish kills?                                 | <i>Heterosigma akashiwo</i> (raphidophyte)   | Coastal/planktonic           | FL, LA                       |   |

|                     |   |  |                                  |                              |   |
|---------------------|---|--|----------------------------------|------------------------------|---|
| Cytolytic toxins?   | Fish kills  | <i>Prymnesium parvum</i> (prymnesiophyte)        | Coastal/estuarine/planktonic     | Gulf-wide                    |   |
| Cytolytic toxins?   | Fish kills?   | <i>Chrysochromulina</i> spp. (prymnesiophyte)    | Coastal/planktonic               | Gulf-wide                    |   |
| ?                   | Benthic animal kills, low dissolved oxygen                    | <i>Schizothrix calcicola</i> (cyanobacterium)    | Estuarine/planktonic             | Tampa Bay, FL                | Toxic in lab studies  |
| Hemolysins          | Sponge mortality?   | <i>Synechococcus elongatus</i> (cyanobacterium)  | Coastal/estuarine/planktonic     | Florida Bay                  | Mechanical damage or toxicity to sponges  |
| Unknown             | Fish kills?/ Physiologically impaired shellfish               | <i>Prorocentrum minimum</i> (dinoflagellate)     | Coastal/estuarine/planktonic     | Gulf-wide, Atlantic coast FL | Toxic in lab studies  |
| ?                   | Fish kills?/Ciguatera   | <i>Trichodesmium erythraeum</i> (cyanobacterium) | Offshore/coastal/planktonic      | Gulf-wide, Atlantic coast FL | Implicated in fish kills, coral mortality in Australia  |
| Debromoaplysiatoxin | Potential for marine animal disease and human health concerns | <i>Lyngbya majuscula</i> (cyanobacterium)        | Marine, benthic, tychoplanktonic | Gulf-wide, Atlantic coast FL | Associated with inflammatory dermatitis and gastrointestinal disorders in humans; debromoaplysiatoxin is a known tumor promoter |

## Known or potentially toxic cyanobacteria (blue-green algae) in fresh and estuarine Florida waters

| Toxin  | Impact  | Species                       | Habitat                                     | Distribution   | Comments  |
|--|---|-------------------------------|---|--|---|
| <b>Hepatotoxins</b>  |   |                               |   |  |   |
| <b>Microcystin (acute toxicity [LD<sub>50</sub> i.p. mouse] of microcystins varies between 50-600 µg/kg)</b> |   |                               |   |  |   |
| Microcystin  | Potential for wildlife and fish kills, loss of habitat, potable water supply problems, tumor promoter in laboratory mice, human health concerns | <i>Anabaena flos-aquae</i>    | Freshwater, planktonic                      | FL, IN, NE, IL, MT; likely found throughout the US                     | Microcystins are inhibitors of protein phosphatases and are considered tumor promoters in laboratory mice; the provisional standard for microcystin-LR in drinking water is 1 µg/L water; no toxic events reported or blooms analyzed for toxin in FL |
| Microcystin  | ?   | <i>Hapalosiphon</i> spp.      | Freshwater, epiphytic (on submerged plants) | FL and likely found throughout the US                                  | <i>H. fontinalis</i> produces an alkaloid substance called Hapalindoles; <i>H. wehrichtii</i> and <i>H. hibernicus</i> known to produce cytotoxins  |
| Microcystin  | Potential for wildlife and fish kills, loss of habitat, potable water supply problems, tumor promoter in laboratory mice, human health concerns | <i>Microcystis aeruginosa</i> | Freshwater, estuarine, planktonic           | FL, CA, WA, WI, ND, MT, OH, WY, IL, LA; likely found throughout the US | Lake Okeechobee, August 1987 – signs of poisoning in lab mice indicated a hepatotoxin; blooms common throughout FL  |
| Microcystin  | ?   | <i>Microcystis viridis</i>    | Freshwater, estuarine, planktonic           | ?  | No toxic events reported or blooms analyzed for toxin in FL   |

|  |  |  |                                       |  |  |   |  |
|--|--|--|---------------------------------------|--|--|---|--|
| Microcystin  | ?  |  | <i>Microcystis wesenbergi</i>         | Freshwater, planktonic   | FL; likely found throughout the US                                     | Implicated in poisoning event; blooms not analyzed for toxin in FL  |  |
| Microcystin  | ?  |  | <i>Nostoc</i> sp.                     | Freshwater, planktonic, tytoplanktonic, benthic, moist soil            | FL; likely found throughout the US                                     | Implicated in poisoning event; blooms not analyzed for toxin in FL  |  |
| Microcystin  | ?  |  | <i>Oscillatoria</i> spp.              | Freshwater, planktonic   | FL; likely found throughout the US                                     | No toxic events reported or blooms analyzed for toxin in FL   |  |
| Microcystin  | ?  |  | <i>Planktothrix agardhii</i>          | Freshwater pools, rivers and on moist soil; planktonic, tytoplanktonic | FL; likely found throughout the US                                     | Formerly <i>Oscillatoria</i> ; no toxic events reported or blooms analyzed for toxin in FL  |  |
| Microcystin  | ?  |  | <i>Planktothrix rubescens</i>         | Freshwater, tytoplanktonic   | FL; likely found throughout the US                                     | Formerly <i>Oscillatoria</i> ; no toxic events reported or blooms analyzed for toxin in FL  |  |
| Microcystin  | ?  |  | <i>Synechocystis</i> sp.              | Freshwater, planktonic   | FL; likely found throughout the US                                     | Picocyanobacterium (0.6-2 µm cell diameter); hepatotoxins and possible neurotoxins produced; no toxic events reported or blooms analyzed for toxin in FL                  |  |
| Microcystin  | ?  |  | <i>Anabaenaopsis milleri</i>          | Freshwater, planktonic   | FL; likely found throughout the US                                     | No toxic events reported or blooms analyzed for toxin in FL   |  |
| <b>Nodularin (acute toxicity [LD<sub>50</sub> i.p. mouse] of nodularin varies between 50-150 µg/kg)</b>  |  |  |                                       |  |  |   |  |
| Nodularin  | Potential for wildlife and fish kills, loss of habitat, tumor promoter in laboratory mice, human health concerns |  | <i>Nodularia spumigena</i>            | Estuarine, planktonic  | FL; likely found along coastal US                                      | Seven different nodularins identified; nodularin isolated from a marine sponge ( <i>Theonella swinhoei</i> ); no toxic events reported or blooms analyzed for toxin in FL |  |
| <b>Cylindrospermopsin (acute toxicity [LD<sub>50</sub> i.p. mouse] of cylindrospermopsin [North Queensland, Australia] was 64 ± 5 mg of freeze-dried culture/kg)</b> |  |  |                                       |  |  |   |  |
| Cylindrospermopsin   | ?  |  | <i>Aphanizomenon ovalisporum</i>      | Freshwater, estuarine, planktonic                                      | FL and likely found along the south Atlantic and Gulf coasts of the US | Cylindrospermopsin responsible for outbreak of severe hepatocenteritis in Australia during the 1970s  |  |
| Cylindrospermopsin   | Alligator, bird, fish, and turtle kills?   |  | <i>Cylindrospermopsis raciborskii</i> | Freshwater, planktonic   | FL, KS, MN; likely found throughout the US                             | <i>Cylindrospermopsis raciborskii</i> blooms occurred during alligator, bird, and fish kills in Lake Griffin, FL; potential toxin production under investigation          |  |
| <b>Neurotoxins</b>   |  |  |                                       |  |  |   |  |
| <b>Anatoxin-a (acute toxicity [LD<sub>50</sub> i.p. mouse] of anatoxin-a is ~250 µg/kg)</b>  |  |  |                                       |  |  |   |  |
| Anatoxin-a   | Potential for wildlife and fish kills, loss of habitat, human health concerns                                    |  | <i>Anabaena circinalis</i>            | Freshwater, estuarine, planktonic                                      | FL, WA, MT, WI, LA, WY, PA; likely found throughout US                 | Lake Istokpoga, September 1988 – signs of poisoning in lab mice indicated a neurotoxin; blooms common throughout FL   |  |
| Anatoxin-a   | ?  |  | <i>Anabaena spiroides</i>             | Freshwater, planktonic   | FL and likely found throughout US                                      | Implicated in poisoning event; uncommon component of algal blooms   |  |

|   |   |  |   |   |  |   |
|---|---|--|---|---|--|---|
| Anatoxin-a  | ?   |  | <i>Cylindrospermum</i> sp.                      | Freshwater, planktonic                    | FL and likely found throughout US                          | No toxic events reported or blooms analyzed for toxin in FL   |
| Anatoxin-a  | ?   |  | <i>Microcystis</i> sp.                          | Freshwater, estuarine, planktonic         | FL and likely found throughout US                          | Lake Okeechobee, August 1987 – signs of poisoning in lab mice indicated a hepatotoxin; <i>Microcystis</i> blooms common throughout FL                               |
| Anatoxin-a(s) (acute toxicity [LD <sub>50</sub> i.p. mouse] of anatoxin-a(s) varies between 30-50 µg/kg)  |   |  |   |   |  |   |
| Paralytic Shellfish Poisons (PSPs)  |   |  |   |   |  |   |
| Saxitoxin (C-toxins are the least potent among the PSPs; the singularly sulfated gonyautoxins have a wide range of toxicities and the nonsulfated saxitoxins are highly potent neurotoxins with an LD <sub>50</sub> [i.p. mouse] of 10 µg/kg) |   |  |   |   |  |   |
| Saxitoxin (Gonyautoxins C1, C2)   | Potential for wildlife and fish kills, loss of habitat, human health concerns |  | <i>Anabaena circinalis</i>                      | Freshwater, estuarine, planktonic         | FL, WA, MT, WI, LA, WY, PA; likely found throughout the US | PSP toxins are common in Australian isolates of <i>Anabaena circinalis</i> ; Lake Istokpoga, September 1988 – signs of poisoning in lab mice indicated a neurotoxin |
| Saxitoxin   | Potential for wildlife and fish kills, loss of habitat, human health concerns |  | <i>Aphanizomenon flos-aquae</i>                 | Freshwater, planktonic                    | FL, WA, ND, MT, NV, NH; likely found throughout the US     | No toxic events reported or blooms analyzed for toxin in FL   |
| Saxitoxin   | Potential for wildlife and fish kills, loss of habitat, human health concerns |  | <i>Cylindrospermopsis raciborskii</i>           | Freshwater, planktonic                    | FL, KS, MN; likely found throughout the US                 | <i>C. raciborskii</i> blooms occurred during alligator, bird, and fish kills in Lake Griffin, FL; potential toxin production in FL under investigation              |
| Saxitoxin (Gonyautoxins 2,3)  | Potential for wildlife and fish kills, loss of habitat, human health concerns |  | <i>Lyngbya wollei</i>                           | Freshwater, estuarine; forms benthic mats | FL, TN; likely found throughout the US                     | No toxic events reported or blooms analyzed for toxin in FL   |
| Neosaxitoxin  |   |  |   |   |  |   |
| Neosaxitoxin  | Potential for wildlife and fish kills, loss of habitat, human health concerns |  | <i>Aphanizomenon flos-aquae</i>                 | Freshwater, planktonic                    | FL, WA, ND, MT, NV, NH                                     | No toxic events reported or blooms analyzed for toxin in FL   |
| Suspect   |   |  |   |   |  |   |
| Purified toxin unavailable  | ?   |  | <i>Anabaena solitaria</i> f. <i>planktonica</i> | Freshwater, planktonic                    | FL; likely found throughout the US                         | Implicated in a poisoning event; occurs as a minor component with other bloom-forming species; no toxic events reported or blooms analyzed for toxin in FL          |
| Purified toxin unavailable  | ?   |  | <i>Coelosphaerium kuetzingianum</i>             | Freshwater, planktonic                    | FL; likely found throughout the US                         | Implicated in a poisoning event; occurs as a minor component with other bloom-forming species; no toxic events reported or blooms analyzed for toxin in FL          |
| Cytotoxic cylindrocyclophane a (1), hepatotoxins likely   | ?   |  | <i>Cylindrospermum</i> sp.                      | Freshwater, planktonic                    | FL; likely found throughout the US                         | No toxic events reported or blooms analyzed for toxin in FL   |
| Purified toxin unavailable  | ?   |  | <i>Microcystis incerta</i>                      | Freshwater, planktonic                    | FL; likely found throughout the US                         | No toxic events reported or blooms analyzed for toxin in FL   |

|                                     |   |  |                               |  |                                    |   |
|-------------------------------------|---|--|-------------------------------|--|------------------------------------|---|
| Purified toxin unavailable          | ? |  | <i>Microcystis wesenbergi</i> | Freshwater, planktonic                           | FL; likely found throughout the US | No toxic events reported or blooms analyzed for toxin in FL |
| Hepatotoxins, nostocyclophane D (2) | ? |  | <i>Nostoc linckia</i>         | Freshwater, planktonic                           | FL; likely found throughout the US | No toxic events reported or blooms analyzed for toxin in FL |
| Purified toxin unavailable          | ? |  | <i>Nostoc paludosum</i>       | Freshwater, benthic, moist soil, tychoplanktonic | FL; likely found throughout the US | No toxic events reported or blooms analyzed for toxin in FL |
| Purified toxin unavailable          | ? |  | <i>Nostoc rivulare</i>        | Freshwater, moist soil                           | FL; likely found throughout the US | No toxic events reported or blooms analyzed for toxin in FL |
| Purified toxin unavailable          | ? |  | <i>Nostoc zetterstedtii</i>   | Freshwater, moist soil                           | FL; likely found throughout the US | No toxic events reported or blooms analyzed for toxin in FL |

**APPENDIX II**  
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