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Bloom-Forming Cyanobacteria and Other Phytoplankton in Northern New Jersey FreshWater Bodies

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BLOOM-FORMING CYANOBACTERIA AND OTHER PHYTOPLANKTON IN NORTHERN NEW JERSEY FRESHWATER BODIES

By

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degree of Master of Science in Biology from the

Department of Biological Sciences of Seton Hall University

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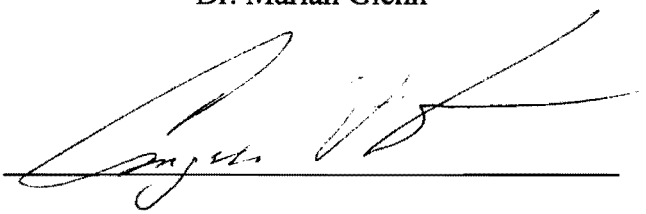

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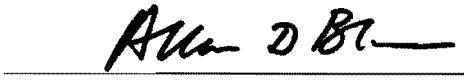

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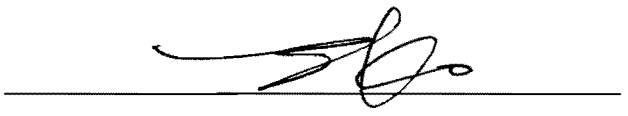

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Abstract

Eutrophication of freshwater ecosystems is a growing problem in urbanized communities. As these freshwater ecosystems receive runoff and pollution from surrounding homes, businesses, industries, and roadways, they become introduced to an excess of nutrients. These nutrients, including nitrogen and phosphorous, allow for microbes and phytoplankton to grow in excess amounts. The overgrowth of phytoplankton, including cyanobacteria and diatoms, is characterized in eutrophication.

Once excess nutrients reach the ecosystem and overgrowth of phytoplankton occurs, a large biomass forms on the surface of the water, known as a Harmful Algal Bloom (HAB). These HABs have several detrimental effects on the water and the living organisms within the water. First, the cyanobacteria and other phytoplankton effectively reduce the dissolved oxygen within the water, limiting life below the water surface. Secondly, the HAB blocks light from penetrating the surface of the water, reducing the amount of photosynthesis needed for plants and other naturally occurring phototrophs. Also, there are several species of cyanobacteria involved in HABs that are known to produce injurious toxins which can harm organisms living in the water as well as humans who come in contact with the water.

In order to prevent the reoccurrence of HABs, the common cyanobacteria and phytoplankton at each ecosystem must be evaluated. In the present study, five freshwater ecosystems (Lakes and Ponds) in Essex County, New Jersey were observed in the summer and fall of 2011. Four sites at each ecosystem were collected and studied for the presence of cyanobacteria, bacteria, and other phytoplankton both through polymerase

chain reaction (PCR) and microscopic observations. Water chemistry was also recorded at each site to determine a correlation between HAB occurrence and pH, dissolved oxygen, and water temperature. Profiles were determined at each of the five ecosystems observed in this study. Flow cytometry-based assays were also carried out. Flow cytometry could potentially be utilized as a complement to microscopic analysis and PCR to identify and profile the cyanobacteria and other phytoplankton among freshwater lakes.

Introduction

Phytoplankton is just as important as it is ubiquitous in the world's freshwater sources. Phytoplankton plays a vast role in the aquatic food chain, as well as a large role in the atmospheric oxygen production (Reynolds, 1984). The term phytoplankton is a general term that can be further subdivided. Phytoplankton includes several groups of algae and bacteria, as well as several other groups. Some of the most common types phytoplankton found in North American freshwater lakes includes species of Bacillariophyceae (diatoms) as well as thousands of species of cyanobacteria.

Diatoms are a type of unicellular phytoplankton that possess several unique contours due to a cell wall composed of silicon dioxide (SiO_2) (Sheath, 2003). The diatoms, or Bacillariophyta, have a distinct structure and thus are easily identifiable in a water sample. The general structure is two halves that fit together, ultimately giving the appearance of a zipper at the midline (Biggs & Kilroy, 2000). Diatomic life is necessary for equilibrium within the lake, providing primary production at the bottom of the food chain. Unlike cyanobacteria, diatoms contain chloroplasts, which possess chlorophyll *a* and *c*. Diatoms can be found in a large range of pH values as well as in ecosystems with a wide concentration of solutes, nutrients, contaminants, and a across a large range of water temperatures (Sheath, 2003). Being so versatile, diatoms are found throughout most freshwaters in the United States and around the world. Along with the ecological parameters of the water body, diatom distribution is also determined by their historical distribution within different geographical regions (Sheath, 2003). Diatom distribution is related to different environmental conditions. Because certain the species are specific to

certain conditions, diatoms have been widely used as environmental indicators and can be used for water quality assessments (Sheath, 2003).

There are many species of cyanobacteria, most commonly found in freshwater lakes and ponds as well as marine environments. Originally called blue-green algae because of their color, cyanobacteria is a phylum of bacteria that uses photosynthesis to obtain energy. Cyanobacteria are prokaryotes. Cyanobacteria do not possess chloroplasts, but all possess the pigment chlorophyll *a,* which is necessary for oxygenic photosynthesis and can be exploited during molecular analysis to detect the presence of cyanobacteria in a sample (Biggs & Kilroy, 2000). Cyanobacteria aided in the transformation of the Earth's atmosphere by producing atmospheric oxygen.

Within the environment, species of cyanobacteria may exist in several forms. Cyanobacteria may exist as unicellular (single-celled) organisms (Synechococcus, Anacystis), filamentous organisms (Anabaena, Oscillatoria), or colonial organisms (Nostoc). Some of the common cyanobacteria found in freshwater sources in North America include Synechococcus, Anabaena, Oscillatoria, Nostoc, and Anacystis (Sheath, 2003).

Although both types of phytoplankton are necessary to maintain equilibrium within freshwater habitats as well as oxygen production, overgrowth in urbanized areas results in the formation of harmful algal blooms, causing deleterious effects to both the aquatic life as well as anything that may use the water. According to the North American Lake Management Society (NALMS), some of the common algal bloom species include

members of the genera Microcystis, Anabaena, and Oscillatoria (Zingone A. & Enevoldsen, 2000).

Essex County, New Jersey, is one of the most densely populated counties in the state of New Jersey, consisting of a population of 783,969 in a land area of 127 square miles (Essex County, 2012). The county includes the municipalities of Bloomfield, Livingston, Milburn, the Oranges, Verona, Belleville, the Caldwells, Cedar Grove, Essex Fells, Fairfield, Glen Ridge, Irvington, Montclair, Roseland, and Nutley, New Jersey. Essex County is a heavily urbanized county located in the New York Metropolitan Area. Essex County contains 12 major highways, as well as three of the nation's major transportation centers (Newark Liberty International Airport, Port Newark, Penn Station), as well as 1,673 miles of public roads (Essex County, 2012). These factors, combined with the massive industrial centers producing goods ranging from chemicals to pharmaceuticals, contribute to the urbanization of the area. Despite being heavily urbanized, Essex County has several parks, freshwater rivers, lakes, and ponds which contribute to the continued efforts in beautification and habitat diversity of the region. The freshwater habitats in this county include Verona Lake in Verona, Clarks Pond in Bloomfield, Branch Brook Lake in Newark, Diamond Mill Pond in Milburn, and South Orange Duck Pond in South Orange, among many other reservoirs throughout the county. These bodies of water, being continually subjected to harmful elements from manmade chemicals and excess nutrient pollution, have seen an increase in phytoplankton blooms. Increases in the amounts of nutrients entering lakes and reservoirs in recent decades in urbanized settings as well as associated changes in the water body's biologics have

contributed to the increase in focus on the problem of nutrient enrichment due to pollution or urbanization, called eutrophication (Reynolds, 1984) (Anderson, 2009).

Eutrophic freshwater ecosystems keep a high average algal biomass which may include phytoplanktons such as cyanobacteria, chlorococcales or dinoflagellates (Watson *et al.*, 1997). Eutrophication is caused by manmade chemicals in urbanized areas, including, but not limited to, nitrates and phosphates from lawn fertilizer runoff. In urbanized areas, the human factor of nutrient introduction to these ecosystems, otherwise known as cultural eutrophication, has recently been considered as one of the most important factors driving the increase in algal bloom frequency as well as intensity(Anderson *et al.*, 2010). Fertilizer runoff, car washing, and pet wastes being discarded into storm drains are three major modern events causing changes that disturb existing equilibrium between phytoplankton and other aquatic life, accelerating eutrophication (Reynolds, 1984). Nutrient enrichment, while mainly caused by human output, may also be caused by decaying plant matter settling in the water. The algal mat that forms at the water's surface can easily prevent sun from penetrating the lower portions of the water. In figure 1 below, an extensive algal bloom is seen in Branch Brook State Park in Newark, NJ.

Eutrophic bodies of water are usually slow-moving ecosystems, with water remaining stagnant at most periods of time. Because the water is not moving, it is not readily oxygenated, further aggravating the problem of oxygen depletion due to algal blooms. Eutrophication and the overgrowth of cyanobacteria and algae in these water

bodies results in depletion of oxygen, which in turn disrupts aquatic life due to a hypoxic environment. The eutrophic freshwater surface, once covered in biomass, poses a significant problem to the ecosystem. The stagnant water eventually becomes deoxygenated, eliminating all oxygen-dependent life below the water surface, and any photosynthetic life below the water surface may also be affected due to the prevention of penetration of sunlight. In healthy, oligotrophic water bodies (Environment that provides few nutrients), the minimal nutrients entering the water can easily be recycled through the food chain or washed out of the pond or lake via natural water movement, at equilibrium. In eutrophic lakes, however, the water becomes overloaded with nutrients, fostering the production of biomass.

Figure 2 below shows the divergence of an oligotrophic lake when compared to a eutrophic lake. In the figure, (L) is considered the nutrient load, while (B) is considered biomass, (R) is considered recycled, (W) is considered washed out of the lake, and (S) is considered sedimented material (Reynolds, 1984).

Figure 1: Harmful Algal Bloom (HAB) was seen at Branch Brook State Park in Newark, New Jersey on June 14th, 2011. HABs are a recurring problem in urbanized freshwater lakes due to excessive eutrophication from pollution run off. As seen in the photograph above, fountains are installed to increase aeration (for oxygenation of stagnant water) in order to clear the area of the HABs.

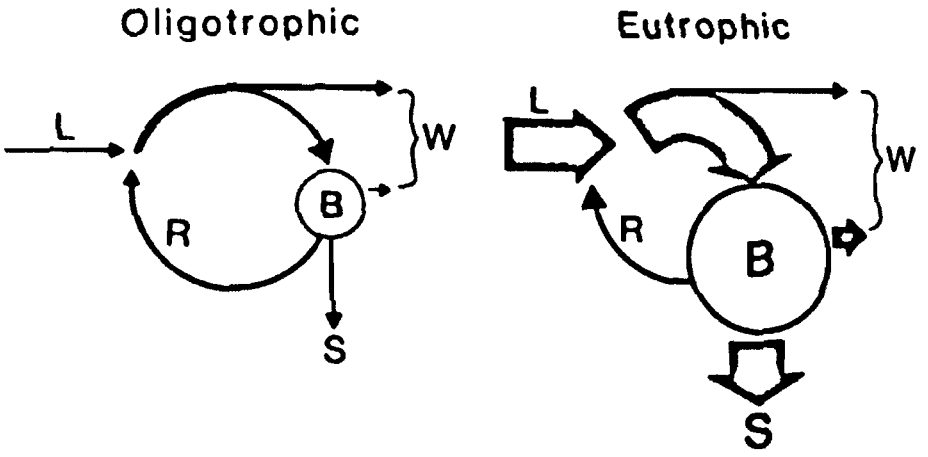


Figure 2: In the oligotrophic lake, there is a light nutrient load (i.e, Nitrogen, Phosphorous, etc) (L) that produces a relatively insignificant biomass (B) and much of this biomass is either washed out of the lake (W) or recycled (R). In a eutrophic lake, the large nutrient load produces a massive biomass, which is less capable of being washed out of the lake or recycled, and is more dependent on depositing into the sediment. (Reynolds, 1984).

Condensed algal blooms are harmful to all living organisms including plants and animals within the water due to the depleted oxygen levels and decreased sunlight levels, but also may affect plants and animals by toxins released by different cyanobacterial species involved in these algal blooms. Many species of cyanobacteria can also produce toxins as a byproduct of their growth, posing a further risk for aquatic life as well as humans, animals, and anything that could come in contact with the water body. Overall, there have been approximately 50 species of cyanobacteria that have been shown to produce toxins which are harmful to invertebrates. Microcystis, Anabaena, Oscillatoria, Aphanizomenon, and Nodularia are just a few genera which contain species known to produce cyanotoxins. There are three main types of cyanotoxins. Neurotoxins (Saxitoxins, Anatoxins) affect the nervous system, hepatotoxins (Microcystins) affect the liver, and dermatoxins (lyngbyatoxin) affect the skin (NALMS) (Codd *et al.*, 2005). These toxins can affect humans and animals severely. In water bodies that both human and animals frequent, or drink from, this is especially an issue. Microcystins and other cyanotoxins are heat stable, thus cannot be destroyed by boiling. Also, many cyanotoxins are not easily separated from drinking water if they are dissolved in the water. Currently, there are several cyanotoxins that are on the US EPA Contaminant Candidate List (CCL2) which are being evaluated for human toxicity (NALMS) (EPA 2012). Exposure routes of these cyanotoxins are dependent on the purpose of the contaminated water. If the contaminated water is part of a reservoir, the exposure route may be ingestion due to improperly filtered drinking water. If the contaminated water is used for recreational use, the exposure route may be skin, ingestion, or inhalation. Human exposure may also

come from ingestion of animals that were living in the contaminated water. Saxitoxins, known neurotoxins secreted by several cyanobacterial species including *Anabaena circinalis*, are also known as paralytic shellfish toxins (PSTs). These neurotoxins infect shellfish, which in turn infect humans who ingest those shellfish (Al-Tebrineh *et al.*, 2010). This neurotoxin, along with the other cyanotoxins produced by these cyanobacteria, has no current cure (CDC 2011).

The need for treatment of freshwater sources across the world is at an all-time high due to the increase in urbanization. Algal blooms occur in all urbanized freshwater sources, and the need to protect both humans and animals remains crucial. In order to prevent harmful algal blooms from forming, it is necessary to understand the delicate balance between cyanobacteria and their viruses, cyanophage. Cyanophage are viruses that infect cyanobacteria in a species specific manner and are just as ubiquitous as cyanobacteria in ecosystems (Lee *et al.*, 2006). The interaction between the cyanophage and cyanobacteria is species specific; a specific cyanophage infects a specific cyanobacterial species. Understanding the control of algal population density by cyanophage is necessary to maintain water quality in freshwater systems (Lee et al., 2006).

The first step in correcting the problems created by harmful algal blooms is to identify the common species at each susceptible freshwater system. Collection of water samples from these systems provides an assorted mixture of eukaryotic and prokaryotic organisms, which may include plant matter. Microscopy can be used to identify the array

of organisms found within the samples. This methodology allows the user to identify not only the organisms present, but also the concentration of cyanobacterial cells within the sample. Microscopy alone is extremely inefficient when attempting to identify species from several sites among several lakes due to the time consumption as well as the ability to only identify to the genus level. For use as a complement to microscopy, the molecular diagnostic procedure Polymerase Chain Reaction (PCR) is a highly useful tool used to amplify specific nucleic acid sequences from samples that may contain many other sequences. PCR can be used to probe for conserved motifs among all phyla of cyanobacteria. These conserved motifs are titled housekeeping genes. Housekeeping genes are defined as genes within a cell that are responsible for the general maintenance and upkeep of the cell. A housekeeping gene which is present in all cells is the small-subunit ribosomal RNA (16s rRNA). In an environmental sample, it is important to first perform PCR using universal cyanobacterial primers in order to determine the presence of cyanobacteria. There have been previous studies in which both universal and phyto-specific primers have been determined to be effective in amplifying the 16s rRNA genes in cyanobacteria (Nübel *et al.*, 1997, Stiller & McClanahan, 2005). After cyanobacterial presence has been confirmed, species – specific primers are then used to effectively determine the profile of the freshwater ecosystem being tested. Using the combined microscopic analysis with molecular techniques allows for an effective and efficient method in determining cyanobacterial profiles among freshwater ecosystems.

Another, extremely efficient method of determining major taxonomic groups of algae is flow cytometry. As mentioned above, cyanobacteria and phytoplankton as a

whole contain the pigment chlorophyll *a,* which allows for flow cytometers detecting cells at a rapid rate by using fluorescence. Because the phytoplankton population in most aquatic systems is highly diverse in composition and size, as well as concentration and shape, flow cytometry offers the ability for automated counting of different phytoplankton by assessing the different physical characteristics of each population (Ziglio & van der Beken, 2002). By exploiting the fact that cyanobacterial cells can be counted using red fluorescence due to the possession of chlorophyll, flow cytometry can be used initially as a rapid monitor of phytoplankton biomass along the water column and its seasonal changes. The most abundant algal species in freshwater all absorb strongly in the blue and green region of the spectrum, and can be distinguished based on their fluorescence emissions. Cyanobacteria can be detected with a laser of 580-630 nm, dinoflagellates and chlorophytes with a laser of 680-690 nm, and other phycocyanin-containing phytoplankton can be distinguished by their red emission at 640-670 nm (Ziglio & van der Beken, 2002). Without using fluorescent probes or DNA stains, it is hard to distinguish individual cells. But, the combination of microscopic analysis, polymerase chain reaction, and flow cytometry can efficiently and successfully develop a profile of freshwater ecosystems.

In the present study, five lakes in Essex County, New Jersey were sampled in the summer and fall of 2011. Samples were subsequently tested for the presence of cyanobacteria and phytoplankton using the three methods described above: Microscopic analysis, polymerase chain reaction, and flow cytometry. Microscopic analysis was performed to identify individual species of cyanobacteria and other phytoplanktons

among each site at each of the five lakes tested. Once cyanobacteria were confirmed and several species identified, polymerase chain reaction was used with universal primers to confirm the presence of cyanobacteria as well as species specific primers to confirm the presence of specific species. Flow cytometry was utilized to compare seasonal profiles as well as to compare the cyanobacterial cell concentrations among the raw water samples, the coarse filter, and the fine filter (used during processing of each sample).

Materials and Methods

Cyanobacterial Cultures. *Synechococcus* sp. IU 625 and *Synechococcus elongatus* PCC 7942 strains were used as controls in this study. Five ml of cells were inoculated in 95 ml of sterilized Mauro's Modified Medium (3M) (Kratz & Meyers, 1955) in 250 ml Erlenmeyer flasks (Chu *et al.*, 2011). The medium was adjusted to a pH of 7.9 using 1 M NaOH or HCl. The cultures were grown under consistent fluorescent lighting and at a temperature of 27° C. The cultures were grown on an Innova™ 2000 Platform Shaker (New Brunswick Scientific, Enfield, CT, USA) with continuous pulsating at 100 rpm.

Environmental Samples. Water samples were collected from several water bodies in Essex County, New Jersey in 2011. A call was made to the Essex County Department of Parks, Recreation and Cultural Affairs to approve collections. There were two collection periods in this study: May 2011-August 2011 (Summer Collections) and October 2011-November 2011 (Fall Collections) to observe microorganism profile seasonal differences. Three to five samples were collected at each body of water, varying in location and water movement. The five bodies of water observed in this study were Diamond Mill Pond (Millburn, NJ, USA) in figure 2, South Orange Duck Pond (South Orange, NJ, USA) in figure 3, Clarks Pond (Bloomfield, NJ, USA) in figure 4, Verona Lake (Verona, NJ, USA) in figure 5, and Branch Brook State Park (Newark, NJ, USA) in figure 6 below. Before collection, each site was tested for pH, dissolved oxygen, and temperature using the ExStickII® pH/Dissolved Oxygen (DO)/ Temperature meter (ExTech® Instruments corp., Nashua, NH, USA). Samples were collected from each water body in 1 L sterile

collection bottles (Nalgene, Rochester, NY, USA). The one liter samples were brought to the lab (Seton Hall University, South Orange, NJ, USA) to be further processed. Each sample was run through a coarse filter with a pore size of 2.7 μm (Denville Scientific, Metuchen, NJ, USA). Filtrate from the coarse filtered sample was run through a fine filter with a pore size of 0.45 μm (Nalgene, Rochester, NY, USA). Both coarse and fine filter from each sample were placed in a Thelco™ Model 2 incubator for drying at 37°C (Precision Scientific, Chennai, India). Aluminum foil was sterilized by UV light using a Purifier Vertical Clean Bench (Labconco, Kansas City, MO, USA). Dried filters were placed on sterilized aluminum foil and placed in -20°C refrigerator for preservation for future studies.

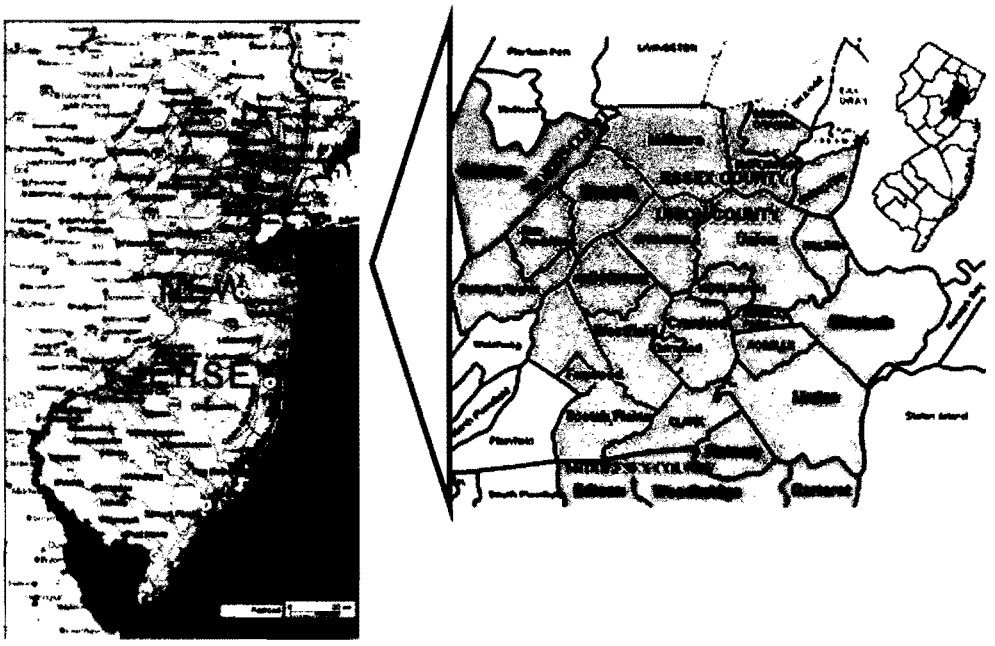
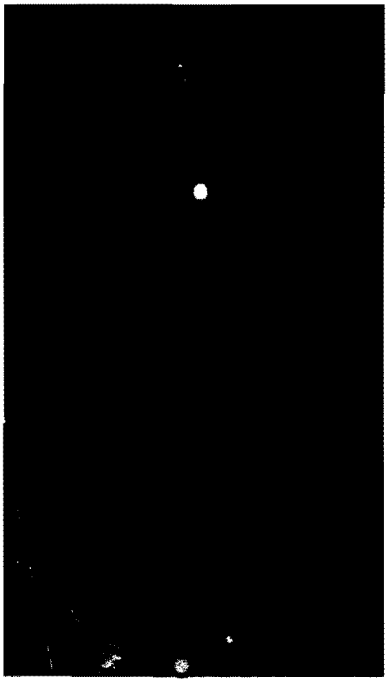
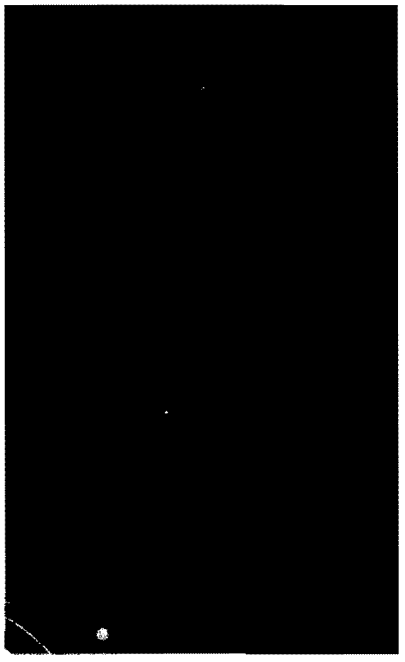


Figure 3: A map of the state of New Jersey, with Essex County enlarged (Geographix, 1997, White, 2009).



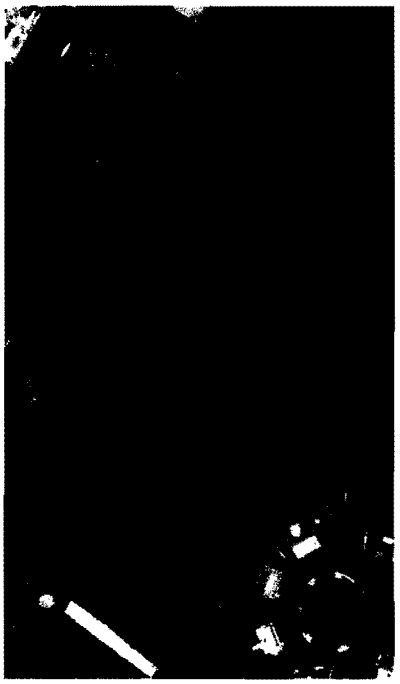
A (Blue)	40°71'2"N, 74°31'9"W
B (Red)	40°69'9"N, 74°31'9"W
C (Orange)	40°71'24"N,74°31'93"W
D (Green)	40°69'9"N,74°31'9"W
E (Light Blue)	40°43'6"N, 74°18'2"W

Figure 4: Diamond Mill Pond (Milburn, NJ) collected on July 13th and November 9th, 2011 ("Diamond Mill, 2011).



A (Blue)	40°45'53"N,74°15'38"W
B (Red)	40°45'51"N,74°15'38"W
C (Orange)	40°44'57"N,74°15'37"W
D (Green)	40°44'56"N,74°15'40"W

Figure 5: South Orange Duck Pond (South Orange, NJ) collected on July 21st and October 4th, 2011("South Orange Duck Pond, 2011).

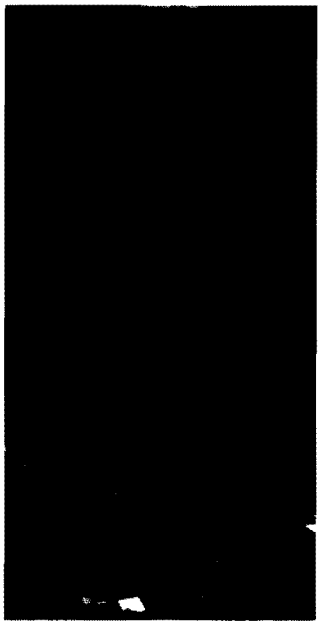


A (Blue)	40°49'30.4"N,74°10'50.5"W
B (Red)	40°49'28.3"N,74°10'52.3"W
C (Orange)	40°49'26.6"N,74°10'52.64"W
D (Green)	40°49'26.5"N, 74°10'52.63W

Figure 6: Clarks Pond (Bloomfield, NJ) collected on July 19th, 2011 and October 24th, 2011 ("Clarks Pond, 2011).

A (Blue)	40°49'40.6"N,74°14'44.5"W
B (Red)	40°49'45.7"N,74°14'41.4"W
C (Orange)	40°49'31.5"N,74°14'47.6"W

Figure 7: Verona Lake (Verona, NJ) collected on May 25th, 2011 and October 4th, 2011. ("Verona Lake, 2011).



A (Blue)	40°45'30.8"N,74°10'99.2"W
B (Red)	40°45'19.8"N,74°11'00.1"W
C (Orange)	40°45'07.0"N,74°10'99.0"W
D (Green)	40°45'21.4"N.74°10'94.6"W

Figure 8: Branch Brook State Park (Newark, NJ) collected on July 14th, 2011 and November 1st, 2011 ("Branch Brook Lake, 2011).

A	Seton Hall University
B (Blue)	South Orange; Duck Pond
C (Red)	Verona; Verona Lake
D (Green)	Newark; Branch Brook Park
E (Orange)	Bloomfield; Clarks Pond
F (Black)	Milburn; Diamond Mill

Figure 9: Lakes studied in Essex County using Seton Hall University as a reference point ("Seton Hall University, 2011).

Genomic DNA Extraction. Genomic DNA of *S.* IU 625 and *S. elongatus* PCC 7942 were extracted using Fermentas® Genomic DNA Purification Kit (Fermentas, Glen Burnie, MD, USA). Ten ml of cyanobacteria cells ($OD_{750\,nm}$ = ~1) were placed in a 15 ml conical tube. The conical tubes were then centrifuged and cells were resuspended in 200 µl of TE Buffer. 200 µl of cells were then mixed with 400 µl lysis solution in an Eppendorf tube (Enfield, CT, USA) and incubated in an Isotemp125D™ Heat Block (Fisher Scientific, Pittsburgh, PA, USA) at 65°C for 5 minutes. 600 µl of chloroform were added and emulsified by inversion. The sample was then centrifuged at 10,000 rpm for two minutes in a Denville 260D microcentrifuge (Denville Scientific, South Plainfield, NJ, USA). While centrifuging, precipitation solution was prepared by mixing 720 µl of deionized water with 80 µl of 10x concentrated precipitation solution. After centrifugation, the upper aqueous phase was transferred to a new tube and 800 µl of the precipitation solution were added. Tube was mixed by several inversions at room temperature for two minutes and centrifuged at 10,000 rpm for two minutes. Supernatant was removed completely and DNA pellet was dissolved by adding 100 µl of 1.2 M NaCl solution with gentle vortexing. 300 µl of cold ethanol (100%) was added to enable DNA precipitation and kept in -20°C for 10 minutes. Tube was then centrifuged at 10,000 rpm for three minutes. Ethanol was discarded and pellet was washed with 70% cold ethanol. The DNA was then dissolved in sterile deionized water, and the DNA concentration and purity were determined with NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

Chelex® DNA Extraction of Environmental Samples. All environmental samples underwent Chelex® DNA extraction as follows. Each filter (for both coarse and fine filters) was hole punched 3-4 times at various spots on the filter to produce three to four disks; disks were placed into 1.5 ml Eppendorf Tubes. 500 µl of deionized water were added to each tube and each tube was vortexed. Tubes were let stand for 10-15 minutes. All tubes were centrifuged for three minutes at 10,000 rpm to concentrate the pellet. Clear supernatant was discarded from each tube, and 200 µl of InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA, USA) were added. Each tube was vortexed for 10 seconds. The tubes were incubated for two hours in a Polyscience© Temperature Controller water bath (Polyscience, Niles, IL, USA) at 56° C, vortexed for 10 seconds, and placed in an Isotemp125D™ Heat Block (Fisher Scientific, Pittsburgh, PA, USA) for 8 minutes at 100°C. The tubes were then centrifuged for 10 minutes at 10,000 rpm, and the supernatant (containing DNA) was transferred to clean Eppendorf tubes. The DNA concentration and purity were determined with NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

PCR Amplification. DNA extracted from the environmental samples, along with the controls (*S.* IU 625, *S. elongatus* PCC 7942) was amplified using general and specific primers to identify the presence of bacteria, cyanobacteria, phytoplankton, and the dominating species. General primers were used to identify bacteria, cyanobacteria, and phytoplankton by utilizing the bacteria-specific 16s rRNA gene primers 27FB and 785R, PSf and PSr, and CPC1f and CPC1r, respectively. Specific primers were used after phytoplankton and cyanobacteria were detected in the samples. PCR was performed

using 6.5 µl nuclease-free deionized water (Promega, Madison, WI, USA), 2.5 µl dimethyl sulfoxide (DMSO), 1 µl of primer in the forward orientation, 1 µl of primer in the reverse orientation, 1.5 µl of DNA sample, and 12.5 µl GoTaq® Hot Start Green Master Mix (Promega). Thermocycling was performed in Veriti 96 Well Thermocycler (Applied Biosystems, Carlsbad, CA, USA). The initial denaturation step was at 95°C for 2 minutes, followed by 35 cycles of DNA denaturation at 95°C for 45 seconds, primer annealing at 50-55°C for 45 seconds, and DNA strand extension at 72°C for 45 seconds, and a final extension step at 72°C for 5 minutes. The amplified DNA was visualized on a 1% agarose gel with ethidium bromide incorporated using TAE electrophoresis buffer (Fermentas). The gel was visualized using a 2UV Transilluminator Gel Docit Imaging System (UVP, Upland, CA, USA).

PCR Primers. Primers used in this study were either developed using NCBI BLAST (http://www.ncbi.nlm.nih.gov/BLAST) or by previous studies in this subject field. General primers included Phytoplankton-Specific PSf (GGGATTAGATACCCCWGTAGTCCT)/PSr (CCCTAATCTATGGGGWCATCAGGA) which identified the 16s rRNA gene in all phytoplankton (Stiller & McClanahan, 2005). Universal primers Uf (GAGAGTTTGATCCTGGTCAG)/Ur (ACGGYTACCTTGTTACGACTT) identified the 16s rRNA gene in all bacteria (Stiller & McClanahan, 2005). General primers 27FB (AGAGTTTGATCCTGGCTCAG) and 785R (ACTACCRGGGTATCTAATCC) were utilized to identify the 16s rRNA in all bacteria, cyanobacteria, and phytoplankton (Barkovskii & Fukui, 2004). Cyanobacteria specific primers used in this study included

CYA106_16sf (CGGACGGGTGAGTAACGCGTGA) and CYA359_16sf (GGGGAATYTTCCGCAATGGG) with CYA781_16sr (GACTACWGGGGTATCTAATCCCWTT) (Nübel et al., 1997) which identified the 16s rRNA subunit conserved in all cyanobacteria. CPC1f (GGCKGCYTGYYTRCGYGACATGGA) /CPC1r (AARCGNCTTGVGWATCDGC) are also cyanobacteria specific primers which identify the β-Subunit of the phycocyanin gene conserved among all cyanobacteria (Stiller). AN3801f (CAAATCACTCAGTTTCTGG) and AN3801r (CAGTAGCAGCTCAGGACTC) are also cyanobacteria specific primers, identifying the DNA polymerase III gene conserved in *S.* IU625 and *S. elongates* PCC 7942. Once cyanobacteria and phytoplankton were identified in a sample, specific primers were obtained and utilized. Primers specific for *Anabaena circinalis* toxin biosynthesis gene cluster were developed using NCBI BLAST searches: ANAf (GATCTAGCCTCACCTGTTGACTT) and ANAr (GGGATCCTTTTTGCTGCGCC). Primers to locate Microcystis were developed in accordance with Herry et al. MSf (ATCCAGCAGTTGAGCAAGC) and MSr (TGCAGATAACTCCGCAGTTG) primers were used to identify the *mycA* gene of *Microcystis* spp. (Herry *et al.*, 2008) . 2156f (ATCACTTCAATCTAACGACT) and 3111r (AGTTGCTGCTGTAAGAAA) primers were utilized to identify the *mycB* gene of *Microcystis* spp. (Herry et al., 2008). PSCf (GCAACATCCCAAGAGCAAAG) and PSCr (CCGACAACATCACAAAGGC) primers were used to identify the *mycC* gene of *Microcystis* spp. (Herry et al., 2008). Diatom presence was identified using primers developed in accordance with Baldi et al. 528f (GCGGTAATTCCAGCTCCAA) with

650r (AACACTCTAATTTTTTCACAG) identified the small subunit ribosomal DNA gene conserved among all diatom species (Baldi *et al.*, 2011).

Microscopic Analysis. Microscopic images were acquired using a Carl Zeiss AxioLab.A1 phase contrast microscope coupled with a Carl Zeiss AxioCam MRc camera (Carl Zeiss Microimaging, Jena, Germany). Coarse filters (2.7 µm pores) were hole punched one time. The fragment was placed into an Eppendorf tube and 100 µl of deionized water were added. The tubes were left at room temperature for 10-20 minutes. 16 µl of the tube's contents were pipetted onto a microscope slide and viewed at 400X power under the phase filter. Images of diatoms, phytoplankton and cyanobacteria were compared to the atlas "Freshwater Algae of North America: Ecology and Classification" (Sheath, 2003). Species of cyanobacteria, diatoms, and phytoplankton were identified for use in specific PCR analysis and amplification.

Flow Cytometry. Flow cytometry was performed on several sites collected from Branch Brook State Park (Newark, NJ) in June 2011 as well as December 2011 by a Guava® EasyCyte™ Plus Flow Cytometry System (Millipore, Billerica, MA, USA). Fluorescence resulting from the excitation with a 488 nm laser was collected using both green and red filters. A 575 nm filter was used to locate carothenoid pigments, while a 675 nm filter was used to locate chlorophyll a pigments, each of which would be indicative of cyanobacterial presence in the water sample. Blanks were created by using Phosphate Buffered Saline (PBS) and deionized water. Tubes were prepared by hole punching both coarse and find filters and placing them in Eppendorf tubes with 100 µl deionized water,

as mentioned above. Cyanobacterial presence was studied in the coarse filter from Branch Brook Park site C from July 2011 (Algal Bloom present), Branch Brook Sites C & D (Raw, unfiltered samples), and both Branch Brook Sites C & D Coarse and Fine filters. Flow Cytometry results were analyzed using FlowJo 7.6.5 Flow Cytometry Analysis Software (Tree Star, Inc., Ashland, OR, USA).

Results

WATER CHEMISTRY

The pH, dissolved oxygen and temperature were analyzed for all sites. Water chemistry provided a connection between pH, dissolved oxygen, temperature and phytoplankton.

Summer

In table 1, the water properties determined at Clarks Pond in Bloomfield, NJ during summer collections are shown. In table 2, the water properties determined at Diamond Mill Pond in Milburn, NJ during summer collections are shown. In table 3, the water properties determined at Verona Lake in Verona, NJ during summer collections are shown. In table 4, the water properties determined at Branch Brook State Park in Newark, NJ during summer collections are shown. In table 5, the water properties determined at South Orange Duck Pond in South Orange, NJ during summer collections are shown.

Clarks Pond Site	pH	Dissolved Oxygen (mg/L)	Temperature (°C)
A	7.28	3.0	27.4
B	8.29	3.0	29.9
C	8.10	3.0	29.3
D	7.94	3.0	29.0

Table 1: Water properties of Clarks Pond in Bloomfield, NJ (July, 2011).

Diamond Mill Pond Site	pH	Dissolved Oxygen (mg/L)	Temperature (°C)
A	7.59	2.0	27.3
B	7.58	2.0	27.5
C	7.75	2.0	25.3
D	7.50	2.0	26.6
E	7.48	1.0	24.9

Table 2: Water properties of Diamond Mill Pond in Milburn, NJ (July, 2011).

Verona Lake Sites	pH	Dissolved Oxygen (mg/L)	Temperature (°C)
A	7.27	N/A	23.5
B	7.55	N/A	24.0
C	7.34	N/A	23.8

Table 3: Water properties of Verona Lake in Verona, NJ (May, 2011).

Branch Brook Lake Sites	pH	Dissolved Oxygen (mg/L)	Temperature (°C)
A	7.83	5.0	25.7
B	8.30	4.0	26.3
C	7.65	4.0	29.3
D	8.10	6.0	26.3

Table 4: Water properties of Branch Brook State Park in Newark, NJ (July, 2011).

South Orange Duck Pond Sites	pH	Dissolved Oxygen (mg/L)	Temperature (°C)
A	7.81	9.0	30.0
B	8.35	10.0	29.3
C	8.45	9.0	29.0
D	9.20	8.0	30.2

Table 5: Water properties of South Orange Duck Pond in South Orange, NJ (July, 2011).

Fall

In table 6, the water properties determined at Clarks Pond in Bloomfield, NJ during fall collections are shown. In table 7, the water properties determined at Diamond Mill Pond in Milburn, NJ during fall collections are shown. In table 8, the water properties determined at Verona Lake in Verona, NJ during fall collections are shown. In table 9, the water properties determined at Branch Brook State Park in Newark, NJ during fall collections are shown. In table 10, the water properties determined at South Orange Duck Pond in South Orange, NJ during fall collections are shown.

Among 20 collected sites, pH values range from 6.60 to 9.25; dissolved oxygen range from 1 mg/L to 11 mg/L; and water temperature range from 9.1°C to 30.2°C. The water chemistry results indicated that the dissolved oxygen and water temperature varied from one water body to another, while the pH ranges appeared to maintain similar levels throughout all water bodies.

Clarks Pond Sites	pH	Dissolved Oxygen (mg/L)	Temperature (°C)
A	7.80	4.0	16.8
B	7.64	4.0	14.5
C	7.57	4.0	14.5
D	7.46	4.0	13.9

Table 6: Water properties of Clarks Pond in Bloomfield, NJ (October, 2011).

Diamond Mill Pond Sites	pH	Dissolved Oxygen (mg/L)	Temperature (°C)
A	7.97	2.0	10.7
B	7.70	2.0	11.4
C	7.49	3.0	10.8
D	8.00	4.0	11.4
E	7.75	4.0	10.8

Table 7: Water properties of Diamond Mill Pond in Milburn, NJ (November, 2011).

Verona Lake Sites	pH	Dissolved Oxygen (mg/L)	Temperature (°C)
A	7.88	10	15.4
B	7.70	10	16.0
C	6.60	10	15.4

Table 8: Water properties of Verona Lake in Verona, NJ are displayed (October, 2011).

Branch Brook Lake Sites	pH	Dissolved Oxygen (mg/L)	Temperature (°C)
A	8.03	6.0	12.4
B	7.42	5.0	12.1
C	7.94	6.0	11.5
D	7.80	7.0	9.1

Table 9: Water properties of Branch Brook State Park in Newark, NJ (November, 2011).

South Orange Duck Pond Sites	pH	Dissolved Oxygen (mg/L)	Temperature (°C)
A	8.70	8.0	17.9
B	8.80	9.0	17.9
C	9.25	10.0	16.8
D	9.21	11.0	16.8

Table 10: Water properties of South Orange Duck Pond in South Orange, NJ (October, 2011).

POLYMERASE CHAIN REACTION (PCR) BASED ASSAYS

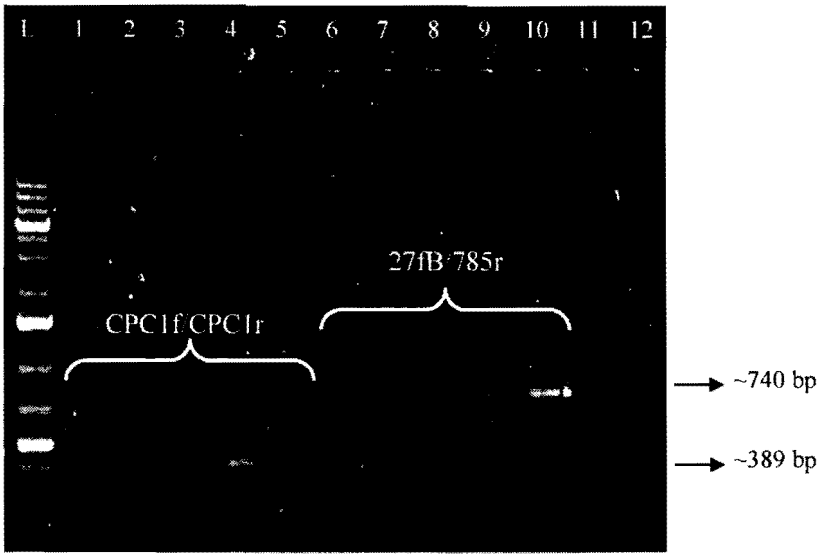
Polymerase chain reaction based assays were performed using DNA extracted from both the coarse and fine filters at each site among all water bodies involved in this study collected in both the summer and fall to identify the presence of bacteria, cyanobacteria, and phytoplankton within each body of water.

Summer Samples (Collected July & August 2011):

Clarks Pond

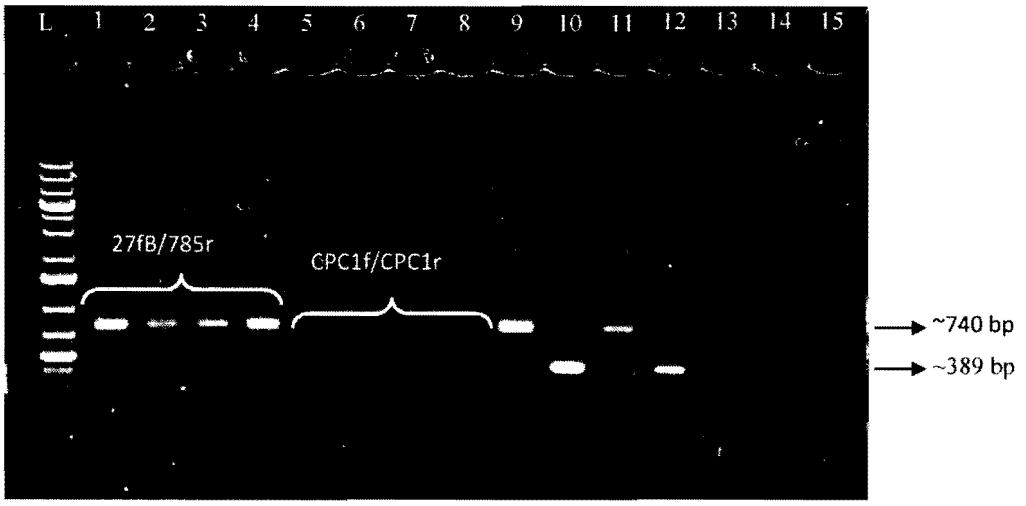
PCR-based assays were performed on the coarse filters from each site collected from Clark's Pond in Bloomfield, NJ, and Branch Brook Lake in Newark, NJ. In figure 10 below, positive results are revealed at Clarks Pond as well as Branch Brook site C (Visible Algal Bloom) using the CPC1f/CPC1r and 27fB/785r primers on a 1% agarose gel. Cyanobacteria was detected in lanes 3 and 4 (Clarks Pond C and D, Coarse Filter) using the CPC1f/CPC1r primer set. Bacteria were detected at all sites tested using the 27fB/785r primer set.

PCR was then performed on the fine filter from each site at Clarks Pond using the CPC1f/CPC1r and 27fB/785r primer sets. In figure 11 below, there appears to be bands among all lanes, indicating that cyanobacteria and bacteria were present at all sites (Fine Filter). *Synechococcus* sp. 1U 625 and *Synechococcus elongatus* PCC 7942, both lab strains, were used as positive controls in this study.



Lane	1	2	3	4	5	6	7	8	9	10	11	12
Waterbody	Clark				BB	Clark				BB	(-) control	
Site	A	B	C	D	C	A	B	C	D	C		

Figure 10: Results from Clarks Pond as well as Branch Brook Park Site C (visible algal bloom) are shown using CPC1f/CPC1r and 27fB/785r primers to detect cyanobacteria and bacteria, respectively. Lanes 3 and 4 indicate the presence of cyanobacteria in these sites. Lanes 6, 7, 8, 9, and 10, indicate the presence of bacteria in these sites.



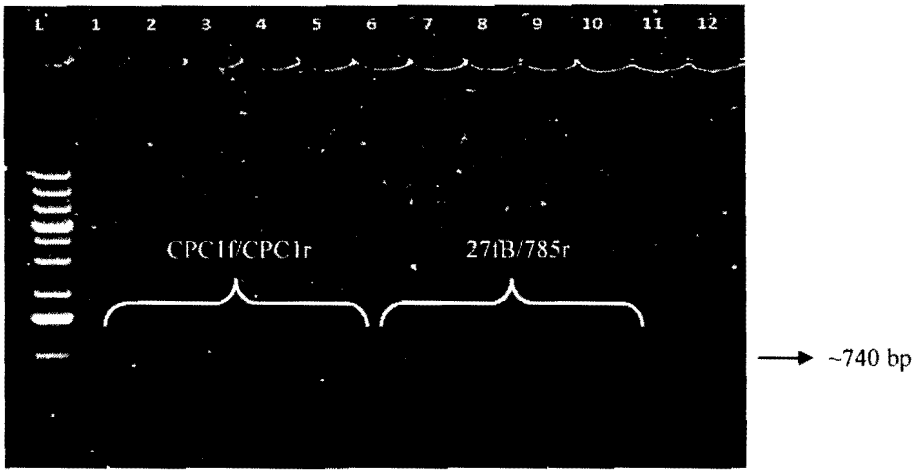
Lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Waterbody	Clark								(+) Control				(-) Control		
Site	A	B	C	D	A	B	C	D							

Figure 11: Results from Clarks Pond fine filters are shown using CPC1f/CPC1r and 27fB/785r primers to detect cyanobacteria and bacteria, respectively. Bands are seen at all lanes in the gel, indicating that cyanobacteria and bacteria were present among all sites.

Diamond Mill Pond

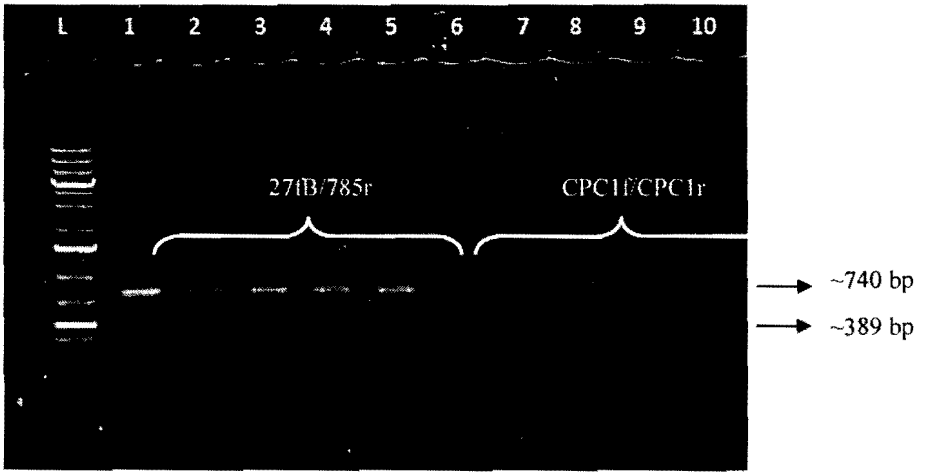
Diamond Mill Pond in Milburn, NJ was tested using PCR and gel electrophoresis with a 1% agarose gel. In figure 12 below, the band appearing in lane 8 shows that Diamond Mill Pond site C has been determined to contain bacteria using the 27fB/785r primer set.

PCR was performed on the fine filters from Diamond Mill Pond. Using the CPC1f/CPC1r primer set and the 27fB/785r primer set, cyanobacteria and bacteria identified among sites tested. In figure 13 below, there appears to be bands in lanes 1, 2, 3, 4, 5, and 6. This is indicative of bacteria being present in sites A, B, C, and D as well as cyanobacteria presence at site A.



Lane	1	2	3	4	5	6	7	8	9	10	11	12
Waterbody	Diamond Mill										(-) control	
Site	A	B	C	D	E	A	B	C	D	E		

Figure 12: Results from Diamond Mill Pond coarse filters are shown using CPC1f/CPC1r and 27fB/785r primers to detect cyanobacteria and bacteria, respectively. The band in lane 8 indicates that bacteria were present at this site.

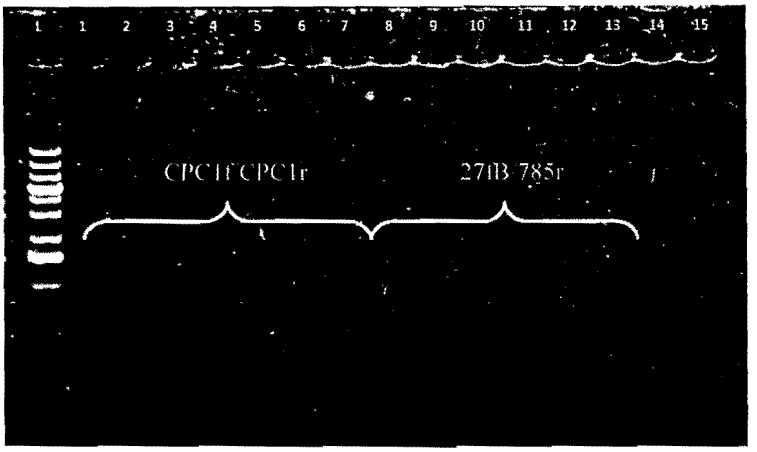


Lane	1	2	3	4	5	6	7	8	9	10	11	12
Water Body	Diamond Mill Pond										(-) control	
Site	A	B	C	D	E	A	B	C	D	E		

Figure 13: Results from Diamond Mill Pond fine filters are shown using CPC1f/CPC1r and 27fB/785r primers to detect cyanobacteria and bacteria, respectively. Bands were in lanes 1 through 5, indicating bacterial presence at all sites. Lane 6 indicates cyanobacterial presence at site A.

Verona Lake

PCR was performed on the DNA extracted from the coarse and fine filters collected from Verona Lake in Verona, NJ using general primers. In figure 14 below, bands were in lanes 7, 9, 10, and 11, indicative that bacteria were identified in site A, site B, and site C.

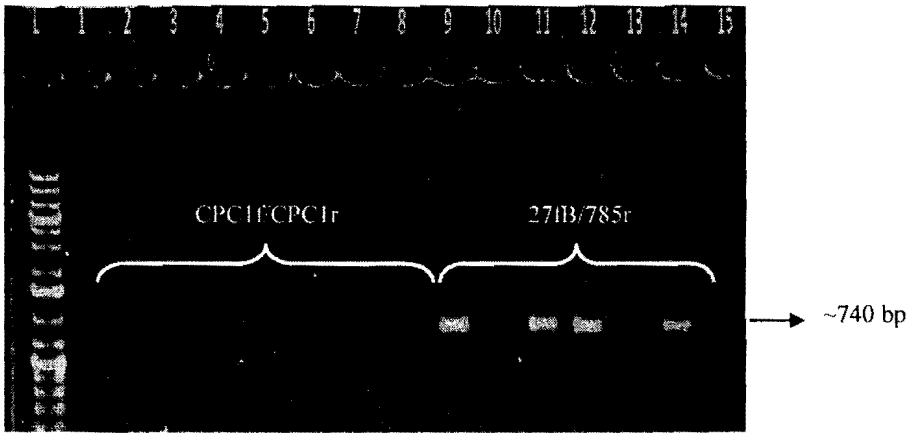


Lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Water Body	Verona												(+) Controls		
Site	A	B	C	D	A	B	C	D	A	B	C	D			
Filter Type	C	C	C	F	F	F	C	C	C	F	F	F			

Figure 14: Results from Verona Lake are shown using CPC1f/CPC1r and 27fB/785r primers to detect cyanobacteria and bacteria, respectively.

Branch Brook State Park

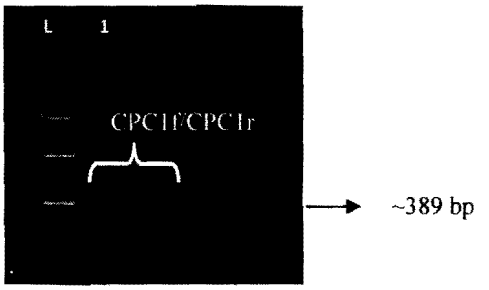
PCR using general primers was used to identify bacteria and cyanobacteria using the CPC1f/CPC1r primer set and the 27fB/785r primer set at Branch Brook State Park (Newark, NJ). In figure 15 below, there are DNA bands on the 1% agarose gel appearing in lanes 9, 11, 12, and 14. These bands indicated that there were bacteria identified at site A (Coarse Filter), site C (Coarse Filter), site D (Coarse Filter), and site B (Fine Filter).



Lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Water Body	Branch Brook														
Site	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C
Filter Type	C	C	C	C	F	F	F	F	C	C	C	C	F	F	F

Figure 15: Results from Branch Brook State Park are shown using CPC1f/CPC1r and 27fB/785r primers to detect cyanobacteria and bacteria, respectively. Bands are seen in lanes 9, 11, 12, and 14, indicating the presence of bacteria at these sites. In column one of the table, the site being tested is established. In column two, the primer set used is stated, and in column three, the amplification size is noted.

South Orange Duck Pond. PCR was performed using the DNA extracted from the coarse filter at sites in the South Orange Duck Pond (South Orange, NJ) using a general cyanobacteria primer (CPC1f/CPC1r). In figure 16 below, a band is seen in lane 1, indicating that cyanobacteria has been identified in site A.

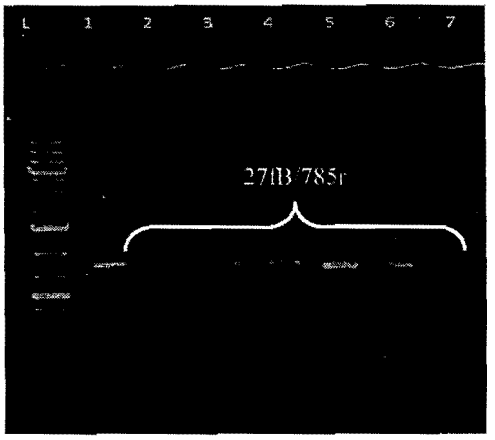


Lane	1
Water Body	S.O. Duck Pond
Site	A

Figure 16: Results from South Orange Duck Pond are shown using the CPC1f/CPC1r primer set to detect cyanobacteria. Lane 1 indicates the presence of cyanobacteria at site A.

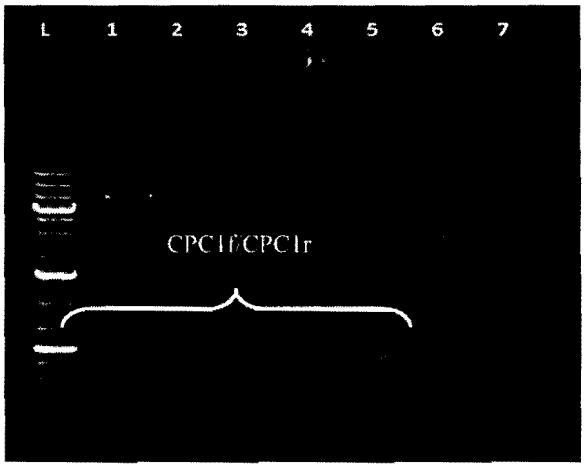
PCR was performed using the DNA extracted from the fine filter at sites in the South Orange Duck Pond (South Orange, NJ) using the general bacteria primer set 27fB/785r. In figure 17, it is evident that all four sites among the fine filter contained bacteria due to bands seen in lanes 1, 2, 3 and 4.

PCR was performed using the DNA extracted from the fine filter at sites in the South Orange Duck Pond (South Orange, NJ) using a general cyanobacteria primer (CPC1f/CPC1r). In figure 18, it is evident that sites A and B contain cyanobacteria among the four sites tested, due to the presence of bands in lanes 1 and 2.



Lane	1	2	3	4	5	6	7
Water Body	S.O. Duck Pond						
Site	A	B	C	D	(+) Control		(-) Control

Figure 17: Results from South Orange Duck Pond are shown using the 27fB/785r primer set to detect bacteria. Lanes 1, 2, 3, and 4 indicate the presence of bacteria at these sites.



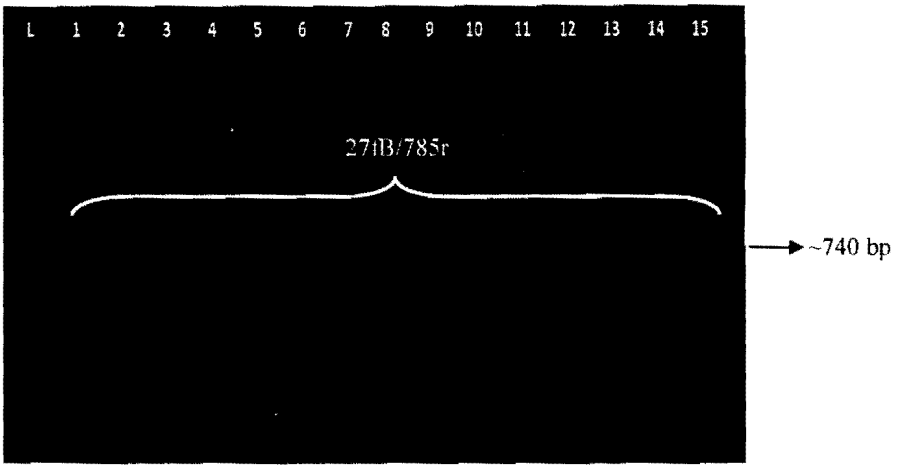
Lane	1	2	3	4	5	6	7
Water Body	S.O. Duck Pond						
Site	A	B	C	D	(+) Control		(-) Control

Figure 18: Results from South Orange Duck Pond are shown using the CPC1f/CPC1r primer set to detect cyanobacteria. Lanes 1 and 2 indicate the presence of cyanobacteria at these sites.

Fall Samples (Collected October & November, 2011)

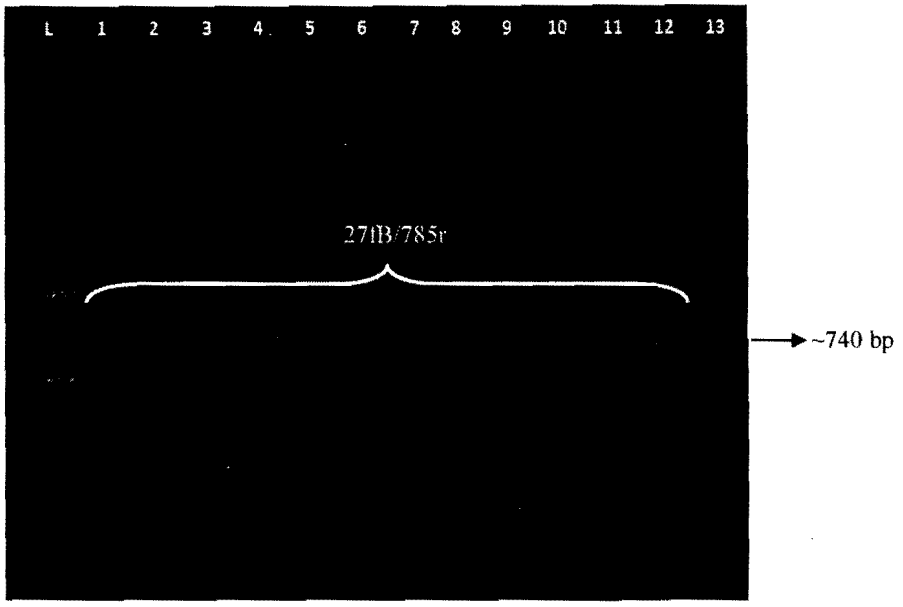
Diamond Mill/Clarks Pond

PCR was performed on DNA extracted from Clarks Pond sites in the fall of 2011, both coarse and fine filters, using the general bacteria primer set 27fB/785r. As seen in figure 31 below, bands are seen in all lanes, indicating bacteria were found among all sites of Clarks Pond (A, B, C, and D). In figure 19 below, bands are seen in all lanes except lane 7, indicating bacterial presence at all sites excluding South Orange Duck Pond site D.



Lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Water Body	Diamond Mill										Clarks				
Site	A	B	C	D	E	A	B	C	D	E	A	B	C	D	A
Filter Type	C	C	C	C	C	F	F	F	F	F	C	C	C	C	F

Figure 19: Results from Diamond Mill and Clarks Pond are shown using the 27fB/785r primer set to detect bacteria. Clarks Pond DNA was run in lanes 11, 12, 13, and 14, giving a band in each lane. This is indicative of bacterial presence among all sites tested.



Lane	1	2	3	4	5	6	7	8	9	10	11	12	13
Water Body	Clarks						S.O. Duck Pond					(+) Control	(-) Control
Site	B	C	D	D	E	A	B	C	D	E	A		
Filter Type	F	F	F	C	C	F	F	F	F	F	C		

Figure 20: Results from Clarks Pond and South Orange Duck Pond coarse filters are shown using the 27fB/785r primer set to detect bacteria. Clarks Pond DNA was run in lanes 1, 2, and 3 giving a band in each lane. This is indicative of bacterial presence among all sites tested at Clarks Pond.

PCR was performed on DNA extracted from Clarks Pond sites in the fall of 2011, both coarse and fine filters, using general cyanobacteria primer sets CPC1f/CPC1r and AN3801f/AN3801r. No positive bands were identified from this PCR.

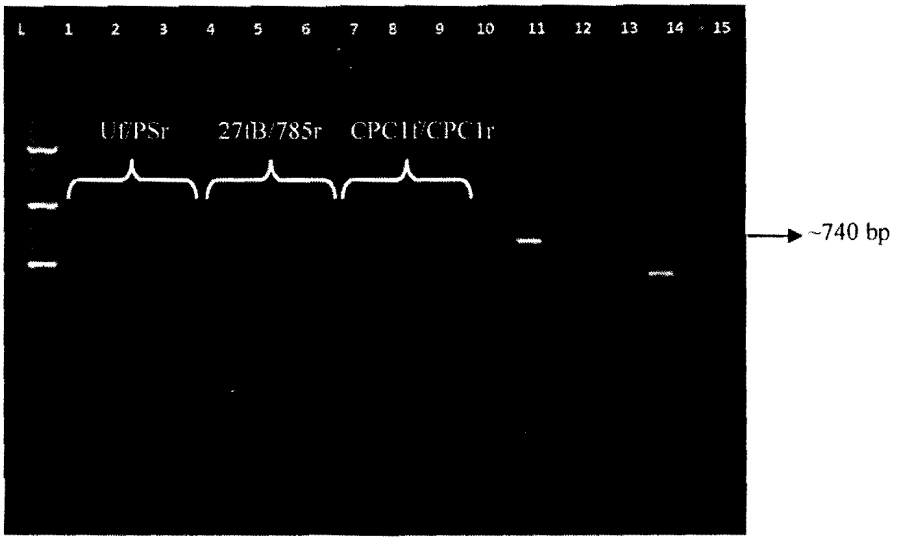
Diamond Mill Pond

PCR was performed on DNA extracted from Diamond Mill sites in the fall of 2011, using general bacteria primer set 27fB/785r. As seen in figure 20 above, bacteria was identified among all sites tested, both coarse and fine filters.

PCR was performed on Diamond Mill coarse and fine filters using cyanobacteria specific general primer sets CPC1f/CPC1r as well as AN3801f/AN3801r. No bands were identified from these sites.

Verona Lake

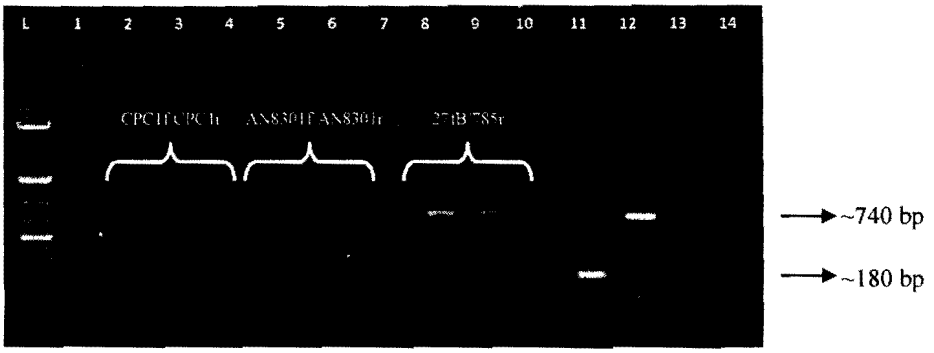
PCR was performed on DNA extracted from Verona Lake sites in the fall of 2011; using general bacteria primer sets 27fB/785r and Uf/PSr, as well as cyanobacteria specific general primer set CPC1f/CPC1r. As seen in figure 21 below, bands appear in lanes 4, 5, and 6, indicative of bacterial presence at all three Verona Lake sites.



Lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Water Body	Verona									(+) Control					(-) Control
Site	A	B	C	A	B	C	A	B	C						

Figure 21: Results from Verona Lake coarse filters are shown using the Uf/PSr and 27fB/785r primer sets to detect bacteria and CPC1f/CPC1r primer set to detect cyanobacteria. Lanes 4, 5, and 6, indicate a bacterial presence at all three sites using the coarse filters.

Next, PCR was performed on the DNA extracted from the fine filters among all sites at Verona Lake. The primer sets used were cyanobacteria and bacteria specific, including CPC1f/CPC1r, AN3801f/AN3801r, and 27fB/785r. In figure 22 below, bands appear in lanes 4, 7, 8, and 9. The band appearing in lane 4 is indicative of cyanobacteria presence, while the bands appearing in lanes 7, 8, and 9 were indicative of bacteria presence among all sites.

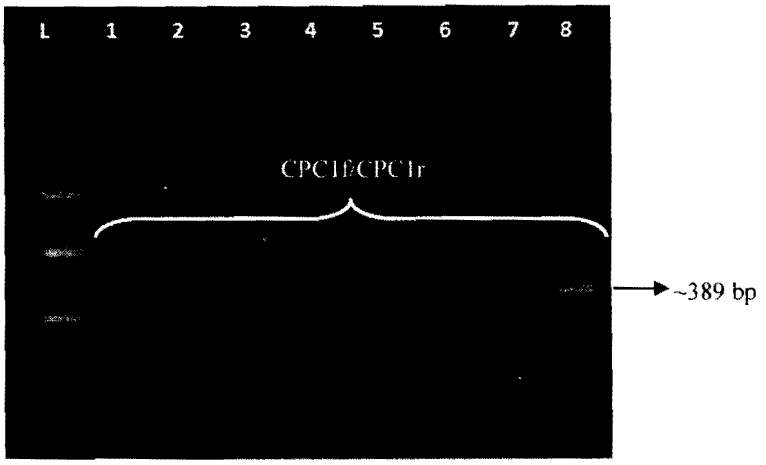


Lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Water Body	Verona									(+) Control			(-) Control	
Site	A	B	C	A	B	C	A	B	C					

Figure 22: Results from Verona Lake fine filters are shown using the CPC1f/CPC1r and AN3801f/AN3801r primer sets to detect cyanobacteria and 27fB/785r primer set to detect bacteria at each site. Bands were seen in lanes 4, 7, 8, and 9. Lane 4 is indicative of cyanobacteria being present at site A, and lanes 7, 8, and 9 are indicative of bacterial presence at all sites.

Branch Brook State Park

PCR was performed on DNA extracted from both coarse and fine filters at all sites at Branch Brook State Park in Newark, NJ. The primer sets used for this assay were the cyanobacteria-specific CPC1f/CPC1r, universal primer set PSf/PSr, and cyanobacteria-specific AN3801f/AN3801r. As seen in figure 2 below, several sites from Branch Brook State Park contain cyanobacteria, including all sites with coarse filter, site A with fine filter, and site D with fine filter.



Lane	1	2	3	4	5	6	7	8
Water Body	Branch Brook							
Site	A	B	C	D	A	B	C	D
Filter Type	C	C	C	C	F	F	F	F

Figure 23: Results from Branch Brook State Park coarse and fine filters are shown using the CPC1f/CPC1r primer set to detect cyanobacteria. Lanes 1, 2, 3, 4, 5, and 8 indicate cyanobacteria at each site with the coarse filter DNA, and at sites A and D with the fine filter DNA.

South Orange Duck Pond

As seen in figure 20 above, DNA extracted from both coarse and fine filters from South Orange Duck Pond were tested using the general bacteria primer 27fB/785r. All sites tested from South Orange Duck Pond showed bands except lane 7, indicative of bacterial presence at all sites but site D with the coarse filter.

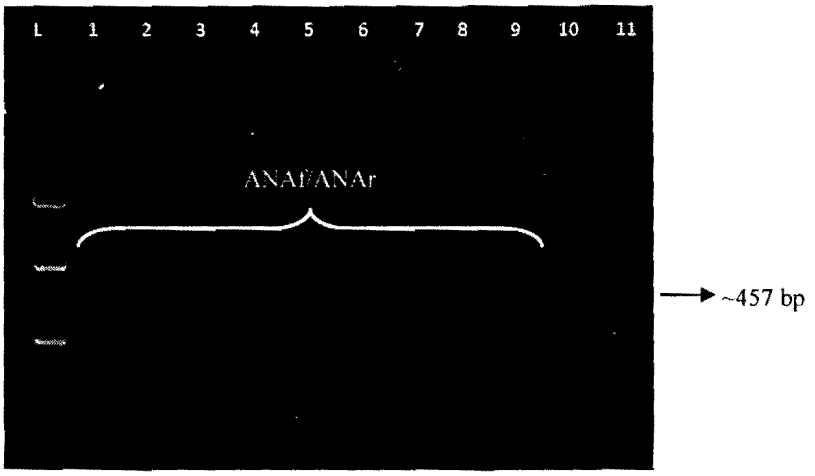
When tested with cyanobacteria specific general primer sets CPC1f/CPC1r and AN3801f/3801r, no bands were identified from any sites.

Specific Primers for Positive Results

Positive results from sites with cyanobacterial presence were tested again with specific primer sets ANAf/ANAr, MSf/MSr, 2156f/2156r, PSCf/PSCr, and 528f/650r.

Summer

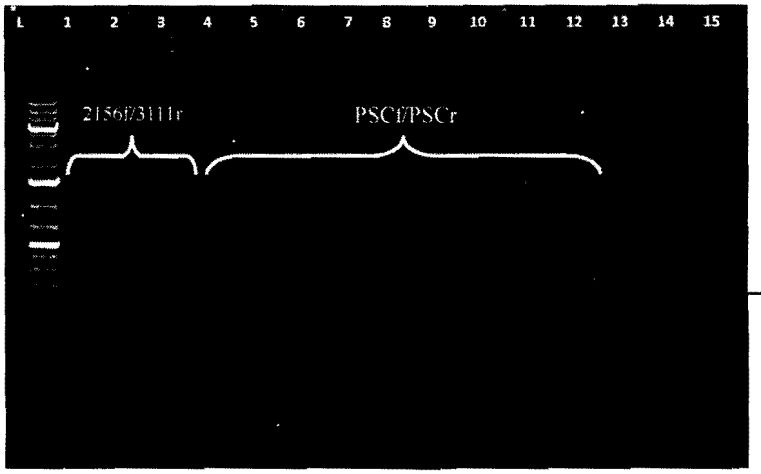
The summer collection sites which were tested with specific primers sets were Clarks Pond Site C (Coarse Filter), Clarks Pond Site D (Coarse Filter), Diamond Mill Site A (Coarse Filter), Clarks Pond Sites A, B, C, and D (Fine Filter), and South Orange Duck Pond Sites A and D (Fine Filter). As seen in figure 24 below, the summer positive sites were tested with ANAf/ANAr primer set, which would be indicative of *Anabaena circinalis*. Positive bands are seen in lanes 2,4,5,6,7,8,9, and 10, indicating that *A. circinalis* is present in all sites except Clarks Pond Site C (Coarse Filter) and Diamond Mill Pond Site A (Coarse Filter).



Lane	1	2	3	4	5	6	7	8	9	10	11
Water Body	Clarks		D.M.	Clarks				S.O.		Blank	(-) Control
Site	C	D	A	A	B	C	D	A	B		
Filter Type	C	C	C	F	F	F	F	F	F		

Figure 24: Results from the summer positive results are shown using the primer set ANAf/ANAr, indicative of *Anabaena circinalis* presence. Lanes 2, 4, 5, 6, 7, 8, and 9, indicate *A. circinalis* presence in all summer positive results except for Clarks Pond Site C (Coarse Filter) and Diamond Mill Site A (Coarse Filter).

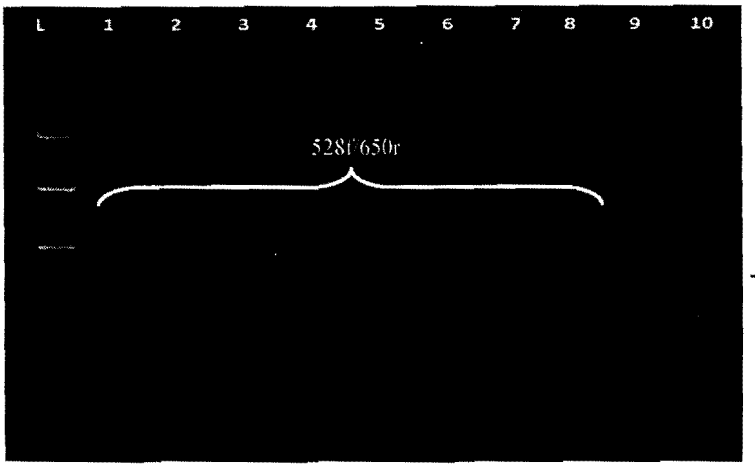
Next, the DNA from the summer positive sites underwent PCR using the primer sets MSf/MSr, 2156f/3111r, and PSCf/PSCr. As seen in figures 25 below, the only site that recorded a band was South Orange Duck Pond site B (Fine Filter), producing a band in lane three.



Lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Water Body	Clarks	S.O.		Clarks		D.M.	Clarks				S.O.		(-) Control		
Site	D	A	B	C	D	A	A	B	C	D	A	B			
Filter Type	F	F	F	C	C	C	F	F	F	F	F	F			

Figure 25: Results from the summer positive results are shown using the primer sets 2156f/3111r and PSCf/PSCr. Lane 3 indicated presence of the *mycB* gene.

The DNA from the summer positive sites underwent PCR using the primer set 528f/650r, which is a general diatom primer set. As seen in figure 26 below, diatoms were present in abundance at most of the sites, including Clarks Pond site C (Coarse Filter), Clarks Pond site D (Coarse Filter), Clarks Pond Site A (Fine Filter), Clarks Pond site D (Fine Filter), and South Orange Duck Pond sites A and B (Fine Filter), as seen by bands identified in lanes 1, 2, 3, 6, 7, and 8.

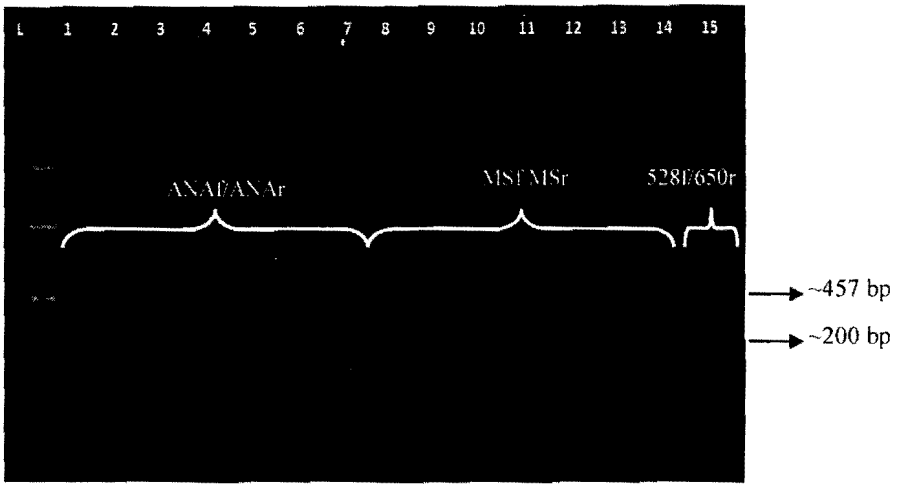


Lane	1	2	3	4	5	6	7	8	9	10	11
Water Body	Clarks		D.M.	Clarks				S.O.		Blank	(-) Control
Site	C	D	A	A	B	C	D	A	B		
Filter Type	C	C	C	F	F	F	F	F	F		

Figure 26: Results from the summer positive results are shown using the primer set 528f/650r for Diatoms. Bands are seen in all lanes except 4 and 5, indicative of diatom presence among all sites except Clarks Pond Site B.

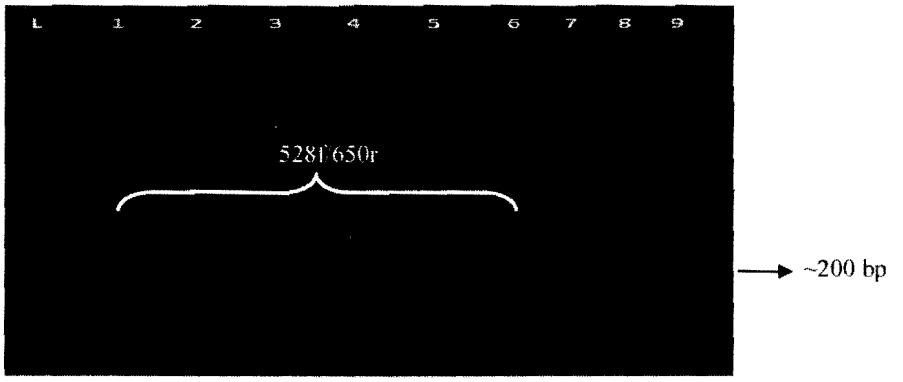
Fall

The fall positive sites were Branch Brook State Park sites A, B, C, and D (Coarse Filter), Branch Brook State Park sites A and D (Fine Filter), and Verona Lake site A (Fine Filter). These sites were tested using PCR with primer sets ANAf/ANAr, MSf/MSr, and 528f/650r. In figures 27 and 28 below, the results from this PCR assay are shown.



Lane	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Water Body	Branch Brook						Verona	Branch Brook						Verona	BB
Site	A	B	C	D	A	D	A	A	B	C	D	A	D	A	A
Filter Type	C	C	C	C	F	F	F	C	C	C	C	F	F	F	C

Figure 27: Results from the fall positive results are shown using the primer sets ANAf/ANAr, MSf/MSr, and 528f/528r. Lanes 1 through 7 indicate Anabaena presence, with lane 15 indicating diatom presence.



Lane	1	2	3	4	5	6	7	8	9
Water Body	Branch Brook					Verona	(-) Control		
Site	B	C	D	A	D	A			
Filter Type	C	C	C	F	F	F			

Figure 28: Results from the fall positive results are shown using the primer set 528f/528r. Bands are seen in all wells, indicative of Diatom presence at all sites.

Table 11: Verona Lake – PCR based assays – Summer

Sites	Diatoms	Cyanobacteria			Photosynthetic Bacteria
	Other	Anabaena	Synechococcus	Other	
A	X	X	X	X	X
B	X				X
C	X				X

Table 12: Diamond Mill Pond – PCR based assays – Summer

Sites	Diatoms	Cyanobacteria			Photosynthetic Bacteria
		Anabaena	Synechococcus	Other	
A	X		X	X	X
B	X				X
C	X		X	X	X
D					X
E	X				X

Table 13: Branch Brook State Park Lake – PCR based assays – Summer

Sites	Diatoms	Cyanobacteria			Photosynthetic Bacteria
		Anabaena	Synechococcus	Other	
A	X	X	X	X	X
B	X	X	X	X	X
C	X	X	X	X	X
D	X	X	X	X	X

Table 14: Clarks Pond – PCR based assays – Summer

Sites	Diatoms	Cyanobacteria			Photosynthetic Bacteria
		Anabaena	Synechococcus	Other	
A	X	X		X	X
B	X	X		X	X
C	X	X	X	X	X
D	X	X		X	X

Table 15: South Orange Duck Pond – PCR based assays – Summer

Sites	Diatoms	Cyanobacteria			Photosynthetic Bacteria
		Anabaena	Synechococcus	Other	
A	X	X		X	X
B	X	X		X	X
C					X
D					X

Table 16: Verona Lake – PCR based assays – Fall

Sites	Diatoms	Cyanobacteria			Photosynthetic Bacteria
		Anabaena	Synechococcus	Other	
A		X	X	X	X
B					X
C					X

Table 17: Diamond Mill – PCR based assays – Fall

Sites	Diatoms	Cyanobacteria			Photosynthetic Bacteria
		Anabaena	Synechococcus	Other	
A					X
B					X
C					X
D					X
E					X

Table 18: Branch Brook State Park Lake – PCR based assays – Fall

Sites	Diatoms	Cyanobacteria			Photosynthetic Bacteria
		Anabaena	Synechococcus	Other	
A	X	X		X	X
B	X	X		X	X
C	X	X		X	X
D	X	X		X	X

Table 19: Clarks Pond – PCR based assays – Fall

Sites	Diatoms	Cyanobacteria			Photosynthetic Bacteria
		Anabaena	Synechococcus	Other	
A					X
B					X
C					X
D					X

Table 20: South Orange Duck Pond – PCR based assays – Fall

Sites	Diatoms	Cyanobacteria			Photosynthetic Bacteria
		Anabaena	Synechococcus	Other	
A					X
B					X
C					X
D					X

Table 21: Verona – Microscopic observations – Summer

Sites	Diatoms				Cyanobacteria		Photosynthetic Bacteria
	Synedra	Cyclotella	Other	Cell # ($x10^6$/ml)	Other	Cell # ($x10^6$/ml)	
A		X	X	6.1	X	9.1	X
B	X			12.3		6.1	X
C			X	3.1		6.1	X

Table 22: Diamond Mill Pond - Microscopic observations - Summer

Sites	Diatoms				Cyanobacteria			Photosynthetic Bacteria
	Amphicampa	Fragilaria	Other	Cell # ($x10^6$/ml)	Synechococcus	Other	Cell # ($x10^6$/ml)	
A	X		X	6.1			3.1	X
B		X		6.1			6.1	X
C			X	3.1	X		9.1	X
D				3.1	X		1.0	X
E			X	1.0	X	X	12.2	X

Table 23: Branch Brook State Park Lake - Microscopic observations – Summer

Sites	Diatoms				Cyanobacteria					Photosynthetic Bacteria
	Synedra	Fragilaria	Other	Cell # ($x10^6$/ml)	Synechococcus	Radiococcus	Oscillatoria	Other	Cell # ($x10^6$/ml)	
A			X	9.1		X		X	9.1	X
B	X		X	6.1				X	9.1	X
C		X	X	15.3		X	X	X	15.3	X
D			X	12.2	X	X		X	12.3	X

Table 24: Clarks Pond - Microscopic observations – Summer

Sites	Diatoms		Cyanobacteria		Photosynthetic Bacteria
	Other	Cell # ($x10^6$/ml)	Other	Cell # ($x10^6$/ml)	
A	X	3.1		3.1	X
B	X	3.1	X	9.2	X
C		0		0	X
D		0		0	X

Table 25: South Orange Duck Pond - Microscopic observations - Summer

Sites	Diatoms						Cyanobacteria			Photosynthetic Bacteria
	Scenedesmus	Fragilaria	Aulacoseria	Stephanocyclus	Other	Cell# (x10^6/ml)	Synechococcus	Other	Cell# (x10^6/ml)	
A			X			9.2		X	6.1	X
B	X				X	9.2		X	24.5	X
C		X		X		9.2	X	X	15.3	X
D			X		X	9.2		X	1.0	X

Table 26: Verona Lake - Microscopic observations – Fall

Sites	Diatoms			Cyanobacteria			Photosynthetic Bacteria
	Fragilaria	Other	Cell # ($x10^6$/ml)	Tetraspora	Other	Cell # ($x10^6$/ml)	
A		X	6.1			6.1	X
B	X		9.1	X		6.1	X
C			0			0	X

Table 27: Diamond Mill Pond - Microscopic observations – Fall

Sites	Diatoms		Cyanobacteria		Photosynthetic Bacteria
	Other	Cell # ($x10^6$/ml)	Other	Cell # ($x10^6$/ml)	
A	X	3.1	X	6.1	X
B	X	3.1	X	6.1	X
C	X	3.1		3.1	X
D		3.1		0	X
E		0		0	X

Table 28: Branch Brook State Park Lake - Microscopic observations – Fall

Sites	Diatoms			Cyanobacteria				Photosynthetic Bacteria
	Asterionella	Other	Cell # (x10^6/ml)	Spirogyra	Tetraspora	Other	Cell # (x10^6/ml)	
A	X	X	9.2	X	X	X	9.1	X
B	X		1.0			X	9.2	X
C			3.1				3.1	X
D			0				3.1	X

Table 29: Clarks Pond - Microscopic observations – Fall

Sites	Diatoms			Cyanobacteria			Photosynthetic Bacteria
	Navicula	Other	Cell # (x10^6/ml)	Synechococcus	Other	Cell # (x10^6/ml)	
A			1.0		X	6.1	X
B	X	X	6.1	X		3.1	X
C		X	9.2	X		3.1	X
D		X	6.1	X		3.1	X

Table 30: South Orange Duck Pond - Microscopic observations – Fall

Sites	Diatoms				Cyanobacteria		Photosynthetic Bacteria
	Synedra	Aulacoseria	Other	Cell# ($x10^6$/ml)	Other	Cell# ($x10^6$/ml)	
A	X	X		9.1	X	9.1	X
B			X	3.1	X	3.1	X
C				3.1	X	3.1	X
D			X	3.1		3.1	X

MICROSCOPIC OBSERVATIONS

Each coarse and fine filter were hole-punched, re-suspended in De-Ionized water, and observed under a phase contrast microscope in order to detect, verify, and determine abundant species among each water body at each site. Images were organized by water body, site, as well as phylogenic classification.

Summer Collections

In figure 29, images of Diatoms from Clarks Pond are shown from coarse filtered sites collected in the summer. In figure 30, images of Diatoms from Diamond Mill Pond are shown from coarse filtered sites collected during the summer. In figure 31, an image of cyanobacteria and a cryptomonad from Diamond Mill Pond are shown from coarse filtered sites collected during the summer. In figure 32, images identified from Diamond Mill Pond fine filtered sites are shown from summer collections. In figure 33, images of Diatoms from Verona Lake are shown from coarse filtered sites collected during the summer. In figure 34, images of cyanobacteria from Verona Lake are shown from coarse filtered sites collected during the summer. In figure 35, images of Diatoms from Branch Brook State Park are shown from coarse filtered sites collected during the summer.

In figure 36, images of cyanobacteria from Branch Brook State Park are shown from coarse filtered sites collected during the summer. In figure 37, images identified from Branch Brook State Park fine filtered sites are shown. In figure 38, images of Diatoms from South Orange Duck Pond are shown from coarse filtered sites collected during the summer. In figure 39, images identified from South Orange Duck Pond fine filtered sites are shown.

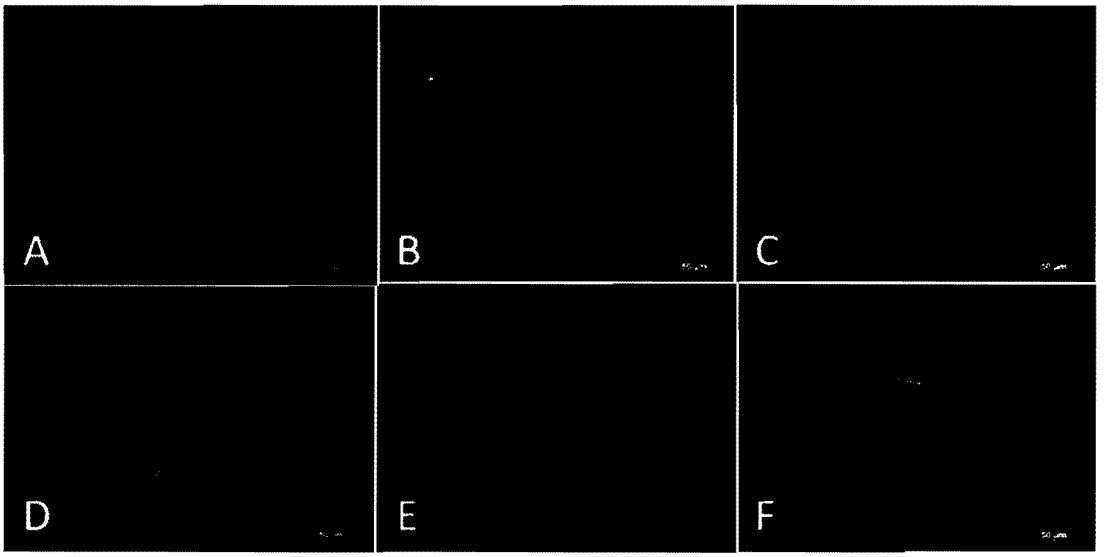


Figure 29: Diatoms located at Clarks Pond in Bloomfield, NJ. (A) Diatom identified from site B. (B) Diatom identified from site B. (C) Diatom identified from site B. (D) Diatom identified from site B. (E) Diatom identified from site B. (F) Diatom identified from site B. (400X)

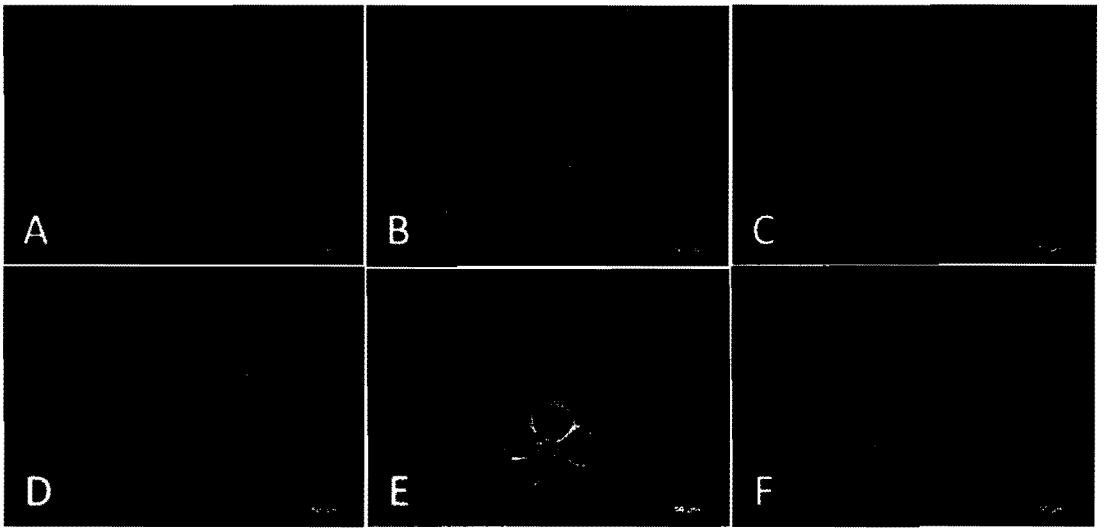


Figure 30: Diatoms located at Diamond Mill Pond in Milburn, NJ. (A) Amphicampa identified from site A. (B) Diatom identified from site B. (C) Diatoms identified from site A. (D) Fragilaria identified from site B. (E) Diatom identified from site C (Coarse Filter). (F) Diatom identified from site A. (400X)

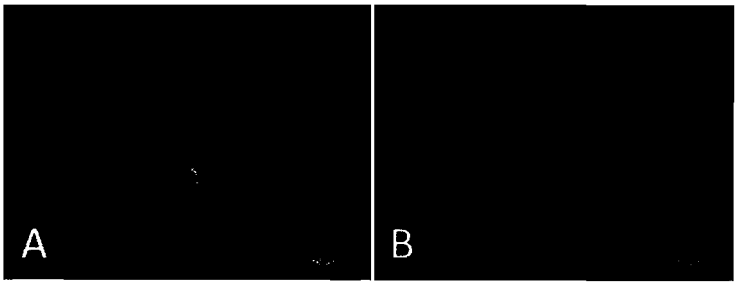


Figure 31: Cyanobacteria identified from Diamond Mill Pond. (A) Cyanobacteria identified from site A. (B) Cryptomonad identified from site B. (400X)

Figure 32: Images identified from Diamond Mill Pond fine filtered sites. (A) Paramecium identified from site C. (B) Synechococcus identified from site C. (C) Synechococcus identified from site D. (D) Diatom identified from site D. (E) Filamentous Cyanobacteria identified from site D. (F) Synechococcus identified from site D. (400X)

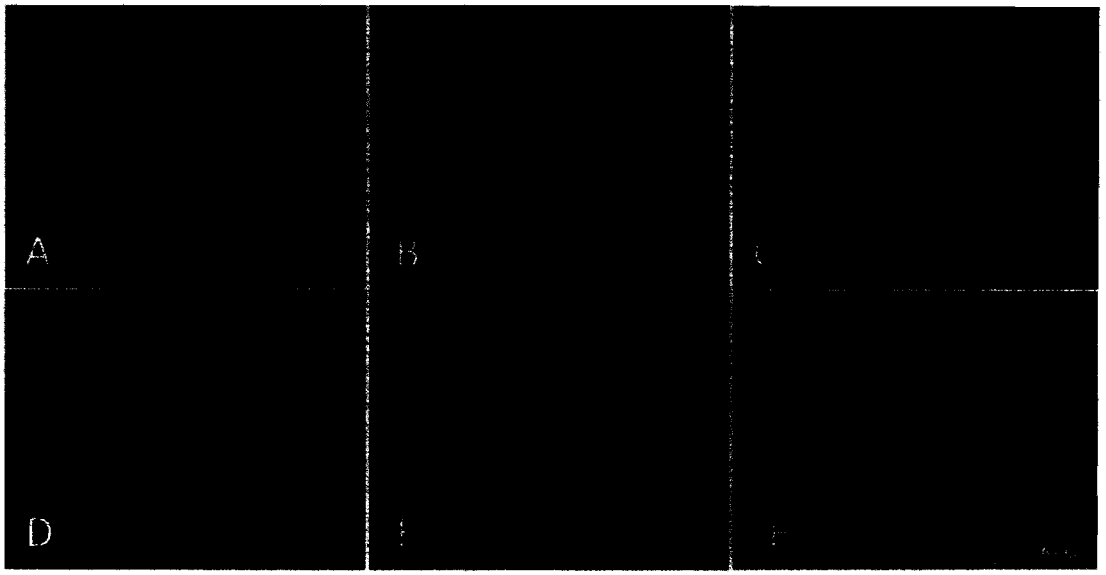


Figure 33: Diatoms located at Verona Lake in Verona, NJ. (A) Diatom identified from site C. (B) Diatom identified from site A. (C) Cyclotella identified from site A. (D) Synedra identified from site B. (E) Diatom identified from site C. (F) Diatom identified from site A. (400X)

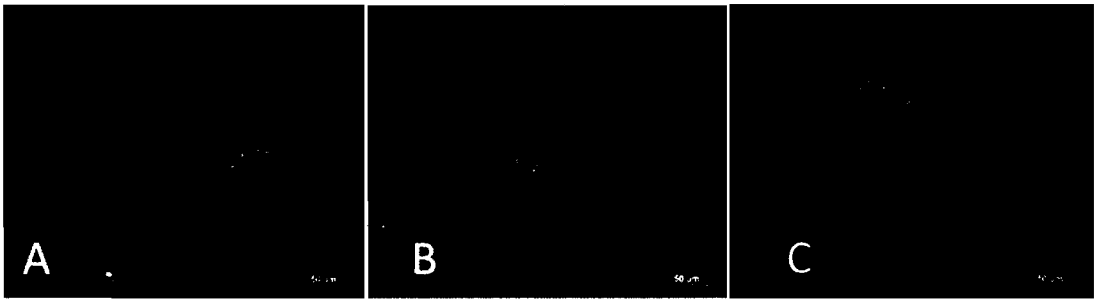


Figure 34: Cyanobacteria located at Verona Lake in Verona, NJ. (A) Cyanobacteria identified from site A. (B) Cyanobacteria identified from site A. (C) Cyanobacteria identified from site A. (400X)

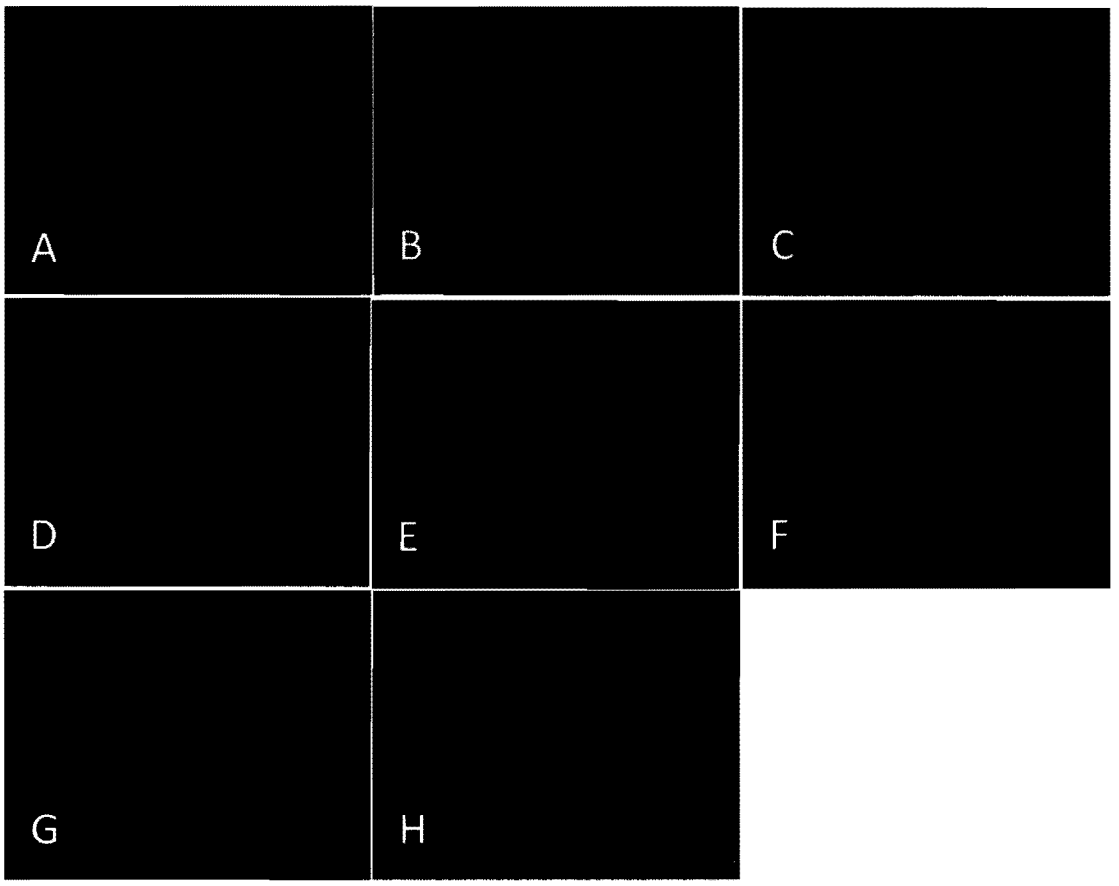


Figure 35: Diatoms collected from Branch Brook State Park in Newark, NJ. (A) Diatom identified from site A. (B) Diatom identified from site A. (C) Diatom identified from site B. (D) Diatom- Synedra identified from site B. (E) Diatom identified from site B. (F) Diatom identified from site C. (G) Diatom identified from site C. (H) Diatom- Fragilaria identified from site C. (400X)

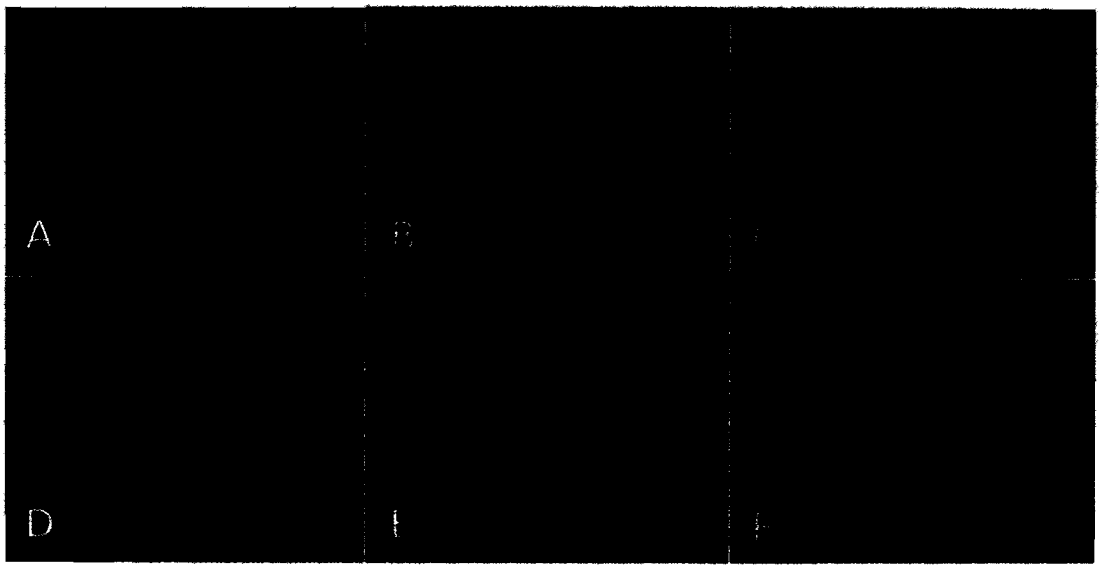


Figure 36: Cyanobacteria collected from Branch Brook State Park in Newark, NJ. (A) Oscillatoria identified from site C. (B) Radiococcus identified from site A. (C) Radiococcus identified from site C. (D) Phytoplankton – Phacus identified from site B. (E) Radiococcus identified from site D. (F) Radiococcus identified from site C. (400X)

Figure 37: Images identified from Branch Brook State Park fine filtered sites. (A) Paramecium identified from site D. (B) Synechococcus identified from site D. (C) Cyanobacteria identified from site D. (D) Synechococcus identified from site D. (E) Radiococcus identified from site D. (F) Synechococcus identified from site D. (1000X)

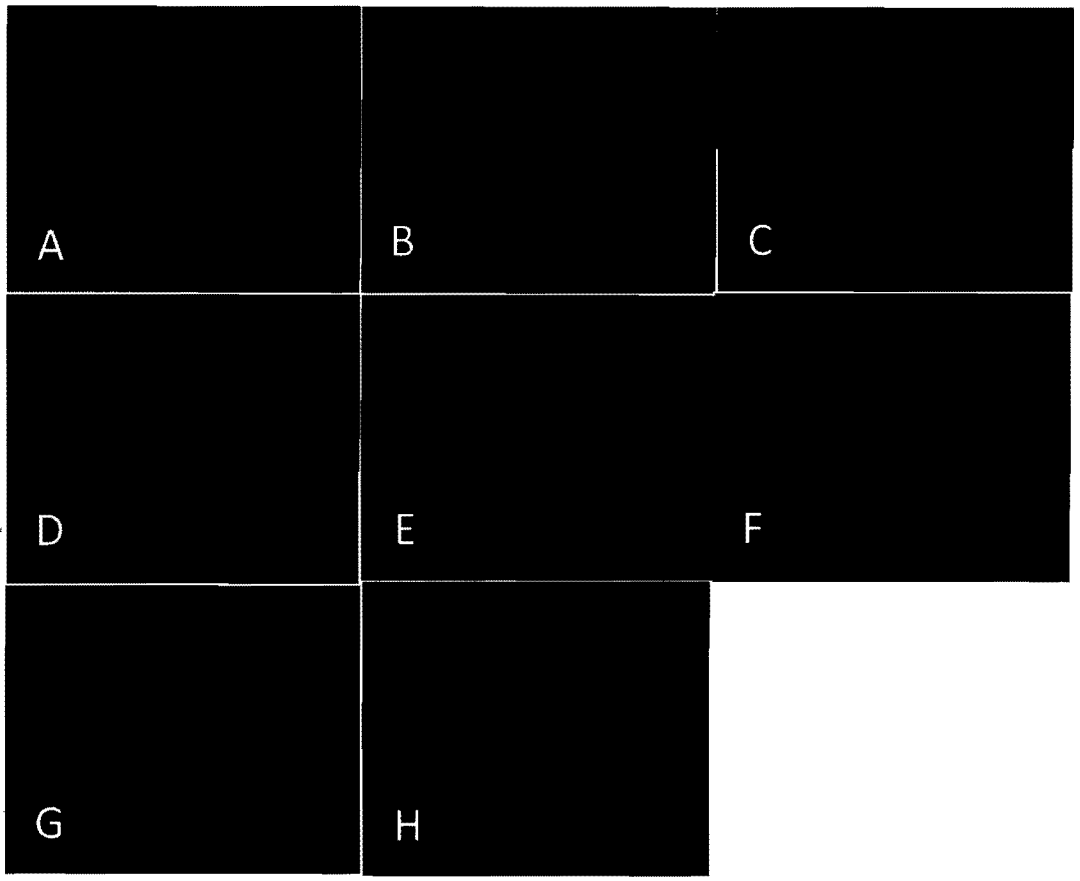


Figure 38: Diatoms collected from South Orange Duck Pond in South Orange, NJ. (A) Diatom- Scenedesmus identified from site B. (B) Diatom- Aulacoseria identified from site A. (C) Diatom identified from site B. (D) Diatom identified from site B. (E) Diatom- Fragilaria identified from site C. (F) Diatom – Stephanocyclus identified from site C. (G) Diatom revered from site D. (H) Diatom- Aulacoseria identified from site D. (400X)

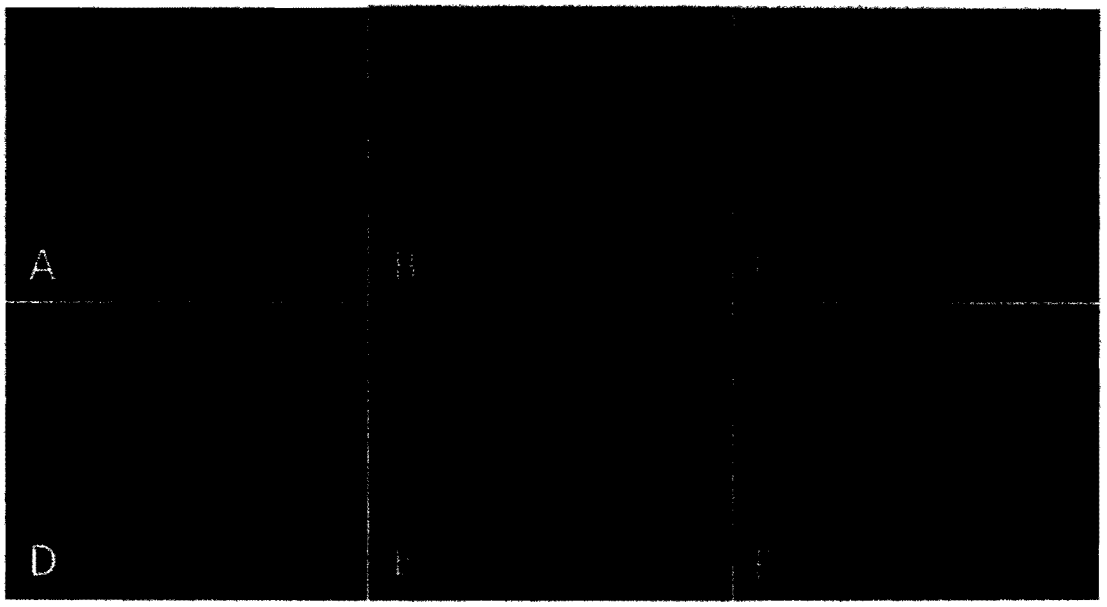


Figure 39: Images identified from South Orange Duck Pond fine filtered sites. (A) Filamentous cyanobacteria identified from site B. (B) Rod-Shaped cyanobacteria identified from site B. (C) Synechococcus identified from site B. (D) Filamentous cyanobacteria identified from site C. (E) Filamentous cyanobacteria identified from site C. (F) Synechococcus identified from site B. (1000X)

Fall Collections

In figure 40, images of Diatoms from Clarks Pond are shown from coarse filtered sites collected in the fall. In figure 41, images of cyanobacteria from Clarks Pond are shown from coarse filtered sites collected in the fall. In figure 42 below, images of Diatoms from Diamond Mill Pond are shown from coarse filtered sites collected in the fall. In figure 43, images of Diatoms from Verona Lake are shown from coarse filtered sites collected in the fall. In figure 44, cyanobacteria are seen identified from Verona Lake during the fall collections. In figure 45 below, images of Diatoms from Branch Brook State Park are shown from coarse filtered sites collected in the fall. In Figures 46, images of cyanobacteria are shown from Branch Brook State Park coarse filtered sites collected in the fall are shown. In figure 47, Diatoms are shown from South Orange Duck Pond coarse filtered sites collected during the fall. In figure 48, images of cyanobacteria identified from South Orange Duck Pond coarse filtered sites from fall collections are shown.

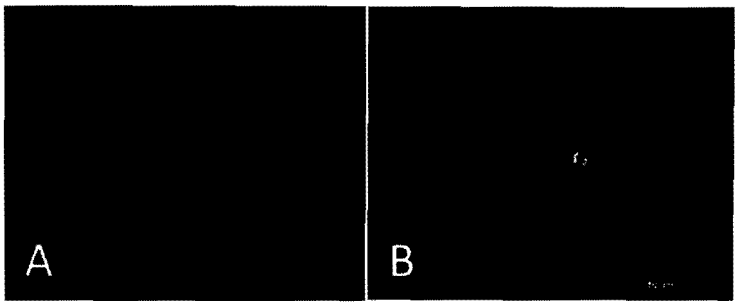


Figure 40: Diatoms identified from Clarks Pond in Bloomfield, NJ. (A) Diatom – Navicula identified from site A. (B) Diatom identified from site A. (1000X)

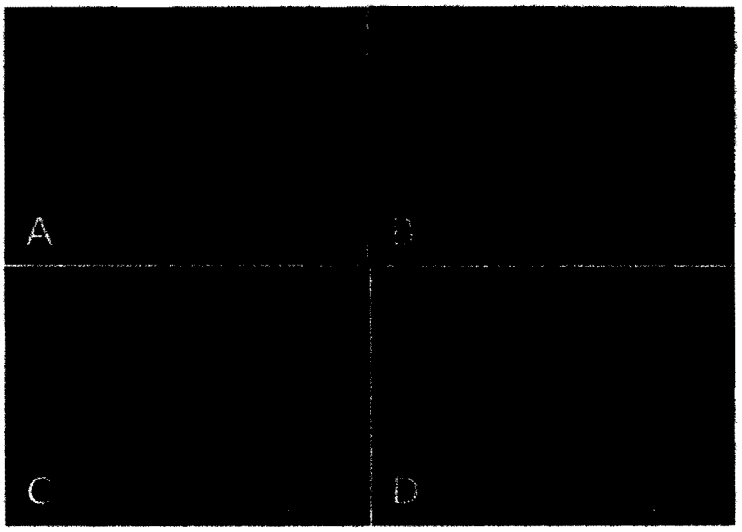


Figure 41: Cyanobacteria identified from Clarks Pond in Bloomfield, NJ. (A) Synechococcus identified from site B. (B) Cyanobacteria identified from site B. (C) Synechococcus identified from site C. (D) Synechococcus identified from site D. (1000X)

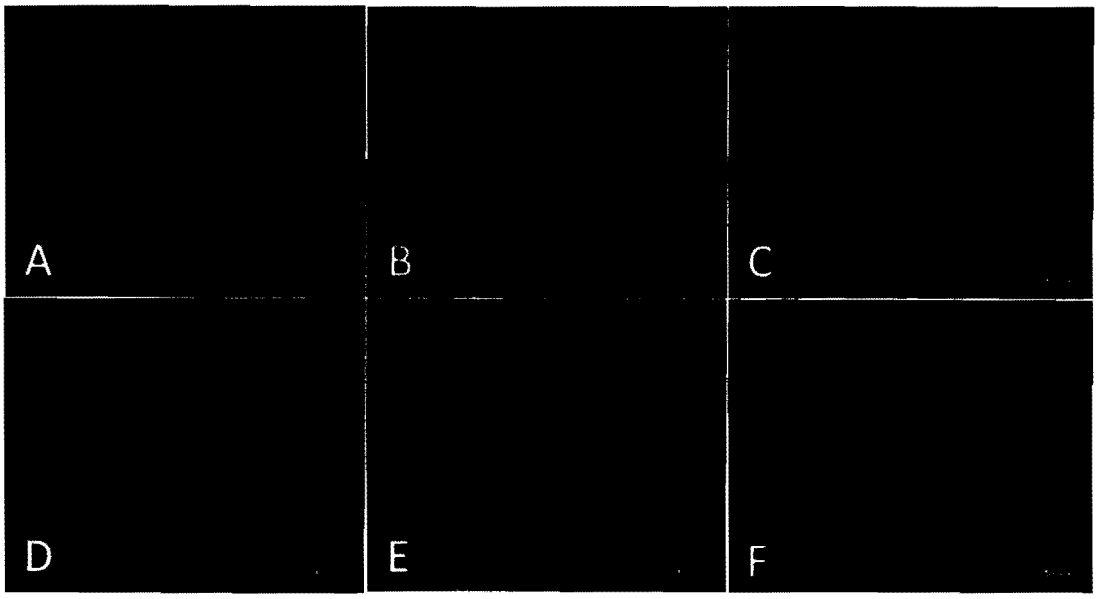


Figure 42: Diatom images identified from Diamond Mill Pond in Milburn, NJ. (A) Diatom identified from site A. (B) Diatom identified from site A. (C) Diatom identified from site B. (D) Diatom identified from site C. (E) Diatom identified from site C. (F) Diatom identified from site A. (400X)

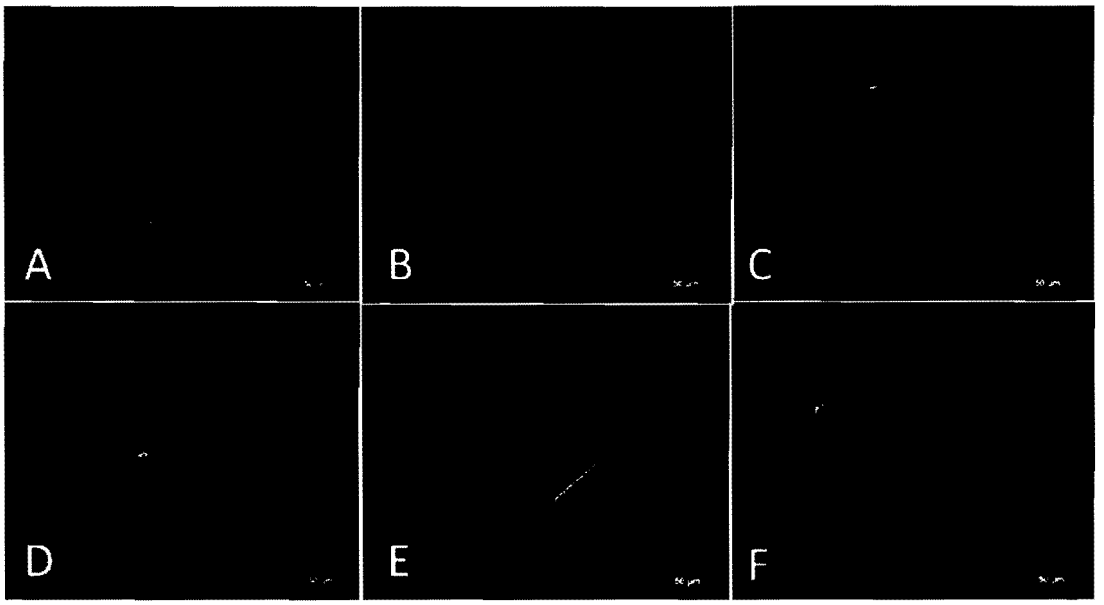


Figure 43: Diatom images identified from Verona Lake in Verona, NJ. (A) Diatom identified from site A. (B) Diatom identified from site B. (C) Diatom identified from site A. (D) Diatom-Fragilaria identified from site A. (E) Diatom identified from site A. (F) Diatom- Fragilaria identified from site B. (400X)

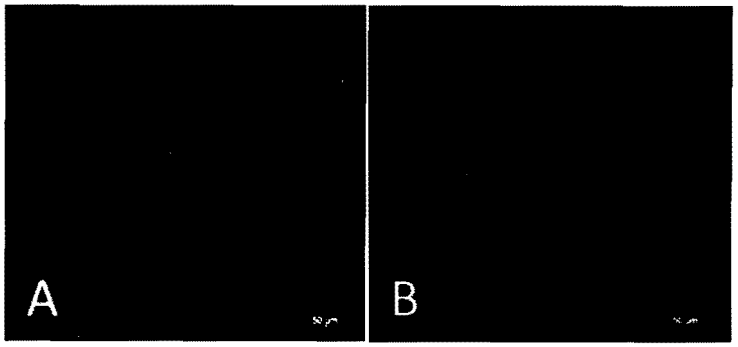


Figure 44: Cyanobacteria images identified from Verona Lake in Verona, NJ. (A) Cyanobacteria identified from site B. (B) Tetraspora identified from site B. (400X)

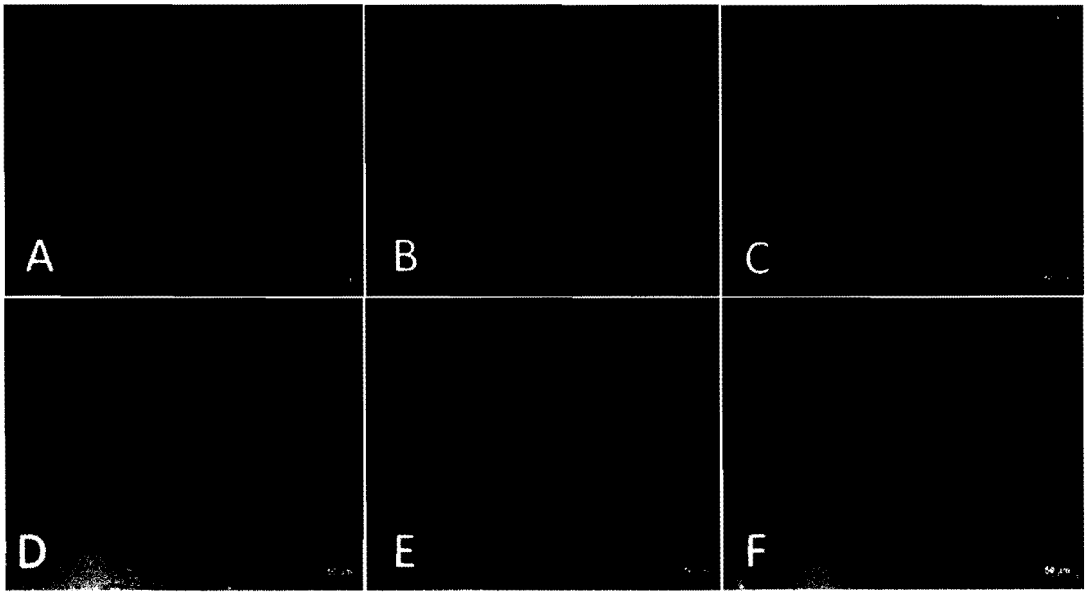


Figure 45: Images of Diatoms at Branch Brook State Park in Newark, NJ. (A) Diatom - Asterionella identified from site A. (B) Diatom - Asterionella identified from site A. (C) Diatom identified from site A. (D) Diatom - Asterionella identified from site B. (E) Diatom - Asterionella identified from site B. (F) Diatom - Asterionella identified from site B. (400X)

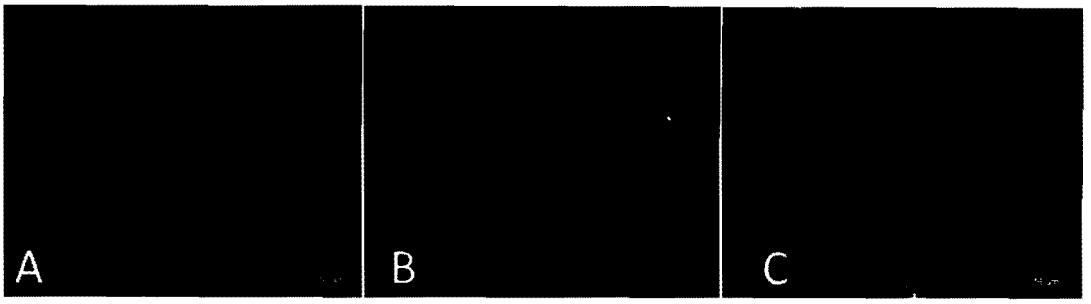


Figure 46: Cyanobacteria identified from Branch Brook State Park in Newark, NJ are shown. (A) Tetraspora identified from site A.(B) Cyanobacteria identified from site A. (C) Spirogyra identified from site A.

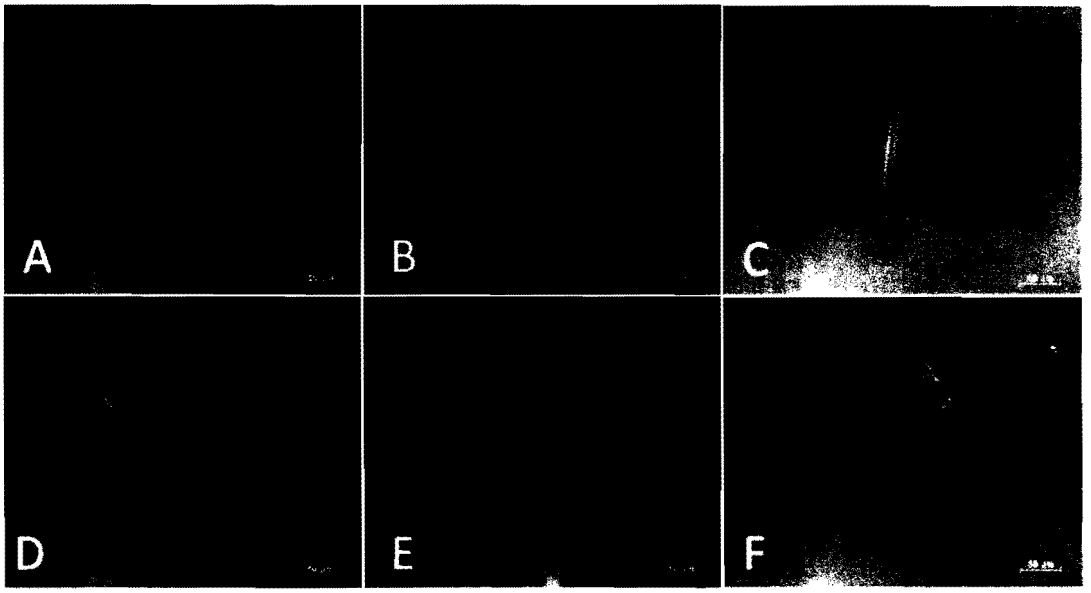


Figure 47: Diatoms identified from South Orange Duck Pond in South Orange, NJ. (A) Diatom- Aulacoseria identified from site A. (B) Diatom- Synedra identified from site A. (C) Diatom identified from site B. (D) Diatom identified from site D. (E) Diatom identified from site D. (E) Diatom- Aulacoseria identified from site A. (400X)

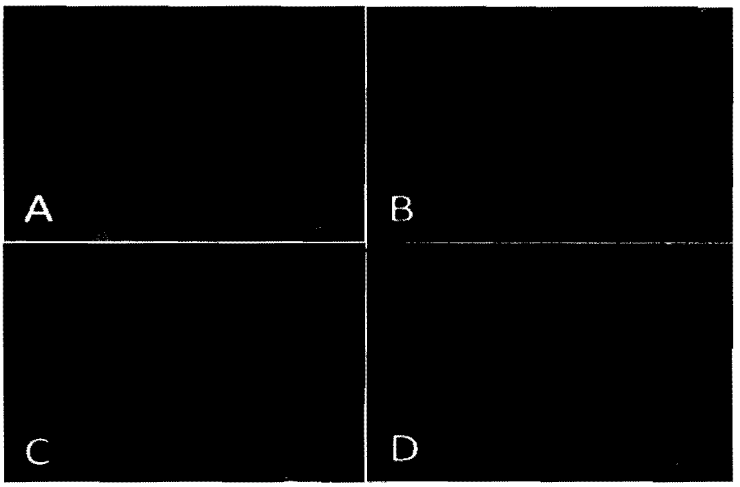


Figure 48: Cyanobacteria identified from South Orange Duck Pond in South Orange, NJ. (A) Cyanobacteria identified from site A. (B) Cyanobacteria identified from site B. (C) Cyanobacteria identified from site C. (D) Cyanobacteria identified from site C. (400X)

Comparison between microscopic observations and PCR based assays results for summer collection

Water Body	Site	Diatoms	Cyanobacteria	Photosynthetic Bacteria
Verona Lake	A	B	B	B
	B	B	M	B
	C	B	M	B
Diamond Mill Pond	A	B	B	B
	B	B	M	B
	C	B	B	B
	D	M	M	P
	E	B	M	B
Branch Brook Lake	A	B	B	B
	B	B	B	B
	C	B	B	B
	D	B	B	B
Clarks Pond	A	P	P	P
	B	P	P	P
	C	P	P	P
	D	P	P	P
South Orange Duck Pond	A	P	B	B
	B	P	P	P
	C	M	M	P
	D	M	M	P

Table 31: The correlation between microscope findings and PCR findings is depicted. PCR detected cyanobacteria in many sites (Tables 11, 12, and 13 above). (B – Highlighted in yellow) indicates detection with PCR and microscope. (P – Highlighted in salmon) indicates detection with PCR. (M – Highlighted in blue) indicates detection with microscopic imaging.

Comparison between microscopic observations and PCR based assays results for fall collection

Water Body	Site	Diatoms	Cyanobacteria	Photosynthetic Bacteria
Verona Lake	A	M	P	B
	B	M	M	B
	C	M	M	B
Diamond Mill Pond	A	M	M	B
	B	M	M	B
	C	M	M	B
	D	M	M	B
	E	M	M	B
Branch Brook Lake	A	B	B	B
	B	B	P	B
	C	P	P	B
	D	P	P	B
Clarks Pond	A	M	M	B
	B	M	M	B
	C	M	M	B
	D	M	M	B
South Orange Duck Pond	A	M	M	B
	B	M	M	B
	C	M	M	B
	D	M	M	B

Table 32: The correlation between microscope findings and PCR findings is depicted. PCR detected cyanobacteria in many sites (Tables 14-16). Upon viewing microscopic images of these sites, the three sites in which cyanobacteria was detected with both PCR and microscopic analysis were Branch Brook Lake sites A and B (Coarse Filter). (B – Highlighted in yellow) indicates detection with PCR and microscope. (P – Highlighted in salmon) indicates detection with PCR. (M – Highlighted in blue) indicates detection with microscopic imaging.

Flow Cytometry

Flow cytometry was performed on samples from Branch Brook State Park. Site C and Site D were sampled in this test. Comparisons were made between raw collected water, coarse filter, as well as fine filter. Comparisons were also made between site C coarse filter from the summer collection as well as site C coarse filter from water collected in December, 2011.

In figure 49, the flow cytometer data Branch Brook State Park site C is shown. In the graph, it is shown that when comparing the raw sample of water with the coarse the amount of phycocyanin-containing cells is reduced. When comparing to the fine filter, the amount of phycocyanin-containing cells is drastically reduced.

In figure 50, the flow cytometer data from site D at Branch Brook State Park is shown. In these graphs, the phycocyanin-containing cells were more concentrated from the coarse filter, resulting in a slightly higher percentage when compared with the raw sample. The fine filter produced a drastically different cell percentage, mirroring the results from site C. Using the auto-fluorescence mechanisms of phycocyanin-containing cells, the flow cytometer was able to produce a profile of phycocyanin-containing cells within the samples.

In figure 51, a comparison was made between the coarse filter from site C collected in the summer and the coarse filter from site C collected in December. As seen in the graphs indicating phycocyanin-containing cell presence, the number of cells is very slightly reduced in the December sample when compared with the summer sample.

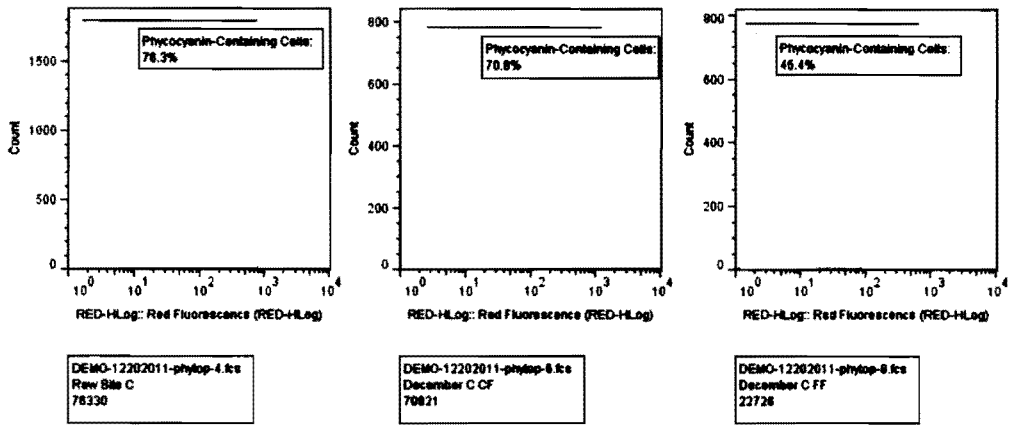


Figure 49: Using red fluorescence to exploit the auto-fluorescence nature of phycocyanin-containing phytoplankton, the flow cytometer determined the amount of phycocyanin-containing cells in the water sample from site C, which is seen at the top, right of each graph. In graph one, the raw water sample, the amount of cells is 76.3%. In graph two, the amount of cells is 70.9%, and in graph three the amount of cells is 45.4%.

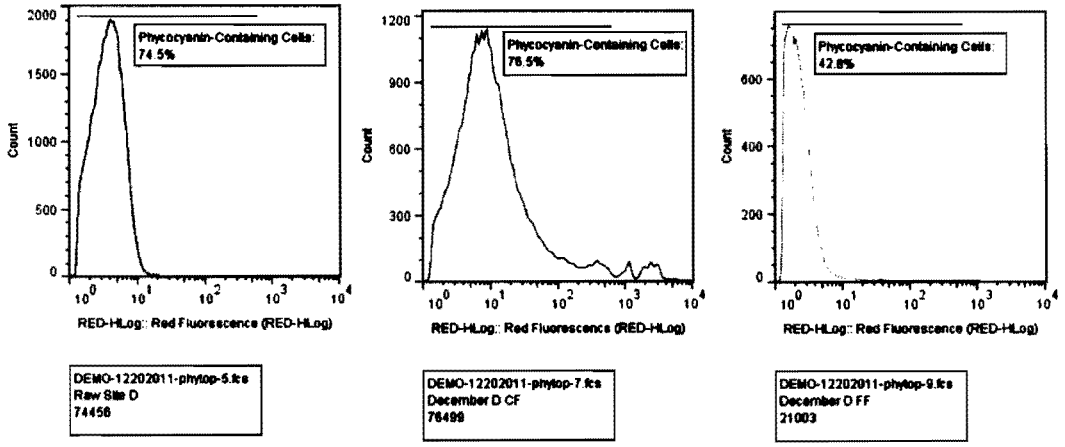


Figure 50: Using red fluorescence to exploit the auto-fluorescence nature of phycocyanin-containing phytoplankton, the flow cytometer determined the amount of phycocyanin-containing cells in the water sample from site D, which is seen at the top, right of each graph. In the left graph, the raw water sample, the amount of cells is 74.5 %. In the middle graph, the amount of cells is 76.5 %, and in right graph the amount of cells is 42.8 %.

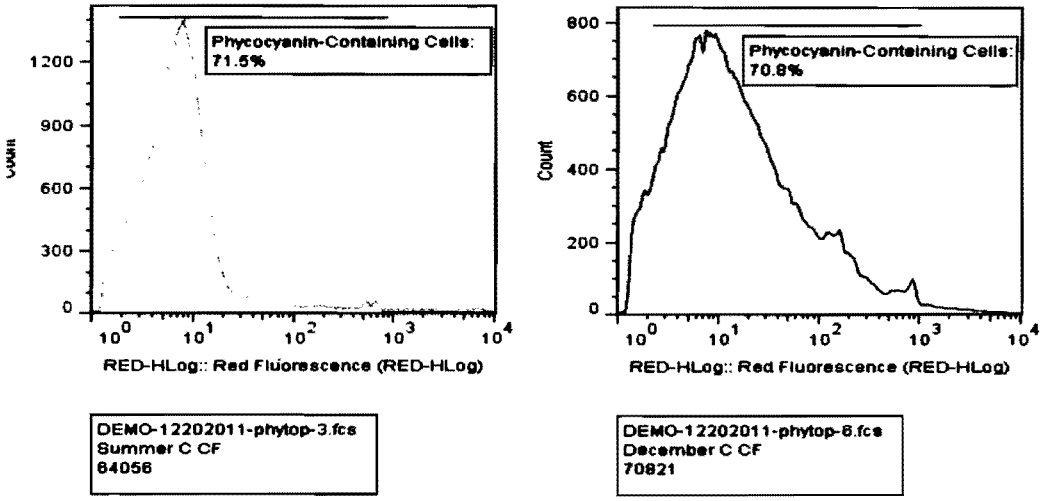


Figure 51: Using red fluorescence to exploit the auto-fluorescence nature of phycocyanin-containing phytoplankton, the flow cytometer determined the amount of phycocyanin-containing cells in the water sample from site C (Both summer and winter samples), which is seen at the top, right of each graph. When comparing the summer and winter collections, there is only a slight reduction in cell percentage, indicating that phycocyanin cells are present in freshwater in both the summer and winter.

Microscopic observation suggested that most microbes among the water sample collected were bacteria, cyanobacteria and diatoms. Cell numbers were determined and recorded during microscopic analysis from each site of the freshwater ecosystems in this study. Cell number was observed and was subsequently plotted against water chemistry parameters, including pH, dissolved oxygen, and temperature for each site during the summer and fall collections. The results are shown in figure 52 to figure 63 below.

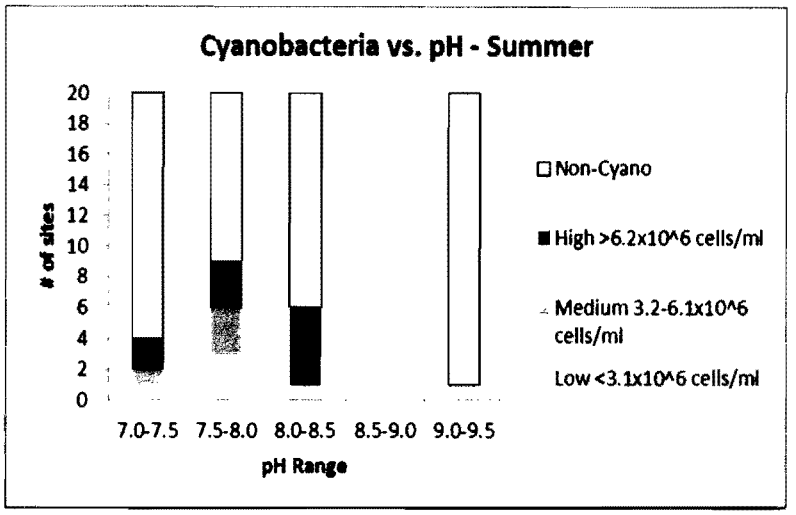


Figure 52: During summer collections, the pH and cell number at each site was recorded. In the given pH ranges above, the graph shows the number of sites with a high cyanobacteria cell number (>6.2x10^{6}cells/ml) (Red), sites with a medium cyanobacteria cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low cyanobacteria cell number (<3.1x10^{6}cells/ml) (Grey).

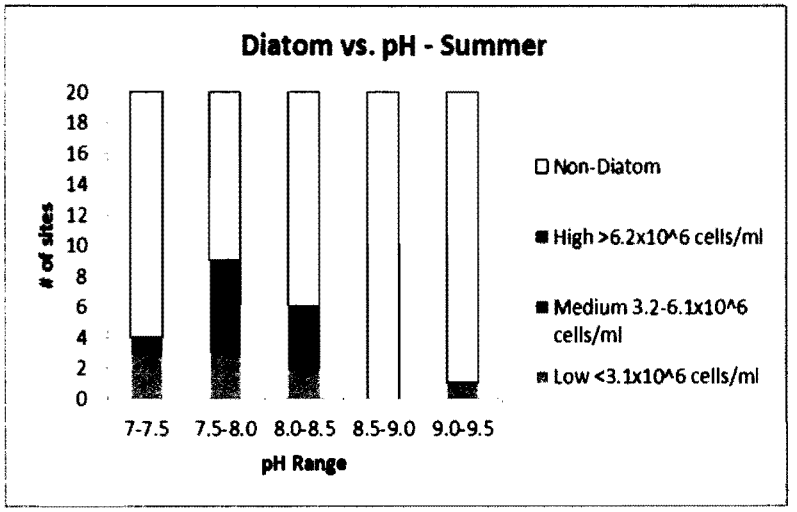


Figure 53: During summer collections, the pH and cell number at each site was recorded. In the given pH ranges above, the graph shows the number of sites with a high diatom cell number (>6.2x10^{6}cells/ml) (Red), sites with a medium diatom cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low diatom cell number (<3.1x10^{6}cells/ml) (Grey).

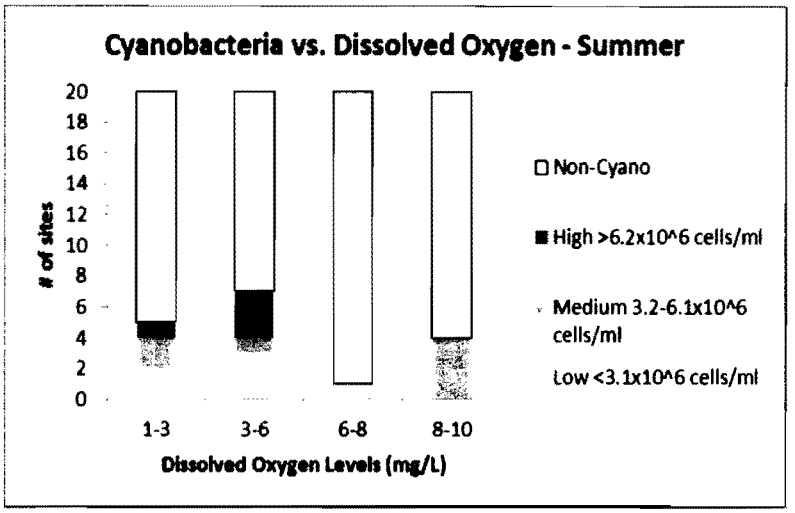


Figure 54: During summer collections, the dissolved oxygen and cell number at each site was recorded. In the given dissolved oxygen ranges above, the graph shows the number of sites with a high cyanobacteria cell number (>6.2x10^{6}cells/ml) (Red), sites with a medium cyanobacteria cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low cyanobacteria cell number (<3.1x10^{6}cells/ml) (Grey).

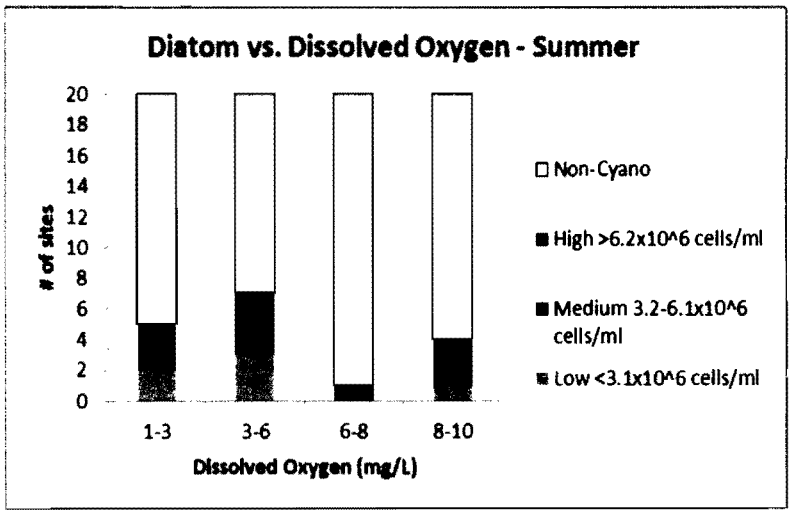


Figure 55: During summer collections, the dissolved oxygen and cell number at each site was recorded. In the given dissolved oxygen ranges above, the graph shows the number of sites with a high diatom cell number (>6.2x10^{6}cells/ml) (Red), sites with a medium diatom cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low diatom cell number (<3.1x10^{6}cells/ml) (Grey).

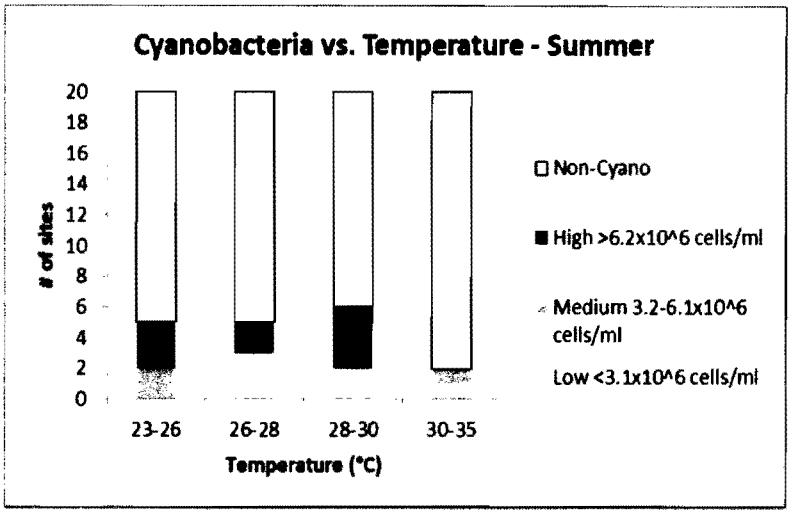


Figure 56: During summer collections, the temperature and cell number at each site was recorded. In the given temperature ranges above, the graph shows the number of sites with a high cyanobacteria cell number (>6.2x10^6cells/ml) (Red), sites with a medium cyanobacteria cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low cyanobacteria cell number (<3.1x10^6cells/ml) (Grey).

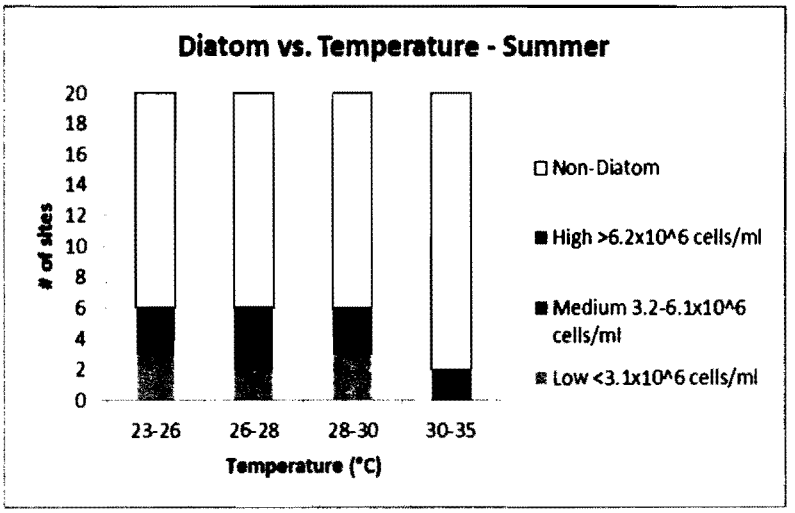


Figure 57: During summer collections, the temperature and cell number at each site was recorded. In the given temperature ranges above, the graph shows the number of sites with a high diatom cell number (>6.2x10^6cells/ml) (Red), sites with a medium diatom cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low diatom cell number (<3.1x10^6cells/ml) (Grey).

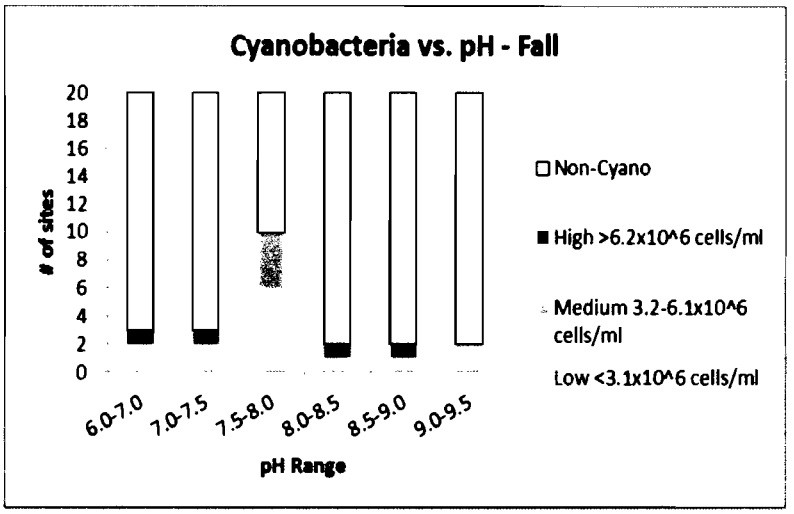


Figure 58: During fall collections, the pH and cell number at each site was recorded. In the given pH ranges above, the graph shows the number of sites with a high cyanobacteria cell number (>6.2x10^{6}cells/ml) (Red), sites with a medium cyanobacteria cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low cyanobacteria cell number (<3.1x10^{6}cells/ml) (Grey).

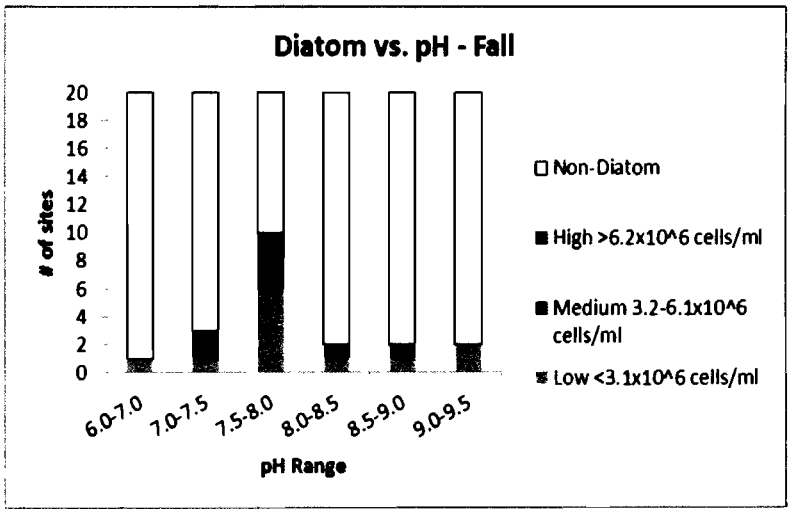


Figure 59: During fall collections, the pH and cell number at each site was recorded. In the given pH ranges above, the graph shows the number of sites with a high diatom cell number (>6.2x10^{6}cells/ml) (Red), sites with a medium diatom cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low diatom cell number (<3.1x10^{6}cells/ml) (Grey).

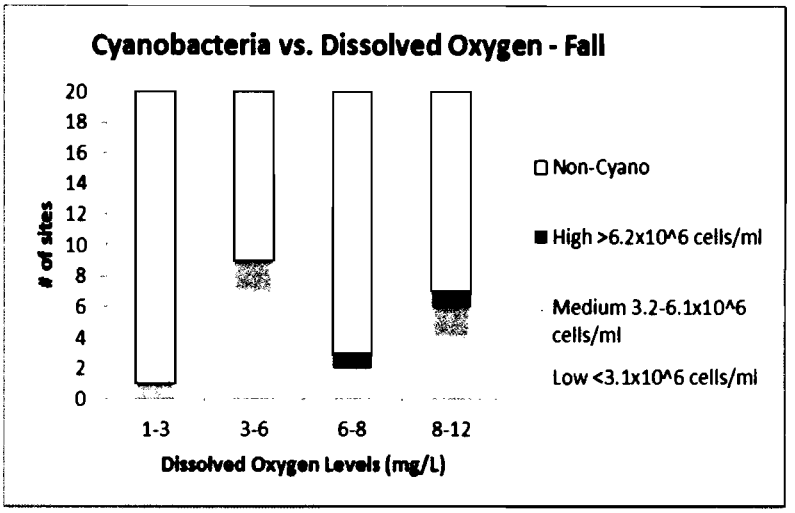


Figure 60: During fall collections, the dissolved oxygen and cell number at each site was recorded. In the given dissolved oxygen ranges above, the graph shows the number of sites with a high cyanobacteria cell number (>6.2x10^6cells/ml) (Red), sites with a medium cyanobacteria cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low cyanobacteria cell number (<3.1x10^6cells/ml) (Grey).

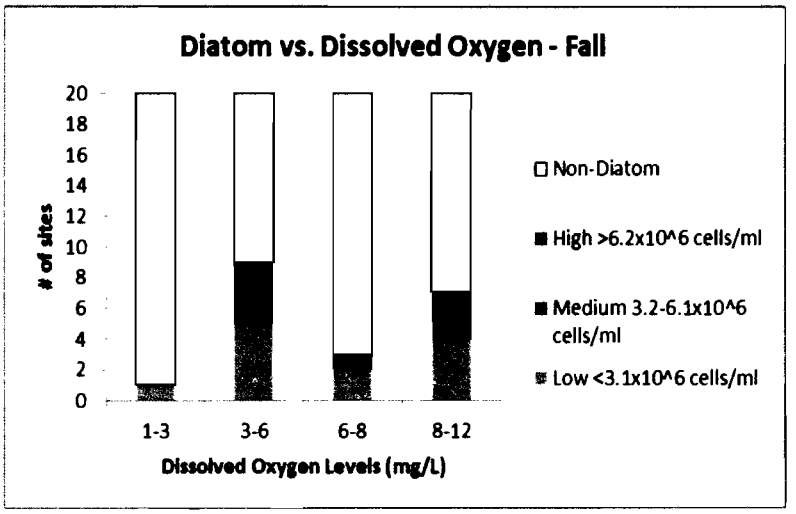


Figure 61: During fall collections, the dissolved oxygen and cell number at each site was recorded. In the given dissolved oxygen ranges above, the graph shows the number of sites with a high diatom cell number (>6.2x10^6cells/ml) (Red), sites with a medium diatom cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low diatom cell number (<3.1x10^6cells/ml) (Grey).

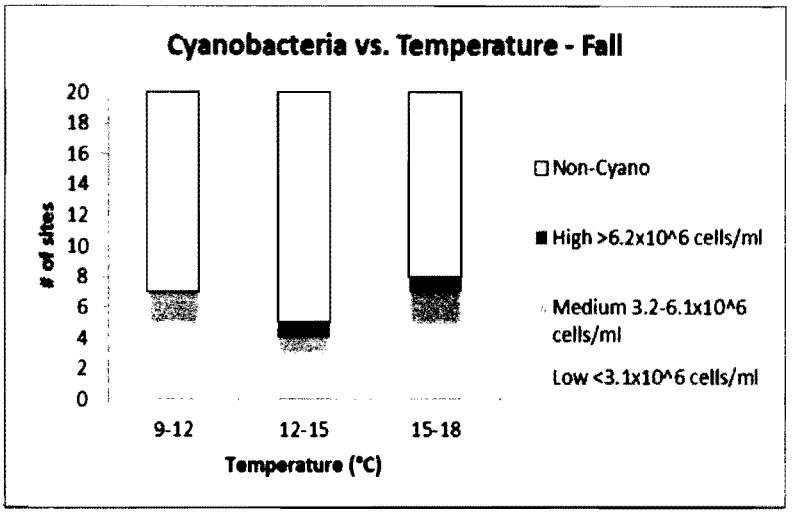


Figure 62: During fall collections, the temperature and cell number at each site was recorded. In the given temperature ranges above, the graph shows the number of sites with a high cyanobacteria cell number (>6.2x10^{6}cells/ml) (Red), sites with a medium cyanobacteria cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low cyanobacteria cell number (<3.1x10^{6}cells/ml) (Grey).

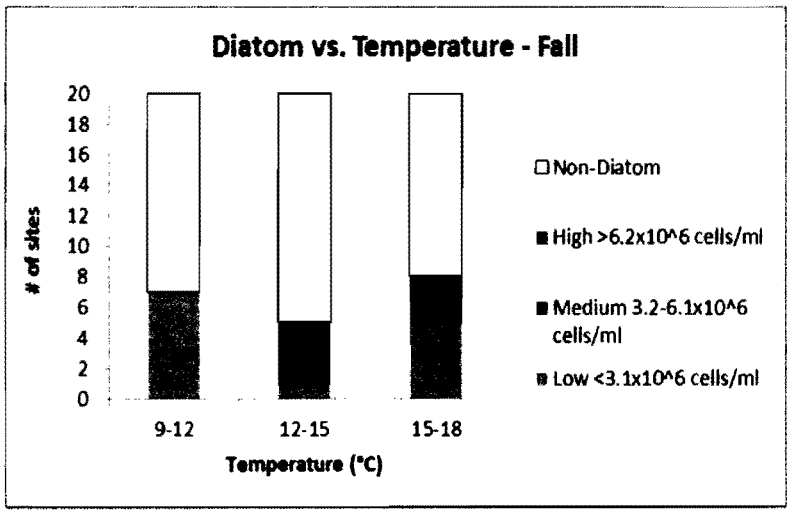


Figure 63: During fall collections, the temperature and cell number at each site was recorded. In the given temperature ranges above, the graph shows the number of sites with a high diatom cell number (>6.2x10^{6}cells/ml) (Red), sites with a medium diatom cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low diatom cell number (<3.1x10^6 cells/ml) (Grey).

Branch Brook Lake was observed for seasonal changes according to water chemistry and cell number (both cyanobacteria and diatom cells). It was found that temperature and dissolved oxygen had a more direct effect on phytoplankton cell number when compared with pH, as the pH ranges remained constant while the cell number fluctuated between seasons. As seen in figures 64 through 69 below, the cell number is seen to significantly decrease with a decreasing water temperature, as well as decreasing with an increase in dissolved oxygen.

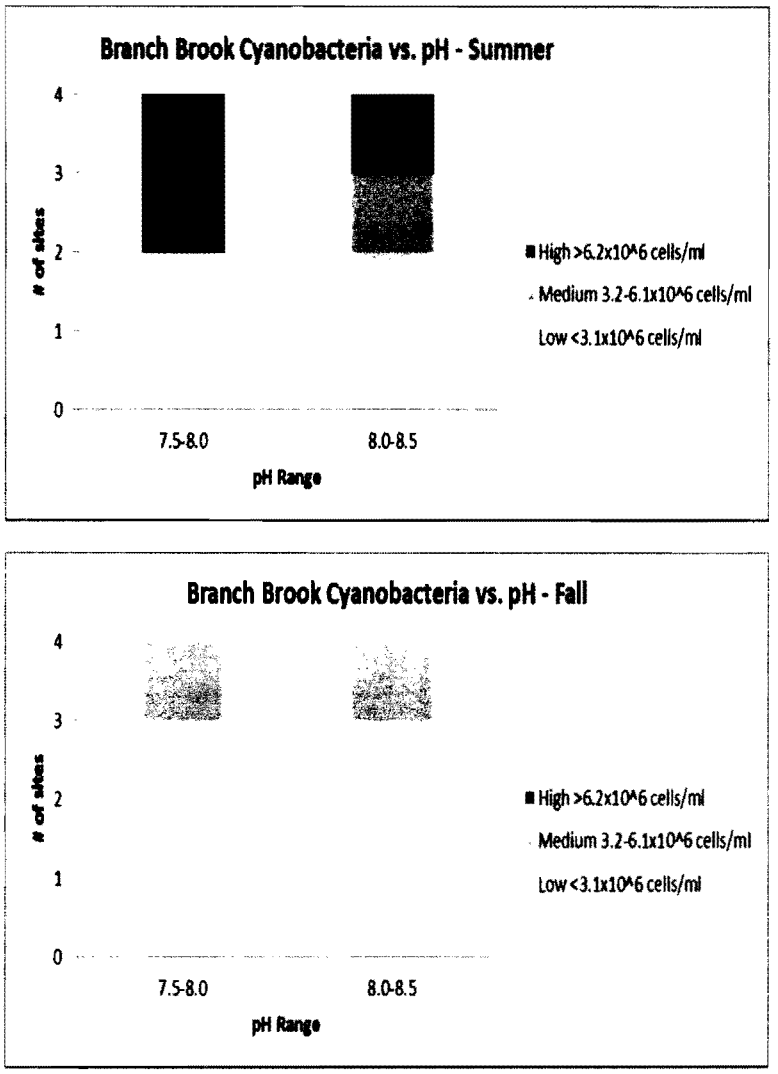


Figure 64: Top - During summer collections, the pH and cell number at each site of Branch Brook Lake was recorded. In the given pH ranges above, the graph shows the number of sites with a high cyanobacteria cell number (>6.2x10^6cells/ml) (Red), sites with a medium cyanobacteria cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low cyanobacteria cell number (<3.1x10^6cells/ml) (Grey). **Bottom** - During fall collections, the pH and cell number at each site of Branch Brook Lake was recorded. In the given pH ranges above, the graph shows the number of sites with a high cyanobacteria cell number (>6.2x10^6cells/ml) (Red), sites with a medium cyanobacteria cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low cyanobacteria cell number (<3.1x10^6cells/ml) (Grey).

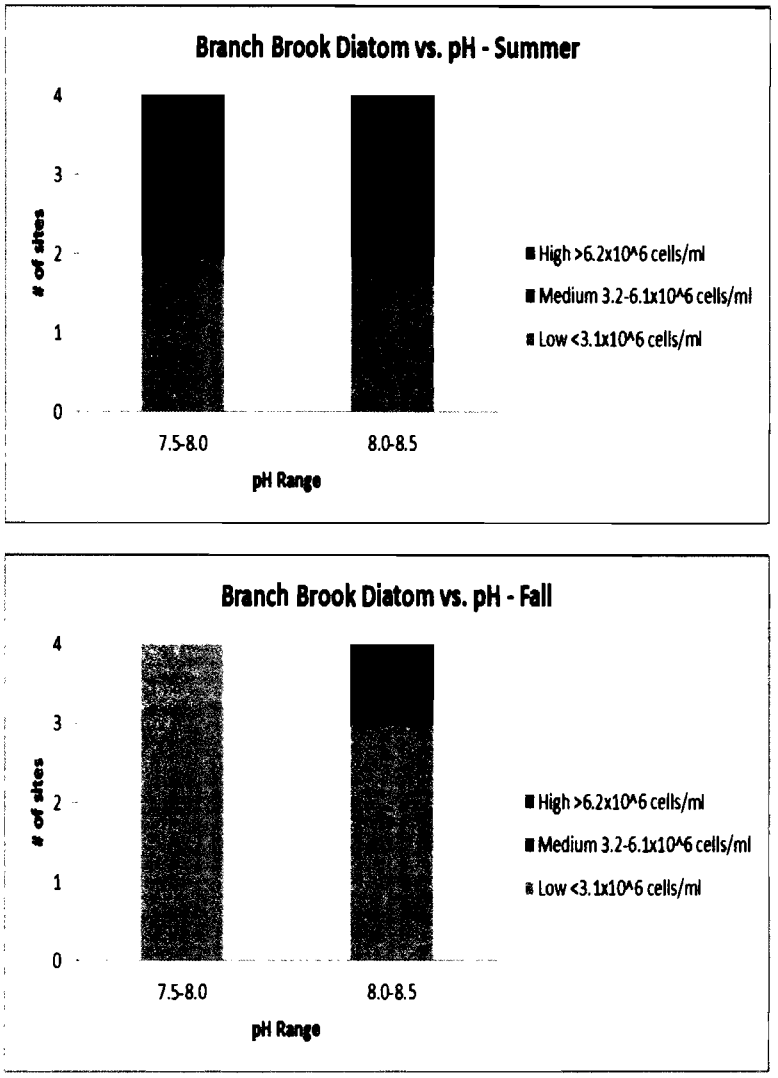


Figure 65: Top - During summer collections, the pH and cell number at each site of Branch Brook Lake was recorded. In the given pH ranges above, the graph shows the number of sites with a high diatom cell number (>6.2x10^6cells/ml) (Red), sites with a medium diatom cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low diatom cell number (<3.1x10^6cells/ml) (Grey). **Bottom** - During fall collections, the pH and cell number at each site of Branch Brook Lake was recorded. In the given pH ranges above, the graph shows the number of sites with a high diatom cell number (>6.2x10^6cells/ml) (Red), sites with a medium diatom cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low diatom cell number (<3.1x10^6cells/ml) (Grey).

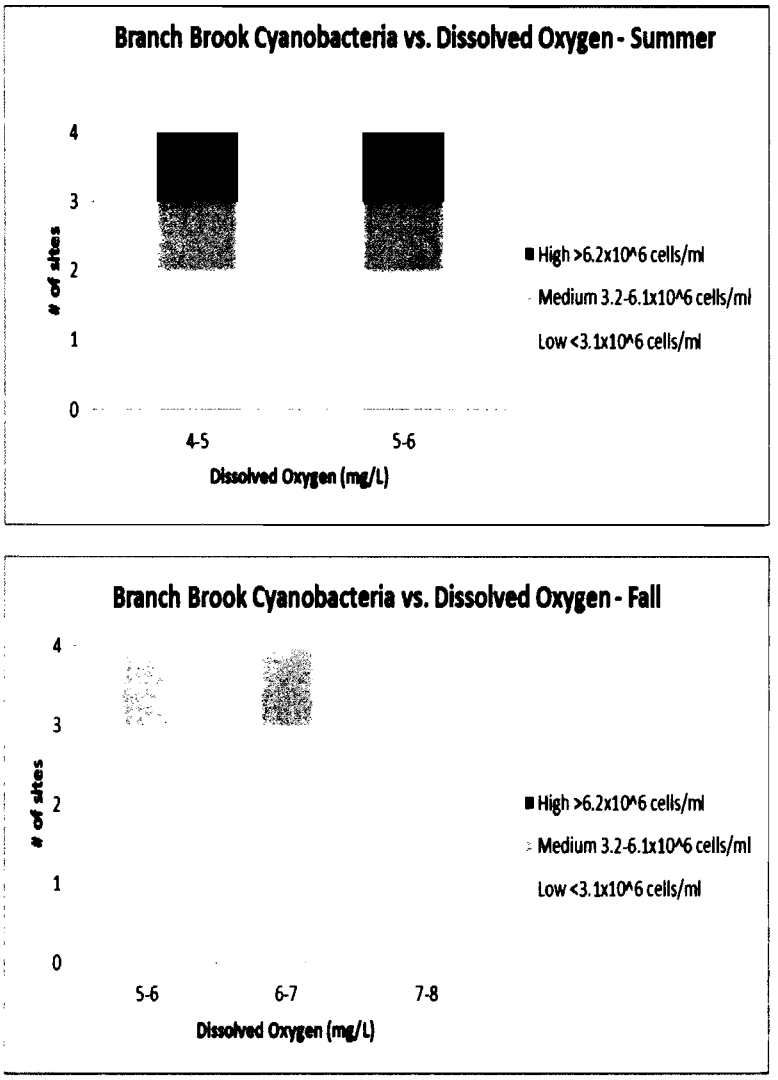


Figure 66: Top - During summer collections, the dissolved oxygen and cell number at each site of Branch Brook Lake was recorded. In the given dissolved oxygen ranges above, the graph shows the number of sites with a high cyanobacteria cell number (>$6.2x10^6$cells/ml) (Red), sites with a medium cyanobacteria cell number ($3.2x10^6$ – $6.0x10^6$ cells/ml) (Green), and sites with a low cyanobacteria cell number (<$3.1x10^6$cells/ml) (Grey). **Bottom** - During fall collections, the dissolved oxygen and cell number at each site of Branch Brook Lake was recorded. In the given dissolved oxygen ranges above, the graph shows the number of sites with a high cyanobacteria cell number (>$6.2x10^6$cells/ml) (Red), sites with a medium cyanobacteria cell number ($3.2x10^6$ – $6.0x10^6$ cells/ml) (Green), and sites with a low cyanobacteria cell number (<$3.1x10^6$cells/ml) (Grey).

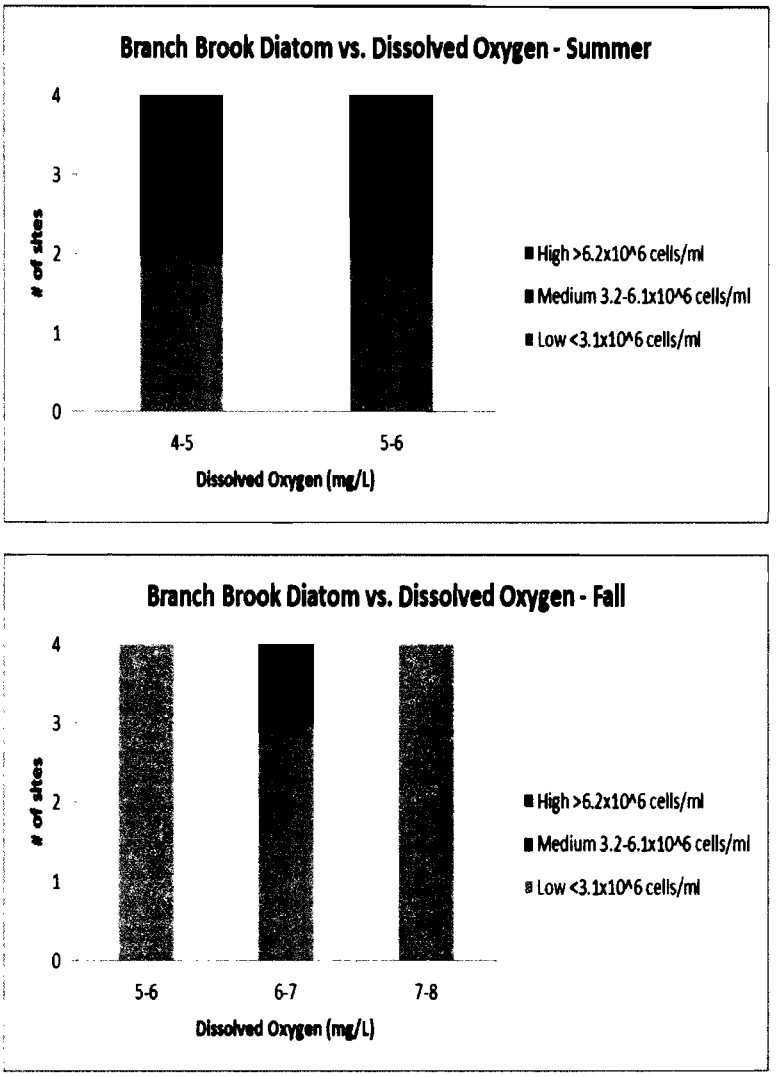


Figure 67: Top - During summer collections, the Dissolved Oxyggen and cell number at each site of Branch Brook Lake was recorded. In the given DO ranges above, the graph shows the number of sites with a high diatom cell number (>6.2x10^{6}cells/ml) (Red), sites with a medium diatom cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low diatom cell number (<3.1x10^{6}cells/ml) (Grey). **Bottom** - During fall collections, the Dissolved Oxyggen and cell number at each site of Branch Brook Lake was recorded. In the given DO ranges above, the graph shows the number of sites with a high diatom cell number (>6.2x10^{6}cells/ml) (Red), sites with a medium diatom cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low diatom cell number (<3.1x10^{6}cells/ml) (Grey).

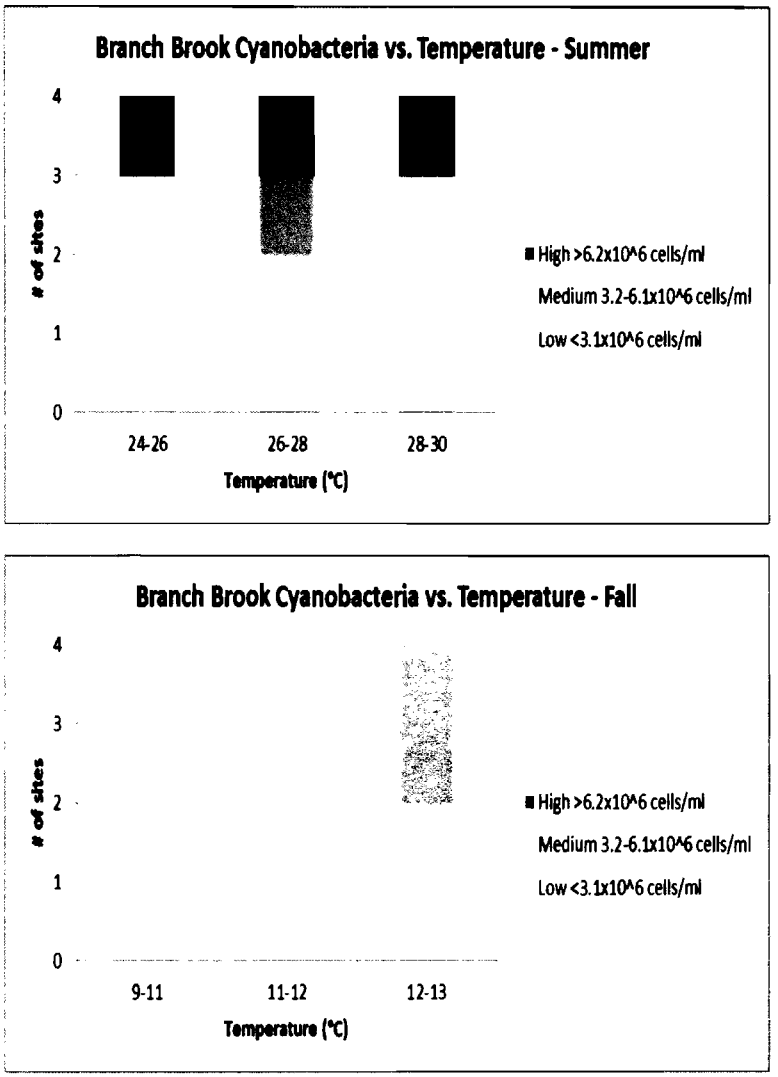


Figure 68: Top - During summer collections, the temperature and cell number at each site of Branch Brook Lake was recorded. In the given temperature ranges above, the graph shows the number of sites with a high cyanobacteria cell number (>6.2x10^6 cells/ml) (Red), sites with a medium cyanobacteria cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low cyanobacteria cell number (<3.1x10^6 cells/ml) (Grey). **Bottom** - During fall collections, the temperature and cell number at each site of Branch Brook Lake was recorded. In the given temperature ranges above, the graph shows the number of sites with a high cyanobacteria cell number (>6.2x10^6 cells/ml) (Red), sites with a medium cyanobacteria cell number (3.2x10^6 – 6.0x10^6 cells/ml) (Green), and sites with a low cyanobacteria cell number (<3.1x10^6 cells/ml) (Grey).

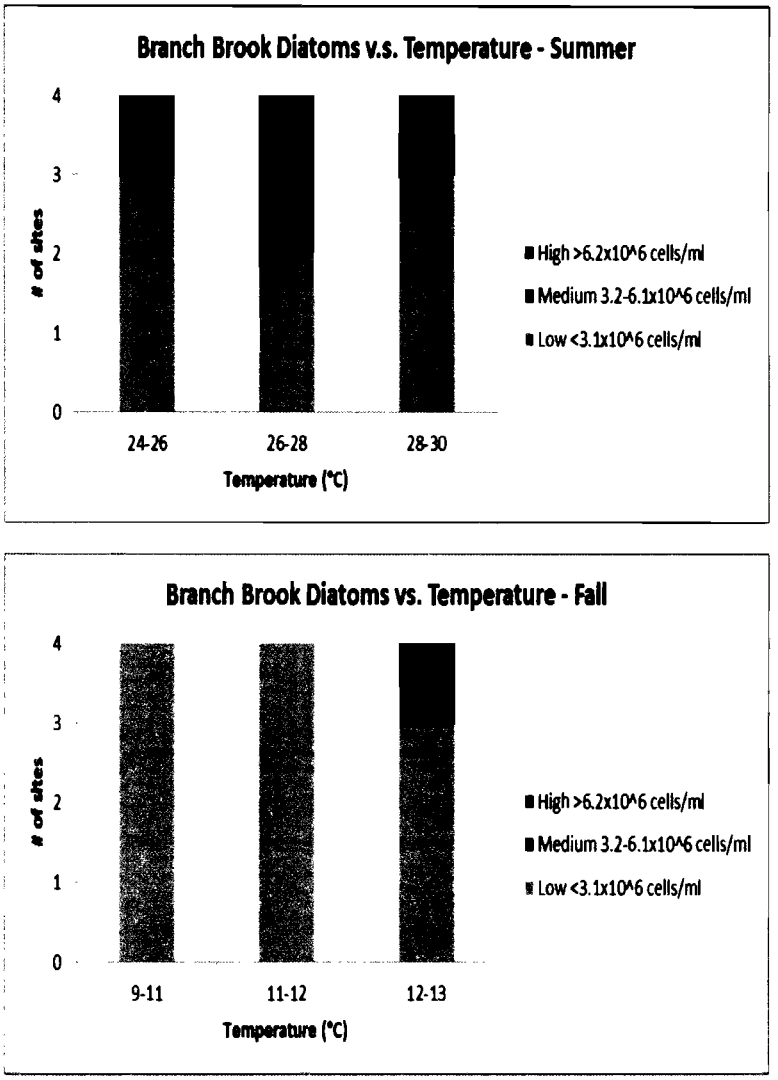


Figure 69: Top - During summer collections, the temperature and cell number at each site of Branch Brook Lake was recorded. In the given temperature ranges above, the graph shows the number of sites with a high diatom cell number (>$6.2x10^6$ cells/ml) (Red), sites with a medium diatom cell number ($3.2x10^6$ – $6.0x10^6$ cells/ml) (Green), and sites with a low diatom cell number (<$3.1x10^6$ cells/ml) (Grey). **Bottom** - During fall collections, the temperature and cell number at each site of Branch Brook Lake was recorded. In the given temperature ranges above, the graph shows the number of sites with a high diatom cell number (>$6.2x10^6$ cells/ml) (Red), sites with a medium diatom cell number ($3.2x10^6$ – $6.0x10^6$ cells/ml) (Green), and sites with a low diatom cell number (<$3.1x10^6$ cells/ml) (Grey).

In the present study, PCR-based assays appeared to be more sensitive when compared with the microscopic observations on cyanobacteria and diatoms. This conclusion can be drawn because PCR-based assays are much more sensitive due to their molecular nature, and PCR-based assays allowed for identification up to the species level, unlike microscopic observations. Although microscopic observation is inefficient, it shows to be useful in cell quantification as well as morphology observations.

Flow cytometry appeared to provide additional insight on the phytoplankton profile when used in conjunction with microscopic analysis and PCR-based assays. With additional experience and the utilization of fluorescent probes to identify cyanobacteria and diatoms to the species level, flow cytometry could be used to significantly enhance and expediate the results of this study.

From the results of the current study, it seems that although PCR is fast and efficient at providing results for identifying DNA from a mixed sample, there are many factors that affect the results, sometimes providing a less sensitive conclusion. Although microscopic analysis is time consuming, there are less factors that can interfere with identifying the species present.

Although all three methods appear to have their limitations, the combination of the three methods can be employed to provide a thorough analysis of the water bodies observed in the study.

Discussion

In this study, five freshwater bodies were sampled during the summer months of May through July and again during the fall months of October and November. At these lakes and ponds, sites were chosen depending on location and geography within the specific lake or pond. Sites were chosen based on coordinates of the previous site as well as water movement within the ecosystem. After each collection, each raw sample was filtered through a coarse filter with a pore size of 2.7μm; the filtrate was then filtered through a fine filter with a pore size of 0.45 μm. DNA was extracted from both the coarse filter and fine filters for polymerase chain reaction (PCR) for detection of cyanobacteria, bacteria, and other phytoplanktons among the DNA that was extracted. Primers for PCR were used for not only the detection of general cyanobacteria, bacteria, and other phytoplankton such as diatoms at each site, but for detection of specific genera such as Anabaena and Microcystis. Microscopic imaging analyses were also carried out with those filters to identify various freshwater phytoplanktons. From the observation of these microscopic images, several genera were identified as well as some detection on the species level. Also from microscopic analysis, cell numbers were derived. Using cell numbers derived from microscope analysis, correlations were detected between cell number and water chemistry, including pH, dissolved oxygen, and temperature at each site. Data analysis revealed that although polymerase chain reaction and microscopic observation of samples are exceptional procedures independently, the efficiency increases with the combination of the two procedures. As tables 23 and 24 indicate, a correlation was established between results observed from PCR and results observed from microscopic analysis.

Each water chemistry factor (pH, DO, temperature) was determined at each site of each water body, during both seasonal collections. Water chemistry at each site provided a correlation

between the pH, temperature, and dissolved oxygen levels and the cell number of cyanobacteria and diatoms among each site. A combination of these physical and chemical properties along with biotic features of the natural water bodies work in a symbiotic manner to determine the sensitivities of these water bodies to eutrophication. (Paerl *et al.*, 2001).

A major chemical water property which is influenced as much by the external environment as it is its own internal environment is the pH of water. The pH of water is partially affected by the CO_2 system components (CO_2, H_2CO_3, and $CO^2{}_{/3}^{-}$). Under basic conditions (pH>7.0), the CO_2 concentration is related to photosynthesis (Moatar *et al.*, 1999). Since most algal cells (cyanobacteria or phytoplankton among these) take in CO_2 during their growth process, the pH of the water body falls within a favorable range for growth of a particular genus or species. Some species, at conditions in which the pH is more than 8.6, may be limited in CO_2 uptake due to inactive ion transport mechanisms. But, it is also known that photosynthesis can occur at a pH of 9-10 in some species. Reduction of photosynthesis is noted at pH above 10 in all species (Reynolds, 1984). A documented case revealing pH dependence of plankton can be found in a small acidic lake, Oak Mere. The lake was at a pH of 4.5 and dominated by chlorophytes (including chlorella) until the pH had risen to 6.5, which in turn allowed cyanobacteria and diatoms (including Microcystis and Asterionella) to become the predominant plankton (Reynolds, 1984). For summer collections, the pH ranged among all sites between 7.27 and 9.20, which reveals an alkaline environment among all water bodies tested. As seen in figures 53 and 54 above, the pH and cell numbers appear to correlate with the findings that there appears to be a particular range in which algal blooms occur more frequently. There are several sites in which the pH was found to be between 7 and 8.5, in which both the highest numbers of cyanobacteria cells per milliliter and the highest numbers of diatom cells per milliliter were

found. As the pH drops below 7, however, there are no visible cyanobacteria or diatom cells. Also, as the pH increases to over 8.5, the cell numbers and the amount of visible cells in the samples visibly decrease. This is important to state because it is understood that the optimal pH range for cyanobacteria growth is found to be between 7.5 and 10 (Giraldez-Ruiz *et al.*, 1997). During fall collections, the pH ranged from 6.60 to 9.25, which again reveals an alkaline environment except for one site (Branch Brook site C). In figures 59 and 60, there again appears to be a correlation between the pH and cell number of both cyanobacteria and diatoms. The sites with pH ranging between 7 and 8.5 appear to contain the highest cell count of both diatom and cyanobacteria.

Dissolved oxygen is another important factor in determining water quality that is affected by both internal environment and external environment of the water body. As algal blooms proliferate, eventually they will exhaust all essential nutrients available in the water body. When this occurs, there is a sudden deterioration in biomass presence, which eventually leads to decaying of the algal bloom, producing a scum that robs the underlying water of oxygen. This depletion of dissolved oxygen can lead to several biological and chemical changes in that particular water body. These changes include hypoxia, in which the dissolved oxygen concentration has dropped below 4 mg/L, or anoxia, in which there is no detectable oxygen levels in the water, leading to death among most finfish and shell fish (Paerl et al., 2001). Among the ponds and lakes tested in the summer collections, dissolved oxygen levels ranged from 1 mg/L to 10 mg/L, constituting a wide range of dissolved oxygen levels. Because it has been previously reported that algal blooms are known to decrease the dissolved oxygen levels (Paerl et al., 2001, Saker *et al.*, 2007), it was important to detect a profile of cells found at each dissolved oxygen level. In figures 55 and 56 above, the graph shows both more cyanobacteria as

well as diatom cell numbers recorded at sites with a lower dissolved oxygen level (<5 mg/L). This result is important because it displays the correlation between cyanobacteria and other phytoplankton growth and the significant depletion of nutrients in the water column. During the fall collections, the dissolved oxygen levels ranged from 2 mg/L to 11 mg/L, representing a slight increase in dissolved oxygen which may be a result of decrease in biomass among the water bodies tested. Appearing in figures 61 and 62 above, there appears to be the same number of cyanobacteria cells over the range of dissolved oxygen levels. Although there are equal numbers among the four sites with countable cell numbers, the cell numbers are minute when compared to the summer collection cell counts. The diatom cell numbers appear to appear at a higher frequency among a lower dissolved oxygen, although there are still some sites in the higher dissolved oxygen range (8-10 mg/L) that contain cyanobacteria cells. Again, the numbers produced by both cyanobacteria and diatoms appear to be much lower in the fall collections when compared to the summer collections, presumably due to lower temperatures and rainfall.

The last factor of water chemistry incorporated into this study was temperature of each site. Temperature has not only been shown to affect the cell size of phytoplankton by controlling enzymatic reactions within the cells, but also to regulate the multiplication rate and standing biomass (phytoplankton population) within the water body. Studies have also shown, though, that it may not be temperature that is limiting the fall and winter growth but the lack of sunlight for photosynthesis (Reynolds, 1984, Alam *et al.*, 2001). Water temperature does seem to dictate the phytoplankton profile. At different temperatures, there seems to be a different phytoplankton profile. For example, the cyanobacteria Anabaena has been found to be severely affected by lower temperatures while the diatom Asterionella is not as affected by temperature but survives in a decrease of nutrients in the water body. During the summer collections, the temperatures

ranged from 23.5 to 30.2 °C. According to figures 57 and 58 above, the amount of cyanobacteria and diatom cells appears to be at a peak between 25 and 30 °C. This is important because it has been previously reported that the optimal growth rate of 'algae' (phytoplankton cells) is between 20 and 25 °C (Reynolds, 1984). As temperature increased from the lowest recorded (23.5 °C), there was a clear increase in both cyanobacteria and diatom cell number, which seemed to decrease after 30 °C. Also, when comparing the phytoplankton distribution between summer collections and fall collections, there is a clear separation in the cell count between the two seasons. This is another finding that corresponds with previous studies that the combination of decreased temperature and decreased light availability for photosynthesis results in decreased phytoplankton growth rate (Sterner & Grover, 1998, Reynolds, 1984). The temperature of sites during the fall fell between the ranges of 9.1 and 17.9 °C. As stated above and seen in figures 63 and 64, the cell count of both diatom and cyanobacteria cells are greatly reduced. The higher cell counts of both cyanobacteria and diatoms in the fall collections appeared to fall in the temperature range of between 13 and 17.9 °C. Below 13 °C, there were no readily countable cyanobacteria or diatom cells. This could be due to limited cell growth below the optimal growth rate temperature. Between 13 and 17.9 °C, there were cyanobacteria and diatoms, although at a clearly decreased level when compared to the summer collections and observations. As temperature increased in fall collections, the amount of cyanobacteria and diatom cells increased.

Clarks Pond in Bloomfield, NJ, has been targeted by the New Jersey Department of Environmental Protection as part of a network to monitor lakes and ponds in order to provide water quality data necessary to assess the ecological health of New Jersey's water resources. The network, which involved testing randomly selected lakes, measured parameters such as

dissolved oxygen, pH, nutrients, and chlorophyll *a* content. According to the information provided by NJDEP, in the summer, the dissolved oxygen content at one station appeared to be 1.41 mg/L. When compared to table 1 above, the dissolved oxygen detected at each of the three sites was 3.0 mg/L. The pH of the pond, according to NJDEP, was found to be 7.17 at the station. From the four sites collected in this study, the pH was recorded at 7.28, 8.29, 8.10, and 7.94. And finally, the temperature of the pond, according to NJDEP, was 25.12 degrees Celsius. According to table 1 above, the temperatures recorded during summer collections were 27.4, 29.9, 29.3 and 29.0. For the fall collections, the NJDEP recorded a dissolved oxygen level of 9.43 mg/L, a pH of 6.92, and a water temperature of 10.2 degrees Celsius. According to table 6 above, dissolved oxygen levels were much lower (4.0 across 4 sites), pH was 7.80, 7.64, 7.57, and 7.46, and temperatures were higher at 16.8, 14.5, 14.5, and 13.9 degrees Celsius. The fluctuation between the NJDEP findings and these findings may have been the timing of collections, as the temperatures recorded in this study appear to be significantly higher than those recorded in the Ambient Lake Monitoring Network (NJDEP, 2012).

Polymerase Chain Reaction (PCR) provided additional verification on the presence of bacteria, cyanobacteria, and algae in the freshwater ecosystems observed. In order to identify and preferentially select cyanobacteria, bacteria, phytoplankton, and specific genera from the mixed samples collected, PCR was used as a highly specific molecular technique that would recover these sequences at high frequencies. In order to identify these sequences, housekeeping gene sequences were exploited. A housekeeping gene within a cell is a gene that is responsible for the general upkeep and maintenance of a cell. Housekeeping genes are constantly active within living cells. The small-subunit ribosomal RNA (16S rRNA) gene segment is a housekeeping gene found within all phototrophs. Because the 16S rRNA gene segment is

always present within the genome of cyanobacteria and bacteria cells, this gene segment served as the target for environmental PCR. The universal primers used in this study (27fB/785r, PSf/PSr, Uf/Ur, CPC1f/CPC1r) utilized the 16s rRNA segment to identify cyanobacteria, bacteria, and phytoplankton have been identified in previous studies (Stiller & McClanahan, 2005, Nübel et al., 1997, Barkovskii & Fukui, 2004). The specific primer used in this study to identify the species *Anabaena circinalis* was developed using NCBI BLAST, while primers for diatoms and Microcystis have been identified in previous studies (Baldi et al., 2011, Herry et al., 2008). In both the summer and fall collections, PCR proved successful in detecting the presence of cyanobacteria, bacteria and phytoplankton by the general "universal" primers identified in previous studies. After detection of these cells within the lakes, a general profile was constructed for each lake. Sites in which cyanobacteria and phytoplankton were detected were subsequently tested with specific primers to create a more specific profile with species and genera. As seen above, all sites were tested with universal primers aimed to detect cyanobacteria, bacteria, and phytoplankton. While the detection of bacteria aided in the primary profile of each site, an attempt was not made to identify bacteria to the species level. At sites where cyanobacteria or phytoplankton were detected, specific primers were then employed in order to identify the specific cyanobacterial or phytoplankton species.

For the summer collections, cyanobacteria and phytoplankton were found at Clark's Pond (Sites A, B, C, and D), Diamond Mill (Site A) and South Orange Duck Pond (Sites A and B). For the fall collections, cyanobacteria and phytoplankton were found at Branch Brook State Park (Sites A, B, C, and D) and Verona Lake (Site A). This large spread of results could be due to several factors.

The water chemistry of the water bodies changed drastically from the summer collections to the fall collections. Water chemistry is known to dictate the profile of cyanobacteria found in the water (Reynolds, 1984). Alterations in temperature, dissolved oxygen, or pH could have dictated the phytoplankton and cyanobacterial profile, shifting from high numbers to low numbers of cells, or diminishing the populations from one site to under-detectable amounts with PCR. Another factor that could have been responsible for the change in profile is the accuracy of the PCR. Although PCR has proven to be an extremely proficient method in obtaining cyanobacterial gene sequences from mixed environmental samples, the process of collecting and extracting DNA from these samples could have had an impact on the results. As mentioned above, each sample was filtered through a coarse and fine filter. Holes were punched in these filters and DNA was subsequently extracted from these filter disks. Although it was attempted to obtain holes from a variety of sections of the filters, there may have been a bias in where the holes were taken from. This may have altered the results because although PCR didn't detect cyanobacteria or phytoplankton at a certain site, it may have been on a separate section of the filter or not on the filter at all. This is an area that may be greatly improved with future studies.

Seasonal pond or Lake Turnover could have had a profound effect on the abundance and population shift of phytoplankton when comparing the summer and fall collections. Lake Turnover is a natural event that results in the mixing of pond and lake waters, caused by the changing temperatures in surface waters during the shifting of seasons (DOC, 2012). The density and weight of water change when temperature changes in the freshwater lakes. Water is most dense at 4°C; it becomes less dense when the temperature drops below 4°C, thus rising to the top (ice floating). This is how fish and other aquatic life can survive during the winter at the floor of the water body, with the warmer water surrounding them towards the sediment (DOC,

2012). This feature, along with the fact that colder water having a higher capacity for dissolved oxygen, can support the fact that phytoplankton numbers are significantly reduced during the colder months. Pond or lake turnover could affect the phytoplankton survival by keeping the colder water at the surface of the water body, where phytoplanktons need to remain for sunlight and photosynthesis. Because colder surface temperatures do not support phytoplankton growth, the phytoplankton cell numbers and algal blooms should be greatly reduced after the fall turnover occurs, and may return after the spring turnover is complete.

Although PCR has its obvious shortcomings in the current project, the molecular technique greatly improved efficiency when compared to morphological studies relying solely on the microscope for observations. Cyanobacterial and phytoplankton presence was first established using PCR as the primary molecular tool. After presence was detected, PCR was used to detect specific genera and species of cyanobacteria and phytoplankton. After these genera and species were confirmed and detected, the microscope was then employed to further expand the species profile by observing specific cell morphologies from each site at each water body. Microscopy alone has been proven to be inefficient and time-consuming. Using microscopy as a complement to the PCR technique for identifying species has, in this study, proven to be an effective method in development of the species profile of each water body. Microscopy confirmed what PCR detected at several sites. In addition to PCR, microscopy aided in detection and identification of several other possible algal bloom forming species at each site. As seen in the results section above, microscopy revealed the dominant genera to be diatoms. It has been previously reported that diatom species have been a major factor in the production of algal blooms (Anderson *et al.*, 2002). Although diatoms have been known to cause algal blooms, the development of harmful algal blooms is due to toxin-producing cyanobacteria.

There have been several reported cases of cyanobacterial blooms leaving behind harmful toxins which affect the ecosystem as well as animals and humans that come in contact with that ecosystem (Anderson et al., 2010).

Branch Brook State Park site C developed a clearly visible algal bloom during the summer collections in July, 2011. During microscopic observation of the coarse filter collected from this site, several species of cyanobacteria were detected. A species of Oscillatoria was detected at site C. Oscillatoria is a type of filamentous cyanobacteria. Oscillatoria, along with other filamentous cyanobacteria, has been previously reported to cause algal blooms (Montealegre *et al.*, 1995). Another cyanobacterium, Radiococcus, was identified at Branch Brook State Park site C. Species of Radiococcus have been detected in small numbers in previous studies (Taft & Taft, 1971), but it has not been recorded to cause algal blooms. Because Radiococcus was seen at increased numbers at this site, this cyanobacterium may have been another factor in this algal bloom production and persistence.

Tables 31 and 32, located above, show the correlation found between morphological microscopy and molecular PCR. Both tables indicate the similarities found between observations made under microscopic observation and PCR. For the summer results, it is shown in table 31 that cyanobacteria were detected by PCR at Diamond Mill Pond sites A and C, and also at South Orange Duck Pond site A. Microscopic observations confirmed the presence of cyanobacteria at these sites (among others). In table 32 above, the fall comparisons are shown. PCR initially detected diatoms and cyanobacteria at Branch Brook Lake site A, while detecting just diatoms at site B. Complementary microscopic analysis confirmed the presence of cyanobacteria and diatoms at these sites. These results prove that although PCR and microscopy may be inefficient on their own, together they are an effective mechanism to develop a

phytoplankton profile for freshwater lakes. These findings correlate with previous studies, which have found that it is difficult to distinguish similar cell morphologies by microscopy (Kormas *et al.*, 2010). Although it is inefficient, microscopy still remains the preeminent means for morphotyping, cell counting, biovolume, viability assays, and life cycle stage observations of cells in a cyanobacterial or phytoplankton bloom (Kormas et al., 2010). The combination of the microscopic technique and the molecular technique provided for a well detailed and wide analysis of the five ecosystems tested in this study.

Flow cytometry provided a rapid analysis for the overall profile of the sites being tested. Flow cytometry was used to detect the overall photoautotroph presence in the sample by exploiting the auto fluorescence mechanisms of all cells containing the photosynthetic pigment chlorophyll *a*. By emitting a 488 nm laser for excitation with a 675 nm filter to locate chlorophyll *a*, a profile was achieved by identifying all cells containing chlorophyll *a*. By doing this, a profile was created comparing the raw sample with the coarse filters and the fine filters. Also, a profile was created comparing Branch Brook Lake site C collections from the summer and the same site collected during the winter (December 20th, 2011). As seen in figure 50 above, it is evident that the raw lake sample contained more phycocyanin-containing cells when compared to the coarse filtered sample. The fine filtered sample contained less phycocyanin-containing cells when compared to both the raw sample and coarse filtered sample. As seen in figure 51, the amount of cells contained on the coarse filter from Branch Brook site D was higher than the number of cells contained in the raw sample. This may have occurred because the coarse filter concentrates cells to one area instead of floating freely within the sample. The fine filter, as in figure 50, had fewer cells when compared to the coarse and raw samples. Figure 52 above provides a seasonal comparison for Branch Brook site C. The summer sample contained

slightly more cells when compared to the winter sample. This does not correlate with the findings comparing the summer and fall collections. It has previously been noted that water temperature has been a main factor in phytoplankton growth and cell number (Reynolds, 1984, Kormas et al., 2010). The deviation from previous results may be from the storage period of the summer sample (July 13th, 2011 to December 20th, 2011). Although the summer sample contained slightly more cells when compared to the winter sample, the photoautotroph cell number should be significantly higher in the summer.

Future Studies

In order to obtain a larger, more complete profile of phytoplankton growth in New Jersey freshwater ecosystems, flow cytometry must be employed at a larger scale. In the current study, it has been established that flow cytometry is successful in the detection of cells containing chlorophyll *a*. In order to develop a rapid yet large profile for many freshwater ecosystems, fluorescent probes must be employed. As used in PCR, the 16s rRNA segments found in all phototrophs can be detected and probed with fluorescence. With these probes, the flow cytometer can successfully identify different species of cyanobacteria and phytoplankton while analyzing mixed microbial populations (Amann *et al.*, 1990).

The New Jersey Department of Environmental Protection (NJDEP) has developed the NJDEP Ambient Lake Monitoring Network, in which lakes and ponds around the state of New Jersey are tested for water quality. The Network tests at least one station and one outlet of each water body. At these stations, the NJDEP tests for total depth, profile depth, Secchi, water temperature, dissolved oxygen, pH, conductivity, phosphorous, nitrates, chlorophyll *a*, and turbidity, among other water quality factors. As mentioned above, phosphates and nitrates are two of the most important elements resulting from pollution that drive the eutrophication in freshwater ecosystems. Also, as the biomass of cyanobacteria and phytoplankton increase, the amount of chlorophyll *a* will increase. These factors are important in monitoring and identifying ecosystems that are threatening for algal bloom formation. In order to gain a complete profile for each freshwater ecosystem, these parameters must be incorporated into the future study.

Identification must continue on this project in order to gain repeated results. Once results are obtained from repeated studies, a program can be developed. The program, following the data obtained from this study and future studies, will take into account all parameters measured

and will ultimately produce an algal bloom formation threat level. With the algal bloom formation threat level, the user will then be able to accurately predict when the bloom will form and which species will be the dominant species in that particular algal bloom. With this information, algal blooms can be predicted, and, eventually, prevented.

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