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entitled

The Ecology of the Nuisance Cyanobacterium, *Lyngbya wollei*, in the Western Basin of Lake Erie

by

Sarah E. Panek

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Master of Science Degree in Biology

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An Abstract of

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While prevalent in the southeastern United States, little is known about the distribution, abundance, and effects of *Lyngbya wollei* in western Lake Erie excluding research by Bridgeman and Penamon (2010). The objective of this study is three-fold: 1) Determine the temporal and spatial distribution of *L. wollei* in the Western Basin of Lake Erie. Also, determine if temporal and spatial patterns of previous growing seasons (2009 summer) are repeated. 2) Determine the relative importance of depth, light intensity, substrate type, and temperature in influencing the establishment and biomass of *L. wollei*. 3) Determine the effects of temperature on *L. wollei* growth/primary production in order to obtain a range of temperatures at which *L. wollei* can grow. Also, account for potential power plant effects (increased water temperature in winter months) which may maintain the minimal temperature conditions in winter months for *L. wollei* growth. Field survey results showed temporal and spatial distributions were consistent in both years sampled. A generalized additive model (GAM) was developed with the environmental factors light at lake bottom, sand, latitude and longitude, temperature, and secchi being the best fit predictors for *L. wollei* density. Laboratory temperature controlled experiments showed
L. wollei growth rate, photosynthetic yield, and electron transport rate decrease with decreasing temperature. L. wollei maintains positive growth rate at temperatures between 7 – 20 °C and a negative growth rate at temperatures \(\leq 5 \, ^\circ\text{C}\). Studying the distribution pattern of L. wollei with accompanying habitat characteristics may be useful in determining which environmental characteristics are most important for the establishment of L. wollei and to predict which areas of Lake Erie and potentially the other Great Lakes may be most affected by L. wollei in the future. Also, by studying the influence of environmental factors I may find ways to reduce L. wollei by altering some human influences such as nutrients, turbidity, or thermal pollution. It is important to understand the potential short term and long term impacts of L. wollei on Lake Erie.
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Chapter 1

Introduction

1.1 Background

Cyanobacteria, commonly referred to as blue-green algae, are true bacteria with a simple prokaryotic cell structure. Cyanobacteria are small, and occur in unicellular, filamentous, and colonial forms, and are abundant components of freshwater and marine phytoplankton. Cyanobacteria are photoautotrophs meaning they utilize light as their energy source and CO₂ as their carbon source, and they are the only prokaryotes which generate oxygen via photosynthesis (Wetzel, 2001; Reece et al., 2011).

Cyanobacteria are the oldest known prokaryotes in Earth’s history originating about 3.5 billion years ago, and they are thought to be the cause of the rapid increase in atmospheric oxygen about 2.7 to 2.3 billion years ago known as the oxygen revolution (Reece et al., 2011). Cyanobacteria also play an important role in nitrogen fixation as they are one of very few groups of organisms that can convert atmospheric nitrogen into an organic form (nitrate or ammonia) via specialized cells called heterocysts (Wetzel, 2001; Reece et al., 2011).
Freshwater cyanobacteria possess both chlorophyll $a$ pigments and the accessory pigment phycocyanin which gives them their characteristic blue-green color (Fay, 1983; Bryant 1981; Sathyendranath et al., 1987; Wetzel, 2001; Becker et al., 2009).

Cyanobacteria differ from other bacteria by the presence of chlorophyll $a$, which is common to chloroplasts of eukaryotic algae and higher plants. Although referred to as blue-green algae, cyanobacteria are not algae; it is only the chloroplast in eukaryotic algae to which cyanobacteria are related (Wetzel, 2001; Reece et al., 2011).

While beneficial in terms of oxygen production and nitrogen fixation, cyanobacteria can also form Harmful Algal Blooms (HABs). HABs can be defined as harmful due to sheer biomass or due to the production of toxins (Backer and McGillicuddy, 2006). HABs can have detrimental public health impacts by contaminating public drinking and recreational freshwater and marine sources (Backer, 2002; Backer and McGillicuddy, 2006). HABs can cause several problems within a water system, such as decreased water quality, fish kills, increased economic costs, and an unaesthetic environment (Doyle and Smart, 1998 and Speziale and Dyck, 1992). A number of factors are thought to contribute to the formation of HABs including, sunlight, water temperature, water depth, water flow, and nutrients (Downing et al., 2001; Havens, 2008; Paerl, 2008; Becker et al., 2009).

There have been several examples of potentially hazardous toxins associated with HABs. In 1987, the diatom *Pseudo-nitzschia* sp. prevalent in the coastal waters of the Pacific Northwest was found to be the source of domoic acid which contaminated mussels (Perl et al., 1990). Domoic acid causes amnesiac shellfish poisoning which has gastrointestinal and neurologic symptoms (Perl et al., 1990; Backer and McGillicuddy,
Outbreaks of the dinoflagellate *Karenia brevis*, or red tide, which causes neurotoxic shellfish poisoning (NSP), have been reported along the Gulf Coast of the southeastern United States since the 1890s (Steidinger, 1993; Backer and McGillicuddy, 2006). Some prevalent toxin-producing cyanobacteria genera include: *Anabena*, *Aphanizomenon*, *Cylindrospermopsis*, and *Microcystis* (Backer and McGillicuddy, 2006).

In 1995, Brittain et al. (2000) documented the first isolation of the microcystin toxin produced by a *Microcystis* strain in Lake Erie since 1966, and *Microcystis* has been prevalent in Lake Erie since (Wang et al., 2009; Chaffin et al., 2011).

### 1.2 *Lyngbya wollei* in Lake Erie

HABs in Lake Erie have been getting worse over the past 15 years. Three major problem species in Lake Erie are *Microcystis aeruginosa* and *Lyngbya wollei*, which are cyanobacteria, and *Cladophora glomerata*, which is a green alga. In recent years, *Microcystis* has become the dominant planktonic cyanobacteria in the Western Basin of Lake Erie and has produced large blooms in the lake (Brittain et al., 2000; Rinta-Kanto et al., 2005; Chaffin et al., 2011). *Microcystis* produces microcystin, a toxin which damages the liver if ingested (Wang et al., 2009), which has caused several animal poisonings (Carmichael and Falconer, 1993; Humpage and Falconer, 1999; Carmichael, 2001).

*L. wollei* (Farlow ex Gomont) Speziale and Dyck is a freshwater nuisance cyanobacterium commonly found in the southeastern United States (Bridgeman and Penamon, 2010 and Speziale and Dyck, 1992). In 2008 *L. wollei* was found to dominate the benthic macroalgae in sections of the Western Basin of Lake Erie (Bridgeman and
Penamon, 2010). A filamentous cyanobacterium, *L. wollei* forms dense benthic and floating mats which can impact aquatic life and recreational water use. *L. wollei* presence in Lake Erie is thought to be due to either changing water chemistry or transfer from boats from the southern United States (Bridgeman and Penamon, 2010).

Algal blooms occurred in Lake Erie during the 1960s due to pollution mainly by phosphorus input from agricultural runoff (Wang et al., 2009; Boegman et al., 2008). In 1972 the United States and Canada signed the Great Lakes Water Quality Agreement to restrict phosphorus inputs to the Great Lakes. This legislation reduced municipal and industrial phosphorus discharge to lakes and limited detergent phosphate concentrations. The decrease of phosphorus inputs reduced the amount of algae and algal blooms, i.e., *Aphanizomenon*, by the 1980s (Wang et al., 2009; Dolan, 1993; Nicholls et al., 1977). However, since then, the appearances of new algal blooms, such as *L. wollei*, have occurred. Property owners and others who live and work on the shorelines are concerned with the sudden occurrence, amount, and endurance of *L. wollei* present in the Western Basin of Lake Erie (Bridgeman and Penamon, 2010).

The widespread distribution of *L. wollei* has potential impacts on large areas of western Lake Erie. Benthic mats can negatively affect aquatic life in the sediments and can lead to the extinction of benthic organisms (Boyce et al., 1987 and Boegman et al., 2008). The dense, floating mats of *L. wollei* clog water intakes (Doyle and Smart, 1998 and Speziale and Dyck, 1992) and decrease both recreational use and aesthetics of Lake Erie. In addition to the nuisance caused by benthic and floating mats, *L. wollei* has been found to produce paralytic shellfish toxins – PSTs (Camacho and Thacker, 2006). PSTs can change feeding behavior thereby affecting survivorship of freshwater zooplankton...
and other aquatic life (Camacho and Thacker, 2006, Gilbert 1996, and Haney et al., 1995). However, *L. wollei* in the western basin of Lake Erie has not been found to produce PSTs to date. One study found *L. wollei* produced a potent neurotoxin which caused decreased movement, convulsion and death from respiratory paralysis in mice (Carmichael and Evans, 1996). It is unknown if there are adverse health risks from *L. wollei* for humans.

1.3 Objectives

The overall goal of this study is to establish the relative importance of various environmental factors (including thermal pollution from power plants) in determining the distribution of *L. wollei* in western Lake Erie. Determining which set of environmental conditions is most highly correlated with *L. wollei* mats will be of future use in predicting which other areas of the Great Lakes may be susceptible to *L. wollei* invasion. To this end, the sub-objectives of the study are as follows: 1) Determine the temporal and spatial distribution of *L. wollei* in the Western Basin of Lake Erie. Also, Determine if temporal and spatial patterns of previous growing seasons (2009 summer) are repeated. 2) Determine the relative importance of depth, light intensity, substrate type, nutrient concentration, and proximity to power plant thermal plume in influencing the establishment and biomass of *L. wollei*. 3) Determine the effects of temperature on *L. wollei* growth/primary production in order to obtain a range of temperatures at which *L. wollei* can grow. Also, to account for potential power plant effects (increased water temperature in winter months) which may maintain the minimal temperature conditions
in winter months for *L. wollei* growth. Potentially, these effects could further determine *L. wollei* seasonal abundance and distribution in western Lake Erie.

1.4 Environmental factors that may influence *L. wollei*

Water depth can play an indirect role in the distribution of *L. wollei*, as depth influences flow conditions and wave action. Shallow, inshore areas receive increased wave action, and as *L. wollei* does not firmly attach to substrate, strong wave and wind influence may inhibit *L. wollei* presence in these areas. Field studies by Cowell and Botts, 1994 showed *L. wollei* in areas with high flow, which provided a greater replenishment of nutrients, was in better physiological condition than *L. wollei* in areas characteristic of low flow and high sediment deposition. Depth may also affect the distribution of *L. wollei* indirectly as light levels reaching the benthos decrease with increasing depth.

Previous studies have shown increased levels of dissolved nutrients, specifically nitrogen and/or phosphorus can stimulate algal growth (Ryan et al., 1972, Baker et al., 1981, Tubea et al., 1981, Canfield, 1983, Carpenter and Lodge, 1986, Cooke et al., 1986, Cowell and Botts, 1994). Maumee Bay, being rich in nutrients (Bridgeman and Penamon, 2010), is expected to provide adequate nutrient concentrations for *L. wollei* abundance as *L. wollei* distributions have been linked with eutrophic water conditions (Speziale et al., 1988). However, field observations performed in Florida springs did not find a strong correlation between water nitrate and phosphate concentrations and *L. wollei* abundance (Cowell and Botts, 1994, Pinowska et al., 2008).
L. wollei has low light requirements for photosynthesis which allow L. wollei to survive beneath aquatic plants or in turbid water (Doyle and Smart, 1998; Dyck, 1994; Beer et al., 1986, 1990). Pinowska et al., 2007 reported average benthic irradiances in the field between 18 and 53 μEm⁻²s⁻¹ for L. wollei. Optimum light for growth of L. wollei under laboratory conditions was reported by Beer et al., 1986, 1990 and Dyck 1994 as 20 – 150 μEm⁻²s⁻¹ and 10 – 100 μEm⁻²s⁻¹ respectively.

Substrate type may play a role in the distribution of L. wollei throughout the Western Basin of Lake Erie. Bridgeman and Penamon (2010) reported the majority of L. wollei presence was found on sand, dreissenid druzes, and crushed dreissenid shell substrate, and very low L. wollei presence on soft, silt sediments and compacted, clay substrate. Unlike Cladophora, a filamentous green alga, which requires attachment to a hard substrate, L. wollei is only loosely associated with substrate and can remain in place by becoming buried or entangled (Bridgeman and Penamon, 2010). Personal observations via snorkeling showed that L. wollei buried within sand substrate could be easily dislodged with a light pull.

The presence of dreissenid mussels may also play a role in the growth and survival of L. wollei. Dreissenid mussels remove particles from the water column, clearing the water which may provide increased light levels for growth of benthic algae (Boegman et al., 2008, Madenjian et al., 1995 and Ackerman et al., 2001). Dreissenid mussels excrete nutrients (phosphorus and ammonia) from the phytoplankton they eat as a part of digestion and metabolic processes. These excreted nutrients in turn serve to fertilize the growth of cyanobacterial blooms (Boegman et al, 2008 and Conroy et al.,
Also, *L. wollei* may become entangled in dreissenid mussel shells and become fixed in a certain area (personal observation).

Power plants may influence benthic algal growth and biomass due to increased water temperature from the presence of thermal effluents discharged from the power plants. Thermal effluents may be especially influential during winter months where increased water temperature and increased light penetration (due to lack of ice cover) may maintain the minimal temperature and light conditions for benthic algal growth (Hickman, 1974; Gallup and Hickman, 1975). There are three prominent power plants located along the shoreline of the Western Basin of Lake Erie, Bayshore power plant, Consumers Energy JR Whiting power plant, and Monroe power plant. The response of cyanobacteria to different temperatures varies and is strongly species-dependent (Ilus & Keskitalo, 2008; Goldman, 1977a, 1977b). Thermal effluents from power plants are about 8 °C to 10 °C higher than the ambient temperatures of surrounding waters (Srivastava et al., 1993; Tison et al., 1981). The increase of temperature from thermal discharges may intensify environmental stress for organisms (Ilus & Keskitalo, 2008) or it may provide a favorable environment for benthic algae growth. I hypothesized that since *L. wollei* is prevalent in the warmer waters of the southeastern United States, the warmer temperatures associated with power plant thermal discharges in Lake Erie may promote its growth in Lake Erie.
1.5 Hypotheses

1) I hypothesize that the temporal and spatial patterns and relationships between *L. wollei* distribution, abundance, and environmental factors observed during the summer of 2009 will be similar to the patterns and relationships observed during the summer of 2010, thus indicating a consistent year-to-year spatial distribution.

2) I hypothesize that a model that includes some or all of the following environmental factors: depth, light intensity, substrate type, nutrient concentration, and proximity to power plant thermal plume will be able to explain a significant amount of the spatial variation in *L. wollei* biomass in western Lake Erie.

3) I hypothesize that *L. wollei* will be able to grow at a wide range of temperatures and low light intensity

1.6 Significance of study

While prevalent in the southeastern United States, little is known about the distribution, abundance, and effects of *L. wollei* in Lake Erie as there is little research with the exception of Bridgeman and Penamon (2010). The importance of this study is to determine how environmental factors are affecting *L. wollei* growth. Studying the relationship between *L. wollei* distribution and habitat characteristics may be useful in determining which environmental factors are most important for the establishment of *L. wollei* in the Western Basin of Lake Erie. The results of this study may help predict which areas of Lake Erie and potentially the other Great Lakes may be most affected by *L. wollei* in the future. Also, by studying the influence of environmental factors I may
find ways to reduce *L. wollei* by altering some human influences such as nutrients, turbidity, or thermal pollution.

In order to gain an understanding of the environmental factors influencing *L. wollei* distribution and abundance, I can create a model which attempts to show the influence of each environmental parameter on *L. wollei* presence and density. The aim of the model is to determine how important each of the environmental parameters (depth, substrate, light, temperature, proximity to power plant, nutrient concentration) is in promoting *L. wollei* presence and biomass.

While there are no models available specific to *L. wollei*, similar models have been created examining the response of *Cladophora glomerata* to changing environmental factors such as temperature, light, and nutrient availability (Canale and Auer, 1982; Tomlinson et al., 2010). Tomlinson et al. (2010) adopted the original *Cladophora* growth model developed by Canale and Auer (1982) and examined the relationship between depth, phosphorus availability, and dreissenid impacts on light availability on *Cladophora* colonization in Lake Michigan. Model results showed increased light availability due to dreissenid influence expanded the depth of colonization by *Cladophora*. In addition, the model also gave phosphorus (P) concentrations (those below 1 μgP/l) at which *Cladophora* would be responsive to P management. The results of the model were used to assist management decisions involving establishment and growth of *Cladophora*. A similar model for *L. wollei* could potentially be used in the future to predict where *L. wollei* will spread in Lake Erie and the rest of the Great Lakes.

In addition to the effects of environmental factors on *L. wollei* distribution and abundance, *L. wollei* within the western basin, can affect and interact with the ecosystem.
Benthic mats can affect aquatic life in the sediments, namely mayfly larvae. By analyzing survey data from both 2009 and 2010 growing seasons of *L. wolleii*, biomass predictions of *L. wolleii* in western Lake Erie can be made which may be more than the biomass of all other algae and phytoplankton combined. This abundance could change the food web, channeling primary production into biomass that may not be edible by grazers. Therefore, it is important to study the effects of *L. wolleii* as to better understand its interactions within the ecosystem.

Factors outside the immediate ecosystem may also play an important role in influencing *L. wolleii* growth and distribution throughout the western basin. Examining the relationship between *L. wolleii* distribution and biomass and power plant thermal effluent presence is important to understanding both *L. wolleii* ecology and potential impacts of the power plants on the Lake Erie ecosystem. It is unknown whether *L. wolleii* appearance in western Lake Erie is due to changing water chemistry (i.e., warmer temperatures similar to those of the southeastern United States) or due to transfer from southern boats (Bridgeman and Penamon, 2010). Global climate change in addition to thermal effluents from shoreline power plants could provide *L. wolleii* with conditions suitable for growth and survival.

It is also important to recognize the impacts of *L. wolleii* distribution and abundance on humans. Several communities comprised of millions of citizens along the shoreline in the United States, Michigan and Ohio, and Canada obtain drinking water from western Lake Erie where these cyanobacteria blooms occur (Wang et al., 2009; Becker et al., 2009). The dense, floating mats of *L. wolleii* clog water intakes and decrease
both recreational use and aesthetics of lakes (Doyle and Smart, 1998 and Speziale and Dyck, 1992).
Chapter 2

Methods

2.1 Description of Study Site

*L. wollei* surveys were conducted in Maumee Bay and the Western Basin of Lake Erie (41°40’40”N to 41°54’0”N; 83°30’0”W to 83°20’0”W) (Figure 2-1). Maumee Bay is shallow, warm, and rich in nutrients (Bridgeman & Penamon, 2010). The surface area of the Western Basin is 3080 km² and extends 60 km east to west and 40 km north to south (Mortimer, 1987; Edwards et al., 2009). The Western Basin is relatively shallow with an average depth of 7.4 m and a maximum depth of 12 m toward the east end of the basin (Mortimer, 1987; Edwards et al., 2009). The substrate of the Western Basin is mostly post-glacial mud and sand mixtures (Verber, 1957; Hartley, 1961; Bolsenga and Herdendorf, 1993; Edwards et al., 2009); however, most of the substrate observed contained *Dreissena* shells (personal observation). The water temperature of the Western Basin ranges from 0 °C during the winter to a maximum of 25 °C during the summer months (Edwards et al., 2009). However, there are three power plants along the shoreline of the Western Basin which emit thermal effluents which are about 8 °C to 10 °C higher
than ambient temperatures of surrounding waters (Tison et al., 1981; Srivastava et al., 1993). Nearshore areas of the basin are often subjected to high turbidity from the Maumee River plume (Bridgeman & Penamon, 2010). Secchi depth is driven by turbidity and varies (30-220 cm) throughout the basin (Wang et al., 2009).

Figure 2-1. Western Basin of Lake Erie. Location of *Lyngbya wollei* field surveys during 2009 and 2010.
2.2 Field Survey: Cyanobacterial Sampling and Preparation

An extensive survey was conducted between June 2009 and September 2009 over an area of approximately 210 km² in the Western Basin of Lake Erie. Benthic samples were georeferenced using GPS in order to create a map of *L. wollei* distribution. In addition to *L. wollei* samples, data were collected on substrate type, water chemistry, light attenuation, temperature, and depth.

A second extensive survey was conducted during the peak growing season of *L. wollei*, July-August 2010. Previous sites of interest and high *L. wollei* density were re-sampled in order to see if patterns and relationships from the summer 2009 survey were repeated. The second survey extended north of Bolles Harbor, Michigan and east toward Sandusky Bay in order to determine if distribution and abundance patterns were similar in different areas of the western basin.

Sampling and collection of submerged *L. wollei* mats was conducted from small boats at different locations throughout the Western Basin of Lake Erie. A benthic rake was used to determine if *L. wollei* was present at depths up to 3.5 m, and an Ekman grab sampler (232 cm²) was used to collect *L. wollei* from each site. Five grabs were taken from each sample site. *L. wollei* from each grab sample was separated from sediments using a sieve bucket (Wildco, 500 μm mesh). In the laboratory, *L. wollei* samples were rinsed with tap water and any remaining sediments, shells, and invertebrates were removed. Samples were then weighed for fresh weight (g) and dried at 60 °C to a constant dry weight for further analyses (Bridgeman and Penamon, 2010 and Panek and Bridgeman, 2009). In addition to *L. wollei* samples, location, depth, secchi, and a multiprobe (YSI 6600 V2 data sonde, Yellow Springs Instruments) was used to measure
water temperature, dissolved oxygen, conductivity, pH, turbidity, and chlorophyll a concentration. Latitude and longitude were recorded at each site using GPS. Depth was measured as the depth in meters of the water column. Light was measured by using a submersible photometer (Li-cor) for light at the bottom measurements and by using secchi depth measurements. Light at the bottom was calculated as percent of surface light. Secchi depth, a measure of water clarity, was recorded in centimeters. Temperature was measured in degrees Celsius at meter intervals throughout the water column, including temperature at the bottom. pH and turbidity were measured using a multiprobe (YSI 6600 V2 data sonde, Yellow Springs Instruments) and measurements were recorded at meter intervals throughout the water column.

Substrate grabs were taken from each site and analyzed in the laboratory using a particle-size analyzer (Mastersizer 2000). Sediments were measured by particle size or a measurement of percent sand, percent gravel, or percent mud. Another substrate measurement that was used was determining *D. polymorpha* (dreissenid mussel) presence or absence in each substrate sample. Water samples were collected at two selected sites along each transect, one site with little or no *L. wollei* present and one site with abundant *L. wollei*, for nutrient analysis by the National Center for Water Quality Research (NCWQR) at Heidelberg University in Ohio. Water samples were analyzed for NH$_3$, NO$_2$, NO$_3$, SiO$_2$, F, Cl, SO$_4$, total Kjeldahl nitrogen (TKN), soluble reactive phosphorus (SRP), total phosphorus (TP), and total soluble phosphorus (TSP) in order to determine if there was a difference in nutrient concentrations where *L. wollei* was absent versus present and abundant.
Temporal and spatial patterns were determined using Geographic Information Systems (GIS) ArcMap 9.3.1 (Environmental Systems Research Institute). Georeferenced data from both 2009 and 2010 seasons were used to create maps of *L. wollei* distribution. Maps were created using *L. wollei* presence and absence measurements as well as *L. wollei* biomass (g/m²) measurements for each sampling season. ArcMap 9.3.1 was used to create maps which were used to visually and analytically evaluate potential temporal and spatial patterns of *L. wollei* in the Western Basin of Lake Erie.

2.3 Model

The model was built with survey data (see Objective 1) collected during the summer growing season of 2010. Several variables, location (latitude and longitude), depth (m), secchi (cm), light at lake bottom (%), sand per substrate sample (%), temperature (°C), pH, turbidity (NTU), and dreissenid presence and absence as well as living or dead, were used while building the model. Each variable was recorded at each site (n = 132) sampled.

Latitude and longitude were included in the model in order to determine if location within the Western Basin of Lake Erie played a role in the abundance and distribution of *L. wollei*. Depth was included in the model because light is attenuated exponentially with depth, and I expect that at a certain depth, there would not be enough light available for *L. wollei* growth. Depth is also used together with K<sub>PAR</sub> to determine light at the lake bottom which was also a factor included in the model. Light at the lake bottom was included in the model because light is necessary for any cyanobacterial
growth. Depth, secchi, and turbidity influence light attenuation, therefore at a certain combination of depth, secchi depth, and turbidity level, there would not be enough light available for *L. wollei* growth. Secchi depth was included in the model because it was used to calculate $K_{\text{PAR}}$ which was used to determine light at the lake bottom which is also used in the model. Substrate type and dreissenid presence and absence were included in the model because *L. wollei* is a benthic cyanobacterium which associates loosely with the substrate and becomes entangled with dreissenid shells and shell fragments (Bridgeman and Penamon, 2010). Also, since *L. wollei* does not attach to hard substrate, unlike *Cladophora*, a green, filamentous alga, there may be regions of the western basin with hard substrate (i.e., rocks) that do not support *L. wollei* abundance. As *L. wollei* is commonly found in warm waters of the southeastern United States, temperature was included in the model to determine if increased water temperatures played a role in the recent abundance of *L. wollei* in the Western Basin of Lake Erie. The presence of thermal effluents of three prominent power plants along the shoreline of the western basin contribute to increased water temperatures and potentially influence *L. wollei* abundance. pH was measured because some cyanobacteria have been found to be sensitive to changes in pH (Speziale et al., 1988; Cowell and Botts, 1994). Turbidity was included as an additional measure of water clarity and influenced light attenuation.
2.4 Laboratory Experiments

The main potential influences on *L. wollei* from the power plant thermal effluents are increased water temperature and increased light penetration during the winter due to lack of ice cover. Growth chamber experiments were performed to manipulate temperature in order to determine the conditions at which *L. wollei* can photosynthesize as well as the minimum temperature range for *L. wollei* to thrive. These experiments also attempted to determine the minimum temperature which *L. wollei* needs to begin photosynthesis.

Based on preliminary experiments (see Appendix A) fresh weight and photosynthetic yield were chosen as the primary indicators of *L. wollei* growth. Glass Mason jars (946 ml) were set up with 500 mL filtered lake water (GFF/ <.45μm) and nutrient solution in a growth chamber. Two grams *L. wollei* fresh weight were added to each jar, and an initial fresh weight was recorded. The biomass of *L. wollei* added to jars reflected the average biomass (g/m²) found in the western basin in my surveys. Nutrient concentrations were based on field data and mimicked concentrations found at the sites in Lake Erie where *L. wollei* biomass was most abundant. 44.3 μL of 435 g/500 ml K₂HPO₄, 44.3 μL micronutrients (4.36 g/L Na₂EDTA, 3.15 g/L FeCl₃, 0.18 g/L MnCl₂, 0.022 g/L ZnSO₄, 0.0046 g/L NaMO₄, 0.012 g/L CoCl₂, 0.01 g/L CuSO₄, and 0.006 g/L H₃BO₃), and 1070 μL of 42.5 g/500 ml NaNO₃, 18.5 g/500 ml MgSO₄, 13.9 g/500 ml CaCl₂, and 12.6 g/500 ml NaHCO₃ were added to each treatment.

Two environmental growth chambers (E7/2 Conviron) were used for the duration of the experiment. The experiment ran for five weeks and had nine temperature conditions, 1 °C, 2 °C, 3 °C, 4 °C, 5 °C, 7 °C, 10 °C, 12 °C, and 20 °C, in order to
determine the temperature at which *L. wollei* breaks dormancy and how *L. wollei* growth responds to increasing temperature. A light regime of 10:14 hours light:dark was used to mimic winter conditions. Light intensity was kept at 100 μEm²s⁻¹ during the light hours which has been shown by literature to be an optimum growing condition for *L. wollei* in a laboratory setting (Doyle and Smith, 1998). Light intensity was manipulated using window screening material as shading. Two HOBO temperature loggers were kept in each chamber for the duration of the experiment to track the stability of chamber temperature. Preliminary experiments were performed to ensure that each chamber could maintain accurate temperatures and light intensity for the entirety of the experiment.

Each of the two chambers began the experiment at 20 °C for the first week because from literature and field observations I know *L. wollei* grows well at this temperature and in order to determine any differences between chambers. For the remaining four weeks, each individual chamber was assigned four different temperature conditions. The first chamber treatments were 20 °C, 12 °C, 7 °C, 3 °C, and 2 °C. The second chamber treatments were 20 °C, 10 °C, 5 °C, 4 °C, and 1 °C. Each chamber contained 8 replicated samples, and water and nutrient solution were replenished on day 1 and day 4 of each week-long treatment, as previous studies by Cowell and Botts (1994) indicate *L. wollei* growth improved with semiweekly media changes versus growth with only weekly media changes.

The response of *L. wollei* to varying temperatures was determined by measuring three parameters: 1) Biomass change over the course of the experiment. Biomass change was determined by recording fresh weight at the beginning and end of each trial. Fresh weight was measured by blotting the sample and pressing the sample using a
constant weight. 2) Photosynthetic light response at indicated by photosynthetic yield (PY). PY was determined using a Pulse Amplitude (PAM) fluorometer (DIVING-PAM, Waltz). In this experiment, differences in PY would indicate differences in photosynthetic electron transport rate (ETR) due to temperature. PY measurements were conducted at both the beginning and end of each temperature treatment. 3) In order to determine encroachment of diatoms or other algal species during the course of the experiment, the composition of algal samples was determine using a Fluoroprobe (bbe Moldaenke, Series 3) capable of distinguishing between major algal divisions. For each sample, 10 Fluoroprobe readings were recorded at 5 second intervals at both the beginning and end of each temperature treatment. The experiment ran for 7 days, and the same L. wollei samples were used throughout all temperature treatments.

2.5 Data Analysis

2.5a Model

Generalized additive models (GAMs) were used to determine any significant relationships and interactions between L. wollei average biomass and environmental factors (latitude and longitude, depth, percent light at lake bottom, secchi, percent sand per substrate sample, temperature, pH, turbidity, and dreissenid presence). A GAM analysis fits generalized additive models to data where the continuous independent variables can be fitted as arbitrary smoothed functions using non-parametric smoothers to determine a best fit model in relationship to a dependent variable (Crawley, 2007).
Preliminary analyses were performed in order to examine each of the environmental factors to determine if they should be included in the final candidate models. First, a correlation matrix was performed in order to determine if any of the factors were highly correlated with one another. Based on the results of the correlation matrix, a Pearson’s product-moment correlation was performed on the highly correlated factors to determine if the correlation was significant. Second, several preliminary candidate models were created in order to determine which factors required a smoothing function versus non-smoothing. Smoothing a parameter introduces a source of error, as smoothing is an estimation of fit (Crawley, 2007); therefore, smoothed parameters should only be used if necessary in order to obtain a best fit model with reduced error.

Model fit was evaluated through analysis of deviance using Akaike’s Information Criterion (AIC). The AIC statistic was used as measure of fit of a model; when comparing models, the smaller the AIC, the better the fit (Crawley, 2007).

Based on the preliminary candidate models, six final candidate models were created for analysis. In order to distinguish a starting point for the final candidate models, I compared the AIC values of each individual factor. For the six final candidate models, \textit{L. wollei} average biomass (dependent variable) was compared to a combination of six environmental factors (latitude and longitude, light at lake bottom, secchi, temperature, sand, and dreissenid presence) selected based on the above preliminary analyses in order to determine the model of best fit. All statistical analyses were performed using R 2.13.1(2011, The R Foundation for Statistical Computing).
2.5b Laboratory Experiments

One-way repeated measures analysis of variance (ANOVA) was used to analyze results, and all statistical analyses were performed using R 2.13.1 (2011, The R Foundation for Statistical Computing).
Chapter 3

Results

3.1 Field Survey

GIS maps showed distribution of all sites sampled across the Western Basin of Lake Erie in both 2009 and 2010 (Figure 3-1). Results indicate widespread distribution of *L. wollei* in the Western Basin. There was a considerable difference in overall biomass between years. In 2009, a maximum biomass of 580 dry weight g/m² was found at a bottom depth of 3.4 m along the Michigan shoreline (Figure 3-1a). In 2010, *L. wollei* biomass was much less with a maximum biomass of 6.0 g dry weight g/m² found at a depth of 3.1 m at a location along the Michigan shoreline about 10 km north of the 2009 maximum (Figure 3-1b). Although the magnitude and distribution of biomass varied between years, the overall spatial distribution of *L. wollei* was similar between years. In a survey that included 64 locations, *L. wollei* was present at 39 sites (60.9 %) in both years (Figure 3-2). *L. wollei* appeared at 7 (10.9 %) sites only in 2009 and at 6 sites (9.4 %) only in 2010. *L. wollei* was absent at 12 (18.8 %) sites in both 2009 and 2010.
Figure 3-1. *Lyngbya wollei* biomass (dry wt. g/m²) in western Lake Erie in (a) 2009 and (b) 2010. N = 154 in 2009 and N = 178 in 2010. Each circle represents average of 5 Ekman grabs at each site.
Figure 3-2. Comparison of *Lyngbya wollei* distribution in 2009 and 2010. Lyngbya Absence = no *L. wollei* at site in 2009 or 2010; Lyngbya Decrease = *L. wollei* present at site in 2009 but not present at site in 2010; Lyngbya Presence = *L. wollei* present at site in 2009 and 2010; and Lyngbya Expansion = *L. wollei* not present at site in 2009 but present at site in 2010. N = 64 sites.

In both 2009 and 2010, benthic mats of *L. wollei* were most commonly found growing at depths between 2.0 and 4.9 m (Figure 3-3). In 2009, *L. wollei* was most commonly found at the 3.0-3.9 depth contour (80% of sites with *L. wollei*; Figure 3-3a), whereas in 2010, *L. wollei* was most commonly found at the 2.0-2.9 m contour (60% of sites with *L. wollei*; Figure 3-3b). In both years, *L. wollei* mats were seldom encountered at depth less than 0.9 m or greater than 6.9 m.
Figure 3-3. (a) 2009 and (b) 2010 distribution of *Lyngbya wollei* at depth contours throughout the Western Basin of Lake Erie. In 2009 the greatest % of sites with *L. wollei* present was between depths of 2-4.9 m. Of 26 sites with depth 3-3.9 m, *L. wollei* was present at 77% of sites. Although *L. wollei* was present at deeper depths (6-6.9 m), biomass was small compared to shallower depths (2-4.9 m). In 2010 the greatest % of sites with *L. wollei* present was between depths of 2 – 4.9 m. ‘n’ indicated the total number of sites in each category.

In 2009, *L. wollei* distribution was most abundant at sites with secchi depth between 41 – 160 cm (Figure 3-4a). Of 20 sites sampled with secchi depth 121 – 160 cm, about 68 % of sites had *L. wollei* present. The majority of sites sampled had a secchi depth of 41 – 80 cm with about 50% of those sites with *L. wollei* present (Figure 3-4a).

In 2010, the majority of sites sampled had a secchi depth between 41 – 120 cm (Figure 3-4b), and of these 109 sites about 60 % of sites had *L. wollei* present.
Figure 3-4. (a) 2009 and (b) 2010 distribution of *Lyngbya wollei* at secchi depth (cm) throughout the Western Basin of Lake Erie. In 2009 the greatest % of sites with *L. wollei* present was between secchi depths of 41 – 160 cm. Of 20 sites sampled with secchi depth 121 – 160 cm, about 65% of sites had *L. wollei* present. In 2010 the greatest % of sites with *L. wollei* present was between depths of 41 – 120 cm. ‘n’ indicated the total number of sites in each category.

Using a large dataset of light attenuation and secchi depth measurements from western Lake Erie (2002-2010, n>200), I was able to convert secchi depth measurements to light attenuation coefficients (K_{PAR}), and then to calculate percent light at the lake bottom. In both 2009 and 2010, the majority of *L. wollei* was distributed in regions receiving less than 15 % light at the lake bottom (Figure 3-5) and could be found at depths receiving as little as 0.05% of surface light.
Figure 3-5. (a) 2009 and (b) 2010 distribution of *Lyngbya wollei* at % of surface light (PAR) at bottom throughout the Western Basin of Lake Erie. In both 2009 and 2010, the majority of *L. wollei* was distributed in regions receiving less than 15 % light at the lake bottom.

During the 2009 sampling season, substrate analysis was purely based qualitative and based on observational data. In 2009, a high percentage of my samples (65%) contained both *Dreissena sp.* shells present with *L. wollei* also present (Figure 3-6a). However, during 2010, substrate samples were collected from each site and particle size analysis was performed. From 2010 substrate samples, the majority of sites with *L. wollei* present had greater than 50% sand substrate composition (Figure 3-6b). Also in 2010, I observed dreissenid mussel presence at each sampling site. Of 174 sites sampled, only eight sites did not have any dreissenid mussels present. Of the eight sites that did
not contain dreissenids, there was zero percent *L. wolleii* presence (Figure 3-7a). Of the 166 sites with dreissenid mussels present, about 50% of sites had *L. wolleii* present. *L. wolleii* presence was highest at sites with living dreissenid mussels present (55%) (Figure 3-7b).

Figure 3-6. (a) 2009 qualitative observations of *Lyngbya wolleii* presence across several substrate types. Greatest % of sites with *Lyngbya wolleii* present on *Dreissena* shell substrate. Of 113 sites with *Dreissena* shell substrate, *L. wolleii* was present at 64% of sites. (b) 2010 particle analysis of *L. wolleii* presence across percent sand substrate composition. The majority of sites with *L. wolleii* present had greater than 50% sand substrate composition. ‘n’ indicates the total number of sites in each category.
Figure 3-7. (a) 2010 distribution of sites with *Lyngbya wollei* presence with dreissenid mussel presence. Of 174 sites samples, only eight sites did not have any dreissenid mussels present, and there was zero percent *L. wollei* present at sites without dreissenid mussels. Of the sites the sites with dreissenid mussels present, about 50% of sites had *L. wollei* present. (b) 2010 distribution of sites with *L. wollei* presence with dreissenid mussels living or dead. *L. wollei* presence was highest at sites with living dreissenid mussels present (57%). ‘n’ indicates the total number of sites in each category.

In both the 2009 and 2010 summer seasons (June – August), *L. wollei* biomass increased and was greatest later in the season (end of July – August) (Figure 3-8). In 2009 the greatest average biomass, calculated by the average of all sites sampled within each month, was 23 dry weight g/m² observed in late July (Figure 3-8a). In 2010 the greatest average biomass was 0.3 dry weight g/m² observed in August (Figure 3-8b).
Figure 3-8. Average biomass (dry weight g/m²) of *Lyngbya wollei* over summer growing season (June – August) for (a) 2009 and (b) 2010. N = 154 for 2009 and N = 175 for 2010. Values are means ± standard error.

3.2 Model

Results of the correlation matrix showed the factors depth and secchi (0.7266272) were positively correlated. The following factors were negatively correlated: Secchi and turbidity (-0.5626262), depth and light at lake bottom (-0.3627647), and depth and turbidity (-0.3807702). Results of the Pearson’s product-moment correlation showed the correlation of the following factors were highly significant: Depth and secchi (p-value = 2.2e-16), secchi and turbidity (p-value = 2.203e-12), depth and light at lake bottom (p-
value = 1.913e-05), and depth and turbidity (p-value = 6.67e-06). As depth was significantly correlated with secchi, light at lake bottom, and turbidity, I chose to remove depth from the model. Turbidity was also removed from the model as it was significantly correlated with both depth and secchi.

pH was removed from the model as it was relatively uniform (8-8.9) throughout all sites sampled in the Western Basin of Lake Erie. ZM1 (dreissenid presence or absence) and ZM2 (dreissenids dead, alive, or dead and alive) were combined into one categorical factor, ZM (dreissenids absent, present-dead, present-alive and dead, present-alive).

I then performed GAM analysis on each of the remaining continuous factors, secchi, light at lake bottom, sand, and temperature, and compared smoothed versus non-smoothed AIC values (Table 3.1). The AIC values of the smoothed GAM were lower for secchi, light at lake bottom, and sand factors; therefore, these factors were smoothed in the model analysis. The AIC value for temperature was identical for smoothed versus non-smoothed (309.3231); therefore, the temperature factor was non-smoothed in the model analysis.

Table 3.1. Comparison of smoothed versus non-smoothed Akaike’s Information Criterion (AIC) values for continuous dependent variables for generalized additive model. Low AIC value indicates best fit.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Smoothed AIC</th>
<th>Non-smoothed AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secchi (cm)</td>
<td>307.8855</td>
<td>311.4467</td>
</tr>
<tr>
<td>Light (% light at lake bottom)</td>
<td>290.88</td>
<td>301.3427</td>
</tr>
<tr>
<td>Sand (% sand per substrate sample)</td>
<td>308.2498</td>
<td>311.8933</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>309.3231</td>
<td>309.3231</td>
</tr>
</tbody>
</table>
Based on the above preliminary analyses, the factors used in the final candidate models were latitude and longitude, secchi, light at lake bottom, sand, temperature, and ZM1 (Table 3.2). In order to distinguish a starting point for the final candidate models, I compared the AIC values of each individual factor (Table 3.3). Light at lake bottom had the lowest AIC value (290.88) of the six factors, ZM had the highest AIC value (310.4181).

Table 3.2. List of original factors (10) and final factors (6) included in candidate generalized additive models.

<table>
<thead>
<tr>
<th>Original Model Factors (10)</th>
<th>Final Model Factors (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latitude and Longitude</td>
<td>Latitude and Longitude</td>
</tr>
<tr>
<td>Depth (m)</td>
<td></td>
</tr>
<tr>
<td>Secchi (cm)</td>
<td>Secchi</td>
</tr>
<tr>
<td>Light (% light at lake bottom)</td>
<td>Light</td>
</tr>
<tr>
<td>Sand (% sand per substrate sample)</td>
<td>Sand</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>Temperature</td>
</tr>
<tr>
<td>pH</td>
<td></td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td></td>
</tr>
<tr>
<td>ZM1 (Presence or absence)</td>
<td>ZM (combine ZM1 and ZM2)</td>
</tr>
<tr>
<td>ZM2 (Dead, Alive, or Dead &amp; Alive)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.3. Akaike’s Information Criterion (AIC) values for each dependent variable of final candidate generalized additive models. Low AIC value indicates best fit.

<table>
<thead>
<tr>
<th>Factors</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light (% light at lake bottom)</td>
<td>290.88</td>
</tr>
<tr>
<td>Latitude and longitude</td>
<td>300.5829</td>
</tr>
<tr>
<td>Secchi (cm)</td>
<td>307.8855</td>
</tr>
<tr>
<td>Sand (% sand per substrate sample)</td>
<td>308.2498</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>309.32311</td>
</tr>
<tr>
<td>ZM</td>
<td>311.9012</td>
</tr>
</tbody>
</table>
The final six candidate models are listed in Table 3.4. The candidate model, G5, was the best fit with the lowest AIC value of 285.8889 and 29.4 % deviance explained. The G5 model contained the following factors: Light at lake bottom (smoothed), sand (smoothed), latitude and longitude, temperature, and secchi (smoothed).

Scatterplots were computed of the three smoothed predictor variable values (light at lake bottom, sand, and secchi) plotted against the partial residuals. The effect of light at lake bottom of *L. wollei* average biomass showed a bimodal humped curve between 0-10 percent light at lake bottom (Figure 3-9a). A straight line, corresponding to 1 degree of freedom, was estimated for the effect of % sand on *L. wollei* average biomass indicating *L. wollei* biomass decreased with increasing percent sand (Figure 3-9b). *L. wollei* average biomass decreased with secchi values greater than 85 cm (Figure 3-9c).

The candidate model G4 had the second lowest AIC value of 298.0080 and 24.5 % deviance explained. The G4 model contained the following factors: Light at lake bottom (smoothed), sand (smoothed), latitude and longitude, and temperature. The full candidate model, G6, had an AIC value of 287.3476 and 29.5 % deviance explained. The G6 model contained all of the same factors as model G5 (light at lake bottom, sand, latitude and longitude, temperature, and secchi) as well as ZM.
Table 3.4. Akaike’s Information Criterion (AIC) value and deviance explained (%) for final six candidate generalized additive models. Low AIC value indicates best fit.

<table>
<thead>
<tr>
<th>Candidate Models</th>
<th>AIC</th>
<th>Deviance explained (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 sLight</td>
<td>290.88</td>
<td>18</td>
</tr>
<tr>
<td>G2 sLight + sSand</td>
<td>289.5781</td>
<td>21.9</td>
</tr>
<tr>
<td>G3 sLight + sSand + LAT + LON</td>
<td>287.406</td>
<td>24.3</td>
</tr>
<tr>
<td>G4 sLight + sSand + LAT + LON + Temp</td>
<td>289.008</td>
<td>24.5</td>
</tr>
<tr>
<td>G5 sLight + sSand + LAT + LON + Temp + sSecchi</td>
<td><strong>285.8889</strong></td>
<td><strong>29.4</strong></td>
</tr>
<tr>
<td>G6 sLight + sSand + LAT + LON + Temp + sSecchi + ZM</td>
<td>287.3476</td>
<td>29.5</td>
</tr>
</tbody>
</table>
Figure 3-9. Scatterplots of partial residuals of three smoothed predictor variables, (a) light (% at lake bottom), (b) sand (% of substrate), and (c) secchi (cm) in best fit candidate generalized model (G5). 95 % confidence intervals.

3.3 Laboratory Experiments

A repeated measures ANOVA showed no significant effect of temperature on change in wet weight (g)/week (p = 0.1094, alpha = 0.05). A pairwise t-test showed no significant differences between temperature treatments. The highest positive change in wet weight (g) per week was observed between 20 °C – 10 °C of the temperature experiment with the highest being at 20 °C with a growth rate change of 0.35 g/wk (Figure 3-10). Although the differences were not statistically significant, growth rate
appeared to decline with decreasing temperature. The greatest negative change in wet weight (g) per week occurred at the lowest temperature treatments of the experiment, 3 – 1 °C (Figure 3-10). The greatest negative change in wet weight (g) per week occurred at 1 °C with a growth rate change of -0.27 g/wk(Figure 3-11).

Figure 3-10. Change wet weight (g/week) of *Lyngbya wollei* versus temperature (°C). Greatest positive growth rate occurred at highest temperature treatments, 20 – 10 °C. Greatest negative growth rate occurred at lowest temperature treatments, 3 – 1 °C. Values are means ± standard error.
Figure 3-11. Change wet weight (g/week) of Lyngbya wollei versus temperature (°C). Growth rate decreased with decreasing temperature. Greatest positive growth rate occurred at highest temperature treatment, 20 °C. Greatest negative growth rate occurred at lowest temperature treatments, 1 °C. Values are means ± standard error.

Photosynthetic yield was highest at 20 °C (0.65). Photosynthetic yield appeared to decline with decreasing temperature until 5 °C reaching 0.15. Photosynthetic yield increased in temperature treatments below 4 °C (Figure 3-12); however, factors other than temperature may have influenced these results. A repeated measures ANOVA showed a significant effect of temperature on photosynthetic yield (p< 0.0001, alpha = 0.05). A pairwise t-test showed significant differences between Temp1 (20 °C) and all other temperature treatments, and a significant difference between Temp 3 (7 °C and 5 °C) and Temp 5 (2 °C and 1°C). This shows the increase in photosynthetic yield at the lower temperature treatments (4 °C – 1 °C) to be significant.
Figure 3-12. Photosynthetic yield of *Lyngbya wollei* versus temperature (°C). Photosynthetic yield was greatest at highest temperature treatment, 20 °C (0.65). Photosynthetic yield decreased with decreasing temperature reaching lowest yield at 5 °C (0.15) and then increased at lowest temperature treatments, 4 – 1 °C. Values are means ± standard error.

Electron transport rate (ETR) followed the same pattern as photosynthetic yield, declining until 5 °C and then increasing at lower temperature treatments (4 °C – 1 °C) (Figure 3-13). A repeated measures ANOVA showed a significant effect of temperature on ETR (p<0.0001, alpha = 0.05). A pairwise t-test comparison showed Temp1 (20 °C) had significant difference when compared to each other temperature treatment, and Temp3 (7 °C and 5°C) significantly differed from Temp5 (2 °C and 1°C).
Figure 3-13. Electron transport rate (ETR) of *Lyngbya wollei* versus temperature (°C). ETR decreased with decreasing temperature. Values are means ± standard error.

Percent blue-green measurements showed that at the beginning of the experiment cyanobacteria was the dominant species present in samples (98%). As the experiment continued, percent blue-green decreased (Figure 3-14). A repeated measures ANOVA showed temperature had a significant effect on percent blue-green (p = 0.0002639, alpha = 0.05). A pairwise t-test showed significant differences between Temp1 (20 °C) and Temp4 (4 °C and 3 °C) and Temp1 (20 °C) and Temp5 (2 °C and 1 °C) which indicates a significant decrease in percent blue-green and an increase in percent diatoms toward the end of the experiment.
Figure 3-14. Percent bluegreen versus week of temperature experiment. Average percent bluegreen decreased toward the conclusion of the temperature experiment (Week 5). Values are means ± standard error.
Chapter 4

Discussion

4.1 Field Survey

*L. wollei* appeared in the Western Basin of Lake Erie in the fall of 2006 when it was noticed washed up on the shoreline (Bridgeman and Penamon, 2010). The western basin may have environmental factors that promote the distribution and abundance of *L. wollei*, such as shallow depths, turbid water and low percent surface light reaching the lake bottom, higher summer water temperature, substrate and dreissenid mussel presence, and high nutrients.

In *L. wollei’s* natural range in the southern United States, it is found in shallow, protected areas where it grows in mats along reservoir and pond bottoms (Speziale and Dyck, 1992; Cowell and Botts, 1994; Stevenson et al., 2004). The shallow depths of the Western Basin of Lake Erie (less than 1 m) experience wave action which may prevent *L. wollei* from becoming established in these areas as it is pushed to greater depths less affected by wave action (Bridgeman and Penamon, 2010). *L. wollei* is only loosely associated with substrate, as it does not attach to hard substrate like other benthic
species, i.e., *Cladophora* (Higgins et al., 2005; Bridgeman and Penamon, 2010). From a previous, less intensive survey in 2007 and 2008 (Bridgeman and Penamon, 2010), *L. wollei* was found to occur at depths between 1.5 and 3.5 m which are similar to my results of 2 – 4.9 m.

*L. wollei* requires much less light for photosynthesis than other benthic alga such as *Cladophora*, and therefore, can be found in turbid areas and areas with low light availability at the lake bottom (Speziale et al., 1988, 1991; Beer et al., 1986, 1990; Doyle and Smart, 1998; Pinowska et al., 2007; Bridgeman and Penamon, 2010). I found the majority of *L. wollei* to occur in regions with a secchi depth between 41 – 160 cm and in areas with less than 15 % surface light reaching the lake bottom. Field observations by Beer et al. (1986) found *L. wollei* established in areas with light saturation levels between 20 and 150 μEm$^{-2}$s$^{-1}$. As the majority of sites with *L. wollei* present had less than 15 % surface light (2000 μEm$^{-2}$s$^{-1}$) the literature supports my field results. The majority of sites with *L. wollei* present had light saturation levels between 0.2 and 300 μEm$^{-2}$s$^{-1}$.

Bridgeman and Penamon (2010) reported *L. wollei* occurring at sites with 4.0 – 0.05 % of surface irradiance in the Western Basin of Lake Erie. Pinowska et al. (2007) reported optimum light levels for southeastern strains of *L. wollei* at 50 μEm$^{-2}$s$^{-1}$.

As Figure 3-5 and previous literature show, *L. wollei* was found in areas of low light (less than 15 % surface light) and relatively shallow depths (Figure 3-3). However, *L. wollei* was not found at greater depths, 7 – 9.9 m, where light is also limited. At these greater depths, other environmental factors, such as nutrient availability or substrate, may be limiting *L. wollei* distribution and abundance. Nutrient concentrations are greater closest to the inflow of the Maumee River (Schwab et al., 2009 and Bridgeman et al.,
2011), and decrease with distance into open water (Moorhead, 2003) limiting the availability to *L. woliei*. Also, at further distances offshore, substrate changes from sandy substrate with dreissenids to softer silt (Verber, 1957; Hartley, 1961; Bolsenga and Herdendorf, 1993; Coakley et al., 2002; and Edwards et al., 2009) which may be unsuitable for *L. woliei* establishment.

*L. woliei* was observed at sites with dreissenid mussels present. Dreissenid mussel correspondence with *L. woliei* could be due to the beneficial effects of dreissenid mussels, increased water clarity and recycling of nutrients to contribute to cyanobacterium growth or due to *L. woliei* becoming entangled within the shells enabling movement (Hecky et al., 2004; Bridgman and Penamon, 2010). Bridgeman and Penamon (2010) survey findings support results from my 2009 and 2010 surveys. They found *L. woliei* present in areas with sand and crushed dreissenid mussel shell-type substrate. Based on our sediment analysis, I found *L. woliei* present in areas with greater than 50% sand substrate composition and in areas with living dreissenid mussels present.

Beer et al. (1986) observed *Lyngbya birgei* biomass to be greatest in the summer months in several lakes and ponds in Florida. The difference in average biomass (dry weight g/m²) between the 2009 and 2010 seasons in my study was unexpected. As shown by Figure 3-1, the distribution pattern was similar in 2009 and 2010. Analysis of the 64 sites sampled in both 2009 and 2010 suggest that *L. woliei* is neither expanding nor decreasing in distribution in the Western Basin of Lake Erie from 2009 to 2010; therefore, my hypothesis that spatial distribution patterns would be consistent between years was supported. However, there is a marked difference in average biomass between 2009 and 2010 (Figure 3-8). The patchiness of *L. woliei* distribution may account for this
difference. As noted, L. wollei only loosely associates with substrate; and therefore, 
distribution can be easily influenced by wave action, wind and water currents (Higgins et 
al., 2005; Bridgeman and Penamon, 2010).

It was also observed that L. wollei washed up on the shoreline in much smaller 
amounts in 2010 than in 2009 (personal observation). As there are several factors that 
can affect the distribution of L. wollei in the western basin, I am not sufficiently confident 
to suggest that L. wollei abundance decreased throughout the entire Western Basin of 
Lake Erie in 2010; however, L. wollei biomass decreased from 2009 to 2010 at the sites 
sampled.

As all of the discussed environmental factors can influence the establishment and 
growth of L. wollei, I created several candidate general additive models in order to 
determine which factor or factors are the most influential for L. wollei distribution and 
abundance in the Western Basin of Lake Erie.

4.2 Model

Model results showed the best fit model for explaining spatial variation of L. 
wollei average biomass was candidate model G5 containing the five of the six dependent 
variables, light at lake bottom, sand, latitude and longitude, temperature, and secchi, with 
the lowest AIC value of 285.8889 which supported my hypothesis that a model including 
several environmental factors would be able to explain the spatial variation of L. wollei in 
western Lake Erie; however, these factors only account for 29.4% of the variation. As 
AIC is a measure of the fit of a model, when comparing models, the smaller the AIC, the
better the fit. AIC is useful because it penalizes any superfluous parameters in the model (Crawley, 2007).

Based on model results, light at lake bottom is an important individual factor for explaining spatial variation of *L. wollei* in western Lake Erie as light availability is important for photosynthesis. However, light at lake bottom only explained 18% of the variation (Table 3.4) indicating there are other factors that explain the spatial variation of *L. wollei*. Several studies show light is an important indicator of *L. wollei* distribution and abundance, as it is a benthic cyanobacterium and can establish in areas with lower light where other benthic algal species may not (Doyle and Smart, 1998). Although *L. wollei* can establish in areas with low light, other factors, such as substrate type, temperature, and nutrient availability, may affect *L. wollei* and prevent it from establishing in areas with satisfactory light requirements.

Light at lake bottom and substrate type together explain 22% of the spatial variability of *L. wollei* biomass in western Lake Erie (Table 3.4). Qualitative observations by Bridgeman and Penamon (2010) found *L. wollei* growth to occur in areas where the substrate consisted of sand. These observations correspond with findings of *L. wollei* distribution as data from the particle-size analysis showed the majority of *L. wollei* distribution to be found in areas with higher percent sand (%); however, the trend results from the model show *L. wollei* abundance decreases with increasing percent sand. *L. wollei* distribution in terms of substrate is widespread over a range of percent sand; however, *L. wollei* density is greatest in areas with lower percent sand. Therefore,
percent sand may be an important driving factor of *L. wollel* abundance, but not necessarily distribution.

According to the model, latitude and longitude may play a significant role in the abundance and distribution of *L. wollel* in the Western Basin of Lake Erie. From the 2009 and 2010 field surveys, I know that *L. wollel* is not established in areas further east or west along the same latitude, nor is *L. wollel* established north along the same longitude (personal observation); therefore, factors other than location are needed to explain *L. wollel* biomass in western Lake Erie. The Eastern Basin of Lake Erie is dominated by *Cladophora* which requires a hard substrate which to attach (Higgins et al., 2005); therefore, the substrate in the eastern basin may not be suitable for *L. wollel* abundance. Further north in western Lake Erie, the water is influenced by the Detroit River, not the Maumee River, which influences turbidity as well as nutrient concentrations. Less turbid conditions allow for greater light penetration to the benthos which may create unsuitable *L. wollel* habitat. It is likely other environmental factors, such as substrate and light availability, at specific latitude and longitude, that explain *L. wollel* abundance and distribution in western Lake Erie.

According to AIC values of the individual environmental factors used within the candidate models, water temperature alone is a poor fit for predicting *L. wollel* biomass in the western basin (Table 3.3). It was unexpected that water temperature did not play a more direct role in *L. wollel* abundance as warmer water temperatures mimic those of the southeastern United States, *L. wollel*’s native range (Yin et al., 1997). In fact, very little *L. wollel* was found in close proximity to power plant thermal plumes (areas of high
water temperature) (Figure 3-1). However, the results of the GAMs show water temperature should be included when predicting the abundance of *L. wollei* in the western basin. These results suggest that there are other factors which also influence the establishment of *L. wollei*, not solely water temperature.

Water temperatures in the western basin measured during the summer ranged from 24 – 33 °C (personal observation) with the greatest percent of *L. wollei* present found between 26 – 27 °C (Figure 4-1). A study by Yin et al. (1997) also found the optimum temperature for *L. wollei* biomass to occur at 26 °C. While Figure 4-1 shows 100 % of sites had *L. wollei* present between 22 – 23 °C, only three sites were found within that temperature range. Of the greatest number of sites (n=83), 50 % of sites had *L. wollei* present between 26 – 27 °C, and at the other 50 % of sites between 26 – 27 °C where *L. wollei* was not present, other environmental factors, such as light availability or substrate, may have ruled out *L. wollei* establishment.

*L. wollei* average biomass decreased with secchi values greater than 85 cm (Figure 3-9c). As secchi plays a role in the amount of light at lake bottom and *L. wollei* has been shown to be found at sites with low light (Beer et al., 1986, 1990; Dyck, 1994; Doyle and Smart, 1998), then *L. wollei* should also be found in areas with low secchi (low water clarity), which was observed.
Figure 4-1. 2010 distribution of *Lyngbya wollei* at temperature ranges (°C) throughout the Western Basin of Lake Erie. In 2010 the greatest % of sites with *L. wollei* present was between 26 – 27 °C. Of 83 sites sampled between 26 – 27 °C, 50% of sites had *L. wollei* present. ‘n’ indicated the total number of sites in each category.

Dreissenid presence and absence did not have a significant effect on the abundance of *L. wollei* at the sites sampled in the Western Basin of Lake Erie most likely due to wide distribution of dreissenids in the basin (personal observation). Dreissenids, either living, dead, or both, were present at the majority (95%) of sites sampled. Observations by Bridgeman and Penamon (2010) found *L. wollei* growth occurred in areas where dreissenids, fragments of dreissenid shells, or a combination of both were present.

The results of the model analysis were not very informative for explaining the spatial variation of *L. wollei* density in the Western Basin of Lake Erie. The five environmental factors included in the best fit model only explained 30 % of the spatial
variation of *L. wolfei*, leaving 70% unexplained. While the environmental factors included in the candidate models were important for establishing *L. wolfei*, I believe they only explained 30% of the spatial variation of *L. wolfei* in western Lake Erie due to additional environmental factors which are required for growth. Environmental factors not included in the candidate models, such as nutrients, water currents, depth, and conductivity, may better explain the spatial variation of *L. wolfei* in western Lake Erie.

Previous studies have shown the importance of nutrient availability, especially nitrogen, phosphorus, and calcium, for *L. wolfei* growth (Cowell and Botts, 1994). As nutrients are an important limiting factor of cyanobacteria growth, they may best explain *L. wolfei* distribution and abundance in western Lake Erie. In areas with ideal habitat characteristics, i.e., adequate light availability, warm water temperature, and high percent sand substrate, *L. wolfei* will not grow if nutrient availability is limited. However, including nutrient concentration in this study would have required a much more comprehensive sampling regime, collecting and analyzing water samples at each site sampled.

Other studies of *L. wolfei* in its native range have shown the importance of environmental factors on *L. wolfei* distribution and abundance. Cowell and Botts (1994) performed multiple regression analysis to compute predictive multiple regression models for a range of environmental factors influencing *L. wolfei* density in Florida springs. According to their analysis, the best model contained alkalinity, conductivity, NH₃, and total phosphate explaining 57.8% of the variability in *L. wolfei* density. Alkalinity,
conductivity, and NH$_3$ were negatively correlated with $L. wollei$ biomass, while total phosphorus was positively correlated.

Several environmental factors have been modeled to partially explain spatial variation of $L. wollei$ in both its native range (Cowell and Botts, 1994) and in western Lake Erie. The complex model created in this study supports that a combination of factors, not one single environmental factor, explains the spatial variation of $L. wollei$ in western Lake Erie; therefore, it will be difficult to predict the spread and distribution of $L. wollei$ in Lake Erie and the other Great Lakes. Further study of environmental factors which are important for explaining $L. wollei$ density, will lead to a better understanding of the distribution and ecology of $L. wollei$ in western Lake Erie.

4.3 Laboratory Experiments

As $L. wollei$ is native to the southeastern United States (Speziale and Dyck, 1992; Pinowska et al., 2008; Bridgeman and Penamon, 2010), I expected the highest growth rate, photosynthetic yield, and electron transport rate to occur at my highest temperature treatment (20 °C) which was supported. A decreased growth rate occurred at temperature treatments between 7 – 1 °C indicating that $L. wollei$ does not grow at these low temperatures. As it has been reported that thermal effluents from power plants are about 8 °C to 10 °C than the ambient temperatures of surrounding waters (Srivastava et al., 1993; Tison et al., 1981), it is possible that the presence of shoreline power plants may raise water temperature to support some $L. wollei$ growth during winter months. While $L. wollei$ still has a decreased growth rate at lower temperatures, then thermal effluents from
the shoreline power plants may provide a survivable temperature threshold for \textit{L. wollei} to survive the winter months leading to the explosive bloom during the summer months.

Benthic mats in South Carolina lakes persisted throughout the entire year, but began growing when water temperatures reached 18 °C (Speziale et al., 1991).

While not studied in this laboratory experiment, the power plant thermal plumes cause decreased ice cover during the winter months which provides the possibility for increased light penetration to the bottom of the lake which can promote cyanobacteria growth (Hickman 1974). Future experiments and field observations could be utilized to examine the effect of light as well as the combination of light and temperature on \textit{L. wollei} growth.

As growth rate decreased with decreasing temperature, I also expected the photosynthetic efficiency and ETR of \textit{L. wollei} to decrease with temperature. Photosynthetic yield was highest at 20 °C and then decreased with decreasing temperature until reaching 5 °C. At the lowest temperature treatments (4 – 1 °C), photosynthetic yield increased. Upon examining results from Fluoroprobe measurements of percent bluegreen, I hypothesize that a decrease in percent bluegreen and an increase in percent diatoms at the lower temperature treatments led to this increase in photosynthetic yield and ETR as Lake Erie diatoms may be more efficient at photosynthesizing than cyanobacteria at colder temperatures (Figure 4-2) (Andersson et al., 1994; Litchman et al., 2003).
A study by Beer et al. (1986) show a linear trend between temperature increasing between 10 and 40 °C and net photosynthesis for *Lyngbya birgei*. They found a positive relationship between temperature and *Lyngbya* photosynthesis where high temperatures (approximately 30 °C) will improve *Lyngbya* photosynthesis.

Other sources of error could also explain this effect such as equipment malfunction of the Diving-PAM, human error when recording measurements, or other experiment factors (i.e. increased CO₂ in the chamber room) which could have affected the results of the experiment. It is also possible that another factor, such as nutrient availability, was limiting *L. wollei* growth and photosynthetic efficiency during the experiment. A study by Cowell and Botts (1994) shows *L. wollei* growth in treatments with frequent (daily or semiweekly) nutrient changes was significantly better than in
treatments with only weekly nutrient changes. I exchanged both water and nutrients biweekly during the temperature experiment, and for future experiments, I will consider changing the nutrient media more frequently to ensure adequate nutrient availability to *L. wollei*.
References


Appendix A

Preliminary Laboratory Experiments

The goal of the preliminary experiments was to measure *L. wollei* growth, photosynthesis, net primary production, oxygen evolution, etc. in order to determine the best method for future experiments.

There are several methods to determine *L. wollei* survival and proliferation. Preliminary experiments were performed in order to determine the most efficient and achievable method to be used in further experimentation (see Objective 3).

Filament length was used as a proxy to measure *L. wollei* growth under light and dark conditions. Methodology was adapted from Pinowska et al. (2008). Individual filaments were carefully separated from *L. wollei* colonies and measured to 10 mm. Filaments were measured by hand with a metric ruler as well as with a microscope with attached camera software (SPOT Advanced). Individual filaments were placed in clear, 2 cm-long capsules and incubated for 5 days. The capsules were filled with lake water. For treatment one, five capsules were kept at ambient temperature and light conditions, and for treatment two, five capsules were kept at ambient temperature in the dark.
Filaments were extremely fragile and difficult to straighten in order to obtain a precise and accurate measurement. Measurements were made by hand-tracing filaments on a computer screen which was difficult and allowed potential for human error. About half of the filaments broke when trying to retrieve them from capsules at the end of the experiment, and of the whole filaments, no growth was observed in either light or dark conditions. For future experiments, several replicates would be needed in order to take into account the inevitability of broken filaments and unusable data. Filament length was used as a measurement of growth because fresh (or wet) weight is a difficult measurement to standardize and error may be too high for accurate results.

The second set of preliminary experiments was performed in a controlled growth chamber (Percival Model E-36HO) which could maintain both temperature and light intensity. Dissolved oxygen (DO) and photosynthetic efficiency were measured. Three custom-made, clear, sealable containers were filled with 400 mL filtered lake water, which was a large enough volume to cover the DO probe. Between 0.7-0.8 g fresh weight of *L. wollei* was added to each chamber. This weight was obtained from average biomass of *L. wollei* present in Lake Erie (Bridgeman and Penamon, 2010). Samples were weighed previous to the start of the experiment in order to obtain an initial fresh weight. Window screen was used to cover each of the containers in order to achieve a light intensity of about 100 μEm⁻²s⁻¹ which has been reported in literature as optimal laboratory light intensity for *L. wollei* growth and photosynthesis (Doyle and Smith, 1998). Five temperatures (5 °C, 10 °C, 15 °C, 20 °C, and 25 °C) representing temperatures experienced throughout the year in the Western Basin of Lake Erie were used as treatments throughout the experiment. Oxygen evolution methodology was
adapted from Necchi (2004) and Necchi and Zucchi (2001). Two containers were kept in light conditions to measure photosynthesis, and one container was covered with a black, felt bag which kept out all light in order to measure respiration and obtain net photosynthesis. Dark samples were taken for 5 °C, 10 °C, and 15 °C only. The three samples were incubated overnight (about 15 hours) at specified temperature prior to any measurements. After 15 hours, initial and final DO (mg/L) was measured over a 15 minute period. DO is a product of photosynthesis. The sample procedure was followed for each of the remaining four temperature conditions.

DO (mg/L) increased over the 15 minute measurement period at all temperatures except 10 °C (Figure A-1). The greatest difference in initial and final DO values was 1.39 experienced at 5 °C. DO decreased as temperature increased, following the trend that warm water holds less dissolved oxygen than cold water.

Potential error with oxygen evolution experiments are the presence of diatoms within the *L. wollei*. While *L. wollei* is washed with filtered lake water and cleaned prior to experiments, it is possible and likely that diatoms will still be present within the *L. wollei*. I was unable to differentiate diatom oxygen evolution from *L. wollei* oxygen evolution. Few diatoms present would not significantly affect *L. wollei* oxygen evolution.

In addition to measuring DO, chlorophyll fluorescence was measured for each sample using an underwater fluorometer DIVING-PAM. Chlorophyll fluorescence can be used as a measurement for photosynthesis, and the DIVING-PAM has the capability to measure photosynthetic yield and can calculate electron transport rate (ETR). Single measurements were taken at each temperature condition for each sample after the 15 hour
incubation period prior to DO measurements. As temperature increased, photosynthetic efficiency and ETR increased (Figure A-2). These results do not agree with the oxygen evolution data. Oxygen evolution data suggests that photosynthesis is greatest at 5 °C; however, there seemed to be no pattern (i.e. increase in DO) as temperature increased.

Figure A-1. Dissolved oxygen (mg/L) versus temperature (°C). DO increased over 15 minute measurement period at all temperatures except 10 °C. The greatest difference in initial and final DO values was experienced at 5 °C.
Figure A-2. Electron transport rate (ETR)/photosynthetic efficiency of *Lyngbya wollei* versus temperature (°C). ETR/photosynthetic efficiency of *L. wollei* increased as temperature increased.

FluoroProbe trials were performed in order to measure chlorophyll (i.e. growth) in addition to photosynthetic efficiency and ETR given by the DIVING-PAM. Trials were performed in order to determine the most efficient way to measure *L. wollei* samples. *L. wollei* samples will be placed in cuvets and placed in the FluoroProbe and an average of multiple measurements will be recorded for chlorophyll. Then, the amount of *L. wollei* will be increased and the same procedure will be followed in order to determine if chlorophyll increased. A second method will utilize the benthic attachment probe of the FluoroProbe to determine the best arrangement of *L. wollei* for measuring chlorophyll.