Assessment of the Public Health Risk from Cyanobacteria and their Related Toxins in Surface Water of the St. Johns River Utilized for Recreational Activities

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by

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This report represents a near continuation of the monitoring scheme employed in 2005. As such, much of this material can also be found in that report.

INTRODUCTION

Cyanobacteria, or blue-green algae, are a group of prokaryotic photosynthetic bacteria that contain chlorophyll *a* and account for up to 40% of planetary oxygen production. Amongst the oldest and most diverse lineages of bacteria, they have radiated into nearly every aquatic and terrestrial ecosystem. However, it is their prevalence in freshwater ecosystems that has caused the greatest amount of interest and concern in recent years.

As anthropological eutrophication escalates and land management practices continue to reduce natural environmental buffer systems, cyanobacteria are an increasingly common yet problematic component of freshwater systems. Excellent competitors at high nutrient levels, they can rapidly form extensive blooms consisting often of a nearly unialgal assemblage, directly causing a number of aesthetic and ecologically significant problems. First, cyanobacteria tend to be inedible or noxious to other aquatic organisms and therefore can cause a shift in established fish communities from a sport to a forage fishery. Second, as blooms senesce they contribute to large-scale hypoxia/anoxia as cells lyse and are degraded, often leading to extensive fish kills and aesthetically unpleasant odors. Thirdly, bloom events can negatively impact the aquatic environment by shading out (blocking sunlight) submerged aquatic vegetation which provide critical habitat to inmature developing animals. Finally, many cyanobacteria naturally produce secondary toxic metabolites that can adversely affect aquatic ecosystems and, potentially, impact human health.

One of the primary concerns regarding cyanobacteria stems from their production and release of cyanotoxins directly into the aquatic environment. Cyanotoxins are a class of potentially harmful chemical compounds typically released during bloom degradation. While over 40 species have been documented to release toxic compounds, the most common genera are species or strains of *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Planktothrix* and *Microcystis*. These toxins are typically differentiated into four major classes based on tissue or organ specificity: hepatotoxins, neurotoxins, dermatotoxins and skin irritants.

The most common types of cyanotoxins reported are the hepatotoxic microcystins (MC), which tend to dominate most freshwaters. MC are produced primarily by species of *Microcystis*, *Anabaena*, *Anabaenopsis*, *Planktothrix* and *Nostoc* and exposure to these compounds can lead to liver toxicity. Microcystins are considered to be tumor promoting compounds (IARQ, 2006). Cylindrospermopsin (CYN), produced by species of *Cylindrospermopsis*, *Aphanizomenon*, and *Umezakia*, predominatly affects the liver and the kidney but has also been shown to interefere with the spleen, the thymus and the heart (Hawkins et al, 1985, 1997). CYN is genotoxic and may also be associated with tumor formation (Humpage et. al, 2000; Falconer and Humpage, 2001). The neurotoxins, anatoxin-a and the saxitoxins, are fast acting toxic compounds produced by species of *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis* and, possibly, by *Lyngbya*. These toxins can be acutely toxic and can cause respiratory failure (Fitzgeorge et al., 1994; Fawell and James, 1994; IPCS, 1984). Given the commonality of eutrophic surface water systems in Florida, coupled with the potential stability of these compounds under normal environmental conditions and the extensive recreational use of these systems, it is essential to monitor for cyanotoxin levels. At present, however, there are no federal or state guidelines or regulatory standards for these compounds.

Based on our monitoring in 2005, a positive correlation between elevated cyanotoxin levels and the presence of associated potentially toxic cyanobacteria (e.g., *M. aeruginosa*, *Anabaena* spp.) did exist. These toxic levels were not confined to a single sampling site nor date, clearly indicating potential ecosystem-wide effects from cyanobacterial blooms. However, the presence of potentially toxic cyanobacteria and elevated cyanotoxin levels was not always correlated (i.e., Lake Jesup during July-September showed moderate microcystin levels without *Microcystis* or *Anabaena* present), so it is not always possible to select monitoring sites based merely on visible algal blooms.

Last year the St. Johns River experienced an extensive *M. aeruginosa* bloom, as did the St. Lucie and the Caloosahatchee Rivers, with microcystin toxins accounting for the majority of cyanotoxins. Microcystin concentrations in excess of 1000 ug/l were reported. However, even within these blooms, other cyanobacteria were detected, including *Cylindrospermopsis* and *Anabaena*, and the co-occurrence of MC with CYN was observed. These blooms, however, exhibited a great degree of temporal and spatial heterogeneity. The causes for such variations are still unclear (although weather and nutrient input are primary factors) but may significantly influence frequency, duration, and the magnitude of toxigenic bloom events. Thus, the purpose of this study was to monitor cyanobacteria and cyanotoxin levels in the St. Johns River and to develop a long-term database to document and better understand the formation and severity of toxigenic bloom events as it relates to and may impact human health.

METHODS

Sample sites

Sample sites were pre-determined and consisted of Doctors Lake, the St. Johns River at the Shands Bridge, the St. Johns River at the Palatka Pier, Crescent Lake, Lake George, Little Lake Harris at Hickory Pt., Lake Monroe, Lake Jesup, and Lake Washington. Latitude and longitudinal coordinates for all sample stations were the same as 2005 are reported in Table 1 and can be viewed with its associated map (Fig. 1). Sample sites were pre-determined based upon recreational use, previous historical occurrence of toxigenic blooms, and potential for being used as a resource for drinking water production.

Sample Collection

Water/plankton collection:

Upon arrival at collection sites, the Geographical Positioning System (GPS, Magellan Map 330X) unit was used to record latitude and longitude of the specific site. Latitude and longitude were then recorded into the sample collection logbook. The date and time when water samples were also collected were recorded. This collection time was used as a sample identification number.

Water samples were collected by using a horizontal Van Dorn (2.2 L) water sampler or by directly immersing sample containers in ambient water. All sample bottles were rinsed with ambient water prior to collection. Samples collected from the mainstem of the northern portion of the St. Johns River (Doctors Lake, Eagle Pt., Palatka Pier, Crescent Lake, Lake George and Lake Washington) were collected by personnel from the St. Johns River Water Management District (SJRWMD). Collections were made bi-weekly to weekly from May-November 2006 at selected sites that are routinely monitored by the Lower St. Johns River Basin Project. Water samples from the southern portion of the St. Johns River (Little Lake Harris, Lake Monroe, and Lake Jesup) were collected by GreenWater Laboratories/CyanoLab and were also sampled bi-weekly to weekly but directly from the shoreline. Once a month, water samples were received from the Upper St. Johns River Basin project/SJRWMD for Lake Washington. All SJRWMD sampled sites have concurrent water chemistry analyses pending.

In general, a total of 1-2 liters per sample site were collected. Collection bottles were stored in coolers under cool conditions (blue ice packs) to ensure that algae in samples remain alive and at low metabolic states until they could be properly processed and/or preserved. Bottles were properly vented, as needed, to maintain algal health. Upon receipt/return of samples to GreenWater Laboratories/CyanoLab, algae were sub-sampled, preserved and the remaining volume stored at $4^{o}C$ for toxin analyses.

Algal Analyses

Raw water sample preservation

Samples were mixed to evenly distribute phytoplankton cells. A 100 mL aliquot was removed and poured into a 125 mL amber polyethylene bottle and preserved with Lugol's solution (0.05-1.0% by volume depending on cell density). Bottles were properly labeled with date, time, sample site, and project identification.

Sample preparation

For each sample to be analyzed, a cleaned Utermöhl counting chamber was constructed. Depending on the phytoplankton density of the sample a settling tower of 5, 10 or 25 mL was used. The tower was secured to the base using a thin film of high vacuum grease. The Lugol's preserved sample was shaken for 20 seconds to evenly distribute phytoplankton cells and the appropriate volume added to the settling tower. A cover glass was placed on top of the tower and the sample allowed to settle in the dark in a vibration-free location. Minimum settling times were between 17 hrs for 5 mL samples, 34 hrs for 10 mL samples and 74 hrs for 25 mL samples. After settling was complete, the tower was slid from the base of the counting chamber to remove overlying water and the chamber covered with a second cover glass. The sample was then ready for microscopic analysis. The date and time of each sample preparation was recorded on the Chain of Custody Form.

Enumeration

Enumeration was performed on a Nikon Eclipse TE200 inverted microscope equipped with phase contrast optics. One ocular was fitted with a whipple disc and used to define the area of the fields to be counted. Specimens that went beyond the left and bottom edges of the whipple grid were not counted.

Before beginning enumeration the slide was scanned at low power to ensure that distribution of cells was relatively uniform. Only intact, viable cells were counted. Counts were made as natural units (cells, filaments, colonies) per a given volume of water.

The goal was to count a total of 400-600 cyanobacterial natural units per sample. A minimum of 10 and a maximum of 50 fields were counted at both 400X and 200X. An additional scan of the entire slide was made at 100X to count large and/or rare taxa. A maximum of 100 fields combined at 200X and 400X was performed on a given sample. Date and time of completion of enumeration was recorded on the Chain of Custody Form.

Data analysis

All counts were performed on counting bench sheets containing the following information: sample ID number, sampling date and location, date of analysis, start and finish times, magnification used, concentration/dilution factor, number of fields counted, species name and number of cells counted. Data was then entered into a spreadsheet that calculated natural units/mL. To calculate cells/mL the average number of cells for colonies and filaments were determined and the units/mL multiplied by these values. Date and time of initial data entry and data check was recorded on the Chain of Custody Form.

Toxin Analyses

Microcystins

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

Anatoxin-a and Cylindrospermopsin

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

RESULTS

Potentially Toxigenic (PTOX) Cyanobacteria

See Figures 2-11 for the monthly estimation of the total number of PTOX (*Microcystis*, *Cylindrospermopsis*, *Anabaena*, *Aphanizomenon*, *Planktothrix*, *Oscillatoria*, and *Snowella*) for each sample site.

See Figures 12-20 for the monthly estimation of the three main genera of PTOX (*Microcystis*, *Anabaena*, and *Cylindrospermopsis*).

Cyanotoxins

Figures 21-22 provide the levels of MC and CYN, respectively, for each site sampled on a monthly basis. Anatoxin-a was not identified in any water sample collected during this time period in any of the lakes sampled

CONCLUSIONS/RECOMMENDATIONS

This is the second consecutive year (2005-2006) that recreational water sites on the St. Johns River were monitored for PTOX cyanobacteria and cyanotoxins. This project is a strong start in developing a comprehensive database that will lead to the better understanding of bloom formation, toxin production dynamics, and assessing the risks involved to human health that might occur during different types (swimming, boating, jet skiing, sun bathing, fishing, etc…) of exposure. The documentation, identification and quantification of toxic algal bloom events is a major advancement from the normal anecdotal descriptions of historical animal mortalities. Even though monitoring, in general, is recognized as a vital strategy at minimizing the potential risks associated with human exposure and for better understanding freshwater toxigenic algal blooms a scarcity of such information exists.

In general, the St. Johns River is a slow moving eutrophic water system that maintains a strong population of potentially toxigenic cyanobacteria throughout the late spring (May) and continuing into the early fall months (October/November) in its freshwater regions. Data collected during the sampling period (May-October) of this project shows that the concentration of total number of potentially toxigenic cyanobacteria increased to levels greater than 20,000 cells/ml (WHO Alert Level 1) and 100,000 cells/ml. (WHO Alert Level 2) in 68% and 36% of all water samples collected, respectively. Population densities (Figs. 2-11) were, however, site specific and not all sites were shown to maintain high concentration levels (> 20,000 cells/ml.). For example, Lake Jesup exhibited levels of greater than 1,000,000 cells/ml from July through September, and never fell below 400,000 cells/ml from June - November (Fig. 9). Similar to last year, the Hickory Pt. site in Little Lake Harris, a permitted bathing water location, exhibited concentration levels of 60,000 cells/ml. or greater throughout the sampling period, with counts approaching 730,000 cells/ml in November (Fig. 11). The SAV CRL20 (Crescent Lake, Fig. 6) site consistently had cells counts close to or greater than 100,000 cells/ml. from June - November. Conversely, the SJSR-16 site (the St. Johns River at the Shands Bridge) exhibited the least amount of cells/ml, never reaching more than 20,000 (Fig. 4). Last year (2005), the Shands Bridge site was an area of high production that exhibited PTOX levels approximating or greater than 100, 000 cells/ml. in 50% of the samples collected between June through September. However, all other sites were >20,000 at least on one sampling occasion, and most had >100,000 cells/ml at least once. These data indicate both the general trends and variability of the St. Johns River sites. Only through continued annual monitoring will a good estimation of how and why these population levels change and how they are associated with toxin production will occur. Such information will be a necessity in developing recreational guideline levels.

In 2006, unlike 2005, no large toxin producing *Microcystis* blooms were reported. The lack of significant, widespread algal blooms in 2006 may have been the result of drought-like conditions. While extensive runoff in 2005 swelled waterways with nutrients and freshwater, the lack of precipitation in 2006 deprived the River of non-point additions. Further, lack of rainfall and evaporation lowered the water level. The St. Johns River has a very shallow slope, thus is highly tidally influenced. As freshwater levels fall saltwater from the Atlantic flows back into the river, elevating salinity well past Palatka. Freshwater cyanobacterial blooms are restricted by elevated salinity levels that may have served to reduce cyanotoxin levels by diminishing freshwater cyanobacterial growth. The St. Johns River does consistently experience large toxigenic blooms (normally *Microcystis* or *Anabaena*) with the last events being reported in 1999 and 2000.

Furthermore, the presence of species of *Anabaena* early in the bloom season (May-October) may have influenced the development of populations of *Microcystis* later in the year (August-October). In 2005, large blooms of *M. aeruginosa* formed in the St. Johns, the St. Lucie, and the Calooshatchee Rivers simultaneously during the month of August. Predictions for 2006, were that bloom events during this year would be as significant or greater than those events that occurred in 2005 (Hendrickson,SJRWMD: personal communication). Large surface blooms never materialized in the St. Johns River and no reports were reported from either the St. Lucie or the Caloosahatchee River as well. If species of *Anabaena* exhausted or diminished nutrient levels early and a lack of rainfall prevented/inhibited new sources of nutrient input to the St. Johns River than species of *Microcystis* may not have had enough nutrition to initiate bloom formation.

As indicated by the presence of cyanotoxins throughout the sampling period, bloom formation is not a prerequisite for toxin production. *C. raciborksii*, unlike *Microcystis* spp., is not a surface floater and therefore is more difficult to recognize as being in a bloom state. Only through consistent monitoring and analyses can high concentrations of either the algae or toxins be identified. Furthermore, *C. raciborksii* can maintain significant population levels throughout the water column, as it is tolerant to low light levels. Because of this ability to utilize the entire water column, this species can produce and maintain extremely high biomass that is unrecognizable by the common recreational user. Higher total biomass could equate to higher toxin production. Under certain environmental conditions, namely calm, static and drought conditions, toxin production could be greater at depth than at the surface further emphasizing the need to monitor both surface and deeper waters.

One again, Lake Jesup water samples contained CYN throughout the sampling period (May-October). The effects that CYN has on the ecology and health of this lake is presently not well understood. In an attempt to initiate such investigations, the Invasive Aquatic Plant Management Program at the Florida Department of Environmental Protection, PhycoTech, Inc. and GreenWater Laboratories are currently collaborating on a project to describe *C. raciborskii* population levels and its potential relationships with morphological compositions (curled vs. straight) of this same species, zooplankton composition, toxin production (surface and at depth), and the effects of toxin on different life history stages of fish. This project should be completed by December of 2006 and data analyzed and finalized by June of 2007.

In 2006, MC were found to be present throughout the sampling period and at all sampling sites, except Doctors Lake, at some point in time during the sampling period. Although high concentrations were not reported (> 5 ug/L, WHO Alert Level 1), as they were in 2005, MC did exhibit the highest levels and highest prevalence of any of the cyanotoxins identified. The lack of MC at Doctors Lake is intriguing as this water system has consistently experienced dense toxic *Microcystis* blooms in previous years. Doctors Lake is a relatively large embayment of the St. Johns River and the lack of MC may be more of a result of sampling in the wrong place at the wrong time than lack of toxin production (sampling is performed by the SJRWMD in a relatively large vessel that cannot adequately sample in-shore populations where cyanobacteria tend to accumulate). It should also be noted that Lake Washington was reported to have MC concentrations close to 1 ug/L and is used as a raw water source for the production of drinking water. Lake Washington is, most of the time, an oligotrophic water system not plagued by high levels of PTOX cyanobacteria. Again, these data indicate the need for consistent monitoring.

Anatoxin-a was not reported at any of these sampling sites in either 2005 or 2006 and therefore is considered less of a priority compound than either the microcystins or cylindrospermopsin. It was, however, identified in a recreational lake in nearby Alachua county, Lake Wauberg, during the summer of 2006 that is used and maintained as a recreational site for the University of Florida. The lack of production of this compound in our study when *Anabaena* levels were, at times, significant (> 100,000 cells/ml) indicates the lack of knowledge we have concerning what initiates toxin production. In general, however, high concentrations (> 100,000 cells/ml) were reported early in the year (May) when water temperatures were low and may have inhibited toxin formation. A caveat to this observation is that Crescent Lake maintained a very strong population of *Anabaena* spp. throughout the year and still was not shown to have antoxin-a present. It should be noted, however, that *A. circinalis*, the blue-green algae most responsible for anatoxin-a production, was only reported at potentially high levels in the month of September. Anatoxin-a is a potent neurotoxin that can cause illness, possibly respiratory failure, rapidly (minutes to hours) and therefore still needs to be monitored on a consistent basis. The last major *A. circinalis* bloom to occur in the St. Johns River was in 2000 when over 40 km of river was covered with a very thick (15-20 cm) bloom formation, anatoxin-a levels were measured at 156 ug/L and a major fish mortality event (predominantly adult menhaden, *Brevoortia tyrannus*) occurred in the Green Cove Springs-Jacksonville Landing region (epicenter of bloom event). Dying fish were not analyzed for toxins so a cause and effect relationship was not identified (although fish showed signs of neurotoxic behavior, personal observation).

Currently, no regulations or safe water guideline levels exist for the management (monitoring, testing, posting and/or closure) of surface waters in the USA for potentially toxigenic cyanobacteria and/or cyanotoxin content. The World Health Organization, however, does suggest concentration levels for the increased monitoring and warning of recreational users that bloom conditions exist and should be avoided due to an increase in the probability of human health risks. These levels are 20,000 cells/ml (alert level 1) and 100,000 cells/ml (alert level 2) which is approximately equivalent to 2-4 ug/L and 20 ug/L of microcystin-LR, respectively (Falconer et al., 1999). The country of Australia has recently developed recreational guidelines for adults and children of 45 ug/L and 15 ug/L for total cyanotoxins, respectively (Burch, 2005). The Aquatic Toxins program at the Florida Department of Health and the Public Health Technical Committee of the Florida Harmful Algal Bloom Task continues to address these issues and develop management strategies relevant to the state of Florida. In 2005, the first public health alert notice was issued by the Duval County Health Department for a toxin producing *Microcystis* bloom. During this same bloom event, the St. Johns River Water Management District sampled daily and issued weekly press releases to the general public on the distribution and toxin content of surface waters of the St. Johns River.

Furthermore, the Center of Disease Control (CDC), in August of 2006, did perform its first epidemiological study aimed at investigating the effects of recreational exposure to environmental MC. The results of this study are not yet finalized. Further studies by the CDC are planned. Also, the USEPA just concluded (January, 2007) a peer review of toxicity data for MC, CYN, and ANTX-a in an attempt to better identify reference dose concentrations for acute, short-term, sub-chronic and chronic oral exposures as well as potentially carcinogenic processes.

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Table 1. Sample Sites for Toxigenic Cyanobacterial Monitoring Project

Sample No.	Sample Site Name	Latitude	Longitude	Sampling Agency
1	Doctors Lake	30-06-35	81-44-47	LSJRB/SJRWMD
2	St. Johns River at Shands Bridge	29-58-25	81-37-25	LSJRB/SJRWMD
3	St. Johns River at the Palatka Pier	29-39-01.7	81-37-18.9	LSJRB/SJRWMD
4	Crescent Lake	29-30-12	81-30-15	LSJRB/SJRWMD
5	Lake George	29-22-42.3	81-39-03.8	LSJRB/SJRWMD
6	Little Lake Harris at Hickory Pt.	28-44.596	81-46.046	GWL
7	Lake Monroe	28-48.952	81-16.372	GWL
8	Lake Jesup	28-42.941	81.14.481	GWL
9	Lake Washington	28-08.825	80-44.043	USJRB-SJRWMD

LSJRB = Lower St. Johns River Basin
USJRB = Upper St. Johns River Basin
SJRWMD = St. Johns River Water Management District
GWL = GreenWater laboratories/CyanoLab

Figure 1. Map of Samples Sites

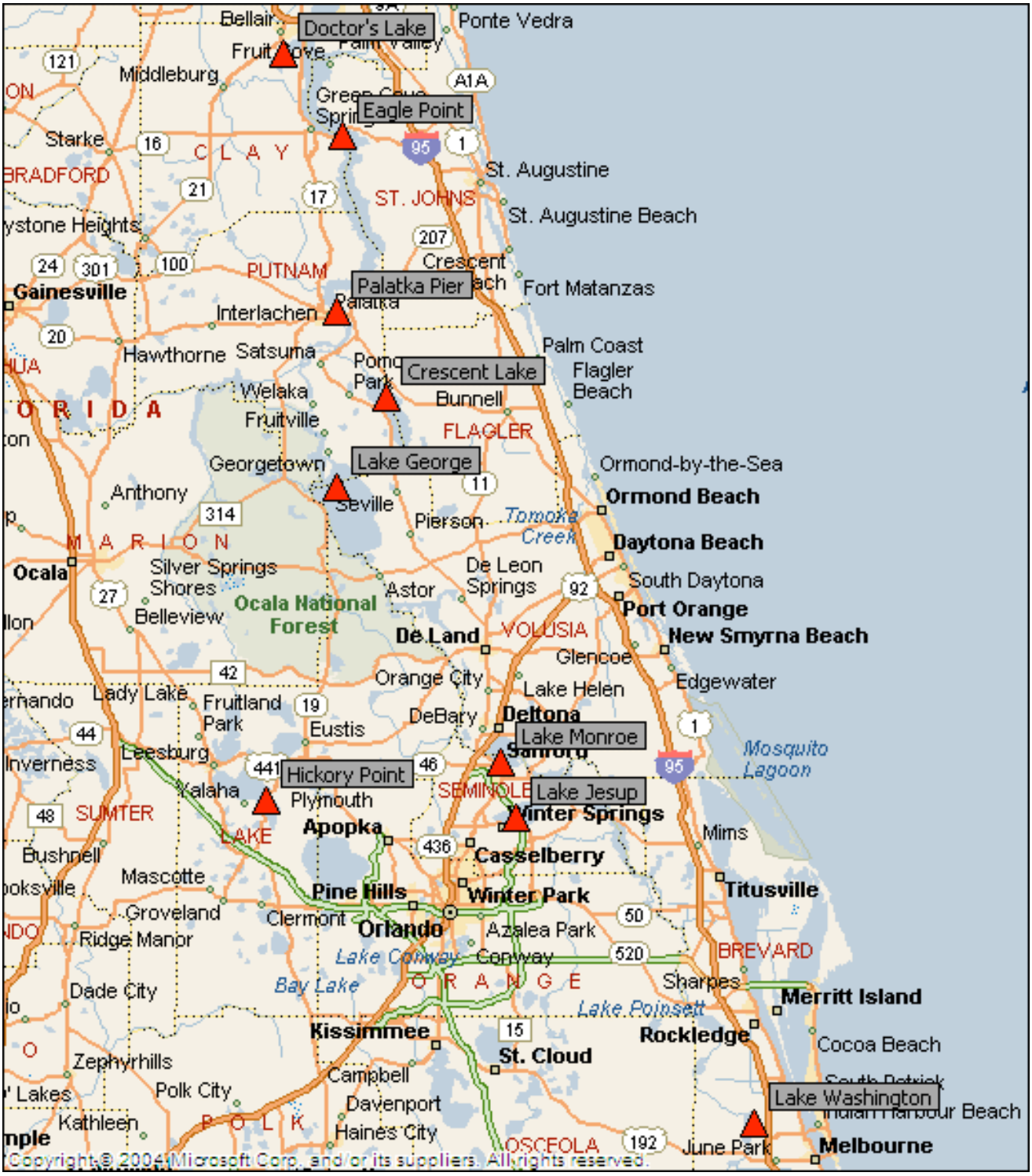

Figure 2: Population Estimates of Potentially Toxigenic Cyanobacteria for 2006

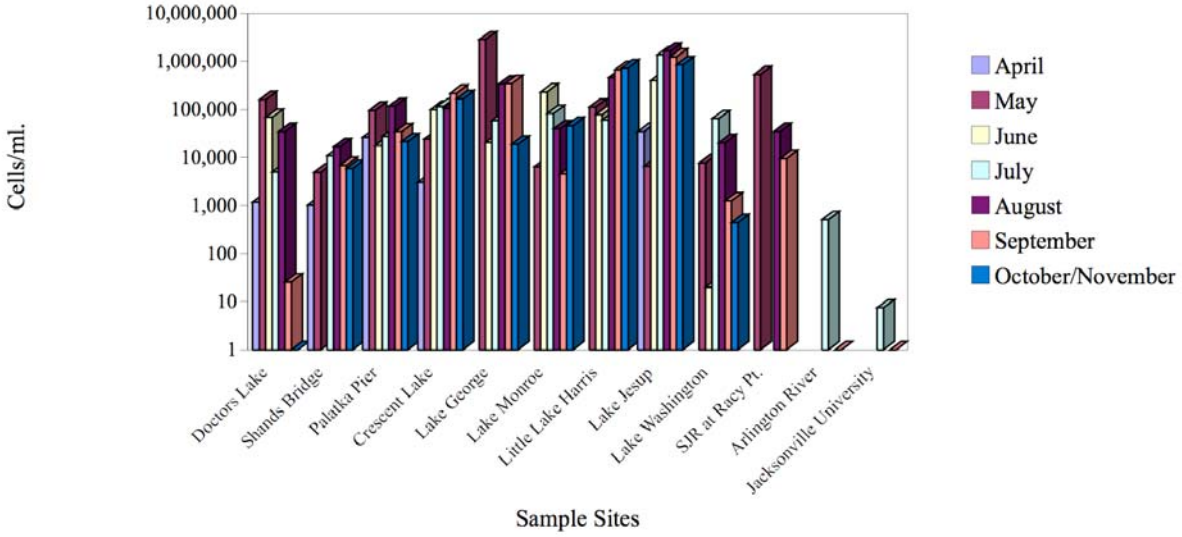

Figure 3: Population Estimates of PTOX Cyanobacteria in Doctors Lake, 2006

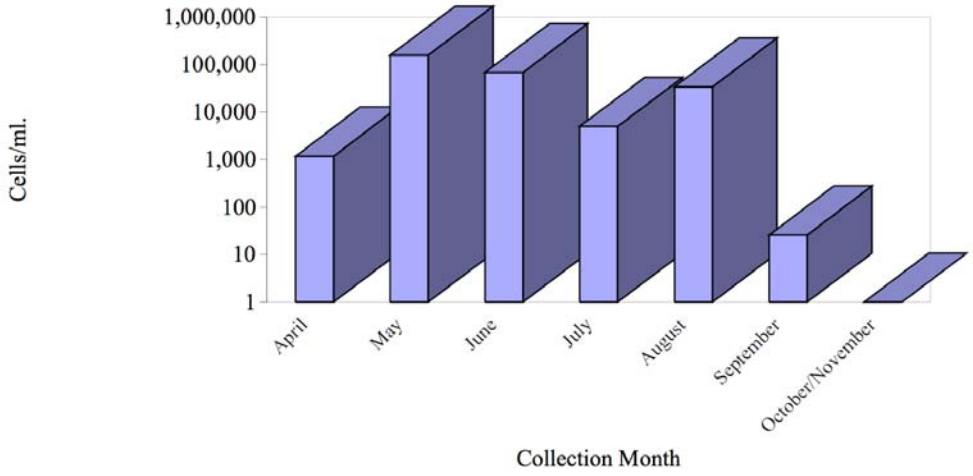

Figure 4: Population Estimates of PTOX Cyanobacteria at the Shands Bridge, 2006

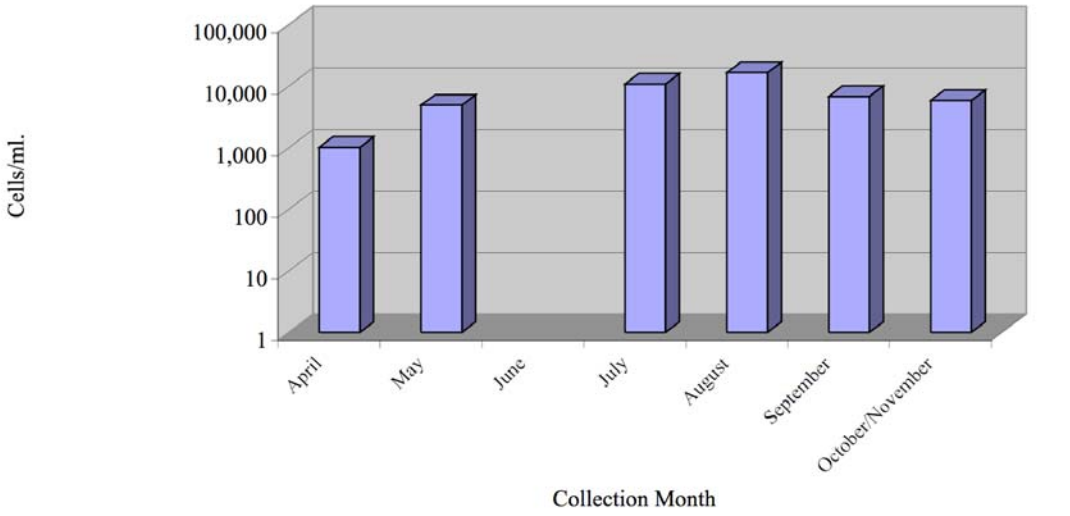

Figure 5: Population Estimates of PTOX Cyanobacteria at the Palatka Pier, 2006

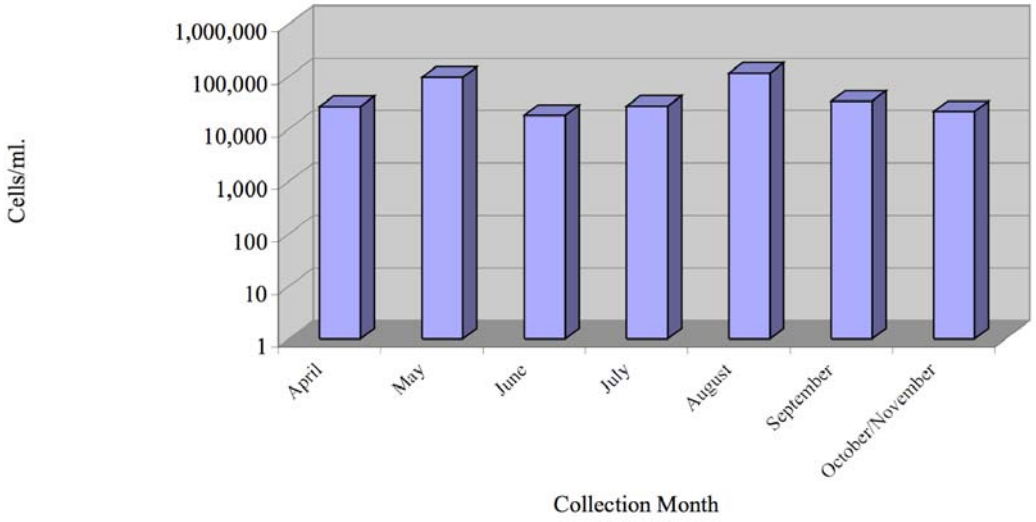

Figure 6: Population Estimates of PTOX Cyanobacteria in Crescent Lake, 2006

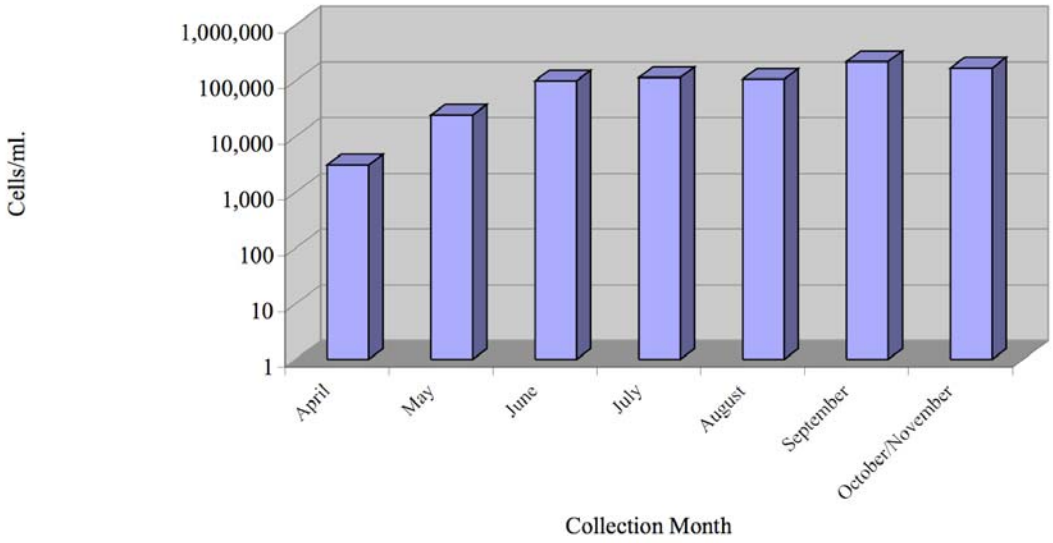

Figure 7: Population Estimates of PTOX Cyanobacteria in Lake George, 2006

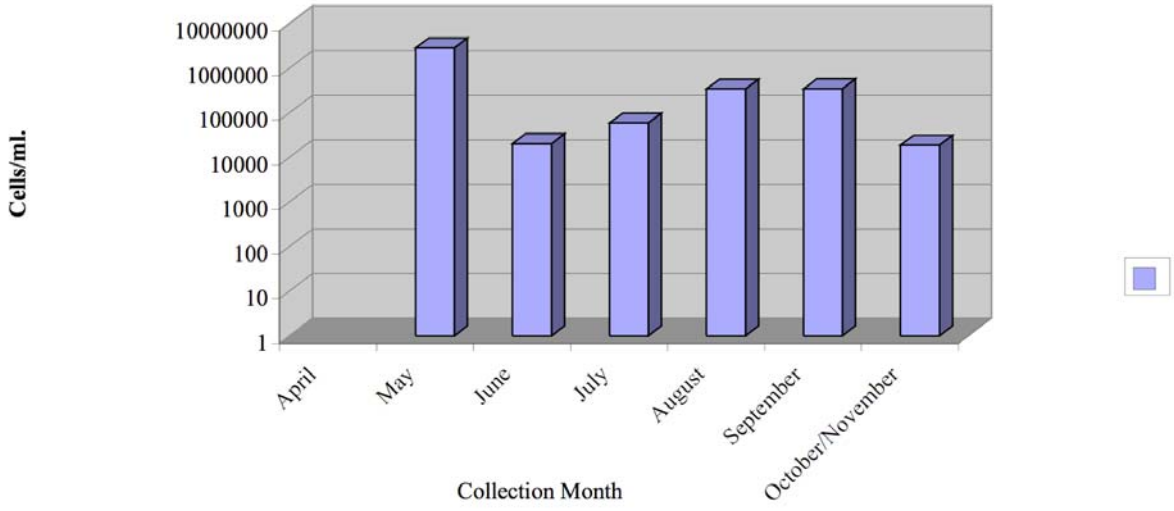

Figure 8: Population Estimates of PTOX Cyanobacteria in Lake Monroe, 2006

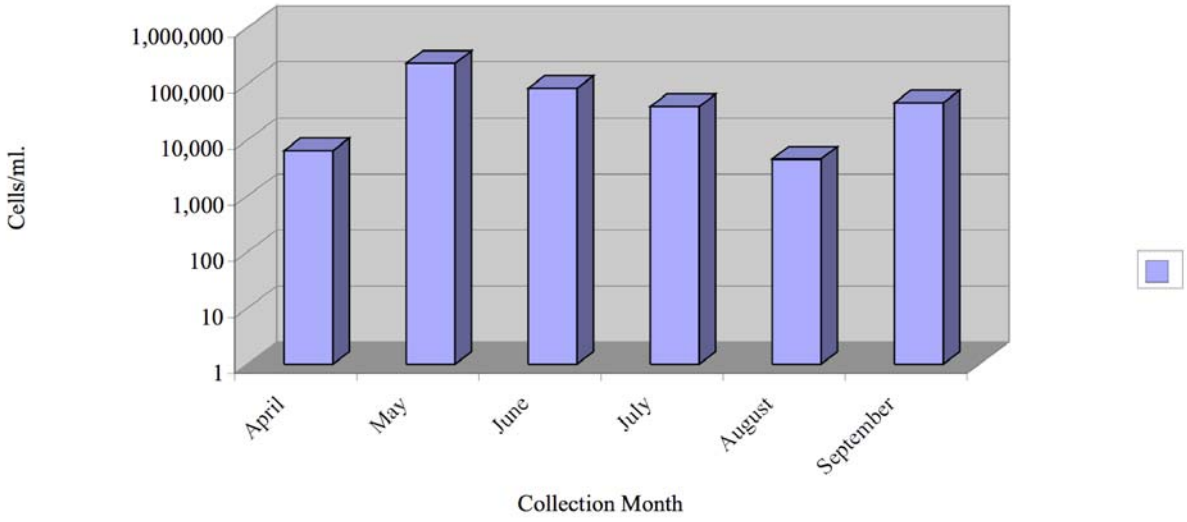

Figure 9: Population Estimates of PTOX Cyanobacteria in Lake Jesup, 2006

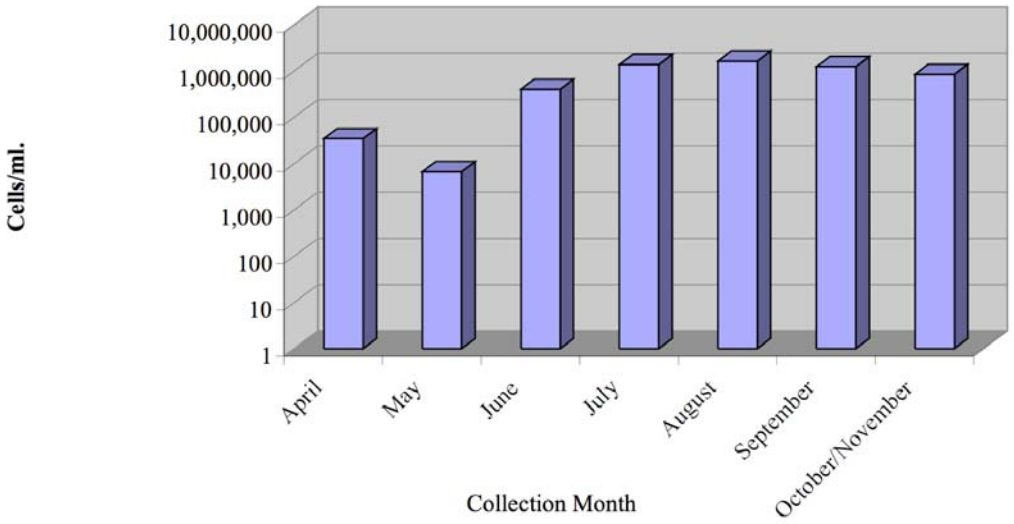

Figure 10: Population Estimates of PTOX Cyanobacteria in Lake Washington, 2006

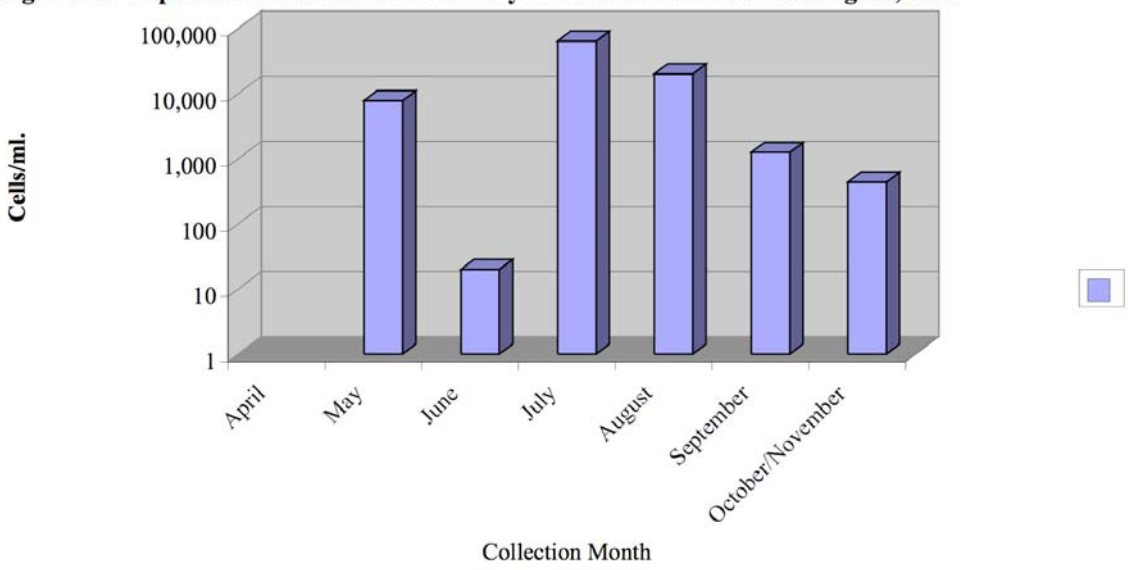

Figure 11: Population Estimates of PTOX Cyanobacteria in Little Lake Harris, 2006

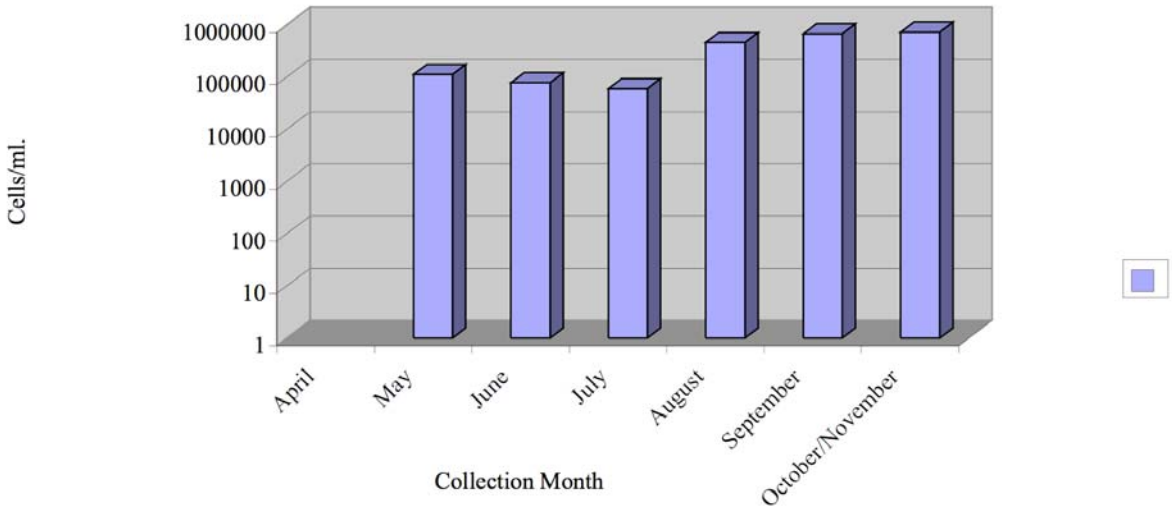

Figure 12: Population Densities of Major PTOX Cyanobacteria Species in Doctors Lake, 2006

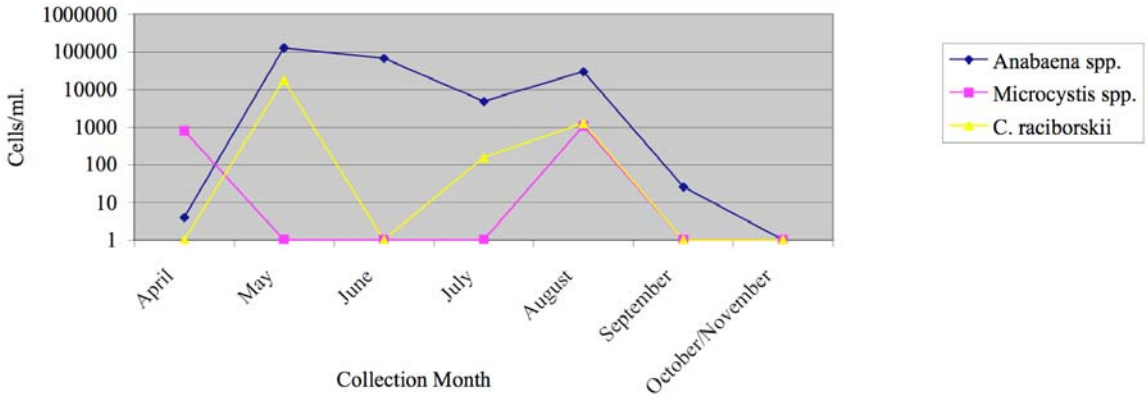

Figure 13: Population Densities of Major PTOX Cyanobacteria Species at the Shands Bridge, 2006

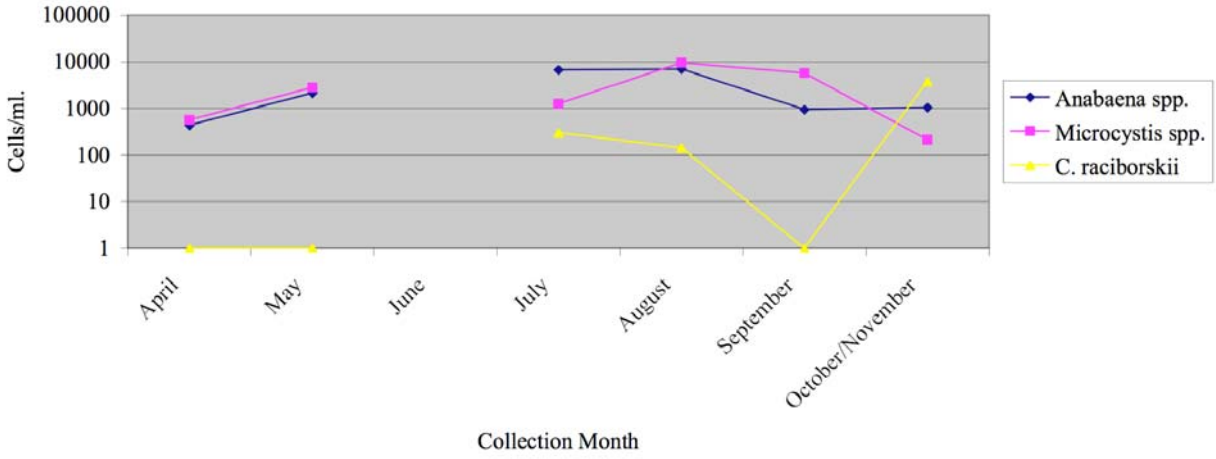

Figure 14: Population Densities of Major PTOX Cyanobacteria Species at the Palatka Pier, 2006

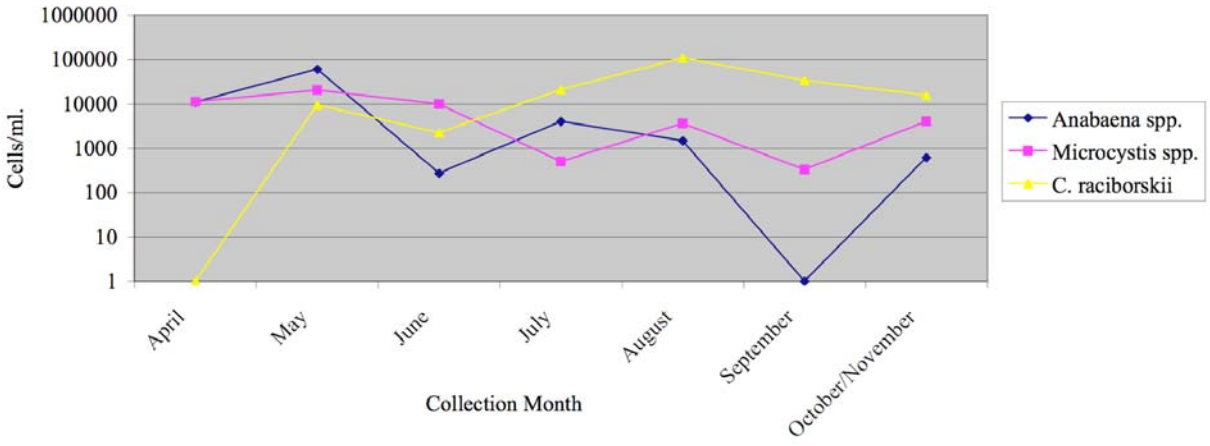

Figure 15: Population Densities of Major PTOX Cyanobacteria Species in Crescent Lake, 2006

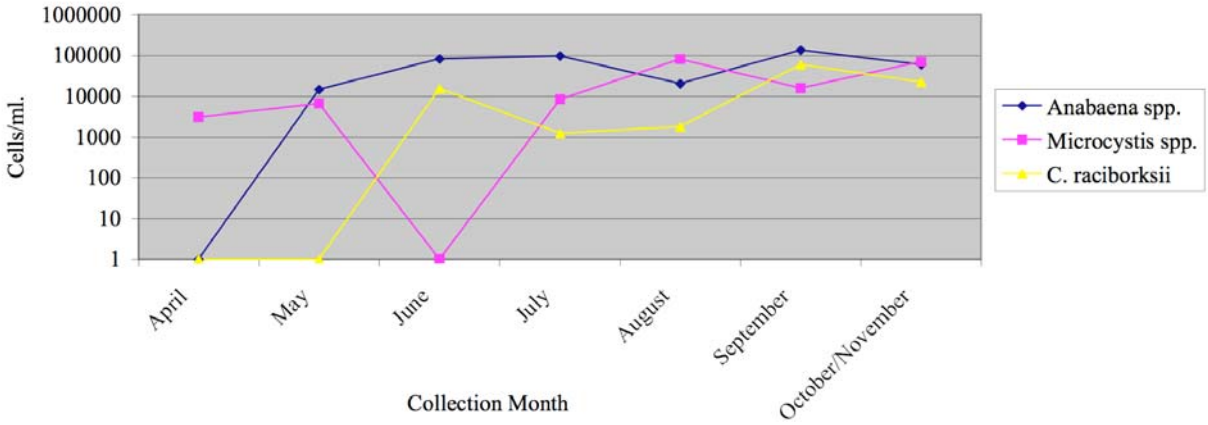

Figure 16: Population Densities of Major PTOX Cyanobacteria Species in Lake George, 2006

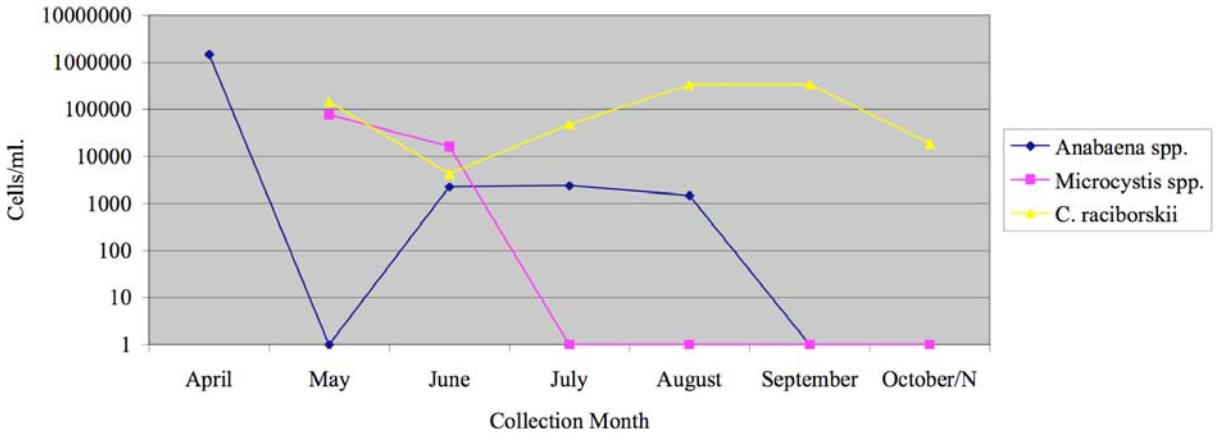

Figure 17: Population Densities of Major PTOX Cyanobacteria Species in Lake Monroe, 2006

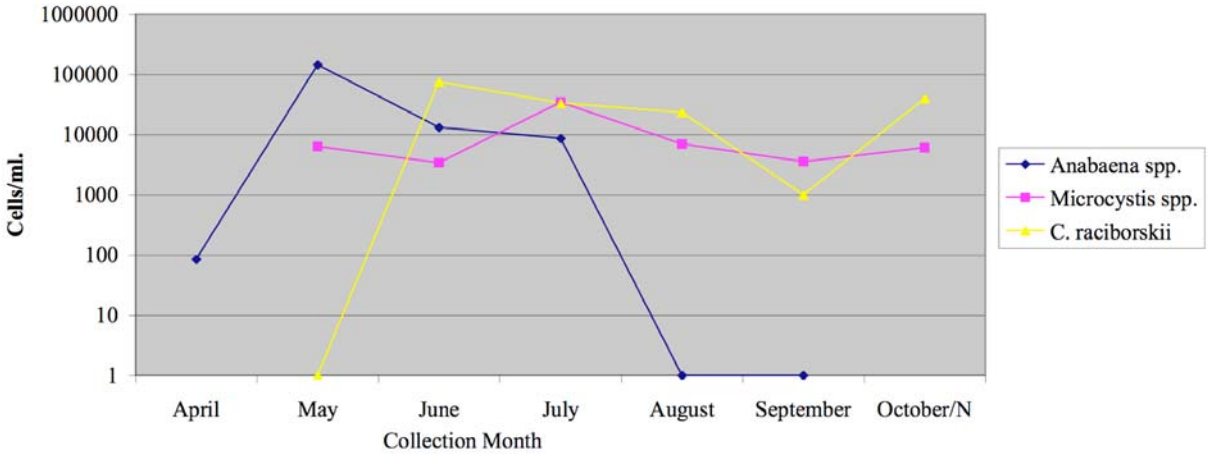

Figure 18: Population Densities of Major PTOX Cyanobacteria Species in Little Lake Harris, 2006

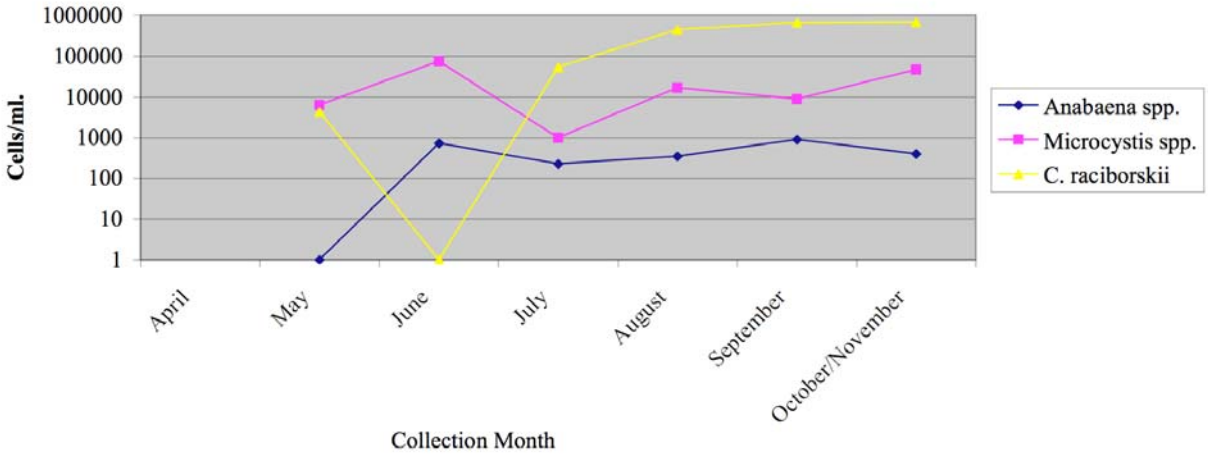

Figure 19: Population Densities of Major PTOX Cyanobacteria Species in Lake Jesup, 2006

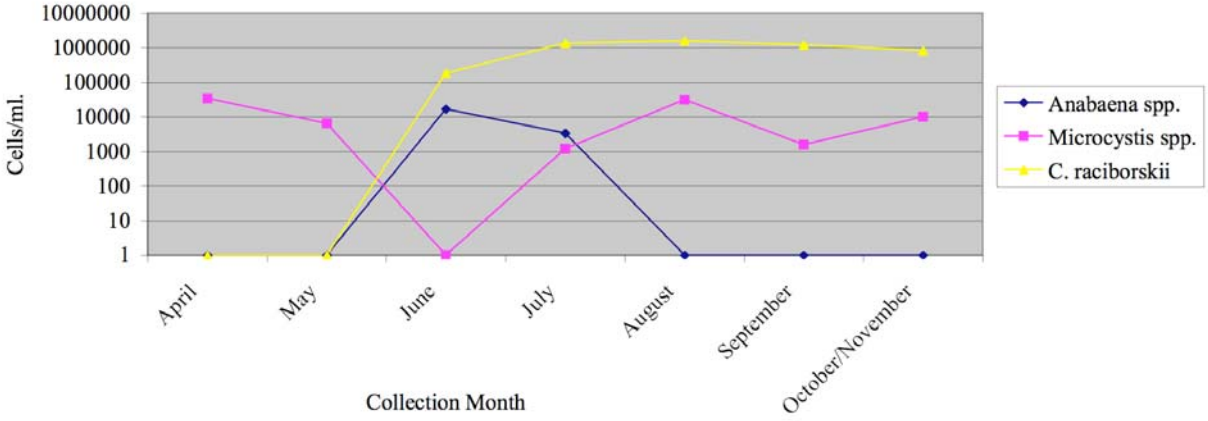

Figure 20: Population Densities of Major PTOX Cyanobacteria Species in Lake Washington, 2006

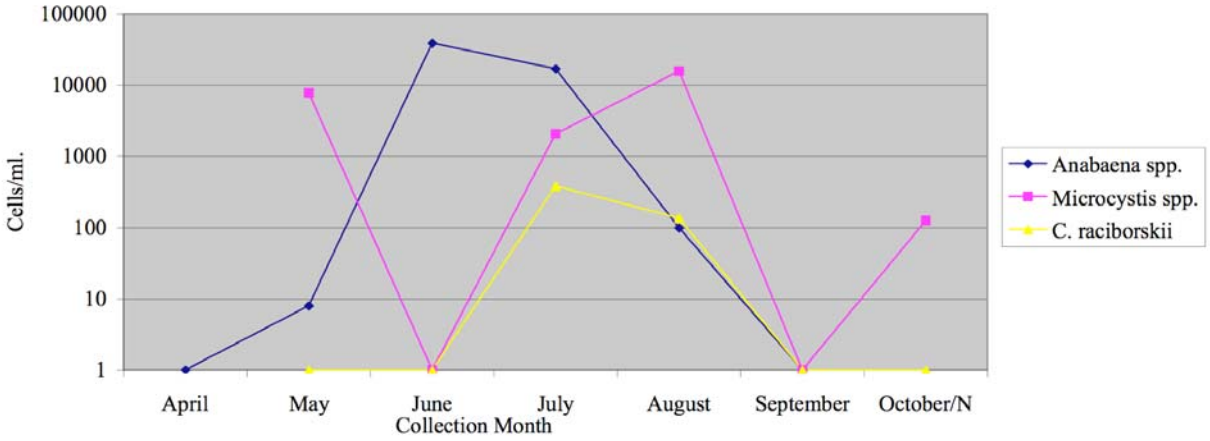

Figure 21: Environmental Microcystin Concentrations

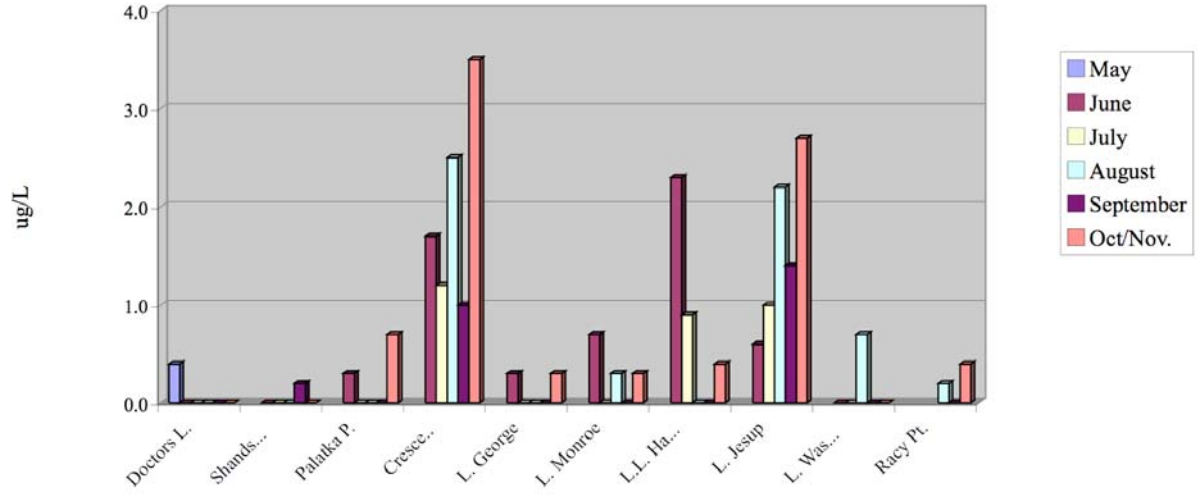

Figure 22: Environmental Cylindrospermopsin Concentrations