Assessment of Cyanotoxins in Florida's Lakes, Reservoirs and Rivers

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EXECUTIVE SUMMARY

Harmful algal blooms (HABs) are population increases of algae above normal background levels and are defined by their negative impacts on the environment, the economy, and human health. Historically, many of Florida's largest and most utilized freshwater and estuarine systems have been plagued by occasional blooms of harmful algae. During the last decade, however, the frequency, duration, and concentration levels of these blooms in freshwater and brackish water have increased significantly, primarily due to changes in land utilization, changes in hydrology, increases in nutrient runoff, loss of aquatic vegetation, and a climate that is very conducive to algal growth and proliferation.

In 1998, the Florida Harmful Algal Bloom Task Force was established to determine the extent to which HABs pose a problem for the state of Florida. Blue-green algae (cyanobacteria) were identified as top research priorities due to their potential to produce toxic chemicals and contaminate natural water systems. In June 1999, the St. Johns River Water Management District (SJRWMD) initiated a collaborative study in conjunction with the Florida Marine Research Institute, the Florida Department of Health, and Wright State University to determine the geographical distribution of various types of toxin-producing blue-green algae in Florida's surface waters and to positively identify any algal toxins present in these waters.

Blue-green algae produce three broad categories of toxins: those that attack the liver, those that attack the nervous system, and those that irritate and cause allergic reactions in the skin. These toxins all are highly toxic and can persist in natural waters for long periods of time. The liver toxins cause liver damage or failure and can contribute to cancer formation. The neurotoxins can cause convulsions, paralysis, and respiratory distress or failure. Other toxins can cause acute dermatitis or "swimmer's itch." Liver and neural toxins, when concentrated during a bloom event, have been reported to cause illness and death in animals following ingestion of bloom material in drinking water. Human illness has also been associated with cyanotoxins following ingestion of toxins in drinking water supplies, exposure to blooms during recreational activities on surface waters, and haemodialysis treatment.

During the summer of 1999, the Cyanobacteria Survey Project at SJRWMD collected a total of 167 water samples from sites throughout the state of Florida. Eighty-eight of these samples, representing 75 individual water bodies, were found to contain significant levels of toxin-producing blue-green algae. Thus far, algal toxins (particularly liver toxins) have been positively identified in all 88 of these water samples, and 80% of these samples have been shown to be lethally toxic to mice.

The widespread distribution and relatively high levels of toxin-producing blue-green algae in Florida's waters, in addition to the demonstrated acute toxicity of the algal toxins, indicate the potential for a significant threat to public health from a poisonous HAB event. Algal toxins were found in water bodies that are presently being used as drinking water resources, which is of particular concern, as these toxins can be highly toxic to humans if not adequately treated. Many cyanobacterial blooms are often detected by the formation of surface scums, the discoloration of water, and off-flavor and odors in drinking water supplies. However, blooms formed by *Cylindrospermopsis raciborskii* in surface water supplies may be more difficult to detect due to the formation of subsurface blooms and the lack of significant levels of taste and odor-causing compounds. Little information currently exists on the long-term effects of chronic exposure to algal toxins. More research is necessary before the possibility of health problems from this type of algal exposure can be ruled out as a public health concern.

Florida may consider addressing cyanotoxins in surface waters by establishing a statewide monitoring program for HABs, identifying appropriate environmental algal toxin guidelines for recreational waters and drinking water sources, and determining potential human health risks associated with algal toxins. Florida may also consider establishing an in-state analytical laboratory for algal toxins and continuing the collaboration of local, state, and federal agencies.

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INTRODUCTION

A diverse assemblage of cyanobacteria (e.g., blue-green algae, cyanoprokaryotes, cyanophytes, myxophyceaens) exists in many of Florida's freshwater and coastal environments. Cyanobacteria are considered to be ancient prokaryotes that represent one of the first living organisms on Earth. They have a long evolutionary history, dominating the Precambrian era as long as 3.5 billion years ago (Schopf 1994a, 1994b). As a group, cyanobacteria are considered to be morphologically and physiologically similar to true bacteria. They are generally defined by their intracellular prokaryotic characteristics, such as the lack of defined nuclei, chloroplasts, and organelles, but also demonstrate the ability to conduct oxygenic photosynthesis in a similar way as do eukaryotic algae and plants. Cyanobacteria often dominate in extreme habitats, such as thermal pools, polar lakes, hot deserts, hypersaline waters and hypereutrophic lakes, and rivers. They are valued for their ability to fix atmospheric nitrogen, bind and enrich soils, and produce medicinally useful compounds.

Despite a long history of cyanobacteria in Florida, a growing trend in the incidence of cyanobacterial blooms has been recognized over the past several decades. The term "bloom" is poorly defined, but typically represents an episodic proliferation of algal biomass that is significantly higher than the known average for a given water body. Cyanobacterial blooms in Florida can be episodic or continuous, occurring most often in hypereutrophic lacustrine and riverine systems or in reservoirs that undergo stratification. Cyanobacterial blooms may be described as nuisance or harmful if they produce toxins, cause toxic effects on humans or other animals, discolor water, produce foul odors, deplete dissolved oxygen, limit underwater light levels or overgrow benthic habitats.

The first toxic cyanobacterial bloom reported in the scientific literature occurred over a century ago in Lake Alexandrina and River Murray, South Australia (Francis 1878). Several hundred animals, including horses, cattle, and sheep, died following the ingestion of algal scum from the lake. Reports of animal and human poisonings consistent with cyanobacterial intoxication have continued since that time, but only recently has the diversity of toxic algae and the toxins produced by them become clear. Worldwide, approximately 42 species of cyanobacteria are known to

produce unique toxins (i.e., cyanotoxins) that can cause acute and chronic health problems in humans and fatal poisonings in other animals, fish, and birds (Table 1). Known Florida reports of cyanotoxins or cyanotoxic events are limited to Lakes Okeechobee, Istokpoga (Carmichael 1992), and Adair (Kennedy 1992). Mouse bioassay results indicated a neurotoxin in Lake Istokpoga and a hepatotoxin in Lake Okeechobee. Following a report of dead birds and a blue-green algal film on Lake Adair, a pathologist examined two cormorants (*Phalacrocorax* sp.) and determined the cause of death to be massive hepatocellular necrosis, suggestive of hepatotoxic poisoning by cyanobacteria.

Cyanobacteria produce a variety of toxins that appear to be less toxic to aquatic biota than to humans and other terrestrial mammals (Sugaya et al. 1990; Vasconcelos 1998; Sivonen and Jones 1999). Cyanotoxins are categorized into three major chemical groups: cyclic peptides, alkaloids, and lipopolysaccharides.

Cyclic hepatotoxic peptides (i.e., liver toxins) are the most common cyanotoxins causing death and illness in animals (Luukkainen et al. 1993, 1994). Cyanobacterial genera known to produce hepatotoxins include *Microcystis, Anabaena, Planktothrix* (*Oscillatoria*), *Nostoc, Hapalosiphon, Anabaenopsis, Nodularia, Cylindrospermopsis, Aphanizomenon,* and *Umezakia.* Over 60 variants of microcystin, the cyclic pentapeptide nodularin and cylindrospermopsin, have been described following initial isolation and characterization of microcystin from *Microcystis aeruginosa* (Bishop et al. 1959; Konst et al. 1965; Carmichael, Biggs et al. 1988; Carmichael, Eschedor et al. 1988; Carmichael, Yu et al. 1988; Harada et al. 1994; Banker et al. 1997). Field poisonings involving cattle, sheep, horses, pigs, ducks, and other wild and domestic animals have been reported. Symptoms or signs of poisoning in laboratory animals include weakness, anorexia, pallor of mucous membranes, vomiting, cold extremities, diarrhea, muscle tremors, and coma. Within a few hours or days, acute hepatotoxicosis can result in death due to intrahepatic hemorrhage and hypovolemic shock. The hepatotoxins are strong inhibitors of types 1 and 2A serine protein phosphatases that are considered vital to cell processes, including growth and tumor suppression (Carmichael 1992, 1994; Luukkainen et al. 1993; MacKintosh et al. 1990; Runnegar et al. 1995). Microcystin is known to be a tumor promoter in laboratory animals and is considered to be one of the

Table 1. Potentially toxic cyanobacteria distributed in fresh and estuarine waters

Toxin(s) Produced	Species	
Hepatotoxin(s)	*Anabaena flos-aquae*	*Nodularia spumigena*
	Anabaenopsis milleri	*Nostoc* sp.
	Aphanizomenon ovalisporum	*Oscillatoria* spp.
	Cylindrospermopsis raciborskii	*Planktothrix agardhii*
	Hapalosiphon spp.	*Planktothrix rubescens*
	Microcystis aeruginosa	*Synechocytis* sp.
	Microcystis viridis	*Umezakia natans*
	Microcystis wesenbergi	
Neurotoxin(s)	*Anabaena circinalis*	*Lyngbya wollei*
	Anabaena flos-aquae	*Microcystis incerta*
	Anabaena lemmermannii	*Microcystis* spp.
	Anabaena solitaria f. *planktonica*	*Microcystis wesenbergi*
	Anabaena spiroides	*Nostoc linckia*
	Aphanizomenon flos-aquae	*Nostoc paludosum*
	Coelosphaerium kuetzingianum	*Nostoc rivulare*
	Cylindrospermopsis raciborskii	*Nostoc zetterstedtii*
	Cylindrospermum sp.	*Phormidium acutissimum*
	Gleotrichia echinulata	*Phormidium formosum*
	Gleotrichia pisum	*Phormidium nigro-viride*
	Lyngbya majuscula	*Planktothrix* spp.
Dermatotoxin(s)	*Anabaena* spp.	*Planktothrix (Oscillatoria)* spp.
	Anabaenopsis spp.	*Schizothrix* spp.
	Anacystis spp.	*Umezakia natans*
	Aphanizomenon spp.	
	Cylindrospermopsis sp.	
	Hapalosiphon spp.	
	Lyngbya spp.	
	Nodularia spp.	
	Nostoc spp.	
	Phormidium sp.	

more potent liver carcinogens yet characterized (Nishiwaki-Matsushima et al. 1992).

Neurotoxic alkaloids have also caused animal poisonings in North America, Europe, and Australia. The alkaloid toxins produced by cyanobacteria are diverse in their chemical structure. They are generally described as heterocyclic nitrogenous compounds containing ring structures with at least one carbon-nitrogen bond. Cyanotoxins known to be neurotoxic include anatoxin-a, homoanatoxin-a, anatoxin-a(s), and saxitoxins (i.e., paralytic shellfish poisons).

Anatoxin-a is produced by the following cyanobacterial genera: *Anabaena, Oscillatoria, Aphanizomenon,* and *Cylindrospermum.* Homoanatoxin-a is a homologue isolated from *Oscillatoria formosa* (*Phormidium formosum*). Anatoxin-a was first described as a "very fast death factor" from bloom samples taken from Canada (Gorham 1964, 1965; Carmichael et al. 1977; Carmichael and Gorham 1977; Carmichael and Gorham 1978). Death due to respiratory arrest occurs within minutes to hours, dependent on concentration of toxin consumed. Signs of poisoning include staggering, muscle fasciculations, gasping, convulsions, and, in birds, opisthotonos (i.e., an abnormal posturing characterized by rigidity and severe arching of the back). Both anatoxin-a and homoanatoxin-a mimic acetylcholine, thereby acting as a postsynaptic depolarizing neuromuscular blocking agent that binds to the nicotinic acetylcholine receptor with a higher affinity than acetylcholine (Carmichael et al. 1975, 1979; Valentine et al. 1991). The lethality of anatoxin-a is primarily due to over-stimulation of muscle that results in muscle fasciculations, fatigue, and paralysis.

Anatoxin-a(s), the *s* denoting viscous hypersalivation in animals, is a unique phosphate ester (cyclic N-hydroxyguanine) that inhibits cholinesterase. It has been isolated from strains of *Anabaena* (Mahmood and Carmichael 1987; Matsunaga et al. 1989) with symptoms of poisoning in animals being similar to anatoxin-a but including ataxia, diarrhea, hypersalivation, and tremors. In ducks, similar conditions occur but also include regurgitation of algae, dilation of cutaneous vessels in the webbed feet, wing and leg paresis, opisthotonos, and clonic seizures prior to death (Cook et al. 1989). The toxin is considered to be a naturally occurring organophosphate and functions similarly to the organophosphate pesticides (e.g., malathion and parathion) (Carmichael 1992, 1994).

Studies of cyanotoxic blooms and isolates of *Anabaena flos-aquae* in Canada found that cyanobacteria were able to produce paralytic shellfish poisons (PSPs) similar to those produced by marine dinoflagellates (e.g., red tide) (Jackim and Gentile 1968; Sawyer et al. 1968; Alam et al. 1973; Ikawa et al. 1982; Mahmood and Carmichael 1987). PSP-toxins are produced by strains of *Anabaena, Aphanizomenon, Lyngbya, Oscillatoria,* and *Cylindrospermopsis.* Symptoms indicative of poisoning include irregular breathing, loss of coordination, twitching, and death by respiratory failure (Carmichael 1992, 1994; Hunter 1995; Keevil 1991). PSP-toxins are a group of carbamate alkaloid neurotoxins that inhibit nerve conduction by blocking sodium channels. The PSP-toxins are structurally characterized as non-sulphated (saxitoins-STX), singly sulphated (gonyautoxins-GTX), or doubly sulphated (C-toxins).

Lipopolysaccharides of cyanobacteria have been suspected to cause inflammatory dermatitis (e.g., swimmer's itch) and bath water fever outbreaks (Muittari et al. 1980; Keleti et al. 1979, 1981). Aplysiatoxin, debromoaplysiatoxin, and lyngbyatoxin-a are produced by the marine cyanobacterium *Lyngbya majuscula* and have also been associated with inflammatory dermatitis and gastroenteritis (Mynderse et al. 1977; Cardellina et al. 1979). Cyanobacteria lipopolysaccharides differ chemically from those found in the outer cell wall of gram negative bacteria and generally have lower biological activity (Keleti and Sykora 1982).

It is not surprising that cyanobacteria tend to be a major component of Florida's aquatic ecosystems, given Florida's subtropical climate, the eutrophic status of many of its surface freshwaters, and significant hydrologic modifications that have occurred following urban development. Cyanobacterial blooms have occurred in many of Florida's largest and most important lakes, rivers, and estuaries, including Lake Okeechobee, Lake Apopka, the St. Johns River, and Florida Bay, over the past several decades. While there is little doubt that occurrences of such blooms pre-date human development in Florida, the recent increase in human population density (~1,000 people/day; Smith 1996) and land use appear to be associated with increased bloom frequency, duration, and intensity.

Cyanobacterial blooms in Florida may represent major threats to water quality, ecosystem stability, surface drinking water supplies, and public health. Hypoxia followed by fish and invertebrate kills is a common

phenomenon associated with bloom degradation. Loss of submerged aquatic habitats also is associated with cyanobacterial blooms where surface scums attenuate underwater irradiance below levels required for plant photosynthesis and survival. Human health may be at risk if cyanobacterial blooms are producing cyanotoxins in recreational and/or surface drinking water supplies. In addition to potential risks to natural resources and human health, significant economic impacts can be attributed to fisheries decline and the decline of recreational value for Florida's lakes and beaches due to the avoidance of poor water quality conditions and the production of algal scums.

In the past, due to the lack of focused research and biomonitoring programs, Florida's understanding of cyanobacterial blooms and their toxins remained largely in the realm of incidental observation and speculation. In order to accurately assess the potential for cyanobacteria to impair water quality, ecosystem stability, and human health, an assessment of cyanobacterial bloom distribution and toxin production was recognized by the Florida Harmful Algal Bloom Task Force (Steidinger et al. 1999).

PROJECT OBJECTIVES

The primary objectives of this project were to identify major water bodies throughout Florida that experience cyanobacterial blooms, collect representative water samples during cyanobacterial blooms, screen collected samples for potentially toxic algal species, and identify/characterize cyanotoxins.

MATERIALS AND METHODS

SAMPLING SITE/TYPE SELECTION

Sample sites were identified and water samples collected throughout the state with the assistance of the Department of Environmental Protection, the water management districts, regional and county water suppliers, and local environmental agencies. Water sampling was attempted from at least 25 sites within each area of the state as designated by water management district boundaries (125 sites total). The number of sites outside of the St. Johns River Water Management District (SJRWMD) was limited by geographical distribution and availability of field personnel capable of sample collection, the frequency and duration of occurrence of algal blooms in water bodies of the region in question, and the costs associated with specific toxin analyses. Site selection was based on the following prioritized designated use classification scheme, as described in the previously accepted Quality Assurance Plan:

Prioritized Site Selection by Designated Use

- Potable surface water supply
- Contact recreation (swimming)
- Agricultural (livestock water supply)
- Outstanding Florida Waters (OFWs)

Water sampling consisted of the collection of 3 liters (L) of surface water for the purposes of cyanobacteria species composition and identification (1 L) and algal toxin detection and characterization (2 L: 1 L for the Florida Department of Health and 1 L for Wright State University). An additional 4 L of water was collected for the storage of an archive sample (1 L) and for the determination of ambient water chemistry (3 L) when proper holding times could be maintained. Macroalgae were also collected if present as surface mats and identified by SJRWMD staff. In general, the concentration of water samples by net tows was not performed due to the inconsistent availability of such equipment and the misrepresentation of natural environmental conditions.

SAMPLING PROCEDURES

General Practices

1. Samples were collected from the bow of the boat, upwind or upstream from the motor (upstream from the boat when wading), from piers, or from the perimeter of the specified water body.

2. Care was taken not to disturb sediments in the immediate area of sample collection.

3. Sample collection equipment was rinsed with sample water prior to sample collection.

4. Sample containers were labeled with the station name and the complete date-time group (YYMMDDHHMM). The time was kept in local time.

Surface Water Sampling

1. The open sampler was lowered into the water perpendicular to the surface of the water until the desired depth was reached (3.0 meters [m], or to within 0.1 m of the bottom in shallow water).

2. The top of the sampler was capped with a rubber stopper.

3. The sampler was briskly removed from the water, and the open end of the sampler was placed over a clean intermediate sample container (churn splitter). The stopper was removed, dispensing the sample.

4. Steps 1–3 were repeated until a sufficient volume was collected in the churn splitter to fill all of the sample bottles.

5. The sample was thoroughly mixed, using the plunger.

6. A small amount of sample from the churn splitter was dispensed into each sample container that was used. The container was capped and agitated so that all internal surfaces were coated with sample, and the contents poured out.

7. Sample containers were filled to the appropriate level. The sample was mixed well in the churn splitter before each bottle was filled. (One 500-

milliliter [mL] sample was collected for taxonomic purposes, two 1-L samples were collected for algal toxin screening and characterization, and one 1-L sample was collected for archiving.)

8. The bottles were placed into a resealable polyethylene bag provided by the laboratory.

All samples were held in a cooler containing wet ice.

Split samples were collected at every site to provide identical samples to both laboratories for analyses, as well as for interlaboratory checks and comparisons.

Sample Preparation

Preparation of all samples for further analyses was performed entirely by the appropriate laboratory, dependent upon the parameter being analyzed.

Water/plankton: A 200-mL sample was filtered through GF/C filter paper (dry weights determined from biomass on filter paper). Cells on filter paper were extracted with 10 mL 100% methanol. Methanol extracts are diluted (1:10 to 1:1000) in 0.01 M PBS buffer for use in the Enzyme Linked Immunosorbent Assay (ELISA) and with distilled water for the Protein Phosphatase Inhibition Assay (PPIA). For the Anticholinesterase Assay (AA), cells were extracted with 10 mL of acidified water (pH 3) and diluted as needed with the same solution for enzyme assay. For anatoxin-a, the method of James et al. (1998) was used. For the mouse bioassay, samples were freeze-thawed two times and filtered through GF/C filter paper.

MEASUREMENT METHODS/TEST PROCEDURES

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

The ELISA 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 in the Microcystin Plate Kit (Envirologix Inc., Catalog No. EP 022). The level of sensitivity for microcystin and nodularin using this method is ~0.5 nanograms per milliliter (ng/mL). Values below or near this level were not considered significant. Freeze-thawed water samples were filtered and dry weights determined from biomass on the filters. Animal tissue was weighed and homogenized. Filtered water (or methanol supernatant of homogenized animal tissue) was concentrated in C-18 Bond Elut cartridges, and the methanol eluate was dried and assayed. Serial dilutions of 10^{-1} to 10^{-4} (in duplicate) were used to run the assay.

MOUSE BIOASSAY

Mouse bioassays incorporated the use of ICR-Swiss male mice (two mice/dose level) injected at 1,000, 500, 250, and 100 milligrams per kilogram body weight, with extracts of freeze-dried sample. When samples contained less than the necessary amount of freeze-dried sample, the mouse bioassay was altered at the discretion of the investigator (i.e., change in concentration levels used and number of mice injected). Mice were observed for 1 hour and then at 1-hour intervals for 8 hours. When possible, mice were observed for longer periods of time up to 48 hours post-injection.

PROTEIN PHOSPHATASE INHIBITION ASSAY (PPIA)

As previously described, the cyclic peptide liver toxins (microcystin and nodularin) have been shown to be specific and potent inhibitors of protein phosphatases 1 and 2A (PP1 and PP2A). Enzyme inhibition has been shown to be correlated with the ability of these toxins to be tumor promoters, especially in the liver. The assay, therefore, was useful in conjunction with the ELISA assay (which tests for the presence of the compounds, not all of which are bioactive) as an activity assay (to measure actual toxic effect). Microcystin and nodularin can bind

covalently to the catalytic subunit of PP1 and PP2A in an irreversible and non-competitive manner. The more microcystin or nodularin contained in the sample, the more PP1 or PP2A will be inhibited. PP1 and PP2A will dephosphorylate p-nitrophenol phosphate (pNPP) and produce paranitrophenol, which has a yellow color (measured at 405 nanometers [nm]). Therefore, PP activity on pNPP can be determined by measuring the rate of color formation from the liberation of pNPP at 405 nm using a microplate reader. The darker the color, the lower the microcystin concentration (or the greater the PP activity), and vice versa. The assay is about 103 times more sensitive than HPLC. It was performed according to the method outlined in An and Carmichael (1994).

ANTICHOLINESTERASE ASSAY (AA)

The anticholinesterase assay for anatoxin-a(s) is based on the colorimetric assay of Ellman et al. (1961) for *in vitro* cholinesterase activity, as adapted by Mahmood and Carmichael (1987) and Matsunaga et al. (1989). The potent toxicity of anatoxin-a(s) is attributed to exceptional anticholinesterase activity. Inhibition of acetylcholinesterase enzyme was measured by a decrease in the color generated and monitored at 412 nm from the reaction of enzyme and the substrate acetylthiocholine. This percent inhibition was compared against a standard curve of percent inhibition vs. log dose of purified anatoxin-a(s). Changes in absorbance were monitored over 1-minute intervals. The sample was run in duplicate. All activities were compared against values obtained with enzyme plus no sample and sample plus no enzyme. Absorbance was measured using a Beckman DU-70 spectrophotometer.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-FLUORESCENCE (HPLC-FL)

The determination of anatoxin-a in natural waters poses an analytical challenge due to the typically low concentrations of this toxin. A highly sensitive HPLC method using fluorometric derivatisation with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) in acetonitrile was developed, which simultaneously determines anatoxin-a and homoanatoxin-a, and their dihydro and epoxy analogues (James et al. 1998). The HPLC uses a C18 column at 35°C. The mobile phase is acetonitrile-water (45:55) with a flow rate of 0.5 mL/minute and fluorometric detection (λ_{ex} = 470 nm, λ_{em} = 530 nm). The limit of detection is 10 nanograms per liter.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-ULTRA VIOLET (HPLC-UV)

An HPLC-MS/MS method was developed for detecting low concentrations of cylindrospermopsin (Eaglesham et al. 1999). An HPLC-UV method was developed based upon the preliminary work of Hawkins et al. (1997) and was applied to this toxin when appropriate.

RESULTS

SAMPLING

A total of 167 water samples (Figure 1; Appendix A) were collected from water bodies throughout the state of Florida between June 10 and November 5, 1999. All samples were analyzed for the presence of toxigenic cyanobacteria, with 81 samples analyzed for cyanotoxins.

SJRWMD was the most extensively sampled water management district (n = 69; 41% of all samples collected), due in large part to the presence and magnitude of the St. Johns River (with 26 sample sites) and a high incidence of blue-green algae blooms. The numbers of water bodies sampled in the other water management districts are as follows: Southwest Florida (n = 29), South Florida (n = 29), Northwest Florida (n = 25), and Suwannee River (n = 15). The last district was sampled the least often due to its relatively small area and the relatively small number of eutrophic lakes located within the region.

ALGAL AND TOXIN DISTRIBUTION

Presence and Distribution of Toxigenic Cyanobacteria

A total of 88 water samples, representing 75 individual water bodies, were dominated by toxigenic cyanobacteria (Tables 2 and 3). Seven genera of cyanobacteria were identified from the water samples collected. Of these, *Microcystis* (43.1%), *Cylindrospermopsis* (39.5%), and *Anabaena* (28.7%) were observed the most frequently and in the greatest concentration levels in samples from throughout the state. The geographic distribution of the dominant three species is shown in Figures 2–4. *Planktothrix* (13.8%), *Aphanizomenon* (7.2%), *Coelosphaerium* (3.6%), and *Lyngbya* (1.2%) were observed less frequently, but at times made significant contributions to the planktonic and macroalgal species composition (Figures 5 and 6).

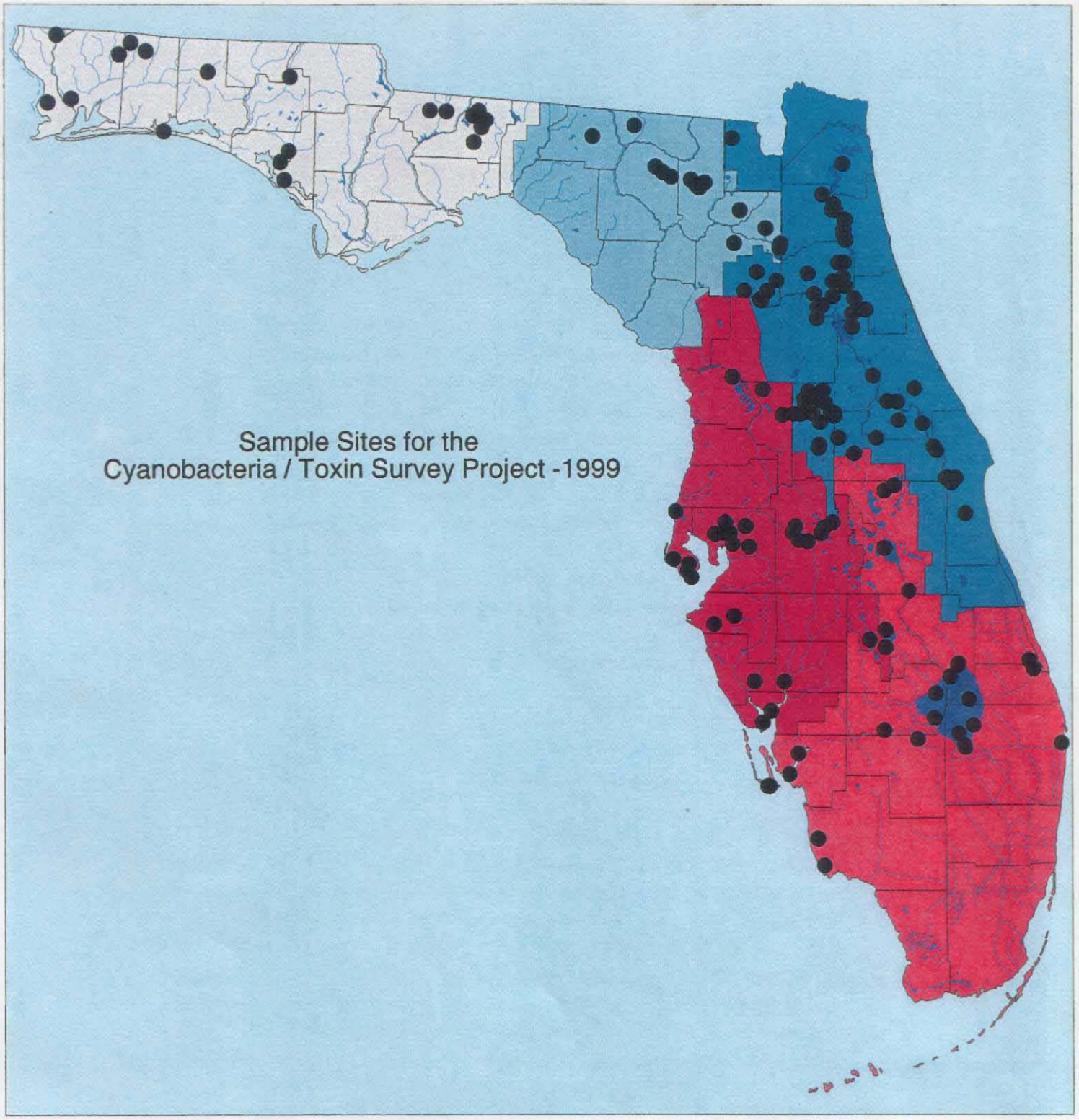

Figure 1

N
W E
S

0 30 60 Miles

1:3772794

- All Sample Sites
- County Boundaries
- Rivers and Canals
- Lakes

WMD Boundaries

- SFWMD
- SWFWMD
- SJRWMD
- SRWMD
- NWFWMD

Table 2. Species list of water samples identified as having potentially toxic cyanobacteria present

Water Body Sampled	Concentration Code	Species of *Cylindrospermopsis*	*Microcystis*	*Anabaena*	*Planktothrix*	*Aphanizomenon*	*Coelosphaerium*
		Suwannee River Water Management District					
Alligator Lake north	1		+		+		
Alligator Lake south	1		+	+			
Lake Francis	1		+	+			
Lake Gwen	1		+		+		
Lake Lowe	1		+	+			
Low Bush Bay	1	+					
Peacock Lake	1		+	+			
Timber Lake	1		+				
Watertown Lake	1	+	+		+		+
Camp County Pond	2						
Lake Montgomery	2			+			
Lake Rowell	2			+			
		Northwest Florida Water Management District					
A.J. Henry Lake	1	+	+	+			
Davis Lake	1		+	+			
Eleven Mile Creek	1				+		
Hurricane Lake	1	+	+			+	
Kell-Aire Lake (Lake Coleman)	1		+	+	+		
Lake Belmont	1	+	+			+	
Kell-Aire Lake (Lake Coleman)	1		+	+			
Lake Olivia	1	+		+		+	
Lake Sommerset	1	+	+	+			
Lake Stone	1	+	+			+	
Martin Lake	1	+					
Quincy Creek 2	1	+					
Quincy Creek	1	+					
Shelly Pond	1		+	+		+	
Blue Heron Lake	2		+				

Table 2—*Continued*

Water Body Sampled	Concentration Code	Species of *Cylindrospermopsis*	*Microcystis*	*Anabaena*	*Planktothrix*	*Aphanizomenon*	*Coelosphaerium*
		Northwest Florida Water Management District—*continued*					
Karick Lake	2	+		+			
Kings Lake	2			+			
Lake Arrowhead	2		+				
Monkey Business Lake	2	+	+	+			
		Southwest Florida Water Management District					
Charlotte Harbor*	1						
East Lake	1	+	+				
Egypt Lake	1	+	+	+	+		
Lake Conine	1	+	+	+			
Lake Forest	1	+	+	+			
Lake Henry	1			+		+	
Lake Hollingsworth	1	+	+	+			
Lake Howard	1	+	+				
Lake Parker	1	+	+				
Lake Persimmon	1	+	+				
Loch Haven	1		+				
Banana Lake	2			+			
Braden River	2			+			
Lake Hancock	2		+	+		+	
Lake Maggiore	2	+					
Lake Seminole	2		+				
Lake Thonotassa	2		+				
Lake Valrico	2			+			
Medard Reservoir	2		+	+			
Moccasin Lake	2	+					
Peace River	2		+				
Shell Creek	2				+		

Table 2—*Continued*

Water Body Sampled	Concentration Code	Species of *Cylindrospermopsis*	*Microcystis*	*Anabaena*	*Planktothrix*	*Aphanizomenon*	*Coelosphaerium*
		South Florida Water Management District					
Kissimmee River north	1	+	+	+			
Lake Istokpoga north	1		+	+			
Lake Kissimmee	1	+	+	+		+	
Lake Okeechobee @ Belle Glade	1	+	+		+		
Lake Okeechobee @ Belle Glade	1	+	+		+		
Lake Okeechobee @ Okeechobee	1	+	+		+	+	
Lake Okeechobee @ South Bay	1	+	+	+	+		
Lake Okeechobee @ South Bay 2	1	+	+	+	+		
Lake Okeechobee east	1						
Lake Okeechobee north	1	+	+		+		
Lake Okeechobee northwest	1						
Lake Okeechobee south	1						
Lake Okeechobee southwest	1						
Caloosahatchie River	2				+		
East Rock Lake	2				+		
Fort Myers WTP	2				+		
Lake Tohopekaligo	2		+				
Little Murex Lake	2			+			
McGregor Wood Lake	2			+			

Table 2—*Continued*

Water Body Sampled	Concentration Code	Species of *Cylindrospermopsis*	*Microcystis*	*Anabaena*	*Planktothrix*	*Aphanizomenon*	*Coelosphaerium*
St. Johns River Water Management District							
Clermont Pond	1		+	+			
Crescent Lake-South	1	+	+	+			
Haines Creek	1	+					
Lake Ashby	1		+	+			
Lake Beauclair	1	+	+				
Lake Bethel	1	+	+			+	+
Lake Carlton	1	+	+				
Lake Crystal	1	+					
Lake Dora	1	+	+				
Lake Eustis	1	+	+				
Lake George	1	+					
Lake Griffin	1	+	+				
Lake Griffin Canal	1	+	+		+		
Lake Griffin center	1	+	+				
Lake Griffin north	1	+	+				
Lake Griffin south	1						
Lake Griffin west	1	+	+				
Lake Harris	1	+	+				
Lake Jesup	1	+	+	+			
Lake Johnson	1	+	+				
Lake Lochloosa	1	+	+				+
Lake Maitland	1	+					+
Lake Orange	1	+	+				+
Lake Poinsett	1	+	+		+		
Lake Wauberg	1	+	+			+	
Lake Yale	1	+	+				
Little Lake Crystal	1	+					
Little Lake Harris	1	+	+				
Newnans Lake	1	+					
SJR @ Cocoa	1		+	+	+		
SJR @ Doctors Lake	1			+			

Table 2—*Continued*

Water Body Sampled	Concentration Code	Species of *Cylindrospermopsis*	*Microcystis*	*Anabaena*	*Planktothrix*	*Aphanizomenon*	*Coelosphaerium*
		St. Johns River Water Management District—*continued*					
SJR @ Dunns Creek	1	+	+	+	+	+	
SJR @ Fort Gates/Buffalo Bluff	1	+	+				
SJR @ Governors Creek	1			+			
SJR @ Jack Wright Island	1			+			
SJR @ Little Lake George	1	+	+				
SJR @ Palatka	1	+	+	+			
SJR @ Picolata	1			+			
SJR @ Scratch Ankle/ Ferreira Point	1	+	+	+			
SJR @ Welaka Springs	1	+					
Crescent Lake center	2	+	+				
Dead Lake	2	+		+			
Farm Pond in Cocoa	2			+	+		
Lake Apopka @ Zellwin Farms Canal	2		+				+
Lake Lochloosa	2	+					
Lake Monroe	2				+		
Lake Orange	2	+					
SJR, north of Russells Point	2	+	+	+			
SJR @ Deland	2	+					
SJR @ Ferreira Point	2	+	+				
SJR @ Hallows Cove	2			+			
SJR @ Rice Creek	2	+					
SJR @ River Mile 18	2				+		
SJR @ River Mile 23	2				+		
SJR @ River Mile 27	2			+	+		
SJR @ Switzerland Point	2			+			

Note: 1 = high biomass
2 = low biomass

*The only samples having species of *Lyngbya*

Table 3. Prevalence of potentially toxic cyanobacteria in samples collected between June and October 1999, by water management district

Species	Water Management District				
	Suwannee River	Northwest Florida	Southwest Florida	South Florida	St. Johns River
Anabaena sp.	26.7	40.0	34.5	20.7	27.3
Microcystis sp.	53.3	44.0	48.3	27.6	47.0
Cylindrospermopsis raciborskii	13.3	40.0	34.5	20.7	57.6
Planktothrix sp.	20.0	8.0	6.9	24.1	13.6
Aphanizomenon sp.	0	20.0	6.9	6.9	4.5
Coelosphaerium kuetzingianum	6.7	0	0	0	7.6
Total samples	15	25	29	29	64

Note: Prevalence = number of samples in which the species was identified as being present / total number of samples collected x 100

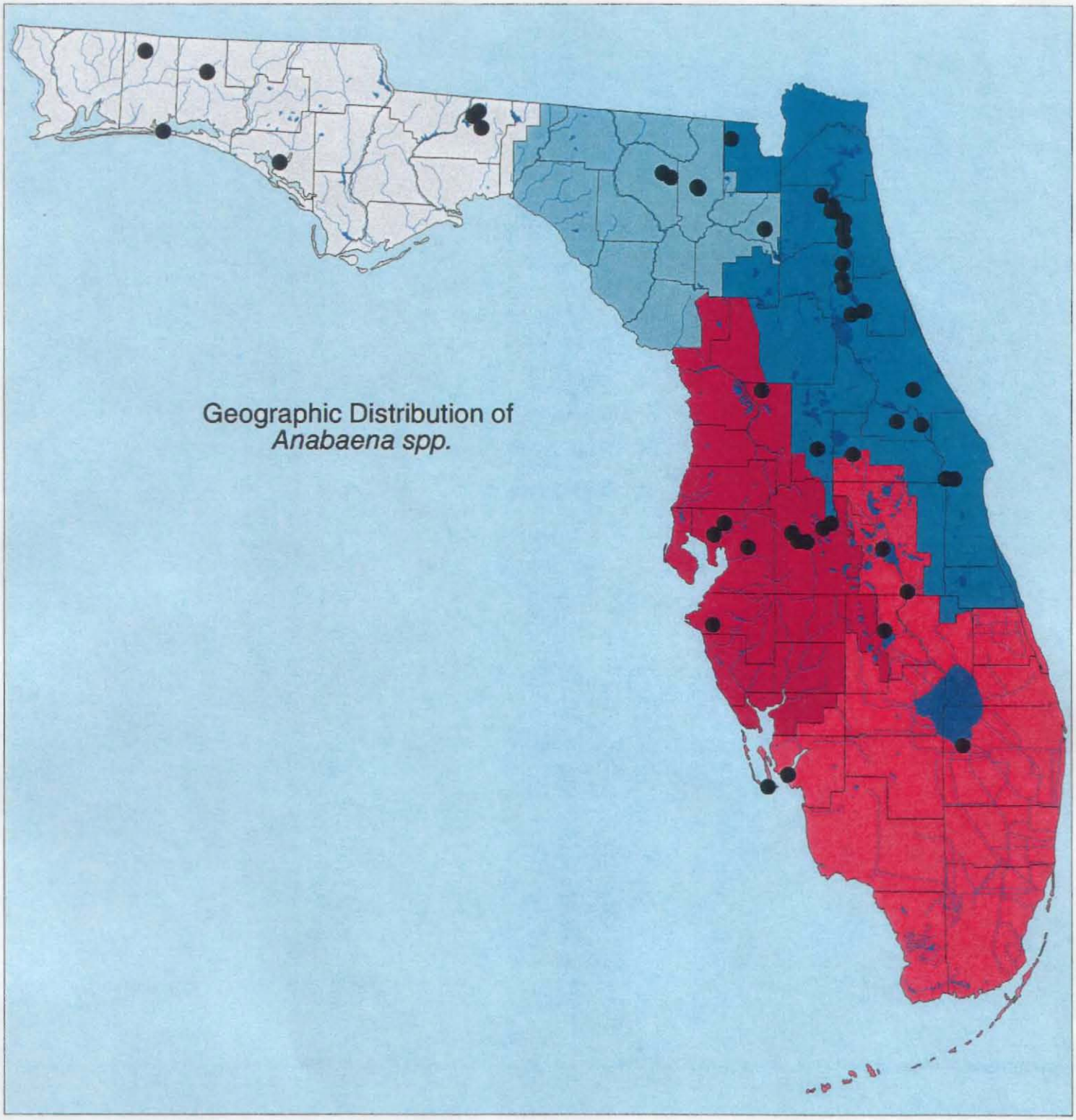

Figure 2

N
W E
S

0 30 60 Miles

1:3772794

- Anabaena
- County Boundaries
- Rivers and Canals
- Lakes

WMD Boundaries

- SFWMD
- SWFWMD
- SJRWMD
- SRWMD
- NWFWMD

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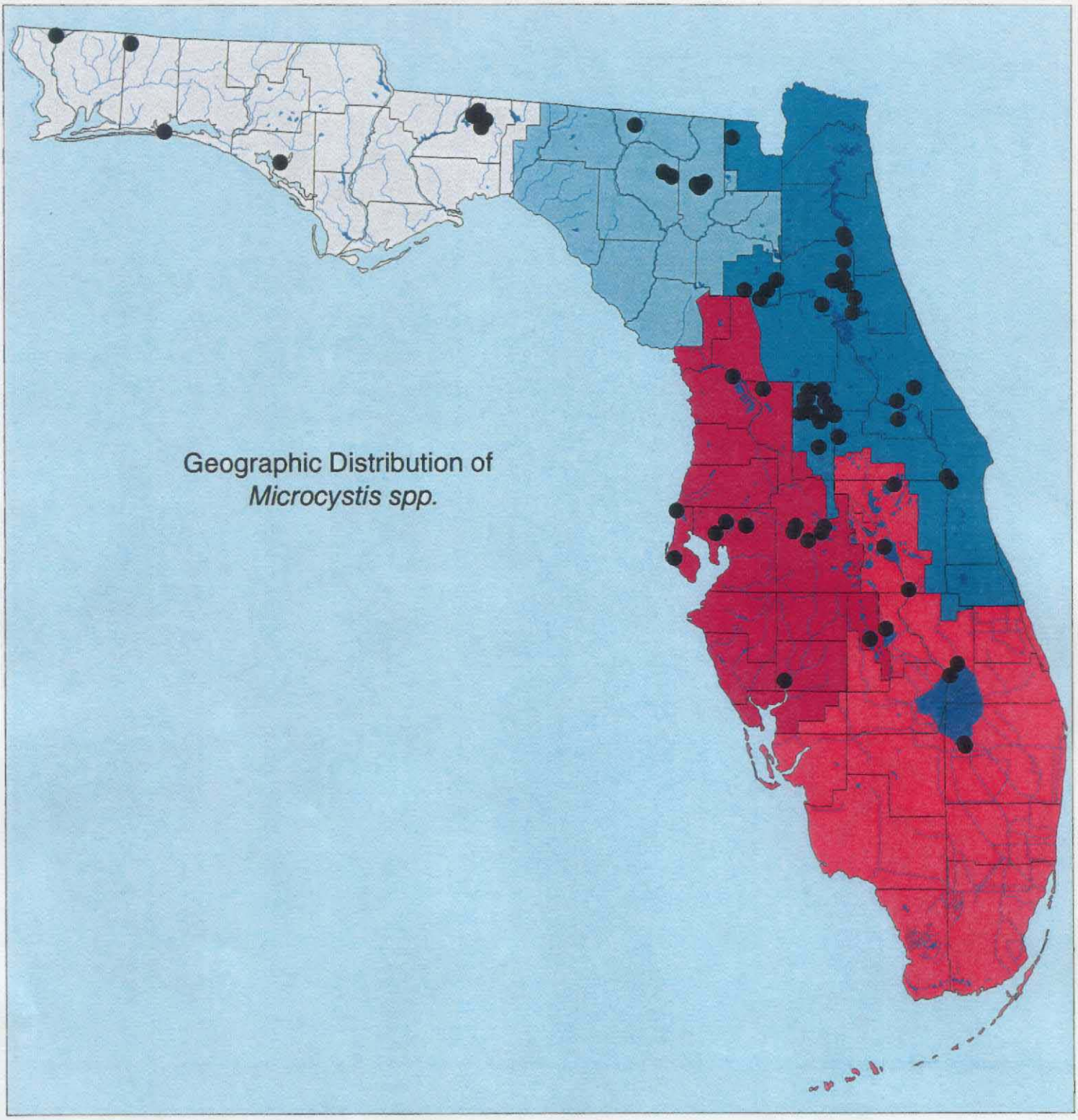

Figure 3

N
W E
S

0 30 60 Miles

1:3772794

- Microcystis
- County Boundaries
- Rivers and Canals
- Lakes

WMD Boundaries

- SFWMD
- SWFWMD
- SJRWMD
- SRWMD
- NWFWMD

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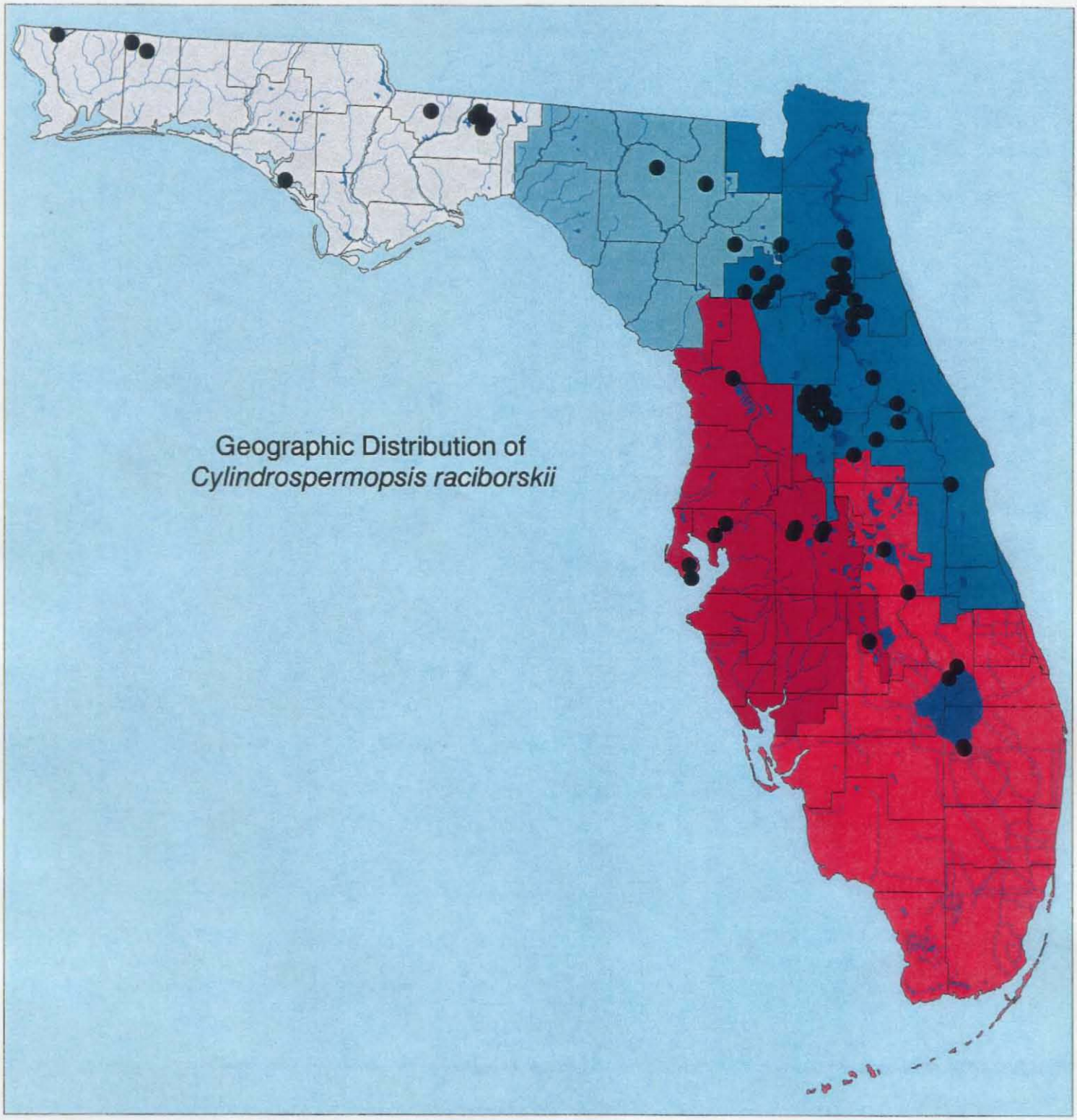

Figure 4

N
W E
S

0 30 60 Miles

1:3772794

- Cylindrospermopsis
- County Boundaries
- Rivers and Canals
- Lakes

WMD Boundaries

- SFWMD
- SWFWMD
- SJRWMD
- SRWMD
- NWFWMD

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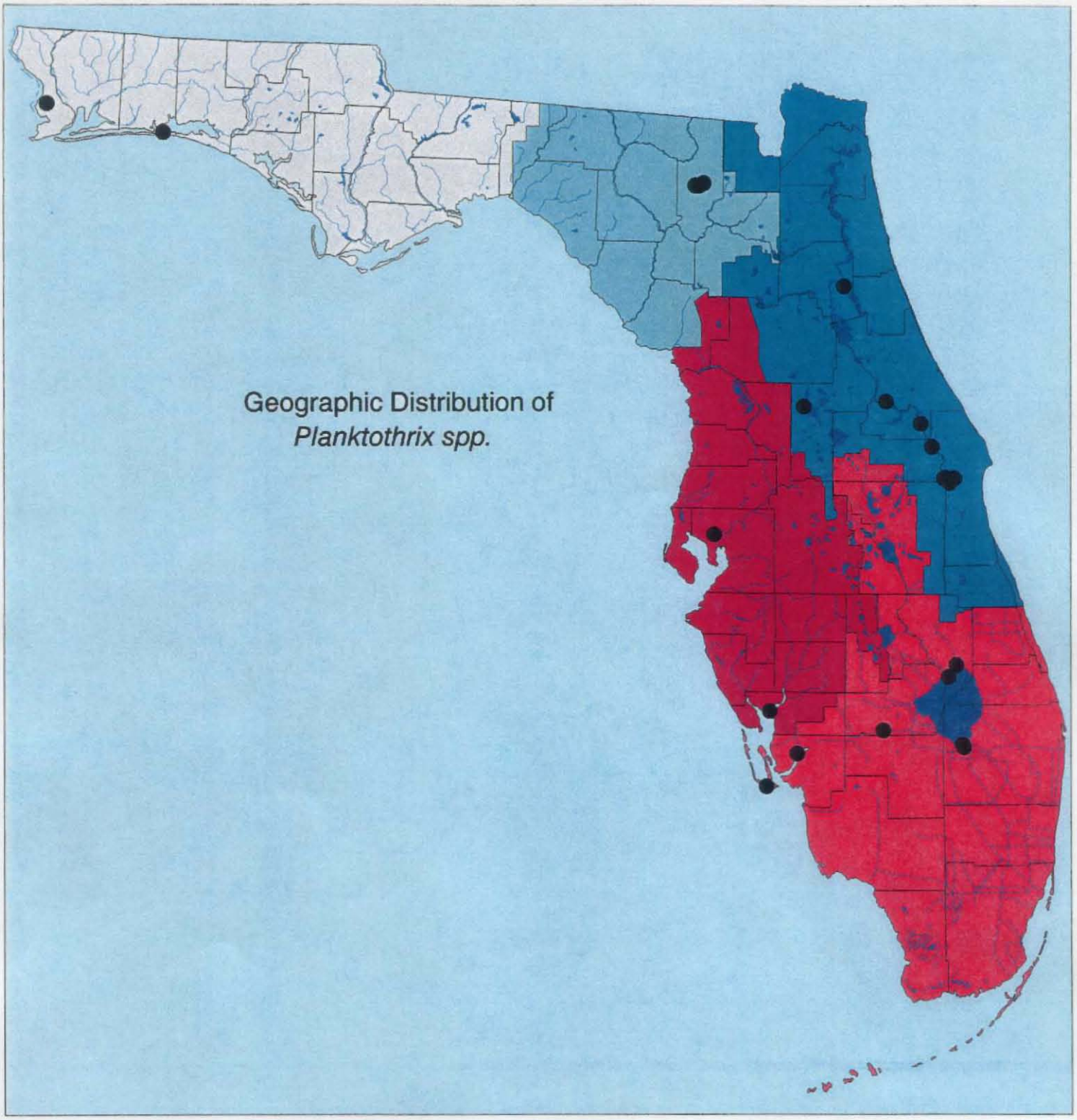

Figure 5

0 30 60 Miles

1:3772794

- Planktothrix
- County Boundaries
- Rivers and Canals
- Lakes

WMD Boundaries

- SFWMD
- SWFWMD
- SJRWMD
- SRWMD
- NWFWMD

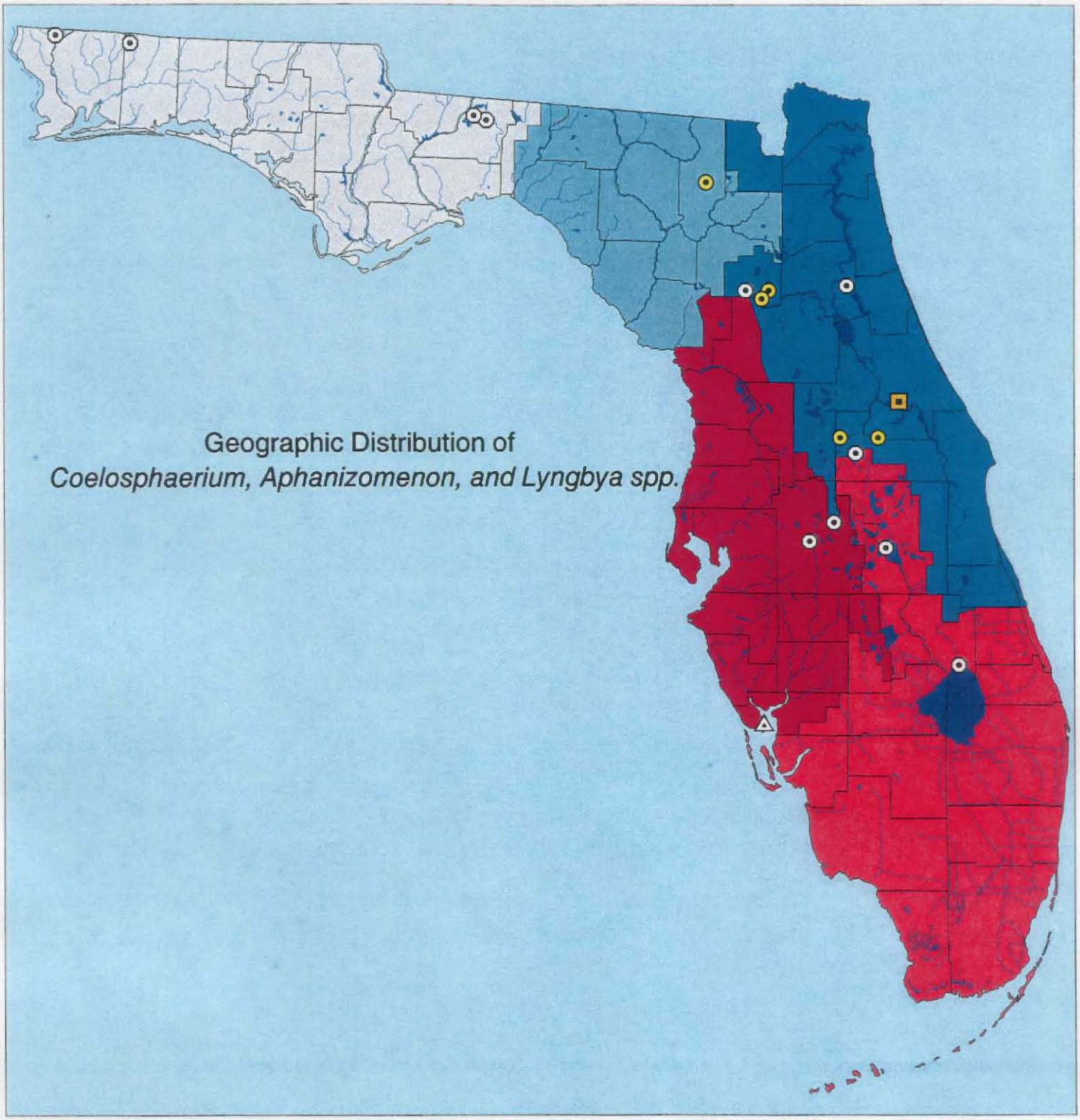

Figure 6

N
W E
S

0 30 60 Miles

1:3773000

- Coelosphaerium and Aphanizomenon
- Coelosphaerium
- Lyngbya
- Aphanizomenon
- County Boundaries
- Rivers and Canals
- Lakes
- SFWMD
- SWFWMD
- SJRWMD
- SRWMD
- NWFWMD

Presence and Distribution of Cyanotoxins

The presence of microcystins was identified at varying levels in all of the 81 samples tested (Figure 7, Table 4). The observed levels of microcystins were highly variable. Significant differences in microcystin levels reported by the two laboratories testing the samples indicate that test methodologies need to be refined. In addition, it should be noted in Table 3 that the two laboratories reported microcystin levels in different units of measure. In general, ELISA values for microcystins are reported in milligrams per milliliter (mg/mL). In an attempt to make the data more comparable, those values reported by the Florida Department of Health (in mg/mL) were converted (division of reported values by the total weight of the lyophilized algal sample added to the ELISA) by SJRWMD to milligrams per nanogram to obtain similar units of measure.

Analyses for protein phosphatase inhibition activity, an index of the microcystin bioactivity, were performed at only one of the analytical laboratories. Samples for analysis were chosen based on their cyanobacterial species compositions. Of the 64 water samples that were analyzed, 44 (69%) exhibited positive signs of microcystin bioactivity (Table 4). The distribution of protein phosphatase activity (and therefore bioactive microcystins) was most noticeable in the central and north-central regions of the state (Figure 8).

A cylindrospermopsin-like compound was discovered in samples dominated by the alga *Cylindrospermopsis raciborskii*. However, positive identification in all water samples that were analyzed for cylindrospermopsin, either the UV-spectra or the UV-spectra and the retention times of similar eluting compounds, did not match that of a cylindrospermopsin standard. All water samples that were analyzed for cylindrospermopsin content and that exhibited toxicity to mice demonstrated behavioral characteristics consistent with those previously observed for cylindrospermopsin poisoning. Recent analysis using HPLC in conjunction with mass spectrometry (MS) has positively identified cylindrospermopsin in isolates collected from Florida water samples made and grown under laboratory conditions. The geographical distribution of this preliminarily identified cylindrospermopsin compound in the state of Florida was found to be consistent with that of *C. raciborskii*. All water samples that contained significant levels of *C. raciborskii* were reported to contain the identified cylindrospermopsin compound (Figure 9, all

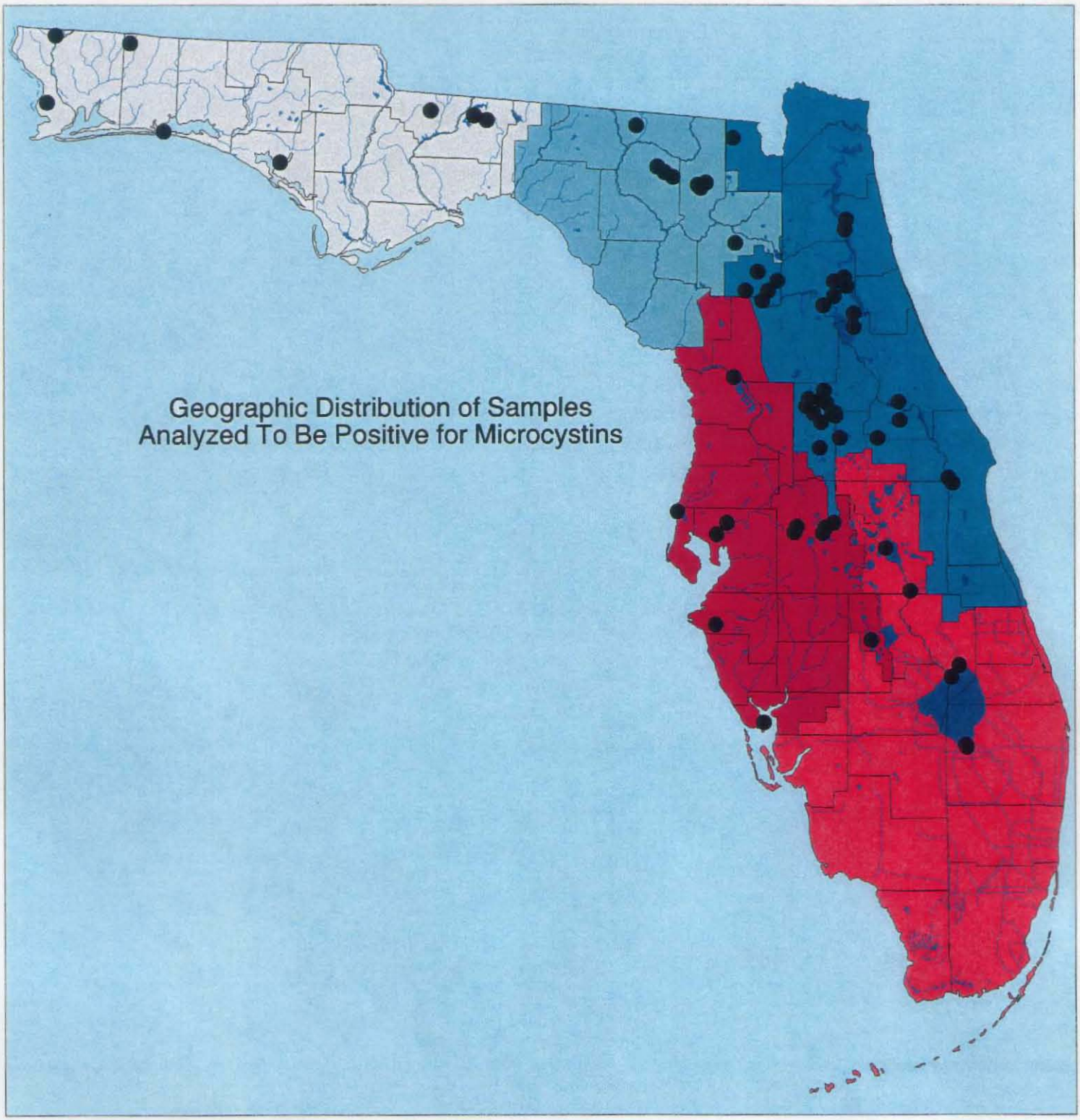

Figure 7

N
W E
S

0 30 60 Miles

1:3772794

- Microcystins
- County Boundaries
- Rivers and Canals
- Lakes

WMD Boundaries

- SFWMD
- SWFWMD
- SJRWMD
- SRWMD
- NWFWMD

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Table 4. Analysis for the presence of microcystins and protein phosphatase inhibition activity in water samples identified as containing a high biomass of toxigenic cyanobacteria

Water Body Sampled	Wright State University			Florida Department of Health			
	Microcystins (ng/mg)[1]	PPIA (ng/mg)	Mouse Bioassay[2,3]	Microcystins (ng/mL)[1,4,5*]	Microcystins (ng/mg)	Mouse Bioassay[2]	Toxic Symptoms
Suwannee River Water Management District							
Alligator Lake north	5.2	1.7		1.0	9.8	+	H
Alligator Lake south	17	10.9	+	0.1	0.4		
Lake Francis	0.4	ND	+	0.1	6.6	NP	
Lake Gwen	5.0	2.9		0.1	0.2		
Low Bush Bay	1.2	1.0	+	1.6×10^5	1.0×10^6	+	H
Low Lake	19.4	10.8	+	1.4×10^3	1.4×10^4	+	H
Peacock Lake	2.8	2.0		0.2	0.6		
Timber Lake	22.0	10.0		0.1	1.3	NP	
Watertown Lake	23.0	15.0		0.1	0.1		
Northwest Florida Water Management District							
Davis Lake	5	ND	+	0.1	0.2		
Eleven Mile Creek	ND	ND	+	0.2	8.2	+	N
Hurricane Lake	1.6	ND	+	1.0	2.0		
Kell-Aire Lake	2.6	1.7		0.1	0.0	+	N
Lake Belmont	NP			0.4	3.3	+	H
Lake Stone	1.2	ND	+	0.2	3.7		
Martin Lake	NP	NP		0.1	39.5	NP	
Lake Olivia	0.3	0.3	NP	0.071[†]	1.4	NP	
Quincy Creek	ND	ND	NP	0.0	0.2	NP	
Quincy Creek 2	NP		+	0.1	1.1	NP	
Shelly Pond	1.0	ND		1.3×10^4	2.6×10^5	NP	
Southwest Florida Water Management District							
Braden River	0.8	ND	NP	0.1	2.2		
Bill Evers Reservoir	NP			NP	NP	NP	
Charlotte Harbor	NP			0.1	0.5	NP	
Charlotte Harbor #2	NP			0.1	2.4	NP	
East Lake	NP			0.1	2.5	NP	
Egypt Lake	NP			0.1	2.3	NP	
Lake Conine	ND	ND	+	0.1	7.1	NP	

Table 4—*Continued*

Water Body Sampled	Wright State University			Florida Department of Health			
	Microcystins (ng/mg)[1]	PPIA (ng/mg)	Mouse Bioassay[2,3]	Microcystins (ng/mL)[1,4,5*]	Microcystins (ng/mg)	Mouse Bioassay[2]	Toxic Symptoms
Southwest Florida Water Management District —*continued*							
Lake Forest	3.7	1.7	+	0.1	2.2		
Lake Henry	ND	ND	+	1.1×10^5	7.2×10^5	+	N
Lake Hollingsworth	1.6	1.0	+	0.6	9.8	+	H
Lake Howard	5.4	2.6	+	0.1	5.8	NP	
Lake Parker	3.2	2.0	+	0.1	7.8	+	H
Lake Persimmon	ND	ND		0.1	1.6		
Loch Haven	1.56	1.0		0.1	2.2		
South Florida Water Management District							
Kissimmee River north	4	2.5	+	1.0×10^3	13.0×10^3	NP	
Lake Kissimmee	2.1	1.5	+	1.2×10^3	4.0×10^4	NP	
Lake Okeechobee @ Belle Glade	1.7	ND	+	0.1	0.3	NP	
Lake Okeechobee @ Belle Glade 2	NP			0.06[†]	2.9	NP	
Lake Okeechobee @ Okeechobee	NP			1.3×10^3	6.6×10^3	+	H
Lake Okeechobee @ Okeechobee 2	NP			NP			
Lake Okeechobee @ South Bay	1.0	ND		0.3	0.1	+	H/N
Lake Okeechobee @ South Bay 2	1.7	ND		0.1[†]	$> 1.1 \times 10^2$		
Lake Okeechobee north	3.0	1.0	+	0.5	6.0	NP	
St. Johns River Water Management District							
Clermont Pond	3.4	2.5	+	0.0	0.1	NP	
Haines Creek	NP	NP		0.1	14.0	NP	
Lake Apopka @ Zellwin Farms	3.7	2.3		NP			
Lake Ashby	2.0	1.5		0.1	1.9	+	N
Lake Beauclair	1.6	1	+	3.6	39.4	+	N
Lake Bethel	0.3	0.2	NP	0.1	1.0	+	N
Lake Carlton	1.4	1.1	+	$1.6 \times 10^{3\dagger}$	4.5×10^3	NP	
Lake Crystal	NP			0.1	26.0	NP	
Lake Dora	3.4	2.9	+	0.1	8.8	NP	
Lake Eustis	2.6	1.8	+	0.1	7.2	NP	
Lake Eustis	13.0	10.0	NP	NP			

Table 4—*Continued*

Water Body Sampled	Wright State University			Florida Department of Health			
	Microcystins (ng/mg)[1]	PPIA (ng/mg)	Mouse Bioassay[2,3]	Microcystins (ng/mL)[1,4,5*]	Microcystins (ng/mg)	Mouse Bioassay[2]	Toxic Symptoms
			St. Johns River Water Management District—*continued*				
Lake George @ Willows Point	NP	NP		0.1	1.2		
Lake Griffin Canal	1.2	1.0	+	0.1	5.2	NP	
Lake Griffin center	0.1	0.1	+	0.1	20.0	NP	
Lake Griffin north	ND	ND	+	NP			
Lake Griffin north	1.0	1.0	+	NP			
Lake Griffin north[6]	72.0	57.0	NP	NP			
Lake Griffin north[6]	26.0	20.0	NP	NP			
Lake Griffin north[6]	78.0	60.0	NP	NP			
Lake Griffin north[6]	15.0	10.0	NP	NP			
Lake Griffin north[6]	42.0	30.0	NP	NP			
Lake Griffin south	ND	ND	+	NP			
Lake Griffin west	ND	ND	+	NP			
Lake Harris	1.6	1.8	+	0.1	3.8	NP	
Lake Harris	10.0	10.0		NP			
Lake Jesup	5.6	3.5	+	0.3	2.0	+	H
Lake Johnson	1.0	1.0	NP	1.2×10^5	4.6×10^5	+	H
St. Johns River							
Lake Lochloosa	2.8	2.7	NP	9.6	64.3	+	H
Lake Maitland	0.7	1.0	NP	1.2	6.0×10^3	+	H
Lake Orange	1.0	0.8	NP	0.2	1.8	+	H
Lake Poinsett	1.4	1.0	+	0.5	48.2	NP	
Lake Wauberg	10.0	8.5	NP	1.5×10^4	1.0×10^5	+	N
Lake Yale	4.0	3.8		0.0	46.0	NP	
Little Lake Harris	1.0	1.0	+	1.0	19.6	NP	
Newnans Lake	1.7	1.0	NP	0.1	1.0	+	N
St. Johns River @ Cocoa	1.0	ND		0.1	8.8	NP	
St. Johns River @ Crescent Lake south	0.1	ND	+	0.1	4.4		
St. Johns River @ Doctors Lake	ND	ND		0.1	1.4	+	H
St. Johns River @ Dunns Creek	0.3	ND	+	0.1	2.1		
St. Johns River @ Fort Gates	ND	ND		1.5×10^5	2.9×10^6	+	H

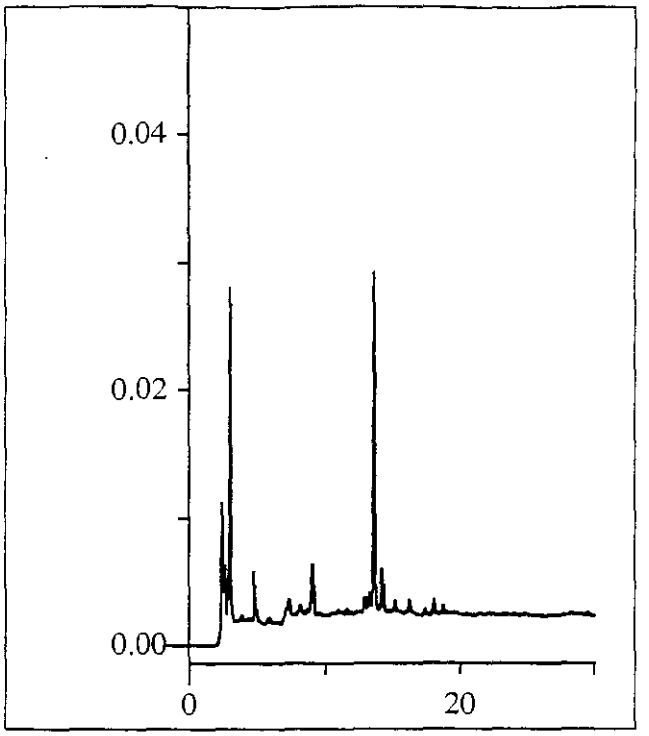
Table 4—*Continued*

Water Body Sampled	Wright State University			Florida Department of Health			
	Microcystins (ng/mg)[1]	PPIA (ng/mg)	Mouse Bioassay[2,3]	Microcystins (ng/mL)[1,4,5*]	Microcystins (ng/mg)	Mouse Bioassay[2]	Toxic Symptoms
			St. Johns River Water Management District—*continued*				
St. Johns River @ Jack Wright Island	0.2	ND	+	0.1	0.3	+	H
St. Johns River @ Little Lake George	8.0	6.5	+	0.5	19.2	+	H
St. Johns River @ Palatka	ND	ND		7.3	1.4×10^3	+	H
St. Johns River @ Picolata	4.0	3.0	+	0.1	0.1		
St. Johns River @ Welaka Springs	0.9	1.0		0.1	3.6	+	H

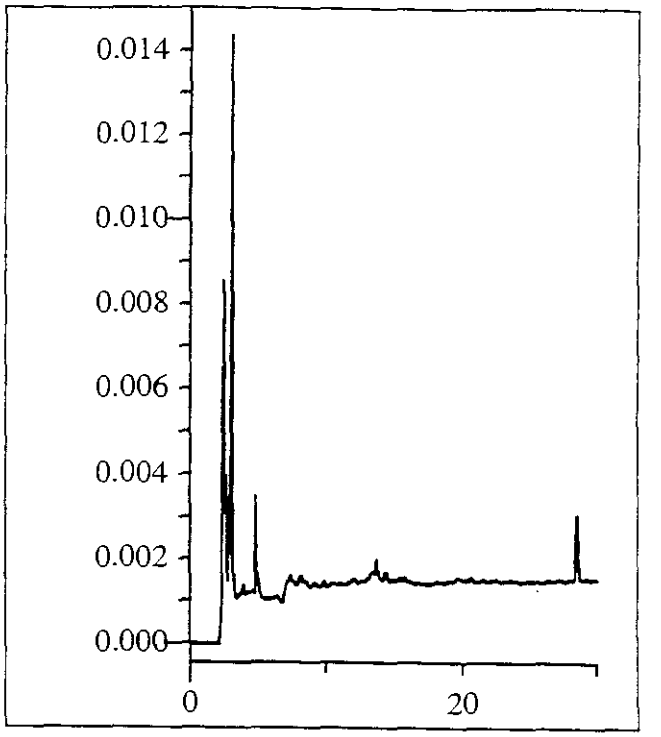
Note: H = hepatotoxic symptoms
N = neurotoxic symptoms
ND = not detectable
NP = not performed

[1]Coefficient of variation < 10% of reported microcystin concentration for all samples
[2]+ = lethal to mice, blank cells = no deaths occurred
[3]All toxic reactions were reported as exhibiting hepatotoxic symptoms
[4]Mean value for negative control = 0.090 nanograms per milliliter (ng/mL)
[5]Mean value for lowest standard = 0.160 ng/mL
[6]Samples analyzed were frozen archived samples and were measured for cyanotoxins in November/December
*Concentration of microcystins in a 1% (0.01) extract of a lyophilized water sample
†Total weight of the lyophilized water sample was less than (<<) 1 milligram (mg)

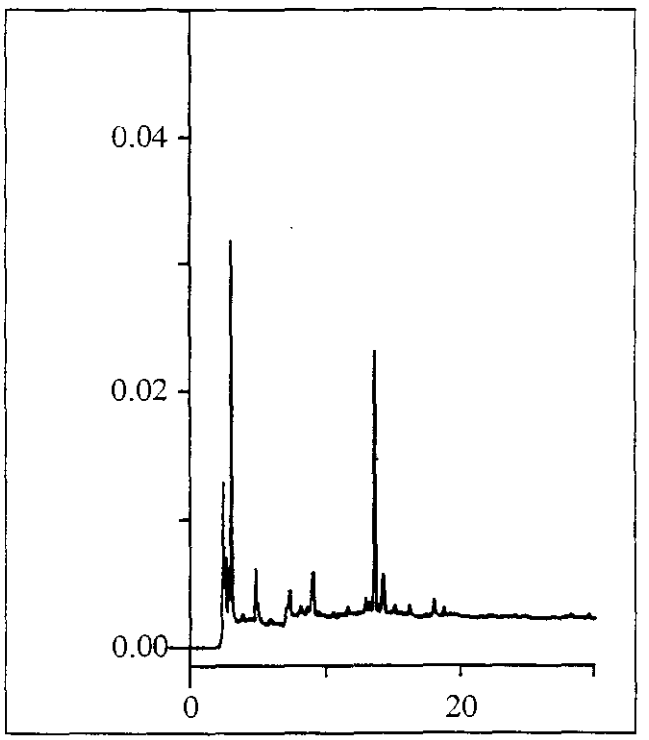
(J.P. Ross, Effects of toxic algae on alligators and alligator egg development, Water Resources Center UF grant 99-012711 and Lake County Water Authority grant)



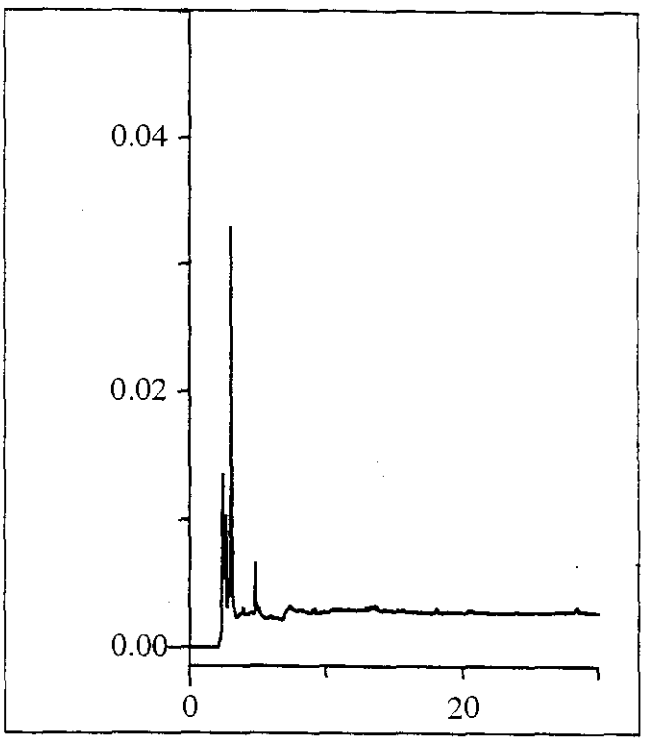
Lake Beauclair, SJRWMD



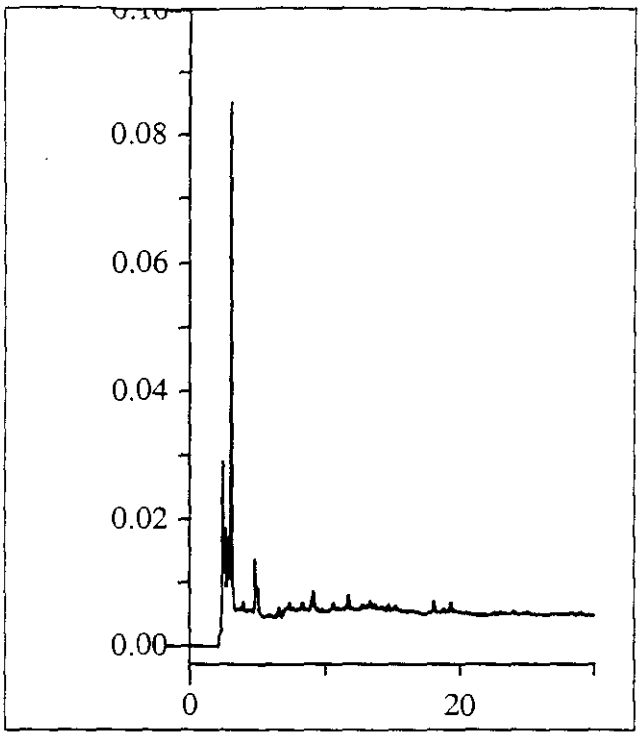
Lake Poinsett, SJRWMD



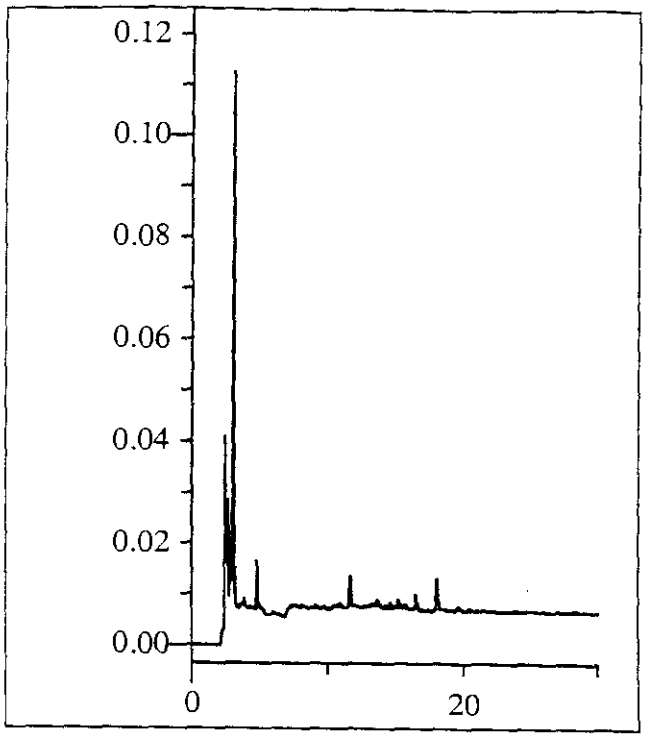
Lake Carlton, SJRWMD



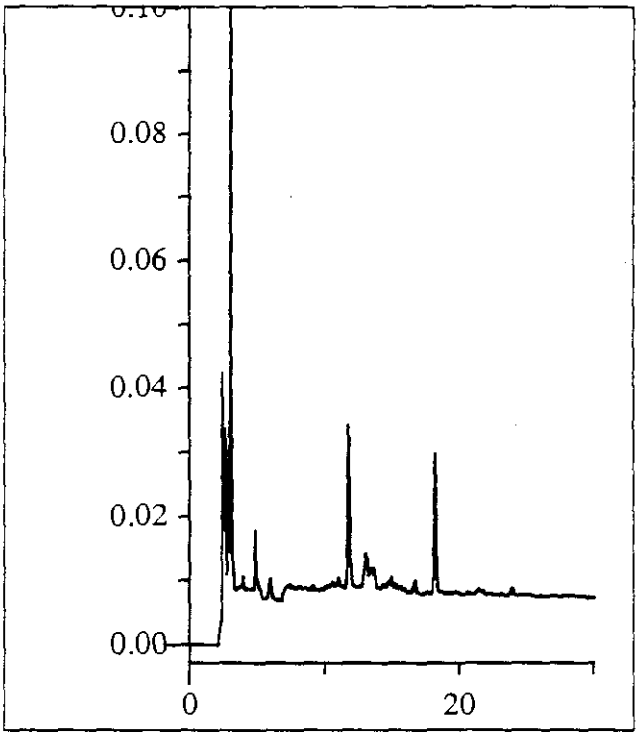
Lake Okeechobee – North, SFWMD



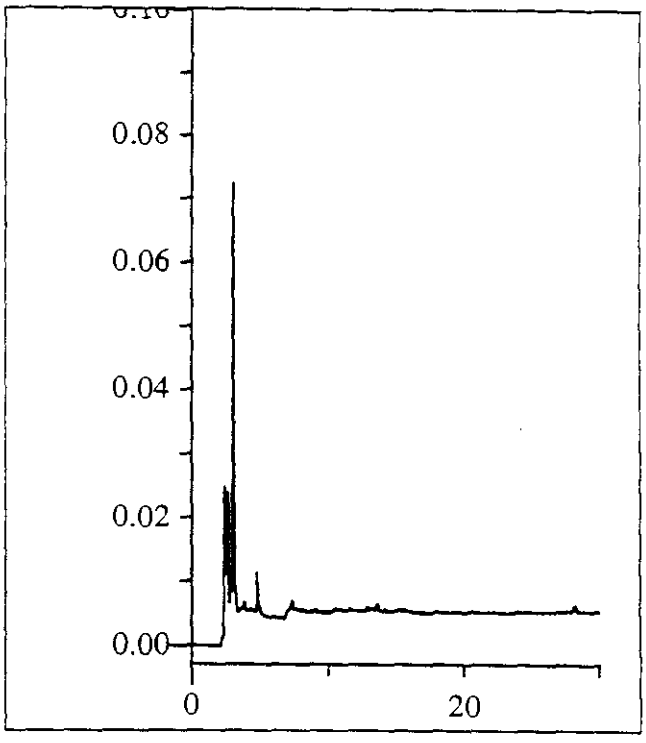
Lake Hollingsworth, SWFWMD



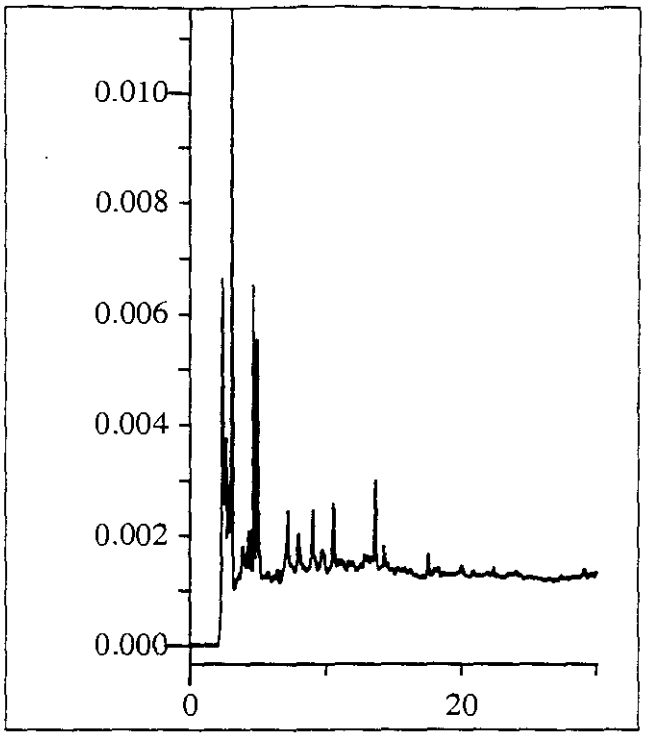
Lake Griffin Canal, SJRWMD



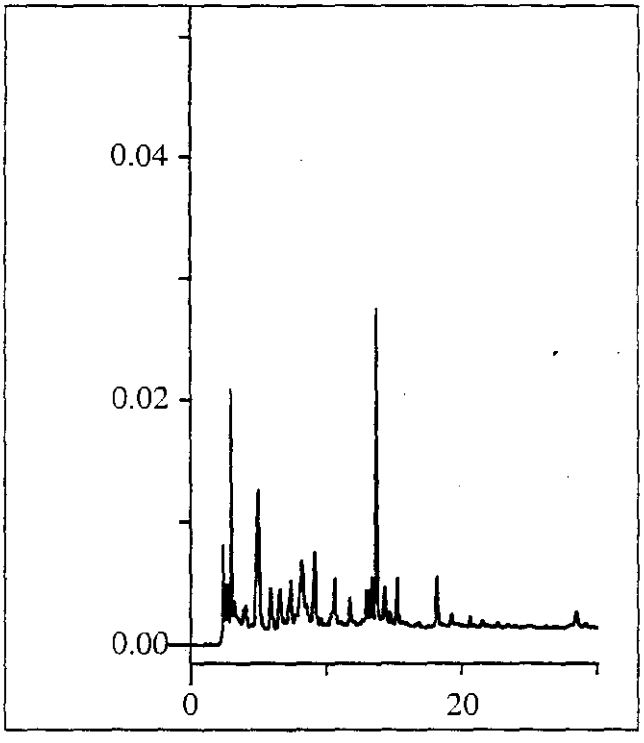
Little Lake Harris, SJRWMD



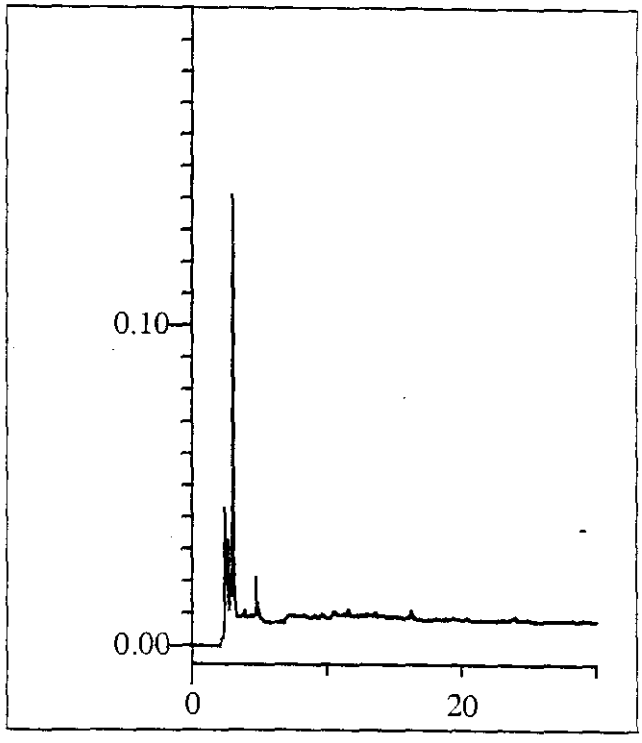
Kissimmee River- North, SFWMD



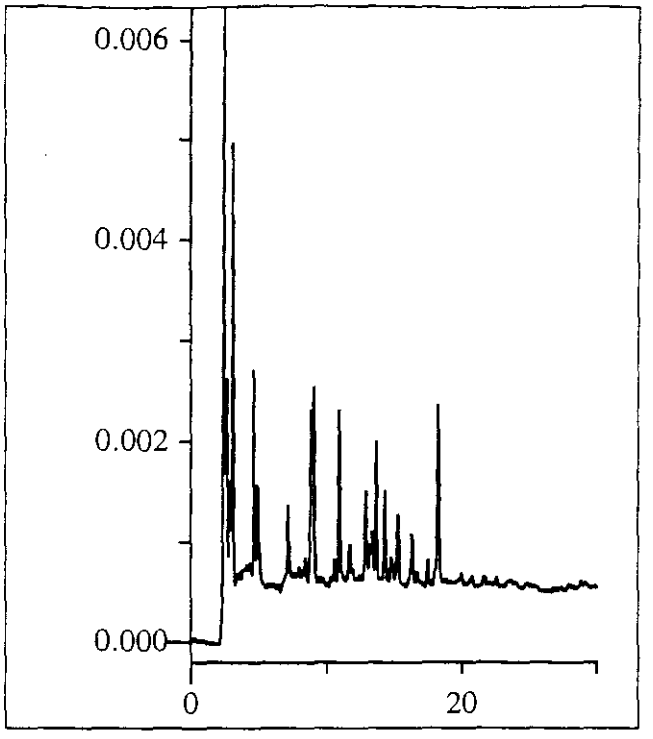
Egypt Lake, SWFWMD



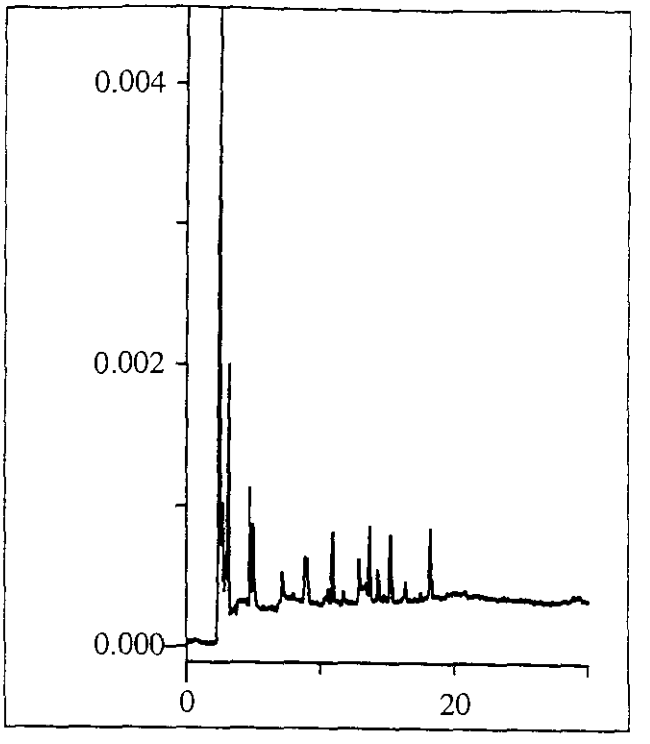
Low Bush Bay, SRWMD



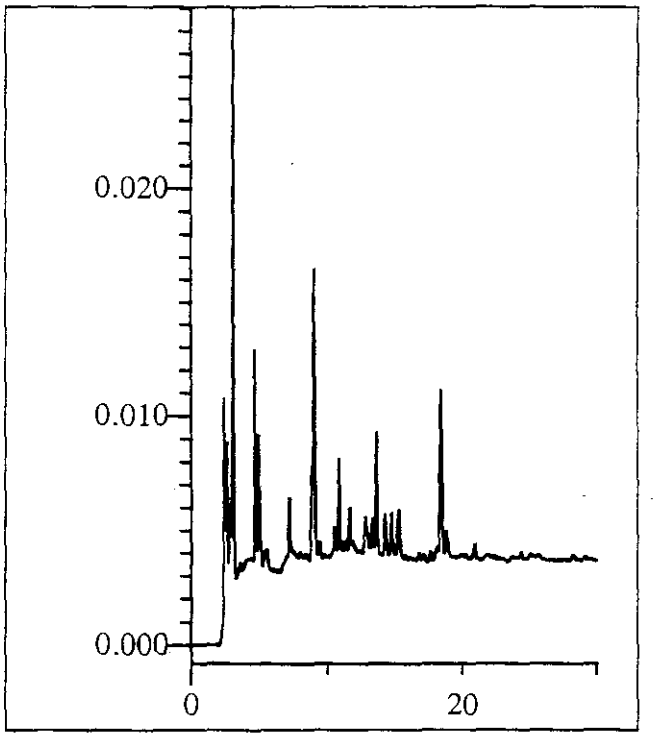
Lake Parker, SWFWMD



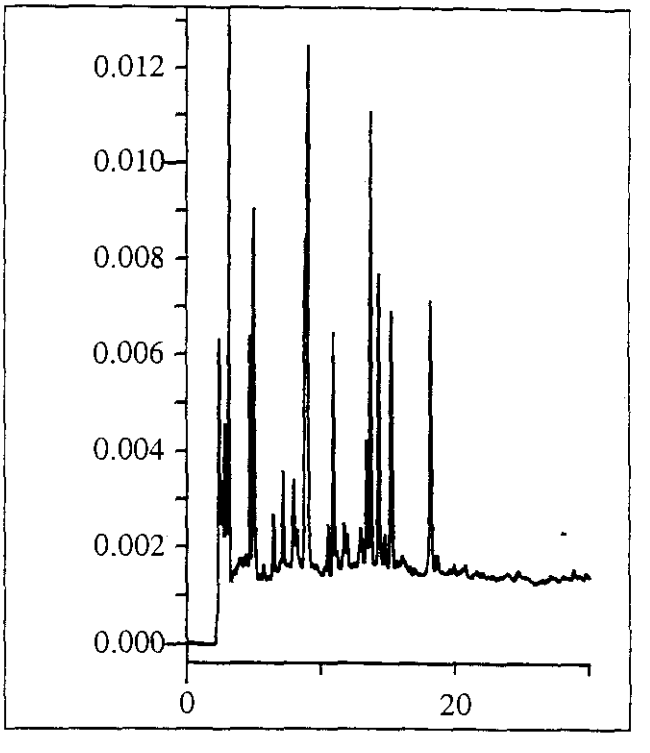
Lake Jesup, SJRWMD



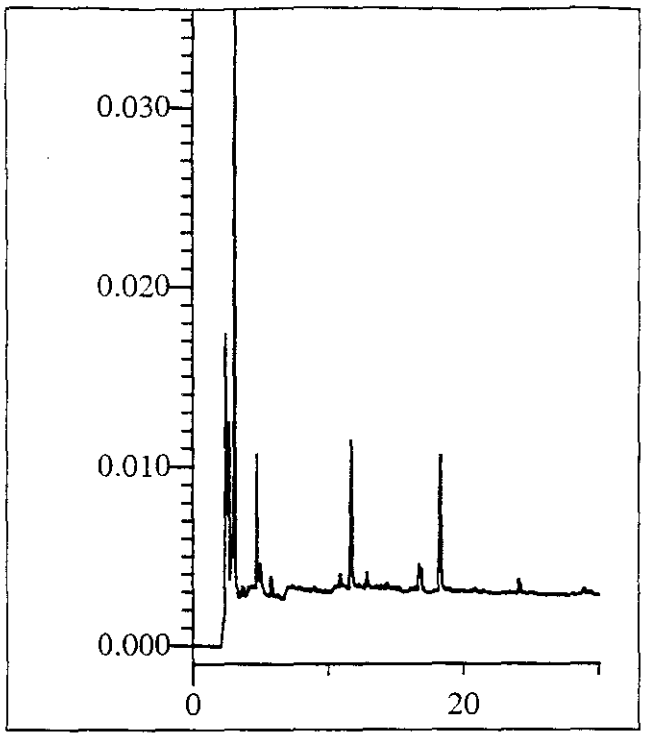
SJR @ Welaka Springs, SJRWMD



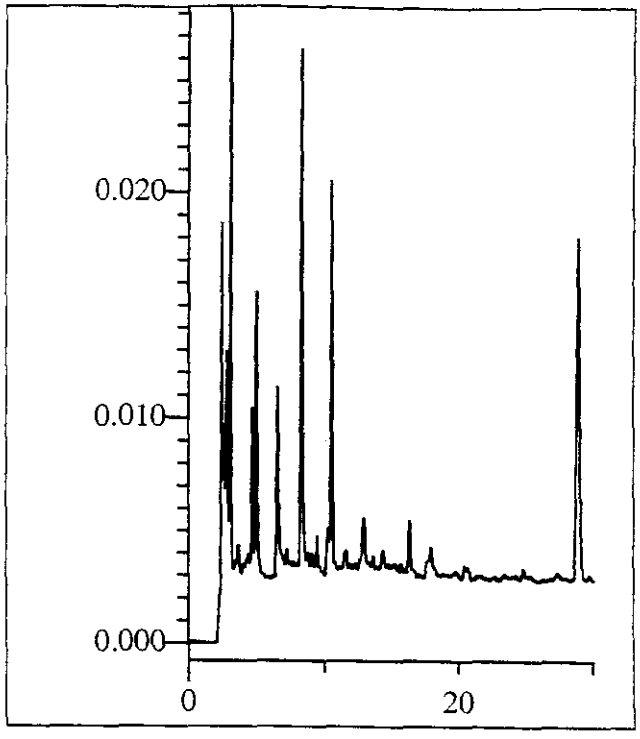
Newnans Lake, SJRWMD



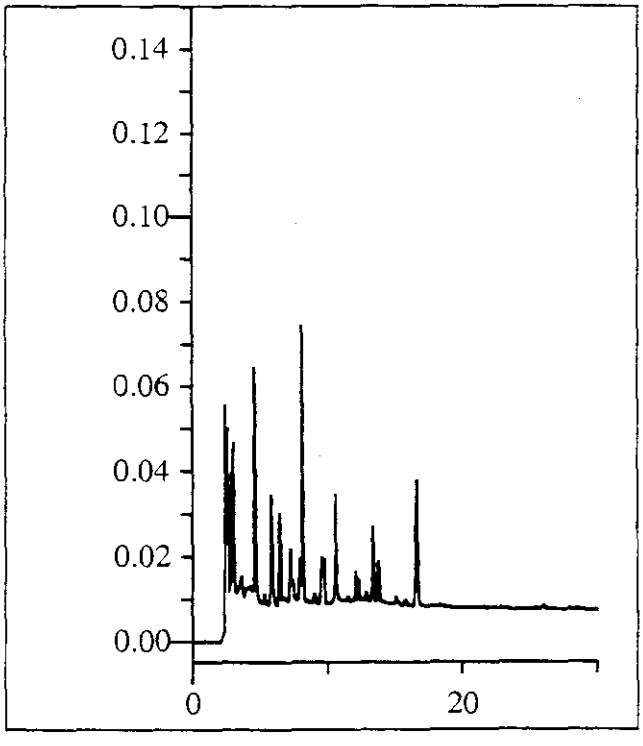
East Lake, SWFWMD



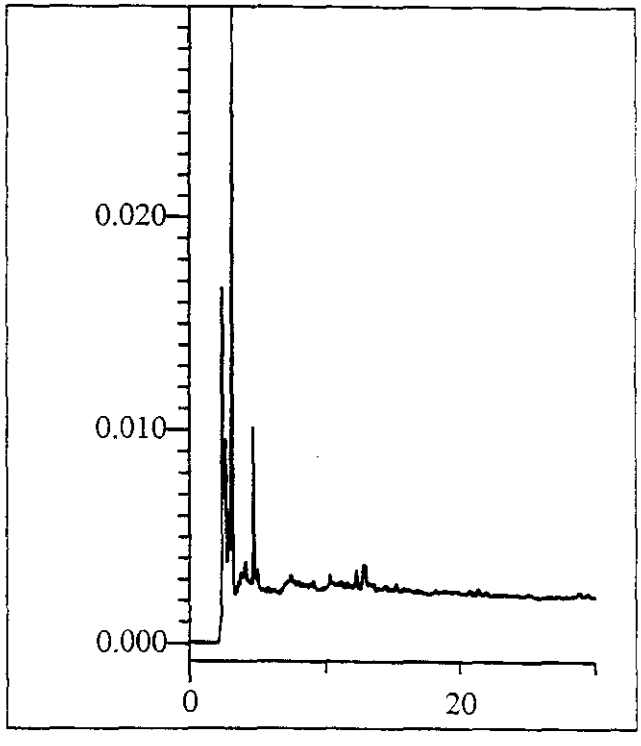
Lake Howard, SWFWMD



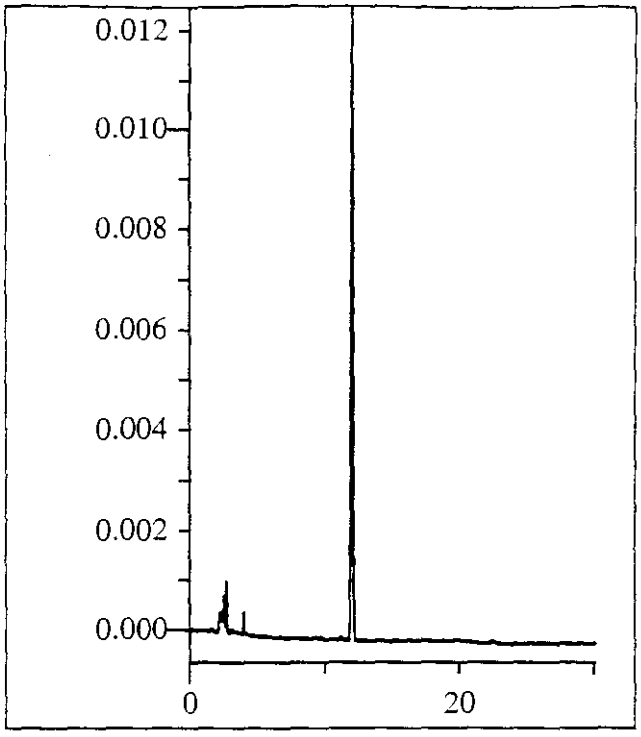
Lake Persimmon, SWFWMD



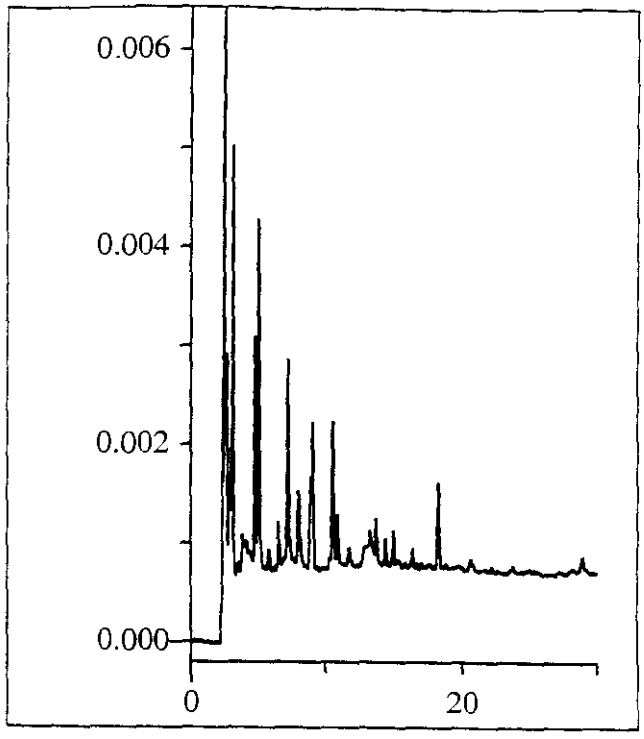
Lake Conine, SWFWMD



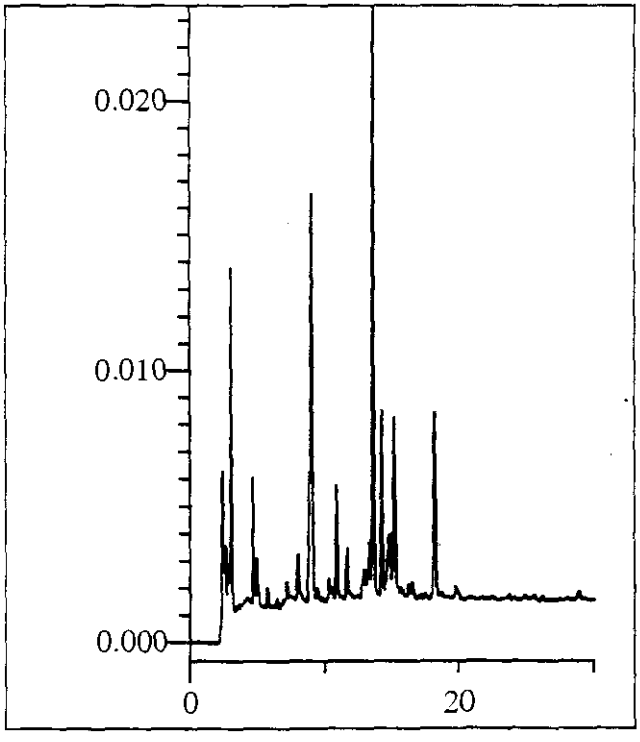
Lake Forest, SWFWMD



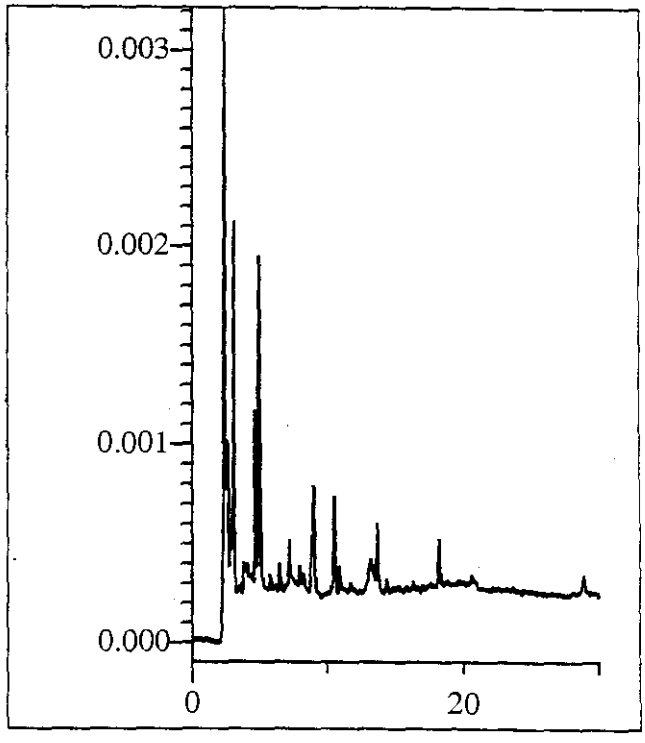
cylindrospermopsin std (50 ng)



Crescent Lake – South, SJRWMD

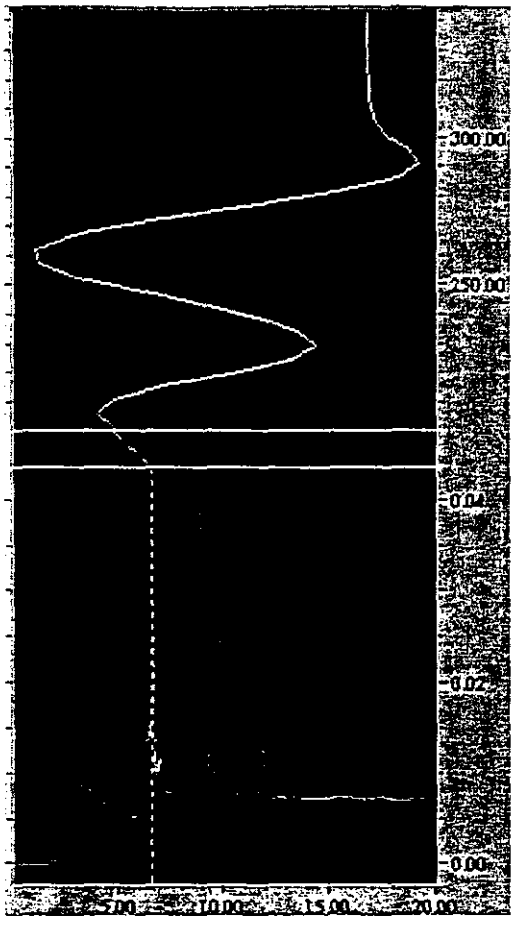


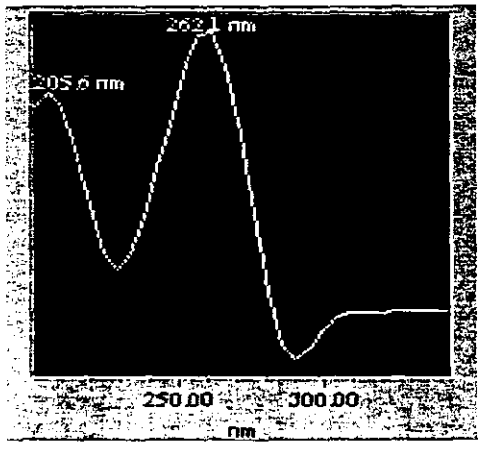
Lake Griffin – center, SJRWMD



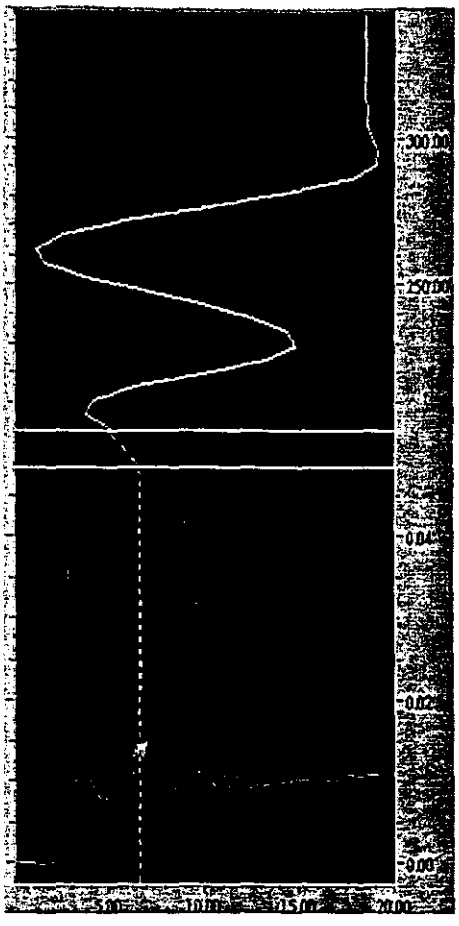
Dunn's Creek, SJRWMD

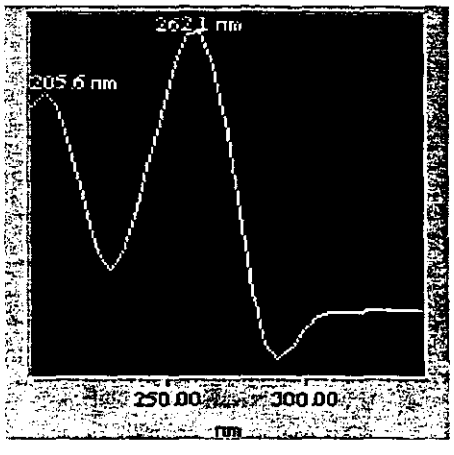
Lake Griffin South (3-10-99)



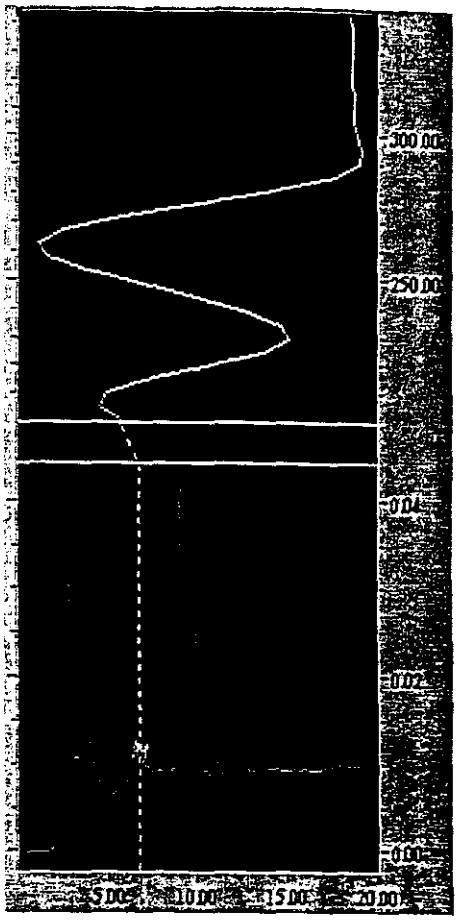


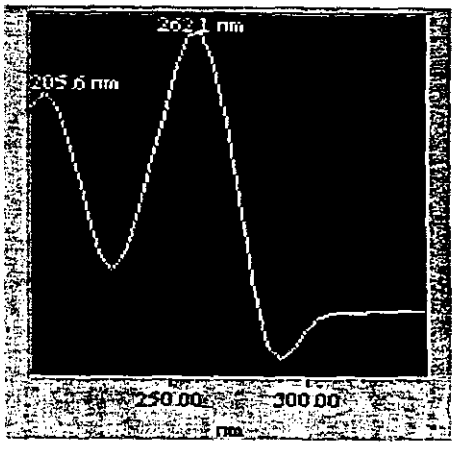
Lake Griffin North (3-10-99)





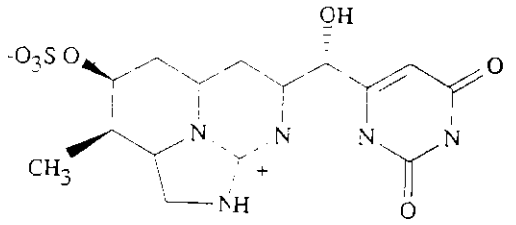
Lake Griffin West (3-10-99)





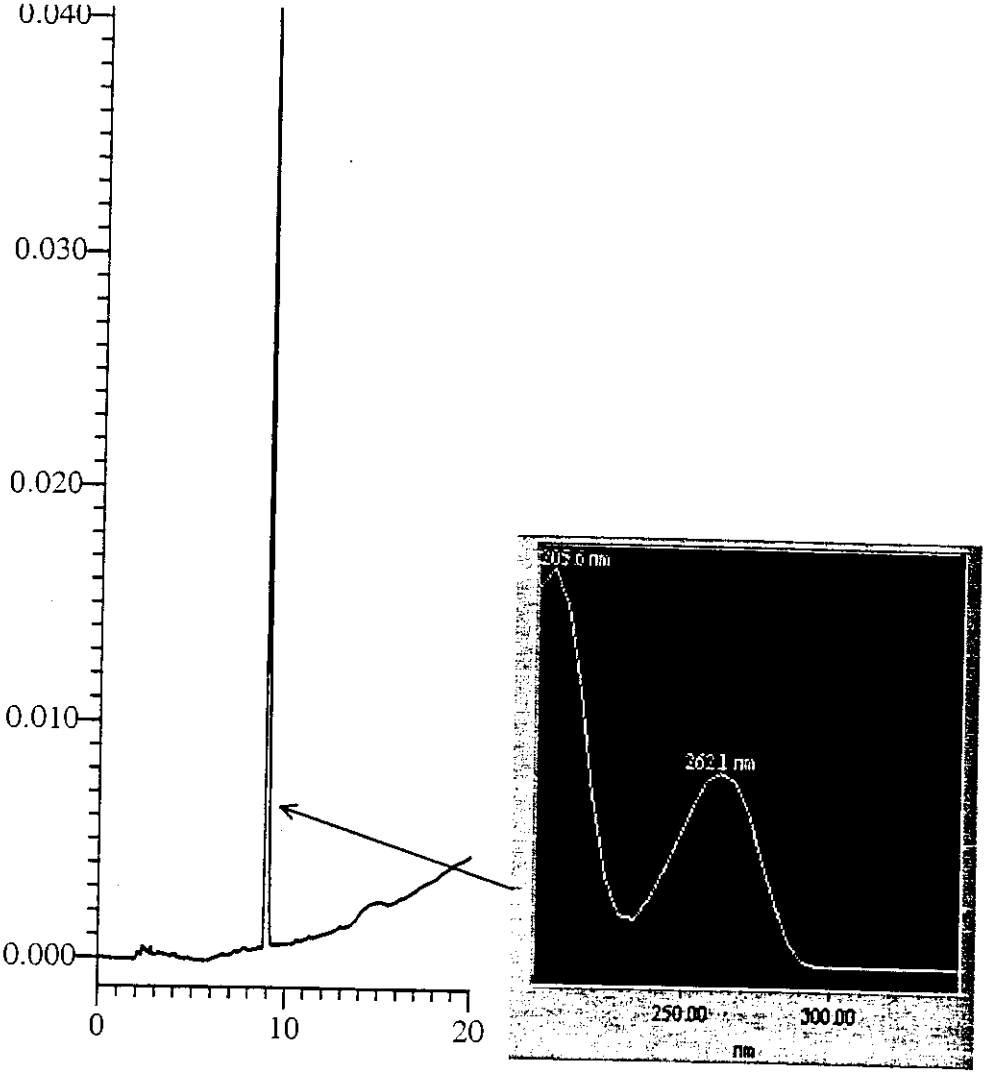
Cylindrospermopsin Identification: High Performance Liquid Chromatography

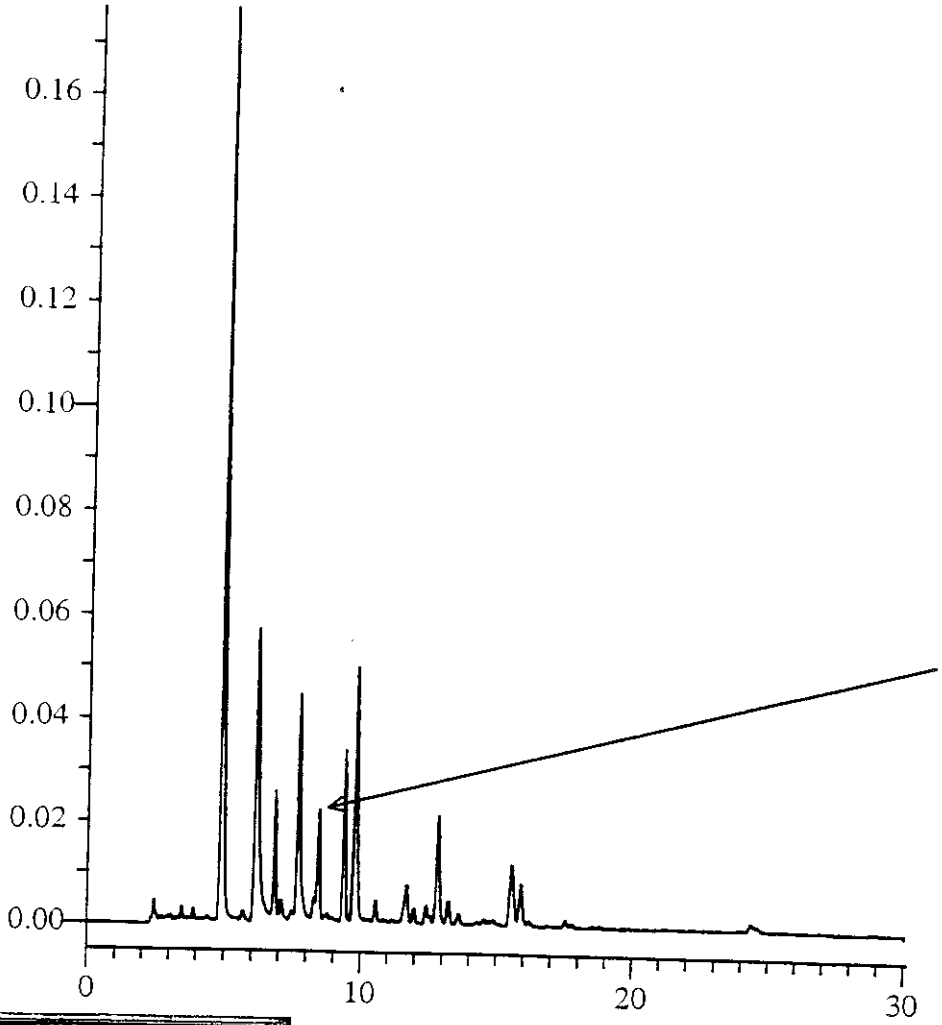
HPLC conditions:
Zorbax SB-C18 4.6x250mm
A: 0.1% TFA (aq.)
B: Acetonitrile + 0.1% TFA
0 to 10%B/30 min 1 mL/min
PDA-UV @ 262 nm



Structure of cylindrospermopsin

cylindrospermopsin (std; 200 ng)

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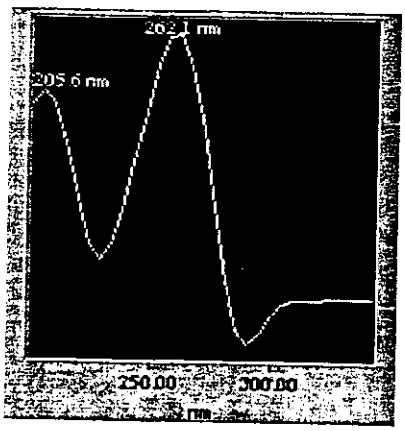
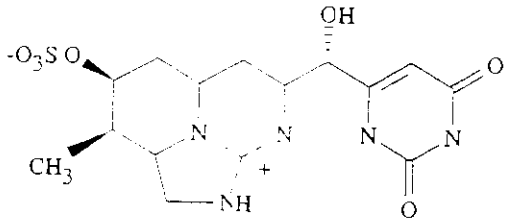
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30


202.1 nm
205.6 nm
250.00
300.00

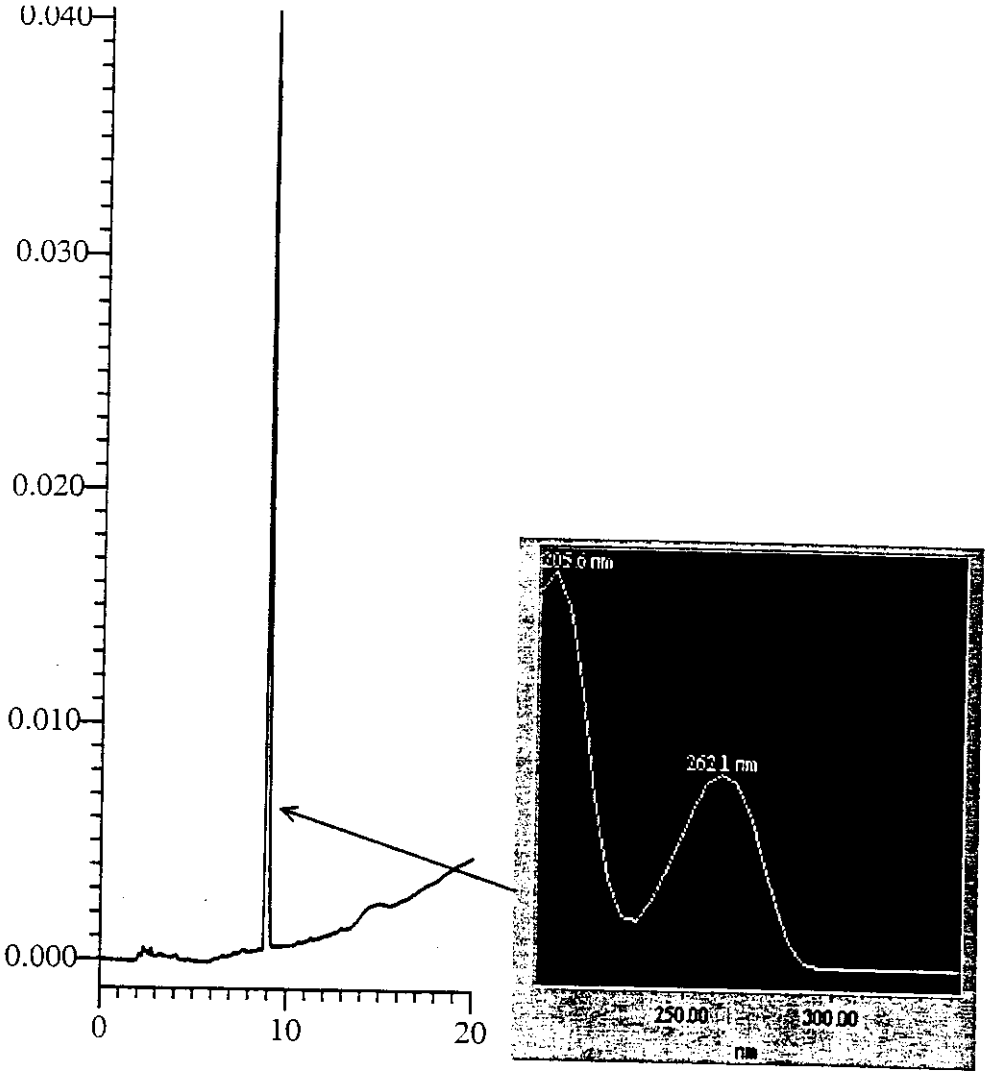
Cylindrospermopsin HPLC Analysis (Lake Griffin, Fla samples)

HPLC conditions:
Zorbax SB-C18 4.6x250mm
A: 0.1% TFA (aq.)
B: Acetonitrile + 0.1% TFA
0 to 10%B/30 min 1 mL/min
PDA-UV @ 262 nm



Structure of cylindrospermopsin

cylindrospermopsin (std; 200 ng)



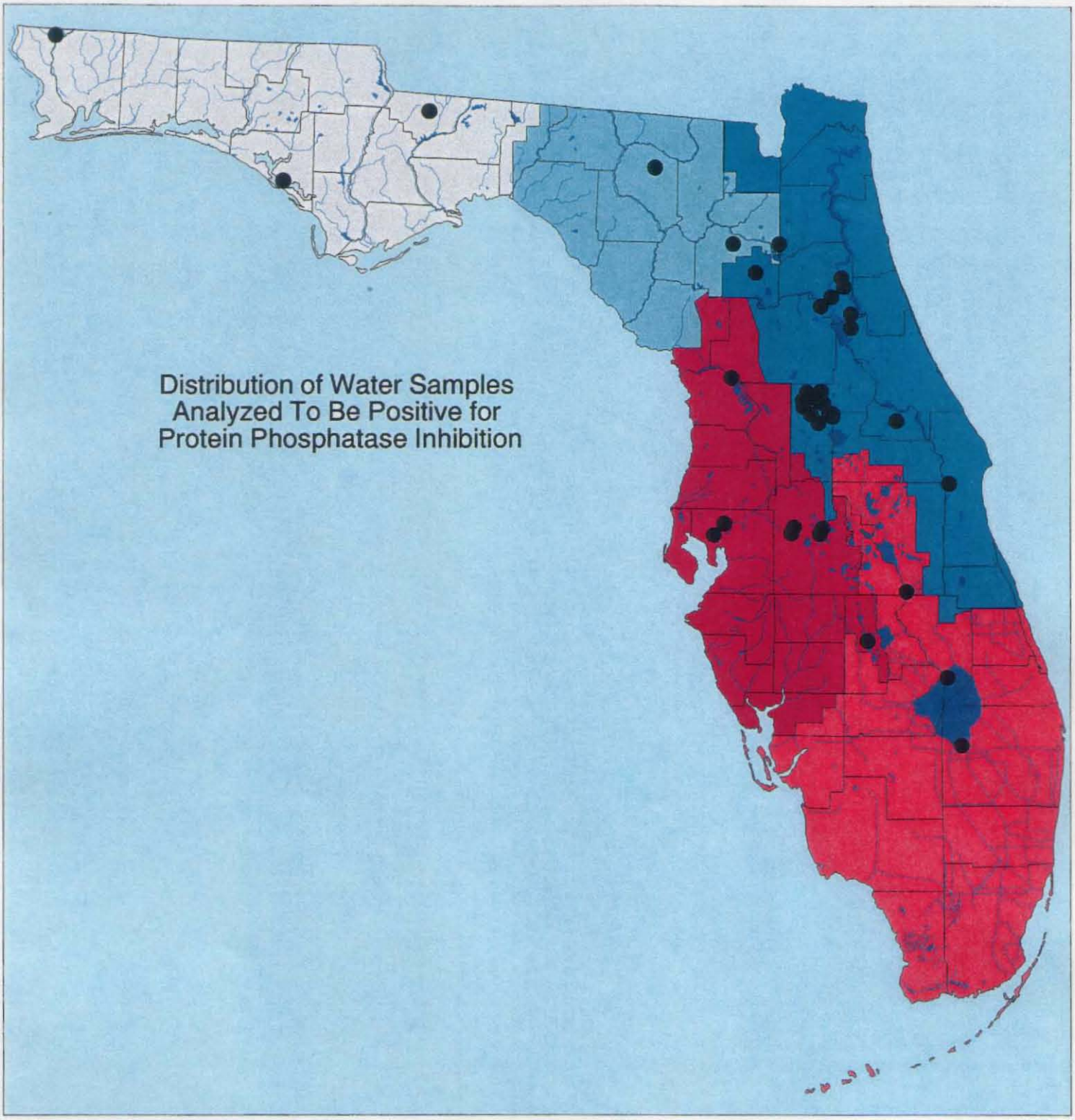


Figure 8

N
W E
S

0 30 60 Miles

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- Hplc.shp
- County Boundaries
- Rivers and Canals
- Lakes

WMD Boundaries

- SFWMD
- SWFWMD
- SJRWMD
- SRWMD
- NWFWMD

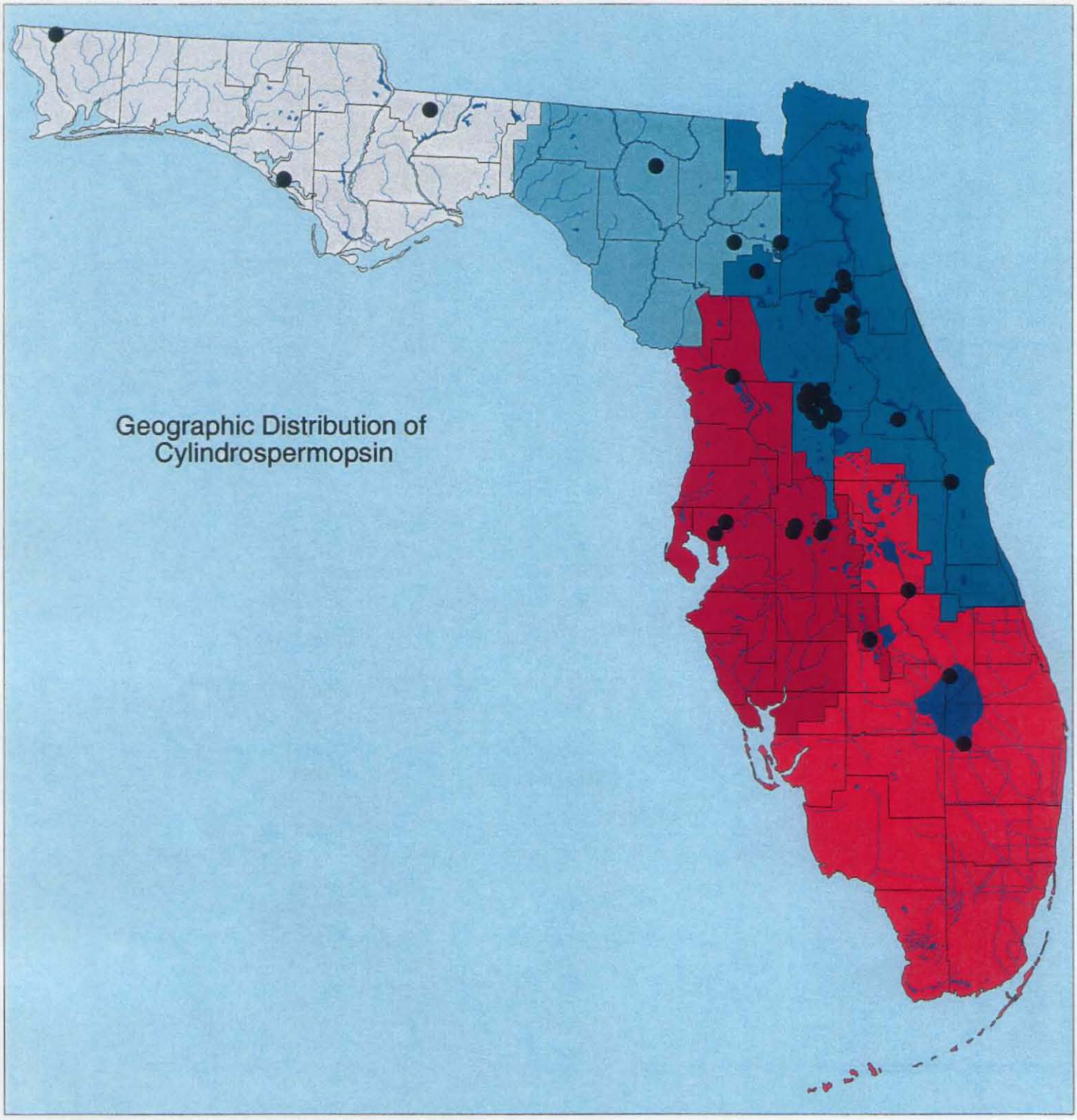

Figure 9

N
W E
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0 30 60 Miles

1:3772794

- CylindrospermopsinToxin
- County Boundaries
- Rivers and Canals
- Lakes

WMD Boundaries

- SFWMD
- SWFWMD
- SJRWMD
- SRWMD
- NWFWMD

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samples tested). No concentration levels were determined for cylindrospermopsin, merely presence or absence was reported. Fourteen of the collected water samples contained cyanobacterial species known to produce anatoxins. However, when these samples were analyzed via HPLC-Fl, no evidence of anatoxins was observed. Consequently, no anticholinesterase assays were performed.

The original intent of this project was to test all samples containing significant levels of toxigenic cyanobacteria for toxicity in mice. However, due to complications resulting from the two test laboratory protocols and insufficient time for sample processing prior to this report, only 68 samples were analyzed. Of these, 54 samples were found to kill mice at algal concentrations between 133 and 1,000 micrograms per kilogram (μg/kg) of mouse weight (Table 5). The predominant behavior observed with the onset of death was that characteristic of hepatotoxic poisoning, although in a few cases, neurotoxic behavior was reported. The geographical distribution of the water samples that were lethally toxic to mice is presented in Figure 10.

The geographical distribution of the compilation of all positive toxin analyses (microcystin, protein phosphatase inhibition activity, cylindrospermopsin, and mouse bioassays) is shown in Figure 11.

Regional Distribution

St. Johns River Water Management District (Northeast Florida)

SJRWMD was the most thoroughly sampled region of Florida and thus may provide the best overall picture of the extent and significance of harmful cyanobacterial blooms in Florida's surface waters. Of the 69 samples collected from this region, 56 (81%) contained potentially toxic cyanobacteria. Forty (58% of total) of these samples contained relatively high algal biomasses. All genera of potentially toxic cyanobacteria observed in samples from this area are reported in Table 2. *Cylindrospermopsis raciborskii* and species of *Microcystis* were the most frequently reported, with *Anabaena* spp., *Planktothrix* spp., *Coelosphaerium kuetzingianum*, and *Aphanizomenon* spp. occurring less often. The individual species identified are as follows: *Cylindrospermopsis raciborskii, Microcystis aeruginosa, M. flos-aquae, M. wesenbergii, M. viridis, Anabaena*

Table 5. Toxicity of water samples as determined by mouse bioassay

Water Body	Maximum Survival Dosage (mg/kg)	Minimum Lethal Dosage (mg/kg)	Time to Death	Toxicity Behavior
Suwannee River Water Management District				
Aligator Lake north		333	10 hr	H
Alligator Lake south		1,000	24 hr	H
Gwen Lake	133			
Lake Francis		1,000	48 hr	H
Low Bush Bay		333	6 hr	H
Lowe Lake		133	10 hr	H
Peacock Lake	67			
Watertown Lake	1,000			
Northwest Florida Water Management District				
Davis Lake		500	24 hr	H
Eleven Mile Creek		133	4 hr	N
Lake Belmont		200	6 hr	H
Lake Davis	667			
Lake Hurricane		500	24 hr	H
Lake Kell-Aire		133	3 hr	N
Lake Stone	67			
Quincy Creek	667			
Shelly Pond		500	24 hr	H
Southwest Florida Water Management District				
Braden River	133			
Lake Conine	1,000			
Lake Forest		500	24 hr	H
Lake Henry		333	5 min	N
Lake Hollingsworth		333	6 hr	H
Lake Howard		500	24 hr	H
Lake Parker		333	6 hr	H
Lake Persimmon		500	24 hr	H
Lock Haven	133			
South Florida Water Management District				
Kissimmee River north		500	24 hr	H
Lake Kissimmee		500	24 hr	H
Lake Okeechobee north		500	24 hr	H
Lake Okeechobee @ Belle Grade		1,000	24 hr	H
Lake Okeechobee @ Okeechobee		333	8 hr	H
Lake Okeechobee @ South Bay		133	4 hr	H/N
Lake Okeechobee @ South Bay 2	133			

Table 5—*continued*

Water Body	Maximum Survival Dosage (mg/kg)	Minimum Lethal Dosage (mg/kg)	Time to Death	Toxicity Behavior
St. Johns River Water Management District				
Clermont Pond		500	24 hr	H
Crescent Lake south		500	24 hr	H
Doctors Lake		133	6 hr	H
Haines Creek	1,000			
Lake Ashby		200	5 min	N
Lake Beauclair		200	30 min	N
Lake Bethel		333	2 min	N
Lake Carlton		500	24 hr	H
Lake Crystal	1,000			
Lake Dora		250	24 hr	H
Lake Eustis		1,000	24 hr	H
Lake George @ Willows Point	1,000			
Lake Griffin canal		500	24 hr	H
Lake Griffin center		500	24 hr	H
Lake Griffin north		250	24 hr	H
Lake Griffin south		500	24 hr	H
Lake Griffin west		500	24 hr	H
Lake Harris		250	24 hr	H
Lake Jesup		333	10 hr	H
Lake Johnson		133	8 hr	H
Lake Lochloosa		133	8 hr	H
Lake Maitland		333	6 hr	H
Lake Orange		133	8 hr	H
Lake Poinsett		1,000	24 hr	H
Lake Wauberg		333	2 min	N
Lake Yale	1,000			
Little Lake Harris		500	24 hr	H
Newnans Lake		200	5 min	N
St. Johns River @ Dunns Creek		500	24 hr	H
St. Johns River @ Fort Gates		133	8 hr	H
St. Johns River @ Jack Wright Island		133	6 hr	H
St. Johns River @ Little Lake George		133	12 hr	H
St. Johns River @ Palatka		333	8 hr	H
St. Johns River @ Picolata		500	24 hr	H
St. Johns River @ Welaka Spring		333	6 hr	H

Note: H = behavior consistent with hepatotoxic poisoning
N = behavior consistent with neurotoxic poisoning

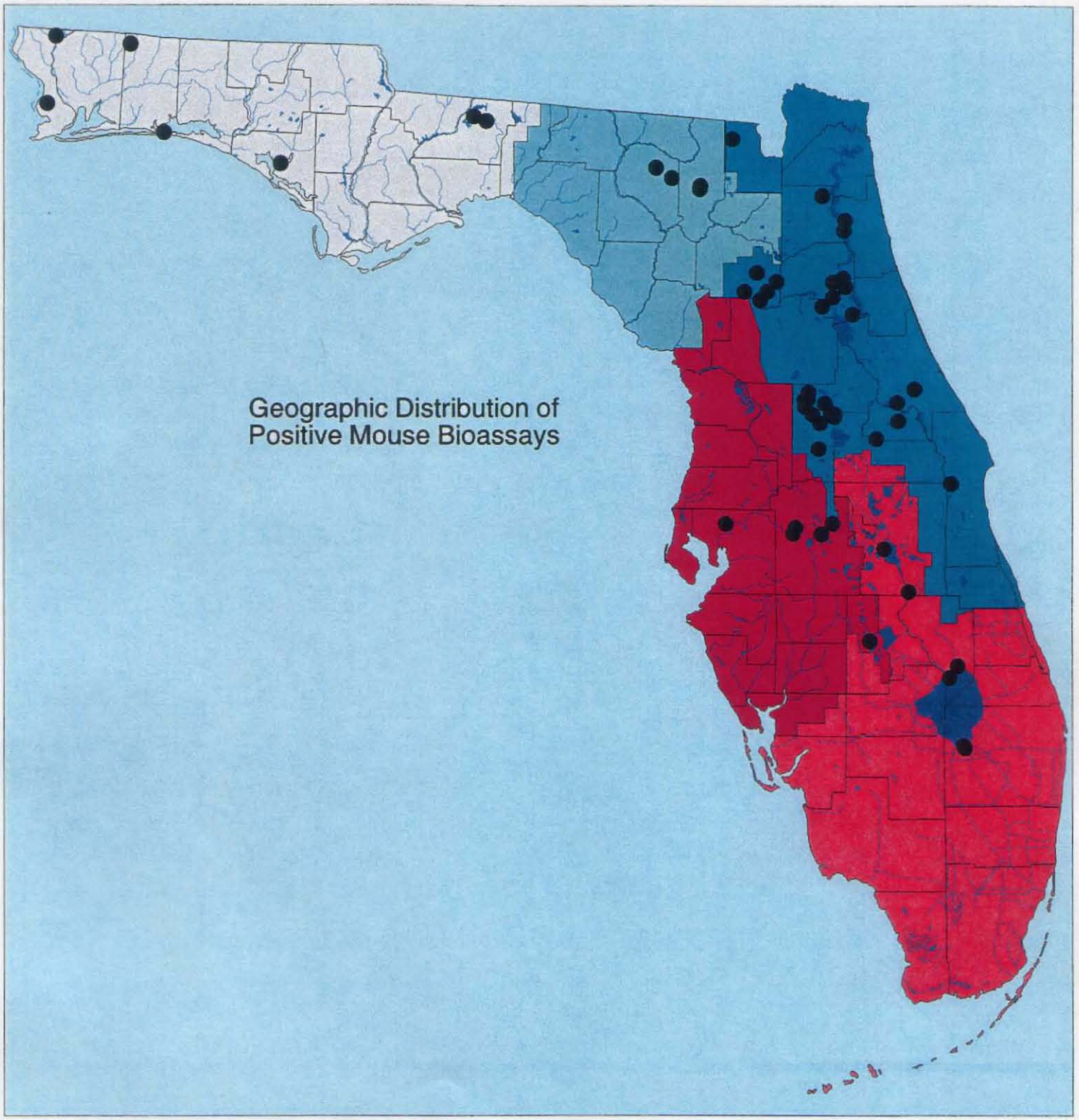

Figure 10

N
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0 30 60 Miles

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Mouse Bioassays
County Boundaries
Rivers and Canals
Lakes

WMD Boundaries
SFWMD
SWFWMD
SJRWMD
SRWMD
NWFWMD

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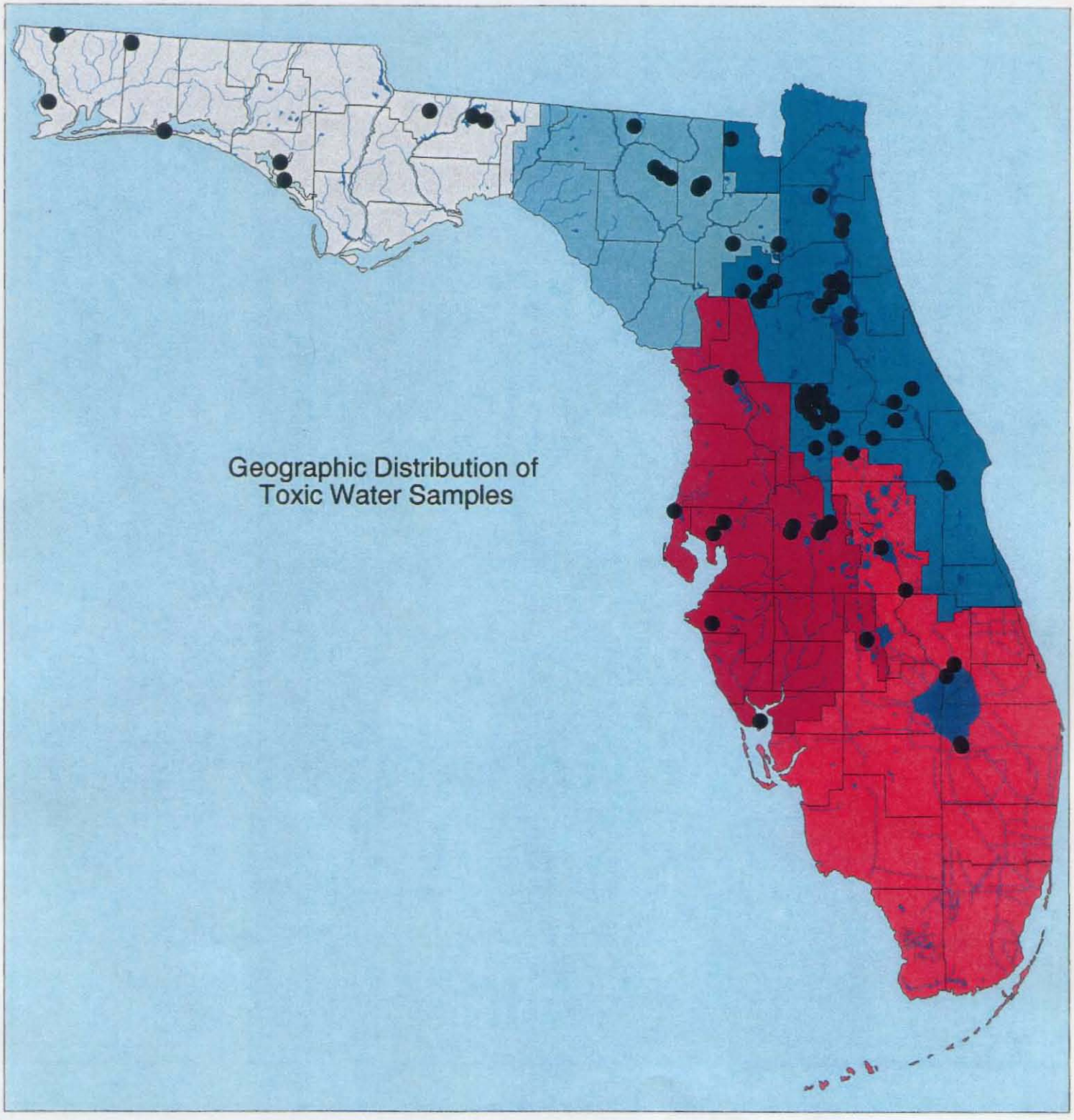

Figure 11

N
W E
S

0 30 60 Miles

1:3772794

- Toxic Water Samples
- County Boundaries
- Rivers and Canals
- Lakes

WMD Boundaries

- SFWMD
- SWFWMD
- SJRWMD
- SRWMD
- NWFWMD

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circinalis, A. compacta/pseudocompacta, A. flos-aquae, A. planctonica, Planktothrix agardhii/rubescens, and *Coelosphaerium kuetzingianum.* Water samples that contained microcystins, displayed protein phosphatase inhibition activity, indicated the presence of cylindrospermopsin, and exhibited toxicity to mice were found extensively throughout the region (Figures 7–10).

Southwest Florida Water Management District

The southwest region of Florida was sampled 29 times, with 22 (76%) of these samples containing potentially toxic cyanobacteria. Only 11 samples (38% of total), however, contained significant levels of toxigenic cyanobacteria. The cyanobacterial genera observed in samples from this region are reported in Table 2. *Anabaena, Microcystis,* and *Cylindrospermopsis* species were the dominant and most consistently observed species, while *Planktothrix* and *Aphanizomenon* were present but had a much lower frequency of occurrence. The individual species identified are as follows: *Anabaena flos-aquae, Anabaena circinalis, Microcystis aeruginosa, Cylindrospermopsis raciborskii,* and *Aphanizomenon flos-aquae* (no individual *Planktothrix* species were identifiable).

Microcystin analyses indicated that all 11 samples containing significant levels of toxigenic cyanobacteria contained microcystins, and four of these samples demonstrated protein phosphatase inhibition activity (Table 4). Eight samples (from Lakes Conine, Howard, Persimmon, Forest, Parker, and Hollingsworth, as well as East Lake and Egypt Lake) contained a cylindrospermopsin-like compound. Results from the mouse bioassays indicated that six samples were toxic to mice; in fact, all mice died when exposed to the two highest concentration levels of all six samples (Table 5). Although most toxic behavior was considered to be hepatotoxic in nature, water from Lake Henry (containing *Anabaena flos-aquae* and *Aphanizomenon flos-aquae*) did elicit neurotoxic responses (Table 5).

South Florida Water Management District

The southern portion of the state was sampled 29 times, with 20 (69%) of these samples containing potentially toxic cyanobacteria. Fourteen of the 20 (48% of total) samples contained significant levels of toxigenic cyanobacteria. The potentially toxic cyanobacterial genera observed in samples from this district are reported in Table 5. As in other regions, *Anabaena* spp., *Microcystis* spp., and *Cylindrospermopsis raciborskii* were the

co-dominant species, but at a much lower frequency of occurrence when compared to the Northwest Florida and Southwest Florida districts (Table 5). Also note that *Planktothrix* spp., which were subdominant in other districts, were observed as frequently as the dominant species. The individual species identified are as follows: *Anabaena circinalis, Microcystis wesenbergi/viridis, M. aeruginosa, Cylindrospermopsis raciborskii, Planktothrix agardhii/rubescens,* and *Aphanizomenon issatschenkoi.*

Microcystin analyses indicated the presence of microcystins in six samples, with three of these samples exhibiting detectable levels of protein phosphatase inhibition activity (Table 4). Samples taken from Lake Okeechobee-North, Kissimmee River-North, and Lake Okeechobee, near the city of Okeechobee, that were contaminated with microcystins also were found to be lethally toxic to mice (Table 4).

Northwest Florida Water Management District

The northwest region of the state was sampled 25 times, with 19 (76%) of these samples containing potentially toxic cyanobacteria. Fourteen of the 18 (56% of total) contained significant levels of toxigenic cyanobacteria. The potentially toxic cyanobacterial genera observed in samples from this region are listed in Table 2. As in other regions, *Microcystis, Anabaena,* and *Cylindrospermopsis* species were the dominant species observed (Table 3). *Planktothrix* and *Aphanizomenon* spp. were observed as well, but less frequently. The individual species that were identified are as follows: *Anabaena f. scheremetievi, A. planctonica, A. compacta/pseudocompacta, A. circinalis, A. flos-aquae, Microcystis aeruginosa, M. flos-aquae, M. wesenbergi, Cylindrospermopsis raciborskii,* and *Aphanizomenon flos-aquae* (no individual *Planktothrix* species were identifiable).

ELISA analysis showed that all 13 samples containing significant levels of toxigenic cyanobacteria were positive for microcystins (Table 4); however, only one sample (from Kell-Aire Lake) was reported to exhibit protein phosphatase inhibition activity. Three samples (Quincy Creek, Martin Lake, and Lake Stone) were found to contain a cylindrospermopsin toxin. Mouse bioassays were performed on 11 of the 13 samples. Mouse bioassay results indicate that six samples were lethal to mice within 24 hours of injection (Table 5). Mice injected with sample material from two samples (including the one from Kell-Aire Lake) indicated signs of neurotoxic poisoning.

Suwannee River Water Management District (North Central Florida)

The Suwannee River region of the state was sampled 15 times, with 12 (75%) samples containing potentially toxic cyanobacteria. Nine of these 12 (60% of total) contained relatively high levels of toxigenic cyanobacteria. Cyanobacterial genera observed in samples from this region are listed in Table 2. The individual species identified are as follows: *Microcystis aeruginosa, M. wesenbergi, M. flos-aquae, Anabaena planctonica, A. circinalis, Cylindrospermopsis raciborskii,* and *Coelosphaerium naegelianum* (*Planktothrix* spp. were observed as well, but were not identifiable at the species level).

Analyses indicated that microcystin was present in all samples that contained high levels of toxigenic cyanobacteria. Particularly high microcystin levels were observed in this region, especially in Lake Lowe (Table 4). The extremely high value of microcystins in Lake Lowe may be a result of the sampling procedure used at that site (i.e., concentration of the plankton in the water by collection with a plankton net). With the exception of Lake Francis, protein phosphatase inhibition activity was observed in all samples containing high levels of toxigenic cyanobacteria. Only one sample (Low Bush Bay) was found to contain cylindrospermopsin. Mouse bioassays indicated that six of the nine

DISCUSSION

SAMPLING

A total of 167 surface water/algal samples were collected within Florida. A review of the geographical distribution of the sampling sites (Figure 1) shows that most of the state was well sampled during the course of this project, with the exception of the southernmost portion of the state near Broward County. This exception was due to both study protocol and weather conditions. Specifically, the initial protocol restricted sample collection to water bodies exhibiting bloom conditions, and the abnormal weather conditions recorded in Broward County in the summer of 1999 reduced the number and the intensity of blooms anticipated in this region. As a result, no water samples were collected for this area. During normal years, algal blooms are a common problem for lakes in this region (K. Carter, Broward County Department of Environmental Protection, pers. com. 1999).

Initial projections called for 25 sites to be sampled in each water management district. In reality, with the exception of the Suwannee River Water Management District (n = 15), all districts were sampled at or above this projected value. SJRWMD was the most frequently sampled region of the state, due to the presence of the St. Johns River (the longest river in the state of Florida), numerous other water bodies in close proximity that were easily sampled, extensive sampling programs already in place that provided additional samples, and full-time personnel dedicated to the collection and identification of water samples. The Suwannee River area was sampled less frequently due to its small size and relative lack of eutrophic water bodies conducive to cyanobacterial blooms. It is interesting to note, however, that many of the water samples collected in this area that had high levels of toxigenic cyanobacteria contained elevated levels of cyanotoxins.

As noted above, the initial focus of the study was to collect samples from water systems experiencing bloom conditions in order to maximize sample collection efficiency and to minimize analytical costs. This initial study was not designed to provide random samples. In reality, however, sampling generally consisted of one-time collections, independent of environmental and bloom conditions, which took place when field

biologists were already scheduled to be at the water body in question. This method of sample collection was a more randomized approach than the planned method and is believed to underestimate the actual frequency, intensity, and duration of harmful cyanobacterial blooms in Florida. Thus, any water samples containing toxigenic cyanobacteria should be considered indicative of a potential for bloom conditions (and thus production of cyanotoxins) to develop. Furthermore, the scope of this project did not allow for the collection of data on the monthly, seasonal, and annual variations of the cyanobacterial blooms observed, nor did it allow for the determination of the absolute concentration levels (cells/mL or gm/L) of cyanobacteria in the affected waters. Such information would prove very useful in providing a quantitative estimate of the biomass of cyanobacteria required to create toxic environmental conditions, identifying patterns in toxin production, and establishing criteria for effective environmental risk management.

CYANOBACTERIAL DISTRIBUTION

The three main cyanobacterial genera observed in water samples from throughout the state were *Anabaena*, *Microcystis*, and *Cylindrospermopsis*. Multiple species of *Anabaena* and *Microcystis* were reported, while *C. raciborskii* was the only observed representative of this genus. Given the widespread distribution and relative high abundance of *Anabaena*, *Microcystis*, and *C. raciborskii* in collected water samples, and the fact that these organisms can produce hepatotoxic and neurotoxic compounds, these three genera are considered to be of the greatest concern in Florida.

Anabaena species were prevalent most often in the Northwest Florida and Southwest Florida water management district regions. Again, it is important to note that the sampling protocol used may underestimate significantly the intensity of most bloom events. For example, although the overall prevalence of *Anabaena* was relatively low in the St. Johns River region (27.3%), an extremely large bloom of *A. circinalis* occurred in the St. Johns River in August 1999. This bloom lasted over 2 months and was estimated to cover nearly 40 miles of the river. During this time period, a large fish kill event (adult menhaden, *Brevoortia tyrannus*, and *B. smithi*) occurred just downstream of the *Anabaena* bloom. Over 10,000 fish, including marine and freshwater species, were estimated to have died. Furthermore, numerous public complaints of algal scums, water discoloration, and foul smells were reported.

Anabaena circinalis has been reported to produce saxitoxins and microcystins (Sivonen and Jones 1999). The water collected from the St. Johns River at Picolata is the most representative sample of the toxic nature of the bloom event described above. Toxin analyses of this sample were positive but inconclusive. Data obtained from Wright State University indicated that microcystins were present, protein phosphatase inhibition activity was relatively high, concentration levels of 1,000 and 500 milligrams per kilogram killed mice, and the mice exhibited behavior consistent with hepatotoxic poisoning. However, results collected by the Florida Department of Health showed that microcystins were relatively absent and that water samples were non-lethal. The apparent discrepancies in these analyses probably were caused by the unavoidable differences in holding times of the water samples at the two laboratories. The Florida Department of Health had to hold all water samples until October due to equipment problems, whereas Wright State University was able to process samples more expediently.

Microcystis species were frequently found at elevated levels in most regions of the state (≥ 44%), with the exception of South Florida (~21%). This cyanobacteria genus was identified in more water samples throughout the state than any of the other toxigenic algae reported. *Microcystis* is known primarily for the production of microcystins (review: Rinehart et al. 1994), but has also been reported to produce anatoxins (Park et al. 1993). Microcystins found in Florida's waters represent a significant challenge to Florida's continued treatment and supply of surface waters used for drinking water. Of all the cyanotoxins, the cyclic peptides represent the greatest threat to human health around the world (Dow and Swoboda 2000). Although acute exposure to high doses may cause death from liver haemorrhage or liver failure, the risk of long-term exposure to comparatively low concentrations of toxin in drinking water is of particular concern. Continual oral exposure to low doses of microcystins have resulted in chronic liver injury, including the possibility of carcinogenesis and tumor growth promotion (Yu 1989, 1995; Fitzgerald and Yamasaki 1990; Falconer 1991). An increase in the geographical distribution of *Cylindrospermopsis raciborskii* and its dominance in eutrophic and hypereutrophic Florida lakes are of concern due to the relative frequency of toxin and toxicity associated with blooms of this alga found throughout the state.

Factors controlling the possible increase in the distribution and dominance of *C. raciborskii* in Florida are unclear. However, *C. raciborskii* may be an

introduced exotic species that has recently gained a competitive edge over other cyanobacteria due to a unique physiological, morphological, and/or ecological advantage. Alternatively, *C. raciborskii* may have been a minor component of Florida's phytoplankton community for some time and/or misidentified as *Anabaenopsis, Aphanizomenon,* or *Rhaphidiopsis* spp. It is now evident that *C. raciborskii* is a common component of freshwater phytoplankton communities throughout the state of Florida (Figure 4). In certain areas, such as Lake Griffin and the Harris chain of lakes, *C. raciborskii* may dominate the phytoplankton community year-round. During periods of prolonged blooms of *C. raciborskii,* Lake Griffin has suffered from failed year-classes of major sport fish and significantly increased alligator, soft-shelled turtle, and Florida gar mortality rates. The possible connection between *C. raciborskii* and these environmental problems (Figure 12) and the potential for similar scenarios to occur in other water systems is still unclear. The St. Johns River, an extremely important ecosystem that provides vital habitat for aquatic and terrestrial animals, provides recreational activities, and which can supply a supplemental source of freshwater to that supplied by the Floridan aquifer, has experienced increasing average concentrations of *C. raciborskii* and more intense blooms (Figure 13) over the past few years. Understanding how *C. raciborskii* will influence the ecology and the quality of water within the river is not known but may be inferred from other systems in Florida or similar river systems in Australia.

The main target of the alkaloid toxin, cylindrospermopsin, is the liver, but it also affects the kidneys, thymus, and heart (Terao et al. 1994). Cylindrospermopsin was first described following a major outbreak of hepatoenteritis among children in Australia who drank water from a surface supply that contained a bloom of *C. raciborskii.* The bloom was treated with 1 ppm of copper sulphate with clinical injury among consumers on that water supply reported the following week. A total of 140 children and 10 adults suffered liver and kidney damage, with the more serious cases requiring treatment for hypovolaemic/acidotic shock. The probability for a similar event in Florida is likely if current management techniques for cyanobacterial bloom monitoring and control,

as well as water treatment methods, are not improved. It is common practice in Florida to control cyanobacterial blooms by applying copper sulphate without conducting toxin analyses. An additional concern regarding *Cylindrospermopsis* in Florida is that it does not typically form surface scums or produce odors (specifically methylisoborneal and geosmin) that are used to alert water managers to cyanobacterial blooms in surface water supplies.

CYANOTOXIN DISTRIBUTION AND POTENTIAL BIOAVAILABILITY

Toxic water samples (defined as those that exhibited lethal effects in the mouse bioassays) were observed from sites throughout the state of Florida, indicating that toxic factors are common components of natural waters. Whether or not these components are bioavailable in their natural state is still unclear. In general, the toxins produced by cyanobacteria are isolated from the environment by the cell wall and membrane of the algal species, which is why most public health concerns are centered around the ingestion of cyanobacterial cells (e.g., via drinking water). Digestion destroys the cell wall and membrane, thereby liberating the toxins and making them bioavailable and thus capable of causing a toxic response. Digestion, however, is not the only means to this end. Cyanobacterial cells die naturally, releasing the toxin component present to the environment, especially during a bloom event.

The importance of the free-toxin component in water samples collected for this project is unknown at the present time, although preliminary measurements (Table 6) indicate that in certain instances, it may be relatively significant. Recent findings by international researchers indicate that up to 20% of the total cyanotoxin content of a healthy algal bloom may be extracellular (Sivonen et al. 1990; Lehtimaki et al. 1997; Negri et al. 1997; Rapala et al. 1997). It is interesting to note that cyanotoxins, in general, are water soluble chemicals and therefore, once released from the cell, could gain entry into an exposed animal's circulatory system. The toxin analyses performed during this project measured the total amounts of toxins present in each water sample, but it was outside the scope of this project to estimate the amount of free toxins in the water column.

Variable cyanotoxin results were obtained from two independent laboratories. Differences between the two analytical laboratories concerning the mouse bioassays and the microcystin analyses indicate that

Table 6. Comparison of freeze-dried (lyophilized) water and unprocessed ambient water samples for the determination of microcystin concentrations

Sample Site	Lyophilized Sample (ng/mL)	Ambient Water (ng/mL)
Alligator Lake north	0.977	* 2.555
Alligator Lake south	0.075	0.094
Gwen Lake	0.073	* 0.106
Lake Timber	0.131	* 1.047
Lowe Lake	1,373.0	3.193
Peacock Lake	0.192	0.091
Watertown Lake	0.083	* 1.877
Eleven Mile Creek	0.164	0.072
Braden River	0.109	0.087
Lake Davis	0.102	* 0.243
Lake Hurricane	0.979	0.076
Lake Kell-Aire	0.077	* 0.170
Lake Stone	0.221	0.077
Martin Lake	0.079	0.073
Quincy Creek	0.033	0.067
Charlotte Harbor	0.103	0.090
Charlotte Harbor #2	0.120	0.058
Lake East	0.101	* 0.126
Lake Hollingsworth	0.590	0.161
Lake Parker	0.101	* 0.174
Little Lake Harris	0.980	0.163
Lake Kissimmee	1,200.0	0.108
Lake Okeechobee @ Belle Glade	0.103	* 0.198
Lake Okeechobee @ Belle Glade #2	0.057	* 0.141
Crescent Lake, St. Johns River	0.128	* 1.643
Doctors Lake, St. Johns River	0.070	* 0.127
Haines Creek	0.140	0.048
Lake Dora	0.088	0.072
Lake Eustis	0.144	0.075
Lake George, St. Johns River	0.123	0.081
Lake Harris	0.075	0.059
Lake Yale	0.046	0.067
Newnans Lake	0.095	* 0.147
St. Johns River @ Welaka Springs	0.108	* 0.155
St. Johns River @ Palatka	7.250	0.060
St. Johns River @ Fort Gates	14,6400.0	0.092
St. Johns River @ Little Lake George	0.481	0.073

*Microcystin levels in ambient water greater than lyophilized samples and higher than negative controls

collection, processing, and analytical methods may need revision. As mentioned earlier, differences in the holding times for water samples probably played a significant role in the differences observed. A state laboratory capable of providing the necessary expertise in cyanotoxin analyses may prove critical to gaining a true understanding of the impact of these compounds on surface waters. At the present time, the project is dependent upon Wright State University in Dayton, Ohio, for the majority of its cyanotoxin analyses; there is no analytical laboratory in Florida dedicated to identification or characterization of cyanotoxins (e.g., microcystins, cylindrospermopsin, anatoxins, or cyanobacterial dermatotoxins).

CYANOTOXIN IDENTIFICATION

Microcystins are a family of toxins that include a number of different chemical compounds and are the most commonly observed cyanotoxins in fresh and estuarine waters. In general, cyanobacteria that produce microcystins can produce several types simultaneously; however, one or two specific chemical types usually are dominant. These compounds are highly stable chemicals that may persist in natural waters for months to years and, in general, are water-soluble. Microcystins are regarded as being highly toxic (lethal in mice at 50–300 μg/kg body weight), are predominantly hepatotoxic in nature, and are documented to bind to and deactivate critical protein phosphatase enzymes which can induce carcinogenesis (Ito et al. 1997). The levels of microcystins reported in this study, except for possibly a few samples from Lake Griffin, are not considered to be high enough to elicit acute toxicity via oral exposure. The effect of chronic exposure to these concentrations is still unknown. The high percentage of protein phosphatase inhibition observed in most samples, however, suggests that these compounds are highly bioactive and therefore may be capable of critical biochemical interference and possibly long-term tumor promotion.

Cylindrospermopsin is highly water-soluble, reasonably stable, hepatotoxic in nature (although it has caused pathology in the kidneys, spleen, thymus, and heart as well), and, biochemically, can block protein synthesis in liver cells. The actual chemical compound of cylindrospermopsin that has been isolated previously from blooms of *C. raciborskii* in Australia was not positively identified by HPLC analysis for samples from Florida, although toxic behavior consistent with cylindrospermopsin poisoning was observed in the mouse bioassays.

Corroborative evidence from scientists in Australia has verified that the chemical observed in this study does not exhibit the same chemical characteristics as does a cylindrospermopsin standard. The chemical compound found in two samples, which displayed a similar retention time but not a matching UV absorbance to purified cylindrospermopsin, might possibly be deoxycylindrospermopsin. Compounds found in other samples that exhibited neither comparable retention times nor matching UV absorbances to purified cylindrospermopsin have recently been identified as cylindrospermopsin by means of HPLC/MS analysis of *C. raciborskii* isolates grown under laboratory conditions. Analysis and identification by HPLC/MS have yet to be performed on ambient water collections. HPLC/MS analysis will permit further characterization and determination of toxin concentrations for cylindrospermopsin and microcystins in future studies.

Microcystins have been shown to bioaccumulate in the tissues of aquatic zooplankton (Watanabe et al. 1992), vertebrates (Carbis et al. 1997; Beattie et al. 1998), and invertebrates (Prepas et al. 1997; Watanabe et al. 1997). Cylindrospermopsin is similar to microcystins in its chemical characteristics and therefore is expected to behave in a similar manner and thus may pose a significant threat to the ecology of any aquatic environment where blooms are large and/or persist long-term. As an example, it has been shown recently that domoic acid, a water-soluble neurotoxin produced by the diatom *Pseudo-nitschia australis*, was trophically transferred through the food web and caused mortality in sea lions (Scholin et al. 2000) during a bloom event.

HUMAN HEALTH IMPLICATIONS

Evidence of toxigenic cyanobacteria and algal toxins in surface supply water, and toxicity demonstrated by mouse bioassay, indicate a possible health concern in Florida (Table 7). Although no human health problems associated with cyanotoxins have been reported in Florida, *Microcystis* and *Anabaena* species were concluded to be the causative factors in the outbreak of a severe gastroenteritis epidemic that occurred in Bahia, Brazil, in 1988. Over 2,000 cases were reported, and 88 people died (Sivonen and Jones 1999). In 1996, a severe case of hepatitis occurred in Caruaru, Brazil—117 patients experienced painful symptoms, 100 patients subsequently developed acute liver failure, and 60 patients eventually

Table 7. Identification of toxigenic cyanobacteria and toxin analyses for water bodies used as drinking water and for alternate drinking water resources

Surface Water Treatment Plant	Water Source	Water Management District	Toxic Algae	Mouse Bioassay	Microcystins	PPI	HPLC
Tampa	Hillsborough River	SWF					
West Palm Beach	Lake Clear	SF					
	Lake Mangonia	SF					
Punta Gorda	Shell Creek	SWF					
Bradenton	Braden River	SWF	+		+		
	Deer Point Reservoir						
Bay County	(Econfina Creek)	NWF					
Quincy	Quincy Creek	NWF	+		+		+
Belle Glade	Lake Okeechobee south	SF	+	+	+		
Pahokee	Lake Okeechobee east	SF					
US Sugar Corporation @ Clewiston	Lake Okeechobee south	SF	NS	NS	NS	NS	NS
US Sugar Corporation @ Bryant Mill	Lake Okeechobee south	SF	NS	NS	NS	NS	NS
Peace River Regional Water Supply Authority	Peace River	SWF					
Manatee County	Manatee River	SWF					
Okeechobee	Lake Okeechobee north	SF	+	+	+		+
South Bay	Lake Okeechobee south	SF	+	+	+		+
Melbourne	Lake Washington	SJR					
Marco Island	Marco Lakes (2)	SF					
North Port	Myakka-Hatchee River	SWF					
Fort Myers	Lake Okeechobee	SF					
Alternate Drinking Water Resources							
SJR @ Cocoa	St. Johns River	SJR	+	NP	+		
SJR @ Deland	St. Johns River	SJR	+	NP			
SJR @ Titusville	St. Johns River	SJR		NP			
SJR @ Switzerland Point	St. Johns River	SJR	+	NP			

Note: NS = not performed—would not agree to sampling but should be assumed to be positive as to the number of other positive samples collected from the same general area
NP = not performed

died. Biological and chemical data concluded that the outbreak was a result of microcystin contamination in water used for hemodialysis treatment (Carmichael 1998; Jochimsen et al. 1998; Pouria et al. 1998). Human illness (e.g., vomiting, painful diarrhoea, central abdominal pain, blistering of the lip, throat ulcers, fever, eye and ear irritation) following accidental swallowing of cyanobacteria during swimming has also been described in Canada (Dillenberg and Dehnel 1960), the United Kingdom (Turner et al. 1990) and Australia (Pilotto et al. 1995).

In general, blooms of blue-green algae are considered to be temporary events that last a few days to a few weeks; however, with the ever-increasing Florida population, the continuous destruction of natural landscapes, and increasing point and non-point source nutrient input to the aquatic environment, the frequency, duration, and intensity of algal blooms has increased (Steidinger et al. 1999). Cyanotoxins have been documented to adversely affect the liver, the spleen, the kidneys, the skin, nerve synapses and axons, and the gastro-intestinal tract (Sivonen and Jones 1999). In China, surface water drinking resources that exhibit high levels of cyanobacteria have been identified as being positively associated with increased cancer rates, specifically hepatocellular carcinoma (Yu 1989, 1995).

Although the effects of long-term exposure of low concentrations of cyanotoxins is unknown, the World Health Organization (1998) has developed a provisional guideline value of 1.0 μg/L (ppb) for the consumption of microcystin-LR. In Australia, a similar concentration level is presently being proposed as a guideline for cylindrospermopsin (G. Shaw, National Research Center for Environmental Toxicology, Queensland, Australia, pers. com. 1999). At several hundred μg/g (ppm) of dry weight cells, acute poisoning of animals consuming such water would occur. Although little is known of the toxic nature of cylindrospermopsin, or cylindrospermopsin-like compounds, it is believed that concentrations in the mg/g (ppt) range would induce acute-lethal toxicity while concentrations in the μg/g (ppm) range could induce chronic toxicity.

Cyanotoxins can be difficult to treat and are expensive to remove from raw water supplies (e.g., some water treatment plants in Florida may spend ~$10,000/day for activated carbon during major algal bloom events). Furthermore, human health may be at risk if cyanobacterial toxins are consumed directly through drinking water or indirectly through

primary contact via recreational exposure. It is anticipated that the dominance and spread of toxic strains of *Cylindrospermopsis*, and the production of other cyanotoxins in Florida's surface waters, may pose significant challenges for water quality and surface supply managers throughout Florida. The identification of cyanotoxins further demonstrates the need for surface water restoration and the lack of separation between water quality and quantity in water supply planning in Florida.

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APPENDIX A—SAMPLE COLLECTION SITES FOR THE CYANOBACTERIA SURVEY PROJECT, 1999

Appendix A: Sample collection sites for the Cyanobacteria Survey Project in the state of Florida for 1999.

Water Management District latitude	longitude	Water Body	County	Date of collection
Suwannee River				
820930	295500	Lake Rowell	Bradford	6/11/99
820330	294740	Keystone Heights, Unknown Pond	Bradford	9/8/99
823745	301030	Alligator Lake - north	Columbia	6/11/99
823800	300930	Alligator Lake - south	Columbia	6/11/99
823920	301030	Lake Gwen	Columbia	6/11/99
824140	301230	Lake Jeffrey	Columbia	8/18/99
823840	301000	Lake Montgomery	Columbia	6/11/99
823550	301130	Watertown Lake	Columbia	6/11/99
825700	301700	Low Bush Bay	Hamilton	8/27/99
830616	303222	Timber Lake	Hamilton	6/10/99
832420	302800	Camp County Pond	Madison	6/10/99
822420	302810	Lake Francis	Madison	8/18/99
825009	301316	Lake Lowe	Suwannee	6/11/99
825350	301500	Peacock Lake	Suwannee	6/11/99
822030	300130	Lake Butler	Union	8/18/99
Northwest Florida				
853831	301444	Davis Lake	Bay	7/7/99
853500	301855	Deer Point Lake	Bay	7/7/99
853631	300817	Martin Lake	Bay	7/7/99
853522	301533	Econfina River	Bay	10/27/99
871948	303252	Eleven Mile Creek	Escambia	6/28/99
871737	305758	Lake Stone	Escambia	6/30/99
842753	303559	Lake Tallavana	Gadsden	9/14/99
843452	303559	Quincy Creek	Gadsden	6/29/99
843452	303559	Quincy Creek WTP	Gadsden	6/29/99
841237	303012	A.J. Henry Lake	Leon	11/04/99
841400	303600	Blue Heron Lake	Leon	10/17/99
841303	303400	Lake Arrowhead	Leon	11/03/99
841045	303301	Lake Belmont	Leon	9/14/99
841549	302442	Lake Lee	Leon	11/04/99
841547	303426	Lake Sommerset	Leon	11/03/99
841356	303621	Lake Monkey Business	Leon	11/03/99
841617	303435	Shelly Pond	Leon	9/15/99
864519	305633	Hurricane Lake	Okaloosa	6/30/99
863834	305343	Karick Lake	Okaloosa	6/30/99
862858	302420	Kell-Aire Lake (Lake Coleman)	Okaloosa	6/28/99
862858	302420	Lake Coleman (Kell-Aire Lake)	Okaloosa	11/03/99
813105	283116	Lake Olivia	Orange	10/12/99
865000	305200	Bear Lake	Santa Rosa	7/7/99
870942	303449	Escambia Bay	Santa Rosa	6/28/99
861121	304705	Kings Lake	Walton	7/7/99
853543	304639	Alligator Creek	Washington	7/7/99

Appendix A (cont.): Sample collection sites for the Cyanobacteria Survey Project in the state of Florida for 1999.

Water Management District latitude	longitude	Water Body	County	Date of collection
Southwest Florida				
820900	265000	Charlotte Harbor	Charlotte	6/17/99
820900	265000	Charlotte Harbor	Charlotte	7/1/99
815959	270526	Peace River	Charlotte	10/05/99
820525	265425	Shell Creek	Charlotte	10/4/99
822248	285924	East Lake	Citrus/Marion	8/17/99
812421	272118	Lake Persimmon	Highlands	7/29/99
822935	280040	Egypt Lake	Hillsborough	8/17/99
822500	280500	Lake Forest	Hillsborough	8/5/99
821640	280339	Lake Thonotassa	Hillsborough	8/4/99
821500	275600	Lake Valrico	Hillsborough	8/4/99
822200	275700	Tampa Bypass Canal	Hillsborough	8/5/99
822300	280100	Tampa Reservoir (Hillsborough R.)	Hillsborough	8/5/99
822528	280056	Hillsborough River	Hillsborough	6/19/99
822931	272635	Braden River	Manatee	6/14/99
822114	272938	Lake Manatee	Manatee	9/30/99
824050	274700	Lake Hewitt	Pinellas	8/11/99
823912	274412	Lake Maggiore	Pinellas	8/4/99
824646	275059	Lake Seminole	Pinellas	8/4/99
824610	280850	Loch Haven	Pinellas	8/11/99
824010	274910	Moccasin Lake	Pinellas	8/11/99
815410	275842	Banana Lake,	Polk	8/17/99
814330	280336	Lake Conine	Polk	7/29/99
815022	275822	Lake Hancock	Polk	8/17/99
814000	280530	Lake Henry	Polk	8/3/99
815644	280133	Lake Hollingsworth	Polk	8/17/99
814440	280126	Lake Howard	Polk	7/29/99
815542	280348	Lake Parker	Polk	8/17/99
821230	270520	Myakka-Hatchee Creek	Sarasota	10/19/99
820958	285454	Medard Resevoir	Sumter	8/17/99

Appendix A (cont.): Sample collection sites for the Cyanobacteria Survey Project in the state of Florida for 1999.

Water Management District latitude	longitude	Water Body	County	Date of collection
South Florida				
814545	260640	Lantern Lake	Collier	7/8/99
815100	255620	Marco Lakes (2)	Collier	10/12/99
811725	271835	Lake Isokpoga-South	Highlands	11/04/99
811730	272500	Lake Istokpoga-North	Highlands	11/04/99
811811	264722	Caloosahatchie River	Lee	8/24/99
820650	262603	East Rock Lake	Lee	10/05/99
815400	263820	Fort Meyers WTP	Lee	8/17/99
820601	262559	Little Murex Lake	Lee	10/05/99
815734	263036	McGregor Wood Lake	Lee	10/05/99
801536	271026	St. Lucie (south fork)	Martin	8/18/99
804730	271200	Lake Okeechobee@Okeechobee	Okeechobee	10/18/99
805047	270733	Lake Okeechobee-North	Okeechobee	8/31/99
804700	271230	Taylor Creek	Okeechobee	11/03/99
811806	275601	Lake Kissimmee	Osceola	9/19/99
811412	281927	Lake Tohopekaligo-East	Osceola	9/9/99
811770	281676	Lake Tohopekaligo-West	Osceola	9/9/99
810803	273944	Kissimmee River -North	Osceola/Polk	8/31/99
805742	271335	Kissimmee River -South	Okeechobee/Highlands	8/31/99
800400	264230	Clear Lake	Palm Beach	9/29/99
810420	264400	Lake Mangonia	Palm Beach	9/29/99
804430	264100	Lake Okeechobee@Belle Glade	Palm Beach	9/17/99
804430	264100	Lake Okeechobee@Belle Glade 2	Palm Beach	9/17/99
804100	264925	Lake Okeechobee@Pahokee	Palm Beach	9/30/99
804445	264200	Lake Okeechobee@South Bay	Palm Beach	9/30/99
804445	264200	Lake Okeechobee@South Bay 2	Palm Beach	9/30/99
804708	264620	Lake Okeechobee-South	Palm Beach	8/30/99
805719	265208	Lake Okeechobee-SW	Palm Beach	8/30/99
804255	265870	Lake Okeechobee-East	Palm Beach/Okeechobee	8/31/99
805645	270123	Lake Okeechobee-NW	Palm Beach/Okeechobee	8/31/99
801719	271340	St. Lucie (north fork)	St. Lucie/Martin	8/18/99

Appendix A (cont.): Sample collection sites for the Cyanobacteria Survey Project in the state of Florida for 1999.

Water Management District latitude	longitude	Water Body	County	Date of collection
St. Johns River				
820430	293530	Lake Johnson	Alachua	10/11/99
820826	293138	Lake Lochloosa	Alachua	6/10/99
820826	293138	Lake Lochloosa	Alachua	10/11/99
821100	292800	Lake Orange	Alachua	6/10/99
821130	292830	Lake Orange	Alachua	10/11/99
821807	293132	Lake Wauberg	Alachua	10/11/99
821310	293835	Newnans Lake	Alachua	8/17/99
804810	282215	Ag. Farm in Cocoa	Brevard	9/1/99
805000	282030	Lake Poinsett	Brevard	9/1/99
804400	280900	Lake Washington	Brevard	10/12/99
805222	282212	SJR@RM18	Brevard/Orange	9/27/99
805729	283359	SJR@RM23	Brevard/Orange	9/27/99
810208	284251	SJR@RM27	Seminole/Volusia	9/27/99
805620	283230	SJR@Titusville	Brevard	8/25/99
822240	294920	Crystal Lake	Clay	9/8/99
820130	284530	Lake Geneva	Clay	9/8/99
820255	294925	Little Lake Crystal	Clay	9/8/99
814510	300737	SJR@Doctor's Lake	Clay	7/7/99
813630	301900	SJR@ the mouth of the Arlington Rive	Duval	8/5/99
812620	292500	Dead Lake	Flagler	7/26/99
814610	283320	Clermont Pond	Lake	8/30/99
814530	285130	Haines Creek	Lake	6/25/99
813930	284630	Lake Beauclair	Lake	8/30/99
813930	284530	Lake Carlton	Lake	8/30/99
815400	284600	Lake Denham - East	Lake	9/19/99
815630	284600	Lake Denham - West	Lake	9/19/99
814100	284700	Lake Dora	Lake	6/25/99
814327	285031	Lake Eustis	Lake	6/25/99
815200	284900	Lake Griffin Canal	Lake	8/25/99
815051	285146	Lake Griffin	Lake	7/28/99
815051	285146	Lake Griffin-Center	Lake	7/28/99
815030	285432	Lake Griffin-North	Lake	8/11/99
815200	284900	Lake Griffin-South	Lake	8/11/99
815221	285140	Lake Griffin-West	Lake	8/11/99
814850	284619	Lake Harris	Lake	6/25/99
814600	283430	Lake Minneola	Lake	8/30/99
814430	285500	Lake Yale	Lake	6/25/99
814530	284300	Little Lake Harris	Lake	8/25/99
813746	283711	Lake Apopka	Lake/Orange	8/19/99
813746	283711	Lake Apopka@Zellwin Farms Canal	Lake/Orange	6/28/99
813746	283711	North Lake Apopka	Lake/Orange	6/28/99
814700	292530	Lake Delancey	Marion	6/30/99
814720	292130	Lake Kerr	Marion	6/30/99
812140	283715	Lake Maitland	Orange	10/13/99
805150	282220	SJR@Cocoa	Orange/Brevard	9/1/99

Appendix A (cont.): Sample collection sites for the Cyanobacteria Survey Project in the state of Florida for 1999.

Water Management District latitude	longitude	Water Body	County	Date of collection
St. Johns River				
813100	292900	Crescent Lake-Center	Putnam	6/23/99
815125	293815	Lake Ida	Putnam	10/11/99
813600	294230	SJR, north of Russel's Point	Putnam	8/9/99
813500	293340	SJR@Dunns Creek	Putnam	7/19/99
814030	293530	SJR@Fort Gates/Buffalo Bluff	Putnam	7/8/99
813920	300150	SJR@Hallows Cove	Putnam	8/3/99
814500	292630	SJR@Little Lake George	Putnam	7/9/99
813610	293700	SJR@Palatka	Putnam	7/2/99
813800	294230	SJR@Rice Creek	Putnam	6/23/99
814020	292950	SJR@Welaka Springs	Putnam	8/17/99
813200	292330	Crescent Lake-South	Putnam/Flagler	7/21/99
814830	293040	Rodman Reservoir	Putnam/Marion	10/11/99
811227	284358	Lake Jesup	Seminole	8/12/99
811700	285100	Lake Monroe	Seminole/Volusia	10/14/99
813600	295300	SJR@Ferreira Point	St. Johns	6/23/99
813530	295830	SJR@Jack Wright Island	St. Johns	8/2/99
813600	295445	SJR@Picolata	St. Johns	7/27/99
813500	295100	SJR@Scratch Ankle/Ferreira Pt.	St. Johns	9/1/99
814030	300400	SJR@Switzerland Point	St. Johns	8/6/99
814100	300200	SJR@Governor's Creek	St. Johns/Clay	8/5/99
810530	285600	Lake Ashby	Volusia	8/30/99
811245	285057	Lake Bethel	Volusia	10/13/99
813200	291830	Lake George	Volusia	6/30/99
812300	290030	SJR@Deland	Volusia	10/12/99

SJR = St. Johns River

APPENDIX B—SAMPLING AGENCIES AND PERSONNEL ASSISTING IN THE COLLECTION OF WATER SAMPLES FOR THE CYANOBACTERIA SURVEY PROJECT, 1999

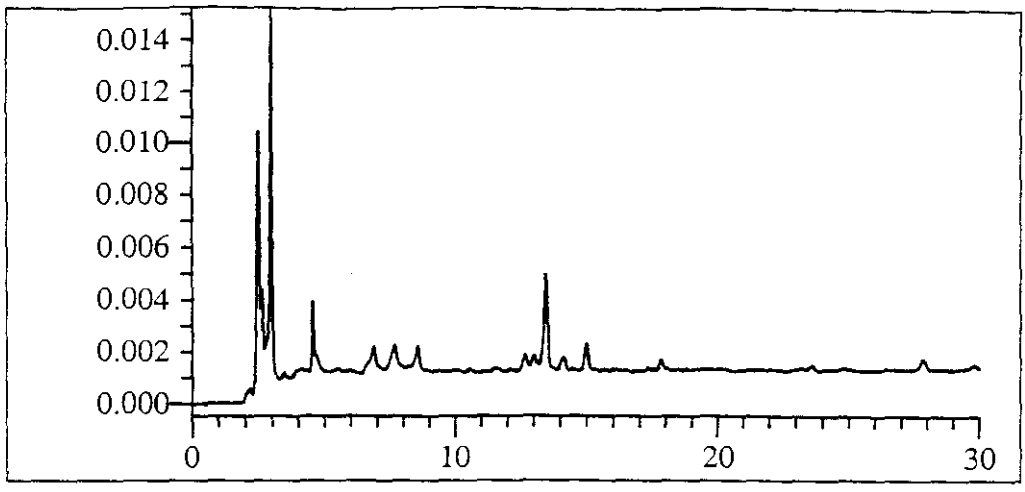
Contact/Agency	Water Body(ies) sampled	
Mr. Louis Aggarat North Port Utilities 5755 North Port Blvd. PO Box 7228 North Port, FL. 34287-5755	Myakka-Hatchee Creek	
Mr. Dan Atkinson Belle Glade Water Works 1016 West Canal Street South Okeechobee, FL. 33430	Lake Okeechobee@Belle Glade	
Mr. Ken Blakeney West Palm Beach Water Department 1009 Banyon Street West Palm Beach, FL. 33401	Lake Clear Lake Mangonia	
Ms. Patricia Burke South Florida Water Management District 1000 NE 40th Avenue Okeechobee, FL. 34972	Lake Okeechobee (5) Kissimmee River (2) Lake Kissimmee Caloosahatchee River	Taylor Creek Lake Tohopekaliga (2) Lake Istokpoga (2) St. Lucie River (2)
Mr. Dwayne Carbonneau Florida Game and Freshwater Fish Commission Wildlife Research Laboratory 4005 S. Main St. Gainesville, FL. 32601	Lake Griffin	
Mr. Roy Carter City of Pahokee Water Department 171 North Lake Avenue Pahokee, FL.	Lake Okeechobee@Pahokee	
Mr. John Casani Lee County Hyacinth Control District 15191 Homestead Road Ft. Myers, FL. 33971	McGregor Wood Lake East Rock Lake Little Murex Lake	
Dr. Bill Crass 13025 S. US Highway 441 Micanopy, FL. 32667	Lake Wauberg Lake Orange Lake Lochloosa	
Mr. Shawn Davis/Dr. Jon Staiger City of Naples, Central Laboratory 380 13th Street - North Naples, FL. 34102	Lantern Lake	
Ms. Lynn Denahan Orange County Environmental Protection Division 2002 E. Michigan St. Orlando, FL. 32806	St. Johns River@RM18 St. Johns River@RM23 St. Johns River@RM27	
Ms. Randie Denker 7600 Bradfordville Rd. Bradfordville, FL. 32308-2019	Lake Belmont	
Ms. Ginny Densmore 9713 Water Meet Drive Tallahassee, FL. 32312	Lake Blue Heron	

Contact/agency	Water bodies sampled	
Ms. Ann Forstchen Florida Marine Research Institute 100 Eighth Avenue S.E. St. Petersburg, FL. 33701	Charlotte Harbor (2)	
Mr. Thomas Frick Florida Department of Environmental Protection Chemistry Department, Laboratory Complex 2600 Blair Stone Road Tallahassee, FL. 32399-2400	Lake Lee Lake Sommerset Lake Monkey Business Lake A.J. Henry Lake Arrowhead	
Mr. Russell Frydenborg Florida Department of Environmental Protection Chemistry Department, Laboratory Complex 2600 Blair Stone Road Tallahassee, FL. 32399-2400	Quincy Creek	
Mr. Jim Griffin Hillsborough County Public Works Department 601 East-Kennedy Blvd. Tampa, FL. 33601	Egypt Lake East Lake Edward Medard Reservoir Lake Forest	Tampa By-Pass Canal Lake Valrico Tampa Hills Reservoir
Mr. Dave Hamilton City of Quincy Water System OMI, 300 North GFA Drive Quincy, FL. 32351	Quincy Creek	
Mr. Bill Harper City of Punta Gorda Water Department 38100 Washington Loop Road Punta Gorda, FL33950	Shell Creek Reservoir	
Mr. Joe Hinkle Department of Environmental Protection 703 North Marion Street Lake City, FL. 32055	Low Bush Bay	
Mr. Frank Kane FWSC/Marco Shores Utilities 300 Main Sail Drive Naples, FL. 34114	Marco Lakes (2)	
Mr. Mike Kelsey 9509 Westover-Roberts Road Windermere, FL. 34786	Lake Olivia	
Mr. Bob Kessler 512 SummerBrooke Drive Tallahassee, FL. 32312	Shelly Pond	
Mr. Joe King Lake Manager Polk County Natural Resources 4177 Ben Durrance Rd. Bartow, FL. 33830	Lake Henry	
Mr. Keith Kolasa Environmental Section Southwest Florida Water Management District Brooksville, FL. 34609-6899	Lake Hancock Banana Lake Lake Hollingsworth Lake Parker Lake Thonotosassa Lake Maggiore	Lake Seminole Lake Conine Lake Howard Lake Persimmon

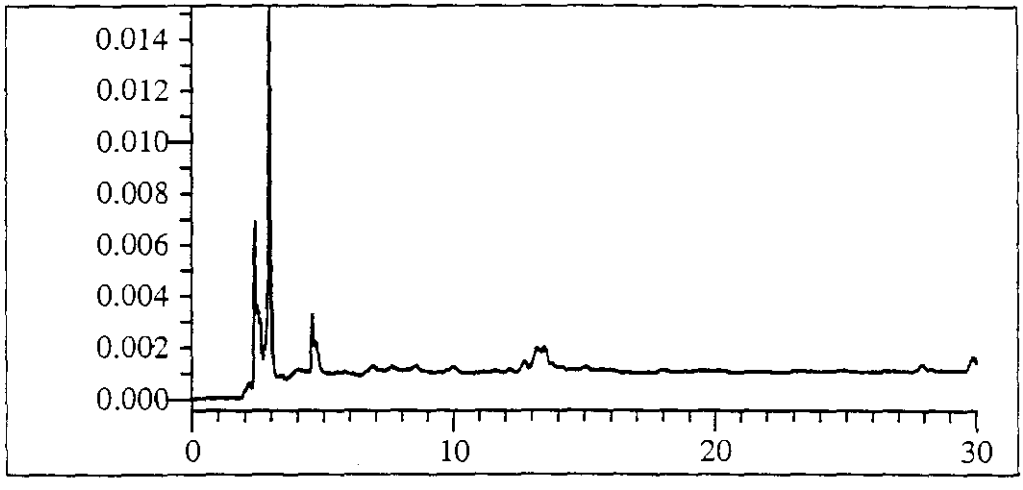
Contact/agency	Water bodies sampled	
Mr. Rob Mattson Suwannee River Water Management District 9225 County Road 49 Live Oak, FL. 32060	Low Bush Bay Lake Jeffrey Lake Francis Peacock Lake Lowe Lake Gwen Lake	Lake Rowell Watertown Lake Alligator Lake-North Alligator Lake-South Lake Montgomery (Hamburg) Camp County Pond Timber Lake
Mr. James McClain P.O. Box 5555 Destin, FL. 32540	Lake Coleman (Kell-Aire)	
Ms. Kathy McDonald/ Mr. Bob Olson County Utility Services Bay County Water System 3400 Transmitter Road Panama City, FL. 32404	Econfina Creek (Deer Point Reservoir)	
Mr. Keith McGum Bradenton City Water Department PO Box 5015 5600 Natalie Way Bradenton, FL. 34206-5015	Bill Evers Reservoir/Braden River	
Mr. Richard Moulton Ft. Myers Water Treatment Plant 2751 Jacksonville Street Ft. Myers, FL. 33916	Caloosahatchie River	
Mr. Rigo Muniz/Todd Larsen 335 SW 2nd Avenue South Bay, FL. 33493	Lake Okeechobee@South Bay	
Ms. Nancy Page Pinellas County Department of Environmental Management 300 South Garden Avenue Clearwater, FL. 33756	Lock Haven Moccasin Lake Lake Hewitt	
Mr. Randy Payne Department of Environmental Protection Suite 308, 160 Governmental Center Pensacola, FL. 32501	Kings Lake, Alligator Creek Deer Point Lake Martin Lake Davis Lake Hurricane Lake	Lake Stone Karick Lake Eleven Mile Creek Kell-Aire (Coleman) Lake Escambia Bay
Mrs. Patricia Powell LakeWatch Volunteer 2423 Tallavana Trail Havana, FL. 32333	Lake Tallavanna	
Mr. Mark Simpson/Bruce McLeod/Eric Larson Manatee County Public Works 18315 Dam Road Bradenton, FL. 34202	Lake Manatee	
Mr. Jerry Tindall, Sam Stone, Grady Sorah Peace River Regional Water Plant 8998 South West County Road 769 Arcadia, FL. 34266	Peace River	

Contact/agency	Water bodies sampled
Mr. Ernesto Laso de la Vega Lee County Hyacinth Control District 15191 Homestead Road Ft. Myers, FL. 33971	McGregor Wood Lake East Rock Lake Little Murex Lake
Mr. Jeff Vilagos City of Tampa Water Department 306 E. Jackson Street, 5 East Tampa, FL. 33602	Hillsborough River
Mr. Doug Wyatt Okeechobee Surface Water Treatment Plant 371 Highway 78 West Okeechobee, FL. 34974	Lake Okeechobee @ Okeechobee
St. Johns River Water Management District Division of Environmental Sciences Highway 100 West Palatka, FL 32177	all collections from this region
St. Johns River Water Management District Lake Apopka Field Station Apopka, FL.	Lake Apopka Harris Chain of Lakes

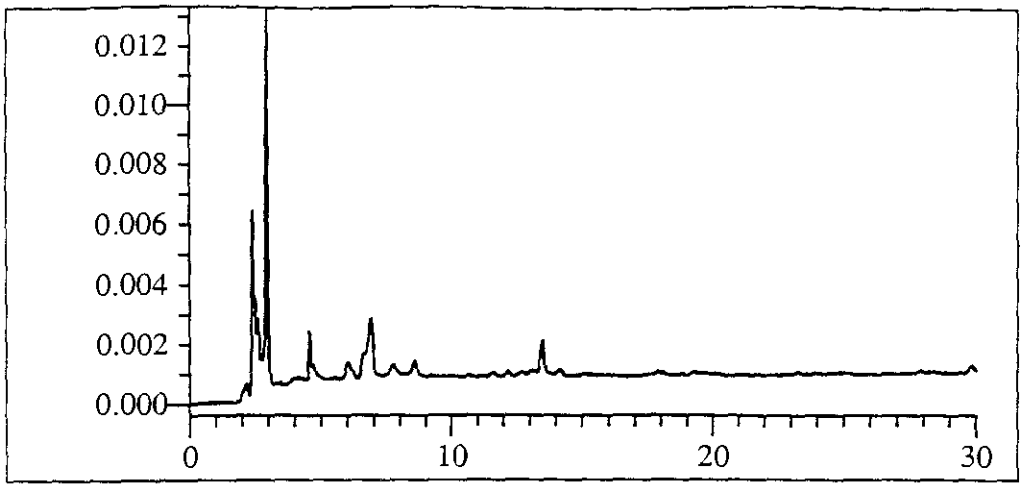
APPENDIX C—SAMPLES OF HIGH PERFORMANCE LIQUID CHROMATOGRAMS FOR THE IDENTIFICATION OF THE CYANOTOXIN, CYLINDROSPERMOPSIN, IN WATER SAMPLES WITH HIGH LEVELS OF *CYLINDROSPERMOPSIS RACIBORSKII*



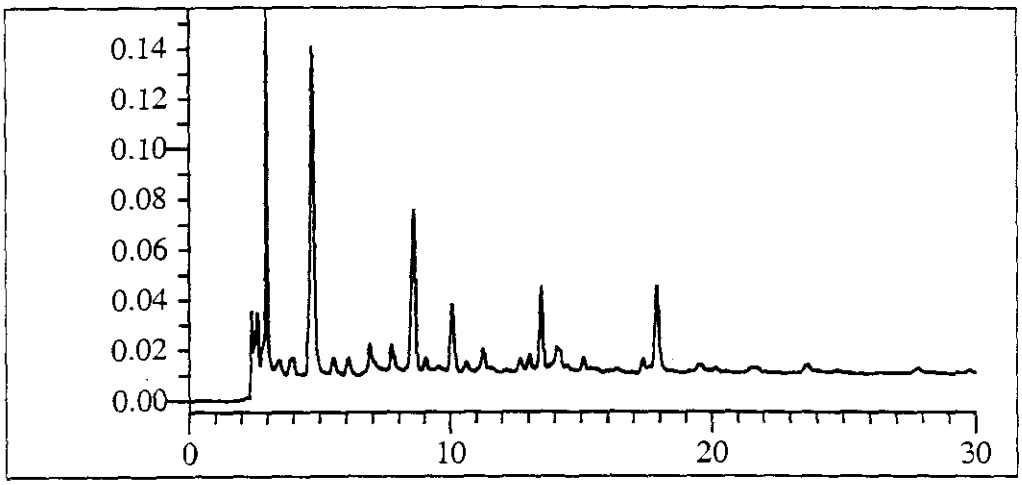
Lake Bethel, SJRWMD



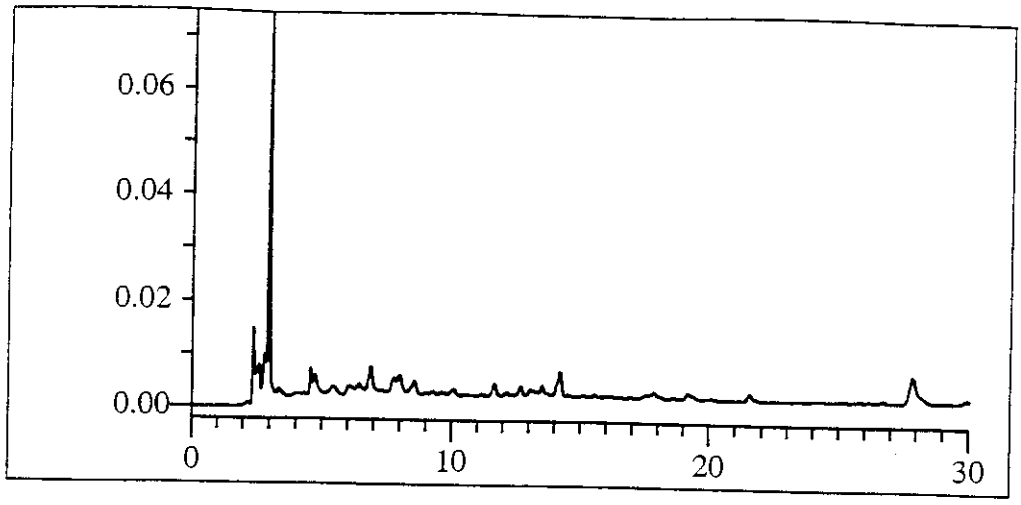
Lake Lochloosa, SJRWMD



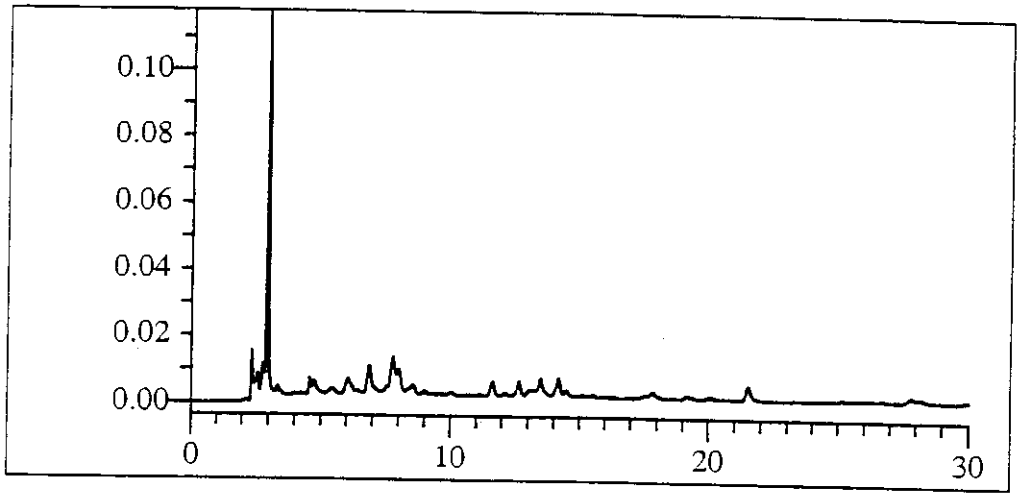
Lake Olivia, NWFWMD



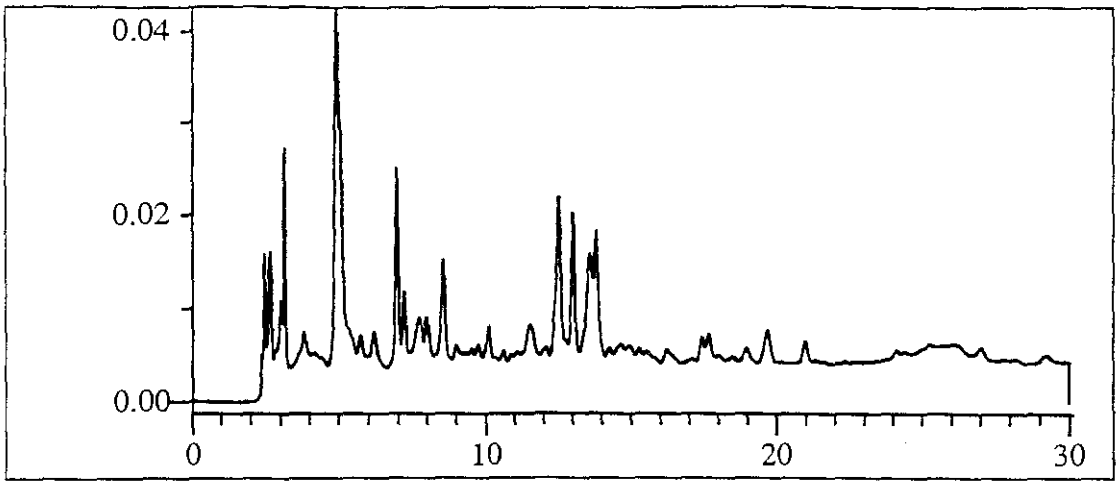
Newnans Lake, SJRWMD



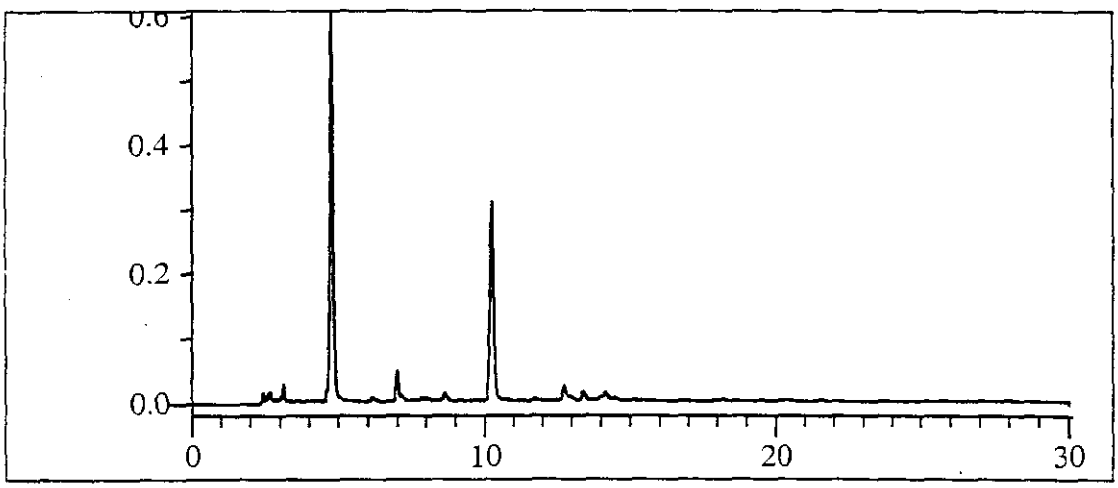
Lake Eustis, SJRWMD



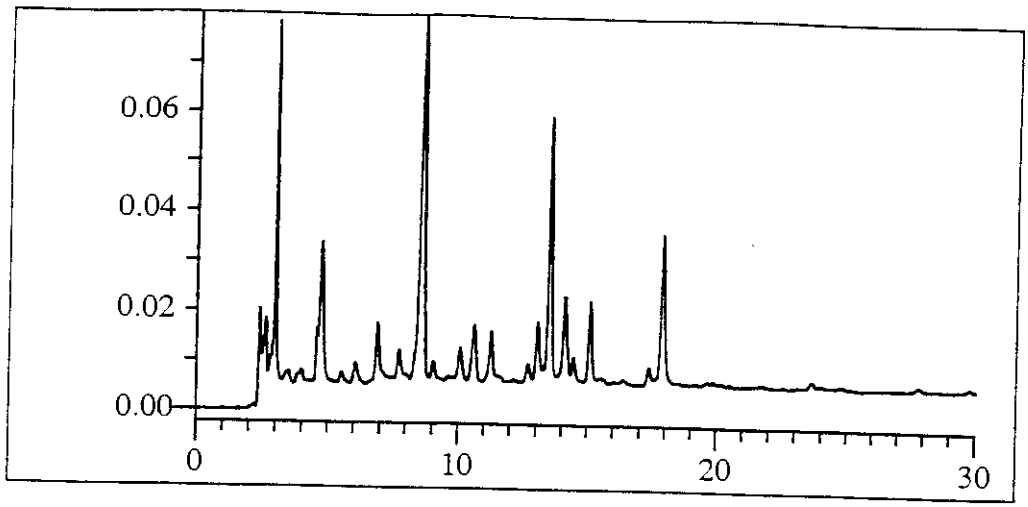
Lake Harris, SJRWMD



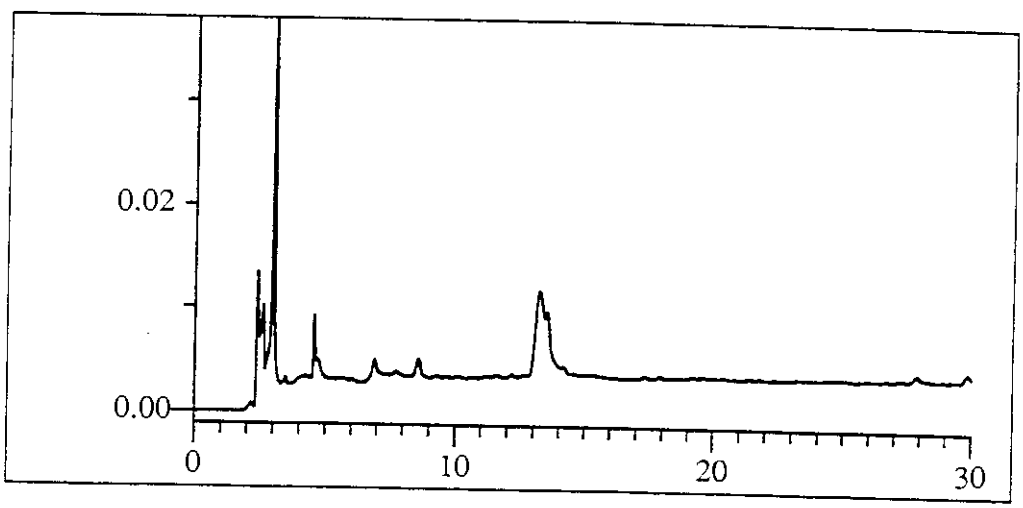
Lake Griffin at Bird Island, SJRWMD



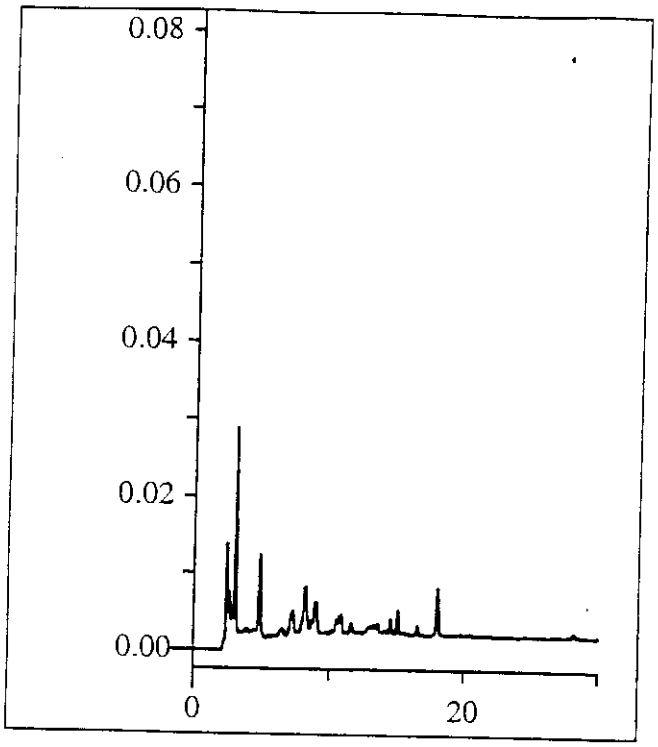
Haines Creek, SJRWMD



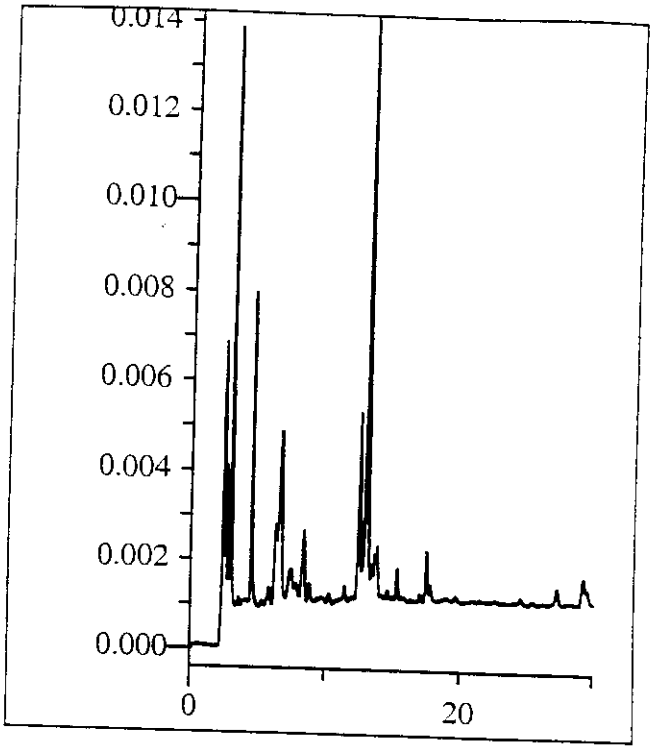
Lake Johnson, SJRWMD



Lake Orange, SJRWMD



Lake Crystal, SJRWMD



Lake Okeechobee @ South Bay, SFWMD