To the Graduate Council:

I am submitting herewith a thesis written by Star Loar entitled "Seasonal Variation in Lake Erie Picoplankton." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Microbiology.

Steven Wilhelm, Major Professor

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Seasonal Variation in Lake Erie Picoplankton

A Thesis
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Star Nicole Loar
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Thank you, Steve, for having insight and the resources to make this opportunity possible, you were everything and exactly what I needed at the right time. To my partner in life and fellow researcher, Lance, thank you for taking this journey with me and for your ongoing encouragement on becoming the best version of myself.

ABSTRACT

Striking rates of environmental changes combined with increased demand make it essential to develop a better understanding of global freshwater resources. Seasonal hypoxia in the central basin of Lake Erie is the result of thermal stratification and lake morphology. Limnetic physics can, however, only explain part of Lake Erie's "behavior": the activity(s) of the ecosystem's biological members can be equally important. The goal of this study was to identify picocyanobacterial community members in the central basin of Lake Erie during summer stratification and the winter season to see how they may vary with season. Identification of microbial communities under the present environmental conditions establishes a relationship (i.e. baseline) from which changes can be seen over time. Seasonal variations in cyanobacterial communities can also offer insight into the biogeochemistry of Lake Erie. Information gained from the microbial ecology of Lake Erie can be applied to other lake systems.

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- CCGS – Canadian Coast Guard
- DNA – Deoxyribonucleic Acid
- DMSO – Dimethyl Sulphoxide
- EDTA – Ethylenediaminetetraacetic Acid
- HCl – Hydrochloric Acid
- PC – phycocyanin
- PCR – polymerase chain reaction
- PE - phycoerythrin
- rpm – revolutions per minute
- RV – Research Vessel
- SDS – Sodium Dodecyl Sulfate
- TE – Tris-HCl and EDTA

INTRODUCTION

History of Lake Erie

The Laurentian Great Lakes of North America contain about 20% of the world's fresh surface water (Grady 2007). Lake Erie is the shallowest and smallest by volume of the Great Lakes with an average depth of 19 m and a maximum depth of 64 m. These dimensions result in Lake Erie having a retention time of ~2.6 years, the shortest of these Great Lakes (Wright 2007). Of these Great Lakes, eutrophication has also most seriously impacted Lake Erie. Effects have been evident in the western and central basin, and include algal blooms and the loss of certain benthic organisms. During the 1950's and 1960's, studies determined that the bottom waters of the central basin were subject to anoxic conditions during summer stratification (Figure 1). In 1970, two extensive studies began to evaluate the status of the lake. The first study was an open lake survey from April to December of 1970 addressing the physical, chemical and biological aspects of the lake. The second study, termed "Project Hypo", was designed to examine the processes involved in the depletion of oxygen from the hypolimnion of the central basin (Boyce 1987).

The reoccurrence of seasonal hypoxia in the central basin of Lake Erie and the concomitant microbial community have been an area of interest to researchers. Previous research has shown that during summer stratification, groups of picocyanobacteria persist within the hypoxic regions of Lake Erie (Wilhelm *et al.* 2006). Cyanobacteria are phototrophic prokaryotes equipped with a variety of strategies which allow for the adaptation to environmental changes (Whitton 1992). These strategies include the ability to adapt to a range in nutrients, temperature and light conditions (Stockner 2000).

Our understanding of the phylogeny of cyanobacteria has changed with the advent of molecular tools. Cyanobacteria had commonly been characterized by morphology but were reclassified in 1979 by Rippka *et al.* into five sections based on structure and development (Rippka 1979). The concept of a species for the cyanobacteria is now based primarily on the 16S rDNA gene and other gene sequences (Nübel 1997). Within the unicellular picocyanobacteria the major freshwater lineages of interest to this study include *Synechococcus* and *Cyanobium*. Also discussed in this research is the marine lineage *Prochlorococcus* (Chisholm 1988; Urbach 1998). The goal of the research presented in this study is to identify and compare picocyanobacteria populations found during summer and winter seasons. To address the future question such as, do winter picocyanobacterial populations influence summer populations and vice versa, these seasonal populations must first be identified. As a second goal we hoped to determine was that if using flow cytometry to select for cyanobacteria that were distinguishable based on their major light-harvesting pigments: phycoerythrin (PE) and phycocyanin (PC) increased genetic richness (Wood 1985).

Morphology of Lake Erie

For over 30 years the formation of a region of seasonal hypoxia, commonly termed the "dead zone," has been recognized in the central basin of Lake Erie (Charlton 2004). Thermal stratification is responsible for the formation of this anoxic region. A review of thermal stratification can be found in the book *Limnology Lake and River Ecosystems* by (Wetzel 2001). Lake Erie is diametric, turning over in the spring and fall. Temperature variations due to seasonal weather conditions result in slight changes within the lake, causing only small changes in density influencing stratification. Thus, there is relatively little thermal resistance to mixing. Wind energy impinging on the lake surface is enough to mix the water column. Convection

currents induced by night cooling and evaporation also aid in the circulation. Following this period of mixing during the spring turnover the surface water of the lake is heated more rapidly than the heat is distributed by mixing. As the surface waters are warmed they become less dense, and there is a relative thermal resistance to mixing with the bottom cooler more dense water. This temperature difference, in some case only a few degrees, is sufficient to prevent homogenous circulation of the water column.

Once stratified, a lake is thermally divided into three regions. The lowest stratum is the hypolimnion, and its initial temperature is determined by the final water temperature during the spring turnover (Hutchinson 1957). The temperature of the hypolimnetic water changes very little throughout the period of summer stratification in larger lakes. During summer stratification, the waters of the upper stratum, or epilimnion, are uniformly warm, freely circulating and fairly turbulent. The epilimnion essentially floats on the cold and undisturbed hypolimnion. The stratum between the epilimnion and hypolimnion is the metalimnion. The metalimnion is defined as the water stratum of steepest thermal gradient, and its boundaries are set by the epilimnion and hypolimnion (Wetzel 2001). The thermocline within the metalimnion refers to the *plane* of maximum rate of decrease of temperature with respect to depth (Hutchinson 1957).

The hypolimnion of the central basin of Lake Erie is about 3 to 4 m thick during most summers, as compared to the eastern basin where the hypolimnion which is 12 to 14 m thick (Figure 2). The difference in productivity between the two basins is small, but the rate of oxygen depletion from the hypolimnion in the central basin is twice that of the eastern basin (Burns 1976). The difference in the rate of oxygen depletion is primarily the result of hypolimnion thickness (Charlton 1980). Hypoxia in the hypolimnion of the central basin occurs when the lake

becomes stratified and the oxygen within this region is consumed by heterotrophic bacteria. Primary production primarily occurs within the photic zone, which in Lake Erie includes the metalimnion. During summer stratification the oxygen produced in the photic zone is unable to diffuse into the hypolimnion. The oxygen in the hypolimnion is consumed by heterotrophic bacteria at a rate much greater than it is replaced resulting in anoxia (Wilhelm *et al.* 2006). That there is some replacement of oxygen is not surprising, however, as there is an abundant community of picocyanobacteria living at the upper boundary of the hypolimnion. To better understand the ecology of these Lake Erie picoplankton communities, they must first be identified. Once the major functional groups comprising the picoplankton communities are identified, the influence that environmental conditions play in the development of these communities could offer insight into the biogeochemistry of the lake during summer stratification.

Seasonality of Cyanobacterial Communities

In 1983, Charlton and Rao published one of the first studies looking at the relationship between hypolimnion oxygen depletion and heterotrophic bacterial population numbers in the central and eastern basins of Lake Erie. Microbial community analysis by PCR based methods were not yet available (though PCR would make its breakthrough the same year). The methods used in this study included total counts by acridine orange epifluorescence microscopy, bright field illumination microscopy to determine respiring bacteria and traditional plate counts to enumerate aerobic heterotrophs. Bacterial estimates were made from May to September of 1979 and were estimated by three methods. This research was significant because it demonstrated seasonal variation in bacterial counts during hypoxic conditions in the central basin (Charlton 1983). Until this time fish kills and algal blooms were the only obvious effects of eutrophication

and hypoxia. This study confirmed the hypothesis that hypoxia was affecting the microbial communities of Lake Erie, even in the bottom waters.

The 2006 publication by Wilhelm *et al.* analyzed the genetic diversity of the picoplankton communities of the hypolimnion in the central basin of Lake Erie. The importance of this study was that it identified some of the members within the cyanobacterial communities during the transition to hypoxic conditions of the central basin. 16S rDNA gene sequence analysis established that a significant population of cyanobacteria was present during the onset of hypoxia in the hypolimnion. Figure 3 illustrates that peak abundances in *Synechococcus* correspond to both the peak in fluorescence and oxygen production. Evidence from this research suggests that hypoxia stimulates the establishment of cyanobacteria in the central basin of Lake Erie, especially at the interface of the thermocline and hypolimnion. Observations, including nutrient dynamics (i.e. nitrate *vs.* ammonia concentrations), also provide insight into how the microbial community may be shaped by hypoxia beyond the direct effects of oxygen availability (Wilhelm *et al.* 2006).

The involvement of microorganisms in carbon cycling of aquatic systems is best be described by the microbial loop (Azam 1983). The aquatic food web is based on primary producers which are almost exclusively photosynthetic organisms that convert CO_2 to organic compounds. These organisms convert light energy into chemical energy which is stored within the organic compounds formed. An important group of these photosynthetic autotrophs are cyanobacteria. The primary producers also convert a portion of the gross primary production back into CO_2 through respiration, and the remaining organic carbon is the net primary production available to heterotrophic consumers. Heterotrophs complete the carbon cycle by converting organic compounds back to CO_2 through respiration (Atlas 1998).

Picoplankton compete throughout the light spectrum resulting in niche differentiation. Picoplankton species are able to utilize different wavelengths with a variety of photosynthetic pigments (Engelmann 1883; Kirk 1994; Stomp 2007). Lab experiments have shown the coexistence of red and green picocyanobacteria results from the partitioning of white light. Figure 4 shows the optical characteristics of red and green picocyanobacteria and the underwater light spectrum of their environment the Baltic Sea. As depth increases, the light spectrum narrows to the green wavelength (Stomp 2007). Light intensity is also an important selective factor in the distribution of picoplankton communities (Huisman 2004). The green picocyanobacteria *Prochlorococcus* are differentiated into several ecotypes, some of which are adapted to high light intensities surface waters whereas others are adapted to low light intensities found at greater depths. Ecotype classification is also complicated by temperature constraints (Johnson 2006).

The most comprehensive research into winter limnology in Lake Erie spanned four years (1938 – 1942) and was reported by David Chandler. The two one-year studies by Chandler included the period of ice coverage in Lake Erie. Under the ice, pulses of phytoplankton (diatoms), were observed in mid-February to late March (Chandler 1940; Chandler 1942a; Chandler 1942b) suggesting microbial growth and development during the winter season. Since then, mixotrophic phytoplankton have been studied during the winter season (Nebaeus 1984; Berninger 1992). Cyanobacteria have been shown to be members of the phytoplankton community found during the winter season in other ice-covered lakes (McKnight 2000). A goal of this study is to identify members of the cyanobacterial community found in Lake Erie during the winter season. The species that comprise the Lake Erie winter cyanobacterial community may have influences that are carried over into the summer season. Identifying the members of

winter community is the first step in understanding how biogeochemical processes and the summer microbial community may be influenced.

Components of the Photosynthetic Apparatus in Cyanobacteria

Observations made by Theodor Engelmann in 1883 stimulated research into photosynthetic pigmentation and the relationship between the solar radiation within an organism's environment relative to its radiant absorption spectrum. Engelmann suggested that the depth at which seaweed grows may be related to its pigment content and the spectrum at which they receive radiation (Engelmann 1883; Raven 2007). Besides chlorophyll, Engelmann suspected that other pigments were present in algae and bacteria. In 1884, Engelmann was the first to determine an action spectrum of photosynthesis in an experiment in which he varied and regulated light intensities used to grow algae (Engelmann 1884; Drews 2005).

The abundance at which cyanobacteria are found in lakes demonstrates that the mechanisms they use to acclimate to changing light conditions are efficient. There are many genetically distinct strains of *Synechococcus* which can be broadly classified into two pigment containing cell types (Ernst 1991). The two main accessory pigments present in cyanobacteria are the blue-colored phycocyanobilin (PC) and the red-colored phycoerythrobilin (PE) (Wedemayer 1991). The colors of the phycobiliproteins arise from covalently attached linear tetrapyrrole prosthetic groups know as phycobilins (Rudiger 1994). Tetrapyrroles are ubiquitous biomolecules that have biological functions including electron transfer, oxygen binding and light absorption. Cyanobacteria can produce a variety of biologically important tetrapyrroles which include the ability to produce hemes, chlorophylls and phycobilins. Like other phototrophic organisms, cyanobacteria have the ability to regulate their tetrapyrrole content within their

photosynthetic apparatus. Changes are made in response to various environmental signals, including light intensity (Beale 1994).

Synechococcus that have PE as its major light-harvesting pigment give yellow autofluorescence and those with PC result in red autofluorescence. *Synechococcus* containing PE or PC can be distinguished by fluorescence emission spectra; PE shows maximal emissions at 578 nm when excited at 520 nm and PC at 648 nm when excited at 600nm (Wood 1985; Ernst 1991; Callieri 1996). The graph in Figure 5.A illustrates flow cytometry results of a water sample collected from Station 84 in the central basin of Lake Erie August 2008. The water sample in graph 5.B was spiked a PE rich strain and 5.C was spiked with a PC rich strain of *Synechococcus*. The PE rich strain of *Synechococcus* is red in color where as the PC rich strain is green, both are similar in size but differ in their fluorescence. The same water sample was also spiked with two ecotypes of *Prochlorococcus*, eMIT-9211 and eNATL2A. The significances of graphs 5.D and 5.E are that they indicate the absence of PE in *Prochlorococcus* and also show how much smaller *Prochlorococcus* are than *Synechococcus*.

Sorting Cyanobacteria by Flow Cytometry

Flow cytometry is a powerful technique that can be used to measure light scattering and fluorescence characteristics of individual particles or in this case cells. Cells are passed coaxially into a stream of sheath fluid that is vibrated in order to form single droplets containing one cell. As the droplet passes through the beam of a laser, optical signals from each event of the cell interacting with the light source are generated. About 50,000 droplets pass through the laser beam each second, and approximately 16,000 events are evaluated each second. However every droplet does not contain a cell. With respect to the research presented here about one in every four droplets contained a cell. If more stringent parameters were needed the flow rate

would be turned down so that one in ten droplets contain a cell, reducing the number of events per second. The measurements taken will be used to evaluate each cell, if the cell falls within the parameters set by the researcher the cell can then be sorted into the selected media or tube. Within the field of phycology flow cytometry has been used by oceanographers to study the smaller members of phytoplankton communities (S. Allman Pers. Com.) (Collier 2000).

Preliminary sequence data from our lab suggested the possible presence of *Prochlorococcus* therefore flow cytometry was initially used in this study when screening lake water samples to increase the chances of isolating *Prochlorococcus* from Lake Erie. *Prochlorococcus* is the smallest most numerically abundant photosynthetic organism found in the ocean between the latitudes of 40˚S and 40˚N (Partensky 1999). *Prochlorococcus* was identified in 1988 as a result of its flow cytometric 'signature'. Forward light scatter signals in general can be used to indicate cell size. The cell size of *Prochlorococcus* is less than one third of the size of *Synechococcus* cells. The fluorescent properties of *Prochlorococcus* along with size allow it to be distinguished from their close neighbor *Synechococcus* (Chisholm 1988). Johnson *et al.* 1999 found that *Prochlorococcus* was frequently found at the oxic-anoxic interface of the euphotic zone in the Arabian Sea. The significance of this research was that there are low-light adapted types of *Prochlorococcus* and in order to reduce oxidative stress *Prochlorococcus* might be stimulated in hypoxic regions (Johnson 1999). The sequence data from our lab came from summer sampling of Lake Erie during conditions of hypoxia. Sequences corresponded with two of the low-light ecotypes of *Prochlorococcus* eMIT9211 and eNATL2A (Figure 6) (Unpublished data, Wilhelm Lab).

Research Objectives

While members of picocyanobacterial communities have been well studied in marine systems, we are just now beginning to understand their richness and the role(s) these communities play in freshwater systems. There is evidence to suggest that environmental conditions influence the community structure of cyanobacteria in Lake Erie. This evidence is derived from a limited number of studies that have investigated the microbial communities of Lake Erie. These studies have yet to determine if there is a difference in PE and PC content between the members of summer and winter populations. Little research has been done looking at the microbial community structure of Lake Erie during the winter season. Specific hypothesizes of this thesis are:

Prochlorococcus is found during summer hypoxia in Lake Erie.

The picocyanobacterial community structure in Lake Erie is different during the summer and winter season.

Flow cytometry can be used to increase the efficiency of genetic identification of picocyanobacteria from aquatic samples.

METHODS

Sampling

Summer environmental sampling occurred during the August 2008 cruise (MELEE XIV) aboard the CCGS RV *Limnos*. Winter environmental sampling occurred during January 2009, February 2008 and 2009 cruises aboard the CCGS *Griffon*. Water column profiles were generated for the 2008 summer and winter 2009 sampling events using a YSI probe. During sampling water was collected in 10 L Niskin bottles on a General Oceanics Rosette sampler. Water was collected from multiple depths in Lake Erie: Central Basin Station 84 (Latitude 41˚ 56' 46" Longitude 80˚ 38' 46"), Western Basin Station 357 (Latitude 41.8˚ Longitude 83.0˚) and Eastern Basin Station 23 (Latitude 42.5˚ Longitude 79.9˚) (Figure 7). At each sampling event 50 ml of the water collected was vacuum aspirated onto a 0.22 μm polycarbonate filter (Millipore) and immediately frozen at -20˚ C. Upon returning to the laboratory the filtered samples were stored at -80˚ C until DNA was extracted (Table 1).

Whole water samples were collected at each sampling event to be sorted later by flow cytometry. 1 ml of whole water was placed in a 1.5 ml cyrovial, to which 75 μl of DMSO (SIGMA) was added, and inverted three times to mix. Samples were immediately placed in liquid nitrogen. After returning to the laboratory samples were stored at -80 ˚C until sorted. Water samples used for flow cytometry were collected in triplicate (Table 2).

Flow Cytometry Sorting Parameters

Upon returning from the summer 2008 Lake Erie cruise the water samples were taken to Oak Ridge National Lab (ORNL) to be sorted by flow cytometry. The initial goal of this project was to search for *Prochlorococcus* if the goal was to construct clone libraries the sorting parameters would not have been modified during this study. Figure 8 is a simplified depiction of

the set up of the InFlux Cell Sorting Flow Cytometer manufactured by Cytopeia Incorporated, model Influx 2L at ORNL. To get the water sample into the flow cytometer the water is transferred to specific 5 ml polypropylene round-bottom tubes (Falcon) that fits into the machine. The sample then becomes pressurized and is streamed into a larger tube of sheath fluid. The sheath fluid containing the sample is passed through the oscillating piezo, which controls the breaks in the fluid resulting in droplet formation. The breaks are made so that there is one cell per droplet. The cell will then pass through a laser that is blocked by a bar in front of a lens. The lens can then detect light changes as each cell containing droplet passes the laser in front of the bar. Other light characteristics such as fluorescence are routed to different detectors, the photomultipliers. Once parameters are set and measurements have been made for each cell, the cell passes through high voltage plates that deflect (*i.e.*, sort) the cell into a tube that corresponds to its unique set parameters. An individual sample can be sorted using up to four parameters at once. A color is associated with each parameter named; Left (green), Half-Left (blue), Half-Right (purple) and Right (red) (Figure 9). A unique parameter is represented by one of four described colors and is set within the detection limits of one of the four photomultipliers. A unique set parameters and its color will always correspond to a specific position on the tube rack (S. Allman Pers. Com.)(Shapiro 2003).

Cell measurements made by the flow cytometry are known as forward and side scattering. In general forward scattering is more sensitive to size or cell shape. Side scattering can indicate characteristics about the inside of the cell (Collier 2000). Initial sorting parameters used in this study are illustrated in Figure 10. By setting four parameters each sample would be sorted select for specific microorganisms; small pico-phytoplankton, *Prochlorococcus*, pico-eukaryotes and *Synechococcus*. The logic used to decide the initial sorting parameters were

based on results from Lake Erie water samples being spiked with known picocyanobacteria (see Figure 5). First, a parameter (purple) was set the bottom of the sample viewing window that represented fluorescence at 670 nm. The purple gate was set to select small pico-phytoplankton with PC fluoresce. Another parameter (red) was set above the purple gate also at fluorescence of 670 nm within the same window to select for *Prochlorococcus*. A third parameter (blue) was set in the upper right of the same viewing window to select for larger PC containing pico-eukaryotes. Lastly, a large parameter (green) was set in the window representing fluorescence at 610 nm. This green gate was set to remove for the presence of chlorophyll, to increase to chances of selecting for *Prochlorococcus*. A list of all the August 2008 Lake Erie water samples sorted October 31, 2008 using the parameters just described are listed in Table 3

All of the sorted samples in Table 3 were examined by PCR amplification and sequencing of the 16S rDNA gene. The results from the first sorting parameters changed how flow cytometry would be used for the remainder of the project. Sorting parameters were evaluated and changed so that the cells within the water samples would sorted based on size, PE and PC content. Each 1 ml water sample was divided into two samples of 500 µl each. One 500 µl sample was sorted to select for different sizes of cells PC rich in content and the other sample was sorted the same way but instead for cells PE rich.

The final parameters used to sort sample are the following: to sort cells by size and PC content three gates were set at a fluorescence of 670 nm, and one large was set around the three smaller gates at a fluorescence of 610 nm. The graph in Figure 11.A illustrates what the content of the sample looked like in the viewing window; each cell is represented by a black dot. To the right of the graph are the sorting parameters set for the sample. The sample in Figure 11.A was collected from Station 84 in the central basin of Lake Erie during the 2009 winter season. A

sorting parameter (blue) was set at a fluorescence of 670 nm, selected for the smallest cells rich in PC content. Another sorting parameter (red) was also set at a fluorescence of 670 nm in the middle, and a parameter (purple) was set for the larger sized cells rich in PC. A large parameter (green) was set at a fluorescence of 610 nm, was set for the following reasons. If a cell happened to meet the criteria set by more than one parameter it would be discarded. In order to make the statement that cells in the three way sort were PC rich and not also rich in PE there needed to be some control in place to select out the PE rich cells, that is why a parameter (green) was set at 610 nm, which was large enough to encompass the three parameters set at 670 nm. The sizes of the parameters varied within these figures because adjustments were made to sorting parameters depending on what the content of each water sample looked like in the main viewing.

To sort cells based on size and PE content three parameters were set at a fluorescence of 610 nm, and one large parameter was set around the three smaller parameters at a fluorescence of 670 nm. Figure 11.B illustrates how samples were sorted based on these parameters. A parameter (blue) was set at a fluorescence of 610 nm selected for the smallest cells rich in PE content. Also at a fluorescence of 610 nm a parameter (purple) was set at in the middle and another parameter (red) was set for the larger sized cells rich in PE. A large parameter (green) was set at a fluorescence of 670 nm to select out cells rich in PC. All of the samples sorted by these final parameters on February 24, 2009 are listed in Table 4.

A final group of samples were sorted on April 16, 2009 using the conditions just described to sort by PC and PE content. Figures 12.A illustrate how samples were sorted under conditions that selected for different cell sizes rich in PC. Figures 12.B represent the samples sorted under conditions that selected for different cell sizes rich in PE. Gate sizes varied within figures 11 and 12 because adjustments were made depending on the content of each water

sample. All of the samples sorted by these parameters on April 16, 2009 are listed in Table 5. Counts were recorded for the number of cells sorted into each gate; counts were done for all samples those sorted under PC or PE selecting parameters.

Extraction of DNA from Filtered and Flow Sorted Samples

DNA was isolated from filtered water samples using a modification of the protocol of Rinta-Kanto *et al.* (2005). Cells were suspended from the filter in 4 ml lysis buffer (10 mM Tris pH 8.0, 1 mM EDTA, 100 mM NaCl). Bacterial cell walls were disrupted through the addition of lysozyme to a final concentration of 1 mg mL^{-1} proceeded by an incubation at 37˚ C for 20 minutes, with shaking at 150 rpm. After incubation, proteinase K was added to a final concentration of 50 μg mL^{-1} and 10% SDS to a final concentration of 0.5%. The cell suspension was incubated at 50˚ C for two hours. Then a phenol/chloroform/isoamyl alcohol (25:24:1) volume equal the cell suspension was added, mixed gently by inverting. The mixture was then transferred to a prepared Phase Lock Gel 15 ml tube (Eppendorf). The aqueous phase was collected by centrifugation at 1,500 × g for 5 minutes (Eppendorf 5810R centrifuge equipped with an Eppendorf F-34-6-38 rotor). DNA was precipitated overnight at -20˚ C after the addition of absolute ethanol (2× aqueous volume) and 3 M sodium acetate (0.1 × the volume of the aqueous phase). DNA was pelleted the next day by centrifugation at 8,000 × g for 30 minutes. The supernatant was carefully removed and the pellet was washed with 1 ml of 70% ice cold ethanol. The DNA pellet was air dried and resuspended in TE buffer (10 mM Tris-HCl and 1 mM EDTA), pH 8. The concentration of the extracted DNA was measured spectrophotometrically (NanoDrop ND-1000, Spectrophotometer).

Cells sorted by flow cytometry were collected into 500 μl microcentrifuge tubes. The number of cells sorted from each sample determined the liquid volume of 1X PBS also known as

sheath fluid collected into each tube. In cases when few cells (i.e. less than 1000) were sorted 50 µl of TE was added for resuspension of the cells and storage. When a large number of cells (i.e. more than 10,000) were sorted into a tube the resulting volume could be greater than 300 µl. Flow sorted samples that resulted in volumes greater than 100 µl were centrifuged in a bench to centrifuge at max speed for 10 minutes. Supernatant was carefully removed by pipetting at the liquid air interface never letting the pipette tip touch the bottom or sides of tube. The remaining volume of 1X PBS left in the tube was estimated to be 50 µl to which 50 µl of TE was so that the volumes of all flow sorted samples were 100 µl or less. DNA was obtained from the sorted cells by through freeze-thaw. Flow sorted samples were placed at -80˚ C for 20 minutes then allowed to thaw at room temperature for 30 minutes. For long term storage samples were stored at -20˚ C.

PCR Amplification

Full length 16S bacterial and cyanobacterial-specific primers (see Table 6) were used to amplify extracted DNA samples and all of the samples generated by flow cytometry. PCR was done in 25 µl volumes: 5X Clear GoTaq buffer with 1.5 mM $MgCl_2$ (Promega), 2.5 mM each deoxynucleotide triphosphates (Fisher), 0.2 µM of each primer (operon), 5 µl DNA, 1 unit of GoTaq DNA polymerase (Promega), 0.1 µg/µl BSA (Sigma) final concentration and water. Cycling condition for 27F and 1522R primers consisted of heating at 95˚ C for 4 minutes, followed by 30 cycles of 95˚ C for 1 minute, 60˚ C for 1 minute, 72˚ C for 1.5 minutes and a final extension time of 10 minutes. Cyanobacterial-specific primers were used to amplify a segment of the 16S rDNA gene. The cycling parameters for CYA106F and CYA781R primers consisted of a heating at 94˚ C for 5 minutes, followed by 30 cycles of 94˚ C for 1 minute, 62˚ C for 1 minute, 72˚ C for 1 minute and a final extension time of 7 minutes. PCR product was run

on a 1.5% agarose gel at 120 volts for 45 minutes. Product lengths were determined after gels were stained with ethidium bromide.

Cloning and Sequencing

PCR amplicons were cloned into the pCR 2.1-TOPO TA Cloning Kit (Invitrogen) using TOP10 competent *Escherichia coli* cells. Colonies were grown on Luria Bertani (LB) plates with 50 μg/ml kanamycin and 40 mg/ml X-gal in dimethylformamide to select for transformants with DNA inserts. Putative colonies were grown in LB liquid with kanamycin and checked for efficiency by colony PCR before being sent for sequencing. The majority of sequencing was performed by the Clemson University Genomics Institute. Colonies were grown up overnight in 96 well plates and 50% glycerol was added before freezing plate at -80˚ C. Plates were then shipped to Clemson were they performed plasmid preps and high-throughput sequencing of the libraries using an ABI 3730xl automated DNA sequencer. All other sequencing reactions were done on DNA isolated by mini-prep which was then sent for sequencing at the UTK Molecular Biology Resource Facility using an Applied Biosystems Hitachi 3730 DNA Analyzer.

Sequence Alignment and Phylogenetic Analysis

Primers were removed from sequences and ambiguous bases were checked and altered using the BioEdit program Version 7.0.9. (Hall 1999). Using the BLASTN search tool on the National Center for Biotechnology Information website sequences were compared to known cultured and environmental sample sequences (Altschul 1990). The Ribosomal Database Project (RDP) Release 10 Classifier was used to characterize sequences by phyla (Cole 2009). Classifier assigns 16S rDNA gene sequences to hierarchical taxa based on a naïve Bayesian rRNA classifier (Wang 2007). Sequences were checked for chimera formation using the RDP II website, which uses the program Chimera Detection that is a part of the SimRank 2.7 package

written by Niels Larsen. All sequences were aligned using the Clustal W program (Thompson 1994). Phylogenetic analysis of the sequences was conducted using MEGA version 4 (Kumar 1994). Reference sequences used for phylogenetic analysis were determined by using BLASTN on sequence data and identifying its nearest ancestral based on DNA sequence patterns. In MEGA 4 Neighbor-Joining analysis with subsequent bootstrapping with 1000 replications was performed on selected reference and sequence data. The phylogenetic method UniFrac was used to compare the cyanobacterial sequence data two ways; the summer to the winter and the flow sorted to the filtered water (environmental) DNA extracted samples (Lozupone 2005).

RESULTS

Oxygen and Lake Conditions

Profiles of the central basin station 84 were taken during the August 2007 and 2008 cruises aboard the CCGS RV *Limnos*. Though no samples were examined from 2007 in the research presented here, a water column profile from station 84 from 2007 (Figure 13) is included because the oxygen probe was not functioning properly when measurements were being made at the same station in 2008 (Figure 14). The temperature measurements taken during August 2007 are similar to the measurements taken in 2008. If accurate measurements for dissolved oxygen in 2008 had been obtained they would likely follow the same trend as recorded for 2007. Both profiles reveal a prominent thermocline due to summer stratification. Temperatures were warmer throughout the metalimnion and cooled as the depth increased, stabilizing around 11˚ C in the hypolimnion. In August 2007, the dissolved oxygen concentration in the hypolimnion reached as low as 1.62 mg/ml and in 2008 our lowest recorded measurement was 5.36 mg/ml. The dissolved oxygen concentration indicated hypoxic, not anoxic, conditions at the time of sampling.

Profiles during the winter season of station 84 were taken during January and February 2009 cruises aboard the CCGS *Griffon*. The profile from January 2009 (Figure 15) showed that temperature changed very little with depth. The temperature did increase with depth which was expected given that the top of the lake had recently frozen and was covered in ice. The final increase in temperature from 0 to almost 25 meters was 0.16˚ C. The initial measurement taken for dissolved oxygen was around 14 mg/ml and peaked at 17.2 mg/ml at a depth of 13.9 meters and returned to almost 14 mg/ml once the probe reached the bottom of the lake. The profile from February 2009 (Figure 16) also showed that the temperature and dissolved oxygen changed

little with depth. In February's profile the temperature decreased less than 0.05˚ C with depth and the concentration of dissolved oxygen decreased less than 0.04 mg/ml with depth.

Summer and Winter 16S Libraries

To assess microbial diversity during summer hypoxia and the winter season, 38 libraries that resulted in 306 clones were generated by primers designed to amplify a cyanobacterial specific region or the full length 16S rDNA gene. Complete sequence reads were not obtained for all 306 clones. Table 7 describes all libraries that yielded sequence data that could be analyzed. It is important to note that for this study all flow sorted and water filtered DNA samples were analyzed by PCR and that for this particular set of experiments 38 libraries were generated. Specific libraries were not selected by choice but as a result of the experimental process presented in this study. A third of the clones were from winter samples and the remaining clones were from summer samples. A small fraction of the clones that were submitted for sequencing were eliminated from further analysis because of poor sequencing. Of the winter clones 61% were identified as chloroplasts or chimeras, and were not considered in this analysis. In contrast, less than 5% of the summer clones were identified as chloroplasts or chimeras. Using the RDP website, Classifier was used to determine the phylum of all the clones at an 80% confidence level. Of the 306 clones sequenced 191 were used in this study. Of the sequences analyzed 154 were from the summer season and 37 from the winter season.

Cyanobacteria was the dominant phylum of the sequences (76.4%) represented in the summer amplicon libraries. Other groups included: Actinobacteria (0.5%), Firmicutes (0.5%), Planctomycetes (1.1%), *γ-proteobacteria* (1.1%), *α-proteobacteria* (0.5%) and unclassified bacteria (0.5%). The dominant phyla of the winter sequence amplicon library was also cyanobacteria (15.2%), other groups included: Verrucomicrobia (0.5%), Acidobacteria (0.5%),

Actinobacteria (0.5%), *β-proteobacteria* (2.2%), and *α-proteobacteria* (0.5%) (Table 8). Cyanobacteria being the dominant phyla of both the summer and winter sequences was expected as the majority of PCR amplification was performed using 16S cyanobacterial specific primers.

Summer Clone Libraries

Seven of the 154 clones from the summer were from samples taken from station 23 in the eastern basin of Lake Erie. The seven clones from station 23 classified as cyanobacteria. Two of the clones from station 23 were from a depth of 1 m, two from 17 m and three from 45 m. The remaining 147 summer clones were generated from samples taken from station 84 in the central basin. Of the clones from station 84, 139 were classified as cyanobacteria: 58 from a depth of 1 m, 9 from 13 m, 34 from 14 m, 10 from 17 m, and 28 from 23 m. The other remaining clones grouped as: 1 Actinobacteria at a depth of 22 m, 1 Firmicute at 22 m, 2 Planctomycetes 1 at 1 m and 1 at 22 m, 2 *γ-proteobacteria* at 22m, 1 *α-proteobacteria* at 1 m and one bacterium as unclassified at 14 m (Table 9).

Due to the fact that the majority of the samples analyzed by PCR were generated using cyanobacterial specific primers and 95% of the summer sequences were classified as cyanobacteria. For a more comprehensive data set to draw conclusions about lake cyanobacteria richness the six sequences identified with other phyla were not included in the trees generated for phylogenetic analysis. Initially all summer cyanobacterial sequences were compiled to construct one large phylogenetic tree and obtaining results from this approach was inconclusive. After collapsing identical and nearly identical sequences into clusters were then results determined. The sequences were then separated by depth upon analyzing the water column profile at the time of sampling. Sequences were separated into three groups that identified their position within the stratified water column, the epilimnion, metalimnion and hypolimnion. Sequences within the

epilimnion group were from a depth on one meter. Sequences assigned to the metalimnion group were from depths of 13, 14 and 17 meters. The hypolimnion group contained sequences from depths of 23 and 45 meters. A phylogenetic tree was constructed for each of the three groups. After sequences were separated by water stratum, a single representative of clones with identical sequences was included in the final phylogenetic trees.

Epilimnion Cyanobacterial Sequences

A total of 59 unique cyanobacterial sequences derived from organisms at surface (1 m) for phylogenetic sequence analysis (Figure 17). Picocyanobacterial phylogenetic tree reference sequences were obtained from GenBank and bootstrap values less than 50 were removed from tree. The phylogenetic tree showed an even distribution of sequences amplified from flow sorted and filtered water DNA extracted samples. The tree topology indicated five clusters/clades, four of which are in general agreement with previous studies by Budinoff *et al.* 2007 and Wilhelm, *et al.* 2006 (Crosbie 2003; Ernst 2003). Of the surfaced derived sequences, 12 clustered with *Synechococcus* sp. LBG2 and LBB3 with a bootstrap value of 71%. This cluster termed, Cluster D by Budinoff *et al.* 2007, is a cluster with high bootstrap support containing freshwater and marine picocyanobacteria. This same cluster was found in Lake Erie during summer hypoxia 2002, termed Clade III by Wilhelm *et al.* 2006. One of the surface sequences clustered with *Synechococcus sp.* MH301 and is being defined in the study as Cluster J. This cluster was unable to be described by Budinoff *et al.* 2007 due to a lack of sequence information at the time. Cluster J was found in Lake Erie as described by Wilhelm *et al.* 2006 and referred to as Clade I. Six of the surface sequences clustered with *Cyanobium* sp. JJR2A5. This cluster groups with *Synechococcus* sp. PS680 and MH305 is termed Cluster A as described by Budinoff *et al.* 2007. This cluster was not described by Wilhelm *et al.* 2006. Five of the sequences clustered with

Synechococcus rubescens SAG3.81. *S. rubescens* SAG3.81 is nearly identical to reference strain BO8807 in Cluster C as described by Budinoff *et al.* 2007. Cluster C was also found by Wilhelm *et al.* 2006 and is referred to as Clade IV. Another cluster found in the phylogenetic analysis is referred to by this study as the *Snowella* Cluster. Reference sequence *Synechocystis* sp. PCC6714 was included in this analysis to illustrate its phylogenetic relationship to *Snowella*. Species from the genera *Snowella* are cyanobacteria commonly found in lakes. *Snowella litoralis* OTU37S04 is blue-green in color and spherical with an average cell diameter of 3.2 μm (Rajaniemi-Wacklin 2006). The three sequences within this cluster were amplified from PE (Qty 1) and PC (Qty 2) flow sorted samples. Three of the sequences cluster with *Synechococcus* sp. PS723 with a bootstrap value of 99%. Though the remaining 29 sequences did not cluster with high bootstrap support to reference sequences in the tree, one of listed reference sequences is a phylogenetic neighbor. The majority of the sequences from the surface were identified as *Synechococcus* which was expected based on the previous findings by Wilhelm *et al.* 2006 that showed the densities of *Synechococcus* to be most abundant at a depth of 1 m from station 84 in Lake Erie, during the of summer 2005.

Metalimnion Cyanobacterial Sequences

A total of 55 clones classified as cyanobacteria from depths of 13, 14 and 17 m and were used for phylogenetic sequence analysis (Figure 18). Picocyanobacterial phylogenetic tree reference sequences were obtained from GenBank and bootstrap values less than 50 were removed from tree. The metalimnion phylogenetic tree was comprised mainly of sequences amplified from DNA extracted from filtered water, with a few sequences amplified from flow sorted samples. Of the three sequence groups this tree indicated the most clusters/clades, six, five of which are in general agreement with previous studies by Budinoff *et al.* 2007 and

Wilhelm, *et al.* 2006 (Crosbie 2003; Ernst 2003). Ten of the metalimnion sequences clustered with a bootstrap support of 99% in Cluster A which included *Cyanobium* sp. JJR2A5. Six sequences with a bootstrap support of 99% grouped with Cluster J/Clade I. One sequence clustered with *Synechococcus* sp. PCC8966 which is described as Cluster F by Budinoff *et al.* 2007 and as Clade VII by Wilhelm *et al.* 2006. Six sequences clustered with *Synechococcus* sp. LBG2 and LBB3 in Cluster D/Clade III with a bootstrap value of 89%. Eleven of the sequences clustered with *S. rubescens* SAG3.81 in Cluster C/Clade IV. Two sequences within the metalimnion group clustered with *Snowella* with a bootstrap support of 100%. The two sequences in the *Snowella* Cluster were amplified from PE and PC flow sorted samples. One sequence clustered with *Synechococcus* sp. PS723 with a bootstrap value of 99%. The remaining 18 sequences did not cluster with high bootstrap support to reference sequences within the tree, but one of listed reference sequences is a phylogenetic neighbor. All samples sorted by flow cytometry were amplified with 16S cyanobacterial specific primers and there was an equal representation of flow sorted samples from the epilimnion, metalimnion and hypolimnion. To increase the chances of obtaining sequence data for community analysis within the metalimnion water samples should be filtered for DNA extraction.

Hypolimnion Cyanobacterial Sequences

A total of 31 clones classified as cyanobacteria from depths of 23 and 45 m and were used for phylogenetic sequence analysis (Figure 19). Picocyanobacterial phylogenetic tree reference sequences were obtained from GenBank and bootstrap values less than 50 were removed from tree. The hypolimnion phylogenetic tree was comprised only of sequences amplified from flow sorted samples. The tree topology indicated five clusters/clades, four of which are in general agreement with previous studies by Budinoff *et al.* 2007 and Wilhelm, *et al.*

2006 (Crosbie 2003; Ernst 2003). Two of the sequence clustered with *Cyanobium* sp. JJR2A5 in Cluster A. Three of the hypolimnion sequences clustered with a bootstrap support of 99% in Cluster J/Clade I which included *Synechococcus sp.* MH301. Ten sequences clustered with *Synechococcus* sp. LBG2 and LBB3 in Cluster D/Clade III with a bootstrap value of 95%. One the sequences clustered with *S. rubescens* SAG3.81 in Cluster C/Clade IV. Four sequences within the hypolimnion group clustered with *Snowella*. The four sequences in the Snowella Cluster were amplified from a PC flow sorted samples. One sequence clustered with *Synechococcus* sp. 1t14s11 with a 97% bootstrap support. Eight sequences clustered with *Cyanobium* sp. JJ19B5 with a 76% bootstrap support. The remaining two sequences did not cluster with high bootstrap support to reference sequences within the tree, but one of listed reference sequences is a phylogenetic neighbor. All of the cyanobacterial sequences in the hypolimnion group were amplified from flow sorted samples. The results of the hypolimnion phylogenetic analysis are similar to the overall cyanobacterial phylogeny identified by Cupp 2006, with one important difference, cyanobacterial sequences were not amplified past a depth of 16.5 m. Community analysis of cyanobacteria in Lake Erie at depths within the hypolimnion flow cytometry could be a valuable tool to select for these communities.

A *Snowella* Cluster Was Identified in All Summer Sequence Groups

There was a reoccurring *Snowella* cluster at the base of three depth specific phylogenetic trees. For better phylogenetic resolution within this cluster, a phylogenetic tree of these nine sequences was constructed (Figure 20). All of the sequences were derived from flow sorted samples. One sequence clustered with *S. litoralis* 1LT47S05. One sequence clustered with *S. litoralis* OTU37S04. Though the remaining sequences did not form a cluster with *S. litoralis* OTU37S04, this reference sequence is the closest phylogenetic relative to the nine sequences.

Winter Cyanobacterial Sequences

A total of 37 sequences were analyzed from winter samples. Thirty three sequences were from station 84 in the central basin of Lake Erie and four were from station 357 in the western basin. The dominant phyla of the winter sequences was cyanobacteria (78.4%), other groups included: Verrucomicrobia (2.7%), Acidobacteria (2.7%), Actinobacteria (2.7%), *β-proteobacteria* (10.8%), and *α-proteobacteria* (2.7%). Of the clones classified as cyanobacteria 12 were from a depth of 1 m and 17 from a depth of 20 m (Table 10). The Verrucomicrobia and Acidobacteria classified clones were from a depth of 22m. The Actinobacteria and *α-proteobacteria* classified clones were from a depth of 1 m. Two of the *β-proteobacteria* clones were from a depth of 1 m and the other two from a depth of 22 m.

A total of 29 clones classified as cyanobacteria were used in the phylogenetic analysis of the winter season Figure 21. All of the sequences used in the analysis were amplified from flow sorted samples. Twelve of the winter sequences grouped with *Synechococcus*. With a bootstrap support of 97% ten sequences were in Cluster D/ Clade III and a bootstrap support of 99% two sequences were in Cluster F/Clade VII. The remaining 17 sequences clustered tightly with *Woronichinia naegeliana* OLE35S01. *Prochlorococcus* was never identified during the phylogenetic analysis of winter sequences. The winter sequence set was not robust.

Phylogenetic Analysis of All Cyanobacterial Sequences

Phylogenetic analysis of all cyanobacterial clones revealed eight clusters/clades (Figure 22). Picocyanobacterial phylogenetic tree reference sequences were obtained from GenBank and bootstrap values less than 50 were removed from tree. All clusters contained sequences amplified from flow sorted and filtered water extracted DNA samples with the exception of Cluster J/Clade I, which only contained sequences amplified from flow sorted samples. The

depth from each sequence is labeled within each cluster. The 16S rDNA sequences of PC and PE rich cyanobacteria can differ by a single nucleotide, so this information about sequences amplified from filtered water within each cluster/clade was not included (Budinoff *et al.* 2007). With a bootstrap support of 56% Cluster J/Clade I contains 12 sequences 1 (1 m), 8 (14 m) and 3 (45 m). This particular tree showed the emergence of one new cluster, Cluster K, not previously described by Budinoff or Wilhelm *et al.*. Cluster K cluster grouped with the reference sequence uncultured cyanobacterium clone LK1mC-31. The reference sequence was amplified from an environmental sample taken from a freshwater lake in Israel, Lake Kinneret and was submitted to GenBank in August 2005 as a new group of *Synechococcus*. A total of 12 sequences, 6 (1 m), 1 (13 m), 4 (17 m) and 1 (23 m), with a bootstrap support of 69% clustered into Cluster K. The closest phylogenetic neighbor of the two sequences branching from the same node as Cluster K is the reference sequence in Cluster K.

Cluster A contained sixteen sequences, six from a depth 1 m and ten from a depth of 14 m. The closest related reference sequence of the two sequences branching from the same node as Cluster A with a bootstrap value of 54% are found within Cluster A, though these sequences did not group with this cluster. Cluster F/Clade VII with a bootstrap support of 65% contained four sequences, two from a depth of 1 m, one from a depth of 17 m and 23 m. Cluster F/Clade VII contained two winter sequences. The majority of the sequences were located in Cluster D/Clade III. There are 64 sequences in Cluster D/Clade III, 26 grouped with uncultured *Synechococcus* (Cluster D) and 38 grouped with *Synechococcus* sp. LBG2 and LBB3 (Cluster D.1). The reference sequence used in Cluster D was Uncultured *Synechococcus* sp. Clone BAN2-04. The sequence was submitted to GenBank in 2005 and was amplified from a sample that was sorted by flow cytometry (Bec 2005). Cluster D is comprised of summer sequences and

Cluster D.1 contains both summer and winter sequences. There are 15 sequences in Cluster C/Clade IV. Four sequences with a bootstrap value of 99% cluster with the reference sequence *Synechococcus* sp. PS723. The remaining 17 sequences did not cluster with high bootstrap support to reference sequences within the tree, but one of listed reference sequences is a phylogenetic neighbor.

Sequences from a novel cluster of cyanobacteria identified in Lake Superior were included in this phylogenetic analysis (Ivanikova 2007). The Lake Superior cluster was included because there were sequences in this study that did not group with previously described clusters/clade. The Lake Superior is extremely oligotrophic and the geography is different from Lake Erie (Nawelajko 1986). None of the clones from this study clustered with the novel Lake Superior cluster. The *Woronichinia* cluster contained 17 winter sequences from a depth of 20 m. The *Snowella* cluster contained nine summer sequences, three from a depth of 1 m, two from 14 m and four from 23 m. *Prochlorococcus* was never identified during the phylogenetic analysis of summer sequences.

UniFrac Phylogenetic Analysis

UniFrac compares the phylogenetic distance between communities as a result of the selected method used to construct the tree. This method compares communities by measuring branch length leading to descendants within a selected community. Clustering and ordination techniques are used to compare multiple communities simultaneously (Lozupone 2005). The standard measure of diversity "evenness" is not represented in UniFrac (Magurran 1988). UniFrac does not consider sequence abundance (Lozupone 2005). UniFrac was used in this study to compare two sets of sequence data, flow sorted vs. environmental cyanobacterial sequences and summer vs. winter cyanobacterial sequences (Table 11). The unweighted UniFrac

score of flow sorted vs. environmental is 0.03 and summer vs. winter is 0.01. A UniFrac score less than 0.001 is considered to be highly significant, a score between 0.001 and 0.01 significant and between 0.01 and 0.05 marginally significant. The *P* test uses parsimony to determine if changes within sequence sets from different environments are greater than to be expected by chance (Martin 2002). For this study a p-value less than 0.05 was considered to be significant. The p-value of flow sorted vs. environmental cyanobacterial sequences was equal to 0.34. The p-value of summer vs. winter cyanobacterial sequences was equal to 0.01.

DISCUSSION

The initial hypothesis that *Prochlorococcus* is found during summer hypoxia in Lake Erie must be rejected for this study since none of the sequences analyzed for this study were identified as *Prochlorococcus*. The second hypothesis that the picocyanobacterial community structure found during the summer and winter season in Lake Erie are different, the null was rejected for this study. The second hypothesis was initially to be addressed by comparing the summer sequence set to the winter sequence set using the statistical method, LIBSHUFF (Singleton 2001). There were two reasons why this approach was not applicable. First, the initial goal of this study was to search for *Prochlorococcus*. This initial approach was the reason for analyzing such a large sample set by PCR and constructing multiple libraries. To research all the libraries generated a small number of clones were investigated from each library to ensure all libraries were analyzed. In order to compare one sequence library to another using the program LIBSHUFF, each library should contain at least 50 sequences (Singleton 2001). However, in this study each library contained anywhere from 1 to 19 clones. Therefore, individual sequence libraries from this study could not be used for statistical analysis. Second, even if sequences were combined into two sets, summer and winter, there were not enough winter sequences to be used for this approach.

Instead of LIBSHUFF the UniFrac program was used to compare the summer cyanobacterial sequences to the winter. The UniFrac phylogenetic analysis confirmed that there was a difference between the summer and winter sequences. This was expected because the phylogenetic trees indicated a *Snowella* cluster in the summer sequences and *Woronichinia* cluster in the winter sequences. *Snowella* related clones were identified in the three summer water strata. All of the *Snowella* related clones were amplified from flow sorted samples. It

cannot be stated by this study if *Snowella* organisms are more easily amplified from flow sorted samples. There were nine sequences that clustered with *Snowella* in this study and seven clones were identified by Cupp 2006 from samples collected from station 84 in Lake Erie, August 2005. The genera *Snowella* and *Woronichinia* belong to the cyanobacterial order Chroococcales (Komàrek 1999). *Snowella* and *Woronichinia* are commonly found in oligotrophic, mesotrophic and eutrophic lakes (Rajaniemi-Wacklin 2006). All of the cyanobacterial winter clones were amplified from flow sorted samples. There were three clusters within the winter cyanobacteria sequences, Cluster F/Clade, Cluster D/Clade III and a *Woronichinia* cluster.

The third hypothesis that flow cytometry can be used to increase the efficiency of genetic identification of picocyanobacteria from aquatic samples, can not be determined by this study. UniFrac analysis determined that there was a marginally significant difference between flow sorted and environmental sequences. The phylogenetic trees suggested there may be a difference between the two. The flow sorted sequences revealed Cluster J/Clade I, *Snowella* and *Woronichinia* clusters that the environmental samples did not. To address hypothesis three more sequence analysis should be done with larger sequence libraries.

Phyla Identified in Libraries

The most prevalent phylum identified from the libraries obtained in this study of Lake Erie was cyanobacteria. Using picocyanobacteria cluster/clade designations presented by Budinoff *et al.* 2007 and Wilhelm *et al.* 2006 based on adaptations by Crosbie and Ernst *et al.* 2003 the diversity of freshwater picocyanobacteria was studied in Lake Erie during the summer and winter season. Budinoff *et al.* 2007 described eight clusters of picocyanobacteria, five of which were present in the summer sequence libraries. As more 16S rDNA sequence data is made available the significance of the phylogeny presented in Budinoff *et al.* 2007, that

phylogenetically related clusters of picocyanobacteria does not imply related physiology between organisms becomes more important. A cluster of picocyanobacteria can be comprised of marine and freshwater organism, as well a PC and PE organism found from different habitats. Previously identified picocyanobacteria phylogeny explains why summer clones in Cluster C/Clade IV were generated from PE and PC flow sorted samples.

The 38 libraries constructed for this study demonstrate the diversity of freshwater picocyanobacteria. It cannot be stated if the diversity of the summer libraries were different from that of the winter. Of the 38 libraries generated 23 summer and 5 winter libraries were included in the phylogenetic analysis portion of this study. Twenty of the summer libraries contained more than one unique sequence type. Within each library, a sequence was considered unique if it clustered with a different Cluster/Clade or if the sequence did not group with a Cluster/Clade. The general trend of the summer libraries was that libraries generated from DNA extracted from filtered water and PE sorted samples contained more unique sequences. This can be explained by two reasons. First, Wilhelm *et al.* 2006 indicated that PE rich cyanobacteria are abundant at a narrow region below the thermocline during hypoxic conditions. All of the summer libraries were generated from samples collected during summer hypoxic conditions. The filtered water samples collected for DNA extractions could contain PC and/or PE rich cyanobacteria. Second, using flow cytometry to select for a specific characteristic of cyanobacteria made it possible to detect which flow sorted samples contained more unique sequences, PE or PC. The three summer libraries containing sequences that clustered into one Cluster/Clade had four or less sequences.

The clones that were classified as phyla other than cyanobacteria are considered planktonic bacteria. Though cyanobacteria was the primary focus of this study, other phyla were

identified and should be discussed. In freshwater lakes planktonic bacteria mediate a large portion of the carbon turnover (del Giorgio 1999). The second most represented phylum in this study was *Proteobacteria*. Three of the five Proteobacteria classes, α, β and γ, are commonly found in aquatic systems (Glöckner 1999). In 2006 eubacterial sequence libraries generated from samples collected prior to hypoxic conditions in Lake Erie *α-proteobacteria* accounted for 23%, *β-proteobacteria* 6% and *γ-proteobacteria* 1% of all the sequences obtained (Cupp 2006).

Another phylum identified in this study was Actinobacteria. Actinobacteria is found in many different habitats, including freshwater ecosystems was identified in the libraries presented in this study (Glöckner 2000; Lindström 2002; Zwart 2002). The most prevalent phylum identified by Cupp 2006 was Actinobacteria, which comprised almost half (47%) of the sequence data. In this study two libraries were constructed using a universal 16S primer set, this explains why Actinobacteria was not the primary phyla identified.

Among the summer sequences were two clones classified as Planctomycetes. Wilhelm, *et al.* 2006 also identified a *Planctomycetales*-related clone. This group of bacteria is capable of novel biogeochemical reactions, including ammonia oxidation under anaerobic conditions (Strous 1999). The conditions of Lake Erie during summer stratification, low oxygen and abundant ammonia and nitrite, explains the presence of these organisms (Mills 2004; Wilhelm *et al.* 2006). One summer clone in this study and several in Cupp 2006 were designated as unclassified bacteria by RDP Classifier.

Overall, this study has provided insight to the community structure present in Lake Erie in the winter season. There is also a difference in the cyanobacterial community structure in summer and winter season. This study has also confirmed findings by Cupp, 2006 that *Prochlorococcus* was not identified in Lake Erie.

REFERENCES

(1998). Lake Erie Pennsylvania's Great Lake. Pennsylvania Angler & Boater**:** 41 - 44.

Altschul, S. F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). "Basic local alignment tool." Journal of molecular biology **15**: 403-410.

Atlas, R. M. a. R. B. (1998). Microbial Ecology Fundamentals and Applications. Menlo Park, Benjamin/Cummings Science Publishing.

Azam, F., T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil and F. Thingstad (1983). "The Ecological Role of Water-Column Microbes in the Sea." Marine Ecology - Press Series **10**: 257 - 263.

Beale, S. I. (1994). Biosynthesis of Cyanobacteria Tetrapyrrole Pigments: Hemes, Chlorophylls, and Phycobilins. The Molecular Biology of Cyanobacteria. D. A. Bryant. Dordrecht, The Netherlands Kluwer Academic Publishers. **1:** 520 - 521.

Bec, B., Masseret,E., Vaquer,A., Souchu,P. and Laugier,T. (2005). Molecular and flow cytometry analyses of picocyanobacteria diversity in Mediterranean coastal lagoons. GenBank.

Berninger, U. G., D. A. Caron, and R. W. Sanders (1992). "Mixotrophic algae in three ice-covered lakes of the Pocono Mountains, U.S.A." Freshwater Biology **28**: 263 - 72.

Bolsenga, S. J. a. C. E. H. (1993). Lake Erie and Lake St. Clair Handbook. Detroit, Wayne State University Press.

Boyce, F. M., M. N. Charlton, D. Rathke, C. H. Mortimer, and J. Bennett (1987). "Lake Erie Research: Recent Results, Remaining Gaps." Journal of Great Lakes Research **13**(4): 826 - 840.

Budinoff *et al.,* C. R. a. J. T. H. (2007). "Ecophysiology of a Mono Lake picocyanobacterium." Limnol. Oceanogr. **52**(6): 2484 - 2495.

Burns, N. M. (1976). "Oxygen depletion in the central and eastern basins of Lake Erie, 1970." Journal of Fish. Res. Board Canada **33**: 512 - 519.

Callieri, C., R. Bertoni, E. Amicucci, M.L. Pinolini and J. Jasser (1996). "Growth rates of freshwater picocyanobacteria measured by FDC: problems and potentials for the estimation of picoplankton organic carbon synthesis " Arch Hydrobiol Spec Issues Advances Limnol **48**: 93 - 103.

Chandler, D. C. (1940). "Limnological studies of western Lake Erie, I. Plankton and certain physical-chemical data of the Bass Island Region, from September, 1938, to November, 1939." The Ohio Journal of Science **XL**(6): 291 - 336.

Chandler, D. C. (1942a). "Limnological studies of western Lake Erie II. Light penetration and its relation to turbidity." Ecology **23**: 41 - 52.

Chandler, D. C. (1942b). "Limnological studies of western Lake Erie: III. Phytoplankton and physical-chemical data from November, 1939. to November, 1940." The Ohio Journal of Science **42**.

Charlton, M. N. (1980). "Hypolimnion oxygen consumption in lakes: discussion of productivity and morphometry effects." Canada Journal Fish. Aquatic Sci. **37**: 72 - 81.

Charlton, M. N., and Milne, J.E. (2004). "Review of thirty years of changes in Lake Erie water quality." National Water Research Institute Contributions: 04-167.

Charlton, M. N. a. S. S. R. (1983). "Oxygen Depletion in Central and Eastern Lake Erie: Relationship With Bacteria, Chlorophyll, POC, and Morphometry." Journal of Great Lakes Research **9**(1): 3 - 8.

Chisholm, S. W., Robert J. Olson, Erik R. Zettler, Ralf Goericke, John B. Waterbury, and Nicholas A. Welschmeyer (1988). "A novel free-living prochlorophyte abundant in the oceanic euphotic zone." Nature **334**: 340 - 343.

Cole, J. R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R. J., Kulam-Syed-Mohideen, A. S., McGarrell, D. M., Marsh, T., Garrity, G. M. and Tiedje, J. M. (2009). "The Ribosomal Database Project: improved alignments and new tools for rRNA analysis." Nucl. Acids Res. **37**(suppl_1): D141-145.

Collier, J. L. (2000). "Flow Cytometry and the Single Cell in Phycology." Journal of Phycology **36**: 628 - 644.

Crosbie, N. D., M. Pockl and T. Weisse (2003). "Dispersal and phylogenetic diversity of nonmarine picocyanobacteria, inferred from 16S rRNA gene and cpcBA-intergenic spacer sequence analyses." Appl. Environ. Microbiol. **69**: 5716 - 5721.

Cupp, A. R. (2006). Photosynthetic Picoplankton and Bacterioplankton in the Central Basin of Lake Erie During Seasonal Hypoxia. Science. Bowling Green, Bowling Green State University. **M.S.:** 90.

del Giorgio, P. A., J. J. Cole, N. F. Caraco and R. H. Peters (1999). "Linking planktonic biomass and metabolism to net gas fluxes in northern temperate lakes." Ecology **80**: 1422 - 1431.

Drews, G. (2005). "Contributions of Theodor Wilhelm Engelmann on phototaxis, chemotaxis, and photosynthesis." Photosynthesis Research **83**: 25 - 34.

Engelmann, T. W. (1883). "Farbe und Assimilation." Bot. *Zeit.* **41**: 1 - 29.

Engelmann, T. W. (1884). "Untersuchungen uber die quantitativen Beziehungen zwischen Absorption des Lichtes and Assimilation in Pflanzenzellen." Bot. Z. **42**: 81 - 93, 97 - 105.

Ernst, A. (1991). "Cyanobacterial picoplankton from Lake Constance. I. Isolation by fluorescence characteristics." Journal of Plankton Research **13**: 1307 - 1312.

Ernst, A., S. Becker, U. I. A. Wollenzien, and C. Postius (2003). "Ecosystem-dependent adaptive radiation of picocyanobacteria inferred from 16S rRNA and ITS-1 sequence analysis." Microbiology **149**: 217 - 228.

Glöckner, F. O., B. M. Fuchs and R. Amann (1999). "Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization." Appl. Environ. Microbiol. **65**: 3721 - 3726.

Glöckner, F. O., E. Zaichikov, N. Belkova, L. Denissova, J. Pernthaler, A. Pernthaler, and R. Amann (2000). "Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters, including an abundant group of actinobacteria." Appl. Environ. Microbiol. **66**: 5053 - 5065.

Grady, W., Ed. (2007). The Great Lakes: The Natural History of a Changing Region

Hall, T. A. (1999). "BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT." Nucleic Acids Symp. Ser. **41**: 95 - 98.

Huisman, J., J. Sharples, J.M. Stroom, P.M. Visser, W.E.A. Kardinaal, J.M.H. Verspagen, *et al.* (2004). "Changes in turbulent mixing shift competition for light between phytoplankton species." Ecology **85**: 2960 - 2970.

Hutchinson, G. E. (1957). A Treatise on Limnology. I. Geography, Physics, and Chemistry. New York, John Wiley & Sons.

Ivanikova, N. V., Linda C. Popels, R. Michael L. McKay, and George S. Bullerjahn (2007). "Lake Superior Supports Novel Clusters of Cyanobacterial Picoplankton." Appl. Environ. Microbiol. **73**: 4055 - 4065.

Johnson, Z., M.L. Landry, R.R. Bidigare, S.L. Brown, L. Campbell, J. Gunderson, J. Marra, C. Trees (1999). "Energetics and growth kinetics of a deep *Prochlorococcus* spp. population in the Arabian Sea." Deep Sea Research II **46**: 1719 - 1743.

Johnson, Z. I., E.R. Zinser, A. Coe, N.P. McNulty, E.M.S. Woodward & S.W. Chisholm (2006). "Niche Partitioning among *Prochlorococcus* ecotypes along ocean-scale environmental gradients." Science **311**: 1737 - 1740.

Kirk, J. T. O. (1994). Light and Photosynthesis in Aquatic Ecosystems. Cambridge, Cambridge University Press.

Komàrek, J. a. K. A. (1999). *Cyanoprokaryota. 1.Teil: Chroococcales*, Jena.

Kumar, S., Tamura, K., and Nei, M. (1994). "MEGA: Molecular Evolutionary Genetics Analysis software for microcomputers." Comput. Appl. Biosci. **10**(189-191).

Lane, D. J. (1991). 16S/23S rRNA Sequencing. Nucleic acid techniques in bacterial systematics. E. S. a. M. Goodfellows. Chichester, John Wiley & Sons**:** 115 - 175.

Lindström, E. S. a. E. L. (2002). "Do neighbouring lakes share common taxa of bacterioplankton? Comparison of 16S rDNA fingerprints and sequences from three geographic regions." Microbial Ecology **44**: 1 -9

Lozupone, C. a. R. K. (2005). "UniFrac: a New Phylogenetic Method for Comparing Microbial Communities." Appl. Environ. Microbiol. **71**(12): 8228-8235.

Magurran, A. E. (1988). Ecological diversity and its measurement. Princeton, N.J., Princeton University Press.

Martin, A. P. (2002). "Phylogenetic approaches for describing and comparing the diversity of microbial communities." Appl. Environ. Microbiol. **68**: 3673 - 3682.

McKnight, D. (2000). "Phytoplankton dynamics in a stably stratified Antarctic lake during winter darkness." Journal of Phycology **36**: 852 - 861.

Mills, H. J., R. J. Martinez, S. Story and P. A. Sobecky (2004). "Identification of members of the metabolically active microbial populations associated with *Beggiatoa* species mat communities from the Gulf of Mexico cold-seep sediments." Appl. Environ. Microbiol. **70**: 5447 - 5458.

Nawelajko, C. a. D. V. (1986). "Effects of environmental variables on growth rates and physiological characteristics of Lake Superior phytoplankton." Canada Journal Fish. Aquatic Sci. **43**: 1163 - 1170.

Nebaeus, M. (1984). "Algal water-blooms under ice-cover." Verh. Int. Verein. Limnol. **22**: 719 - 24.

Nübel, U., Ferran Garcia-Pichel and Gerard Muyzer (1997). "PCR Primers To Amplify 16S rRNA Genes from Cyanobacteria." Applied and Environmental Microbiology **63**(8): 3327 - 3332.

Partensky, F., W. R. Hess, and D. Vaulot (1999). "*Prochlorococcus*, a Marine Photosynthetic Prokaryote of Global Significance." Microbiology and Molecular Biology Reviews **63**(1): 106 - 127.

Rajaniemi-Wacklin, P., Anne Rantala, Maria A. Mugnai, Silvia Turicchia, Stefano Ventura, Jarka Komárková, Liisa Lepistö and Kaarina Sivonen (2006). "Correspondence Between Phylogeny and Morphology of Snowella spp. and Woronichinia Naegeliana, Cyanobacteria Commonly Occurring in Lakes." Journal of Phycology **42**(1): 226-232.

Raven, J. (2007). "Photosynthesis in watercolours." Nature **448**: 418.

Rinta-Kanto, J. M., A. J. A. Ouellette, G. L. Boyer, M. R. Twiss, T. B. Bridgeman, and S. W. Wilhelm (2005). "Quantification of Toxic *Microcystis* spp. during the 2003 and 2004 Blooms in Western Lake Erie using Quantitative Real-Time PCR." Environmental Science and Technology **39**(11): 4198 - 4205.

Rippka, R., Josette Deruelles, John B. Waterbury, Michael Herdman and Roger Y. Stanier (1979). "Generic Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria." Journal of General Microbiology **111**: 1 - 61.

Rudiger, W. (1994). Progress in Phycological Research. F. E. R. a. D. J. Chapman. Bristol, UK, Biopress. **10**.

Shapiro, H. M. (2003). Practical Flow Cytometry. Hoboken, John Wiley & Sons.

Singleton, D. R., Furlong, M.A., Rathbun, S.L. and Whitman, W.B. (2001). "Quantitative comparisons of 16S rRNA gene sequence libraries from environmental samples." Appl. Environ. Microbiol. **67**: 4374 - 4376.

Stockner, J., Cristiana Callieri and Gertrud Cronberg (2000). Picoplankton and Other Non-Bloom-Forming Cyanobacteria in Lakes. The Ecology of Cyanobacteria. B. A. W. a. M. Potts. Netherlands, Kluwer Academic Publishers**:** 195 - 231.

Stomp, M., Jeff Huisman, Lajos Voros, Frances R. Pick, Maria Laamanen, Thomas Haverkamp, Lucas J. Stal (2007). "Colourful coexistence of red and green picocyanobacteria in lakes and seas." Ecology Letters **10**(4): 290-298.

Strous, M., J. G. Kuenen, and M. Jetten (1999). "Key Physiology of Anaerobic Ammonium Oxidation." Appl. Environ. Microbiol. **65**(7): 3248-3250.

Thompson, J. D., Higgins, Desmond G. and Gibson, Toby J. (1994). "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice." Nucl. Acids Res. **22**(22): 4673-4680.

Urbach, E., David J. Scanlan, Daniel L. Distel, John B. Waterbury and Sallie W. Chisholm (1998). "Rapid Diversification of Marine Picophytoplankton with Dissimilar Light-Harvesting Structures Inferred from Sequences of *Prochlorococcus* and *Synechococcus*." Journal of Molecular Evolution **46**: 188 - 201.

Wang, Q., George M. Garrity, James M. Tiedje, James R. Cole (2007). "Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy." Appl. Environ. Microbiol. **73**(16): 5261-5267.

Wedemayer, G. J., D.E. Wemmer and A.N. Glazer (1991). "Phycobilins of cryptophycean algae. Structures of novel bilins with acryloyl substituents from phycoerythrin 566." Journal of Biol. Chem. **266**: 4731 - 4741.

Wetzel, R. G. (2001). Limnology Lake and River Ecosystems. San Diego, Academic Press.

Whitton, B. A. (1992). Diversity, ecology, and taxonomy of the cyanobacteria. Biotechnology Handbooks: Photosynthesis Prokaryotes. M. N. a. C. NG. New York, Plenum Press. **6:** 1 - 52.

Wilhelm *et al.*, S. W., George S. Bullerjahn, Melanie L. Eldridge, Johanna M. Rinta-Kanto, Leo Poorvin and Richard A. Bourbonniere (2006). "Seasonal Hypoxia and the Genetic Diversity of Prokaryote Populations in the Central Basin Hypolimnion of Lake Erie: Evidence for Abundant Cyanobacteria and Photosynthesis." Journal of Great Lakes Research **32**: 657-671.

Wood, A. M., P.K. Horan, K. Muirhead, D.A. Phinney, C.M. Yentsch and J.B. Waterbury (1985). "Discrimination between types of pigments in marine *Synechococcus spp.* by scanning spectroscopy, epifluorescence microscopy and flow cytometry." Limnol. Oceanogr. **30**: 1303 - 1315.

Wright, J. W., Ed. (2007). The New York Times Almanac.

Zinser, E. R., A. Coe, Z. I. Johnson, A. Martiny, N. J. Fuller, D. J. Scanlan and S. W. Chisholm (2006). "Prochlorococcus Ecotypes Abundance in the North Atlantic Ocean as Revealed by an Improved Quantative PCR Method." Appl. Environ. Microbiol. **72**(1): 723 - 732.

Zwart, G., B. C. Crump, M. P. Kamst-van Agterveld, F. Hagen and S. K. Han (2002). "Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers." Aquatic Microbial Ecology **28**: 141 - 155.

APPENDICES

APPENDIX A: Tables

Table 1. All Lake Erie environmental samples taken for DNA extraction were prepared by filtering 50 ml of water onto a 0.2 μm filter. After collection all samples were stored at -20˚ C.

DATE COLLECTED	STATION	DEPTH (m)
August, 2008	84	1
August, 2008	84	13
August, 2008	84	17
August, 2008	84	23
August, 2008	23	1
August, 2008	23	17
August, 2008	23	45
February, 2008	84	1
February, 2008	84	22
February, 2008	340	surface
January, 2009	84	1
January, 2009	84	20
January, 2009	357	1
February, 2009	84	1
February, 2009	84	20
February, 2009	357	1
February, 2009	357	8

Table 2. Lake Erie water samples were collected in triplicate for flow cytometry. All samples were collected in 1 ml aliquots to which 75 μl of DMSO was added and stored in liquid nitrogen upon arrival to the lab. Long term storage for samples was at -80˚ C.

DATE COLLECTED	STATION	DEPTH (m)
August, 2008	84	1
August, 2008	84	13
August, 2008	84	14
August, 2008	84	22
August, 2008	84	23
August, 2008	23	1
August, 2008	23	17
August, 2008	23	45
January, 2009	84	1
January, 2009	84	20
January, 2009	357	1
January, 2009	452	1
February, 2009	84	1
February, 2009	84	20
February, 2009	357	1
February, 2009	357	8

Table 3. Initial set of sorting parameters. All samples were collected August 2008 from Station 84 in the central basin of Lake Erie. All samples were sorted on October 31, 2008.

Name	Pigment	Depth (m)	How The Sample Was Sorted
38L	PE	1	Green gate set at 610, *Synechococcus*
38HL	PC	1	Blue gate set at 670, pico-eukaryotes
38HR	PC	1	Purple gate, set at 670, pico-phytoplankton
38R	PC	1	Red gate, set at 670, *Prochlorococcus*
39L	PE	1	Green gate set at 610, *Synechococcus*
39HL	PC	1	Blue gate set at 670, pico-eukaryotes
39HR	PC	1	Purple gate, set at 670, pico-phytoplankton
39R	PC	1	Red gate, set at 670, *Prochlorococcus*
40L	PE	1	Green gate set at 610, *Synechococcus*
40HL	PC	1	Blue gate set at 670, pico-eukaryotes
40HR	PC	1	Purple gate, set at 670, pico-phytoplankton
40R	PC	1	Red gate, set at 670, *Prochlorococcus*
41L	PE	14	Green gate set at 610, *Synechococcus*
41HL	PC	14	Blue gate set at 670, pico-eukaryotes
41HR	PC	14	Purple gate, set at 670, pico-phytoplankton
41R	PC	14	Red gate, set at 670, *Prochlorococcus*
42L	PE	14	Green gate set at 610, *Synechococcus*
42HL	PC	14	Blue gate set at 670, pico-eukaryotes
42HR	PC	14	Purple gate, set at 670, pico-phytoplankton
42R	PC	14	Red gate, set at 670, *Prochlorococcus*
43L	PE	14	Green gate set at 610, *Synechococcus*
43HL	PC	14	Blue gate set at 670, pico-eukaryotes
43HR	PC	14	Purple gate, set at 670, pico-phytoplankton
43R	PC	14	Red gate, set at 670, *Prochlorococcus*
44L	PE	23	Green gate set at 610, *Synechococcus*
44HL	PC	23	Blue gate set at 670, pico-eukaryotes
44HR	PC	23	Purple gate, set at 670, pico-phytoplankton
44R	PC	23	Red gate, set at 670, *Prochlorococcus*
45L	PE	23	Green gate set at 610, *Synechococcus*
45HL	PC	23	Blue gate set at 670, pico-eukaryotes
45HR	PC	23	Purple gate, set at 670, pico-phytoplankton
45R	PC	23	Red gate, set at 670, *Prochlorococcus*
46L	PE	23	Green gate set at 610, *Synechococcus*
46HL	PC	23	Blue gate set at 670, pico-eukaryotes
46HR	PC	23	Purple gate, set at 670, pico-phytoplankton
46R	PC	23	Red gate, set at 670, *Prochlorococcus*
47L	PE	23	Green gate set at 610, *Synechococcus*
47HL	PC	23	Blue gate set at 670, pico-eukaryotes
47HR	PC	23	Purple gate, set at 670, pico-phytoplankton
47R	PC	23	Red gate, set at 670, *Prochlorococcus*

Table 4. Final set of sorting parameters. All samples were collected from the central basin of Lake Erie Station 84. Samples were sorted on February 24, 2009. L – left, HL - half left, HR – half right and R – right indicating the position in sorting rack.

Name	Pigment	Date Collected	Cells/mL	Depth (m)	How The Sample Was Sorted
10-PC-L	PE	January 2009	37,740	1	Green gate, remove or gate out fluorescence at 610 - 20
10-PC-HL	PC	January 2009	78,710	1	Blue gate, bottom gate selecting fluorescence at 670 - 30
10-PC-HR	PC	January 2009	2,132	1	Purple gate, top gate selecting fluorescence at 670 - 30
10-PC-R	PC	January 2009	12,032	1	Red gate, middle gate selecting fluorescence at 670 - 30
10-PE-L	PC	January 2009	55,418	1	Green gate, remove or gate out fluorescence at 670 - 30
10-PE-HL	PE	January 2009	5,104	1	Blue gate, bottom gate selecting fluorescence at 610 - 20
10-PE-HR	PE	January 2009	548	1	Purple gate, middle gate selecting fluorescence at 610 - 20
10-PE-R	PE	January 2009	140	1	Red gate, top gate selecting fluorescence at 610 - 20
13-PC-L	PE	January 2009	17,416	20	Green gate, remove or gate out fluorescence at 610 - 20
13-PC-HL	PC	January 2009	48,254	20	Blue gate, bottom gate selecting fluorescence at 670 - 30
13-PC-HR	PC	January 2009	1,594	20	Purple gate, top gate selecting fluorescence at 670 - 30
13-PC-R	PC	January 2009	5,958	20	Red gate, middle gate selecting fluorescence at 670 - 30
13-PE-L	PC	January 2009	44,850	20	Green gate, remove or gate out fluorescence at 670 - 30
13-PE-HL	PE	January 2009	3,002	20	Blue gate, bottom gate selecting fluorescence at 610 - 20
13-PE-HR	PE	January 2009	374	20	Purple gate, middle gate selecting fluorescence at 610 - 20
13-PE-R	PE	January 2009	94	20	Red gate, top gate selecting fluorescence at 610 - 20
25-PC-L	PE	February 2009	13,150	1	Green gate, remove or gate out fluorescence at 610 - 20
25-PC-HL	PC	February 2009	8,872	1	Blue gate, bottom gate selecting fluorescence at 670 - 30
25-PC-HR	PC	February 2009	1,222	1	Purple gate, top gate selecting fluorescence at 670 - 30
25-PC-R	PC	February 2009	1,682	1	Red gate, middle gate selecting fluorescence at 670 - 30
25-PE-L	PC	February 2009	16,588	1	Green gate, remove or gate out fluorescence at 670 - 30
25-PE-HL	PE	February 2009	117,772	1	Blue gate, bottom gate selecting fluorescence at 610 - 20
25-PE-HR	PE	February 2009	1,758	1	Purple gate, middle gate selecting fluorescence at 610 - 20
25-PE-R	PE	February 2009	368	1	Red gate, top gate selecting fluorescence at 610 - 20

Table 4. Continued

Name	Pigment	Date Collected	Cells/mL	Depth (m)	How The Sample Was Sorted
28-PC-L	PE	February 2009	74,694	20	Green gate, remove or gate out fluorescence at 610 - 20
28-PC-HL	PC	February 2009	38,594	20	Blue gate, bottom gate selecting fluorescence at 670 - 30
28-PC-HR	PC	February 2009	4,360	20	Purple gate, top gate selecting fluorescence at 670 - 30
28-PC-R	PC	February 2009	2,998	20	Red gate, middle gate selecting fluorescence at 670 - 30
28-PE-L	PC	February 2009	36,832	20	Green gate, remove or gate out fluorescence at 670 - 30
28-PE-HL	PE	February 2009	101,416	20	Blue gate, bottom gate selecting fluorescence at 610 - 20
28-PE-HR	PE	February 2009	10,700	20	Purple gate, middle gate selecting fluorescence at 610 - 20
28-PE-R	PE	February 2009	3,034	20	Red gate, top gate selecting fluorescence at 610 - 20
30-PC-L	PE	February 2009	22,900	20	Green gate, remove or gate out fluorescence at 610 - 20
30-PC-HL	PC	February 2009	35,574	20	Blue gate, bottom gate selecting fluorescence at 670 - 30
30-PC-HR	PC	February 2009	350	20	Purple gate, top gate selecting fluorescence at 670 - 30
30-PC-R	PC	February 2009	1,332	20	Red gate, middle gate selecting fluorescence at 670 - 30
30-PE-L	PC	February 2009	283,936	20	Green gate, remove or gate out fluorescence at 670 - 30
30-PE-HL	PE	February 2009	918,968	20	Blue gate, bottom gate selecting fluorescence at 610 - 20
30-PE-HR	PE	February 2009	200,000	20	Purple gate, middle gate selecting fluorescence at 610 - 20
30-PE-R	PE	February 2009	45,024	20	Red gate, top gate selecting fluorescence at 610 - 20
61-PC-L	PE	August 2008	12,352	1	Green gate, remove or gate out fluorescence at 610 - 20
61-PC-HL	PC	August 2008	27,560	1	Blue gate, bottom gate selecting fluorescence at 670 - 30
61-PC-HR	PC	August 2008	3,372	1	Purple gate, top gate selecting fluorescence at 670 - 30
61-PC-R	PC	August 2008	7,002	1	Red gate, middle gate selecting fluorescence at 670 - 30
61-PE-L	PC	August 2008	49,390	1	Green gate, remove or gate out fluorescence at 670 - 30
61-PE-HL	PE	August 2008	6,900	1	Blue gate, bottom gate selecting fluorescence at 610 - 20
61-PE-HR	PE	August 2008	750	1	Purple gate, middle gate selecting fluorescence at 610 - 20
61-PE-R	PE	August 2008	224	1	Red gate, top gate selecting fluorescence at 610 - 20

Table 4. Continued

Name	Pigment	Date Collected	Cells/mL	Depth (m)	How The Sample Was Sorted
63-PC-L	PE	August 2008	19,332	13	Green gate, remove or gate out fluorescence at 610 - 20
63-PC-HL	PC	August 2008	15,346	13	Blue gate, bottom gate selecting fluorescence at 670 - 30
63-PC-HR	PC	August 2008	1,296	13	Purple gate, top gate selecting fluorescence at 670 - 30
63-PC-R	PC	August 2008	2,026	13	Red gate, middle gate selecting fluorescence at 670 - 30
63-PE-L	PC	August 2008	21,188	13	Green gate, remove or gate out fluorescence at 670 - 30
63-PE-HL	PE	August 2008	7,784	13	Blue gate, bottom gate selecting fluorescence at 610 - 20
63-PE-HR	PE	August 2008	728	13	Purple gate, middle gate selecting fluorescence at 610 - 20
63-PE-R	PE	August 2008	142	13	Red gate, top gate selecting fluorescence at 610 - 20
70-PC-L	PE	August 2008	4,818	23	Green gate, remove or gate out fluorescence at 610 - 20
70-PC-HL	PC	August 2008	7,502	23	Blue gate, bottom gate selecting fluorescence at 670 - 30
70-PC-HR	PC	August 2008	864	23	Purple gate, top gate selecting fluorescence at 670 - 30
70-PC-R	PC	August 2008	528	23	Red gate, middle gate selecting fluorescence at 670 - 30
70-PE-L	PC	August 2008	5,064	23	Green gate, remove or gate out fluorescence at 670 - 30
70-PE-HL	PE	August 2008	2,122	23	Blue gate, bottom gate selecting fluorescence at 610 - 20
70-PE-HR	PE	August 2008	80	23	Purple gate, middle gate selecting fluorescence at 610 - 20
70-PE-R	PE	August 2008	66	23	Red gate, top gate selecting fluorescence at 610 - 20

Table 5. Final set of sorting parameters. All samples were collected from Lake Erie and sorted on April 16, 2009.

Name	Station	Pigment	Date Collected	Cells/mL	Depth (m)	How Sample Was Sorted
1-PC-L	357	PE	Jan. 2009	1,144	1	Green gate, remove or gate out fluorescence at 610 - 20
1-PC-HL	357	PC	Jan. 2009	1,920	1	Blue gate, bottom gate selecting fluorescence at 670 - 30
1-PC-HR	357	PC	Jan. 2009	540	1	Purple gate, top gate selecting fluorescence at 670 - 30
1-PC-R	357	PC	Jan. 2009	338	1	Red gate, middle gate selecting fluorescence at 670 - 30
1-PE-L	357	PC	Jan. 2009	2,034	1	Green gate, remove or gate out fluorescence at 670 - 30
1-PE-HL	357	PE	Jan. 2009	152	1	Blue gate, bottom gate selecting fluorescence at 610 - 20
1-PE-HR	357	PE	Jan. 2009	14	1	Purple gate, middle gate selecting fluorescence at 610 - 20
1-PE-R	357	PE	Jan. 2009	18	1	Red gate, top gate selecting fluorescence at 610 - 20
16-PC-L	452	PE	Jan. 2009	1,060	1	Green gate, remove or gate out fluorescence at 610 - 20
16-PC-HL	452	PC	Jan. 2009	2,176	1	Blue gate, bottom gate selecting fluorescence at 670 - 30
16-PC-HR	452	PC	Jan. 2009	424	1	Purple gate, top gate selecting fluorescence at 670 - 30
16-PC-R	452	PC	Jan. 2009	392	1	Red gate, middle gate selecting fluorescence at 670 - 30
16-PE-L	452	PC	Jan. 2009	1,768	1	Green gate, remove or gate out fluorescence at 670 - 30
16-PE-HL	452	PE	Jan. 2009	86	1	Blue gate, bottom gate selecting fluorescence at 610 - 20
16-PE-HR	452	PE	Jan. 2009	14	1	Purple gate, middle gate selecting fluorescence at 610 - 20
16-PE-R	452	PE	Jan. 2009	10	1	Red gate, top gate selecting fluorescence at 610 - 20
1-PC-L	357	PE	Feb. 2009	8,128	1	Green gate, remove or gate out fluorescence at 610 - 20
1-PC-HL	357	PC	Feb. 2009	2,564	1	Blue gate, bottom gate selecting fluorescence at 670 – 30
1-PC-HR	357	PC	Feb. 2009	444	1	Purple gate, top gate selecting fluorescence at 670 – 30
1-PC-R	357	PC	Feb. 2009	226	1	Red gate, middle gate selecting fluorescence at 670 – 30
1-PE-L	357	PC	Feb. 2009	20,194	1	Green gate, remove or gate out fluorescence at 670 – 30
1-PE-HL	357	PE	Feb. 2009	5,326	1	Blue gate, bottom gate selecting fluorescence at 610 – 20
1-PE-HR	357	PE	Feb. 2009	100	1	Purple gate, middle gate selecting fluorescence at 610 – 20
1-PE-R	357	PE	Feb. 2009	210	1	Red gate, top gate selecting fluorescence at 610 – 20

Table 5. Continued

Name	Station	Pigment	Date Collected	Cells/mL	Depth (m)	How Sample Was Sorted
4-PC-L	357	PE	Feb. 2009	8,618	8	Green gate, remove or gate out fluorescence at 610 – 20
4-PC-HL	357	PC	Feb. 2009	2,998	8	Blue gate, bottom gate selecting fluorescence at 670 – 30
4-PC-HR	357	PC	Feb. 2009	1,098	8	Purple gate, top gate selecting fluorescence at 670 – 30
4-PC-R	357	PC	Feb. 2009	372	8	Red gate, middle gate selecting fluorescence at 670 – 30
4-PE-L	357	PC	Feb. 2009	7,088	8	Green gate, remove or gate out fluorescence at 670 – 30
4-PE-HL	357	PE	Feb. 2009	1.584	8	Blue gate, bottom gate selecting fluorescence at 610 – 20
4-PE-HR	357	PE	Feb. 2009	162	8	Purple gate, middle gate selecting fluorescence at 610 – 20
4-PE-R	357	PE	Feb. 2009	156	8	Red gate, top gate selecting fluorescence at 610 – 20
74-PC-L	23	PE	Aug. 2008	16,860	1	Green gate, remove or gate out fluorescence at 610 – 20
74-PC-HL	23	PC	Aug. 2008	10,420	1	Blue gate, bottom gate selecting fluorescence at 670 – 30
74-PC-HR	23	PC	Aug. 2008	2,342	1	Purple gate, top gate selecting fluorescence at 670 – 30
74-PC-R	23	PC	Aug. 2008	3,006	1	Red gate, middle gate selecting fluorescence at 670 – 30
74-PE-L	23	PC	Aug. 2008	38,356	1	Green gate, remove or gate out fluorescence at 670 – 30
74-PE-HL	23	PE	Aug. 2008	7,444	1	Blue gate, bottom gate selecting fluorescence at 610 – 20
74-PE-HR	23	PE	Aug. 2008	132	1	Purple gate, middle gate selecting fluorescence at 610 – 20
74-PE-R	23	PE	Aug. 2008	292	1	Red gate, top gate selecting fluorescence at 610 – 20
77-PC-L	23	PE	Aug. 2008	21,548	17	Green gate, remove or gate out fluorescence at 610 – 20
77-PC-HL	23	PC	Aug. 2008	7,930	17	Blue gate, bottom gate selecting fluorescence at 670 – 30
77-PC-HR	23	PC	Aug. 2008	2,142	17	Purple gate, top gate selecting fluorescence at 670 – 30
77-PC-R	23	PC	Aug. 2008	2,384	17	Red gate, middle gate selecting fluorescence at 670 – 30
77-PE-L	23	PC	Aug. 2008	34,952	17	Green gate, remove or gate out fluorescence at 670 – 30
77-PE-HL	23	PE	Aug. 2008	13,592	17	Blue gate, bottom gate selecting fluorescence at 610 – 20
77-PE-HR	23	PE	Aug. 2008	522	17	Purple gate, middle gate selecting fluorescence at 610 – 20
77-PE-R	23	PE	Aug. 2008	614	17	Red gate, top gate selecting fluorescence at 610 – 20

Table 5. Continued

Name	Station	Pigment	Date Collected	Cells/mL	Depth (m)	How Sample Was Sorted
80-PC-L	23	PE	Aug. 2008	314	45	Green gate, remove or gate out fluorescence at 610 – 20
80-PC-HL	23	PC	Aug. 2008	216	45	Blue gate, bottom gate selecting fluorescence at 670 – 30
80-PC-HR	23	PC	Aug. 2008	48	45	Purple gate, top gate selecting fluorescence at 670 – 30
80-PC-R	23	PC	Aug. 2008	80	45	Red gate, middle gate selecting fluorescence at 670 – 30
80-PE-L	23	PC	Aug. 2008	240	45	Green gate, remove or gate out fluorescence at 670 – 30
80-PE-HL	23	PE	Aug. 2008	90	45	Blue gate, bottom gate selecting fluorescence at 610 – 20
80-PE-HR	23	PE	Aug. 2008	2	45	Purple gate, middle gate selecting fluorescence at 610 – 20
80-PE-R	23	PE	Aug. 2008	10	45	Red gate, top gate selecting fluorescence at 610 – 20

Table 6. Full length and cyanobacterial specific 16S rDNA primers with corresponding sequences and amplicon lengths.

Primer	Sequence 5' - 3'	Target Site	Product Length	Reference
27F	AGA GTT TGA TCM TGG CTC AG	~27		
1522R	AAG GAG GTG ATC CAN CCR CA	~1522	~1495 bp	(Lane 1991)
CYA106F	CGG ACG GGT GAG TAA CGC GTG A	106-127		
CYA781R	GAC TAC WGG GGT ATC TAA TCC CWT T	781-805	~675 bp	(Nübel 1997)

Table 7. Library Table – The name of the library matches the clone name used for sequence analysis. The clone name is then followed by the clone number. This table indicates the number of sequenced clones that had complete sequence reads. This table does not indicate the number of clones removed due to the identification of a chimera. Chloroplast in, the # of unique column, indicates that the sequence set for that library was not used for further analysis (chloroplast libraries were not renamed to denote the station, season or depth). Flow Sort (FS). *Sample was amplified with full length universal 16S primers. All other samples were amplified using cyanobacterial specific partial 16S primers.

Library Name	DNA Isolated From	# of Clones	# of Unique	Station	Depth (m)	Collected
84-SynFSAug14m	*Synechococcus* FS	10	3	84	14	Aug-08
84-SynFSAug1m	*Synechococcus* FS	9	2	84	1	Aug-08
84-Aug1m	filtered water sample	8	3	84	1	Aug-08
84-Aug13m	filtered water sample	9	5	84	13	Aug-08
84-Aug17m	filtered water sample	10	5	84	17	Aug-08
84-Aug1mB	filtered water sample	8	3	84	1	Aug-08
84-Aug1m-PE	PE flow sort	8	6	84	1	Aug-08
84-Aug1mPC	PC flow sort	9	3	84	1	Aug-08
84-Aug1m-PCB	PC flow sort	9	3	84	1	Aug-08
84-Aug1m-PCC	PC flow sort	9	4	84	1	Aug-08
84-Aug14m-PE	PE flow sort	11	7	84	14	Aug-08
84-Aug14m-PC	PC flow sort	12	3	84	14	Aug-08
84-Aug14m-PCB	PC flow sort	1	1	84	14	Aug-08
84-Aug14m-PCC	PC flow sort	4	1	84	14	Aug-08
84-Aug23-PE	PE flow sort	4	3	84	23	Aug-08
84-Aug23m-PC	PC flow sort	8	3	84	23	Aug-08
84-Aug23m-PCC	PC flow sort	9	2	84	23	Aug-08
84-Aug23m-PCD	PC flow sort	8	3	84	23	Aug-08
84-Aug1m-PCD	pico-eukaryote FS	4	2*	84	1	Aug-08
84-Aug22m-PC	*Prochlorococcus* FS	5	4*	84	22	Aug-08
23-Aug17m-PE	PE flow sort	3	2	23	17	Aug-08
23-Aug45m-PC	PC flow sort	3	1	23	45	Aug-08
23-Aug1m-PC	PC flow sort	3	2	23	1	Aug-08
84-Feb1m	filtered water sample	5	3*	84	1	Feb-08
84-Feb22m	filtered water sample	4	3*	84	22	Feb-08
3DNAFeb08	filtered water sample	1	chloroplast	84	1	Feb-08
84-Jan20m-PC	PC flow sort	9	1	84	20	Jan-09
84-Jan20m-PE	PE flow sort	10	2	84	20	Jan-09
13PE-HR	PE flow sort	9	chloroplast	84	20	Jan-09
25PC-R	PC flow sort	5	chloroplast	84	1	Jan-09
84-Jan1m-PE	PE flow sort	8	1	84	1	Jan-09
74DNA-Feb09	filtered water sample	19	chloroplast	84	1	Feb-09
75DNA-Feb09	filtered water sample	5	chloroplast	84	20	Feb-09
49-340Feb08	filtered water sample	10	chloroplast	340	1	Feb-08
357-Feb1m-PC	PC flow sort	2	1	357	1	Feb-09
357-Jan1m-PC	PC flow sort	2	1	357	1	Jan-09
4PE-L	PE flow sort	1	chloroplast	357	8	Feb-09
16PE-L	PE flow sort	1	chloroplast	452	1	Jan-09

Table 8. Phyla of all clones determined by RDP Classifier.

Phyla	Summer Season	Winter Season
Cyanobacteria	146	29
Verrucomicrobia	0	1
Acidobacteria	0	1
Actinobacteria	1	1
Firmicutes	1	0
Planctomycetes	2	0
β-proteobacteria	0	4
γ-proteobacteria	2	0
α-proteobacteria	1	1
Unclassified Bacteria	1	0
	154	37

Table 9. Phyla of summer clones from station 84 by depth determined by RDP Classifier.

	Depth (m)						
Phyla	**1**	**13**	**14**	**17**	**22**	**23**	**Total**
Cyanobacteria	58	9	34	10	0	28	139
Actinobacteria	0	0	0	0	1	0	1
Firmicutes	0	0	0	0	1	0	1
Planctomycetes	1	0	0	0	1	0	2
γ-proteobacteria	0	0	0	0	2	0	2
α-proteobacteria	1	0	0	0	0	0	1
Unclassified Bacteria	0	0	1	0	0	0	1
							147

Table 10. Phyla of winter clones from by depth determined by RDP Classifier.

	Depth (m)			
Phyla	**1**	**20**	**22**	**Total**
Cyanobacteria	12	17	0	29
Verrucomicrobia	0	0	1	1
Acidobacteria	0	0	1	1
Actinobacteria	1	0	0	1
β-proteobacteria	2	0	2	4
α-proteobacteria	1	0	0	1

Table 11. UniFrac phylogenetic analysis of cyanobacterial sequences, flow sorted sequences compared to environmental sequences and summer compared to winter sequences.

	Unweighted UniFrac	P-test of Difference
Flow Sorted vs. Environmental Samples	0.03	0.34
Summer vs. Winter	0.01	0.01

APPENDIX B: Figures

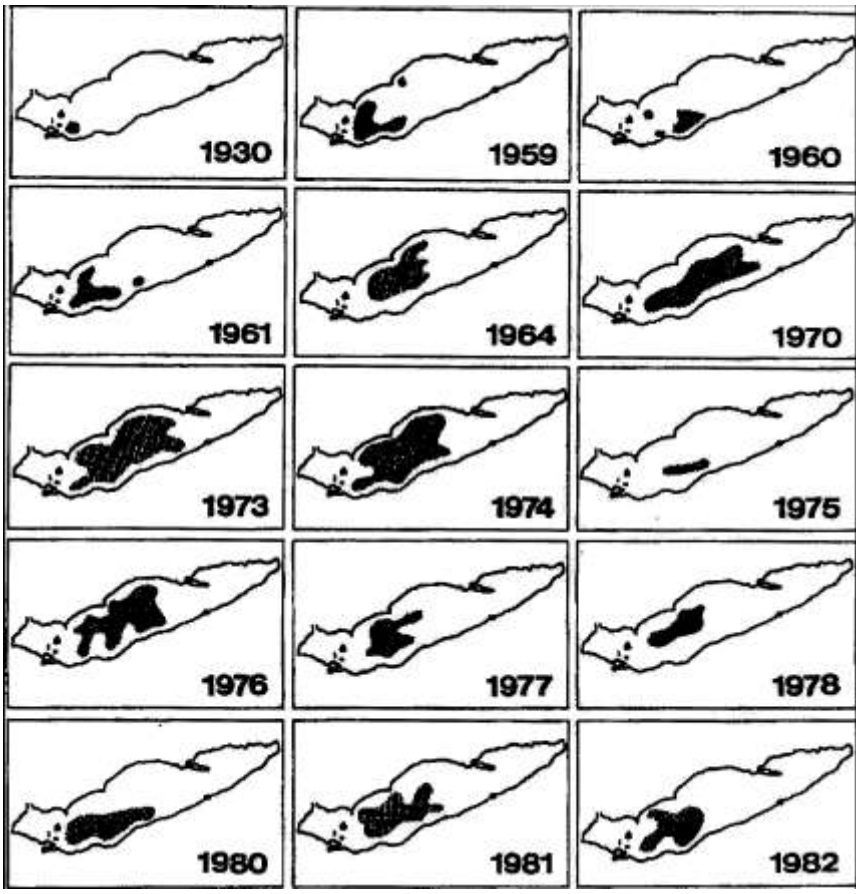


Figure 1. Seasonal anoxia in the central basin of Lake Erie, indicated in black (Bolsenga 1993). Bolsenga, Pater T. and Charles E. Herdendorf. Figure 5.27 "Distribution of hyolimnion anoxia in central Lake Erie (1930-1982)." /Lake Erie and Lake St. Clair Handbook. /Copyright © 1993 Wayne State University Press, with permission of Wayne State University Press.

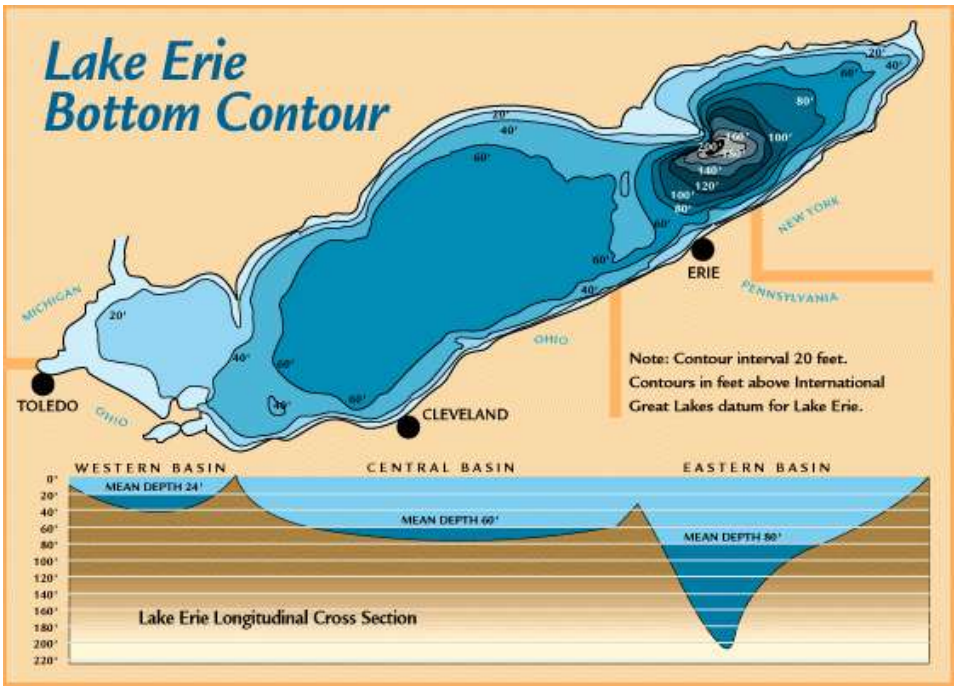


Figure 2. Morphology of Lake Erie (1998). Permission granted on July 8, 2009 to use this image in this thesis from Pennsylvania Angler & Boater who is responsible for the production and content of this magazine.

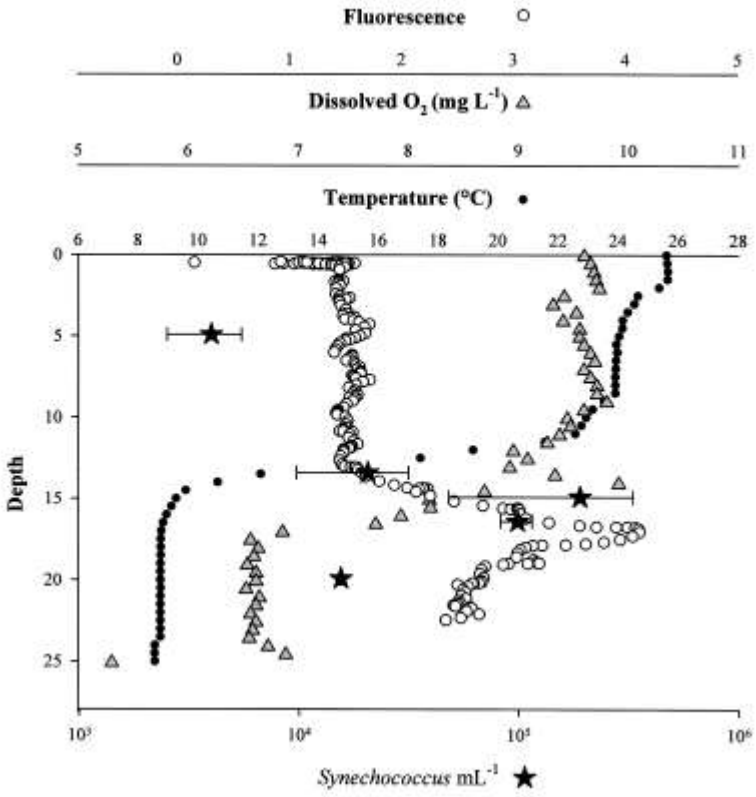


Figure 3. Water Column profile from July 2005, demonstrating the distribution of *Synechococcus*. Peaks in chlorophyll a fluorescence and hypolimnetic oxygen coincide with the abundance of cells (Wilhelm *et al.* 2006). Permission to reproduce image given by Elsevier on July 6, 2009, license number 2223120536281.

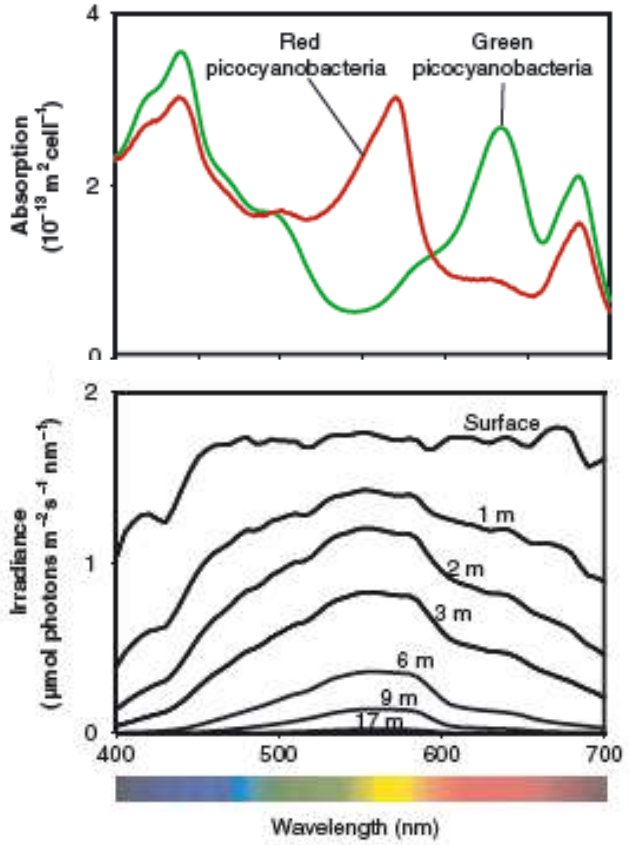


Figure 4. The top graph indicates the optical characteristics of red and green picocyanobacteria. The bottom graph represents the underwater light spectrum of the Baltic Sea. As depth increases the spectrum narrows to the green wavelength. Figure based on an adaptation by (Stomp 2007).

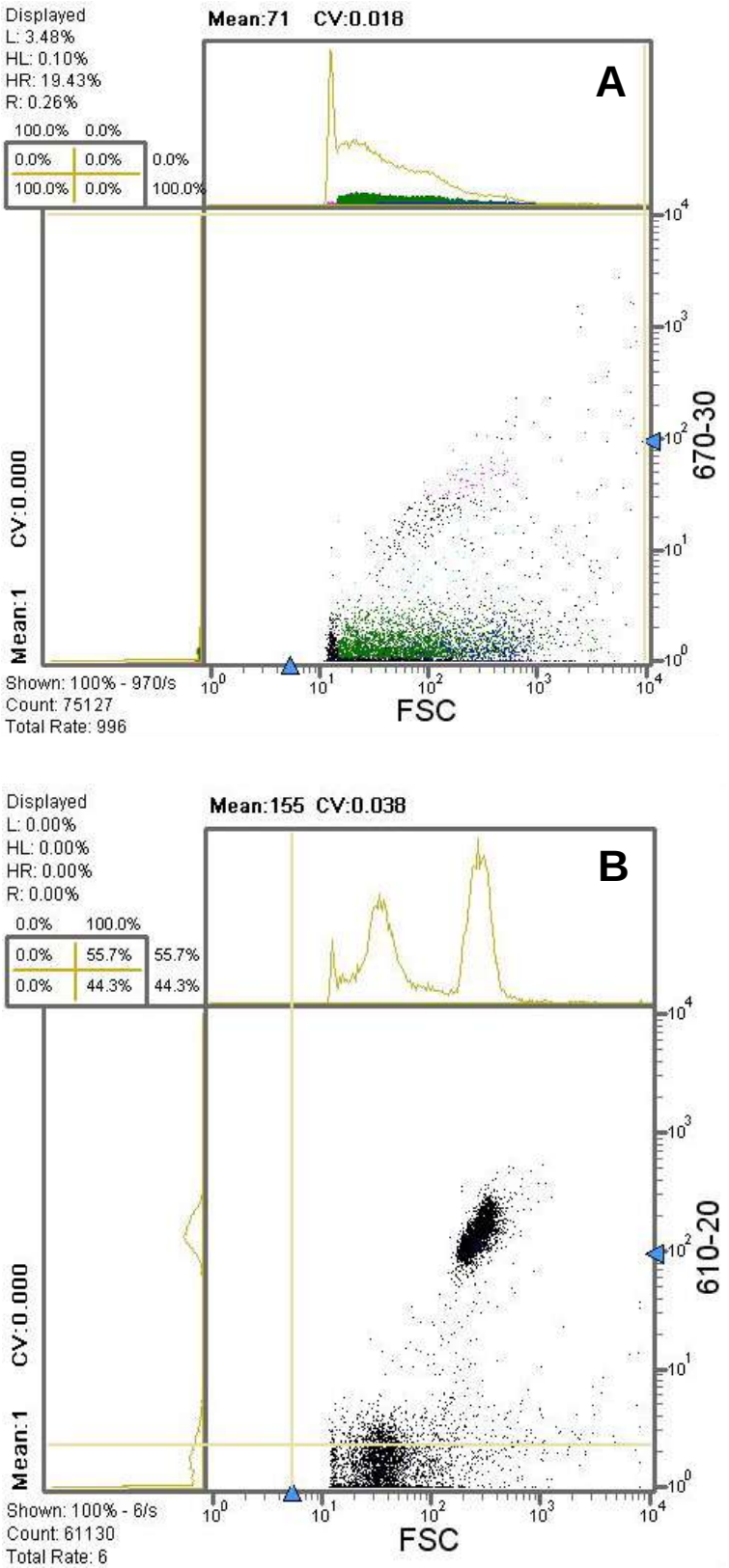
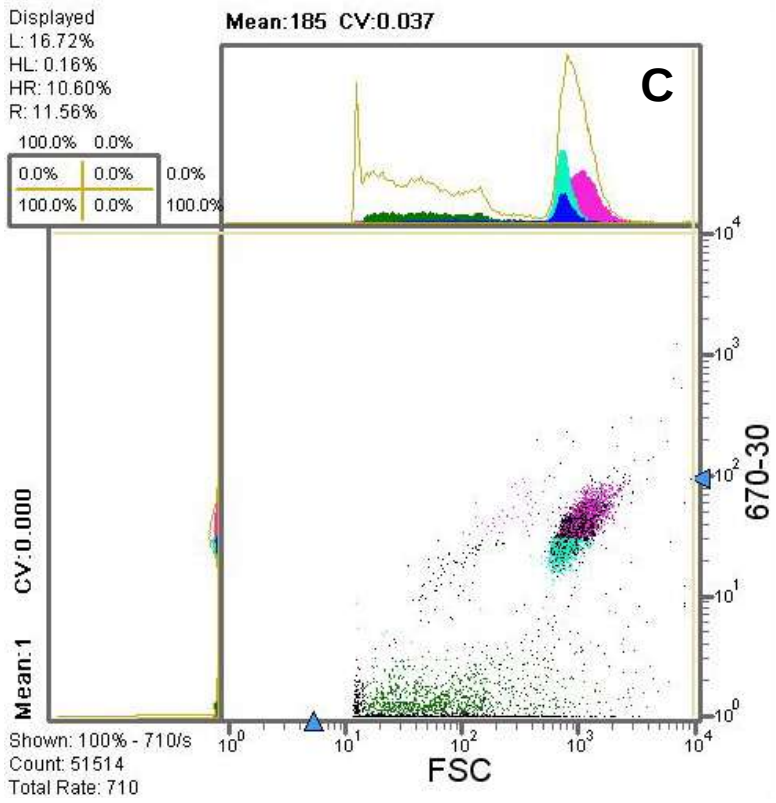


Figure 5. Continued



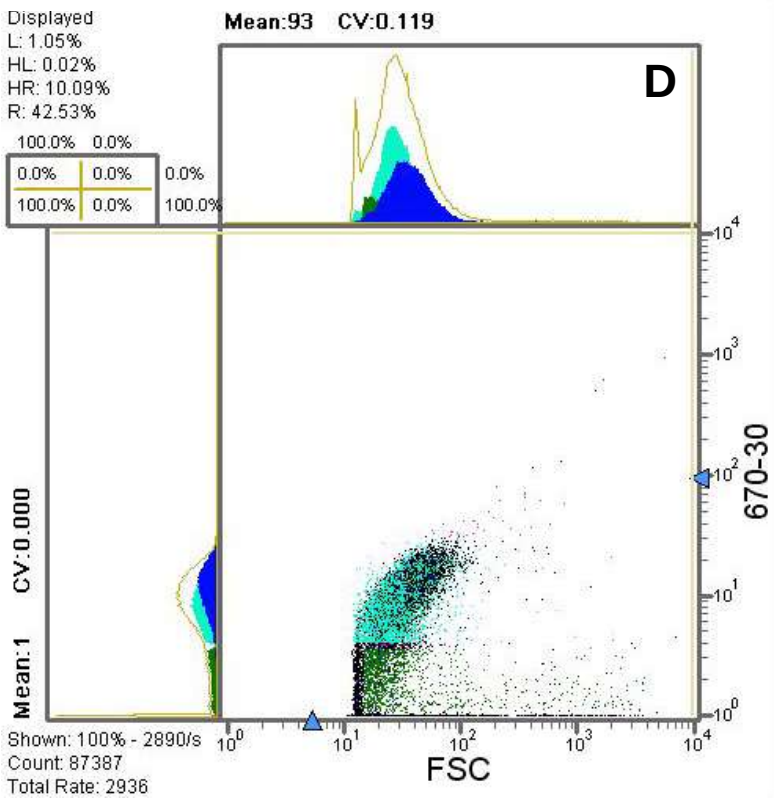


Figure 5. Continued

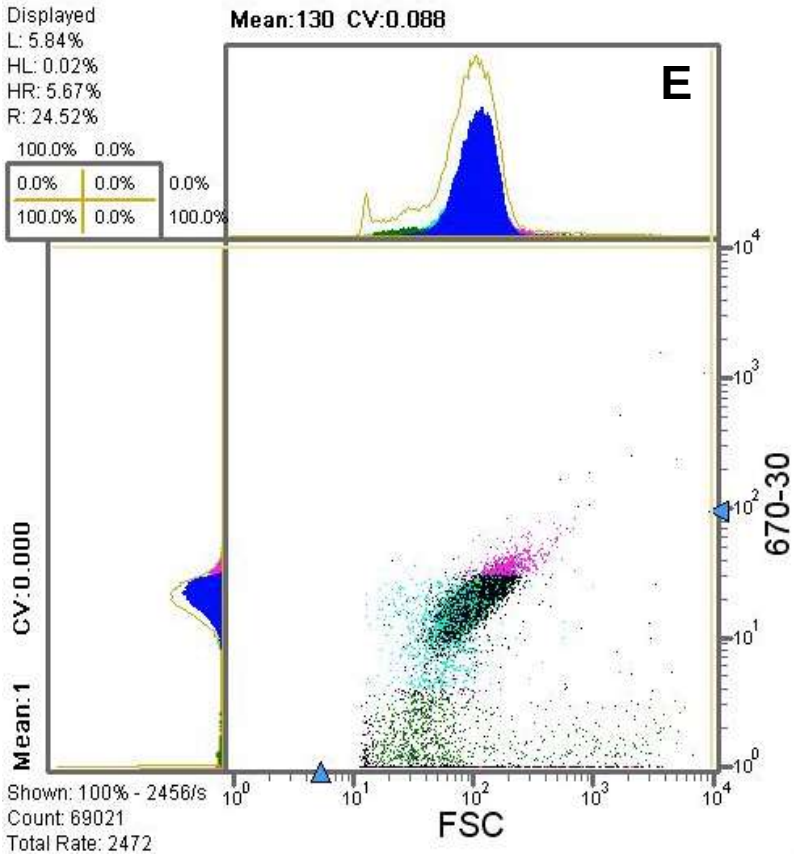


Figure 5. Flow cytometry results from a water sample taken from Station 84 in the central Basin of Lake Erie August 2008. Forward scattering indicating size is on the x-axis and fluorescence on the y-axis. (A) unspiked water sample, (B) water sample spiked with cultured isolate *Synechococcus* ARC-21 PE-rich, (C) water sample spiked with cultured isolate *Synechococcus* ARC-11 PC-rich, (D) water sample spiked with *Prochlorococcus* ecotype eMIT-9211, (E) water sample spiked with *Prochlorococcus* ecotype eNATL2A.

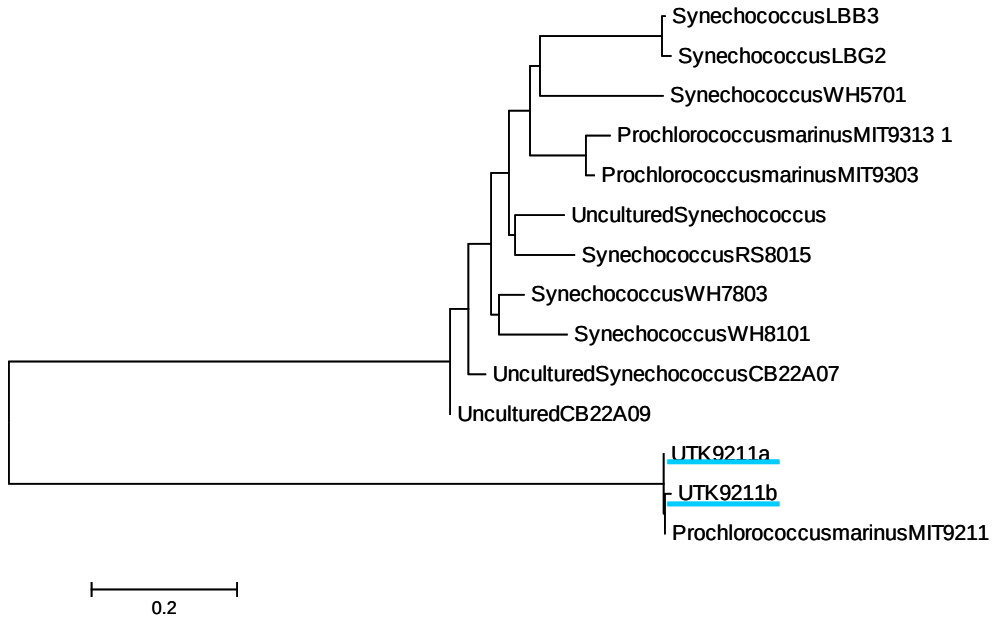


Figure 6. Neighbor-joining analysis of cyanobacterial sequences. PCR was performed on DNA extracted from water filtered from Lake Erie August 2004 Station 84 using *Prochlorococcus*-ecotype 9211 specific ITS primers (Zinser 2006). Sequence underlined in blue represent the sequences found indicating the presence of eMIT9211 (unpublished data).

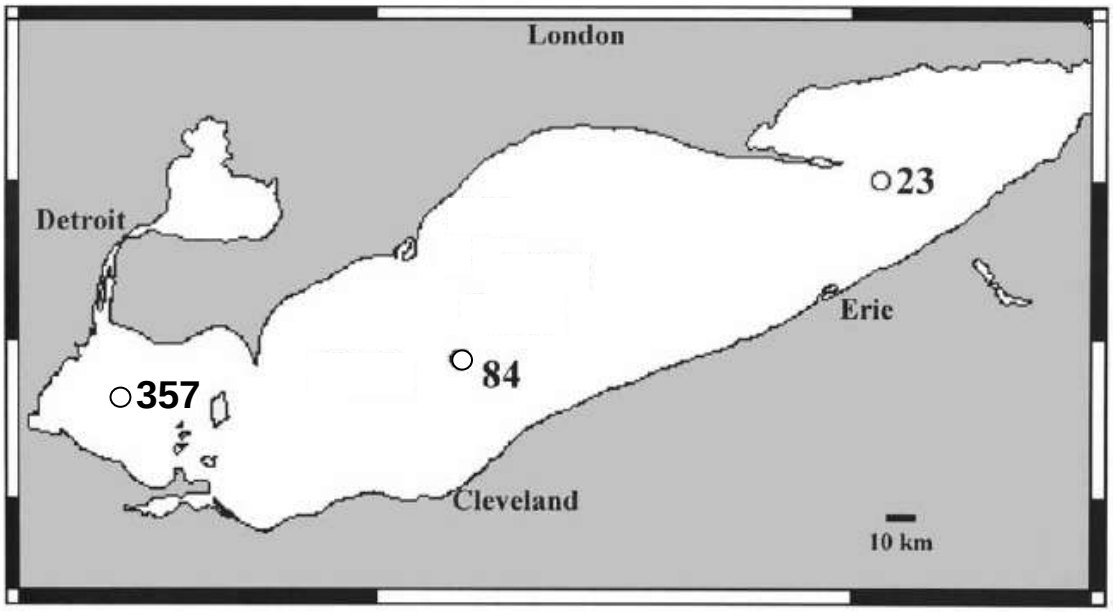


Figure 7. Stations in Lake Erie where sampling events occurred. Station 357 is in the western basin, Station 84 in the central basin and Station 23 in the eastern basin. Figure based on an adaptation from (Wilhelm *et al.* 2006).

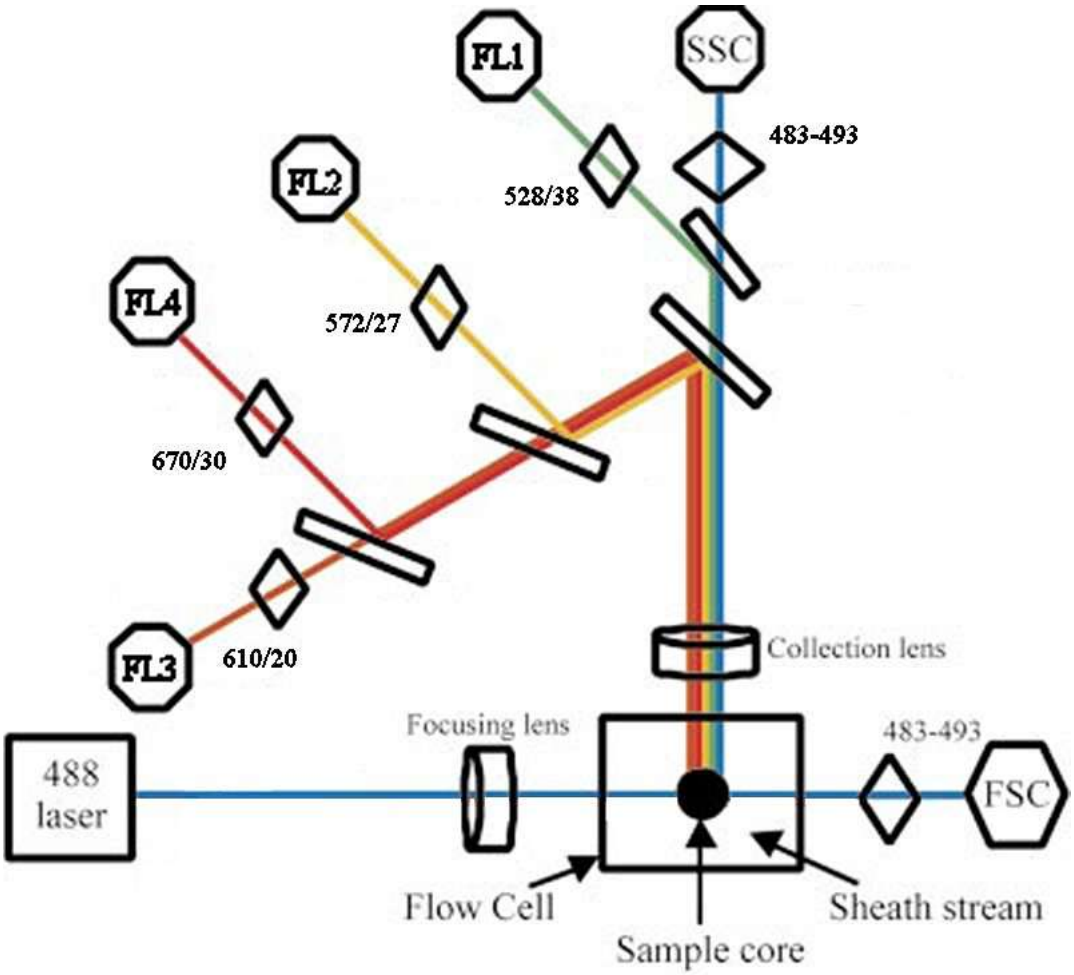


Figure 8. This figure is based on an adaptation from (Collier 2000) to illustrate the set up of the flow cytometer used to sort the samples in this study. The InFlux Cell Sorting Flow Cytometer at ORNL is manufactured by Cytopeia Incorporated model Influx 2L. SSC – side scatter, FAC – forward scatter and FL – photomultiplier.

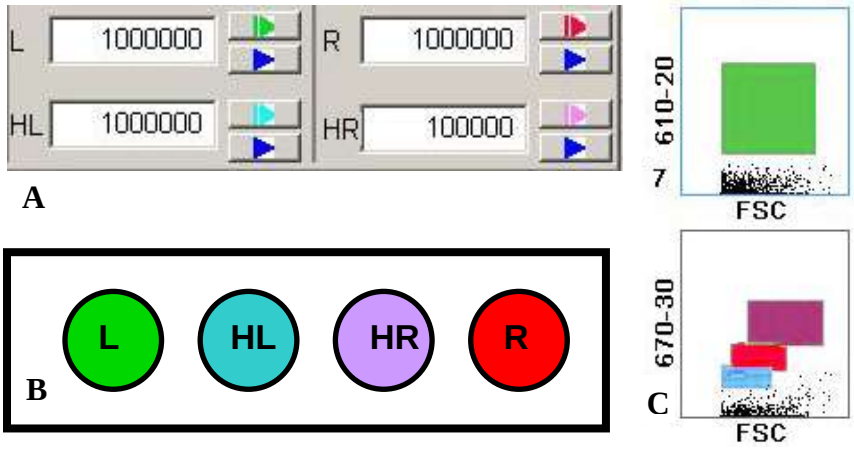


Figure 9. Four parameters can be set when sorting a sample. Each sorting parameter is represented by a specific color. A. Indicates the number of events recorded for each parameter. B. The contents of each parameter will be deflected into a tube at a specific position on the collection rack. Collection Rack: L – Left (green parameter), HL – Half-Left (blue parameter), HR – Half-Right (purple parameter) and R – Right (red parameter). The numbers of events sorted by each parameter are recorded. C. An example of how parameters can be set within viewing windows.

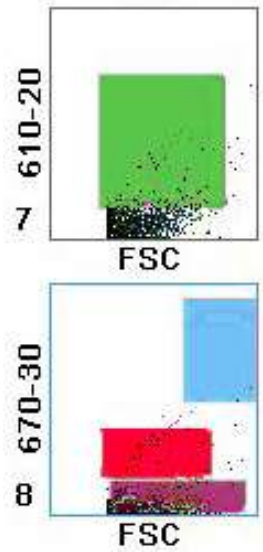


Figure 10. The initial sorting parameters set for samples sorted on October 31, 2008. A parameter (green) was set at a fluorescence of 610 nm to select for PE rich *Synechococcus*. Three parameters were set at a fluorescence of 670 nm: one parameter (purple) to select for pico-phytoplankton, one parameter (red) was set to select for *Prochlorococcus* and another parameter (blue) to select for pico-eukaryotes.

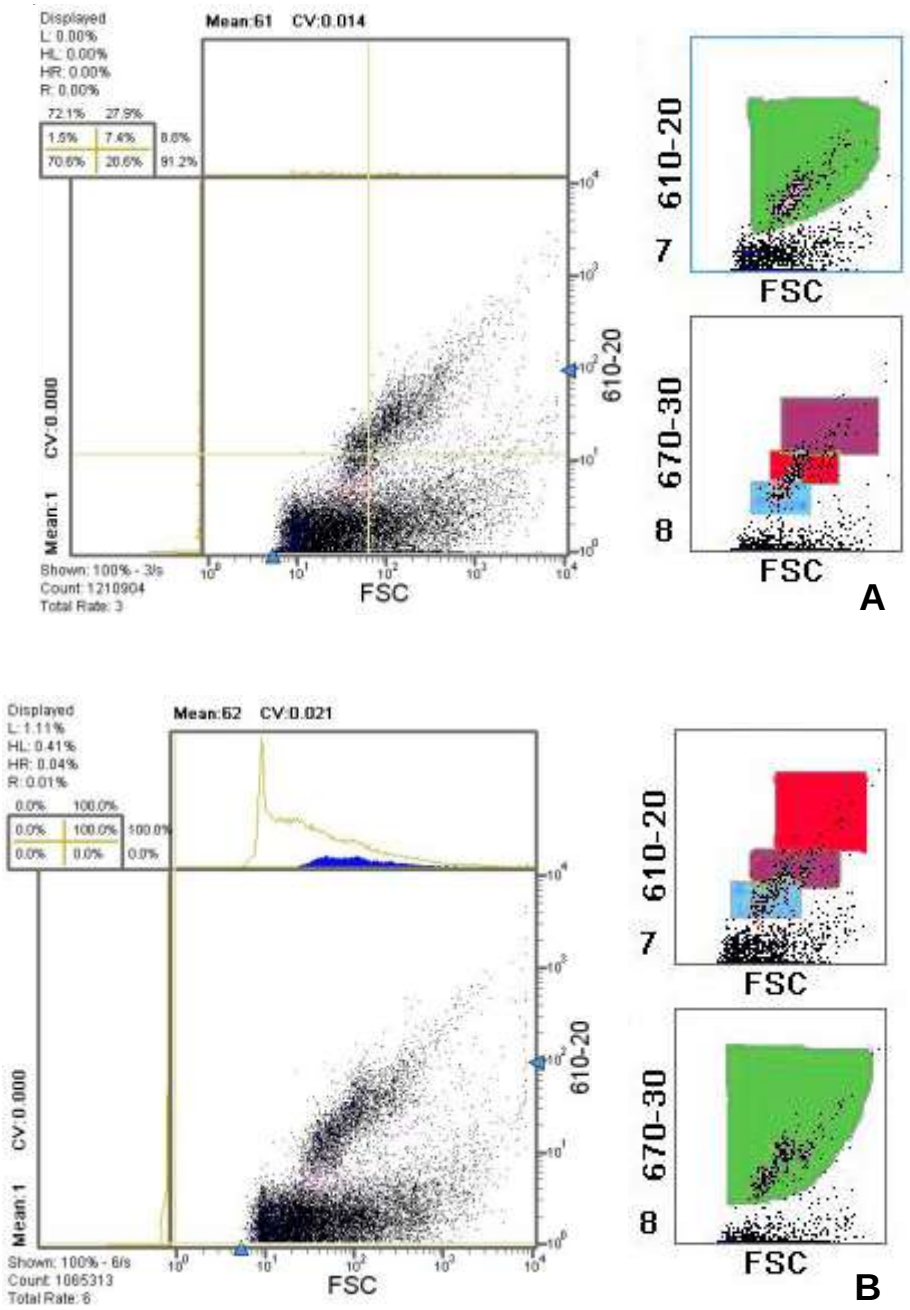


Figure 11. The final sorting parameters set for samples sorted on February 24, 2009. All samples were collected from Station 84 in the central basin of Lake Erie. The first graph in the figure shows the content of each water sample in main viewing window of the flow cytometer. The second part of the figure illustrates the parameters that were set to sort each sample. PC samples were sorted so that PC rich cells were sorted three ways. PE samples were sorted so that PE rich cells were sorted three ways. (A) Sample 63PC collected August 2008 at a depth of 13m, (B) Sample 63PE collected August 2008 at a depth of 13m.

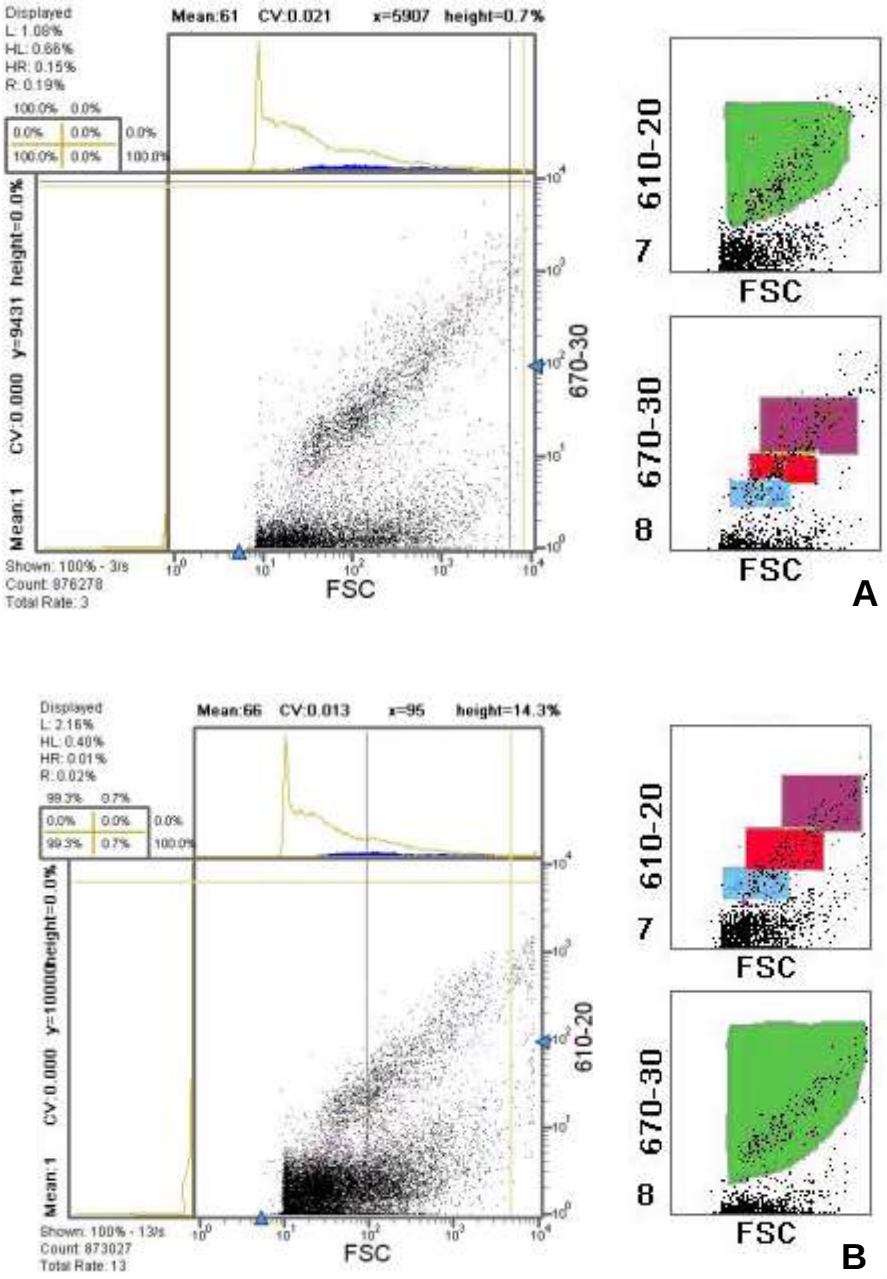


Figure 12. Sorting parameters used for samples sorted on April 16, 2009. All samples were collected from Lake Erie. The first graph in the figure shows the content of each water sample in main viewing window of the flow cytometer. The second part of the figure illustrates the parameters that were set to sort each sample. PC samples were sorted so that PC rich cells were sorted three ways. PE samples were sorted so that PE rich cells were sorted three ways. (A) Sample 74PC collected August 2008 from Station 23 at a depth of 1 m and (B) Sample 74PE collected August 2008 from Station 23 at a depth of 1 m.

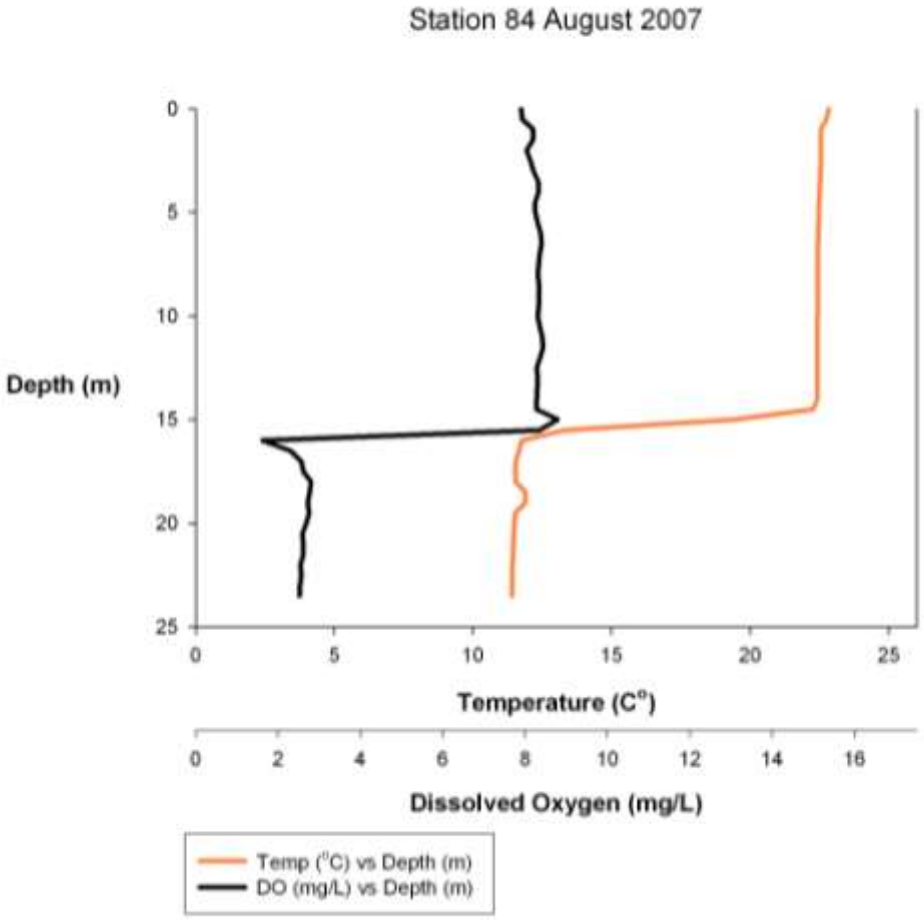


Figure 13. A water column profile from Lake Erie station 84 during summer stratification August 2007. The orange line represents temperature in ˚C vs. depth in meters. The black line represents dissolved oxygen in mg/L vs. depth in meters. This water column profile from 2007 was included because the oxygen probe used to make measurements in August 2008 was not functioning properly. Graph was constructed using Sigma Plot.

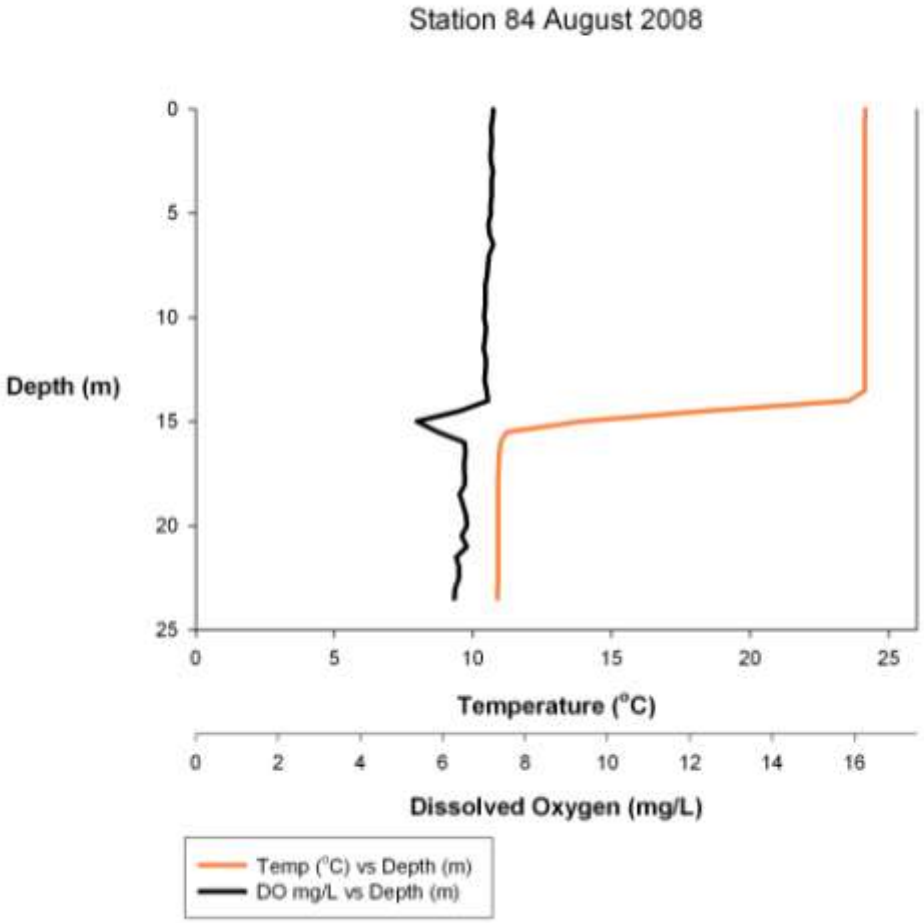


Figure 14. A water column profile from Lake Erie station 84 during summer stratification August 2008. The orange line represents temperature in ˚C vs. depth in meters. The black line represents dissolved oxygen in mg/L vs. depth in meters. The oxygen probe used to make measurements was not functioning properly. Graph was constructed using Sigma Plot.

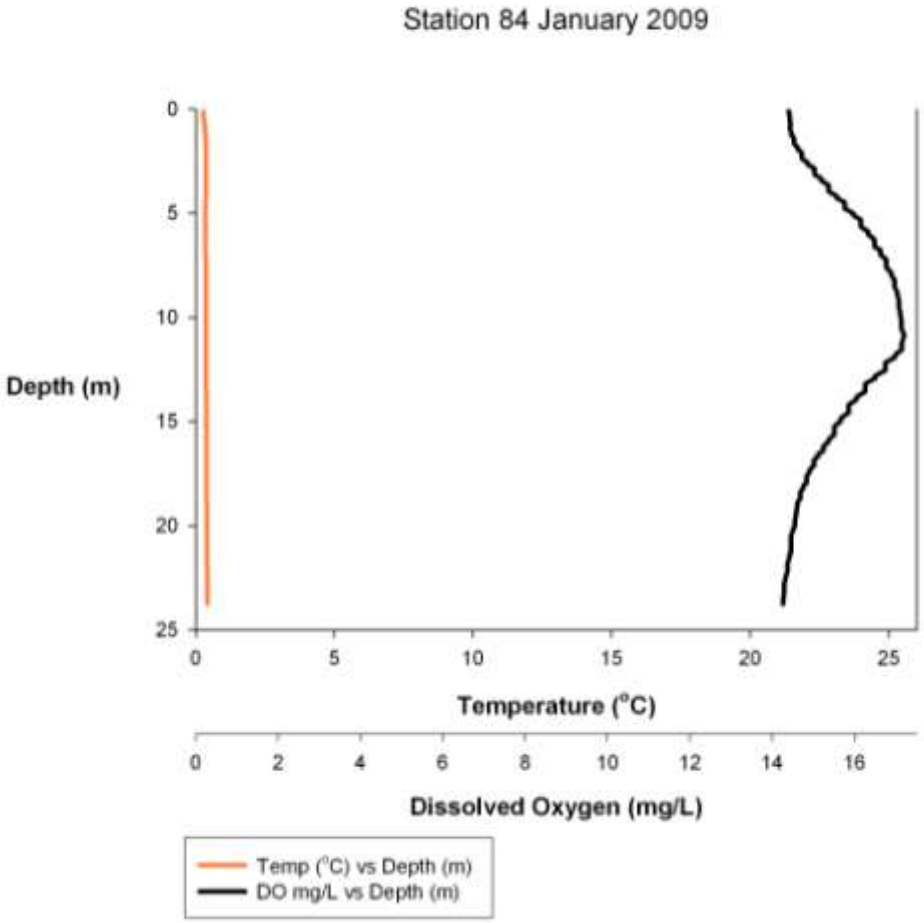


Figure 15. A water column profile from Lake Erie station 84 during January 2009. The orange line represents temperature in ˚C vs. depth in meters. The black line represents dissolved oxygen in mg/L vs. depth in meters. Graph was constructed using Sigma Plot.

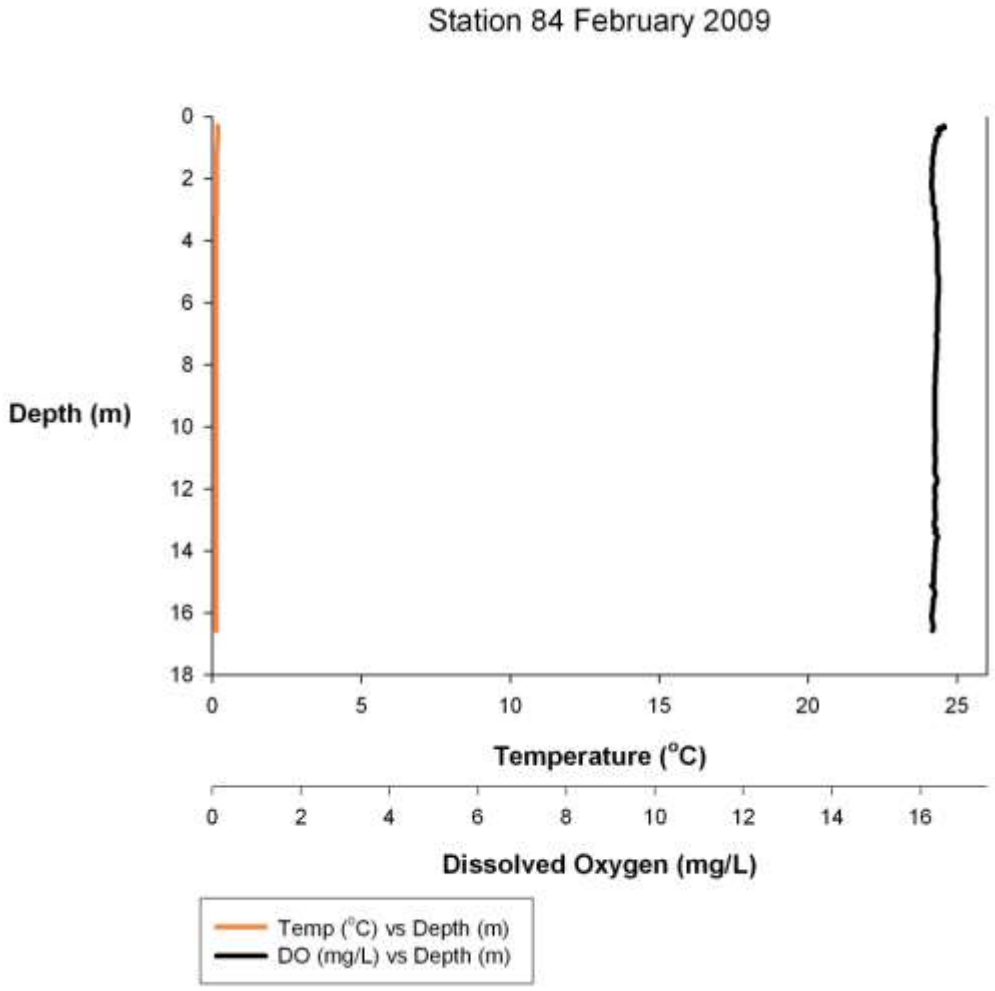


Figure 16. A water column profile from Lake Erie station 84 during February 2009. The orange line represents temperature in ˚C vs. depth in meters. The black line represents dissolved oxygen in mg/L vs. depth in meters. Graph was constructed using Sigma Plot.

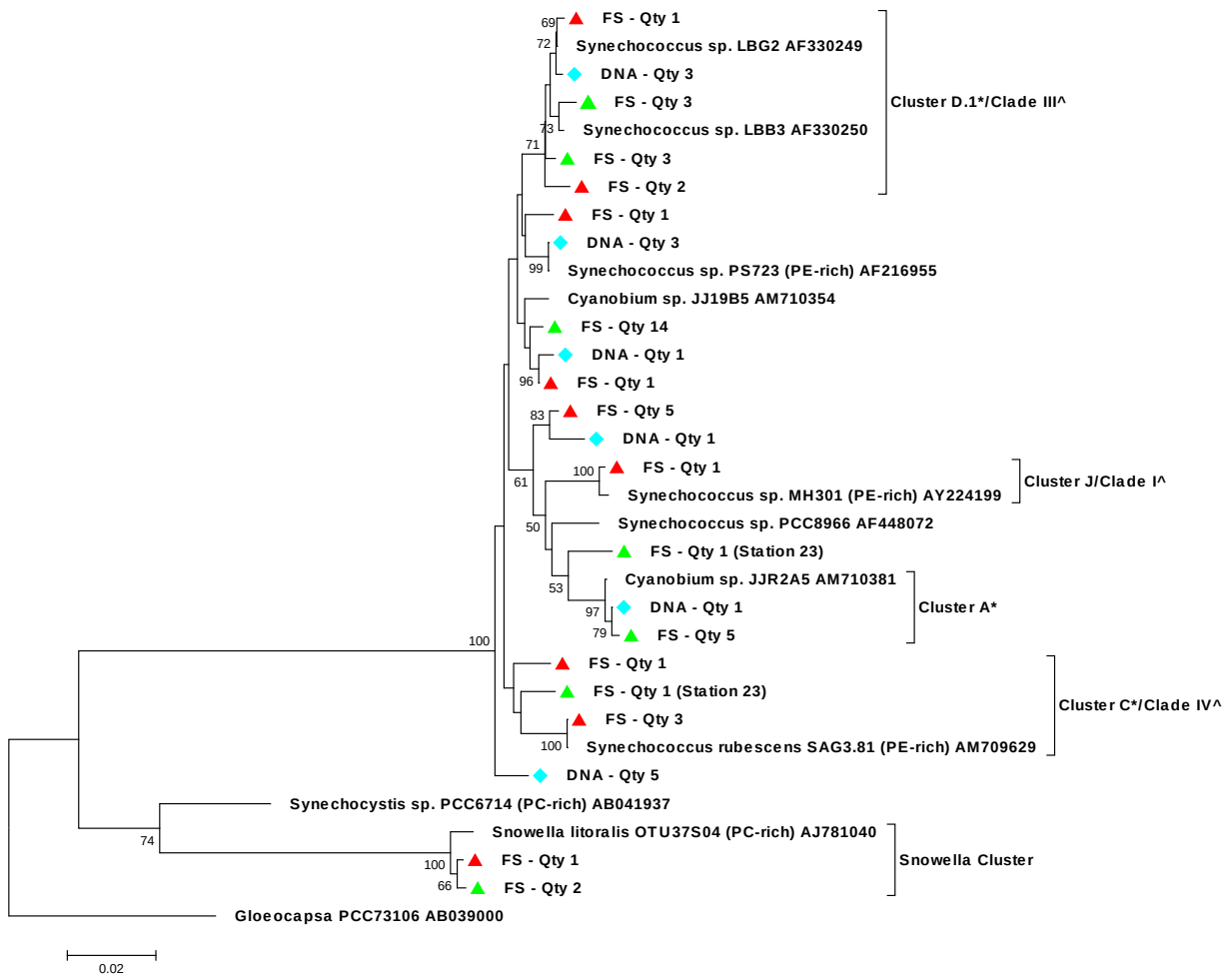


Figure 17. Neighbor-joining analysis of summer cyanobacterial sequences. Bootstrap values less than 50 were removed. Summer sequences are from the epilimnion group at a depth of 1m. Triangle shape indicates that the sequences was amplified from a flow sorted (FS) sample. The color indicates if the sample was sorted to select for PE (▲) or PC (▲) rich cells. The blue diamond (♦) indicates that the sequence was amplified from a DNA extracted sample. The quantity (Qty) of each sequence is indicated numerically. All sequences were from samples collected during August 2008 from the central basin of Lake Erie station 84, with the exception of the two sequences from station 23 as indicated in the labeling. * Clusters identified as in Budinoff, *et al.* 2007. ^ Clades identified as in Wilhelm, *et al.* 2006.

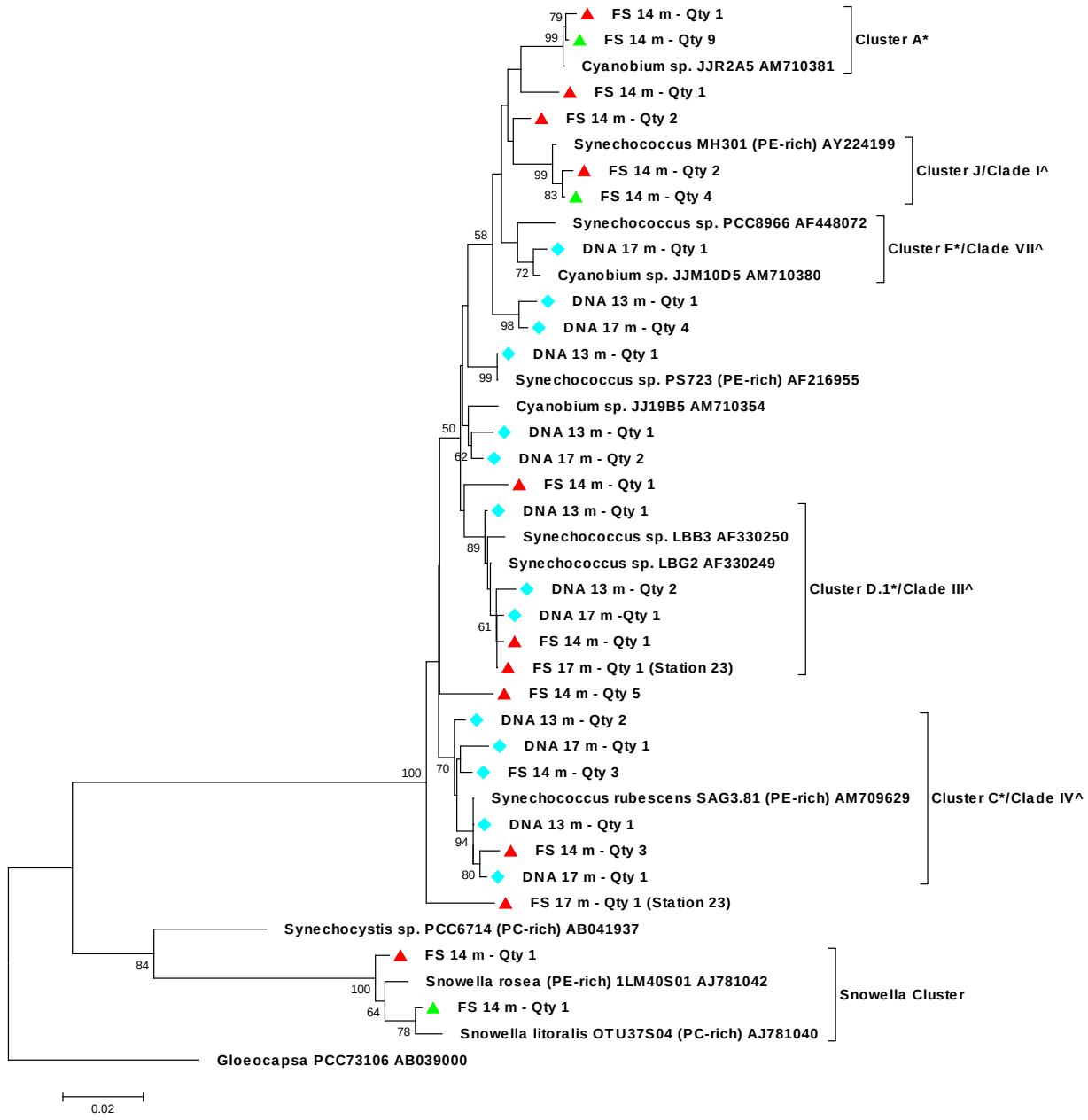


Figure 18. Neighbor-joining analysis of summer cyanobacterial sequences. Bootstrap values less than 50 were removed. Summer sequences are from metalimnion group from depths of 13, 14 and 17 m. Triangle shape indicates that the sequences was amplified from a flow sorted (FS) sample. The color indicates if the sample was sorted to select for PE (▲) or PC (▲) rich cells. The blue diamond (♦) indicates that the sequence was amplified from a DNA extracted sample. The quantity (Qty) of each sequence is indicated numerically. All sequences were from samples collected during August 2008 from the central basin of Lake Erie station 84, with the exception of the two sequences from station 23 as indicated in the labeling. * Clusters identified as in Budinoff, *et al.* 2007. ^ Clades identified as in Wilhelm, *et al.* 2006.

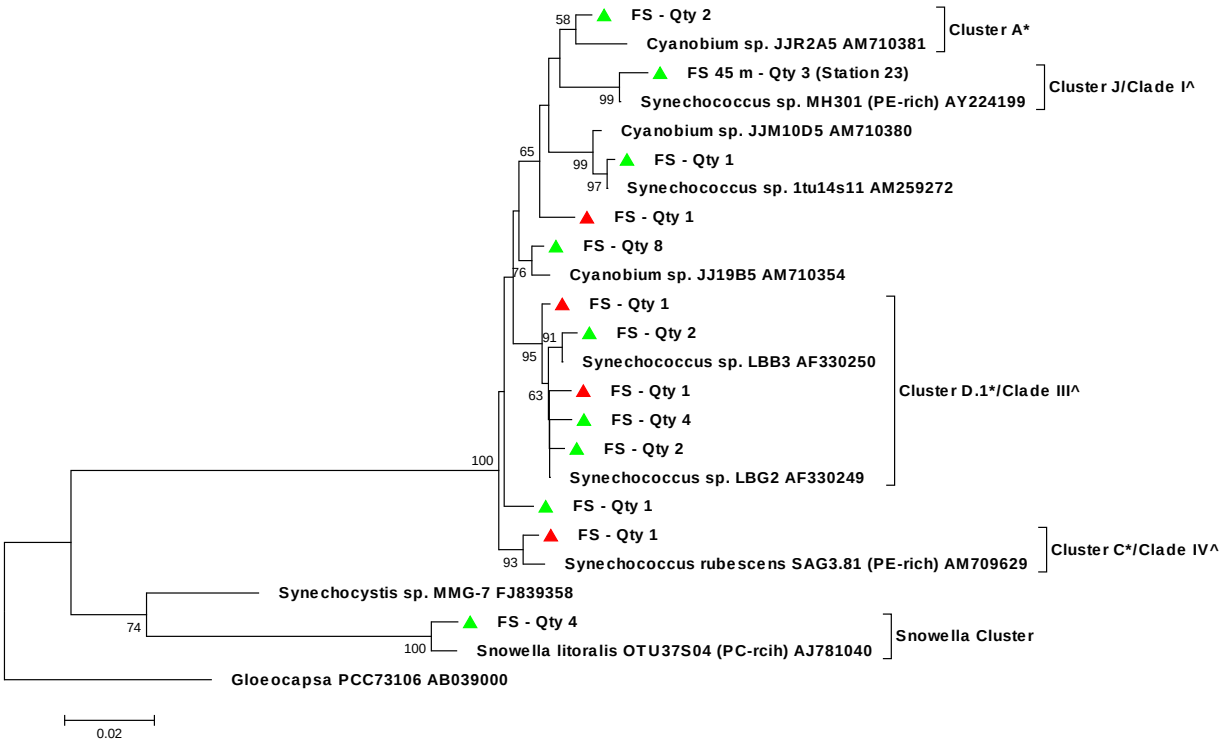


Figure 19. Neighbor-joining analysis of summer cyanobacterial sequences. Bootstrap values less than 50 were removed. Summer sequences in the hypolimnion group are from depths of 23 and 45 m. All sequences are from a depth of 23 m except for the one that is indicated to be from a depth of 45 m. Triangle shape indicates that the sequences was amplified from a flow sorted (FS) sample. The color indicates if the sample was sorted to select for PE (▲) or PC (▲) rich cells. The quantity (Qty) of each sequence is indicated numerically. All sequences were amplified from samples collected during August 2008 from the central basin of Lake Erie station 84, with the exception of the one sequences from station 23 which is also from a depth of 45 m. * Clusters identified as in Budinoff, *et al.* 2007. ^ Clades identified as in Wilhelm, *et al.* 2006.

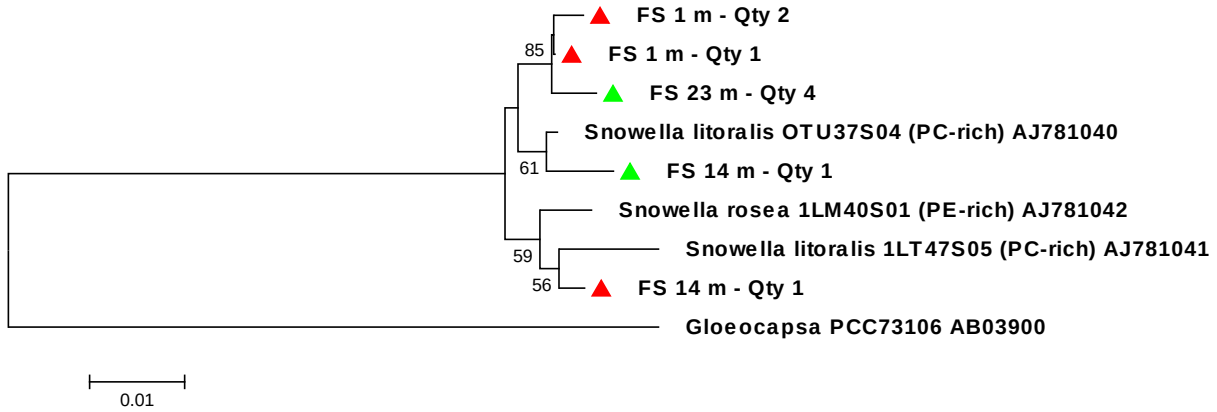


Figure 20. Neighbor-joining analysis of summer *Snowella* cyanobacterial sequences. Bootstrap values less than 50 were removed. Triangle shape indicates that the sequences was amplified from a flow sorted (FS) sample. The color indicates if the sample was sorted to select for PE (▲) or PC (▲) rich cells. The quantity (Qty) of each sequence is indicated numerically. All sequences were from samples collected during August 2008 from Lake Erie station 84.

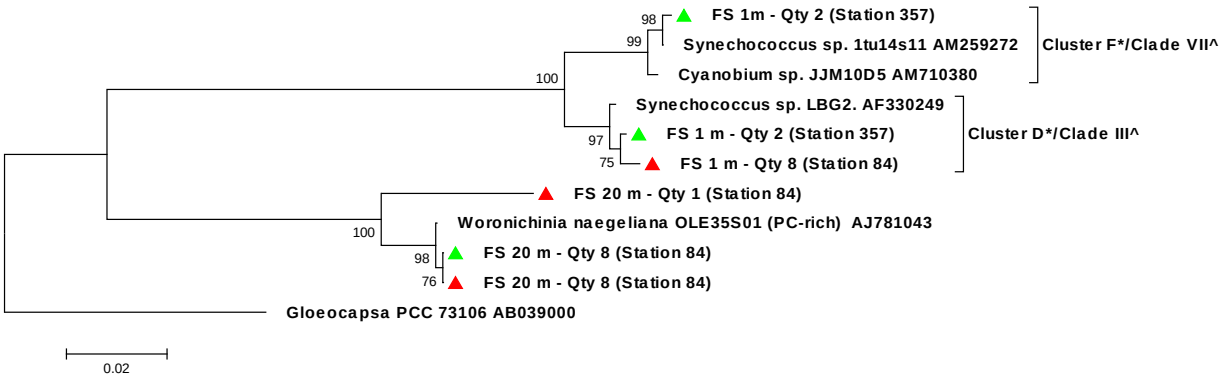


Figure 21. Neighbor-joining analysis of winter cyanobacterial sequences. Bootstrap values less than 50 were removed. Triangle shape indicates that the sequences was amplified from a flow sorted (FS) sample. The color indicates if the sample was sorted to select for PE (▲) or PC (▲) rich cells. The quantity (Qty) of each sequence is indicated numerically. All sequences were from samples collected during the winter season from Lake Erie. * Clusters identified as in Budinoff, *et al.* 2007. ^ Clades identified as in Wilhelm, *et al.* 2006.

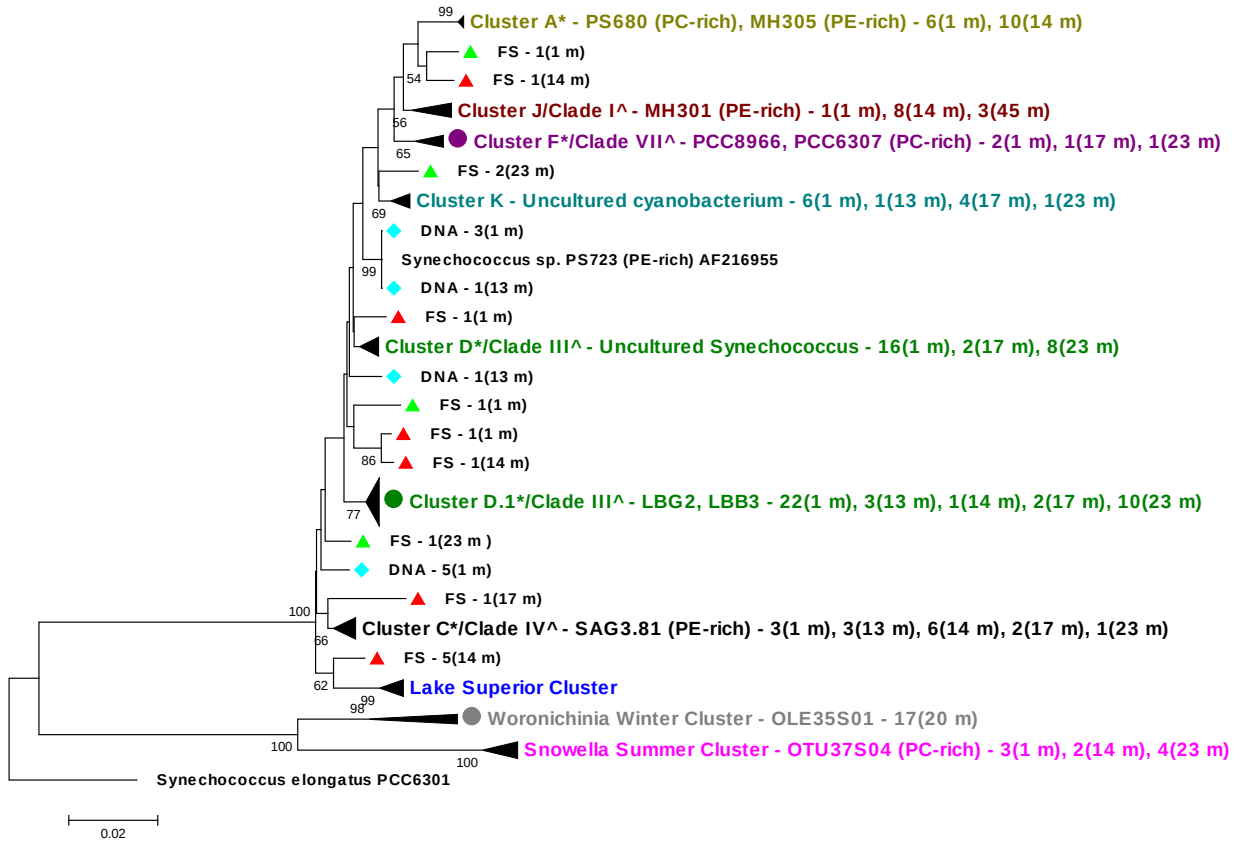


Figure 22. Neighbor-joining analysis of all cyanobacterial sequences with the Snowella Cluster removed. Bootstrap values less than 50 were removed. Triangle shape indicates that the sequences was amplified from a flow sorted (FS) sample. The color indicates if the sample was sorted to select for PE (▲) or PC (▲) rich cells. The blue diamond (♦) indicates that the sequence was amplified from a water filtered DNA extracted sample. The quantity (Qty) of each sequence is indicated numerically. All sequences were from samples collected during August 2008 from Lake Erie. A circle before the cluster name indicates that the cluster contains sequences from the winter season. * Clusters identified as in Budinoff, *et al.* 2007. ^ Clades identified as in Wilhelm, *et al.* 2006.

VITA

Star Loar was born. She went to school at The University of Tennessee. She wrote a thesis. She graduated.