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Identification and Characterization of Phytoplankton in Barnegat Bay, New Jersey

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IDENTIFICATION AND CHARACTERIZATION OF PHYTOPLANKTON IN BARNEGAT BAY, NEW JERSEY

By

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Abstract

Barnegat Bay is a marine estuary that encompasses 670-square miles of the eastern coast of New Jersey. The bay serves as a home to thousands of species of plants, animals, and microorganisms. During the summer months, the population of the bay increases from 576,000 to over 1.5 million residents. In 2011, the Barnegat Bay Partnership reported that environmental conditions of the bay are declining due to increased pollutants from nutrient fertilizers and runoff. Increased nitrogen levels have led to eutrophication and proliferation of phytoplankton algal blooms. Harmful algal blooms (HABs) cause a decrease in dissolved oxygen levels in the water as well as the release of harmful toxins that affect organisms inhabiting the bay, including humans.

To research and develop methods to prevent HABs from occurring, 16 Barnegat Bay sites ranging from the northern to southern regions of the bay were studied in order identify species of cyanobacteria, such as *Synechococcus* and *Prochlorococcus,* and other phytoplankton such as diatoms and dinoflagellates. Polymerase chain reaction (PCR), microscopy, and flow cytometry were utilized to generate profiles of each site. Collectively, the utilization of the three methods detected phytoplankton species such as *Synechococcus*, *Prochlorococcus,* and *Cylindrotheca fusiformis*. Viral plaque assays using viral lysate from each site detected the presence of cyanophage as a natural control for phytoplankton populations.

Introduction

Eutrophication results when the rate of supply of nutrients to an ecosystem is increased and organic matter accumulates. Allochthonous organic matter is transported from outside of the ecosystem via watershed runoff, riverine inflow, or tidal inlets. Autochthonous organic matter is generated within the ecosystem by photosynthetic microorganisms such as cyanobacteria and other phytoplankton (Pinckney *et al.,* 2001). Catastrophic events such as hurricanes, floods, and earthquakes may also influence the input of organic matter into an estuary. Nitrogen (N) is the most common nutrient that aids phytoplankton growth and production and it is also able to influence the cell-size distribution of phytoplankton communities. Other nutrients such as phosphate (PO_4^{-3}), Iron (Fe) and silicon (Si) are essential nutrients for diatom growth. As a result, high nutrient concentrations may produce algal blooms (Pinckney *et al.,* 2001).

Barnegat Bay, New Jersey is a lagoonal estuary. It is a semi-enclosed feature which contains a mix of fresh and salt water. Salinity is highest at the southern inlets of the bay and lowest at rivers and streams which include Metedeconk River, Toms River, and Westeconk Creek which provide the flow of freshwater into Barnegat Bay. In 1995, the EPA designated Barnegat Bay as an Estuary of National Significance. Barnegat Bay is fed by a 670-square-mile watershed that covers 7.7 percent of New Jersey's 8,721-square-mile land area. The watershed is home to over 576,000 residents year round, and over 1.5 million residents during the summer months in 33 towns in Ocean, Monmouth, and Burlington Counties. The economic value of Barnegat Bay exceeds $4 billion in water quality, water supply, fish/wildlife, recreation, agriculture, forests, and public parks (Kauffman & Cruz-Ortiz, 2012).

In August of 2011, New Jersey Governor Chris Christie signed several bills to authorize funds of $16 million for a 10-point Comprehensive Action Plan for the cleanup of Barnegat Bay (Kauffman & Cruz-Ortiz, 2012). Some of the 10 point Action Plan efforts include the following:

reducing nutrient pollution from fertilizer, funding storm water runoff mitigation projects, and closing Oyster Creek Nuclear Power Plant. In addition, a comprehensive public education program was developed to raise awareness of the state of Barnegat Bay in conjunction with a biological research agenda to implement studies of the bay (NJDEP, 2014).

Marine phytoplankton, although small in size, are large in abundance and contribute 45% of the world's net primary production. Phytoplankton includes the prokaryote cyanobacteria and the microalgae diatoms and dinoflagellates. A large contribution of oxygen to the oceans and atmosphere comes from marine phytoplankton. Marine phytoplankton are ingested by zooplankton, marine grazers, and consumed indirectly by the predators of these two types of organisms (Simon *et al.*, 2009).

Many species of marine phytoplankton produce cysts which are able to germinate to form algal blooms. Toxic or harmful species of marine phytoplankton use cysts as a strategy for survival. Non-toxic species of marine phytoplankton are still likely to cause harm by forming dense algal blooms that result in anoxia (Anderson *et al.*, 1995). Marine phytoplankton, although providing many benefits, can have harmful effects. This occurs when dense algal blooms are formed or when they are toxic to other organisms. Dinoflagellates are more likely to cause harmful algal blooms by producing cysts. These cysts are known to cause red tides that result in fish death and harmful health effects (Anderson *et al.*, 1995). Diatoms are unicellular organisms that make up the largest group among different types of algae. The cell wall of a diatom is made up of a silica-based scaffold and organic macromolecules (Kroger *et al.*, 1997).

Cyanobacteria are the dominant phototrophic bacteria in both marine and freshwater ecosystems. Cyanobacteria proliferate in nitrogen and phosphorus-rich environments which indicates eutrophication in the environment (Kim et al. 2004). Some species of cyanobacteria are

able to grow in both marine and freshwater environments. This is a possible result of run-off from rivers or from the ability of the same species to thrive in both freshwater and marine environments (Burja *et al.,* 2001).

Bloom-forming cyanobacteria produce harmful toxic compounds such as microcystins and saxitoxins (Kim *et al.,* 2004). Freshwater cyanobacteria blooms have been extensively researched and found to cause neuro-, cyto-, and hepatotoxicity to mammals (including humans), birds, fish, livestock, and invertebrates. These harmful blooms also occur in tropical and sub-tropical regions. Species of cyanobacteria can produce secondary metabolites which are classified as biotoxins. The cytotoxic activity of cyanobacteria biotoxins are associated with anti-cancer, antifungal, anti-HIV, antimicrobial, and antimalarial activities. It has been observed that organisms lacking an immune system are more likely to release secondary metabolites as a defense against predators such as zooplankton. The secreted toxins are potentially lethal to cyanobacteria predators along with reducing the number of predator offspring (Burja *et al.,* 2001).

Cyanophage are viruses that infect cyanobacteria and control cyanobacterial populations. Cyanophage are ubiquitous in both marine and freshwater environments, with a high abundance of viral particles that is estimated at 10^7 per ml. Viral infection can affect microbial populations through lytic and lysogenic cycles. The lytic cycle results in the destruction of host cells, while the lysogenic cycle results in the viral genome inserted into the host cell as a prophage (Lee *et al.,* 2006). For some time, viruses which infect freshwater cyanobacteria have been known. However, in recent years it has been realized that cyanophages that infect *Synechococcus* species in seawater are extremely abundant. Studies show that cyanophages are also a dynamic

component of marine planktonic communities because they were found to be responsible for the lysis of a portion of *Synechococcus* populations on a daily basis (Suttle & Chan, 1994).

In order to promote and improve the health of the Barnegat Bay ecosystem, it is necessary to identify the species of phytoplankton that are at risk of proliferating and forming algal blooms. Several scientific methods are useful in identifying an array of microorganisms within a collection of water samples. One method is polymerase chain reaction (PCR), which is useful in the amplification of nucleic acid sequences of extracted genetic material from microorganisms located within the water samples. PCR can be used to identify generic and specific phytoplankton in Barnegat Bay. Also, microscopy allows for the direct detection of species within the water samples, although the method is more time-consuming and less efficient than molecular methodologies. In addition to PCR and microscopy, flow cytometry is a method that is efficient in determining the cell count, cell size, and cell complexity. The flow cytometer is equipped with fluorescence detectors, which is useful in discriminating phytoplankton that contain an auto-fluorescing chlorophyll component (Veldhuis & Kraay, 2000).

The current study used water samples from 16 different sites ranging from the northern to southern locations of Barnegat Bay, New Jersey. Each sample was tested and analyzed to detect the presence of cyanobacteria and other phytoplankton using PCR, microscopy, and flow cytometry. After the detection of species of phytoplankton was confirmed, an additional study on the cyanophage present in the samples was carried out using viral plaque assays. The detection of cyanophage in Barnegat Bay may be useful in the control of algal population density and maintenance of the health state of the bay (Lee *et al.*, 2006).

Materials and Methods

Environmental Samples Collection and Processing

Water samples were collected from 16 sites of Barnegat Bay, New Jersey in August 2012. The water sample collections were kindly provided by Dr. Jack Gaynor and Dr. Paul Bologna of Montclair State University. On August 7, 2012 the Northern sites were collected along with measurement of dissolved oxygen (DO), salinity (ppt), temperature (°C), and tide heights (ft), the order of collection is as follow: Metedeconk River West, Metedeconk River East, Silver Bay West, Toms River West, Forked River West, Double Creek West, Double Creek East, Forked River East, Toms River East, and Silver Bay East. On August 8, 2012 the remaining Southern sites were collected along with the measurements of the same variables, the order of collection is as follows: Harvey Cedars West, Westeconk Creek West, Tuckerton Creek West, Tuckerton Creek East, Westeconk Creek East, and Harvey Cedars East. Samples were collected and placed in 1 L sterile collection bags. The 16 water samples were initially filtered through a 100 µm filter and the filtrate was filtered through a 0.45 µm filter (Nalgene, Rochester, NY, USA). The filter from each site was removed and dried in a 37°C incubator for 1-2 hours. The dried filters were then wrapped in the sterile aluminum foil and stored in the 4°C refrigerator for future study.

Chelex® DNA Extraction of Environmental Samples

The following protocol modified by Chu and Rienzo (2013) was used to extract the DNA from environmental samples using Chelex® (Chu & Rienzo, 2013). The filtered discs from the 16 sites were folded in half and hole punched 3 times at random spots around the paper using a sterile hole puncher to make 6 pieces of small filter paper. The 6 small filter discs were then placed into a 1.7 ml microfuge tube, one for each site, using sterile forceps. 200µL of 5%

Chelex® was added to each Eppendorf tube and vortexed for 10 seconds using a VWR Scientific Vortex-Genie 2. The tubes were then incubated at 56°C for 2 hours using a Polyscience© Temperature Controller water bath (Polyscience, Niles, IL, USA), vortexed for 10 seconds, and then incubated at 100°C for 8 minutes in an Isotemp125D™ Heat Block (Fisher Scientific, Pittsburgh, PA, USA). The tubes were then centrifuged for 3 minutes at 10,000 rpm to remove the pellet. The supernatant was removed from each tube, with care to avoid disturbing the pellet, and transferred into clean 1.7 ml microfuge tubes. The DNA concentration and purity of the Chelex extraction from each site was measured with a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) at 260 and 280 nm.

PCR Primers

The primers that were used in the study for PCR assays were developed by previous studies (Chu & Rienzo, 2013) and from literature research. A range of primer sets were used in the study to identify genes of universal bacteria, general photosynthetic bacteria, cyanobacteria, dinoflagellates, and diatoms. Universal primers Uf (GAGAGTTTGATCCTGGTCAG)/ Ur (ACGGYTACCTTGTTACGACTT) were used to identify the 16s rRNA gene in all species of bacteria (Stiller & McClanahan, 2005). General primers include the PSf (GGGATTAGATACCCCWGTAGTCCT)/ PSr (CCCTAATCTATGGGGWCATCAGGA) primer set that identified the 16s rRNA gene in all photosynthetic bacteria (Stiller & McClanahan, 2005). Another general primer set used in the study is the OXY107F (GGACGGGTGAGTAACGCGTG)/ OXY1313R (CTTCAYGYAGGCGAGTTGCAGC) which identified the 16S rDNA sequence of oxygenic phototrophs (Fuller *et al*., 2003). General primer set 27Fb (AGAGTTTGATCCTGGCTCAG)/ 785R (ACTACCRGGGTATCTAATCC) identified the 16s rRNA in all bacteria, cyanobacteria, and other phytoplankton (Kim *et al*.,

2004). To identify the 16s rRNA subunit that is conserved in all cyanobacteria, the primer set CYA359F (CGGACGGGTGAGTAACGCGTGA)/ CYA781R (GACTACTGGGGTATCTAATCCCATT) was used (Nübel *et al.*, 1997). The primers cpc1F (TAGTGTAAAACGACGGCCAGTTG(C/T) (C/T)T(G/T)CGCGACATGGA)/ cpc1R (TAGCAGGAAACAGCTATGACGTGGTGTA (G/A)GGGAA(T/C)TT) were used to identify the β-Subunit of the phycocyanin gene in cyanobacteria (Robertson *et al.*, 2001). After the identification of cyanobacteria was confirmed, several specific primer sets were used to identify species of cyanobacteria, *Synechococcus* and *Prochlorococcus*. The RNA polymerase core subunit gene *rpoC1* was identified in *Synechococcus* using the primers rpoC1-39F (GGCATYGTYTGYGAGCGYTG)/ rpoC1-426R (CGYAGRCGCTTGRTCAGCTT) (Mühling *et al.*, 2006). Developed by Lavin *et al.* in 2008, the DINAf (GAA TCT GCC CTC AGG AGG GGG)/DINAr (GGG TTG CCC CAT TCG GAA AT) primer set was used to identify the 16S rRNA gene and the 16S-23S internal transcribed spacer (ITS) region of *Prochlorococcus* and *Synechococcus* cyanobacteria species. Two primer sets were developed using NCBI BLAST searches. The first primer set, AN3801f (CAAATCACTCAGTTTCTGG)/AN3801r (CAGTAGCAGCTCAGGACTC), identifies the DNA polymerase III gene that is conserved in the following two *Synechococcus* species: *S*. IU 625 and *S. elongates* PCC 7942 (Chu & Rienzo, 2013). The second primer set is ANAf (GATCTAGCCTCACCTGTTGACTT)/ ANAr (GGGATCCTTTTTGCTGCGCC) which is specific for the toxin biosynthesis gene cluster of *Anabaena circinalis* (Chu & Rienzo, 2013). In order to identify species of dinoflagellates, dinoSLg-F (cgagagtatcAGCCATTTTGGCTCAAG)/ dinoSLg-R (acagaacaAGCCAAAATGGCTACGG) were used to reveal presence of the trans-splicing spliced leader (SL) region of dinoflagellate RNA (Zhang *et al.*, 2009). The DANAT1F

(CACCGCTTTCTTCATCTGGTTYGCNAT)/DANAT1R(GCACACAGCGCCGGTRTTNCCNCC) primer set was used to identify amino acids within the DNA sequence of the marine diatom *Cylindrotheca fusiformis* (Song & Ward, 2007). A second primer set was used to identify a gene that is conserved among diatom species involving small subunit ribosomal DNA. This primer set is 528f (GCGGTAATTCCAGCTCCAA)/650r (AACACTCTAATTTTTTCACAG) (Baldi *et al.*, 2001).

Table 1. Complete list of primer sets used in PCR-based assays found in literature research and developed using NCBI BLAST. Table shows nucleotide sequence, annealing temperature (°C), and amplicon size (bp). General and specific primers were used to identify species of cyanobacteria, diatoms, and dinoflagellates.

Primer Name	Sequence	Tm (°C)	Amplicon Size (nt)
CPC1F	TAGTGTAAAACGACGGCCAGTTG(C/T)(C/T)T(G/T)CGCGACATG	45	389
CPC1R	TAGCAGGAAACAGCTATGACGTGGTGTA(G/A)GGGAA(T/C)TT	45	389
rpoC1-39F	GGCATYGTYTGYGAGCGYTG	51	403
rpoC1-462R	CGYAGRCGCTTGRTCAGCTT	51	403
CYA359F	CGGACGGGTGAGTAACGCGTGA	60	446
CYA781R	GACTACTGGGGTATCTAATCCCATT	60	446
OXY107F	GGACGGGTGAGTAACGCGTG	55	951
OXY1313R	CTTCAYGYAGGCGAGTTGCAGC	55	951
PSf	GGGATTAGATACCCCWGTAGTCCT	50	955
Ur	ACGGYTACCTTGTTACGACTT	50	955
DANAT1F	CACCGCTTTCTTCATCTGGTTYGCNAT	63.4	1053
DANAT1R	GCACACAGCGCCGGTRTTNCCNCC	69.1	1053
dinoSLg-F	cgagagtatcAGCCATTTTGGCTCAAG	58	175
dinoSLg-R	acagaacaAGCCAAAATGGCTACGG	58	175
27Fb	AGAGTTTGATCCTGGCTCAG	58	740
785R	ACTACCRGGGTATCTAATCC	58	740
DINAf	GAA TCT GCC CTC AGG AGG GGG	58	548
DINAr	GGG TTG CCC CAT TCG GAA AT	58	548
ANAF	GATCTAGCCTCACCTGTTGACTT	53	457
ANAR	GGGATCCTTTTTGCTGCGCC	53	457
AN3801F	CAAATCACTCAGTTTCTGG	55	171
AN3801R	CAGTAGCAGCTCAGGACTC	55	171
528f	GCGGTAATTCCAGCTCCAA	50	200
650r	AACACTCTAATTTTTTCACAG	50	200

PCR Amplification

General and specific primers were used with the environmental DNA samples to identify the presence of general bacteria, cyanobacteria, dinoflagellates, and diatoms. Initial detection of general bacteria, cyanobacteria and other phytoplankton utilized general primers such as the

CPC1F and CPC1R, Uf and Ur, and the rpoC1-39F and rpoC1-426R primer set. Once the presence of cyanobacteria and other phytoplankton species was confirmed, specific primers were then utilized using the PCR method. The PCR reaction was carried out in 0.2 mL MicroAMP® reaction tubes by mixing 7.5 µL nuclease-free deionized water (Promega, Madison, WI, USA), 2 µL dimethyl sulfoxide (DMSO), 1 µL of forward primer, 1 µL of reverse primer, 1 µL of DNA sample, and 12.5 µL GoTaq® Hot Start Green Master Mix (Promega). The prepared PCR tubes were then placed into a Veriti 96 Well Thermocycler (Applied Biosystems, Carlsbad, CA, USA). The initial step was a 95°C denaturation step for 2 minutes, followed by further denaturation of 35 cycles at 95°C for 40 seconds, 35 cycles of primer annealing at 50-65°C for 40 seconds, and 35 cycles for DNA strand extension at 72°C for 40 seconds followed by a final extension step at 72°C for 5 minutes. The cycle ended with a 4°C cooling step so amplified DNA could be stored at low temperatures. Gel electrophoresis was used to visualize the amplified DNA on a 1% agarose gel containing ethidium bromide and placed in TAE electrophoresis buffer (Fermentas). A 2UV Transilluminator Gel DocIt Imaging system was used to visualize the gel (UVP, Upland, CA, USA).

Microscopic Analysis

The 1.5 mL plastic bags that contained the water samples from each site were stored at 4°C in the refrigerator to prevent any remaining droplets of sample from drying out. The plastic bags were each rinsed out with 30 mL of 1X BG-11 media combined with vitamin mix. The rinse from each site was placed in a conical tube and left under light to promote organism growth for imaging analysis. The original bags were disregarded after rinsing. 10 µL of the rinse was pipetted onto a glass microscope slide and covered with a glass cover slip. Images were viewed at 400x objective and acquired using a Carl Zeiss AxioLab.A1 phase contrast microscope along

with a Carl Zeiss AxioCam MRc camera (Carl Zeiss Microimaging, Jena, Germany). Images were also acquired using an Olympus BH2 Research Microscope at 400x and 200x objectives (Olympus America, Inc., Melville, New York, USA). Images obtained of diatoms were compared to images of microorganisms present in the atlas "Freshwater Algae of North America: Ecology and Classification" (Sheath, 2003).

Flow Cytometry

Flow cytometry was carried out to analyze the water samples collected from the 16 sites. A mixed marine cyanobacteria culture that contains five species of marine cyanobacteria (*Anabaena, Synechococcus, Gloeotrichia, Nostoc, Oscillatoria*) and each of the five species of the cyanobacteria used as controls for the analysis. Each site was prepared for flow cytometry by hole punching the 0.45 µm filters six times at various locations on the filter disc and placing them into 1 mL of sterile deionized water. A MACSQuant® Analyzer (Miltenyi Biotec, Inc., San Diego, CA, USA) was used to perform flow cytometry and the results were analyzed using FlowJo vX.0.7 Flow Cytometry Analysis Software (Tree Star, Inc., Ashland, OR, USA).

Viral Plaque Assay

The filtered water from each site was concentrated by undergoing multiple runs using a Sorvall RC5C centrifuge (DuPont, Fayetteville, NC, USA). 30 ml of filtered water was placed into the Sorvall tube at a time and spun at 17,000 rpm for 15 minutes. Flow through was discarded and the pellet was resuspended into an additional 30 ml of water until the entire volume of water (total volumes ranged depending on sample filtrate) was concentrated. The concentrated water containing viral lysate from Silver Bay West, Silver Bay East, Forked River West, Double Creek West, Westeconk West, and Westeconk East was then used in viral plaque

assays to detect the presence of cyanophage. For each site with viral lysate, 10 ml of mixed marine cyanobacteria in 99.5 ml of 1X BG11 media with 0.5 ml vitamin mix was placed into a 15 ml conical tube and centrifuged at 5,000 rpm for 10 minutes. 8 ml of the supernatant was removed from the conical tube and the remaining 2 ml of supernatant was used to resuspend the pellet. After the resuspension, 2 ml of the culture per site was transferred into a 4 ml cryogenic vial (Corning Incorporated, Corning, NY, USA) and 100 µl of the viral lysate from each site was added to each individual vial. The vials were gently shaken on an Innova 2000 platform shaker (New Brunswick Scientific, Enfield, CT, USA) for one hour for adsorption. 1 ml of 1% 3M soft top agar was added to each vial and vortexed for 10 seconds. Immediately following the addition of the 1% 3M soft top agar, the entire contents of each vial was poured and spread on to individual 3M agar plates designated for each site with viral lysate. Plates were allowed to dry and inverted under white light at room temperature. Presence of viral plaques were observed and photographed daily.

Results

BARNEGAT BAY SITES

Water samples were collected from the 16 sites labeled with stars in Figure 1 below. Table 2 shows the geographical coordinates of the sixteen sites ranging from the northern to southern sites. The time and height of high tides and low tides were recorded for both evening and night on the day of collection for each of the 16 sites, as shown in Table 3. Table 4 includes salinity, temperature, dissolved oxygen, and percent dissolved oxygen of the sixteen sites in order from northern to southern sites. Sample collection took place over a two day period in August of 2012.

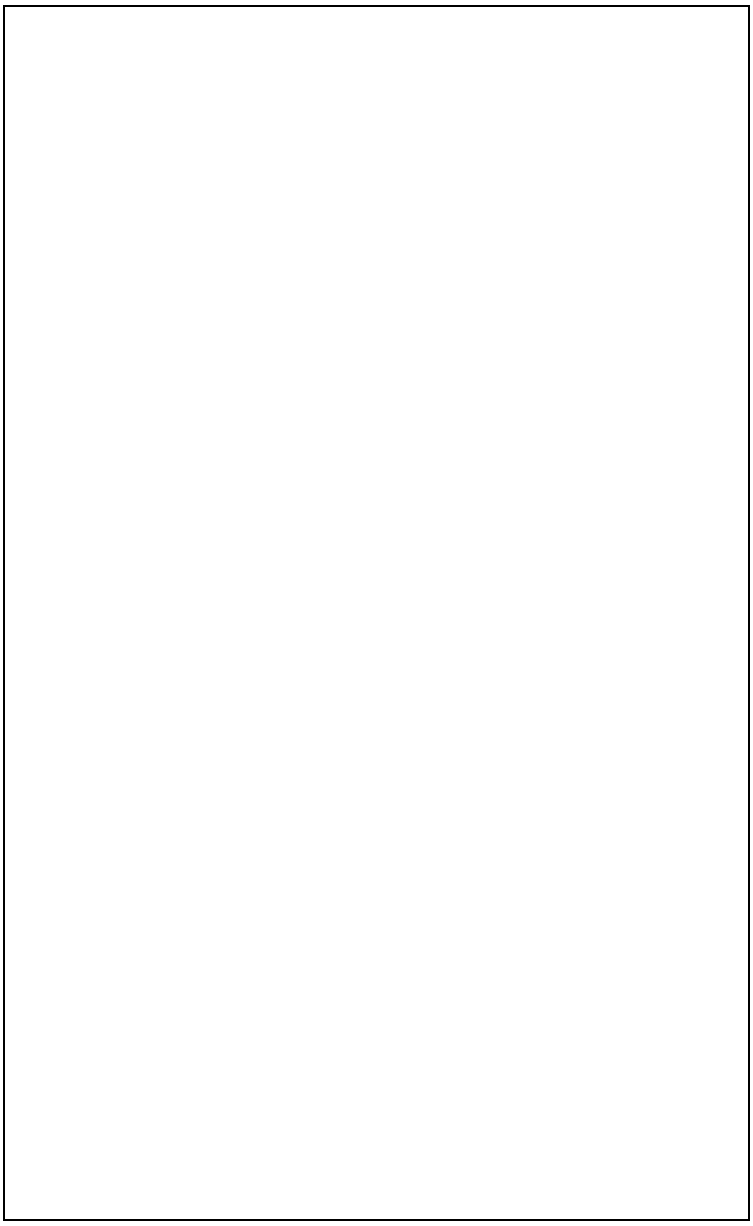

Figure 1. The site map of the Barnegat Bay estuary. Sites of water collection are labeled with a star symbol.

Table 2: Geographical coordinates of 16 collected sites in Barnegat Bay, NJ (August 2012).

Name	Coordinates	
Metedeconk East	40.04694° N	074.05470° W
Metedeconk West	40.05001° N	074.06485° W
Silver Bay East	39.98664° N	074.08652° W
Silver Bay West	39.99290° N	074.11984° W
Toms River East	39.92672° N	074.08454° W
Toms River West	39.93283° N	074.11005° W
Forked River East	39.81578° N	074.12276° W
Forked River West	39.82100° N	074.15955° W
Double Creek East	39.78765° N	074.15394° W
Double Creek West	39.78606° N	074.18314° W
Harvey Cedar East	39.66914° N	074.14600° W
Harvey Cedar West	39.70118° N	074.16106° W
West Creek East	39.59874° N	074.22974° W
West Creek West	39.61984° N	074.25923° W
Tuckerton Creek East	39.55717° N	074.25449° W
Tuckerton Creek West	39.57924° N	074.32362° W

Table 3: Tide collection data of Barnegat Bay, NJ (August 2012).

Collection Date	Site	Time of Collection	High Tide Morning	High Tide Height	Low Tide Morning	Low Tide Height	High Tide Evening	High Tide Height 2	Low Tide Evening	Low Tide Height 3
Aug 7, 2012	Metedeconk River West	8:15 AM	4:30 AM	0.34ft	10:44 AM	0.02 ft	5:00 PM	0.35 ft	11:19 PM	0.06 ft
Aug 7, 2012	Metedeconk River East	8:38 AM	4:30 AM	0.34ft	10:44 AM	0.02 ft	5:00 PM	0.35 ft	11:19 PM	0.06 ft
Aug 7, 2012	Silver Bay East	9:18 AM	4:28 AM	0.39 ft	10:44 AM	0.02 ft	4:58 PM	0.39 ft	11:19 PM	0.07 ft
Aug 7, 2012	Toms River West	9:55 AM	3:41 AM	0.39 ft	10:10 AM	0.02 ft	4:12 PM	0.39 ft	10:45 PM	0.07 ft
Aug 7, 2012	Forked River West	10:33 AM	3:09 AM	0.34 ft	9:25 AM	0.02 ft	3:40 PM	0.35 ft	10:00 PM	0.06 ft
Aug 7, 2012	Double Creek West	11:02 AM	3:04 AM	0.34 ft	9:38 AM	0.02 ft	3:35 PM	0.35 ft	10:13 PM	0.06 ft
Aug 7, 2012	Double Creek East	11:22 AM	3:04 AM	0.49 ft	9:44 AM	0.02 ft	3:34 PM	0.49 ft	10:19 PM	0.08 ft
Aug 7, 2012	Forked River East	11:48 AM	3:09 AM	0.34 ft	9:25 AM	0.02 ft	3:40 PM	0.35 ft	10:00 PM	0.06 ft
Aug 7, 2012	Toms River East	12:24 PM	3:41 AM	0.39 ft	10:10 AM	0.02 ft	4:12 PM	0.39 ft	10:45 PM	0.07 ft
Aug 7, 2012	Silver Bay East	12:53 PM	4:28 AM	0.39 ft	10:44 AM	0.02 ft	4:58 PM	0.39 ft	11:19 PM	0.07 ft
Aug 8, 2012	Harvey Cedars West	9:06 AM	3:39 AM	1.22 ft	10:36 AM	0.15 ft	4:08 PM	1.30 ft	10:36 PM	0.15 ft
Aug 8, 2012	Westeconk Creek West	10:44 AM	2:44 AM	1.95 ft	9:21 AM	0.23 ft	3:13 PM	2.06 ft	10:08 PM	0.47 ft
Aug 8, 2012	Tuckerton Creek West	11:12 AM	2:21 AM	2.04 ft	8:44 AM	0.24 ft	2:50 PM	2.16 ft	9:31 PM	0.49 ft
Aug 8, 2012	Tuckerton Creek East	11:42 AM	2:30 AM	2.09 ft	8:56 AM	0.25 ft	2:59 PM	2.21 ft	9:43 PM	0.5 ft
Aug 8, 2012	Westeconk Creek East	12:12 PM	2:54 AM	2.04 ft	9:22 AM	0.24 ft	3:23 PM	2.16 ft	10:09 PM	0.49 ft
Aug 8, 2012	Harvey Cedars East	2:00 PM	3:36 AM	1.22 ft	10:24 AM	0.15 ft	4:05 PM	1.30 ft	11:11 PM	0.30 ft

Table 4: Water properties of 16 sites collected from Barnegat Bay, NJ (August 2012).

Name	salinity (ppt)	temp (°C)	D.O. (mg/L)	D. O. saturation (%)
Metedeconk East	20.2	27	5.14	84.7
Metedeconk West	17.8	26.1	5.7	78.1
Silver Bay East	19	27	5.66	79.1
Silver Bay West	18	28.4	6.58	84.5
Toms River East	21.2	28.3	6.13	89.2
Toms River West	15.2	27.3	6.74	88.3
Forked River East	28.6	27.3	6.32	98.1
Forked River West	28	29	5.6	86.5
Double Creek East	29.6	27.3	7.93	114.1
Double Creek West	28.6	28.8	5.58	85.2
Harvey Cedar East	29.4	28.4	6.87	106
Harvey Cedar West	29.1	27.2	6.82	93.4
West Creek East	30.3	27.7	7.06	109
West Creek West	26.5	27.4	6.07	92.5
Tuckerton Creek East	30.7	26.7	7.83	128.8
Tuckerton Creek West	29.1	27.6	6.67	94.6

CHELEX® DNA EXTRACTION

Chelex® extraction was performed twice in two different areas selected randomly from the collected filter (disk region 1 and disk region 2) in order to analyze a greater surface area of the fine filter disks to confirm the presence of marine phytoplankton. DNA extractions were analyzed using a NanoDrop to determine the DNA concentration and purity with a 260/280 ratio. Samples were used in PCR extraction when the DNA concentration was over 10 ng/µl. Ideal DNA purity was considered a 1.8 from the 260/280 nm ratio. Table 4 and table 5 below show the NanoDrop readings of the northern and southern sites, respectively. All samples analyzed have concentrations exceeding 10 ng/µl. DNA concentrations among the samples range from 10.91 to 65.74 ng/µl. The purity of DNA using a 260/280 nm ratio ranges from 1.44 to 1.73.

Table 5. NanoDrop readings of the DNA extractions of the northern sites. The concentrations are calculated in ng/µl. DNA purity was determined by 260/280 ratio.

Northern Sites	ng/µl	260/280
Metedeconk East #1	44.46	1.69
Metedeconk East #2	37.08	1.66
Metedeconk East #3	32.07	1.69
Metedeconk West #1	27.14	1.6
Metedeconk West #2	29.26	1.62
Silver Bay East #1	32.98	1.5
Silver Bay East #2	31.92	1.53
Silver Bay East #3	29.99	1.44
Silver Bay West #1	50.77	1.52
Toms River East #1	27.46	1.59
Toms River East #2	31.27	1.63
Toms River West #1	37.88	1.58
Toms River West #2	55.1	1.56
Forked River East #1	13.38	1.69
Forked River East #2	12.9	1.68
Forked River West #1	13.29	1.63
Forked River West #2	14.32	1.71

Table 6. NanoDrop readings of the DNA extractions of the northern sites. The DNA yield was measured in ng/µl. DNA purity was determined by 260/280 ratio.

Southern Sites	ng/µl	260/280
Double Creek East #1	10.91	1.53
Double Creek East #2	11.75	1.56
Double Creek West #1	20.18	1.57
Double Creek West #2	23.66	1.64
Harvey Cedar East #1	14.29	1.76
Harvey Cedar East #2	13.13	1.52
Harvey Cedar West #1	17.31	1.62
Harvey Cedar West #2	28.72	1.59
West Creek East #1	21.79	1.64
West Creek East #2	22.33	1.66
West Creek West #1	59.86	1.47
West Creek West #2	26.96	1.49
West Creek West #3	65.74	1.44
Tuckerton East #1	42.58	1.73
Tuckerton East #2	35.59	1.73
Tuckerton West #1	17.02	1.67
Tuckerton West #2	26.57	1.7

POLYMERASE CHAIN REACTION (PCR) ASSAYS

After the determination of DNA concentration and purity from Chelex® extraction for each sample, the samples were then utilized in PCR-based assays in the identification of general species of bacteria, cyanobacteria, diatoms, and dinoflagellates. The ubiquitous species of cyanobacteria, *Synechococcus* and *Prochlorococcus,* were detected using specific primers. In many of the primer sets, the 16S rRNA gene was targeted for amplification as it is a shared gene among many types of marine phytoplankton.

Figures 2 through 23 show the gel electrophoresis images of the PCR products for each primer set. PCR based-assays were conducted for disk region 1 and disk region 2 for each of the 16 sites to provide a more complete profile of each site using randomly selected areas of the collected filter.

Psf/Ur

The PSf/Ur primers were used in combination to identify photosynthetic bacteria. In Figure 2, the gel electrophoresis image showed negative results for all sites on a 1% agarose gel using the PSf/Ur primer set from disk region 1.

PCR was performed on disk region 2 using the PSf/Ur primers as seen in Figure 3. The DNA extractions from the sixteen sites came from different sections of the fine filter which may include differences in types of species or abundance of photosynthetic bacteria. Therefore, results show a bright band at the positive control lane as well as the Tuckerton East site. The positive control for this assay was *Synechococcus* sp. IU 625.

Figure 2. Results from PSf/Ur primers for the first set of Chelex® extracts from the Barnegat Bay sites. No presence of photosynthetic bacteria was detected using the primer set.

Figure 3. Results from the second set of DNA Chelex® for the 16 Barnegat Bay sites using the PSf/Ur primers. Lane 17 indicates the presence of photosynthetic bacteria in Tuckerton East.

OXY107F/OXY1313R

The general primer set OXY107F/OXY1313R was used to identify the 16S rDNA sequence of oxygenic phototrophs. The 16 sites were tested using PCR and gel electrophoresis with a 1% agarose gel. Lanes 2, 4, 5, 6, 11, and 14 revealed positive results as shown in Figure 4. The DNA extract used in this PCR assay came from disk region 1 of Chelex® extractions performed for the 16 Barnegat Bay sites.

PCR using the OXY107F/OXY1313R primer set was performed on disk region 2 for all 16 sites. The presence of oxygenic phototrophs was detected in lanes 2, 13, 16, and 17 as seen in Figure 5.

27Fb/785R

Bacteria, cyanobacteria, and other phytoplankton were detected using the primer set 27Fb/785R. PCR was performed on disk region 1 DNA extracts from the sixteen sites. The image shows gel electrophoresis results on a 1% agarose gel. In Figure 6, bands were detected in lanes 4, 7, 8, 9, 10, 12, 13, 14, and 15. The brightness and density of the bands varied amongst the sites where positive results are shown.

Figure 7 shows the gel electrophoresis image of a 1% agarose gel using disk region 2 PCR from the Barnegat Bay sites. Primer set 27Fb/785R detected positive results in lanes 1, 7, 8, 10, 11, 12, 13, 14, 15, and 16.

Figure 4. PCR assays of the OXY107F/OXY1313R primer set detects the presence of oxygenic phototrophs in Double Creek West, Forked River East, Harvey Cedar East, Harvey Cedar West, Toms River East, and Tuckerton West.

Figure 5. Gel electrophoresis image of the second OXY107F/OXY1313R primer set of the 16 sites. The presence of bands were detected in Metedeconk East, Harvey Cedars East, Tuckerton West, and Tuckerton East sites.

Figure 6. Gel electrophoresis image of 27Fb/785R primer set. Presence of bacteria, cyanobacteria, and other phytoplankton detected at Forked River West, Metedeconk East, Metedeconk West, Silver Bay East, Tuckerton East, Toms River East, Toms River West, Tuckerton West, and Westeconk East.

Figure 7. PCR results for disk region 2 DNA extracts and 27Fb/785R. Positive results for bacteria, cyanobacteria, and other phytoplankton detected in Metedeconk East, Harvey Cedars East, Tuckerton West, and Tuckerton East.

CYA359F/CYA781R

The CYA359F/CYA781R primer set was used to detect cyanobacteria presence in the water samples using PCR and gel electrophoresis. Figure 8 shows the presence of cyanobacteria in all of the sites tested, as indicated by a band in lanes 1 through 15. Brightness of the bands indicates a strong presence of cyanobacteria species.

The disk region 2 DNA extracts from the sixteen sites were analyzed using CYA739F/CYA781R primers. Unlike the first set of PCR, positive results were only detected for lanes 1, 8, 10, 12, and 17, shown in Figure 9.

cpc1F/cpc1R

An additional primer set, cpc1F/cpc1R, was utilized to detect cyanobacteria within the disk region 1 of the collected filter. As shown in Figure 10, positive results were detected in lanes 2 through 6, and lanes 8 through 10. The positive control for this primer set was DNA extract from *Synechococcus* sp. IU 625.

Disk region 2 DNA extracts of the sixteen sites were analyzed using the cpc1F/cpc1R primers. Cyanobacteria were detected in lanes 1, 4, 10, and 17 as seen in Figure 11 below.

Figure 8. PCR analysis of sites using CYA359F/781R primer set. Positive results detected in all lanes to confirm the presence of cyanobacteria. Sites Silver Bay East and Silver Bay West are not included in this assay.

Figure 9. PCR analysis of the disk region 2 Chelex® extracts. Presence of cyanobacteria detected in Metedeconk West, Forked River East, Double Creek West, Harvey Cedars West, and Tuckerton East.

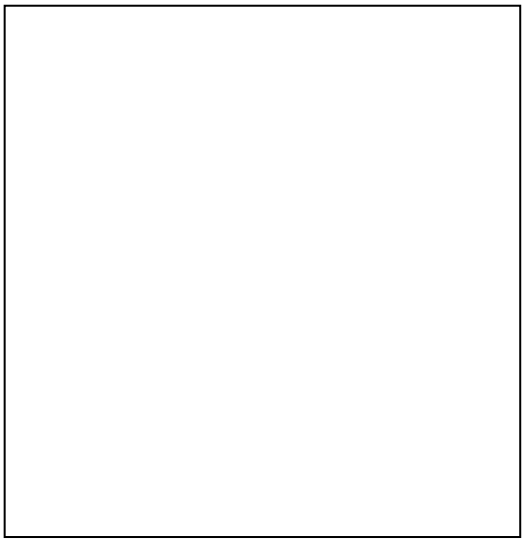

Figure 10. cpc1F/cpc1R primer set shows bands for Metedeconk West, Forked River East, Forked River West, Harvey Cedar East, Tuckerton East, Westeconk East, and Toms River East. Bands are brightest for the Westeconk East and Toms River East sites.

Figure 11. Cyanobateria presence detected using cpc1F/cpc1R primers for disk region 2. Bands show positive results at Metedeconk West, Silver Bay East, Double Creek West, and Tuckerton East.

rpoC1-39F/rpoC1-426R and AN3801f/AN3801r

Following the detection of cyanobacteria using general primers, primer sets for specific species of cyanobacteria were used to analyze the Barnegat Bay water samples. The rpoC1-30F/rpoC1-426R and AN3801f/AN3801r primer sets were used to detect genes conserved *Synechococcus* species. Figure 12 below shows the rpoC1-39F/rpoC1-426R primer assay for disk region 1 DNA extracts from water samples. Lanes 1 through 5, 7 through 9, and 11 through 15 detect positive results for the presence of *Synechococcus*. The disk region 2 DNA extracts using the same primer set shows bands in lanes 1, 2, 4 through 6, 8m and 15 as shown in Figure 13.

The AN3801f/AN3801r primer set did not detect the presence of *Synechococcus* in any of the DNA extracts from disk region 1 collected filters, as shown from the negative results in Figure 14. However, in the disk region 2 DNA extracts show in Figure 15, a positive result was detected in lane 16 along with dull, barely detectable bands present in lanes 10 and 11.

Figure 12. Positive results for *Synechococcus* detected by the rpoC1-39F/rpoC1-426R primer set in Double Creek East, Double Creek West, Forked River East, Forked River West, Harvey Cedar West, Harvey Cedar East, Metedeconk West, Silver Bay East, Toms River East, Toms River West, Tuckerton East, Tuckerton West, and Westeconk East.

Figure 13. Positive results for *Synechococcus* detected by rpoC1-39F/rpoC1-426R primer set for the disk region 2 DNA extracts from water samples. Positive results detected in Metedeconk West, Metedeconk East, Silver Bay East, Toms River West, Toms River East, Forked River West, Forked River East, Double Creek West, and Westeconk East.

Figure 14. AN3801F/AN3801R primer set shows no positive results for any of the disk region 1 DNA extracts from the water samples.

Figure 15. The disk region 2 DNA extracts show a positive result for *Synechococcus* in Tuckerton West as detected by the AN3801F/AN3801R primer set.

DINAf/DINAr

To detect the presence of another abundant species of cyanobacteria, *Prochlorococcus*, the primer set DINAf/DINAr was utilized on the two sets of DNA extracts, as shown in Figure 16 and Figure 17. The first set of DNA extracts for the water samples shows negative results in all lanes excluding the positive control which shows a weak band in lane 17.

The second PCR assay using the DINAf/DINAr primer analyzed the disk region 2 Chelex® extracts from the sixteen sites and showed similar results to the first assay shown in Figure 16. Figure 17 shows negative results in all lanes with the exception of lane 8 which shows a weak band.

dinoSLgf/dinoSLgR

The detection of dinoflagellate species using PCR assays was carried out using the dinoSLgf/dinoSLgr primer set. In Figure 18, the disk region 1 DNA extracts from water samples was analyzed for dinoflagellates. Positive results were detected in lanes 2, and lanes 11 through 14.

For disk region 2 DNA extracts using the dinoSLgf/dinoSLgr primers, positive results were detected in lanes 1, 6, 9, 11, 15, and 16 as shown in Figure 19.

Figure 16. No bands detected for *Prochlorococcus* cyanobacteria species for all sites analyzed.

Figure 17. PCR assay of disk region 2 DNA extracts using DINAf/DINAr primer set. All lanes show no detection of bands with the exception of lane 8, Forked River East.

Figure 18. PCR assay using the dinoSLgf/dinoSLgr primer set. Positive results detected in Double Creek West, Toms River East, Toms River West, Tuckerton East, and Tuckerton West.

Figure 19. Detection of *Prochlorococcus* species in Metedeconk West, Toms River East, Double Creek West, Harvey Cedars East, Tuckerton East.

DANAT1F/DANAT1R and 528f/650r

In order to identify diatom species within the water samples from Barnegat Bay, two primer sets DANAT1F/DANAT1R and 528f/650r were used for analysis. The DANAT1F/DANAT1R primer set was used to identify the marine diatom *Cylindrotheca fusiformis* as shown in Figures 20 and 21 below. The disk region 1 DNA extracts, as shown in Figure 20 below, lacks positive results for the DINAT1F/DINAT1R primers, however a band is shown in lane 16 in Figure 21.

Diatom species were better detected using the 528f/650r primer set for both sets of Chelex® for the water samples. Figure 22 below shows positive results for lanes 1, 2, 4 through12, and 14. Figure 23 shows the DNA extracts for the disk region 2 DNA extracts. Positive results are detected in all lanes analyzed.

Figure 20. No bands were detected using the DANAT1F/DANAT1R primer set.

Figure 21. Positive result for diatom species detected in Tuckerton West using the primer set DANAT1F/DANAT1R.

Figure 22. Positive results for diatom species detected in Metedeconk West, Metedeconk East, Silver Bay East, Toms River West, Toms River East, Forked River West, Forked River East, Double Creek West, Double Creek East, Harvey Cedars West, Harvey Cedars East, and Tuckerton West.

Figure 23. Diatom species detected in all sites from disk region 2 DNA extractions of Barnegat Bay sites.

Table 7. Complete table of PCR assay results with all primer sets analyzed in the study.

Northern Site Name	CPC1F/ CPC1R	PSf/Ur	CYA359F/ CYA781R	ANAf/ ANAr	dinoSLgf/ dinoSLr	27Fb/ 785R	DANAT1F/ DANAT1R	OXY107F/ OXY1313R	DINAf/ DINAr	AN3801F/ AN3801R	rpOc1f/ rpOc1r	528f/ 650r
	Synechococcus	Phytoplankton	Cyanobacteria	*Anabaena circinalis*	Dinoflagellates	Universal bacteria	Diatoms	Oxygenic Phototrophs	*Synechococcus, Prochlorococcus*	*Synechococcus*	*Synechococcus*	Diatoms
Metedeconk East	x		x		x	x		x			x	xx
Metedeconk West	xx		xx			xx		x			xx	xx
Silver Bay East	x					x		x			xx	xx
Silver Bay West								x				x
Toms River East	x		x		xx	x		x			xx	xx
Toms River West	x		x		x	x		x			xx	xx
Forked River East	x		xx			x		x	x		xx	xx
Forked River West	x		x			xx					x	xx
Southern Sites												
Double Creek East			x			x					x	xx
Double Creek West	xx		xx		x	x		x			xx	xx
Harvey Cedars East	x		x		x	x		x			x	xx
Harvey Cedars West	x		xx			x					x	xx
Westeconk East	x		x			xx					xx	xx
Westeconk West			x			x						x
Tuckerton East	x	x	xx		xx	x		x			x	xx
Tuckerton West	x		x	x	x	xx	x	xx		x	x	xx

Table 8. Northern sites PCR assays.

Northern Sites	Universal bacteria	Oxygenic phototrophs	Cyanobacteria	Dinoflagellates	Diatoms
Metedeconk West	x	x	x		x
Metedeconk East	x	x	x	x	x
Silver Bay West		x			x
Silver Bay East	x	x	x		x
Toms River West	x	x	x	x	x
Toms River East	x	x	x	x	x
Forked River West	x		x		x
Forked River East	x		x		x

Table 9. Southern sites PCR assays.

Southern Sites	Universal bacteria	Oxygenic phototrophs	Cyanobacteria	Dinoflagellates	Diatoms
Double Creek West	x	x	x	x	x
Double Creek East	x		x		x
Harvey Cedars West	x		x		x
Harvey Cedars East	x	x	x	x	x
Westeconk West	x		x		x
Westeconk East	x		x		x
Tuckerton West	x	x	x	x	x
Tuckerton East	x	x	x	x	x

Table 10. Western sites PCR assays.

Western Sites	Universal bacteria	Oxygenic phototrophs	Cyanobacteria	Dinoflagellates	Diatoms
Metedeconk West	x	x	x		x
Silver Bay West		x			x
Toms River West	x	x	x	x	x
Forked River West	x		x		x
Double Creek West	x	x	x	x	x
Harvey Cedars West	x		x		x
Westeconk West	x		x		x
Tuckerton West	x	x	x	x	x

Table 11. Eastern sites PCR assays.

Eastern Sites	Universal bacteria	Oxygenic phototrophs	Cyanobacteria	Dinoflagellates	Diatoms
Metedeconk East	x	x	x	x	x
Silver Bay East	x	x	x		x
Toms River East	x	x	x	x	x
Forked River East	x		x		x
Double Creek East	x		x		x
Harvey Cedars East	x	x	x	x	x
Westeconk East	x		x		x
Tuckerton East	x	x	x	x	x

MICROSCOPY

The sixteen water samples were analyzed using phase contrast microscopy and bright-field microscopy using a compound light microscope. Samples were prepared by hole-punching the fine filter for each site and placing the pieces of filter into 1 mL of sterile, deionized water. Sites for analysis were chosen based off of the appearance of the turbidity of the water and the amount of debris present on the fine filter.

In Figure 24, 25, and 26 images obtained with a phase contrast microscope are identified from Tuckerton East. In Figure 27, a phase contrast image of a diatom known as *Cylindrotheca fusiformis* is identified from Tuckerton West. Figure 28 includes images obtained from a compound light microscope for Harvey Cedars East, Harvey Cedars West, Metedeconk East, Metedeconk West, Tuckerton East, Tuckerton West, Silver Bay East, Silver Bay West, and Westeconk West. Figure 29 shows images of Tuckerton East that were obtained from a phase contrast microscope.

Figure 24. Phase contrast microscope image from Tuckerton East identified as algae *Spirogyra* (400x).

Figure 25. Phase contrast microscope image for Tuckerton East identified as a diatom (400x).

Figure 26. Phase contrast image of a pinnate diatom from Tuckerton East (400x).

Figure 27. Phase contrast image of diatom *Cylindrotheca fusiformis* from Tuckerton West.

Figure 28. Images obtained from a compound light microscope. **(A)** Double Creek West (400x). **(B)** Harvey Cedars East (400x). **(C)** Harvey Cedars West (400x). **(D)** Harvey Cedars West (400x). **(E)** Metedeconk East (200x). **(F)** Metedeconk West (200x). **(G)** Silver Bay East (200x). **(H)** Silver Bay West (200x). **(I)** Tuckerton East (400x). **(J)** Tuckerton East (400x). **(K)** Tuckerton West (400x). **(L)** Westeconk East (400x).

Figure 29. Phase contrast images of Tuckerton East site with three unknown species. All with 400x.

FLOW CYTOMETRY

Flow cytometry was performed on a sample of mixed marine cyanobacteria which included *Nostoc, Gloeotrichia, Oscillatoria, Anabaena,* and *Synechococcus*. Figures 30 through 32 show a dot plot and histogram of the mixed marine cyanobacteria sample. After analysis of the mixed sample, samples of each individual cyanobacteria species was analyzed using flow cytometry. All of the known samples were analyzed using the same forward scatter (FSC) and side scatter (SSC) voltages to analyze cell size and cell granularity, respectively.

To analyze the 16 Barnegat Bay sites, flow cytometry was used to produce dot plots and histograms. Due to the diversity of the size range in the marine microorganisms, it is difficult to observe all sizes at a fixed voltage. Sites were analyzed using optimal voltages for FSC and SSC that provided the best representation of each individual site as shown in Table 12. In Figures 33 through 40, a dot plot and histogram is shown for each of the 16 sites. Voltages were then adjusted to 236 V FSC and 387 V SSC to compare sites and known cyanobacteria cultures for detection of species. Figures 41 through 45 show the offset histograms comparing the sites and known cyanobacteria cultures using the same FSC voltage setting of 236 V and 387 V for SSC.

In order to understand more about the cells within the water samples, FSC and SSC were utilized to create bar graphs as shown in Figures 46 through 48. Forked River West shows a significant number of cells, much greater than the other 15 sites, with an estimated cell count of 1.119×10^7 cells/µl. Metedeconk West contained the smallest number of cells with an estimated 6.2×10^5 cells/µl. FSC, or cell size, was analyzed is Figure 47 and it was observed that Tuckerton East contains the largest cells, and Silver Bay East contains the smallest cells. Figure 48 shows a bar graph that compares cell granularity, or complexity, among all of the 16 sites. According to the pattern in the bar graph, cell granularity is lower in the Northern sites and higher in the Southern sites.

The 488 nm blue laser on the flow cytometer to detect GFP for the identification of phycocyanin-containing cells. The *Synechococcus* species *S.* IU 625 was used as a control at various voltages to detect the percentage of phycocyanin-containing cells within the gated sample. The gates were then

superimposed on the flow cytometer results of the Barnegat Bay samples to produce a profile of phycocyanin-containing cells within the samples. Figures 49 and 50 show the histograms of the gated control compared to the water samples. Two bar graphs show in Figures 51 and 52 show the percent of phycocyanin-containing cells in the Barnegat Bay samples when compared to control cultures of *S.* IU 625 and mixed marine cyanobacteria.

Table 12. Fixed voltage settings of forward scatter (FSC) and side scatter (SSC) with all control organisms during analysis with the flow cytometer.

Site	FSC (V)	SSC (V)
Metedeconk West	286	339
Metedeconk East	306	359
Silver Bay West	296	374
Silver Bay East	296	374
Toms River West	306	428
Toms River East	306	458
Forked River West	386	588
Forked River East	386	588
Double Creek West	296	388
Double Creek East	296	408
Harvey Cedars West	296	408
Harvey Cedars East	306	428
Westeconk West	306	458
Westeconk East	336	378
Tuckerton West	336	328
Tuckerton East	386	538
Sample	**FSC (V)**	**SSC (V)**
Mixed Marine Cyanobacteria	236	387
Anabaena	236	387
Nostoc	236	387
Anacystis nidulans	236	387
Oscillatoria	236	387
S. IU 625	236	387

Figure 30. Left: Dense plot of FSC/SSC for mixed marine cyanobacteria at 236 V (FSC) and 387 V (SSC). Right: Corresponding histogram of FSC indicates that there are two major clusters from the sample based on size variation.

A

B

C

D

E

Figure 31. (A) *Anabaena*. Left: Dense plot. Right: Corresponding histogram of FSC indicates that there are two major clusters from the sample based on size variation. **(B)** *Anacystis nidulans*. Left: Dense plot of FSC/SSC. Right: Corresponding histogram of FSC indicates that there are two major clusters from the sample based on size variation. **(C)** *Oscillatoria.* Left: Dense plot. Right: Corresponding histogram of FSC indicates that there are three major clusters from the sample based on size variation. **(D)** *Nostoc.* Left: Dense plot of FSC/SSC. Right: Corresponding histogram of FSC indicates that there are two major clusters from the sample based on size variation. **(E)** *Synechococcus* sp. IU 625. Left: Dense plot of FSC/SSC. Right: Corresponding histogram of FSC indicates that there is one major cluster from the sample based on size variation.

Figure 32. Overlapping histogram of known cyanobacteria cultures analyzed at 236 V (FSC) and 387 V (SSC). Known cyanobacteria species include *Oscillatoria*, *S.* IU 625, *Anacystis nidulans*, *Nostoc*, Mixed marine cyanobacteria, and *Anabaena*.

A

B

Figure 33. Metedeconk sites flow cytometry data. **(A)** Metedeconk West analyzed at 286 V (FSC) and 339 V (SSC). Left: Dense plot of FSC/SSC. Right: Overlapping histogram of FSC of three repeatings show three major clusters within the sample. **(B)** Metedeconk East analyzed with 306 V (FSC) and 359 V (SSC). Left: Dense plot of FSC/SSC. Right: Overlapping histogram of FSC of three repeatings show three major clusters within the sample.

A

B

Figure 34. Silver Bay sites flow cytometry data. **(A)** Silver Bay West analyzed at 296 V (FSC) and 374 V (SSC). Left: Dense plot of FSC/SSC. Right: Overlapping histogram of FSC of three repeatings show two major clusters within the sample. **(B)** Silver Bay East analyzed with 296 V (FSC) and 374 V (SSC). Left: Dense plot of FSC/SSC. Right: Overlapping histogram of FSC of three repeatings show three major clusters within the sample.

A

B

Figure 35. Toms River sites flow cytometry data. **(A)** Toms River West analyzed at 306 V (FSC) and 428 V (SSC). Left: Dense plot of FSC/SSC. Right: Overlapping histogram of FSC of three repeatings show three major clusters within the sample. **(B)** Toms River East analyzed with 306 V (FSC) and 458 V (SSC). Left: Dense plot of FSC/SSC. Right: Overlapping histogram of FSC of three repeatings show three major clusters within the sample.

A

B

Figure 36. Forked River sites flow cytometry data. **(A)** Forked River West analyzed at 386 V (FSC) and 588 V (SSC). Left: Dense plot of FSC/SSC. Right: Overlapping histogram of FSC of three repeatings show one major cluster within the sample. **(B)** Forked River East analyzed with 386 V (FSC) and 588 V (SSC). Left: Dense plot of FSC/SSC. Right: Overlapping histogram of FSC of three repeatings show two major clusters within the sample.

A

B

Figure 37. Flow cytometry results of Double Creek sites. **(A)** Double Creek West analyzed at 296 V (FSC) and 388 V (SSC). Left: Dense plot of FSC/SSC. Right: Overlapping histogram of FSC of three repeatings show three major clusters within the sample. **(B)** Double Creek East analyzed with 296 V (FSC) and 408 V (SSC). Left: Dense plot of FSC/SSC. Right: Overlapping histogram of FSC of three repeatings show two major clusters within the sample.

A

B

Figure 38. Flow cytometry data of Harvey Cedars sites. **(A)** Harvey Cedars West analyzed at 296 V (FSC) and 408 V (SSC). Left: Dense plot of FSC/SSC. Right: Overlapping histogram of FSC of three repeatings show three major clusters within the sample. **(B)** Harvey Cedars East analyzed with 306 V (FSC) and 428 V (SSC). Left: Dense plot of FSC/SSC. Right: Overlapping histogram of FSC of three repeatings show three major clusters within the sample.

A

B

Figure 39. Flow cytometry data of Westeconk sites. **(A)** Westeconk West analyzed at 306 V (FSC) and 458 V (SSC). Left: Dense plot of FSC/SSC. Right: Overlapping histogram of FSC of three repeatings show three major clusters within the sample. **(B)** Westeconk East analyzed with 336 V (FSC) and 378 V (SSC). Left: Dense plot of FSC/SSC. Right: Overlapping histogram of FSC of three repeatings show five major clusters within the sample.

A

B

Figure 40. Flow cytometry data of Tuckerton sites. **(A)** Tuckerton West analyzed at 336 V (FSC) and 328 V (SSC). Left: Dense plot of FSC/SSC. Right: Overlapping histogram of FSC of three repeatings show four major clusters within the sample. **(B)** Tuckerton East analyzed with 386 V (FSC) and 538 V (SSC). Left: Dense plot of FSC/SSC. Right: Overlapping histogram of FSC of three repeatings show three major clusters within the sample.

Figure 41. Offset histogram of all known cyanobacteria species in comparison to all 16 Barnegat Bay sites. FSC is 236 V and SSC is 397 V. Tuckerton East (red) shows peaks similar in size and location to the peaks shown for *Anabaena* (magenta) indicating presence of *Anabaena* species at the site. Cell count is the number of cells per 50 µl.

Figure 42. Offset histogram of sites analyzed with 296 V FSC. Cell count is the number of cells per 50 µl.

Figure 43. Offset histogram of sites with 306 V FSC setting. Cell count is the number of cells per 50 µl.

Figure 44. Offset histogram of sites with the 386 V FSC setting. Cell count is the number of cells per 50 µl.

Figure 45. Offset histogram of sites with 336 V FSC setting. Cell count is the number of cells per 50 µl.

Figure 46. Bar graph representing the cell count of the 16 Barnegat Bay sites using the flow cytometer. Forked River West shows the largest cell count of 1.119×10^7 cells/ml. The majority of sites fall around 1.00×10^5 cells/ml while Metedeconk West showed the smallest number of cells/µl with a cell count of 6.2×10^4 cells/ml.

Figure 47. Bar graph representing the mean FSC values, cell size, of cells within the Barnegat Bay sites. Cell size ranges from 91 to 443. The pattern visible from the graph shows larger cell sizes present in the Southern sites than those present in the Northern sites.

Figure 48. Bar graph representing the mean SSC values, granularity, of cells within the Barnegat Bay sites. Cell granularity ranges from 127 (Forked River West) to 1266 (Tuckerton East). Although granularity varies among the sites, the pattern on the graph shows higher granularities present in Southern sites than those in Northern sites.

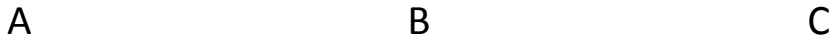

Figure 49. (A) *S.* IU 625 control contains 77.5% phycocyanin-containing cells. **(B)** Silver Bay West site contains 52.0% phycocyanin-containing cells. **(C)** Silver Bay East contains 65.4% phycocyanin-containing cells. Compared to the control as a whole, Silver Bay West contains 67.1% of the total phycocyanin-containing cells while Silver Bay East contains 84.4% of the total phycocyanin-containing cells.

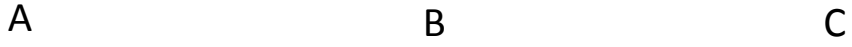

Figure 50. (A) *S.* IU 625 control contains 88.0% phycocyanin-containing cells. **(B)** Forked River West site contains 63.4% phycocyanin-containing cells. **(C)** Forked River East site contains 64.2% phycocyanin-containing cells. Compared to the control as a whole, Forked River West contains 72.0% of the total phycocyanin-containing cells while Forked River East contains 80.0% of the total phycocyanin-containing cells.

Figure 51. *S.* IU 625 was used as a control to obtain the range of phycocyanin containing cells using the flow cytometer. The bar graph above shows the percentage of phycocyanin-containing cells present within each Barnegat Bay water sample.

Figure 52. Mixed marine cyanobacteria culture containing five species of cyanobacteria (*Anabaena,Oscillatoria, Gloeotrichia, Anacystis nidulans,* and *Gloeotrichia*) was used as a control to obtain the range of phycocyanin-containing cells using the flow cytometer. The bar graph shows the percentage of phycocyanin-containing cells (cyanobacteria) within each Barnegat Bay sample.

Viral Plaque Assay

After the filtration of the water samples using the 0.45 μm filters, the filtrates were concentrated exponentially in order to produce viral lysate. The viral lysate from each site was used to perform viral plaque assays to detect the presence of marine cyanophage within the water samples. The following six sites were analyzed in the viral plaque assay: Silver Bay West, Silver Bay East, Forked River West, Double Creek West, Westeconk West, and Westeconk East. Plates were analyzed over a 16 day period, with photographs taken each day to monitor the occurrence of viral plaques, as shown in Figure 53.

Viral plaques were visible beginning on day 6. Many of the plaques are similar in shape and size as they grow in various places on the assay plates. In Figure 53c, many of the plaques on the plates begin to fuse together until the plates show the majority of clearance due to plaque formation by day 16 shown in Figure 53f. Figures 54 through 59 show the progression of the viral plaque clearances throughout a 16 day period for each individual site analyzed. The viral plaque assays confirm the presence of cyanophage that is specific to one or more of the cyanobacteria species within the mixed marine cyanobacteria culture.

A

B

C

D

E

F

Figure 53. Viral plaque assay of six of the 16 sites using concentrated viral lysate combined with mixed cyanobacteria culture. Plates were observed over a 16-day period. Plaques were visible by day 6. The order of sites from left to right is as follows: Westeconk West, Westeconk East, Silver Bay East, Silver Bay West, Double Creek West, and Forked River West. **(A)** Day 2. **(B)** Day 5. **(C)** Day 6. **(D)** Day 8. **(E)** Day 10. **(F)** Day 16.

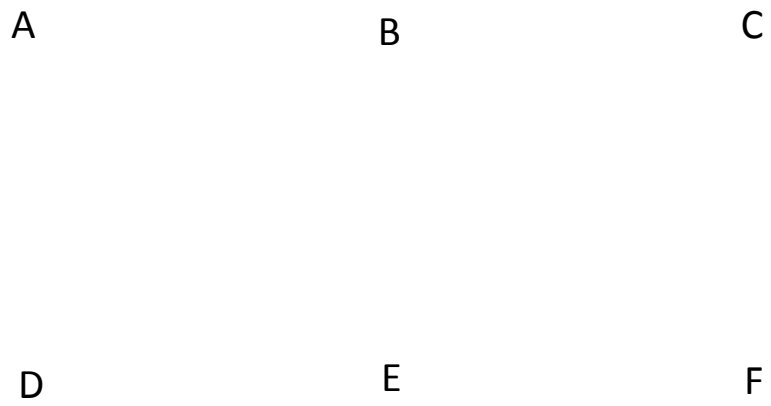

Figure 54. Silver Bay West viral plaque assay. **(A)** Day 2. **(B)** Day 5. **(C)** Day 6. **(D)** Day 8. **(E)** Day 10. **(F)** Day 16. Plaques begin to appear on day 6. The green pigment of the mixed cyanobacteria culture on the plate loses the rich green pigment beginning on day 8 until there is clearance on the plate by day 16.

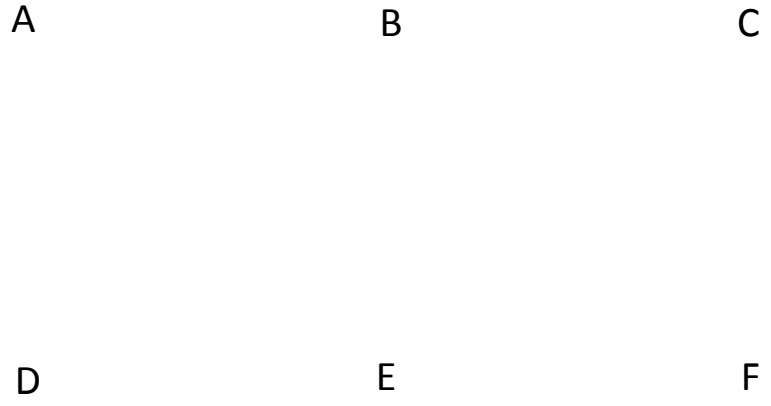

Figure 55. Silver Bay East viral plaque assay. **(A)** Day 2. **(B)** Day 5. **(C)** Day 6. **(D)** Day 8. **(E)** Day 10. **(F)** Day 16. The presence of plaques are detected by day 5. Plaques begin to fuse by day 10 until the plate is almost completely cleared by day 16.

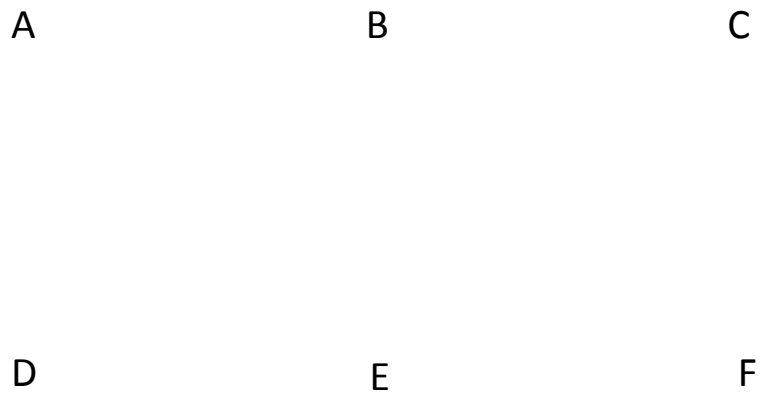

Figure 56. Forked River West viral plaque assay. **(A)** Day 2. **(B)** Day 5. **(C)** Day 6. **(D)** Day 8. **(E)** Day 10. **(F)** Day 16. The presence of viral plaques were detected on day 6. Plaques begin to fuse by day 8. About 70% of the plate is cleared by day 16.

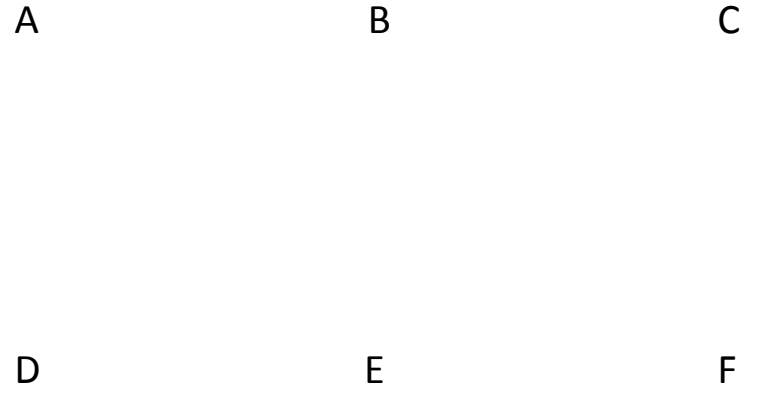

Figure 57. Double Creek West viral plaque assay. **(A)** Day 2. **(B)** Day 5. **(C)** Day 6. **(D)** Day 8. **(E)** Day 10. **(F)** Day 16. Viral plaques were detected on day 6. The mixed marine cyanobacteria culture began to lose the rich green pigment on day 10. The plate showed a majority of clearance on day 16.

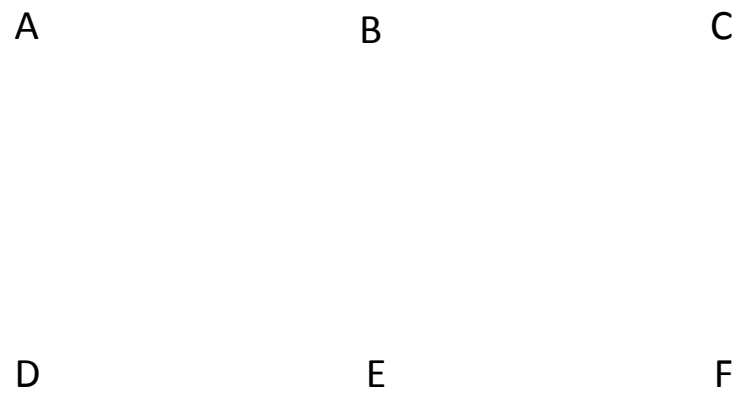

Figure 58. Westeconk West viral plaque assay. **(A)** Day 2. **(B)** Day 5. **(C)** Day 6. **(D)** Day 8. **(E)** Day 10. **(F)** Day 16. Viral plaques were detected on day 6. Plaques began to fuse by day 10 until there was about 60% plate clearance on day 16.

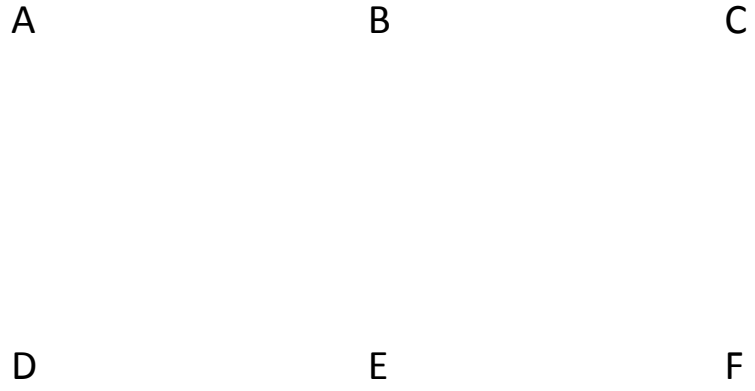

Figure 59. Westeconk East viral plaque assay. **(A)** Day 2. **(B)** Day 5. **(C)** Day 6. **(D)** Day 8. **(E)** Day 10. **(F)** Day 16. Viral plaques were detected by day 6. On day 10, it was evident that the mixed cyanobacteria culture was losing the rich green pigment. The plate showed about 80% clearance by day 16.

Figure 60. Site map of the 16 Barnegat Bay sites summarized with detected species using PCR, microscopy, and flow cytometry. Of the 16 sites analyzed, Tuckerton West was the only site which provided a complete profile of the types of species analyzed using the three methods. This figure shows the diversity among the sites ranging from Northern locations to Southern locations as well as Eastern and Western locations.

Sixteen sites obtained from various locations throughout Barnegat Bay were collected and used for the analysis of algal-bloom causing cyanobacteria and species of marine diatoms and dinoflagellates. Collected water samples were filtered through a filter with a 100 µm pore size and again through a fine filter with a 0.45 µm pore size. The 0.45 µm were dried and hole punched to be used for DNA extraction using a Chelex® extraction method (Chu and Rienzo, 2013). The DNA extracted for each site was used for polymerase chain reaction (PCR) assays. The primer sets used for PCR were both general and specific. General primers detected universal bacteria, cyanobacteria, and oxygenic phototrophs. Specific primer sets were used in the detection of species of cyanobacteria such as *Synechococcus*, *Prochlorococcus*, *Anabaena*, and *Anacystis nidulans* as well as other phytoplankton such as diatoms and dinoflagellates. The same fine filter was hole punched in various locations again and used for microscopic observation as well as flow cytometry. Microscopic imaging analysis with a phase contrast microscope and a compound light microscope was used to identify the cyanobacteria and other phytoplankton within the water samples. Flow cytometry was useful in understanding the cell size and granularity of the cells within the samples as well as the detection of the percentage of phycocyanin-containing cells for each site. The results of the three methods combined provided an initial outlook of the 16 sites which is summarized in Figure 60 to better understand the species of algal-bloom causing microorganisms in the Barnegat Bay estuary.

Discussion

The study purpose of the study is to conduct pilot experiments to detect and identify species of marine phytoplankton that are capable of causing algal blooms in Barnegat Bay, New Jersey. Water samples from 16 sites were obtained from varying locations throughout Barnegat Bay that ranged from northern to southern latitudes as well as western and eastern longitudes. The northern sites were considered as Metedeconk West, Metedeconk East, Silver Bay West, Silver Bay East, Toms River West, Toms River East, Forked River West, and Forked River East listed in order from northernmost to southernmost, respectively. The southern sites were considered as Double Creek West, Double Creek East, Harvey Cedars West, Harvey Cedars East, Westeconk West, Westeconk East, Tuckerton West, and Tuckerton East listed in order from northernmost to southernmost, respectively. Barnegat Bay is a lagoonal estuary which contains a mix of freshwater and salt water. The northern sites were expected to contain a higher ratio of freshwater when compared to the southern sites that were located nearest the area where the bay leads into the ocean. In addition, the western sites were also believed to contain a higher ratio of freshwater than the eastern locations due to the many rivers and streams that originate inland on the western coast of the bay. Table 4 shows the water properties during collection which includes salinity, temperature, and dissolved oxygen. The data from the table shows that the southern sites have higher salinities that range from 28.6 to 30.7 ppt than the northern sites that range from 15.2 to 28.6 ppt. In comparison to the western sites, the salinities of the eastern sites were higher for each site with similar latitude, suggesting that the southern sites and eastern sites contain a higher mixture of salt water than the northern and western sites.

In May 2013, the United States Environmental Protection Agency's Office of Water issued "Impacts of Climate Change on the Occurrence of Harmful Algal Blooms." The article

suggests that impacts of climate change, such as changes in salinity, promote the growth and dominance of algal bloom-forming species, such as phytoplankton. There are species of toxin-producing cyanobacteria, those which cause HABs, which are able to tolerate environments with high salinity. However, salt stress can lead to leakage of cells that results in the release of toxins that are harmful to humans, fish, and other organisms exposed in the habitat (EPA, 2013). In the open ocean, the salinity of seawater remains constant around 35 ppt. As salinity increases to levels higher than 35 ppt, chemical conditions within the estuary are affected, particularly dissolved oxygen solubility which decreases during high salinity conditions ("Salinity", 2008). Therefore, understanding more about the algal-bloom causing organisms in the high salinity environments can be beneficial in preventing their proliferation and toxin release that can lead to further detrimental effects in the ecosystem.

An additional factor of the water chemistry during collection of the samples was temperature. Temperature effects microbial diversity directly and indirectly. During the summer months when temperatures are high, levels of evaporation are increased, thus increasing salinity. Increased salinity affects water chemistry, decreasing dissolved oxygen levels that will also affect pH and result in hypoxic conditions ("Salinity," 2008). In past studies, temperature was observed to regulate the multiplication rate of natural phytoplankton populations (Alam *et al.*, 2001). In addition to multiplication, many phytoplankton cellular processes depend upon temperature as it was observed that rates accelerated exponentially as temperature increased. The maximal rate values of cellular processes occurred between 25 and 40°C. Cyanobacteria species grow optimally at temperatures greater than 20°C; while optimal temperatures for other phytoplankton groups tend to be lower (Robarts and Zohary, 1987). Table 4 shows the water temperature at the time of sample collection which shows a narrow variance in temperatures

among all of the sites. Temperatures ranged from 26.1 to 29°C. Interestingly, Forked River West, a site located near the center of the bay, has the highest temperature among the 16 sites. Forked River West is also one of the 16 sites that contains the most diversity. Further research as to why this temperature increase exists discovered that the Oyster Creek Nuclear Generating Station is located in Lacey Township, nearest Forked River West (BBP, 2011). The plant is the oldest operating plant in the United States, posing many ecological threats to Barnegat Bay due to the plant's cooling system. As a result of the cooling system, the bay is exposed to thermal pollution caused by the plant's discharge of water at significantly higher temperatures (Dutzik & O'Malley, 2010). Figure 46 shows the cell count in cells/µl of each site determined by flow cytometry. Interestingly, Forked River West has a cell count of 1.119×10^7 cells/ml, a number that is exponentially larger than the remaining 15 sites which range between 6.2×10^4 and 2.115×10^7 cells/ml. Additional flow cytometry data that analyzed the cell size and cell granularity of each site, shown in Figures 47 and 48, found that Forked River West, although high in cell numbers, showed smaller cell sizes and granularities when compared to the other sites in Barnegat Bay. This data indicates that Forked River West contains small cells with little complexity in their shape, similar to species of unicellular bacteria and cyanobacteria that are typically smaller in size (~2 µm) when compared to other species of phytoplankton such as diatoms and other algae (20-200 µm) depending on the species (Veldhuis & Kraay, 2000). Aside from the data obtained from the flow cytometer, the variation of species detected by PCR and microscopy does not appear to be affected by the differences in temperatures between the 16 sites.

The three methodologies utilized in the present study were PCR-based assays, microscopic observations, and analysis using a flow cytometer. PCR-based assays were useful in

the detection of phytoplankton on a molecular level which allows for greater specificity in not only identifying general types of microorganisms, but identifying organisms within the water samples on a species level. Although PCR proved to be a fast and efficient method in this study using samples of mixed DNA, the utilization of this method has its limitations due to the risk of many factors that could de-sensitize the results. Limitations of PCR include the need for troubleshooting the amounts of DNA or primers included in the PCR reactions. Additional limitations include applying the optimum melting temperature for primers as well as applying the proper number of cycles needed for the PCR reactions in the ThermoCycler. Human error and the risk of contamination while preparing the PCR tubes are two additional limitations to the PCR method. Microscopic analysis is a less efficient method compared to the other two in terms of time consumption; however it is beneficial in directly identifying cell morphologies with few factors that can interfere with the study, such as human error. Flow cytometry assays proved to be a useful method for this study to provide additional information for each site regarding the cell quantification, cell size, and cell granularity. With additional knowledge and experience of the flow cytometer and its applications to environmental samples, this method will provide significant insights into the phytoplankton profile of Barnegat Bay as well as increase the speed and efficiency of the generation of results. Although each method had its own drawbacks, when the three were combined they provided an understanding and appreciation of the ubiquity and diversity of phytoplankton species within Barnegat Bay.

PCR was useful in identifying species of phytoplankton from a mixture of DNA extracted from the fine filters of each water sample. PCR is a molecular technique which provides high specificity to identify species based off of specific DNA sequences that are able to be identified using general and specific sets of primers. The common gene shared among phototrophs is the

small-subunit ribosomal RNA (16S rRNA) gene that is also referred to as a housekeeping gene because they are utilized for basic cellular functions and maintenance. General and specific primers such as the PSf/Ur, 27Fb/785R, OXY107F/OXY1313R, CYA359F/CYA781R, rpoC1-39F/rpoC1426R primer sets identified the 16S rRNA gene present in universal bacteria, cyanobacteria, and *Synechococcus* species (Stiller & McClanahan, 2005, Fuller *et al.,* 2003, Kim *et al.,* 2004, Nübel *et al.,* 1997, Muhling *et al.,* 2006). Additional primer sets were identified in previous studies to detect other types of general and specific species such as *Prochlorococcus*, diatoms, and dinoflagellates. These primer sets included cpc1F/cpc1R, DINAf/DINAr, AN3801f/AN3801r, ANAf/ANAr, dinoSLg-F/dinoSLg-R, DANAT1F/DANAT1R, and 528f/650r which were used to identify specific sequences among the different species of phytoplankton (Robertson *et al.,* Lavin *et al.,* 2008, Chu & Rienzo, 2013, Zhang *et al.,* 2009, Song & Ward, 2007, Baldi *et. al.*, 2001). As shown in tables 7 through 11 and summarized in Figure 60, PCR was an efficient method in detecting several types of marine phytoplankton in each of the 16 sites.

The PCR-based assays were conducted twice for each primer set using two sets of Chelex® DNA extractions from the fine filters for each site. The fine filters were hole-punched in random, varying locations around the filter to obtain data from random distributions of populations throughout the filter. From an ecological perspective, the method for PCR assays used in this study is used to better understand the biodiversity within each sample. Classical measures of biodiversity include quantification of the number of species (species richness), total abundance, and evenness, which determine the proportion of each species within a community. An additional method of measuring biodiversity is the geometric mean of relative abundance. The use of the geometric mean of relative abundance has many advantages because it is not

prone to bias if detectability varies by species, it reflects a trend in evenness, measures trends in abundance, and can combine different surveys to monitor biodiversity (Buckland *et al.,* 2011). The geometric mean could therefore be beneficial and applicable to the study to evaluate the current status of biodiversity in Barnegat Bay as well as any changes that may occur in biological diversity.

Flow cytometry was useful in analyzing different aspects of the water samples. Using the forward scatter (FSC) settings, cell sizes were obtained and compared between sites. Side scatter (SSC) was also utilized to determine cell granularity and complexity. The flow cytometer also provided the cell count in cells/µl to provide a better understanding of the abundance of cells present in each sample. Collectively, the data provided by the flow cytometer helped construct a profile of each of the 16 sites and more information about the cells present in the samples. Flow cytometry was also utilized to detect the types of species, specifically phycocyanin-containing cells. Detection of phytoplankton using flow cytometry was carried out by exploiting the presence of fluorescent pigments, such as phycocyanin, of cyanobacteria ("Flow cytometry", 2014). Green fluorescent protein, GFP, is a biological fluorophore that is used as a gene expression reporter for various research applications. A derivative of GFP is phycocyanin, the pigment found in blue-green algae. A 488 nm laser was used for excitation and emission to detect the presence of phycocyanin-containing cells (Snider, 2014). The control used for this study was the *Synechococcus* sp. *S.* IU 625. Each site was compared to the control and it was found that all sites contained over 50% phycocyanin-containing cells. Therefore, the sites in Barnegat Bay contain an abundance of cells that are able to carry out photosynthesis, thus having the capability to contribute to the formation of algal blooms.

The final study of the Barnegat Bay sites was to detect the presence of cyanophage within the water samples using viral plaque assays. Earlier reports of marine cyanophages suggest that the viruses are able to regulate primary productivity as well as phytoplankton community structure. The abundance of ocean viruses affects the evolution of microbial communities in a marine ecosystem through host mortality (Sullivan *et al.,* 2010). The population regulation of phytoplankton by phage could influence the flux of nutrients and energy within an ecosystem (Wilson *et al.,* 1993). Currently, little is known about marine cyanophage and their characteristics and mechanisms. However, by concentrating the filtrate from each water sample in Barnegat Bay, the viral lysate was utilized in viral plaque assays to detect the presence of marine cyanophage in Barnegat Bay. Figures 53 through 59 show images of viral plaque assays of viral lysate obtained from six different sites in Barnegat Bay. The assay was monitored over a 16 day period, and cyanophage presence was detected by day 6 in all sites that were analyzed. The viral lysate caused more than 70% plaque clearance of each plate, indicating an actively lysing virus(es) that was/were specific to one or more of the cyanobacteria species within the mixed cyanobacteria culture used in the viral plaque assay. Detection of the marine cyanophage(s) within the Barnegat Bay samples is significant because the cyanophage can be used as a natural method to control phytoplankton populations, thus reducing the risk of algal bloom formation (Lee *et al.,* 2006). The possibility of phage treatment to Barnegat Bay is a safer, natural alternative to chemical means of cleaning the bay that may cause harm to the ecosystem.

Future Studies

To detect and identify more of the phytoplankton species within Barnegat Bay, several methods and techniques can be altered and improved. PCR conditions could be improved by optimizing PCR conditions by increasing the amount of DNA used in the PCR reaction as well as the concentration of DNA using purer extracts. More species-specific primers must be used to identify phytoplankton on a species level to identify the organisms capable of causing algal blooms or those which release toxins and form HABs. By successfully identifying the species of phytoplankton that cause algal blooms, more can be understood about the conditions that cause their proliferation by monitoring water conditions, such as temperature.

Flow cytometry was useful in learning different aspects of the water samples; however the method should be utilized in future studies through the employment of antibodies to detect various cyanotoxin, such as microcystin. Microcystins are produced by several genera of cyanobacteria, including *Anabaena*, and are responsible for many acute poisonings through contamination of drinking water supplies ("Microcystins & Other Cyanobacterial Toxins," 2014). The use of control cultures to develop profiles of phytoplankton will make it beneficial when analyzing unknown environmental samples from Barnegat Bay on the flow cytometer.

The New Jersey Department of Environmental Protection (NJDEP) and Governor Chris Christie issued a comprehensive action plan to assess the ecological health of Barnegat Bay and begin the steps to improve the conditions of the bay's ecosystem. One of the goals of the action plan is to shut down Oyster Creek Nuclear Power Plant, which causes thermal pollution of the bay. Future studies of Barnegat Bay will contribute to the improvement and survival of Barnegat Bay and serve as an indication that the action plan is successful.

Future studies should also include comparison of the Barnegat Bay site variations between seasons to assess variations in climate and the effects on the distribution of phytoplankton populations. An additional study that will be beneficial to the profiling of the bay is to compare the water samples prior to Hurricane Sandy and those obtained post Hurricane Sandy.

There is still much to be identified and learned about Barnegat Bay, therefore further research and repeatings must be conducted in order to produce a method or program to anticipate algal blooms prior to their formation in hopes that treatments can be developed to prevent algal bloom formation from occurring.

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