Chapter 5: Toxic Cyanobacteria in Florida Waters

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Cyanobacteria represent a common component of algal assemblages in Florida's lakes, springs, estuaries, and other marine environments. The frequency of cyanobacterial bloom reports has recently become more common and algal toxin data are just beginning to be collected with the advent of monitoring efforts and the availability of laboratories in the state capable of conducting algal toxin analyses.

Given Florida's growing population, subtropical environment, and the eutrophic nature of its lakes, rivers, and estuaries, it is not surprising that cyanobacteria are a common feature of aquatic ecosystems. However, cyanobacterial blooms may have always been common in some regions that include water bodies influenced by underlying phosphate deposits. Phosphate deposits cover much of peninsular Florida and are mined throughout the west–central part of the state (FIPR 2006; FDEP 2006).

Although there is little doubt that the phenomenon of cyanobacterial blooms predates human development in Florida, the recent acceleration in population growth and associated changes to surrounding landscapes has contributed to the increased frequency, duration, and intensity of cyanobacterial blooms and precipitated public concern over their possible harmful effects to aquatic ecosystems and human health. Toxic cyanobacterial blooms in Florida waters 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 are increasingly impacted by toxic cyanobacterial blooms, including the St. Johns River (SJRWMD 2001), Lake Okeechobee (Havens et al. 1996), St. Lucie River (FFWCC 2005), Caloosahatchee River (Gilbert et al. 2006), and the Harris Chain of Lakes (LCWA, personal communication). Cyanobacteria, such as *Lyngbya*, are also common in many of Florida's springs

and along coastal reef tracts where blooms form extensive mats and blanket corals and submersed aquatic vegetation. Shifts in phytoplankton composition to potentially toxic cyanobacteria taxa have also occurred in some eutrophic Florida lakes (Chapman and Schelske 1997). Historically, atopic sensitivity to cyanobacteria has been reported following exposure to algae in lakes. For example, Heise (1949) found blue–green algae responsible for seasonal rhinitis following exposure to algae while swimming in lakes. Human sensitivity to cyanobacteria may be related to a hereditary predisposition toward developing certain hypersensitivity reactions when exposed to specific antigens.

There is a growing need in Florida and the other areas of the United States to define the specific relationships among freshwater cyanobacterial blooms, the production of secondary blooms in estuaries and marine coastal systems, and potential ecological and human health consequences associated with prolonged toxic bloom events. Moreover, because Florida lacks sufficient research and biomonitoring programs for toxic cyanobacteria, and little coordination exists between surface water managers and public health officials, relationships between toxic cyanobacteria and their environmental consequences remain largely in the realm of incidental observation and speculation.

Historically, reports of cyanobacterial blooms in Florida are often associated with fish kill events in freshwater and estuarine systems. These reports often lacked specific algal identification information and toxin data was not collected. The earliest known record of algal toxins in Florida was reported following the death of cattle near Lake Okeechobee (Carmichael 1992). Toxic cyanobacterial blooms in Florida were first recorded by Wayne Carmichael in Lake Okeechobee (1987, 1989) and Lake Istokpoga (1988). Dead cattle, signs of poisoning in laboratory mice, and contact irritation were found associated with Anabaena and Microcystis blooms. The toxin microcystin and an unidentified neurotoxin were attributed to the toxic effects found in Florida lake samples. Although toxic cyanobacterial blooms had become a major concern throughout the world, and the World Health Organization had set provisional guidelines for the consumption of microcystin– LR (WHO 1998), little information on toxic cyanobacteria in Florida waters had been published since those first toxic events identified in 1992.

No Florida survey of cyanobacterial blooms or cyanobacterial toxins had been conducted until the advent of the creation of the Florida Harmful Bloom Task Force 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." In March 1999, a technical advisory group to the Harmful Algal Bloom Task Force produced a report that identified known existing harmful algal species in the state and made recommendations for research and monitoring priorities (Steidinger et al. 1999). The report provided the following recommendations to the Task Force regarding toxic blue–green algae: 1) Determine distribution of toxic and non–toxic strains in Florida waters; 2) Develop epidemiological studies to determine what public health threats are involved; 3)Develop economic impact studies to properly evaluate losses by locale or industry; and 4) Determine the roles of nutrient enrichment and managed freshwater flow in bloom development; 5) Investigate the applicability and efficacy of control and mitigation methods.

Following the above recommendations, the Task Force funded an investigation of the occurrence and distribution of cyanobacteria and their toxins in 1999. The goals of the state–wide survey for the cyanobacteria survey included: 1) identification of toxic cyanobacteria throughout Florida; 2) identify and characterize level of cyanotoxins; 3) assist with the development of analytical capability for algal toxin analysis within the Florida Department of Health laboratory in Jacksonville, Florida; and evaluate the presence of cyanobacterial toxins at water treatment plants that utilize surface water resources.

The FHABTF, Florida Marine Research Institute, and St. Johns River Water Management District initiated a collaborative study with the Florida Department of Health and Wright State University to identify potential cyanobacterial toxins in Florida's lakes, rivers, reservoirs, and estuaries. Samples were collected and analyzed during 1999 and then extended to better understand the potential impacts of cyanobacterial toxins detected in waters currently utilized for drinking water or identified as a potential future drinking water source.

Methods employed to identify alga taxa included microscopic examination and epifluorescence. Algal toxins were characterized and quantified by enzyme linked immunosorbant assay (ELISA), protein phosphatase inhibition assay (PPIA), HPLC–Fl, HPLC–UV, and LC/MS/MS. Mouse bioassays were used to characterize toxicity by intraperitoneal injection of freeze–dried sample extracts into ICR–Swiss male mice.

With the assistance of numerous state and local agencies in 1999, a total of 167 samples were collected throughout Florida, eighty–eight of these samples, representing 75 individual water bodies, were found to contain cyanotoxins. Approximately 80% of the samples containing cyanotoxins were found to be lethal to mice following intraperitoneal injection. Most bloom forming cyanobacteria genera were distributed throughout the state, but water bodies such as Lake Okeechobee, Harris Chain of Lakes, Lower St. Johns River, Calooshatchee River, Lake Seminole, Lake George, Crescent Lake, Doctors Lake, and the St. Lucie River were water bodies that supported extensive cyanobacterial biomass. Seven genera of cyanobacteria were identified from water samples collected. *Microcystis* (43.1%), *Cylindrospermopsis* (39.5%), and *Anabaena* (28.7%) were observed most frequently and in greatest concentration. *Planktothrix* (13.8%), *Aphanizomenon* (7.2%), *Coelosphaerium* (3.6%) and *Lyngbya* (1.2%) were found less frequently, but at times accounted for a significant proportion of the planktonic and macroalgal species composition. *Aphanizomenon* and *Anabaenopsis* were also found consistently during the 2000 survey. Cyanobacterial blooms were common throughout the state, some of which form long lasting or continuous blooms in eutrophic and hypereutrophic systems. Many of the water bodies affected by cyanobacterial blooms were identified by water management agencies as areas of current concern or were being addressed by ongoing or proposed restoration efforts.

Algal toxins identified from bloom material during the study included hepatotoxic microcystins, neurotoxic anatoxin–A, and the cytotoxic alkaloid cylindrospermopsin. Subsequent identification of lyngbyatoxin–A and debromoaplysiatoxin were found associated with *Lyngbya* blooms collected from Florida springs and coastal embayments. Microcystins were the most commonly found toxins in Florida waters, occurring in all 87 samples analyzed during 1999. During the 2000 survey, microcystins were detected in pre– and post–treated drinking water. Finished water microcystin concentration ranged from below detection levels to 12.5 $\mu g\ L^{-1}$. Microcystins are considered the most frequently found cyanobacterial toxins around the world. Over 60 structural variants of this cyclic peptide have been reported, causing considerable concern due to their high chemical stability, high water solubility, environmental persistence and exposure to humans in surface water bodies. The World Health Organization has set a provisional consumption limit of 1 $\mu g\ L^{-1}$ for microcystin–LR (WHO 1998). The mammalian toxicity of microcystin occurs by active transport across membrane boundaries and is mediated through binding to protein phosphatases (Runnegar et al. 1991; Falconer et al. 1992). Analysis of protein phosphatase inhibition activity, an index of microcystin bioactivity, was found to be positive in 44 (69%) of 64 Florida samples tested. There

is limited evidence of tumor promotion (Sato et al. 1984; Falconer 1991, Nishiwaki–Matsushima et al. 1992, Wang and Zhu 1996) and clastogenic dose–related increases in chromosomal breakage by microcystin (Repavich et al. 1990), but no mutagenic evidence has been reported (Runnegar and Falconer 1982, Repavich et al. 1990).

Anatoxin–a is a potent neurotoxic alkaloid that has been frequently implicated in animal and wildfowl poisonings (Ressom et al. 1994). It is considered a nicotinic agonist that binds to neuronal nicotinic acetylcholine receptors which leads to depolarization and a block of electrical transmission in the body (Soliakov et al. 1995). At sufficiently high doses (oral LD50 = >5,000 μg kg^{-1} body weight), it can lead to paralysis, asphyxiation, and death (Fitzgeorge et al. 1994, Carmichael 1997). Although alternative hypotheses exist to help explain alligator mortalities in Florida lakes, unexplained bird and alligator mortality events during cyanobacterial blooms may be due to exposure to neurotoxic compounds produced by species of *Anabaena*, *Aphanizomenon*, and *Cylindrospermopsis*. Anatoxin–a was found in three finished water samples and in tissues from Blue Tilapia and one White Pelican during surveys in 2000. Anatoxin–a was found in the gut and liver of a White Pelican and in Blue Tilapia (0.51 to 43.3 μg g^{-1}) and in finished drinking water (below detectable limits to 8.46 μg L^{-1}).

All 1999 samples containing the organism *Cylindrospermopsis* were positive for the toxin cylindrospermopsin. Nine (9) finished drinking water samples collected during the 2000 survey were positive for cylindrospermopsin and ranged in concentration from 8.07 to 97.12 μg L^{-1}. Identification of the algal toxin cylindrospermopsin during this study represented the first record of this hepatotoxic alkaloid in North America. This toxin primarily affects the liver, but extracts given orally or injected in mice also induce pathological damage to kidneys, spleen, thymus, and heart (Hawkins et al. 1985, 1997). Cylindrospermopsin is a potential important contaminant of drinking waters in Australia, Central Europe, South America, and the United States. The toxin was identified after 138 children and 10 adults were poisoned following a *Cylindrospermopsis* bloom and copper sulfate applications in a water supply reservoir on Palm Island, Australia (Hawkins et al. 1985). Over 69% of the affected individuals required intravenous therapy for electrolyte imbalance, and the more severe cases for hypovolemic and acidotic shock (Byth 1980). The oral toxicity or lethal dose of cylindrospermopsin has been reported between 4.4 and 6.9 mg kg^{-1} mouse body weight with death occurring 2–3 days after treatment (Humpage and Falconer 2002). In experiments where cell free extract of *Cylindrospermopsis* was administered to mice in drinking water over 90 days, no pathological symptoms were recorded up to a maximum dose of 150

mg kg^{-1} day^{-1} (Shaw et al. 2001). Humpage and Falconer (2002) suggest a Tolerable Daily Intake and Guideline Value for cylindrospermopsin in drinking water of 1 μg L^{-1}based on an oral No Observed Adverse Effect Level of 30 μg kg^{-1} day^{-1} and a Lowest Observed Adverse Effect Level of 60 μg kg^{-1} day^{-1}.

Lyngbyatoxin–a and debromoaplysiatoxin have been identified from *Lyngbya* samples collected from Florida springs and marine embayments, respectively (J. Burns, N. Osborne, and G. Shaw, unpublished data). The aplysiatoxins and lyngbyatoxins are considered dermatotoxic alkaloids, causing severe dermatitis among swimmers and other recreational users of water bodies that come into direct contact with the organism (Mynderse et al. 1977, Fujiki et al. 1982). Aplysiatoxins are lethal to mice at a minimum dose of 0.3 mg kg^{-1} (Moore 1977). Aplysiatoxins and lyngbyatoxins are also considered potent tumor promoters and protein kinase C activators (Fujiki et al. 1990). Osborne et al. (2001) reviewed the human and ecological effects of *Lyngbya majuscula* blooms and reported acute contact dermatitis in Hawaii, Japan, and Australia. One potential death due to exposure via ingestion of turtle meat containing lyngbyatoxin– a was reported by Yasumoto (2000). Severe dermatitis has also been reported in Florida following recreational activities in waters supporting *Lyngbya* blooms in Florida's springs (John Burns, Ian Stewart and G. Shaw, unpublished data). *Lyngbya* mats have been detected along coral reef tracts adjacent to the southeast coast of Florida near Dade and Broward counties. Although no toxins have been detected from the limited number of samples analyzed, thick mats of *Lyngbya* have smothered corals, causing severe damage to the reef.

To date, toxic cyanobacterial blooms continue to occur throughout Florida and no state–wide monitoring program for cyanobacteria or cyanobacterial toxins exists. No Florida guidelines for recreational exposure to toxic cyanobacteria or cyanobacterial toxins in drinking water are available. However, several independent monitoring efforts for cyanobacteria and their toxins have been initiated and the Florida Harmful Algal Bloom Task Force has helped fund the following efforts, "Cyanobacteria Automated Detection Workshop", "*Cylindrospermopsis* Culture for Production of Cylindrospermopsin", and "Cyanobacteria Public Health Issues: Education and Epidemiologic Study Monitoring and response efforts have been initiated by the St. Johns River Water Management District (Lower St. Johns River Basin), South Florida Water Management District (Lake Okeechobee), Lake County Water Authority (Harris Chain of Lakes), Florida Lake Watch, and several water utilities in the state that utilize surface waters for

drinking water supply. One of the most severe blooms ever recorded in Florida occurred in the Lower St. Johns River during the summer of 2005 (Fig. 1,2,3 see Color Plate 3), extending from Lake George to the mouth of the river at Mayport, Florida. Large rafts of toxic algal scum were slowly transported north through the city of Jacksonville to the Atlantic Ocean by tide. *Microcystis* and *Cylindrospermopsis* were the dominant bloom forming species, with microcystin detected as high as ~1,400 μg L^{-1} (SJRWMD 2005). One human death was reported during the bloom event following recreational contact (i.e., jet skiing) with surface algal scums. It was reported that a young female with an open leg wound contracted a lethal *Vibrio* infection following recreational contact with waters of the St. Johns River near Jacksonville, Florida. Although the *Vibrio* infection was not related to algal toxin poisoning, it is important to recognize that the presence of cyanobacterial blooms and the concentration of algal scums along shorelines may increase the likelihood of human exposure to other bacteria and pathogens that thrive in such conditions. The Florida Department of Health released a public health advisory that warned the public to refrain from use of the river during the bloom event. During the summer of 1995, toxic *Microcystis* blooms also occurred in Lake Okeechobee, Calooshatchee River, St. Lucie River, and the West Palm Beach Canal (C–51). Canal gates near the entrance to an existing water supply for a water treatment plant in south Florida were temporarily closed during the bloom event to protect the quality of existing surface water supplies.

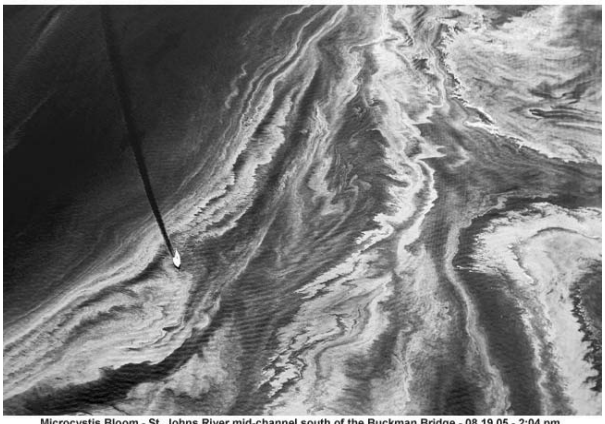

Fig. 1. Mycrocystis Bloom—St. Johns River mid-channel south of the Buckman Bridge. (See Color Plate 3).

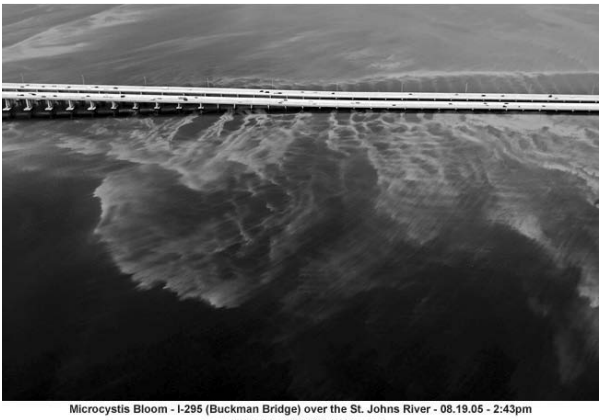

Fig. 2. Microcystis Bloom—I295 (Buckman Bridge) over the St. Johns River. (See Color Plate 3).

Fig. 3. Microcystis Bloom – East bank of the St. Johns River – Mandarin. (See Color Plate 3).

Summary

The occurrence of toxic cyanobacterial blooms in Florida waters have become more prominent following increased growth, declining groundwater supplies, and identification of impaired surface waters as future drinking water sources. Cyanobacterial toxins have been identified in source waters used for drinking water supply and in post–treated drinking water during algal bloom events. Algal toxin concentrations in post–treated drinking water have exceeded existing and proposed World Health Organization guidelines for the oral consumption of microcystin and cylindrospermopsin. Severe dermatitis has also been reported by swimmers in Florida springs where *Lyngbya* mats have expanded.

The prevalence and toxicity of cyanobacteria should be considered when developing appropriate Total Maximum Daily Loads for impaired Florida waters that do not currently meet their designated use. It could also support further efforts to characterize potential ecological and human health risks due to toxic cyanobacterial blooms. Identification of algal toxins in finished drinking water and reports of severe skin irritation following contact with toxic cyanobacteria should be utilized for justification and implementation of increased monitoring of potentially toxic cyanobacterial blooms by surface water managers and water utilities. Epidemiological studies may also be required in Florida to assess potential human health risks due to algal toxin consumption at the tap and for those exposed to cyanotoxic blooms during recreational use of lakes, springs and rivers.

Without adequate water treatment and coordinated state–wide monitoring efforts, it is anticipated that the likelihood for human exposure to cyanobacteria and their toxins will increase as Florida becomes more dependent upon surface waters to supply a growing population and an expanding urban environment. Coordination and communication between surface water managers and public health officials at the local level will be critical to the overall protection of the environment and public health during toxic cyanobacterial bloom events.

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