Toxin Producing Blue-Green Algae at Recreational Sites in the St. Johns River, Florida

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by

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INTRODUCTION

Cyanobacteria, or blue-green algae, are a group of oxygenic-photosynthetic bacteria containing chlorophyll *a* and accounting 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, cyanobacteria are an increasingly common component of freshwater systems, especially in southern, sub-tropical systems. Excellent competitors at high nutrient levels, they can rapidly form extensive blooms consisting often of a nearly unialgal assemblage, presenting a number of problems especially for potable waters. First, cyanobacteria tend to be inedible or noxious to other aquatic organisms. Second, as blooms senesce they contribute to large-scale anoxia as cells lyse and are degraded, often leading to extensive fish kills. Finally, many cyanobacteria produce secondary metabolites that can adversely impact aquatic ecosystems.

One of the primary concerns with cyanobacteria stems from the production of cyanotoxins. Cyanotoxins are a class of chemical compounds typically released during lysis of mature colonies. While over 40 species have been documented to release toxic compounds, the most common genera are species or strains of *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis* and *Microcystis*, with several new genera recently being confirmed as toxin producing. These toxins are typically differentiated into five major classes based on tissue or organ specificity: hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins.

Of these, the most common are hepatotoxic microcystins (MC), which tend to dominate most freshwaters. Produced primarily by *Microcystis aeruginosa*, several other genera including *Anabaena*, *Anabaenopsis*, *Planktothrix* and *Nostoc* have recently also been documented to produce these toxins which can lead to skin irritation, kidney problems and most significantly liver toxicity. Given the preponderance of eutrophic lentic and lotic water systems in Florida, coupled with the extensive recreation value of these systems, it is essential to monitor the cyanotoxin levels. However, at present there is no federal drinking water standard for these compounds. The purpose of this study was to monitor cyanotoxin levels in the St. Johns River.

METHODS

Sample sites

Sample sites were pre-determined and consisted of Doctors Lake, Eagle Point (Shands Bridge), the Palatka Pier, Crescent Lake, Lake George, Little Lake Harris at Hickory Pt., Lake Monroe, Lake Jesup, and Lake Washington. Geographical distribution of sample sites are shown in Figure 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.

Algal Analyses

Water samples were collected by using a horizontal Van Dorn (2.2 L) water sampler or by directly immersing sample containers in ambient water. Water samples that were analyzed for toxigenic cyanobacteria were preserved with 10% lugols solution and viewed microscopically using an inverted microscope equipped with phase contrast optics.

Toxin Analyses

Microcystins

Enzyme Linked Immunosorbent Assay (ELISA) was utilized for the determination of the concentration of total microcystins (MCs) present. Antibody-coated plates, standards, and all reagents were supplied by Abraxis LLC (Product No. 520011). The level of sensitivity for microcystin(s) using this method was approximately 0.15 μg/L.

Anatoxin-a and Cylindrospermopsin

A liquid chromatographic/mass spectrometric (LC/MS/MS) system was utilized for the identification and quantification of cylindrospermopsin (CYN) and anatoxin-a (ANTX-A) for all water samples analyzed.

RESULTS and OBSERVATIONS

Potentially Toxigenic Cyanobacteria

Currently, no guideline levels exist for the management (monitoring, testing, posting and/or closure) of surface waters for potentially toxigenic cyanobacteria and/or cyanotoxin content for water bodies that support recreational activities in the US. The World Health Organization, however, does suggest concentration levels for the increased monitoring and warning of recreational users that bloom conditions exist 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). 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 Florida Department of Health and the Public Health Technical Committee of the Florida Harmful Algal Bloom Task hopes to address these issues relevant to the state of Florida in the very near future.

In general, the St. Johns River maintains a strong population of potentially toxigenic cyanobacteria throughout the late spring (May) and continuing into the early fall months (October/November). Data collected during the sampling period (May-October) of this project shows that the concentration of potentially toxigenic cyanobacteria, on several occasions, increased to greater than 500,000 cells/ml (Table 1). Population densities were, however, site specific and not all sites were shown to maintain high concentration levels (> 20,000 cells/ml.). For example, the Hickory Pt. site in Little Lake Harris, a permitted bathing water location, exhibited concentration levels of greater than 100,000 cells/ml. throughout the sampling period (100%) while Lakes Washington and Monroe were never observed to have population levels above 100,000 cells/ml. and were only observed to have concentration levels above 20,000 cells/ml twice (Lake Monroe in mid/late May). Most water bodies showed typical "up and down" fluctuations in population levels as weather, nutrient input, and environmental conditions varied during the year, which emphasizes the need for continuous monitoring with short intervals between monitoring events. It should be noted, however, that patterns of occurrence are impossible to determine with such a limited time frame of monitoring and usually need multiple years (5-10) of frequency data before a true understanding can be extracted. Inter-annual variation in algal population dynamics and subsequently toxin production can be significant.

During the term of this project, species of *Microcystis* and *Cylindrospermopsis raciborskii* were the dominant toxin producers observed.

Cyanotoxins

Microcystins and cylindrospermopsin, but not anatoxin-a, were positively identified at samples sites in the St. Johns River. Figures 2 and 3 provide monthly levels of MC and CYN, respectively. In most cases, the presence of toxin was indicative of a significant level of associated cyanobacteria. One exception to this correlation is with the MC data found for Lake Jesup. Moderate levels (2-4 µg/L) of MC were determined for July-Sept. without significant levels (> 50,000 cells/mL) of either *Microcystis* or *Anabaena* (MC sources) present.

In September of 2005 a major *Microcystis* (primarily *M. aeruginosa*) bloom occurred in the St. Johns River between Jacksonville and Crescent City, Florida, a span of over 100 river miles. Microcystin concentrations ranged from non-detectable to over 1400 ug/L (70x greater than the World Health Organization alert level 2) indicating the patchiness of bloom events. Figure 3 shows the frequency of toxin levels reported for this event. This bloom event persisted from late August through the end of October and was concentrated on the eastern shore of the St. Johns River due to westerly winds. For the first time in the state of Florida, the St. Johns County Health Department issued a health advisory for the St. Johns River informing citizens to be aware of bloom scum formations and describing potential health risks. Also during this bloom event, the St. Johns River Water Management District sampled several times per week and issued weekly press releases to the media to alert the public as to where the bloom had concentrated and what stretches of the river may need to be avoided. Concurrent blooms of *M. aeruginosa* developed in the St. Lucie River, Lake Okeechobee, and the Caloosahatchee River. It should also be noted that cylindrospermopsin was positively identified at sample sites located within this bloom area and shows the possibility of multiple toxins being produced simultaneously. Figures 4 and 5 show bloom accumulations at Governors' Creek and Doctors Lake in the St. Johns River system.

MCs are a family of liver toxins and potentially can act as tumor promoting agents (Falconer, 1991). MCs can be produced by numerous species of *Microcystis* as well as species of *Planktothrix*, and *Anabaena*. The most widely observed genus in Florida is, by far, *Microcystis*. MCs have been shown to cause animal mortalities during bloom events (as reviewed in Ressom et al. 1994) and were the causative agent responsible for the death of three dogs last year in Minnesota (unpublished data, Steve Heiskary; Environmental Outcomes Division, Minnesota Pollution Control Agency).

As a rule, CYN is not observed nearly as often as MC in the St. Johns River and its basin and any detectable level (> 0.05 µg/L) is usually evidence for the presence of *Cylindrospermopsis*. As seen with MC, CYN was found more frequently during July-October. Lake Jesup had the highest (0.4 – 1.6 µg/L) levels of CYN (July-October) of any site monitored during this study. This data correlated well with the levels of *Cylindrospermopsis* observed during the same period . Palatka Pier , Lake Monroe, Lake George and Eagle Pt. were all found to have detectable levels (> 0.05 µg/L) of CYN present and significant levels (> 40,000 cells/mL) of *Cylindrospermopsis*. What was of particular interest was that Lake Jesup, with the highest CYN levels, did not have the highest *Cylindrospermopsis* cell count. Palatka Pier, Lake George, and Eagle Pt. all had cell counts higher than Lake Jesup and Drs. Lake not only had the highest *Cylindrospermopsis* cell count (> 375,000 cells/mL), but no detectable level of CYN.

CYN is produced mainly by *Cylindrospermopsis raciborskii*. CYN is classified as a hepatotoxin but can affect other tissues such as the kidneys, adrenal glands, lungs, and intestines (Hawkins et al., 1985). CYN has also been shown to be genotoxic (Humpage et al., 2000) and, potentially, can act as a tumor promoter similar to that of MCs (Falconer and Humpage, 2001).

The predominant cyanobacteria species responsible for the production of ANTX-A in northeast Florida is *Anabaena circinalis* but species of *Aphanizomenon* can also produce this compound. ANTX-A has been the least observed of the three toxins, but as a neurotoxin can be of greatest concern and has caused the only reported human death in the U.S. attributed to cyanotoxins. ANTX-A was not found at any detectable level (> 0.05 µg/L) for any of the sample sites included in this study, but species of *Anabaena* and other sources of ANTX-A continue to be documented. Palatka Pier , Lake George and Drs. Lake had *Anabaena* (primary ANTX-A source) cell counts > 50,000 cells/mL. This information, in addition to some of the *Cylindrospermopsis* data, reinforces the need for toxin monitoring as the only way of accurately determining the presence and level of cyanotoxins.

CONCLUSIONS/RECOMMENDATIONS

Multiple hurricane events in the state of Florida complicated normal seasonal variation in 2004. The significance of those events to the severe *Microcystis* blooms of 2005 is uncertain, but given the occurrence of *Microcystis* blooms throughout Florida in 2005 and the precipitation levels associated with the hurricanes; the increased run-off may have significantly increased nutrient loads and coupled with the heat of the summer primed the middle and lower St Johns River Basin for the large blooms and toxin levels observed.

As previously documented, populations of toxigenic cyanobacteria do persist in the St. Johns River. The effect that these toxins have on human health and the overall health of the river is not well understood. It is clear, based on the recent events, that the St. Johns River and its basin maintain the potential to experience significant toxin producing blooms when the necessary environmental conditions arise. A continuation of this sampling and monitoring study or something similar is recommended. If feasible, both an inshore and offshore sampling program should be implemented to cover the areas of greatest risk for human exposure. One way this might be performed is by the use of a public information system hotline that can accept phone calls from people who observe algal cell accumulations and instruct field personnel, for sampling purposes, as to where bloom events are occurring. GreenWater Laboratories does maintain a toll free number (1-877-TOX-ALGA) for such reports.

The identification and quantification of dominant MC variants (i.e. LR, RR, etc.), particularly with *Microcystis* blooms of the size observed in 2005, would be highly recommended. In addition to providing further data it would also help in evaluating the level of toxicity and potential risk levels due to differences in toxicity among the different MC isomers.

Production of β-N-methylamino-L-alanine (BMAA), a neurotoxic amino acid, has recently been associated with the majority of cyanobacteria genera. *Microcystis*, *Anabaena,* and *Cylindrospermopsis raciborskii* are certainly included in this group as well as any other cyanobacteria found in the St. Johns River and its basin. *Cylindrospermopsis raciborskii* actually produces some of the highest levels of BMAA. BMAA has been suggested as a possible cause of the amyotrophic lateral sclerosis/parkinsonism – dementia complex (ALS/PDC). BMAA was recently discovered in the brain tissues of nine Canadian Alzheimer's patients, but was not detected in the brain tissues of 14 other Canadians who died of causes unrelated to neurodegeneration. (Cox et al., 2005). With the increased interest in BMAA due to its potential global human exposure, some preliminary determinations of BMAA levels in the St. Johns River should be of interest.

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Figure 1: Cyanobacteria in Doctor's Lake (2005)

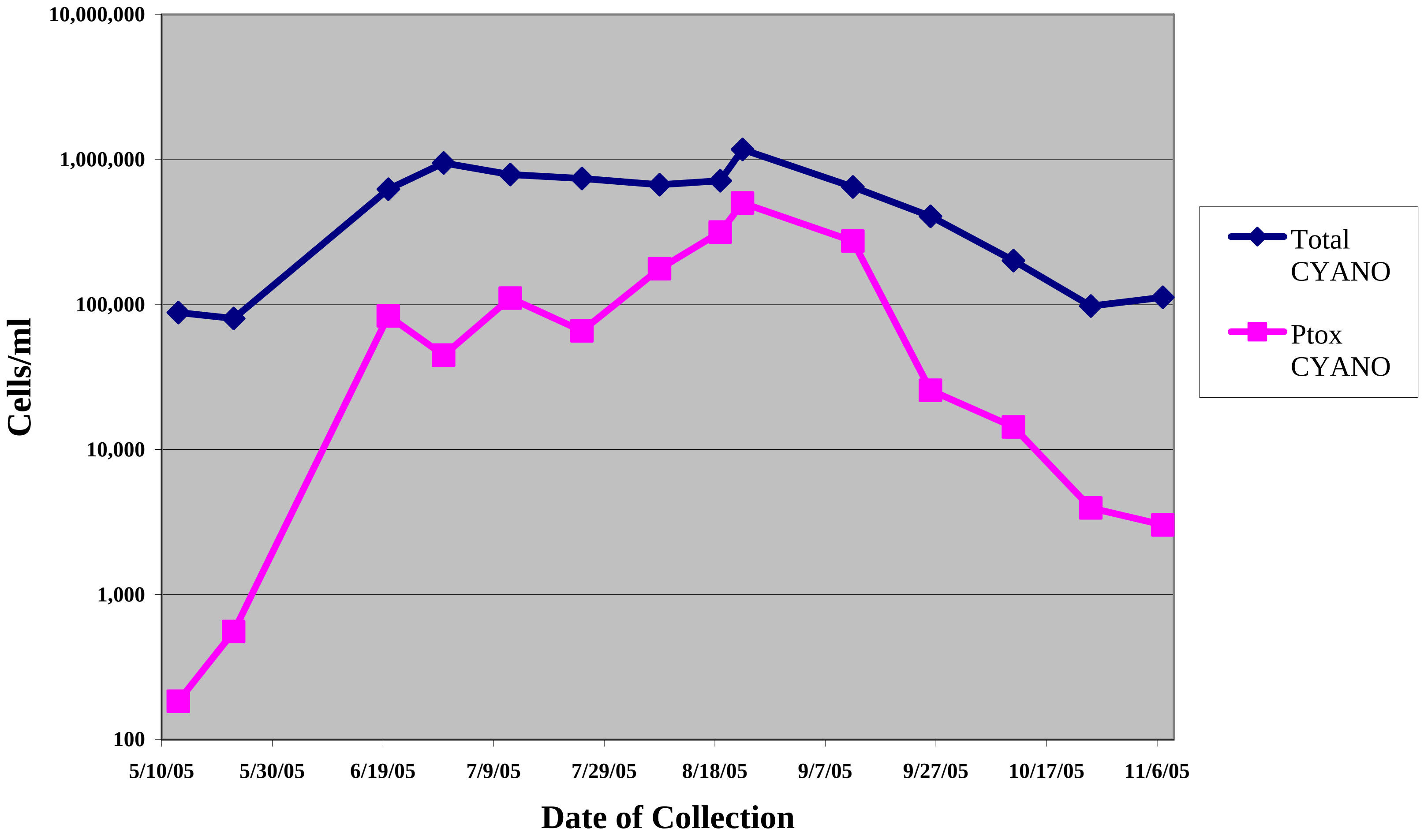


Figure 2: Cyanobacteria in Eagle Harbor (2005)

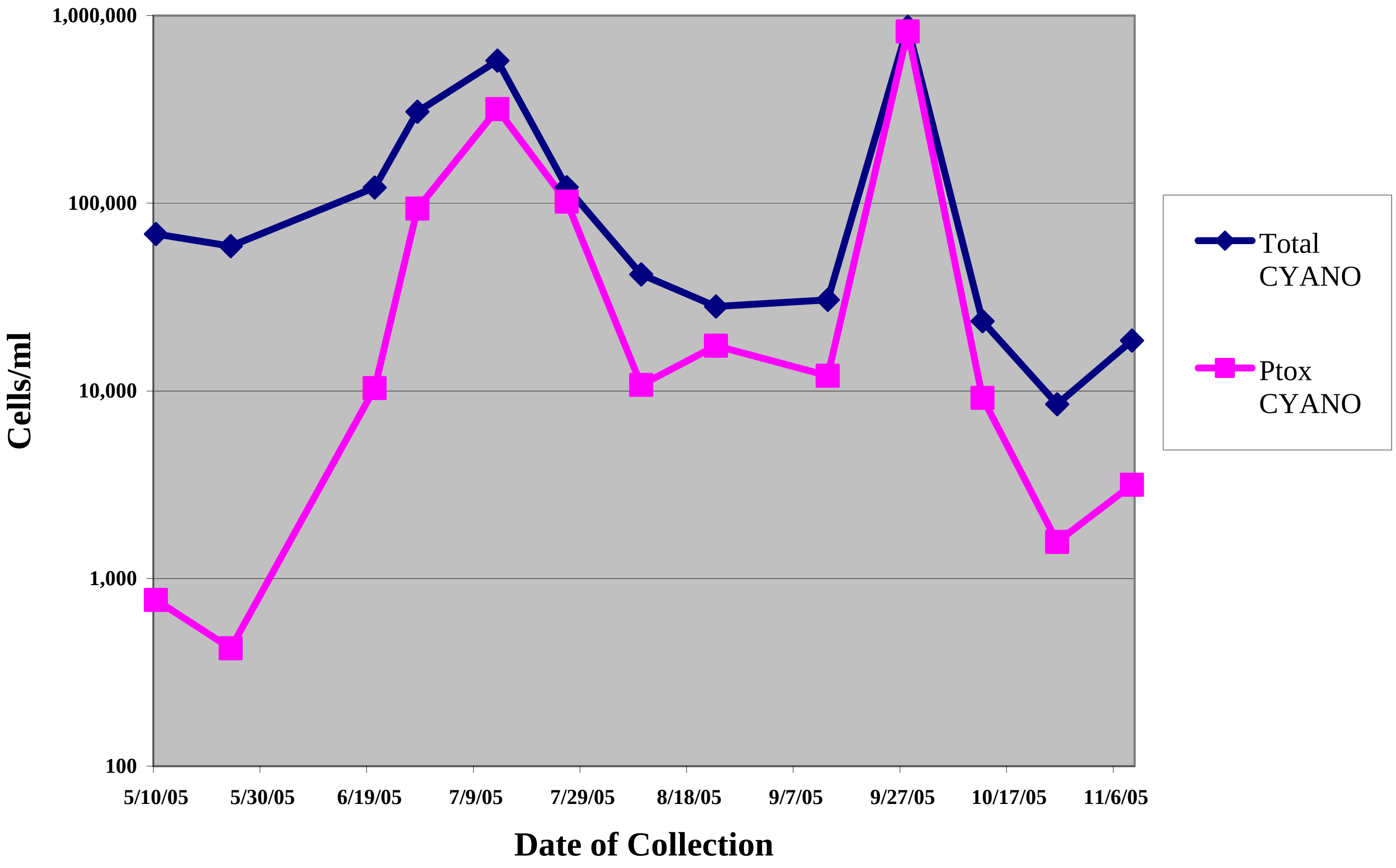


Figure 3: Cyanobacteria from Palatka Pier (2005)

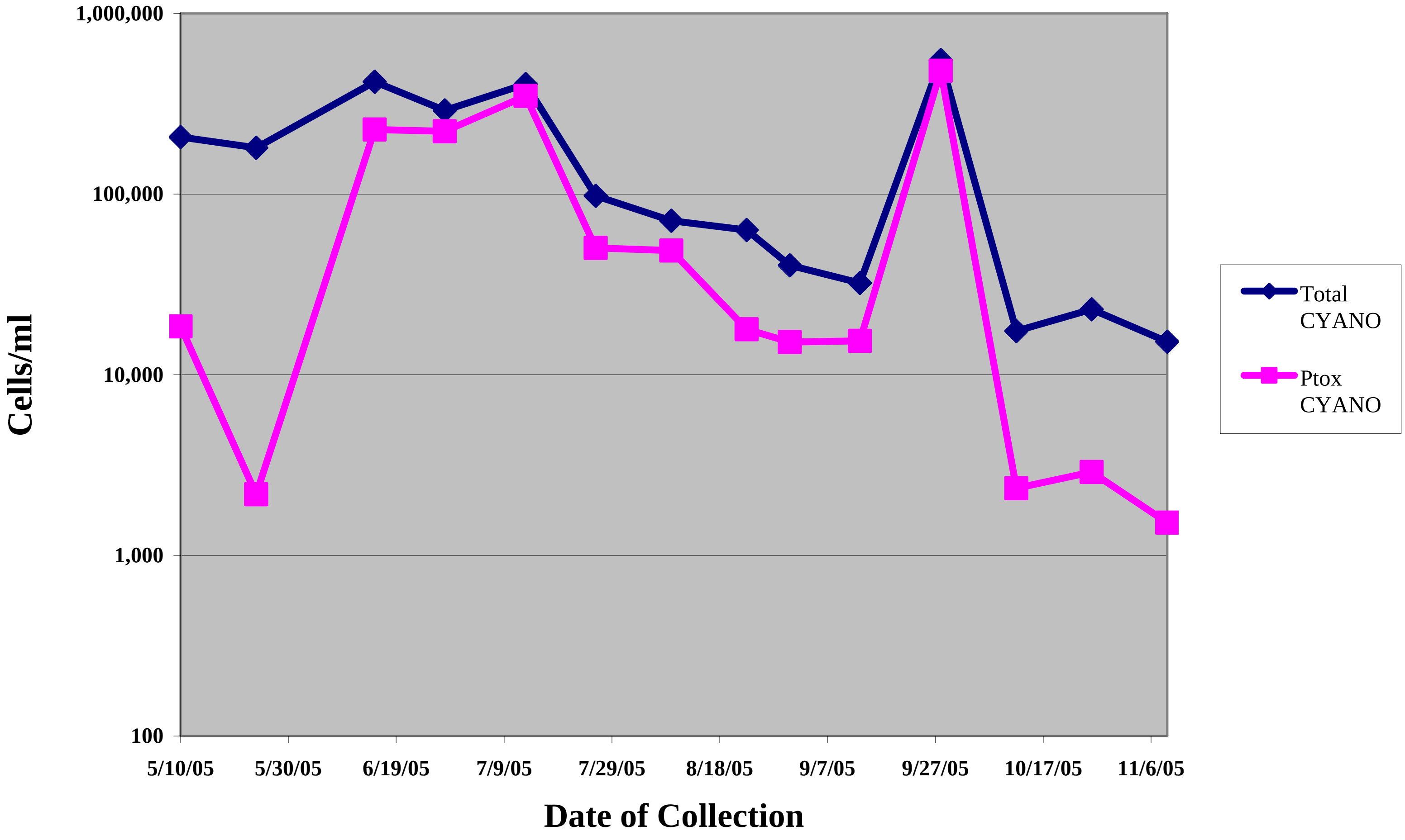


Figure 4: Cyanobacteria in Crescent Lake (2005)

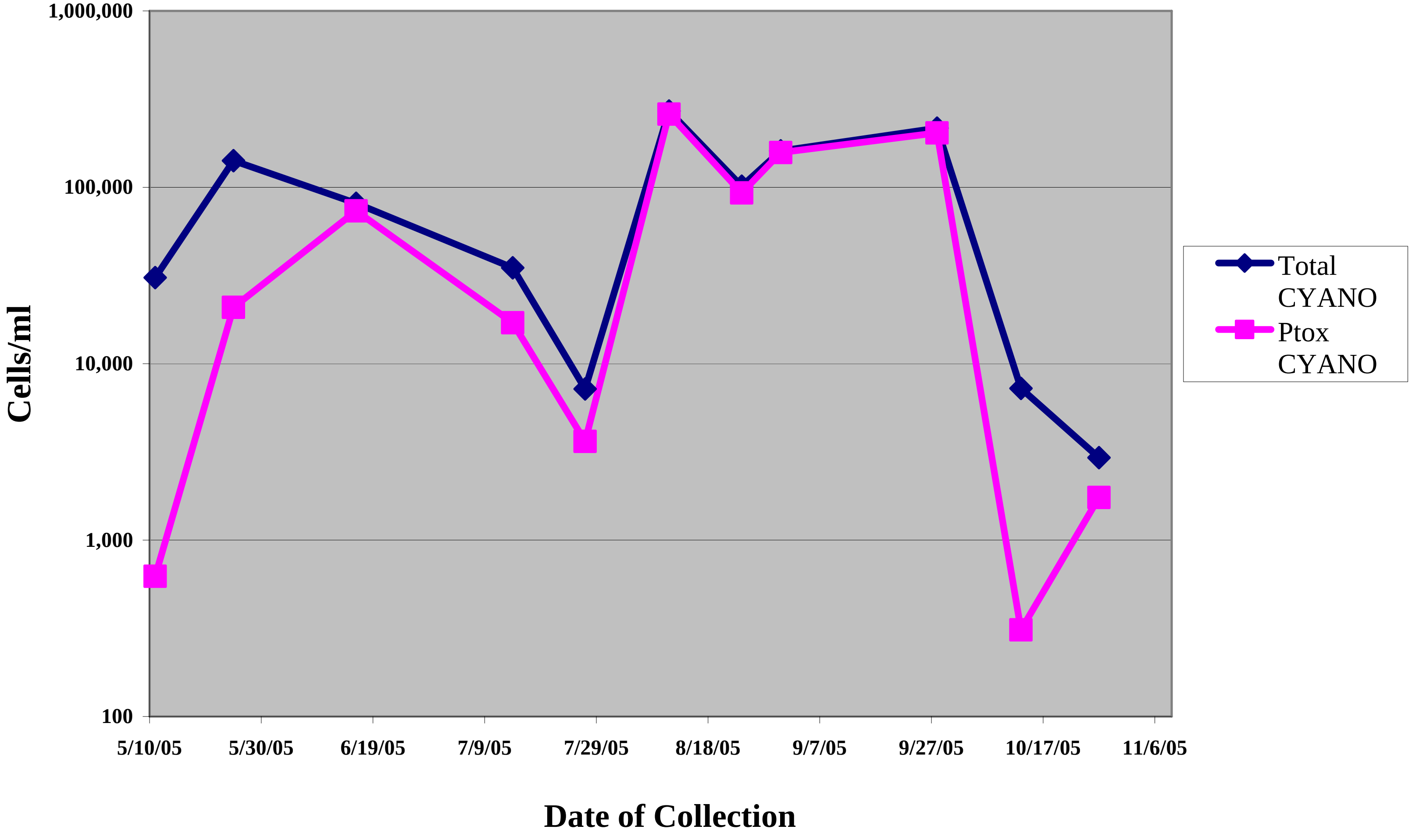


Figure 5: Cyanobacteria in Lake George (2005)

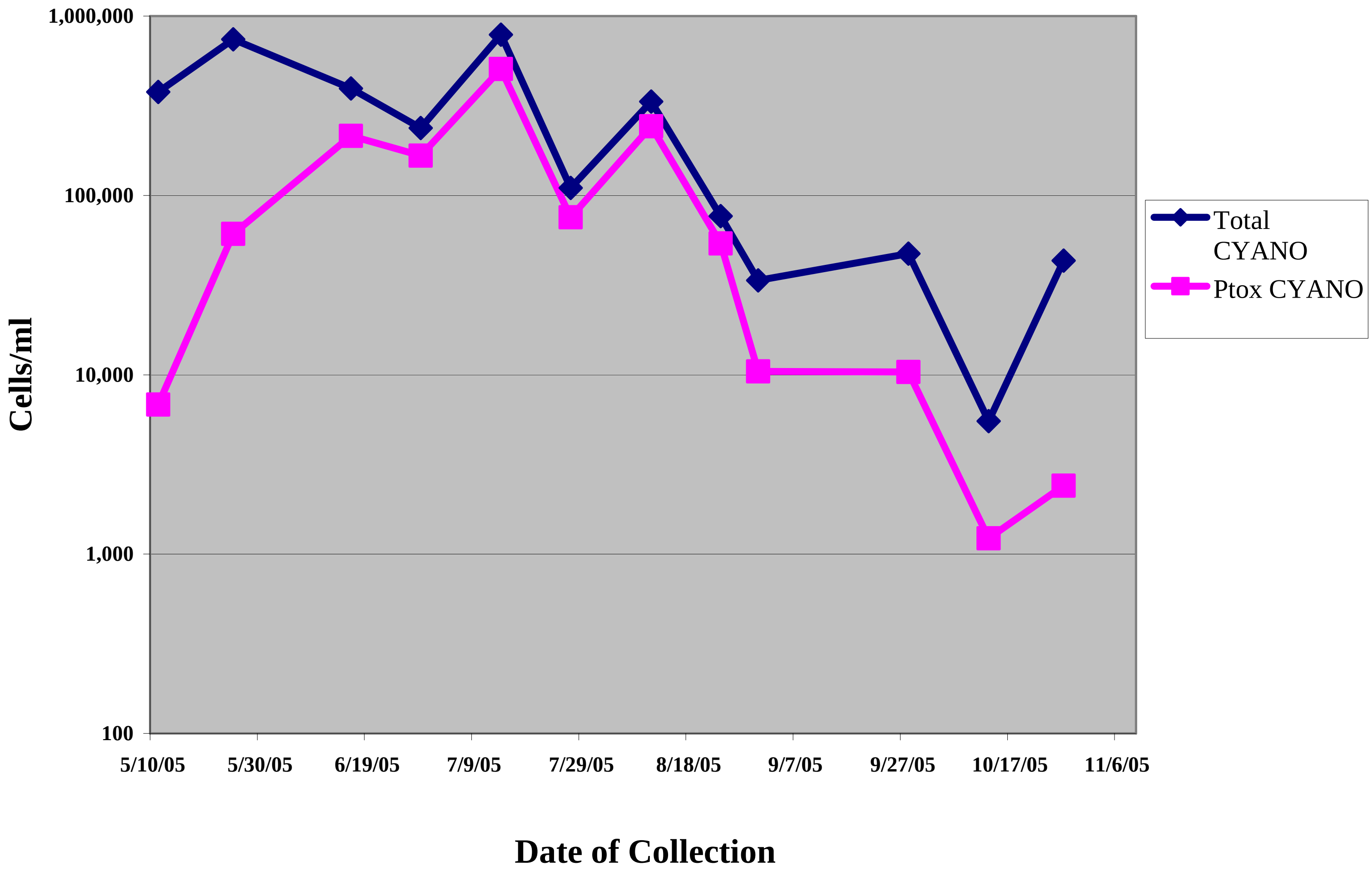


Figure 6A: Cyanobacteria in Hickory Point (Bath) (2005)

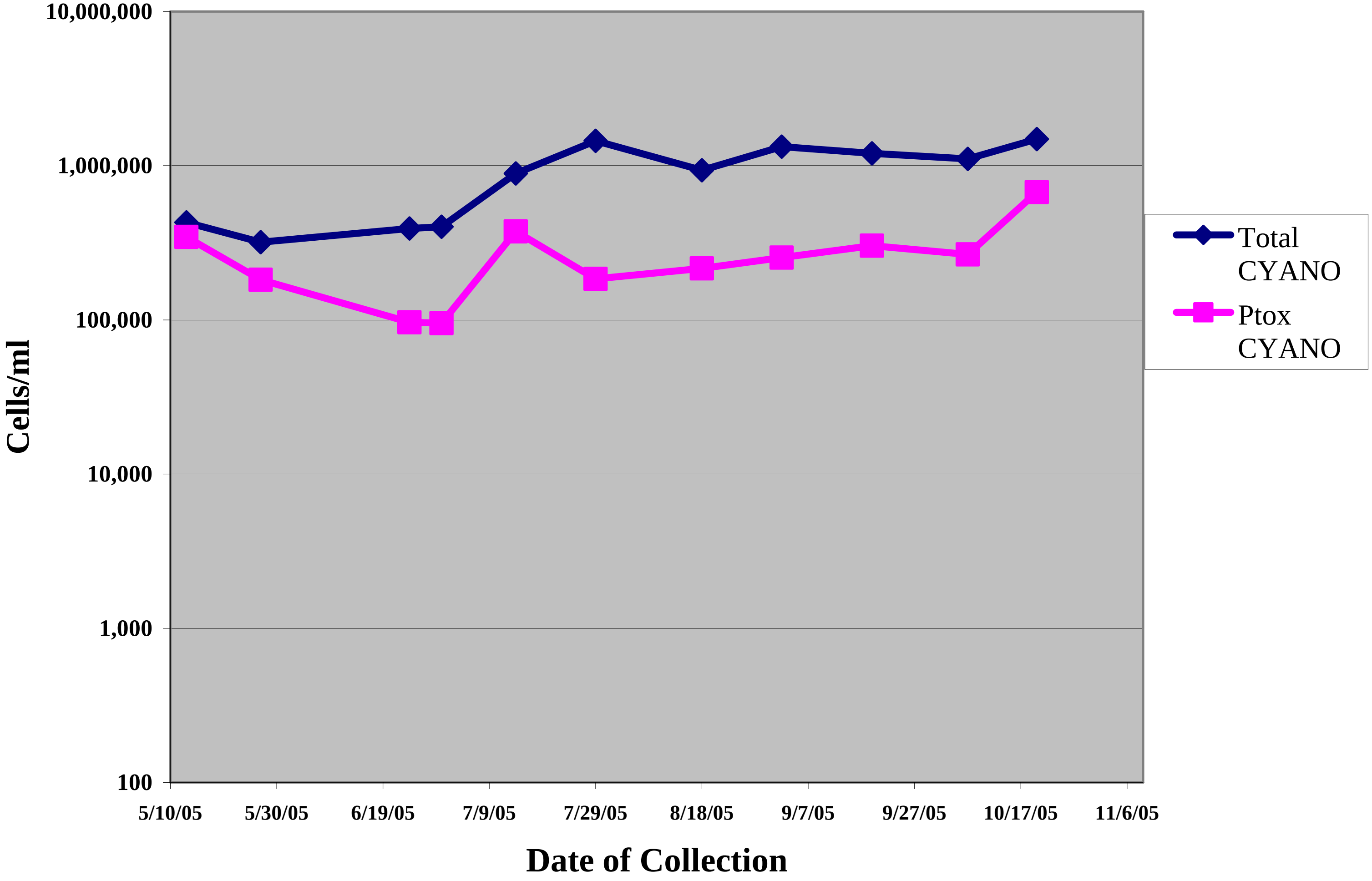


Figure 6B: Cyanobacteria at Hickory Point (Dock) (2005)

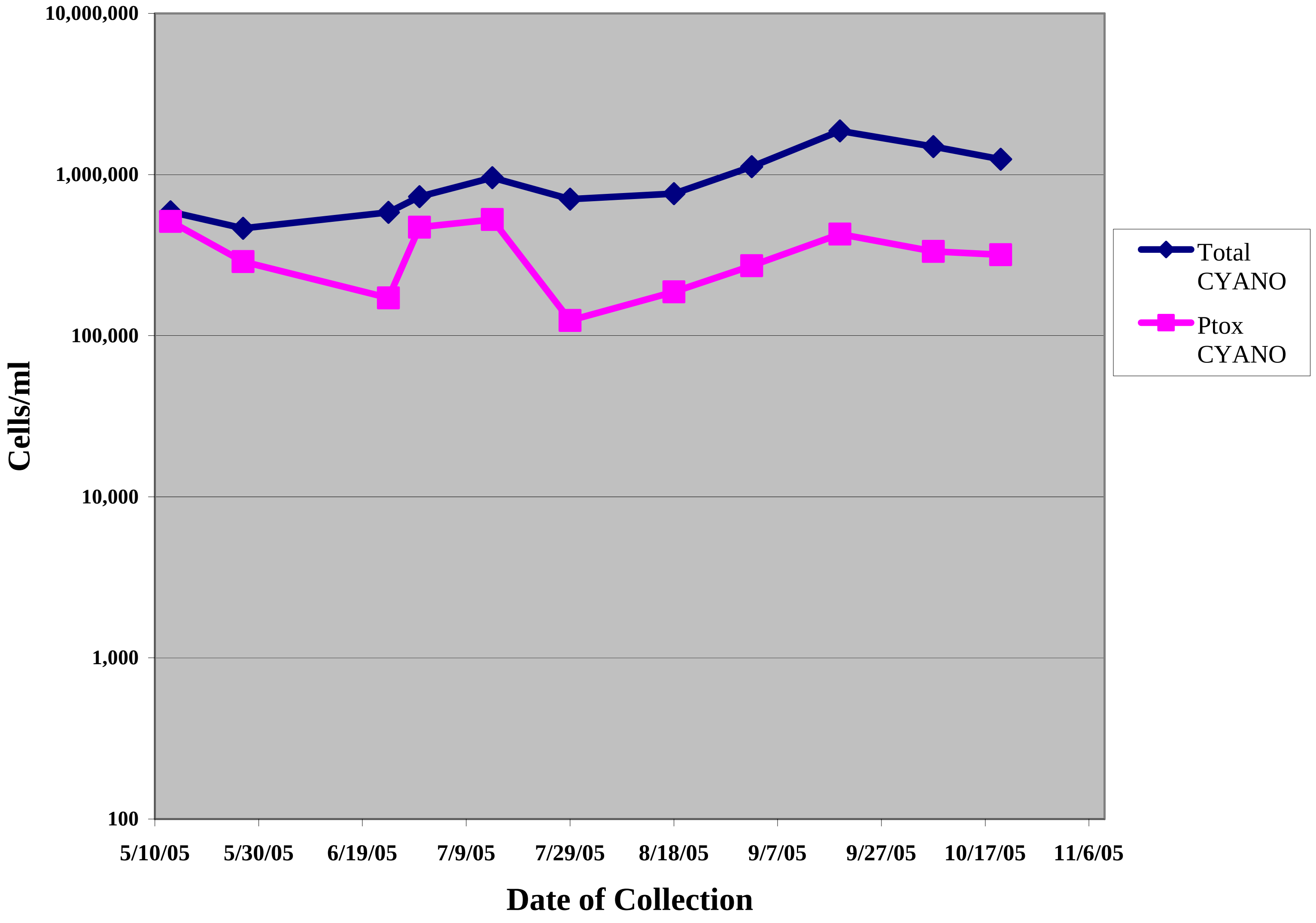


Figure 7: Cyanobacteria in Lake Monroe (2005)

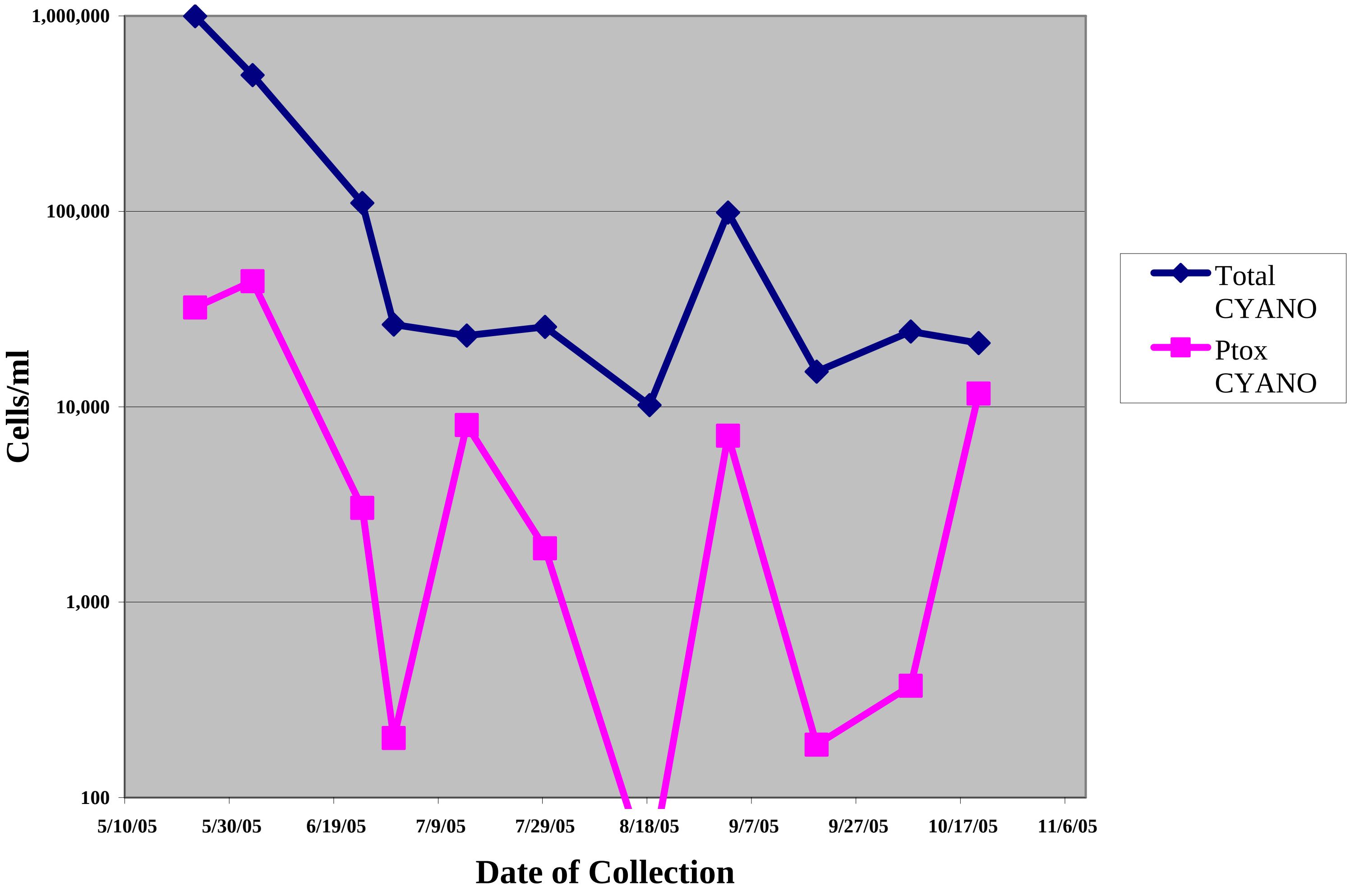


Figure 8: Cyanobacteria in Lake Jesup (2005)

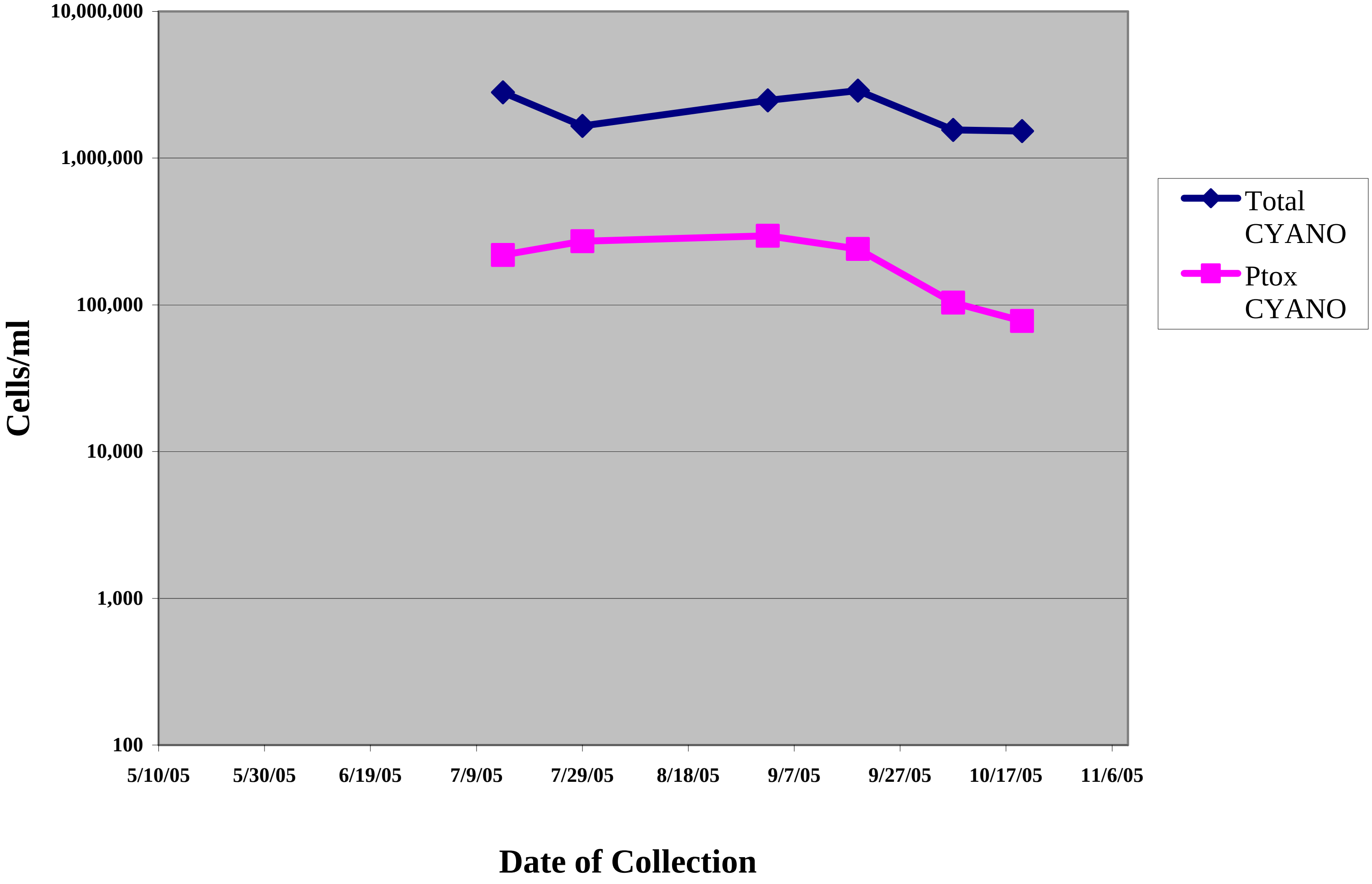


Figure 9: Cyanobacteria in Lake Washington (2005)

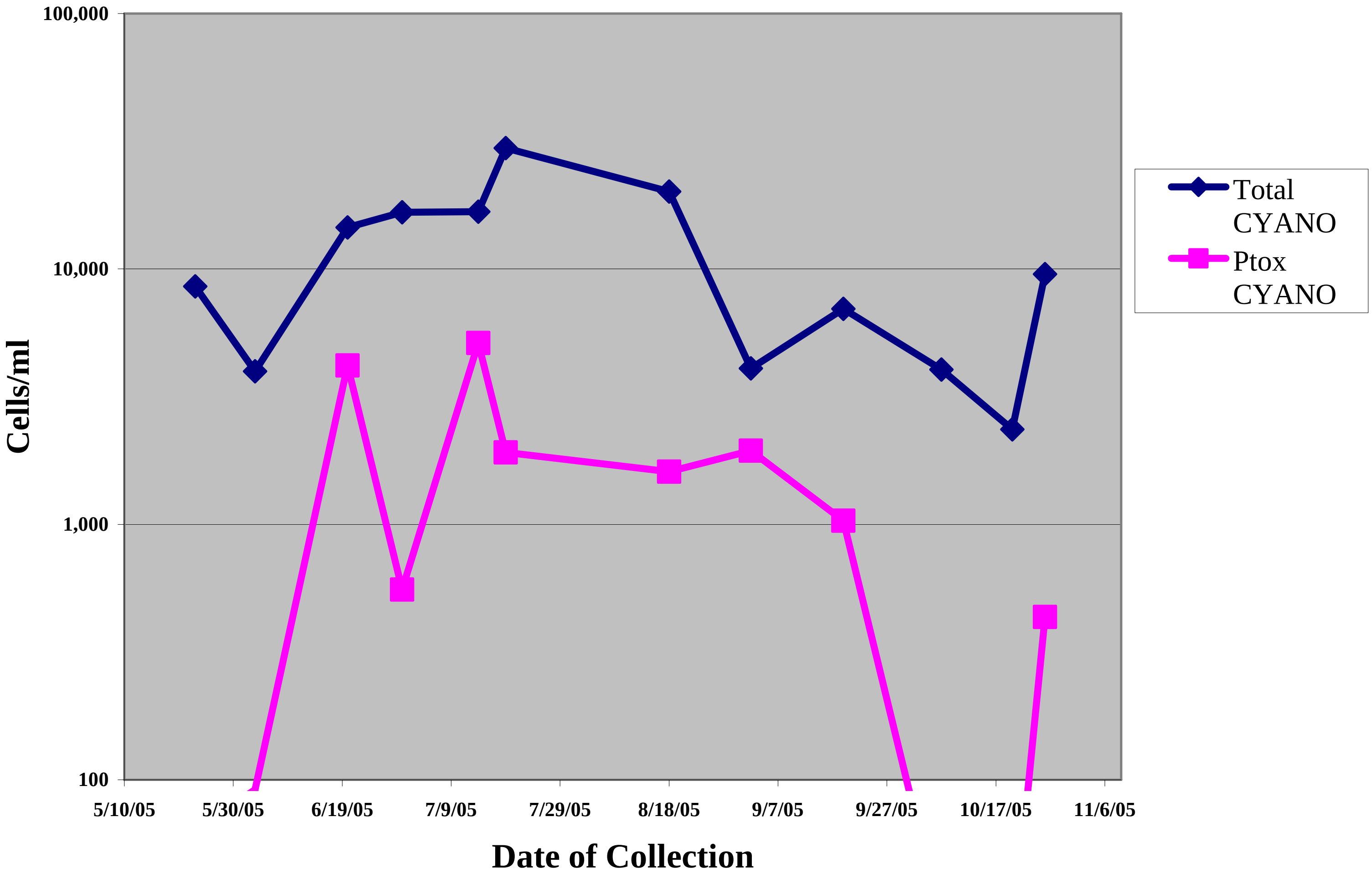


Figure 10: Potentially Toxigenic Algae at Palatka Pier (2005)

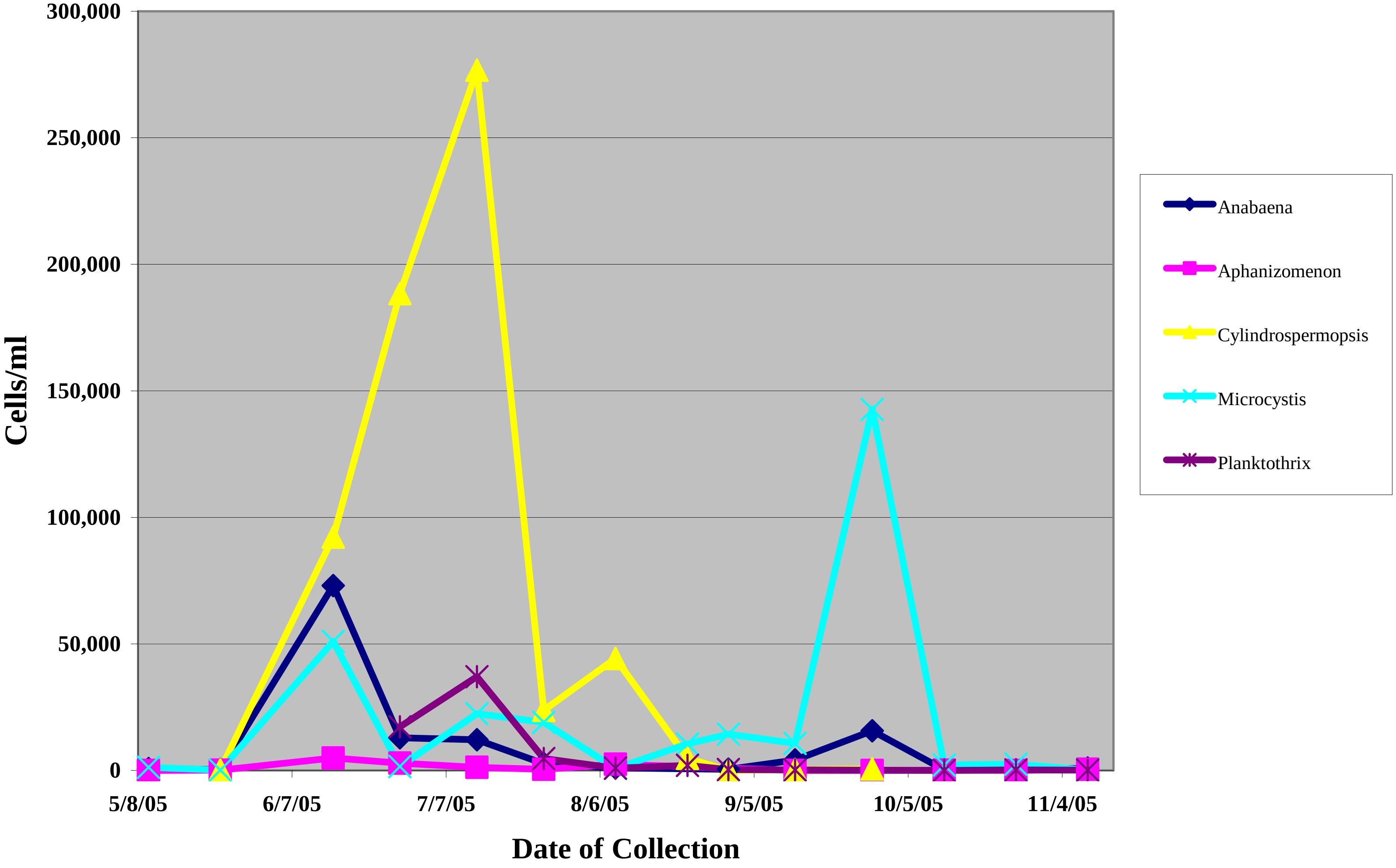


Figure 11: Potentially Toxigenic Algae in Lake Washington (2005)

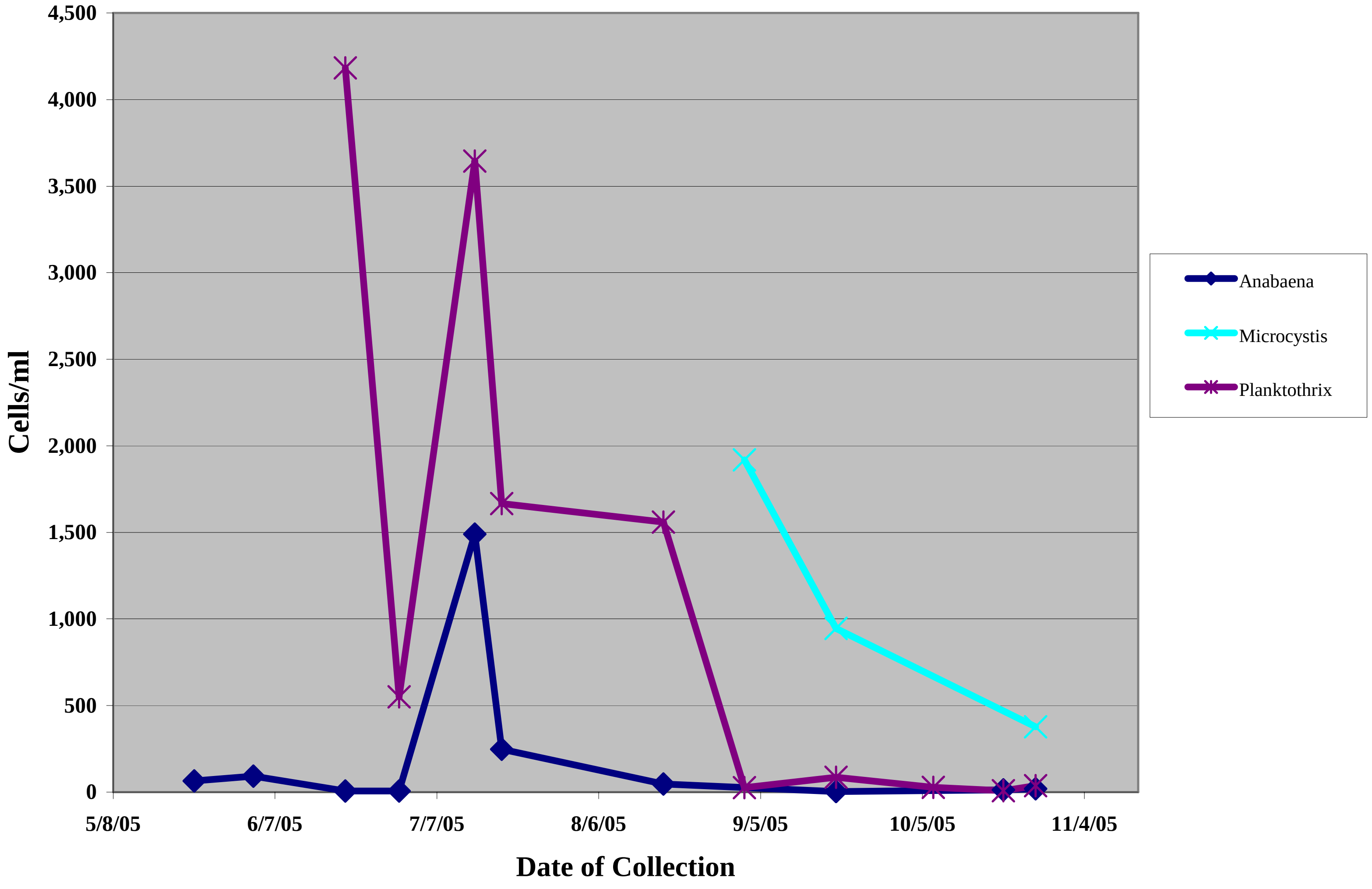


Figure 12: Potentially Toxigenic Algae in Lake Monroe (2005)

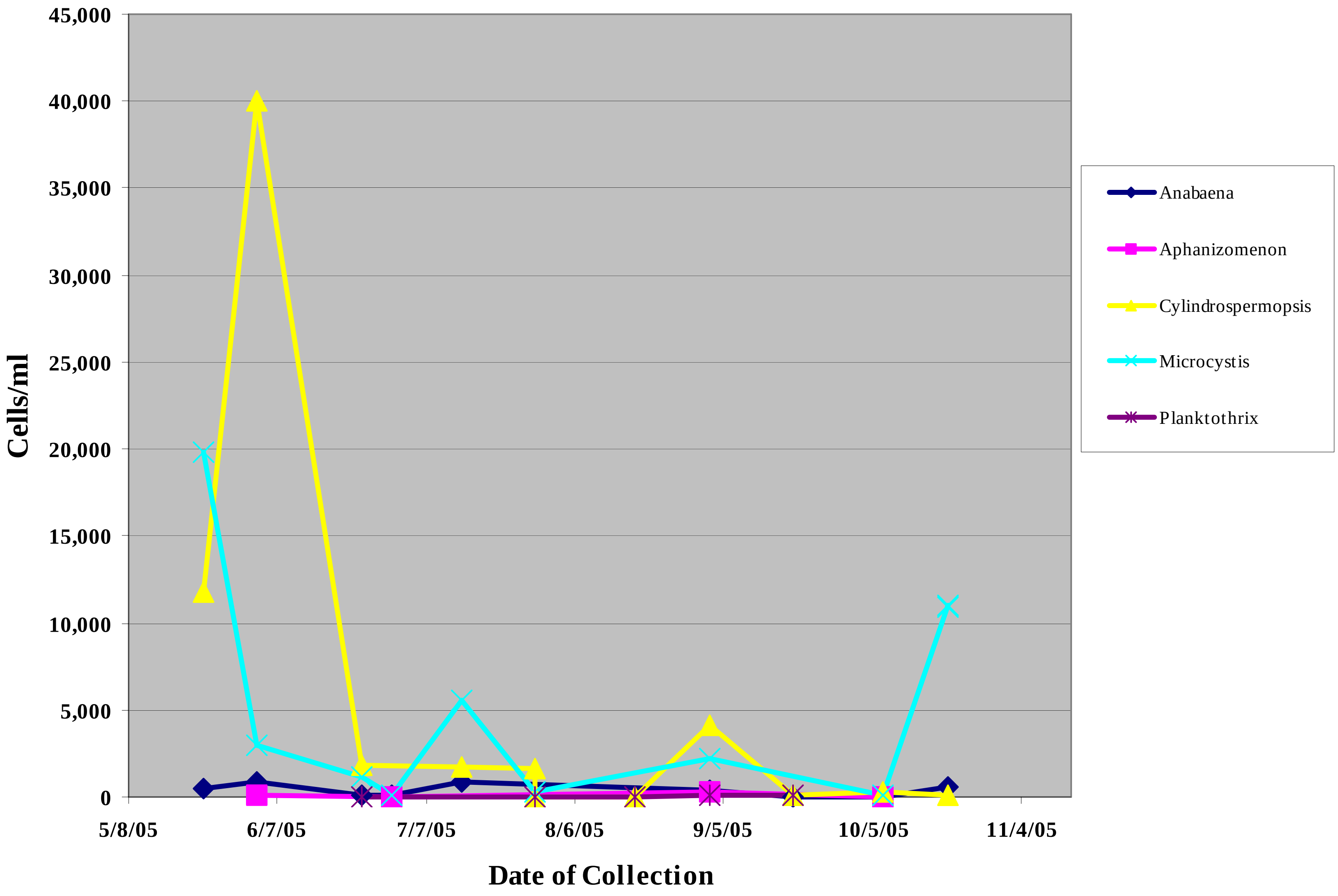


Figure 13: Potentially Toxigenic Algae in Lake Jesup (2005)

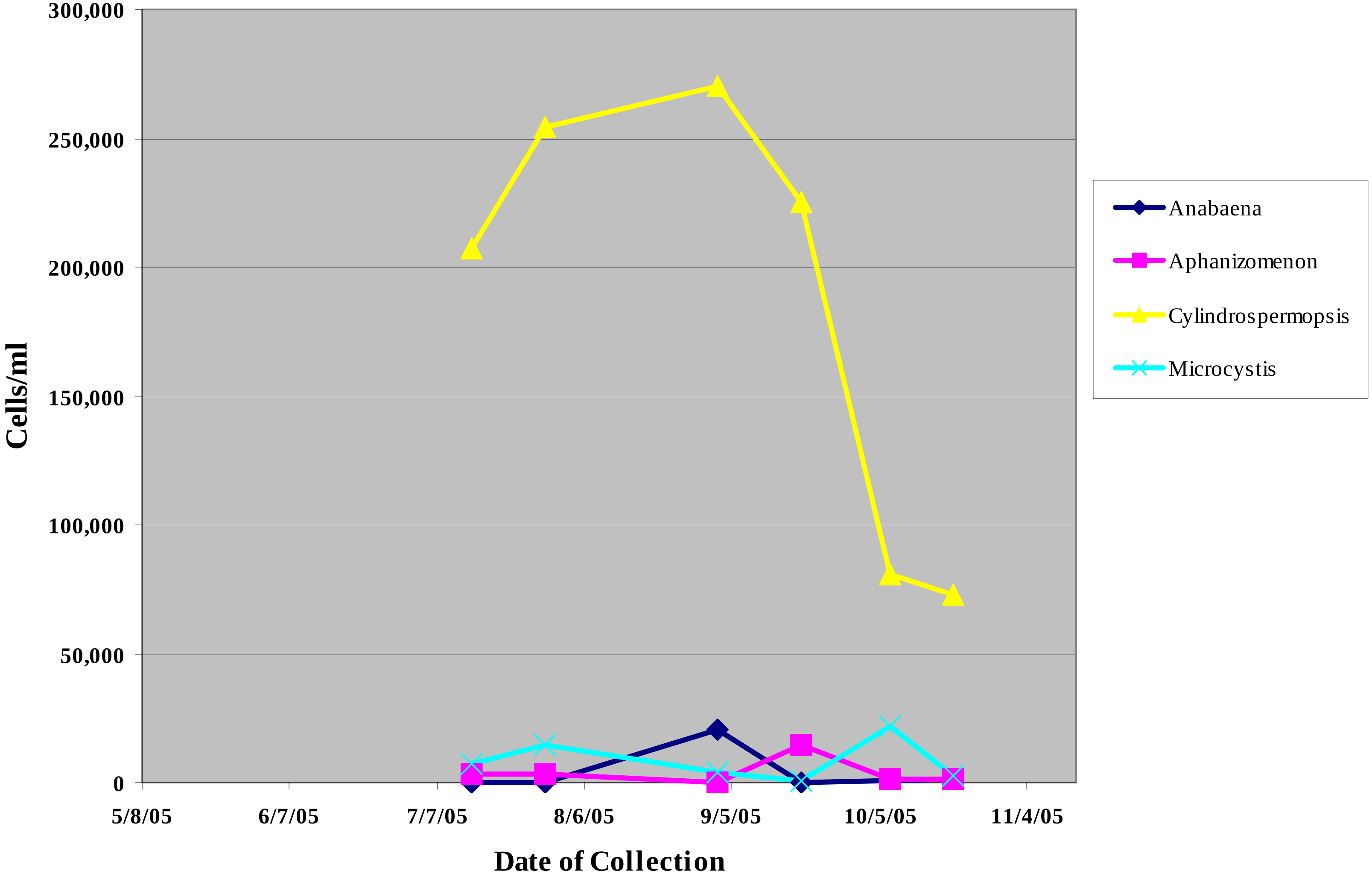


Figure 14: Potentially Toxigenic Algae in Lake George (2005)

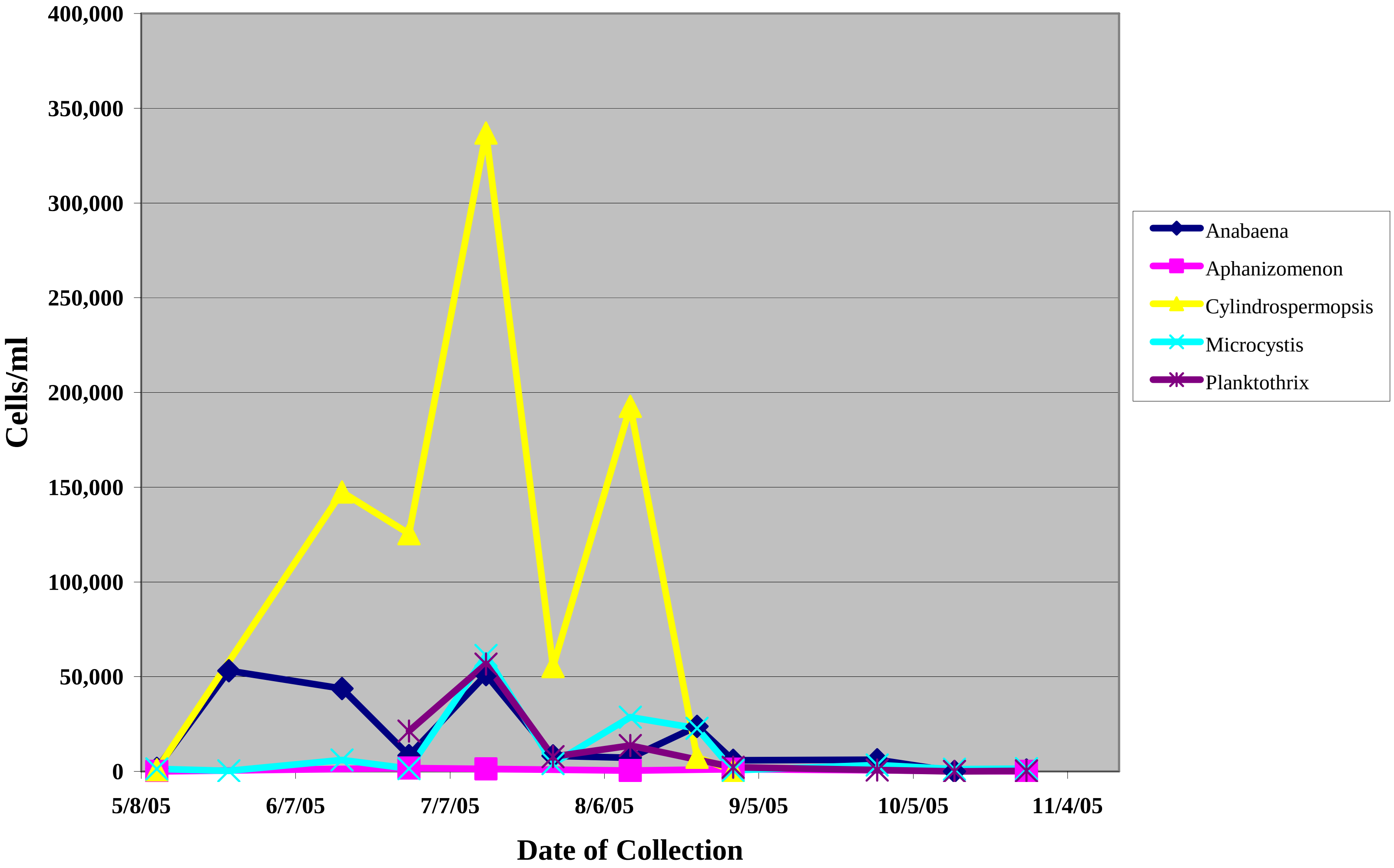


Figure 15A: Potentially Toxigenic Algae at Hickory Point (Bath) (2005)

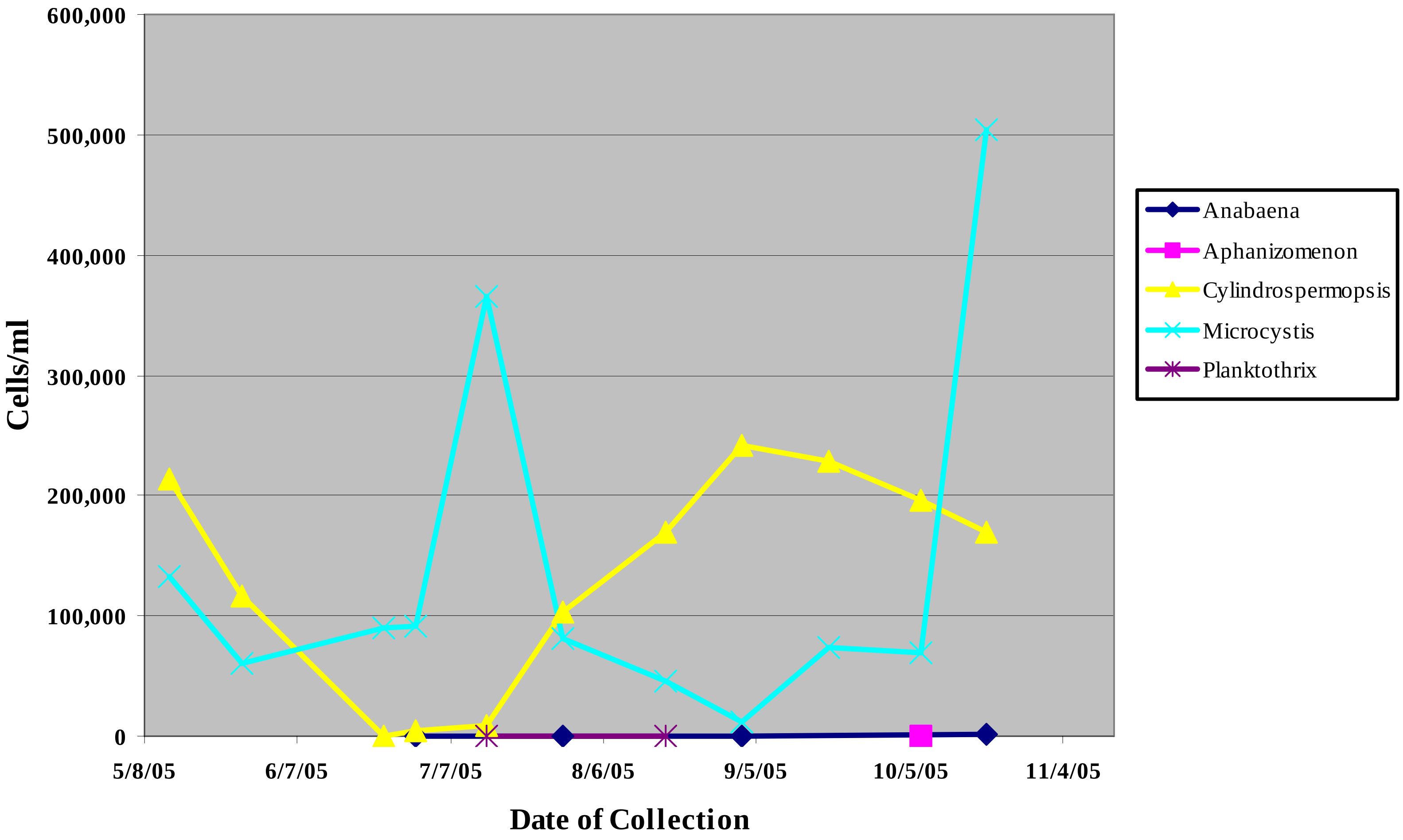


Figure 15B: Potentially Toxigenic Algae at Hickory Point (Dock) (2005)

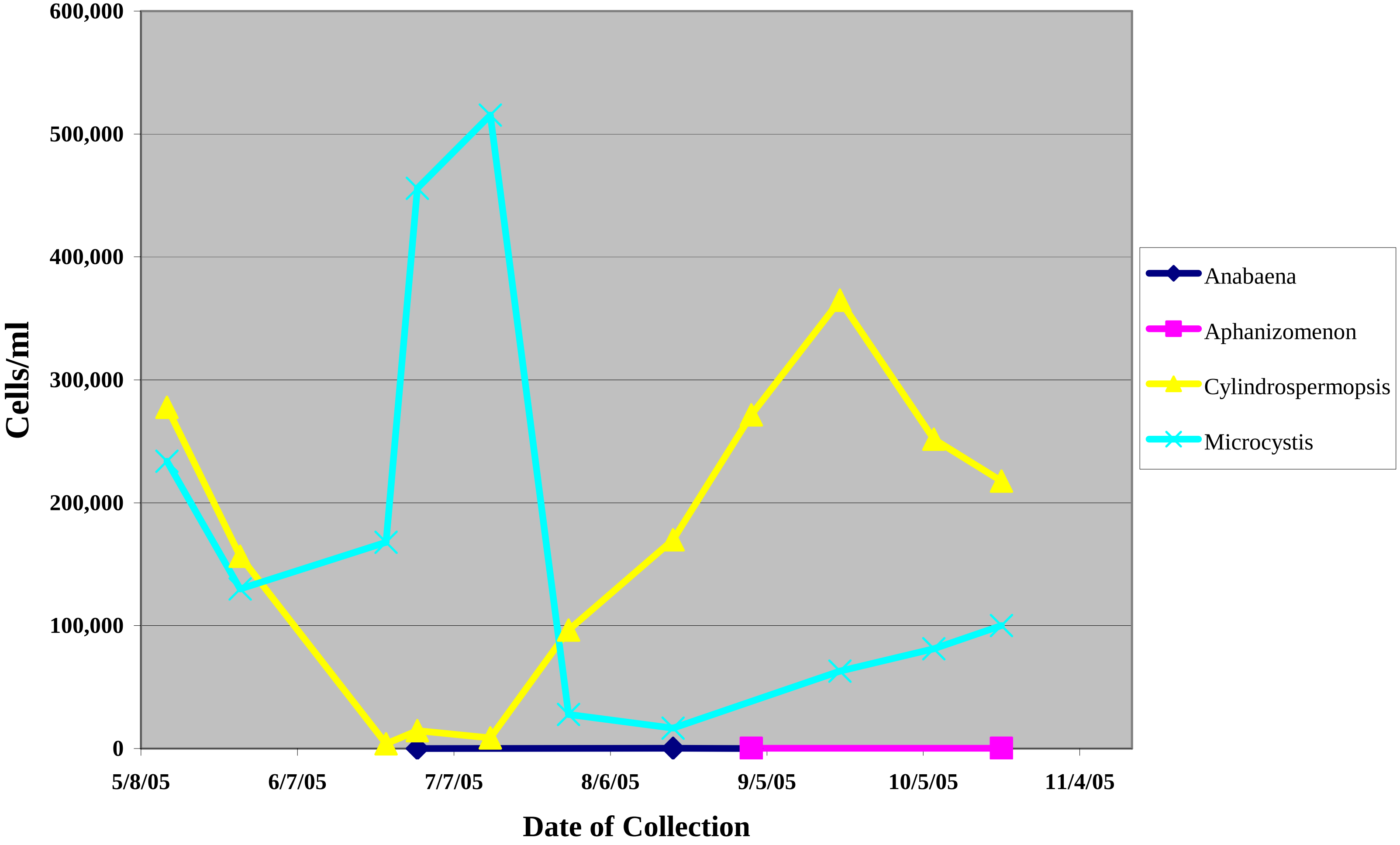
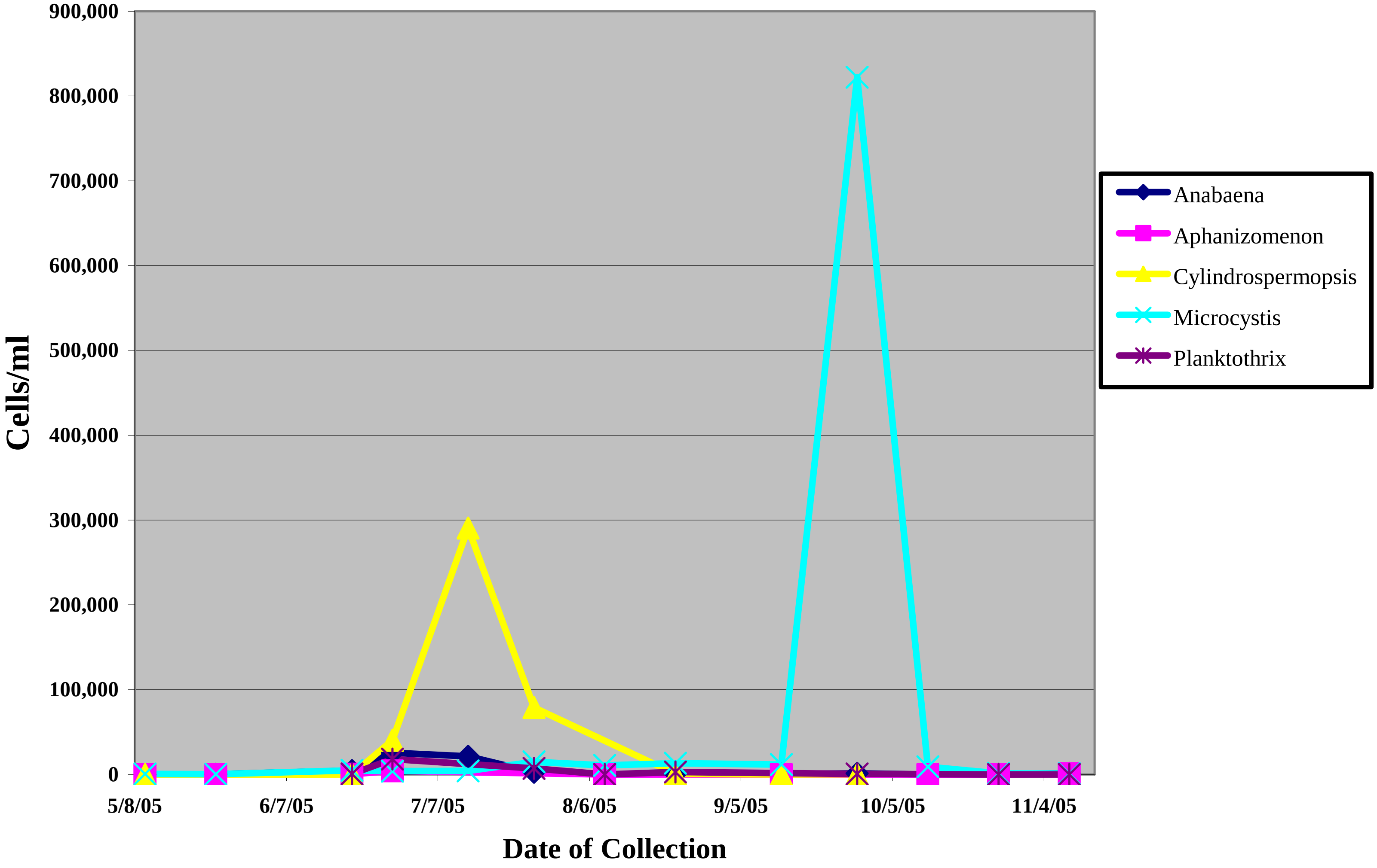


Figure 16: Potentially Toxigenic Algae in Eagle Harbor (2005)



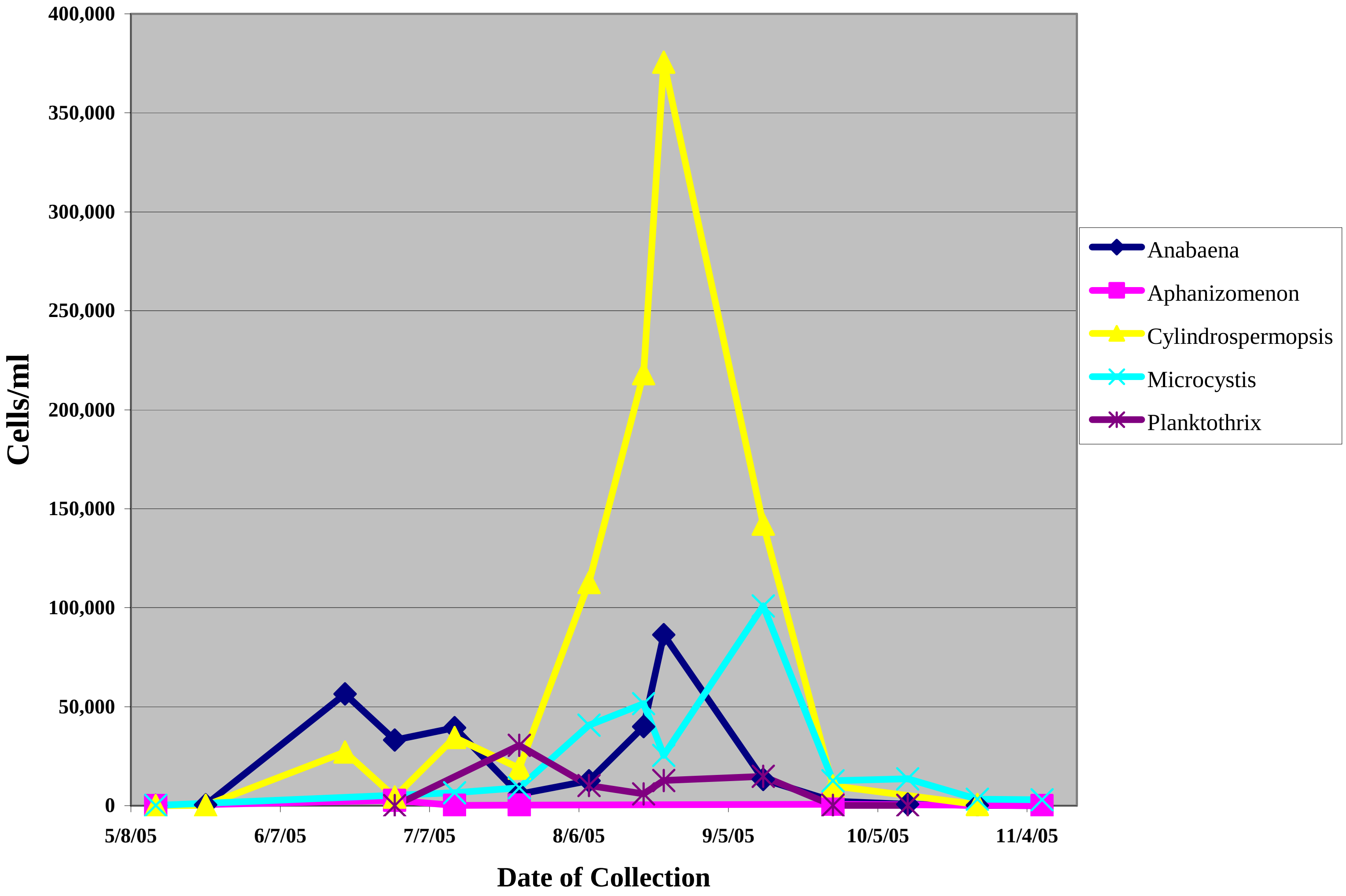


Figure 17: Potentially Toxigenic Algae in Doctor's Lake (2005)

Figure 18: Potentially Toxigenic Algae in Crescent Lake (2005)

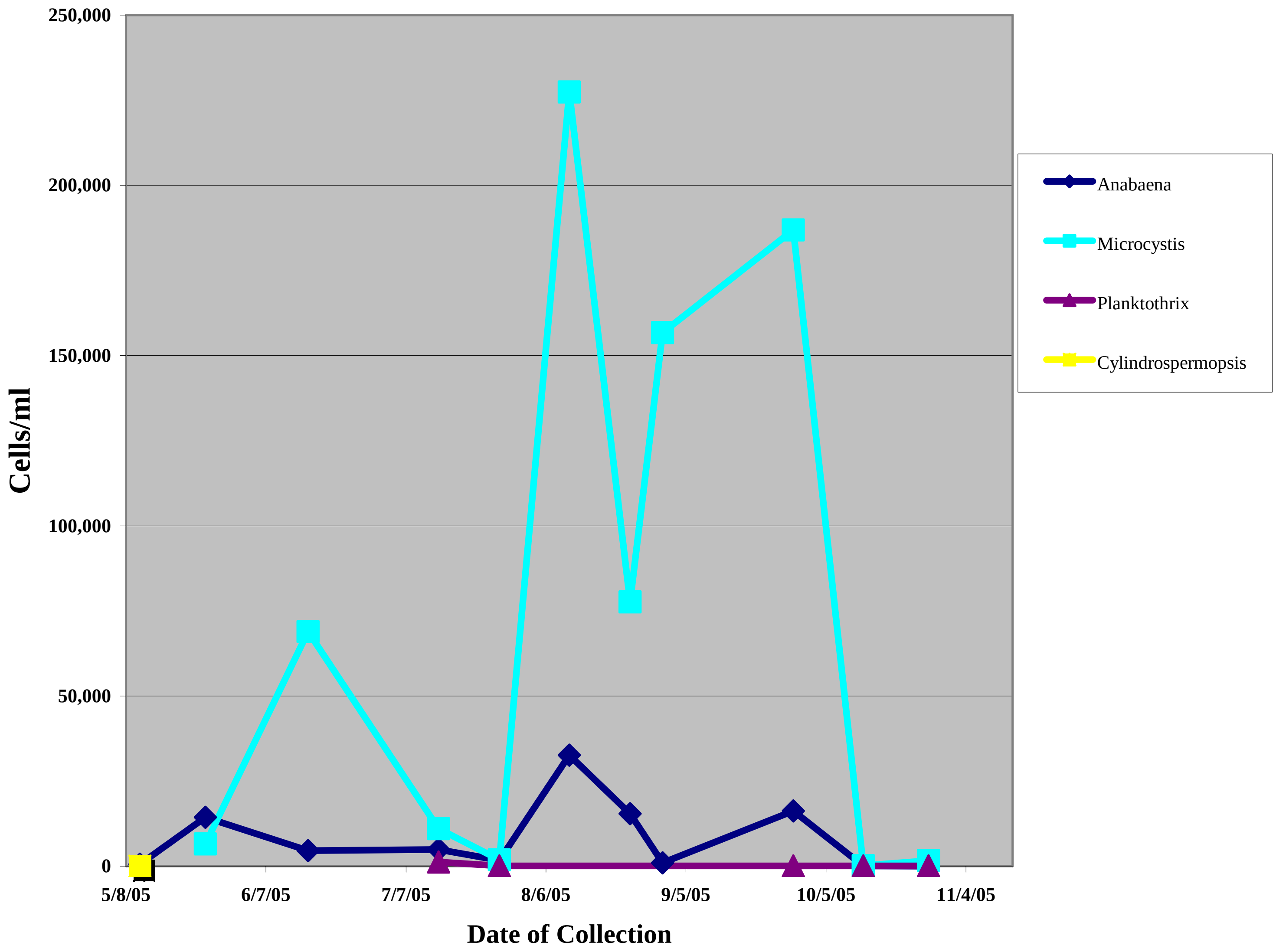


Figure 19: Microcystin Concentration Levels

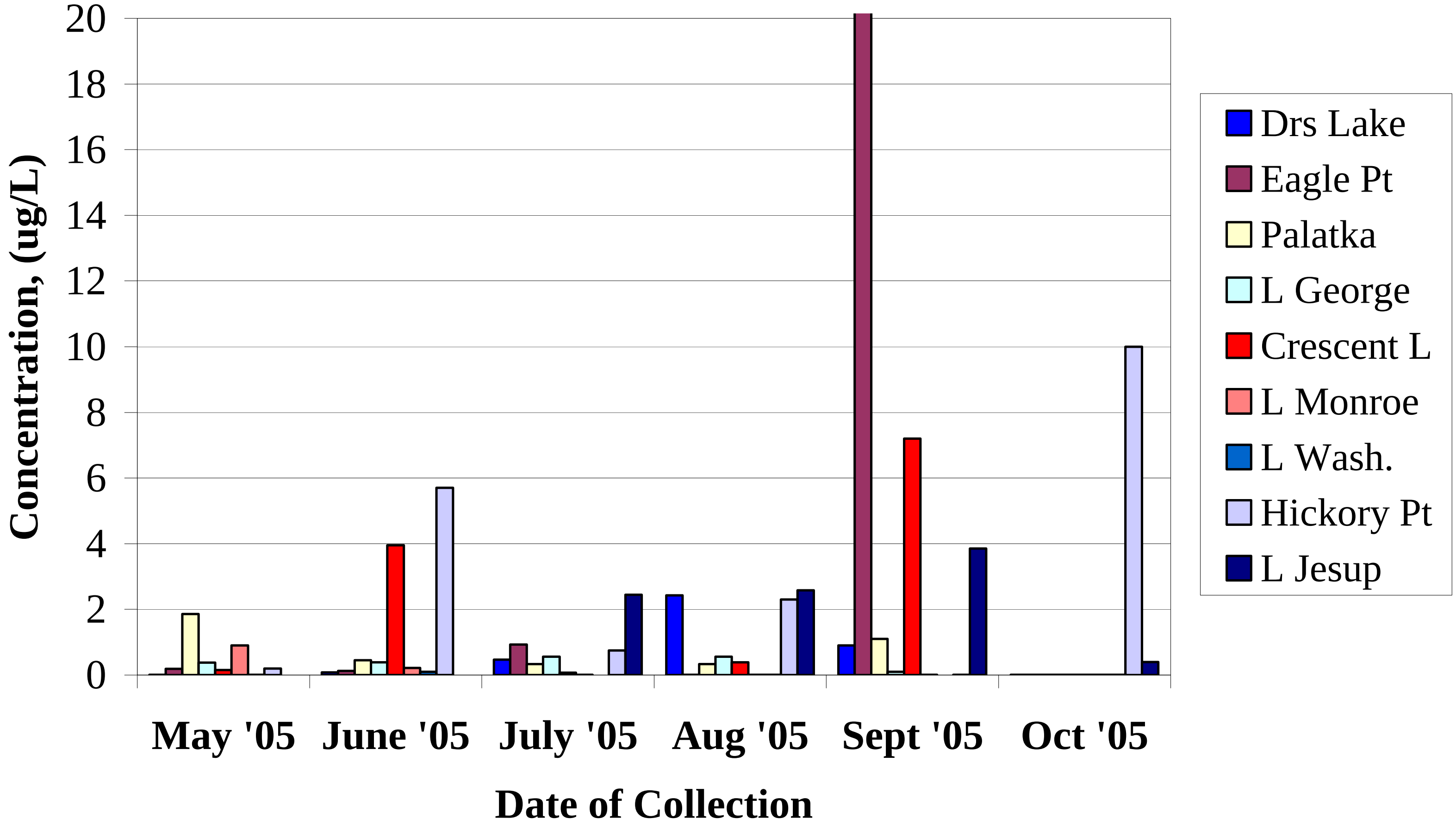


Figure 20: Cylindrospermopsin Concentration Levels

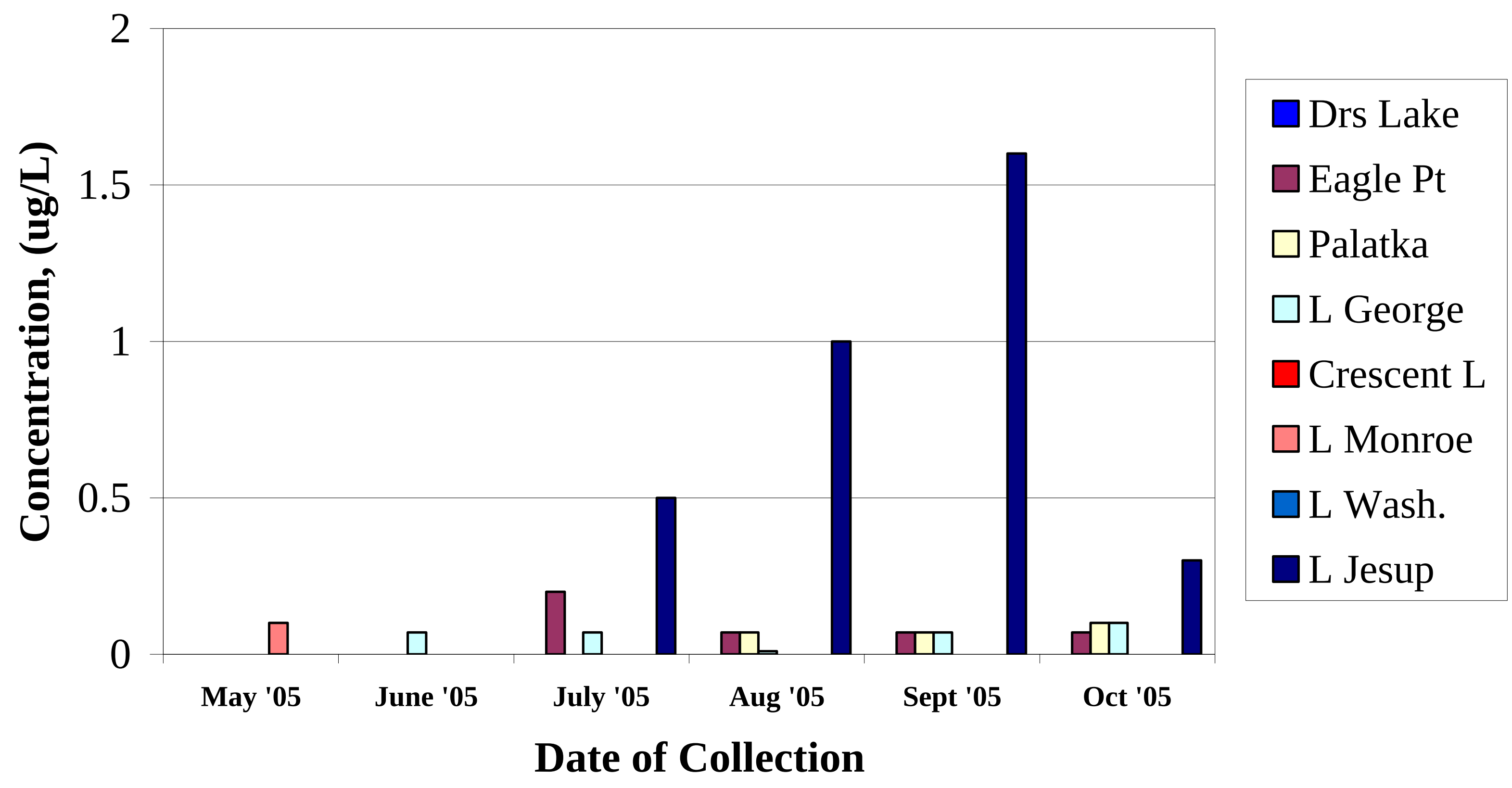
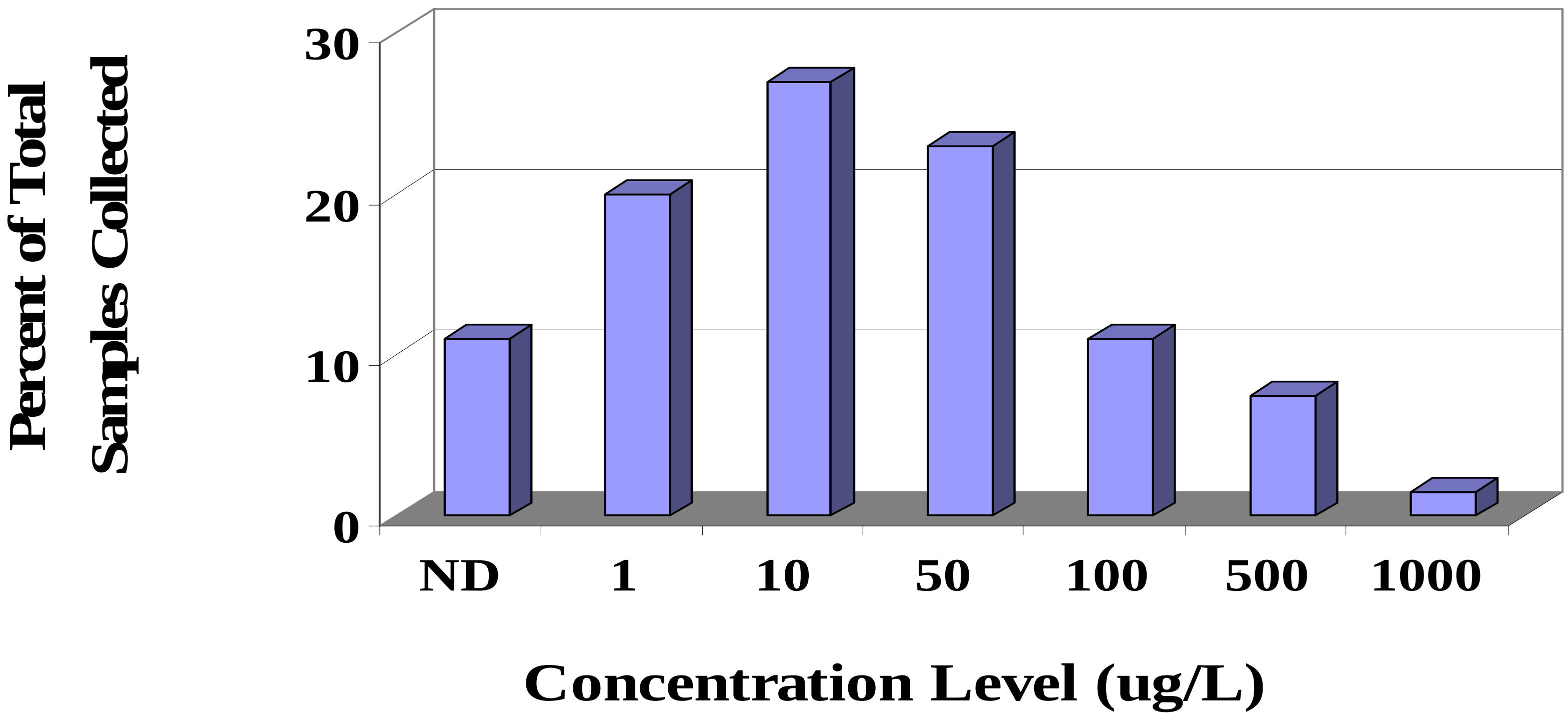


Figure 21: Frequency of Microcystin Concentrations Analyzed from Water Samples Collected by the St. Johns River Water Management District from a *Microcystis aeruginosa* bloom in the St. Johns River, September - October 2005.



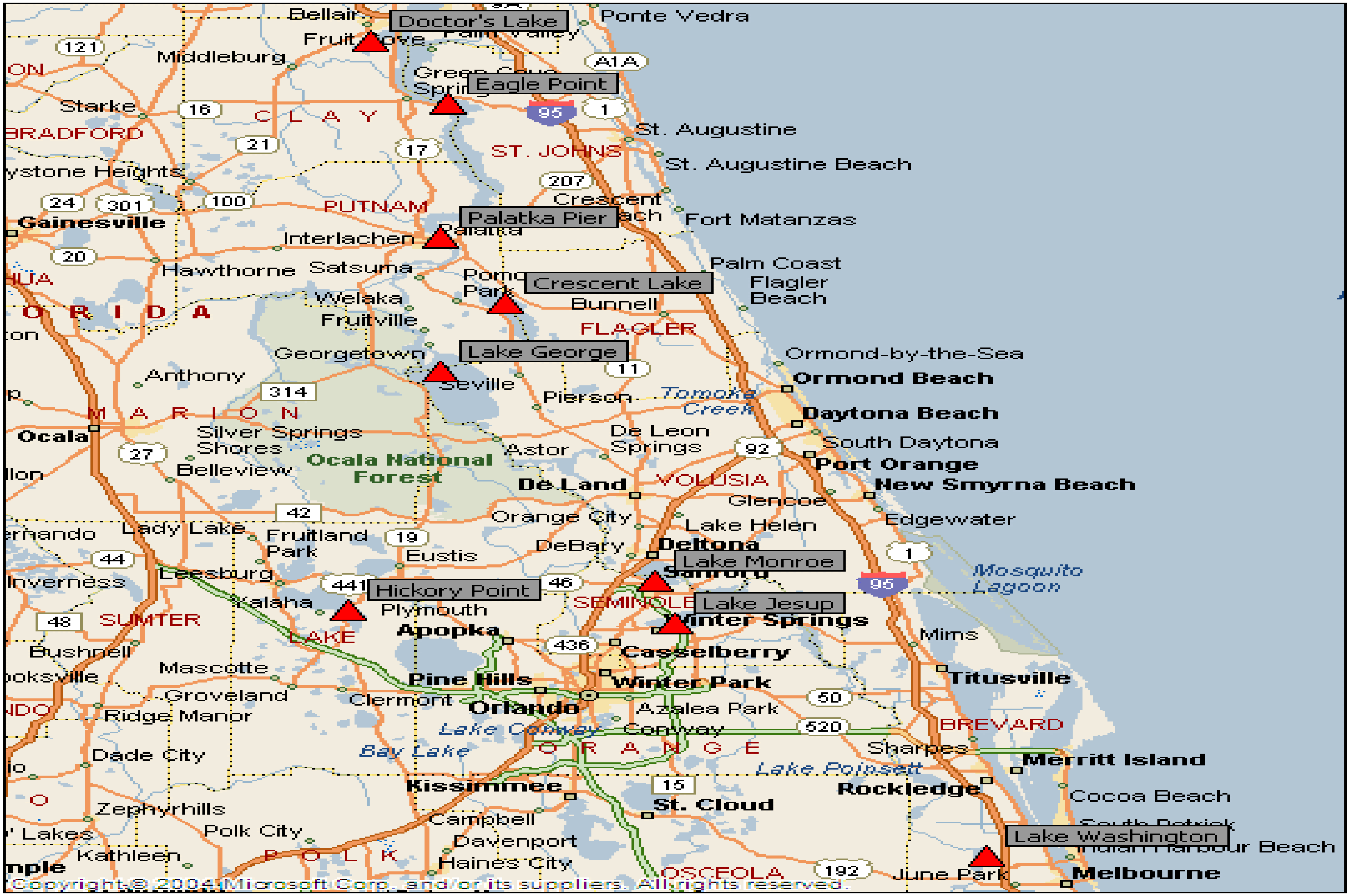
Note: Values on the X-axis are a range of values up to but not including the next higher number

Sample size (n) = 66 water samples

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	Eagle Pt. (Shands Bridge)	29-58-25	81-37-25	LSJRB/SJRWMD
3	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 (Hickory Pt. - 2)	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	GWL/USJRB-SJRWMD

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



Map of Samples Sites

Table 2: Frequency of Water Samples Above Suggested World Health Organization Guideline/Monitoring Levels

Sample No.	Sample Site Name	Frequency (%) ≥ 20,000 cells/ml	Frequency (%) ≥ 100,000 cells/ml	Maximum Concentration	Sampling Events
1	Doctors Lake	64	36	500,000	14
2	Eagle Pt. (Shands Bridge)	31	31	823,000	13
3	Palatka Pier	43	29	481,000	14
4	Crescent Lake	55	27	260,000	11
5	Lake George	58	33	507,000	12
6	Little Lake Harris (Hickory Pt. - Beach)	100	100	675,000	11
	Little Lake Harris (Hickory Pt. - Docks)	100	100	527,000	11
7	Lake Monroe	18	0	44,000	11
8	Lake Jesup	100	83	295,000	6
9	Lake Washington	0	0	5,100	11