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Flow Cytometric Analysis for Cyanobacteria in 36 New Jersey Freshwater Bodies

Ruchit Patel
ruchit.patel@student.shu.edu

FLOW CYTOMETRIC ANALYSIS FOR CYANOBACTERIA

IN 36 NEW JERSEY FRESHWATER BODIES

By

RUCHIT PATEL

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APPROVED BY

MENTOR

Dr. Tin-Chun Chu

COMMITTEE MEMBER

Dr. Daniel B. Nichols

COMMITTEE MEMBER

Dr. Constantine Bitsaktsis

DRECTOR OF GRADUATE STUDIES

Dr. Angela V. Klaus

CHAIRPERSON, DEPARTMENT OF BIOLOGICAL SCIENCES

Dr. Jane L. Ko

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Abstract

Eutrophication, a process that occurs due to excessive accumulation of nutrients such as nitrogen and phosphorous is primarily from anthropogenic nitrogen and natural occurrences. This phenomenon causes cyanobacterial overgrowth which can potentially lead to toxic algal blooms that affect public drinking water sources and recreational usage. An immediate need to detect bloom forming cyanobacteria in freshwater bodies early hand is critical to implement prevention strategies. These microorganisms contain phycobiliproteins such as phycoerytrhin, and allophycocyanin as part of the phycobillisome that allow autofluorescence. In this study, 36 freshwater bodies from 14 New Jersey counties were collected and processed for flow cytometric analysis for forward- scatter, phcyoerthrin and allophycocyanin parameters. Pure cultures of *Synechococcus* sp. IU 625 (S. IU 625), *Cylindrospermum* spp. and *Microcystis aeruginosa* (*M. aeruginosa*) were utilized as references. Polymerase chain reaction (PCR)-based assay was performed for the validation of the flow cytometric analysis. The results revealed 17 out of the 36 sites showed all three reference species and their related. 6 waterbodies showed Cylindrospermum like species, 7 waterbodies showed Microcystis and Cylindrospermum like species, 4 waterbodies showed Microcystis and Cylindrospermum like species and 2 sites showed Microcystis like species. PCR results validated these results by showing positive results for phytoplankton, cyanobacteria and Synechococcus. Flow cytometry has high potential for the rapid detection of cyanobacteria in live form due to their autofluorescence properties from the phycobilisome.

Introduction

Cyanobacteria, previously known as blue green algae, are one of the earliest dated oxygen releasing prokaryotes that originate ~ 3.5 billion years ago (Schopf, 2002). Cyanobacteria may have very different cellular arrangement such as unicellular *Synechococcus* spp., filamentous *Cylindrospermum* spp., and potential colonial *Microcystis* spp. Their sizes range from 0.5 to > 50 µm. These cyanobacteria contain a thicker peptidoglycan layer than gram negative bacteria and possess complex metabolic pathways that have allowed the evolutionary advantage of surviving various environmental conditions such as the ability of the electron flow from both photosynthesis and respiration to occur in the thylakoid membrane (Campbell et al., 1998; Vermaas, 2001). These photosynthetic microorganisms are located in marine waters and freshwater environments such as lakes, ponds, rivers, brackish water and water reservoirs. Moreover, since cyanobacteria have some evolutionary metabolic advantages and the ability to tolerate various environmental conditions, they are able to survive as well as outcompete other biological species in eutrophied water bodies. Therefore, cyanobacteria can over-grow and form an algal bloom because of the accelerated accumulations of nutrients such as nitrogen and phosphorus. These sources are introduced into water due to anthropogenic nitrogen, manmade situations and natural occurrences such as fertilizers, humans waste, agricultural waste, urbanization, housing developments (especially lake houses); decaying plants, storms, flooding and water runoffs (Paerl et al., 2013). The bloom masses may cover the water surface, lowering the dissolved oxygen which leads to hypoxia in the water. In addition, many cyanobacteria

possess the ability to release toxins. Both cases are considered as harmful algal blooms (HABs) since the ecosystems are affected. An example of an algal bloom from Clark Reservoir in Clark, NJ is shown in Figure 1.

HAB that is toxic becomes a serious issue when the ecosystem is affected and when it becomes dangerous towards the public that utilize the water for drinking and recreational purposes. Some cyanobacteria produce secondary metabolites known as cyanotoxins that are toxic to other cells, organisms, fish, animals and humans. However, not all cyanobacteria are toxic and not every toxic species will always secrete cyanotoxins based on the cell's condition and the environment. The toxicity can also vary from one bloom to another where specific species can secrete a certain toxin or even multiple species can release different cyanotoxins (Funari and Testai, 2008; USEPA 2015a). Intracellular toxins for most cyanobacteria remain internal unless an environmental stress factor induces cell lysis (ILS, 2000; USEPA 2015a). One reason intracellular toxins remain balanced is because daughter cells are lost during cell division (Paerl et al., 2013). These cyanotoxins can be exposed to humans and animals from skin contact and inhalation during recreational activities such as swimming or from ingesting contaminated drinking water from cyanotoxins. 80% of human exposure tends to be through ingestion of contaminated water (WHO, 1998; Merel et al., 2013). Cyanotoxins are broadly organized as hepatotoxins, neurotoxins and dermatoxins. Cyanotoxins have also been categorized based on United States Environmental Protection Agency's (USEPA) contaminate candidate list that primarily affect the public drinking water. These include the toxins microcystin, cylindrospermopsin and the anatoxin-a- group.

Microcystins, primarily found in fresh and brackish water, contain about 80 variations in their amino acids. The toxic mechanism involves inhibiting eukaryotic protein phosphatases 1 and 2A (Jungblut et al., 2006). Exposure to this toxin causes liver inflammation and or liver hemorrhage (Jungblut et al., 2006; Van Apeldoorn et al., 2006). Common species include Microcystis, Anabaena, Planktothrix, Anabaenopsis and Aphanizomenon (USEPA, 2014).

Anatoxin- a is a neurotoxin that contains 2 to 6 variances in the amino acids. The mechanism involves the binding of this toxin to neuronal pre-synaptic acetylcholine receptor where nicotine receptors are mimicked, thus, causing neurological effects such as paralysis and even death from respiratory arrest (Carmichael et al., 1992; WHO, 1999; Wonnacott and Gallagher, 2006; Farrer et al., 2015; USEPA, 2014; USEPA 2015b). Just like microcystin this toxin is water soluble, however unstable at pH > 10 and becomes nontoxic and unstable from long duration of sunlight exposure (Merel et al., 2013). Without sunlight the toxin from the bacteria can survive days to several months (Stevens and Krieger, 1990; US EPA 2015b). Common species for this group include Anabaena, Planktothrix, Aphanizomenon, Cylindrospermopsis, Cylindrospermum and Oscillatoria (Blaha et al., 2009; USEPA, 2014).

Cylindrospermopsin, a hepatotoxin with 3 variants or analogues found further below the water surface, can have the toxin released extracellularly up to 50%. It's found in brackish, marine waters, freshwater ponds, rivers, reservoirs and eutrophied lakes (Chiswell et al., 1999; USEPA, 2014; USEPA 2015c). The toxic mechanism involves inhibiting any forms of protein synthesis which can cause kidney and liver failure (Van

Apeldoorn et al., 2007).

Guidelines need to be established since no U.S Federal guidelines are currently available and cyanotoxins contaminate drinking water. The World Health Organization has established a recommendation for microcystin- LR of 1 µg/L in drinking water. According to USEPA, only 18 countries and 3 states in the U.S have established a toxin ingestion guideline for drinking water. For instance, 16 countries contain a guideline value of 1.0 µg/L, Australia and Canada contain values of 1.3 and 1.5 µg/L respectively (USEPA, 2015d). Only Minnesota, Ohio and Oregon in the United States have guidelines values of 0.04, 1 and 1 µg/L respectively (USEPA, 2015d). EPA states that Australia, New Zealand and Brazil have cylindrospermopsin guideline values of 1, 1, and 15 µg/L respectively (USEPA, 2015e) while Ohio and Oregon both contain cylindrospermopsin guideline values of 1 µg/L (US EPA, 2015e). Children are at greater risks than adults because of their body size to weight ratio. Moreover, EPA has stated guidelines for children less than six of 0.3 and 0.7 µg/L for microcystin and cylindrospermopsin respectively (USEPA, 2015f; Farrer et al., 2015).

Treatments are in dire need to help avoid harmful algal blooms. In 2014 Toledo, Ohio banned their drinking water and asked civilians not to drink or boil water because the microcystin toxin doesn't disintegrate from boiling (Rao et al., 2002; USEPA 2015d). In addition, it is vital to remove intracellular as well as possible extracellular toxins to fully eradicate these cyanotoxins. Algaecides such as copper sulfates have been used because it affects electron transport in photosystem II and stops some fundamental enzyme activities of many cyanobacteria (WHO, 1999; Le Jeune et al., 2006). However,

it still leads to cell lysis, thus causing secretion of toxins. In addition, this issue also occurs for chlorination treatment (Daly et al., 2007; Weirich et al., 2014). Microfiltration and ultrafiltration have been effective towards the prevention of intracellular toxins with up to 98% removal of *Microcystis aeruginosa*, but still ineffective towards the removal of extracellular toxins (Chow et al., 1997; USEPA, 2014). Cyanophages, a host specific bacteriophage have the ability to infect species specific cyanobacteria, is a possible biological prevention approach for cyanobaterial blooms. Knowledge of cyanophage can be used as a means to reduce bloom forming cyanobacteria and increase water quality and health of the ecosystem (Lee et al., 2006).

Finding solutions and awareness of harmful algal blooms is an immediate need as rapid detections can avoid or halt further issues and reduce the need for chemical treatments which will cause cell lysis and toxin secretion. New Jersey along with other states are actively coming up with efficient monitoring methods. New Jersey Department of Environmental Protection (NJDEP) in 2005 created an ambient lake monitoring network plan to determine and assess water quality and ecological health of lentic water resource to meet the Clean Water Act and the Total Maximum Daily Load requirements. NJDEP is making an effort into improving water quality to ensure it does not affect the public. NJDEP has a network station of 200 water bodies that are monitored periodically and approximately 40 waterbodies are sampled every year.

In summer 2015, collaboration with NJDEP was established. A total of 36 freshwater bodies in 14 New Jersey Counties were processed from their water collection. Cyanobacteria contain phycobillisomes that are attached to the thylakoid membrane and

utilized as a light harvesting and energy transfer complex toward photosystem II reaction centers (MacColl et al., 1998; Teldford et al., 2001). Phycobillisomes are composed of highly organized phycobiliproteins such as phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC) where the energy flow will follow in the order of these auto fluorescent pigments respectively (MacColl et al., 1998). Furthermore, many flow cytometers allow detection of PE and APC fluorescence and cyanobacteria have the ability to autofluorescence as they contain the natural pigments chlorophyll a, phycoerythrin and allophycocyanin (Telford et al. 2001; Dennis et al., 2011). Therefore, these natural pigments in photosynthetic microorganisms including cyanobacteria can be rapidly detected for real time monitoring without additional staining. Cyanobacterial profiling can be determined by comparing the flow cytometric results from water samples to pure cultures. *Synechococcus* sp. IU 625, a unicellular cyanobacterium, was used as a general algal bloom indicator and *Microcystis aeruginosa* as well as *Cylindrospermum* spp. were used as toxic algal bloom indicators (WHO, 1999; USEPA 2015a). Thus, this method can potentially help detect early harmful algal blooms on freshwater bodies that are provided for public drinking water and recreational activities.

Flow cytometry can be implemented as a tool for analyzing routine environmental water samples for the prevention of algal blooms (Dennis et al., 2011). Its sensitivity allows accurate measurements and detection because it's sufficient to even analyze submicron particles (Dubelaar et al., 2000; Read et al., 2014). Therefore, a high throughput of environmental water samples can run through the flow cytometer to quickly reveal a profile of species. In addition, this instrument can even be used to detect

low level toxic species to help find and identify an early harmful algal bloom (Cellamare et al., 2010). Polymerase Chain Reaction (PCR) can also be utilized as a supplement to flow cytometry to validate and verify the specific cyanobacterial presence in the water. Thus, flow cytometry can be used to rapidly determine a profile of cyanobacteria from water samples and PCR can help confirm and validate these results.

Figure 1: A cyanobacterial bloom with a dense green mat appearance is evident at Clark Reservoir, Clark Township, Middlesex County, NJ during summer-fall 2015.

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Materials and Methods

Water Sample Collection and Processing

Water samples from 36 water bodies in New Jersey were collected by NJDEP in 250 ml Nalgene bottles during summer 2015. The GPS coordinates as well as water temperature (°C), pH and dissolved oxygen (mg/L) were measured and recorded on site. The water samples were refrigerated until filtration.

Cyanobacteria Culture Maintenance

5 ml of American Type Culture Collection (ATCC) Axenic *Synechococcus* sp. IU 625 (S. IU 625) strain was maintained in 95 ml sterilized Mauro's Modified Medium (3M with a pH of 7.9) in a 250 ml Erlenmeyer flask (Chu et al., 2012). The cells were grown in a Gyromax 747T incubator shaker (Amerex Instruments, Lafeyette, CA) that contained continuous fluorescent lighting, a temperature of 26°C and a rotation of 100 rpm. *Microcystis* and *Cylindrospermum* were obtained from Carolina Biological (Carolina Biological Supply Company, Burlington, NC) in a stock culture with Alga-Gro® Freshwater medium. 3 ml was inoculated from this stock into a 50 ml Erlenmeyer flask along with 5 ml of 3M and these cultures were each grown separately on an Innova 2000 shaker (New Brunswick Scientific, Enfield, CT, USA). A Pharmacia Ultraspec III Spectrophotometer (Pharmacia LKB, Sweden) was utilized to monitor the growth at an OD_{750nm} until the middle of log phase was reached. Then, 5 ml of these separate cultures were inoculated into a 250 ml Erlenmeyer flask along with 95 ml of 3M and these cultures were now grown under the Gyromax 747 T incubator shaker. A mixed

cyanobacteria culture containing S. IU 625, *Microcystis*, *Cylindrospermum*, *Synechococcus elongatus* PCC 7942 and *Synechocystis* PCC 6803 were also maintained in a 250 ml Erlenmeyer flask that contained 5 ml of culture and 95 ml of 1X BG-11 medium (Sigma Aldrich, St. Louis, MO).

Filtration

A Thermo Scientific™ Nalgene™ (Thermo Scientific, Rochester, NY) vacuum filtration unit was assembled and polycarbonate membrane filters (Sterlitech Corporation, Kent, WA) were placed onto the filter holder using sterile forceps. The water samples that were stored in a Nalgene bottle were mixed thoroughly and poured through the 30 µm membrane of the filtration unit. The filter was then placed on sterile aluminum foil using sterile forceps and once dried the aluminum foil was folded and put into the 4°C refrigerator. The filtrate was mixed thoroughly and poured into a 50 ml conical tube. Most of the remaining filtrate was then poured onto another filtration unit that contained a 5 µm membrane and this whole process was repeated for 0.4µm and 0.1µm membranes.

Chelex DNA Extraction

A Chelex DNA extraction was performed utilizing a modified protocol from (Chu and Rienzo, 2013). For the DNA extractions of the water samples, 1.5 ml of the sample was added into a 1.7 ml microcentrifuge tube and then centrifuged at 10,000 rpm for 3 minutes using a Denville 260D microcentrifuge (Denville Scientific, South Plainfield, NJ, USA) and 200 µl of Chelex InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA)

was added. The samples were then vortexed for 10 seconds and placed into a Polyscience Temperature Water Bath (Polysciences, Niles, IL) for two hours at 56°C. Thereafter, the tubes were placed into an ISOTEMP125D Heat Block (Fisher Scientific, Pittsburg, PA) that was set to 100°C and incubated for about 8 minutes. These samples were then centrifuged at 14,000 rpm for 12 minutes. The supernatant (DNA) was then transferred into a different 1.7 ml microcentrifuge tube. The DNA samples were then measured for the concentration yield and purity (A260/280 nm) using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). The same process also occurred for genomic DNA extractions for the standards except 500 μl of the standard cultures were added directly into a microcentrifuge tube and were then immediately centrifuged. In addition, during the heat block step the incubation occurred for 12 minutes instead of 8 minutes.

PCR (Polymerase Chain Reactions)

PCR was conducted for the amplification of the environmental DNA samples. The specific primers utilized for this purpose were found through literature for the presence of specific cyanobacteria or for specific toxins induced by cyanobacteria. The lists of primers used for this study are located in Table 1. Each PCR reaction tube contains 6 μl sterile deionized water, 2.5 μl dimethyl sulfoxide (DMSO), 1 μl of forward primer, 1 μl of reverse primer, 2 μl of DNA (~10 ng) and 12.5 μl of 2X GoTaq® Hot Start Green Master Mix (Promega), a total of 25 μl reaction. A Veriti 96 well Thermocycler (Applied Biosystems, Carlsbad, CA, USA) was utilized with the primary

denaturation of 95°C for 2 minutes followed by a secondary denaturation of 95°C for 45 seconds. Thereafter, the PCR continued with annealing between 50 to 60°C based on primer set for 45 seconds, first extension at 72°C for 45 to 50 seconds and a final extension step at 72°C for 5 minutes. An additional step of 4°C occurred to ensure the amplified PCR product did not degrade.

Gel Electrophoresis

Gel Electrophoresis was conducted for the visualization of the amplified DNA samples. A 1% agarose gel was created with SYBR® Safe (Invitrogen) DNA intercalating agent. This gel was then placed into a Denville MIDI (Denville Scientific Inc.) gel electrophoresis apparatus that was submerged with 1X TAE buffer (Fermentas ThermoFisher Scientific) with a voltage setting of 100 to 105V. The gel was visualized under a 2UV Transilluminator Gel Docit System (UVP, Upland, CA).

Flow Cytometry

Flow cytometry was conducted by using a MACSQuant® Analyzer (Miltenyi Biotec, Inc., SanDiego, CA). The three standard control cultures *Microcystis*, *S*. IU 625 and *Cylindrospermum* spp. with OD_{750nm} of 0.591, 0.521 and 0.640 respectively were run through the flow cytometer at fixed voltage settings with various dilutions. The water samples for each site and the serial filtrates of < 30 µm, < 5 µm and < 0.4 µm were run through the flow cytometer with the same voltage settings as the standard controls. The analysis was conducted using the software FlowJo vX.0.7 (Tree Star, Inc., Ashland, OR).

Table 1. A list of primer sets utilized to perform PCR that were found in literature studies. The table displays the primer name, the nucleotide sequence (5'-3'), annealing temperature (°C), amplicon size (nt), gene, species and the reference source.

Primer	Sequence (5'-3')	Tm (°C)	Amplicon (nt)	Gene	Species	Reference
PSf	GGGATTAGATACCCCWGTAGTCCT	50	735	16s rRNA	General phyto-specific	Stiller & McClanahan, 2005
Ur	ACGGYTACCTTGTTACGACTT					
CYA106_16sf	CGGACGGGTGAGTAACGCGTGA	50	665	16s rRNA	General Cyanobacteria	Nubel et al., 1997
CYA781_16sr	GACTACWGGGGTATCTAATCCCWTT					
16s_19f	AAGCCTGACGGAGCAACGCC	50	393	ssu rDNA	General cyanobacteria *Synechococcus*	Sanchez-Baracaldo et al., 2008
16s_409r	GGTATCTAATCCCTTTCGCTCC					
MICf	ATGTGCCGCGAGGTGAAACCTAAT	55	200	16s rRNA	Microcystis	Hotto et al., 2007
MICr	TTACAAYCCAARRRCCTTCCTCCC					
MSf	ATCCAGCAGTTGAGCAAGC	50	1369	*mcy*A	Various	Tillet et al., 2001
MSr	TGCAGAAAACTCCGCAGTTG					
CPC1f	GGCKGCYTGYYTRCGYGACATGGA	50	389	Phycocyanin gene β-subunit	General for Cyanobacteria	Kim et al., 2006
CPC1r	AARCGNCTTGVGWATCDGC					

Results

Water collection sites

Water samples from the 36 waterbodies, 14 counties were collected by NJDEP throughout summer and fall in 2015. Water properties were measured and recorded for all sites. Table 2 lists the information about 36 water collection sites including the site name, county, municipal, latitude, longitude, water temperature (°C), pH, and dissolved oxygen (mg/L). Among all water sample collected and tested, the water temperature ranged from 16.97 (NJLM 0213) to 30.9°C (Alloway Lake). The pH ranged from 4.09 (Mt. Misery Lake) to 9.75 (Cooper Lake) with majority sites were between 6 and 9. The dissolved oxygen ranged from 1.66 (NJLM 0213) to 19.1 mg/L (Cooper Lake).

Table 2.   Information on the 36 water bodies displaying the site name, county, municipal, latitude, longitude, water temperature (°C), pH, and dissolved oxygen (mg/L).

Site Name	County	Municipal	Latitude	Longitude	Water Temp (°C)	pH	Dissolved O$_2$ (mg/L)
Atlantic City Reservoir	Atlantic	Egg Harbor Township	39.4395711	-74.55689	20.73	7.16	9.95
NJLM-1286	Bergen	Franklin Lakes Borough	41.0281391	-74.19290831	28.49	9.18	14.64
Deverson Lake	Burlington	Pemberton Township	39.90935	-74.57618	26.84	5.03	5.97
Lake Pachoango		Evesham Township	39.807790	-74.880049	29.35	6.79	11.96
Lake Pemberton		Pemberton Township	39.95848	-74.66996	28.72	6.38	10.21
Mt. Misery Lake			39.9257820	-74.5260940	18.41	4.09	6.35
Mirror Lake			39.97477	-74.56182	21.65	5.89	7.13
NJLM-0315		Shamong Township	39.77161957	-74.71541127	22.35	6.24	7.05
NJLM-0754		Medford Township	39.816796	-74.860840	22.95	6.92	9.81
Hands Mill Pond	Cumberland	Maurice River Township	39.2432870	-74.9015740	28.08	4.44	7.65
Union Lake		Millville City	39.41864	-75.06378	27.29	7.48	9.2
Cooper Lake	Gloucester	Logan Township	39.801655	-75.332575	24.88	9.75	19.17
Iona Lake		Franklin Township	39.598586	-75.084098	24.89	6.33	5.07
Franklinville Lake			39.621302	-75.077138	26.78	5.53	3.09
McCarthys Lakes			39.573972	-74.925978	26.45	6.27	8.21
NJLM-0489		Logan Township	39.7495	-75.3807	30.09	9.38	10.77
Amwell Lake	Hunterdon	West Amwell Township	40.405095	-74.91248	28.25	8.7	9.97

Site Name	County	Municipal	Latitude	Longitude	Water Temp (°C)	pH	Dissolved O$_2$ (mg/L)
Roosevelt County Park Lake	Middlesex	Edison Township	40.5529	-74.3394	29.2	8.73	13.42
NJLM-1034	Monmouth	Upper Freehold Township	40.166283	-74.496989	20.32	6.95	9.87
Cifrese Lake (NJLM-1045)	Morris	Randolph Township	40.818504	-74.608268	28.17	9.2	10.18
Lake Morski Oko		Jefferson Township	41.027069	-74.542093	22.64	7	6.57
Prospertown Lake	Ocean	Jackson Township	40.133337	-74.4412	27.43	6.64	7.44
Green Turtle Lake	Passaic	West Milford Township	41.148516	-74.329033	28.89	8.92	8.92
Alloway Lake		Alloway Township	39.572417	-75.351820	30.9	8.03	9.59
Parvin Lake	Salem	Pittsgrove Township	39.5098	-75.13545	27.8	6.86	8.17
Rainbow Lake			39.49055	-75.11241	28.1	6.83	7.11
Great Gorge Lake		Vernon Township	41.161202	-74.523444	26.57	9.24	8.89
Hunts Pond		Fredon Township	41.004037	-74.846306	26.67	8.25	8.95
Laidlaw Pond		Sparta Township	41.005938	-74.626666	27.01	8.43	8.1
Mashipacong Pond		Montague Township	41.269306	-74.726483	26.56	6.2	7.04
NJLM-0213		Hampton Township	41.1419850	-74.8365110	16.97	6.03	1.66
NJLM-0378	Sussex	Montague Township	41.28154931	-74.80019941	25.8	6.21	2.65
Silver Lake		Hardyston Township	41.12171900	-74.5532404	26.7	6.39	3.32
Upper East Highland Lake		Vernon Township	41.16415	-74.4553	26.87	8.51	7.92
Watchu Pond		Byram Township	40.930143	-74.769397	25.17	6.83	3.69
Deer Park Pond	Warren	Allamuchy Township	40.9062	-74.7947770	28.2	7.15	6.81

Figure 2: A map of 36 water collection sites in New Jersey. Each of the 14 counties is color-coded along with corresponding site number.

Flow cytometry

Flow cytometric analysis of *Microcystis aeruginosa*, *Synechococcus* sp. IU 625 and *Cylindrospermum* spp.

The three reference species were measured and analyzed through flow cytometry. The results revealed that *Microcystis aeruginosa* when measured and analyzed with Phycoerythrin and Allophycocyanin is detectable at an accurate range from an OD_{750nm} of 0.001 and 4,196 cells/ml up to an OD_{750nm} of 0.591 and 2.0×10^6 cells/ml (Figure 3). *Synechococcus* sp. IU 625, when measured and analyzed with Phycoerythrin and Allophycocyanin is detectable at an accurate range from an OD_{750nm} of 0.008 and cell count of 2,787 cells/ml up to an OD_{750nm} of 0.521 and 187,800 cells/ml (Figure 4). *Cylindrospermum* spp., when measured and analyzed with Phycoerythrin and Allophycocyanin is detectable at an accurate range from an OD_{750nm} of 0.001 and cell count of 1,479 cells/ml up to an OD_{750nm} of 0.640 and 7.21×10^5 cells/ml (Figure 5).

Figure 3. Overlay histogram of Phycoerythrin (left) and Allophycocyanin (right) for *Microcystis aeruginosa*. The range is detectable from an OD_{750nm} of 0.001 and 4,196 cells/ml up to an OD_{750nm} of 0.591 and 2.0 x 10^6 cells/ml..

Figure 4. Overlay histogram of Phycoerythrin (left) and Allophycocyanin (right) for *Synechococcus* sp. IU 625. The range is detectable from an OD_{750nm} of 0.008 and cell count of 2,787 cells/ml up to an OD_{750nm} of 0.521 and 187,800 cells/ml.

Figure 5. Overlay histogram of Phycoerythrin (left) and Allophycocyanin (right) for *Cylindrospermum* spp. The range is detectable from an OD_{750nm} of 0.001 and cell count of 1,469 cells/ml up to an OD_{750nm} of 0.640 and 7.21 x 10^5 cells/ml

Flow cytometric analysis of water samples collected from Atlantic County

Atlantic City Reservoir water sample from Atlantic County was collected and analyzed. This water site indicated the presence of phycoerythrin-rich species while there is little to no allo-phycocyanin species detected (Figure 6).

Three pure cyanobacterial strains, *Cylindrospermum* spp., *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 were included in the forward scatter-phycoerythrin profile analysis. The result showed the phytoplanktons in Atlantic City Reservoir could contain mostly *Microcystis aeruginosa* and *Cylindrospermum* spp. related species (Figure 7).

Figure 6. Histogram of phycoerythrin and allophycocyanin among 3 water samples from Atlantic City Reservoir. Results showed Atlantic City Reservoir contain phycoerythrin-rich species but no significant allophycocyanin was detected.

Figure 7. Forward scatter-phycoerythrin profile analysis of Atlantic City Reservoir in Atlantic County and 3 reference species: Cylindrospermum (Red) Microcystis aeruginosa (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). Atlantic City Reservoir showed mostly *Microcystis aeruginosa* and *Cylindrospermum* spp. related species.

Flow cytometric analysis of water samples collected from Bergen County

NJLM 1286 (Shadow Lake) water sample from Bergen County was collected and analyzed. This water site indicated the presence of phycoerythrin-rich species, while little to no allophycocyanin species detected (Figure 8).

Three pure cyanobacterial strains, *Cylindrospermum* spp., *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 were included in the forward scatter-phycoerythrin profile analysis. The result showed the phytoplanktons in NJLM 1286 (Shadow Lake) could contain mostly Microcystis and *Synechococcus* sp. IU 625 related species (Figure 9).

Figure 8. Histogram of phycoerythrin and allophycocyanin for NJLM 1286 (Shadow Lake) water sample in Bergen County. The results suggested this site contains phycoerythrin-rich species and little to no allophycocyanin.

Figure 9. Forward scatter-phycoerythrin profile analysis of NJLM 1286 (Shadow Lake) and *Cylindrospermum* spp. (Red), *Microcystis aeruginosa* (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). NJLM 1286 (Shadow Lake) showed mostly Microcystis and *Synechococcus* sp. IU 625 related species.

Flow cytometric analysis of water samples collected from Burlington County

A total of seven water bodies from Burlington County were collected and analyzed. All sites indicated the presence of phycoerythrin-rich species, while there is little to no allophycocyanin species detected (Figures 10 and 11).

Three pure cyanobacterial strains, *Cylindrospermum* spp., *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 were included in the forward scatter-phycoerythrin profile analysis. The result showed the phytoplanktons in Deverson Lake, Lake Pachoango and Lake Pemberton could contain all three groups and their related species (Figure 12). Meanwhile, Mt. Misery mostly has *Microcystis aeruginosa* related species (Figure 13). Mirror Lake, NJLM 0315, and NJLM 0754 showed mostly *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 related species. (Figure 14).

Figure 10. Histogram of phycoerythrin among 16 water samples from 7 freshwater bodies in Burlington County. The results suggested all sites contain phycoerythrin-rich species.

Figure 11. Histogram of allophycocyanin among 16 water samples from 7 freshwater bodies in Burlington County. No significant allophycocyanin was detected in Burlington County water samples.

Figure 12. Forward scatter-phycoerythrin profile analysis of sites #3-5 in Burlington County and Cylindrospermum (Red) Microcsytis (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). (A) Deverson Lake, (B) Lake Pachoango and (C) Lake Pemberton showed all three related species.

Figure 13. Forward scatter-phycoerythrin profile analysis of Mt. Misery Lake (site #6) in Burlington County and Cylindrospermum (Red) Microcsytis (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). Mt. Misery Lake showed mostly *Microcystis aeruginosa* related species.

Figure 14. Forward scatter-phycoerythrin profile analysis of sites #7-9 in Burlington County and Cylindrospermum (Red) Microcsytis (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). (A) Mirror Lake, (B) NJLM 0315, and (C) NJLM 0754 showed mostly *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 related species.

Flow cytometric analysis of water samples collected from Cumberland County

A total of two water bodies from Cumberland County were collected and analyzed. All sites indicated the presence of phycoerythin-rich species, while allophycocyanin species were only detected in McCarthys Lakes (Figure 15).

Three pure cyanobacterial strains, *Cylindrospermum* spp., *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 were included in the forward scatter-phycoerythrin profile analysis. The result showed the phytoplanktons in Hands Mill and Union Lake could contain all three groups and their related species (Figure 16).

Figure 15. Histogram of phycoerythrin among 4 water samples from 2 freshwater bodies in Cumberland County. The results suggested all sites contain phycoerythrin-rich species and no significant allophycocyanin was detected.

Figure 16. Forward scatter-phycoerythrin profile analysis with sites #10-11 and Cylindrospermum (Red) Microcsytis (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). (A) Hands Mill Pond and (B) Union Lake showed all three related species.

Flow cytometric analysis of water samples collected from Gloucester County

A total of five water bodies (sites #12-16) from Gloucester County were collected and analyzed. All sites indicated the presence of phycoerythin-rich species (Figure 17), while allophycocyanin species were only detected in McCarthys Lakes (Figure 18).

Three pure cyanobacterial strains, *Cylindrospermum* spp., *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 were included in the forward scatter-phycoerythrin profile analysis. The result showed the phytoplanktons in Cooper Lake could contain all three groups and their related species (Figure 19). Meanwhile, Franklinville Lake potentially has mainly *Microcystis aeruginosa* related species and NJLM 0489 revealed primarily *Cylindrospermum* spp. related species (Figure 20). Iona Lake, showed mostly *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 related species and McCarthys Lake showed mostly *Microcystis aeruginosa* and *Cylindrospermum* spp. related species (Figure 21).

Figure 17. Histogram of phycoerythrin among 8 water samples from 5 freshwater bodies in Gloucester County. The results suggested all sites contain phycoerythrin-rich species.

Figure 18. Histogram of allophycocyanin among 8 water samples from 5 freshwater bodies in Burlington County. Allophycocyanin was detected in McCarthys Lake water sample.

Figure 19. Forward scatter-phycoerythrin profile analysis of Cooper Lake (site #12) and Cylindrospermum (Red) Microcsytis (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). Results showed that Cooper Lake contained all three related species.

Figure 20. Forward scatter-phycoerythrin profile analysis of sites 14, 16 and Cylindrospermum (Red) Microcsytis (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). (A) Franklinville Lake contained mostly *Microcystis aeruginosa* related species and (B) NJLM 0489 contained primarily *Cylindrospermum* spp. related species.

Figure 21. Forward scatter-phycoerythrin profile analysis of sites #13, 15, and Cylindrospermum (Red) Microcsytis (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). (A) Iona Lake showed primarily *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 related species. (B) McCarthys Lake contained mostly *Microcystis aeruginosa* and *Cylindrospermum* spp. related species.

Flow cytometric analysis of water samples collected from Hunterdon County

Amwell Lake from Hunterdon Country was collected and analyzed. This water site indicated the presence of phycoerythin-rich species, while there is little to no allo-phycocyanin species detected (Figure 22).

Three pure cyanobacterial strains, *Cylindrospermum* spp., *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 were included in the forward scatter-phycoerythrin profile analysis. The result showed the phytoplanktons in Amwell Lake could contain mainly *Microcystis aeruginosa* and *Cylindrospermum* spp. related species (Figure 23).

Figure 22. Histogram of phycoerythrin and allophycocyanin for Amwell Lake water sample in Hunterdon County. The results suggested this site contains phycoerythrin-rich species and little to no significant allophycocyanin.

Figure 23. Forward scatter-phycoerythrin profile analysis of Amwell Lake (site #17) and Cylindrospermum (Red) Microcsytis (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). Amwell Lake showed mostly *Microcystis aeruginosa* and *Cylindrospermum* spp. related species.

Flow cytometric analysis of water samples collected from Middlesex County

Roosevelt County Park Lake water sample from Middlesex County was collected and analyzed. This water site indicated the presence of phycoerythrin-rich species and some allophycocyanin species detected (Figure 24).

Three pure cyanobacterial strains, *Cylindrospermum* spp., *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 were included in the forward scatter-phycoerythrin profile analysis. The result showed the phytoplanktons in Roosevelt Lake could contain mostly *Cylindrospermum* spp. related species (Figure 25).

Figure 24. Histogram of phycoerythrin and allophycocyanin for Roosevelt Lake water sample in Middlesex County. The results suggested this site contains phycoerythrin-rich species and some significant allophycocyanin.

Figure 25. Forward scatter-phycoerythrin profile analysis of Roosevelt County Park Lake (site # 18) and Cylindrospermum (Red) *Microcystis aeruginosa* (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). Roosevelt County Park Lake showed mainly *Cylindrospermum* spp. related species.

Flow cytometric analysis of water samples collected from Monmouth County

NJLM 1034 water sample from Monmouth County was collected and analyzed. This water site indicated the presence of phycoerythrin-rich species while there is little to no allophycocyanin species detected (Figure 26).

Three pure cyanobacterial strains, *Cylindrospermum* spp., *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 were included in the forward scatter-phycoerythrin profile analysis. The result showed the phytoplanktons in NJLM 1034 water body could contain mainly *Cylindrospermum* spp. related species and an undetermined group (Figure 27).

Figure 26. Histogram of phycoerythrin and allophycocyanin for 2 water samples from NJLM 1034 water boy in Monmouth County. The results suggested this site contains phycoerythrin-rich species and little to no allophycocyanin

Figure 27. Forward scatter-phycoerythrin profile analysis of NJLM 1034 (site #19) and Cylindrospermum (Red) *Microcystis aeruginosa* (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). NJLM 1034 water body showed mainly *Cylindrospermum* spp. related species as well as an undetermined group.

Flow cytometric analysis of water samples collected from Morris County

NJLM 1045 (Cifrese Lake) and Lake Morski Oko water sample from Morris County were collected and analyzed. This water site indicated the presence of phycoerythrin-rich species and some allo-phycocyanin species detected for Lake Morski Oko (Figure 28).

Three pure cyanobacterial strains, Cylindrospermum sp., *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 were included in the forward scatter-phycoerythrin profile analysis. The result showed the phytoplanktons in NJLM 1045 (Cifrese Lake) could contain *Microcystis aeruginosa* as well as *Synechococcus* spp. related species (Figure 29A). Meanwhile, Lake Morski Oko showed all three and their related species (Figure 29B).

Figure 28. Histogram of phycoerythrin and allophycocyanin for 3 water samples from NJLM 1045 (Cifrese Lake) and Lake Morski Oko in Morris County. The results suggested these site contains phycoerythrin-rich species and some significant allophycocyanin for Lake Morski Oko.

Figure 29. Forward scatter-phycoerythrin profile analysis of sites #20-21 and Cylindrospermum (Red) *Microcystis aeruginosa* (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). (A) NJLM 1045 showed mostly *Microcystis aeruginosa* and *Synechococcus* spp. related species and (B) Lake Morski Oko shows all three and its related species as well as an additional undetermined group.

Flow cytometric analysis of water samples collected from Ocean County

Prospertown Lake water sample from Ocean County was collected and analyzed. This water site indicated the presence of phycoerythrin-rich species while there is little to no allophycocyanin species detected (Figure 30).

Three pure cyanobacterial strains, *Cylindrospermum* spp., *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 were included in the forward scatter-phycoerythrin profile analysis. The result showed the phytoplanktons in Prospertown Lake could mainly contain Cylindrospermum related species (Figure 31).

Figure 30. Histogram of phycoerythrin and allophycocyanin for 2 water samples from Prospertown Lake in Ocean County. The results suggested this site contains phycoerythrin-rich species and little to no allophycocyanin.

Figure 31. Forward scatter-phycoerythrin profile analysis with Prospertown Lake (site #22) and Cylindrospermum (Red) *Microcystis aeruginosa* (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). Prospertown Lake showed mostly *Cylindrospermum* spp. related species.

Flow cytometric analysis of water samples collected from Passaic County

Green Turtle Lake water sample from Passaic County was collected and analyzed. This water site indicated the presence of phycoerythrin-rich species and allo-phycocyanin- rich species (Figure 32).

Three pure cyanobacterial strains, *Cylindrospermum* spp., *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 were included in the forward scatter-phycoerythrin profile analysis. The result showed the phytoplanktons in Green Turtle Lake could contain mostly *Cylindrospermum* spp. related species (Figure 33).

Figure 32. Histogram of phycoerythrin and allophycocyanin for 2 water samples from Green Turtle Lake in Passaic County. The results suggested this site contained phycoerythrin and allophycocyanin-rich species.

Figure 33. Forward scatter-phycoerythrin profile analysis of Green Turtle Lake (site #23) and Cylindrospermum (Red) *Microcystis aeruginosa* (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). Green Turtle Lake showed mainly *Cylindrospermum* spp. related species and an additional undetermined group.

Flow cytometric analysis of water samples collected from Salem County

A total of three water bodies from Salem County were collected and analyzed. All sites indicated the presence of phycoerythin-rich species, while allophycocyanin species were only detected in Rainbow Lake (Figures 34-35).

Three pure cyanobacterial strains, *Cylindrospermum* spp., *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 were included in the forward scatter-phycoerythrin profile analysis. The result showed the phytoplanktons in these three water bodies could contain all three groups and their related species (Figure 36).

Figure 34. Histogram of phycoerythrin among 6 water samples from 3 freshwater bodies in Salem County. The results suggested all sites contained phycoerythrin-rich species.

Figure 35. Histogram of allophycocyanin among 6 water samples from 3 freshwater bodies in Burlington County. The results suggested Allophycocyanin was detected in Rainbow Lake.

Figure 36. Forward scatter-phycoerythrin profile analysis of sites #24-26 and Cylindrospermum (Red) *Microcsytis aeruginosa* (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). (A) Alloway Lake, (B) Parvin Lake and (C) Rainbow Lake show all three reference species and their related species.

Flow cytometric analysis of water samples collected from Sussex County

A total of nine water bodies from Sussex County were collected and analyzed. All sites indicated the presence of phycoerythin-rich species, while there is little to no allo-phycocyanin species detected (Figure 37 and 38).

Three pure cyanobacterial strains, *Cylindrospermum* spp., *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 were included in the forward scatter-phycoerythrin profile analysis. The result showed the phytoplanktons in Great Gorge Lake, NJLM 0213, NJLM 0378 (Figure 39-1) as well as Silver Lake, Upper East Highland and Watchu Pond (Fig. Figure 39-2) could contain all three groups and their related species. Meanwhile, Hunts Pond mostly contained *Cylindrospermum* spp. related species (Figure 40A). Laidlaw Pond showed mainly *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 related species (Figure 40B), whereas Mashipacong Pond showed mostly *Microcystis aeruginosa* and *Cylindrospermum* spp. related species (Figure 40C).

Figure 37. Histogram of phycoerythrin among 13 water samples from 9 freshwater bodies in Sussex County. The results suggested all sites contain phycoerythrin-rich species

Figure 38. Histogram of allophycocyanin among 14 water samples from 9 freshwater bodies in Burlington County. No significant allophycocyanin was detected in Sussex County water samples.

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Figure 39-1. Forward scatter-phycoerythrin profile analysis of sites #27, 31, 32 and Cylindrospermum (Red) *Microcystis aeruginosa* (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). (A) Great Gorge Lake, (B) NJLM 0213, and (C) NJLM 0378 showed all three references and their related species.

Figure 39-2. Forward scatter-phycoerythrin profile analysis of sites #33-35 and Cylindrospermum (Red) *Microcystis aeruginosa* (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). (D) Silver Lake, (E) Upper East Highland, and (F) Watchu Pond showed all three references and their related species.

Figure 40. Forward scatter-phycoerythrin profile analysis of sites #28-30 and Cylindrospermum (Red) Microcystis aeruginosa (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). (A) Hunts Pond contained mostly *Cylindrospermum* spp. related species. (B) Laidlaw Pond showed mostly *Microcsytis aeruginosa* and *Synechococcus* sp. IU 625 related species. (C) Mashipacong Pond contained mostly *Microcystis aeruginosa* and *Cylindrospermum* spp. related species.

Flow cytometric analysis of water samples collected from Warren County

Deer Park Pond water sample from Warren County was collected and analyzed. This water site indicated the presence of phycoerythrin-rich species, while little to no allo-phycocyanin species detected (Figure 41).

Three pure cyanobacterial strains, *Cylindrospermum* spp., *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 were included in the forward scatter-phycoerythrin profile analysis. The result showed the phytoplanktons in Deer Park Pond could contain mostly *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 related species (Figure 42).

Figure 41. Histogram of phycoerythrin and allophycocyanin of 2 water samples from Deer Park Pond in Warren County. The results suggested this site contains phycoerythrin-rich species and little to no allophycocyanin.

Figure 42. Forward scatter-phycoerythrin profile analysis of Deer Park Pond (site #36) and Cylindrospermum (Red) *Microcystis aeruginosa* (Orange) as well as *Synechococcus* sp. IU 625 (Light blue). Deer Park Pond shows mostly *Microcystis aeruginosa* and *Synechococcus* sp. IU 625 related species.

Figure 43. Flow cytometric summary on map for the profile analysis of Atlantic City Reservoir from Atlantic County. M represents *Microcystis aeruginosa* like species, S represents *Synechococcus* sp. IU 625 related species, and C represents *Cylindrospermum* spp. like species.

Figure 44. Flow cytometric summary on map for the profile analysis of NJLM 1286 (Shadow Lake) from Bergen County. M represents *Microcystis aeruginosa* like species, S represents *Synechococcus* sp. IU 625 related species, and C represents *Cylindrospermum* spp. like species.

Figure 45. Flow cytometric summary on map for the profile analysis of the sites from Burlington County. M represents *Microcystis aeruginosa* like species, S represents *Synechococcus* sp. IU 625 related species, and C represents *Cylindrospermum* spp. like species.

Figure 46. Flow cytometric summary on map for the profile analysis of the sites from Cumberland County. M represents *Microcystis aeruginosa* like species, S represents *Synechococcus* sp. IU 625 related species, and C represents *Cylindrospermum* spp. like species.

Figure 47. Flow cytometric summary on map for the profile analysis of the sites from Gloucester County. M represents *Microcystis aeruginosa* like species, S represents *Synechococcus* sp. IU 625 related species, and C represents *Cylindrospermum* spp. like species.

Figure 48. Flow Cytometric summary on map for the profile analysis of Amwell Lake from Hunterdon County. M represents *Microcystis aeruginosa* like species, S represents *Synechococcus* sp. IU 625 related species, and C represents *Cylindrospermum* spp. like species.

Figure 49. Flow Cytometric summary on map for the profile analysis of Roosevelt County Park Lake from Middlesex County. M represents *Microcystis aeruginosa* like species, S represents *Synechococcus* sp. IU 625 related species, and C represents *Cylindrospermum* spp. like species.

Figure 50. Flow cytometric summary on map for the profile analysis of NJLM 1034 from Monmouth County. M represents *Microcystis aeruginosa* like species, S represents *Synechococcus* sp. IU 625 related species, and C represents *Cylindrospermum* spp. like species.

Figure 51. Flow cytometric summary on map for the profile analysis of sites from Morris County. M represents *Microcystis aeruginosa* like species, S represents *Synechococcus* sp. IU 625 related species, and C represents *Cylindrospermum* spp. like species.

Figure 52. Flow cytometric summary on map for the profile analysis of Prospertown Lake from Ocean County. M represents *Microcystis aeruginosa* like species, S represents *Synechococcus* sp. IU 625 related species, and C represents *Cylindrospermum* spp. like species.

Figure 53. Flow cytometric summary on map for the profile analysis of Green Turtle Lake from Passaic County. M represents *Microcystis aeruginosa* like species, S represents *Synechococcus* sp. IU 625 related species, and C represents *Cylindrospermum* spp. like species.

Figure 54. Flow cytometric summary on map for the profile analysis of sites from Salem County. M represents *Microcystis aeruginosa* like species, S represents *Synechococcus* sp. IU 625 related species, and C represents *Cylindrospermum* spp. like species.

Figure 55. Flow cytometric summary on map for the profile analysis of sites from Sussex County. M represents *Microcystis aeruginosa* like species, S represents *Synechococcus* sp. IU 625 related species, and C represents *Cylindrospermum* spp. like species.

Figure 56. Flow cytometric summary on map for the profile analysis of Deer Park Pond from Warren County. M represents *Microcystis aeruginosa* like species, S represents *Synechococcus* sp. IU 625 related species, and C represents *Cylindrospermum* spp. like species.

DNA Extraction

Chelex DNA extraction was conducted for all 36 collected water samples. The concentration and purity were determined using a NanoDrop (Table 3). These samples were then utilized to perform PCR reactions.

Polymerase Chain Reactions (PCR)

PCR-based assays were carried out with a total of 6 general and specific primer sets (Table 1). 1% Agarose gel electrophoresis were run for all PCR products. The results indicated that phyto-specific microorganisms and Synechococcus related species were detected for all water sites (Figures 57 and 59). General cyanobacteria were detected for most sites except for 3, #3 Deverson Lake, #10 Hands Mill Pond and #14 Franklinville Lake (Figure 58). No microcystin was detected from any site. The was performed for the amplification of DNA from the 36 water body samples to determine and identify the presence of general phytoplankton/photosynthetic bacteria, general cyanobacteria and general *Synechococcus* spp.

Table 3. DNA yield and purity for 36 collected water samples.

Site Name	County	ng/µl	A260/280
Atlantic City Reservoir	Atlantic	3.49	1.8
NJLM 1286	Bergen	4.53	1.57
Deverson Lake	Burlington	22.61	1.91
Lake Pachoango		3.62	1.71
Lake Pemberton		5.71	1.63
Mt. Misery Lake		18.8	1.46
Mirror Lake		21.86	1.52
NJLM 0315		6.27	1.61
NJLM 0754		3.59	1.45
Hands Mill Pond	Cumberland	16.67	1.57
Union Lake		5.32	1.53
Cooper Lake	Gloucester	4.08	1.88
Iona Lake		14.87	1.51
Franklinville Lake		46.95	1.51
McCarthys Lake		9.24	1.66
NJLM 0489		3.77	1.75
Amwell Lake	Hunterdon	3.64	1.66
Roosevelt County Park Lake	Middlesex	5.41	1.54
NJLM 1034	Monmouth	2.25	1.59
NJLM 1045 (Cifrese Lake)	Morris	2.94	1.79
Lake Morski Oko		2.52	2
Prospertown Lake	Ocean	3.79	2.44
Green Turtle Lake	Passaic	2.23	1.86
Alloway Lake	Salem	6.7	1.75
Parvin Lake		5	1.87
Rainbow Lake		4.74	1.68
Great Gorge Lake	Sussex	7.74	1.70
Hunts Pond		4.64	1.56
Laidlaw Pond		4.8	1.8
Mashipacong Pond		6.61	1.65
NJLM 0213		7.42	1.82
NJLM 0378		3.56	2.19
Silver Lake		5.12	1.79
Upper East Highland		3.07	1.81
Watchu Pond		4.48	1.54
Deer Park Pond	Warren	4.13	2.18

Figure 57. 1% agarose gel for phyto-specific species detection with primer set PSf and Ur. All sites showed positive detection.

Figure 58. 1% agarose gel for general cyanobacteria detection with primer set CYA106_16sf and CYA781_16sr. All sites showed positive detection except for 3 sites (#3 Deverson Lake, #10 Hands Mill Pond and #14 Franklinville Lake).

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Figure 59. 1% agarose gel for *Synechococcus* spp. detection with primer set 16s_19f and 16s_409r. All sites showed positive detection for *Synechococcus* spp.

Table 4. Summary of the PCR and flow cytometry results for the 36 water sites. ND stands for non-detectable.

Microorganisms / Site Name	Phytoplankton		Cyanobacteria		*Synechococcus* spp.	
	Flow cytometry	PCR	Flow cytometry	PCR	Flow cytometry	PCR
Atlantic City Reservoir	X	X	X	X	X	X
NJLM-1286 (Shadow Lake)	X	X	X	X	X	X
Deverson Lake	X	X	X	ND	X	X
Lake Pachoango	X	X	X	X	X	X
Lake Pemberton	X	X	X	X	X	X
Mt. Misery Lake	X	X	X	X	X	X
Mirror Lake	X	X	X	X	X	X
NJLM-0315	X	X	X	X	X	X
NJLM-0754	X	X	X	X	X	X
Hands Mill Pond	X	X	X	ND	X	X
Union Lake	X	X	X	X	X	X
Cooper Lake	X	X	X	X	X	X
Iona Lake	X	X	X	X	X	X
Franklinville Lake	X	X	X	ND	X	X
McCarthys Lakes	X	X	X	X	X	X
NJLM-0489	X	X	X	X	X	X
Amwell Lake	X	X	X	X	X	X
Roosevelt County Park Lake	X	X	X	X	X	X
NJLM-1034	X	X	X	X	X	X
Cifrese Lake (NJLM-1045)	X	X	X	X	X	X
Lake Morski Oko	X	X	X	X	X	X
Prospertown Lake	X	X	X	X	X	X
Green Turtle Lake	X	X	X	X	X	X
Alloway Lake	X	X	X	X	X	X
Parvin Lake	X	X	X	X	X	X
Rainbow Lake	X	X	X	X	X	X
Great Gorge Lake	X	X	X	X	X	X
Hunts Pond	X	X	X	X	X	X
Laidlaw Pond	X	X	X	X	X	X
Mashipacong Pond	X	X	X	X	X	X
NJLM-0213	X	X	X	X	X	X
NJLM-0378	X	X	X	X	X	X
Silver Lake	X	X	X	X	X	X
Upper East Highland Lake	X	X	X	X	X	X
Watchu Pond	X	X	X	X	X	X
Deer Park Pond	X	X	X	X	X	X

For flow cytometry the three reference species *Microcystis aeruginosa*, *Synechococcus* sp. IU 625 and *Cylindrospermum* spp. were measured, detected, and analyzed. Based on the result, *Microcystis aeruginosa* is detectable when measured and analyzed with Phycoerythrin and Allophycocyanin from an OD_{750nm} of 0.001 and 4,196 cells/ml up to an OD_{750nm} of 0.591 with 2.0×10^6 cells/ml (Figure 3). *Synechococcus* sp. IU 625 is detectable at an accurate range from an OD_{750nm} of 0.008 and cell count of 2,787 cells/ml up to an OD_{750nm} of 0.521 and 187,800 cells/ml (Figure 4). *Cylindrospermum* spp. is detectable at an accurate range from an OD_{750nm} of 0.008 and cell count of 1,469 cells/ml up to an OD_{750nm} of 0.640 and 7.21×10^5 cells/ml (Figure 5). Thus, these three reference species were utilized as standards to help identify these species or ones related in the water samples.

The initial flow cytometric histogram analysis with the sites compared to standards showed all 36 sites contained pytoplanktons and cyanobacteria with phycoerythrin. McCarthys Lake from Glocuester County, Roosevelt County Park Lake from Middlesex County, Lake Morski Oko from Morris County, Green Turtle Lake from Passaic County and Rainbow Lake from Salem County also contained species with allophycocyanin as seen in Figures 15, 24, 28, 32 and 35, respectively.

A further analysis involving the phycoerythrin/ forward scatter with the water sites compared to the standards allowed the identification of the undetermined water samples to the three references. 17 out of the 36 sites showed all three and their related species as seen in Figures 12A-C, 16A-B, 19, 29B, 36A-C, 39A-F and 42. This is also summarized in Figures 45-47, 51, 55-56. Seven sites showed mostly *Microcystis*

aeruginosa and *S.* IU 625 like species as seen in Figures 8, 13A-C, 21A, 29A and 40B. Six sites showed *Cylindrospermum* spp. like species as seen in Figures 20B, 25, 27, 31, 33, 40A; Green Turtle also showed an undetermined group that may possibly be micro algae due to its large size and phycoerythrin fluorescence (Figure 33). Atlantic City Reservoir (Figure 7), McCarthys Lake (Figure 21), Amwell Lake (Figure 23), and Mashipacong Pond (Figure 40C) showed Microcystis and *Cylindrospermum* spp. like species as seen in Mt. Misery Lake and Franklinville Lake (Figure 20A), showed mostly *Microcystis aeruginosa* like species.

Discussion

In the present study water samples from 36 water bodies throughout 14 New Jersey counties were studied and analyzed. The water chemistry of the water temperature (ºC), pH, and dissolved oxygen were measured by NJDEP as seen in Table 2. Out of all sites NJLM 0213 contained the lowest water temperature and dissolved oxygen, 16.97 ºC and 1.66 mg/L, respectively. The highest water temperature was 30.9 ºC from Alloway Lake. The highest pH and dissolved oxygen was found in Cooper Lake with 9.75 and 19.1 mg/L respectively. The lowest pH was 4.09 from Mt. Misery Lake. The optimal pH for cyanobacteria and their blooms is between 6 and 9 (USEPA, 2015a). Most sites fall within this range as seen again in Table 2, thus indicating most of these sites contain the pH ranges for cyanobacteria to grow and possibly accumulate. The dissolved oxygen is a water property that is crucial where concentrations less than 4 mg/L can be considered hypoxic to the water ecosystem (Paerl et al., 2001). Interestingly, though 4 sites from Sussex County, NJLM 0213, NJLM 0378, Silver Lake and Watchu Pond, contained very little dissolved oxygen of 1.66, 2.65, 3.32 and 3.69 mg/L respectively (Table 2). These sites all contain all three and related species from flow cytometric results as seen in Figures (39 1-2)

Furthermore, based on conducting flow cytometric measurements of the 36 water sites it is possible to obtain results in about 4 to 5 minutes per sample. Thus, this suggests that flow cytometry can rapidly process and accurately detect a large volume of environmental water samples. In addition, a profile of cyanobacteria species can be detected in their live form because of autofluorescence. Moreover, this is highly efficient

because early preventive measurements can be implemented if detection is found early, thus time and money can be saved in the long run if this method becomes a routine for sampling. PCR-based assays were conducted as a complementary method to help validate the flow cytometric analysis results. PCR was performed on the mixed population of DNA from water samples can help determine the presence or absence using general and specific primers for detection. It is evident that all 36 sites contained phytoplankton species when Psf/Ur primer set was utilized as seen in (Figures 57-59) and summarized in (Table 4). Phyto-specific species, cyanobacteria and *Synechococcus* spp. were successfully detected in all 36 collected water sites with both flow cytometry and PCR-based assay, except for 3 sites. PCR was carried out on slightly more specific primer set CYA106_16sf/ CYA781_16sr for the detection of general cyanobacteria and 33 out of 36 sites showed positive bands as seen in (Figure 58) and summarized in Table 4. However, when PCR was conducted for an even more specific primer set 16s_19f/ 16S_409R to detect Synechococcus all 36 sites showed positive bands indicated the genus Synechococcus is present. Since these bands showed up most likely the three bands that didn't show up for the general cyanobacteria could be from the fact that the DNA is from a water site that contains a mixed population so there could be variances. Interestingly, the bands for Msf/ Msr, MICf/MICr and CPC1f/CPC1r were not at detectable levels because many of the sites did contain low DNA concentrations. The primer set Msf/Msr for *mcy*A gene did not contain detectable levels of microcystin which can actually indicate that this is beneficial to the environment and the public health. Moreover, when PCR was utilized on the primer set CPC1f/CPC1r there was no detectable levels of

general cyanobacteria that contained beta subunit of phycocyanin and this correlated fairly well with the flow cytometric analysis as only 5 out of 36 sites revealed phytoplankton and cyanobacteria with allophycocyanin. As mentioned above most of the cyanobacteria or phytoplankton species were mainly phycoerythrin rich.

Future studies can involve the utilization of flow cytometry, PCR and even fluid imaging detection technology from FlowCAM. Flow cytometry can be implemented for rapid routine water sampling, thus sampling larger volume and quantities of fresh water bodies can be achieved. In addition, portable flow cytometry are widely available and can be implemented to detect cyanobacterial species on boat on the various water bodies to allow even more accurate measurements as this will be on site. Moreover, this can allow detection at the same moment of time as sampling to help conduct analysis and various depths of water sample can be collected and analyzed. Furthermore, FlowCAM and its imaging technology can allow detection of larger size species to avoid the possibility of clogging a flow cytometer and circumvent filamentous species that can mistakenly be considered as multiple cells. In other words, flow cytometry can be utilized to detect cyanobacteria from fairly large to as low as submicron particles and FlowCAM can detect large cells (Dubelaar et al., 2000; Read et al., 2014).

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