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Assessment of Phytoplankton Assemblages and Harmful Algal Blooms in New Jersey

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ASSESSMENT OF PHYTOPLANKTON ASSEMBLAGES AND HARMFUL ALGAL BLOOMS IN NEW JERSEY

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Abstract

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by Yaritza Acosta Caraballo

Urbanization, industrialization, and climate change have played a major role in the pollution of waterways, leading to a global increasing trend of harmful algal blooms (HAB) while jeopardizing water quality. Phytoplankton and HAB were evaluated within the highly urbanized and historically polluted state of New Jersey to help provide statewide baseline data for HAB and water quality management. A total of three studies were included in this dissertation. In the first study, phytoplankton communities were characterized in freshwaters of New Jersey during the cyanobacterial HAB season and their relationships to water quality at both statewide and ecoregion levels were examined. This information was critical since there existed little knowledge of freshwater phytoplankton in New Jersey. Results showed that cyanobacteria were present in most of the selected waterbodies with urbanized ecoregions having higher nutrients and cyanobacteria. Furthermore, results showed that the fluorescence of phycocyanin could be used as a proxy for cyanobacterial HAB conditions. Continuous cyanobacterial HAB monitoring efforts should extend to include colder seasons to help improve management strategies. The second study documented cyanobacteria and cyanotoxins in selected source waters of five New Jersey drinking water treatment plants. Results showed that cyanobacteria were present in all source waters along with high total phosphorus concentrations exceeding the New Jersey Surface Water Quality Standards, and suggesting these waters are susceptible to future cyanobacterial HAB events. Active monitoring of New Jersey source waters is crucial to lessen the public

health's risk of exposure to cyanobacterial HAB. Lastly, in the third study, water quality and HAB were evaluated along the five rivers and two bays across the Hudson-Raritan Estuary (Estuary). Results showed that most rivers in the Estuary have more favorable water quality conditions for phytoplankton and HAB to grow. To improve management strategies of the Estuary, focus should be placed on addressing water quality and pollution in these rivers and bays while conducting long-term monitoring. Overall, the results of this study provide insight into the statewide phytoplankton and HAB conditions as an attempt to address eutrophication factors and water quality degradation in the highly urbanized state of New Jersey.

Keywords: phytoplankton, New Jersey, water quality, harmful algal blooms, cyanotoxins, management

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Chapter 1: Introduction

Phytoplankton are commonly described as microscopic photosynthetic organisms that drift in water and have the ability to affect many ecosystem processes such as nutrient cycling, energy flow, and food web dynamics (Santhanam et al., 2019). These organisms have been documented to occupy a wide variety of habitats and salinity gradients, from freshwater to saltwater (Harris, 2012). Due to their role as primary producers, they comprise an important part of aquatic ecosystems and have been estimated to generate half of the atmosphere's oxygen (Falkowski, 2012; Paerl & Justić, 2011; Reynolds, 2006). They play a crucial role in the Earth's carbon cycle since, through the process of photosynthesis, phytoplankton transform inorganic carbon in the atmosphere and water into organic compounds (Reynolds, 2006). While phytoplankton are critical to biogeochemical cycles, some can form blooms and impact humans and other organisms (Chorus & Welker, 2021). Phytoplankton are capable of forming algal blooms when their biomass concentration increases through biological and physical processes (Shumway et al., 2018). Some taxa such as *Aphanizomenon*, *Dolichospermum*, *Nodularia*, and *Cylindrospermopsis* have the capability to fix nitrogen and access nitrogen supplies in nitrogen-limited conditions, providing a competitive advantage over non-nitrogen fixing phytoplankton (Cottingham et al., 2015).

1.1 Harmful Algal Blooms

While cyanobacterial blooms have existed for billions of years, there is a documented increasing global trend in cyanobacterial dominance over the past 200 years, with the most rapid increase occurring after 1945 (Taranu et al., 2015). Harmful algal blooms (HAB) occur when phytoplankton grow in excess, become dominant, and cause harmful effects on people, flora, and

fauna (Sellner et al., 2003). HAB can produce toxic effects through toxin production of some phytoplankton species, such as cyanobacteria (cyanotoxins), dinoflagellates (dinotoxins), and diatoms (diatom toxins) (Granéli, 2006). Furthermore, algal biomass overabundance can have non-toxic effects through increased turbidity, blocking out sunlight needed for other organisms to grow, and clogging fish gills which can lead to respiratory failure (Bruno et al., 1989; Meriluoto et al., 2017; Potts & Edwards, 1987; Weiss et al., 2016). Anoxia can also occur when oxygen consumption is greater than production due to decomposition of large amounts of biomass and organic matter (Zingone & Wyatt, 2005). When the algae die and decompose, they consume oxygen in the water leading to hypoxic or anoxic conditions, further affecting fish and bottom-dwelling organisms (Lester et al., 2016; Weiss et al., 2016). Hence, HAB can have deleterious effects on aquatic environments, affecting food webs, habitat and trophic structures, ecological integrity, and ecosystem services (Havens, 2008; Kleinteich et al., 2012). Adverse impacts on wildlife include but are not limited to illness and death (Anderson et al., 2012). Cyanobacteria such as *Aphanizomenon*, *Cylindrospermopsis*, *Dolichospermum*, *Microcystis*, *Nodularia*, and *Planktothrix* are commonly reported as dominating cyanobacterial HAB (Huisman et al., 2018).

1.1.1 Economic Impacts

In addition to human health and ecological impacts, HAB also produce substantial economic impacts. However, quantifying the true economic impact of HAB is difficult due to complications when estimating their effects on human health and ecosystem services (Kudela et al., 2015). Impacted human activities that can be quantified include tourism, fisheries, drinking water supply, health care, desalination plants, aquaculture, etc. (Kudela et al., 2015). Other costs relating to HAB monitoring, mitigation, and research are important to estimate and should be

included in the operation budget since they help reduce annual economic losses (Kudela et al., 2015). Annual expenses related to HAB impacts on US freshwaters have been estimated at $4.6 billion in 2014; costs used to estimate this number included those related to recreational activities, drinking water utilities, real estate, and recovery of endangered species (Bernard et al., 2014). These costs are expected to rise considerably due to inflation and the increasing global trend of cyanobacterial HAB. Other estimates of HAB have reported that global impacts on human health cost over $4 billion per year (Berdalet et al., 2016; GESAMP, 2001). Annual estimated losses related to HAB in the US have increased from $50 to $82 million dollars per year; costs are generally attributed to public health, commercial fisheries, tourism and recreation, and HAB monitoring and management (Anderson et al., 2012; Hoagland & Scatasta, 2006).

1.1.2 Ecological Effects

When compared to other phytoplankton groups, high cyanobacteria presence can further reduce the energy flow to upper trophic levels due to their poor nutritional value (Brett et al., 2000). During a HAB event, algae and cyanobacteria that have aggregated towards the surface block the sunlight that other aquatic organisms need to grow and survive (O'Hare et al., 2018). The reduction of light in the littoral zone can affect prey who need ample light for detecting predators, even influencing habitat choice and weight in some organisms such as larval pike *Esox lucius* (Engström-Öst & Mattila, 2008; Ferrari et al., 2010). Food web dynamics are further influenced by cyanobacterial blooms since they have poor nutritional value, produce toxins and can interfere with large zooplankton feeding (Vanni & Lampert, 1992). This in turn excludes large zooplankton and can lead to a shift in the community towards small zooplankton taxa such as cladocerans which have different food sources like picoplankton (Gilber, 2022; Karpowicz et al., 2020). Overall, heterotrophic biomass is decreased and the efficiency of transfer between

phytoplankton and zooplankton is reduced (Karpowicz et al., 2020; Moustaka-Gouni & Sommer, 2020). Furthermore, cyanobacterial blooms can negatively affect fish dynamics. In the presence of cyanobacteria dominated HAB, if the dominant fish do not feed on cyanobacteria, such as cyprinids, it can lead to a high quantity of cyanobacteria biomass present at the end of a bloom, which decays and releases dissolved organic carbon (DOC) (Moustaka-Gouni & Sommer, 2020). This availability of DOC increases bacterial growth leading to a shift in the carbon and energy sources of organisms atop the food web (Work et al., 2005). Overall, in the absence of cyanobacteria-feeding fish, the food web can extend and lead to a lower ratio of fish production to primary production (Sommer et al., 2002).

Cyanobacteria dominated HAB effects on waterbodies can fluctuate throughout the day. During the daylight, high photosynthetic activity by cyanobacteria can exhaust the dissolved CO_2 concentrations causing increases in pH and dissolved oxygen concentrations (Ibelings & Maberly, 1998; Verschoor et al., 2013; Vos & Roos, 2005). This diurnal increase in pH has been documented to impair chemoreception in aquatic organisms such as *Physa acuta*, affecting individual fitness (Turner & Chislock, 2010). At night, they switch to respiration and reduce water pH and dissolved oxygen concentrations which can cause kills of fish and invertebrates (Griffith & Gobler, 2020). When HAB die, microbial decomposition of cyanobacteria generates either hypoxic or anoxic conditions resulting in unsuitable environments for most organisms to survive, ie. Dead zones (Vos & Roos, 2005).

One of the world's largest dead zones is located in the Gulf of Mexico where benthic hypoxia can reach an area up to 23,000 km^2 (Rabalais & Tuner, 2019). Hypoxia in the Gulf of Mexico is promoted by strong temperature and salinity-driven stratification and decomposition of organic matter (Rabalais et al., 1991). This organic matter originates from the Mississippi

River and phytoplankton blooms which are promoted by river nutrients (Eadie et al., 1994; Turner & Rabalais, 1994). Agricultural nutrient pollution, urban runoff, and wastewater are the main contributors of excess river nutrients in the Mississippi River (Rabalais & Turner, 2019). Hypoxia/anoxia conditions have caused large mortality events due to lack of oxygen required for survival of some aquatic organisms (Rabalais et al., 2001a; Diaz & Rosenberg, 2008). Other organisms such as fish, crabs, squid, and shrimp, have been known to move towards higher oxygenated waters inland waters, deeper waters, or higher in the water column (Rabalais et al., 2001a). The organisms that can survive in low oxygenated conditions are then reduced which can ultimately affect ecosystem structure and function, ie. lower secondary production (Rabalais et al., 2001b). Additionally, hypoxia/anoxia can also affect human health through toxic blooms and the economy from fishery production losses (Diaz, 2001; Diaz & Rosenberg, 2011).

Due to these hypoxia impacts in the Gulf of Mexico, water quality improvement efforts have gone underway. While water quality has been improved in some areas of the Mississippi River through conservation practices, other areas experience increased nutrient loads (Rabalais & Turner, 2019). Initiatives like the Mississippi River Nutrient/Gulf of Mexico Hypoxia Task Force (2001) proposed to reduce the size of the hypoxic zone to 5000 km^2 or less in 5-years by 2015. However, this goal was not reached and, consequently, has been extended to 2035 with a 20% reduction of nitrogen and phosphorus loads in areas surrounding the Mississippi River (Mississippi River/Gulf of Mexico Watershed Nutrient Task Force, 2015). Overall, the dead zone in the Gulf of Mexico has not experienced a significant improvement as of 2019 and no solution is available to help create significant change (Rabalais & Turner, 2019).

1.1.3 Climate Change

Waterbodies worldwide are under threat due to the impending effects associated with climate change trends and increasing surface temperatures which are predicted to have long-lasting negative impacts on recreational activities and water quality (Woolway et al., 2020). HAB incidents have seen an increased trend over the past years in freshwater, brackish and marine systems, stimulated by anthropogenic nutrient pollution and climate change trends (Taranu et al., 2015). Similarly, New Jersey reports indicate that there has been an increase in frequency and duration of HAB events in recreational waters over the last four years (NJDEP, 2022a). Humans substantially contribute to the eutrophication of waters through fertilization of agricultural crops, industrial and sewage waste, etc. (Hamilton et al., 2016).

Climate change can be an additional catalyst for the expansion of HAB due to contributing factors such as increasing global temperatures, sea level rise, drought, and changing precipitation patterns (Paerl & Huisman, 2009; Paerl & Paul, 2012; Paul, 2008). At higher water temperatures, some cyanobacteria are capable of rapid growth since they have the capability to increase the abundance of photosynthetic proteins and shift state transitions (Mackey et al., 2013). Additionally, increased water temperatures can prolong thermal stratification which can decrease the presence of competing phytoplankton and increase internal phosphorus loading from sediments (McCarthy et al., 2007; Woolway et al., 2021). Studies have suggested that HAB can increase the water surface temperature of individual waterbodies, creating a positive feedback loop that favors cyanobacteria dominance (Paerl & Huisman, 2008). Moreover, increased water temperatures in the spring season have been associated with phytoplankton utilizing nutrients earlier which can facilitate nitrogen limitation and subsequently support diazotrophic cyanobacteria (Stal, 2009). Extreme rainfall events are predicted to increase in

frequency which increase runoff and therefore discharge large amounts of nutrients from anthropogenic sources into coastal and marine waters (Fong et al., 2020; Paerl & Huisman, 2009; Paerl & Otten, 2013; Paerl et al., 2011). Nutrient enrichment combined with increased residence time can lead to greater nutrient sequestration, cyanobacterial dominance, and reduced biodiversity (Paerl & Huisman, 2009; Paerl & Otten, 2013; Paerl et al., 2011). Moreover, rising sea levels, droughts, and freshwater usage have contributed to a global increase in salinization (Paerl & Otten, 2013). Freshwater cyanobacterial taxa that dominate HAB such as *Anabaena*, *Anabaenopsis*, *Microcystis*, *Nodularia*, and *Lyngbya* are salt-tolerant and are often found present in brackish waters (Moisander et al., 2002; Paerl & Fulton, 2006; Tonk et al., 2007; Whitton & Potts, 2007). This creates concerns for brackish water and marine organisms, and humans due to their possible exposure to greater cyanotoxin concentrations (Paerl & Paul, 2012). Excess nutrients lead to increased growth and dominance of these taxa in brackish water systems (Paerl & Otten, 2013). In addition, some cyanobacteria taxa such as *Cylindrospermopsis*, *Aphanizomenon*, and *Lyngbya* have been documented to expand their geographical range due to climate change trends (Conley, 2009; Padisak, 1997). *Cylindrospermopsis* can only grow and proliferate in warm waters above 20°C which indicates a possible link to global warming trends (Cirés et al., 2013). Similarly, some *Aphanizomenon* species possess akinetes which are suggested to be activated by temperature increases (Conley, 2009).

1.2 Health Impacts of Cyanotoxins

Cyanotoxins can be intracellular, i.e. produced and retained within the cyanobacterial cells, or extracellular i.e. when toxins are released during cell death and lysis (USEPA, 2015a-e). These toxins can be classified by their toxicological targets: the nervous system (neurotoxins), liver (hepatotoxins), both neurotoxic and hepatotoxic effects (cytotoxins), or the skin

(dermatoxins) (USEPA, 2015a-e). Toxins such as hepatotoxins (i.e., microcystins) and neurotoxins (i.e., anatoxin-a) can produce both acute and chronic health effects (Paerl & Huisman, 2009). For example, microcystin-LR can cause a wide range of health effects such as abdominal pain, diarrhea, vomiting, and pneumonia (USEPA, 2021). The neurotoxin anatoxin-a can cause effects such as numbness, drowsiness, and respiratory paralysis leading to death (USEPA, 2021). Further, some cyanobacterial taxa such as the commonly reported North American freshwater cyanobacterium *Dolichospermum* contains several species capable of producing a variety of toxins including anatoxin-a and saxitoxins (neurotoxins), microcystins (hepatotoxins), and cylindrospermopsin (cytotoxins) (USEPA, 2015a-e). Other cyanotoxins, such as the amino acid β-*N*-methylamino-L-alanine (BMMA), are of particular interest due to ongoing investigations of its possible health risk on human neurodegenerative diseases such as amyotrophic lateral sclerosis/parkinsonism dementia complex (ALS/PDC) (Ra et al., 2021).

Common recreational activities associated with HAB exposure include swimming, boating, bathing, water sports, etc. (Dietrich et al., 2008); humans can be exposed to cyanotoxins in recreational waters through the inhalation or ingestion of HAB-impacted water, or through skin exposure (Backer et al., 2010; Carmichael & Boyer, 2016; De Figueiredo et al., 2004; Hudnell, 2010; USEPA, 2021). Stewart et al., (2006) reported that cyanotoxin exposure through ingestion and inhalation of aerosolized toxins could produce respiratory symptoms, such as pneumonia. Furthermore, the ingestion of cyanotoxins could cause damage to internal organs such as non-alcoholic liver disease (Zhang et al., 2015). Skin exposure has been reported to cause skin irritation and allergies (Dionysiou, 2010). Among the most commonly reported symptoms related to cyanobacteria exposure are gastrointestinal symptoms, skin rashes, and fever (Stewart et al., 2006). Giannuzzi et al. (2011) reported a case of acute intoxication of a man

after being submerged in a recreational lake which had a bloom with 48.6 ug/L of microcystins. Symptoms after exposure ranged from abdominal pain, fever, and pneumonia to hepatotoxicosis and multiple organ failure (Giannuzzi et al., 2011). In a survey study of illnesses related to cyanobacteria dominated HAB events in 15 US states, results suggested that 3,194 illnesses occurred when cyanobacterial toxins were detected (Backer et al., 2015). Most of these illnesses occurred through ingestion of seafood that had been exposed to cyanobacteria dominated HAB and 176 events occurred due to recreational activity exposure (Backer et al., 2015). It is important to note that there are limitations in assessing recreational exposure to cyanobacteria and their toxins due to the variation of exposure in terms of intensity and duration, HAB toxicity, and cyanotoxins present and their concentrations (Koreivienė et al., 2014). In addition, reports on HAB exposure in recreational waters are limited due to their typically low sample size of exposed humans and their range of sensitivity to the cyanotoxins (Koreivienė et al., 2014).

Animal deaths have been reported within minutes to hours of exposure to cyanotoxins, with 368 verified cases of pet dog deaths in the US between 1920 and 2012 (Backer et al., 2015; Carmichael & Boyer, 2016; Foss et al., 2019). Microcystins have been associated with wildlife, cattle, and dog deaths (Foss et al., 2018; Puschner et al., 1998). Bengis et al. (2016) reported several mortality events of white rhinoceroses, zebras and wildebeest that occurred during confirmed HAB events with high microcystin concentrations. Sublethal effects of saxitoxins and anatoxin-a on *Daphnia similis* and *Daphnia magna* were documented to produce both physiological and behavioral changes, respectively, such as decreased swimming speed (Bownik & Pawlik-Skowrońska, 2019; Ferrão-Filho & da Silva, 2020).

1.3 Cyanotoxin Advisories

The World Health Organization (WHO) has proposed drinking water advisories for microcystin-LR at 1 µg/L for lifetime drinking water and 12 µg/L for short-term drinking water (WHO, 2020). Recreational guidelines of cyanobacterial mass indicator values from the WHO are categorized into three alert levels: vigilance level (1–4 mm^3/L biovolume or 1–12 µg/L chlorophyll a with dominance of cyanobacteria), alert level 1 (4–8 mm^3/L biovolume or 12–24 µg/L chlorophyll a with dominance of cyanobacteria), and alert level 2 (scum or transparency <0.5–1 m) (WHO, 2021). The WHO has also proposed recreational guideline values for the following cyanotoxins: microcystin (24 µg/L), cylindrospermopsin (6 µg/L), anatoxin-a (60 µg/L), and saxitoxin (30 µg/L) (WHO, 2021). The USEPA has developed drinking water health advisories (over a 10-day period) for cylindrospermopsin (0.7 µg/L for infants; 3.0 µg/L for adults and school-age children) and microcystins (0.3 µg/L for infants; 1.6 µg/L for adults and school-age children) (USEPA, 2015b, 2015c). In terms of recreational criteria for cyanotoxins over a 10-day assessment period, the USEPA has proposed total microcystins of 8 µg/L and cylindrospermopsin of 15 µg/L (USEPA, 2019). Within the US, states such as Ohio, Pennsylvania, Oregon, Minnesota, and Vermont, among others, have adopted their own guidance levels or regulations for cyanotoxins (USEPA, 2023). For example, Ohio set recreational advisory thresholds for microcystins (8 µg/L), cylindrospermopsin (15 µg/L), anatoxin-a (8 µg/L), and saxitoxins (0.8 µg/L) (ODH, n.d.). Cyanotoxin testing is required in Ohio drinking waters with advisories for microcystins (0.3 µg/L for infants and people at higher risk; 1.6 µg/L for pets, livestock, and all people), cylindrospermopsin (0.7 µg/L for infants and people at higher risk; 3.0 µg/L for pets, livestock, and all people), anatoxin-a (0.3 µg/L for infants and people at higher risk; 1.6 µg/L for pets, livestock, and all people), and saxitoxins (0.3 µg/L for infants and

people at higher risk; 1.6 µg/L for pets, livestock, and all people) (ODH, n.d.). The Pennsylvania Department of Environmental Protection has an advisory system for recreational activities with two response levels: advisory and avoid contact (PE DEP, 2023). An advisory level has thresholds set for microcystins (8 µg/L), cylindrospermopsin (15 µg/L), anatoxin-a (80 µg/L), saxitoxins (0.8 µg/L), and cyanobacteria colony density (300 cells/mL). The avoid contact level thresholds include microcystins (20 µg/L), cylindrospermopsin (20 µg/L), anatoxin-a (300 µg/L), saxitoxins (3.0 µg/L), and cyanobacteria colony density (1,500 cells/mL) (PE DEP, 2023). Moreover, drinking water testing is optional in Pennsylvania and advisories are based on the US EPA values.

The New Jersey Department of Environmental Protection (NJDEP) began implementing their HAB response strategy in 2017 (NJDEP, 2021). The recreational thresholds for freshwater HAB were based on cyanobacterial cell densities (>20,000 cells/mL) and/or microcystins (3 µg/L), cylindrospermopsin (8 µg/L), and anatoxin-a (27 µg/L) concentrations (NJWSA, 2019). In 2021 the NJDEP updated the recreational guidance for HAB, implementing a six tier (none, watch, alert, advisory, warning and danger) system, each tier being designated with specific criteria and recommendations (NJDEP, 2022b). Beach closures and/or advisories were recommended when cyanotoxins exceeded new threshold concentrations of 2 µg/L, 5 µg/L, 15 µg/L, and 0.6 µg/L for microcystins, cylindrospermopsin, anatoxin-a, and saxitoxins, respectively (NJDEP, 2022b). In 2021, 63.6% (35 of 55 suspected) waterbodies surveyed in New Jersey had sites with a watch advisory or greater (NJDEP, 2022a). Of the 2021 reported HAB alerts, 70 were reported as Watch, 51 as Advisory, and 1 as Warning. Lastly, 12 waterbodies were confirmed with an alert level of Watch or above during December 2021 indicating the

persistence of HAB through winter months. Drinking water advisories in New Jersey treatment facilities are optional.

1.4 HAB Impacts on Water Treatment Facilities

Drinking water facilities that utilize surface water as a source experience a multitude of complications due to HAB including filter clogging, difficulty of cyanotoxin removal during high bloom events, and difficulty of cyanotoxin removal due to multiple cyanotoxins present (He et al., 2016). In addition to toxin production, highly-dense HAB can cause issues in both drinking and recreational waters through taste and odor issues with impacted water often being described as musty or earthy (Zamyadi et al., 2015). Most cyanobacteria produce compounds such as 2-methylisoborneol (MIB) and Geosmin, causing taste and odor issues and often significant economic cost (Srinivasan & Sorial, 2011; Burr et al., 2012). Drinking water consumers lose confidence in the water utilities to provide safe drinking water since they can detect taste and odor issues at a concentration as low as 10 ng/L (Zamyadi et al., 2015). Moreover, drinking water purveyors might encounter difficulties in supplying safe drinking water to their customers at a reasonable rate when high cyanobacterial densities and cyanotoxins are detected in their source waters. Seven drinking water sources in New Jersey were confirmed with an HAB alert level of Watch or above during 2021 (NJDEP, 2022a).

While treatments for cyanobacterial and cyanotoxin removal exist, the costs are high and can potentially jeopardize water quality due to the low cyanotoxin removal efficiency which could be as low as 60% (USEPA, 2015; Zamyadi et al., 2012). For example, in an Ohio water treatment plant, treatment using powdered activated carbon cost greater than $200,000 per month (Cheung et al., 2013). Due to the high cost associated with HAB treatment, drinking water source management strategies need to incorporate a range of assessments to reduce the

probability of cyanobacterial cell lysing and cyanotoxin release (Hitzfeld et al., 2000). This could help alleviate costs associated with cyanotoxin removal (Hitzfeld et al., 2000). Management strategies include but are not limited to dominant cyanobacterial taxa, relevant water treatment system, and cyanotoxin location inside the cell (intracellular) or within the water column (extracellular) (Westrick et al., 2010).

1.5 Objective

Characterizing cyanobacteria assemblages and cyanotoxins in freshwaters is crucial for management and monitoring of HAB. The overall goal of this dissertation is to provide much needed data in cyanobacteria and phytoplankton communities across New Jersey. Three case studies are included in chapters 2, 3, and 4:

Chapter 2: Summer Phytoplankton Assemblages and Harmful Algal Blooms in Freshwaters of New Jersey. Phytoplankton assemblages are of great importance as indicators of water quality. This study systematically surveyed waterbodies across New Jersey to document the distribution and occurrence of phytoplankton. Variations in phytoplankton communities among the five New Jersey ecoregions were also examined. The results of this study provide insight into the freshwater phytoplankton communities during the cyanobacterial HAB season and their relationship with water quality conditions in New Jersey. The manuscript of this chapter is currently under review by the Northeastern Naturalist journal.

Chapter 3: An Investigation of Cyanobacteria, Cyanotoxins and Environmental Variables in Select Drinking Water Treatment Plants in New Jersey. Upward trends of cyanobacterial Harmful algal blooms (HAB) events in drinking water sources are impacted by eutrophication. These cyanobacterial HAB have the potential to impact human health primarily through their possible cyanotoxins production. This study examined cyanobacteria and cyanotoxins in both

source and finished waters at five drinking water treatment plants in New Jersey. To our knowledge, this is the first study that examines cyanobacteria and cyanotoxins in drinking water sources of New Jersey and documents water quality parameters. This study suggests that drinking water sources in New Jersey are vulnerable to forthcoming cyanobacterial HAB.

Chapter 4: Phytoplankton Dynamics Across the Spatial and Temporal Gradients in the Hudson-Raritan Estuary. Estuaries supply many ecological, environmental, and economic benefits. However, worldwide trends for estuaries suggest a decline in water quality partly due to nutrient over-enrichment and eutrophication. This study documents spatial and temporal changes in phytoplankton and cyanobacterial communities in the Hudson-Raritan Estuary. This study documented the relationship between the environmental gradients along the river-estuary continuum, and phytoplankton and cyanobacterial composition.

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Chapter 2 Summer Phytoplankton Assemblages and Harmful Algal Blooms in Freshwaters of New Jersey

2.1 Introduction

Anthropogenic activities such as agriculture and industry can negatively impact waters through nutrient over-enrichment which can lead to the sudden overgrowth or bloom of phytoplankton such as cyanobacteria (Bergstrom & Jansson, 2006, Elser et al., 2009; Maberly & Elliot, 2012; Wolfe et al., 2001). Aside from water quality impacts, cyanobacterial harmful algal bloom (HAB) generally have detrimental effects on the economy and public health (Brooks et al., 2016; Hoagland et al., 2002; Hoagland et al., 2006; Sellner et al., 2003). These anthropogenic effects will be further worsened by climate change trends. The predicted increases of water temperature, duration of thermal stratification, and precipitation can promote HAB growth (Beardall & Raven, 2004; Elser et al., 2009; Griffith & Gobler, 2020; Richardson et al., 2018). Thus, phytoplankton and HAB are imperative to study due to increasing anthropogenic disturbances.

Cyanobacteria form an important component of aquatic foodwebs and of the global carbon and nitrogen cycles (Kriss, 1954; Buford et al., 2020). However, HAB are of great public health concern since they can produce cyanotoxins that have a wide range of harmful health effects to aquatic and terrestrial biota. These toxins can affect the nervous system (neurotoxins), liver (hepatotoxins), or the skin (dermatoxins) and produce symptoms such ranging from mild rashes to death (USEPA, 2019). HAB can vary greatly in toxin production, with some blooms that have reported undetectable cyanotoxin levels (Bolch et al., 1997). Moreover, eutrophication can disrupt aquatic ecosystems by altering food webs, nutrient cycles, resilience and regime shifts of lentic systems, and lowering biodiversity (Cook et al., 2018; Karpowicz et al., 2020; Cottingham

et al., 2015; Sterner & Elser, 2003). In eutrophic lakes, HAB form dense layers at the water surface which could restrict sunlight and create a shift in the phytoplankton community towards a dominance of cyanobacteria (Feuchtmayr et al., 2009). Shifts in the phytoplankton community could be influenced by several cyanobacteria taxa through buoyancy regulation and vertical migration, which allow for access to sunlight and nutrients (Brookes & Ganf, 2001; Overman & Wells, 2022).

Phytoplankton community composition can be influenced by both biotic and abiotic factors. Abiotic factors such as the geologic setting of an area impacts its land use and determines the hydrology of a system, e.g., an area with nutrient rich soils is often used for agriculture (Rimet et al., 2007). Hence, hydrologically and geologically similar waterbodies are likely to house similar compositions of phytoplankton (Abonyi et al., 2014; Rimet, 2009). The ecoregions concept, which classifies areas with similar environmental resources and ecosystems, is often applied while designing regional level integrated monitoring and management (Gerritsen et al., 2000; Omernik & Griffith, 2014).

New Jersey freshwaters, encompassing roughly 1,900 lentic waterbodies, experience HAB and cyanotoxins which can impact human health (Brooks et al., 2016; NJDEP, 2020). Report estimates show a 26% increase in confirmed blooms between 2019 and 2020 (NJDEP, 2020). However, this could reflect the slight increase in the total number of waterbodies sampled from 2019 (68) to 2020 (83) (NJDEP, 2020). Moreover, annual precipitation in New Jersey decreased between 2019 and 2020 from 51.55 in to 49.85 in, respectively, suggesting other environmental conditions possibly influenced this increase (ONJSC, n.d.).

To better protect public health from the effects of HAB, the NJDEP developed a five-tier alert index based on cyanobacterial cell count and/or cyanotoxin concentrations with suggestions

on recreational uses for affected waterbodies (NJDEP, 2020). A watch level indicates a suspected HAB through visual survey, or a confirmed HAB defined by a cyanobacterial cell count between 20,000 and 80,000 cells/mL and no cyanotoxins detected (NJDEP, 2020). According to the HAB alert index, a total of 47 (of 83) waterbodies surveyed were confirmed as a watch level or greater in 2020, while 102 (of 130) events were confirmed in 2021 (NJDEP, 2020). Furthermore, data suggests that HAB are recurring in a great number of freshwaters and remaining until the winter months (NJDEP, 2020).

To date, there are a limited number of studies whose objective focus on documenting information about select taxonomic groups in a few New Jersey waterbodies (Chu et al., 2013). A comprehensive phytoplankton community level study is lacking for New Jersey freshwaters. Thus, there is an urgency to document and make publicly available information on freshwater phytoplankton community assemblages in New Jersey. This study systematically surveyed waterbodies across New Jersey to document the distribution and occurrence of phytoplankton. Variations in phytoplankton communities among the five New Jersey ecoregions were also examined. An ecoregion analysis provides holistic ecosystem management strategies which might be more efficient than assessing individual waterbodies in the same ecoregion that are experiencing similar problems. This analysis helps integrate information from various stakeholders that are responsible for different resources in the same area which can ultimately help improve HAB forecasting. The objective extends to identify an environmental stressor that have the potential to trigger HAB in New Jersey aquatic ecosystems.

2.2 Materials and methods

A total of 110 water bodies were selected for this study (Figure 2.1). The selected study sites were either chosen randomly (14 out of 110 or 12.7%), as a part of the routine lake monitoring program by the New Jersey Department of Environmental Protection (NJDEP) (45 out of 110 or 40.9%), or as a part of the NJDEP's HAB response program (51 out of 110 or 46.4%). Study sites consisted of lakes (75 out of 110 or 68.2%), ponds (19 out of 110 or 17.3%), reservoirs (10 out of 110 or 9.1%), and rivers (6 out of 110 or 5.5%) in New Jersey. These waterbodies spread across 18 New Jersey counties, with Sussex (21 out of 110 or 19.1%), Passaic (14 out of 110 or 12.7%), and Morris (10 out of 110 or 9.1%) counties comprising the greatest percentage of waterbodies included in this study.

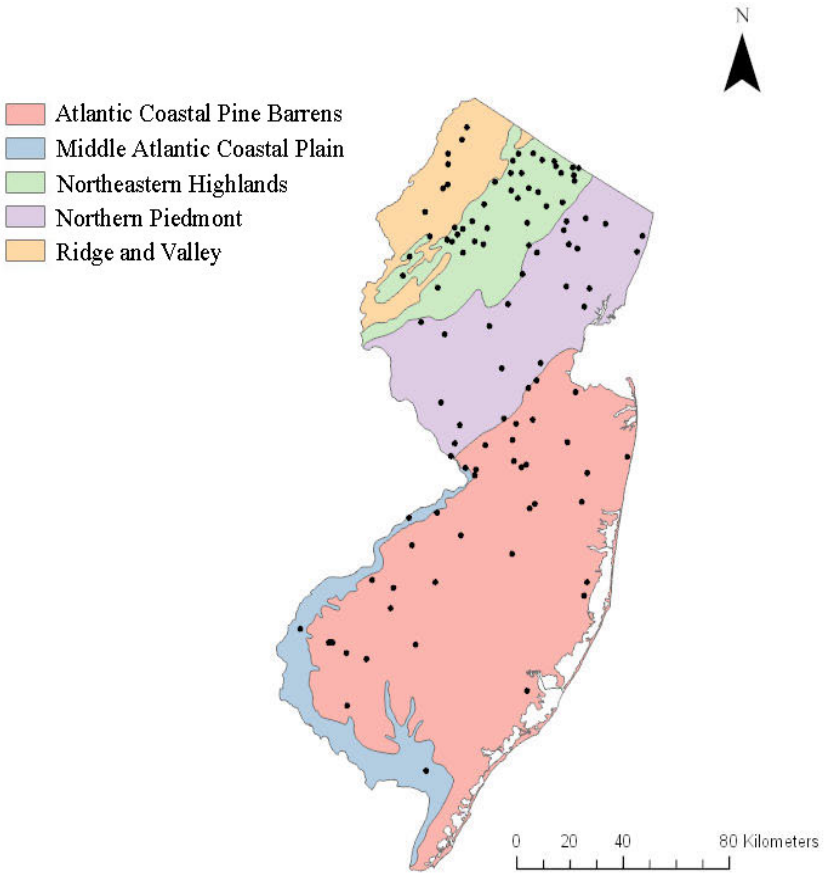

Figure 2.1 A total of 110 waterbodies were selected for this study across the five New Jersey ecoregions: Northeastern Highlands (n=37), Northern Piedmont (n=25), Ridge and Valley (n=7), Middle Atlantic Coastal Plain (n=4), and Atlantic Coastal Pine Barrens (n=37).

The U.S. Environmental Protection Agency (U.S. EPA) designated the State of New Jersey into five ecoregions: *Northeastern Highlands* (refer to as *Highlands*), *Middle Atlantic Coastal Plain* (refer to as *Coastal Plain*), *Northern Piedmont* (refer to as *Piedmont*), *Ridge and Valley* (refer to as *Ridge and Valley*), *and Atlantic Coastal Pine Barrens* (refer to as *Pine Barrens*) (Omernik & Griffith, 2014). The selected waterbodies were located in all five ecoregions: *Highlands* (37 out of 110 or 33.6%), *Pine Barrens* (37 out of 110 or 33.6%), *Piedmont* (25 out of 110 or 22.7%), *Ridge and Valley* (7 out of 110 or 6.4%), and *Coastal Plain* (4 out of 110 or 3.6%). The *Highlands* is composed of gneiss, limestone and shale and thus can have either

nutrient-poor or nutrient-rich soils ecoregion (U.S. EPA ORD, 2012). Streams in most of the ecoregion have been characterized as having higher calcium, magnesium, and bicarbonate ions and lower total suspended solids (TSS) due to low erosion rates (Anderson & George, 1966). However, TSS values are amongst the highest in the State towards the western portion of the ecoregion (90-250 ppm) in which streams are influenced by limestone and dolomite deposits (Anderson & George, 1966). Procopio & Zampella (2023) reported median nutrient concentrations in this ecoregion of dissolved organic carbon (DOC) (2.9 mg/L), phosphorus (0.018 mg/L), TN (0.923 mg/L). This ecoregion is characterized by forested, farmland and rural land types.

The *Coastal Plain* houses gravels, sands and silts largely dominated by wetlands and pine forests while streams have higher calcium, magnesium, and sulfate chloride (Anderson & George, 1966; U.S. EPA ORD, 2012). Streams in this ecoregion have highly variable TSS concentrations ranging from 10 to 500 ppm (Anderson & George, 1966). The study sites selected within the *Coastal Plain* ecoregion in this study were located in both the *Inner* and *Outer Coastal Plain* province reported in Procopio and Zampella (2023). Low nutrient concentrations have been reported for the *Inner* and *Outer Coastal Plain* provinces with median concentrations of 3.7 mg/L and 5.7 mg/L (DOC), 0.024 mg/L and 0.013 mg/L (phosphorus), and 1.4 mg/L and 0.6 (TN) (Procopio & Zampella 2023). The *Pine Barrens* study sites were also located in both the *Inner* and *Outer Coastal Plain* provinces, and have been characterized by low pH and low nutrients (mean 5.9) (Procopio & Zampella 2023). The *Pine Barrens* have widespread low nutrient soils, and is composed of clays, silts, marls, sands, gravels and shell beds (U.S. EPA ORD, 2012). Woodlands dominate and occur along with wetlands, agricultural and urban land use types. Streams are characterized by higher sodium potassium and sulfate chloride, and high

iron concentrations (10 ppm) due to groundwater influence (Anderson & George, 1966). Lower TSS values in this ecoregion are partially due to the high permeability of its soils (Anderson & George, 1966).

The *Piedmont* is comprised of shale, sandstone and argillite with highly fertile soils (U.S. EPA ORD, 2012). Streams are similar to the *Coastal Plain* with higher calcium, magnesium, and sulfate chloride (Anderson & George, 1966). TSS values are higher (ranging from 40 to 120 ppm) due to higher silt and clay concentrations present in the sediment and lower vegetation (Anderson & George, 1966). However, streams in this ecoregion that are located in the eastern portion, close to New York City, are characterized by higher percentage of chloride and nitrate ions, and have higher TSS (130-450 ppm) concentrations (Anderson & George, 1966). Some streams closer to New York City also contain higher iron concentrations (>1.0 ppm), possibly due to wastewater influence and decomposition of vegetation (Anderson & George, 1966). Median nutrient concentrations in the *Piedmont* province have been documented at 3.8 mg/L (DOC), 0.042 mg/L (phosphorus), and 1.47 mg/L (TN) (Procopio & Zampella 2023). Land use types that dominate this ecoregion are agriculture and urban land.

Ridge and Valley is a diverse ecoregion with low fertility ridge soils composed of sandstone and higher fertility valley soils composed of limestone, shale or glacial till (U.S. EPA ORD, 2012). The streams in this ecoregion have higher calcium, magnesium, and bicarbonate ions (Anderson & George, 1966). Lower TSS (30-90 ppm) concentrations characterize this ecoregion's streams due to both high runoff rates and low erosion rates (Anderson & George, 1966). Median nutrient concentrations in the *Ridge and Valley* province have been reported at 2.9 mg/L (DOC), 0.015 mg/L (phosphorus), and 0.8 mg/L (TN) (Procopio & Zampella, 2023). Ridges are dominated primarily by forest land type and valleys by agriculture and farming.

Ecoregion level III data was obtained from the U.S. EPA website and analyzed using the ArcGIS Pro software (U.S. EPA ORD, 2012).

2.2.1 Phytoplankton community

Selection of grab sample numbers and location were determined by the NJDEP following the state *Field Sampling Procedures Manual*, 125 mL water samples were collected in amber plastic bottles from up to four sampling locations per water body (NJDEP, 2005). Grab samples from multiple locations within a waterbody were not combined or average, they were each treated as a single sample. The frequency of sampling per waterbody was determined by the purpose of collection. Waterbodies within the randomized program were collected only once. Routine lake monitoring program samples were collected one to two times in each waterbody. Whereas waterbodies within the NJDEP's HAB response program were sampled up to 24 times. A total of 196 water samples were collected in summer (June) and early fall (October) between 2016 and 2019. Grab samples were collected at 0.5m depth in amber bottles, wrapped in foil, kept in the cold (4°C), and transported to a Montclair State University laboratory. Samples were then preserved in Lugol's Iodine solution and stored in cold and dark conditions.

For cell identification and enumeration, the unconcentrated samples were homogenized by carefully inverting the container 25 times prior to processing while reducing damage of phytoplankton structural features. For each replicate, there was 10µl of a sample that was pipetted into a hemocytometer chamber (Fisher Scientific 02-671-51B) and allowed it to settle for one minute prior to being observed under a compound light microscope (Fisher Scientific™ AX800). Due to the small pipette opening, this method might have resulted in bias towards phytoplankton with smaller cell sizes. The hemocytometer had three different sizes of counting

grids: small, medium and large. The grid was selected based on the dominant particle size and density. If a sample contained large, sparse cells, the large-size grid containing four large squares were used. On the contrary, if a sample presented small, dense cells, a small-size grid containing five small squares would be utilized. Cells with GALD (greatest axial linear dimension) of less than 2µm were not recorded. Cells were counted and identified at 400x to the genus level or the lowest taxonomic level possible using published freshwater phytoplankton identification materials including *Freshwater algae of North America: ecology and classification* (Wehr et al., 2015), *Cyanoprokaryota-1. Teil/Part 1: Chroococcales* (Komárek & Anagnostidis, 2008), *Cyanoprokaryota-2. Teil/Part 2: Oscillatoriales* (Komárek & Anagnostidis, 2008), and *Cyanoprokaryota-3. Teil/Part 3: Heterocytous Genera* (Komárek, 2013). For quality control, samples were counted in triplicates to account for natural variability. The triplicate results from each sample were averaged. Averages of all phytoplankton taxa (Phyto) and cyanobacteria (Cyano) only taxa were reported in this study. Percent cyanobacteria dominance per site was defined as the sum of cyanobacteria taxa divided by the total number of phytoplankton individuals in that site. To further study the distribution of each taxon, if a taxon was observed it was documented as present. Distribution was calculated by dividing the number of waterbodies in which the taxon was observed by the total number of waterbodies (110) examined in this study.

When a taxon was observed in a waterbody, it was considered as present in that waterbody. Frequency of presence for each taxon was calculated in this study by dividing the number of waterbodies present with the taxon by the total of waterbodies observed (110).

The Shannon Wiener Diversity Index (DI) was used to measure phytoplankton diversity within the phytoplankton community in each waterbody. The following formula was used to calculate the Shannon Wiener Index:

$$H = -\sum p_i \ln p_i$$

Where p_i = proportion of total sample represented by species $_i$

2.2.2 Water quality data

The water quality of 181 water samples were analyzed by the NJDEP. We explored water quality parameters commonly used to assess HAB conditions. Chlorophyll is essential to perform photosynthesis, present in all phytoplankton and commonly used as an indicator of primary production (Smyth et al., 2004; Zeng & Li, 2015). Chlorophyll-a (Chl-a) is the primary photosynthetic pigment for phytoplankton; Chl-a is commonly used as a proxy of phytoplankton primary production in freshwater ecosystems (Boyer et al., 2009; Cullen, 1982; Gregor & Maršálek, 2004; Riley, 1937). The following parameters were measured in situ using a multiparameter field meter: dissolved oxygen (DO), pH, water temperature (T), specific conductance (SPC) (HQ40D portable multi meter, Hach; Loveland, CO). The fluorescence of chlorophyll (Chl) and fluorescence of phycocyanin (PC) were measured in situ using a handheld fluorometer (CyanoFluor, Turner Designs; San Diego, CA). Secchi depth (Secchi) was measured in situ using a secchi disk. Laboratory analyses were conducted for the following parameters: total phosphorous (TP) (USGS-I-4650-03), total nitrogen (TN) (USGS-I-4650-03), alkalinity (Alk) (SM 2320 B-11), hardness (Hard) (SM 2340 C-11), and chlorophyll-a (Chl-a) (EPA 445.0). Toxin analysis for microcystins (MC), cylindrospermopsin, and anatoxin-a was

conducted using the Enzyme-Linked ImmunoSorbent Assay (ELISA), EPA method 546, an automated plate reader and ABRAXIS kits (NJDEP, 2022).

The water quality data of the 181 samples was included in the analysis of both statewide and ecoregion-specific environmental conditions to identify the environmental triggers of HAB. In order to help protect the public from the risks associated with cyanotoxin exposure, the NJDEP provides thresholds for toxins such as microcystins (NJDEP, 2020). This threshold was modified on February 2022 to 2 µg/L (NJDEP, 2022). Prior to this date, the NJDEP recreational microcystins threshold was 3 µg/L. This study used the 3 µg/L microcystins threshold since the samples used in the analysis were collected between 2016 and 2019. Microcystins concentrations below the detection limit of 0.15 µg/L were reported as undetectable. TP concentrations below the detections limit of 0.01 mg/L were reported as undetectable. TN concentrations below the detection limit of 0.1 mg/L were reported as undetectable.

2.2.3 Statistical analysis

Shapiro-Wilk's test showed that data was not normally distributed, thus we conducted non-parametric tests. Bivariate plots were used to discern relationships between water quality parameters and phytoplankton and cyanobacterial density. Spearman's Correlation was also conducted to test correlations between water quality parameters and phytoplankton and cyanobacterial density ($\alpha = 0.05$). The significance threshold was set at 0.05 and the Spearman's rank correlation coefficient was denoted by the letters rs. A multivariate statistical method, the Principal Component Analysis (PCA), was used to examine similarities between environmental variables and phytoplankton and cyanobacterial density ($\alpha = 0.05$). Significant differences between the means of water quality parameters and phytoplankton and cyanobacterial density in

different ecoregions and waterbody type were determined using a Kruskal-Wallis test ($\alpha = 0.05$). A Dunn's post hoc test was used to determine which means were significantly different. All statistical analyses were done using JMP Pro 15.

Cylindrospermopsins and anatoxin-a were excluded from statistical analyses due to the limited number of data points above the lower detection limit. If a detected concentration was below the parameter's lower detection limit, one half of the detection limit was used for the purpose of statistical analysis. For example, if microcystins concentrations were below its lower detection limit of 0.15 μg/L, the data was analyzed as 0.075 μg/L.

2.3 Results

2.3.1 Water quality conditions and HAB advisory in New Jersey

Due to the increasing incidence of HAB in New Jersey, a statewide analysis of cyanobacteria was performed. Cyanobacteria were found present in 87.2% of the water samples, with cell densities ranging from 150 to 11,730,000 cells/mL, and a medium density of 8,700 cells/mL. The median cyanobacterial dominance between all sites was 71.8%, with 62.8% of samples (124 of 196) having cyanobacteria dominance greater than 50%, and 5.6% (11 of 196) of samples at 100% dominance. Approximately 38.8% of samples (76/196) had cell densities in the 20,000 to 80,000 cells/mL range. Microcystins were detectable in 58.0% of samples (105/181) with a median of 0.22 μg/L (Table 2.1). According to the NJDEP recreational health advisory criteria, 13.2% (24/181) of samples analyzed between 2016 and 2019 were above microcystins thresholds demonstrating a potential health risk (NJDEP, 2020). Furthermore, yearly trends indicated that samples exceeded thresholds from 10.2% (12/118) in 2018 to 19.0.% (12/63) in 2019. Other

toxins analyzed were detected less frequently than microcystins. Anatoxin-a was detected in 11 samples with the highest concentration at 5 µg/L, while all cylindrospermopsin concentrations were below the lowest detection limit.

Water quality results indicated Chl-a concentrations of 0.60 to 152.80 µg/L with a median of 10.60 µg/L (Table 2.2). As cyanobacterial biomass increases, so do the fluorescence of phycocyanin (Brient et al., 2008). Results showed PC values ranging from 4.53 to 3,773.00 RFU (median 30.07 RFU). Nutrients, including nitrogen and phosphorus, fuel cyanobacteria growth in waterbodies leading to HAB. Statewide, New Jersey waterbodies were characterized by TP ranging from UD to 143.13 mg/L (median 0.02 mg/L), and TN concentrations ranging from 0.23 to 2.44 mg/L (0.39 mg/L). A total of 6.6% (12 of 181) of samples did not meet the New Jersey Surface Water Quality Standards (SWQS) for TP (Table 2.1). While not statistically significant, there was a decrease observed between samples that exceeded TP thresholds from 9.3% (11 of 118) in 2018 to 1.6% (1 of 63) in 2019. With increased nutrients leading to increased HAB frequency and duration, water quality will be affected through diel impacts of HAB on DO and pH. A total of seven (of 181; 3.9%) samples failed to meet the SWQS for DO. A greater percentage of samples (8.8%; 16 of 181) did not meet SWQS for pH. Water quality standards were not met in some waterbodies in New Jersey, indicating that the health of these waters should be protected.

Table 2.1. Frequency of samples (n=181 water quality; n=196 cyanobacterial density) that exceed water quality thresholds for both statewide and five New Jersey ecoregions: Northeastern Highlands (Highlands; n=67), Northern Piedmont (Piedmont n=34), Ridge and Valley (Ridge and Valley; n=35), Middle Atlantic Coastal Plain (Coastal Plain; n=4), and Atlantic Coastal Pine Barrens (Pine Barrens; n=41). Dissolved oxygen (DO), total phosphorus (TP), cyanobacteria cell density (Cyano), and microcystins (MC). Number of samples that exceed thresholds indicated in parenthesis.

	Statewide	Ecoregions				
		Highlands	*Piedmont*	*Ridge and Valley*	*Coastal Plain*	*Pine Barrens*
DO (mg/L)	1.7% (3)	4.5% (3)	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)
pH	9.9% (18)	9.0% (6)	0.0% (0)	22.9% (8)	0.0% (0)	9.8% (4)
TP (mg/l)	6.6% (12)	1.5% (1)	2.9% (1)	5.7% (2)	0.0% (0)	19.5% (8)
Cyano (cells/ mL)	40.3% (73)	28.4% (19)	61.8% (21)	14.3% (5)	50.0% (2)	63.4% (26)
MC (µg/L)	13.8% (25)	11.9% (8)	26.5% (9)	5.7% (2)	25.0% (1)	12.2% (5)

2.3.2 Statewide phytoplankton assemblages

Among the 196 samples collected between 2016 and 2019 from 110 New Jersey waterbodies, ninety-one (91) phytoplankton taxa were documented with medium density of 14,325 cells/mL (ranging from 300 to 11,730,000 cells/mL). The most diverse taxa groups were green algae (38; 41.8%), cyanobacteria (21; 23.1%), and diatoms (20; 22.0%). Less diverse taxa groups included golden algae (4; 4.4%), dinoflagellates (3; 3.3%), euglenoids (3; 3.3%), and cryptomonads (2; 2.2%). Statewide DI values ranged from 0.01 to 8.01 with a median value of 1.26. Phytoplankton composition was observed by waterbody type. Lakes had the highest percentage of diatoms and the highest median DI values at 1.30 (ranging from 0.02 to 3.70). Rivers had a median DI value of 8.01 (ranging from 0.06 to 8.01) and no golden algae were recorded present. Ponds had median DI values at 1.21 (ranging from 0.01 to 6.22) and the highest percentage of green algae.

The highest percentage of cyanobacteria was recorded in reservoirs, which exhibited the lowest median DI values at 1.11 (ranging from 0.11 to 3.65). No golden algae were recorded present in reservoirs.

The most widely distributed phytoplankton taxa were the cyanobacteria. Among all the cyanobacteria, the most widely distributed taxa were *Synechococcus* 74.5%*, Aphanocapsa* 40.9%*, Microcystis* 36.4%, and *Dolichospermum* 31.8% (Table 2.3). Green algae were also found widely distributed in New Jersey waters, with *Chlorella* (73.6%), *Chlamydomonas* (54.5%, *Selenastrum* 47.3%, *Scenedesmus* 45.5%, and *Oocystis* 40.0% among the most frequently seen (Table 2.3). Diatoms such as *Cyclotella* (21.8%), *Synedra* 18.2%, *Navicula* 16.4% were observed at a lower frequency than green algae and cyanobacteria (Table 2.3). Less distributed phytoplankton groups included cryptomonads (*Cryptomonas* (20.9%), and *Chroomonas* 10.0%), and euglenoids (*Phacus* (20.0%) and *Trachelomonas* 20.9%) (Table 2.3). Furthermore, the dinoflagellates (*Gymnodinium* (3.6%)), cryptomonads (*Dinobryon* (6.4%)), and golden algae (*Mallomonas* (11.8%)) observed in New Jersey waters were scarcely distributed and primarily documented in lentic systems (Table 2.3).

Phytoplankton composition was observed by waterbody type. Lakes were the most diverse waterbodies with eighty-three total taxa, comprised primarily of green algae, cyanobacteria, and diatoms (Figure 2.2). Ponds were the second highest diverse waterbodies with sixty-five taxa and the highest percentage of green algae of all waterbodies (Figure 2.2). Rivers had a total of thirty-five taxa present with the highest percentage of cyptomonads (Figure 2.2). Reservoirs were the least diverse waterbodies with thirty-nine taxa recorded. They showed the highest percentage of cyanobacteria (Figure 2.2). No golden algae were recorded present in reservoirs nor rivers.

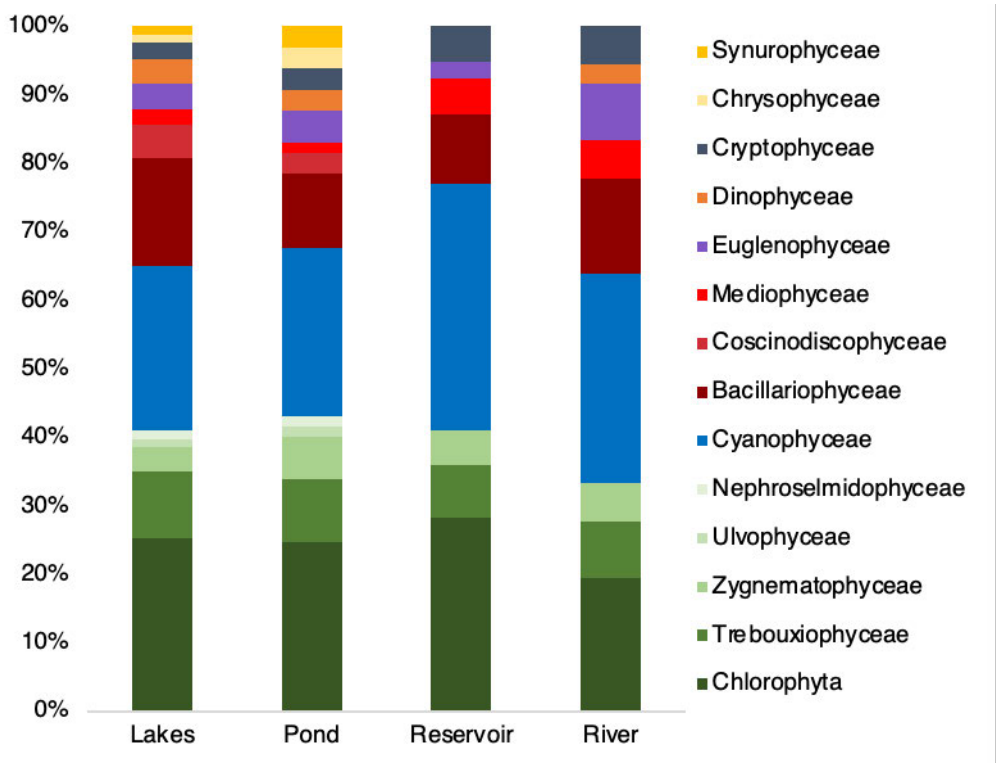

Figure 2.2. Phytoplankton composition among the four waterbody types, lakes (n=75), ponds (n=19), reservoirs (n=10), and rivers (n=6) in New Jersey from 2016-2019.

2.3.3 Water quality conditions and phytoplankton communities at five New Jersey ecoregions

The highest number of waterbodies were selected in the *Highlands* (37), *Pine Barrens* (37), and *Piedmont* ecoregions (25) (Table 2.2). Only four waterbodies were included within the *Coastal Plain* and 7 in the *Ridge and Valley*, thus results might not necessarily best represent the entire ecoregion. Forested land was the most dominant LULC statewide (28.7%) and for the *Pine Barrens* (26.0%), *Highlands* (54.0%), and *Ridge and Valley* (53.2%) (Appendix A). Whereas urban land types were prominent in the *Piedmont* ecoregion (53.4%) (Appendix A). The *Coastal Plain* is characterized by marsh (30.0%), urban (12.5%) and forest (11.0%) land types (Appendix A). A high percentage of marsh land type in this ecoregion could be influenced by the inclusion

of the Delaware Bay in the LULC analysis. Furthermore, the *Coastal Plain* ecoregion comprises a small and narrow area.

Waters exhibited DO values sufficient to support aquatic life in *Ridge and Valley*, *Pine Barrens*, and *Piedmont* (Table 2.2). The lowest median DO concentrations were observed in the *Coastal Plain* (Table 2.2). Waterbodies in the *Northeastern Highlands* ecoregions had samples that exceeded DO thresholds (Table 2.1). Samples that exceeded pH thresholds (4.5 to 7.5 for the Lower Delaware River Basin, Atlantic Coastal Basin, Lower Raritan River and Raritan Bay Basin, and 6.5 to 8.5) for all other freshwaters were observed only in the *Ridge and Valley*, *Highlands*, and *Pine Barrens* (Table 2.1) (N.J.A. C. 7:9B-1.14). Yearly trends in the *Ridge and Valley* ecoregion indicate that the percentage of samples exceeding pH thresholds decreased from 25.8% (8 of 31) in 2018 to 0% (0 of 4) in 2019. Median TP and TN concentrations were lowest in the *Highlands*, *Coastal Plain*, and *Ridge and Valley* (Table 2.2). The *Pine Barrens* and *Piedmont* were the ecoregions with the highest median TP and TN concentrations (Table 2.2). The highest single TP concentration was documented in the *Pine Barrens* from Deal Lake at 143.13 mg/L and corresponded with Cyano of 125,700 cells/mL and MC of 0.37 µg/L, indicating non-toxic bloom conditions occurred (Appendix B). Waterbodies in the *Highlands, Piedmont*, *Ridge and Valley*, and *Pine Barrens* ecoregions had samples that exceeded TP thresholds (Table 2.2). While not statistically significant, there was a decrease in TP samples that exceeded thresholds from 26.9% (7 of 26) to none in 2019 which could be influenced by a decrease in annual precipitation between these years (ONJSC, n.d.).

Green algae and cyanobacteria had the highest richness in each ecoregion (Figure 2.3). *Synechoccoccus* and *Chlorella* were frequently observed at all five ecoregions (Table 2.3). Phytoplankton taxa (32) and diversity (median DI 1.19; ranging from 0.78 to 4.77) were lowest

in *Coastal Plain* while the *Pine Barrens* had the highest number of taxa (72) and high median DI of 1.40 (ranging from 0.11 to 8.01) (Table 2.2). Furthermore, median Phyto and Cyano counts were low in the *Highlands* and *Ridge and Valley*, with corresponding low median Chl-a and PC values (Table 2.2). In contrast, high median Phyto and Cyano counts were recorded in *Coastal Plain*, *Piedmont*, and *Pine Barrens*, supported by high Chl-a and PC median values (Table 2.2). Waters in *Piedmont* had low MC median concentration, while *Northeastern Highlands* and *Coastal Plain* had concentration below the detection limit (Table 2.2). However, an MC concentration of 254.20 μg/L from Saddle River County Park in the *Piedmont* was the second highest of all samples in all ecoregions and corresponded with a high Cyano count of 11,730,000 cells/mL (Appendix B). According to the NJDEP recreational advisory, this waterbody would be classified as a Warning alert level due to the cyanotoxin content. This alert level indicates a high risk of adverse health effects to both humans and animals due to the high toxin concentrations.

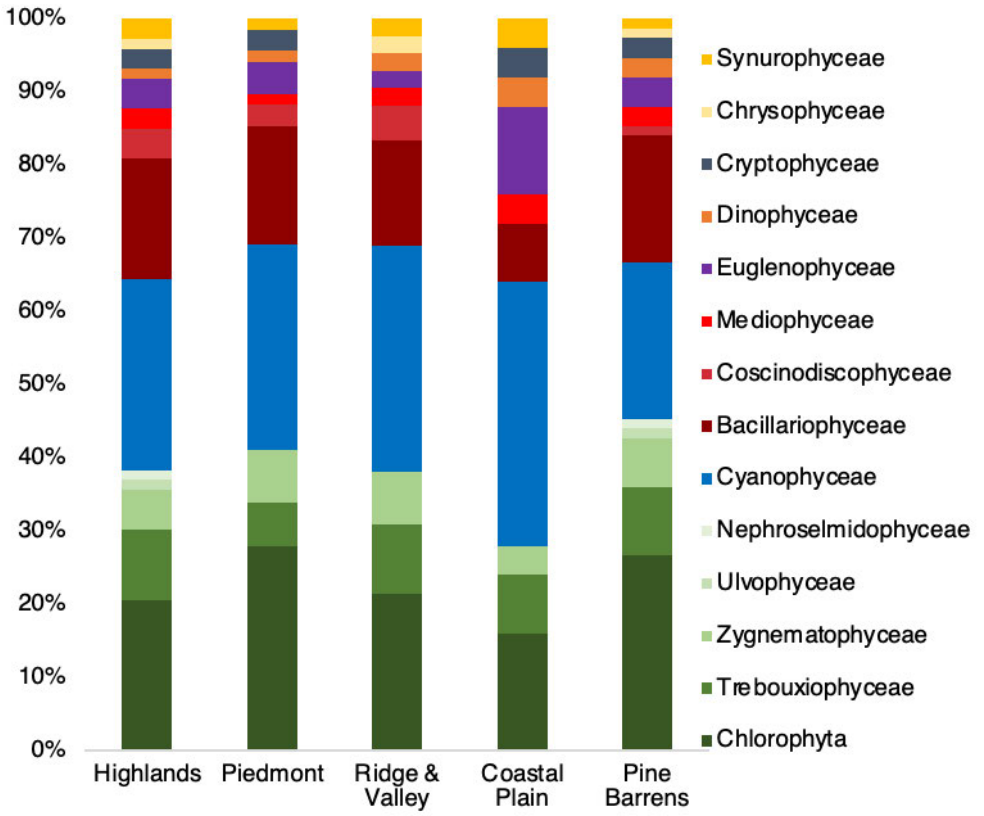

Figure 2.3. Phytoplankton composition among the five New Jersey ecoregions, Northeastern Highlands (*Highlands*) (n=37), Northern Piedmont (*Piedmont*) (n=25), Ridge and Valley (*Ridge and Valley)* (n=7), Middle Atlantic Coastal Plain (*Coastal Plain*) (n=4), and Atlantic Coastal Pine Barrens (*Pine Barrens*) (n=37) from 2016–2019.

One sample in the *Coastal Plain* from Amico Island Park Pond exceeded thresholds at 336.8 µg/L, which was the highest microcystin concentration of all samples included in this study (Table 2.2; Appendix B). Following the criteria in the NJDEP alert tier system, it would correspond to the Warning level which indicates a high risk of adverse health effects due to the cyanotoxin concentrations. This microcystin concentration corresponded with a high Cyano of 952,500 cells/mL and PC value of 3,773 RFU. These parameters suggest a highly toxic HAB occurred in this waterbody posing a great threat to both humans and animals. The *Ridge and Valley* and *Pine Barrens* exhibited among the highest median MC concentrations (Table 2.2).

Additionally, MC samples had concentrations above the recreational threshold in the *Northeastern Highlands*, *Piedmont*, *Ridge and Valley*, and *Pine Barrens* (Table 2.1). There was an increase in the percentage of samples that exceeded the MC threashold between 2018 from 2.8% (1 of 26) and to 6.7% 2019 (4 of 15) which could have been influenced by environmental conditions such as increased light or nitrogen availability (Hellweger, 2022).

2.3.4 Statewide HAB and water quality relationships

Results showed that both Phyto and Cyano positively correlated with MC and aligned with published literature (Kotak et al., 1995; Oh et al., 2001; Rolland et al., 2005). Our results document an increase in Phyto counts with higher PC values ($r_s = 0.8018$, $p < 0.0001$), Chl values ($r_s=0.7243$, $p < 0.0001$), and Chl-a concentrations ($r_s = 0.5742$, $p < 0.0001$) (Table 2.4). Oh et al. (2001) further suggested using Chl-a as an indicator for MC levels. Our study observed a similar trend, Chl-a concentrations were found to be higher with increasing MC concentrations ($r_s = 0.3307$, $p = 0.0012$; Table 2.4). On the other hand, PC values are often used to predict cyanobacterial biomass and HAB status (Brient et al., 2008; Gregor et al., 2007; Izydorczyk et al., 2005; Lee et al., 1994; Yacobi et al., 2015). Our results showed that Cyano cells positively correlated with PC concentrations ($r_s = 0.8005$, $p < 0.0001$). Previous studies have also concluded phycocyanin can be used as a proxy for microcystins, which was supported by our results showing high MC concentrations increasing with high PC values ($r_s = 0.5955$, $p < 0.0001$) (Wong & Hobbs, 2019). Hence, based on the results of this study, PC could be used as a proxy to indicate Cyano, MC, and HAB conditions. Nutrient results found Phyto counts to increase with TP concentrations ($r_s = 0.2638$, $p = 0.0136$) but not TN ($r_s = 0.1544$, $p = 0.1607$). However, Cyano was found to increase with both TN ($r_s = 0.2154$, $p = 0.0491$) and TP ($r_s =$

0.3433, p = 0.0011); similar patterns were also reported in previous studies (Kotak et al., 1995, 2000; Søndergaard et al., 2011). A Principal Component Analysis was performed to investigate the similarities between water quality parameters and HAB parameters, including MC, Phyto, and Cyano. PCA results support the findings that Phyto, Cyano and MC are associated with the first component's environmental variables such as TN, TP, Chl, PC, and Chl-a (Figure 2.4).

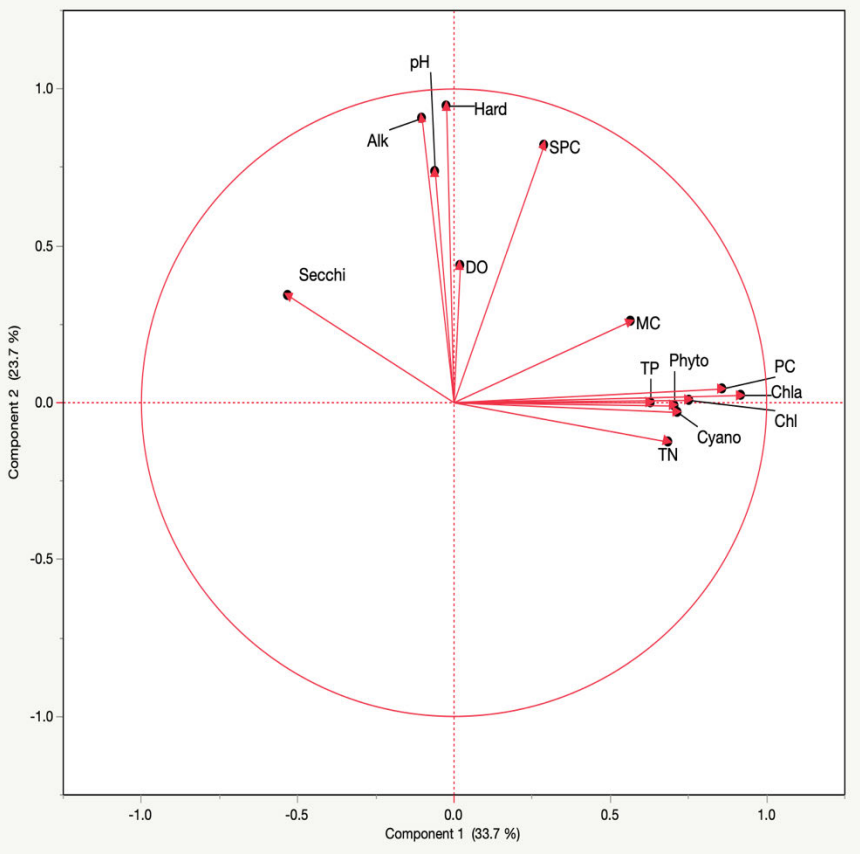

Figure 2.4. Principal components analysis of water quality data in 110 selected waterbodies in New Jersey documented from 2016–2019. Parameters included specific conductance (SPC), dissolved oxygen (DO), pH, temperature (T), secchi depth (Secchi), alkalinity (Alk), hardness (Hard), total nitrogen (TN), total phosphorus (TP), fluorescence of phycocyanin (PC), fluorescence of chlorophyll (Chl), chlorophyll-a (Chl-a), phytoplankton cell density (Phyto), cyanobacteria cell density (Cyano), and microcystins (MC).

Dunn's post hoc results showed MC concentrations were significantly lower in rivers than in ponds (p = 0.0045) and lakes (p < 0.0387). Analyses were also performed to investigate water

quality and HAB trends at the ecoregion level (Table 2.5). Some significant differences in water quality were found between ecoregions. While the *Coastal Plain* was found to have similar water quality to the other four ecoregions, the results suggest that the *Highlands* and *Ridge and Valley* ecoregions were found to have significantly better water quality than the other three: *Pine Barrens, Coastal Plain,* and *Piedmont* (Table 2.5). Moreover, *Piedmont* and *Pine Barrens* were similar in nutrient and HAB water quality (Table 2.5). Both TN and TP were significantly lower in the *Highlands* ($p < 0.01$ and $p < 0.01$, respectively) and the *Ridge and Valley* ($p < 0.01$ and $p < 0.01$, respectively) than the nutrients in the *Pine Barrens* (Table 6). The *Ridge and Valley* had significantly lower Phyto ($p = 0.01$), Cyano ($p = 0.01$), PC ($p = 0.04$), and Chl ($p = 0.01$) than the *Pine Barrens* and lower Chl ($p=0.04$) than the *Coastal Plain*. However, no significant difference was found in Phyto, Cyano or Chl between *Pine Barrens* and the *Highlands* ecoregions. Additionally, TN ($p= 0.01$), PC ($p < 0.01$, Phyto ($p < 0.01$), Cyano ($p < 0.01$), and Chl ($p < 0.01$) were all significantly lower in the *Ridge and Valley* than the *Piedmont* (Table 6). The *Highlands* ecoregion had significantly lower MC ($p < 0.01$), Cyano ($p < 0.01$), PC ($p = 0.01$), and Phyto ($p < 0.01$) than the *Piedmont* ecoregion.

2.4 Discussion

This is the first documentation of freshwater phytoplankton community assemblages in New Jersey waters that incorporates phytoplankton and cyanobacteria communities, water chemistry data, and cyanotoxins measurements. Results of this study show that a total of 91 phytoplankton taxa were present in freshwater waterbodies of New Jersey with seven major taxa groups documented including green algae, cyanobacteria, diatoms, golden algae, dinoflagellates, euglenoids, and cryptomonads. Most waterbodies (87.2%) in New Jersey had cyanobacteria

present, with cyanobacteria being the most widely distributed phytoplankton group statewide and across all five ecoregions. When cyanobacteria dominate phytoplankton assemblages, they affect interactions between phytoplankton taxa through altering ecological process rates (Hillenbrand et al., 2008). The high presence and dominance of cyanobacteria documented in this study could have been biased due to a number of reasons. For example, all sampling events took place during the HAB seasons, in summers and autumns, which provided favorable conditions, such as increased sunlight and surface water temperature, for cyanobacteria to proliferate (Buzzi, 2002). In addition, a high percentage of samples collected by the NJDEP were the results of responding to suspicious HAB events where visual blooms were detected and reported to the NJDEP; thus, observance of higher cyanobacteria dominance was expected.

Some of the cyanobacteria present in the assemblages of the documented waterbodies share morphological and behavioral adaptations such as small size and motility (e.g. flagella and buoyancy), which help increase their time in the photic zone, and aid in their dominance over other phytoplankton groups (Buzzi, 2002). Our results showed that *Synechococcus* was the most frequently documented phytoplankton statewide and found in all ecoregions and all waterbody types. This taxa's small size and ability to exchange nutrients with the environment give *Synechococcus* unique competitive advantages over other phytoplankton (Liu et al., 2019). Scanlan and Carr (1993) reported that when phosphorus is limited in a waterbody, *Synechococcus* can produce a polypeptide that aids in binding phosphorus, which enables this cyanobacterium to survive in phosphorus limited environments.

In terms of being present in waterbodies, across all five ecoregions, Cyanophytes and Chlorophytes were the most frequently observed phytoplankton. Similarities in phytoplankton composition of different ecoregions have also been reported in previous studies of freshwater

lakes. Literature suggested that those similarities might have been caused by environmental resemblances between lakes of close proximity that are situated in two ecoregions, such as physico-chemical characteristics, landscape features, and morphometry (Griffith et al., 1999; Sodre et al., 2020; Whittier et al., 1988). Ecoregion differences and similarities were examined using its land use and land cover patterns. Land use and land cover (LULC) of a drainage area can greatly impact the water quality such that increased runoff can increase TP and TN concentrations, stimulating Cyano growth, and HAB events (Beaver et al., 2012; Cross & Jacobson, 2013; Huang et al., 2016; Katsiapi et al., 2012; Paul & Meyer et al., 2001; Omernik et al., 2016; Schindler, 2006; Shi et al., 2017; Wagner et al., 2011). Our results demonstrated similar patterns to previous studies (Beaver et al., 2012; Marion et al., 2017; Paul et al., 2012) which have documented that as forest land cover increased, concentration of nutrients, Cyano and HAB events decreased. The forest-dominated *Highlands* was found to have lower nutrients than the urban-dominated *Piedmont* consistent with previous results (Paul et al., 2012). When compared to other ecoregions, the *Ridge and Valley* and *Highlands* had the greatest percentage of forest cover (>50%) while percent urbanization was at least half of the forest cover (<25%), which could influence nutrient inputs relative to other ecoregions (U.S. EPA ORD, 2012). Moreover, the *Ridge and Valley* is largely dominated by soils that are nutrient poor, possibly contributing to overall low nutrients (U.S. EPA ORD, 2012). The high TN concentrations documented in the *Piedmont* ecoregion could be associated with the relatively high human population density, its history of urbanization and industrialization, as well as its land use and land cover, dominated by agriculture and urban (U.S. EPA ORD, 2012). All of the above could lead to elevated nutrients in runoff, over-growth of cyanobacteria, and HAB events. Results for the forest-dominated *Pine Barrens* were not in accordance with previous studies which have

documented low nutrient concentrations in this area (Zampella & Procopio, 2023). The high nutrient concentrations reported in this study could be influenced by the focus on HAB response in the study site selection as well as possible watershed disturbance upstream from the selected sites.

Although published literature has mixed conclusions about the effectiveness of describing aquatic systems through ecoregions (Beaver et al., 2012; Sodré et al., 2020; Soranno et al., 2008), the results of this study indicated that land use and land cover is a major factor influencing water quality and HAB formation. We recognize there are limitations in this study with a wide array of sampling events across a wide spatial distribution across the State of New Jersey. However, the study sites were not selected randomly or systematically but opportunistically; hence, some ecoregions were better represented than others. Specifically, less numbers of waterbodies were surveyed within the *Coastal Plain* and *Ridge and Valley* ecoregions, which might cause biased results.

In addition to the phytoplankton community composition and ecoregion variations, relationships between environmental conditions were studied. The results of this study suggest that PC can be used as a proxy for Cyano, MC, and HAB conditions in New Jersey waterbodies. Our results are in agreement with the findings of Izydorczyk et al. (2005), Francy et al. (2016), and Marion et al. (2012) who suggested that PC values are an effective water quality parameter to estimate MC concentrations. Previous studies have suggested that PC values could be used as an HAB monitoring proxy for cyanobacterial concentrations (Ahn et al., 2007; Izydorczyk et al., 2005; Mchau et al., 2019).

In this study, sampling activities were limited to phytoplankton community assemblage and dominance during the HAB season. Community assemblage and dominance are likely to exhibit

seasonal changes (Figueredo & Giani, 2009; Hilaluddin et al., 2020; Jiang et al., 2014; Mengxu et al., 2005). The results of the study were not intended to represent the year-round community dynamics. Year-round sampling is suggested to provide a greater understanding of seasonal variation in phytoplankton and cyanobacterial communities in New Jersey. Particularly, some HAB in New Jersey were documented to continue into the late fall or last throughout the winter with some taxa such as *Planktothrix* being capable of blooming under ice and possibly producing cyanotoxins (Messineo et al., 2006; Nürnberg et al., 2003). Thus, it would be advantageous to study phytoplankton seasonal succession in New Jersey waterbodies since summer phytoplankton compositions are likely to be influenced by winter phytoplankton communities (Babanazarova et al., 2013). Phytoplankton community succession and waterbody environmental conditions can be used to project future phytoplankton compositions and HAB.

Across the state, and within each of the five ecoregions, cyanobacteria were found to be the most dominant phytoplankton. Based on published literature, most cyanobacteria taxa documented are capable of producing cyanotoxins. However, only a portion of the samples were analyzed for microcystins and an even smaller portion for cylindrospermopsin and anatoxin-A. We suggest further incorporating a comprehensive cyanotoxin and molecular analysis into future monitoring plans to better assess human health risk.

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Table 2.2. Median and range of water quality parameters recorded (n=181) and phytoplankton and cyanobacteria (n=196) at the selected waterbodies in New Jersey from 2019–2019 including specific conductance (SPC; µS/cm), dissolved oxygen (DO; mg/L), pH, temperature (T; °C), secchi depth (Secchi; m), alkalinity (Alk; mg/L), hardness (Hard; mg/L), total nitrogen (TN; mg/L), total phosphorus (TP; mg/L), fluorescence of phycocyanin (PC; RFU), fluorescence of chlorophyll (Chl; RFU), chlorophyll-a (Chl-a; µg/L), phytoplankton cell density (Phyto; cells/mL), cyanobacteria cell density (Cyano; cells/mL), and microcystins (MC; µg/L). (UD: under detection limit).

	Statewide		Ecoregion									
			Pine Barrens		*Coastal Plain*		*Highlands*		*Piedmont*		*Ridge and Valley*	
	Range	Med	Range	Med	Range	Med	Range	Med	Range	Med	Range	Med
SPC	17.50–710.80	188.70	31.90–710.80	142.50	44.60–45.80	45.20	28.80–591.50	138.35	107.00–602.30	189.40	17.50–494.40	262.75
DO	0.84–13.01	7.57	4.36–13.01	7.11	4.58–5.24	4.91	0.84–10.13	7.78	6.53–8.93	6.79	5.96–9.91	8.31
pH	2.00–9.01	7.43	2.00–7.98	6.10	4.43–4.51	4.47	5.70–9.01	7.50	6.71–7.92	6.97	5.74–8.70	8.31
T	19.25–30.05	24.31	21.30–29.21	24.31	24.00–25.46	24.73	19.25–30.05	24.88	21.51–29.35	26.79	19.96–27.60	23.86
Secchi	0.20–8.34	1.80	0.20–2.00	1.00	1.20–1.20	1.20	0.35–4.10	2.10	1.30–1.50	1.40	0.80–8.34	2.60
Alk	1.00–225.00	44.50	1.00–40.00	10.00	1.00–2.00	1.50	4.00–211.00	42.00	11.00–72.00	28.00	3.00–225.00	75.00
Hard	2.15–235.00	58.65	2.15–70.80	21.40	3.85–5.85	4.85	11.90–226.00	55.50	20.40–95.80	37.90	5.75–235.00	87.45
TN	0.23–2.44	0.39	0.38–2.44	0.82	0.50–0.67	0.59	0.20–1.32	0.39	0.73–1.03	0.77	0.24–0.68	0.30
TP	< 0.01–143.13	0.02	< 0.01–143.13	0.04	0.02–0.04	0.03	< 0.01–0.11	0.02	0.02–0.06	0.03	< 0.01–0.10	0.02
PC	4.53–3773.00	30.07	13.86–759.70	50.37	17.87–3773.00	47.24	12.52–2818.00	26.77	13.63–2884.00	66.31	4.53–861.80	21.30
Chl	1.16–298.00	10.56	2.09–298.00	14.51	8.19–176.40	46.56	1.16–142.70	8.89	1.96–158.00	19.77	2.48–91.23	6.06
Chl–a	0.60–152.80	10.60	0.60–152.80	14.30	3.20–84.10	43.65	2.60–72.90	9.80	4.80–28.30	19.15	2.20–24.10	9.05
Phyto	225–11,730,000	14,325	225–535,500	35,588	2,700–954,000	37,425	300–6,990,000	10,575	825–11,730,000	32,400	1,200–868,500	8,588
Cyano	0–11,730,000	8,700	0–444,000	27,225	0–952,500	36,225	0–6,990,000	5,625	0–11,730,000	22,725	0–838,500	4,163
MC	< 0.15–336.80	0.22	< 0.15–246.40	0.19	< 0.15–336.80	< 0.15	< 0.15–7.48	< 0.15	< 0.15–254.20	0.53	< 0.15–35.00	0.31

Table 2.3. Frequencies of phytoplankton taxa documented in the selected New Jersey waterbodies (n=110) as well as frequencies by five New Jersey ecoregions: Northeastern Highlands (Highlands; n=37), Northern Piedmont (Piedmont; n=25), Ridge and Valley (Ridge and Valley; n=7), Middle Atlantic Coastal Plain (Coastal Plain; n=4), and Atlantic Coastal Pine Barrens (Pine Barrens; n=37). "--" marks the absence of taxa.

Taxa			Statewide	Ecoregion				
Group	**Class**	**Genus**		***Highlands***	***Piedmont***	***Ridge and Valley***	***Coastal Plain***	***Pine Barrens***
Golden Algae	Synurophyceae	*Synura*	0.9%	2.70%	--	--	--	--
		Mallomonas	11.8%	8.10%	8.00%	14.30%	50.00%	13.50%
Cryptomonads	Chrysophyceae	*Bitrichia*	0.9%	2.70%	--	--	--	--
		Dinobryon	6.4%	10.80%	--	28.60%	--	2.70%
	Cryptophyceae	*Chroomonas*	10.9%	10.80%	16.00%	14.30%	--	8.10%
		Cryptomonas	21.8%	32.40%	12.00%	14.30%	50.00%	21.62%
Dinoflagellates	Dinophyceae	*Ceratium*	0.9%	2.70%	--	--	--	--
		Gymnodinium	3.6%	--	12.00%	--	--	2.70%
		Peridinium	2.7%	--	--	14.30%	25.00%	2.70%
Euglenoids	Euglenophyceae	*Phacus*	17.3%	18.90%	8.00%	42.90%	25.00%	16.20%
		Euglena	15.5%	10.80%	12.00%	14.30%	25.00%	24.30%
		Trachelomonas	20.9%	18.90%	20.00%	--	25.00%	27.00%
Diatoms	Mediophyceae	*Cyclotella*	20.9%	8.10%	20.00%	42.90%	25.00%	35.14%
		Chaetoceros	0.9%	--	--	--	--	2.70%
		Stephanodiscus	2.7%	5.40%	4.00%	--	--	--
	Coscinodiscophyceae	*Coscinodiscus*	1.8%	--	4.00%	--	--	2.70%

Table 2.3. Continued.

Taxa			Statewide	Ecoregion				
Group	**Class**	**Genus**		***Highlands***	***Piedmont***	***Ridge and Valley***	***Coastal Plain***	***Pine Barrens***
Diatoms	Coscinodiscophyceae	*Rhizosolenia*	2.70%	2.7%	5.40%	--	14.30%	--
		Melosira	6.40%	5.5%	2.70%	8.00%	--	--
		Aulacoseira	5.50%	4.5%	5.40%	4.00%	--	--
	Bacillariophyceae	*Achnanthes*	1.80%	2.7%	2.70%	8.00%	--	--
		Cocconeis	9.10%	9.1%	5.40%	12.00%	--	--
		Nitzschia	11.80%	12.7%	16.20%	12.00%	14.30%	--
		Cymbella	3.60%	20.0%	2.70%	4.00%	--	--
		Synedra	18.20%	20.0%	18.90%	16.00%	42.90%	--
		Navicula	16.40%	16.4%	10.80%	24.00%	--	25.00%
		Pinnularia	7.30%	7.3%	5.40%	12.00%	14.30%	--
		Gyrosigma	0.90%	0.9%	--	--	--	--
		Stauroneis	6.40%	7.3%	2.70%	16.00%	14.30%	--
		Asterionella	6.40%	6.4%	5.40%	8.00%	--	--
		Fragilaria	8.20%	8.2%	5.40%	12.00%	14.30%	--
		Tabellaria	8.20%	8.2%	7.90%	8.00%	14.30%	--
		Gomphonema	0.90%	0.9%	2.60%	--	--	--
Cyanobacteria	Cyanophyceae	*Chrococcus*	30.90%	29.1%	29.70%	24.00%	28.60%	50.00%
		Gomphosphaeria	5.50%	4.5%	2.70%	8.00%	--	--
		Microcystis	36.40%	34.5%	29.70%	52.00%	--	50.00%

Table 2.3. Continued.

Taxa			Statewide	Ecoregion				
Group	**Class**	**Genus**		***Highlands***	***Piedmont***	***Ridge and Valley***	***Coastal Plain***	***Pine Barrens***
Cyanobacteria	Cyanophyceae	*Dolichospermum*	31.80%	31.8%	24.30%	32.00%	28.60%	25.00%
		Aphanizomenon	21.80%	21.8%	16.20%	36.00%	28.60%	25.00%
		Limnoraphis	10.9%	16.20%	8.00%	28.60%	--	5.40%
		Synechococcus	74.5%	78.40%	64.00%	57.10%	100.00%	83.78%
		Merismopedia	10.0%	--	20.00%	--	--	16.20%
		Aphanocapsa	40.9%	48.60%	32.00%	42.90%	75.00%	35.10%
		Aphanothece	8.2%	2.70%	8.00%	--	--	16.20%
		Cuspidothrix	2.7%	5.40%	4.00%	--	--	--
		Cyanodictyon	2.7%	2.70%	--	14.30%	--	2.70%
		Oscillatoria	2.7%	2.70%	--	14.30%	25.00%	--
		Phormidium	10.0%	13.50%	8.00%	14.30%	--	8.10%
		Planktolyngbya	11.8%	10.80%	12.00%	--	25.00%	13.50%
		Planktothrix	6.4%	2.70%	4.00%	14.30%	25.00%	8.10%
		Pseudanabaena	11.8%	8.10%	12.00%	--	25.00%	16.20%
		Raphidiopsis	16.4%	13.50%	16.00%	28.60%	50.00%	16.22%
		Rhabdoderma	0.9%	--	4.00%	--	--	--
		Snowella	2.7%	2.70%	4.00%	--	--	2.70%
		Woronichinia	5.5%	2.70%	12.00%	28.60%	--	--
Green Algae	Nephroselmidophyceae	*Nephroselmis*	4.5%	8.10%	4.00%	--	--	2.70%

Table 2.3. Continued.

Taxa			Statewide	Ecoregion				
Group	**Class**	**Genus**		***Highlands***	***Piedmont***	***Ridge and Valley***	***Coastal Plain***	***Pine Barrens***
Green Algae	Ulvophyceae	*Ulothrix*	2.7%	2.70%	4.00%	--	--	2.70%
	Zynematohyceae	*Closterium*	29.1%	5.40%	4.00%	42.90%	25.00%	29.70%
		Cosmarium	4.5%	5.40%	12.00%	14.30%	--	2.70%
		Pleurotaenium	0.9%	--	--	--	--	2.70%
	Zynematohyceae	*Staurastrum*	10.9%	5.40%	8.00%	28.60%	--	13.50%
		Euastrum	0.9%	2.70%	--	--	--	--
		Spirogyra	0.9%	--	4.00%	--	--	--
	Trebouxiophyceae	*Chlorella*	71.8%	75.70%	60.00%	85.70%	75.00%	78.40%
		Micractinium	2.7%	2.70%	--	14.30%	--	2.70%
		Oocystis	39.1%	45.90%	48.00%	28.60%	--	32.40%
		Dictyosphaerium	7.3%	--	4.00%	14.30%	--	16.20%
		Actinastrum	10.9%	--	--	42.90%	25.00%	13.50%
		Botryococcus	0.9%	2.70%	--	--	--	--
		Crucigenia	2.7%	2.70%	4.00%	--	--	2.70%
		Nephrocytium	4.5%	5.40%	4.00%	--	--	5.40%
	Chlorophyta	*Chlamydomonas*	54.5%	56.80%	44.00%	71.40%	50.00%	56.80%
		Eudorina	10.9%	8.10%	16.00%	--	--	13.50%
		Pediastrum	5.5%	--	12.00%	--	--	8.10%
		Coelastrum	9.1%	2.70%	12.00%	14.30%	--	13.50%
		Scenedesmus	46.4%	51.40%	48.00%	71.40%	50.00%	35.10%

Table 2.3. Continued.

Taxa			Statewide	Ecoregion				
Group	**Class**	**Genus**		***Highlands***	***Piedmont***	***Ridge and Valley***	***Coastal Plain***	***Pine Barrens***
Green Algae	Chlorophyta	*Oedogonium*	2.7%	2.70%	4.00%	--	--	2.70%
		Tetraedron	10.9%	5.40%	20.00%	14.30%	–	10.80%
		Ankistrodesmus	20.9%	24.30%	16.00%	14.30%	--	27.00%
		Selenastrum	48.2%	48.60%	48.00%	28.60%	25.00%	54.10%
		Gonium	6.4%	--	8.00%	28.60%	--	8.10%
		Pandorina	14.5%	13.50%	8.00%	28.60%	25.00%	16.20%
		Volvox	5.5%	--	--	14.30%	--	13.50%
		Asterococcus	2.7%	--	8.00%	--	--	2.70%
		Desmodesmus	6.4%	2.70%	12.00%	--	--	13.50%
		Gloeocystis	0.9%	--	--	14.30%	--	--
		Golenkinia	4.5%	5.40%	4.00%	--	--	5.40%
		Monoraphidium	12.7%	10.80%	20.00%	--	25.00%	10.80%
		Schroederia	4.5%	--	12.00%	--	--	5.40%
		Sphaerocystis	0.9%	--	4.00%	--	--	--
		Stauridium	0.9%	--	--	--	--	2.70%
		Tetraspora	1.8%	2.70%	--	--	--	2.70%
		Tetrastrum	3.6%	5.40%	4.00%	--	--	2.70%

Table 2.4. Spearman's correlation coefficient (rs) and probability (p) between water quality parameters in the selected 110 waterbodies in New Jersey from 2016-2019 including specific conductance (SPC), dissolved oxygen (DO), pH, temperature (T), secchi depth (Secchi), alkalinity (Alk), hardness (Hard), total nitrogen (TN), total phosphorus (TP), phycocyanin (PC), fluorescence of chlorophyll (Chl), chlorophyll-a (Chl-a), phytoplankton cell density (Phyto), cyanobacteria cell density (Cyano), and microcystins (MC). (ns: not significant; p > 0.05; * p<0.05; **p<0.01; *** p<0.001).

	SPC		DO		pH		T		Secchi		Alk		Hard		TN	
	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p
SPC	–	–	0.349	**	0.575	***	0.142	ns	0.005	ns	0.763	***	0.305	**	0.058	ns
DO	0.349	**	–	–	0.657	***	0.176	ns	0.341	**	0.227	*	0.701	***	-0.085	ns
pH	0.575	***	0.657	***	–	–	0.163	ns	0.382	**	0.688	***	0.701	***	-0.344	**
T	0.142	ns	0.176	ns	0.163	ns	–	–	-0.065	ns	-0.095	ns	-0.025	ns	0.202	ns
Secchi	0.005	ns	0.341	**	0.382	**	-0.065	ns	–	–	0.243	ns	0.213	ns	-0.583	***
Alk	0.763	***	0.227	*	0.688	***	-0.095	ns	0.243	ns	–	–	0.953	***	-0.206	ns
Hard	0.870	***	0.305	**	0.701	***	-0.025	ns	0.213	ns	0.953	***	–	–	-0.127	ns
TN	0.058	ns	-0.085	ns	-0.344	**	0.202	ns	-0.583	***	-0.206	ns	-0.127	ns	–	–
TP	0.115	ns	-0.329	**	-0.371	**	-0.016	ns	-0.617	***	-0.073	ns	-0.020	ns	0.593	***
PC	0.358	**	0.098	ns	0.151	ns	-0.022	ns	-0.404	**	0.116	ns	0.166	ns	0.252	*
Chl	0.161	ns	-0.119	ns	-0.115	ns	0.174	ns	-0.504	***	-0.050	ns	0.054	ns	0.390	**
Chl-a	0.286	**	0.011	ns	0.035	ns	-0.023	ns	-0.387	**	0.144	ns	0.206	ns	0.332	**
Phyto	0.187	ns	0.139	ns	0.114	ns	0.068	ns	-0.220	ns	-0.050	ns	0.023	ns	0.154	ns
Cyano	0.123	ns	0.005	ns	0.093	ns	-0.011	ns	-0.331	**	-0.053	ns	-0.019	ns	0.215	*
MC	0.374	**	0.095	ns	0.337	**	0.093	ns	-0.086	ns	0.436	***	0.444	***	-0.028	ns

Table 2.4. Continued.

	TP		PC		Chl		Chl-a		Phyto		Cyano		MC	
	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p	rs	p
SPC	0.115	ns	0.358	**	0.161	ns	0.29	**	0.187	ns	0.123	ns	0.374	**
DO	-0.329	**	0.098	ns	-0.119	ns	0.01	ns	0.139	ns	0.005	ns	0.095	ns
pH	-0.371	**	0.151	ns	-0.115	ns	0.03	ns	0.114	ns	0.093	ns	0.337	**
T	-0.016	ns	-0.022	ns	0.174	ns	-0.02	ns	0.068	ns	-0.011	ns	0.093	ns
Secchi	-0.617	***	-0.404	**	-0.504	***	-0.39	**	-0.220	ns	-0.331	**	-0.086	ns
Alk	-0.073	ns	0.116	ns	-0.050	ns	0.14	ns	-0.050	ns	-0.053	ns	0.436	***
Hard	-0.020	ns	0.166	ns	0.054	ns	0.21	ns	0.023	ns	-0.019	ns	0.444	***
TN	0.593	***	0.252	*	0.390	**	0.33	**	0.154	ns	0.215	*	-0.028	ns
TP	–	–	0.524	***	0.500	***	0.56	***	0.264	*	0.343	**	0.188	ns
PC	0.524	***	–	–	0.770	***	0.83	***	0.802	***	0.801	***	0.596	***
Chl	0.500	***	0.770	***	–	–	0.78	***	0.724	***	0.642	***	0.437	***
Chl-a	0.562	***	0.829	***	0.778	***	–	–	0.574	***	0.474	***	0.331	**
Phyto	0.264	*	0.802	***	0.724	***	0.57	***	–	–	0.943	***	0.472	***
Cyano	0.343	**	0.801	***	0.642	***	0.47	***	0.943	***	–	–	0.476	***
MC	0.188	ns	0.596	***	0.437	***	0.33	**	0.472	***	0.476	***	–	–

Table 2.5. Dunn's post-hoc results for analysis of five New Jersey ecoregions. Parameters selected included: specific conductance (SPC), dissolved oxygen (DO), pH, temperature (T), secchi depth (Secchi), alkalinity (Alk), hardness (Hard), total nitrogen (TN), total phosphorus (TP), phycocyanin (PC), fluorescence of chlorophyll (Chl), chlorophyll-a (Chl-a), phytoplankton cell density (Phyto), cyanobacteria cell density (Cyano), and microcystins (MC). (ns: not significant, $p > 0.05$).

Ecoregions	SPC	DO	pH	Temp	Secchi	Alk	Hard	TN	TP	PC	Chl	Chl-a	Phyto	Cyano	MC
Coastal Plain vs Highlands	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Coastal Plain vs Piedmont	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Coastal Plain vs Pine Barrens	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Coastal Plain vs Ridge and Valley	ns	ns	ns	ns	ns	0.0184*	0.0323*	ns	ns	ns	0.0470*	ns	ns	ns	ns
Highlands vs Piedmont	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.0192*	ns	ns	0.0056*	0.0070*	0.0035*
Highlands vs Pine Barrens	ns	ns	0.0034*	ns	0.0025*	0.0123*	0.0464*	0.0008*	0.0036*	ns	ns	ns	ns	ns	ns
Highlands vs Ridge and Valley	ns	ns	ns	ns	ns	0.0184*	ns	ns	ns	ns	ns	ns	ns	ns	ns
Piedmont vs Pine Barrens	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Piedmont vs Ridge and Valley	ns	ns	ns	ns	ns	ns	ns	0.0184*	ns	0.0004*	0.0013*	ns	0.0013*	0.0006*	ns
Pine Barrens vs Ridge and Valley	ns	ns	<.0001*	ns	<.0001*	<.0001*	<.0001*	<.0001*	<.0001*	0.0429*	0.0173*	ns	0.0148*	0.0115*	ns

Chapter 3 An Investigation of Cyanobacteria, Cyanotoxins and Environmental Variables in Select Drinking Water Treatment Plants in New Jersey

3.1 Introduction

Potable water is vital for human health and survival. However, in recent years, drinking water resources worldwide are increasingly affected by cyanobacterial harmful algal blooms (HAB) (Mohan et al., 2019). A HAB forms when algal cells grow in excess, affecting the ecological integrity and degrading the water quality of a waterbody (Paerl & Otten, 2013). Bloom events can be influenced by several factors such as: nutrient availability, stratification, climate conditions, flushing and residence time, light intensity, dissolved organic matter, and grazing (Paerl & Otten, 2013). For instance, eutrophication occurs when there is excess of nutrients in a waterbody that stimulates the growth of algae which can lead to blooms causing hypoxia/anoxia and fish kills (Paerl & Otten, 2013).

Many lakes, rivers, and reservoirs affected by HAB are utilized as source waters by drinking water treatment facilities. The overabundance of algal biomass and cyanotoxins that it produced in these source waters are likely to require additional treatment processes in order to supply safe drinking water, such as increases in coagulants, use of chlorine, management of disinfection by-products, microbial regrowth in the distribution systems, as well as shortening filter runs via clogging (Knappe et al., 2004; Gray, 2008; Westrick et al., 2010). However, these treatment processes will result in large-scale cell lysis, causing high concentrations of cyanotoxins to be released into the water (Ibelings & Maberly, 1998). Westrick et al. (2010) found that about 95% of anatoxin-a, saxitoxins, and microcystins were present intracellularly before bloom senesce. The high intracellular presence of these frequently observed cyanotoxins

highlights the importance of removing intact cyanobacterial cells without lysing. Cyanotoxin removal becomes more complicated once dissolved in water which can occur via cell lysis, excretion, or deterioration (Cheung et al., 2013). Thus, water purveyors have added complications in the water treatment process to remove multiple cyanotoxins in relatively high concentrations (He et al., 2016). Consequently, cyanotoxins break-through in finished waters have been documented at multiple drinking water facilities in concentrations above which adverse health effects could occur (USEPA, 2015a; Yen et al., 2011). For instance, in 2014, a cyanobacterial bloom near the water intake of a drinking water facility in Toledo, Ohio led to total microcystins concentrations in the finished water up to 2.5 µg/L which affected 500,000 customers (USEPA, 2015a). Yen et al. (2011) reported that cylindrospermopsin was detected in finished waters on Kinmen Island, Taiwan in 2007 at concentrations of 1.3 and 8.6 µg/L. In order to minimize the risk from cyanotoxins, drinking water source management strategies need to incorporate a range of assessments to reduce the probability of cyanobacterial cell lysing and cyanotoxin release (Hitzfeld et al., 2000). These strategies include but are not limited to dominant cyanobacterial taxa, relevant water treatment system, and cyanotoxin location inside the cell (intracellular) or within the water column (extracellular) (Westrick et al., 2010).

HAB can also affect drinking water quality through odor, taste, and appearance (Knappe et al., 2004; Gray, 2008). Geosmin and 2-methylisoborneol (MIB) are the most frequently reported taste and odor compounds in both finished and source waters (Chorus & Welker, 2021). Taxa such as *Dolichospermum*, *Oscillatoria*, *Phormidium*, *Lyngbya*, *Planktothrix*, *Pseudanabaena*, and *Synechococcus* are known producers of geosmin and/or MIB (Jüttner & Watson 2007; Krishnani et al., 2008). Conventional water treatment methods such as coagulation, flocculation, sedimentation, and filtration, as well as common disinfectants and

oxidants do not completely remove taste and odor compounds (Bruchet et al., 2004; Chorus & Welker, 2021; Ghernaout et al., 2010). However, these taste and odor compounds can be noticed by humans at very low concentrations of about 4 ng/L and 6 ng/L for geosmin and MIB, respectively (Young et al., 1996). Once detected, water is deemed unacceptable by consumers; thus, monitoring of HAB is considered the most effective approach in managing the taste and odor compounds (Ghernaout et al., 2010; Srinivasan et al., 2011).

During the water treatment process, challenges in managing source water HAB often involve repairing clogged filters and pipes, and removing suspended algal biomass (Chorus & Welker, 2021; He et al., 2016). Algal biomass removal can be difficult due to poor flocculation of algal cells which is influenced by the motility of each algae taxon (Petruševski et al., 1996). Needle-shaped diatoms such as *Synedra* and *Asterionella* have been reported to attach to flocs, efficiently reducing their density, to an extent that they overflow the sedimentation basin (Joh et al., 2011). Small-celled, colonial, and mucilaginous algae (i.e., cyanobacteria such as *Microcystis*) are less dense and have been documented to float to the surface, overflow the sedimentation basin, and clog the filter (Joh et al., 2011).

Algae can also cause high concentrations of soluble or biodegradable organic compounds which, after chlorination, can be a source of trihalomethanes which are potential carcinogens (Hoehn et al., 1980; Richardson, 2003). Cyanotoxins such as microcystin-LR and cylindrospermopsin have been reported to react with disinfectants to form disinfection by-products, potentially creating additional toxicity through new pathways during the treatment processes (Merel et al., 2010). Overall, the best strategy on HAB management is to focus on decreasing algae abundance at the source water before initiating the treatment process since conventional treatment processes (coagulation, flocculation, sedimentation, and filtration) have

been reported as not effective at removing all algal cells, cyanotoxins and the treatment by-products (Mouchet & Bonnélye et al., 1998; Henderson et al., 2008; OEPA, 2001; Westrick et al., 2010). Large-scale solutions to manage HAB in source water include catchment-scale nutrient management plans, investment in sewage system improvement, investment in sewage system improvement, and political changes relating to nutrient loads (Chorus & Welker, 2021). However, source water HAB management is complex and there is no one simple solution, thus treatment will most likely be necessary for safety usage (Nwankwegu et al., 2019). Ultimately, more effective treatment methods and advancements in treatment technology are needed to remove cyanobacteria and their toxins.

3.1.1 Treatment Costs

The human population is expected to rise to 10 billion by the middle of this century (Chorus & Welker, 2021). As human population increases, so does the demand for safe drinking water and drinking water sources (Watson & Lawrence, 2003). To help meet this demand, monitoring drinking water sources such as reservoirs, lakes, and rivers is crucial, including HAB water quality parameters (Treuer et al., 2021). Reservoirs are of special interest to drinking water managers due to their longer water residence times and water column stratification which help promote HAB events (Paerl et al., 2018; Tundisi et al., 1999). While targeted treatments for cyanobacteria and cyanotoxins are commercially available, they can be costly and may potentially jeopardize drinking water availability due to its low cyanotoxin removal efficiency as low as 60% (USEPA, 2015b; Zamyadi et al., 2012). Economic loss in water treatment facilities can be associated with backwash frequency such that if high densities of empty algal cells are found in backwash water, it's deemed unsuitable for reuse and water production is reduced (Joh et al., 2011). Joh et al. (2011) reported a daily loss of 18% of the daily volume (60,000 m3) of

treated water due to discarded backwash affected by high concentrations of empty algal cells and other debris.

HAB can cause significant losses in both water resources and the economy (Joh et al., 2011). Purcell et al. (2013) reported that harmful algal blooms increased treatment costs by 25%. Results from a public water systems survey of HAB events at Lake Erie, Ohio estimated annual HAB control costs of $417,200 (Weicksel & Lupi, 2013; Bingham et al., 2015). The treatments utilized by public water systems to control HAB included powdered activated carbon, chlorine dioxide, and potassium permanganate (Weicksel & Lupi, 2013). Of the 15 public water systems surveyed, 10 reported using additional treatments due to HAB events (Weicksel & Lupi, 2013). In a single HAB event in 2013, Toledo, Ohio reported treatment costs of $1 million related to cyanotoxins in source water obtained from Lake Erie (Walker, 2014).

3.1.2 Effects on Human Health

Under the current trend of climate change, HAB events are predicted to increase in frequency, intensity, and duration, posing serious public health risks worldwide due to some cyanobacteria's capacity to produce cyanotoxins (Paerl, 2016; Paerl et al., 2011; Figgatt et al., 2016; Paerl & Huisman, 2009; Paul, 2008). In large quantities, cyanobacteria and the cyanotoxins they produce can have significant negative impacts on water quality, biotic health and economy (Srinivasan & Sorial, 2011; Landsberg, 2002; Hoagland & Scatasta, 2006; Adams et al., 2018). Cyanotoxins are secondary metabolites that have a wide range of molecular structures and toxicity, and can cause adverse health effects to humans such as abdominal pain, fever, headache, vomiting, nausea, diarrhea, pneumonia, tingling, numbness, incoherent speech, salivation, respiratory paralysis, and possible death (Szlag et al., 2015; USEPA, 2019). In lethal

doses, cyanotoxins are considered among the compounds with the highest toxicity in nature (Chorus & Welker, 2021). Cyanotoxin classes and concentrations in HAB can vary since one cyanobacterium can potentially produce multiple toxins, and multiple cyanobacteria can produce the same toxin (Davis et al., 2009; Chorus et al., 2021). The commonly documented cyanotoxins in freshwaters of the United States are microcystins, cylindrospermopsin, anatoxins, and saxitoxins (Szlag et al., 2015). Microcystins, a class of cyanotoxins with more than 200 congeners, can be produced by a number of cyanobacteria including *Anabaenopsis*, *Aphanocapsa*, *Aphanizomenon*, *Dolichospermum*, *Limnothrix*, *Microcystis*, *Nostoc*, *Oscillatoria*, *Phormidium* and *Planktothrix* (Chernoff et al., 2020; Buratti et al., 2017; Žegura et al., 2011). Microcystins are hepatotoxins with the potential to cause acute health effects in humans ranging from abdominal pain and headaches to pneumonia and liver disease (Jochimsen et al., 1998; Carmichael et al., 2001). Microcystins are regarded as possible human carcinogens (Nishiwaki-Matsushima et al., 1992; USEPA, 2015a). Previous studies such as, Yu et al. (2001), have reported that increasing microcystins concentrations in the river and pond sources positively correlated with the incidence of colorectal cancer. Zhou et al. (2001) also reported that the number of people drinking microcystin-contaminated water correlated with liver cancer cases in China. Another hepatotoxin, cylindrospermopsins, have also been documented as possibly carcinogenic and mutagenic, and can be produced by taxa such as *Aphanizomenon*, *Dolichospermum, Oscillatoria, Raphidiopsis,* and *Umazekia* (Buratti et al., 2017; Kinnear et al., 2010; US EPA, 2015c; Žegura et al., 2011). However, there is no sufficient data available to determine cylindropsermopsin's carcinogenic potential, although some studies have found preliminary evidence of tumor-initiating activities in mice such as immunotoxic effects in the

thymus and spleen (Diez-Quijada et al., 2022; Falconer & Humpage, 2001; Humpage et al., 2005; Maire et al., 2010; Puerto et al., 2018; Shen et al., 2002; USEPA, 2015c).

3.1.3 Thresholds

Potable water regulations are of importance to help safeguard human health against harmful effects of contaminants (USEPA, 2022). The United States Environmental Protection Agency (US EPA) has not imposed a regulation on cyanotoxins for potable waters (USEPA, 2021), and instead issued Health Advisories (HA) for cyanotoxins providing concentrations at which detrimental effects on human health are not anticipated (USEPA, 2021). HA for microcystins in bottle-fed infants and pre-school children is 0.3 µg/L and 0.7 µg/L for cylindrospermopsin over a ten-day period (USEPA, 2021). The HA for school-aged children and adults is 1.6 µg/L over a ten-day period while cylindrospermopsin is 3.0 µg/L (USEPA, 2021). Although no guideline at the federal level, states can implement their own regulations for cyanotoxins in potable waters. New Jersey does not currently have drinking water standards for cyanotoxins however, there is a pending act under public review which has an implementation schedule. A draft was developed on drinking water health advisory values for microcystins based on the US EPA's advisory at 0.07 µg/L for people six years of age and older (DWQI, 2022). Thus, investigating potentially toxin-producing HAB in source and finished waters of New Jersey is of importance since cyanotoxins are unregulated.

3.1.4 Climate Change Impacts

Climate change is predicted to increase extreme weather events such as heavy rainfall, heat and cold waves, and droughts (Fischer et al., 2015; Pörtner et al., 2022; Pall et al, 2011;

Rahmstorf et al., 2011; Reidmiller et al., 2018). Patterns of these extreme weather events, such as drought followed by heavy rainfall and flooding can cause excess nutrients and sediment to be transported in runoff, encouraging phytoplankton growth and formation of HAB (Chapra et al., 2017; HaRa et al., 2020; Kaushal et al., 2014). Moreover, intensifying lake stratification is another documented climate change trend contributing to the formation of HAB (Moore et al., 2008; Paerl et al., 2011). Increased thermal stratification and salinization-induced stratification suppress the mixing within the water column, and cause low oxygen in the hypolimnion which, when anoxic, can lead to a complex process where sediment-bound phosphorus is released into the hypolimnion (Behrenfeld et al., 2006; Smayda, 2010). This phenomenon creates a favorable environment for some phytoplankton taxa that are equipped with adaptations to have competitive advantages. For example, some cyanobacteria taxa such as *Microcystis* and *Dolichospermum* are able to regulate their gas vacuoles and change their position in the water column to reach the hypolimnion and utilize nutrients only available in this bottom layer (Behrenfeld et al., 2006; Smayda, 2010; Bormans et al., 1999). Additionally, taxa such as *Cylindrospermopsis* and *Dolichospermum* possess the capability to fix nitrogen which allows for their survival under nitrogen-limited waters (Willis et al., 2016; Wood et al., 2010). Other taxa such as *Synechococcus* are able to uptake orthophosphate at higher rates than eukaryotic algae; a competitive advantage in phosphorous-depleted waters (Moutin et al., 2002). Additionally, climate change has been suggested as a cause for some phytoplankton taxa to expand beyond their historical ecological ranges. Wiedner et al. (2007) suggested the toxin-producing cyanobacteria *Raphidiopsis raciborskii* expanded their range from subtropical to temperate water due to the earlier warming enabling earlier germination of *R. raciborskii*. This expansion led to

early population growth and establishment of *R. raciborskii* before the establishment of other phytoplankton taxa (Wiedner et al., 2007).

Eutrophication might be further intensified by the current trend of climate change resulting in even greater frequency and intensity of HAB events, and the negative effects they cause, posing a greater risk for drinking water safety (Manabe, 2019; Paerl & Huisman, 2009). To our knowledge, no studies have been published describing the harmful algal blooms, cyanobacteria and cyanotoxins in drinking water sources in New Jersey, which further highlights the need for public knowledge on HAB of New Jersey drinking water sources. The objective of this study is to assess the water quality conditions in drinking water sources in New Jersey. Five drinking water treatment facilities were selected; water quality and harmful algal bloom parameters were examined. Finished water was also tested to determine efficacies of cyanotoxin removal.

3.2 Materials and methods

3.2.1 Study sites

Five water treatment plants in New Jersey that pump water from a reservoir or directly from a river source were included in this study. Due to the sensitivity of identity, the names and specific locations of these treatment plants were not disclosed; instead, sites are referred to as sites A through E in this chapter. Site A draws its source water from the confluence of Millstone River and Raritan River, serves as a drinking water supply for more than 1 million residents, and produces an average of 132 million gallons per day (MGD) (NJDEP, 2017). Site B draws its source water from a reservoir that is fed by the following bodies of water: Ramanessin Brook, Fourth Creek, Bordens Brook, Willow Brook, Hopp Brook, Big Brook, Fulling Mill Brook,

Barren Neck Brook, Trout Brook, Yellow Brook, Miry Bog Brook, Mine Brook, Slope Brook, Hockhockson Brook, and Pine Brook (NJDEP, 2017). This 2.3 billion-gallon (BG) reservoir has a catchment basin of 125.6 km. Site C receives source water for its reservoir from the Shark River and Jumping Brook with a catchment area of 41.4 km and a storage capacity of 1 BG (NJDEP, 2017). Sites B and C serve as a drinking water supply to a combined total of 366,000 people (NJDEP, 2017). Site D is sourced directly from the Pompton River and the Passaic River and has an off-stream reservoir with a storage capacity of 2.9 BG. It produces an average of 83 MGD and serves about 1 million people (NJDEP, 2017). Site E is sourced from a different section of the Passaic River and serves 2.7 million people (NJDEP, 2017). The water from Site E is pumped and collected into three raw water reservoirs before the treatment process and has a combined storage capacity of 2.84 BG.

3.2.2 Field sampling

Samples were collected once a month between May and October 2019 from two locations at each treatment plant: source (at 0.5 m water depth in a reservoir/river) and finished. For source waters, in-situ water characteristics such as water temperature (T), pH, dissolved oxygen (DO), and specific conductance (Cond) were recorded on location using a multi-parameter sonde (Yellow Springs Instrument Professional Plus; Yellow Springs, OH). Fluorescence of phycocyanin (PC) and fluorescence of chlorophyll (Chl) were recorded on locations in source waters using a hand-held fluorometer (CyanoFluor, Turner Designs; San Jose, CA). Water samples were collected, stored on ice in coolers (4°C), and transferred to Montclair State University laboratories for further analysis. Parameters analyzed at the laboratory included total suspended solids (TSS), total dissolved solids (TDS), chlorophyll-a (Chl-a), total nitrogen (TN), total phosphorus (TP), ammonia (AM), non-purgeable dissolved organic carbon (NPDOC),

phytoplankton cell density (Phyto), cyanobacterial cell density (Cyano), microcystins (MC), cylindrospermopsin (CY), *Microcystis* 16S rRNA gene (MCS), microcystin-producing *mcyB* gene (MCN), *Cylindrospermopsis raciborskii-specific* genes *rpoC1* (CYS), and cylindrospermopsin-producing *pks* gene (CYN). Finished water samples were only analyzed for MC and CYN. Samples for TSS, TDS, TN, NH_3, NPDOC, Phyto, and Cyano were collected in plastic bottles cleaned with phosphorus-free soap, while Chl-a were collected in the same containers and wrapped in foil to protect from light. MC and CY samples were collected in amber glass containers cleaned with phosphorus-free soap. Finished water CY samples were preserved using ascorbic acid and pH was adjusted between 4 and 7. TP samples were collected using a plastic bottle cleaned with 1:1 hydrochloric acid and rinsed with deionized water. Polypropylene bottles were washed with phosphorus-free soap and autoclaved for sterilization prior to collecting MCS, MCN, CYS, and CYN samples.

3.2.3 Phytoplankton identification and enumeration

Phytoplankton water samples were preserved by adding Lugol's iodine solution as soon as they were back in the lab. Samples were kept in a cold room until processing. Phytoplankton samples were identified via sedgwick-rafter chamber under a light microscope with phase contrast using a long-distance working objective using the following taxonomic keys: *Freshwater algae of North America: ecology and classification* (Wehr et al., 2015), *Cyanoprokaryota-1. Teil/Part 1: Chroococcales* (Komárek & Anagnostidis, 2008), *Cyanoprokaryota-2. Teil/Part 2: Oscillatoriales* (Komárek & Anagnostidis, 2008), and *Cyanoprokaryota-3. Teil/Part 3: Heterocytous Genera* (Komárek, 2013). Samples were homogenized by gently inverting the sample container 25 times. For each replicate, 1mL of sample was pipetted into the chamber and settled for fifteen minutes before counting. Cells were

counted and identified at 400x to the genus level until at least 200 natural units or 40 fields of view were reached. Cells with GALD (greatest axial linear dimension) of <2µm were not recorded due to identification difficulty. Zooplankton and nonviable cells were excluded from counts. For quality control, samples were counted in triplicates to account for natural variability.

3.2.4 Laboratory analyses

3.2.4.1 Water quality parameters. TSS and TDS were analyzed within 24 hours according to *Standard Methods for the Examination of Water and Wastewater* (Method 2540D and 2540C). Chl-a were filtered within 24 hours. The filters were then stored in a freezer and analyzed within 24 days following the EPA Method 445.0. TN, NH_3, and TP were preserved to pH less than 2 with concentrated sulfuric acid and kept at or below 6°C. These samples were analyzed within 28 days, where pH was adjusted to 7 with 5.0 N sodium hydroxide solution, and the results were later corrected for dilution. TN was determined using the Total Nitrogen by the Persulfate Digestion Test 'N Tube method (Hach Method 10071), TP with the Total Phosphorus by the PhosVer® 3 Ascorbic Acid method with Acid Persulfate Digestion (Hach Method 8190), and NH_3 with Ammonia Nitrogen by the Salicylate method (Hach Method 8155) using a UV–Vis spectrophotometer (Hach, DR 5000, CO). NPDOC were analyzed within 24 hours, and the concentrations were determined using a Total Organic Carbon Analyzer (Shimadzu TOC-L CPH, Columbia, MD). MC and CY samples were kept frozen and analyzed using the Microcystins-ADDA ELISA (Microtiter Plate) kit for Microcystins/Nodularins and Abraxis Cylindrospermopsin ELISA kit (Eurofins Abraxis, Warminster, PA) following the manufacturer's instructions within three months. Quantified MC concentrations less than 0.15 µg/L, the lowest limit of quantification and CY concentration less than 0.04 µg/L were reported as undetectable (UD). Copy numbers of MCS less than 117 copies/mL, MCN less than 228

copies/mL, CYS less than 141copies/mL, and CYN less than 187 copies/mL were reported as undetectable.

3.2.4.2 Real-time PCR. Two hundred mL of water samples were filtered within 24 hours with mixed cellulose ester filters (0.45 mm, 47mm) for MCS, MCN, CYS, and CYN analysis. The filters were kept in a freezer and analyzed within six months. DNeasy Power Water Kits (Qiagen, Germantown, MD) were used for DNA extraction. The final eluate was 100 mL. The DNA concentrations and purity were checked using a NanoDropTM 2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Real-time polymerase chain reaction (qPCR) analysis was performed using StepOnePlus Real-Time PCR System (Thermo Fisher Scientific). Primers used in this study, including MCS, MCN, CYS, and CYN, were shown in Table 3.1. Known concentrations of recombinant plasmid including gene targets were used to establish calibration curves against cycle threshold values (C_T). DNA extracts from *Microcystis aeruginosa* UTEX B2662 culture or water samples identified with presence of *Cylindrospermopsis* were used as template for cloning. Detailed process of cloning was described elsewhere in Hsu et al., (2019). Contents of Master Mix stock included 1X PowerUp SYBR Green Master Mix (Applied Biosystems, Foster City, CA) and 10 mM of forward and reverse primers (Table 3.1). Programs started at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 sec and polymerization at 60°C for 1 min. Quality control measures included duplicate sample analysis, no-template-control, coefficient of determination of calibration curves greater than 0.99 as well as melting curve programs to confirm the specificity of amplification. Levels of genetic markers were reported in copies/mL of water. Reporting limit for MCS was 117 copies/mL, 228 copies/mL for MCN, 141 copies/mL for CYS, and 187 copies/mL for CYN.

Table 3.1. Primers selected for qPCR quantifications in the analysis of water samples collected from five New Jersey drinking water treatment facilities at three locations: source and finished.

Target Gene	Primer/Probe	Sequence	Size (bp)	Literature Cited
Microcystis 16S rRNA	Micr184F Micr431R	GCCGCRAGGTGAAAMCTAA AATCCAAARACCTTCCTCCC	247	Rinta-Kanto et al., 2005; Chiu et al., 2017
Microcystin *mcy*B	*mcy*B#04F *mcy*B#04R	TGTGGAGTCTATTTATCCTCTTTCC GAGTTTGACTACAATAAATCCCTGAAT	95	Yen et al., 2012; Chiu et al., 2017
Cylindrospermopsis raciiborski rpoC1	Cyl2 Cyl4	GGCATTCCTAGTTATATTGCCATACTA GCCCGTTTTTGTCCCTTTGCTGC	308	Rasmussen et al., 2008; Wilson et al., 2000; Chiu et al., 2017
Cylindrospermopsin pks	m4 k18	GAAGCTCTGGAATCCGGTAA CCTCGCACATAGCCATTTGC	422	Schembri et al., 2001; Rasmussen et al., 2008; Fergusson & Saint, 2003; Chiu et al., 2017

3.2.5 Statistical Analyses

Assumptions of normality were not met in all variables, thus non-parametric tests were performed. Spearman's Rank Correlation coefficient was used to assess the relationship between the parameters. Kruskal-Wallis tests were performed to show significant differences between the parameters by sites and month. All statistical analyses were completed using JMP Pro 11 software. For MC concentrations less than 0.15 µg/L, the lowest limit of quantification, 0.075 µg/L was used for statistical analyses. For CY concentrations less than 0.04 µg/L, the lowest limit of quantification, 0.02 µg/L was used for statistical analyses. CY, CYS, and CYN were excluded from statistical analysis because the majority of values for these parameters were at or below the detection or reporting limit.

3.3 Results

3.3.1 Water Quality at Five Study Sites

Site A: Results showed that there was low cyanobacterial cell count and undetected cyanotoxins at Site A within the duration of the study. Seasonal patterns indicate that HAB water quality results were higher in the fall. This waterbody had the highest median TSS concentration of all sites at 4.4 mg/L (ranging from 2.6 in October to 25.9 mg/L in July); which was above thresholds and indicates water is not clear (Table 3.2). NPDOC concentrations were lowest at 3.3 in June and highest at 17.8 mg/L in October. The lowest median NPDOC value of all sites was recorded in Site A at 7.3 mg/L with other studies that have found similar concentrations (<0.2 to 36 mg/L) of organics in rivers (Table 3.2; Canham et al., 2004, Kretser et al., 1989). Overall, this site had the lowest Cyano cell count documented among all sites (0 in all months except September at 27 cells/mL) (Figure 3.1). MCS had the lowest median copy numbers among all sites at 1,425 copies/mL (minimum of 364 in September and maximum of 5,572 copies/mL in October) (Figure 3.2). All MC concentrations were found to be below the detection limit (Table 3.3). Molecular results supported the observations of low MC concentrations with MCN having the lowest median copy numbers among all sites at 511 copies/mL (lowest was undetected in August and highest was 922 copies/mL in July) (Figure 3.2). *Raphidiopsis* was detected at this site, however CY concentrations for all samples were below the detection limit. As for *Cylindrospermopsis* and cylindrospermopsin-producing genes, low CYS and CYN copy numbers were observed, the highest median CYS value among all sites was reported at 1,131 copies/mL (ranging from undetected in May, June, July, and October to 2,033 copies/mL in September), while CYN was not detected in any sample from this study site.

With regards to the nutrient water quality, site A had elevated nutrient concentrations. Seasonal patterns indicate that nutrient levels were higher in the summer. This site had the second lowest median NH_3 concentration among all sites at 0.05 mg/L (minimum of 0.01 in June to maximum of 0.11 mg/L in August). TN concentrations ranged from 0.85 in October to 1.56 mg/L in June and had the second highest median TN concentration among all sites at 1.26 mg/L (Figure 3.3). TP concentrations were lowest at 0.15 mg/L in September and highest at 0.60 mg/L in July (median 0.22mg/L), and all concentrations documented were above the thresholds for rivers (equal to or less than 0.1 mg/L) under the New Jersey Surface Water Quality Standards (SWQS).

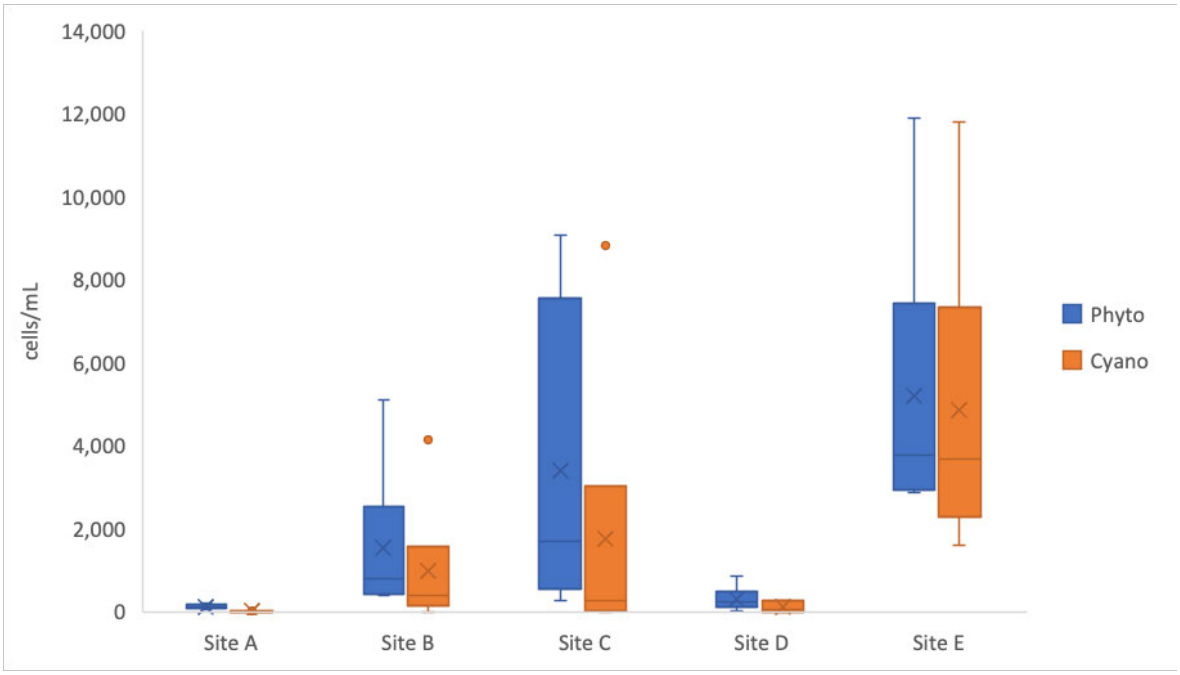

Figure 3.1. Side by side box and whisker plot of phytoplankton cell density (Phyto) and cyanobacterial cell density (Cyano) in five New Jersey drinking water sources. The x represents the median; the whiskers represent the minimum and maximum values.

Site B: Similar to Site A, low cyanobacterial cell count and undetected cyanotoxins were reported in Site B during the study period. HAB water quality results were higher during the

summer and fall seasons. This site was characterized by clear water, with the lowest median TSS concentration among all sites at 1.9 mg/L (minimum of 0.9 in May to maximum of 3.1 mg/L in September) (Table 3.2). NPDOC concentrations peaked at 14.0 mg/L in October and dropped down to 4.0 in July (median 8.2 mg/L) which were similar to those previously reported (<0.2 to 36 mg/L) for lentic systems (Table 3.2; Canham et al., 2004; Kretser et al., 1989). Cyano cell densities reported in Site B were the second highest median among all sites at 398 cells/mL (ranging from 0 in June to 4,131 cells/mL in September) which was below the 20,000 cells/mL cyanobacteria threshold enforced during the study period by the NJDEP (Figure 3.1; Table 3.3; NJDEP, 2019). MCS had the second lowest median copy numbers at 3,039 copies/mL (minimum of 571 in May to maximum of 6,018 copies/mL in October). The low cyanobacterial cell densities reported were supported by low toxin and molecular values (Figure 3.2). This site had undetected MC concentrations for all samples. MCN copy numbers ranged from undetected in May to 2,606 copies/mL in June (median 988 copies/mL). As for *Cylindrospermopsis* and cylindrospermopsin-producing genes, low CYS and CYN copy numbers were observed. The lowest CYS value was undetected in May, June, August, and October and the highest was 1,627 copies/mL in September (median 941 copies/mL). CY not detected in any Site C water sample, however *Raphidiopsis* was observed.

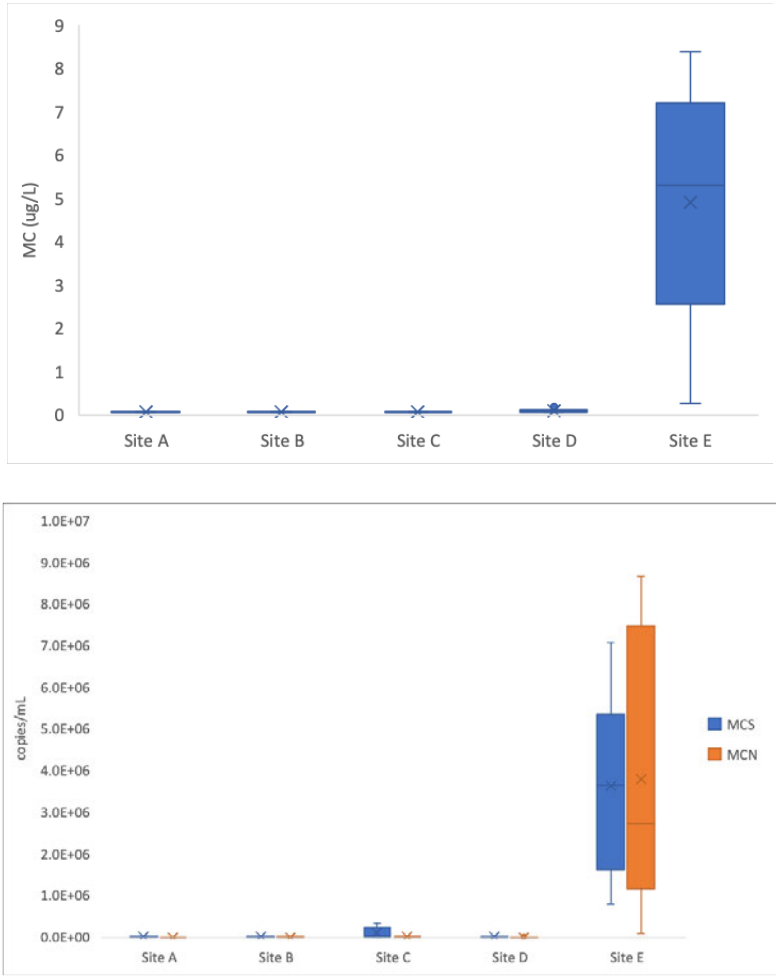

Figure 3.2. Box and whisker plot comparison between microcystins (MC), *Microcystis* 16S rRNA (MCS), and microcystin-producing *mcyB* gene (MCN) in five New Jersey drinking water sources. The x represents the median; the whiskers represent the minimum and maximum values.

In regard to the nutrient water quality, site B had excess nutrients. Nutrients concentrations at this site were higher in the spring and summer seasons. NH_3 concentrations ranged from 0.01 in June and July to 0.48 mg/L in August, TN had the second lowest concentration among all sites at 0.75 mg/L (minimum of 0.35 in July to maximum of 1.30 mg/L in August) (Figure 3.3). TP concentrations had the lowest median concentration at 0.15 mg/L (0.13 in June to 0.25 mg/L in May); however, these values were above the NJ SWQS TP threshold for lakes (equal to or less than 0.05 mg/L).

Site C: Cyanobacterial cell densities were low and toxins were undetected in Site C, however cyanobacteria taxa were documented. Overall, HAB parameters were higher in the summer season. This site had TSS lowest concentrations at 1.4 in May to highest concentrations at 5.2 mg/L in August (median 2.8 mg/L), suggesting clear water. However, the highest median NPDOC concentration was reported at 12.4 mg/L (ranging from 11.5 in June to 13.9 mg/L in September), which previous reports have suggested concentrations greater than 12 mg/L as indicative of eutrophication (Table 3.2; Canham et al., 2004; Kretser et al., 1989). Cyano cell densities were low at this site and ranged from 0 in June to 8,844 cells/ mL in October (median 269 cells/mL) while all MC concentrations recorded for this site were undetected (Figure 3.2, Table 3.3). Molecular results showed MCS and MCN had the second highest median copy numbers among all sites at 64,277 copies/mL (minimum of undetected in May to maximum of 336,077 copies/mL in August) and 6,347 copies/mL (ranging from undetected to 13,583 copies/mL in July), respectively. In contrast, CYS had the lowest median copy numbers among all sites at 141 copies/mL with lowest values at undetected in May, June, July, September, and October to highest values at 292 copies/mL in August. Although *Raphidiopsis* was present, all of the waters tested in this site had undetected CY concentrations.

Nutrient water quality at this site suggested substantial concentrations present. Seasonal trends in this site suggested nutrients were higher in the spring and summer. Among the five sites, NH_3 had the second highest median concentration at 0.11 mg/L, with the lowest value of 0.03 in August and highest value of 0.19 mg/L in June. TN had the lowest reported median concentration among all sites at 0.73 mg/L (ranging from 0.35 in October to 1.31 mg/L in May) (Figure 3.3). While TP had the second lowest median concentration at 0.20 mg/L (minimum of 0.13 in August to maximum of 0.57 mg/L in May) and above the SWQS threshold.

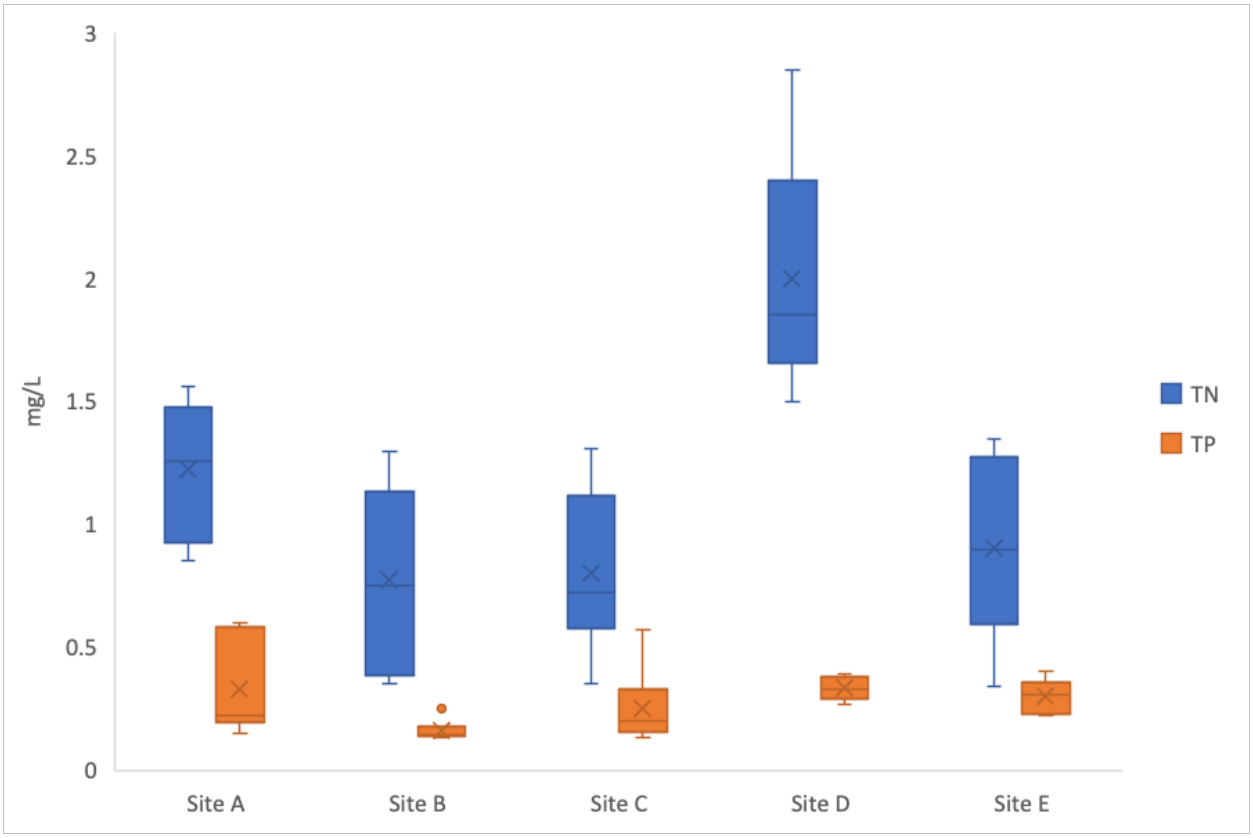

Figure 3.3. Side by side box and whisker plot of total nitrogen (TN) (mg/L) and total phosphorus (TP) (mg/L) in five New Jersey drinking water sources. The x represents the median; the whiskers represent the minimum and maximum values.

Site D: The results showed that Site D had low cyanobacterial cell densities and undetected cyanotoxins within the duration of the study. HAB parameters in this site were higher in the summer. The second highest median TSS concentration among all sites was reported at 3.8 mg/L (ranging from 0.8 in October to 11.4 mg/L in May); however, these concentrations were below thresholds, suggesting clear waters (Table 3.2). NPDOC concentrations were second lowest among all sites with a median of 8.2 mg/L, with the lowest value at 4.0 in May and the highest value at 20.7 mg/L in October (Table 3.2). Cyano cell densities were low, with Site D having the second lowest value among all sites at 56 cells/mL (minimum of 0 in June and August to maximum of 259 cells/mL in October) (Figure 3.1, Table 3.3). MCS copy numbers ranged

from undetected in May, June, and September to 7,555 copies/mL in July (median 5,116 copies/mL). These low cyanobacterial counts were supported by low toxin and molecular results (Figure 3.2). This site had MC concentrations that ranged from undetected in May, July, August, September, and October to 0.18 µg/L in June. None of the finished water was detected with MC. MCN and CYS had the second lowest median copy numbers at 808 copies/mL (minimum of undetected in May, June, September, and October to maximum of 1,285 copies/mL in August) and 211 copies/mL (minimum of undetected in May, June, July, August, and October to maximum of 211 copies/mL in September), respectively. While *Raphidiopsis* was observed at this site, CY was undetected in source and finished waters.

Nutrient water quality in Site D was elevated with nutrients being the highest among all sites; nutrients were higher in the summer and fall. Site D had more nutrient issues than all other sites. The highest median NH_3, TN, and TP concentrations among all sites were reported at 0.14 mg/L (ranging from 0.06 in August to 0.36 mg/L in October), 2.85 mg/L (ranging from 1.50 in July to 2.85 mg/L in September), and 0.33 mg/L (ranging from 0.27 in August to 0.39 mg/L in June), respectively (Figure 3.3). All TP concentrations recorded at this site exceeded SWQS thresholds.

Site E: Among all sites surveyed, the only site that exhibited HAB issues, specifically elevated microcystins levels, was Site E. Specifically, HAB parameters were highest in the summer and fall. The highest median TSS concentration among all sites was reported at 4.9 mg/L (minimum of 2.5 in May and maximum of 5.6 mg/L in June), which is below threshold and indicates clear waters (Table 3.2). This site had the second highest NPDOC median concentration at 11.5 mg/L (ranging from 5.1 in July to 20.1 mg/L in October) (Table 3.2). The highest median Cyano cell densities were documented at this site at 3,687 cells/mL (ranging

from 1,603 in September to 11,807 cells/mL in October), with *Microcystis* cells present (Figure 3.1, Table 3.3). These results were supported by MCS values with the highest median copy number among all sites at 3,648,401 copies/mL (lowest at 794,435 in May to highest at 7,076,480 copies/mL in July (Figure 3.2).

MC concentrations were documented at all months at this site, with a median of 5.31 µg/L (ranging from 0.26 in May to 8.39 µg/L in September) (Table 3.3). The treatment processes were able to effectively remove MC and it was not detected at finished water samples. Monthly MC concentrations from source to finished waters in Site E were: May from 0.26 µg/L to UD, June from 4.80 µg/L to UD, July 3.32 µg/L to UD, August 5.81 µg/L to UD, September 8.39 µg/L to UD, and October 6.80 µg/L to UD. Molecular analyses supported these cell count and toxin results with the highest median MCN numbers at 2,723,562 copies/mL (minimum of 82,437 in May and maximum of 8,660,900 copies/mL in September. CY concentrations were only detected in Site E at 0.09 µg/L in July and were undetected in finished waters. This suggests that they were being effectively removed by treatment processes. CYS had the second highest median value of all sites at 958 copies/mL (ranging from undetected in May, June, and July to 2,794 copies/mL in September).

Site E nutrient water quality exhibited considerable nutrient concentrations. Of all sites documented, site E had greater HAB issues with the highest values of HAB conditions. Seasonal trends indicate that nutrients were high in all seasons. This site had the lowest median NH_3 concentrations at 0.04 mg/L (minimum of 0.01 in August and maximum of September to 0.10 mg/L in July). TN concentrations ranged from 0.34 in May to 1.35 mg/L in September (median 0.90 mg/L) while the second highest median TP concentration among all sites was reported at

0.31 mg/L (lowest at 0.22 in October and highest at 0.40 mg/L in May) (Figure 3.3). TP concentrations exceeded SWQS thresholds in this site.

3.3.2 Comparison of Source Water Quality

A Principal Component Analysis was performed to examine the association between HAB parameters (the first component) and water quality parameters (the second component). The PCA results showed that the first component explains 31.4% of the variation and the second component explains 16.6% of the variation (Figure 3.4). PC, MC, and MCS had large positive loadings on the first component suggesting these three HAB parameters were associated with one another. TN had a large positive loading on the second component with TP and NH_3, suggesting a positive relationship with the HAB parameters. Temporal analysis of water quality conditions of all five sites combined were analyzed to determine any statistically significant difference. Results indicated that NPDOC was the only parameter that varied significantly by month ($p=0.0037$).

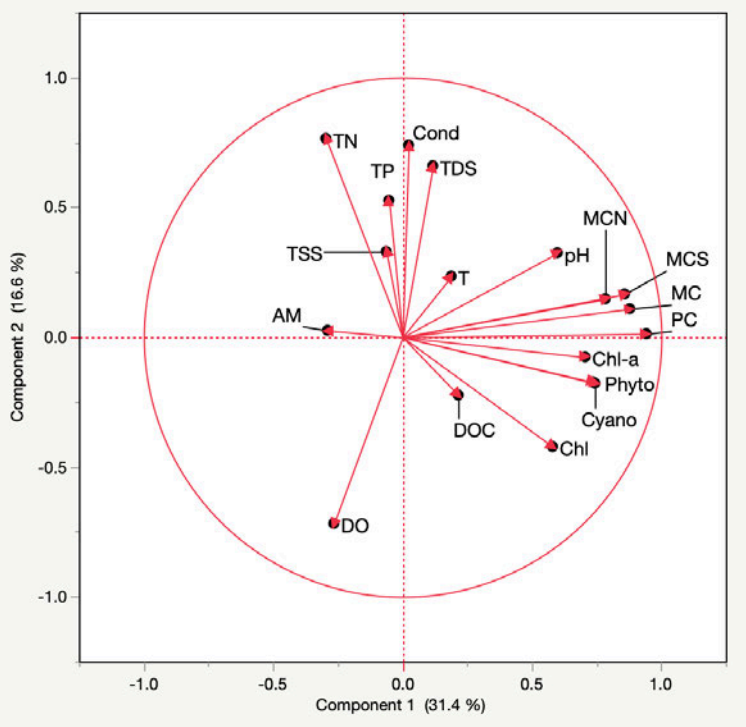

Figure 3.4. Principal components analysis of water quality data in five New Jersey drinking water sources. Conductivity (Cond), dissolved oxygen (DO), pH, temperature (T), total suspended solids (TSS), total dissolved solids (TDS), non-purgeable dissolved organic carbon (DOC), ammonia (NH_3), total nitrogen (TN), total phosphorus (TP), fluorescence of phycocyanin (PC), fluorescence of chlorophyll (Chl), chlorophyll-a (Chl-a), phytoplankton cell density (Phyto), cyanobacterial cell density (Cyano), microcystins (MC), *Microcystis* 16S rRNA (MCS), and microcystin-producing *mcyB* gene (MCN).

In addition to temporal analysis, water quality results of source waters were compared between sites to discern statistically significant differences. Results showed that there was a significant difference in the TSS ($p=0.0416$), Cond ($p=0.0004$), and pH ($p=0.0009$) values between source sites, suggesting unique water chemistry characteristics of each drinking water source. With respect to nutrients, results showed that both TN ($p=0.0016$) and TP ($p=0.0118$) concentrations were statistically significant. This indicates some sites have significantly higher nutrients than others thus, a post hoc test was used to identify which sites were different. Lastly, various HAB water quality parameters differed significantly by site including Chl ($p=0.0028$), PC ($p=0.0003$), Chl-a ($p=0.0026$), Phyto ($p=0.0003$), MC ($p<0.0001$), MCS ($p=0.0026$), and

MCN (p=0.0025). These results suggest some sites had significantly greater HAB issues than others. This was further confirmed by Dunn's post-hoc test results which investigated the difference in source water quality among the five selected study sites (Table 3.4). Dunn's post-hoc test results showed that Site D had elevated nutrient water quality; TN concentrations in Site D were significantly higher than in Sites B (p=0.0061), C (p=0.0077) and E (p=0.0255). On the other hand, TP concentrations were not significantly different among sites with an exception that Site D had significantly higher concentrations than Site B (p=0.0162).

Although not the most eutrophied site, Site E demonstrated the most severe HAB water quality with the highest average cyanobacterial cell densities, microcystins concentrations, and microcystin-producing *mcyB* gene copies. Cyano cell densities were significantly higher in Site E than sites A (p=0.0009) and D (p=0.0363). Overall, MC concentrations were significantly higher in Site E than all other sites: Site A (p=0.0005), Site B (p=0.0005), Site C (p=0.0005), and Site D (p=0.0040). Site E had significantly higher copy numbers of MCN than Sites A (p=0.0220), B (p=0.0419) and D (p=0.0024). Additionally, Site E had significantly higher PC values (p=0.001) and Phyto (p= 0.0008) than Site A. Similar patterns were found at site D, where PC values (p=0.0037) and Phyto (p=0.0184) were also greater than Site A. Among all five sites, D and E were of the greatest concern due to their elevated nutrient and/or HAB water quality.

Table 3.2. Post-Hoc Dunn Method results for Kruskal-Wallis test of drinking water sources by study site. (nd: not significant; $p > 0.05$). Conductivity (Cond), dissolved oxygen (DO), pH, temperature (T), total suspended solids (TSS), total dissolved solids (TDS), non-purgeable dissolved organic carbon (DOC), ammonia (NH_3), total nitrogen (TN), total phosphorus (TP), fluorescence of phycocyanin (PC), fluorescence of chlorophyll (Chl), chlorophyll-a (Chl-a), phytoplankton cell density (Phyto), cyanobacteria cell density (Cyano), microcystins (MC), *Microcystis* 16S rRNA (MCS), and microcystin-producing *mcyB* gene (MCN).

Sites	Cond	DO	pH	T	TSS	TDS	DOC	NH_3	TN	TP	PC	Chl	Chl-a	Phyto	Cyano	MC	MCS	MCN
A vs B	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.0183	nd	nd	nd	nd	nd	nd
A vs C	nd	nd	0.0284	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.0255	nd	nd	nd	nd
A vs D	0.0131	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
A vs E	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.0010	0.0205	0.0170	0.0008	0.0009	0.0005	0.0115	0.0220
B vs C	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B vs D	0.0012	nd	nd	nd	nd	nd	nd	nd	0.0061	0.0162	nd	nd	nd	nd	nd	nd	nd	nd
B vs E	0.0460	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.0005	nd	0.0419
C vs D	nd	nd	nd	nd	nd	nd	nd	nd	0.0077	nd	nd	nd	nd	nd	nd	nd	nd	nd
C vs E	nd	nd	0.0004	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.0005	nd	nd
D vs E	nd	nd	nd	nd	nd	nd	nd	nd	0.0255	nd	0.0037	nd	0.0057	0.0184	0.0363	0.0040	0.0037	0.0024

3.3.3 Impact of Environmental Conditions on HAB

HAB were detected in some of the study sites at the level posing a threat to human recreation safety and drinking water quality. Thus, we sought to identify environmental conditions that might have been influencing the formation and development of HAB. The impacts of environmental conditions on the growth of cyanobacteria, *Microcystis*-specific genes, and microcystin-producing genes were examined using Spearman correlations. Results showed that as TN increased, Cyano cell densities were lower (r_s=-0.5137, p=0.0037), whereas TP concentrations were not found to significantly affect cyanobacterial cell densities. Furthermore, TN concentrations were lower with greater MCS copy numbers (r_s=-0.4498, p=0.0126),

supported by previous studies, suggesting nitrogen could be a nutrient source used by *Microcystis* (Conradie & Barnard, 2012; Rinta-Kanto et al., 2009). Our study results also showed that microcystin-producing *mcyB* gene copy numbers (MCN) decreased with increasing TN concentrations (rs= -0.4462, p=0.0135). Conradie & Barnard (2012) reported that MCN copies were lower when TN concentrations were higher, indicating that nitrogen could be used during microcystin production. In addition, NH_3 was found to increase with the decreasing *Microcystis* MCS copy numbers (rs=-0.4211, p=0.0205) and microcystin-producing *mcyB* gene MCN copy numbers (rs=-0.5639, p=0.0012).

While previous studies have documented high Cyano cell densities associated with high MC concentrations, a high cyanobacterial density sample could have no toxins detected (Bolch et al., 1997; Rolland et al., 2005). Toxin detection can depend on different toxin-producing cyanobacteria taxa and their wide range in capacity to produce MCs (Chorus, 2001). Furthermore, there are particular environmental conditions, such as temperature and nutrients, that affect the MC production of each toxin-producing cyanobacteria taxa (Mowe et al., 2015b; Vézie et al., 2002). In our study, increasing Cyano cell densities were associated with higher MC concentrations (rs=0.5211, p=0.0032) and higher MCN copy numbers (rs=0.5725, p=0.0009). Furthermore, we sought to investigate if certain cyanobacterial taxa, such as *Microcystis*, were related to toxin concentrations. Our results showed that as MC concentrations increased with the abundance of *Microcystis* 16S rRNA copies (rs=0.5903, p=0.0008) and microcystin-producing *mcyB* genes (rs=0.6037, p=0.0004). Higher MC concentrations with increasing *Microcystis* 16S rRNA copies and microcystin-producing *mcyB* genes were also reported by Conradie and Barnard (2012), Rinta-Kanto et al. (2009), Ha et al. (2009), Otten et al. (2012), and Te and Gin (2011). Our results aligned with published literature (Beversdorf et al., 2015; Francy et al., 2016;

Izydorczyk et al., 2005) demonstrating that PC values increase with MC concentrations (rs=0.5675, p=0.0011) (Table 3.5).

3.4 Discussion

Cyanobacteria were found at the source waters of all five drinking water facilities selected for this study. *Microcystis* was the most dominant cyanobacteria taxon. Previous studies have shown contradicting results regarding the positive correlations between *Microcystis* and cyanotoxin concentrations since toxin-producing genes can be present or absent in *Microcystis* (Dai et al, 2006; Kurmayer et al., 2003; Orr & Jones, 1998; Rinta-Kanto et al., 2009; Via Ordorika et al., 2004). Furthermore, even when toxin-producing genes are present, toxins might not be produced which is believed to possibly be controlled by environmental factors (Dai et al., 2006; Kurmayer et al., 2003; Orr & Jones, 1998; Rinta-Kanto et al., 2009; Via-Ordorika et al., 2004). Studies such as Kardinaal & Visser (2005) reported that high *Microcystis* densities does not necessarily indicate high toxicity in water. Thus, quantifying MCS and MCN copy numbers can be alternative parameters that provide closer estimations of the true toxicity in waters and a valuable tool for monitoring blooms (Bittencourt-Oliveira et al., 2010; Conradie & Barnard, 2012; Mankiewicz-Boczek et al., 2006; Rinta-Kanto et al., 2009; Te & Gin, 2011; Wood et al., 2021). Results from this study also confirmed that higher MCS gene copy numbers and MCN copy numbers presence were associated with higher MC concentrations. Furthermore, our results suggest that MCN copy numbers were more sensitive and had lower detection when compared with MC concentrations. Thus, MCN copy numbers can be used for early detection of potentially toxic HAB before MC accumulates to a detectable concentration using current available quantification methods.

PC is the primary photosynthetic pigment found mainly in freshwater cyanobacteria and has been widely used as a proxy to estimate cyanobacterial biomass and HAB in freshwater in-situ (Ahn et al., 2002; Basheva et al., 2018; Brient et al., 2008; Cotterill et al., 2019; Izydorczyk et al., 2005; Lee et al., 1994). In our study sites, fluorescence of PC values were significantly correlated with various cyanobacterial parameters including MC concentrations, MCS copy numbers, and MCN copy numbers. These results support the concept that fluorescence of PC is a good proxy to estimate HAB conditions at our study sites. However, it is important to consider that PC has limitations since certain environmental conditions and pigment cell content can interfere with the in-situ measurement of PC fluorescence and thus, these interferences need to be accounted for (Beutler et al., 2003; Chang et al., 2012; Zamyadi et al., 2016). The same phycocyanin pigments can result in different RFU measurements at different temperatures and light conditions (Rousso et al., 2021; Zamyadi et al., 2016). For example, light intensity influences cyanobacteria fluorescence emission by light harvesting regulation processes, thus darker environments have higher production rates per unit volume than brighter ones (Erickson et al., 2012; Rousso et al., 2021). This suggests that cyanobacteria that occupy the top of the water column during the day would have a reduction in pigment production and less fluorescence emitted by each cell due to intense light availability (Hofstraat et al., 1991; Zamyadi et al., 2016). The effects of light stress on phytoplankton cellular fluorescence have been documented to be correlated to contractions and movement of chloroplasts associated with photosynthesis photoinhibition and fluctuations in cellular fluorescence (Kiefer, 1973). In contrast, cyanobacteria located at the bottom of the water column with reduced light might increase their phycocyanin pigment production to have a greater ability to process light and lead to an increase in their phycocyanin fluorescence per cell (Zamyadi et al., 2016). Furthermore, fluorescence

intensity has been documented to decrease as water temperature increases (Downing et al., 2012; Watras et al., 2011). Similarly, Henderson et al. (2009) concluded that organic matter fluorescence intensity decreased with increased temperature. Thus, the effects of water temperature on phycocyanin fluorescence and field measurements are a subject that should be further studied. While fluorescence of PC has been reported as a good real-time proxy for cyanobacterial biomass, it should not be used as a HAB indicator.

In this study, high Chl-a concentrations, and MCS and MCN copy numbers in the selected drinking water sources had a significant inverse correlation with concentrations of total nitrogen and NH_3. Additionally, high Phyto and Cyano densities were negatively correlated with TN. While nitrogen is an essential macronutrient needed for cyanobacterial growth, helps regulate metabolic activity, and promotes cyanobacterial growth, phytoplankton and cyanobacteria can differ in their preference and reactions to various forms and concentrations of nitrogen (Dai et al., 2012). Freshwater cyanobacteria mostly take up and use nitrate, ammonia, and urea, with NH_3 being the most favorable nitrogen source (Bothe et al., 1982; Yates et al., 1980; Wu et al., 2006). This is so since NH_3 assimilation is less energetically costly than NO_{3-} reduction and N_2 fixation (Schubert, 1982). However, NH_3 has toxic effects on cyanobacteria at higher concentrations, pH values, and light intensity (Markou & Muylaert, 2016; Peccia et al., 2013; Yuan et al., 2011). NH_3 toxicity can suppress the enzyme activity, photosynthesis, denitrification, and growth of cyanobacteria (Azov & Goldman, 1982; Belkin & Boussiba, 1991; Gao et al., 2012; Thomas et al., 1980). Previous studies have reported that Chl-a concentrations in freshwaters are inversely correlated to NH_3 and have concluded that NH_3 could be a factor that limits phytoplankton growth (Ooi et al., 2010). Temporal analyses suggest that low NH_3 concentrations and high phytoplankton and cyanobacteria presence is observed during the

growing seasons (Carvalho, 1994; Dai et al., 2012). Ooi et al. (2010) reported that when NH_3 concentrations are low NO_{3-} is utilized by phytoplankton thus leading to an inverse correlation between NO_{3-} and phytoplankton. Certain cyanobacteria taxa, such as the non-N-fixing *Microcystis*, have also been reported to be efficient at taking up NH_3 during the summer (Dai et al., 2012). Blomqvistet al. (1994) hypothesized that due to the lack of nitrogen-fixing capabilities, cyanobacteria taxa such as *Microcystis* have higher competitiveness for ammonia nitrogen than other phytoplankton and thus ammonia concentrations could influence cyanobacterial dominance.

In addition to the associations between TN concentrations and cyanobacteria, there was a presence of high TP concentrations across all study sites. All drinking water treatment sources had TP concentrations above New Jersey DEP SWQS (>0.05µg/L for lakes, 0.01µg/L for rivers). The availability of phosphorus can lead to an increase in cyanobacterial growth and ultimately have detrimental effects on the surrounding biota and water quality (Huisman et al., 2018). Phosphorus concentrations thus should be reduced to achieve target concentrations at the study sites. Future studies should focus on land use and land cover analysis in drinking water sources to identify phosphorus sources and help create more effective and updated nutrient management. Moreover, HAB intensity and frequency in these sites could be further exacerbated by climate change. Factors such as increased water stratification and warmer temperatures can lead to increased cyanobacterial population growth and their dominance in the phytoplankton assemblages, which can be worsened when combined with nutrient enrichment (Carey et al., 2012; Paerl, 2008). Specifically, thermal stratification favors cyanobacteria with some mobility that can change their positions within the water column. Those cyanobacteria are able to move downward in the water column to be closer to the nutrient-rich deeper water while other

phytoplankton taxa are without access to the nutrients (Carey et al., 2012; Cottingham et al., 2015; Walsby, 1994). In contrast, mixing events during spring and fall turnovers can disrupt cyanobacteria scums, and transport cyanobacterial cells to the profundal zone where no sunlight is available. Additionally, the pressure from increasing depths can cause cyanobacteria's gas vesicles to collapse (Visser et al., 1996; Zhao et al., 2017); cells are unable to control buoyancy and sink to the bottom where light and temperature conditions negatively affect their survival (Huisman & Hulot, 2005; Kinsman et al., 1991; Oliver & Walsby, 1984; Pfeifer, 2012). It has been hypothesized that one of the main causes for increases in cyanobacterial blooms is increased stratification influenced by climate change (Carey et al., 2012; Joehnk et al., 2008). With impending climate change affecting multiple drivers of HAB formation, all sites present in this study are vulnerable for future eutrophication and HAB events (O'neil et al., 2012).

Among all five study sites, Site E is of particular concern since it exhibited a prevalence of HAB and impaired water quality conditions throughout the duration of the study. Overall, this was the only site with detectable MC concentrations during all months, with some MC concentrations above the 8µg/L US EPA recreational water Health Advisories (HAs) for source waters. However, toxin concentrations in the finished waters were below HAs post-treatment. This suggests that Site E is equipped to effectively remove cyanobacteria and MC from the source waters. While the MC levels in the finished water were below drinking water health advisory guidelines in Site E, this site is still with the greatest risk of future HAB events and ultimately greater treatment costs associated with cyanobacteria and cyanotoxin removal. Additionally, while the other four drinking water sources did not have detectable CYN genes present, CY was detected in Site E although at very low concentrations. However, our results also showed that CY concentrations were effectively reduced to acceptable drinking water levels

after treatment. Similarly, during a four-year sampling period in the neighboring state of New York CY was rarely detected in natural waterbodies (Boyer, 2007). Boyer (2007) reported CY were detected in five eutrophic lakes in New York with concentrations ranging from 0.1 ug/L to 0.25 ug/L.

HAB when dominated by cyanobacteria bring additional issues for water treatment plants. *Microcystis* are taxa known to form surface scums which can be further concentrated by wind (Chorus & Welker, 2021; Konopka, 1989). The specialized adaptations (i.e., akinetes, buoyancy, nitrogen-fixation, luxury phosphorus uptake and storage) that cyanobacteria possess relating to nutrient stress aid in their survival and dominance within their communities and create further difficulties in managing *Microcystis* for drinking water facilities (Carey et al., 2012). *Microcystis* is a cyanobacterial taxon known to dominate eutrophic lakes with sufficient stratification which aids in their buoyancy regulation throughout the water column (Dokulil & Teubner, 2000; Reynolds, 1987). Water column stability allows for cyanobacteria to either grow somewhere in the middle with favorable conditions or move towards the surface to access light and CO_2 (Dokulil & Teubner, 2000). These varying depth positions can impact drinking water facilities by having to make adjustments to the intake depth if a bloom is occurring to avoid excess cyanobacterial cells (Brookes et al., 2021; Westrick et al., 2010). Furthermore, adjusting intake depth is not practical for all drinking water treatment facilities since most have a fixed intake depth. A study by Boyer (2007) concluded that the 1 m depth sampling method for analyzing CY was biased since the CY-producing *Cylindrospermopsis raciborskii* blooms are dispersed throughout the water column (Boyer, 2007; Padisak, 1997). Thus, future CY sampling should consider that *C. raciborskii* population peaks in stratified lakes occur at various depths within the epilimnion (Padisak, 1997). Similarly, dispersal and toxin concerns apply to other

cyanobacteria taxa and toxins with these adaptations. However, research on diurnal vertical buoyancy patterns of cyanobacteria and impacts on water quality and treatment is limited and should be further examined (Kim et al., 2007; Westrick et al., 2010). Due to the presence of possibly toxic cyanobacteria and future susceptibility of all drinking water sources to HAB events, routine monitoring and management is suggested to reduce the risk of cyanotoxin contamination. Routine monitoring would provide early bloom detection, lower treatment costs, and lower public health risk to the community.

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Table 3.3. Water quality data of the drinking water sources in New Jersey: maximum (Max), minimum (Min) and median (Med), sample number (N), 25th percentile and 75th percentile of conductivity (Cond; µS/cm), dissolved oxygen (DO; mg/L), pH, temperature (T; °C), total suspended solids (TSS; mg/L), total dissolved solids (TDS; mg/L), non-purgeable dissolved organic carbon (DOC; mg/L), ammonia (NH_3; mg/L), total nitrogen (TN; mg/L), and total phosphorus (TP; mg/L); (--: not tested).

	Site A						Site B						Site C						Site D						Site E					
	Min	Max	Med	N	25th	75th	Min	Max	Med	N	25th	75th	Min	Max	Med	N	25th	75th	Min	Max	Med	N	25th	75th	Min	Max	Med	N	25th	75th
Cond	261.4	362.8	313.6	6	280.6	350.1	252.1	299.2	281.0	5	260.8	290.7	–	–	–				375.0	609.0	492.6	6	422.0	592.5	363.4	424.1	405.7	6	381.9	420.1
DO	6.3	9.7	7.3	6	6.8	8.8	6.3	9.7	8.1	5	6.8	9.1	–	–	–				2.8	7.7	5.2	6	4.3	6.2	2.9	8.6	6.8	6	3.8	7.6
pH	7.35	7.63	7.54	6	7.46	7.61	6.57	8.64	7.19	6	6.91	7.81	6.23	7.12	6.33	6	6.29	6.88	7.07	7.72	7.30	6	7.15	7.57	7.32	9.32	8.41	6	8.06	9.20
T	15.0	25.5	21.1	6	18.6	22.9	15.5	27.8	22.2	6	19.0	25.0	17.0	23.8	20.9	6	19.3	22.5	14.7	24.5	20.6	6	17.9	22.9	15.1	28.2	23.0	6	20.6	24.5
TSS	2.6	25.9	5.5	6	3.0	15.5	0.9	3.1	1.9	6	1.0	3.0	1.4	5.2	2.8	6	1.8	5.0	0.8	11.4	3.8	6	1.8	9.5	2.5	5.6	4.9	6	3.0	5.3
TDS	9	19	12	6	9	15	9	17	13	6	12	15	5	17	13	6	4	15	7	27	24	6	18	26	8	25	14	6	12	19
DOC	3.3	17.8	7.3	6	3.8	16.0	4.0	14.0	8.2	6	4.2	11.1	11.5	13.9	12.4	6	11.8	13.6	4.0	20.7	8.7	6	4.2	17.7	5.1	20.1	11.5	6	6.3	19.3
NH_3	0.01	0.11	0.05	6	0.02	0.10	0.01	0.48	0.06	6	0.01	0.19	0.03	0.19	0.11	6	0.04	0.17	0.06	0.36	0.14	6	0.11	0.20	0.01	0.10	0.04	6	0.01	0.08
TN	0.85	1.56	1.26	6	0.93	1.48	0.35	1.30	0.75	6	0.39	1.14	0.35	1.31	0.73	6	0.58	1.12	1.50	2.85	1.86	6	1.66	2.40	0.34	1.35	0.90	6	0.60	1.28
TP	0.15	0.60	0.22	6	0.20	0.59	0.13	0.25	0.15	6	0.14	0.18	0.13	0.57	0.20	6	0.15	0.33	0.27	0.39	0.33	6	0.29	0.38	0.22	0.40	0.31	6	0.23	0.36

Table 3.4. Harmful algal bloom data of the drinking water sources in New Jersey: maximum (Max), minimum (Min) and median (Med), sample number (N), 25th percentile and 75th percentile of fluorescence of phycocyanin (PC; µg/L), fluorescence of chlorophyll (Chl; RFU), Chlorophyll-a (Chl-a; µg/L), phytoplankton cell density (Phyto; cells/mL), cyanobacterial cell density (Cyano; cells/mL), microcystins (MC; µg/L), *Microcystis* 16S rRNA (MCS; copies/mL), microcystin-producing *mcyB* gene (MCN; copies/mL), cylindrospermopsin (CY; µg/L), *Cylindrospermopsis raciborskii-specific* genes *rpoC1* (CYS; copies/mL), and cylindrospermopsin-producing *pks* gene (CYN; copies/mL). (UD: under detection/reporting limit).

	Site A						Site B						Site C						Site D						Site E					
	Min	Max	Med	N	25th	75th	Min	Max	Med	N	25th	75th	Min	Max	Med	N	25th	75th	Min	Max	Med	N	25th	75th	Min	Max	Med	N	25th	75th
PC	0.1	0.5	0.3	6	0.1	0.4	1.6	5.0	3.1	6	1.8	3.9	0.2	37.6	1.5	6	0.4	15.4	0.1	1.5	0.4	6	0.2	0.7	3.5	46.0	28.8	6	16.1	44.1
Chl	0.6	3.6	1.1	6	0.8	2.8	5.1	22.6	16.5	6	12.0	19.2	1.1	39.5	7.5	6	1.9	24.1	0.9	8.6	1.5	6	1.1	7.3	5.6	20.1	16.6	6	9.6	18.4
Chl-a	2.0	8.0	4.7	6	2.1	6.8	5.7	14.6	13.1	6	10.0	14.3	3.1	26.1	7.7	6	3.5	20.9	1.2	11.5	2.7	6	1.8	8.3	10.6	51.4	46.3	6	17.0	50.0
Phyto	37	218	102	6	73	167	378	5,111	805	6	429	2,533	262	9,065	1,684	6	533	7546	17	851	237	6	109	471	2,862	11,903	3,786	6	2,926	7,444
Cyano	0	27	0	6	0	7	0	4,131	398	6	131	1,574	0	8,844	269	6	13	3012	0	259	56	6	0	254	1,603	11,807	3,687	6	2,292	7,349
MC	UD	UD	UD	6	0.08	0.08	UD	UD	UD	6	0.08	0.08	UD	UD	UD	6	0.08	0.08	UD	0.18	UD	6	0.08	0.10	0.26	8.39	5.31	6	2.56	7.20
MCS	364	5,572	1,425	6	689	3096	571	6,018	3,039	6	855	4525	UD	336,077	64,277	6	466	237778	UD	7,555	5,116	6	59	5726	794,435	7,076,480	3,648,401	6	1617931	5339390
MCN	UD	922	511	6	216	895	UD	2,606	988	6	179	1527	UD	13,583	6,347	6	3436	8526	UD	1,285	808	6	114	569	82,437	8,660,900	2,723,562	6	1160060	7475182
CY	UD	UD	UD	6	UD	UD	UD	UD	UD	6	UD	UD	UD	UD	UD	6	UD	UD	UD	UD	UD	6	UD	UD	UD	0.09	UD	6	UD	0.05
CYS	UD	2033	1131	6	UD	679	UD	1627	941	6	UD	598	UD	292	141	6	UD	178	UD	211	211	6	UD	158	UD	2794	958	6	UD	1417
CYN	UD	UD	UD	6	UD	UD	UD	UD	UD	6	UD	UD	UD	UD	UD	6	UD	UD	UD	UD	UD	6	UD	UD	UD	UD	UD	6	UD	UD

Table 3.5. Spearman's Correlation Results for drinking water sources. Conductivity (Cond), dissolved oxygen (DO), pH, temperature (T), total suspended solids (TSS), total dissolved solids (TDS), non-purgeable dissolved organic carbon (DOC), ammonia (NH_3), total nitrogen (TN), total phosphorus (TP), fluorescence of phycocyanin (PC), fluorescence of chlorophyll (Chl), chlorophyll-a (Chl-a), phytoplankton cell density (Phyto), cyanobacterial cell density (Cyano), microcystins (MC), *Microcystis* 16S rRNA (MCS), and microcystin-producing *mcyB* gene (MCN). (*: $p<0.05$)

	Cond	DO	pH	T	TSS	TDS	PC	Chl	Chl-a	Phyto	Cyano	MC	MCS	MCN	DOC	NH_3	TN	TP
	rs	rs	rs	rs	rs	rs	rs	rs	rs	rs	rs	rs	rs	rs	rs	rs	rs	rs
Cond	–	-0.5188*	-0.0010	-0.2683	0.1240	0.5080*	-0.0603	-0.0806	-0.1746	0.0983	0.0511	0.3874	-0.0069	0.0015	0.1215	0.2770	0.5868*	0.5137*
DO	-0.5188*	–	-0.1522	-0.2619	-0.2575	-0.4981*	-0.0889	-0.1092	-0.0458	-0.0301	0.0531	-0.2322	0.0752	-0.0897	0.0939	-0.2755	-0.3900	-0.3562
pH	-0.0010	-0.1522	–	0.4055*	0.2807	0.0901	0.1480	-0.0065	0.3007	0.0590	0.1802	0.4900*	0.1569	0.2341	-0.3397	-0.3781*	0.0327	0.3631*
T	-0.2683	-0.2619	0.4055*	–	0.2042	-0.1809	0.3703*	0.2300	0.3950*	0.2435	0.1778	0.1951	0.2981	0.4265*	-0.2982	-0.2305	-0.2175	-0.0645
TSS	0.1240	-0.2575	0.2807	0.2042	–	-0.2037	0.1859	0.1432	0.3316	0.0859	0.0313	0.2471	0.2088	0.2344	-0.1436	-0.1348	0.1899	0.3352
TDS	0.0580*	-0.4981	0.0901	-0.1809	-0.2037	–	-0.1333	-0.1164	-0.1299	0.0443	0.0413	0.1596	-0.0704	0.0186	-0.0892	-0.0630	0.3196	-0.0900
DOC	0.1215	0.0939	-0.3397	-0.2982	-0.1436	-0.0892	0.0990	0.0031	0.0473	0.0768	0.3550	0.1239	0.2347	0.1168	–	0.1737	-0.0899	-0.0682
NH_3	0.2770	-0.2755	-0.3781*	-0.2305	-0.1348	-0.0630	-0.3902*	-0.3068	-0.4383*	-0.2178	-0.0929	-0.2665	-0.4211*	-0.5639*	0.1737	–	0.2269	0.2922
TN	0.5868*	-0.3900	0.0327	-0.2175	0.1899	0.3196	-0.5161*	-0.4538*	-0.4961*	-0.4566*	-0.5137*	-0.0912	-0.4498*	-0.4462*	-0.0899	0.2269	–	0.4088*
TP	0.5137*	-0.3562	0.3631*	-0.0645	0.3352	-0.0900	-0.2874	-0.2643	-0.2136	-0.2534	-0.0985	0.2996	-0.0783	-0.0812	-0.0682	0.2922	0.4088*	–
PC	-0.0603	-0.0889	0.1480	0.3703*	0.1859	-0.1333	–	0.8466*	0.6204*	0.8017*	0.8177*	0.5675*	0.6925*	0.7113*	0.0990	-0.3902*	-0.5161*	-0.2874
Chl	-0.0806	-0.1092	-0.0065	0.2300	0.1432	-0.1164	0.8466*	–	0.5501*	0.6775*	0.6912*	0.3334	0.5917*	0.6004*	0.0031	-0.3068	-0.4538*	-0.2643
Chl-a	-0.1746	-0.0458	0.3007	0.3950*	0.3316	-0.1299	0.6204*	0.5501*	–	0.6020*	0.5213*	0.5903*	0.5182*	0.5042*	0.0473	-0.4383*	-0.4961*	-0.2136
Phyto	0.0983	-0.0301	0.0590	0.2435	0.0859	0.0443	0.8017*	0.6775*	0.6020*	–	0.7491*	0.5170*	0.5433*	0.5905*	0.0768	-0.2178	-0.4566*	-0.2534
Cyano	0.0511	0.0531	0.1802	0.1778	0.0313	0.0413	0.8177*	0.6912*	0.5213*	0.7491*	–	0.5211*	0.6264*	0.5725*	0.3550	-0.0929	-0.5137*	-0.0985
MC	0.3874	-0.2322	0.4900*	0.1951	0.2471	0.1596	0.5675*	0.3334	0.5130*	0.5170*	0.5211*	–	0.5770*	0.6109*	0.1239	-0.2665	-0.0912	0.2996
MCS	-0.0069	0.0752	0.1569	0.2981	0.2088	-0.0704	0.6925*	0.5917*	0.5182*	0.5433*	0.6264*	0.5770*	–	0.8959*	0.2347	-0.4211*	-0.4498*	-0.0783
MCN	0.0015	-0.0897	0.2341	0.4060*	0.2344	0.0186	0.7113*	0.6004*	0.5042*	0.5905*	0.5725*	0.6037*	0.8959*	–	0.1168	-0.5639*	-0.4462*	-0.0812

Chapter 4 Phytoplankton Dynamics Across the Spatial and Temporal Gradients in the Hudson-Raritan Estuary

4.1 Introduction

Estuaries are essential ecosystems which provide a myriad of services, from feeding and nursery habitats to the capability of processing and filtering a large portion of the watershed discharge from the rivers and coastal areas on Earth (Costanza et al., 1997; Paerl, 2006). Dürr et al. (2011) estimated about 57% of water discharge from rivers that drain to the ocean is filtered by estuaries. Specifically, estuaries can exclude and hold materials such as sediments, nutrients and pollutants from rivers and oceans that are both suspended and in solution and provide cleaner water for humans and wildlife (NRC, 2000; Kennedy, 2013). The waters discharged into the estuaries can be greatly impacted by anthropogenic activities which can increase nutrient inputs and aggravate eutrophication of rivers and coastal waters (O'Neil et al., 2012; Paerl et al., 2018; Wurtsbaugh et al., 2019). Among the anthropogenic activities that can lead to an increase in nutrients are urbanization, agriculture, wastewater, and aquaculture (Adams et al., 2020; Heisler et al., 2008).

4.1.1 Hudson-Raritan Estuary

The Hudson-Raritan Estuary (Estuary) provides important economical, ecological, and recreational resources to the surrounding populations (Pendleton, 2008; Pirani et al., 2018). Moreover, it provides many ecological benefits such as a nursery to many juvenile fishes, habitat for shellfish, and resting areas for migratory and non-migratory birds (Able, 1999; Adams et al., 1998; Duffy-Anderson et al., 2003). The Estuary is a complex ecosystem located in New York and New Jersey which is influenced by both tidal and wind generated currents (Bagheri et al., 2002). These currents are altered by discharges from four major rivers: the Hackensack, Hudson,

Passaic, Arthur, and Raritan rivers (Bagheri et al., 2002). A long history of urbanization and industrialization of surrounding cities such as Newark, New Jersey and New York City, New York have contributed both point and non-point sources of pollution and lead to a decline in water quality of the rivers and bays within the Hudson-Raritan Estuary (Bloom et al., 2008; Feng et al., 1998; Jung et al., 2021; USEPA, 2007). High concentrations of pollutants such as Cu and Pb have been documented in the sediments of the Hudson River Estuary and have been suggested to originate from urban runoff and wastewater effluent (Feng et al., 1998). Other consequences of urbanization in the Estuary include habitat loss, reduced recreation, and a decrease in biodiversity (Studholme, 1988).

The many impacts of human activities on water quality have ultimately led to eutrophication symptoms in the Estuary. Harmful algal blooms (HAB) have been a concern in the New Jersey coastal area and rivers that drain to the Estuary (Gastrich, 2000; Mahoney et al., 1990; Van, 2016). The Passaic River has been reported to have higher phosphorus concentrations than other freshwaters in New Jersey (Van, 2016). Studies have suggested sewage waters are the main source of phosphorus inputs to the Hudson, Raritan, Hackensack, and Passaic rivers (Jung, 2017; Obropta et al., 2008; Pearce et al., 1988). Similarly, O'Shea et al. (2000) suggested that Raritan Bay water quality had declined and HCBs were increasing, possibly due to wastewater effluents and an increase in nonpoint source pollution. Moreover, the study documented an increase in surface dissolved oxygen supersaturation and stratification (O'Shea et al., 2000). These findings are supported by worldwide trends where estuaries have seen a decline in water quality partly due to nutrient enrichment and eutrophication (O'Neil et al., 2012; Paerl et al., 2014).

4.1.2 Economic and Ecological Impacts

The Hudson-Raritan Estuary has been greatly impacted by urban and industrial development since the early 1800's (Suszkowski et al., 1990). Greater urbanization can ultimately increase runoff and transport greater amounts of nutrients into waterbodies (Sugianto et al., 2022). The Estuary is located in one of the most densely populated areas in the United States and possesses the largest shipping port of the east coast (Pirani et al., 2018). This shipping port is of importance to the regional economy since the value of the cargo in the Hudson-Raritan Estuary has been estimated at $200 billion per year and provides around 190,000 local jobs (Pirani et al., 2018). Moreover, the Estuary is of great economic value since its waters have been estimated to serve as a public resource for 14 million people (Pirani et al., 2018).

The Passaic, Hudson, and Raritan rivers in the Estuary are utilized to supply drinking water to surrounding populations (Cosgrove et al., 2005; Hall & Dietrich, 2000; McLaughlin et al., 1988; Palmer et al., 2011). These water systems are vulnerable to eutrophication conditions. The costs of HAB treatment on drinking water facilities have been estimated at $70 million in a 10-year span in Texas (Dunlap et al., 2015). Furthermore, treatment processes such as powdered activated carbon have been estimated at more than $1 million during the summer season only (Walker, 2014). There are numerous economic costs associated with human activities in the Estuary.

In addition to economic impacts, habitat loss, fragmentation, wetland filling, and legacy toxic contaminants have influenced local biota by reducing diversity and abundance of organisms (Crawford et al., 1994; Pearce, 1988). Furthermore, a rise in eutrophication events have contributed to the increasing frequency and intensity of HAB, and subsequently produced a negative effect on dissolved oxygen levels (Wurtsbaugh et al., 2019). HAB impacts on wildlife have been reported in the rivers and bays of the Estuary related to toxins and eutrophication.

Brown tides events have been associated with affecting the mortality of eelgrass beds (*Zostera marina*), which provides habitat for juvenile finfish and shellfish (Dennison et al., 1989). Moreover, HAB were suggested to facilitate hypoxia in the Hudson-Raritan Estuary (Olsen & Mulcahy, 1991). While HAB in the Estuary have been mostly documented in the coastal and marine ecosystems, research that focuses on the comparison between the rivers and bays of the Estuary is limited.

4.1.3 Public Health and Recreational Impacts

Toxin-production by various HAB taxa can have both acute and chronic impacts on human health (Gastrich, 2000). These health impacts from HAB can occur through various routes of exposure including ingestion, skin contact, and aerosolization (Gastrich, 2000). In New Jersey, coastal blooms have been associated with reports of moderate illness and discomfort from New Jersey bathers. (Gastrich, 2000; USEPA, 1986). Reports of acute toxicity from exposure HAB have been reported by the New Jersey Department of Environmental Protection, with reported adverse effects such as respiratory issues, fever, skin reactions, and nausea after bloom exposure (NJDEP, 1998; USEPA, 1986). Blooms in the Estuary have also been reported to affect recreation such as decreased aesthetic value of the beaches (Mahoney & McLaughlin, 1977). While freshwater HAB have been confirmed by the NJDEP in the Passaic and Hackensack rivers, reports with information on public health impacts are lacking (NJDEP, 2021).

4.1.4 HAB and Phytoplankton Communities

Phytoplankton are photosynthetic organisms that contribute to approximately half of the primary production in aquatic coastal ecosystems (Sigman & Hain, 2012). The physical and biogeochemical processes that occur in the river to ocean continuum are influenced primarily by freshwater flow and river-ocean mixing (Wang & Zhang, 2020). This suggests that observed

poor water quality in estuaries could be occurring as a result of eutrophication and HAB from freshwaters upstream or waterbodies downstream (Wang & Zhang, 2020). Eutrophication can facilitate a rapid increase in the algae concentration causing HAB and can result in detrimental effects to other living organisms and water quality (Hallett et al., 2016). These detrimental effects can be attributed to the algal toxins and/or large biomass produced (Anderson et al., 2002). Some HAB have been documented to cause adverse ecological effects such as hypoxic/anoxic conditions and fish kills (Paerl et al., 2018; Wurtsbaugh et al., 2019). Thus, eutrophication and HAB can interfere with the ecosystem services that estuaries provide (Elliot & Whitfield, 2011). In addition, climate change trends are predicted to exacerbate extreme weather events such as flooding and droughts, which could stimulate HAB and worsen detrimental impacts on water quality and wildlife (IPCC, 2014; O'Neil et al., 2012; Philps, 2020; Wetz & Yoskowitz, 2013).

Various studies have observed a strong influence of freshwater discharge on the distribution of phytoplankton biomass (Day et al., 2009; Pilkaitytė & Razinkovas, 2007). This suggests that freshwater discharge transports nutrients from surrounding watersheds which support primary production and influence phytoplankton growth. Freshwater discharge can also affect phytoplankton composition by altering flushing characteristics and residence times in estuaries (Crump et al., 2022). Similarly, Qian et al. (2000) suggested that phytoplankton growth within the river-ocean continuum, and exacerbating eutrophication in estuaries could be due to a shift from nitrogen to phosphorus limitation. Other water quality conditions have been hypothesized to alter phytoplankton communities. For instance, previous studies have suggested that the shift in phytoplankton community composition from freshwater to marine taxa could be influenced by salinity and turbidity (Fortunato et al., 2012; Keller et al., 2014). As freshwater

phytoplankton move towards the ocean, they can die as they access the oligohaline and mesohaline zones (Wang & Zhang, 2020). This could be due to osmotic stress or turbidity increases along the river-ocean continuum (Wang & Zhang, 2020). Thus, the transport and fate of phytoplankton from freshwater to brackish waters should be further studied and incorporated for creating and implementing effective water quality management strategies that integrate rivers, bays, and estuarine systems.

As previously mentioned, the highly productive waters within the Estuary are influenced by factors such as winds and turbidity which could in turn impact its phytoplankton assemblages. Furthermore, freshwater discharge to the bays could impact the physical, chemical, and biological features of the Estuary (Hickey et al., 2010). While water quality and its relation to HAB have been a focus of research in both freshwater and brackish water systems, fluvial studies of both systems in spatial and seasonal scales are scarce (Fortunato et al., 2012, 2013). Due to the complex nature of the Estuary, management strategies should incorporate data from its rivers and bays. To our knowledge, there is no systematic study of the inputs and transports of cyanobacteria and pollution in the Hudson-Raritan Estuary. This study sought to document the variation and trends in water quality and HAB conditions between the rivers and bays of the Estuary. Moreover, this study reported the environmental factors that influence the spatial and temporal changes in both the phytoplankton and cyanobacterial communities within the Estuary.

4.2 Materials and methods

4.2.1 Site Description

A total of 31 sampling sites were selected for this study which spanned across the 5 rivers and 2 bays in the Hudson-Raritan Estuary including 14 sites in the Passaic River, 4 in the Hackensack River, 3 in the Hudson River, 2 in the Arthur Kill, 2 in the Raritan River, 3 sites in the Newark Bay, and 3 in the Raritan Bay (Table 4.1; Figures 4.1 and 4.2). The Pompton, Saddle, and Second rivers are three tributaries that were incorporated into the Passaic River sampling sites for the purpose of this study. These waterbodies had a wide range of salinities from fresh to brackish water (Figure 4.3). We divided the waterbodies into tidal and non-tidal waters with non-tidal waters defined, for the purpose of our study, as sampling sites upstream of the salt line.

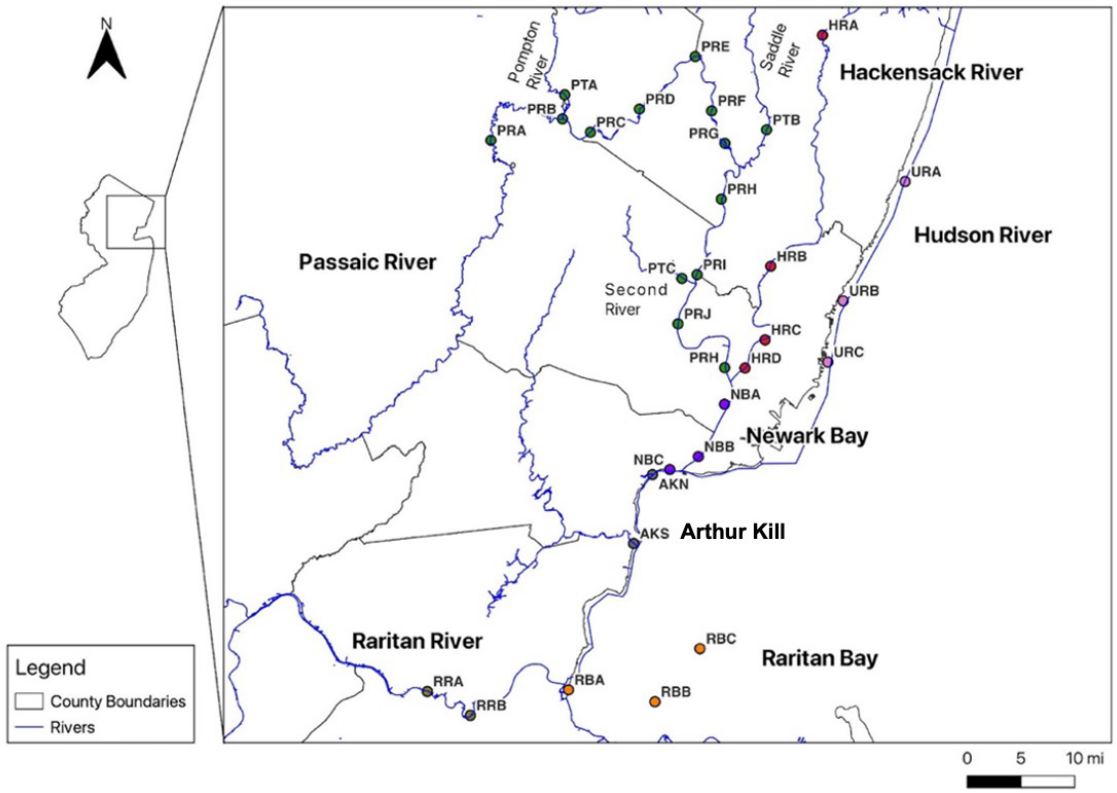

Figure 4.1. A total of 31 sites were selected for this study across the Hudson-Raritan Estuary in New Jersey.

Passaic River: The Passaic River is located in Northern New Jersey, approximately 30 miles long, has a daily average discharge of 47 m^3/s, and ends at its confluence with Newark Bay (Brydon, 1974; Shrestha et al., 2014). A history of severe pollution from industrialization and urbanization have degraded the water quality of the river, especially the lower 6 to 7 miles, and have led to its designation as a Superfund site (Iannuzzi et al., 2002; Olson & Tharp, 2020; USEPA, 1998). Pollutants such as 2,3,7,8-tetrachlorodibenzo-p-dioxins, among others, have contaminated its sediments, fish, and crustaceans to the extent where harvesting is banned from the lower portion of the river (Iannuzzi et al., 2004).

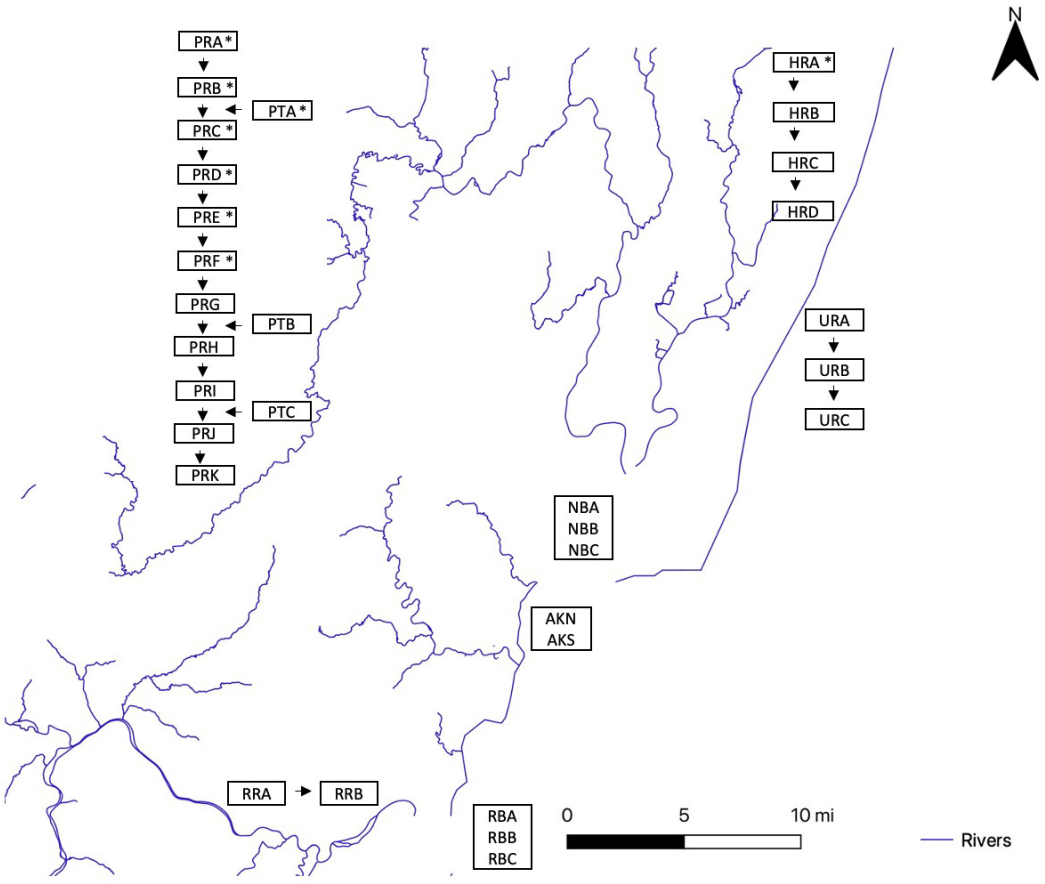

Figure 4.2 Concept map of water flow of study sites of the Hudson-Raritan Estuary. (*= indicates non-tidal waters).

Hackensack River: Another river that drains into Newark Bay is the Hackensack River which is approximately 45 miles long, with a daily discharge of 6 m^3/s, and is located both in New York and New Jersey (Shrestha et al., 2014; USACE, 2016). Similar to the Passaic River, the water quality in the lower portion of the Hackensack River has been worsened by industrialization and urbanization of the Hackensack meadowlands (Crawford et al., 1994; Long et al., 1995). Severe pollution from chemical waste resulted in one of its tributaries, Berry's Creek, having the highest sediment concentrations of methyl mercury recorded in a waterbody in the United States (Cardona-Marek et al., 2007; Lipsky & Harkov, 1980; Schartup et al., 2003).

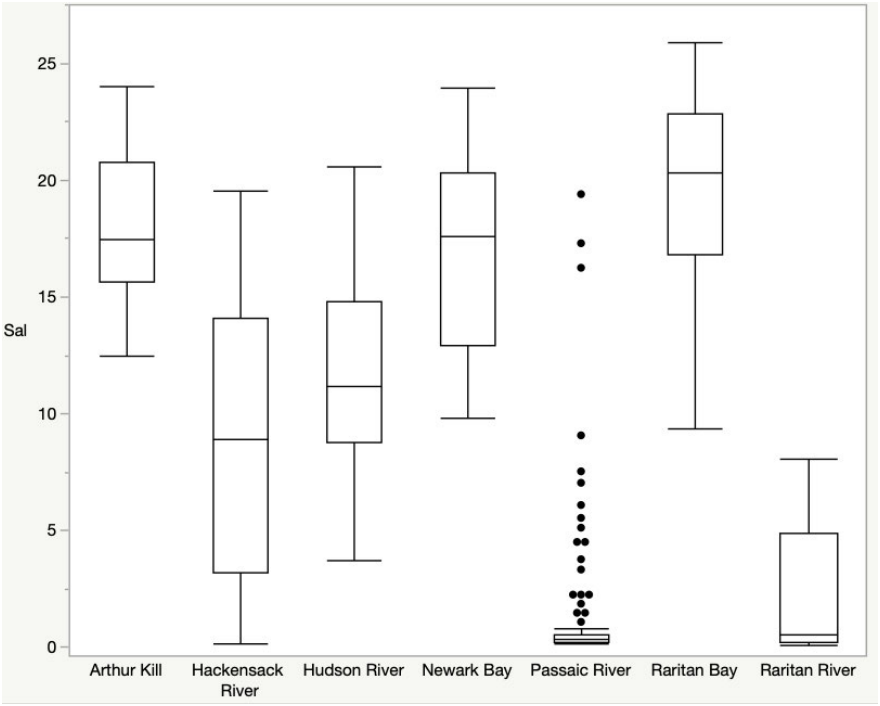

Figure 4.3. Box (median) and whiskers (maximum and minimum) plot of the salinity (ppt) of five rivers and seven bays within the Hudson- Raritan Estuary in New Jersey. Outliers are represented by black dots.

Hudson River: The 315-mile-long Hudson River drains into the Lower New York Bay, with its lower stretch being a tidal estuary and has a daily average discharge of 620 m^3/s (Dunwell, 2011). Its utilization as a main transportation passageway has subsequently increased local

population and further affected water quality (Dunwell, 2011). This river was also impacted by industrial pollution, especially polychlorinated biphenyls (PCBs) (Dunwell, 2011; Williams et al, 1978; Olsen et al., 1978).

Newark Bay: The Newark Bay is approximately 6.21 miles long and located at the confluence of the Hackensack and Passaic Rivers (Crawford et al., 1994). It is economically valuable, being designated as a major commercial port in the United States (Suszkowski et al., 1990). However, this tidal bay has been severely impacted by pollution from its port and discharges from the Passaic and Hackensack rivers (Bonnevie et al., 1994; Dimou et al., 2006). Pollutants such as dioxins, mercury, lead, and PCBs have been reported in high concentrations at Newark Bay (Bopp et al., 1991; Crawford et al., 1995; Saba & Su, 2013; Wolfskill & McNutt, 1998).

Arthur Kill: The Arthur Kill, a 15 miles long river or strait, connects to the Newark and Raritan bays and is an important navigable estuarine channel worldwide (Burger, 1994). The majority of its riverbanks have been altered for industrial purposes (Burger, 1994). Historically, it has experienced a higher frequency of oil spills than any other waterbodies in the Hudson-Raritan Estuary with an estimated average spill volume of 2,541,939 US gallons (Gunster et al., 1993). Huntley et al. (1995) showed severely high polyaromatic hydrocarbons (PAH) concentrations in the Arthur Kill sediments. Other reports have shown heavy metal concentrations were highest at the Arthur Kill (Grieg & McGrath, 1977).

Raritan River: The Raritan River is the largest interstate river system in New Jersey at 30 miles long, which has an average discharge of 34 m^3/s and drains to the Raritan Bay (Ashley et al., 1998). Between the 1920s and 1980s, industrial wastes were discharged directly to the river such as formaldehyde, phenols, arsenic, and copper (Rudolfs & Fletcher, 1951). Other contaminants such as sulfate, chloride and nitrate ions concentrations significantly increased in the 1970's,

indicating waste-water discharge and agricultural runoff inputs (Anderson & Faust, 1974). Furthermore, it flows through approximately 18,000 contaminated industrial sites (Li et al., 2020). The surrounding population utilize the river for drinking water, transportation, and recreation (Reiser, 2001).

Raritan Bay: At a length of 12 mi, the Raritan Bay is a drowned river estuary which receives freshwater from the Hudson and Raritan Rivers and effluents from the Hackensack and Passaic Rivers (Jeffries, 1962; MacKenzie, 1990). The bay has been impacted by a long history of landscape alterations, ocean pollution, industrial chemicals, and increased population density (Pearce, 1979). These activities have led to an increase in heavy metals (mercury, cadmium, copper, and lead), and hydrocarbons (PCBs) concentrations at the bay (Greig & McGrath, 1977; Staiken, 1979; Waldhauer et al., 1978). It has been considered as one of the most deteriorated estuaries in the United States (Pearce, 1979).

4.2.2 Phytoplankton and HAB Density

Samples for phytoplankton and HAB taxa analysis were collected at 0.5m depth in glass jars which were wrapped in foil, kept cold, and transported to a Montclair State University laboratory. Lugol's Iodine solution was used to preserve the samples which were then stored in a cold room. A FlowCam 8000 Series Dynamic Imaging Particle Analyzer (referred as FlowCam in this paper) (Yokogawa Fluid Imaging Technologies, Scarborough, ME) was used to process phytoplankton samples for identification and enumeration. Samples were homogenized by gently inverting 25 times, and for each analysis, 300 µl of sample was processed by the FlowCam. Samples were run in triplicates. The FlowCam's AutoPilot software was used for categorizing and counting of phytoplankton and HAB particles. Images generated by FlowCam were then further processed for taxa identification and cell density. Cells were identified and counted to the

lowest taxonomic level possible using identification materials such as *Freshwater algae of North America: ecology and classification* (Wehr et.al, 2015), *Cyanoprokaryota-1. Teil/Part 1: Chroococcales* (Komárek & Anagnostidis, 2008), *Cyanoprokaryota-2. Teil/Part 2: Oscillatoriales* (Komárek & Anagnostidis, 2008), and *Cyanoprokaryota-3. Teil/Part 3: Heterocytous Genera* (Komárek, 2013). Phytoplankton cell density (Phyto) refers to the total density of all phytoplankton taxa. Harmful algal bloom cell density (HAB) was calculated by adding freshwater cyanobacteria taxa and red tide phytoplankton taxa.

4.2.3. Water quality data

Data was downloaded from the US EPA Water Quality Portal (https://www.waterqualitydata.us/) including the following parameters: pH, dissolved oxygen (DO), temperature (Temp), salinity (Sal), total suspended solids (TSS), total phosphorus (TP), nitrate (NO_3^-), nitrite (NO_2^-), and Total Kjeldahl Nitrogen (TKN), fand chlorophyll-a (Chl-a). The TN concentrations were calculated by adding nitrate (NO_3^-), nitrite (NO_2^-), and Total Kjeldahl Nitrogen (TKN) concentrations.

4.2.4. Statistical analysis

The data were not normally distributed (Shapiro-Wilk's test), thus non-parametric tests were conducted. The non-parametric Spearman's Correlation test was conducted to observe correlations between water quality and phytoplankton and HAB taxa density. For this test, a p-value of 0.05 was chosen as the threshold for statistical significance. Dunn's post hoc test was conducted to determine differences between water quality parameter means of bays and rivers and seasons. The Principal Component Analysis (PCA) was used to discern patterns among environmental parameters and phytoplankton and HAB taxa density. Statistical analyses were done using the JMP Pro 15 software.

4.3 Results

4.3.1 Water Quality at the Selected Study Sites

Arthur Kill

The lowest number of taxa was recorded in the Arthur Kill with a total of 35 phytoplankton taxa. Major phytoplankton taxa groups present in this river include the diatoms (19; 54.3%), green algae (7; 20.0%), cyanobacteria (5; 14.3%), dinoflagellates (2; 5.7%), euglenoids (1; 2.9%), and cryptomonads (1; 2.9%) (Appendix C). The diatoms *Cyclotella* were present in 18 of 22 samples (81.8%) and *Thalassiosira* in 12 of 22 of samples (54.5%) in the Arthur Kill. The Arthur Kill phytoplankton community was dominated by HAB taxa. While phytoplankton densities were the lowest amongst all rivers and bays at a median density of 96 cells/mL (ranging from 22 to 1,281 cells/mL), the HAB densities were found the highest ranging from 13 to 842 cells/mL (Table 4.2). Low Chl-a concentrations were also recorded with a median of 2.32 ug/l (ranging from 0.8 to 18.8 ug/l). The median water temperature in this river was 14.5 °C (ranging from 3.0 to 24.3 °C) while Sal concentration had a median of 17.5 ppt, ranging from 12.5 to 24.0 ppt.

Hudson River

A total of 39 phytoplankton taxa were observed in the Hudson River with five major phytoplankton groups present: diatoms (19; 48.7%), cyanobacteria (7; 18.0%), green algae (10; 25.6%), dinoflagellates (2; 5.1%), and cryptomonads (1; 2.6%) (Appendix C). The diatom *Cyclotella* were found in 30 of 33 samples (90.9%) while the cryptomonad *Cryptomonas* was found in 22 of 33 of samples (66.7.%). Similar to Arthur Kill, results in the Hudson River showed lower phytoplankton and HAB taxa density. The Hudson River had low phytoplankton densities with a range from 16 to 365 cells/mL and a median of 113 cells/mL, while HAB counts were amongst the lowest in the Estuary ranging from 16 to 365 cells/mL (Table 4.2). Chl-a concentrations supported these results with the lowest median concentration of all sites at 1.96

ug/l (ranging from 0 to 13.6 ug/l). Median water temperature was among the lowest of all sites at 12.9 °C (ranging from 2.3 to 25.6 °C) while Sal concentrations ranged from 3.7 to 20.6 ppt (median of 11.2 ppt).

Newark Bay

Similar to the Hudson River, a total of 39 phytoplankton taxa were recorded in the Newark Bay. The Newark Bay results showed some of the lowest phytoplankton and HAB taxa in the Estuary. Seven major phytoplankton taxa groups were recorded in this bay and included the diatoms (15; 38.5%), green algae (14; 35.9%), cyanobacteria (6; 15.4%), dinoflagellates (1; 2.6%), euglenoids (1; 2.6%), golden algae (1; 2.6%), and cryptomonads (1; 2.6%) (Appendix C). Of the phytoplankton taxa recorded, the diatom *Cyclotella* was detected in 26 of 33 samples (78.8%) and *Thalassiosira* was detected in 17 of 33 of samples (51.5%) at Newark Bay. Median Phyto densities were the third lowest in the Estuary at 135 cells/mL (ranging from 24 to 650 cells/mL) and HAB counts were low ranging from 13 to 429 cells/mL (Table 4.2). These results were supported by low median Chl-a concentrations 2.54 ug/l (ranging from 0.57 to 12.5 ug/l). The Newark Bay had median water temperature at 14.2 °C (ranging from 3.1 to 24.9 °C) while median Sal concentration were the second highest of all sites at 17.6 ppt (ranging from 9.8 to 24.0 ppt).

Passaic River

The highest taxa richness in the Estuary was recorded in the Passaic River with a total of 82 phytoplankton taxa. Furthermore, this river had seven phytoplankton taxa groups: diatoms (30; 36.6%), green algae (27; 32.9%), cyanobacteria (18; 22.0%), golden algae (3; 3.7%), dinoflagellates (2; 2.4%), euglenoids (1; 1.2%), and cryptomonads (1; 1.2%) (Appendix C). Of the 82 taxa, the diatom *Navicula* was observed in 141 of 157 samples (89.9%) while the

cryptomonad *Cryptomonas* in 111 of 157 of samples (70.7%). Overall, higher phytoplankton and HAB density were observed when compared to other waterbodies in the Estuary. Phyto densities ranged from 5 to 10,393 cells/mL with a median of 275 cells/mL while HAB counts ranged from 4 to 10,244 cells/mL with a median of 138 cells/mL (Table 4.2). Chl-a concentrations were also higher ranging from 0.49 to 32.8 ug/L with a median of 3.36 ug/L. However, water temperature and Sal were found to be the lowest in the Estuary among all sites, with a median Temp at 9.7 °C (ranging from 1.4 to 26.5 °C) and a median Sal concentration of 0.3 ppt (ranging from 0.2 ppt recorded at upstream freshwater sites to 1.94 ppt observed at the tidal section of the Passaic River).

Hackensack River

The Hackensack River had the second highest phytoplankton taxa richness at 65. Similar to the Passaic River, there were seven major phytoplankton groups present including the diatoms (28; 43.1%), green algae (18; 27.7%), cyanobacteria (10; 15.4%), dinoflagellates (3; 4.6%), golden algae (3; 3.1%), euglenoids (2; 3.1%), and cryptomonads (1; 1.5%). Similar to the Passaic River, the diatom *Navicula* was present in 30 of 43 samples (69.8%) and the cryptomonad *Cryptomonas* in 29 of 43 samples (67.4%) (Appendix C). Phytoplankton and HAB taxa density in the Hackensack River was amongst the highest in the Estuary. The phytoplankton densities had a median of 261 cells/mL (36 to 18,147 cells/mL) while HAB densities had a median of 72 cells/mL (ranging from 4 to 17,235 cells/mL) (Table 4.2). The higher phytoplankton densities were supported by the third highest median Chl-a median concentrations among all sites at 4.93 ug/l (ranging from 0.51 to 50.6 ug/l). Furthermore, the median water temperature and Sal were the second highest in the Estuary at 15.4 °C (ranging from 2.6 to 27.2 °C) and 8.9 ppt (ranging from 0.2 to 19.5 ppt), respectively.

Raritan Bay

Similar to the Newark Bay, a total of 39 phytoplankton taxa were recorded in the Raritan Bay. Major phytoplankton taxa groups present in this bay were the diatoms (22; 56.4%), cyanobacteria (5; 12.8%), dinoflagellates (4; 10.3%), green algae (4; 10.3%), golden algae (2; 5.1%), euglenoids (1; 2.6%), and cryptomonads (1; 2.6%) (Appendix C). The phytoplankton taxa in this bay included the diatoms *Cyclotella* which was found in 19 of 31 samples (61.3%) and *Thalassiosira* in 18 of 31 samples (58.1%). Phytoplankton densities were high amongst all rivers and bays ranging from 18 to 2,932 cells/mL and a median density of 803 cells/ml while HAB densities ranged from 15 to 1,642 cells/mL (Table 4.2). Median Chl-a concentration was the highest in the Estuary at 19.75 ug/L and ranging from 1.14 to 83.4 ug/L. The median water temperature was higher at 14.8 °C (ranging from 2.5 to 29.2 °C) and the highest median Sal concentration in the Estuary was recorded at 20.3 ppt (ranging from 9.4 to 25.9 ppt).

Raritan River

Forty-six phytoplankton taxa were recorded in the Raritan River, the third highest in the Estuary. All seven taxa groups were present in this river with the diatoms (16; 34.8%), green algae (13; 28.3%), cyanobacteria (10; 21.74%), dinoflagellates (3; 6.5%), golden algae (2; 4.3%), cryptomonads (1; 2.8%), and euglenoids (1; 2.8%) (Appendix C). The diatom *Navicula* was recorded in 21 of 22 samples (95.5%) and the green algae *Monoraphidium* in 14 of 22 samples (63.6%) were amongst the highest number of taxa detected in the Raritan River samples. The Raritan River had the second highest median phytoplankton and HAB densities when compared to other waterbodies in the Estuary at 329 cells/ml (ranging from 42 to 2,400 cells/mL) and 200 cells/mL (ranging from 4 to 1,833 cells/mL), respectively (Table 4.2). Median Chl-a concentrations were also the second highest in the Estuary at 6.26 ug/l and ranging from 0.65 to

33.6 ug/l. The highest median temperature in the Estuary was recorded at 15.8 °C (ranging from 2.6 to 27.3 °C) while median Sal concentrations were second lowest at 0.5 ppt (ranging from 0.1 ppt to 8.1 ppt).

4.3.2 Water Quality in the Hudson-Raritan Estuary

Water quality data was analyzed to obtain a greater comprehension of the environmental conditions across the five rivers and two bays in the Hudson-Raritan Estuary. Across the Estuary, Phyto densities were low during the study period ranging from 18 to 18,147 cells/mL with a median of 238 cells/mL; as well as low HAB densities with a range from 4 to 17,235 cells/mL (median of 138 cells/mL) suggesting low to no HAB risk to the human health (Table 4.3). Certain parameters such as Chl-a have been commonly utilized to determine phytoplankton biomass estimations. The results of this study showed Chl-a concentrations of 0 to 83.4 µg/L with a median of 3.66 µg/L. According to the US EPA guidance values for estuaries, these Chl-a concentrations indicated that the selected study sites in the Hudson-Raritan Estuary ranged from oligotrophic to eutrophic (USEPA, 2001). Any Chl-a concentrations above 10 µg/L could indicate impacted water quality (NJDEP, 2011; USEPA, 2001; WHO, 2003). During the study period, among the 31 study sites, 19.1% of samples (50 of 262) were above 10 µg/L. Among those 50 samples with Chl-a concentrations above 10 µg/L, the Raritan Bay had the highest number of samples exceeding the threshold at 40% (20 of 50), followed by the Hackensack River at 30% (15 of 50), the Passaic River at 20% (10 of 50), and the Raritan River at 18% (9 of 50). The Newark Bay, Hudson River, and Arthur Kill each had less than 7% of samples whose Chl-a concentrations were above 10 µg/L.

Both phytoplankton and HAB growth can be impacted by excess or lack of TN and TP; nutrient data can be useful to assess water quality degradation due to nutrient pollution. Using

historical and recent nutrient data as well as reference site conditions, the US EPA established ecoregional nutrient recommendations as guidelines for states to establish water quality standards (USEPA, 2000a). These nutrient recommendations indicate conditions where surface waters are least affected by anthropogenic activities, and where water quality is protected for recreation and to support aquatic organisms (USEPA, 2000a). Two sub ecoregions span the study sites in the Hudson-Raritan Estuary: the Northern Piedmont and the Atlantic Coastal Pine Barrens. The Passaic River, Hudson River, Hackensack River, Arthur Kill, and the Newark Bay sites were located in the Northern Piedmont ecoregion. Nutrient criteria conditions for this ecoregion ranged from 0.07 to 1.0 mg/L for TN and 0.022 to 0.1 mg/L for TP (USEPA, 2000a). More recent studies have reported nutrient concentrations for reference waterbodies in New Jersey, defined as <10% altered land use/land cover (Procopio & Zampella, 2023). We compared the Hackensack, Passaic, Hudson, and Arthur rivers and the Newark Bay TN and TP concentrations with the median TN and TP reference conditions of the northern reference streams as reported in Procopio & Zampella (2023) at 0.26 mg/L and 0.01 mg/L, respectively. TN was calculated by summing the medians of NH_3 +ON and NO_3 +NO_2 concentrations. The TN results of the northern study sites ranged from 0.0001 to 0.530 mg/L with the Hackensack River and Passaic River having the only samples exceeding TN reference conditions at 14.3% and 1.5%, respectively (Tables 4.3, 4.4). TP concentrations in the northern study sites ranged from 0.050 to 1.150 mg/L, with all waterbodies having 100% of samples exceeding TP reference conditions (Tables 4.3, 4.4).

The Raritan River and Raritan Bay were located in the Atlantic Coastal Pine Barrens ecoregion which had nutrient criteria ranging from 0.48 to 0.87 mg/L for TN and 0.006 mg/L to 0.052 mg/L for TP (USEPA, 2000b). The Raritan River and Raritan Bay were compared with

reference conditions for the southern region of New Jersey at 0.19 mg/L for TN and <0.01 mg/L for TP (Zampella & Procopio, 2023). However, the draining waters of the Raritan River and Raritan Bay primarily originate in the Piedmont and Highlands physiographic regions in the northern area of New Jersey. Results showed that TN concentrations from the study sites in the southern NJ area ranged from 0.001 to 0.059 mg/L, with no samples exceeding either the northern or southern TN reference conditions (Table 4.4). TP concentrations at the Atlantic Coastal Pine Barrens ecoregion ranged from 0.050 to 0.290 mg/L and all waterbodies having 100% of samples exceeding both northern and southern TP reference conditions (Tables 4.3, 4.4).

We conducted a Principal Components Analysis (PCA) in order to identify environmental conditions associated with phytoplankton and HAB taxa density at all seven waterbodies in the Estuary. The first and second principal components explained 49.8% of the total variance, with Phyto densities making the greatest contribution to the first axis (Figure 4.4). The first component measures phytoplankton and harmful algal bloom indicators such as Phyto, HAB, and Chl-a. While the second component measures water quality conditions such as Temp, TN, TP, and TSS. Spearman's correlation results showed that as TP ($p<0.01$) and TN ($p<0.01$) concentrations increased, Chl-a concentrations decreased, which has been reported in previous studies (Liang et al., 2020; Saraswathy et al., 2012) (Table 4.5).

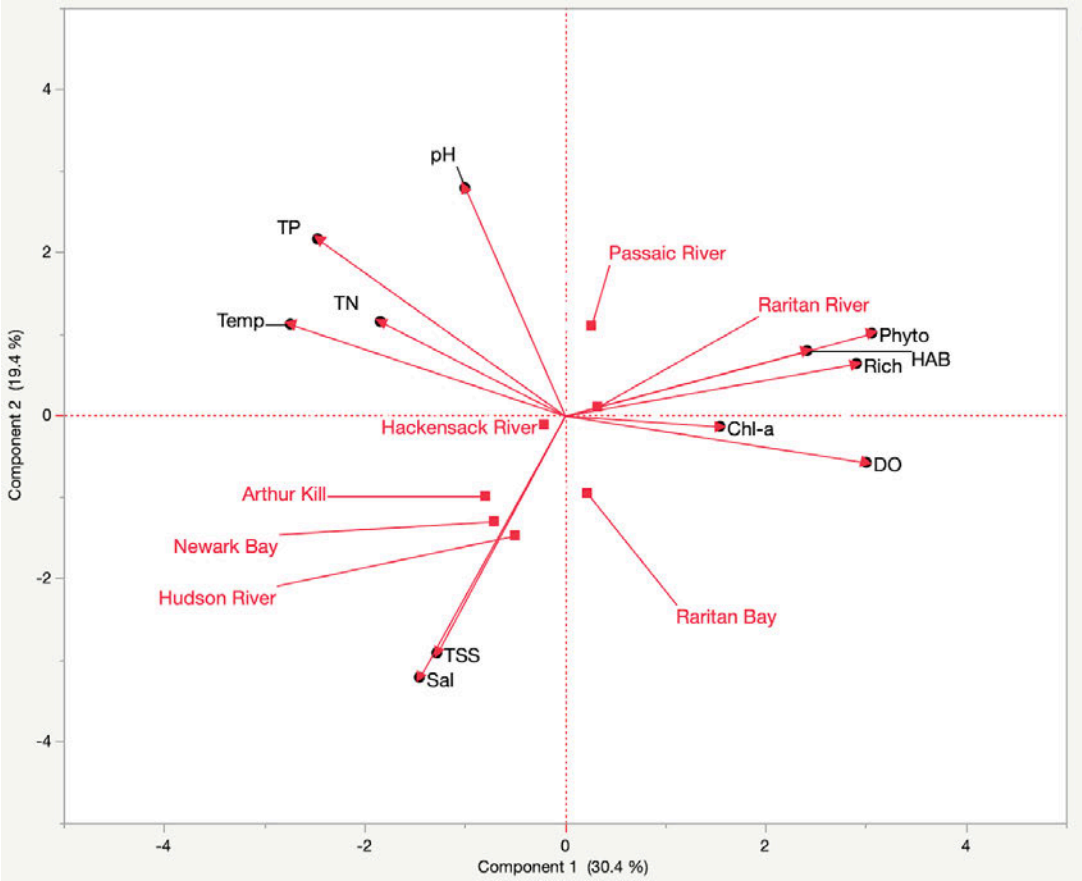

Figure 4.4. Principal Component Analysis of water quality parameters and phytoplankton and cyanobacterial densities of 31 sites in the Hudson-Raritan Estuary. Parameters: pH, dissolved oxygen (DO), salinity (Sal), temperature (T), total suspended solids (TSS), Total Nitrogen(TN), Total Phosphorus (TP), harmful algal bloom cell density (cells/mL) (HAB), Chlorophyll-a (Chl-a), phytoplankton cell density (Phyto), and richness (Rich).

Moreover, Phyto densities also decreased with increasing TP ($p<0.01$) concentrations (Table 4.5). However, this result contradicts previous studies showing that phytoplankton densities increase with higher nutrient concentrations (Buford & O'Donohue, 2006; Sirunda et al., 2021). This negative correlation could indicate that phytoplankton could be experiencing some functional constraints such that if the competitive ability for nitrate is higher, it can cause the competitive ability for phosphate to be lower (Edwards et al., 2011).

Seasonal and Temporal Patterns

Seasonal patterns were examined to observe the influence of weather conditions on the water quality in the Hudson-Raritan Estuary. In order to discern which water quality parameters were significantly different between seasons, a Dunn's post-hoc test was conducted. Among all seasons, the fall had the lowest Phyto densities ranging from 18 to 3,761 cells/mL (median 86 cells/mL) ($p<0.001$, $p<0.001$, $p<0.001$), lowest HAB densities ranging from 8 to 3,548 cells/mL (median 72 cells/ml), and lower Chl-a concentrations ($p<0.001$, $p<0.001$) (Tables 4.6, 4.7). The fall was also characterized by having higher nutrients with TP concentrations ranging from 0.10 to 1.15 mg/L (median 0.17 mg/L) ($p<0.001$, $p<0.001$) and the highest TN concentrations ranging from 0.00 to 7.90 mg/L (median 1.36 mg/L) ($p<0.001$, $p<0.001$, $p<0.001$) (Tables 4.6, 4.7). The winter season showed higher Phyto densities ranging from 40 to 10,393 cells/mL (median 261 cells/mL), the second highest median HAB densities at 194 cells/ml (ranging from 5 to 10,244 cells/mL), while lower Chl-a concentrations ranging from 0.49 to 54.20 µg/L (median 2.09 µg/L) ($p<0.001$, $p=0.019$) (Tables 4.6, 4.7). Lower nutrient concentrations were observed in the winter with TP ranging from 0.06 to 0.58 mg/L (median 0.11 mg/L) along with lower TN concentrations ranging from 0.00 to 6.83 mg/L (median 0.92 mg/L). Results showed that the spring season had higher Phyto densities ranging from 62 to 3,061 cells/mL (median 344 cells/mL), HAB densities ranging from 4 to 1,833 cells/mL (median 198 cells/mL), and higher Chl-a concentrations ranging from 0.00 to 83.40 µg/L (median 6.67 µg/L). The spring season also had lower TP concentrations ranging from 0.05 to 0.42mg/L (median 0.08 mg/L) and TN concentrations ranging from 0 to 4.87 mg/L (median 1.28 mg/L). When compared to all other seasons, the summer season results showed lower Phyto densities ranging from 22 to 18,147 cells/mL (median 220 cells/mL) ($p=0.036$) and TP concentrations ranging from 0.05 to 0.41 mg/L (median 0.15 mg/L) ($p<0.001$, $p<0.001$), while lower Chl-a concentrations ranging from

0.54 to 65.2 µg/L (3.72 µg/L) and TN concentrations ranging from 0 to 4.25 mg/L (median 1.25 mg/L) (Tables 4.6, 4.7).

Spatial Patterns

The PCA results showed that the Passaic River, Raritan River, and the Raritan Bay were on the positive side of the first component, indicating similarities in phytoplankton and HAB indicator parameters such as Chl-a concentrations, Phyto densities and HAB densities (Figure 4.4). In contrast, Arthur Kill, Hackensack River, Hudson River, and Newark Bay were on the negative side of the first component, suggesting they were dissimilar from the Passaic River, and Raritan River, and Raritan Bay with lower phytoplankton and HAB indicator parameters. Moreover, a Dunn's post-hoc test was conducted to determine which parameters were significantly different between waterbodies in the Estuary. Phytoplankton and HAB densities were the greatest in lower salinity waterbodies with an exception of the Raritan Bay, which had the highest salinity in the Estuary. Results showed that the Raritan Bay was characterized by higher phytoplankton and HAB densities than other waterbodies in the Estuary. For example, results showed that the Raritan Bay had significantly greater Chl-a concentrations than the Arthur Kill ($p<0.001$), Hudson River ($p<0.001$), Newark Bay ($p<0.001$), and Passaic River ($p<0.001$) (Table 4.8).

Similarly, Phyto densities were significantly higher in the Raritan Bay than the Arthur Kill ($p<0.01$), Hudson River ($p<0.001$), and Newark Bay ($p<0.001$) (Table 4.8). After the Raritan Bay, the Raritan River had the greatest phytoplankton densities. Additionally, Chl-a concentrations ($p=0.036$) and Phyto densities ($p<0.01$) were significantly higher in the Raritan River than the Hudson River (Table 4.8). Furthermore, Phyto densities ($p=0.0302$) and Chl-a concentrations ($p<0.01$) were significantly greater in the Raritan River than the Newark Bay.

Following the Raritan Bay and Raritan River, the Hackensack River differed from various waterbodies in the Estuary when comparing phytoplankton and HAB parameter values. Both Chl-a concentrations ($p=0.025$) and Phyto densities ($p<0.001$) were significantly greater in the Hackensack River than the Hudson River. Similarly, Chl-a concentrations were significantly higher in the Hackensack River than the Newark Bay ($p=0.031$), and the Passaic River ($p=0.033$). Lastly, the Passaic River had significantly higher Phyto densities than both the Hudson River ($p<0.001$) and Newark Bay ($p=0.019$).

Similar to phytoplankton and HAB densities, nutrients were the highest in waterbodies with lower salinity such as the Raritan River, Passaic River, and Hackensack River, except the Raritan Bay which had the highest salinity, high phytoplankton and HAB densities, and low nutrient concentrations. The lowest nutrient concentrations were observed in the Hudson River while the Raritan Bay had the second lowest TP concentrations. Results showed that the Hudson River had significantly lower TN concentrations than the Raritan River ($p<0.001$), Passaic River ($p<0.001$), and Hackensack River ($p<0.001$) (Table 4.8). The Hudson River also had lower TP concentrations than Raritan River ($p<0.01$), Passaic River ($p<0.001$), Hackensack River ($p<0.001$), and Arthur Kill ($p=0.026$). The Raritan Bay had significantly lower TN than the Passaic River ($p<0.01$), and Hackensack River ($p<0.01$). Furthermore, the Raritan River and Passaic River were not statistically significant ($p>0.05$) from each other in terms of nutrients.

Lastly, to explore the effect of salinity on water quality and HAB conditions in the Estuary, samples were grouped into tidal and non-tidal waters. Tidal waters were characterized by low Phyto and HAB densities. Results showed that median Phyto densities were higher in non-tidal waters ($p>0.05$) at 279 cells/mL (ranging from 25 to 18,147 cells/mL) when compared to the tidal median of 218 cells/ml (ranging from 18 to 10,393 cells/mL) (Table 4.9). However,

Chl-a concentrations were slightly higher in the tidal waters ($p>0.05$) with a median concentration of 3.70 µg/L (ranging from 0.00 to 83.40 µg/L) (Table 4.9). Non-tidal waters had a median Chl-a concentration of 3.42 µg/L (ranging from 0.58 to 27.10 µg/L). Similar to Phyto densities, HAB densities were higher in the non-tidal waters ($p>0.05$) ranging from 4 to 17,235 cells/mL and tidal counts ranged from 4 to 10,244 cell/mL. Results from the Hudson-Raritan Estuary showed that the lowest TN concentrations were observed in the non-tidal waters ranging from 0.00 to 4.88 mg/L while tidal waters had higher TN concentrations ($p<0.0001$) ranging from 0.00 to 7.90 mg/L (Table 4.9). Reference nutrient concentrations in the northern part of New Jersey had a median of 1.1 mg/L for TN (Procopio & Zampella, 2023). Thus, the higher ranges reported in both tidal and non-tidal waters were higher than reference conditions. TP concentrations were higher in tidal waters ($p=0.035$) ranging from 0.05 to 1.15 mg/L while non-tidal water concentrations ranged from 0.05 to 0.18 mg/L. Procopio & Zampella (2023) reported reference conditions for TP at 0.02 mg/L, suggesting that higher TP concentrations were reported in our study. A PCA was conducted to further explore the relationships between water quality parameters in samples from tidal and non-tidal waters in the Estuary. The first and second principal components explained 49.8% of the total variance, with Phyto densities making the greatest contribution to the first axis (Figure 4.5). Results showed that tidal and non-tidal water quality parameters were dissimilar since they were located on the opposite sides of both principal 1 and 2 components. When compared to non-tidal waters, tidal waters were associated with lower Phyto densities, DO concentrations, and higher Temp, TN, TP, and TSS concentrations.

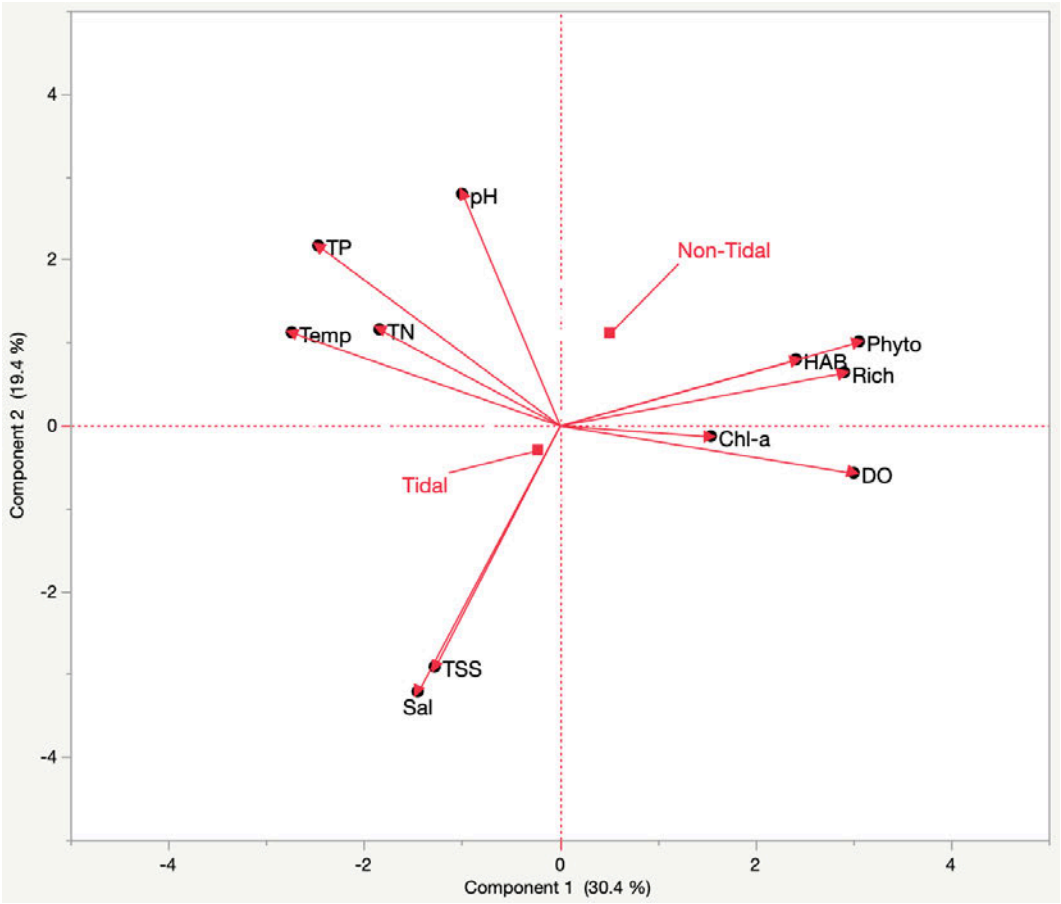

Figure 4.5. Principal Component Analysis of water quality parameters and phytoplankton and cyanobacterial densities of 31 tidal and non-tidal sites in the Hudson-Raritan Estuary. Parameters: pH, dissolved oxygen (DO), salinity (Sal), temperature (T), total suspended solids (TSS), Total Nitrogen (TN), Total Phosphorus (TP), Chlorophyll-a (Chl-a), harmful algal bloom cell density (cells/mL) (HAB), phytoplankton cell density (Phyto), and richness (Rich).

4.4 Discussion

While various water quality parameters in the Estuary have gradually improved (i.e., lower total suspended solids and higher dissolved oxygen) in response to sewage discharge regulations, its pollution history, and continuously growing population are likely to continue influencing nutrient loads; impacting water quality and ecological integrity (Hetling et al., 2003; Howarth et al., 2006; Fizabadi et al., 2002; Brosnan & O'Shea, 1996; O'Shea & Brosnan, 2000). For example, untreated sewage and sewer overflows have been reported as a major source of

pollution for the Estuary even after the Clean Water Act regulations regarding pollutant discharge have been in place for decades (Shu, 2004; USEPA, 2007). Furthermore, no nutrient or DO water quality standards exist in New Jersey for tidal rivers which further complicates management of these systems (NJDEP, 2018). For the purpose of our study, we used reference nutrient conditions (<10% altered land) from Procopio & Zampella (2023) in which conditions were assessed only for non-tidal waters since this was the only data available for comparison. This lack of regulations, failure to comply with regulations, accompanied by pollution from local municipal discharges could further contribute to the decline in water quality of the tidal rivers and bays (Howarth et al., 2006). However, the USEPA (2001) has reported regional nutrient criteria for rivers and streams to be used as a baseline for States to develop water quality standards for estuaries and coastal waters. Establishing nutrient criteria for New Jersey estuaries is of importance to protect the water quality of these complex systems. The planning of nutrient criteria in Barnegat Bay and Atlantic Coastal Estuaries are currently ongoing, thus have not been adopted by NJDEP's Water Quality Standards (NJDEP, 2018). Additionally, our study showed that more than half of the total phosphorus concentrations in the Passaic River, Hudson River, Hackensack River, Arthur Kill, and the Newark Bay were higher than the regional nutrient criteria range from the USEPA. In the Raritan River and Raritan Bay, the majority of samples were above the regional TP nutrient criteria. This indicates that the Estuary could be possibly subject to increasing nutrient loadings, greater phytoplankton primary productivity, and decline in water quality. Future studies should focus on implementing nutrient criteria for tidal rivers as well as within the Hudson-Raritan Estuary to help lessen nutrient enrichment.

4.4.1 Phytoplankton Assemblages in the Hudson-Raritan Estuary

Results showed a total of 99 phytoplankton genera were observed in the five rivers and two bays in the Estuary. For example, the diatom *Cyclotella* was the most frequently observed phytoplankton taxon in all samples across the Estuary. This taxon is known to inhabit fresh, brackish, and marine waters and requires NO_3^- as its nitrogen source in brackish and marine environments (Kipp et al., 2023). The elevated total nitrogen concentrations observed in the Estuary could indicate favorable conditions present for *Cyclotella* growth. Another important diatom taxa observed was *Thalassiosira* which can inhabit waters of varying salinities, with rapid growth rates reported at higher salinities (Vrieling et al., 1999). While this taxon is non-toxic, it has been associated with phytoplankton assemblages that produce red tides and polluted regions with high nutrient concentrations (Raman & Prakash, 1989; Yamaoka et al., 1998). It has the potential for climate change adaptations since it can grow in elevated CO_2 concentrations, temperatures, and high pH levels (Ishida et al., 2000; Lomas & Gilbert, 1998). Thus, this taxon could be of interest for future research in the Hudson-Raritan Estuary.

While HAB taxa such as *Gymnodinium*, *Amphidinium*, and *Chaetoceros* were present in the Hudson-Raritan Estuary, no blooms were recorded. The genus *Gymnodinium* is of note since some species are capable of producing neurotoxins and saxitoxins (Negri et al., 2007). Human illnesses associated with the consumption of toxic shellfish include Paralytic Shellfish Poisoning (saxitoxins) and Neurotoxic Shellfish Poisoning (neurotoxins) and can cause symptoms ranging from tingling and numbness to difficulty in breathing (Negri et al., 2007; Rodrigues et al., 2012; WHOI, 1999). Similarly, some *Amphidinium* species possess the capability of producing ciguatoxins which are known to cause the foodborne illness Ciguatera fish poisoning and have a range of symptoms from nausea to tingling of fingers and toes (Hallegraeff, 1993; NJDEP, 1998; WHOI, 1999). Previous reports have mentioned some common HAB taxa in New Jersey coastal

waters such as *Prorocentrum* spp.*, Ceratium tripos, Cochlodinium heterolobatum*, *Katodinium rotundatum*, and *Olisthodiscus luteus* (Gastrich, 2000). However, these taxa were not observed in this study which could be influenced by the natural variability of the phytoplankton community or study sampling techniques.

4.4.2 Temporal Trends

Our results showed that Chl-a concentrations were higher in the summer than winter, however cell densities were lower. While previous studies have suggested that temperature could be a factor affecting phytoplankton growth rates, the lower phytoplankton densities observed could have been impacted by a bias in counting methodology (Boynton et al., 1982; Eppley, 1972; Muylaert et al., 2000; Nixon, 1981). For example, the FlowCam has been reported to have lower counts when compared to microscopic methods (Brzezinski et al., 2011; Owen et al., 2022). These differences could be due to preservation impacts in some phytoplankton groups or size limitations from the FlowCam, with smaller-sized cells (<5 μm), such as picoplankton, being excluded (Brzezinski et al., 2011). Future studies should incorporate manual microscopic methods in order to validate the FlowCam results (Owen et al., 2022). Temporal trends suggested that the winter season was characteristic of lower nutrients and Chl-a concentrations. Previous studies have suggested that phytoplankton growth in the winter and fall can be limited by low temperatures, light, and high river discharge (Muylaert et al., 2000). Furthermore, the spring season in this study exhibited higher total nitrogen and Chl-a concentrations, consistent with findings from Muylaert et al. (2000). Previous studies have suggested that spring destratification can increase nutrient inputs from the benthos into the surface water, making the nutrients more accessible for phytoplankton growth (Webb & D'Elia, 1980). Overall, the spring and summer seasons in this study had the most favorable conditions for phytoplankton to grow in

the Hudson-Raritan Estuary. Future research could focus on collecting continuous data on water quality and HAB of the rivers and bays of the Estuary and analyzing trends of multi-year studies to assess the impacts of climate change on these seasonal patterns.

4.4.3 Spatial Trends

The results from this study suggested that the Raritan River, Hackensack River, and Passaic River had more advantageous conditions for phytoplankton and HAB to flourish. Most of the rivers in the Hudson-Raritan Estuary, except the Hudson River and Arthur Kill, showed higher Chl-a concentrations than the Newark Bay, which agrees with previous studies suggesting that higher nutrients and light increase phytoplankton accumulation in upstream systems of the turbidity zone (Harding et al., 1986; Fisher et al., 1988). In contrast to the Newark Bay, the Raritan Bay had some of the highest Chl-a concentrations in the Estuary. Previous studies have suggested high Chl-a concentrations present either landward or seaward of the turbidity maximum in an estuary and related to a decrease in phosphate, ammonium, and nitrate in this zone (Anderson, 1986; Harding et al., 1986; Fisher et al., 1988). The Raritan Bay sampling locations were further removed from the confluence of its rivers when compared to the Newark Bay locations; phytoplankton communities at the Raritan Bay could be less impacted by high turbidity.

The least advantageous phytoplankton and HAB conditions in the Hudson-River Estuary were reported at the Hudson River, the Newark Bay, and the Arthur Kill. Both sunlight and salinity changes have been reported to impact phytoplankton assemblages and mortality in the turbidity maximum (Cloern et al., 1983; Wofsy, 1983; Harding et al., 1986; Pennock & Sharp, 1994; Filardo & Dunstan, 1985), which could have influenced the low Chl-a values observed at the Newark Bay sites when compared to the riverine sites. The Hudson River and Arthur Kill

were two rivers that did not follow the same pattern as the Passaic River, Hackensack River, and Raritan River. Low Chl-a concentrations were reported in both the Hudson River and Arthur Kill. The Hudson River results showed both low Chl-a and TN concentrations, suggesting this waterbody could be less eutrophied than the other rivers in the Estuary (Desmit et al., 2020; Irvine & Murphy, 2009). Hansey and Rattray (1966) suggested that phytoplankton dynamics are more complicated in the river-estuary transition due to the impacts of river discharge on water mixing and high turbidity. Similar to the trend observed at the Arthur Kill with low Chl-a concentrations and higher nutrients, Li et al. (2018) found similar results with a tidal strait that had high nutrients and low chlorophyll concentrations and suggested that phytoplankton growth could be limited by reduced light availability from suspended silt (Li et al., 2018).

Nutrient analysis in most of the rivers from the Estuary, except the Hudson River, showed higher nitrogen concentrations when compared to both the Newark Bay and Raritan Bay which could indicate that these bays could be a sink for nitrogen. Tao et al. (2021) reported similar results with higher nutrient concentrations in rivers than in bays. Furthermore, the Raritan River has been documented as a source of nitrogen to the Raritan Bay in periods of high discharge (Reiser, 2004). While rivers can be sources of nutrients via downstream transport to the Estuary, the lower concentration of TN in the bays suggest a possibility that processes, such as denitrification, could be acting as a removal mechanism, and sedimentation taking place in the bay could serve as a nitrogen sink (An & Gardner, 2002; Tobias et al., 2001). Moreover, the Hudson River had lower nutrient concentrations than the Newark Bay. This could suggest that Newark Bay could be a source of nitrogen to the Hudson River (Prastka et al., 1988). The Hudson River has also been subject to water quality improvement efforts which could have possibly influenced the lower nutrient concentrations observed in our study (Brosnan & O'Shea,

1996; Taillie et al., 2020). However, median nitrogen concentrations in the Hudson River were still within the regional nutrient criteria (USEPA, 2001a, 2001b). While nutrient concentrations between rivers and bays showed some similar patterns, overall median nutrient concentrations recorded in the Estuary were higher than regional nutrient criteria for TP (USEPA, 2001a, 2001b).

Shallcross et al. (2002) suggested that the main sources of pollution entering the Hudson-Raritan Estuary are increased urbanization, urban runoff, and storm drain discharges. Due to the increasing population surrounding the highly urbanized Hudson-Raritan Estuary, combined restoration and remediation efforts are crucial for water quality improvement of the Hudson-Raritan Estuary. In order to address water quality and ecological health, focus on assessment and improvement of the Estuary has persisted since the 1970's. Previous ecosystem restoration research between the 1990's and 2000's focused on various studies of which three were within the Estuary including the Hackensack Meadowlands, Lower Passaic River, and a more comprehensive study of the Hudson-Raritan Estuary (NJSEA, 2019; (USEPA, 2001a, 2001b). In 2020, the USACE proposed a new restoration project combining the data from these studies to restore various ecosystems throughout the Estuary (USACE, 2020). The main goals of these projects were to restore ecological function and structure of estuarine, freshwater riverine, marsh island habitats, and increase oyster reefs (USACE, 2020).

While environmental variables are commonly studied in relation to HAB, future research in the Hudson-Raritan Estuary could extend to examine the relationships of environmental and biotic parameters due to its complex nature and need for comprehensive management strategies. For example, Rothenberger et al. (2023) suggested that biotic factors could be associated with HAB expansion and toxin production. Their results showed positive relationships between biotic

factors (such as diatoms, dinoflagellates, and zooplankton) and HAB taxa (Rothenberger et al., 2023). Thus, their research implies that a better understanding is needed to possibly utilize biotic factors in HAB monitoring and management. Moreover, research regarding the interactions between bacterial communities and HAB is needed. Bertrand and Allen (2012) reported that various bloom-forming cyanobacteria taxa rely on bacteria to obtain vitamin B_{12}. Future research on these bacterial relationships with HAB-forming phytoplankton taxa could provide new and useful information for future HAB and water quality management. Molecular analysis in future studies could also help support and complement microscopic methods while providing new opportunities for HAB forecasting and monitoring.

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Table 4.1. Site information of the selected study sites in the Hudson-River Estuary in New Jersey and New York. (*= indicates non-tidal waters).

Location	Site Description	Latitude	Longitude	County/State
PTA*	Pompton River, Tributary of Passaic River	-74.27063	40.91442	Passaic/NJ
PRA*	Passaic River	-74.33985	40.88211	Essex/NJ
PRB*	Passaic River	-74.27277	40.89726	Morris/NJ
PRC*	Passaic River	-74.24668	40.88773	Passaic/NJ
PRD*	Passaic River	-74.20066	40.90416	Passaic/NJ
PRE*	Passaic River	-74.1482	40.9413	Passaic/NJ
PRF*	Passaic River	-74.133	40.90266	Bergen/NJ
PRG	Passaic River	-74.12066	40.8795	Passaic/NJ
PTB	Saddle River, Tributary of Passaic River	-74.08166	40.889	Bergen/NJ
PRH	Passaic River	-74.12433	40.83983	Passaic/NJ
PRI	Passaic River	-74.14733	40.78616	Hudson/NJ
PTC	Second River, Tributary of Passaic River	-74.1615	40.7835	Essex/NJ
PRJ	Passaic River	-74.1653	40.7512	Essex/NJ
PRK	Passaic River	-74.12183	40.71983	Essex/NJ
HRA*	Hackensack River	-74.0288	40.9561	Bergen/NJ
HRB	Hackensack River	-74.07837	40.7919	Hudson/NJ
HRC	Hackensack River	-74.084	40.7395	Hudson/NJ
HRD	Hackensack River	-74.10283	40.7195	Hudson/NJ
NBA	Newark Bay	-74.12216	40.69383	Essex/NJ
NBB	Newark Bay	-74.14683	40.65666	Union/NJ
NBC	Newark Bay	-74.1735	40.6475	Union/NJ
AKN	Arthur Kill, North	-74.18961	40.64395	Union/NJ
AKS	Arthur Kill, South	-74.20745	40.59497	Middlesex/NJ
RRA	Raritan River	-74.4	40.49	Middlesex/NJ
RRB	Raritan River	-74.36	40.473	Middlesex/NJ
RBA	Raritan Bay	-74.26856	40.49097	Middlesex/NJ
RBB	Raritan Bay	-74.18808	40.48232	Middlesex/NJ
RBC	Raritan Bay	-74.146	40.52	Richmond/NY
URA	Hudson River	-73.9522	40.8516	New York/NY
URB	Hudson River	-74.01083	40.76701	Hudson/NJ
URC	Hudson River	-74.02553	40.72351	Hudson/NJ

Table 4.2. Medium and range of water quality parameters recorded at each of the five rivers and two bays in New Jersey in 2019 including pH, dissolved oxygen (mg/L) (DO), salinity (ppt) (Sal), temperature (°C) (T), total suspended solids (mg/L) (TSS), total nitrogen (mg/L) (TN), total phosphorus (mg/L) (TP), chlorophyll-a (ug/L (Chl-a), harmful algal bloom cell density (cells/mL) (HAB), phytoplankton cell density (cells/mL) (Phyto), and richness (Rich).

	Arthur Kill			Hudson River			Newark Bay			Hackensack River			Passaic River			Raritan Bay			Raritan River		
	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med
pH	5.1	8.2	7	5.3	8.9	7	5.1	8	7	5.5	7.9	7	6.3	8.4	7.5	5.6	8.4	7.1	5.5	7.9	6.9
DO	4.5	13.7	7.7	5.2	14.9	10.5	4.9	13.2	7.9	2.6	16.9	8.1	3.5	17.7	12	3.7	17.1	11.6	4.8	16.1	10.2
Sal	12.5	24	17.5	3.7	20.6	11.2	9.8	24	17.6	0.2	19.5	8.9	0.2	19.4	0.3	9.4	25.9	20.3	0.1	8.1	0.5
Temp	3.0	24.9	14.5	2.3	25.6	12.9	3.1	24.9	14.2	2.6	27.2	15.4	1.4	26.5	9.7	2.5	29.2	14.8	2.6	27.3	15.8
TSS	6	37	16	8	52	22	6	32	17	3	37	16	1	44	6	12	32	25	8	16	11
TP	0.06	0.31	0.15	0.06	0.16	0.08	0.05	0.25	0.11	0.05	0.41	0.17	0.05	1.15	0.14	0.05	0.17	0.10	0.07	0.3	0.13
TN	0.58	1.84	1.2	0.3	1.28	0.73	0	1.69	1.14	0.34	4.87	1.48	0	7.9	1.28	0	1.76	1.08	1.49	2.64	1.93
Rich	3	17	6	3	13	5	2	13	6	4	20	8	2	17	8	2	15	5	3	15	9
Chl-a	0.8	18.8	2.3	0	13.6	2.0	0.6	12.5	2.5	0.5	50.6	4.9	0.5	32.8	3.4	1.1	83.4	19.8	0.7	33.6	6.3
Phyto	22	1,281	96	40	568	113	24	650	135	36	18,147	261	25	10,393	275	18	2,932	803	42	2,400	329
HAB	13	842	218	16	365	82	13	429	111	4	17,235	72	4	102,44	138	15	1642	76	4	1,833	200

Table 4.3. Medium and range of water quality parameters recorded across the Hudson-Raritan Estuary including pH, dissolved oxygen (mg/L) (DO), salinity (ppt) (Sal), temperature (°C) (T), total suspended solids (mg/L) (TSS), total nitrogen (mg/L) (TN), total phosphorus (mg/L) (TP), chlorophyll-a (ug/L (Chl-a), harmful algal bloom cell density (cells/mL) (HAB), phytoplankton cell density (cells/mL) (Phyto), and richness (Rich).

Parameter	Min	Max	Med
DO	2.5	17.7	10.5
pH	5.0	8.9	7.2
TP	0.05	1.15	0.12
Rich	2	20	7
Sal	0.1	25.9	2.6
Temp	1.4	29.2	13.6
Chl-a	0.0	83.4	3.6
HAB	4	17,235	138
Phyto	18	18,147	238
TSS	1	52	14
TN	0.00	7.90	1.23

Table 4.4. Number of samples (N), total nitrogen mg/L (TN), total phosphorus mg/L (TP), and percent samples exceeding TN and TP reference conditions for five rivers and two bays within the Hudson-Raritan Estuary.

Waterbody	N	TN reference (mg/L)	%exceeding TN reference	N	TP reference (mg/L)	%exceeding TP reference
Raritan River	15	0.01	0	20	0.19	100
Raritan Bay	22	0.01	0	26	0.19	100
Arthur Kill	14	0.358	0	22	0.008	100
Hackensack River	35	0.358	14.3	37	0.008	100
Hudson River	25	0.358	0	33	0.008	100
Newark Bay	21	0.358	0	32	0.008	100
Passaic River	68	0.358	1.5	69	0.008	100

Table 4.5. Spearman's correlation coefficient (rs) and probability (p) between water quality parameters of in the Hudson-Raritan Estuary from January to December 2019 including pH, dissolved oxygen (DO), salinity (Sal), temperature (T), total suspended solids (TSS), total nitrogen (TN), total phosphorus (TP), Chlorophyll-a (Chl-a), harmful algal bloom cell density (cells/mL) (HAB), phytoplankton cell density (Phyto); (ns: not significant; $p > 0.05$; **$p<0.01$; *** $p<0.001$.

	Phyto		HAB		Chl-a	
	rs	p	rs	p	rs	p
DO	0.320	***	0.209	**	-0.008	ns
pH	0.005	ns	-0.014	ns	-0.034	ns
Temp	-0.205	**	-0.133	ns	0.102	ns
Sal	-0.246	***	-0.192	**	0.044	ns
TSS	-0.182	**	-0.061	ns	-0.002	ns
TN	-0.114	ns	-0.058	ns	-0.189	**
TP	-0.168	**	-0.149	ns	-0.205	**
Rich	0.713	***	0.463	***	0.372	***

Table 4.6. Seasonal median and range of water quality parameters recorded from the Hudson-Raritan Estuary, New Jersey between January and December 2019 including pH, dissolved oxygen (mg/L) (DO), salinity (ppt) (Sal), temperature (°C) (T), total suspended solids (mg/L) (TSS), total nitrogen (mg/L) (TN), total phosphorus (mg/L) (TP), chlorophyll-a (ug/L (Chl-a), harmful algal bloom cell density (cells/mL) (HAB), phytoplankton cell density (cells/mL) (Phyto), and richness (Rich).

	Fall			Spring			Summer			Winter		
	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med
pH	6.4	8.3	7.4	5.0	8.1	6.9	6.3	8.9	7.4	5.2	8.1	7.2
DO	3.7	10.5	6.5	4.9	17.5	12.8	2.5	13.5	6.9	8.0	17.7	13.3
Temp	17.6	23.5	20.7	2.4	16.8	8.9	14.7	29.2	24.4	1.4	10.6	4.4
Sal	0.1	25.9	9.9	0.1	22.0	4.2	0.1	25.0	3.3	0.1	25.0	0.7
TSS	6	37	20	1	34	9	1	51	16	1	52	15
TN	0.00	7.90	1.36	0.00	4.87	1.28	0.00	4.25	1.25	0.00	6.83	0.92
TP	0.10	1.15	0.17	0.05	0.42	0.08	0.05	0.41	0.15	0.06	0.58	0.11
Chl-a	0.5	33.6	2.6	0.0	83.4	6.6	0.5	65.2	3.7	0.4	54.2	2.0
Rich	2	16	4	3	17	9	3	20	6	2	17	7
HAB	8	3,548	72	4	1,833	198	4	17,235	99	5	10,244	194
Phyto	18	3,761	86	62	3,061	344	22	18,147	220	40	10,393	261

Table 4.7. Post-Hoc Dunn Method results for analysis of water quality between seasons in the Hudson-Raritan Estuary in 2019. pH, dissolved oxygen (DO), salinity (Sal), temperature (T), total suspended solids (TSS), total phosphorus (TP), total nitrogen (TN), Chlorophyll-a (Chl-a), harmful algal bloom cell density (cells/mL) (HAB), phytoplankton cell density (Phyto), richness (Rich) (ns: not significant, $p > 0.05$; * indicates statistical significance).

Seasons	Chl-a	HAB	DO	pH	Phyto	TP	Rich	Sal	Temp	TN	TSS
Fall vs Summer	<.001*	ns	<.001*	ns	<.001*	ns	<.001*	ns	<.001*	<.001*	ns
Fall vs Spring	<.001*	ns	<.001*	<.001*	<.001*	<.001*	<.001*	ns	<.001*	<.001*	ns
Fall vs Winter	ns	ns	<.001*	ns	<.001*	<.001*	<.001*	0.0409*	<.001*	<.001*	ns
Summer vs Spring	ns	ns	<.001*	<.001*	0.0365*	<.001*	<.001*	ns	<.001*	ns	ns
Summer vs Winter	0.0190*	ns	<.001*	<.001*	ns	<.001*	0.0263*	ns	<.001*	ns	ns
Spring vs Winter	<.001*	ns	ns	ns	ns	ns	ns	ns	<.001*	ns	ns

Table 4.8. Post-Hoc Dunn Method results for one way analysis of study sites in the Hudson-Raritan Estuary in 2019. pH, dissolved oxygen (DO), salinity (Sal), temperature (T), total suspended solids (TSS), total phosphorus (TP), total nitrogen (TN), Chlorophyll-a (Chl-a), harmful algal bloom cell density (cells/mL) (HAB), phytoplankton cell density (Phyto), richness (Rich) (ns: not significant, $p > 0.05$; * indicates statistical significance). Arthur Kill= AK, Hackensack River= HR, Hudson River= UR, Newark Bay= NB, Raritan River= RR, Raritan Bay= RB, and Passaic River= PR.

River/Bay	Chl-a	HAB	DO	pH	Phyto	TP	Rich	Sal	Temp	TN	TSS
AK vs HR	ns	ns	ns	ns	ns	ns	ns	<.001*	ns	ns	ns
AK vs UR	ns	ns	ns	ns	ns	0.026*	ns	ns	ns	ns	0.023*
AK vs NB	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
AK vs PR	ns	ns	ns	<.001*	ns	ns	ns	<.001*	ns	ns	<.001*
AK vs RB	<.001*	ns	ns	ns	<.01*	ns	ns	ns	ns	ns	ns
AK vs RR	ns	ns	ns	ns	ns	ns	ns	<.001*	ns	ns	ns
HR vs UR	0.025*	ns	ns	ns	<.001*	<.001*	ns	ns	ns	<.001*	<.001*
HR vs NB	0.031*	ns	ns	ns	ns	ns	ns	<.001*	ns	ns	ns
HR vs PR	0.033*	ns	0.017*	<.001*	ns	ns	ns	<.001*	ns	ns	<.001*
HR vs RB	ns	ns	ns	ns	ns	ns	ns	<.001*	ns	<.001*	ns
HR vs RR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UR vs NB	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
UR vs PR	ns	ns	ns	<.001*	<.001*	<.001*	ns	<.001*	ns	<.001*	<.001*
UR vs RB	<.001*	ns	ns	ns	<.001*	ns	ns	ns	ns	ns	ns

Table 4.8. Continued.

River/Bay	Chl-a	HAB	DO	pH	Phyto	TP	Rich	Sal	Temp	TN	TSS
UR vs RR	0.036*	ns	ns	ns	<.01*	<.001*	ns	<.001*	ns	ns	<.001*
NB vs PR	ns	ns	ns	<.001*	0.019*	ns	ns	<.001*	ns	ns	<.001*
NB vs RB	<.001*	ns	ns	ns	<.001*	ns	ns	ns	ns	ns	ns
NB vs RR	<.01*	ns	ns	ns	0.030*	ns	ns	<.001*	ns	ns	ns
PR vs RB	<.001*	ns	ns	<.001*	ns	ns	0.0401*	<.001*	ns	<.001*	<.001*
PR vs RR	ns	ns	ns	<.001*	ns	ns	ns	ns	ns	ns	ns
RB vs RR	ns	ns	ns	ns	ns	ns	ns	<.001*	ns	ns	0.038*

Table 4.9. Medium and range of water quality parameters recorded in tidal and non-tidal waters in New Jersey in 2019 including pH, dissolved oxygen (mg/L) (DO), salinity (ppt) (Sal), temperature (°C) (T), total suspended solids (mg/L) (TSS), total nitrogen (mg/L) (TN), total phosphorus (mg/L) (TP), chlorophyll-a (ug/L (Chl-a), harmful algal bloom cell density (cells/mL) (HAB), phytoplankton cell density (cells/mL) (Phyto), and richness (Rich).

	Non-tidal			Tidal		
	Min	Max	Med	Min	Max	Med
DO	3.4	17.7	12.0	2.5	17.4	10.4
pH	6.3	8.3	7.4	5.0	8.9	7.1
TP	0.05	0.18	0.11	0.05	1.15	0.12
Rich	2	20	8	2	17	6
Sal	0.1	0.7	0.2	0.1	25.9	10.9
Temp	1.4	26.5	9.4	2.1	29.2	14.2
Chl-a	0.5	27.1	3.4	0.0	83.4	3.7
HAB	4	17,235	199	4	10,244	134
Phyto	25	18,147	279	18	10,393	218
TSS	1	7	4	1	52	16
TN	0.00	4.88	0.00	0.00	7.90	1.43

Chapter 5 Conclusion and Environmental Management Implications

Harmful algal blooms (HAB) have been increasing globally over the last three decades, simultaneously impacting the environment, society, and economy (Paul, 2008; Hudnell, 2010). This increase in global HAB can be affected by increased detection of blooms, nutrient enrichment, ship ballast water transport, and climate change. Some HAB are fueled by anthropogenic pollution from industrial, agricultural, and wastewater discharges (Benayache et al., 2019; Gatz, 2017; Lapointe et al., 2015; Scavia et al., 2014). However, other areas experience HAB without a direct relationship with pollution which could reflect the natural dispersal of indigenous algae populations (Carey, Weathers, & Cottinghamn, 2008; Reinl et al., 2021). The detection of blooms has increased over the past decades due to improved methods of detection and increased awareness and surveillance (Benson et al., 2019; Esenkulova et al., 2021; Hallegraeff et al., 2021; Hanlon et al., 2022; Schmale et al., 2019; Stauffer et al., 2019). Research has shown that ship ballast water has contributed to the global transportation and expansion of toxic algal taxa around the world (Smayda, 2007). For example, Hallegraeff & Bolch (1992) reported that 50% of ballast tank sediment samples from 343 vessels contained dinoflagellate resting spores, including some toxic taxa. Similarly, Wang et al. (2022) showed that ship ballast water from 40 commercial ships in the Yangshan deep water port had 21 bloom forming taxa with 4 potentially toxin-producing taxa.

5.1 Mitigation and Management of HAB

Due to the global increase in HAB, strategies are needed to help prevent and control these blooms. These strategies are complicated by the diverse nature of the blooms (Sukenik & Kaplan, 2021; Zingone & Enevoldsen, 2000). Current HAB management strategies focus on the simultaneous protection of the ecology, economy, and public health impacts (Meriluoto et al.,

2017). The mitigation of HAB can involve taking action of current or ongoing blooms as well as decreasing their negative effects (Howard et al., 2022). For example, routine monitoring of cyanobacteria and cyanotoxins can help determine when waters are unsafe for fish and shellfish harvesting, recreation, and drinking water consumption (Anderson et al., 2001). In addition to addressing current blooms, strategies that help prevent the occurrence of a bloom would be greatly beneficial due to the reduced impacts from nuisance algae (Anderson, 2004). However, the prevention of HAB is difficult due to the existing knowledge gaps in HAB occurrence, physiology, and ecology (O'Keeffe, 2019; Schmale et al., 2019). Nonetheless, various studies show that several human activities such as increases in fertilizer, fossil fuel combustion, and sewage disposal have contributed significantly to nutrient pollution of our waters and subsequently HAB (Howarth, 2008; Paerl & Scott, 2010; Wurtsbaugh et al., 2019). These activities can be a focus of HAB prevention through legislative or policy changes (Castro et al., 2016; Davis et al., 2019; Hudnell, 2010).

HAB management strategies can be focused on prevention and control of blooms. Prevention measures can include environmental manipulation such as pollution control policies which can offer long-term reduction of nutrients that contribute to HAB (Kudela et al., 2005). Prevention strategies in recreational and drinking waters such as aeration focus on changes in stratification while dredging can create a shift in residence times to create less favorable conditions for cyanobacteria proliferation (Kibuye et al., 2021; Paerl et al., 2016). The sedimentation control strategy, utilized in both drinking and recreational waters, moves cyanobacterial cells to the bottom sediments by adding clay particles to the water surface (Beaulieu et al., 2005). These clay particles help HAB cells to coagulate and flocculate, overall increasing the sedimentation of the cells and removing them from the water surface (Beaulieu et

al., 2005). However, factors such as environmental clearances, permits, and economic costs hinder the application of this technique in the United States (Sengco & Anderson, 2004). Management strategies incorporating biological, genetic, and chemical control measures are not as extensively utilized since they pose various logistical problems including negative consequences of non-indigenous organisms on other aquatic organisms (Pal et al., 2020). There are numerous options for the management and mitigation of HAB. Strategy selection should also take into consideration physical attributes (i.e., hydrology, and temperature) of the waterbodies experiencing HAB.

5.2 Drinking Water Supply

The mitigation and management of cyanobacterial HAB (HAB) extends to drinking water supplies since many lakes, streams, rivers, and reservoirs are used for drinking water facilities (Falconer, 1999). Some consequences of the presence of cyanobacteria and cyanotoxins in drinking water facilities include increased microbial growth, disinfection by-product (DBP) formation, and treatment chemicals, among others (Foreman et al., 2021; He et al., 2016; Zhang, 2018). HAB can also produce taste and odor issues which can affect finished water quality for consumers in drinking water facilities that do not have proper treatment technologies (Watson, 2004). The management of taste and odor compounds should focus on monitoring and prevention efforts (Dietrich et al., 2020; Suffet et al., 1995). Another management strategy that would help improve water treatment is the prediction of odor events (Chong et al., 2018; Srinivasan & Sorial, 2011; Watson et al., 2008). Cyanobacteria and their associated toxins can pose a risk to water facilities and affect the course of treatment, especially since there are no federal regulations set in place in the United States (USEPA, 2022). Thus, since there exist only advisories, states can decide whether to actively monitor and treat HAB in drinking waters,

possibly increasing public health risk. However, New Jersey is currently finalizing cyanotoxin drinking water regulation. Subsequently, many drinking water facilities have unambiguous monitoring protocols which could pose a health risk to consumers potentially exposed to cyanotoxins (Mchau et al., 2019; Rashidiet al., 2021; Treuer et al., 2021).

Impacts regarding this lack of HAB management have been focused greatly on economic and public health effects (Brooks et al., 2016; Hoagland & Scatasta, 2006). Economic concerns from HAB are associated with increased treatment costs and water bans (Schinck et al., 2020). For example, HAB related treatment costs in 10 drinking water facilities in Ohio were estimated at $417,200 (Loadings & Blooms, 2014). A single HAB event from Toledo, Ohio in 2014 exposed residents to levels of microcystins above drinking water thresholds and affected the health of more than 100 persons (Gill and Joshi 2018). Treatment costs associated with this incident were estimated over $200,000 per month for activated carbon treatment (Liu & Klaiber et al., 2022). Other countries have also experienced drinking water crises with Qin et al. (2010) reporting two million people being affected for a week after a *Microcystis* bloom in Wuxi, Jiangsu Province, China contaminated the city's only water supply. Another public health concern for drinking water facilities is the delay between occurrence, detection, and public advisories (Stroming et al., 2020). Because it takes a few days for drinking water facilities to obtain toxin concentration results, consumers could potentially be exposed to cyanotoxins in the drinking water (Cheung et al., 2013; O'Keeffe, 2019). Many drinking water supplies could be at risk of HAB events and a proper course of action is needed to reduce public health risk.

5.3 Climate Change Predictions

The mitigation and management of HAB is further complicated by climate change trends. Climate change trends such as increased lake stratification, length of growing season,

temperatures, and alteration of the hydrologic and nutrient cycles can create beneficial conditions for cyanobacteria to proliferate which is predicted to increase the frequency and intensity of bloom events (Moore et al., 2008; Paerl 2016; Wells et al., 2020). In New Jersey, studies have shown long-term changes in the average annual temperature which has increased significantly by 1.9°C over the last 100 years while the winter season showed the highest temperature increase, which can create more favorable conditions for HAB growth (NJDEP, 2020). Predictions also suggest that New Jersey will have an average annual temperature increase of 4.1°F to 5.7°F and annual precipitation increase between 4% and 11% by 2050 (NJDEP, 2020). Other precipitation events such as droughts, floods, and tropical storms are also expected to increase in frequency and intensity in New Jersey (NJDEP, 2020). Moreover, estimates show that there is a 50% chance that sea-level in New Jersey will rise at or above 1.4 feet by 2050 (NJDEP, 2020). Sea-level rise can create shallower and more stable coastal waters in which HAB can proliferate (Paerl, 2016). Lastly, global predictions suggest that if the current CO_2 emission rates continue, ocean pH conditions will worsen and possibly create more acidic conditions than has been recorded in the past 20 million years (NJDEP, 2020). Ocean acidification is expected to have significant impacts in New Jersey since the economy of coastal communities depend on shellfish resources which are highly vulnerable to acidification impacts (NJDEP, 2020).

Warmer temperatures are predicted to favor some cyanobacteria taxa such as *Microcystis* since conditions such as increased stratification are preferred (Deng et al., 2014). Many cyanobacteria possess climate change related adaptations such as photo-protective pigments that allow for some cyanobacteria to withstand high irradiance levels (Paerl et al., 1983). Climate change trends have been predicted to impact the spatial and temporal ranges of HAB taxa, including the expansion of cyanobacteria (Gobler et al., 2017; Paerl & Paul, 2012; Visser et al.,

2016; Weyhenmeyerh, 2001). Conditions such as earlier summers and later falls have contributed to this cyanobacteria expansion (Wiedner et al., 2007). In addition to affecting cyanobacteria taxa directly, climate change is predicted to affect conditions which are favorable for cyanobacteria and bloom growth. For example, changes in precipitation, hydrology, and increased stratification are predicted to affect nutrient composition and supply (Koltz et al., 2022; Moss 2012). Kosten et al. (2012) reported an increase in cyanobacteria dominance and frequency with increasing temperatures and total nitrogen concentrations.

All HAB mitigation planning is affected by climate change trends, thus a shift towards new and improved strategies is imperative. Climate-change focused mitigation approaches should implement interactions between changes in hydrology, nutrient transport, rising temperatures, and agricultural and industrial impacts (Paerl et al., 2016). As HAB growth conditions (i.e., temperature, and nutrients) are altered by climate change, new guidelines will have to be changed accordingly to reduce nutrient inputs into waterbodies (Paerl et al., 2016). Furthermore, several studies have shown that watershed-scale remediation approaches could provide long-term nutrient reduction from urban and agricultural sources (Gao et al., 2014; Vymazal, 2007). For example, Vymazal (2007) concluded that constructed wetlands reduced approximately 50% of total nitrogen and total phosphorus from an agricultural watershed. With impending climate change trends, HAB mitigation and management strategies will require constant adjustments.

5.4 Future Directions

This study aimed to provide statewide baseline information for fresh and brackish water phytoplankton, cyanobacteria, and cyanotoxins in New Jersey with the implication to help inform management and monitoring strategies for HAB and ultimately help improve water

quality. Our field studies assessed water quality conditions of various waterbodies used for recreational and drinking purposes in New Jersey with a focus on cyanobacteria detection. Most of the selected study sites were deemed vulnerable to future HAB events. As the global population increases, continued impacts from human activities are expected to increase blooms and impact water resources (Bramburger et al., 2023; Ekstrom et al., 2020; Holland & Leonard et al., 2020; Oberle et al., 2019). Thus, we suggest that future HAB management strategies should include dual control of N and P along with continued and/or routine monitoring to obtain long-term data trends for improved bloom prediction. The relationship between nutrients and cyanobacteria dynamics are another important area of management research. For example, (Higgins et al., 2018) reported that residence times in lentic waterbodies could impact nitrogen-fixing cyanobacteria which could help balance the availability of phosphorus (Higgins et al., 2018). While planktonic cyanobacteria research is extensive, there is information lacking on benthic cyanobacteria communities and their impacts on eutrophication. Results from McCarthy et al. (2016) and Tobias et al. (2003) suggest that benthic cyanobacteria help reduce nitrogen deficits in some oligotrophic lakes and estuaries. Research on this topic could help gain a better understanding of HAB ecology and inform management strategies.

Current HAB mitigation strategies do not discriminate between problematic taxa and other phytoplankton present in the waters. Thus, future mitigation tools that target toxic HAB taxa would be beneficial for aquatic ecosystem health. Wells et al. (2020) suggested that viruses and nanotechnology tools are two new possible HAB mitigation strategies of interest that should be investigated. Future HAB management directions should also address technology issues. Due to the large amounts of data generated by HAB and the large number of reported incidents in the water sector, water cybersecurity is an important component of HAB management (DHS, 2015;

Schmale et al., 2019). Panguluri et al. (2017) recommended that water cybersecurity programs are necessary to secure public health and limit service disruptions to water and wastewater utilities. Furthermore, revising and improving the ways in which various sectors such as municipal, domestic, industrial, and recreational utilize water would help alleviate water quality degradation. A focus on current and ongoing HAB research is essential to implement new and improved monitoring, mitigation, and management policies (Gobler, 2020). For example, there is insufficient research on deep cyanobacteria layers which could affect risk management actions (Erratt et al., 2022). Moreover, public education, outreach, and communication of HAB science to citizens is essential to inform their understanding and increase their trust in the science and policies derived to protect human health and the ecosystem. Ultimately, a holistic social-ecological research approach to managing HAB is necessary to better understand the interactions between humans and the environment and further improve resource management (Bauer et al., 2010).

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Appendix A. Land-use and land-cover of the statewide and five New Jersey ecoregions: Northeastern Highlands (*Highlands*; n=37), Northern Piedmont (*Piedmont*; n=25), Ridge and Valley (*Ridge and Valley*; n=7), Middle Atlantic Coastal Plain (*Coastal Plain*; n=4), and Atlantic Coastal Pine Barrens (*Pine Barrens*; n=37). Ecoregion data was sourced from the U.S. EPA website (U.S. EPA ORD 2012).

	Land Use/Land Cover					
Ecoregion	**Agriculture**	**Barren Land**	**Forest**	**Urban**	**Water**	**Wetlands**
Highlands	7.30%	0.70%	54.00%	24.50%	4.60%	8.90%
Coastal Plain	10.30%	1.20%	11.00%	12.50%	34.90%	30.00%
Piedmont	10.20%	0.90%	20.40%	53.40%	5.00%	10.10%
Ridge and Valley	17.30%	0.60%	53.20%	14.70%	3.00%	11.30%
Pine Barrens	10.00%	1.00%	26.00%	24.80%	18.00%	20.30%
Statewide	10.4%	0.9%	28.7%	28.1%	14.5%	17.4%

Appendix B. Water quality parameters (N= sample size, Median) of 181 samples collected in New Jersey from 2016-2019. specific conductance (SPC; µS/cm), dissolved oxygen (DO; mg/L), pH, temperature (T; °C), secchi depth (Secchi; m), alkalinity (Alk; mg/L), hardness (Hard; mg/L), total nitrogen (TN; mg/L), total phosphorus (TP; mg/L), fluorescence of phycocyanin (PC; RFU), fluorescence of chlorophyll (Chl; RFU), chlorophyll-a (Chl-a; µg/L), phytoplankton cell density (Phyto; cells/mL), cyanobacteria cell density (Cyano; cells/mL), and microcystins (MC; µg/L). (-- indicates data not collected).

Site Name	Phyto N	Phyto Median	Cyano N	Cyano Median	MC N	MC Median	Chl-a N	Chl-a Median	PC N	PC Median	Chl N	Chl Median	DO N	DO Median	pH N	pH Median	SPC N	SPC Median	T N	T Median	Secchi N	Secchi Median	Alk N	Alk Median	Hard N	Hard Median	TP N	TP Median	TN N	TN Median
Allamuchy Pond	1	4080000	1	4077000	1	0.07	0	--	1	40.35	1	23.18	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Amico Island Park Pond	1	954000	1	952500	1	336.80	0	--	1	3773.00	1	176.40	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Amwell Lake	1	7200	1	0	1	0.07	0	--	1	19.79	1	9.31	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Assupink Lake	1	8775	1	2475	1	0.07	1	14.80	1	45.65	1	7.24	1	7.10	1	5.97	1	127.20	1	21.62	1	1.20	1	15.00	1	26.10	1	0.04	1	0.88
Bells Lake	1	11025	1	225	1	0.07	1	37.30	1	28.12	1	22.93	1	9.96	1	6.63	1	230.40	1	24.42	1	1.75	1	23.00	1	42.20	1	0.04	1	0.78
Birchwood Lake	1	3825	1	0	1	0.07	1	6.60	1	15.62	1	4.89	1	3.42	1	6.65	1	133.70	1	27.80	1	2.40	1	38.00	1	55.30	1	0.02	1	0.39
Bontoon Reservoir	1	32400	1	31875	1	0.65	0	--	1	166.90	1	9.41	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Branch Brook Park Lake	2	36525	2	36000	2	0.07	0	--	2	113.62	2	11.09	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Budd Lake	2	132038	2	128325	2	4.40	0	--	2	10040.40	2	42.09	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Canistear Reservoir	1	14625	1	13500	1	0.35	1	21.40	1	31.92	1	8.40	1	8.08	1	7.41	1	90.80	1	20.60	0	--	1	25.00	1	27.30	1	0.02	1	0.36
Castings Pond	1	14325	1	2625	1	0.07	0	--	1	73.39	1	142.70	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Clinton Reservoir	1	9000	1	0	1	0.07	1	4.80	1	15.21	1	6.67	1	8.36	1	7.09	1	65.70	1	24.91	1	3.60	1	4.00	1	13.80	1	0.01	1	0.31
Colliers Lake	1	9750	1	5325	1	0.07	1	6.10	1	17.08	1	11.52	1	4.36	1	5.79	1	124.40	1	26.44	1	1.10	1	6.00	1	16.90	1	0.02	1	0.58
Cranberry Lake	1	5325	1	0	1	0.07	1	5.60	1	14.87	1	5.92	1	8.07	1	7.78	1	277.80	1	29.13	1	3.10	1	40.00	1	58.60	1	0.01	1	0.42

Appendix B. Continued.

Site Name	Phyto N	Phyto Median	Cyano N	Cyano Median	MC N	MC Median	Chl-a N	Chl-a Median	PC N	PC Median	Chl N	Chl Median	DO N	DO Median	pH N	pH Median	SPC N	SPC Median	T N	T Median	Secchi N	Secchi Median	Alk N	Alk Median	Hard N	Hard Median	TP N	TP Median	TN N	TN Median
Crystal Springs Pond	2	127 136 4	2	126 753 9	2	2.3 2	0	--	2	590. 57	2	39. 76	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Cupsaw Lake	2	397 725 0	2	397 725 0	2	2.4 4	0	--	2	181 0.75	2	65. 78	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Curney's Point Game Creek Pond	1	538 500	1	375 000	1	1.1 3	1	72. 90	1	85.9 7	1	49. 90	1	6. 0 7	1	6. 9 5	1	574 .30	1	30. 05	1	0. 3 5	1	40. 00	1	64. 70	1	0.1 1	1	1. 3 2
Daretown Lake	1	106 125	1	980 25	1	1.1 4	0	--	1	151. 20	1	8.2 2	0	--	0	--	0	--	0	--	0		0	--	0	--	0	--	0	--
Deal Lake	8	164 738	8	146 550	8	0.2 2	4	76. 65	8	99.3 1	8	42. 33	1	7. 1 2	1	6. 9 1	1	710 .80	1	25. 31	1	0. 4 5	0	--	0	--	2	135 .16	0	--
Deer Park Pond	3	746 3	3	446 2	2	0.2 0	2	8.7 0	2	18.3 8	2	6.8 2	2	2. 7 3	2	6. 3 3	2	74. 45	2	24. 79	1	1. 7 0	2	35. 00	2	37. 15	1	0.0 2	2	0. 3 8
Dramesi Park Lake	1	618 75	1	583 50	1	18. 80	0	--	1	374. 10	1	23. 28	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Duck Pond	4	569 25	4	471 75	4	0.2 9	1	24. 10	4	45.1 6	4	22. 18	1	6. 5 3	1	6. 8 4	1	107 .00	1	27. 24	1	1. 3 0	1	28. 00	1	37. 90	1	0.0 6	1	0. 7 7
East Lake	1	291 675	1	282 825	1	40. 10	0	--	1	702. 40	1	64. 96	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Echo Lake	7	607 5	7	487 5	7	0.3 7	1	25. 90	7	39.7 5	7	21. 30	1	9. 5 9	1	9. 0 1	1	187 .70	1	27. 60	0	--	0	--	0	--	0	--	0	--
Elmer Lake Dam	1	330 000	1	330 000	1	246 .40	0	--	1	759. 70	1	48. 25	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Furnace Lake	2	626 3	2	225 0	2	0.0 7	2	10. 25	2	19.7 5	2	10. 33	2	9. 5 1	2	8. 8 4	2	184 .65	2	24. 16	2	2. 6 5	2	44. 00	2	61. 40	2	0.0 1	2	0. 2 9
Ghost Lake	2	776 2	2	405 0	2	0.0 7	2	14. 40	2	20.2 1	2	19. 32	1	2. 6 6	1	6. 7 4	1	241 .20	1	20. 86	1	1. 2 0	1	125 .00	1	140 .00	1	0.0 3	1	0. 6 0
Great Gorge Lake	3	427 5	3	450	3	0.0 7	3	7.6 0	3	19.8 4	3	3.0 0	3	7. 7 8	3	7. 6 5	3	84. 60	3	21. 79	2	3. 1 0	3	40. 00	3	42. 40	3	0.0 2	3	0. 3 0
Green Turtle Lake	4	162 00	4	228 00	4	0.0 7	3	3.1 0	4	18.9 3	4	4.6 4	3	8. 3 2	3	7. 5 7	3	129 .10	3	27. 00	2	3. 9 0	3	14. 00	3	27. 70	3	0.0 1	3	0. 2 5

Appendix B. Continued.

Site Name	Phyto N	Phyto Median	Cyano N	Cyano Median	MC N	MC Median	Chl-a N	Chl-a Median	PC N	PC Median	Chl N	Chl Median	DO N	DO Median	pH N	pH Median	SPC N	SPC Median	T N	T Median	Secchi N	Secchi Median	Alk N	Alk Median	Hard N	Hard Median	TP N	TP Median	TN N	TN Median
Greenwood Lake	2	562 87	2	456 75	2	3. 33	0	--	2	140. 05	2	15. 38	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Haledon Reservoir	1	171 75	1	162 00	1	0. 25	0	--	1	25.5 4	1	4.1 6	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Hands Mill Pond	2	273 8	2	270 0	2	0. 07	2	43. 65	2	23.1 5	2	23. 86	2	4. 91	2	4. 47	2	45.2 0	2	24. 73	1	1. 20	2	1.5 0	2	4.8 5	2	0. 03	2	0. 59
High Crest Lake	1	173 25	1	108 00	1	0. 07	1	27. 90	1	53.0 8	1	7.7 8	1	9. 95	1	8. 83	1	337. 70	1	26. 67	1	1. 60	1	45. 00	1	58. 70	1	0. 02	1	0. 39
Kennedy Pond	1	855 0	1	847 5	1	0. 07	0	--	1	20.4 7	1	3.5 8	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Lake Ashroe	1	125 25	1	697 5	1	0. 07	1	24. 10	1	25.7 9	1	17. 24	1	7. 08	1	7. 13	1	62.6 0	1	25. 16	1	1. 50	1	24. 00	1	26. 50	1	0. 03	1	0. 39
Lake Carasaljo	1	100 800	1	995 25	1	0. 13	1	10. 60	1	24.2 0	1	8.4 2	1	7. 32	1	6. 33	1	149. 50	1	24. 14	0	--	1	10. 00	1	23. 40	1	0. 04	1	1. 05
Lake Ceva	1	530 25	1	507 00	1	7. 28	0	--	1	202. 90	1	15. 49	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Lake Hopatcong	3	784 50	3	750 00	4	0. 40	0	--	4	46.8 9	4	28. 13	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Lake McCormack	1	241 50	1	123 75	1	0. 26	1	14. 20	1	25.6 5	1	12. 83	1	8. 93	1	7. 92	1	135. 70	1	29. 35	1	1. 50	1	11. 00	1	20. 40	1	0. 02	1	0. 73
Lake Meone	1	851 25	1	786 75	1	0. 17	0	--	1	226. 60	1	38. 09	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Lake Mohawk	1	336 00	1	327 00	1	4. 94	0	--	1	214. 70	1	15. 17	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Lake Musconetcong	3	720 0	3	165 0	3	0. 27	2	20. 40	3	26.3 5	3	15. 63	2	9. 04	2	7. 96	2	541. 60	2	22. 93	2	1. 25	2	48. 00	2	91. 25	2	0. 04	2	0. 51
Lake Norrsken	1	268 50	1	256 50	1	0. 07	1	13. 80	1	23.7 4	1	7.5 7	1	8. 34	1	6. 13	1	142. 50	1	22. 54	0	--	1	6.0 0	1	21. 40	1	0. 03	1	0. 72
Lake Owassa	1	106 50	1	102 00	1	3. 39	0	--	1	245. 40	1	12. 98	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Lake Pocahontas	1	975	1	0	1	0. 07	1	4.8 0	1	17.2 9	1	1.9 6	1	6. 56	1	7. 09	1	602. 30	1	22. 80	0	--	0	--	0	--	0	--	0	--
Lake Rogerene	1	284 25	1	278 25	1	0. 12	0	--	1	67.3 5	1	13. 57	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Lake Stockholm	1	335 25	1	270 00	1	0. 45	0	--	1	1131 .00	1	94. 44	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Lake Sylvan	1	108 825	1	104 850	1	0. 66	0	--	1	64.2 6	1	14. 51	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--

Appendix B. Continued.

Site Name	Phyto N	Phyto Median	Cyano N	Cyano Median	MC N	MC Median	Chl-a N	Chl-a Median	PC N	PC Median	Chl N	Chl Median	DO N	DO Median	pH N	pH Median	SPC N	SPC Median	T N	T Median	Secchi N	Secchi Median	Alk N	Alk Median	Hard N	Hard Median	TP N	TP Median	TN N	TN Median
Lake Topanemus	1	8400	1	0	1	0.07	0	--	1	30.07	1	18.37	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Lake Tranquility	2	9188	2	3150	2	0.07	2	10.35	2	20.46	2	10.26	2	8.18	2	8.09	2	456.60	2	25.59	2	2.10	2	175.00	2	183.50	2	0.02	2	0.50
Lake Washington	1	5775	1	2025	1	0.17	1	26.10	1	32.00	1	5.58	1	7.01	1	6.71	1	164.70	1	22.72	0	--	1	30.00	1	34.60	1	0.03	1	0.55
Lincoln Park Community Lake	5	21975	5	8100	5	0.87	1	28.30	5	37.95	5	25.42	1	7.01	1	6.71	1	465.20	1	26.79	0	--	1	72.00	1	95.80	1	0.03	1	1.03
Little Swartswood Lake	1	580500	1	553500	1	35.00	0	--	1	861.80	1	51.25	0	--	0		0	--	0	--	0	--	0	--	0	--	0	--	0	--
Lower Lake	1	4500	1	0	1	0.07	1	2.00	1	15.57	1	5.67	1	7.58	1	4.07	1	75.80	1	24.06	1	1.00	1	1.00	1	6.84	1	0.00	1	0.41
Manalapan Lake	1	375	1	150	1	0.11	0	--	1	29.75	1	4.06	0	--	0	--	0	--	0	--	0	--	0		0		0	--	0	
Manasquan Reservoir	4	7237	4	6450	4	0.13	3	5.30	4	21.90	4	4.64	2	6.91	2	4.99	1	221.40	1	27.74	1	2.00	0		0		2	8.70	0	
Mary Elmers Lake	1	57825	1	54225	1	0.20	1	38.90	1	46.52	1	22.93	1	13.01	1	7.90	1	155.10	1	28.33	1	0.70	1	25.00	1	53.60	1	0.03	1	2.44
Mashipacong Pond	3	5700	3	6188	3	0.07	3	5.40	3	16.70	3	4.85	3	7.15	3	5.95	3	18.80	3	23.30	2	3.85	3	3.00	3	6.58	3	0.01	3	0.28
Memorial Lake	2	300375	2	138000	2	2.41	0	--	2	170.90	2	229.00	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Mercer Lake	2	65738	2	62025	2	0.58	1	53.90	2	48.20	2	18.72	1	6.47	1	7.16	1	187.90	1	22.79	1	0.80	1	29.00	1	45.00	1	0.06	1	0.86
Monksville Reservoir	1	16650	1	16350	1	0.24	0	--	1	28.62	1	6.51	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Mount Laurel Lake	1	3300	1	1350	1	0.15	1	6.50	1	15.62	1	4.93	1	7.77	1	7.47	1	298.40	1	27.13	1	1.30	1	45.00	1	58.80	1	0.02	1	0.38
Mountain Creek Lake	1	40575	1	39075	1	0.07	0	--	1	57.65	1	8.01	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Mountain Lake	1	8475	1	7575	1	0.11	1	21.90	1	33.62	1	8.54	1	7.75	1	7.53	1	438.90	1	28.80	0	--	1	54.00	1	79.60	1	0.03	1	0.53
Mt. Misery Lake	3	4500	3	2100	3	0.07	3	1.00	3	14.26	3	3.21	3	5.90	3	4.19	3	33.90	3	21.75	2	1.00	3	1.00	3	2.90	3	0.02	3	0.63
Mud Pond	1	9675	1	8700	1	0.07	1	15.80	1	30.29	1	3.76	1	7.59	1	7.74	1	591.50	1	20.91	0	--	1	211.00	1	226.00	1	0.02	1	0.51
Oak Ridge Reservoir	1	11400	1	10950	1	0.07	1	4.10	1	19.83	1	3.27	1	8.74	1	7.69	1	141.70	1	24.85	0	--	1	34.00	1	37.00	1	0.01	1	0.32
Ocean Twp. Bathing Beach	1	11475	1	2250	1	0.07	1	4.60	1	13.86	1	8.02	1	7.39	1	6.07	1	99.70	1	28.22	1	1.40	1	6.00	1	13.00	1	0.02	1	0.71

Appendix B. Continued.

Site Name	Phyto N	Phyto Median	Cyano N	Cyano Median	MC N	MC Median	Chl-a N	Chl-a Median	PC N	PC Median	Chl N	Chl Median	DO N	DO Median	pH N	pH Median	SPC N	SPC Median	T N	T Median	Secchi N	Secchi Median	Alk N	Alk Median	Hard N	Hard Median	TP N	TP Median	TN N	TN Median
Papaianni Lake	1	37050	1	36675	1	3.93	0	--	1	186.10	1	18.89	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Rahway River	5	27675	5	35100	5	2.47	0	--	5	44.48	5	9.80	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Rosedale Lake	1	88725	1	81300	1	0.73	0	--	1	202.10	1	20.65	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Saddle River County Park	1	11730000	1	11730000	1	254.20	0	--	1	1103.00	1	110.20	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Salem River	1	64575	1	63075	1	15.20	0	--	1	95.91	1	7.90	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Shepard Lake	2	5512	2	2250	2	0.07	0	--	2	18.91	2	4.04	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Sheppard Pond	1	10875	1	9975	0	--	1	16.10	1	26.77	1	3.64	1	9.26	1	8.77	1	99.00	1	27.90	0	--	1	27.00	1	31.20	1	0.02	1	0.51
Silver Lake	2	4350	2	2925	2	0.07	2	7.20	2	16.25	2	7.14	2	2.90	2	5.84	2	44.00	2	24.90	1	1.60	1	7.00	1	11.90	1	0.01	1	0.40
Smithville Lake	1	212288	1	161288	1	0.24	0	--	1	146.00	1	71.71	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Southard Park Pond	1	554250	1	516000	1	0.25	0	--	1	2884.00	1	158.00	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Splitrock Reservoir	1	21600	1	19575	1	0.07	1	11.50	1	20.56	1	6.31	1	8.68	1	6.95	1	51.10	1	21.63	0	--	1	11.00	1	15.10	1	0.02	1	0.35
Spring Lake	1	40800	1	36225	1	0.07	0	--	1	66.05	1	53.59	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Spruce Run Reservoir	3	376425	3	375525	3	3.04	0	--	3	469.80	3	23.01	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Stacy Pond	2	478215	2	463763	2	0.46	0	--	2	63.67	2	34.83	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--
Stewart Lake	1	54375	1	27975	1	0.71	1	75.00	1	120.90	1	37.97	1	8.66	1	6.98	1	382.40	1	29.21	1	0.20	1	40.00	1	70.80	1	0.11	1	1.16
Strawbridge Lake	1	27000	1	27000	1	0.40	1	21.80	1	50.37	1	10.17	1	6.89	1	6.60	1	274.30	1	22.01	1	0.50	1	35.00	1	55.70	1	0.13	1	1.16
Sunset Lake	1	1915500	1	1911000	1	0.10	0	--	1	249.60	1	39.15	0	--	0	--	1	189.40	1	21.51	0	--	0	--	0	--	0	--	0	--
Swartswood Lake	27	7875	27	3825	27	0.31	24	9.70	27	21.33	27	6.33	18	8.39	18	8.42	18	263.10	18	23.86	18	2.40	24	75.00	24	87.60	24	0.02	24	0.30
Timber Lake	1	4725	1	0	1	0.20	1	11.00	1	19.89	1	11.09	1	6.83	1	5.74	1	69.60	1	25.43	1	1.10	1	10.00	1	17.80	1	0.03	1	0.82
Upper Erskine Lake	1	24300	1	6750	1	0.29	0	--	1	147.80	1	11.50	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--	0	--

Appendix B. Continued.

Site Name	Phyto N	Phyto Median	Cyano N	Cyano Median	MC N	MC Median	Chl-a N	Chl-a Median	PC N	PC Median	Chl N	Chl Median	DO N	DO Median	pH N	pH Median	SPC N	SPC Median	T N	T Median	Secchi N	Secchi Median	Alk N	Alk Median	Hard N	Hard Median	TP N	TP Median	TN N	TN Median
Watchu Pond	3	6300	3	1575	3	0.13	3	19.00	3	16.93	3	8.36	3	2.41	3	6.67	3	96.10	3	22.95	2	1.25	3	54.00	3	55.70	3	0.03	3	0.48
Wawayonda Lake	1	4200	1	1950	1	0.26	1	6.60	1	21.71	1	7.90	1	7.56	1	7.64	1	360.60	1	25.93	1	2.10	1	65.00	1	86.20	1	0.03	1	0.49
Weeqahic Lake	1	3855 00	1	3855 00	1	1.90	0	--	1	272.40	1	15.04	0	--	0		0	--	0	--	0	--	0	--	0	--	0	--	0	--
White Lake	2	6000	2	0	1	0.15	2	10.03	2	9.10	1	3.35	2	9.88	2	8.05	2	475.70	2	23.78	2	4.70	2	214.50	2	226.50	2	0.01	2	0.51
Willow Pond	1	14250	1	12900	1	0.07	0	--	1	22.77	1	7.32	0	--	0		0	--	0	--	0	--	0	--	0	--	0	--	0	--
Wright Pond	1	5625	1	0	1	0.07	1	3.80	1	16.38	1	3.90	1	6.31	1	6.91	1	230.00	1	19.51	1	4.10	1	66.00	1	82.50	1	0.02	1	0.24

Appendix C. Phytoplankton taxa presence and absence and overall number of taxa in the Hudson-Raritan Estuary (X indicates taxon present; * indicates taxa found only in non-tidal waters) (F=freshwater, B=brackish water, and M=marine).

			River					Bay		
Groups	**Taxonomy**	**Habitat**	**Passaic**	**Hackensack**	**Hudson**	**Arthur**	**Raritan**	**Newark**	**Raritan**	**Overall**
Cryptomonads	*Cryptomonas*	F, M	X	X	X	X	X	X	X	7
Cyanobacteria	*Aphanizomenon*	F, B, M	X	X	X		X			4
	*Arthrospira**	F	X							1
	Chroococcus	F, M	X	X	X		X	X	X	6
	*Chrysosporum**	F	X							1
	Cuspidothrix	F, B					X			1
	*Cylindrospermum**	F	X							1
	Dolichospermum	F	X	X	X	X		X	X	6
	*Gloeothece**	F		X						1
	*Gomphosphaeria**	F	X							1
	*Jaaginema**	F	X							1
	*Komvophoron**	F	X			X	X			3
	Leptolyngbya	F, M	X	X	X	X	X	X	X	7
	*Limnoraphis**	F					X			1
	Merismopedia	F, M	X	X			X			3
	*Microcystis**	F	X							1
	Nodularia	F, B, M							X	1
	Nostoc	F	X	X	X		X	X		5
	*Planktolyngbya**	F	X	X						2
	*Planktothrix**	F	X							1
	Pseudanabaena	F, M	X	X	X	X	X	X	X	7
	Raphidiopsis	F	X	X		X	X	X		5
	*Rhabdoderma**	F	X							1
	*Rivularia**	F, M			X					1

Appendix C. Continued.

			River					Bay		
Groups	**Taxonomy**	**Habitat**	**Passaic**	**Hackensack**	**Hudson**	**Arthur**	**Raritan**	**Newark**	**Raritan**	**Overall**
Diatom	*Achnanthes*	F, M	X	X	X	X	X		X	6
	*Asterionella**	F	X	X						2
	Asterionellopsis	M	X	X	X	X	X	X	X	7
	*Aulacoseira**	F	X	X				X		3
	*Caloneis**	F, B, M	X							1
	Cerataulina	F, B	X	X				X		3
	Chaetoceros	F, M	X	X	X			X	X	5
	Cocconeis	F, M	X	X	X	X	X	X	X	7
	Coscinodiscus	M				X		X		2
	Cyclotella	F, B, M	X	X	X	X	X	X	X	7
	Cymbella	F, M	X	X	X		X	X	X	6
	*Diatoma**	F	X	X						2
	Ditylum	M			X	X	X	X	X	5
	Entomoneis	F, B, M						X		1
	*Eunotia**	F	X							1
	Fragilaria	F	X	X		X	X	X		5
	Gomphonema	F	X			X				2
	Gyrosigma	F, B, M	X	X	X	X			X	5
	Lauderia	M						X		1
	Leptocylindrus	M	X	X	X	X	X	X	X	7
	Melosira	F, M	X	X		X				3
	Navicula	F, M	X	X	X	X	X	X	X	7
	Nitzschia	F, M	X	X	X	X	X	X	X	7
	*Pinnularia**	F	X	X						2

Appendix C. Continued.

			River					Bay		
Groups	**Taxonomy**	**Habitat**	**Passaic**	**Hackensack**	**Hudson**	**Arthur**	**Raritan**	**Newark**	**Raritan**	**Overall**
Diatom	*Pleurosigma**	F, B, M		X						1
	Rhizosolenia	M	X	X	X	X	X	X	X	7
	*Rhoicosphenia**	F	X	X					X	3
	Skeletonema	M		X				X	X	3
	Stauroneis	F, B, M	X	X		X	X	X	X	6
	Stephanodiscus	F, M	X	X	X	X	X	X	X	7
	*Surirella**	F	X							1
	*Synedra**	F	X	X						2
	Thalassionema	M	X	X	X	X	X	X	X	7
	Thalassiosira	F, M	X	X	X	X	X	X	X	7
	Ulnaria	F, M	X	X		X	X	X	X	6
	*Urosolenia**	F	X	X						2
Dinoflagellates	*Amphidinium**	F, M		X						1
	Boreadinium	M						X		1
	Gymnodinium	F, M	X	X	X	X	X	X	X	7
	Gyrodinium	F, M					X	X		2
	Peridinium	F, M	X	X		X	X	X	X	6
Euglenoids	*Euglena*	F, M	X	X	X	X	X	X		6
	*Phacus**	F		X						1
Golden Algae	*Dinobryon*	F, M	X	X	X		X	X		5
	Mallomonas	F, M	X	X				X		3
	Synura	F, B, M	X	X			X			3
Green Algae	*Actinastrum**	F	X	X						2
	*Actinotaenium**	F	X	X						2

Appendix C. Continued.

			River					Bay		
Groups	**Taxonomy**	**Habitat**	**Passaic**	**Hackensack**	**Hudson**	**Arthur**	**Raritan**	**Newark**	**Raritan**	**Overall**
Green Algae	*Ankistrodesmus**	F	X	X	X		X			4
	Chlamydomonas	F, M	X	X	X	X	X	X	X	7
	Chlorella	F, M	X	X	X	X	X	X	X	7
	Closterium	F	X	X	X		X		X	5
	*Coelastrum**	F	X	X	X	X				4
	Cosmarium	F, B, M	X	X	X	X		X		5
	*Crucigenia**	F	X	X						2
	Desmodesmus	F	X	X	X		X		X	5
	*Dictyosphaerium**	F	X			X	X			3
	*Eudorina**	F		X						1
	*Golenkinia**	F	X	X						2
	*Gonatozygon**	F	X							1
	Micractinium	F, M	X		X			X		3
	Monoraphidium	F	X	X	X	X	X		X	6
	*Mougeotia**	F	X							1
	*Oocystis**	F	X	X	X					3
	*Pandorina**	F	X				X			2
	Pediastrum	F	X	X	X		X		X	5
	*Roya**	F	X							1
	Scenedesmus	F	X	X	X		X		X	5
	Schroederia	F	X	X	X				X	4
	Spirogyra	F, B	X							1
	*Spirotaenia**	F	X							1
	Staurastrum	F, B	X	X			X		X	4

Appendix C. Continued.

			River					Bay		
Groups	**Taxonomy**	**Habitat**	**Passaic**	**Hackensack**	**Hudson**	**Arthur**	**Raritan**	**Newark**	**Raritan**	**Overall**
Green Algae	*Tetraedron*	F	X		X	X	X		X	5
	*Tetrastrum**	F	X							1
	*Volvox**	F					X			1

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