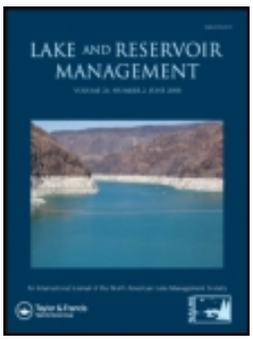

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Identification of cyanobacterial toxins in Florida's freshwater systems

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Abstract

Williams, C.D., M.T. Aubel, A.D. Chapman and P.E. D'Aiuto. 2007. Identification of cyanobacterial toxins in Florida's freshwater systems. Lake and Reserv. Manage. 23:144-152.

Toxigenic cyanobacteria are common components of Florida's surface waters and may pose a threat to their ecology and a risk to human health. The Lake County Water Authority, the Florida Department of Health, the St. Johns River Water Management District, the South Florida Water Management District, and the Lee County Health Department have all recently monitored local waters for cyanotoxins. In 2004, 6 central Florida lakes were sampled monthly for 12 months at centrally located open water sample sites. Toxin data (n = 72 for all toxin analyses) showed that microcystins (0.1-3.6 µg/L) were present in all 12 months; multiple bloom events per lake were observed; bloom concentrations ranged from 5-7,500 µg/L; and blooms occurred throughout the entire year. Cylindrospermopsin, ranging in concentration from 0.05 to 0.2 µg/L, was reported in 22% of all samples analyzed and occurred predominantly between August and December. Anatoxin-a was not reported in 2003/2004 but was identified in 2002/2003 (30%, 0.05-7.0 µg/L). Data from the St. Johns River was similar to that of the central Florida lakes. In general, microcystins ranged between 0.1 and 31 µg/L and were detected in all samples (n = 50), while cylindrospermopsin was observed less frequently (30%, n = 48) and at lower concentrations (0.07-1.6 µg/L). Anatoxin-a was not reported (n = 48). In contrast, the major *Microcystis* spp. blooms that occurred in the St. Johns, the St. Lucie, and the Caloosahatchee rivers in 2005 were reported to contain relatively high levels of microcystins ($range_{max.\ conc.}$= 278-5700 µg/L) and persisted for approximately 2 months. Data indicate that cyanotoxin production can be a year-round phenomenon in Florida and can occur at levels that may cause ecological and human health problems.

Key words: anatoxin-a, blue-green algal toxins, cyanotoxins, cylindrospermopsin, microcystins, toxigenic cyanobacteria

Cyanobacteria (blue-green algae) are common, naturally occurring organisms in Florida's lakes, rivers, streams, and canals. Dense populations of cyanobacteria can occur in surface water resources due to Florida's climate, a strong and historical agricultural industry, high anthropogenic nutrient loads, major reductions in the natural buffering capacity around water bodies due to land development, and natural environmental characteristics (high retention times, relatively shallow waters, high sedimentary phosphorus content, and highly mixed water column). Dense populations of algae (blooms) can be detrimental to the environment by limiting light penetration, causing hypoxic to anoxic conditions upon degradation of bloom conditions, inhibiting the presence of submerged aquatic vegetation, and producing aesthetically unpleasant odor and taste compounds, such as 2-methylisoborneol (MIB) and geosmin, as well as toxic chemical metabolites such as cyanotoxins (Ressom *et al.* 1994, Chorus and Bartram 1999, Whitton and Potts 2000).

Cyanotoxins are water-soluble compounds that can be categorized into 4 distinct groups: hepatotoxins (microcystins and cylindrospermopsin); neurotoxins (anatoxins and saxitoxins); dermatotoxins (lyngbyatoxins and aplysiatoxin); and skin irritants (all cyanotoxins). Microcystins (a family of toxins with differing toxicities) and cylindrospermopsin are both reported as being potential tumor promoters (Falconer 1991, Falconer and Humpage 2001), and cylindrospermopsin as being genotoxic (Humpage *et al.* 2000). In general, cyanotoxins are found inside healthy algal cells and thus are not directly bioavailable within the aqueous environment. Algal blooms, however, are usually transient in nature, and as algae die their toxins are released into the surrounding environment. Cyanotoxins are relatively stable compounds and, depending

on environmental conditions, can persist for weeks to months (Matsunaga *et al.* 1989, Smith and Sutton 1993, Tsuji *et al.* 1993, Harada *et al.* 1996, Jones and Negri 1997, Chriswell *et al.* 1999). Cyanotoxins can severely sicken and/or kill animals (review in Ressom *et al.* 1994, Carbis *et al.* 1997 Negri *et al.* 1995, Henriksen *et al.* 1997) and humans (review in Ressom *et al.* 1994, Jochimsen *et al.* 1998, Carmichael 1999, Pouria *et al.* 1998).

Cyanotoxins are potent and can be fast acting compounds if they reach significant concentrations and are directly ingested. In November 2004, 4 dogs died within 30 minutes of swimming in a Minnesota lake that was experiencing a heavy algal bloom. No autopsies were performed because the Minnesota Department of Natural Resources and the residents of the lake concluded the deaths were clearly caused by the ingestion of algal cells (Peterson 2004). In September 2004, farmers in Vernal, Utah, experienced a cow mortality event (3 adults and 15 calves), and these deaths were attributed to the ingestion of waters that contained microcystins (www.TriCountyHealth.com 2004). During 2004-2005, several states (Oregon, Nebraska, Wisconsin, Minnesota and Iowa) decided that the presence of blue-green algal toxins pose a significant threat to public safety and therefore initiated or are in the process of developing lake monitoring programs to determine and track toxin levels as well as to inform and caution recreational users.

Previously, potentially toxigenic cyanobacteria and cyanotoxins were identified in surface waters throughout the state of Florida (Williams *et al.* 2001, 2006. Potentially toxigenic species of *Microcystis*, *Anabaena*, *Cylindrospermopsis*, *Planktothrix*, and *Aphanizomenon* were frequently observed. Microcystins (0-107 µg/L), cylindrospermopsin (0-202 µg/L), anatoxin-a (0-156 µg/L) and debromoaplysiatoxin (0-0.3ug/g) were all positively identified and quantified. Saxitoxins were not reported. Numerous water samples (80%, n = 70) were found to be lethal to mice following intraperitoneal injection of ambient cyanotoxin containing water samples, and both neurotoxic and hepatotoxic symptoms were reported.

Monitoring of state surface waters for the presence of cyanotoxins ceased in 2000; however, since 2000 several independent monitoring projects have occurred. Between 2001 and 2004, the Lake County Water Authority monitored the Harris Chain of Lakes for cyanotoxins due, in part, to a prolonged alligator mortality event that occurred in Lake Griffin. In May 2005 the Florida Department of Health, in conjunction with the Center for Disease Control, monitored the St. Johns River for cyanotoxins at popular recreational sites. In September and October 2005, the St. Johns River and the South Florida Water Management Districts (SJRWMD; SFWMD) monitored large *Microcystis* spp. blooms in the St. Johns and the St. Lucie Rivers for the production of microcystins. Furthermore, the Lee County Department of Health monitored the Caloosahatchee River for microcystins during this same time period. The results of these projects are summarized in this report.

Materials and methods

Sample collection

Lake County Water Authority/Harris Chain of Lakes

Six lakes (Beauclair, Dora, Eustis, Harris, Griffin, and Yale) were monitored for cyanotoxins (Table 1; Fig. 1). Two-L water samples, integrated from 0-0.5m, were collected at the center of each lake by GreenWater Laboratories/CyanoLab every 2 weeks from 1 October 2003 through 10 November 2004. If an algal surface scum was present, a bloom sample was also collected. Samples were collected in opaque 1-L sample bottles, immediately stored in coolers with ice, and transported back to the laboratory on the day of collection. Upon arrival at the laboratory, all samples were stored immediately at 4°C. Samples were analyzed for total microcystins via enzyme linked immunosorbent assay (ELISA) and for cylindrospermopsin and anatoxin-a by liquid chromatography/mass spectrometry (LC/MS/MS).

Florida, Department of Health/St. Johns River Recreational Sites

Nine fixed sample sites (Doctors Lake; Shands Bridge at Eagle Point; Palatka; Crescent Lake; Lake George; Lake Monroe; Lake Jesup; Little Lake Harris at Hickory Point, part of the Harris Chain of Lakes with a permitted bathing beach; and Lake Washington, a drinking water supply) within the Lower, Middle, and Upper St. Johns River Basin were monitored for cyanotoxins (Table 1; Fig. 1). Water samples were collected by GreenWater Laboratories/CyanoLab and the St. Johns River Water Management District on a monthly basis from 1 May through 30 October 2005. All sample sites, except Lake Monroe (shoreline), were sampled from open water areas. Samples were collected and analyzed in the same manner as samples collected from the Harris Chain of Lakes.

St. Johns River Water Management District/St. Johns River *Microcystis* Bloom Event

Samples were collected weekly from August through October 2005 by the Lower St. Johns River Basin program from SJRWMD. Samples were from Crescent Lake/Lake George (southern section) to Mayport, Florida (northern section; Table 1; Fig. 1). Sample sites were not fixed and were contingent on the distribution of the surface accumulation of algal cells (some sites were sampled on multiple occasions). In general, 250-1000 ml were collected in opaque

Table 1.-General Description of Florida Projects Involved with the Analysis of Water Samples for the Quantification of Cyanotoxins

Water System	Project Objective	Sampling Frequency	Collection Organization	Sample Type	Sample Sites	*Analyses Performed
Harris Chain of Lakes	Recreational Monitoring	Bi-weekly	GWL-CL	Open Water/ Bloom Events	Fixed/ Shoreline Accumulations	M,C,A
St. Johns River	Recreational Monitoring	Bi-weekly	LSJRB/GWL-CL	Open Water	Fixed	M,C,A
St. Johns River	Bloom Tracking	Weekly	LSJRB	Bloom Event	Open Water Accumulations	M
Lake Okeechobee	Water Quality Monitoring	Monthly	LOMP-SFWMD	Open Water	Fixed	M,C,A
St. Lucie River	Bloom Tracking	Weekly	SFWMD	Open Water	Fixed	M
Caloosahatchee River	Bloom Tracking	Weekly	LCPHS	Bloom Events	Bloom Accumulations	M

* GWL-CL: GreenWater Laboratories-CyanoLab
LSJRB: Lower St. Johns River Basin Program
SJRWMD: St. Johns River Water Management District
LOMP-SFWMD: Lake Okeechobee Monitoring Program
SFWMD: South Florida Water Management District
LCPH: Lee County Public Health Services

M = total microcystins
C = total cylindrospermopsin
A = total anatoxin-a

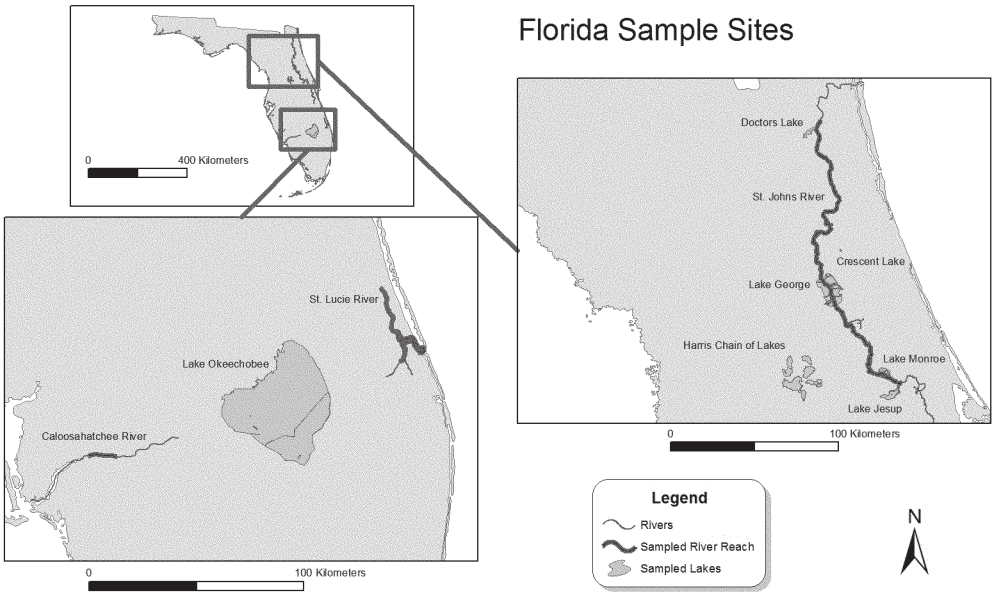

Figure 1.-Geographical Distribution and Location of Water Bodies and Sample Sites.

sample containers and transported on the day of collection to GreenWater Laboratories/CyanoLab for analyses. Water samples were analyzed for total microcystin concentrations by ELISA.

South Florida Water Management District/St. Lucie River *Microcystis* Bloom Event

Sets of samples were collected bi-weekly from August through October 2005 by the SFWMD (Table 1; Fig. 1). Sample sites (n = 42) were fixed and a part of previously established monitoring programs. In general, 250 ml were collected and placed into precleaned amber glass bottles. Samples were immediately placed in a cooler with ice and at the end of the day transported to GreenWater Laboratories/CyanoLab via overnight mail. Samples were analyzed for total microcystin concentrations by ELISA.

South Florida Water Management District/Lake Okeechobee

The Lake Okeechobee Monitoring Program of the SFWMD collected monthly samples during May-September 2005 at 6 fixed sample locations within the lake (Table 1; Fig. 1). Two-L samples were collected from surface waters and placed into precleaned amber plastic bottles. Samples were immediately placed into a cooler with ice and transported to GreenWater Laboratories/CyanoLab via overnight mail the day after collection. Samples were analyzed for total microcystin concentrations by ELISA and for cylindrospermopsin and anatoxin-a by LC/MS/MS.

Lee County Health Department/Caloosahatchee River

During August-October 2005, 2 samples per week were collected by the Lee County Health Department from the eastern section of the Calooshatchee River (Table 1; Fig. 1). In general, 1000 ml were collected and placed into precleaned opaque plastic containers. Samples were immediately chilled and placed in the dark; samples were transported to GreenWater Laboratories/CyanoLab via overnight mail the day after collection. Samples were analyzed for total microcystins by ELISA.

Toxin analyses

Sample preparation

For microcystin analysis, water samples were thoroughly mixed, aliquotted into a 250-ml subsample, sonicated, and filtered via a 0.45 µm centrifuge filter. Samples for cylindrospermopsin and anatoxin-a analyses were thoroughly mixed, aliquotted into a 300-ml subsample, sonicated, and frozen overnight at -20°C in lyophilization freeze flasks. Frozen samples were lyophilized to dryness and extracted with acidified ethanol. Extraction solutions were then dried down to a final volume of <1 ml under a stream of nitrogen and heat. In general, all samples were processed within 72 hrs after receipt and stored at 4°C until final analyses were performed.

Analytical protocols

Total microcystins

Enzyme Linked Immunosorbent Assay (ELISA) was utilized for the determination of the concentration of total microcystins (MCs) present. The ELISA assay is based on the polyclonal antibody method described by Chu *et al.* (1990) and adapted by An and Carmichael (1994). Antibody-coated plates, standards, and all reagents were supplied by Abraxis LLC (Product No. 520011). The level of sensitivity for microcystin(s) using this method was approximately 0.15 µg/L. The Abraxis ELISA kit is a competitive colorimetric assay and recognizes most microcystin variants. Microcystins were quantified using a Stat Fax 303+ spectrophotometer at a wavelength of 450 nm in conjunction with a reference wavelength of 630 nm. A final estimate for microcystin content was obtained and calculated as the mean of at least 2 subsamples (2 replicates per subsample).

Anatoxin-a and Cylindrospermopsin

A Thermo Finnigan LCQ Advantage liquid chromatographic/mass spectrometric (LC/MS/MS) system was used for the identification and quantification of cylindrospermopsin (CYN) and anatoxin-a (ANTX-A) in select samples from the Harris Chain of Lakes. An aliquot of each reconstituted lyophilized sample (at 100×) was directly analyzed. An additional aliquot was analyzed following the use of solid phase extraction (SPE) for clean-up of the lyophilized sample. Samples were eluted using both a normal phase/hydrophilic interactive column (ANTX-A) and a C18 reverse phase column (CYN) coupled with a mobile phase of water, acetonitrile, formic acid, and ammonium acetate (Aversano *et al.* 2004). Run times were 10 and 15 min for ANTX-A and CYN, respectively. The $[M+H]^+$ ions for ANTX-A (*m/z* 166) and CYN (*m/z* 416) were fragmented (MS/MS), and the major product ions for ANTX-A (*m/z* 149, 131, 107, and 91) and CYN (*m/z* 336, 318, 274, and 194) provided both specificity and sensitivity. Limits of quantification were established at 0.1 µg/L for both CYN and ANTX-A with a detection limit of 0.05 µg/L

Results

Harris Chain of Lakes

Open water microcystin concentrations in 6 central Florida lakes ranged from 0.05 to 3.6 µg/L and were reported in all lakes year-round (n = 90). Cylindrospermopsin, however, was identified in 18% of all water samples analyzed (n = 90) and ranged in concentration from 0.05 to 0.2 µg/L. Anatoxin-a was not positively identified in any water samples (n = 90) during the duration of this monitoring project. Microcystins and cylindrospermopsin co-occurred in 9 out of the 15 months (60%) when samples were collected.

Microcystis spp. bloom events (visible accumulations of cells onshore while performing sampling) had microcystin concentrations that ranged from 5 to 7,500 µg/L (Fig. 2). All lakes had at least one identifiable bloom event over the 15-month sampling period, while several lakes experienced up to 4 identified events. Bloom events occurred throughout the year, but the highest concentrations of microcystins occurred between October and December in 2003/2004. One lake had noticeable small clusters of *Microcystis* colonies in almost all months sampled. Bloom events were not tested for cylindrospermopsin or anatoxin-a due to the predominance of *Microcystis* spp.

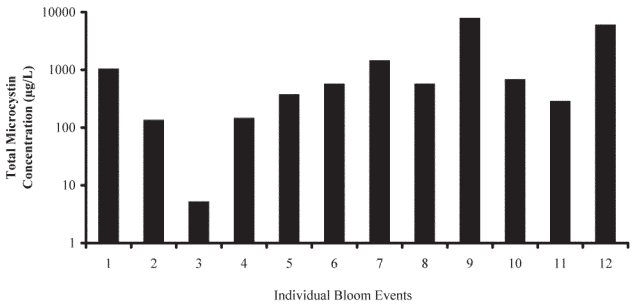

Figure 2.-Total Microcystin Concentrations during 2004 *Microcystis* Bloom Events in Central Florida Lakes (Harris Chain of Lakes).

St. Johns River recreational sites

Microcystin concentrations ranged from 0.01 to 31 µg/L (n = 50). Microcystins were reported from all sites throughout the monitoring period (May-Oct 2005). Of all samples analyzed, 24% had concentrations >1 µg/L while 4% had concentrations >10 µg/L.

Cylindrospermopsin concentrations ranged from nondetectable to 1.6 µg/L (n = 48). Of all samples analyzed, 35% were positive for the presence of cylindrospermopsin and 4% had concentrations ≥ 1.0 µg/L. Anatoxin-a was not identified (n = 48) in any samples analyzed.

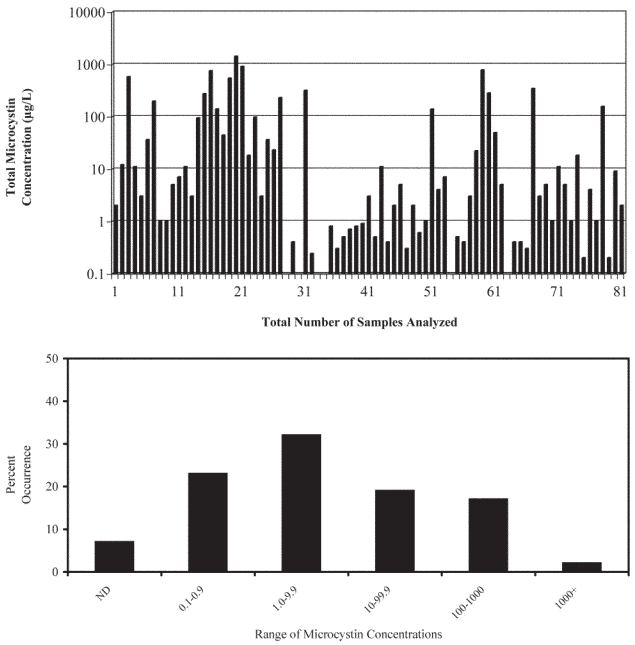

Figure 3.-A. Total Microcystin Concentrations Collected in Northeast Florida from a *Microcytsis* spp. Bloom in the St. Johns River, August/October 2005 (1-81, August water samples to September water samples) B. Frequency of Total Microcystin Concentrations Collected from a *Microcytsis* spp. Bloom in the St. Johns River, August/October 2005.

*St. Johns River and St. Lucie River/***Microcystis** ***spp. bloom event***

M. aeruginosa was the dominant species present in both bloom events, even though several different species of *Microcystis* were present. Both events started in August and persisted through October 2005. Microcystin concentrations in the St. Johns River ranged from nondetectable to 1,413 µg/L (n = 84; Fig. 3A). Of all samples analyzed, 32% contained microcystin concentrations >1 µg/L, 19% >10 µg/L, 17% >100 µg/L, and 2% >1,000 µg/L (Fig. 3B).

Microcystin concentrations in the St. Lucie River (and its associated watershed) ranged from nondetectable to 927 µg/L (n = 171; Fig. 4A). Of all samples reported, 53% had concentrations >1 µg/L, 12% >10 µg/L, and 5% >100 µg/L (Fig. 4B). Due to the dominant nature of *M. aeruginosa*, analyses were not performed for cylindrospermopsin or anatoxin-a for either bloom event.

Lake Okeechobee monitoring program

Fifty Samples were collected from January through September 2005 and analyzed for total microcystins, cylindrospermopsin, and anatoxin-a. Microcystins were reported in 100% of samples and ranged in concentration from 0.1 to 95 µg/L.

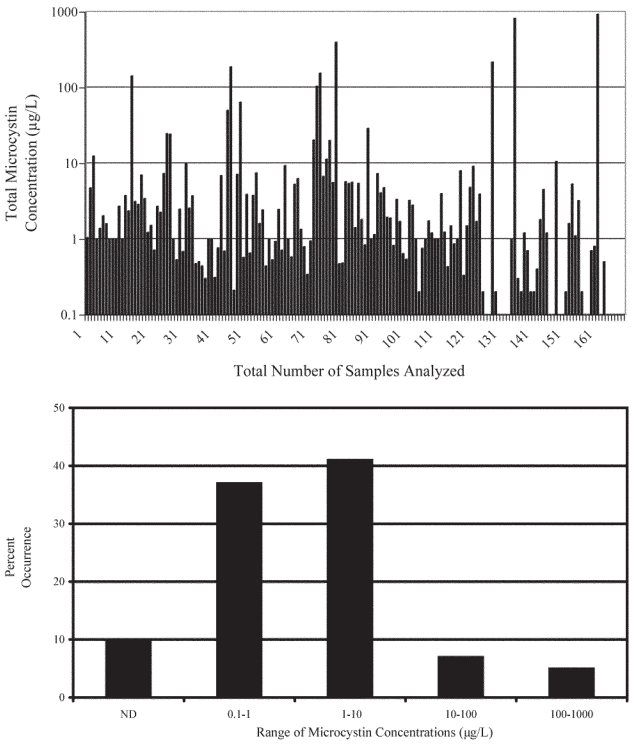

Figure 4.-A. Total Microcystin Concentrations in South Florida during a *Microcystis* spp. Bloom Event in the St. Lucie River, August/October 2005 (1-185, August water samples to September water samples) B. Frequency of Total Microcystin Concentrations in South Florida during a *Microcystis* spp. Bloom Event in the St. Lucie River, August/October 2005.

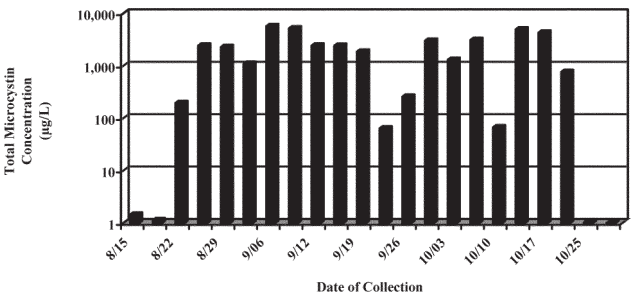

Figure 5.-Total Microcystin Concentrations in Southwest Florida during a *Microcystis* spp. Bloom Event in the Caloosahatchee River, August/October 2005.

Peak microcystin concentrations were observed in August. Cylindrospermopsin was reported in 2% of all samples and was detectable but not quantifiable. Anatoxin-a was not positively identified in any of the samples provided.

Caloosahatchee River

Microcystin concentrations ranged from 0.42 µg/L, collected at the end of the sampling period (Oct 2005), to 5,680 µg/L collected during the peak of the bloom event (Sept; 2005 n = 22; Fig. 5). In general, water samples were collected at sites where cell accumulations had occurred. Of the samples analyzed, 54% were reported at levels >1000 µg/L, 73% >100 µg/L, 82% >10 µg/L, and 95% >1 µg/L. Analyses for cylindrospermopsin and anatoxin-a were not performed.

Discussion

In general, species of *Microcystis*, *Cylindrospermopsis*, *Anabaena*, *Aphanizomenon*, *Planktothrix*, and *Lyngbya* (*Plectonema*) are the most common potentially toxigenic cyanobacteria observed in freshwaters in the state of Florida (Table 2; Williams *et al.* 2001, 2006. *Lyngbya* (*Plectonema*) *wollei* is usually most abundant in Florida springs and is benthic and macroscopic, while the other genera are primarily planktonic and microscopic (C. Williams pers. observ.). *Microcystis aeruginosa*, *Anabaena circinalis*, and *Cylindrospermopsis raciborksii* are considered to be the primary producers of microcystins, anatoxin-a, and cylindrospermopsin, respectively, in Florida surface waters (A. Chapman, pers. comm.). Data collected from the St. Johns River, Lake Okeechobee and the Harris Chain of Lakes, the long-term projects that analyzed the 3 main cyanotoxins, resulted in a priority listing (based on prevalence and concentration levels observed) of toxins for Florida: (1) microcystins, (2) cylindrospermopsin, and (3) anatoxin-a (Table 3).

Microcystis blooms are the most noticeable (high density floating algal accumulations), the most prevalent, and contain the highest concentrations of cyanotoxins (microcystins) in

Table 2.-List of commonly observed potentially toxigenic cyanobacteria in Florida's surface water.

Genera	Species	Toxins Produced
Anabaena	*circinalis*, *flos-aquae*, *planctonica lemmermannii*	microcystins, anatoxin-a, saxitoxin
Aphanizomenon	*ovalisporum*, *gracile*, *flos-aquae issatchenkoi*	anatoxin-a, cylindrospermopsin, saxitoxin
Cylindrospermopsis	*raciborskii*	cylindrospermopsin, saxitoxin
Lyngbya (*Plectonema*)	*wollei*, *majuscula*	aplysiatoxin, lyngbyatoxins
Microcystis	*aeruginosa*, *wesenbergii*, *flos-aquae viridis*	microcystins
Planktothrix	*agardhii*	microcystins, anatoxins, saxitoxins, aplysiatoxins

Table 3.-Comparison of total microcystin concentrations and total cylindrospermopsin concentrations for different monitoring projects performed in the state of Florida in 2005. Anatoxin-a was not detected in samples collected during 2005.

Water System	Months of Collection	Microcystins					Cylindrospermopsin				
		n	min	max	median	freq. (%)	n	min	max	median	freq. (%)
Open Water Samples											
*Harris Chain of Lakes	Sept. - Nov.	90	0.05	3.6	0.5	100	90	ND	0.2	0.1	18
St. Johns River Recreational Sites	May - Oct.	50	0.01	31	0.6	100	48	ND	1.6	0.1	35
Lake Okeechobee Monitoring Program	Jan. - Dec.	67	0.1	95	0.1	100	50	ND	0.05	ND	2
Bloom Events											
*Harris Chain of Lakes	Jan. - Dec.	12	5	7,500	550	100					
St. Johns River	Aug. - Oct.	81	ND	1,413	3.0	93					
St. Lucie Watershed	Aug. - Oct.	169	ND	927	1.2	88					
Caloosahatchee River	Aug. - Oct.	22	0.42	5,680	1,584	100					

* sample collection performed between Sept. 2003 through Nov. 2004.
ND = non-detect

Florida. Blooms in the St. Johns, the Caloosahatchee, and the St. Lucie Rivers all persisted 45-60 days. Blooms started to develop at the end of August 2005, maintained high densities and toxin production through September/October, and terminated at the end of October coinciding with hurricane activity and falling temperatures. It is unclear if blooms would have naturally dispersed without the aid of heavy storm activity. Exposure durations of >45 days to toxin-producing blooms may be problematic to both recreational use and ecological health of impacted water systems.

Microcystis spp. blooms in Florida have the potential to produce high concentrations of microcystins (>20 µg/L). In Falconer *et al.*, (1999) a concentration of 20 µg/L is recommended as the advisable level to post and close-off recreational water sites, inform citizens of current conditions, avoid high density algal surface formations, and to monitor for toxin levels on a daily basis due to a significantly higher risk for health problems due to exposure. The St. Johns River, the Caloosahatchee River, and several of the lakes in the Harris Chain had numerous sample concentrations above this guideline. Recently, Australia developed recreational exposure values for children (~15 µg/L) and adults (~45 µg/L; Burch 2005). Microcystin levels in Florida have, on numerous occasions, exceeded these thresholds by 100× or more (Table 3). In 2005, the first official health alert for a toxigenic algal bloom was issued by the St. Johns County Department of Health. Consequently, the SJRWMD sampled daily and issued weekly press releases to inform the public of areas of high microcystin concentrations. Currently, there are no federal public health guideline concentrations in the United States for any of the cyanotoxins regarding recreational exposure or drinking water safety; however, USEPA recently organized (Jan 2007) an expert panel to review published peer-reviewed toxicity data regarding acute, short-term, subchronic, chronic and carcinogenicity potential for the microcystins, cylindropspermoposin, and anatoxin-a. Furthermore, these 3 compounds are currently on the USEPA's chemical contamination list II for potential regulation as water quality parameters for drinking water resources.

Differences in microcystin concentrations between similar types of blooms in the St. Johns, St. Lucie, and Caloosahatchee Rivers as well as Lake Okeechobee probably represent differences in sampling protocols and objectives. The St. Johns River was sampled in an attempt to track the bloom and identify "hot spots," the St. Lucie River, Lake Okeechobee, and associated water systems in south Florida were sampled at previously established SFWMD monitoring sites that may or may not have been close in proximity to bloom events, while collections in the Caloosahatchee River were predominantly at locations where cell accumulations occurred. The differences observed in total concentrations depict the importance of sampling methods (surface vs. integrated), sampling location (boat vs. shoreline, within/near the bloom vs. outside the bloom), sample volume (L vs. mls), and sampling objectives (maximum levels vs. geographical distribution). Data within individual databases were also variable, depicting the patchiness and the temporal variability of bloom events, especially surface cell accumulations that are

wind transported and concentrated (Fig. 3-5; Table 3). Proper sampling techniques and strategies to be employed depend on the intent and the objectives of the project.

C. raciborskii and cylindrospermopsin also need to be monitored closely. During 2000 cylindrospermopsin concentrations were commonly reported between 50 and100 µg/L and as high as 202 µg/L (Williams *et al.* 2006) in some Florida lakes. Data from the Harris Chain of Lakes show that the frequency of cylindrospermopsin occurrence increased from 2002/2003 to 2003/2004 from approximately 14% to 22%, although concentrations remained relatively low (<0.2 µg/L) during both sampling periods. *C. raciborskii* can dominate Florida phytoplankton assemblages in a near bloom state year-round, maintain equal or greater densities at depth (0.8-0.9 z_{max}) relative to surface concentrations, and may be equally distributed throughout the mainstem of the entire lake (Upper Ocklawaha River Basin/SJRWMD; Williams *et al.* 2006). Furthermore, cylindrospermopsin, although classified as a hepatotoxin, acts more as a general tissue toxin than do microcystins that specifically act upon only the liver. Both cylindrospermopsin and microcystins have been implicated as potential tumor-promoting compounds (Falconer 1991, Falconer and Humpage 2001), while cylindrospermopsin has been shown to have genotoxic activity as well (Humpage *et al.* 2000). The co-occurrence of these compounds further complicates the potential risks involved with cyanotoxin production and indicates the importance of analyzing for both (all) compounds when microcystin-producing species and *C. raciborskii* (other toxin-producing species) co-exist.

Recently (2003-2005), the presence of anatoxin-a in Florida surface waters was uncommon but has historically been reported at elevated concentrations. In 2000, a bloom of *A. circinalis* was reported to contain 156 µg/L of anatoxin-a and was associated with a major fish mortality event in the St. Johns River (Williams *et al.* 2006. In 2002/2003, the Harris Chain of Lakes were reported to contain anatoxin-a in 31% of all samples analyzed (n = 60, range 0.1-7 µg/L), while in 2003/2004 anatoxin-a was not identified in any samples collected (n = 72). Likewise, anatoxin-a was not identified in either the Florida Department Health study or the Lake Okeechobee monitoring program. Anatoxin-a is a neurotoxin that is relatively fast-acting and has been implicated in the death of numerous animal species (Ressom *et al.* 1994) worldwide. Although not observed as frequently as the microcystins and cylindrospermopsin in Florida surface waters, anatoxin-a is still a compound of major concern due to its fast-acting nature and potential to induce paralysis and respiratory failure (Carmichael 1997).

More long-term (5-10 yr) cyanotoxin monitoring projects need to be designed and implemented by water and environmental management agencies in conjunction with routine water quality monitoring programs. Different water bodies respond differently to similar types of environmental influences/conditions and therefore baseline data need to be established for individual water systems. Long-term monitoring is a key component to better understanding potential initiation factors, toxin production dynamics, trends in occurrence, and the potential ecological and human health risks and impacts of toxigenic cyanobacteria.

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