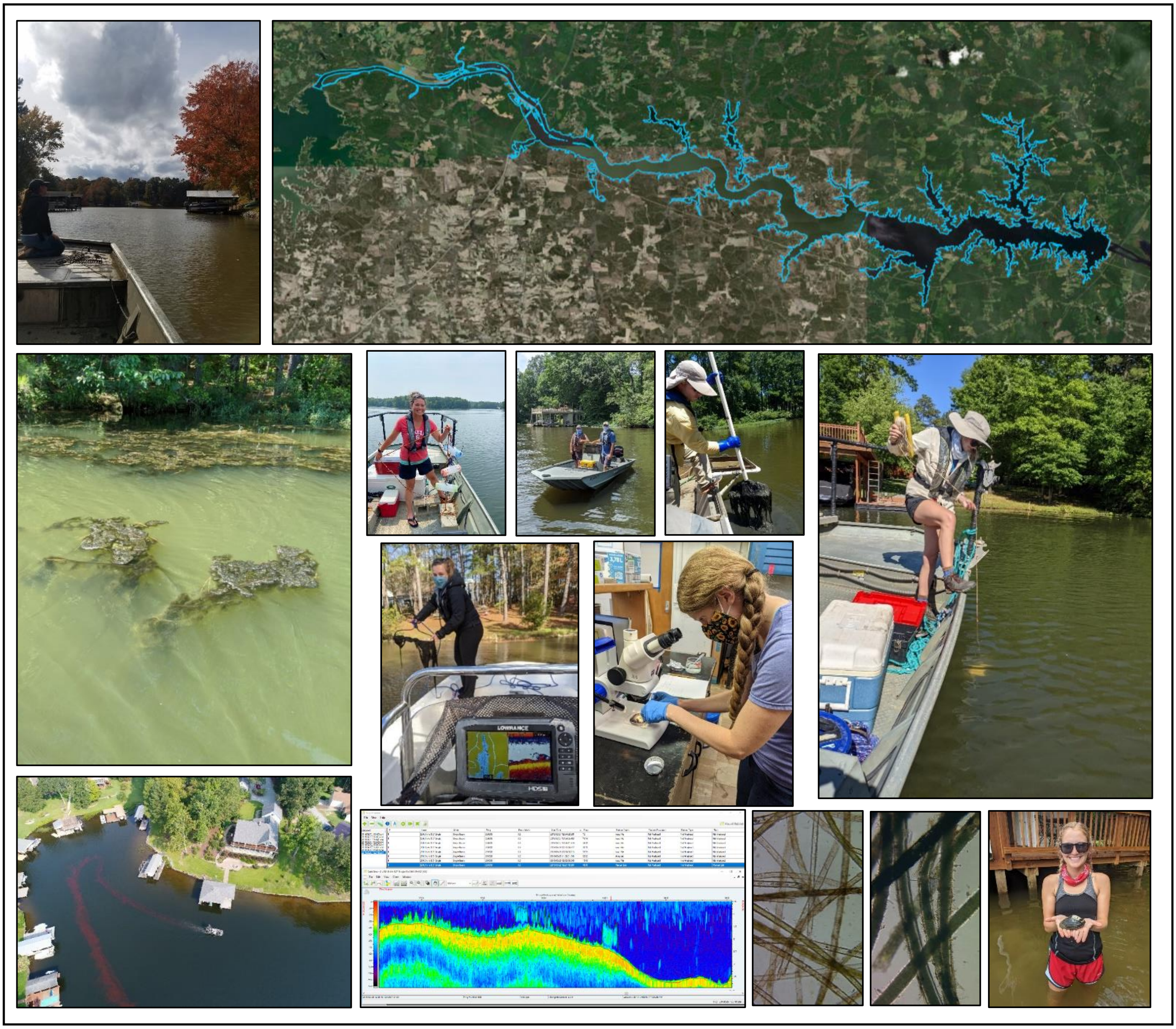
Integrated Research to Address Lake Gaston's Water Quality, Biodiversity, and Noxious Weed Management



NC STATE UNIVERSITY

Aquatic Plant Management Program

Integrated Research to Address Lake Gaston's Water Quality, Biodiversity, and Noxious Weed Management

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Executive Summary

The following report details research conducted by North Carolina State University (NCSU) Aquatic Plant Management Program's directed at management of the free-suspension filamentous cyanobacterium, *Lyngbya wollei* (hereinafter, lyngbya), within Lake Gaston. This research was funded by the Lake Gaston Weed Control Council. The goals of this research were to 1) develop a comprehensive document of previous research regarding phenological descriptions and management efforts directed towards lyngbya, 2) develop a predictive model that will identify how varying physical features, water quality parameters, and biotic dynamics relates to the distribution and density of lyngbya throughout Lake Gaston, 3) identify a chemical control method that is effective in reducing lyngbya growth and determine appropriate methods for monitoring treatment success, 4) apply all of the best management practices developed during this project to implement an expanded, operational level treatment program targeting lyngbya in Lake Gaston, 5) identify and reduce any potential harmful impacts to native aquatic fauna that might be presented by lyngbya targeting treatment protocols, and 6) identify any potential human health risk posed by lyngbya infestations within Lake Gaston.

Lyngbya has become increasingly problematic among southeastern waterways. It was first identified in Lake Gaston in the mid 1990's, but over the past decade has displayed increased expansion across the system. Nuisance populations of lyngbya have the ability to produce robust mat-like formations that emit foul odors and negatively impact aquatic ecosystems, recreational activities, and the aesthetic value of shoreline homes. These negative impacts associated with lyngbya have increased the demand for a better understanding of what environmental conditions drive increased growth and the development of effective management strategies that target lyngbya within multi-use reservoirs.

Although lyngbya is distributed throughout Lake Gaston, there is a high level of geographical variability in regards to the extent of distribution and the level of benthic mat proliferation (growth). Increased lyngbya proliferation has been attributed to a host of environmental drivers and physiological factors in other systems, however growth responses to these various drivers vary from system to system. For Lake Gaston, we found that factors that drive the distribution of lyngbya throughout the system differ from those that drive increased proliferations of benthic mat formations. We found that the direction in which a shoreline is facing, increased turbidity, and the presence of certain native floating leaf aquatic plant fauna all resulted in a positive association with increased occurrence of lyngbya within an area. We also found that the presence of a native macroalgae species that can form dense beds along the benthos resulted in a decreased level of lyngbya occurrence within an area. In regards to increased growth experienced by lyngbya, we found that flow regimes and nutrient dynamics both had positive relationships with the level of proliferation experienced within benthic mat formations. Understanding the drivers of lyngbya growth within individual systems is critical for managers in allowing them to develop appropriate response and management plans that will minimize the overall impact of lyngbya infestations.

Efficacy of field based algaecide applications have demonstrated some success in smaller systems (< 12,500 acres), however successful treatment program have not been documented in large dynamics systems, such as Lake Gaston. For this study, we utilized a combination of laboratory and field based trials to evaluate the efficacy of multiple treatment protocols and determine the best treatment program for lyngbya within Lake Gaston. To identify the most appropriate chemical treatment protocol, we evaluated multiple algaecide formulations, including Algimycin®, Captain XTR®, Cutrine Ultra®, and Hydrothol®, in combination with surfactants of AMP®, Green Clean®, and Reward®. For our field based trials, we evaluated both a drop down hose and autonomous hydraulic injection system application method. The morphological features of lyngbya mats, including high variability in both physical and spatial characteristics, makes evaluating the effectiveness of treatment protocols very difficult. Therefore, we developed evaluation methods that allowed for a more holistic evaluation of the lyngbya mat material for both the laboratory and field based applications. With this approach, we were able to capture the response of lyngbya to the varying herbicide protocols and determined that the use of an autonomous application system that targets the benthos with a combination of Captain XTR® + AMP® resulted in effective lyngbya control for Lake Gaston. We feel that through this study we have improved not only the lyngbya treatment program for Lake Gaston, but can now appropriately evaluate the efficacy of any future program modifications.

Along with evaluating management related issues regarding lyngbya within Lake Gaston, this project was able to address concerns of potential harmful impacts from interactions with lyngbya treatment protocols to native aquatic fauna. Application methods that utilize chelated copper based algaecides, like the ones found to be most effective in the management of lyngbya at Lake Gaston, are highly toxic to aquatic organisms, especially freshwater mussels. The Tidewater Mucket is a North Carolina state threatened mollusk that is well established within Lake Gaston, however little is known about this populations overall abundance or distribution within the system. A mussel mortality event within areas of Lake Gaston that were being actively treated for lyngbya resulted in direct survey efforts of impacted areas both within and outside of the treatment areas. This effort resulted in modified treatment protocols that minimized the potential of a negative interaction with mussel beds by eliminating shallow water (<4ft) applications. However, this protocol allows lyngbya to continue to establish and expand within the shallow coves of areas receiving treatment applications decreasing the overall effectiveness of treatments. This problematic situation highlights the need for further understanding of Tidewater Mucket distribution and abundance within Lake Gaston, especially within active treatment areas.

In addition to the aforementioned research, this study was also able to incorporate a pilot study looking at the potential for toxin production related to lyngbya within Lake Gaston. Previous studies looking at toxin production for freshwater lyngbya species extracted samples from the physical benthic mat material, however the mechanism for toxin release and subsequent leaching of those toxins into the ecosystem is unknown. Therefore, this study utilized a method called Solid Phase Adsorption Toxin Tracking (SPATT) to provide a cost-effective way to monitor the potential of ambulant cyanotoxins directly from the water column over a continuous time period. The scope of this project was small due to a nationwide shortage of the required toxin absorbent, however we were still able to detect possible cyanotoxin production and determine proof of concept for using SPATT technology within Lake Gaston for future monitoring efforts.

Management Implications

Overall, this study was able to establish a successful lyngbya treatment program for Lake Gaston, NC/VA. Our findings were similar to previous studies that documented the efficacy of chelated-copper based algaecides to produce favorable control responses in lyngbya; however, we were able to translate our results into a larger, field-based scenario and improve on methods used to evaluate the efficacy of such large-scale treatments. With the use of novel sonar imagery and evaluation of cellular response, we were able to determine that the use of an autonomous application system that targets the benthos with a combination of Captain XTR® + AMP® resulted in effective lyngbya control for Lake Gaston. We recommend that this treatment protocol be continued for future lyngbya directed management efforts. However, we also recommend continued evaluation of this protocol to maintain best management practices in regards to minimized impacts to native aquatic fauna and relief of budgetary constraints on management efforts.

This study has identified several areas of further research that would provide beneficial information in regards to the impacts of lyngbya within Lake Gaston and improve overall management efforts. Future research topics include: 1) increase the knowledge base for Tidewater Mucket distribution, abundance, and habitat preference in Lake Gaston and use that knowledge to develop a more effective lyngbya treatment protocol that will minimize negative impacts to native mussel species while increasing the efficacy of the treatment design, 2) understand the potential toxin production capabilities of lyngbya within Lake Gaston to better assess the environmental and human health risks posed by this cyanobacteria, and 3) continue to evaluate the efficacy of varying treatment protocols and the potential ecological impacts of lyngbya interactions with native aquatic fauna, resulting in best management practices for Lake Gaston's lyngbya treatment program.

1.2 LITERATURE REVIEW

Adapted from Emily Vulgamore's final report requirement of M.R. degree

Introduction

This review focuses on an organism widely known among aquatic resource managers and scientists as the cyanobacterium or "alga" *Lyngbya wollei* (Farlow ex Gomont) comb. nov. That name is retained here, but recently a proposed name change, to *Microseria wollei* was recently accepted by Algaebase and other key taxonomic authorities (and see McGregor et al. 2015, Smith et al. 2019). In current status, *Lyngbya wollei* is regarded as a synonym of *Microseira wollei* (Farlow ex Gomont).

Lyngbya wollei is mostly considered a filamentous cyanobacterium, considered mostly benthic in habit (Speziale and Dyck 1992). Though present in Australia, India, and Europe (Macbeth 2004), populations in the southeastern U.S. have gained notoriety for excessive biomass production, taste and odor problems, and water quality degradation (Hudon et al. 2014 and references therein, Speziale and Dyck 1992). The remainder of this text addresses literature regarding *L. wollei* as a cryptic, benthic cyanobacterium.

General biology and ecology of cyanobacteria

Cyanobacteria (syn. blue-green algae, cyanophytes) are a ubiquitous, old and diverse group (division or phylum) of photosynthetic, gram-negative microbes that play a major role in the function and quality of aquatic ecosystems (Vincent 2012). Fossil records suggest that they have been on the Earth for at least 3.5 billion years, and they are credited for oxygenating the Earth's atmosphere (Graham and Wilcox 2000, and references therein). Thus, cyanobacteria are considered to have "paved the way" for the evolution of eukaryotic life, including other algal phyla and higher plants.

Although initially classified as algae under the Botanical Nomenclature, for the past ~50 years cyanobacteria have been included as a bacterial clade at the division (phylum) level (Stanier et al. 1978; and see Garcia-Pichel et al. 2019) due to their prokaryotic nature. Here, cyanobacteria are defined as organisms in the domain Bacteria with similar photosynthetic biochemistry as higher plants, able to carry out oxygenic photosynthesis with water as an electron donor and to reduce CO_2 as a source of carbon, or those secondarily evolved from such organisms (Garcia-Pichel et al. 2019). As a classic example of "nature mocking at human categories," this definition is at odds with the ecological function of the group, mainly as primary producers (Lewin 1979, Golubec 1979), so cyanobacteria are ecologically often included as "algae" which are, themselves defined as primitive organisms mostly with the same photosynthetic biochemistry as higher plants (Graham et al. 2008), At present, there are roughly 2,000 species in 150 genera that range in shape from unicellular to colonial or filamentous.

Cyanobacteria are of special interest to aquatic resource managers because they can produce taste and odor compounds, toxins, and high-biomass outbreaks ("blooms"). Benthic taxa that form loose or compact mats are considered ecologically important because mats can disrupt the food web, compound water quality degradation, and alter the species diversity of a system (Graham and Wilcox 2000).

Multiple physical and chemical adaptations allow for cyanobacteria to persist for prolonged time periods under unfavorable conditions (Nienow et al. 2003). A mucilaginous, polysaccharide sheath supports gliding mobility and physical defense. Sheaths, consisting of up to 98% water and 70% of the total filament biomass, can act as creating a water reserve for hours to days, retarding evaporation and retaining moisture (Nienow et al. 2003). Light-harvesting complexes, or phycobiliproteins, allow for growth in low, incident light conditions (Graham et al. 2008). Cyanobacteria are also well known for carbon-concentrating mechanisms, allowing for improved photosynthetic efficiency (Stevenson et al. 2007).

Cyanobacterial ecology mostly has been assessed for planktonic taxa (floating at or near the water surface, or within the water column). Success in a given aquatic ecosystem is often attributed to their buoyancy regulation and, for some species, the ability to “fix” atmospheric nitrogen gas into inorganic nitrogen as ammonia (Hyenstrand et al. 1998). Buoyancy regulation allows them to sink, at night, to lower depths where nutrients are more abundant, and then to move back up toward the surface for photosynthesis during the day (Reynolds and Walsby 1975). Nitrogen fixation affords them a competitive advantage over other primary producers when inorganic N supplies are low (Bothe 1982, Hyenstrand et al. 1998).

Benthic cyanobacteria occur attached to various substrata in the photic zones of aquatic environments (Stevenson et al. 1996). Because of their cryptic nature and position in the water column, they have been poorly studied in comparison to planktonic forms (Cantonati and Lowe 2014). They are usually filamentous forms that attach to various substrata. *Lyngbya* is considered a benthic cyanobacterial genus because it establishes at the solid-liquid substrata interface, though it can move to the surface or near-surface of the water column as its growing season progresses. Although they can be microscopic, *Lyngbya* spp. typically are regarded as macroalgae following the definition of Sheath and Cole (1992), which includes filamentous, colonial, tuft-forming masses that are visible to the naked eye (also see Lapointe et al. 2018 and references therein). Filamentous forms can take longer to manifest in community development depending on the biomass attained (Stevenson et al. 2007). Filaments may establish on or attached to benthic substratum and can remain undetected unless dislodged (Stevenson et al. 2007).

Another term, periphyton, is commonly used to describe benthic algae and cyanobacteria. This term generally refers to all microflora (algae and bacteria) growing on, or attached to, substrata (Wetzel 2001). Here, periphyton is used to refer to biofilms and thicker matrices of microscopic organisms in association with or on *Lyngbya* filaments. Filaments may be covered with algae and heterotrophic fungi and bacteria. *Lyngbya* can grow epiphytically (on algae or higher plants), epipsammically (on or among sand grains), epilithically (on rocks), or epipelically (on or among sediments). As *Lyngbya* grows from these substrata up into the water column, filaments that are not attached to the substrata but remain in the photic zone are considered metaphytic (floating near the bottom). Thus, the nature of the habitats *Lyngbya* may experience are diverse dependent substrata availability and the amount of growth (Stevenson et al. 2007).

The growth habit of algae and cyanobacteria confers different competitive advantages. For example, taxa that extend into the water column experience a different environment with more available light and but often lower nutrient availability than benthic forms (Wetzel 2001). The upper eulittoral zone is often characterized by higher-energy wave action, and tends to be dominated by organisms that strongly attach to substrata. On the other hand, a species closely related to *Lyngbya wollei*, *Lyngbya diguetti* (Gomont) Anagnostidis & Komárek, is an example of an epiphytic organism that grows in deep littoral and eulittoral zones (Stevenson et al. 2007). Thus, *Lyngbya* as a genus includes species that are both upper littoral and deeper littoral/eulittoral zones.

Historical perspectives

Lyngbya wollei was first formally documented in the United States in the nineteenth century by Reverend Francis Wolle (Speziale and Dyck 1992). Francis Wolle belonged to a familial, musical dynasty that brought Bach’s music to America (Larson 2002). Though he was an educator, his interest in freshwater algae was thought to have been kindled by the industrial revolution or more specifically, the family iron and railway company located along the river in Bethlehem, Pennsylvania, and led him to Drs. H.C. Wood and Farlow. In his obituary, Reverend Wolle was remembered as an algal artist, a successful educator, and the contributor of the first patent of a paper bag-making machine. In his authorship years, Wolle was first to formally introduce the species, citing Farlow:

> *Forms smaller or larger tufts attached to river stones; the more sluggish the water the larger the extent of the growth, even to masses yards in diameter and a foot or more in thickness. Usually brownish black; but when older somewhat faded, more olive or brown or yellowish. Articulation primarily about 4 to diameter, after division 6-8. Sheath firm, and lamellate in older forms.*
>
> *Diameter of filaments variable, from 40-45 u to 50-60 u. Widely distributed from Massachusetts to Florida, and westward to Minnesota. The largest masses occurred to personal observation, in ponds, New Jersey. In one instance in pond near Stanhope, the floating mass was fully ten years long, 2-3 yards wide, a foot or more in thickness, and so densely matted, it was impossible to break through a rowboat.*
>
> (Adapted from Fresh-water Algae of The United States, Francis Wolle, page 297)

At that time, the genus *Lyngbya* included the presently recognized separate genera *Phormidium, Siphoderma, Leptothrix, Hypheothrix, Amphithrix,* and *Leibleinia.*

Taxonomy and Species Description

Over the course of a century, this genus included other genera as well, such as *Plectonema*, and was subjected to further taxonomic changes. Speziale and Dyck (1992) classified multiple species of *Lyngbya* into one genus using what are now known to be polymorphic morphological features (Table 1). Despite recognizing *Lyngbya* as a cyanobacterium under the Bacteriological Code of Nomenclature, the Botanical and Bacteriological Codes were confusingly, inappropriately "mixed" in that taxonomic classification:

Empire: Prokaryota

Kingdom: Eubateria

Phylum: Cyanobacteria, Cyanophyta (common name: blue-green algae)

Class: Cyanophyceae

Order: Oscillatoriales

Family: Oscillatoriaceae

Genus: *Lyngbya*

As noted above, *Lyngbya wollei* is a benthic filamentous cyanobacterium found in freshwater systems (Speziale and Dyck 1992). It initially attaches to substrata but can dislodge and float to or near the surface (Hudon et al. 2014 and references therein). Visually, it appears as dense, dark-colored filaments that have a coarse, wool-like texture. Surface mats can appear orange or yellow due to degradation by ultraviolet light or to increasing production of carotenoid pigments for photoprotection (Figure 1.2.1, and see below).

This cyanobacterium is microscopically identified as straight filaments consisting of trichomes (linear filaments), generally within a sheath that extends beyond the cells (Figure 1.2.2). The sheath, a mesh-like microbial "glue" (Martin and Wyatt 1974) as described above, is hyaline or colored due to absorption of metals such as iron, and varies in thickness depending on environmental conditions (Speziale and Dyck 1992) (Figure 1.2.3). The cells are uniformly pigmented and large in comparison to those of other cyanobacteria within the order Oscillatoriales (Speziale and Dyck 1992). Morphological plasticity (polymorphism) is well known throughout species within this order and likely influenced by both abiotic and biotic factors, but most of the related mechanisms, causes, and effects are largely speculated and unknown.

Microscopically, *Lyngbya wollei* appearance can be similar to that of some *Phormidium* spp. and certain other genera within the Oscillatoriales, though visual diagnostics do not necessarily equate to cladistic relationships. Although morphological diagnostics utilizing microscopy remain the most widely used in identification, recent advances in molecular genomics have addressed the polymorphism induced by environment and response. In general, more than 500 cyanobacterial genomes have been sequenced (Garcia-Pichel et al. 20190), few of which are *Lyngbya* or close relatives. Joyner (2008) considered *Lyngbya wollei* to be at least two cryptic species, or 2-3 individual strains within one species. The extent of strain variation within *L. wollei* is largely unknown within and among regions.

In asexual reproduction, trichomes may exit the sheath, becoming hormogonia or small motile filamentous fragments with dividing cells (Stevenson et al. 2007) (Figure 1.2.4). Fragmentation can also occur when filaments are stressed (Adamec at al. 2005). Sexuality in *L. wollei* is unknown, as in other cyanobacteria (Graham et al. 2008), but genetic recombination can occur through viral transformation, conjugation (Cassier-Chauvat et al., 2016) or electroporation (Flores et al., 2008). Cyanobacteria have a widely diverse genome and possess *E.coli*-like DNA repair and recombination genes (Cassier-Chauvat et al. 2016). Vectors used in successful bacterial to cyanobacterial conjugation have been marked by antibiotic-resistance genes passed on from *E. coli* (Flores et al. 2008).

Autecology

Often described as an opportunistic macroalga, *L. wollei* is thought to have various mechanisms that allow for efficient establishment and function in suboptimal conditions. Under certain conditions, surface mats can form with excessive biomass in summer to late fall (Stevenson et al. 2007). Under these circumstances, an infestation can be considered as a harmful algal bloom (Shumway et al. 2018). Bloom formation is influenced by physical-chemical factors such as light, temperature, depth, wave action and disturbances, nutrient sequestration and availability, grazing, residence time, and desiccation (Levesque et al. 2015, Vis et al. 2008, Stevenson et al. 2007). Wave and current exposure can cause sloughing which is thought to regulate spatial biomass accumulation (Levesque et al. 2015). Proliferation of benthic macroalgal blooms has also been linked to increased nutrient loading and system eutrophication (Lapointe et al. 1997, 2018; Stevenson et al. 2007). The mat consortium, whether in a floating or benthic habit, has within it steep chemical gradients where nutrients can be recycled independently from the surrounding environment (Stevenson et al. 2007). The accumulation of N and P from sediment or microbial decomposition is a suggested mechanism for sustained growth within a mat (Sickman et al. 2009).

Water chemistry

Infestations of *L. wollei* typically are reported from alkaline waters or calcium-rich (7-15 mg/L) spring-fed aquifers (Stevenson et al. 2007). The addition of calcium may serve in growth and buffering capacity. Nevertheless, *L. wollei* has been reported across a broad pH range from 5.9-9.3, and it has the capability to influence the pH of its micro-environment (Hudon et al. 2014, Vijayavel et al. 2013).

Temperature

Spatial and temporal variation in temperature strongly influences benthic algae, where temperature affects algal photosynthetic metabolism and enzymatic reaction rates (Stevenson et al. 2007). Unfortunately, little research has been conducted about temperature effects on *L. wollei*. Positive growth rates have been observed for temperatures ranging from 7-20°C (Yin et al. 1997). Negative growth rates occurred at <5°C, though *L. wollei* can persist under ice in a perennial state (Panek 2012). In the southeastern U.S., excessive biomass of *L. wollei* is produced when temperatures approach 18°C and higher in the summer season (Speziale et al. 1991). In contrast, Florida springs are consistently around 22°C and produce comparable biomass year-round (Joyner et al. 2008, Hudon et al. 2014). In laboratory studies (Yin et al. 1997), maximum biomass was observed at 26°C and declined at 10°C. The ability to withstand and grow under a range of temperatures with maximum biomass production under warmer temperatures supports biomass was observed at 26°C and declined at 10°C. The ability to withstand and grow under a range of temperatures with maximum biomass production under warmer temperatures supports the likelihood of increased *L. wollei* proliferation under warming trends in climate change (Hudon et al. 2014 and references therein).

Pigmentation and light

Light is a vital resource for primary producers, and it attenuates with depth in the water column as well as within an algal mat or biofilm (Stevenson et al. 2007). Light attenuation is affected by both suspended abiotic particles (turbidity, sediments) and phytoplankton. Although chlorophyll *a* is the "universal plant pigment" required for oxygenic photosynthesis, it reflects green light which tends to penetrate most deeply into turbid waters (Wetzel 2001). Benthic cyanobacteria have adaptations to compensate for low light and the greenish-yellow wavelengths that commonly attenuate to deeper waters (Wetzel 2001). Such adaptations include secondary pigments or "light antennae" that absorb greenish-yellow light and fluoresce the energy at lower- energy red wavelengths that can be absorbed by chlorophyll *a*. This allows for growth under low incident light in conditions that are not favorable for potential competitors lacking accessory pigments (Stevenson et al. 2007).

Benthic cyanobacteria have adaptations to compensate for low light and the greenish- yellow wavelengths that commonly attenuate to deeper waters (Wetzel 2001). Such adaptations include secondary pigments or "light antennae" that absorb greenish-yellow light and fluoresce the energy at lower-energy red wavelengths that can be absorbed by chlorophyll *a*. This allows for growth under low incident light in conditions that are not favorable for potential competitors lacking accessory pigments (Stevenson et al. 2007). Cells of *L. wollei* are darkly pigmented (Speziale and Dyck 1992) and contain accessory pigments—most notably, phycobilins (phycobiliproteins) phycoerythrin, phycocyanin, and allo-phycocyanin—that can capture greenish/greenish-yellow light and fluoresce it to chlorophyll *a*. With the ability to absorb and utilize nearly the entire visible light spectrum, *L. wollei* can optimally grow in < 0.1% of incident photosynthetically active radiation or the low photosynthetic light compensation point of 32 uEinst $m^{-2}s^{-1}$ (Speziale et al., 1991).

Opposite stressors are encountered by *L. wollei* growth at or near the water surface. As mentioned, surface mats subjected to high light intensity may appear yellow or orange with increased UV-exposure. This phenomenon is thought to be caused by production of carotenoids such as myxoxanthophyll production (Speziale et al. 1991) that serve as a repair mechanism for the mat consortium (Yin et al. 1997). The outer growth shades the rest and may be genetically altered to better withstand UV-exposure.

Depth in relation to biomass

The depth at which *L. wollei* grows is variable and dependent on the system (Table 2). Maximum biomass has been reported at depths between 1.5 and 3.5 m in the Great Lakes (Bridgeman and Penamon 2010). Benthic irradiance corresponded to 4.0-0.05% of surface irradiance at these depths. In several systems little biomass has been found near the shoreline at depths of less than 1 m (Hudon et al., 2014 and references therein).

Nutrients

Established benthic cyanobacterial and algal mats have steep, geo-chemical gradients of light, pH, iron, phosphorus, nitrogen and other nutrients with dark, anoxic conditions next to the sediment (Hudon et al. 2014, Stal 1995). It is hypothesized that nutrients are regenerative within benthic biofilms such as *Lyngbya* mats (Stevenson et al. 2007 and references therein). As mats grow, they can accumulate N and P from living and dead organisms and debris (Burkholder 1996). As mats thicken, the interior portion dies, and microbial decomposition may release the N and P via advection or diffusion. This process allows for nutrient availability and subsequent uptake by nearby living tissue. Internal regeneration of nutrients may support growth when nutrients are depleted, or a physical disturbance otherwise prevents growth. It should be notes that when algal mats die or senesce quickly, they can cause localized depletion of oxygen and release ammonia and hydrogen sulfide, leading to declines in water quality (Sickman et al. 2009).

Nitrogen (N) and phosphorus (P) are most emphasized in cyanobacterial research because they are essential macronutrients that are often most important in controlling growth of primary producers (Hudon et al. 2014). In the southern U.S., enriched field conditions associated with *L. wollei* blooms reached up to 5,800 µg NO3-N/L (Yin et al., 1997 and references therein) and ~280 µg total P/L (Cowell and Botts 1994). *Lyngbya* bloomed under similarly enriched water- column conditions in northern geographic regions as well. In Quebec, however, blooms were reported at much lower NO3-N (210 ug N/L and total P (86 µg/L) (Levesque et al. 2012). It is suggested that *Lyngbya* can regenerate or recycle as well as take up nutrients within a mat, as known for other benthic algae (Moeller et al. 1988, Burkholder 1996). A study on first-order springs in Florida (Sickman et al. 2009) found that *Lyngbya* N and P requirements were met from the high external nutrient inputs, hypothesized to have been supplied by nearby agro- industrial and urban inputs. Advection of nutrients from mats to the water column were less than 12% and 0.05% of daily net N and P uptake, respectively. Furthermore, NO3-N concentrations have been shown to decline over mats, suggesting that under some conditions *Lyngbya* relies on its external environment at least partially for nutrient sources. In contrast, Stevenson (2004) found only a weak relationship between *Lyngbya* abundance in mat formations and P from the overlying water column or the underlying sediments, and no relationship between abundance and external NO3-N concentrations.

Despite lacking specialized cells, *L. wollei* has the ability to fix nitrogen gas into inorganic N as ammonia (Phlips et al. 1992), with reported nitrogenase activity under low light or anoxic conditions (Beer et al. 1990 and references therein). In general, cyanobacteria have high iron requirements when N is limiting (Hudon 2014 and references therein). Iron, a constituent for the synthesis of nitrogenase during nitrogen fixation (Paerl 1990), has a negative effect on *L. wollei* growth at high concentrations (>600 µg Fe/L) (Pinowska et al. 2007).

Nevertheless, low growth rates were observed when no iron was added to culture media, suggesting that iron is an important nutritive component of growth. In contrast, the marine *Lyngbya majuscula* Harvey ex Gomont has a high iron requirement (1400-6000 µg Fe/L) for growth (Hudon et al. 2014 and references therein).

Metabolism

Increased levels of dissolved nutrients (N and P) stimulate *L. wollei* growth (Panek et al. 2012 and references therein), though multiple regenerative mechanisms may influence *Lyngbya* growth. Unlike C-3 photosynthetic metabolism which is thought to have evolved from cyanobacterial endosymbiosis, *L. wollei* exhibits an efficient C-4-like mechanism equipped for productivity regardless of dissolved CO2 availability (Beer at al. 1990). However, it is unknown whether *Lyngbya* possess C-4 mechanism genes. Related species *Lyngbya birgei* G.M. Smith appears to use internal CO2 levels and a carbon concentrating system such as carboxysome granules to suppress inefficient photorespiration (Beer et al. 1992). The research further suggests that *Lyngbya* resembles micro-cyanobacteria, or cyanobacteria invisible to the naked eye, in its ability to take up HCO3-, which increases internal CO2 levels within filament aggregates or mats. These interstitial water conditions would support saturated photosynthesis by HCO3- during the day while CO2 levels remained low.

Biologically Active Compounds and Toxins

More than 70 biologically active compounds, many of which are carcinogenic or otherwise toxic, have been isolated from the genus *Lyngbya* (Sickman et al. 2009, Osborne et al. 2001). Geosmin and 2-methylisoborneol produced "earthy" and musty off-flavors but are not known to be toxic (Schrader and Blevins 1993). Various cyanotoxin-encoding genes have been identified in *L. wollei* from various geographic locations. Anatoxins, cylindrospermopsins, and paralytic shellfish poisoning toxins (PSTs) have been identified including CYN and deoxy-CYN (Seifert et al. 2007), LWTX-1-6 (Lajeunesse et al. 2012), dcSTX, and dcGTX-2 and-3 (Odonera et al. 1997). Saxitoxins are neurotoxins commonly referred to as paralytic shellfish poisons and are more often associated with dinoflagellates than with freshwater cyanobacteria (Foss et al. 2012, Burkholder et al. 2018 and references therein). There are more than 57 structural variants that function in blocking voltage-gated sodium and calcium channels, causing illness, paralysis and potential death (Foss et al. 2012 and references therein). In laboratory studies, low N:P ratios and higher calcium levels increased biomass and toxin production (Yin et al. 1997). In north temperate natural lakes, the nutrient (N and P) and chlorophyll *a* concentrations of *L. wollei* filaments have been shown to vary depending on environmental conditions (Smith et al. 2019). In general, the conditions that control toxin production in *L. wollei* under field conditions are largely unknown. In the laboratory (Yin et al. 1997), maximum biomass and PSTs production occurred at 26°C under low light conditions (11–22 µEinst m^{-2} s^{-1}). Further, toxin production was observed with high nutrient concentrations of PO4-3P (0.55–5.5 mg P/L) and NO3-N (83 mg/L) (Yin et al. 1997, Hudon et al. 2014).

Some authors have considered *L. wollei* to be best described as an indicator alga for accelerated eutrophication caused by point and nonpoint source pollution (Hudon et al. 2014 and references therein). Historic records have associated infestations with agro-industrial and chemical inputs from storm disturbances (Stevenson et al. 2007). Information is sparse, however, and limited to selected aquatic systems.

This cyanobacterium may serve as a structural habitat for other aquatic organisms, and as a catchment for bacterial and fecal particulates (Vijayavel et al. 2013) settling out from the water column. Filaments of *L. wollei* often have epiphytic material covering or attached to the sheath (Figure 1.2.5). It has been proposed that the sheath serves as a defense against crustacean and amphipod grazing (Camacho and Thacker 2006), considering that such organisms do not feed or only minimally feed on *Lyngbya*. In some cases, it has been reported as toxic to potential grazers (Gélinas et al. 2013, Hudon et al. 2014).

Harmful *L. wollei* outbreaks (blooms) are often associated with impairments to the function and aesthetics of waterbodies (Hudon et al. 2014). Negative impacts associated with bloom conditions may include taste and odor problems caused by the secondary metabolites, geosmin and 2,methylisoborneol (MIB); excessive biomass restricting sonar navigation and recreational accessibility; decreased biodiversity and habitat suitability for aquatic flora and fauna; and economic depreciation of shoreline property (Levesque et al. 2012 and references therein). The following text considers potential control mechanisms for management of *L. wollei.*

Biological control

Biological control refers to the stocking of natural enemies that will feed on or out- compete the managed species (Gettys et al., 2014). The presence of some aquatic vegetation may assist in controlling the proliferation of *Lyngbya wollei* through competition. Additionally, the stocking of triploid grass carp is a common method of biological control utilized in the management of aquatic macrophytes. However, there is no evidence that triploid grass carp (*Ctenopharyngodon idella)* feed on *L. wollei* (Kasinak et al. 2015). Grass carp can change the disturbance regime through physical removal of submersed aquatic vegetation (SAV) (Sickman et al. 2009). *L. wollei* has been documented to co-occur with *Vaucheria* (Sickman et al., 2009; Stevenson et al. 2009), but the nature of this potential competition is not well understood. Other research suggests that macrophytes *Pontederia cordata* and *Potamogeton nodosus* may limit *L. wollei* growth (Doyle and Smart 1998). In systems where *Hydrilla verticillata* and *Lyngbya wollei* are both present*, Hydrilla* has been shown to prevail under increased salinity concentrations, though *L. wollei* is not known to grow in salinity concentrations above salinity 5.25 (Cowell and Botts 1994). Replacement of SAV with nuisance algae and cyanobacteria has negative impacts on environmental quality and operational uses of aquatic systems (Sickman et al. 2009).

Chemical Control

Chemical control refers to the utilization of selective or non-selective algaecides to limit the growth of algae and cyanobacteria in impounded waters, lakes, ponds, reservoirs, stock tanks and irrigation systems (Gettys et al. 2014). Chemical treatments on *L. wollei* can be efficacious in controlling biomass (Table 4) (Nagai and Taya 2015, and references therein). Algaecides may alter species composition and community structure, specific to benthic algal assemblages in natural aquatic ecosystems. Sensitivities may significantly differ, however, depending on the mode of action of an herbicide (Nagai and Taya 2015, and references therein).

Chemical control studies have shown chelated copper algaecides provide some level of biomass reduction in laboratory and field studies (Bishop et al. 2015, Calomenti et al. 2015, and Anderson et al. 2019). A multi-year study in Lay Lake, Jordan Lake and Lake Mitchell documented up to 94.7% reduction in total nuisance acreage treated for *L. wollei* (Anderson et al. 2019). The study further indicated that operational shifts toward a double-chelated copper algaecide with added surfactants and emulsifiers are more efficacious and economic compared to repeated single or combination treatments. Laboratory experiments from Lay Lake populations of *L. wollei* indicate that infused copper is a critical component of toxicity (Bishop et al. 2015). Phycomycin® SCP at concentrations of 23, 46, 92 and 184 mg/L decreased its biomass compared to biomass in untreated controls (Bishop et al. 2015). A treatment of peroxide (10.1 mg/L) followed by Cide-Kick® II (0.2 mg/L) and Algimycin®-PWF (0.26 mg/L) could offer control of *L. wollei* (Calomeni et al. 2015).

One aspect of management is to ensure that non-target species are not injured or at risk of injury with algaecide applications. Studies on mussels and other sensitive, water-filtering taxa have shown that chemical applications at lower water temperatures reduce the chance of non- target injury or die-off (Buczek et al. 2018). Exposure and risk assessments to other non-target species such as *Daphnia* (Bishop et al. 2018) can be used to continue to evaluate methods for operational treatments and chemical efficacy.

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Figures

Figure 1.2.1. *Lyngbya wollei* can grow attached to benthic substrata and produce floating surface or near-surface mats. Healthy filaments appear as darker blackish-green colors with a dense, wool texture. Filaments subjected to high light intensity may turn to a warm-toned color.

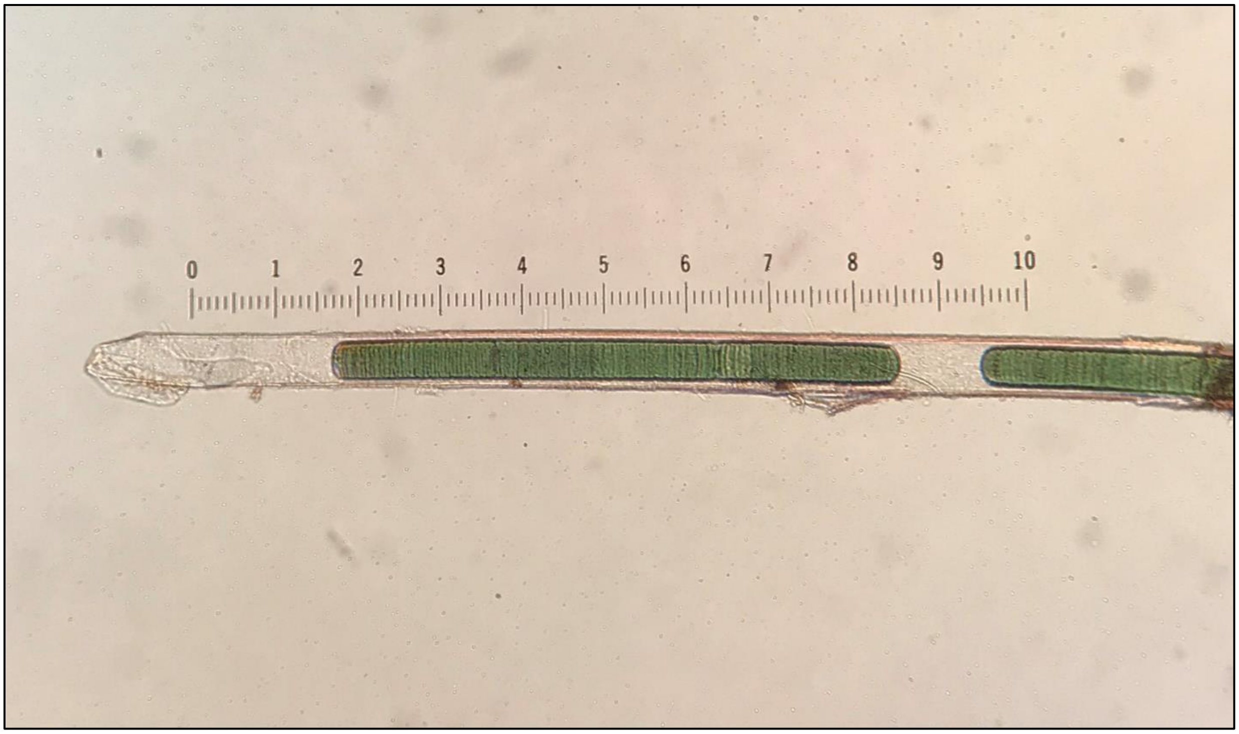

Figure 1.2.2. *Lyngbya wollei* can be morphologically distinguished from other *Oscillatoriales* cyanobacteria by a sheath that extends beyond trichomes of cells (200x).

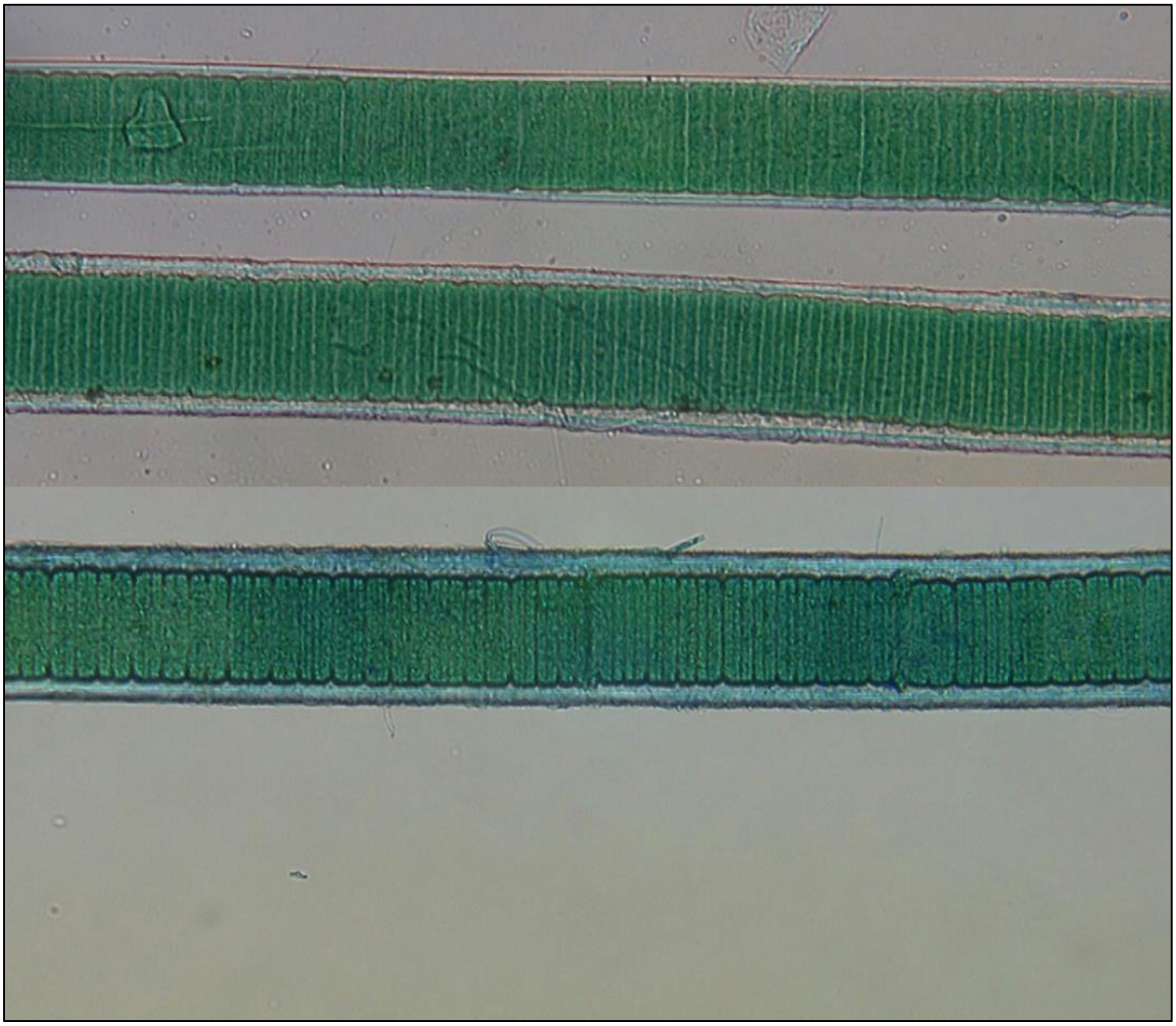

Figure 1.2.3. Sheath variability in thickness and color may be due to environmental conditions (400x).

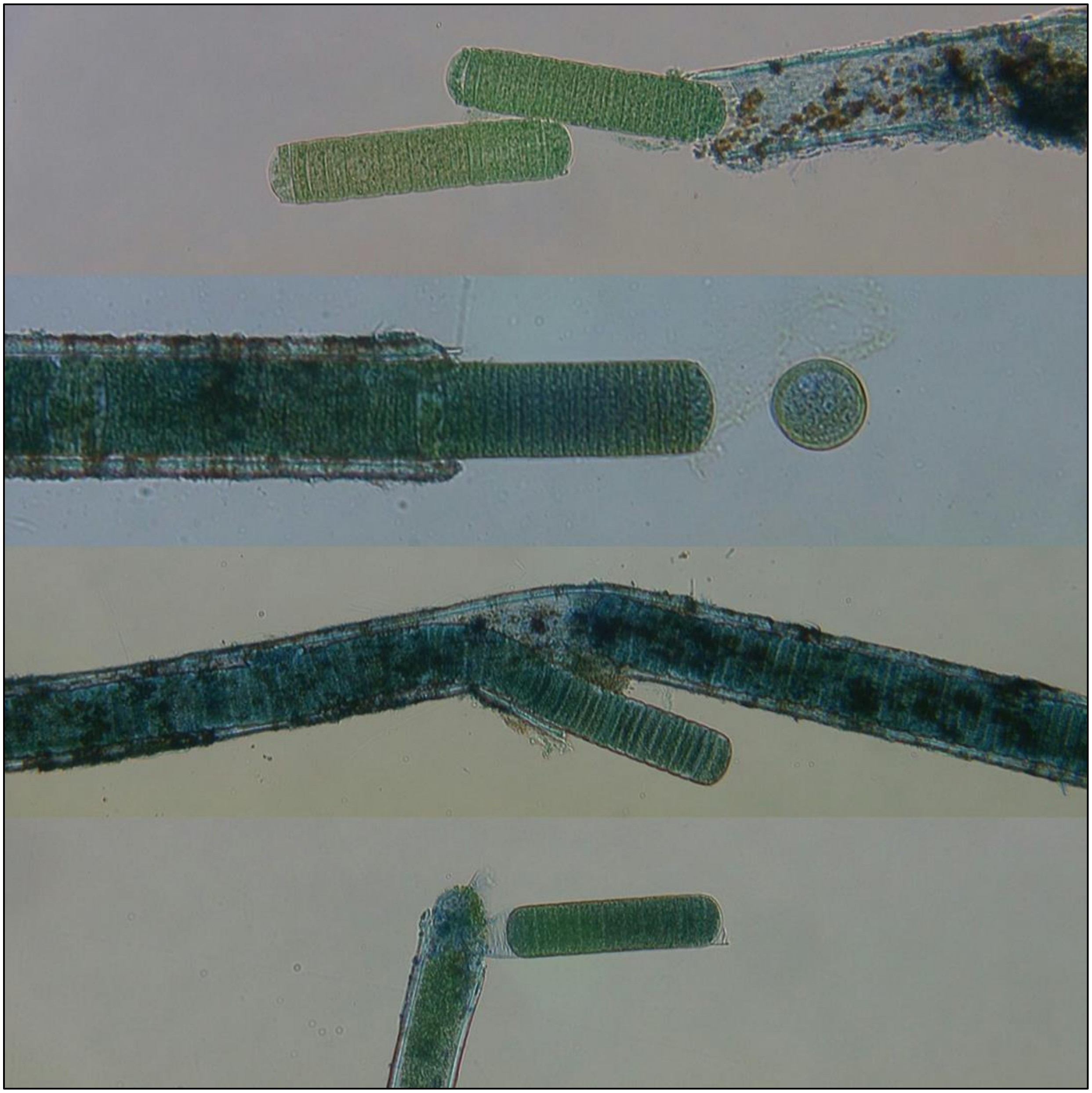

Figure 1.2.4. Hormogonia are motile offspring filaments that depart from the parental filament and sheath (400x).

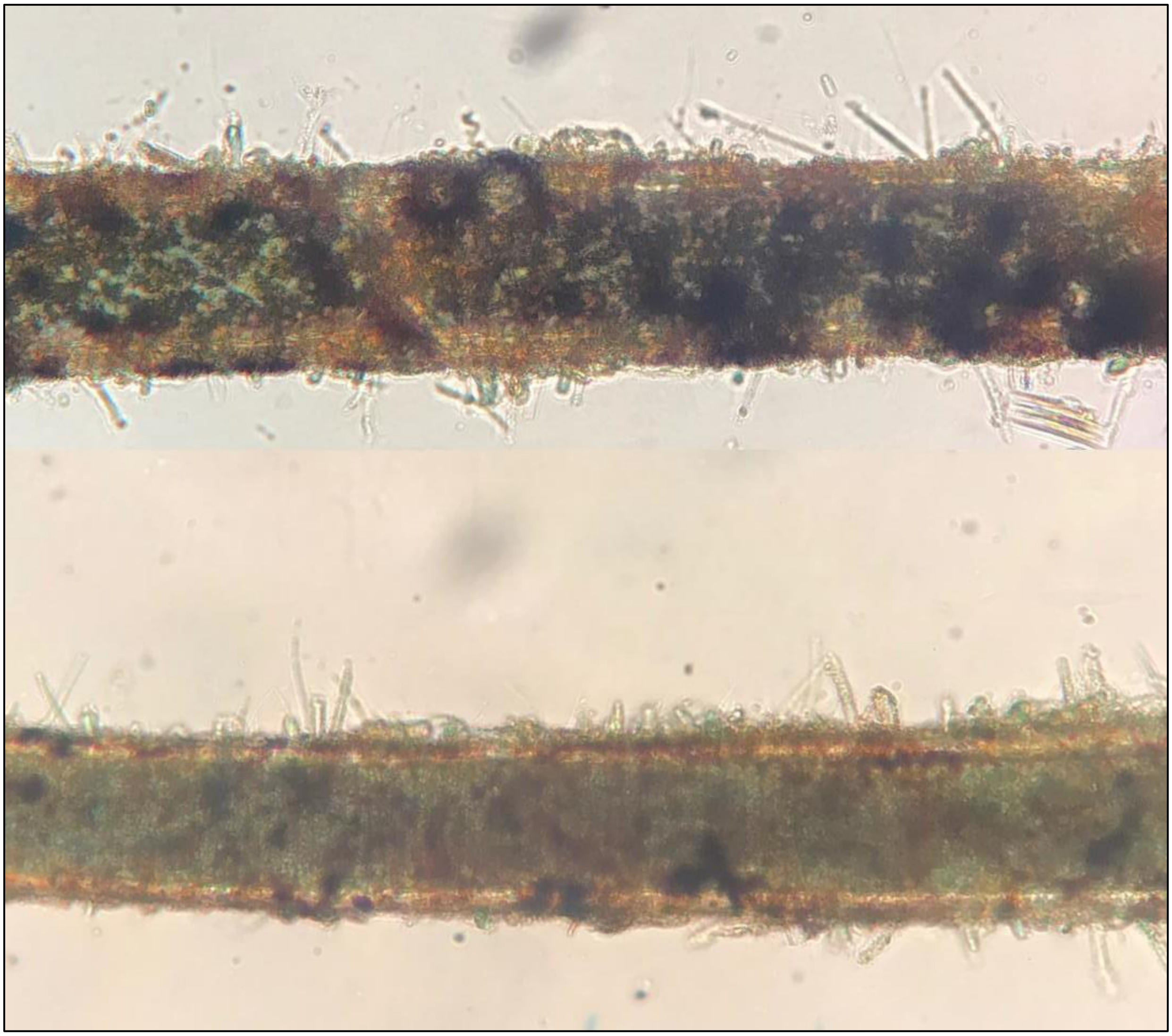

Figure 1.2.5. Sheaths may be covered in periphytic material (400x).

1.3 Predictive Modeling of Lyngbya Occurrence and Proliferation

Introduction

The growth potential of aquatic plant communities is closely associated with spatial and temporal trends in the chemical, biological, and physical characteristics of their surrounding environments (Lacoul and Freeman 2006). Light, water temperature, flow regimes, nutrient levels, and sediment characteristics have all been attributed to the distribution of aquatic plant communities (Bornette and Pijulon 2011). And while all of these environmental factors contribute to the ability of aquatic plants to establish and distribute themselves throughout a system, identifying individual factors that contribute to overall success is difficult (Parinet et al. 2004). Determining specific factors that drive growth and distribution for non-native or noxious species compounds the problem because they are not naturally limited, have the ability to alter water quality, and can reduce overall biodiversity (Hussner et al. 2017; Lolis et al. 2019).

Lyngbya was first identified in Lake Gaston during the 1990's, but over the past decade has displayed increased expansion across the system. Although lyngbya is distributed throughout Lake Gaston, there is a high level of geographical variability in regards to the extent of distribution and the level of proliferation. Increased lyngbya proliferation has been attributed to a host of environmental drivers and physiological factors, however growth responses to these various drivers vary from system to system. Therefore, understanding the drivers of lyngbya growth within individual systems is critical for managers in allowing them to develop appropriate response and management plans that will minimize the overall impact of lyngbya infestations.

Identifying direct relationships between aquatic plant communities and surrounding environmental factors can be difficult. There are a plethora of individual parameters that may directly or indirectly influence the relationship, including physico-chemical, biological, morphological, and hydrological factors associated with a particular system (Parinet et al. 2004). Therefore, a multidimensional statistical treatment of all associated variables should be utilized to capture the complexity of an aquatic system. Principal Components Analysis (PCA) is a well-known method that has been used to describe complex relationships between the water quality, physical habitat, and flow regime of a system to the overall dynamics of an aquatic plant community (Daniel et al. 2006; Li et al. 2017; Nunes et al. 2022). PCA data will not explain direct relationships between individual variables, but yet explains the relationship among all the identified environmental factors.

For our study, the objective was to use PCA modeling to identify how varying physical features, water quality parameters, and aquatic plant community dynamics related to the distribution and density of lyngbya across Lake Gaston.

Methods

Sampling

The aquatic plant community of Lake Gaston was documented on a yearly basis using a boat-based point-intercept method. Surveys were conducted during the months of September, October, and November using staff from North Carolina State University, as well as a volunteer based effort from a local organization, The Lake Gaston Association. Depending on the year, the point-intercept survey captured between 5,000 and 6,000 sampling locations which completely covered the entire 350 miles of shoreline contained within the eight major sub-watersheds of Lake Gaston (Figure 1.3.1). At each sample site, a double-sided throw rake was tossed towards the shoreline to collect any submersed aquatic plant species that was present. Surveyors also recorded the presence of any emergent or floating leaf species that were visually observed. The frequency of specific aquatic plant species was calculated for each individual survey year by dividing the number of survey sites in which the target species was present by the total number of sites surveyed. If lyngbya was encountered at a survey site, additional metrics for density were recorded. A density rating (1-4) was assigned to each rake toss based on the amount of biomass present on the rake head (Figure 1.3.2).

Water quality parameters were collected monthly at 35 sites that were distributed across the geological extent of Lake Gaston (Figure 1.3.1) and represented every major sub-watershed and tributary. Average discharge rates for Kerr Lake dam were reported by the US Army Corps of Engineers – Wilmington District and mean monthly flow averages were calculated.

At each sample site, a surface sample was taken to measure nutrient levels and physical water chemistry parameters. A Eurika multiprobe water quality meter measured physical parameters in the field including temperature, dissolved oxygen, pH, chlorophyll-a, and conductivity. Nutrient parameters were measured from water samples that were collected and stored for later chemical analysis at the Weaver Laboratory at North Carolina State University and included total Kjeldahl nitrogen, ammonia, total nitrate/total nitrite, total phosphorus, and orthophosphorus. Total nitrogen levels were calculated by combining total Kjeldahl nitrogen and total nitrate/total nitrite levels. Nutrient samples were kept on ice in the field and then stored frozen until processed. Secchi depths were collected using standard secchi disk methods.

Data processing

Physical characteristics of a system that can influence aquatic fauna distribution, including water depth, shoreline slope, and aspect were exported from sonar data using ciBioBase programing (Valley 2016) and separately interpolated within the waterbody's surface using the "Spline with Barriers" tool in ArcMap's Spatial Analyst toolbox. Water depth was exported in grid format, entered as XY data in ArcMap, and subjected to interpolation using the "Spline with Barriers" (Spatial Analyst) (Foley 2020 and Valley 2016). Shoreline slope and aspect (cardinal direction of shoreline) was determined through manipulation of the rasterized depth data with the appropriate "Slope" and "Aspect" tools in ArcMap's Spatial Analyst toolbox (Foley 2020).

Statistical analyses

All statistical analysis was accomplished in JMP 14.3.0[4] and watershed delineation was performed using ArcGIS Pro. A yearly average for each parameter was reported for all eight major sub-watersheds and were included in the model. A principal component analysis (PCA) was then performed to evaluate the environmental variability among sites and their relationship to lyngbya occurrence and benthic mat densities.

Results

A biplot of principal component analysis (PCA) performed on a set of varying physical features, water quality parameters, and aquatic plant community dynamics found across individual major sub-watersheds within the Lake Gaston system captured 41% of the total variance in the data (Figure 1.3.3). The frequency of lyngbya occurrence across Lake Gaston had close relationships with the distribution of other aquatic plant communities and water quality parameters, while the density of lyngbya mats was more closely related to water quality parameters and physical bathymetric features.

Yearly point-intercept shoreline vegetation surveys conducted between 2019 and 2021 documented the occurrence and distribution of a plethora of aquatic plant species across Lake Gaston. Along with lyngbya, five other species (chara, hydrilla, spatterdock, white water lily, and water willow) were consistently documented across Lake Gaston and represent both beneficial, native species and undesirable, noxious species. Water willow was the dominant species for all three survey years and represented approximately 80% of the aquatic plant community each year. Other beneficial species represented during all three survey years were spatterdock (5%) and white water lily (3%). Chara, a native beneficial microalgae, steadily declined throughout the three year study period representing 27% of the aquatic plant community in 2019 and only 7% in 2021. Lyngbya and hydrilla represent two noxious species found at Lake Gaston and are the focus of significant management efforts within the system. Lyngbya has been the second most abundant species since 2020, consistently representing approximately 30% of Lake Gaston's aquatic plant community. Hydrilla was once the dominant species within the system, however after intense management efforts it now only represents approximately 2% of the aquatic plant community.

Principal component analysis indicated that the frequency of lyngbya occurrence within a watershed was most closely related to the frequency of two native species, spatterdock and white water lily (Figure 1.3.3). This indicates that increases in either of those populations related to an increase in lyngbya frequency. The opposite relationship was found with the occurrence of chara, and increases within the chara community had a strong negative correlated to lyngbya occurrence (Figure 1.3.3; Figure 1.3.4). Water willow was closely associated to the origin of the PCA model, indicating that it did not have a strong influence on any relationships for the other environmental factors (Figure 1.3.3).

A wide range of water chemistry characteristics and nutrient dynamics were studied during the three year study period (See section 2 of final report). The parameters that had the most influence on data variance included Chlorophyll a, ammonia, total phosphorous, Total Kjeldahl Nitrogen, orthophosphorus, pH, and secchi depth. Water quality parameters that were strongly related with lyngbya frequency included Total Kjeldahl Nitrogen (TKN), which exhibited a strong positive correlation with the occurrence of lyngbya, and secchi depth which was negatively correlated to occurrence (Figure 1.3.3). Chlorophyll a and ammonia levels were the most strongly related variables to the density of lyngbya mats, while orthophosphorus was not highly associated with mat density (Figure 1.3.3). Of these water quality parameters, only chlorophyll-a and secchi depth displayed significant differences among watersheds (Figures 1.3.5 – 1.3.8; also see Section 2 of this report, Table 2.2.3).

The PCA also incorporated several physical features of the Lake Gaston system, including flow rates of water entering the system from Kerr Dam and shoreline features such as aspect (the cardinal direction of the shoreline) and bathometric slope of the shoreline. These physical features had a strong correlation to either lyngbya frequency and mat densities. Flow rates were positively correlated with the density of the lyngbya mats (Figure 1.3.3), but displayed a weaker correlation than that of the nutrient levels. For the shoreline features, the cardinal direction of the shoreline displayed a strong negative correlation to the frequency of lyngbya within a watershed. Since aspect values range from 0 to 360(in which 0 is north and in a clockwise direction, 90 is east, 180 is south, and 270 is west), the negative correlation between aspect and density indicates that dense lyngbya mats are correlated to those shorelines that face more in a north and/or eastern direction. Shoreline slope was positively correlated to the density of lyngbya mats, however the close proximity to the PCA model's origin indicates that it is a weak relationship (Figure 1.3.3).

Discussion

We found strong relationships between the distribution and growth levels of lyngbya and the chemical, biological, and physical characteristics of its surrounding environment within Lake Gaston. Individual parameters were strongly associated with either occurrence of lyngbya throughout the system or the density of the benthic mat formations, however no single parameter was closely associated with both. Therefore, those factors that drive the distribution of lyngbya throughout Lake Gaston differ from those that drive increased proliferations of benthic mat formations.

Physical characteristics and water quality parameters of Lake Gaston were both identified as contributing drivers for the distribution of lyngbya throughout the system, while flow regimes were positively associated with mat density levels. Although PCA analysis cannot give us direct correlations between individual parameters, we found that those shorelines facing in a northern and/or eastern direction with low levels of light availability are strongly associated with increased lyngbya frequency within Lake Gaston. These trends are reflected in the contrast between the Pea Hill watershed (located on the northern shoreline of the lake) that has both the highest water clarity and the lowest levels of lyngbya infestation and Smith Creek watershed (located on the southern shoreline) which contained a high occurrence of lyngbya and very low water clarity levels. Similar relationships with flow regime and light availability were reported for lyngbya distribution within the St. Lawrence River system (Lévesque et al. 2012).

Nutrient dynamics within Lake Gaston had varying relationships with both the frequency of lyngbya and the density of benthic mat growth. Hudon et al. (2014) reported on the inconsistency of lyngbya in response to ambient nitrogen and phosphorous concentrations across multiple systems and laboratory settings under varying nutrient gradients. One possible explanation for this variability, is that studies have shown ambient nutrient concentrations of both nitrogen and phosphorous do not reflect levels reported within lyngbya's benthic filamentous mats and therefore lyngbya may be establishing favorable conditions through internal nutrient recycling or release from anoxic sediments (Doyle and Smart 1998; Stevenson et al 2007; Phlips et al. 1992). Our model found that the relationship between lyngbya occurrence and these nutrient parameters were similar to those reported in the St. Lawrence River system (Lévesque et al. 2012), in that high nutrient concentrations did not seem to be the drivers in lyngbya occurrence as seen with other harmful algal blooms and eutrophication. We found that only one of the four nutrient parameters included in the model reported a strong positive relationship to lyngbya occurrence (Total Kjeldahl Nitrogen) and that the other included nutrient parameters produced neutral responses. However, our study did show that these nutrient parameters had more of a relationship on the level of proliferation (density) occurring within benthic mats. Orthophosphorus displayed a negative relationship to the density of benthic mats and could possibly contribute to suppression of mat proliferation. Alternatively, both ammonia and total phosphorous displayed a positive relationship to mat density, indicating that increased levels of both these nutrient forms are related to increased lyngbya mat proliferation in Lake Gaston. While this study did not include factors that could distinguish nutrient loading pathways, both freshwater and marine species of lyngbya have been reported to display a reliance on nutrient inputs from the surrounding watershed (Albert et al. 2005; Lévesque et al. 2012). This reliance on external nutrients, combined with high occurrence levels in areas of reduced light availability, supports the theory that lyngbya can exhibit heterotrophic growth (Burkholder et al. 2008; Lévesque et al. 2012) and could explain the persistence of benthic filaments over the winter months.

We found strong relationships between lyngbya and native aquatic plant community dynamics within Lake Gaston. Previous studies have attributed negative relationships between lyngbya and aquatic macrophytes as either the result of opportunistic establishment of lyngbya into unused niches created by a rapid reduction of aquatic plant biomass (Dick 1989) or that reductions within the macrophyte community were a direct result of increased proliferation of lyngbya (Lévesque et al. 2012). Our study found that the only species to display a negative relationship with lyngbya occurrence was Chara, a native macroalgae species that can form dense beds along the benthos and ranges in height from inches to several feet. Much like lyngbya, chara is not rooted to the benthos and instead uses holdfasts to secure itself to the benthic substrate. Since PCA analysis cannot give us direct correlations between individual parameters, it is unclear if this negative relationship is the result of differing preferences in environmental conditions between these two species or if the presence of chara is somehow impacting the ability of lyngbya to establish and proliferate. Previous studies have attributed the negative relationships between lyngbya and native macrophytes to disrupted nutrient dynamics along the benthos, reduction of light conditions due to shading, and the physical displacement of lygbya mats by macrophyte biomass (Wells et al. 1997; Doyle and Smart 1998). All of these factors could help explain why chara and lyngbya are negatively associated with each other in Lake Gaston. In contrast, we found positive relationships between several floating leaf species within Lake Gaston and lyngbya occurrence. White water lily and spatterdock are both broad leaf species that provide high levels of shade and a root system that is capable of disrupting the nutrients dynamics along the benthos, so it is unclear why these two species are positively related to lyngbya occurrence. One possible explanation is that during the summer and fall months, large floating surface mats of lyngbya are observed drifting across Lake Gaston, and these floating mats then become entangled with any aquatic plant species that it encounters (personal observation of NCSU staff). Therefore, in addition to preferring the same environmental conditions, the positive relationship found between floating leaf species and lyngbya could be strengthened by this observed entanglement. The most prolific species found within the system was water willow, but its high abundance level resulted in it being a poor indicator for lyngbya occurrence.

Although PCA analysis cannot give us direct correlations between individual parameters and lyngbya growth dynamics, it can give us an indication of how lyngbya is responding to the system as a whole. Lyngbya's ability to establish itself over a wide range of geographically and environmentally diverse ecosystems demonstrates why it's such a robust invader and why the parameters of individual systems must be taken into account when trying to manage this species. Similar to previous studies that used Principal Components Analysis (PCA) to identify driving factors for the expansion of aquatic plant species

(Daniel et al. 2006; Lévesque et al. 2012; Nunes et al 2022), we were able to attribute spatial characteristics, system flow, light availability, nutrient dynamics, and native aquatic plant community dynamics to the distribution and density of lyngbya mats. This knowledge will help drive further management efforts by presenting additional factors to consider when identifying and prioritizing treatment areas.

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Figures

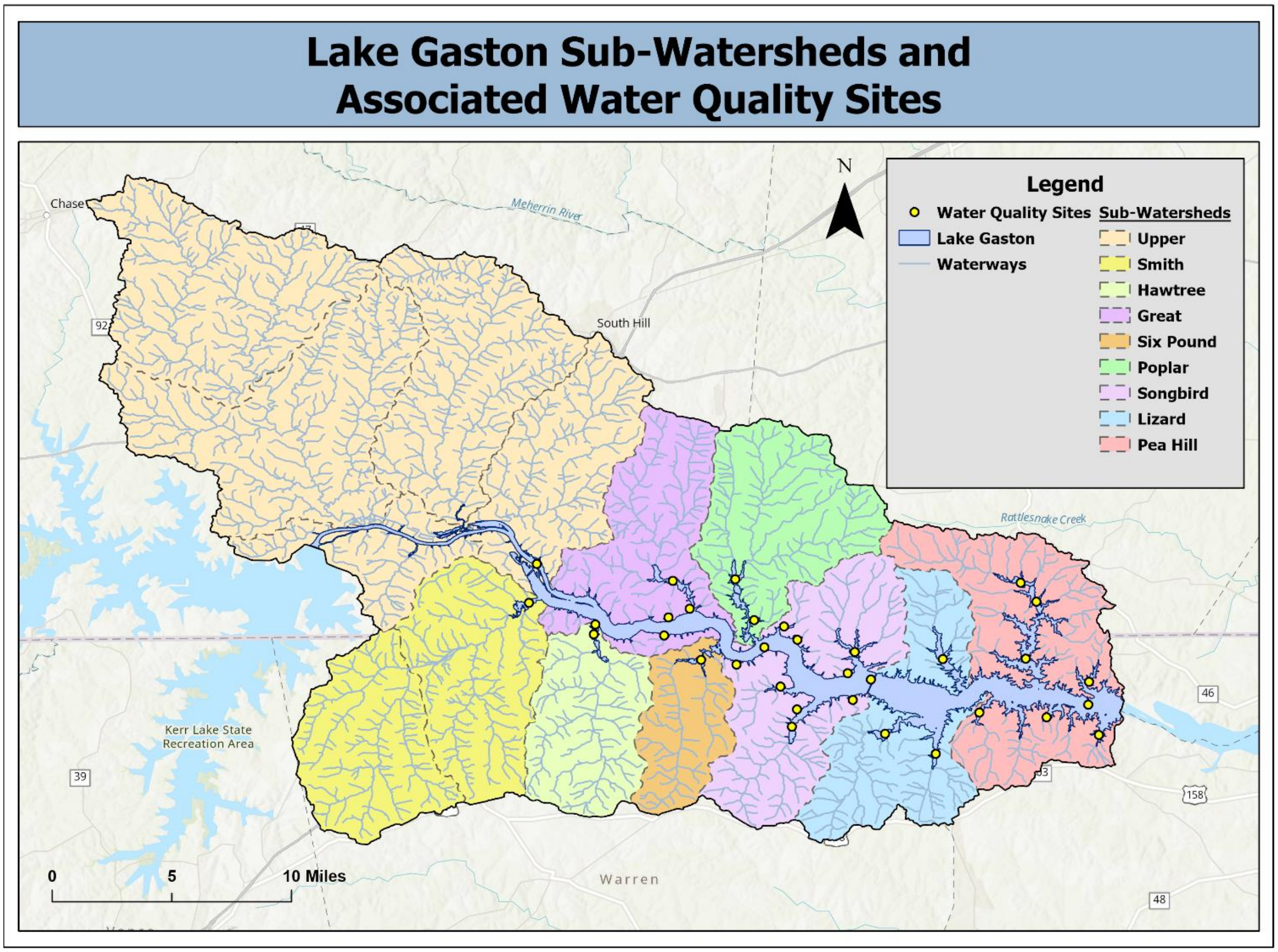

Figure 1.3.1. Map of Lake Gaston's Sub-Watershed and associated water quality sites.

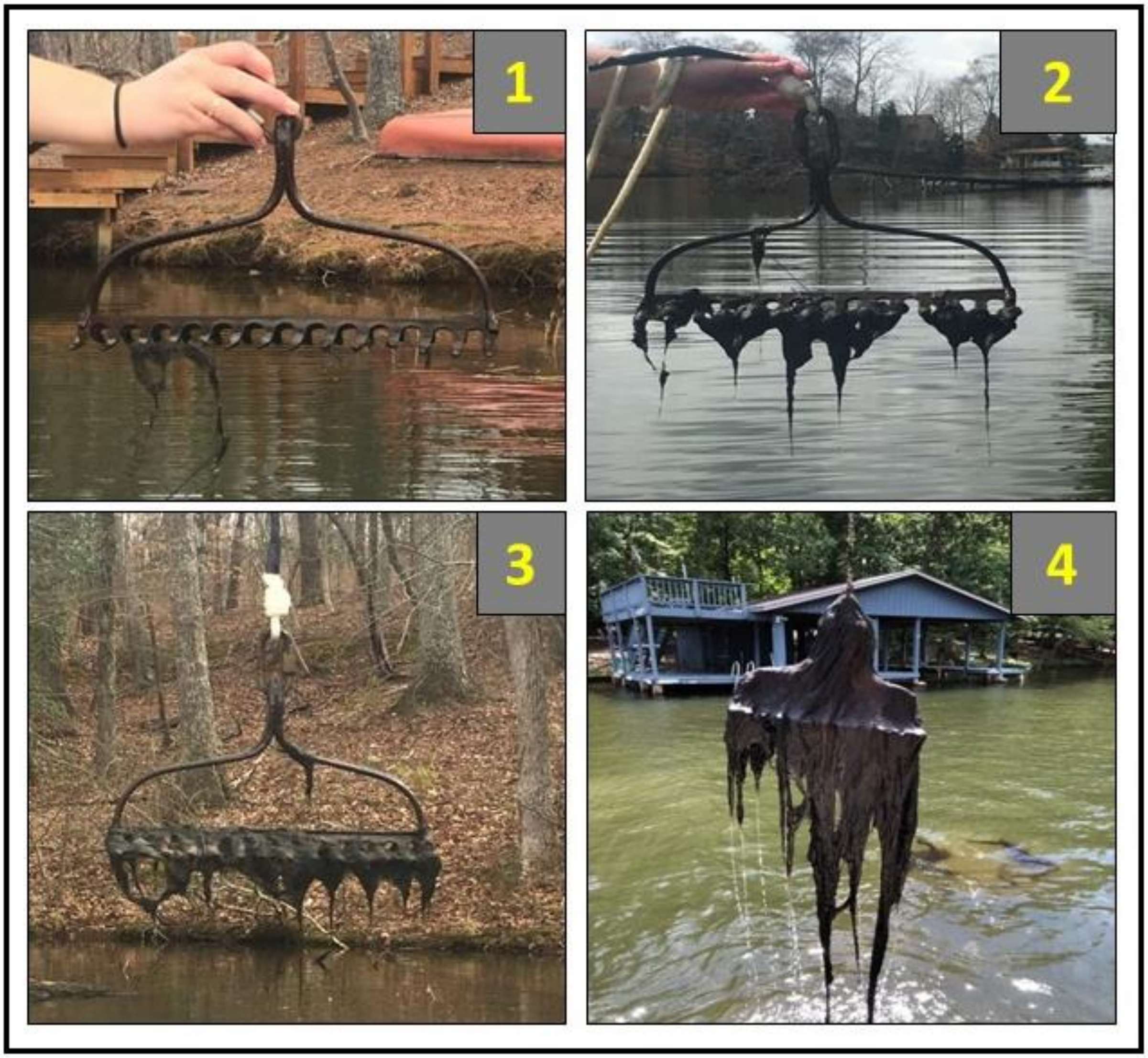

Figure 1.3.2. Images of the four rankings that were assigned to throw rakes to identify the severity of lyngbya benthic mat densities. Rankings were assigned in order of magnitude, with a ranking of 1 indicating that mats were presents but displaying little proliferation and a ranking of 4 indicating dense benthic mats with high levels of proliferation.

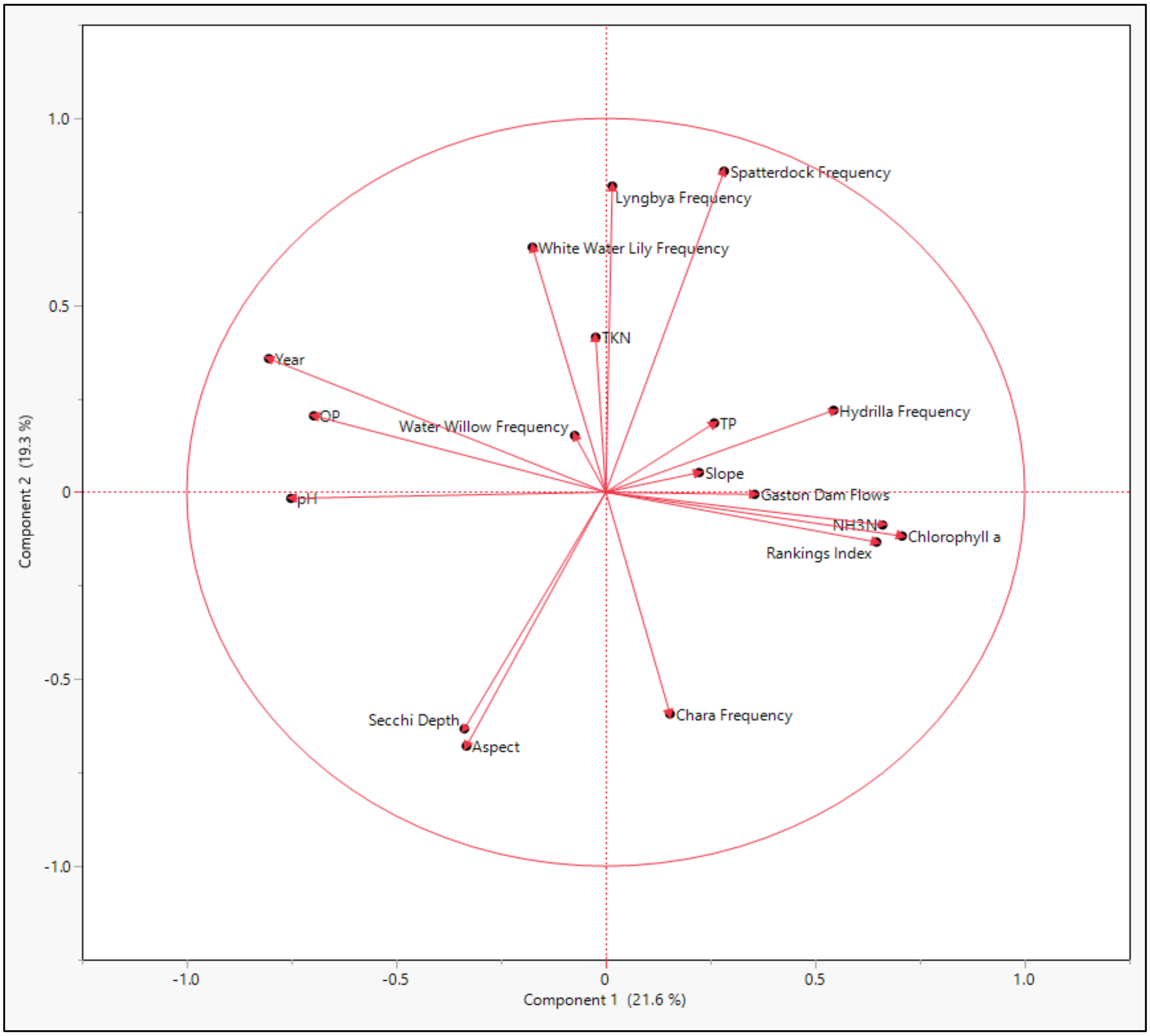

Figure 1.3.3. Biplot of the principal component analysis (PCA) based on correlations. Arrows represent environmental variables. The axes represent 41% of the data variance. TKN (Total Kjeldahl Nitrogen), TP (Total Phosphorus), NH3N (Ammonia), and OP (Orthophosphorus) represent nutrients found in aquatic systems that support the growth of aquatic plants. Ranking index refers to the density of lyngbya identified at various SAV point-intercept survey sites. Slope refers to the bathymetry of the system and aspect refers to the cardinal direction in which the shoreline is facing.

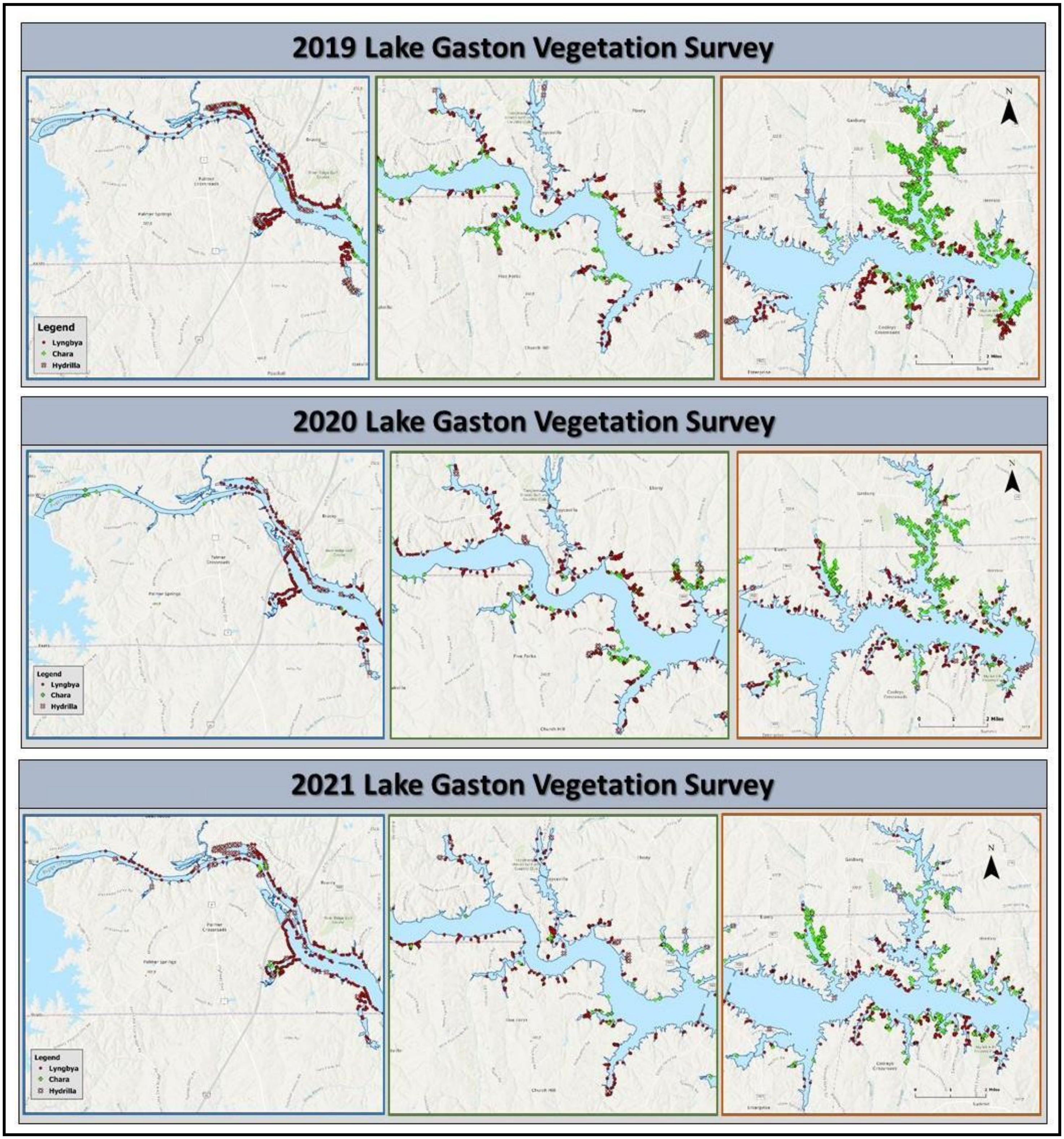

Figure 1.3.4. Results from 3 consecutive years of SAV point-intercept survey at Lake Gaston, NC/VA. Results show temporal differences between lyngbya (noxious species), chara (native species), and hydrilla (federally listed invasive species).

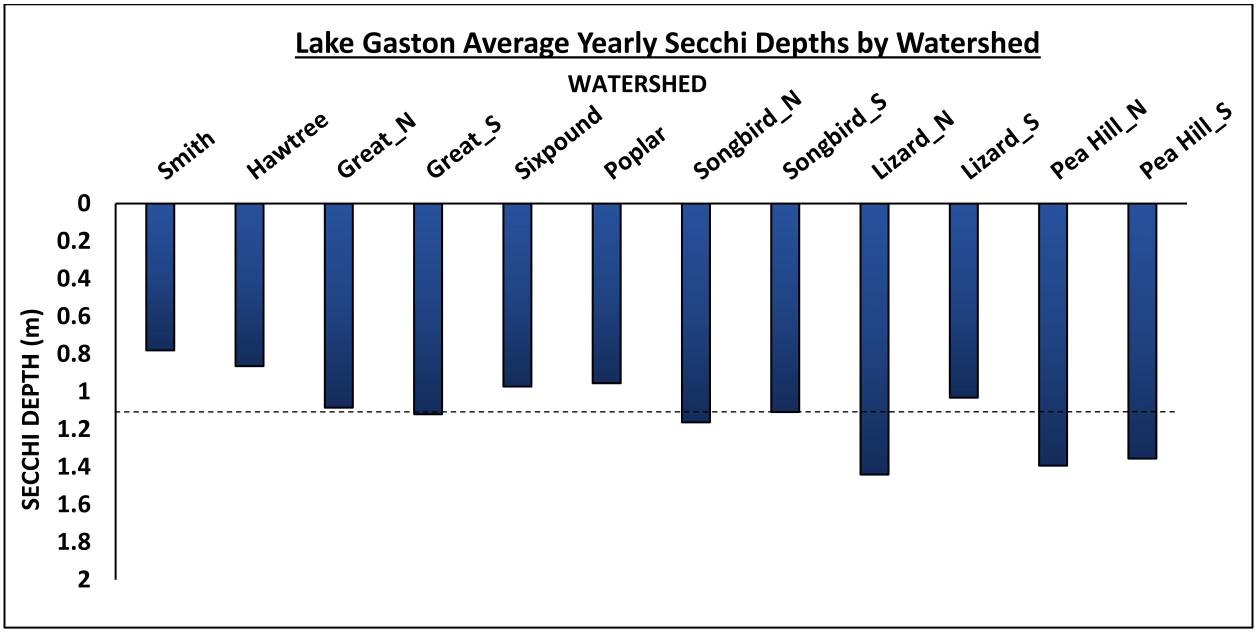

Figure 1.3.5. Average secchi depths reported for Lake Gaston's associated watersheds between 2019 and 2021. The dotted line represents the overall lake average for secchi depths (1.2 m). Watershed data is reported in order of most upstream to downstream locations and watersheds that encompass both the northern and southern shorelines are divided into two independent sub-watersheds (N, S).

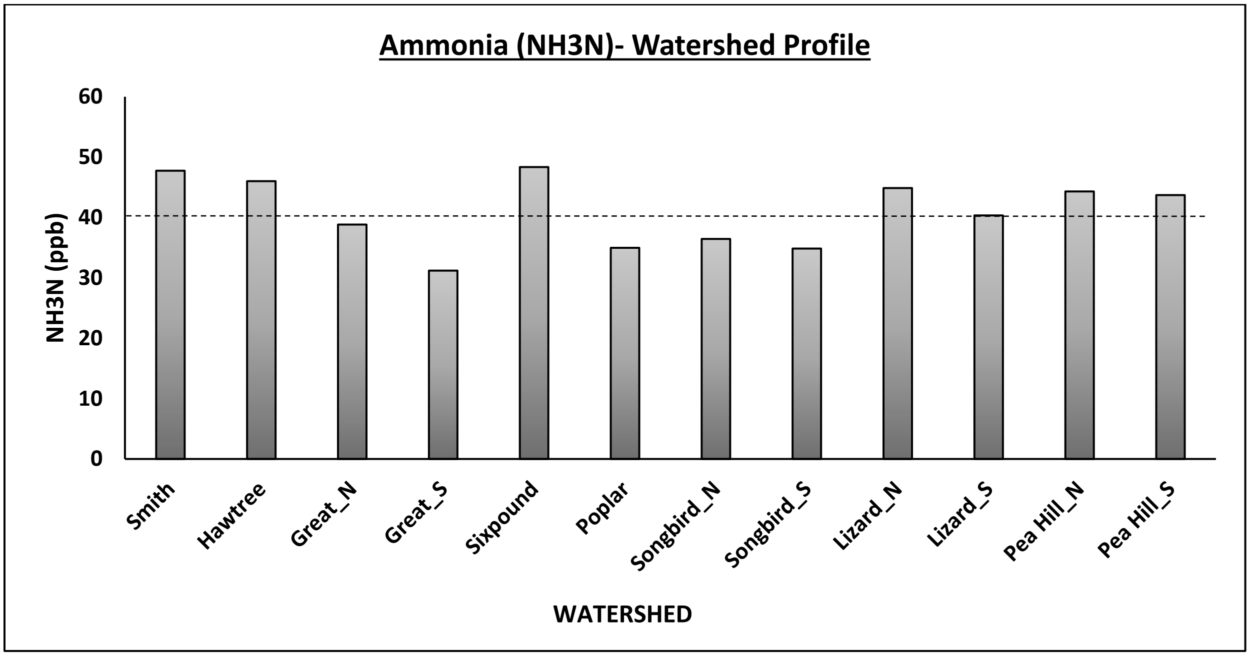

Figure 1.3.6. Average ammonia (NH3N) values reported for Lake Gaston's associated watersheds between 2019 and 2021. The dotted line represents the overall lake average for total ammonia (40 ppb). Watershed data is reported in order of most upstream to downstream locations and watersheds that encompass both the northern and southern shorelines are divided into two independent sub-watersheds (N, S).

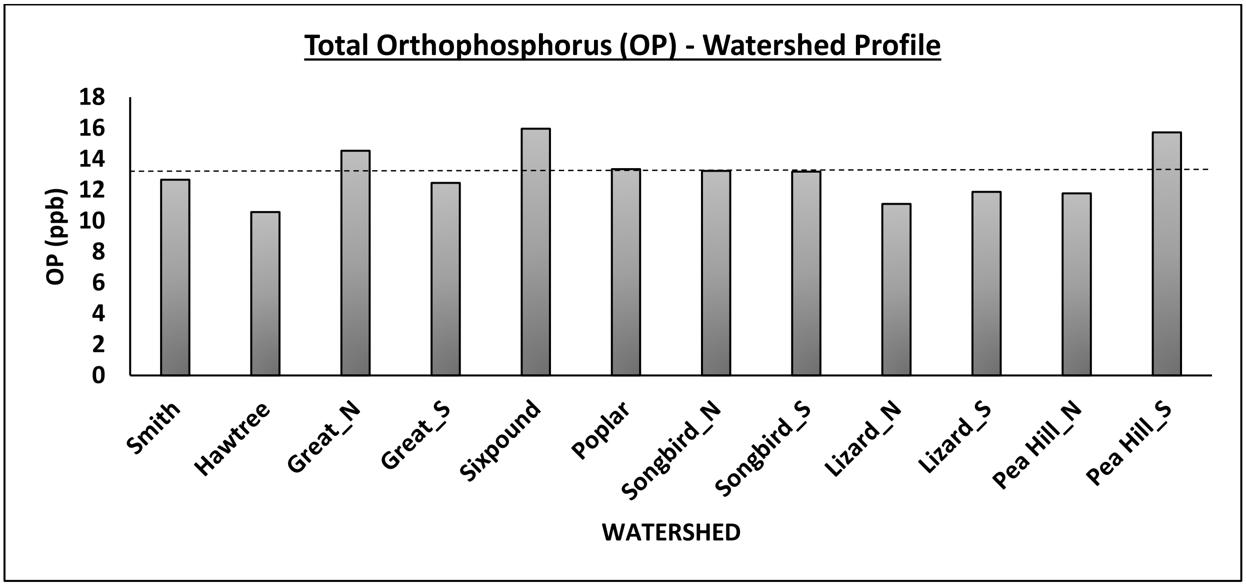

Figure 1.3.7. Average orthophosphorus (OP) values reported for Lake Gaston's associated watersheds between 2019 and 2021. The dotted line represents the overall lake average for total orthophosphorus (13 ppb). Watershed data is reported in order of most upstream to downstream locations and watersheds that encompass both the northern and southern shorelines are divided into two independent sub-watersheds (N, S).

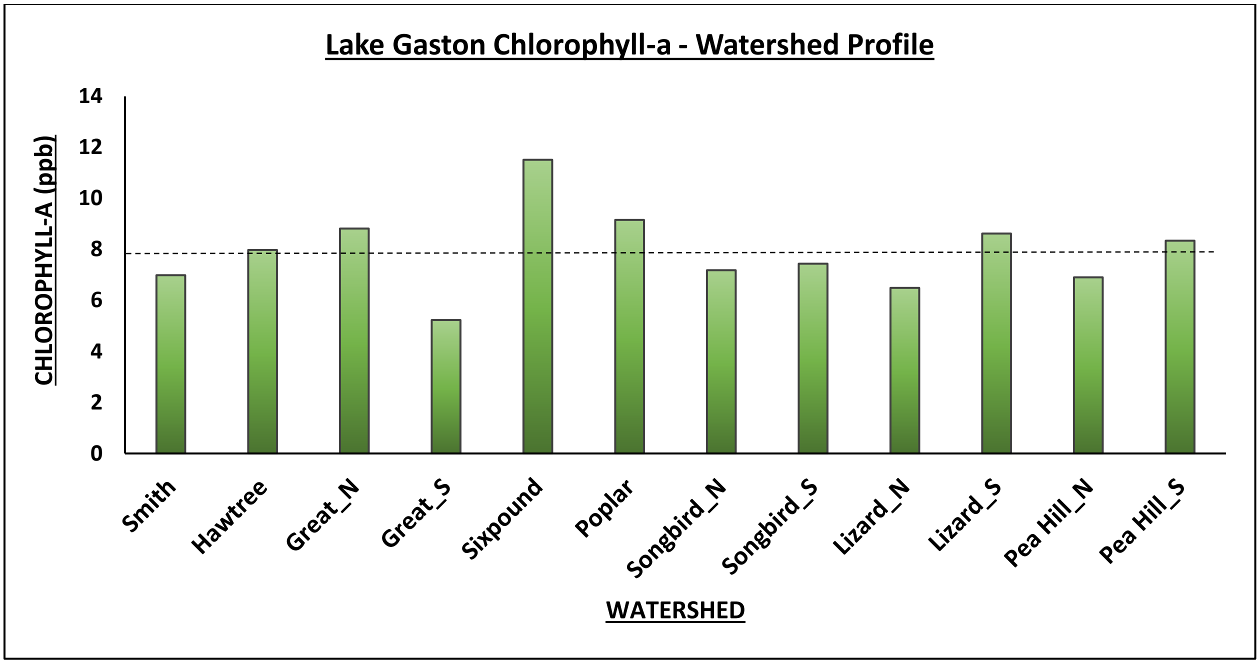

Figure 1.3.8. Average chlorophyll-a values reported for Lake Gaston's associated watersheds during 2020 and 2021. The dotted line represents the overall lake average for chlorophyll-a (7.9 ppb). Watershed data is reported in order of most upstream to downstream locations and watersheds that encompass both the northern and southern shorelines are divided into two independent sub-watersheds (N, S).

1.4 EFFICACY OF LYNGBYA TREATMENT PROGRAM

Introduction

The free-suspension filamentous cyanobacterium, *Lyngbya wollei* (hereinafter, lyngbya), has become increasingly problematic among southeastern waterways (Anderson et al. 2019; Smith et al. 2019). Nuisance populations of lyngbya have the ability to produce robust mat-like formations which negatively impact aquatic ecosystems by reducing availability of light to the benthos, altering water quality (e.g., pH and dissolved oxygen), and decreasing available habitat for fish, macroinvertebrates, and submersed flora (Mastin et al. 2002; Tourville Poirier and Cattaneo 2010). Additionally, littoral abundance of lyngbya often impedes recreational activities (e.g., boating; swimming; fishing) and depreciates the aesthetic value of shoreline homes due to unappealing surface mats. The cyanobacterium can also produce organic compounds that create foul odors and negatively alter the taste of drinking water (Tabachek and Yurkowski 1976); thus, further impacting the anthropogenic use of a reservoir system and prompting human health concerns (Smith et al. 2019). The negative impacts associated with lyngbya have increased demands for an effective management strategy within highly developed, multi-use reservoirs such as Lake Gaston (Tabachek and Yurkowski 1976; Anderson et al. 2019; Hudson et al. 2014).

Algaecides have remained the preferred option for filamentous algae management for more than a century (Moore and Kellerman 1904), and have been widely utilized to control algal growth among complex lentic and lotic systems (Rogers and Pietruszewski 2021). Prior research has shown chelated-copper based algaecides provide > 90% control of lyngbya in laboratory evaluation settings (Bishop et al. 2015; Calomeni et al. 2018). However, influences of algae morphology and ecological factors in field scenarios, such as dense filamentous mat formations and benthic conditions, can greatly alter algaecide efficacy (Lembi 2000). The success of algaecide treatments has been directly correlated with lyngbya growth and colonization, with treatment success declining as algal biomass increases (Bishop and Rodgers 2012; Calomeni et al. 2018). Lyngbya populations commonly mimic the perennial growth patterns expected of vascular plants since location and seasonality (i.e., winter to summer) influences lyngbya presence and abundance (Beer et al. 1986, Bridgeman et al. 2012). The timing of growth and proliferation varies regionally with maximum lyngbya biomass accumulation occurring during the warmer summer months (Bridgeman and Penamon 2010; Speziale et al. 1991).

Efficacy of field based algaecide applications have demonstrated some success in smaller systems (< 12,500 acres), such as within the Coosa River system (AL) when copper complexes were applied at 22.7 to 64.4 L of product (Captain XTR®, SePRO Corporation) per surface acre (Anderson et al. 2019). A similar study investigating copper algaecide treatments on lyngbya showed 0.3 mg Cu L^{-1} effectively reduced algae viability by ~90% in Lay Lake, AL (Bishop et al. 2015). Nevertheless, varying results can occur in larger lake systems due to factors that remain under investigation. These factors include environmental parameters, algaecide application techniques, and possible genotypic variation in algal populations (Lake Gaston, NC; personal observation). There remains a clear need to evaluate the currently available algaecide formulations, rates, and application techniques (e.g., treatment frequency) under operational field conditions that perform similar to previous small-scale laboratory evaluations.

While small-scale laboratory studies generally investigate lyngbya filament response to algaecides on a small scale under controlled conditions, several complexities exist with field-based assessments. In general, detecting and quantifying filamentous algal species has proven challenging, as phenological shifts and fluvial patterns can occur with frequency (Depew et al. 2009). Past studies evaluating algaecide treatment success in lake settings have utilized traditional rake-toss techniques commonly deployed for vascular plant surveys (e.g., point or line intercept techniques). Bishop et al. (2015) and Anderson et al. (2019) used rake-toss assessments to gauge lyngbya biomass flux pre- and post-treatment to evaluate algaecide performance. However, these traditional survey techniques can be inconsistent and may not be the most appropriate evaluation tactic. Unlike submersed aquatic vegetation (SAV) like hydrilla (*Hydrilla verticillata* L.f. Royle), filamentous algae are not rooted and continuously move within and around the waterways they occupy via gliding mobility on a small scale and hydrodynamics of the system on a larger scale (Hoiczyk and Hansel 2000). Therefore, rake-toss evaluations made within a given algaecide treatment zone may not only represent algal response to treatment but also represent shifts in mat locations due to environmental, diurnal, or phenotypic variability. Inherent variability is expected in field-based surveys; however, variability could be compounded due to subjectivity when conducting rake-toss surveys since rake biomass represents a finite area within the surveyed area. Assessment methods that use point-intercept rake-toss and biomass rake sampling commonly used for SAV evaluation (Madsen 1999; Johnson and Newman 2011) are likely limited in the spatiotemporal assessment ability for lyngbya due to the complex and varying distribution patterns of the mats.

As mentioned, the scale of traditional point-intercept evaluations ultimately limits repeated measurements that could reduce accurate estimates of lyngbya phenological shifts or response to management. Visual assessment of filamentous algae is permissible when mats are at, or near, the surface of the water column. Still, nuisance populations of lyngbya are often undetected until surface mats emerge thus, current detection techniques limit the scale of traditional survey techniques. Over the past several decades, echosounding techniques have been successfully utilized to quantify SAV (e.g., invasive hydrilla) (Maceina et al. 1984; Valley and Drake 2005; Howell and Richardson 2019). Depew et al. (2009) successfully detected and characterized benthic filamentous algal stands of *Cladophora* spp. on hard benthic surfaces using high-frequency echosounding measures. However, literature is limited on the ability to identify and quantify nuisance cyanobacteria populations over time within lentic environments.

Our objectives for evaluating algaecide efficacy of the Lake Gaston treatment program were to 1) identify a algalcide treatment method that is effective in reducing lyngbya growth and that translate success into operational field scenarios, 2) determine appropriate methods for monitoring field treatment success, and 3) identify temporal population shifts of lyngbya that influence treatment application success.

Efficacy Evaluations

I. Laboratory Evaluation

Laboratory evaluations of algaecide treatment protocols allow managers to address the complexity of factors that can influence the efficacy of a treatment program and help direct field-based applications operations. Treatments performed in a controlled setting can determine the effectiveness of specific chemical treatment protocols without the compounding environmental factors such as turbidity, flow rates, pH, and conductivity that can negatively impact overall efficacy (Richardson and Haug 2018). Chelated-copper based algaecides have proven effective in negatively impacting lyngbya in a laboratory setting (Bishop et al. 2015; Calomeni et al. 2018), however few studies have translated those positive results into field based scenarios (Bishop et al. 2015).

The objectives for laboratory evaluations were to 1) determine efficacy of algaecide treatment protocols used within Lake Gaston's treatment program in a controlled environment and 2) determine if similar responses would be observed with lyngbya located in systems that are geographically separated from Lake Gaston.

Laboratory Methods

Lyngbya for this trial was collected from three separate North Carolina reservoirs located within two independent watersheds (Figure 1.4.1). Tuckertown Reservoir (2,500 acres) and Badin Lake (5,350 acres) are located in series on the Yadkin-Pee Dee River, with the Tuckertown Reservoir dam located at the uppermost end of Badin Lake. Lake Gaston is a 20,000 acre lake that is located on the main stream of the Roanoke River and is second in series between Kerr Lake (50,000 acre) and Roanoke Rapids Reservoir (4,600 acre).

Lyngbya and site water samples were collected from all three lakes. For Badin Lake and Lake Gaston samples, lyngbya was collected utilizing a double sided rake that gently pulled mat material off the benthic substrate. Due to the reduced health levels of benthic mats from Tuckertown Reservoir, surface mats were collected and used for this trial. After field collection, lyngbya material and associated hydrosoils were transferred to a ziplock bag, placed on ice, and brought back to the lab where it was thoroughly washed to remove detritus and soil from mat filaments. Lyngbya samples were placed in site water and acclimated within a growth chamber that was maintained at 25 °C with a 14:10-h light:dark cycle for the duration of the experiment.

To ensure the overall health of lyngbya prior to treatment, 0.25 g fresh weight of clean lyngbya mat material was evaluated at 40x magnification. Filaments were spread evenly over a 12x12 cm area and filaments that contained sheaths lacking viable cells or predominately chlorotic cells were removed and replaced with healthy living material. A final visual assessment was performed by two independent readers in which each sample was evaluated at 40x magnification and viability levels were averaged across samples. A criterion of > 90% viability for each sample was established for treatment to occur. The 0.25 g fresh weight of mat material was then transferred to a 40 mL test tube containing 30 mL of respective site water and allowed to acclimate for 7 days prior to treatment.

Lyngbya collected from each of the three lakes was exposed to a sequential series of herbicide treatment exposures utilizing chelated copper-based algaecides and adjuvants. Treatment units and an untreated control unit for each of the three lakes was represented by four replicates per treatment exposure. Additional vials containing untreated lyngbya were evaluated prior to the beginning of the experiment to establish a baseline of health, referred to as a pre-treatment harvest (PH). Treatment combinations included Captain XTR® + AMP, Cutrine Ultra® + AMP, and Captain XTR® + Reward®. Both Captain XTR® and Cutrine Ultra® are chelated copper formulations with copper ethanolamine complex as the active ingredient, AMP® has a blend of proteins and surfactants as active ingredients, and Reward® has an active ingredient of diquat dibromide. All were applied at maximum label rates with an exposure time of 48 hours. Following the herbicide exposure period, lyngbya was gently removed from each vial, dabbed dry, suspended and washed in a beaker of fresh DI water, removed, and patted dry prior to being placed in sterile 40 mL test tube containing 30 mL of untreated site water**.** Evaluation of injury to material was performed 10-days post-treatment to assess lyngbya response to each treatment combination and control. A series of three treatments were performed on each sample unit in consecutive order and occurred 14-days after the previous treatment date.

ANOVA followed by Tukey's HSD tests were performed to identify potential differences in treatment efficacy between lakes. Temporal changes were also determined over the span of three consecutive treatments within each lake. As previously discussed, temporal responses were determined for individual herbicide treatments and control units by assessing viability levels for each lake ten days following every round of treatment. These three evaluations will be further referred to as T-1, T-2, and T-3. Viability levels were then further compared to baseline samples. Lyngbya fresh weight measurements were taken both prior to the first treatment and following the last treatment in the three treatment series. A percent change in fresh weight for each sample unit was calculated.

Laboratory Results

All treatment combinations significantly decreased lyngbya viability 10-days following the third treatment exposure when compared to the pre- treatment harvest (PH) measurements ($p < 0.001$). Lyngbya collected from Lake Gaston and Tuckertown Reservoir showed a significant response in terms of decreased viability following the third round for all three treatment combinations when compared to the control ($p < 0.001$) (Figure 1.4.2). For Lake Gaston lyngbya, no individual treatment combination impacted viability at a significantly greater level when compared to other treatments (Figure 1.4.2). The same response is shown for Tuckertown Reservoir lyngbya (Figure 1.4.2). Lyngbya collected from Badin Lake experienced overall stress following the third round of treatment exposure and displayed a reduction in viability for both the treated and control units. No single chemical combination showed a significant reduction in viability for Badin Lake lyngbya 10-days following the third treatment exposure when compared to the control (Figure 1.4.2). Decreased viability of Lyngbya visible at x40 magnification was not visible at a macroscale (Figure 1.4.3).

Temporal responses to individual treatment exposures varied among treatment combinations and lakes (Figure 1.4.4). Viability for individual herbicide treatment combinations was classified for each lake following every round of treatment exposure (T-1, T-2, and T-3).

Lyngbya collected from Lake Gaston and treated with a combination of Captain XTR® + Reward® or Cutrine Ultra® + AMP® displayed decreases in viability between each round of treatments (Figure 1.4.4). Response in viability to the herbicide combination of Captain XTR® + AMP® was only noted 10-days post the first and third exposure events (Figure 1.4.4). Viability within the control units remained at or above 89% for the entirety of the experiment (Figure 1.4.4).

Lyngbya collected from Tuckertown Reservoir and treated with a combination of Captain XTR® + Reward® or Cutrine Ultra® + AMP® displayed decreases in viability following the first treatment exposure but no subsequent treatments produced additional injury (Figure 1.4.4). A decreased response in viability to the herbicide combination of Captain XTR® + AMP® was only noted 10-days following the first and third treatment exposures (Figure 1.4.4). After an initial slight decrease in viability within the control units, levels steadily increased throughout the remainder of the study (Figure 1.4.4).

Lyngbya collected from Badin Lake displayed a decrease in viability following the first treatment exposure for all treatment combinations (Figure 1.4.4). Unexpectedly, following the second algaecide exposure, viability measurements improved across all treatment combinations (Figure 1.4.4). However, all units, including the control, decreased significantly in viability levels 10-days post the third treatment exposure (Figure 1.4.4).

Changes in lyngbya fresh weights (% change) varied between lakes and treatment combinations (Figure 1.4.5). All treatment units for Lake Gaston lyngbya experienced an increased change in fresh weights indicating growth, with the exception of Captain XTR® + Reward® which decreased slightly, indicating a reduction in total lyngbya biomass (Figure 1.4.5). All treatment units and the control unit for Badin Lake experienced decreased percentages in fresh weight (Figure 1.4.5). For Tuckertown Reservoir, the untreated control unit displayed an increased change in lyngbya fresh weight and all treatment combinations experienced a decreased change in fresh weight (Figure 1.4.5). These results for Tuckertown Reservoir are what we would expect to see in terms of changes in biomass. However, it should be noted that biomass alone does not indicate the health of the sample. Empty sheaths without viable cells will still have biomass.

The results presented here are from the first of two planned runs using identical treatment protocols. The second run completed the three consecutive treatment series, however when lyngbya was retrieved 10-days post the third treatment exposure it was discovered that the incubator thermostat unit had malfunctioned. All lyngbya units had been exposed to air temperatures up to 55°C (131 °F) for an unknown amount of time. This resulted in all data collected from the second run becoming invalid, however there was an interesting observation made from this run. Visually, it was observed that all control units from each rep experienced a greater negative impact of the extreme temperatures than the treated units (Figure 1.4.6). Treated units were still visibly green indicating living cells were present while control units were brown and chlorotic. These visual results were confirmed under 40x magnification (Figure 1.4.6). It is unclear as to why the response of treated versus untreated lyngbya varied so drastically, but the potential of a defensive response of lyngbya to chemical algacide treatments should be explored further.

II. Field Evaluation – Treatment Applications

Chemical Treatment Protocols

This study evaluated the effectiveness of multiple distinct algaecide treatments for lyngbya control applied at Lake Gaston between 2019 and 2021. Algaecides used for treatments originated from the manufacturers: SePRO, Applied Biochemist (formally Lonza and currently SePRO), and UPL (formally UPI)/Biosafe. Treatment protocols included using the following herbicides in varying combinations: AMP®, Algimycin®, Captain XTR®, Cutrine Ultra®, Green Clean®, Hydrothol®, and Reward®. Captain XTR® and Cutrine Ultra® are chelated copper formulations with copper ethanolamine complex as the active ingredient. Algimycin® is also a chelated copper formulation, but has the active ingredients of chelates of copper gluconate and copper citrate. All chelated copper formulations were applied in combination with either AMP®, which has a blend of proteins and surfactants as active ingredients, or Reward®, which has an active ingredient of diquat dibromide. Hydrothol® has an active ingredient of Mono (N,N-dimethylalkylamine) salt of endothall and was used in combination with Green Clean® which has an active ingredient of hydrogen peroxide. Specific treatment attributes followed manufacturer guidelines while sites were determined by mutual decision between NCSU and manufacturer. Each product was applied monthly (approximately every 30 days) and exact application methods are detailed in the yearly treatment summary and recommendations reports prepared by each algaecide applicator. The timing of treatment initiation, application methods, application rates, site location, and product protocol varied from year to year as methods were modified and improved upon based on the previous year's results (Table 1.4.1). Treatments levels became operational during the 2021 treatment season and treatment expansion sites were prioritized by proximity to boat ramps and dry hydrants, density of boat docks, public complaints, and level of lyngbya infection. Locations of treatment sites evaluated for this study can be found in Figure 1.4.7 to Figure 1.4.12.

Application Methods

Traditional methods of herbicide application in an aquatic system include use of a weighted trailing hose system in which herbicides are pumped through a series of heavy submersed trailing hoses and injected into the water column at a specific depth from the water surface. This methods has proven successful in controlling other submersed aquatic plant species, such as hydrilla, and was utilized during the 2019 and 2020 lyngbya treatment seasons. However, lyngbya is highly associated with the benthos and this method may lose efficacy for deeper treatment sites based on the bathymetry of treatment areas and hose length limitations. One major modification to the lyngbya treatment protocol was a switch in application methods during the 2021 treatment season to an autonomous system in which a hydraulic injector system deployed product based on the bathymetry of the treatment area and algaecide was injected within the bottom 2 feet of the water column (Table 1.4.1; Figure 1.4.13). This autonomous system was also deployed using airboat technology as opposed to the traditional trailing hose method which was applied by an outboard motor powered fiberglass boat.

Application Methods – Evaluation of Copper Fate

Following an algaecide application, the ultimate fate of copper within a treatment area can be dependent on algae biomass, suspended sediments, and water movement (Willis and Bishop 2016). With the use of two distinctly different injection systems for algaecide application during this study, we wanted to determine if application method would also influence how copper dispersed post algaecide application. Previous studies (Buczek et al. 2019) evaluated the movement of copper within Lake Gaston following algaecide application that utilized the traditional weighted trailing hose method applied by an outboard motor powered fiberglass boat. Temporal changes in copper concentrations post application by an autonomous system that utilized an airboat were evaluated during the course of this study.

<u>**Methods**</u>

For each application method, ambient water samples were taken at the surface, middle, and bottom of the water column at 12 site locations throughout the treatment areas at multiple time periods post application (0 hours after treatment (HAT), 1 HAT, 2 HAT, 3 HAT, 4 HAT, 6 HAT, 24 HAT, 48, HAT, and 96 HAT). These individual samples were transferred back to the laboratory where analysis was performed and copper concentrations were determined. Buczek et al. (2019) evaluated copper concentrations within treatment areas at Lake Gaston that received applications of Captain XTR® + Reward® at target copper concentration rates of 0.25 and 0.5 mg/L during the 2018 treatment season. The autonomous system was evaluated based on a 2021 application of Captain XTR® + AMP® with a target copper concentration rate of 0.8 mg/L. There is one difference to note regarding the collection method for water samples between the two studies, Buczek et al. (2019) utilized a bilge pump method in which water samples were pulled from a specific water depth and our study utilized a Van Dorn water sampler for deep water collections.

Copper analysis can be a costly way to determine the impacts water movement and exchange have on aquatic herbicide dispersion. Therefore, Rhodamine dye was used in conjunction with the algaecide application during the evaluation of the autonomous application system to determine if dispersal patterns and concentrations could be predicted in-situ using this inert tracer fluorescent dye. Studies have shown the dispersal of Rhodamine dye through aquatic applications significantly mimics applications of common aquatic herbicides including fluridone and endothall (Fox et al. 1991; Fox et al. 1993; Getsinger 2013), however, to the authors knowledge, this correlation has not been determined with chelated-copper based applications.

Results

Overall, copper concentrations did not meet or exceed the targeted rates using either the traditional trailing hose application method or the autonomous system (Buczek et al. 2019)(Figure 1.4.14). Concentration levels for the trailing hose deployment system were closer to the targeted rates than the autonomous system, reaching 50% of the targeted rate at 2 HAT versus 11% of the targeted rate at 1 HAT for the autonomous system. However, the low concentration of ambient copper detected with use of the autonomous system could be a reflection of using the adjuvant AMP® in combination with Captain XTR® instead of Reward® which was used during the 2018 treatment application. AMP® is used to increase the adhesiveness of copper to lyngbya filaments and thus would concentrate copper in the benthos and not suspended in the water column where samples were collected. Low concentration levels were also reflected within the Rhodamine dye data with concentrations at 0 HAT measuring approximately 35% of expected levels despite having dye that was visually present following application (Figure 1.4.15). This low concentration rate could be a result of dye suspension located at a water depth that was not directly sampled. Although concentrations were below expected levels, similar results were found regarding the fate of copper within the treatment areas for both studies. Buczek et al. (2019) reported that copper concentration and movement was site dependent, but overall copper was not highly available at 48 to 96 hours post application. Similar results were found with the autonomous system and at 48-hours post application copper was not highly available and Rhodamine dye concentrations were deemed insignificant in terms of detection (1 ppb) (Figure 1.4.14).

III. Field Evaluation – Treatment Efficacy

Sampling Methods

Several sampling methods were utilized to evaluate the efficacy of lyngbya treatments within Lake Gaston during the 2019, 2020, and 2021 seasons (Figure 1.4.16). Traditional assessment methods used to evaluate submersed aquatic vegetation, including rake-tosses and biomass rakes (Madsen 1999; Johnson and Newman 2011), were evaluated. In addition to traditional sampling methods, two novel sampling methods were also evaluated. A BioSonics MX Scientific Echosounder (sonar unit) (hereinafter, BioSonics) was used to provide a quantitative assessment of water column occupancy (e.g. height and dimensions of lyngbya mat) and an unmanned aerial system (UAS) was used to assess surface mat formations. During the 2020 treatment season, an additional method, cellular viability, was incorporated into the evaluation protocol.

During preliminary surveys, it was determined that two of the aforementioned methods produced inadequate data and were therefore removed from the sampling protocol. The first method utilized traditional rake-tosses and was removed due to the variability and subjectivity that comes from dragging rakes over non-rooted algal mats. Figure 1.4.16 (A) highlights the level of mat material sampled using a traditional rake-toss in comparison with the level of lyngbya detected in the benthos using sonar. The second method was use of UAS which did not provide adequate metrics for lyngbya detection due to environmental constraints.

Biomass Sampling

Lyngbya biomass was collected using a double-sided twist rake approach. The double rake head, created by welding two steel garden rake heads together, was attached to a PVC pole and lowered to the benthos before being rotated 360 degrees to collect any lyngbya biomass that was present. The sample was brought to the surface, washed thoroughly over a mesh sieve to remove extra soil and detritus present in the mat material, and then all water was wrung out of the mat before an overall fresh weight was collected for the biomass sample. This process was repeated twice at each site and the average of those two biomass weights was calculated to determine the overall biomass for each site. All sample sites were contained within the treatment areas and were sampled within 10 feet of the original GPS coordinates. Biomass was sampled multiple time throughout the treatment season, including pre and post treatment samples, at sites located within all algaecide treatment protocols. Biomass samples were also collected within control areas located outside of the treatment areas that were devoid of any direct control measures.

Hydroacoustic Sampling

Utilizing a BioSonics MX Scientific Echosounder (BioSonics), a quantitative measurement of water column occupancy (e.g. height and area dimensions of lyngbya mat) were recorded pre- and post-treatment at selected intervals. The echosounding unit utilizes a 204.8-kHz single frequency transducer with 8.5° beam angle that is capable of 5-Hz refresh rate. This system uses a narrow sensing swath which delivers greater detail of objects in the water column than what might traditionally be found using a wider beam angle found on consumer units (e.g. 20° beam angle found on Lowrance echosounders). The internal DGPS receiver allows for acoustically derived data to be georeferenced over time with other field-based sampling measures conducted throughout the treatment period. BioSonics sampling occurred monthly to estimate lyngbya growth, or succession, following each algacide treatment interval.

Prior to initial sampling, parallel GPX tracks were developed using mapping software and were then uploaded to the research vessel autopilot navigation system (Lowrance HDS-9 Carbon). Prearranged transects allowed for direct spatial occupancy comparisons over time within each treatment zone. Within scanned sites, the serpentine transect with 20 m (65.6 ft) spacing was navigated at a boat speed of ˜6.4 ± 2 km h^{-1} (4-6 MPH) to delineate area interpolation of bathymetry and lyngbya mat abundance. The DT4 sonar logs representing each site and time point provided unique identification for post-processing analysis. All logged data collected from BioSonics Visual Acquisition software were viewable and saved via a linked Panasonic Toughbook in real-time (Figure 1.4.16). To ensure outputs of the echosounder accurately assessed lyngbya mat locations, randomly selected locations in each plot were sampled for lyngbya presence using a throw rake. Lyngbya abundance, or absence, was notated in the sonar log to aid confirmation of lyngbya mats during analysis.

Viability Sampling

Lyngbya was collected post treatment season (2020 and 2021) within a subset of treatment and control areas to determine the overall cellular health of the filaments (Figure 1.4.17). Although samples were taken from a subset of treatment areas, both Captain XTR® and Cutrine Ultra® treated areas were represented. Samples were collected by gently pulling a throw rake across the upper portion of the benthic mat material, transferring that material into ziplock bags, and holding it on ice for transport back to the lab. To prevent damage, samples were imaged within 24 hours of collection and therefore a limited number of samples were processed due to time constraints associated with utilization of laboratory equipment. Images for the 2020 treatment season were captured by the NCSU Center for Applied Aquatic Ecology lab and images for the 2021 treatment season were analyzed by the NCSU Aquatic Plant Management Program. Samples for the 2020 treatment season were simply classified as viable or non-viable to determine overall treatment efficacy between treated and control areas. However in 2021, this method was modified to allow for quantitative comparison between lyngbya samples that were treated with Captain XTR® and those treated with Cutrine Ultra®. Methods from the above section labeled "Laboratory Evaluation" were replicated for field samples to calculate filament viability.

<u>Data Analysis</u>

Lyngbya Biomass

For the 2019 treatment season, an analysis of variance (ANOVA) model was performed to examine the effect of site and sampling period on the difference in mean biomass weight sampled prior to treatment, mid-point of treatment, and post treatment for each individual product's treatment areas. Due to the COVID-19 restrictions put in place by NC State University biomass data was not collected prior to the 2020 treatment season. Therefore, an ANOVA was performed using data points from July, August, September, and October for the 2020 treatment season.

All models were subjected to analysis of variance (ANOVA) with a significant probability ($\propto = 0.05$).

Lyngbya BioSonics

All echosounding data was manually analyzed using BioSonics Visual Habitat computer application. Using this processing method, input thresholds were tailored to provide benthic criteria, or suggested lyngbya mat height and presence (e.g. amend water column depth due to soft substrate or known non-lyngbya mats from the sonar output). Adjusted sonar logs were then exported as CSV records of spatial location, mat height, water column occupancy, and bathymetric readings. Using similar methods to Howell and Richardson (2019), representative plots among treatment sites were imported into ESRI ArcGIS 10.5.1 software for further post-processing and analysis. Georeferenced point features were then used to interpolate raster grids with 5 m (16.4 ft) cell size and masked to each respective treatment zone. The resulting raster grids were used to calculate summary statistics of lyngbya occupancy, spatial coverage, and provide visual representation of mat progression over time. A false positive limiting depth of 0.76 m (2.5 ft) was assigned as the minimum depth threshold used for analysis due to heavy noise and backscatter from the transducer in shallow areas (Duarte, 1987; Valley et al., 2015). Likewise, mat heights < 7.62 cm (3 in) were removed during analysis to protect from false positive detections.

Statistical analyses ensued by extracting values from interpolated raster grids to assign a mat height spatial comparison among each treatment cove over time. Interpolated grid point features were also used to provide a holistic analysis of lyngbya acreage among each site over time by summing all cells which met lyngbya presence thresholds (>3 in). Non-parametric statistical comparisons were used to evaluate treatment mean distributions with Kruskal–Wallis one-way analysis of variance, and Tukey's HSD mean separation approaches ($\alpha = 0.05$) using RStudio 4.0.3. There was inherent spatial autocorrelation of treatment sites based on design, therefore sites are not grouped by treatment and instead analyzed independently over time.

Cellular Viability

Samples for the 2020 treatment season were simply classified as viable or non-viable to determine overall treatment efficacy between treated and control areas. However in 2021, this method was modified to allow for quantitative comparison between lyngbya samples that were treated with Captain XTR® and those treated with Cutrine Ultra®. All models in 2021 were subjected to analysis of variance (ANOVA) with a significant probability ($\propto = 0.05$).

Results

2019

Overall, neither sampling method (biomass or BioSonics) revealed a significant response of lyngbya to treatments in terms of displaying control during the 2019 treatment season (Table 1.4.2; Table 1.4.3). It is worth noting that neither method explained variability in lyngbya mats well, however when the two methods were compared similar overall trends were observed. Both methods indicated that protocols involving Captain XTR® or Algimycin® did not show significant changes that would provide evidence of control due to treatments, but protocols utilizing Hydrothol® and Green Clean® appeared to display evidence of increased lyngbya proliferation within treatment areas (Table 1.4.2; Table 1.4.3; Figure 1.4.18). Although data collected using the two separate sampling methods produced similar final results, biomass data collected at multiple time points throughout the study period did not reveal a significant temporal interaction among sampling dates ($p < 0.05$) with the exception of Smith North ($p > 0.05$) (Table 1.4.2) while data collected using BioSonics was able to determine significant temporal variation in vertical mat occupancy among individual sampling periods (Table 1.4.3).

2020

Due to Covid-19 restrictions put in place by North Carolina State University (NCSU), BioSonics was the only method utilized prior to and throughout the entire treatment season. Biomass and viability samples were collected once NCSU restrictions were lifted in July 2020.

Similar to results displayed in 2019, evaluation methods involving lyngbya biomass and BioSonics did not detect a significant response of lyngbya to treatments in terms of displaying control for any of the treatment protocols utilized in 2020 (Table 1.4.4; Table 1.4.5; Figure 1.4.19; Figure 1.4.20). However, in 2020 an additional method was utilized to determine efficacy of lyngbya treatments by performing a qualitative evaluation of the overall viability of lyngbya at a cellular level. Results from this method determined that samples collected within sites treated with either Captain XTR® or Cutrine Ultra® contained a high percentage of filaments that displayed decreased viability, with a majority of the samples being represented by empty sheaths containing no cellular material, while samples collected from untreated areas displayed a high percentage of green, healthy lyngbya (Figure 1.4.17). Although protocols involving Captain XTR® and Cutrine Ultra® did not show significant changes that would provide evidence of control due to treatments, BioSonics data showed that the vertical occupancy of lyngbya within the water column following the treatment season was similar or below levels detected prior to the initiation of treatments (Figure 1.4.19; Figure 1.4.20). However, sites treated with Algimycin® increased overall occupancy when compared to pre-treatment levels (Figure 1.4.20).

Methods that utilized data collected from lyngbya biomass to determine both temporal and spatial responses of benthic lyngbya mats to treatment protocols proved unsuccessful (Table 1.4.4; Figure 1.4.21). While methods that included the use of BioSonics were able to determine monthly variations in vertical occupancy during the 2020 treatment season (Table 1.4.5; Figure 1.4.19; Figure 1.4.20), those temporal changes were not detected using data collected from lyngbya biomass (Table 1.4.4).

2021

Overall, there was a positive response in terms control displayed for lyngbya located within treatment areas that received monthly application of either Captain XTR® or Cutrine Ultra® in 2021. When compared to control sites, lyngbya within treatment areas displayed decreased benthic mat proliferation and reduced cellular viability following the 2021 treatment season (Table 1.4.6; Figure 1.4.22; Figure 1.4.23; Figure 1.4.24; Figure 1.4.25).

BioSonics data showed that vertical occupancy within the water column of benthic lyngbya mats increased significantly during the months of July, August, and September for control areas that contained lyngbya not subjected to control measures (Table 1.4.6; Figure 1.4.22). Benthic lyngbya mats in untreated control areas also displayed a significant decrease in overall vertical occupancy during the month of October (Table 1.4.6; Figure 1.4.22). For treatment areas that received monthly applications of Captain XTR®, increased proliferation during the month of July, August, and September was only documented within Lyon's Creek (September) (Table 1.4.6; Figure 1.4.23; Figure 1.4.24). All other sites that received applications of Captain XTR® remained at or below levels detected in April, prior to the initiation of treatments (Table 1.4.6; Figure 1.4.23; Figure 1.4.24). All sites displayed a significant decrease in overall vertical occupancy during the month of October. Treatment areas that received monthly applications of Cutrine Ultra® were variable in the level of control displayed and only one site (Site 18) displayed decreased mat proliferation during the July, August, and September time period (Table 1.4.6; Figure 1.4.23; Figure 1.4.24). Increased benthic mat proliferation was documented for all other sites that received Cutrine Ultra® during this time period and was not directly related to the concentration rate at which copper was applied. Smith Creek and Site 18 both received applications at the highest rate of copper used in 2021 (2.4 gal/AF), but unlike the results found in Site 18, Smith Creek displayed elevated vertical occupancy levels throughout the entire treatment season (Table 1.4.6; Figure 1.4.23).

The treatment sites within Hawtree Creek were designed for comparison of chemical treatment protocols exposed to similar environmental conditions. However, it is worth noting that the area designated as an untreated control site was impacted by aggressive jet skiing aimed at dislodging lyngbya mats. This was determined after the conclusion of the study and can be seen with the consistent decrease in vertical occupancy displayed by benthic lyngbya mats within this area throughout the 2021 treatment season (Table 1.4.4; Figure 1.4.24). Direct comparisons between treatment protocols are unable to be completed as originally designed due to the compromised untreated control site, however trends in benthic mat proliferation for areas treated with Captain XTR® and Cutrine Ultra® were similar to those previously reported for single treatment site locations (Table 1.4.6; Figure 1.4.23; Figure 1.4.24).

Overall, cellular viability was significantly reduced for lyngbya treated by both Captain XTR® and Cutrine Ultra® ($p < 0.001$) (Figure 1.4.25). Lyngbya within control sites void of any direct lyngbya control measures displayed high viability (95%) and were significantly healthier than lyngbya that was treated with an algaecide ($p < 0.05$). Although both treatment protocols significantly reduced cellular viability, lyngbya that received treatments of Captain XTR® displayed a significantly greater decrease in viability (39% viable) when compared to lyngbya that received treatments of Cutrine Ultra® (65% viable) (Figure 1.4.25).

Discussion

Previous studies that have reported on the efficacy of lyngbya treatment programs have utilized traditional rake toss methods to evaluate the response of benthic lyngbya mats to algaecide applications (Bishop et al. 2017 and Anderson et al. 2019). However, we found that localized sampling methods using lyngbya biomass as an indicator of efficacy were unable to produce results that could identify differences between treatment combinations. Only two, of 29 total, sites evaluated using biomass data collected by rakes produced significant temporal changes over the 2019 and 2020 treatment seasons. Unlike biomass data, BioSonics was able to detect temporal changes in all but six of the 29 total sites evaluated during the 2019 and 2020 treatment seasons. The ineffectiveness of biomass data to evaluate benthic lyngbya mats may be a product of evaluating benthic mat formations that are not continuous in nature and that can display high variability in both spatial and physical characteristics.

Our study expanded on the findings of previous studies that evaluated the effectiveness of algaecide combinations (Bishop et al. 2017 and Calomeni et al. 2018); however we modified our methods to be more representative of field based algaecide applications and to allow for a more holistic evaluation of the lyngbya mat material. Previous laboratory studies assessed algaecide efficacy by evaluating subsamples of the overall treated lyngbya mass (e.g., evaluation of individual filaments or single microscope views), however we found these methods to be potentially bias due to the variability in individual filament response to treatment exposure. Therefore, we feel we improved on previous methods by taking into account the morphological factors that can alter algaecide effectiveness including the matrix of interstitial spacing created by lyngbya mat formations (Lembi 2000). Our laboratory method allowed us to evaluate the response of lyngbya as a whole to treatment exposures, incorporating the influence that the physiological makeup of lyngbya mat formation has on treatment efficacy and how location within the matrix can influence the level of algaecide exposure to individual filaments. This holistic approach to evaluating treatment efficacy could explain why Lake Gaston lyngbya required multiple treatment events to produce control level reductions in viability, unlike other studies that saw an immediate response in lyngbya to a single algaecide application (Calomeni et al. 2018). This approach could also explain how our laboratory evaluations were able to highlight the differences in temporal responses to different algaecide combinations for Lake Gaston lyngbya. While all treatment combinations were equally effective post the third and final treatment exposure, there was varying temporal responses when exposed to treatment combinations of Captain XTR® + Reward® and Cutrine Ultra® + AMP® when compared to exposure to Captain XTR® + AMP. For the former two combinations, lyngbya responded to each treatment exposure and displayed a gradual decrease in viability. For the latter Captain XTR® + AMP® treatment, lyngbya responded to the initial treatment but then required a third treatment exposure to produce further cellular damage. Understanding these delayed responses to algaecide exposure in lyngbya is not only critical in developing an effective treatment program, but also in developing an appropriate field monitoring program.

The efficacy of a treatment program depends not only on an appropriate chemical combination, but also on application techniques that are able to address the ecological factors that can greatly alter algaecide efficacy. Adjustments in the timing of treatment season initiation and chemical combinations made throughout this study made it difficult to perform a direct comparison of the historical trailing hose method to the autonomous system. The level of control displayed during the 2019 treatment season could have been negatively impacted by increased biomass from pre-treatment proliferation of the benthic mats due to a later in season treatment initiation (Lembie 2000). However, the seasonal timing of increased proliferation for benthic lyngbya mats, in the form of vertical occupancy, varied between months and years for designated control sites during the 2020 and 2021 treatment seasons. During the 2020 and 2021 treatment seasons BioSonics sampling began in April and increased proliferation for control sites was reported to begin in either May, June, or July depending on site, therefore it is unclear what impacts lyngbya biomass levels had on the level of control displayed during the 2019 treatment season. As previously mentioned the timing of treatment initiation for the 2020 and 2021 treatment seasons were similar, however treatment combinations were modified between the two seasons. The 2021 treatment season was the first to utilize the treatment combination of Captain XTR® + AMP, which produced the most consistent results of both the 2020 and 2021 treatment seasons. Lab studies showed the effectiveness of Captain XTR® at reducing the viability of lyngbya in a controlled environment and the replacement of Reward® with AMP, an adjuvant that is intended to increase the adhesiveness of copper to targeted aquatic species, could have resulted in increased exposure time. Our results were similar to those found in Alabama reservoirs that received applications of Captain XTR® by the same autonomous system as utilized in our study, however managers for the Alabama reservoirs did not incorporate AMP® into their treatment protocol (Anderson et al. 2019). Therefore, it is difficult to determine if the improved efficacy demonstrated during the 2021 treatment season is a result of application technique, the chemical protocol of treatment, or a combination of both.

With this study we confirmed the efficacy of chelated copper based algaecides on reducing lyngbya viability levels in a laboratory setting and then translated those positive results to a large scale operational field scenario. Throughout the study we evaluated traditional methods of algaecide application and submersed vegetation sampling techniques, but it was the incorporation of several novel techniques of both application and evaluation that proved successful.

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Tables

Table 1.4.1. Timing, acreage, total number of sites, and application method for treatments during the 2019, 2020, and 2021 Lake Gaston lyngbya treatment seasons.

Chemical Protocol	**Application Method**	**Treatment Timing**	**Application Rates *(gal/AF)***	**Total Treatment Acreage**	**Total Treatment Sites**
		2019			
Algimycin® & AMP®	Drop Down Hoses / Outboard Motor	June - October	Algimycin® *(2.13)* AMP® *(0.5)*	26.41	3
Captain XTR® & Reward®	Drop Down Hoses / Outboard Motor	June - October	Captain XTR® *(0.75 / 1.5)* Reward® *(0.5)*	41.16	3
Hydrothol® & Green Clean®	Drop Down Hoses / Outboard Motor	June - October	Algimycin® *(2.13)* AMP® *(0.15)*	30.52	3
		2020			
Algimycin® & AMP®	Drop Down Hoses / Outboard Motor	April - September	Algimycin® *(2.13)* AMP® *(0.5 / 0.75)*	12.13	4
Captain XTR® & Reward®	Drop Down Hoses / Outboard Motor	April - September	Captain XTR® *(0.75 / 1.50 / 2.40)* Reward® *(0.15)*	48.87	5
Cutrine Ultra® & AMP®	Drop Down Hoses / Outboard Motor	April - September	Cutrine Ultra® *(1.4)* AMP® *(0.50)*	29.24	3
		2021			
Captain XTR® & AMP®	Hydraulic Injection / Airboat	April - September	Captain XTR® *(1.5 / 2.4)* AMP® *(0.25 / 0.5)*	163.50	17
Cutrine Ultra® & AMP®	Hydraulic Injection / Airboat	April - September	Cutrine Ultra® *(0.75 / 1.5 / 2.4)* AMP® *(0.25 / 0.5)*	136.11	21

Table 1.4.2. Results of ANOVA and Tukey's multiple-contrast tests on lyngbya biomass weights (grams) sampled in April (Pre), August (Mid), and November (Post) of the 2019 Lake Gaston lyngbya treatment season. Values given for each sampling period are the mean biomass weight calculated for each treatment overall and each treatment site individually during those times periods. All analysis shown (*P*-values and Tukey's results) are comparing pre, mid, and post samples for each product and treatment site individually. Sampling periods with common letters are not significantly different (α = 0.05).

2019 Treatment Season – Lyngbya Biomass			
	April (*pre*)	**August (*mid*)**	**November (*post*)**
Control	139 *a*	266 *a*	166 *a*
Control 1	197 *a*	292 *a*	98 *a*
Control 2	96 *a*	246 *a*	216 *a*
SePRO	42 *a*	35 *a*	41 *a*
Lyons	69 *a*	65 *a*	60 *a*
Pretty – Lower	25 *a*	23 *a*	34 *a*
St. Tammany	36 *a*	15 *a*	28 *a*
Applied Biochemist	139 *a*	224 *a*	124 *a*
Lees 1	109 *a*	234 *a*	98 *a*
Lees 2	237 *a*	312 *a*	155 *a*
Lees 3	28 *a*	34 *a*	27 *a*
Pretty – Upper	167 *a*	302 *a*	173 *a*
Rocky Branch	4 *a*	4 *a*	3 *a*
UPL / Biosafe	285 *b*	497 *ab*	643 *a*
Hawtree N	566 *a*	257 *a*	591 *a*
Hawtree E	95 *a*	356 *a*	374 *a*
Hawtree W	233 *a*	745 *a*	569 *a*
Smith N	328 *b*	592 *ab*	1029 *a*
Smith S	217 *a*	878 *a*	896 *a*
Great Creek	36 *a*	195 *a*	111 *a*

Table 1.4.3. Mean lyngbya vertical occupancy (feet) of benthic mat material during the 2019 Lake Gaston lyngbya treatment season. Vertical occupancy was determine using BioSonics echosounding measurements recorded using the same coordinates as biomass sampling points (n=4 per treatment area). All analysis shown are comparing monthly samples for each treatment site individually and means within the same row followed by the same letter do not significantly differ ($\alpha = 0.05$).

2019 Treatment Season – Lyngbya Vertical Occupancy						
	April	***June***	***July***	***August***	***September***	***November***
Control						
Control 2	0.26 *a*	0.30 *a*	0.15 *a*	0.36 *a*	0.20 *a*	0.88 *b*
Captain XTR®						
Lyons	0.21 *a*	0.42 *bc*	0.46 *bc*	0.51 *c*	0.22 *a*	0.31 *ab*
Pretty – Lower	0.25 *a*	0.17 *a*	0.21 *a*	0.29 *a*	0.25 *a*	0.25 *a*
St. Tammany	0.27 *a*	0.33 *a*	0.28 *a*	0.28 *a*	0.28 *a*	0.22 *a*
Algimycin®						
Lees 1	0.23 *a*	0.38 *a*	0.43 *a*	0.29 *a*	0.22 *a*	0.26 *a*
Lees 2	0.25 *ab*	0.29 *ab*	0.39 *ab*	0.43 *a*	0.13 *b*	0.24 *ab*
Lees 3	0.17 *a*	0.54 *a*	0.38 *a*	0.22 *a*	0.44 *a*	0.32 *a*
Pretty – Upper	0.22 *a*	0.47 *ab*	0.61 *b*	0.40 *ab*	0.39 *ab*	0.24 *a*
Rocky Branch	0.24 *ab*	0.23 *ab*	0.35 *a*	0.05 *b*	0.13 *ab*	0.38 *a*
UPL / Biosafe						
Hawtree N	0.27 *a*	0.28 *a*	0.31 *a*	0.26 *a*	0.40 *a*	0.67 *a*
Hawtree E	0.23 *a*	0.19 *a*	0.35 *a*	0.42 *ab*	0.37 *a*	0.75 *b*
Hawtree W	0.22 *a*	0.20 *a*	0.24 *a*	0.28 *a*	0.33 *a*	0.48 *a*
Smith N	0.13 *a*	0.28 *ab*	0.15 *a*	0.25 *a*	0.31 *ab*	0.73 *b*
Smith S	0.23 *a*	0.33 *ab*	0.83 *b*	0.36 *ab*	0.22 *a*	0.38 *ab*
Great Creek	0.23 *a*	0.22 *a*	0.27 *a*	0.41 *ab*	0.36 *b*	0.26 *a*

Table 1.4.4. Results of ANOVA and Tukey's multiple-contrast tests on Lyngbya biomass weights sampled monthly between July and October during the 2020 Lake Gaston Lyngbya treatment season. Values given for each sampling period are the mean biomass weight calculated for each treatment site individually. All analysis shown (*P*-values and Tukey's results) are comparing samples within individual treatment sites. Means within the same row followed by the same letter do not significantly differ ($\alpha = 0.05$).

2020 Treatment Season – Lyngbya Biomass				
	July	**August**	**September**	**October**
Experimental Designs				
Smith				
Cutrine Ultra® & AMP®	117 *a*	55 *a*	131 *a*	120 *a*
Captain XTR® & Reward®	348 *a*	261 *a*	113 *a*	327 *a*
Control	231 *a*	306 *a*	39 *a*	71 *a*
Hawtree				
Cutrine Ultra® & AMP®	1 *a*	127 *a*	0 *a*	0 *a*
Captain XTR® & Reward®	119 *a*	129 *a*	225 *a*	170 *a*
Control	268 *a*	290 *a*	159 *a*	327 *a*
Pretty				
Algimycin® & AMP®	14 *a*	23 *a*	98 *a*	77 *a*
Captain XTR® & Reward®	16 *a*	92 *a*	20 *a*	22 *a*
Control	1.4 *a*	44 *a*	50 *a*	6 *a*
Single Treatments				
Rocky Branch - Algimycin® & AMP®	8 *a*	38 *a*	42 *a*	25 *a*
Lyons - Captain XTR® & Reward®	9 *b*	25 *ab*	57 *a*	29 *ab*
Single Control Site				
Control Site	n/a	563 *a*	767 *a*	1376 *a*

Table 1.4.5. Mean lyngbya vertical occupancy (inches) of benthic mat material during the 2020 Lake Gaston lyngbya treatment season. Vertical occupancy was determine using BioSonics echosounding measurements. All analysis shown are comparing monthly samples for each treatment site individually and means within the same row followed by the same letter do not significantly differ (α = 0.05).

2020 Treatment Season – Lyngbya Vertical Occupancy									
	April	**May**	**June**	**July**	**Aug.**	**Sept.**	**Oct.**	**Nov.**	**Dec.**
Experimental Designs									
Smith									
Cutrine Ultra® & AMP	5.5 *bc*	5.9 *a*	3.9 *e*	6.1 *a*	5.9 *a*	5.7 *b*	4.9 *d*	5.4 *c*	0.0 *f*
Captain XTR® & Reward®	4.8 *bc*	5.0 *b*	3.5 *e*	4.9 *cd*	5.3 *b*	5.1 *b*	4.6 *d*	5.6 *a*	0.0 *f*
Control	5.7 *bc*	6.0 *a*	3.0 *f*	6.0 *ab*	5.4 *c*	5.4 *c*	4.1 *e*	4.9 *d*	0.0 *g*
Hawtree									
Cutrine Ultra® & AMP	4.7 *c*	4.9 *bc*	5.4 *a*	5.2 *ab*	4.7 *c*	4.9 *c*	3.4 *d*	5.3 *ab*	0.0 *e*
Captain XTR® & Reward®	5.1 *cd*	4.6 *d*	4.6 *d*	5.9 *a*	5.4 *b*	5.0 *c*	5.0 *c*	5.5 *b*	0.0 *e*
Control	6.1 *a*	5.9 *a*	6.4 *b*	6.4 *b*	5.7 *c*	5.0 *d*	4.8 *d*	5.9 *e*	0.0 *f*
Pretty									
Algimycin® & AMP®	4.2 *d*	5.2 *c*	5.6 *a*	5.8 *ab*	5.9 *ab*	5.5 *b*	5.2 *c*	5.5 *b*	0.0 *e*
Captain XTR® & Reward®	5.0 *c*	5.8 *a*	5.1 *c*	4.8 *d*	5.4 *c*	4.3 *e*	4.8 *d*	5.7 *b*	0.0 *g*
Control	5.1 *c*	5.9 *ab*	6.1 *a*	5.7 *b*	4.5 *c*	4.9 *c*	5.0 *c*	6.0 *ab*	0.0 *d*
Single Treatments									
Algimycin® & AMP®									
Rocky Branch	4.1 *e*	5.1 *bcd*	5.8 *a*	5.0 *cd*	5.6 *abc*	5.5 *ab*	5.3 *bcd*	5.1 *d*	0.0 *f*
Lee 1	2.5 *e*	3.5 *d*	4.1 *c*	5.4 *b*	6.5 *a*	4.3 *cd*	3.9 *cd*	3.4 *d*	0.0 *f*
Lee 2	3.6 *d*	4.1 *c*	4.2 *c*	6.8 *a*	6.0 *b*	5.5 *b*	4.6 *c*	4.2 *c*	0.0 *e*
Lee 3	4.4 *bc*	3.8 *d*	4.3 *bc*	--	4.2 *bcd*	4.4 *cd*	4.8 *a*	5.4 *b*	0.0 *e*
Captain XTR® & Reward®									
Lyons	4.8 *de*	4.8 *cd*	10.2 *a*	5.5 *b*	6.1 *b*	5.0 *c*	4.7 *ef*	4.5 *f*	0.1 *g*
St. Tammany	6.1 *b*	5.3 *e*	5.5 *cd*	6.2 *a*	5.9 *b*	5.8 de	4.9 *e*	5.8 *bc*	0.0 *f*
Control Site									
Control	--	--	--	--	5.2 *a*	4.9 *b*	4.8 *c*	5.2 *d*	0.0 *e*

Table 1.4.6: Mean lyngbya vertical occupancy (inches) of benthic mat material during the 2021 Lake Gaston lyngbya treatment season. Vertical occupancy was determine using BioSonics echosounding measurements. All analysis shown are comparing monthly samples for each treatment site individually and means within the same row followed by the same letter do not significantly differ (α = 0.05).

2021 Treatment Season – Lyngbya Vertical Occupancy							
	April	**May**	**June**	**July**	**August**	**September**	**October**
Experimental Designs							
Hawtree							
Captain XTR® & AMP	6.1 *ab*	5.8 *c*	6.2 *a*	5.3 *d*	5.8 *c*	5.8 *bc*	4.6 *e*
Cutrine Ultra® & AMP	4.8 *c*	5.5 *b*	6.1 *a*	5.6 *b*	4.8 *c*	5.5 *b*	3.8 *d*
Control	6.8 *a*	6.4 *b*	6.4 *b*	5.9 *c*	5.4 *cd*	5.3 d	3.9 *e*
Single Treatments							
Captain XTR® & AMP							
Lees Creek	5.2 *c*	5.6 *a*	5.2 *c*	5.2 *c*	5.2 *c*	4.5 *d*	2.3 *f*
Lyons	5.1 *c*	5.5 *b*	5.9 *a*	4.9 *d*	4.9 *d*	5.4 *b*	3.6 *e*
Site 19	5.2 *b*	5.5 *a*	4.9 *c*	4.4 *e*	4.5 *de*	4.3 *e*	1.9 *g*
Smith	5.5 *a*	4.8 *c*	5.4 *a*	5.3 *ab*	5.2 *b*	5.4 *a*	3.8 *d*
Cutrine Ultra® & AMP							
Stillhouse	5.5 *c*	6.2 *a*	6.1 *a*	5.5 *c*	5.4 *c*	5.7 *b*	4.3 *d*
Hamlin	5.3 *b*	5.1 *c*	--	5.6 *a*	5.6 *a*	4.8 *d*	2.4 *e*
Site 18	5 *a*	5.1 *a*	4.9 *a*	4.5 *b*	5 *a*	4.4 *b*	1.6 *d*
Smith	5.4 *b*	5.6 *a*	5.9 *a*	5.9 *a*	5.9 *a*	5.8 *a*	4.8 *c*
Control Sites							
Upper Control	5.5 *c*	5.6 *bc*	5.8 *b*	6.3 *a*	6.3 *a*	6.2 *a*	4 *d*
Lower Control	5 *c*	5 *c*	4.9 *c*	6.4 *b*	6.9 *a*	6.6a *b*	4 *e*

Figures

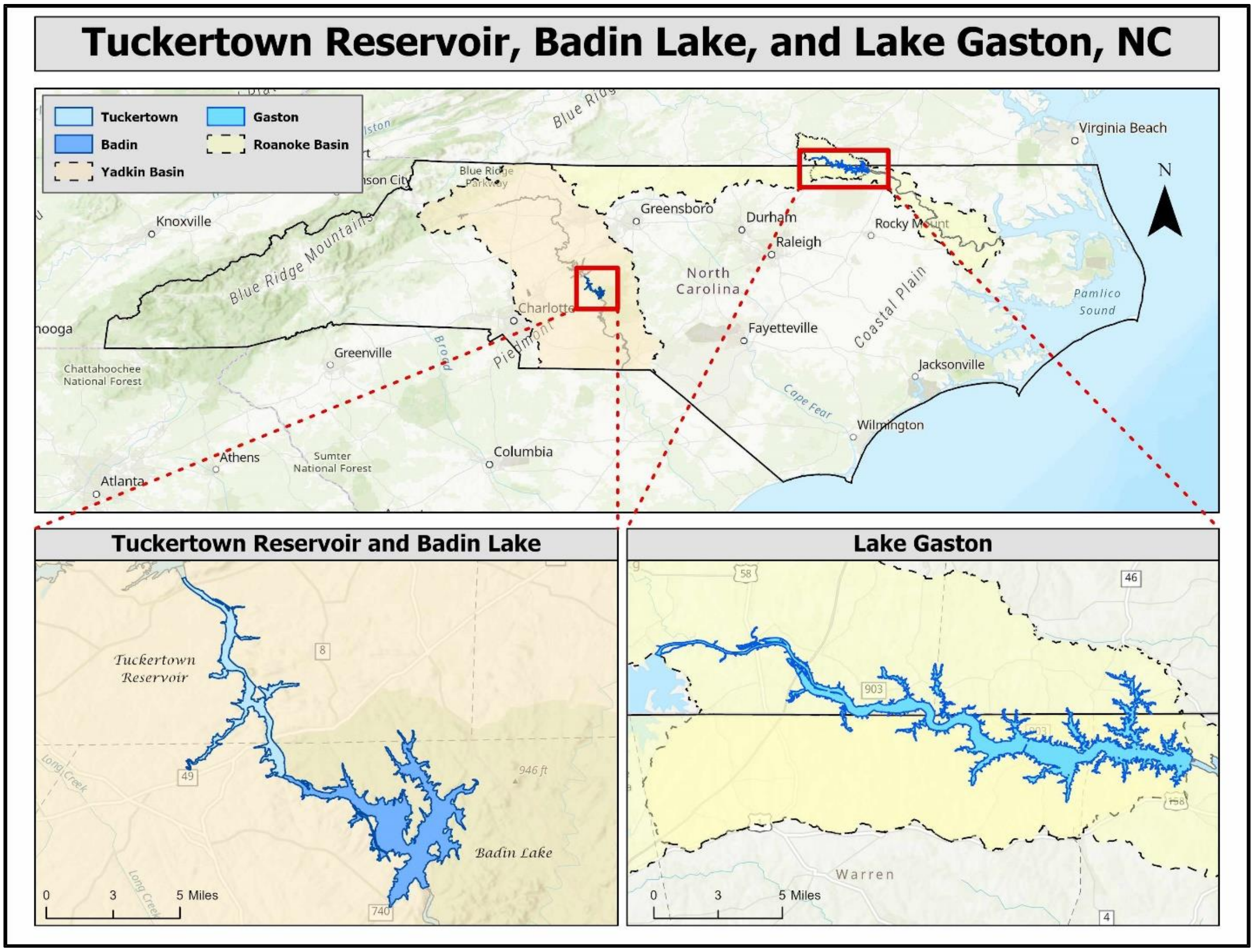

Figure 1.4.1. Map showing locations of Tuckertown Reservoir, Badin Lake, and Lake Gaston NC.

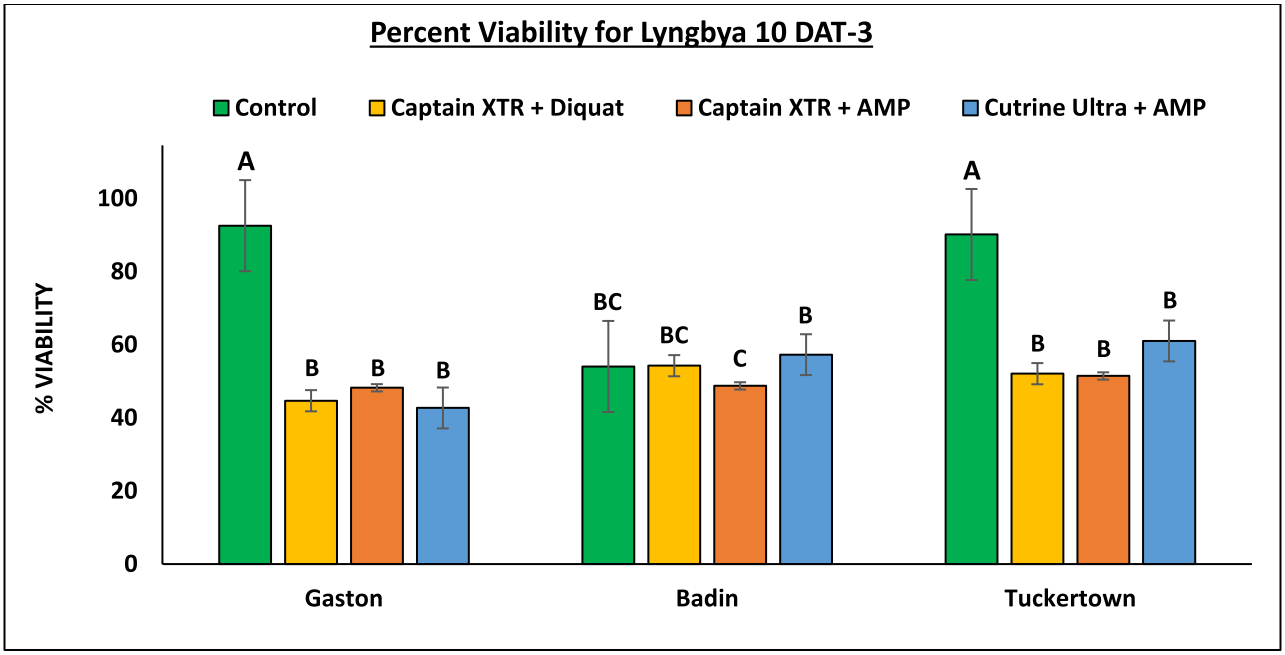

Figure 1.4.2. Changes in the percent viability of lyngbya in response to a series of three sequential herbicide treatments using combinations of either Captain XTR® + Reward®, Captain XTR® + AMP, or Cutrine Ultra® + AMP. Viability was determined 10-days post third treatment exposure. Letters indicating the results of Tukey's HSD ($p < 0.05$) identify the significance of viability measurements when compared to the control for each lake. If measurements share a common letter, viability was not significantly different. Each treatment combination was performed on lyngbya collected from Lake Gaston, NC/VA, Tuckertown Reservoir, NC and Badin Lake, NC.

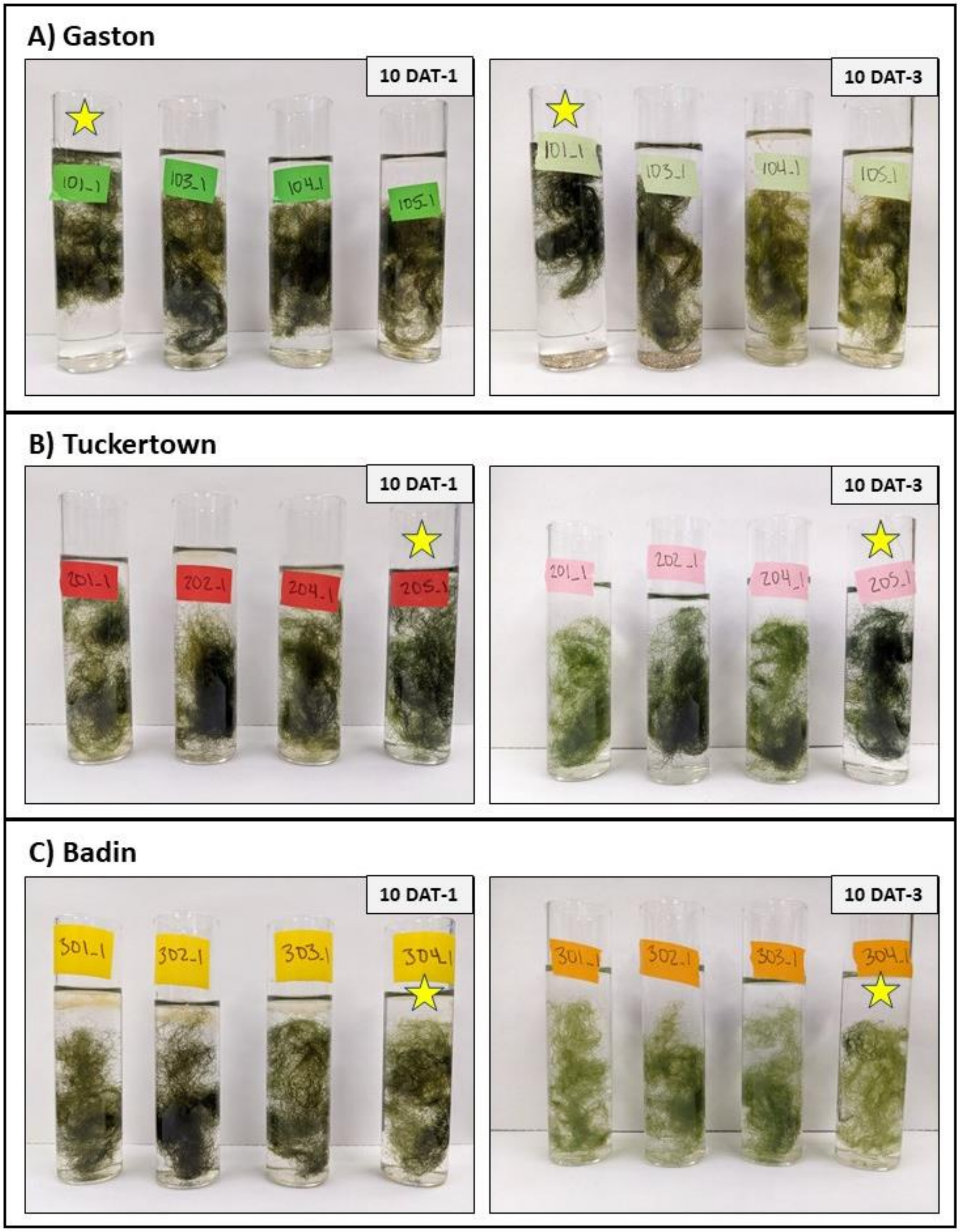

Figure 1.4.3. Images of lyngbya collected from A) Lake Gaston, B) Tuckertown Reservoir, and C) Badin Lake 10-days post exposure to treatment combinations of either Captain XTR® + Reward®, Captain XTR® + AMP, or Cutrine Ultra® + AMP® during a series of three consecutive treatment rounds. Images are showing one rep example (four total) from each lake both 10-days post the first (left) and third (right) treatment exposure. Control units from each rep are indicated by a star.

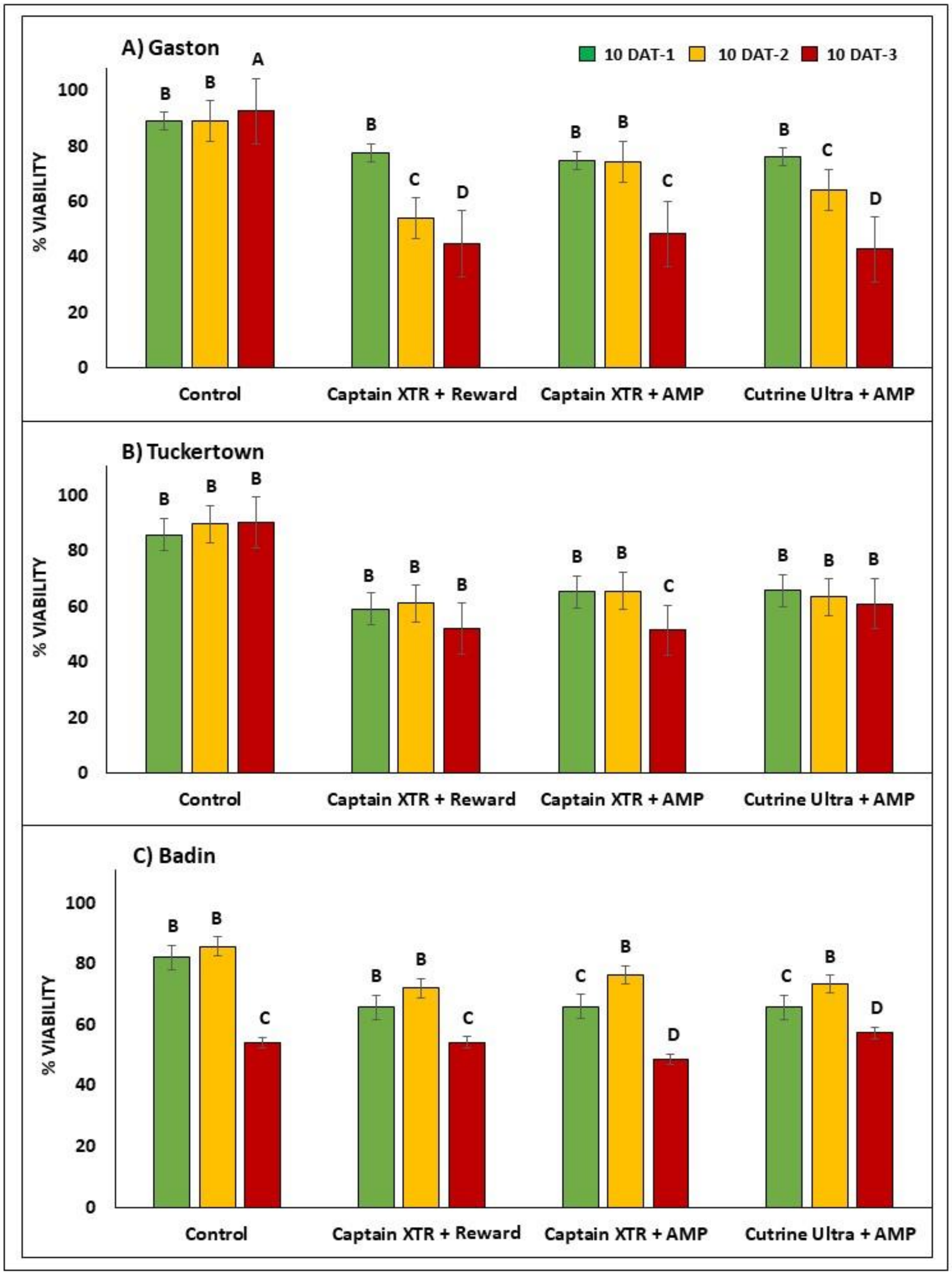

Figure 1.4.4. Changes in the percent viability of lyngbya in response to a series of three sequential herbicide treatments using treatment combinations of either Captain XTR® + Reward®, Captain XTR® + AMP, or Cutrine Ultra® + AMP. All samples were deemed >95% viable prior to the initiation of treatments and subsequent levels of viability were determined 10-days post each treatment exposure (10 DAT-1, 10 DAT-2, and 10 DAT-3). Each treatment combination was performed on lyngbya collected from A) Lake Gaston, NC/VA B) Tuckertown Reservoir, NC and C) Badin Lake, NC.

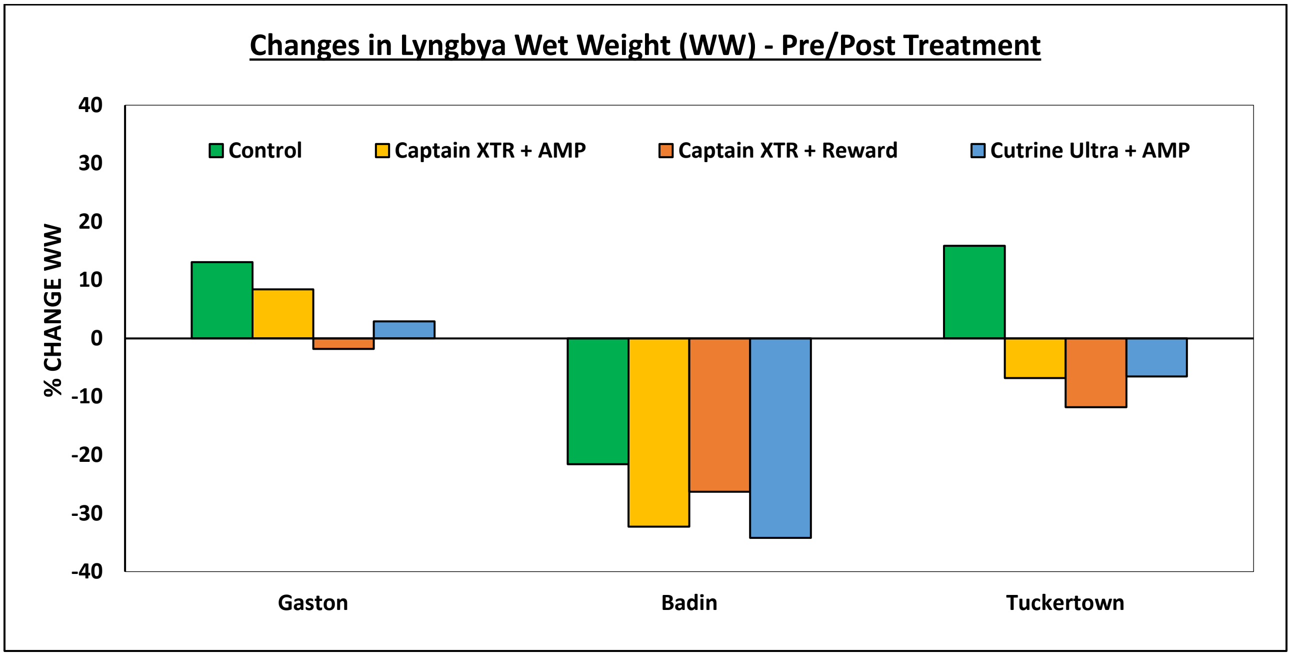

Figure 1.4.5. Changes in lyngbya fresh weights in response to a series of three sequential herbicide treatments using combinations of either Captain XTR® + Reward®, Captain XTR® + AMP, or Cutrine Ultra® + AMP. Each treatment combination was performed on lyngbya collected from Lake Gaston, NC/VA, Tuckertown Reservoir, NC and, Badin Lake, NC.

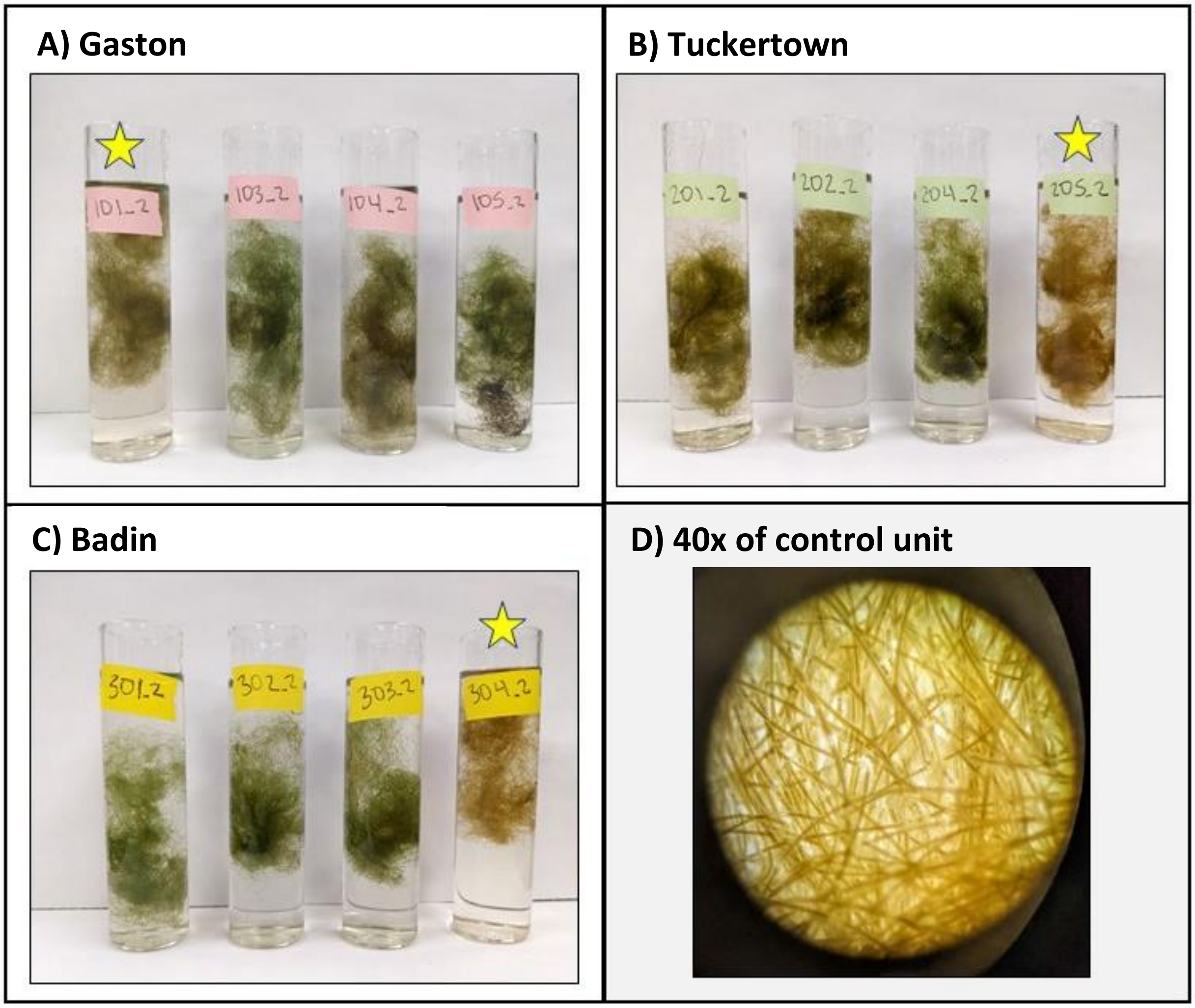

Figure 1.4.6. Images taken post extreme heat exposure (55°C / 131 °F) of lyngbya that completed three consecutive treatment exposures using a combination of either Captain XTR® + Reward®, Captain XTR® + AMP, or Cutrine Ultra® + AMP. Control units from each rep are indicated by a star. Image of control unit at 40x magnification is also shown (D).

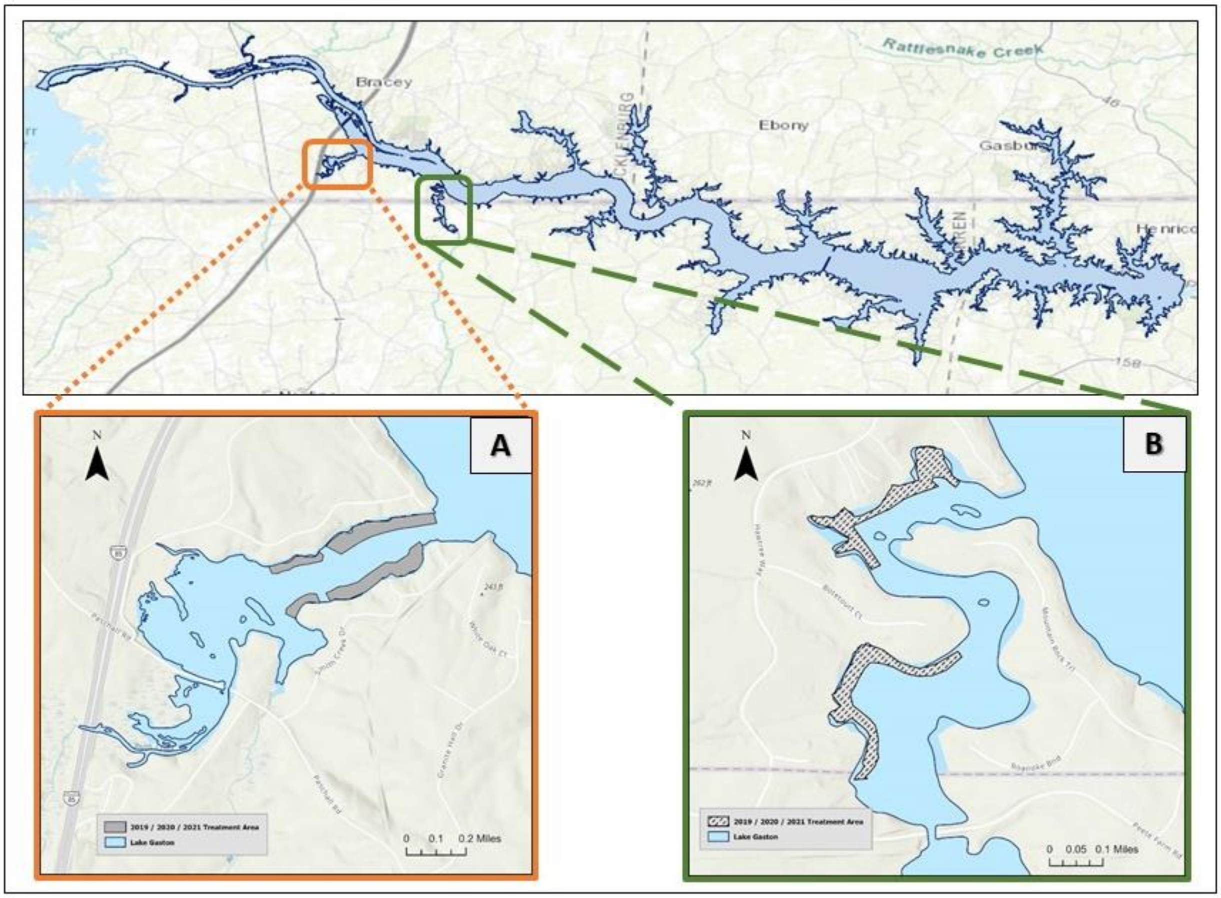

Figure 1.4.7. Map indicating sites within Smith (A) and Hawtree (B) creeks that were selected for treatment evaluation during the 2019, 2020, and 2021 treatment seasons.

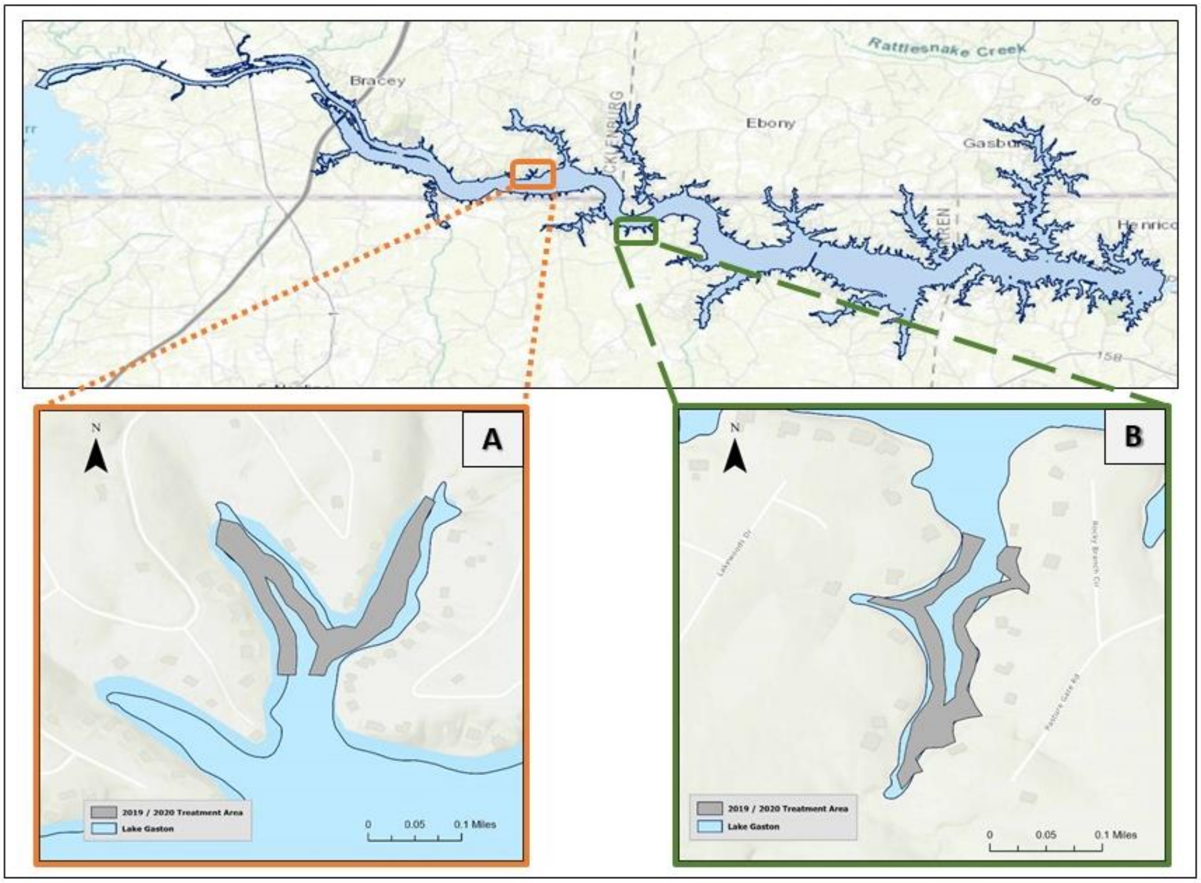

Figure 1.4.8. Map indicating sites within St. Tammany (A) and Rocky Branch (B) that were selected for treatment evaluation during the 2019, 2020, and 2021 treatment seasons.

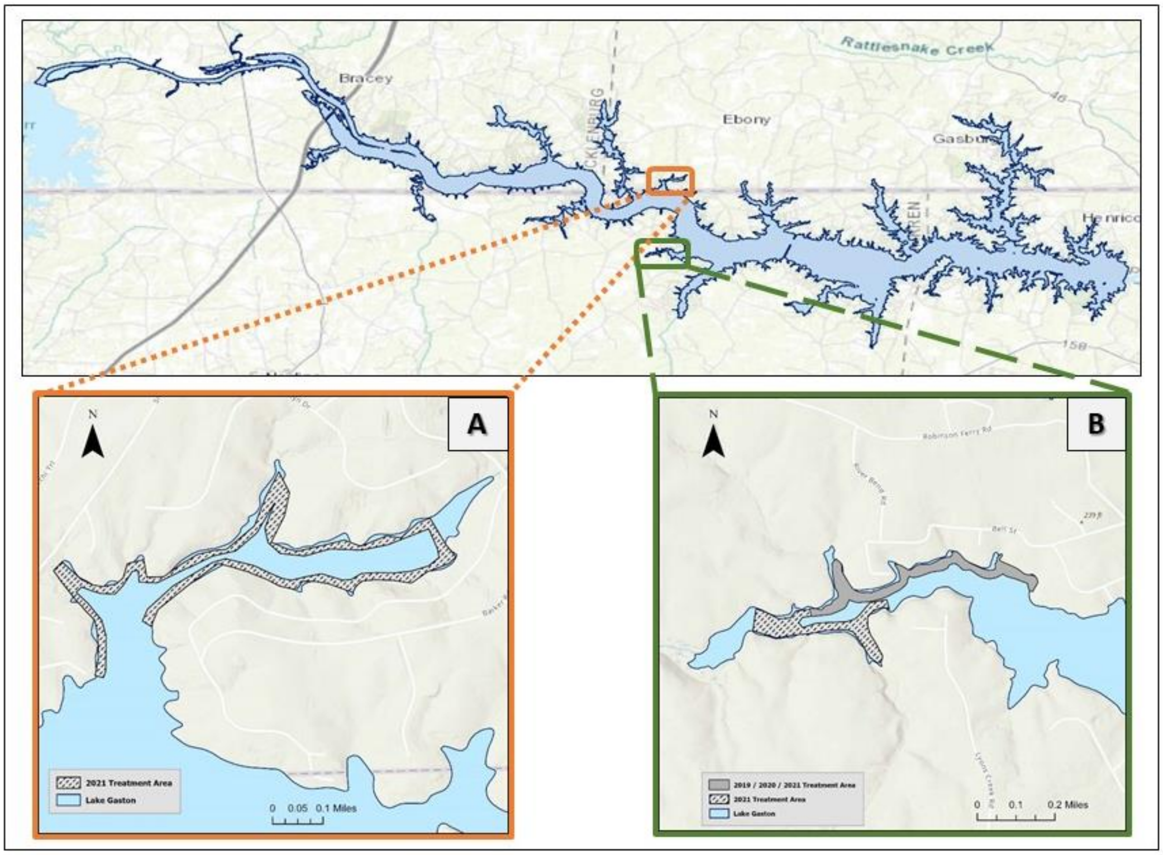

Figure 1.4.9. Map indicating sites within Stillhouse (A) and Lyons Creek (B) that were selected for treatment evaluation during the 2019, 2020, and 2021 treatment seasons.

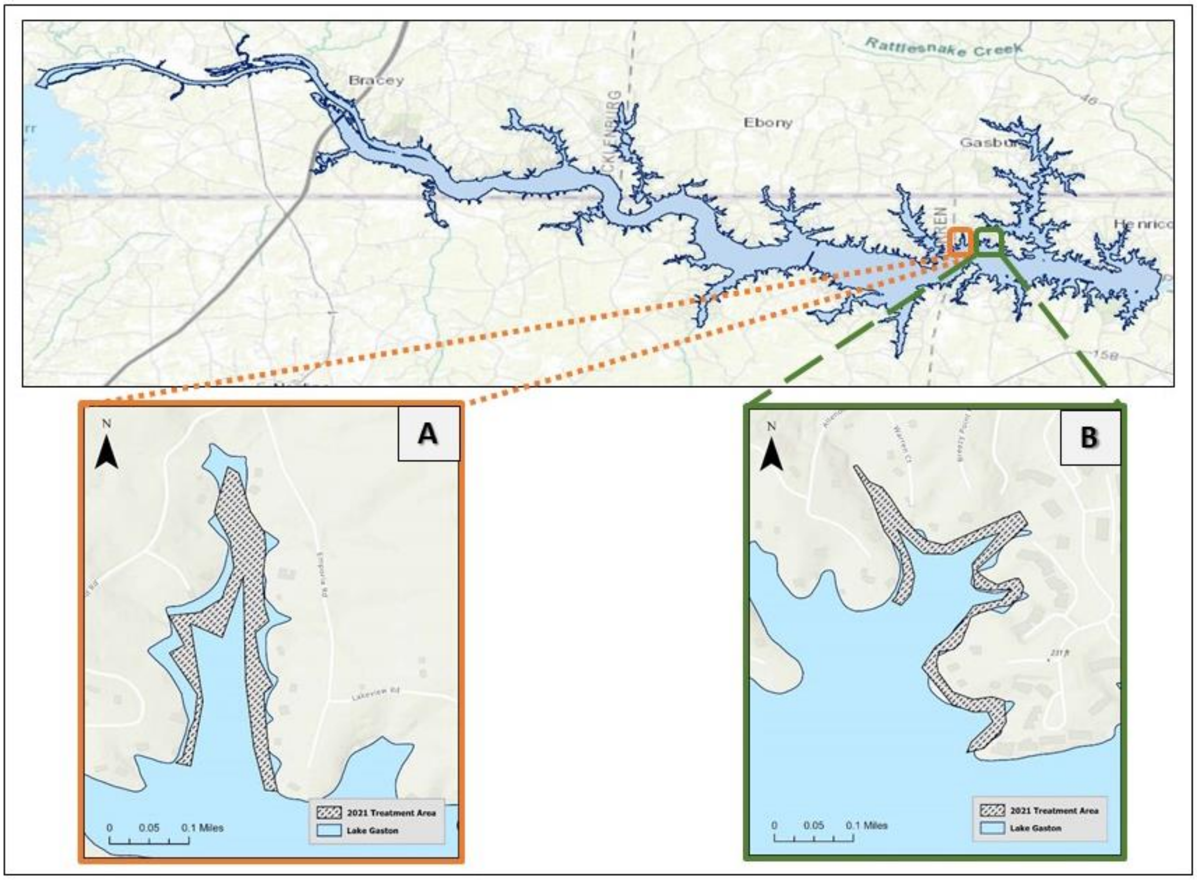

Figure 1.4.10. Map indicating sites on the northeastern shoreline of the main lake that were selected for treatment evaluation during the 2021 treatment season. Sites were labeled as Site 19 (A) and Site 20 (B).

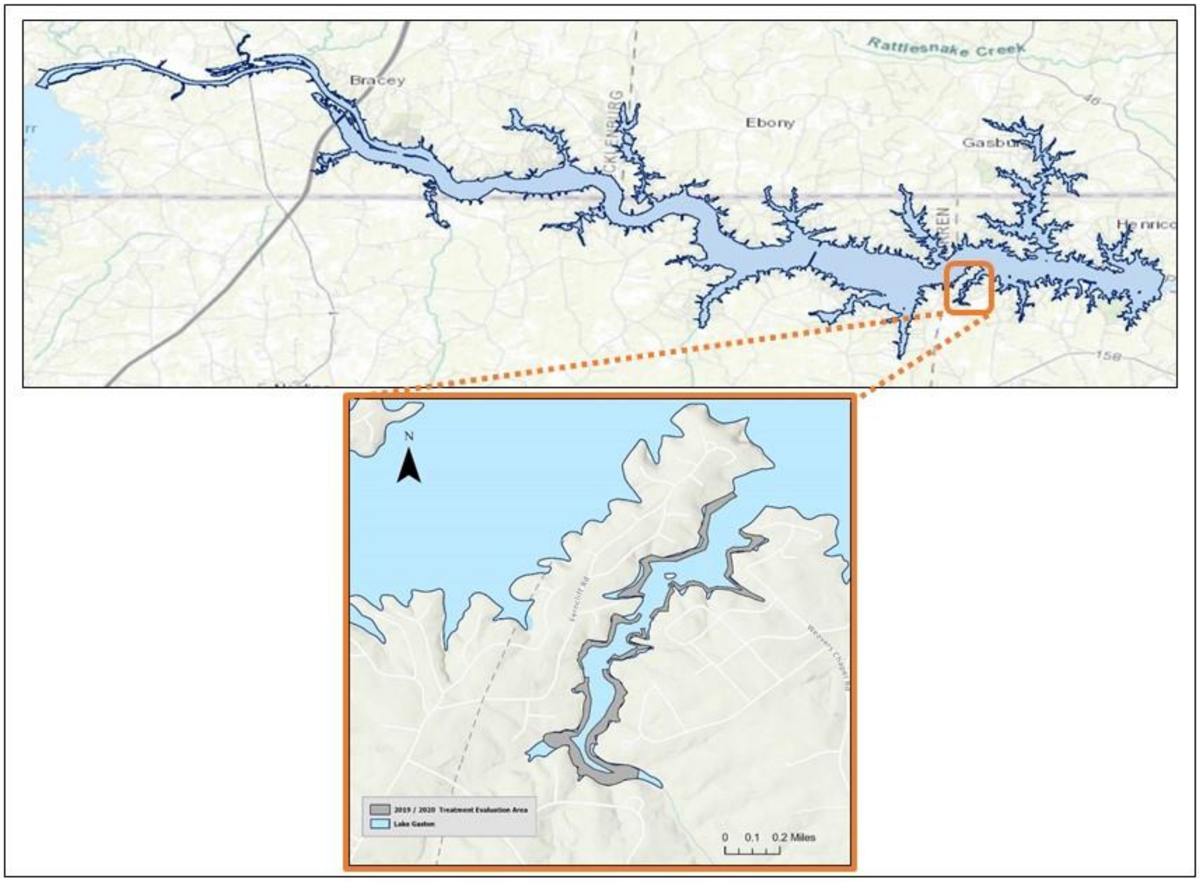

Figure 1.4.11. Map indicating sites in Pretty Creek that were selected for treatment evaluation during the 2019 and 2020 treatment seasons

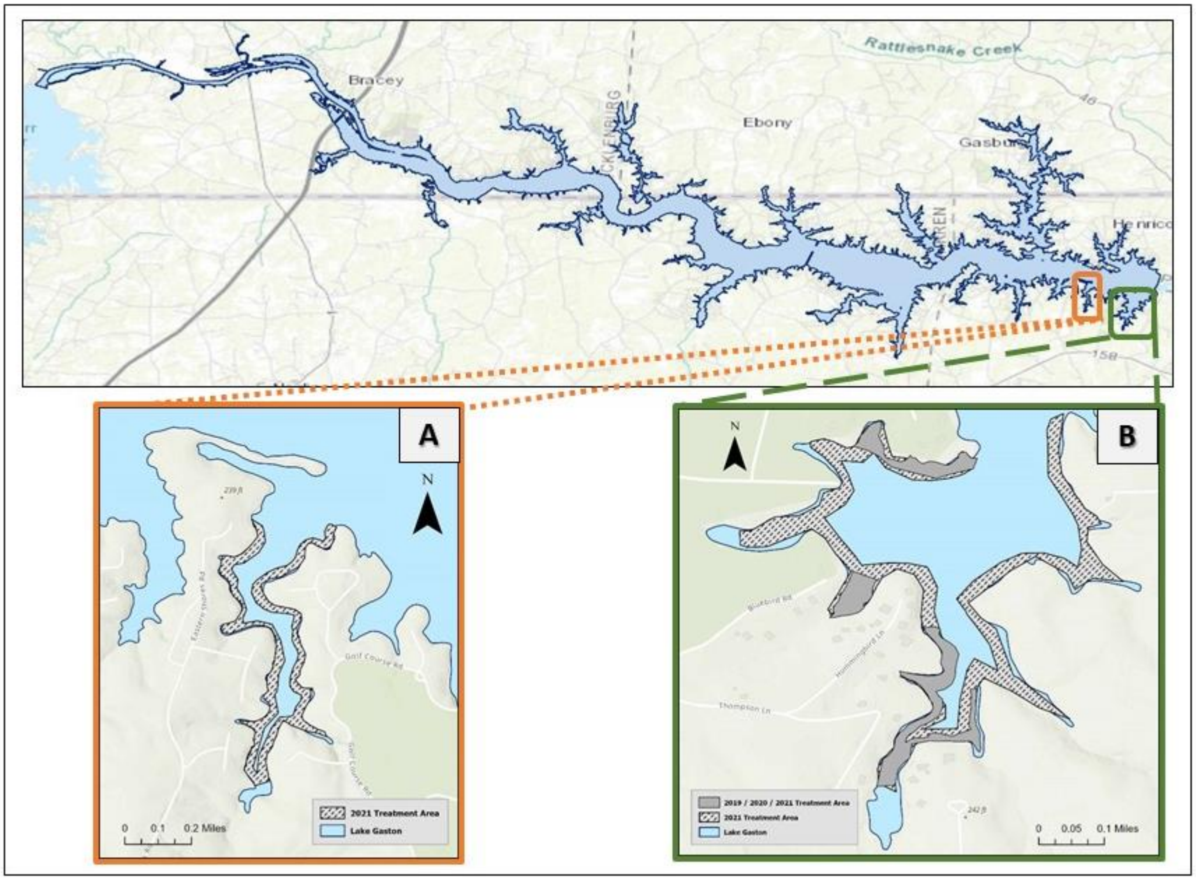

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Figure 1.4.12. Map indicating sites within Sledge (A) and Lees Creek (B) that were selected for treatment evaluation during the 2019, 2020, and 2021 treatment seasons.

Figure 1.4.13. Images of two application techniques utilized during the evaluation of lyngbya treatments on Lake Gaston (2019-2021). The left image shows the traditional application method used during the 2019 and 2020 treatment season that utilizes a drop down weighted hose system on a fiberglass boat powered by an outboard motor. The right images shows the autonomous system used during the 2021 treatment season that utilizes a hydraulic injector system that targets the benthos and is located on the back of an airboat.

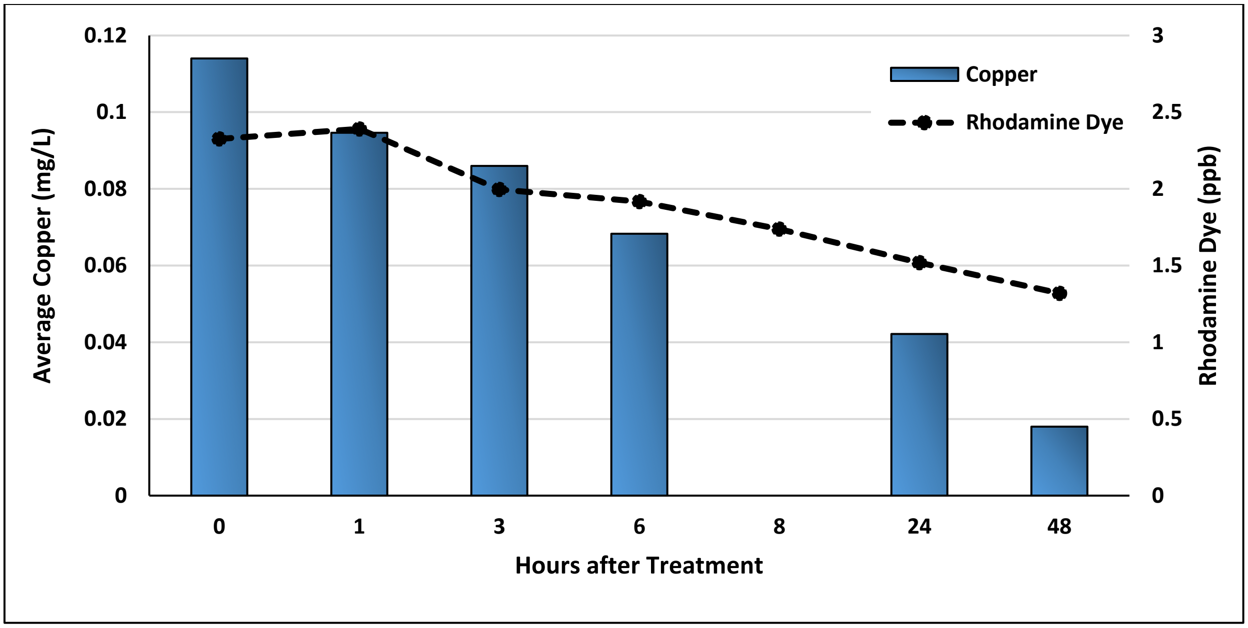

Figure 1.4.14. Concentrations of both copper (mg/L) and Rhodamine dye (ppb) post algaecide treatment using an autonomous application system that targets the benthos of a system. The algaecide protocol for this treatment application was Captain XTR® + AMP® with a targeted copper concentration rate of 0.80 mg/L.

Figure 1.4.15. Drone images capturing the visual trials of Rhodamine dye that was used in conjunction with a 2021 Captain XTR® and AMP® treatment applied by the autonomous injection system.

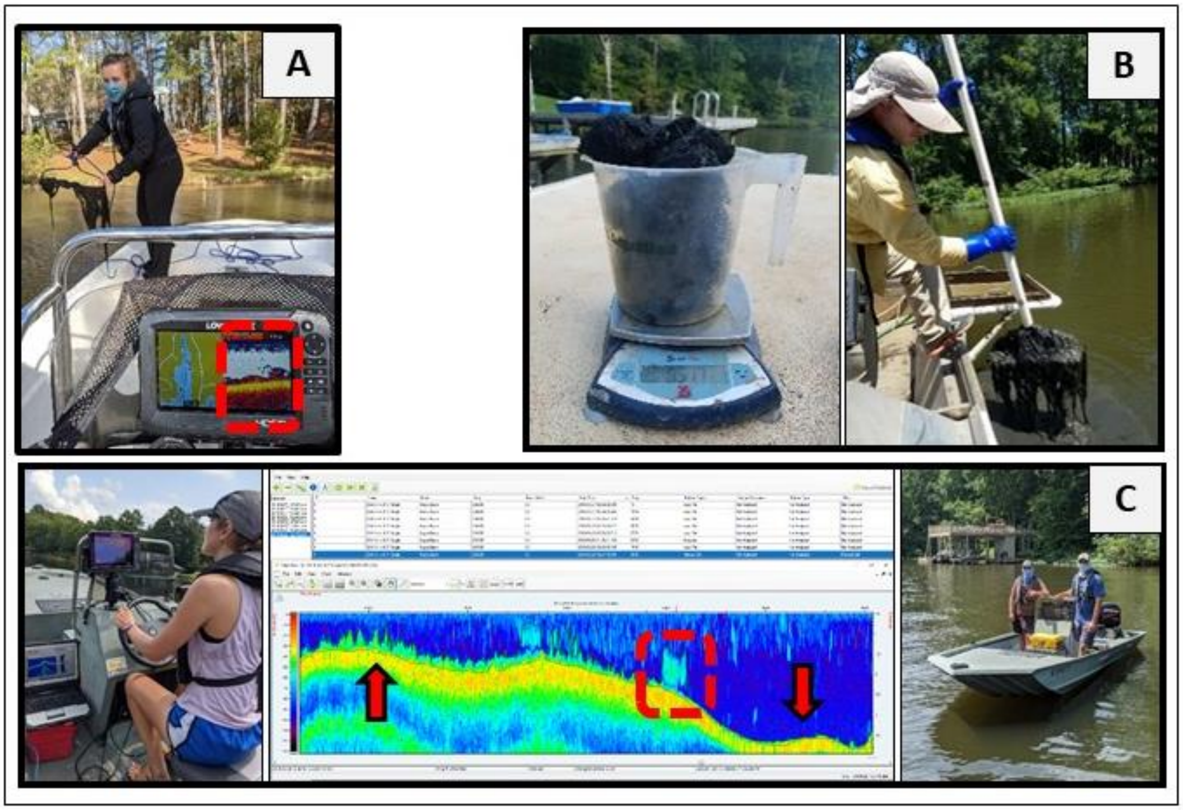

Figure 1.4.16. Sampling methods utilized to evaluate the efficacy of lyngbya targeting algaecide applications within Lake Gaston during the 2019, 2020, and 2021 treatment seasons. Included were traditional methods used to evaluate submersed aquatic vegetation including rake-tosses (A) and biomass rakes (B) and a novel method of utilizing a BioSonics MX Scientific Echosounder (sonar unit) (C). Red arrows and boxes highlight the growth or absence of lyngbya located in the benthos.

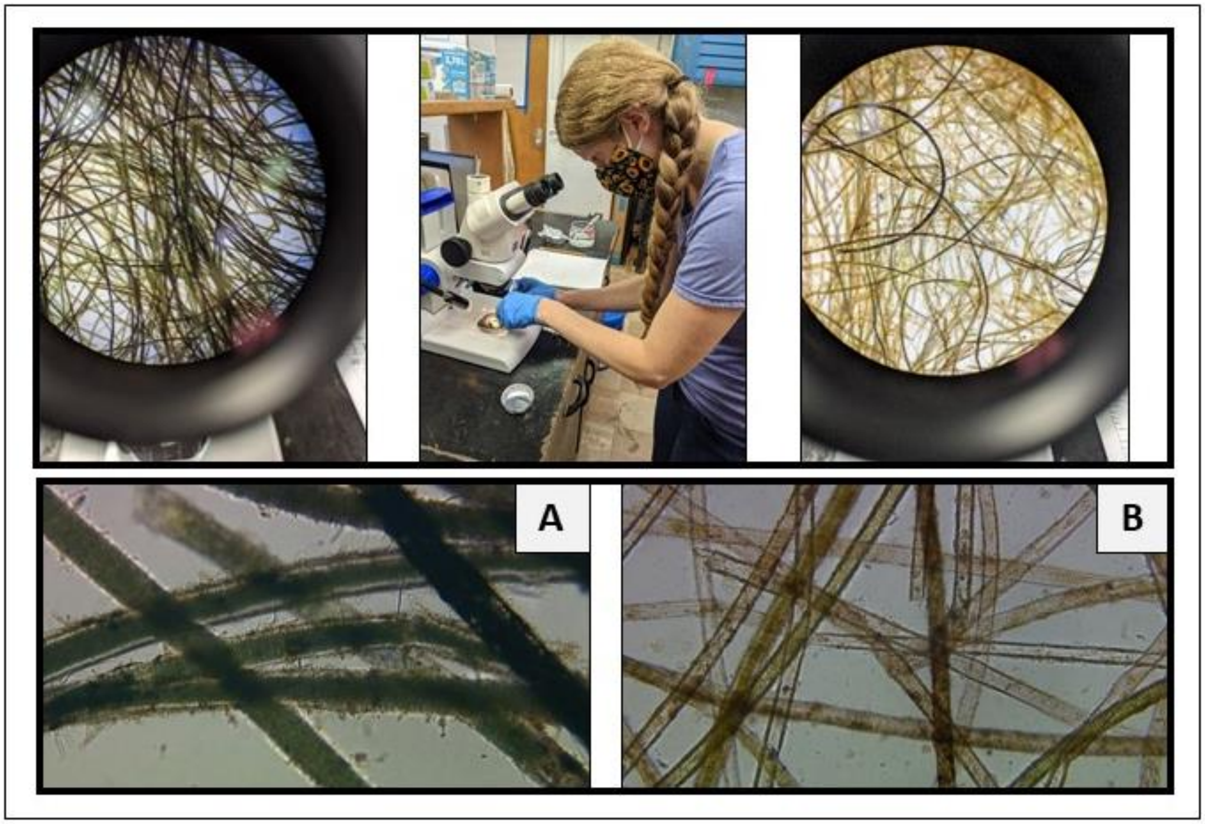

Figure 1.4.17. Lyngbya samples collected within a subset of algaecide treatment areas and control areas within Lake Gaston. Filaments were examined at 40x magnification to determine the overall cellular health of the filaments. The top left image shows highly viable lyngbya filaments and the top right images shows an example of lyngbya in poor condition and represented mostly by empty sheaths. The bottom images show samples collected from a control site (A) and an area that received algaecide applications (B) post lyngbya treatment season. Bottom images were captured by the NCSU Center for Applied Aquatic Ecology lab using an Olympus AX70 Research Microscope at 300x magnification and imaged with a Microcast HD Pro-Lite digital camera system.

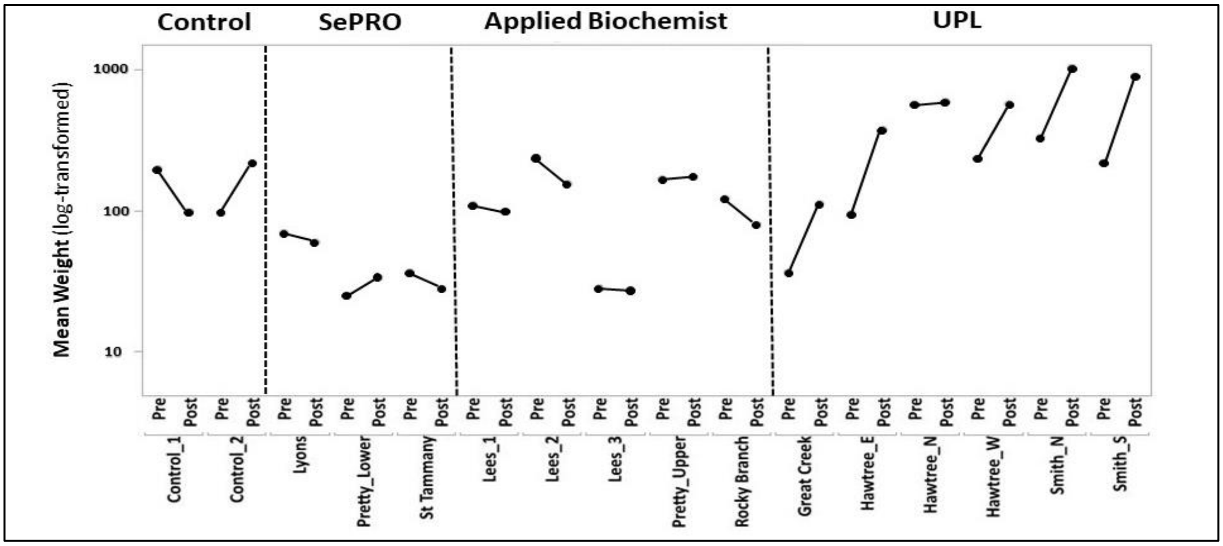

Figure 1.4.18. Changes in lyngbya biomass from pre-treatment *(April)* to post-treatment *(November)* sampling for treatment and control sites during the 2019 treatment study at Lake Gaston.

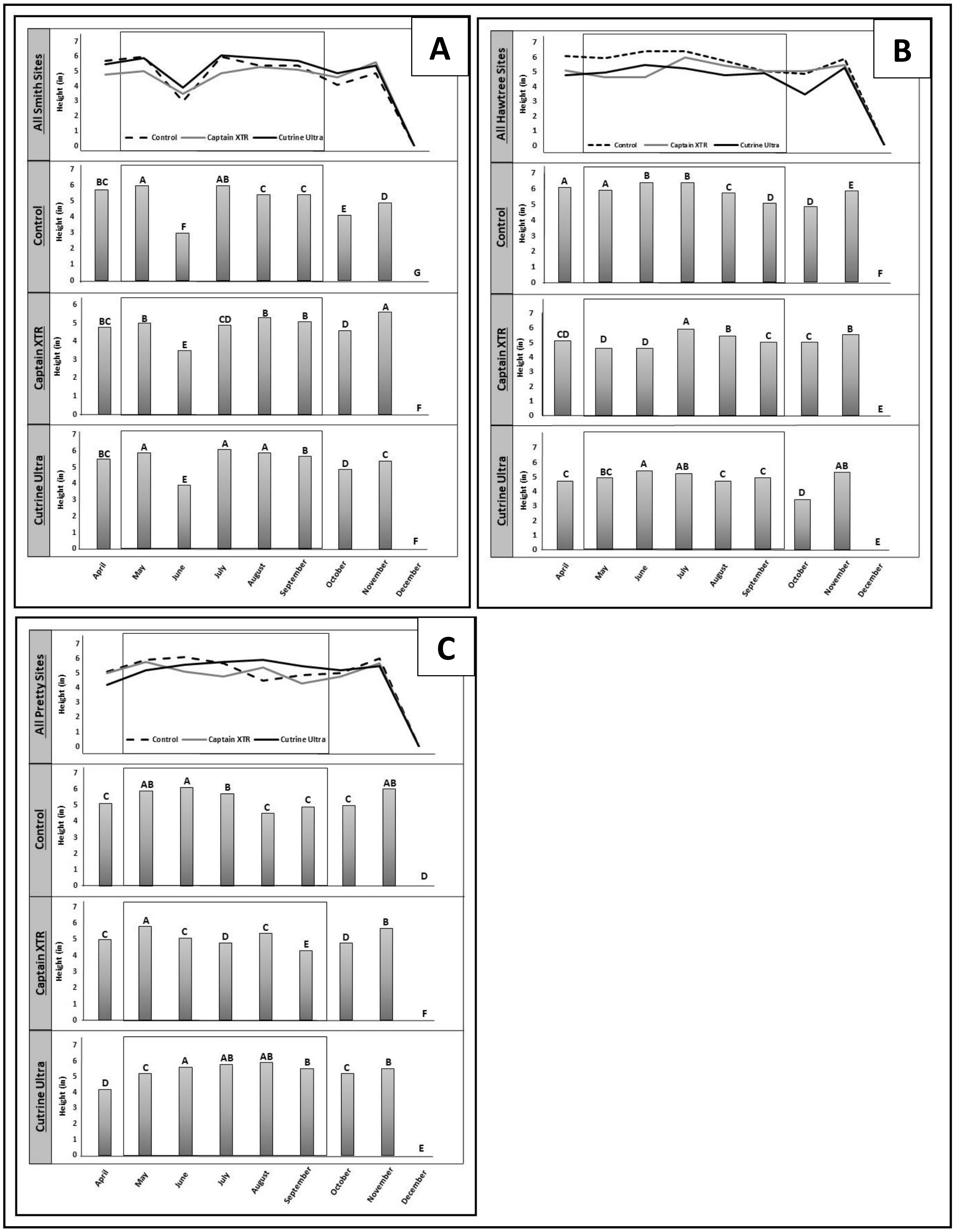

Figure 1.4.19. Mean vertical water column occupancy derived from BioSonics echosounding measures over the 2020 lyngbya treatment period from creeks that contained sites for both Captain XTR® and Cutrine Ultra®, as well as, a control site. These sites included Smith (A), Hawtree (B), and Pretty (C) and each received monthly treatments between May and September. Means that have the same letter above them do not significantly differ according to Tukey's HSD ($p < 0.05$). Data points that received treatment within 30 days of the sampling event are indicated by the black box.

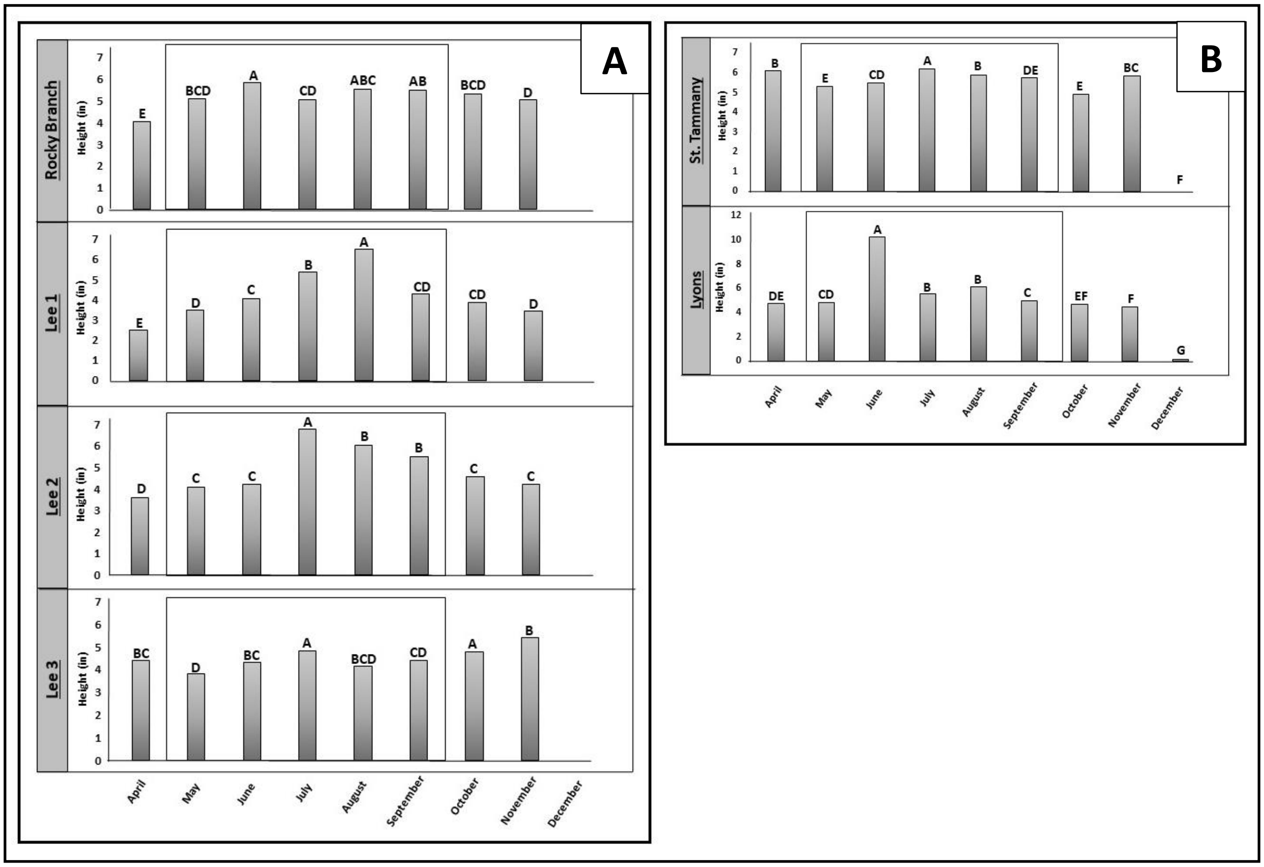

Figure 1.4.20. Mean vertical water column occupancy derived from BioSonics echosounding measures over the 2020 lyngbya treatment period using algaecide treatment algaecide treatment that included Algimycin® (A) and Captain XTR® (B). Means that have the same letter above them do not significantly differ according to Tukey's HSD ($p < 0.05$). Data points that received treatment within 30 days of the sampling event are indicated by the black box.

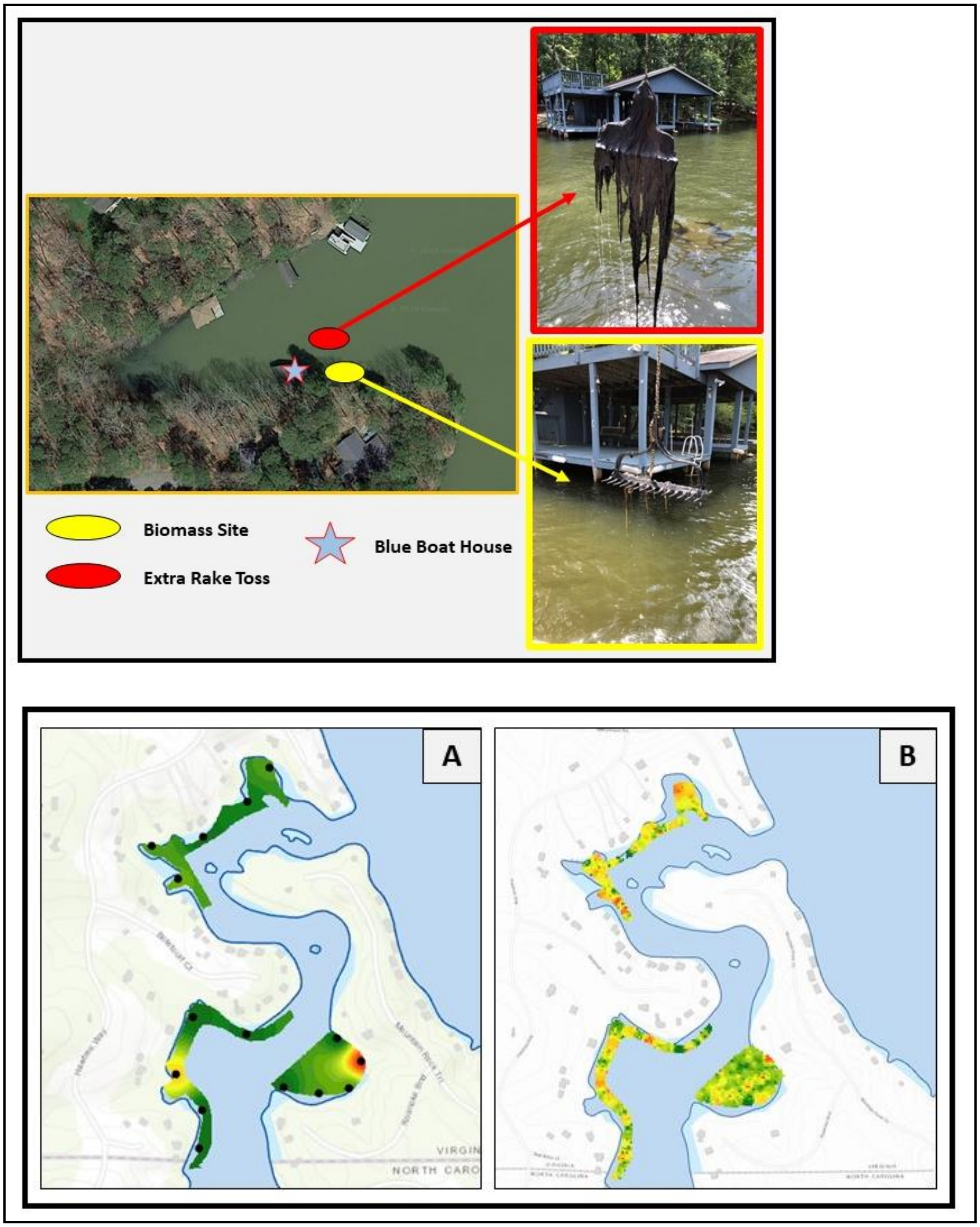

Figure 1.4.21. Examples of spatial difficulties encountered while evaluating benthic lyngbya mats to determine the efficacy of treatment protocols. The top image shows a rake toss performed at a biomass sampling site (yellow) and one thrown in close proximity to it (red). The bottom images shows interpolated maps of lyngbya mats collected from biomass sampling sites (A) and BioSonics echosounding data (B).

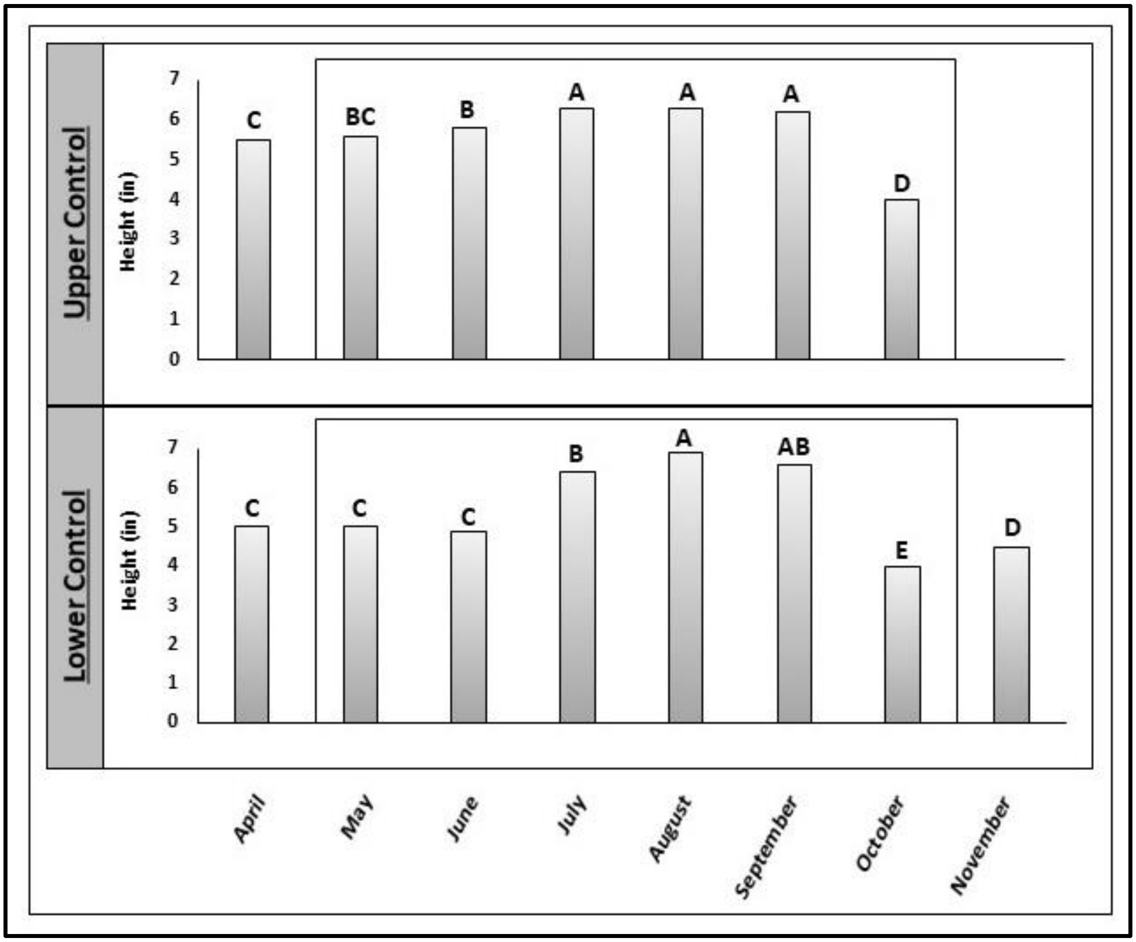

Figure 1.4.22. Mean vertical water column occupancy derived from BioSonics echosounding measures over the 2021 lyngbya treatment period from areas that were designated as control sites and were not targeted with lyngbya control methods in 2021. Means that have the same letter above them do not significantly differ according to Tukey's HSD ($p < 0.05$). Data points that coincide temporally with lyngbya treatments are indicated by the black box.

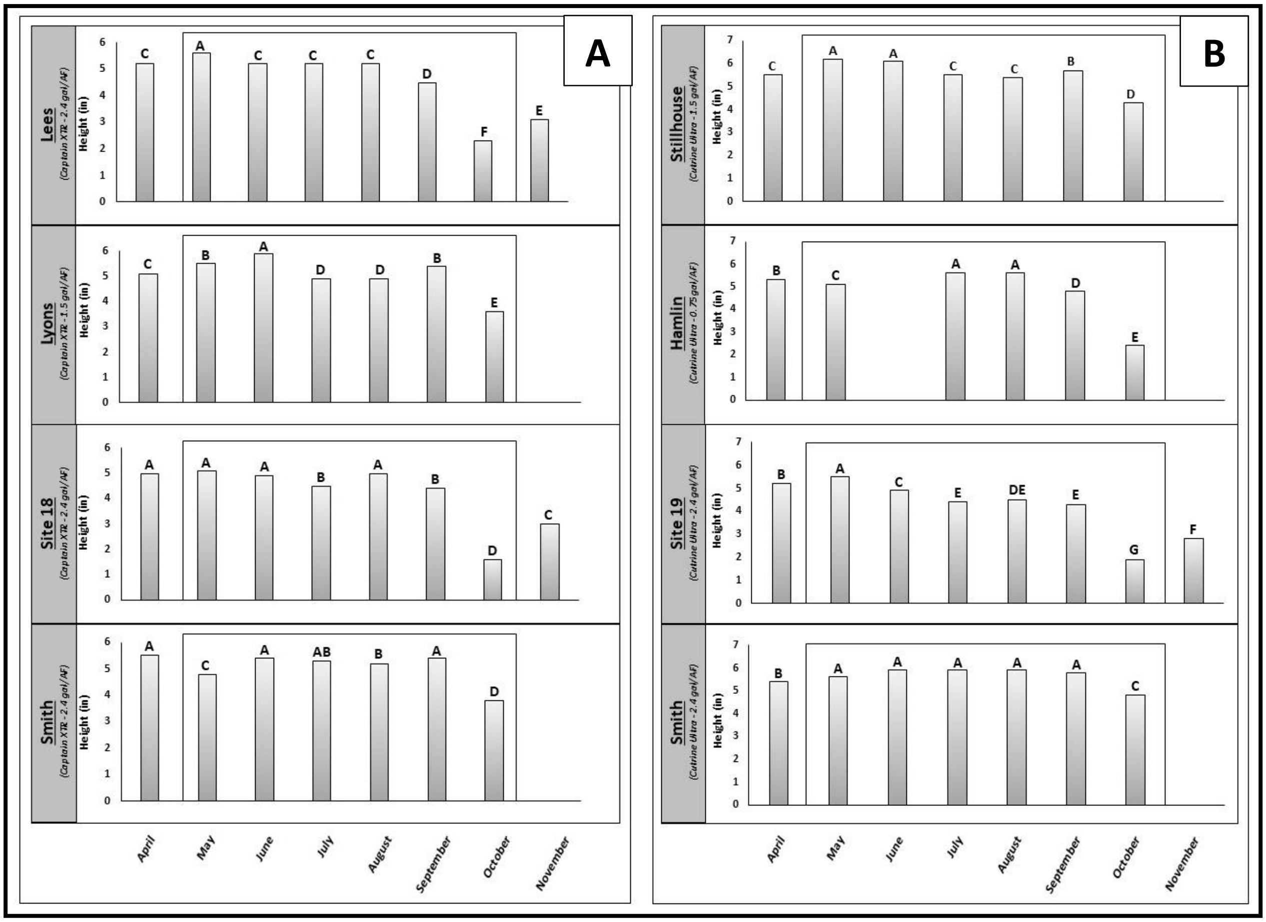

Figure 1.4.23. Mean vertical water column occupancy derived from BioSonics echosounding measures over the 2021 lyngbya treatment period from creeks that received monthly treatments using algaecide treatmentalgaecide treatment that included Captain XTR® (A) and Cutrine Ultra® (B). Means that have the same letter above them do not significantly differ according to Tukey's HSD ($p < 0.05$). Data points that received treatment within 30 days of the sampling event are indicated by the black box.

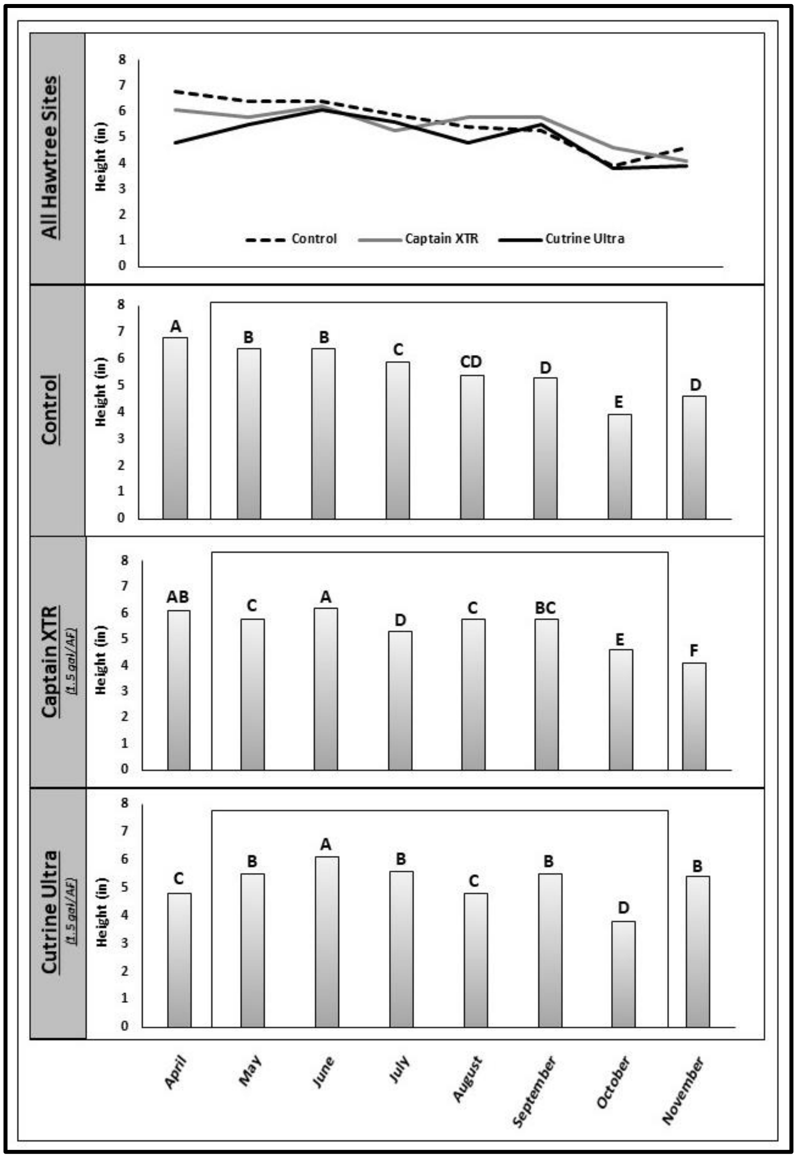

Figure 1.4.24. Mean vertical water column occupancy derived from BioSonics echosounding measures over the 2021 lyngbya treatment period from Hawtree Creek which contained treatment sites for Captain XTR® and Cutrine Ultra®, as well as, a control site. Means that have the same letter above them do not significantly differ according to Tukey's HSD ($p < 0.05$). Data points that received treatment within 30 days of the sampling event are indicated by the black box.

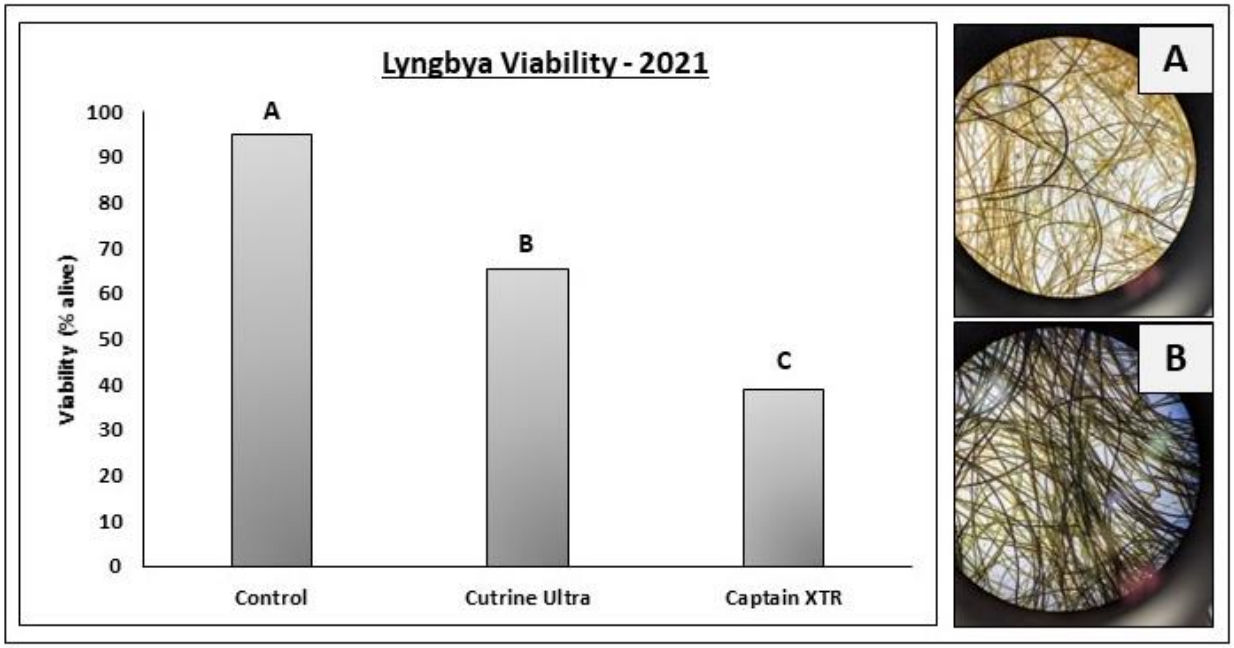

Figure 1.4.25. Results of viability measurements indicating the percent of lyngbya that was viable from samples collected at sites that received monthly treatments of Captain XTR® and Cutrine Ultra® from April to September of 2021 and a control site that did not receive lyngbya control measures in 2021. Means that have the same letter above them do not significantly differ according to Tukey's HSD ($p < 0.05$). Images show examples of samples that displayed low viability (A) and high viability (B).

1.5 POTENTIAL ENVIRONMENTAL IMPACTS OF TREATMENTS

Introduction

As described in the *Lyngbya Treatment Overview* section of this report, application methods that utlize chelated copper based algaecides directly to the benthic lyngbya mat have been proven most effective in the management of lyngbya at Lake Gaston. However, the toxicity of copper to aquatic organisms, especially freshwater mussels, has been well documented (Keller and Zam 1991, Jacobson et al. 1993, Jacobson et al. 1997, Gillis et al. 2008, and Jorge et al. 2013). Naturally encountered environmental stressors such as ammonia and water temperature have also been associated with acute toxicity in mussels (Augspurger et al. 2003, Wang et al. 2007a, and Wang et al. 2007b) and so has the compounding effects of copper with environmental stressors (Pandolfo et al. 2010 and Wagner et al. 2017). The Tidewater Mucket (*Leptodea ochracea*) is a North Carolina state threatened mollusk that is native to the Roanoke River drainage (NCWRC) and is well established within Lake Gaston. The reservoir has provided a unique refugee for this species, protecting it from the increased risks associated with climate change and rising sea levels that negatively impact populations located in the coastal region further downstream (Dr. Greg Cope, NCSU, personal communication). However, this reservoir population is now co-occurring with an expanding lyngbya infestation at Lake Gaston and little is known about this reservoir population including distribution and abundance estimates (Michael Fisk, NCWRC, personal communication). The steady increase of lyngbya within Lake Gaston and potential negative interactions that can occur with mussel beds and those treatments targeting lyngbya has highlighted the importance of addressing this knowledge gap for Lake Gaston's Tidewater Mucket population.

In June 2021, a mussel mortality event occurred in several areas of Lake Gaston that were actively being treated for lyngbya. While the additional stress of exposure to the copper-based algaecide likely exacerbated mortality in the treatment areas, the role of other environmental factors is unclear. Timing of lyngbya treatments in Lake Gaston coincides with the occurrence of all the aforementioned environmental stressors. Herbicide application techniques allow pathways for fish and other highly mobile aquatic species to egress from a treatment area, however benthic mollusks are confined to the impacted sediment. The extent of impact to existing mussel beds is unclear since little is known about the overall population dynamics of Tidewater Mucket in Lake Gaston.

Methods

In response to the reported mortality , staff from NC State University and the NC Wildlife Resources Commission surveyed several locations and documented recent mussel mortality which occurred both inside and outside of the treatment areas. Three sites were identified for further investigation in September 2021 based on a visual assessment of overall impact. Jimmie's Creek was identified as a reference site due to its robust mussel population and lack of herbicide treatments applications. Two sites that experienced high levels of mortality in June 2021, Lee's Creek and Site 18, were also identified to be further surveyed. Sites ranged from less than 1 foot to 13 feet in water depth and were surveyed with equal amounts of effort using a quadrat sampling method with both snorkeling and scuba gear (3 hrs. total effort per site). Living mussels were counted, sexed, and length measurements were recorded for each site. Logistic restraints associated with the collaborative effort between NCSU and NCWRC prevented additional sites from being included in the survey effort.

Results

Of the four native freshwater mussels that were identified during the September 2021 survey, Tidewater Muckets were the most abundant species at all sites (Figure 1.5.1 and Figure 1.5.2). Tidewater Muckets were predominately found in shallow water depths (< 6.5 ft), with no individuals found in depths greater than 6.5 feet at Site 18 (Figure 1.5.3). The highest abundance of Tidewater Mucket's were found at the Jimmie's Creek reference site (n = 142) (Figure 1.5.2). Although Site 18 and Lee's Creek were both equally impacted by mussel mortality, Site 18 had a higher abundance of Tidewater Muckets (n = 77) than Lee's Creek (n = 22) three months post the event (Figure 1.5.2). It is worth noting that without data prior to June 2021, impacts to overall abundance for individual sites cannot be determined. However of the impacted sites, Site 18 appeared to show more recovery from June to September 2021 than did Lee's Creek.

Routine water quality monitoring of Lake Gaston (see section 2 – Water Quality) included sites that were adjacent to areas in which mussel survey occurred for both Jimmie's Creek and Lee's Creek. Overall, mussels were experiencing decreased dissolved oxygen levels and increased temperatures during June 2021 (Figure 1.5.4). Ammonia levels were also elevated during the month of June to the second highest level of the year (0.057 ppb). Levels were well below acute toxicity levels reported for freshwater mussels and ammonia (Augspurger et al. 2003; Wang et al. 2007a; and Wang et al. 2007b), however the compounding effects of increased ammonia, decreased dissolved oxygen, and increased temperature may have contributed to the June mortality event. Hydrosoil data (see section 2 – Water Quality) also showed increased levels of both copper and ammonia in Lee's Creek when compared to Jimmie's Creek (Copper: 74 mg/kg and 43.7 mg/kg respectively; Ammonia: 3.19 mg/L and 1.48 mg/L respectively) which could have increased stress levels for the Lee's Creek mussel population. There was no water quality data directly associated with Site 18.

Discussion

Lack of distribution and abundance data has hindered manager's ability to effectively treat lyngbya due to potential negative impacts to the Tidewater Mucket population in Lake Gaston. Determining the distribution of Tidewater Mucket beds in relation to the expanding lyngbya infestation is one of the first steps to balancing the control of lyngbya and mitigating negative impacts to Tidewater Mucket populations. After evaluation of the mussel mortality event it was determined that Tidewater Muckets were found predominately in shallow water depths (Figure 1.5.3) and therefore future algaecide application were restricted to deep water habitats in all treatment areas to minimize the potential of a negative interaction between mussel beds and treatment. However, this protocol allows lyngbya to continue to establish and expand within the shallow coves of areas receiving treatment applications decreasing the overall effectiveness of treatments. While negative impacts of copper based algaecides is well documented, the impacts of freshwater mussels co-occurring with a benthic mat forming and potentially toxin producing cyanobacteria (lyngbya) is not known (Figure 1.5.5). Replacement of aquatic macrophyte beds by lyngbya has been shown to result in shifts of aquatic macroinvertebrate communities and decreases in gastropod abundance (Tourville Poirier and Cattaneo 2010), but no such study could be found that focused on the impact of lyngbya on freshwater mollusks. Chelated copper based herbicide treatments have a potential to negatively impact native freshwater mussel populations, however allowing infestations of lyngbya within a system to expand could result in even more ecological harm. To better assist managers that are balancing the control of a noxious algae species with trying to minimize negative impacts to a listed freshwater mussel, future research needs to focus on the direct impacts that lyngbya has on freshwater mussel populations including possible toxin production and habitat alteration.

The inability to determine overall impacts to mussel bed abundance from the 2021 mussel mortality event highlights the need of a more robust understanding of Lake Gaston's Tidewater Mucket population. One of the challenges to accurately assessing mussel populations relates to the logistical issues that are involved in surveying a benthic dwelling species. Once a potential habitat has been identified, visual observations that require methods such as snorkeling and scuba are combined with tactile methods by actively hand grubbing in benthic substrates to assess mussel populations. Lake Gaston's turbid environment, extensive shoreline, and growing lyngbya infestation provides limitations on resources that are needed to acutely determine the distribution of Tidewater Mucket populations within the reservoir. To address limitations associated with water clarity and shoreline area, a method such as side-scan sonar that does not have light requirements, can increase the total survey area, and decrease time spent in the field can be utilized to image and classify benthic habitats. The ability of side-scan sonar to map a variety of benthic aquatic habitats has been well established (Kaeser et al. 2013; Litts and Kaeser 2016). Possible limitations for identifying Tidewater Mucket mussel bed sites based solely on benthic habitat features are that sandy/silt substrates have been noted as preferred by the species (NCWRC website) and a large amount of Lake Gaston shoreline would likely fall into this category. However, side-scan sonar has also been shown to be an effective tool for identifying individual mussel beds (Powers et al. 2015). Powers et al. 2015 successfully distinguish mussel shells that were located in habitats that consisted of sand and clay substrates and significantly reduce sampling efforts as compared to traditional field sampling methods (Christian and Harris 2005). Using this technology to map mussel beds within treatment zones targeting lyngbya would allow managers to help protect Tidewater Muckets from direct exposure to chelated copper based treatments. The presence and extent of mussel beds could also become a determining factor for future treatment locations both in terms of treating lyngbya that is encroaching on identified Tidewater Mucket beds or exclusion of areas that contain extensive mussel beds. Identifying and estimating the size of active mussel beds would also assist in assessing other population dynamics such as abundance and habitat preference.

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Figures

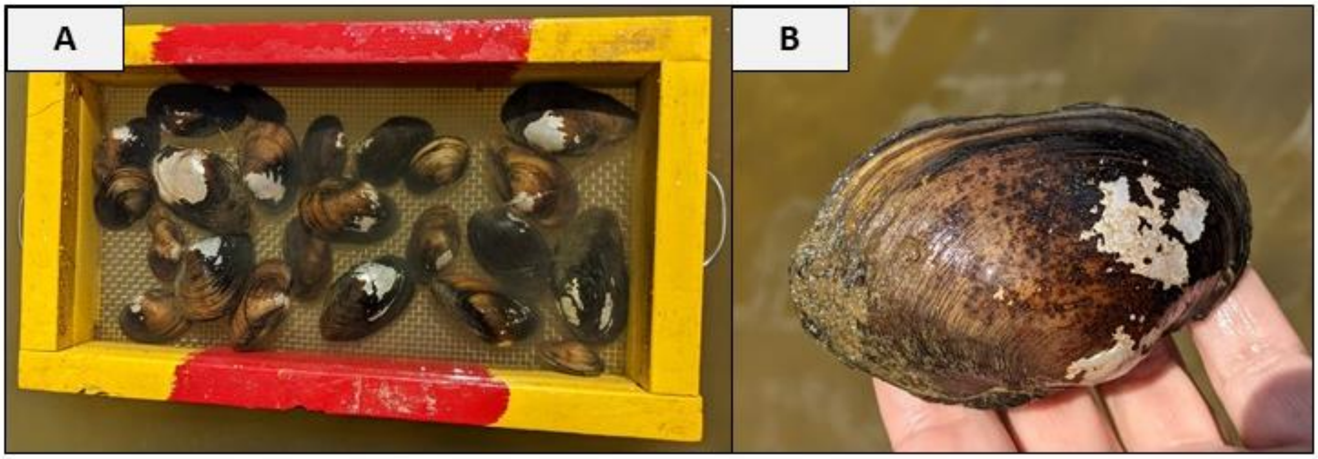

Figure 1.5.1. Images showing A) the variety of freshwater mussels found in Lake Gaston and B) a Tidewater Mucket (Leptodea ochracea).

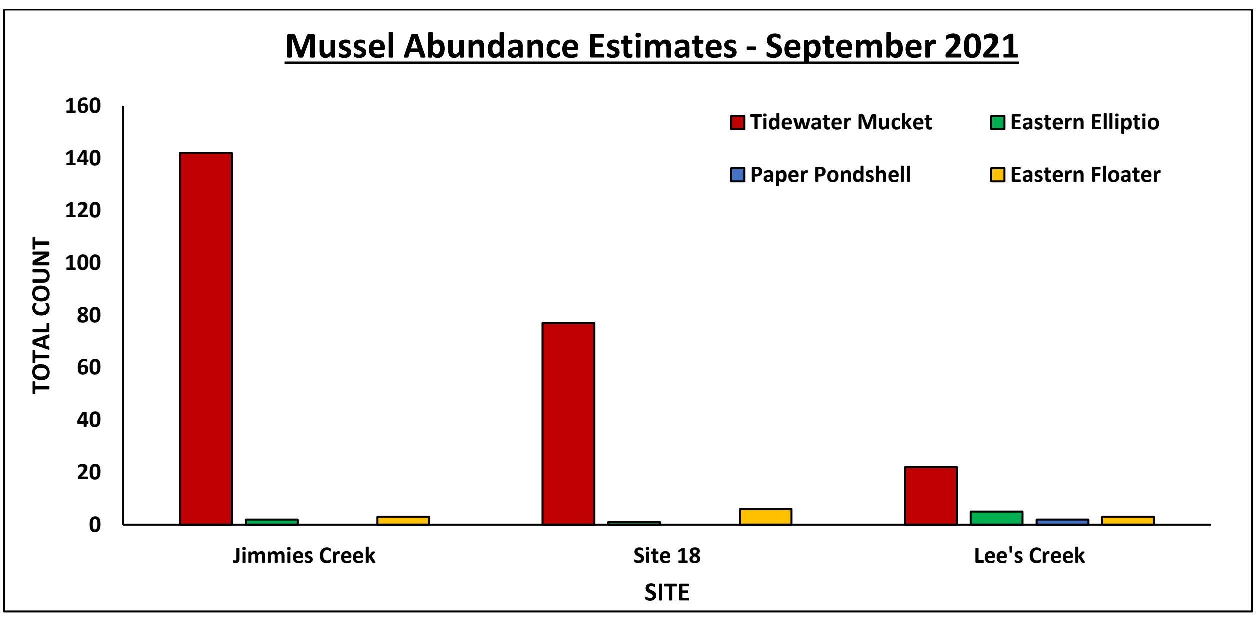

Figure 1.5.2. Total abundance estimates of four freshwater mussel species found in Lake Gaston surveyed in September 2021. Abundance estimates were calculated three months post a reported mussel mortality event in June 2021. Sites represent a reference site (Jimmies Creek) and two impacted sites (Site 18 and Lee's Creek).

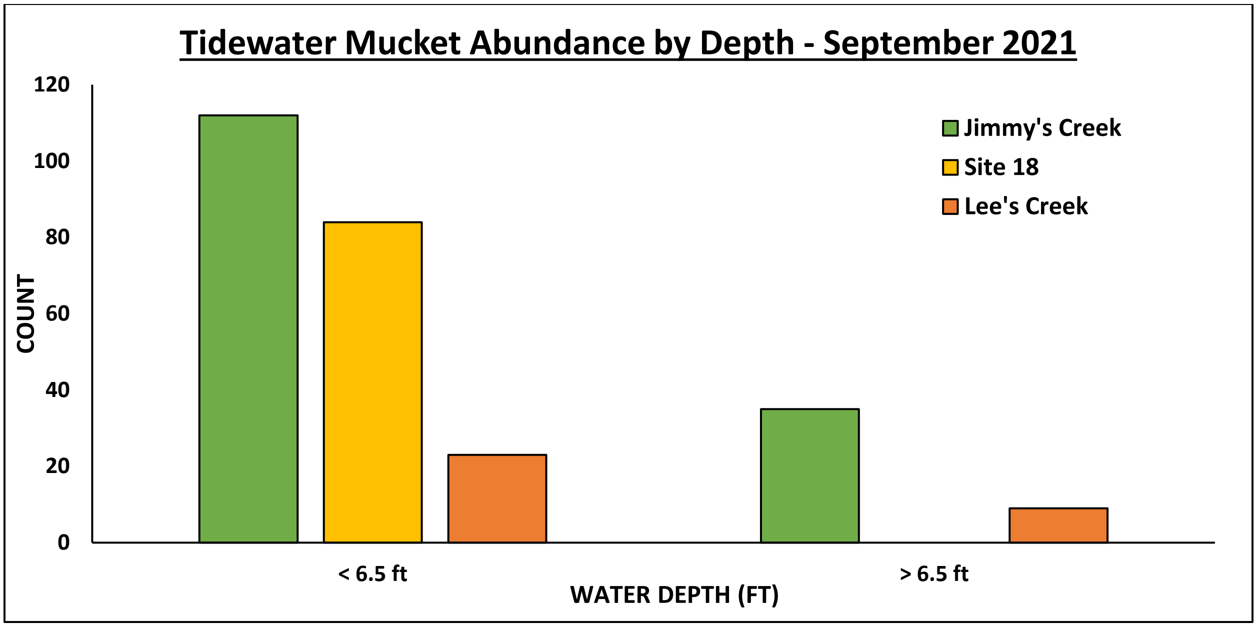

Figure 1.5.3. Total abundance estimates of Tidewater Mucket sites surveyed in September 2021. Abundance estimates were divided into those individuals that were collected in shallow water (<6.5 ft) and those that were collected in deep water (> 6.5 ft). Sites represent a reference site (Jimmies Creek) and two impacted sites (Site 18 and Lee's Creek).

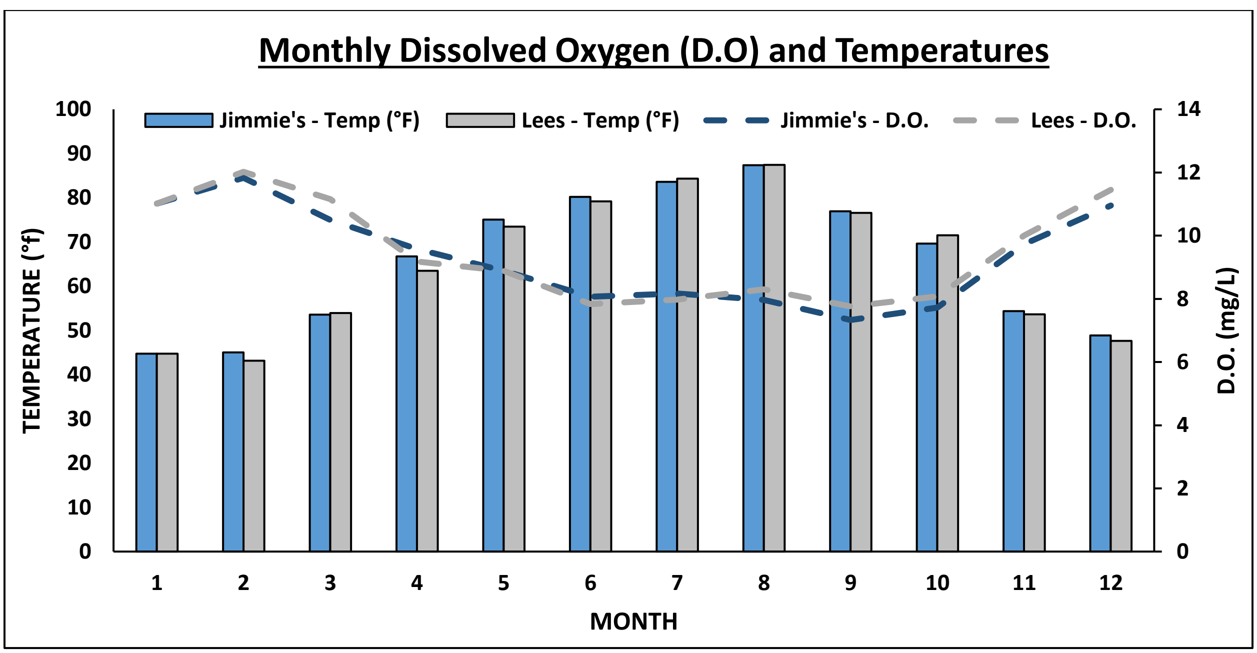

Figure 1.5.4. Monthly values reported in 2021 for dissolved oxygen levels (mg/L) and temperature (°F) for water quality sites located within Jimmie's Creek and Lee's Creek.

Figure 1.5.5. An image showing the proximity of a dense lyngbya benthic mat to a large Tidewater Mucket mussel bed.

1.6 LYNGBYA TOXIN POTENTIAL

Introduction

Harmful Algal Blooms (HABs) can produce a plethora of ecological stresses that range from decreased water clarity that can suppress native macrophyte communities to toxic blooms that can threaten aquatic ecosystems and negatively impact human health (Paerl and Otten 2013). A major concern regarding cyanobacteria is their ability to produce cyanotoxins, including hepatotoxins (those that impact liver function) and neurotoxins (those that impact nervous system function). The concern is even greater when these species occur in freshwater systems that are utilized for recreational activities and are sources of drinking water. Due to the potential of harmful interactions between cyanobacteria and human health, governmental HAB response protocols have been established in many states. These response protocols primarily focus on planktonic forms of cyanobacteria due to their high toxicity potential, however the increased levels of lyngbya infestations in freshwater environments has raised concerns over the possible toxin production of this benthic cyanobacteria.

One of the early studies to identify potential toxin production in lyngbya species found in freshwater environments occurred in the early 1990's in Guntersville Reservoir on the Tennessee River in Alabama (Carmichael et. al 1997 and Yin et. al 1997). Carmichael et al. (1997) discovered that Guntersville lyngbya produced toxic compounds that were typical of either antx-A or Paralytic Shellfish Poison (PSP) neurotoxins. More recent studies support and expand on the toxin producing ability of freshwater lyngbya to include detection of an analogue to the PSP neurotoxin, saxitoxin, within the benthic lyngbya mat material collected from the St. Lawrence River, Canada (Lajeunesse et al. 2012), Butterfield Lake, NY (Smith, Z. et al. 2019), and Lake Wateree, SC (Smith, M. et al. 2019). Increased toxin concentrations within freshwater lyngbya has been positively correlated with increased biomass, active growth, and the physiological heath of lyngbya (Yin et al. 1997 and Hudon et al. 2016). With all of the aforementioned studies, toxin concentrations were extracted directly from lyngbya filaments collected from benthic mat material. However, since the mechanism for toxin release from lyngbya cells is unknown and it is not clear how the presence of a thick protective sheath impacts the leaching of those toxins into the ecosystem, mat material may not give a true indication of toxin concentrations within the surrounding water column.

Recent developments in cyanotoxin collection methods could help further our understanding of the relationship between the ability of lyngbya in a freshwater environment to produce toxins and the potential of ambient release of those toxins. A method called Solid Phase Adsorption Toxin Tracking (SPATT) was developed in 2004 by MacKenzie et al (2004) to provide a cost-effective way to monitor toxic algal blooms in New Zealand. This technology deploys a synthetic resin with the ability to adsorb toxins directly from the water column and has been shown to be useful in detection of a wide range of phycotoxins (Roué et al. 2018 and Onofrio et al. 2021). This technique provides a sampling design that can increase the understanding of toxin release both spatially and temporally by identifying toxin presence over a several week period instead of at the single moment in time that traditional water sample grabs provide.

The objective of this pilot study was to determine if the use of SPATT technology could be used to monitor cyanotoxins related to lyngbya within Lake Gaston. This was a collaboration between NCSU's Aquatic Plant Management Program and Old Dominion University's (ODU) Phytoplankton Analysis

Laboratory in which NCSU designed, deployed, and collected the SPATTS, and provided the needed ELISA kit and ODU extracted and provided analysis of the cyanotoxin concentrations.

Methods

Study Site and Field Sampling

SPATT technology was deployed at four sites within Lake Gaston between the dates of July 20th and August 16th, 2021 (Figure 1.6.1). Sites were selected for physiological properties within the benthic lyngbya mat material and its geographical relationship to ongoing lyngbya treatment areas. Prior to SPATT deployment, lyngbya mat material was collected from multiple sites (Figure 1.6.1) utilizing a double sided rake that pulled mat material off the benthic substrate. These samples were transferred to a ziplock bag, placed on ice, and shipped overnight to the Phytoplankton Analysis Laboratory at Old Dominion University, Norfolk VA for toxin analysis. Three SPATT sites were then located within areas where toxin analysis confirmed that saxitoxin-a was present within the filamentous material of those benthic mats. Of these three sites, two (T1 and T2) were also located in areas that were being treated with Captain XTR & AMP on a monthly basis as part of the Lake Gaston Weed Control Council (LKWCC) lyngbya treatment program. These monthly treatments were aimed at controlling lyngbya growth within the lake and occurred on a monthly basis between the months of April and September. A third site (C1) that contained lyngbya was located in an area that was excluded from the monthly treatment program and did not receive any form of herbicide treatment from the LGWCC. The final site (R1) was used as a reference site and was located in an area that was completely void of lyngbya.

The SPATT design was based off the Maryland Department of Natural Resources Tidewater Ecosystem Assessment standard operating protocol. Resin bags (SPATTs) were constructed using 100um Nitex bolting cloth that was cut and heat sealed to form a (115 mm x 115 mm) bag that contained 3g (dry weight) of Supelco HP20 Diaion resin (Figure 1.6.2). Prior to field deployment, each bag was activated during a soaking period of 48 hours in 100% methanol, rinsed in de-ionized water, and the stored submerged in de-ionized water at 4 – 6 °C. During field deployment, each SPATT was housed in a protective plastic case with large holes to allow for sufficient water exchange (Figure 1.6.2). SPATTs were suspended and oriented within the housing unit to ensure that the resin was dispersed evenly throughout the entire bag for maximum absorption potential. During retrieval from field deployment, resin bags were placed in individual ziplock bags on ice for transport to the laboratory and then stored at -18 °C until toxin analysis occurred.

Timing of initial deployment coincided with the fourth treatment round of the LKWCC's 2021 lyngbya treatment program which occurred on July 20th, 2021. Each site contained up to three SPATT units that were vertically aligned and deployed for varying lengths of time (Figure 1.6.3). SPATT sites were located nearshore (<2.5 m in depth) and all units were suspended at locations that were either 0.91 m, 1.06 m, or 1.21 m from the bottom substrate. Each SPATT site (T1, T2, C1, and R1) contained individual units that were deployed for the one week period (7/20 – 7/27) and two week period (7/20 – 8/3) post treatment. Sites T2 and R1 contained units that were deployed for a second two week period (8/3 – 8/16) and sites T1 and C1 contained units that were deployed during the entire four week period (7/20 – 8/16). Ambient water quality samples were also collected from each SPATT site (T1, T2, C1, and R1) on July 27th, August 3rd, and August 16th.

Toxin Analysis

Toxin extraction - Benthic mat material

Upon arrival, mat material was removed from storage bags and cleaned of debris (ie: freshwater clams and invertebrates, rocks, and sand) using forceps, then rinsed clear of mud, sand, and sediment using Milli-Q water. Two subsamples of cleaned material from each sample were viewed at 400x on an Olympus inverted microscope under brightfield and Phase contrast illumination to ensure that the lyngbya was free from other epiphytic cyanobacteria taxa or material that might interfere with the toxins assays. If necessary, material was rinsed a second or third time to remove dirt and sediment, and checked under the microscope before proceeding to the next step. Cleaned lyngbya mat material was weighed out and 50 grams was placed into a centrifuge tube and frozen overnight at -80°C prior to toxin analysis.

Benthic mat material was processed for toxins analysis using indirect competitive enzyme-linked immunosorbent assay (ELISA) at the Phytoplankton Analysis Laboratory at Old Dominion University, Norfolk VA.

Toxin extraction - SPATT

Due to its confirmed presence in previous studies and in the benthic mat analysis performed at Lake Gaston, saxitoxin-a was used for the SPATT analyses. In preparation for extraction, SPATTs were thawed and resin was transferred into a 50mL conical tube. Resin was rinsed with a 75% methanol solution prior to a two-step extraction process that included a 30 minute shaker table period and centrifugation at 3,894 rpm for 10 minutes. The SPATT extract was then diluted to a 5% methanol solution prior to storage at -20 °C until toxin analysis.

Solid Phase Adsorption Toxin Tracking (SPATT) bags were processed for toxins analysis using indirect competitive enzyme-linked immunosorbent assay (ELISA) at the Phytoplankton Analysis Laboratory at Old Dominion University, Norfolk VA.

Results

Toxin analysis of benthic mat material resulted in positive detections of saxitoxin-a and cylindrospermopsin at sites T1, T2, and C1. Saxitoxin-a concentrations exceeded the upper test limit for the ELISA kits at a 1:2000 dilution rate for all three sites, so true concentration values could not be determined. High concentration levels are likely due to the processing methods of extracting toxins directly from mat material and are more representative of intercellular toxin levels. Due to the high levels of saxitoxin-a present within lynbya mat material at these sites, T1, T2, and C1 were chosen as deployment sites for the SPATTs.

The ability to detect the cyanotoxin saxitoxin-a through the use of SPATTs was confirmed during this pilot study. A single SPATT site (T2) resulted in saxitoxin-a detection levels greater than the limit of the saxitoxin-a ELISA kit (2.75) (Figure 1.6.4). This individual SPATT was deployed two weeks post lyngbya treatment application for a two week period between August 3rd and August 16th of 2021. During the collection of SPATT (T2), a water sample was also collected and toxin analysis resulted in a positive detection of saxitoxin-a (0.074 ppb). All other SPATTs were retrieved in good condition but did not result in toxin levels greater than the ELISA lower limit.

Discussion

This is the first study to our knowledge that has utilized SPATTs to detect possible cyanotoxin production by freshwater lyngbya. SPATT technology gives an indication of toxin presence and not concentration since the amount of water transfer needed to produce a positive response is unknown. Therefore, it is important to note that toxin concentrations calculated from SPATT technology can not be correlated with EPA standards or other methods of toxin analysis that calculate concentrations from a known volume of water. The scope of this project was small due to a shortage of Supelco HP20 Diaion resin that resulted from the ongoing global COVID-19 pandemic. However, we were still able to determine proof of concept for using SPATT technology to detect lyngbya cyanotoxins within a freshwater environment.

The EPA has published drinking water health advisories for the cyanotoxins microcystins and cylindrosoermopsin, but no such standard exists for saxitoxins. Saxitoxin is a neurotoxin that impacts the nervous system and has a suite of symptoms ranging in severity. However, the saxitoxin derivatives associated with lyngbya is less potent than other PSP-producing cyanobacteria (Yin et al. 1997, Lajeunesse et al. 2012, and Hudon et al. 2016) and presents a relatively low risk to humans and animals. However, Hudon (2016) notes that chronic exposure through drinking water could be a concern for long-term public health and that changing environmental factors related to climate change could impact lyngbya toxin production. Smith, M. et al. (2019) notes that accurate risk assessments are difficult to determine due to the lack standards for other lyngbya produced toxins that results in a lack of understanding in regards to the possible compounding effects of multiple toxins being released simultaneously in a system. It is worth noting that even if toxin-producing cyanobacteria are present within a system, it does not mean they are producing toxins. The environmental and physiological factors that drive toxin production and release in lyngbya are still not well understood (EPA and Poirier-Larabie et al. 2020).

Higher levels of cyanotoxins found in the benthic mat material of lyngbya of this study were not surprising as these toxins naturally exist within cyanobacteria's cytoplasm and are retained within the cell wall (EPA). Poirier-Larabie (2020) also found higher levels of intercellular toxins when compared to ambient water samples collected directly above benthic lyngbya mats and noted a weak correlation between the two toxin concentrations. Release of intercellular toxins into the surrounding aquatic environment occurs when cyanobacteria's cellular walls are ruptured (lysing) and it has been hypothesized that disturbance of benthic lyngbya mats related to wind and wave action could stimulate toxin release in lyngbya (Poirier-Larabie et al. 2020). The positive detection of saxitoxin-a in this study could indicate a possible driver of stress induced lysing for lyngbya. Saxitoxin was detected within an area that was actively being treated for lyngbya using a chelated-copper based algaecide, in addition, this area was directly adjacent to new construction that was occurring along the shoreline, including a new boat dock. Either of these actions could have resulted in disruption of the benthic mat material and therefore have been a driver for stress induced toxin production. Due to the small scope of this project, further investigation would be required to better understand that relationship.

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Figures

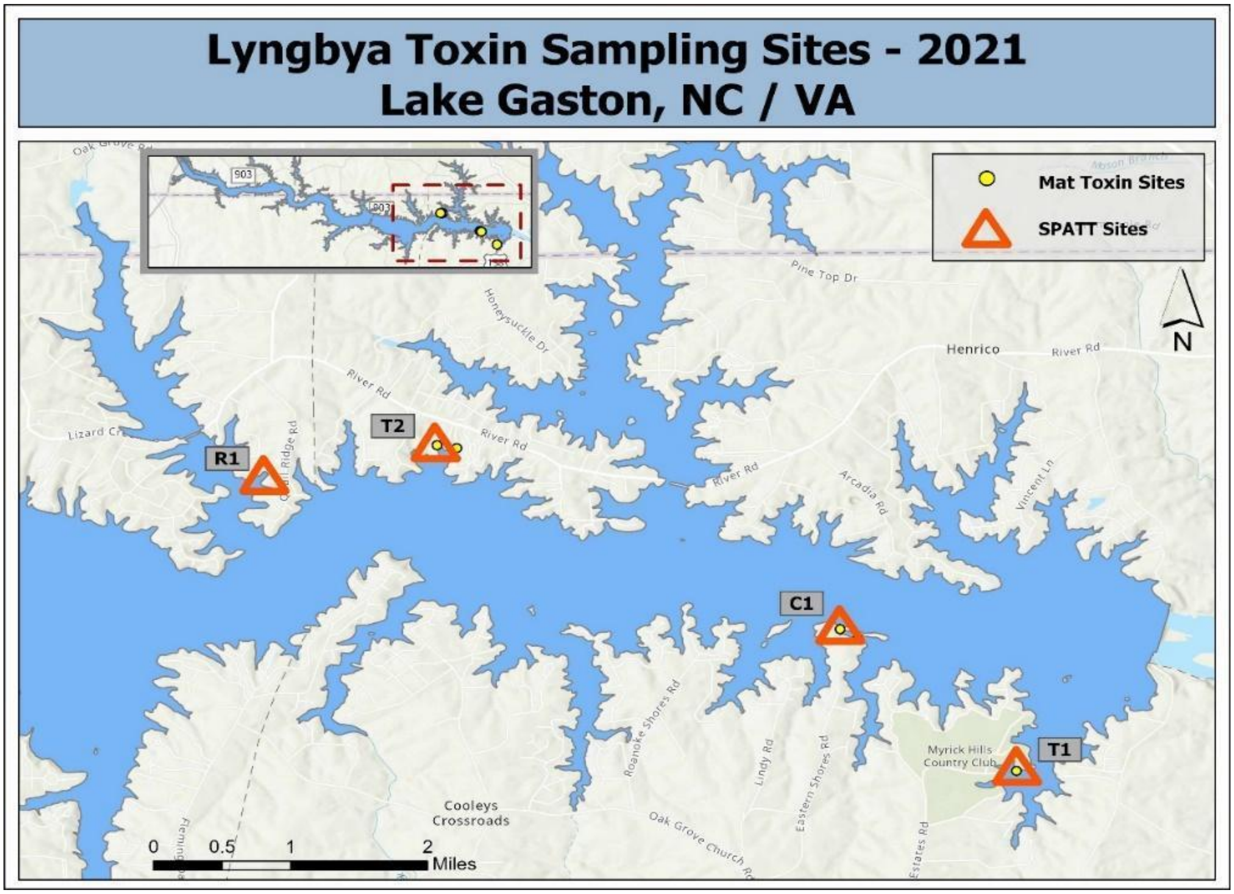


Figure 1.6.1. A map indicating sites where cyanotoxin samples were collected at Lake Gaston from both benthic lyngbya mat material and through the use of SPATT technology.

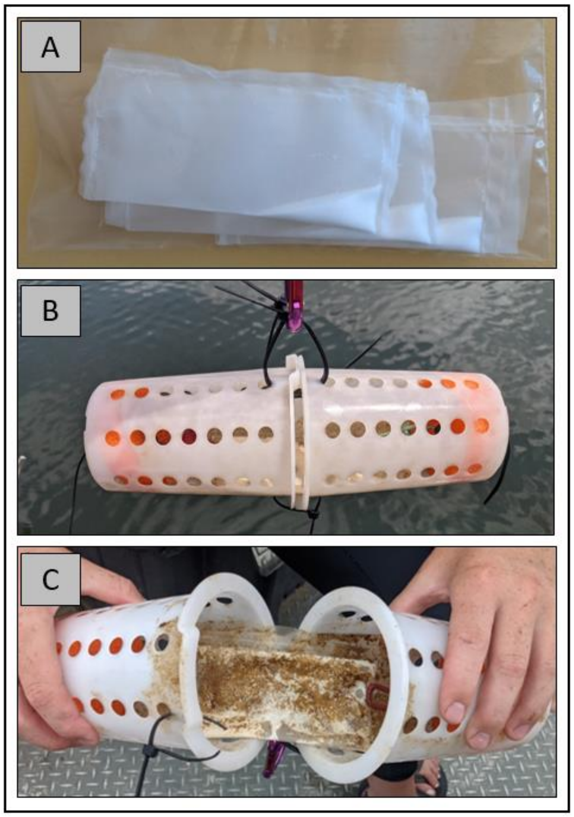
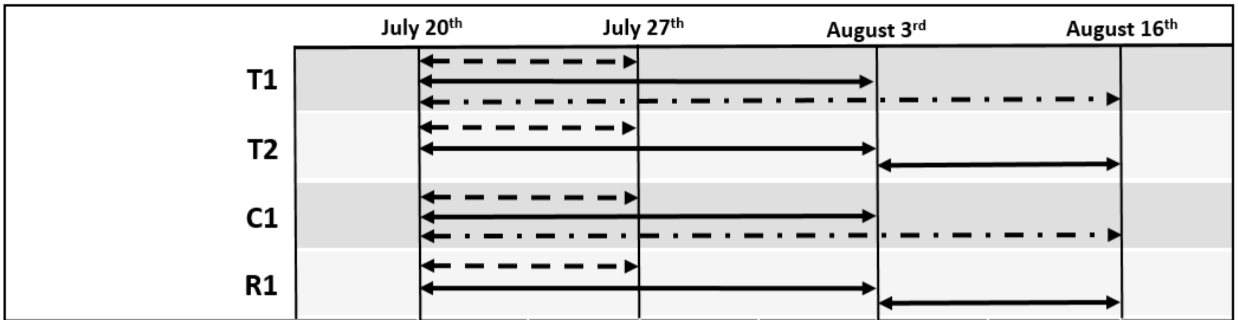


Figure 1.6.2. SPATT resin bags (A) were deployed housed in a protective plastic case (B) to collect cyanotoxin samples at Lake Gaston. Imagine C shows bag after a one week deployment.

Figure 1.6.3. Images of SPATT units deployed at Lake Gaston. Each site contained up to three SPATT units that were deployed in vertical sequence for varying lengths of time**.**



			SPATT (7/27)	H20 (7/27)	SPATT (8/3)	H20 (8/3)	SPATT (8/16)	H20 (8/16)
T1 *(lyngbya - treatment)*			*Below Limit*	*Below Limit*	*Below Limit*	*Below Limit*	*Below Limit*	*Below Limit*
T2 *(lyngbya - treatment)*			*Below Limit*	*Below Limit*	*Below Limit*	*Below Limit*	**3.42** *(8/3 – 8/16)*	**0.074** ***ppb***
Control *(lyngbya - no treatment)*			*Below Limit*	*Below Limit*	*Below Limit*	*Below Limit*	*Below Limit*	*Below Limit*
Reference *(lyngbya void)*			*Below Limit*	*Below Limit*	*Below Limit*	*Below Limit*	*Below Limit*	*Below Limit*

Figure 1.6.4. Results of saxitoxin analysis performed on SPATT technology and ambient water samples collected between July 27th and August 16th at Lake Gaston. All sites contained individual units that were deployed for a one week period (dotted line) and two week period (solid line). Sites T2 and R1 contained units that were deployed for a second two week period (solid line) and sites T1 and C1 contained units that were deployed for a four week period (dot-dash line).

1.7 FUTURE LYNGBYA RELATED RESEARCH RECOMMENDATIONS

1.7.1 Tidewater Mucket Distribution

BACKGROUND:

The main focus of this project will be to increase the understanding of Tidewater Mucket distribution, abundance, and habitat preference in Lake Gaston and use that knowledge to develop a more effective lyngbya treatment protocol that will minimize negative impacts to native mussel species while increasing the efficacy of the treatment design. Lyngbya treatments in Lake Gaston are currently being restricted to areas of deep water where mussel bed densities are assumed to be low. However, this protocol allows for unregulated growth of benthic lyngbya mats in shallow water areas of treatment plots, decreasing the overall efficacy of the treatments.

OBJECTIVES:

The objectives of this project will be to 1) determine if side-scan sonar can be utilized for locating freshwater mussel beds in Lake Gaston, 2) determine if mussel beds can be properly identified and mapped within lyngbya treatment zones, and 3) determine overall distribution patterns and habitat preferences of Tidewater Mucket populations within Lake Gaston. A collaboration with the North Carolina Wildlife Resources Commission (NCWRC) will further expand the scope of this research to include data on overall abundance estimates and mussel distribution within identified beds.

SCOPE:

- Develop reference images of known mussel beds to determine if side-scan sonar can be utilized to scan and identify the locations of previously unknown mussel beds.
- Develop appropriate treatment protocols addressing the presence or absence of mussel beds within treatment areas.
- Collaborate with NCWRC to validate and survey identified mussel bed locations to collect community data and measure habitat characteristic associated with mussel-bed presence.

POTENTIAL BENEFITS:

If Tidewater Mucket distribution could be determined within treatment areas then proper application techniques could be put in place to maximum the efficacy of treatments while ensuring minimal contact with mussel beds. This knowledge would allow for the removal of protective restrictions that are currently in place on treatment protocols, allowing applicators to target areas in which unregulated growth of lyngbya benthic mats is occurring. Identifying potential Tidewater Mucket populations within treatment areas would also decrease the potential of a large mortality event which could result in agency shut down of the treatment program. Better documentation of mussel populations could also reduce public and agency concerns that treatments negatively impact the overall mussel population.

1.7.2 Potential Lyngbya Toxin Production

BACKGROUND:

A common theme found throughout the publications related to the potential of lyngbya to produce toxins is the need for continued monitoring to better assess the environmental and human health risks posed by this cyanobacteria in freshwater environments. We have shown through our pilot study that the use of SPATT technology can be used successfully to detect cyanotoxin release from freshwater lyngbya. Expanding on this study to address various factors that promote lyngbya growth and/or increase stress levels could help increase our understanding of the environmental and physiological factors that drive toxin production and release for this species.

OBJECTIVES:

Perform regular monitoring for cyanotoxin production related to lyngbya with the goal of 1) characterizing spatial and temporal variation in potential toxin production within Lake Gaston and 2) identify potential stress drivers for production and release of toxins from benthic lyngbya mats.

SCOPE:

- Deploy SPATTs at sites representing various environment conditions that could potentially influence growth conditions of lyngbya (temperature, light intensity, flow, etc.) and therefore toxin production.
- Deploy SPATTS at sites that could pose increased stress and drive toxin production (lyngbya treatment sites and high traffic areas).
- Conduct sampling efforts over a 12 month period to identify any temporal variations in toxin production that could be associated with active growth and/or the senescence of lyngbya.

POTENTIAL BENEFITS:

The environmental and physiological factors that drive toxin production and release in freshwater lyngbya are still not well understood. This research would be cutting-edge and allow for collaborations with other universities, such as The University of South Carolina, who are researching toxin production and its impacts to human health. One concern of researchers is chronic exposure to lyngbya through drinking water sources. Documentation of toxin production or the potential for toxin production could result in greater funding for lyngbya control efforts due to the significant volume of drinking water that is removed from Lake Gaston. Better understanding of the potential for toxin production would also assist in addressing public concerns on the potential threat level present regarding lyngbya within Lake Gaston.

1.7.3 Laboratory Evaluation of Lyngbya Treatment Efficacy

OBJECTIVES:

Continue evaluating chelated copper algaecides in a laboratory setting with the goals of 1) determining how consecutive treatment exposures impacts lyngbya viability, 2) determining how timing of consecutive treatment exposures impacts lyngbya viability, 3) determining how varying water temperatures impacts the efficacy of lyngbya treatments, and 4) continue exploring the efficacy of different algaecide combinations.

SCOPE:

- Perform laboratory trials that evaluate the viability response of lyngbya following repeated exposure to algaecides treatments. This will be performed using two separate trials that will focus on a.) chronic exposure (years) and b.) acute exposure (single treatments).
    a. A trial will be run utilizing lyngbya collected from several locations within Lake Gaston that have received different exposure levels to lyngbya treatments. Collection sites will include areas that have been treated for one to four seasons.
    b. Lyngbya viability will be evaluated following individual treatments during consecutive exposure events. The trial will continue until a predetermined level of viability has been achieved.
- Perform a laboratory trial that evaluates the response of lyngbya to algaecide applications when exposed during varying water temperatures.
- Perform a laboratory trial that evaluates the response of lyngbya to newly reported effective algaecide combinations.

POTENTIAL BENEFITS:

Laboratory trials will allow NCSU to evaluate the efficacy of varying treatment protocols in a cost effective manner, resulting in best management practices for Lake Gaston's lyngbya treatment program. Identifying new treatment protocols (e.g., Captain XTR alone) could result in cost savings for the LKWCC by reducing overall chemical costs. Trials could also help predict potential impacts to the overall efficacy of the treatment program that could result from changes in environmental conditions or physiological responses of lyngbya to the current treatment protocol. This knowledge will allow for more timely modifications to the treatment protocol in an attempt to reduce any negative impacts these factors may have to the overall efficacy of the treatment program.

2. Water Quality

2.1 Executive Summary and Management Implications

Executive Summary

The following report details complimentary research that was completed in relation to the lyngbya research outlined in Section 1 of this final report. This research was performed by North Carolina State University (NCSU) Aquatic Plant Management Program's and funded by the Lake Gaston Weed Control Council. This research was directed at providing a comprehensive summary of water quality parameters within Lake Gaston for use as baseline data in future water quality monitoring efforts and to incorporate into the predictive modeling of lyngbya occurrence and proliferation detailed in section 1.3 of this final report. The objectives for this research were to 1) characterize basic water quality parameters of Lake Gaston for future monitoring efforts, 2) identify any potential negative impacts to water quality, 3) monitor coliform bacteria levels for possible indications of fecal contamination, 4) determine hydrosoil (lake sediment) characteristics and relate them to water quality parameters, and 5) provide a comprehensive data set to be utilized in the development of a predictive model correlating lyngbya presence with environmental conditions.

Monthly water quality samples were collected to monitor both the nutrient dynamics and overall water chemistry of Lake Gaston. Sites were distributed across the geological extent of Lake Gaston and represented every major sub-watershed and tributary, as well as the main body of the lake. Monitoring these various water quality parameters allows managers to develop baseline data sets that will help indicate the overall health and nutrient production, or trophic level, of a system. Monitoring changes in these parameters could provide early indications of impacts due to anthropogenic influences such as nutrient loading or point source pollution. The aforementioned parameters are also drivers for many natural aquatic processes and can help predict growth patterns of various aquatic organisms.

Overall, water chemistry and nutrient parameters for Lake Gaston fall within or below the levels recommended by the EPA for the protection of aquatic life and recreation. Lake Gaston experiences expected seasonal fluctuations in temperature, dissolved oxygen levels, flow rates, water clarity, and nutrient dynamics. The average pH for Lake Gaston was considered neutral and within the optimal range for aquatic organisms. Water clarity experienced geographical difference, with sites located further downstream experiencing greater clarity than those located within the upper portion of the system. Significant geographical differences within nutrient parameters (nitrogen and phosphorous) were not detected across different watersheds, indicating that point source nutrient pollution is not occurring on a large scale. Results from the trophic state index determined that there is a close relationship between phosphorous and secchi depth which indicates that Lake Gaston's water clarity is not driven by algal blooms, but instead by phosphorous bound particulate matter. Overall, Lake Gaston was classified as eutrophic system, which indicates the ability of the system to support a healthy and diverse population of aquatic organisms.

Due to Lake Gaston's highly developed shoreline and concerns over potential nutrient loading, coliform bacteria sampling was conducted at sites within all major sub-watersheds. Although E. coli was positively detected at multiple sites within Lake Gaston, the population was never recorded at a level that would constitute a violation from a human health perspective. This study also found that overall

E.coli counts varied between years and sites and no single site displayed consistently high levels of E. coli that would indicate a point source problem.

The ability of a reservoir to retain sediments at high levels results in the accumulation of sediment-associated nutrients, including nitrogen and phosphorus. Monitoring nutrient levels associated with these hydrosoils (reservoir benthic sediment) allows managers to identify areas that could be at greater risk for future eutrophication related issues. For Lake Gaston, hydrosoil samples were collected at sites that represented all of Lake Gaston's associated sub-watersheds. This study found that nutrient dynamics within hydrosoil samples followed similar spatial trends as reported in water column samples and did not indicate any potential increased nutrient loading within the sediment. This dataset will be utilized as baseline data for any future sampling efforts.

Management Implications

This research was able to establish a robust dataset for any future water quality monitoring efforts performed at Lake Gaston. While current parameters are stable and within EPA recommended levels, changes to these baseline levels will provide early indications of negative impacts to the system such as nutrient loading or point source pollution. Changes could also indicate increased growth potential within nuisance aquatic species populations. Continued regular monitoring of all water quality and hydrosoil parameters will allow managers to identify any potentially negative impacts to water quality for Lake Gaston.

2.2 WATER CHEMISTRY AND NUTRIENT LEVELS

Introduction

Water chemistry parameters, including temperature, dissolved oxygen, pH, alkalinity, and conductivity are drivers for many natural aquatic processes and can help indicate the overall health of a system. Fluctuations in these parameters can be influenced by rainfall, flow rates, naturally occurring events. For Lake Gaston, monthly fluctuations in water chemistry parameters could also be a result of discharge flows from the upstream Kerr Lake Dam. Most notably, discharge from Kerr Lake can impact the rate of water flowing through the Lake Gaston system and in the summer months can impact oxygen levels due Kerr Lake dam's hypolimnetic withdrawal. While some seasonal fluctuations are expected, large or unexpected fluctuations in these parameters could indicate harmful impacts of anthropogenic influences.

Natural seasonal fluctuations are expected for temperature and dissolved oxygen and are major drivers for natural processes in an aquatic system. Rising water temperatures in the summer months cause the development of a thermocline within the water column. This thermocline splits the water column into an area of warm, highly oxygenated water located close to the surface and an area of cold, low-oxygenated water located beneath the thermocline. This area of low-oxygenated water can be a stress factor to many aquatic organisms and plays a major role in natural processes that influence the nutrient dynamics of a system. Water chemistry parameters, including pH, alkalinity, and conductivity remain fairly constant within an aquatic system but are monitored for temporal changes due to possible harmful anthropogenic impacts. pH is an indicator of carbon dioxide levels in a system, which can be influenced by natural plant processes such as photosynthesis and decomposition or anthropogenic influences such as air pollution. Since many aquatic chemical reactions are pH dependent, measuring alkalinity, or the system's ability to resist changes in pH, is also important. Conductivity is the level of ionic substances that are dissolved in the water and remains fairly constant within individual aquatic systems. Changes to any of these parameters could indicate possible negative impacts to the system, such as point source pollution or erosion issues.

Nutrients are an essential component to the energy flow of an aquatic system and fuels its level of primary production and trophic status. However, increased nutrient levels can alter ecological processes and impact aquatic ecosystems in a plethora of different ways. Monitoring temporal changes to the trophic status of an aquatic system allows managers to determine the level of primary nutrients and primary producers within a system, identify shifts in the overall productivity of a system, and help identify possible factors impacting ecosystems processes. Nine individual parameters were monitored to indicate the trophic status of Lake Gaston: total phosphorous, orthophosphorus, total nitrogen, total nitrogen / total phosphorous ratio, total Kjeldahl nitrogen, ammonia, total nitrate / total nitrite, chlorophyll-a, and secchi depth. Several of the aforementioned parameters are temperature dependent, therefore seasonal variations are expected due to seasonal fluctuations in water temperatures. However, variations between watersheds could identify potential water quality issues occurring within individual watersheds.

Phosphorous is a major component in processes involved in the formation of cell membranes, cellular energy, and growth of aquatic plants. However, phosphorous can be a limiting nutrient within a system. Limiting nutrients within an aquatic system are those nutrients that drive the growth of aquatic communities, however are not readily available within the system. Biologically available forms of phosphorous are limited in surface water but can bind to sediment particles or be excreted by aquatic organisms such as zooplankton. Most phosphorous enters an aquatic system from point and non-point sources such as atmospheric deposition, inflow from streams, runoff, or erosion. Internal loading of phosphorous into a system can also occur through the release of phosphorous from sediment to water column (Wu et al. 2017). Loss of phosphorous within a system occurs through sedimentation of bound particles, however environmental factors such as oxygen levels and physical turbulence can re-release this nutrient back into the water column. We report on two forms of phosphorous within Lake Gaston. The measure of all phosphorous present within a system, including those forms that are not biologically available for use by aquatic organisms, is reported as total phosphorous. Orthophosphate is a soluble form of phosphorous and is the only directly utilizable form that is readily available for use by algae and other aquatic plants for growth.

Nitrogen, along with phosphorous, is a major nutrient that affects the productivity of an aquatic system. For plants and animals, nitrogen is needed to synthesize protein and is essential to the production of cellular tissues (citation?). Nitrogen has numerous forms, both organic and inorganic, and can be introduced into aquatic systems externally through fallout from atmospheric sources or surface water run-off and internally through nitrogen fixation. Organic forms of nitrogen are present through the excreted waste of living organisms or the decomposition process of dead organisms. Densities of nitrogen fixing algae have been positively correlated with concentrations of dissolved organic nitrogen in the water (Wetzel 1983). Inorganic forms include nitrate, nitrite, or ammonia and commonly enter a system through point and non-point source pollution. These forms of nitrogen can be toxic to both humans and aquatic organisms at elevated levels. Inorganic forms of nitrogen are good indicators of nutrient pollution within a system due to their naturally occurring low levels and their high solubility in run-off water. Nitrogen levels within a system can display dynamic variations both spatially and temporally due to nitrogen fixation either through aquatic organisms or through nitrogen's ability to naturally transform from one form to another given proper aquatic environmental conditions. Nitrification along the water-sediment interface can be a major source of internal nitrogen loading within a system (Wu et al. 2017; Gautreau et al. 2020). We report on four forms of nitrogen within Lake Gaston. Measurements for inorganic nitrogen (nitrate/nitrite nitrogen and ammonia) can be taken directly, while organic nitrogen levels are calculated through total Kjeldahl nitrogen measurements. Total nitrogen is calculated by combining the levels inorganic nitrogen (nitrate/nitrite nitrogen) with the level of organic nitrogen (total Kjeldahl nitrogen) within a system.

Chlorophyll-a is a specific form of chlorophyll used in oxygenic photosynthesis of plants, including algae. Therefore, it can be used to estimate community levels of primary producers within a system.

Secchi depth is an indicator of water clarity and can be affected by suspended sediment, algae abundance, or overall color of the water. Since a change in average secchi depths can reflect algal blooms or impacts of sediment loading into a system, secchi depths are often compared to both total phosphorous and chlorophyll-a to determine what factors are influencing water clarity.

To further understand the dynamics of an aquatic system, managers must understand the relationship between these nutrient factors and how they correlate with limiting or promoting growth within the aquatic communities. The trophic state index (TSI) model developed by Carlson (1977) assumes algal biomass to be the basis for trophic classification (Carlson and Havens 2005). This model uses deviations in the expected levels of chlorophyll-a, secchi depth, and total phosphorous to classify the productivity level of aquatic systems and identify limiting factors within a system (Carlson 1977). These models were designed to take complex relationships that drive productivity within an aquatic ecosystems and present them in a format that is simple to understand and disseminate (Carlson and Simpson 1996). These models transform nutrient measurements into a continuous index that allows for direct comparison of the relationships occurring between the varying nutrient parameters and ultimately allows for the classification of the trophic status of a system (Carlson and Simpson 1996). Aquatic systems with clear water and low productivity are classified as oligotrophic, a mesotrophic system has increased productivity but maintains a moderately clear appearance, and eutrophic systems are highly productive which drastically decreases the clarity of the water.

Aquatic systems need healthy levels of nutrients, such as nitrogen and phosphorous, to support the growth of aquatic plants and animals. However, when levels become out of balance or an overabundance of these nutrients occurs, a system can experience harmful ecological impacts. Monitoring these nutrient dynamics and the chemical properties that drive them can provide early warnings of increased eutrophication within the lake and identify possible point sources of excess nutrients into the system.

Methods

Water quality parameters at 35 sites were sampled monthly at Lake Gaston (Figure 2.2.1). The sites were distributed across the geological extent of Lake Gaston and represented every major sub-watershed and tributary, as well as the main body of the lake. Due to restrictions put in place by North Carolina State University in response to the global COVID-19 pandemic, water quality was not collected in April 2020. Due to equipment failure and repair delays related to the pandemic, water quality was also not conducted in August 2020. Average discharge rates for both Kerr Lake dam and Lake Gaston dam were reported by the US Army Corps of Engineers – Wilmington District and their data has been incorporated into this report.

At each sample site, a surface sample was taken to measure nutrient levels and physical water chemistry parameters. At sites with water depths greater than 6 feet, a second sample was collected from the bottom of the water column (1.5 feet above the substrate) and used to evaluate the conditions in areas beneath the thermocline. Parameters that require water samples to be taken from benthic environments were collected using a Van Doran sampler. A Eurika multiprobe water quality meter measured physical parameters in the field including temperature, dissolved oxygen, pH, chlorophyll-a, and conductivity at both surface and benthic depths. Nutrient parameters were measured from water samples that were collected and stored for later chemical analysis at the Weaver Laboratory at North Carolina State University and included total Kjeldahl nitrogen, ammonia, total nitrate/total nitrite, total phosphorus, and orthophosphorus. Total nitrogen levels were calculated by combining total Kjeldahl nitrogen and total nitrate/total nitrite levels. Nutrient samples were kept on ice in the field and then stored frozen until processed. Secchi depths were collected using a standard secchi disk that was lowered into the water column and then raised to a depth where it could be visually observed.

Following the completion of the 2020 water quality season, all collected Parameters were statistically evaluated to identify any temporal or geospatial variations across watersheds. A 2-way factorial ANOVA determined that there was no significant difference between surface and benthic samples for all reported nutrient parameters ($p < 0.05$). Therefore, all results are based on surface sample collections.

A 2-way factorial ANOVA determined that nutrient values varied significantly between individual watersheds for most parameters, therefore results were further analyzed to a sub-watershed level (Table 2.2.3). Unless stated, there was no significant interaction between individual watersheds and collection year ($p < 0.05$), therefore reported values for all parameters have been averaged over the three years period.

Results

The US Army Corps of Engineers – Wilmington District reports average discharge rates for both Kerr Lake dam and Lake Gaston dam (Figure 2.2.2). Flow rates reported for Lake Gaston's dam varied significantly between years ($p < 0.001$) and results of Tukey's multiple-contrast test indicated that 2020 (14,419 cfs) experienced a significantly greater amount of flow than both 2019 (9,415 cfs) and 2020 (8,416 cfs) (Figure 2.2.3). Monthly flow rates varied significantly between years ($p < 0.001$) (Figure 2.2.3). A Tukey's multiple-contrast test indicated that the flow rates for the months of May, June, September, October, and November were significantly higher in 2020 than in 2019 or 2021 and that flow rates were significantly different for the months of January, June, and December during all three years of the study.

Expected seasonal fluctuations in both temperature and dissolved oxygen levels were observed between 2019 and 2021 (Figure 2.2.4 and Figure 2.2.5). Seasonal water temperatures were lowest during the winter months (mean = 46 °F), peaked in the summer (mean = 77°F), and were similar during the spring and fall seasonal months (spring: mean = 61°F; fall mean = 62°F). Dissolved oxygen levels followed a similar seasonal pattern, however dissolved oxygen levels were the highest during cold winter months (11 mg/L) and lowest during the warm summer months (7 mg/L). Dissolved oxygen levels for the spring and fall months were both reported to be 9 mg/L. These seasonal trends were also reported for individual watersheds, but no single watershed experienced major variations in either temperature or dissolved oxygen (Figure 2.2.6; Figure 2.2.7). All seasonal dissolved oxygen levels were above the level needed to sustain aquatic life (EPA standard = 3 mg/L). Seasonal stratification of the water column and development of a thermocline was indicated by divergent temperature and dissolved oxygen levels at shallow and deep readings between the months of May and September (Figure 2.2.8).

The average pH for Lake Gaston was 7.04 (Table 2.2.1) which is considered neutral and within the optimal range for aquatic organisms (6.5 – 9) set by the US Environmental Protection Agency (EPA). pH has experienced a slight increase each year from 6.83 reported in 2019 to 7.04 in 2021. Average lake wide alkalinity level was 29.24 mg/L and is above the EPA's recommended minimum buffering capacity of 20 mg/L to protect aquatic life from swings in pH (Table 2.2.1). Lake Gaston's average conductivity was 78.57 µs/cm (Table 2.2.1) and increased slightly from the reported 73 µs/cm in 2020 to 83.61 µs/cm in 2021.   Data was not collected for alkalinity or conductivity in 2019 and the EPA does not provide standards for conductivity.

Overall, all nutrient parameters were within the range of water quality standards recommended by the EPA (Table 2.2.2). Due to the dynamic nature of aquatic systems, yearly variations were expected and did occur across all reported nutrient parameters. For phosphorous, nitrogen, and chlorophyll-a, 2020 reported values were the highest across the three year study period and corresponded to decreased water clarity and increased flow rates reported for that year.

For the two forms of phosphorous reported, total phosphorous and orthophosphorus, spatial and temporal variations were detected. Although watershed was determined to be a significant factor in total phosphorous levels ($p = 0.032$) and orthophosphorus levels ($p = 0.004$), a Tukey's multiple-contrast test did not detect individual watersheds that influenced those differences for either parameter (Figure 2.2.9; Figure 2.2.10). Significant temporal variations were reported for both forms of phosphorous ($p < 0.001$), however a seasonal pattern was not determined from a Tukey's multiple-contrast test. Yearly variations in phosphorous levels occurred, and values reported in 2020 were the highest for both total phosphorous and orthophosphorus.

Nitrogen levels for Lake Gaston were reported in the form of total nitrogen, total Kjeldahl nitrogen, ammonia, and total nitrate/ total nitrite. Total Kjeldahl nitrogen and ammonia values were collected during all three years of the study and were both highest during the 2020 sampling season (Table 2.2.2). Total nitrogen and total nitrate/total nitrite were additional parameters included to the sampling protocol in 2021 (Table 2.2.2). Nitrogen levels did not vary spatially across watersheds for total nitrogen ($p = 0.718$; Figure 2.2.11), total Kjeldahl nitrogen ($p = 0.876$; Figure 2.2.12), or ammonia ($p = 0.079$; Figure 2.2.13) (Table 2.2.2). Total nitrate and total nitrite also showed no spatial variability across most watersheds, with the exception of Poplar and the southern shoreline of Songbird ($p < 0.001$; Table 2.2.3; Figure 2.2.14). Significant temporal variations were detected for all forms of nitrogen ($p<0.001$), however a seasonal pattern was not determined. The ratio of total nitrogen to total phosphorous was determine for each watershed in 2021 and there was no significant differences detected between watersheds ($p=0.115$; Figure 2.2.15).

Lake Gaston's water clarity fell well within the EPA standard range between 2019 and 2020 (Table 2.2.2). Temporal and geographical differences in water clarity were displayed throughout the system (Table 2.2.2; Figure 2.2.16; Figure 2.2.17) and average lake wide secchi depths varied significantly by watershed ($p < 0.0001$) and by month ($p < 0.0001$). Although 2020 displayed decreased water clarity when compared to 2019 and 2020, there was no significant interaction between watersheds and year ($p = 0.6217$) and values were pooled over the three year period for individual watersheds (Table 2.2.3). Temporal variations are expected due to increased seasonal rainfall and reservoir turnover events and geographical variation were based on the watersheds distance from the Lake Gaston dam. Pea Hill watershed is located directly adjacent to the dam and was the only watershed that exhibited significantly higher water clarity during all three years. Hawtree and Smith watersheds are both located in the upper most part of Lake Gaston and both were categorized with the lowest overall water clarity within the system. All watersheds located mid-system displayed similar water clarities throughout the three year period.

Average lake wide chlorophyll-a concentrations reported for 2020 and 2021 were within the EPA standard range (Table 2.2.2). Overall, there were significant differences in lake wide chlorophyll-a concentrations between watersheds ($p < 0.0001$), however this was mainly represented by increased concentrations within the Sixpound watershed (Figure 2.2.18). Results of Tukey's multiple-contrast test indicated that there was not a significant difference in chlorophyll-a concentrations among the other eleven watersheds. Monthly variations were detected ($p < 0.0001$) and were driven by high chlorophyll-a concentrations during the months of January and February. Due to missing data related to equipment failure, chlorophyll-a measurements were not reported for 2019.

Results of the trophic state index classified Lake Gaston as a eutrophic system (Figure 2.2.19). TSI values for total phosphorous, secchi depth, and chlorophyll-a were calculated for individual watersheds and are reported in Table 2.2.4. For all watersheds, the TSI values for total phosphorous and secchi depth were closely related and both index values were greater than the TSI values reported for chlorophyll-a (Figure 2.2.19).

Monthly individual water chemistry and nutrient parameters at each water quality site are reported in the respective end of year final reports.

Discussion

Overall, water chemistry and nutrient parameters fall within or below the levels recommended by the EPA for the protection of aquatic life and recreation. Lake Gaston experiences expected seasonal fluctuations in temperature, dissolved oxygen levels, and flow rates. All of which can drive responses in natural aquatic processes and impact the trophic dynamics of a system. Although dissolved oxygen levels decreased as water temperatures increased during summer months, levels never reached a critical point of negatively impacting aquatic life on a large scale. Thermocline development can impact nutrient dynamics by driving the natural input of nitrogen and creating anoxic conditions, however differences in seasonal swings of nitrogen levels were not identified between the surface and benthic environments. Changes in nutrient dynamics seem to be closely related to overall flow rates, with 2020 experiencing the highest levels in all of the aforementioned parameters.

Certain parameters should remain fairly constant within individual aquatic systems and include pH, alkalinity, and conductivity. These parameters are used to indicate major temporal changes over multiple years that could be a potential result of anthropogenic impacts such as pollution. Overall, Lake Gaston exhibited a neutral pH for all three years of the study and this consistency is expected due to the buffering capacity that a higher alkalinity level will allow.

Lake Gaston was classified as eutrophic system, which indicates the ability of the system to support a healthy and diverse population of aquatic organisms. Nutrient parameters were not significantly different across watersheds, indicating that point source nutrient pollution is not occurring on a large scale. Results from the trophic state index determined that there is a close relationship between phosphorous and secchi depth which indicates that Lake Gaston's water clarity is not driven by algal blooms, but instead by phosphorous bound particulate matter. Therefore, use of secchi depths to monitor trophic production levels may not be appropriate in Lake Gaston.

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Tables

Table 2.2.1. Average pH, alkalinity, and conductivity levels for Lake Gaston 2019 - 2021. Alkalinity and conductivity were not collected during 2019. The water quality standards recommended by the US Environmental Protection Agency (EPA) for each parameter are also listed.

Year	Average pH	Average Alkalinity (mg/L)	Average Conductivity (µs/cm)
EPA standard	*6.5 - 9*	*> 20 mg/L*	*n/a*
Lake Average	**7.04**	**29.24**	**78.57**
2021	7.21	25.22	83.61
2020	7.08	33.26	73.53
2019	6.83	n/a	n/a

Table 2.2.2. Lake wide average nutrients levels for Lake Gaston for 2019 – 2021. Total nitrogen and phosphorous, chlorophyll-a, and secchi depths (A) and individual nitrogen and phosphorous parameters (B). The water quality standards recommended by the US Environmental Protection Agency (EPA) for each parameter are also listed.

(A)

Year	*Total Nitrogen (ppb)*	*Total Phosphorus (ppb)*	*TN:TP Ratio*	*Chlorophyll-a (ppb)*	Secchi Depth (m)
EPA standard	*2000 – 6000*	*10 – 62.5*	*n/a*	*1.87 – 12.95*	*0.46 – 2.04*
2021	1039.23	39	31:1	7.55	1.2
2020	n/a	60	n/a	9.81	1.0
2019	n/a	40	n/a	n/a	1.3

(B)

Year	*Total Kjeldahl Nitrogen (ppb)*	*Ammonia (ppb)*	Total Nitrate / Total Nitrite (ppb)	*Orthophosphorus (ppb)*
EPA standard	*n/a*	*n/a*	*n/a*	*n/a*
2021	849	40	87	13
2020	906	41	n/a	16
2019	790	40	n/a	12

Table 2.2.3. Average yearly parameter values reported for Lake Gaston's associated watersheds for 2019 - 2021. Total nitrogen and phosphorous, chlorophyll-a, and secchi depths (A) and individual nitrogen and phosphorous parameters (B). Watersheds that contained main lake sections of Lake Gaston were divided and classified as either the north or south side of the Roanoke River. Results of Tukey's multiple-contrast test are indicated by letters and compare watersheds within a single year. Means within the same column followed by the same letter do not significantly differ (α = 0.05) and columns without letters indicate no significant difference was detected within any single watershed.

(A)

Watershed	Total Nitrogen (ppb)	Total Phosphorous (ppb)	TN:TP Ratio	Chlorophyll –a (ppb)	Secchi Depth (m)
Smith	1066.67	39.16	22.70	6.98 *ab*	0.78 *f*
Hawtree	1276.09	43.37	32.25	7.97 *ab*	0.86 *ef*
Great_N	1125.78	44.96	36.79	8.81 *ab*	1.08 *cde*
Great_S	1003.63	34.64	26.60	5.23 *b*	1.12 a*bcde*
Sixpound	902.47	49.31	26.49	11.51 *a*	0.97 *bcde*
Poplar	867.30	41.11	17.29	9.15 *ab*	0.95 *def*
Songbird_N	1063.46	39.86	39.10	7.18 *b*	1.16 *b*
Songbird_S	1031.18	36.42	29.86	7.43 *b*	1.11 *cdef*
Lizard_N	1114.60	27.87	33.69	6.49 *b*	1.44 *ab*
Lizard_S	1092.07	34.80	33.71	8.62 *ab*	1.03 *cdef*
Pea Hill_N	966.99	32.73	31.84	6.90 *b*	1.39 *a*
Pea Hill_S	990.98	45.33	26.27	8.34 *ab*	1.36 *ab*

(B)

Watershed	Total Kjeldahl Nitrogen (ppb)	Ammonia (ppb)	Total Nitrate / Total Nitrite (ppb)	Orthophosphorus (ppb)
Smith	870.89	47.75	95.33 *abc*	12.66
Hawtree	941.16	46.02	121.64 *ab*	10.57
Great_N	900.21	38.83	89.31 *abc*	14.53
Great_S	720.30	31.20	117.33 *abc*	12.46
Sixpound	789.26	48.35	74.00 *abc*	15.96
Poplar	859.22	34.98	40.78 *c*	13.35
Songbird_N	831.38	36.45	98.69 *abc*	13.23
Songbird_S	841.33	34.86	114.28 *a*	13.19
Lizard_N	917.48	44.88	28.36 *abc*	11.10
Lizard_S	875.63	40.34	91.45 *abc*	11.87
Pea Hill_N	844.66	44.32	51.70 *bc*	11.78
Pea Hill_S	813.61	43.71	95.85 *abc*	15.72

Table 2.2.4. Average trophic state index values reported for chlorophyll-a, secchi depth, total phosphorous for Lake Gaton's associated watersheds. Combined TSI is an average of all three reported TSI parameters. Watersheds that contained main lake sections of Lake Gaston were divided and classified as either the north or south side of the Roanoke River.

Watershed	Chlorophyll-a TSI	Secchi Depth TSI	Total Phosphorous TSI	Combined TSI
Smith	50	64	57	57
Hawtree	51	62	59	57
Great_N	52	59	59	57
Great_S	47	58	55	53
Sixpound	55	60	60	58
Poplar	52	61	58	57
Songbird_N	50	58	57	55
Songbird_S	50	59	56	55
Lizard_N	49	55	52	52
Lizard_S	52	60	55	56
Pea Hill_N	50	55	54	53
Pea Hill_S	51	56	59	55

Figures

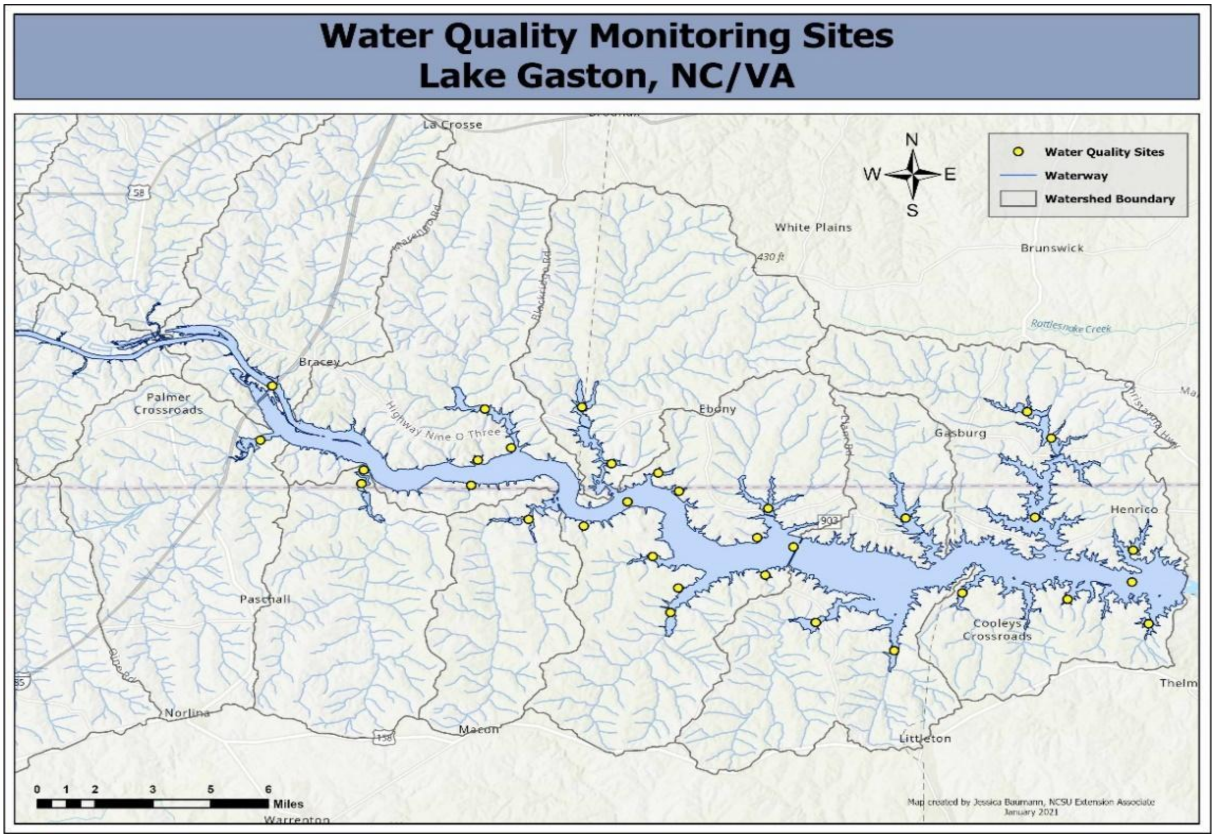

Figure 2.2.1. A map of Lake Gaston's water quality monitoring sites.

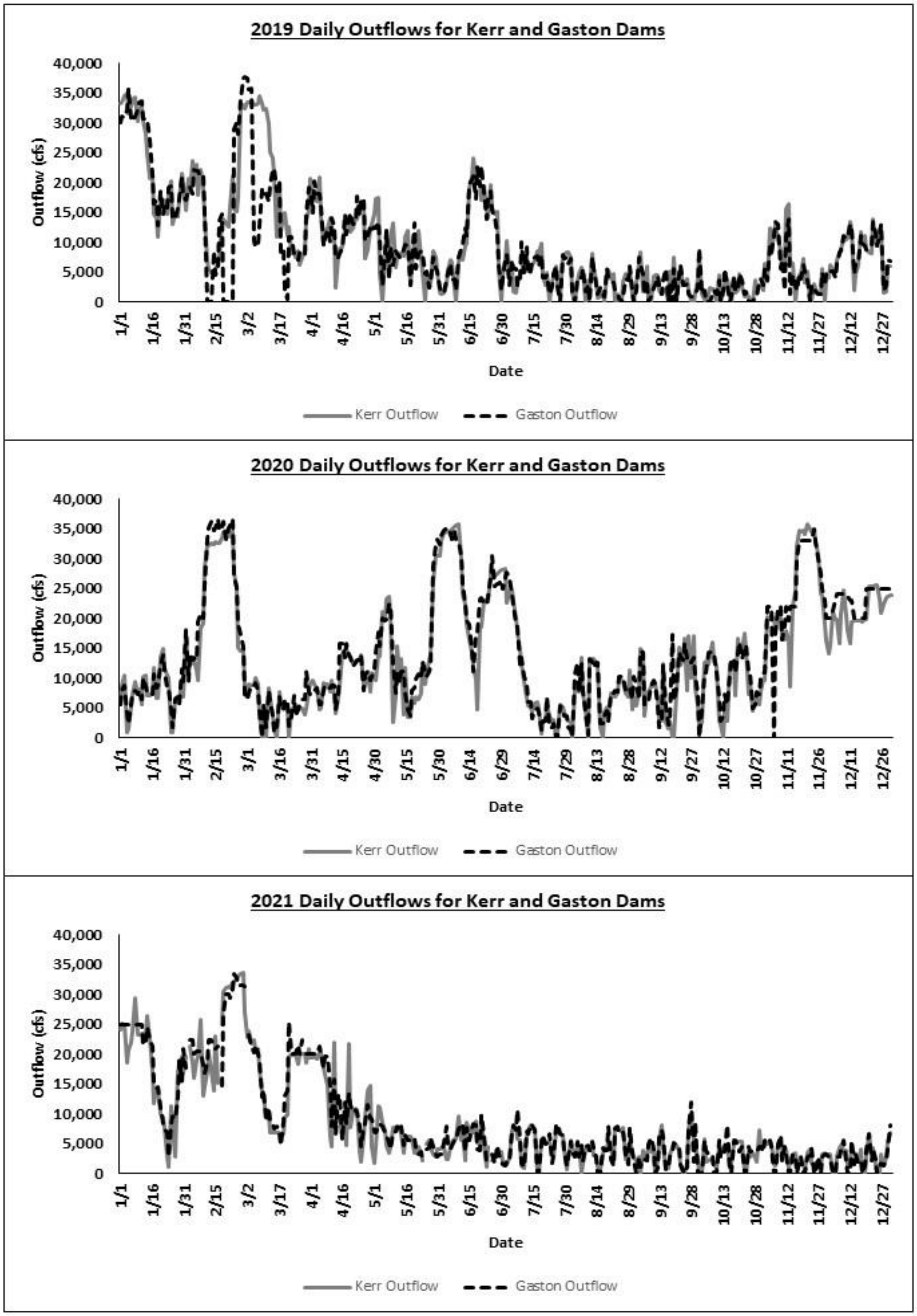

Figure 2.2.2 Average daily discharge values recorded at Kerr Lake dam and Lake Gaston dam for 2019, 2020, and 2021.

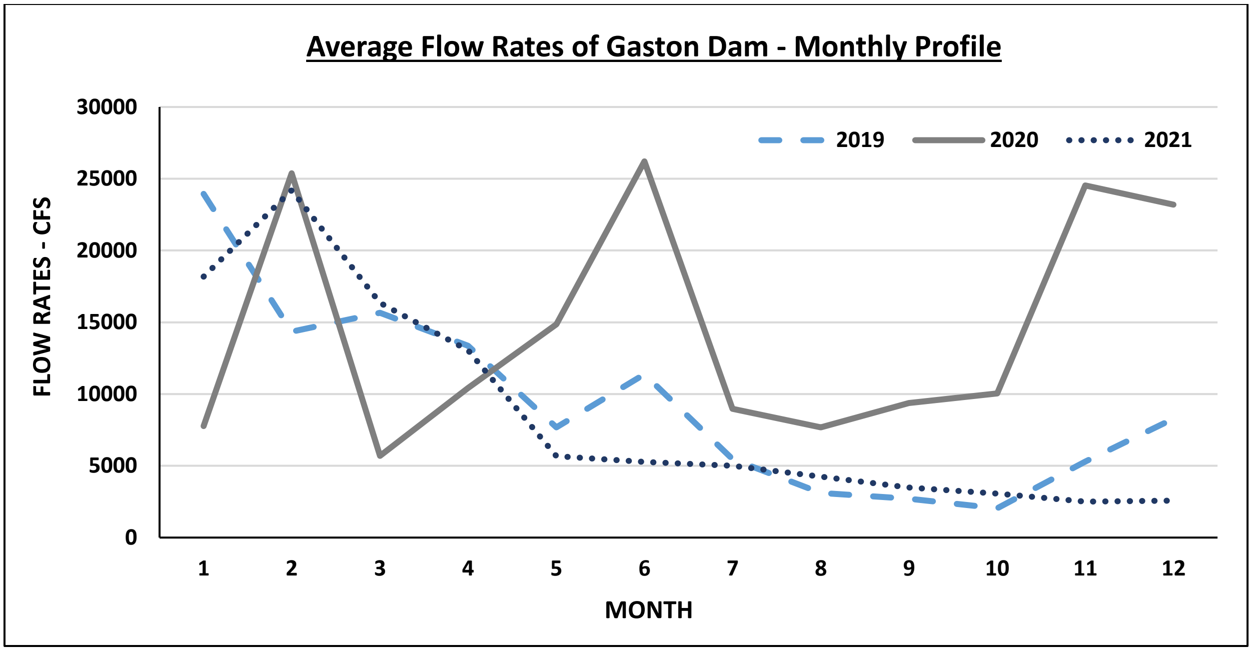

Figure 2.2.3. Average monthly flow rates reported for Lake Gaston dam between 2019 and 2021.

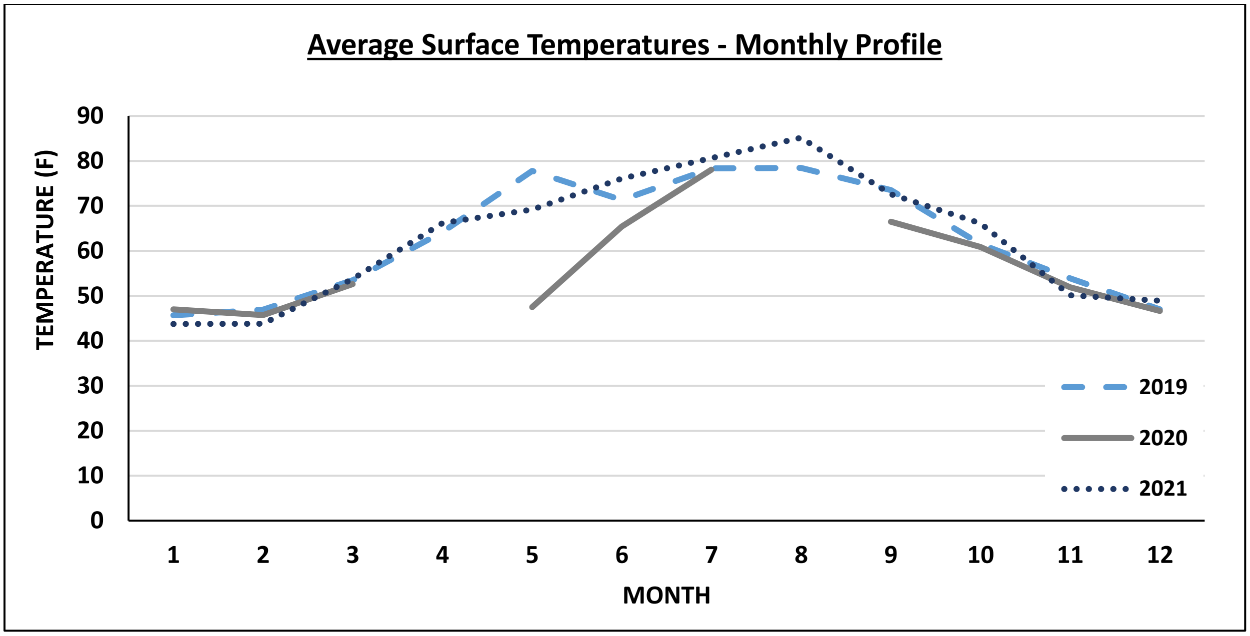

Figure 2.2.4. Average monthly surface temperatures reported for Lake Gaston between 2019 and 2021.

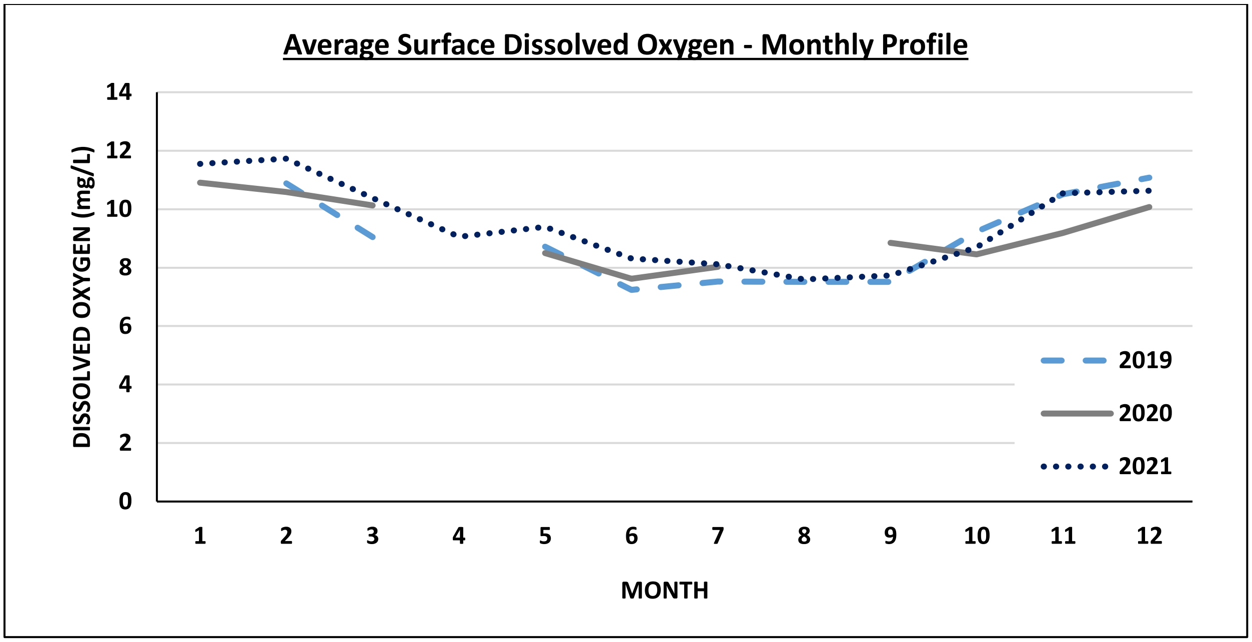

Figure 2.2.5. Average monthly surface dissolved oxygen reported for Lake Gaston between 2019 and 2021.

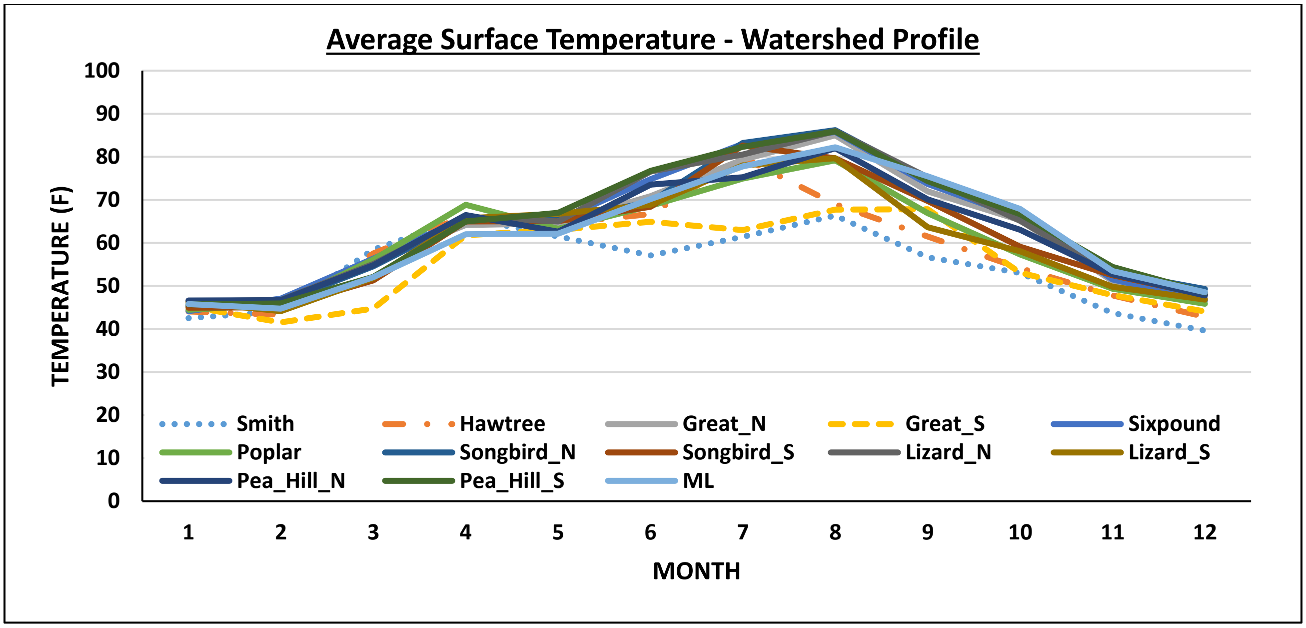

Figure 2.2.6. Average monthly surface temperatures reported for Lake Gaston's associated watersheds between 2019 and 2021.

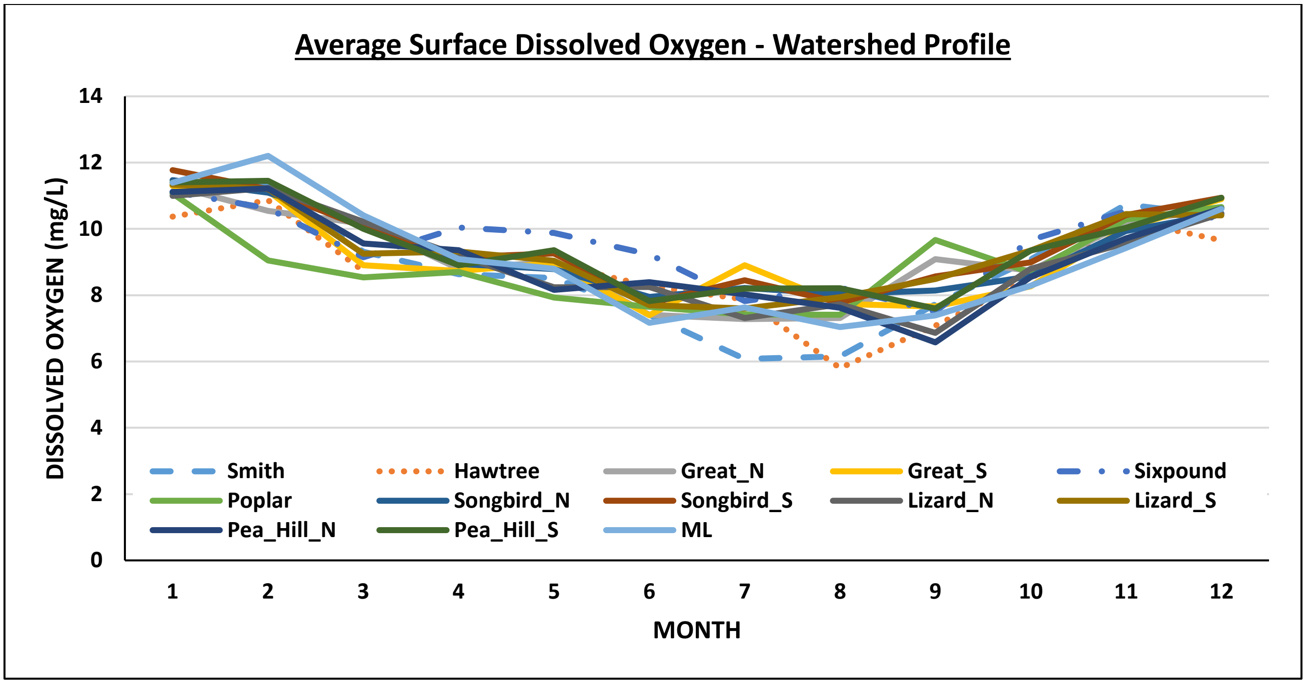

Figure 2.2.7. Average monthly surface temperatures reported for Lake Gaston's associated watersheds between 2019 and 2021.

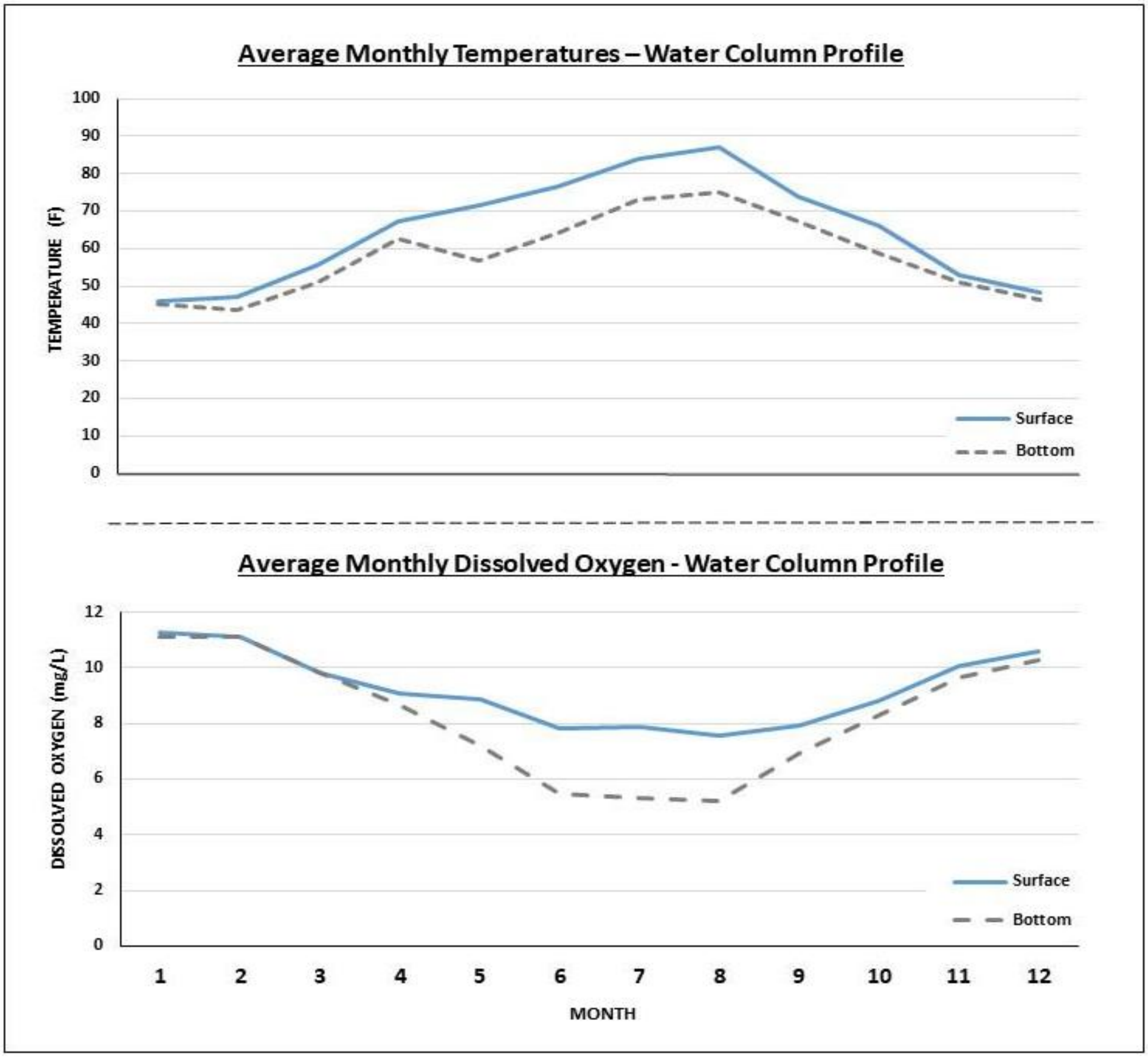

Figure 2.2.8. Average monthly temperatures and dissolved oxygen collected from both the surface and the bottom of the water column between 2019 and 2021.

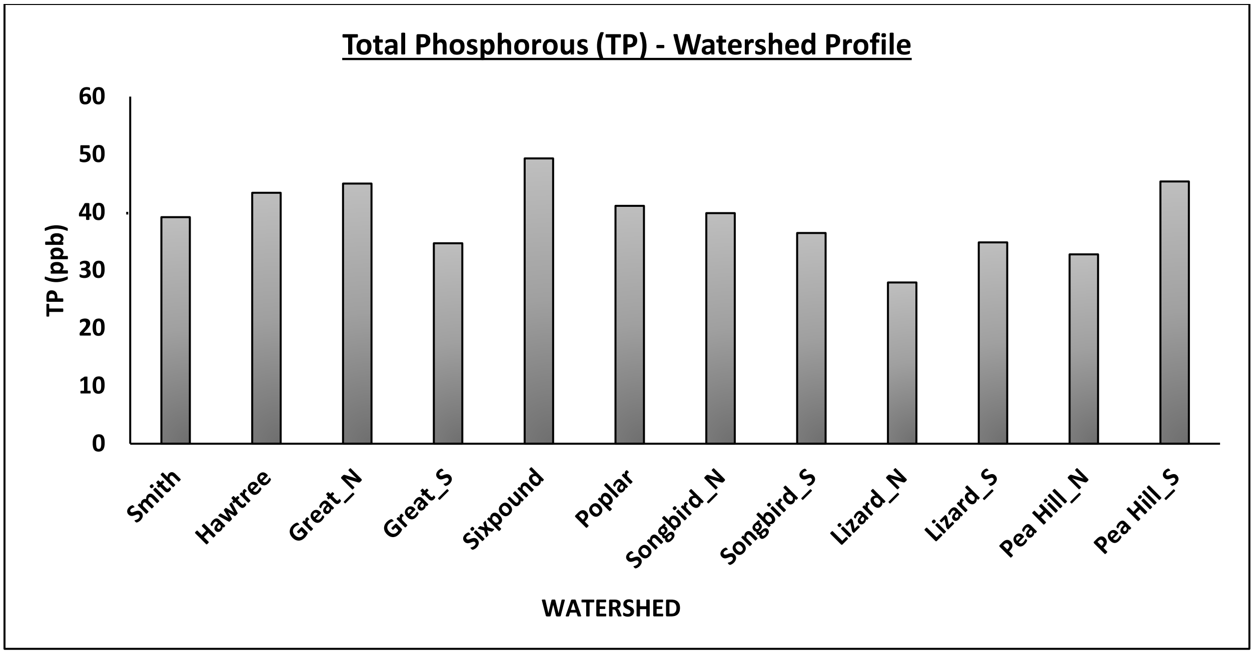

Figure 2.2.9. Average total phosphorous (TP) values reported for Lake Gaston's associated watersheds between 2019 and 2021. The dotted line represents the overall lake average for total phosphorous (39 ppb). Watershed data is reported in order of most upstream to downstream locations and watersheds that encompass both the northern and southern shorelines are divided into two independent sub-watersheds (N, S).

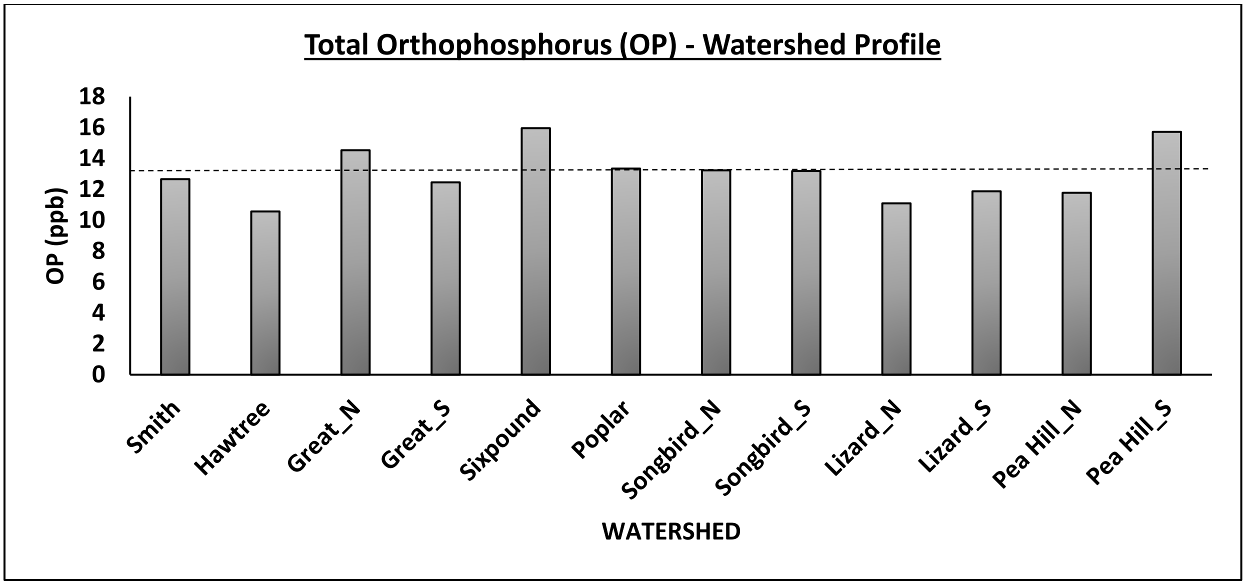

Figure 2.2.10. Average orthophosphorus (OP) values reported for Lake Gaston's associated watersheds between 2019 and 2021. The dotted line represents the overall lake average for total orthophosphorus (13 ppb). Watershed data is reported in order of most upstream to downstream locations and watersheds that encompass both the northern and southern shorelines are divided into two independent sub-watersheds (N, S).

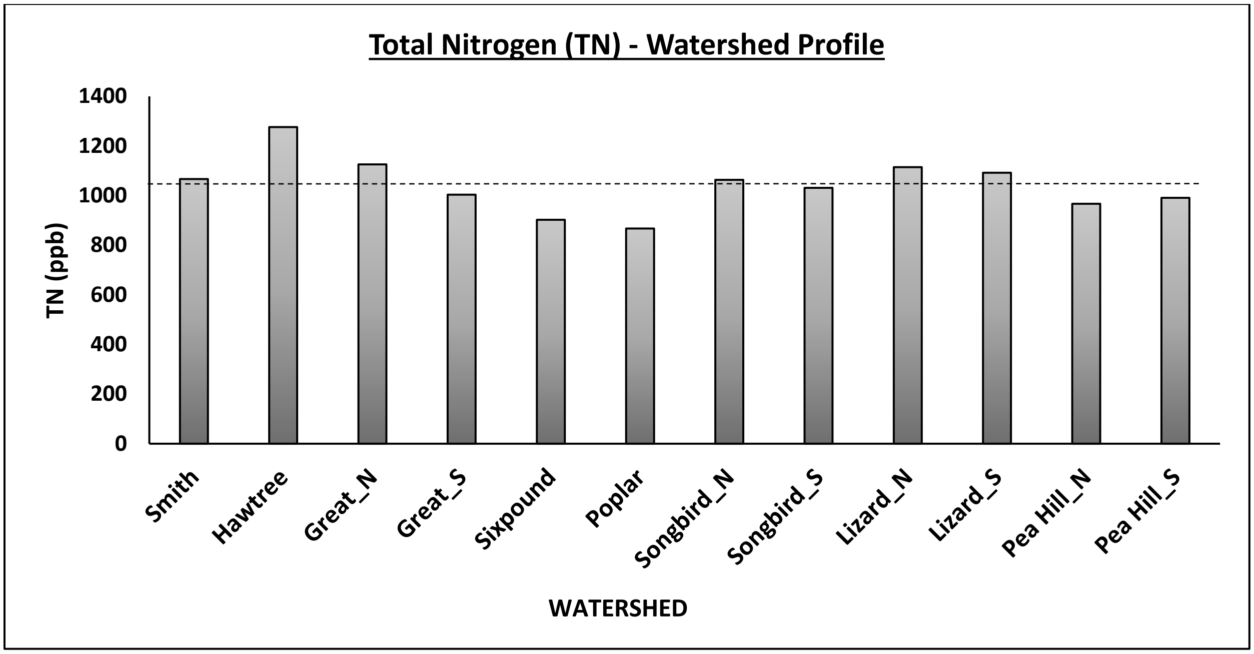

Figure 2.2.11. Average total nitrogen (TN) values reported for Lake Gaston's associated watersheds in 2021. The dotted line represents the overall lake average for total nitrogen (1,039 ppb). Watershed data is reported in order of most upstream to downstream locations and watersheds that encompass both the northern and southern shorelines are divided into two independent sub-watersheds (N, S).

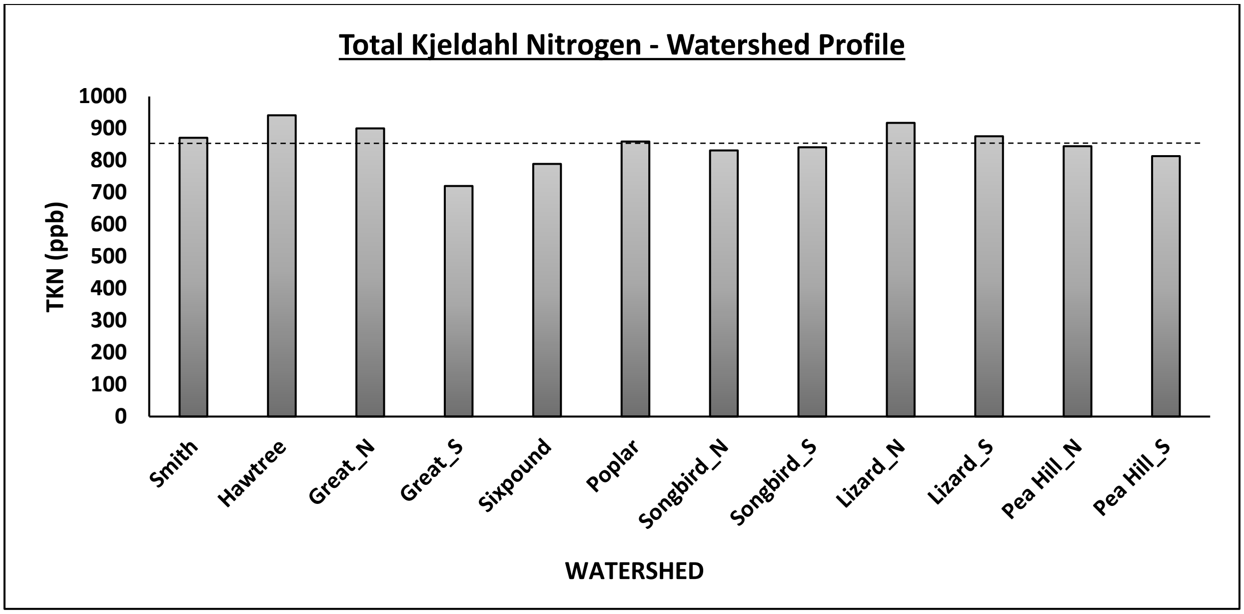

Figure 2.2.12. Average total Kjeldahl nitrogen (TKN) values reported for Lake Gaston's associated watersheds between 2019 and 2021. The dotted line represents the overall lake average for total Kjeldahl nitrogen (849 ppb).Watershed data is reported in order of most upstream to downstream locations and watersheds that encompass both the northern and southern shorelines are divided into two independent sub-watersheds (N, S).

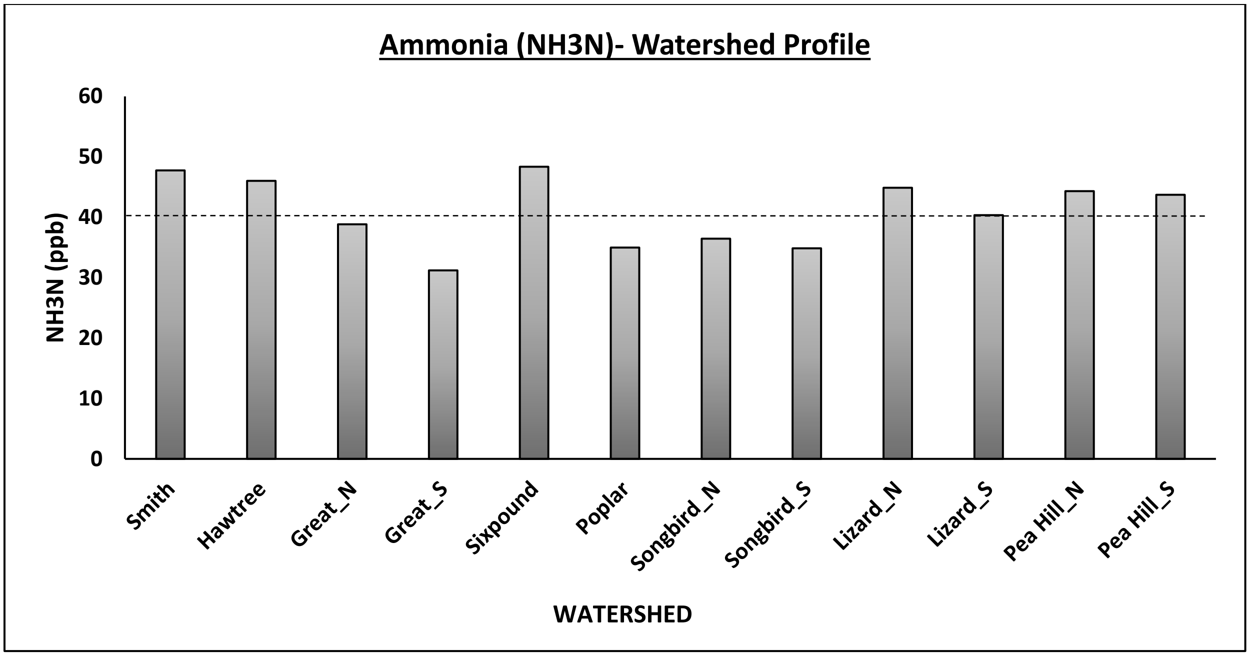

Figure 2.2.13. Average ammonia (NH3N) values reported for Lake Gaston's associated watersheds between 2019 and 2021. The dotted line represents the overall lake average for total ammonia (40 ppb). Watershed data is reported in order of most upstream to downstream locations and watersheds that encompass both the northern and southern shorelines are divided into two independent sub-watersheds (N, S).

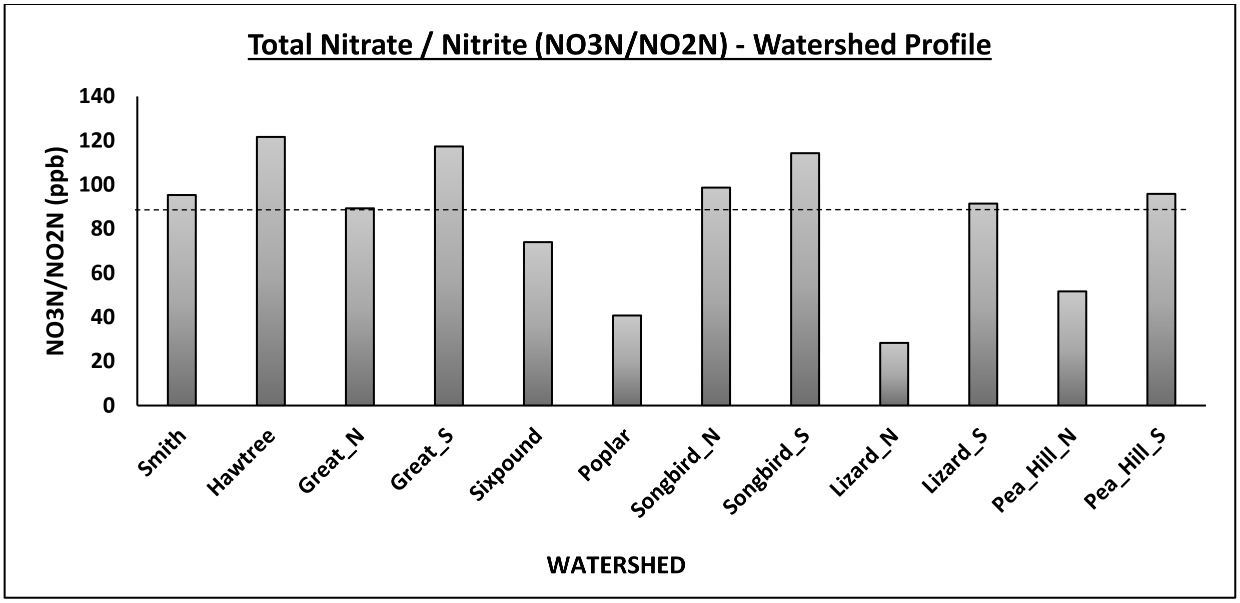

Figure 2.2.14. Average total nitrate and total nitrite (NO3N/NO2N) values reported for Lake Gaston's associated watersheds in 2021. The dotted line represents the overall lake average for total nitrate/nitrite (87 ppb). Watershed data is reported in order of most upstream to downstream locations and watersheds that encompass both the northern and southern shorelines are divided into two independent sub-watersheds (N, S).

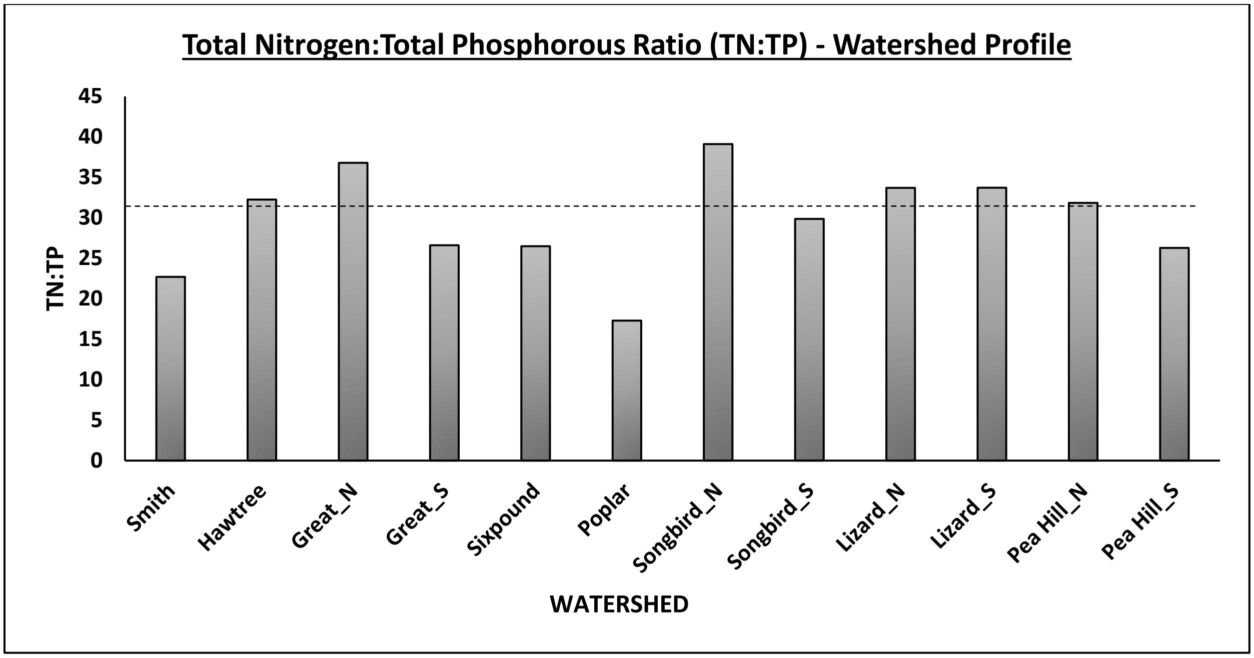

Figure 2.2.15. Average total nitrogen (TN) and total phosphorous (TP) ratios reported for Lake Gaston's associated watersheds during 2021. The dotted line represents the overall lake average for total nitrogen and total phosphorous ratio (31 ppb).Watershed data is reported in order of most upstream to downstream locations and watersheds that encompass both the northern and southern shorelines are divided into two independent sub-watersheds (N, S).

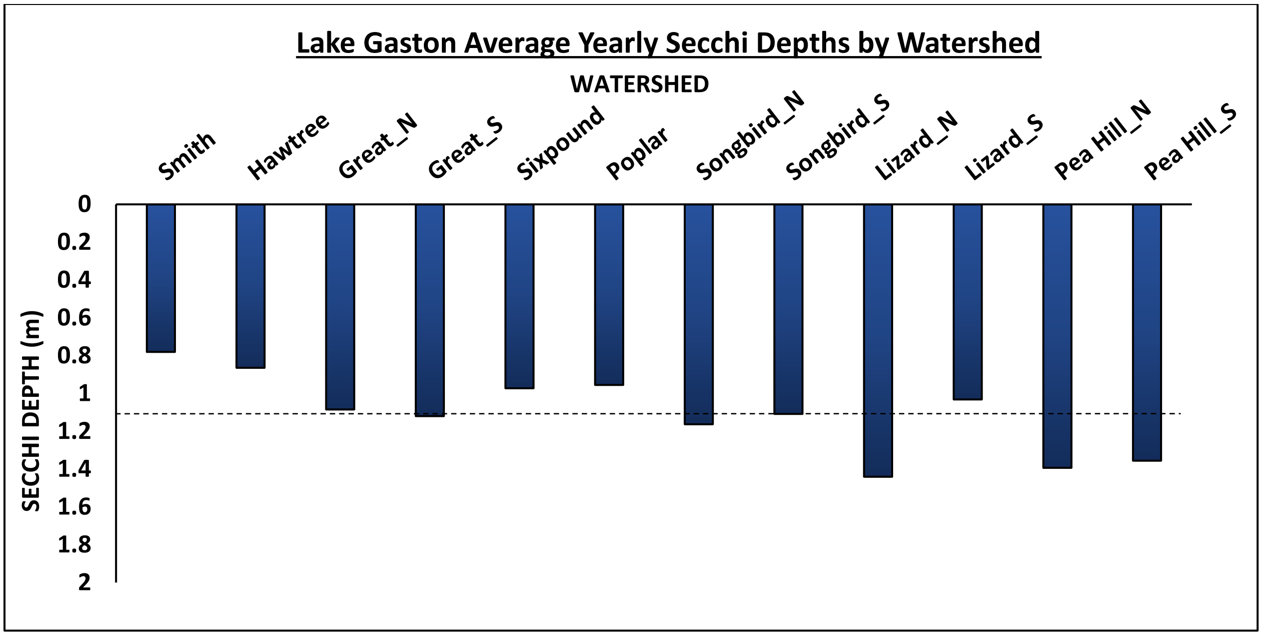

Figure 2.2.16. Average secchi depths reported for Lake Gaston's associated watersheds between 2019 and 2021. The dotted line represents the overall lake average for secchi depths (1.2 m). Watershed data is reported in order of most upstream to downstream locations and watersheds that encompass both the northern and southern shorelines are divided into two independent sub-watersheds (N, S).

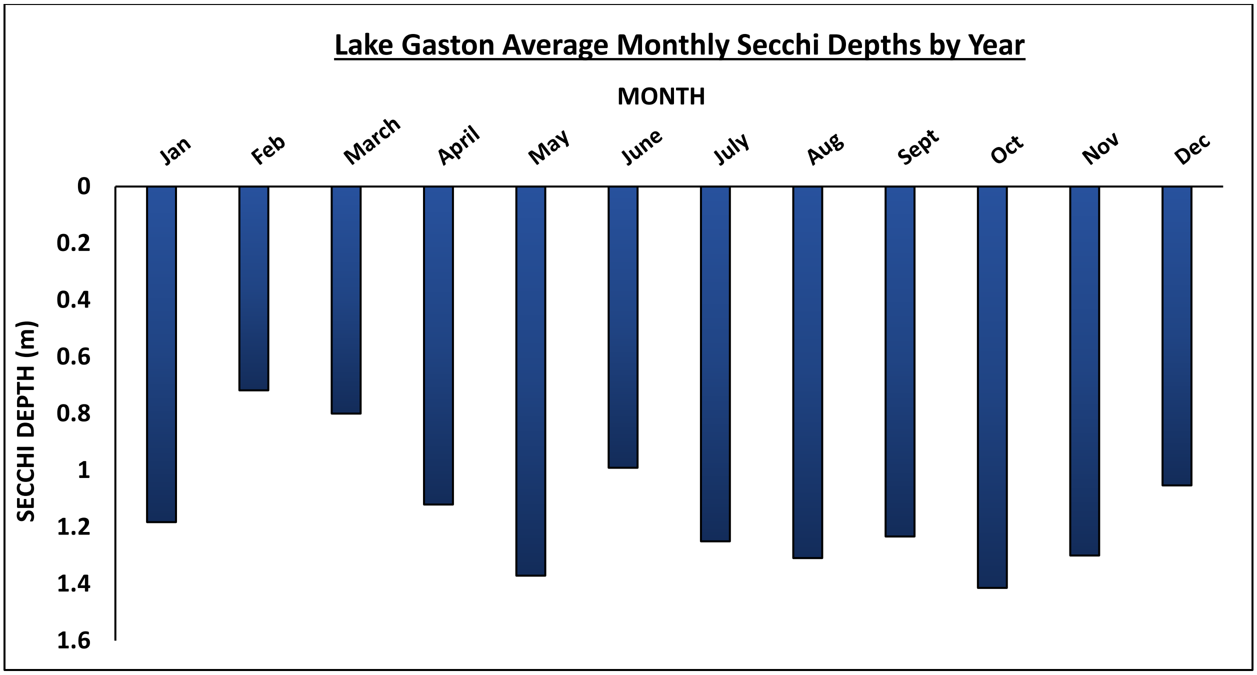

Figure 2.2.17. Average monthly secchi depths recorded for Lake Gaston between 2019 and 2021.

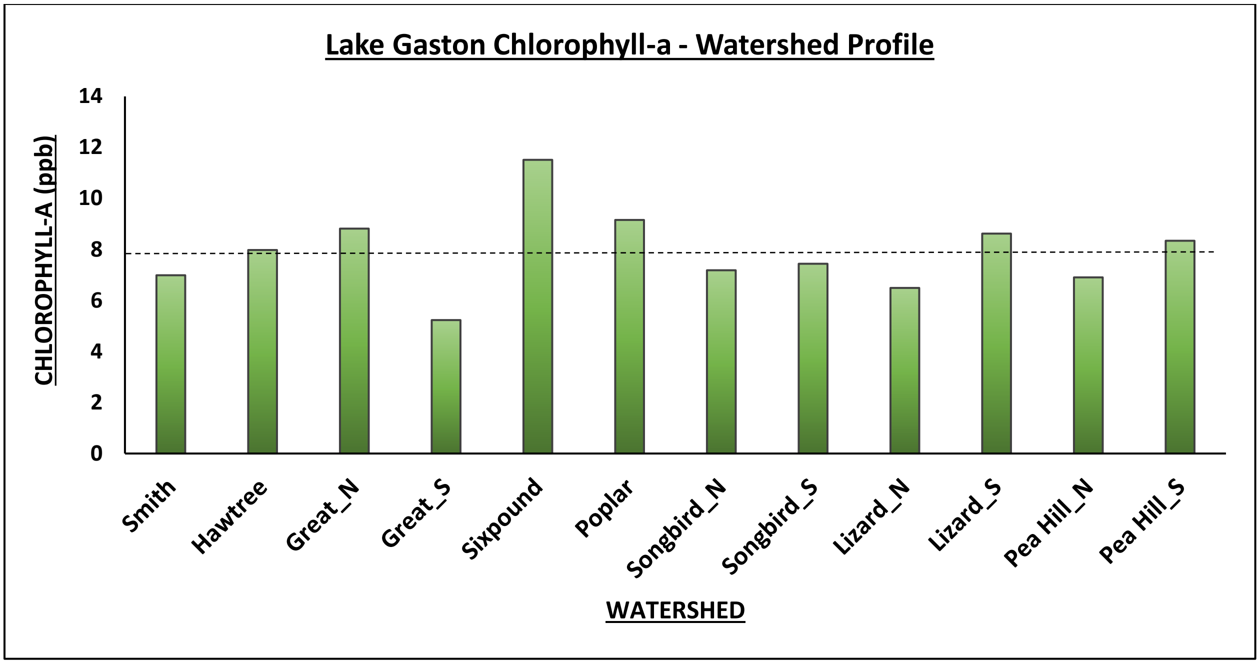

Figure 2.2.18. Average chlorophyll-a values reported for Lake Gaston's associated watersheds during 2020 and 2021. The dotted line represents the overall lake average for chlorophyll-a (7.9 ppb). Watershed data is reported in order of most upstream to downstream locations and watersheds that encompass both the northern and southern shorelines are divided into two independent sub-watersheds (N, S).

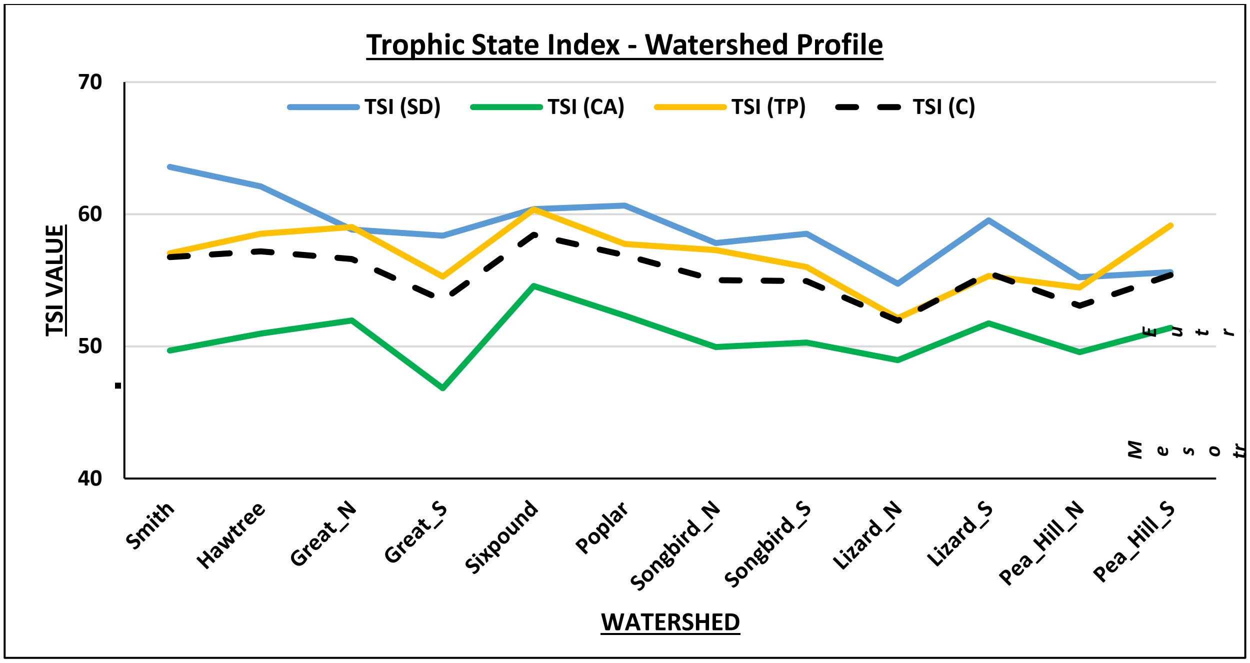

Figure 2.2.19. Trophic State Index values reported for chlorophyll-a (CA), secchi depth (SD), and total phosphorous (TP) for Lake Gaston's associated watersheds. The average of all the three reported TSI parameters are indicated by the black dotted line. The solid black line indicates the value (50) that separates a system from being classified as mesotrophic or eutrophic.

2.3 BACTERIA MONITORING

Introduction

Coliform bacteria are organisms that are commonly found in the environment and typically do not pose a human health treat. However, fecal coliform bacteria originates from the intestines of warm-blooded animals and their presence could indicate fecal contamination within a system and an increased potential of pathogenic organisms. The US Environmental Protection Agency uses bacterial indicators, including the fecal coliform, *E. coli*, as part of their recreational water quality criteria (RWQC). This criteria is intended to protect the public from exposure to harmful levels of pathogens if engaging in high water contact recreation where immersion and ingestion are likely. The EPA RWQC sets two E.coli count threshold recommendations based on illness rates per 1,000 individuals (32/1,000 and 36/1,000). For an illness rate of 32/1,000, the EPA recommends that E.coli counts (CFU = colony-forming unit) should not exceed a geometrical mean of 100 CFU/100mL or a statistical threshold value of 320 CFU/100mL in more than 10 percent of samples collected within a 30-day period. Virginia's Health Standard for recreational waters and North Carolina's surface water standards for Class B freshwater bodies (primary source of recreation) have minimum standards requirements for levels of coliform bacteria of 126 CFU/100mL and ≤ 200 CFU/100mL respectively.

Methods

Coliform bacteria samples were collected on a monthly basis at 18 of the 35 water quality sites. In 2020, E. coli samples were collected during the months of June, July, September, and October. In 2021, monthly sampling occurred from April through September. E. coli sampling sites represent all major tributaries and associated watersheds within the system. Surface water samples were collected using sterile amber polypropylene bottles, stored on ice, and transferred back to the lab for same day plating using a Coliscan® Easygel® medium. For each site, 5 mL of lake water were transferred and placed directly into a bottle of Coliscan® Easygel® using a sterile syringe. Bottles were swirled to evenly distribute the water/medium mixture, poured directly into a petri dish, and then swirled again to ensure complete coverage of the dish. Petri dishes were sealed with lids and incubated for 48 hours at room temperature. Bacterial colonies that displayed a dark blue/purple color indicated a positive result for E. coli. Colonies that displayed a pink to red color or a teal color indicated other forms of coliform bacteria. E.coli colonies were counted and reported in terms of colony forming units (CFU) by the number of E.coli per 100mL.

Results

Although the EPA RWQC recommendation is based on the geometrical mean of multiple samples within a 30 day period, no single sample collected from Lake Gaston in 2020 exceeded the EPA's threshold mean of 100 CFU/100mL for an illness rate of 32 per 1,000 individuals (Figure 2.3.1). Individual site counts ranged from 0 to 30 CFU/100mL. In 2021, two samples displayed elevated counts that exceeded the EPA mean threshold (Figure 2.3.1). Elevated samples were collected in Smith Creek and Songbird Creek during June of 2021 and both sites displayed E. coli counts of 140 CFU/100mL (Figure 2.3.2). Three samples collected in 2021 reported E.coli counts of 100 CFU/100mL and occurred in June (Sixpound), July (St. Tammany), and August (Pretty). For both sampling seasons, there was not a single site that contained E.coli at every monthly sampling event. In 2020, five sites did not present positive E. coli detections during any of the monthly samples and in 2021 that count was reduced to a single site. Pea Hill is the only site that did not display any E.coli activity during either the 2020 or 2021 sampling season.

Overall, there was a significant difference in average E. coli counts reported by year ($p = 0.0007$) and month ($p = 0.0034$), but not by site ($p = 0.8460$). The average E. coli counts for Lake Gaston were greater during the 2021 sampling season (18.15 CFU/100mL) than those reported in 2020 (9.4 CFU/100mL) (Figure 2.3.3). In 2020 the monthly E.coli counts were variable over time, with October exhibiting the highest number of sites producing positive E. coli detections ($n = 9$) and an overall count average of 20 (Figure 2.3.3). In 2021, E.coli counts steadily increased until counts peaked in June where 12 sites produced positive E. coli detections and averaged 39 CFU/100mL (Figure 2.3.3). Yearly E. coli count averages varied between sites, however with the exception of two sites (Hawtree and Main Lake Cove) average counts were higher per site in 2021 than in 2020 (Figure 2.3.4). Maps showing site variability for E.coli detection rates and average overall counts can be found in Figure 2.3.5 (2020) and Figure 2.3.6 (2021).

<u>Discussion</u>

The presence of E.coli within a system can be attributed to anthropogenic sources such as failing septic tanks, and natural sources such as increased waterfowl presence. Lake Gaston's highly developed shoreline has raised concerns over aging septic tanks and the potential of increased levels of E. coli. Land use practices within a watershed can also increase the potential of E. coli breakouts within the system. Therefore, E. coli sampling sites were chosen to represent all major watersheds associated with Lake Gaston with land uses that include highly developed areas and agricultural fields.

Although E. coli was positively detected in Lake Gaston at multiple samples throughout 2020 and 2021, the population was never recorded at a level that would constitute a violation from a human health perspective. Overall, sites downstream of Eaton's Ferry Bridge displayed lower overall E. coli counts and did not report levels above 15 CFU/100 mL. As for individual sites, Lyons Creek exhibited the highest overall E.coli counts over the combined sampling seasons and the highest monthly detection rates. Hawtree Creek, in the upper most section of the lake, displayed the largest decrease in counts between sampling seasons and Pea Hill was the only site that did not display E. coli activity in either 2020 or 2021. We found that overall E.coli counts varied between years and sites and no single site displayed consistently high levels of E. coli that would indicate a point source problem.

Figures

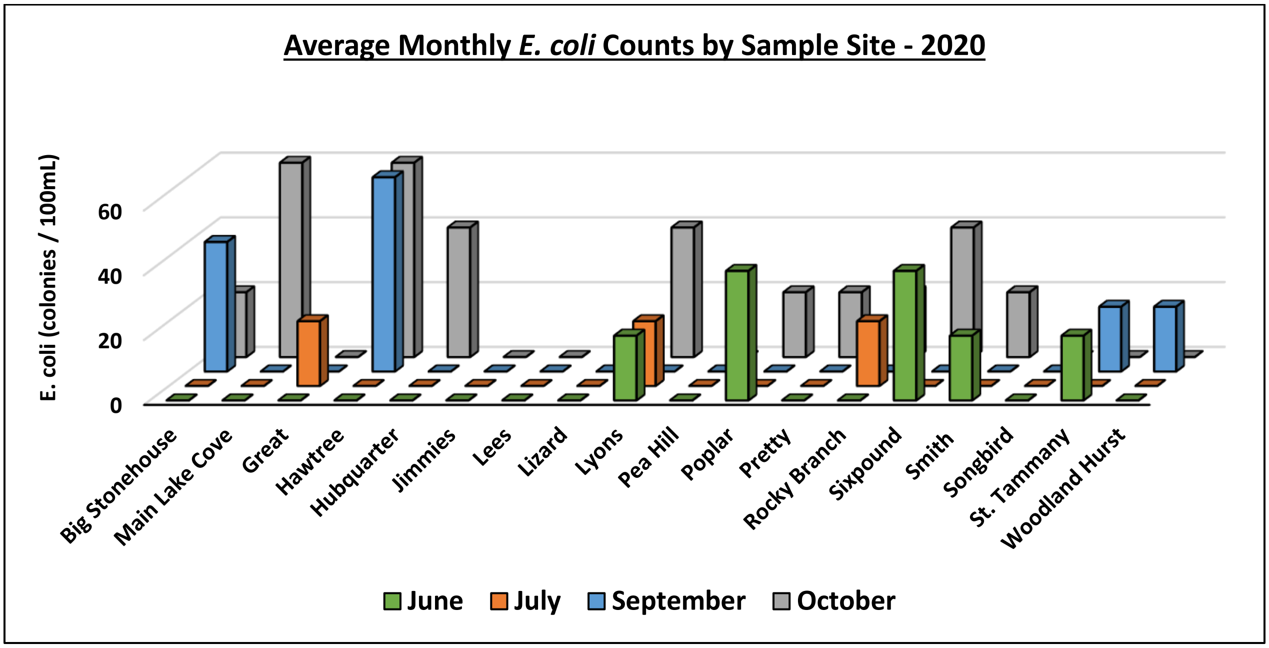

Figure 2.3.1. Average monthly E.coli counts for each of the 18 water quality sites sampled for E.coli on Lake Gaston in 2020.

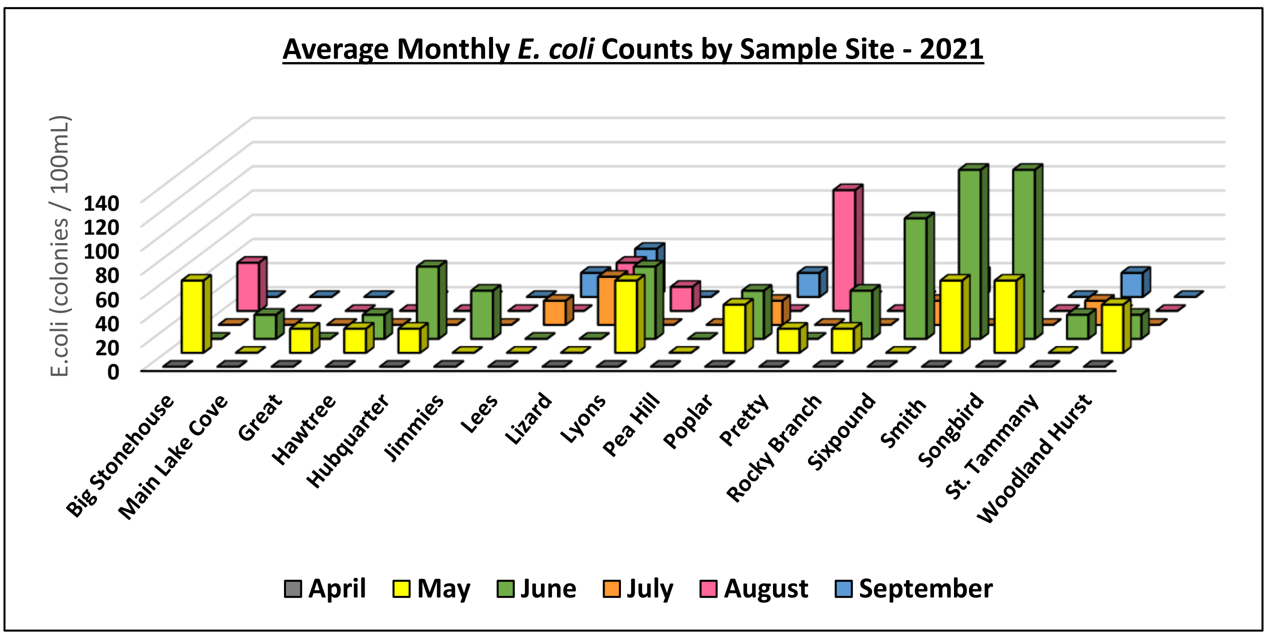

Figure 2.3.2. Average monthly E.coli counts for each of the 18 water quality sites sampled for E.coli on Lake Gaston in 2021.

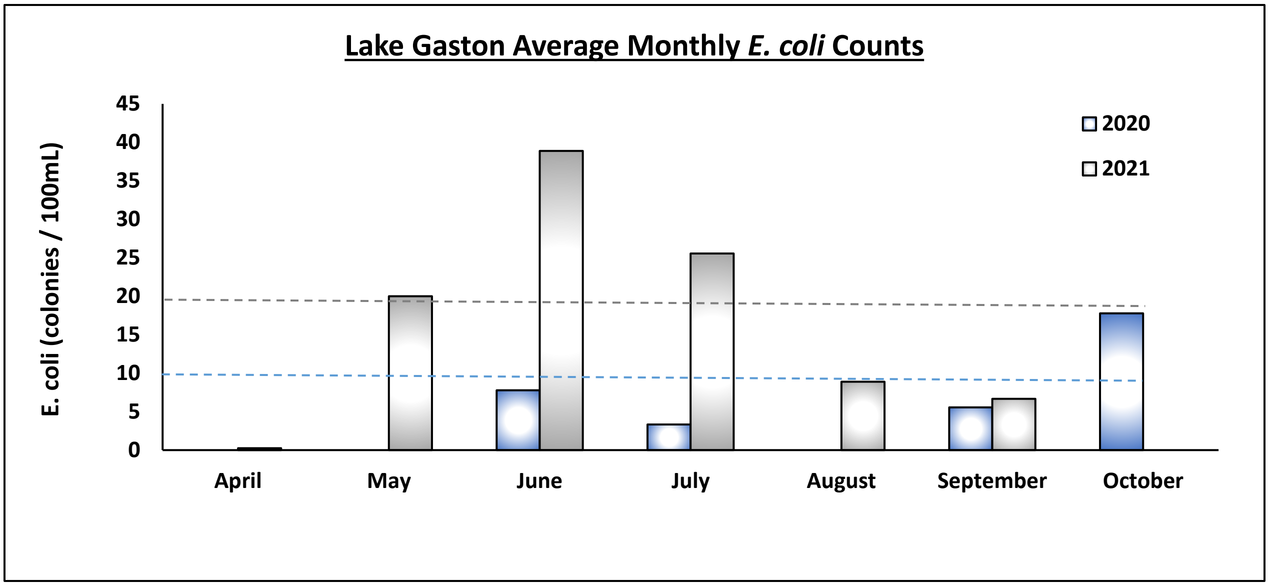

Figure 2.3.3. Average monthly E.coli counts for 18 water quality sites sampled in Lake Gaston during 2020 and 2021. The dotted line represents the overall lake average for E.coli of 9.4 in 2020 (grey) and 18.15 in 2021 (blue).

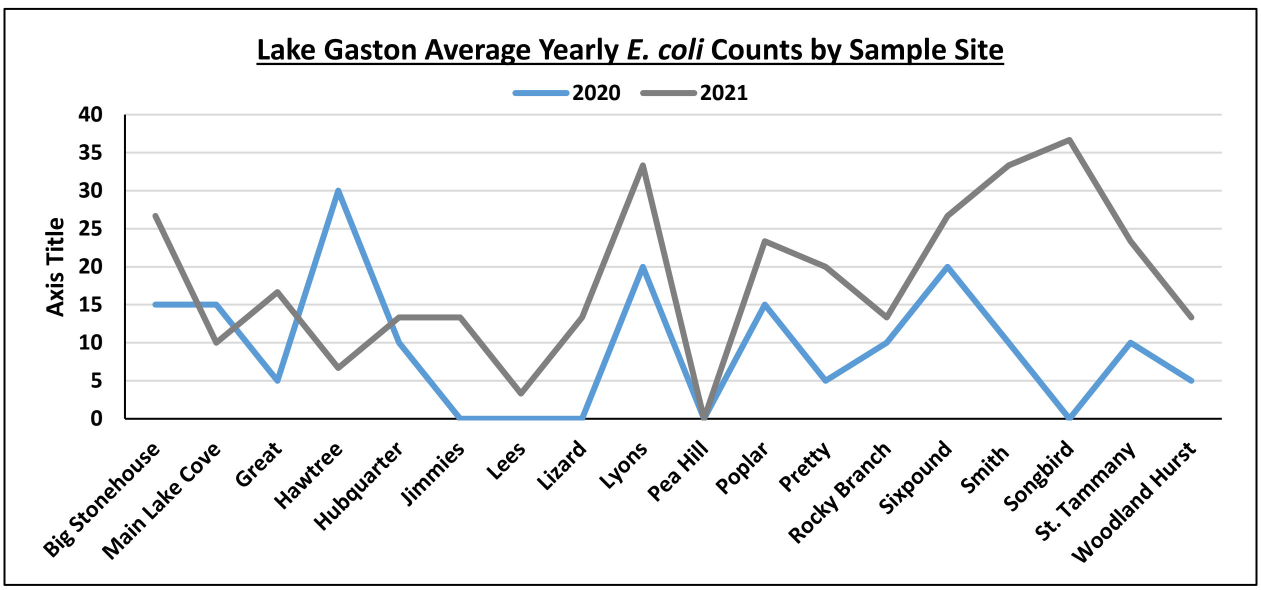

Figure 2.3.4. Average yearly E.coli counts for 18 water quality sites sampled in Lake Gaston during 2020 and 2021.

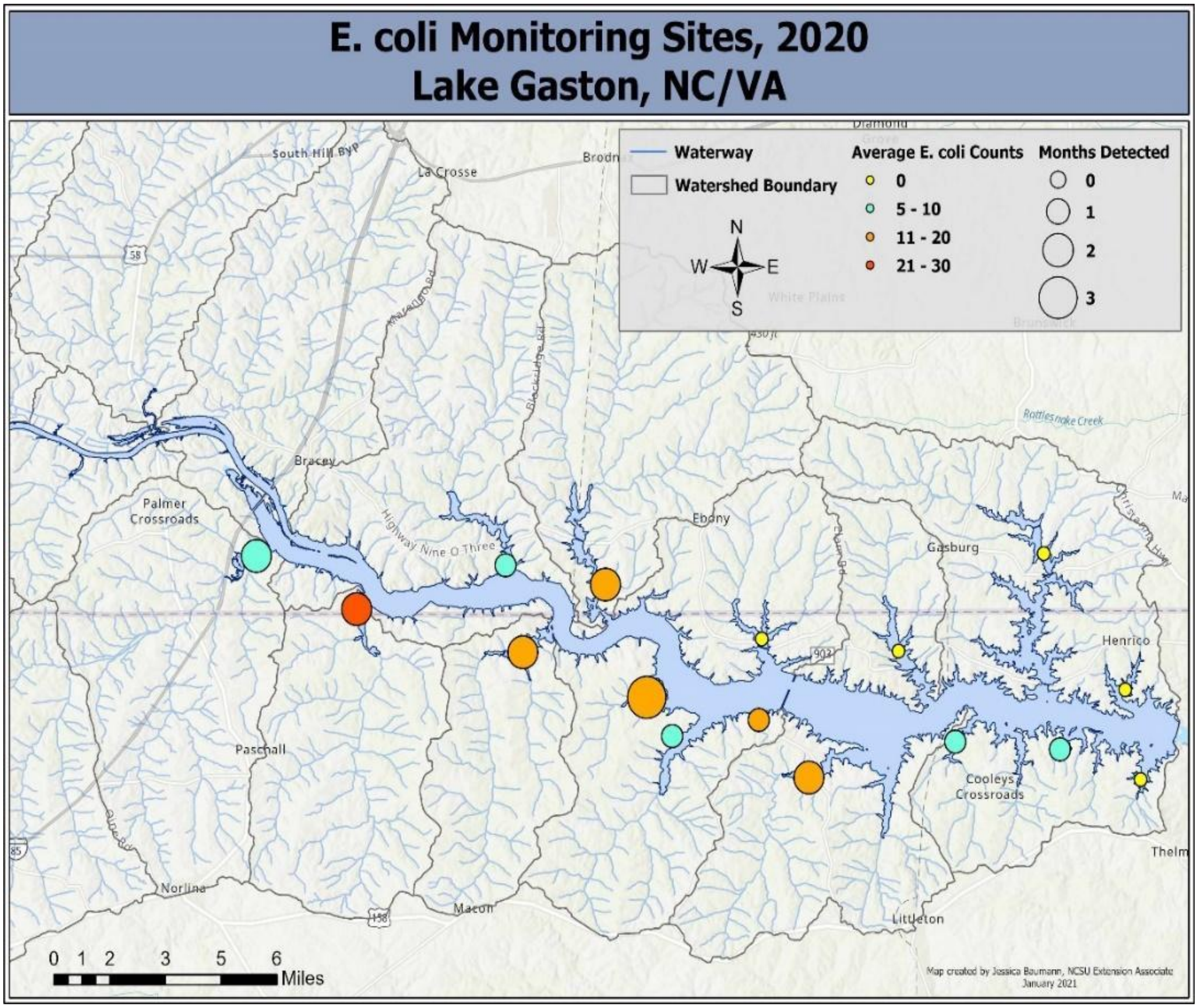

Figure 2.3.5. Map of E.coli sampling sites in Lake Gaston, NC/VA for 2020. Symbol color represents average E.coli counts for each site and symbol size represents number of positive E. coli detections over the 2020 sampling season.

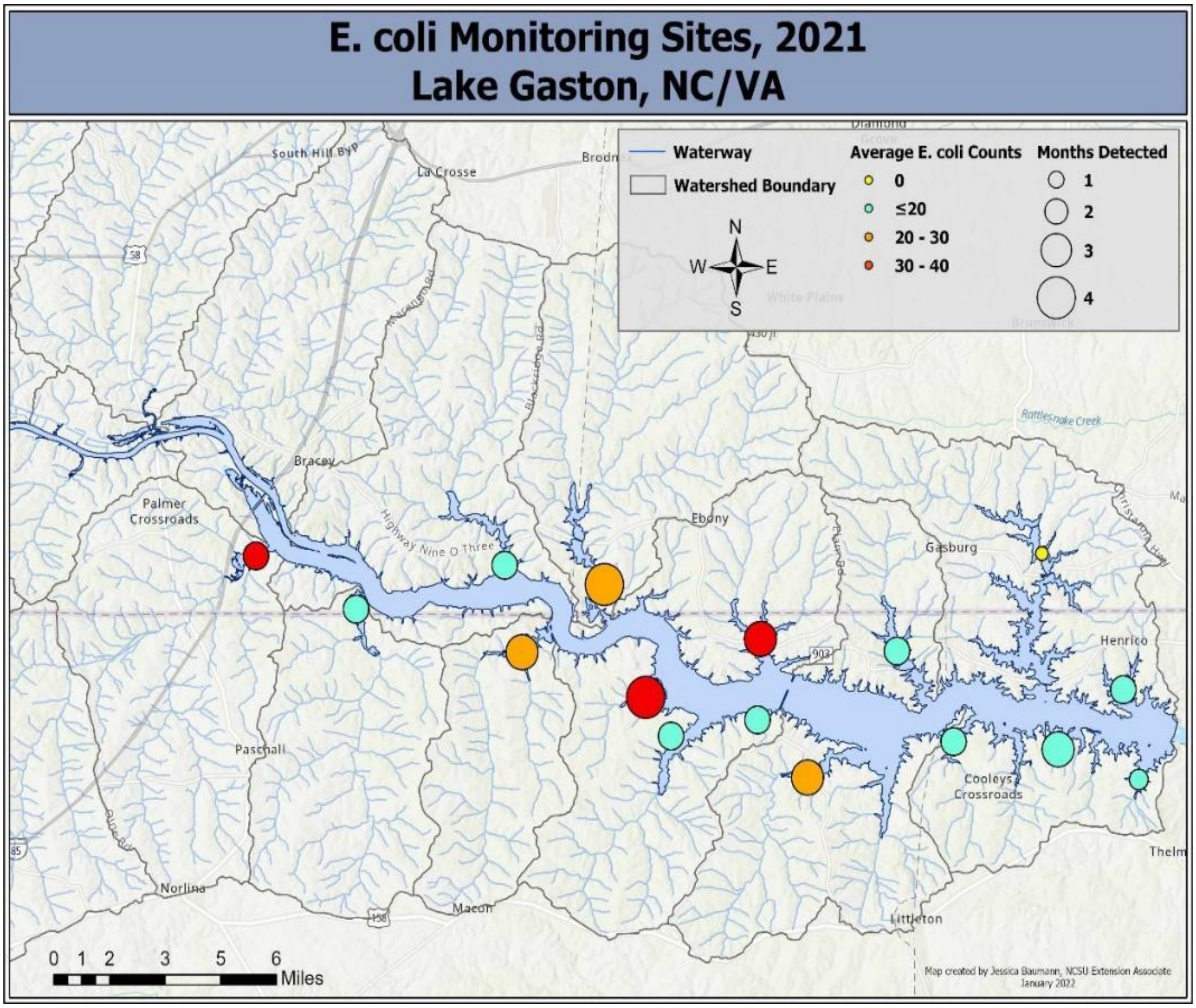

Figure 2.3.6. Map of E.coli sampling sites in Lake Gaston, NC/VA for 2021. Symbol color represents average E.coli counts for each site and symbol size represents number of positive E. coli detections over the 2021 sampling season.

2.4 HYDROSOIL CHARATERISTICS

Introduction

Sediment characteristics play an important role in the nutrient dynamics of a reservoir. The ability of a reservoir to retain sediments at high levels results in the accumulation of sediment-associated nutrients, including nitrogen and phosphorus (Gautreau 2020). This nutrient accumulation has the potential to influence the nutrient dynamics of a system as much as anthropogenic impacts and compromise directed efforts to improvement a system's water quality (Søndergaard et al. 2003; Wu et al. 2017). Through various ecological processes, freshwater systems can experience internal fertilization by releasing nutrients from the sediment back into the water column, making them available for the primary producers of the system (Wetzel 1983; Wu et al. 2017). Nutrient deposits can be a product of distribution of nutrient bound particles suspended in the water column or high levels of biological activity, including benthic microbial and macroinvertabrate communities, which recycle nutrients through metabolic activities and bioturbation (Wetzel 1983; Gautreau 2020). In addition, nutrient dynamics of both the sediment and water column within the littoral zone are impacted by the establishment of aquatic macrophyte communities. While most accessible nutrients for aquatic plant growth are located within the water column, rooted macrophytes can utilize phosphorous and nitrogen sources bound within the sediment. These macrophytes also provide a source of organic matter and nutrients during senescence and death.

Chemical factors, such as dissolved oxygen levels and temperature, and the physical characteristic of a reservoirs benthic environments can impact nitrogen and phosphorus fluxes at the sediment-water column interface (Kristensen 2000; Lavery et al. 2001; and Ni and Wang 2015). During warm summer months benthic environments develop anoxic conditions which triggers the internal loading of nitrogen and phosphorous (Gautreau 2020). These warm summer months also coincide with increased primary production within a system and the combination could contribute to eutrophication. Monitoring nutrient levels within the water column gives managers an indication of the current state of nutrient dynamics within a system. However, monitoring the nutrient levels within the sediment allows managers to identify areas that could be at greater risk for future increased nutrient loading and sources for potential eutrophication.

Methods

A single hydrosoil sample was collected at 18 water quality sites during September 2021 and represent all of Lake Gaston's associated watersheds. Hydrosoil sites corresponded to bacteria monitoring sites and GPS location information can be found in Appendix A.1. A total of five sediment samples were collected per site using a petite ponar stainless steel grab. A stainless steel spoon was used to collect the hydrosoil sample from the ponar grab and transfer it to an amber I-Chem certified clean bottle. All five hydrosoil samples were aggregated into a single jar to represent the sample for an individual collection site. Samples were brought back to the lab and allowed to completely air dry prior to laboratory analysis. Samples were then processed by North Carolina State University's Environmental and Agricultural Testing Service laboratory to determine the following parameters: Carbon (% weight), Nitrogen (% weight), Nitrate (mg/L), Ammonia (mg/L), Phosphorous (mg/kg), Copper (mg/kg), and Iron (mg/kg).

Results

Results for hydrosoil nutrient levels for Lake Gaston's associated watersheds can be found in Table 2.4.1 Carbon ranged between 2.51 and 3.97 in % weight per sample, with Lizard – N reporting the highest levels and Pea Hill – N reporting the lowest levels. Nitrogen and phosphorous followed similar spatial trends per watershed as was reported with water samples (Figure 2.4.1 and Figure 2.4.2). Hawtree reported the highest levels of nitrogen in the water column (1,276 ppb) and the second highest in percent per sample for hydrosoils (0.34). Lizard – N reported the highest percent of nitrogen per sample for hydrosoils (0.40). Sixpound watershed reported the highest levels of phosphorus within the water column (49.31 ppb), but was fourth highest in hydrosoil levels (611.5 mg/kg). Inorganic forms of nitrogen, nitrate and ammonia, varied between watersheds. Nitrate was highest in the Great – N watershed (5 mg/L) and lowest in Lizard – S (0.03 mg/L). Ammonia levels were reported at their highest level in Lizard – N (10.29 mg/L) and lowest in Smith (2.01 mg/L). Metal levels reported for hydrosoils were iron and copper. Iron was highest in Songbird – N (85,932 mg/L) and lowest in Pea Hill – N (35,949 mg/kg). Copper levels ranged from 21.79 mg/kg in the Smith watershed to 93.28 mg/kg in Pea Hill – S. Since all samples were collected in 2021, no temporal comparisons could be made.

Discussion

Understanding the level of nutrients that are bound within the sediment of a system allows managers to evaluate potential sources of internal nutrient loading that could result in negative trophic impacts. Hydrosoil samples followed similar spatial trends as reported in water column samples and did not indicate any potential increased nutrient loading within the sediment for any individual watershed. There was also no spatial indication of nutrient bound sediments falling out of the water column as water moved downstream. No temporal comparison can be made at this time, however values reported here can be utilized as baseline data for future sampling.

References

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Tables

Table 2.4.1. Nutrient parameters for hydrosoils collected in September 2021 at Lake Gaston, NC/VA.

Watershed	Carbon (% wt.)	Nitrogen (% wt.)	Phosphorous (mg/kg)	Nitrate (mg/L)	Ammonia (mg/L)	Iron (mg/kg)	Copper (mg/kg)
Smith	2.82	0.22	496.53	0.75	2.01	46,184.84	21.79
Hawtree	3.79	0.34	607.00	0.07	8.10	68,878.69	33.37
Great - N	3.57	0.33	717.63	5.00	2.76	66,572.71	67.27
Sixpound	3.86	0.33	611.50	3.08	2.77	66,123.66	31.01
Poplar	3.25	0.30	750.25	2.78	3.24	77,964.41	40.57
Songbird -N	3.55	0.33	697.25	1.36	2.22	85,932.19	41.89
Songbird - S	3.20	0.28	553.54	1.89	2.80	57,799.35	53.54
Lizard - N	3.97	0.40	576.25	1.24	10.29	68,815.74	87.90
Lizard - S	2.79	0.26	414.80	0.03	8.30	51,783.45	33.32
Pea Hill - N	2.51	0.22	384.14	0.22	2.54	35,949.60	37.96
Pea Hill - S	3.22	0.31	598.03	2.38	4.06	48,865.01	93.28

Figures

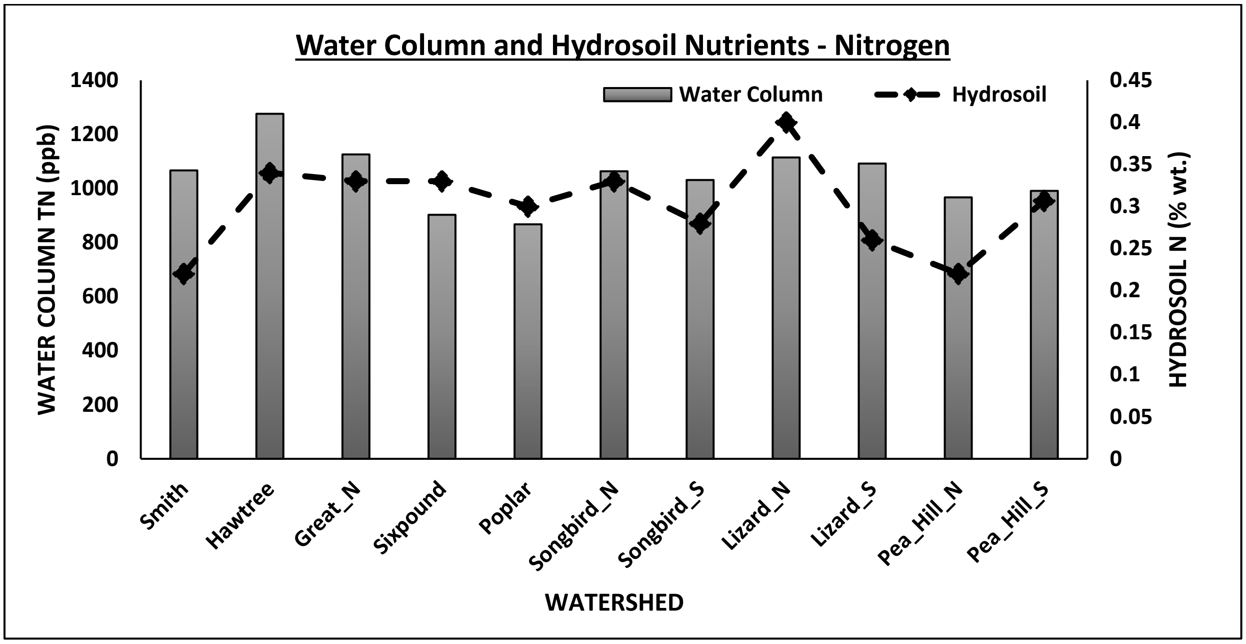

Figure 2.4.1. Hydrosoil nitrogen levels and average total nitrogen (TN) values reported for water column samples between 2019 and 2020 for Lake Gaston's associated watersheds. Watershed data is reported in order of most upstream to downstream locations and watersheds that encompass both the northern and southern shorelines are divided into two independent sub-watersheds (N, S).

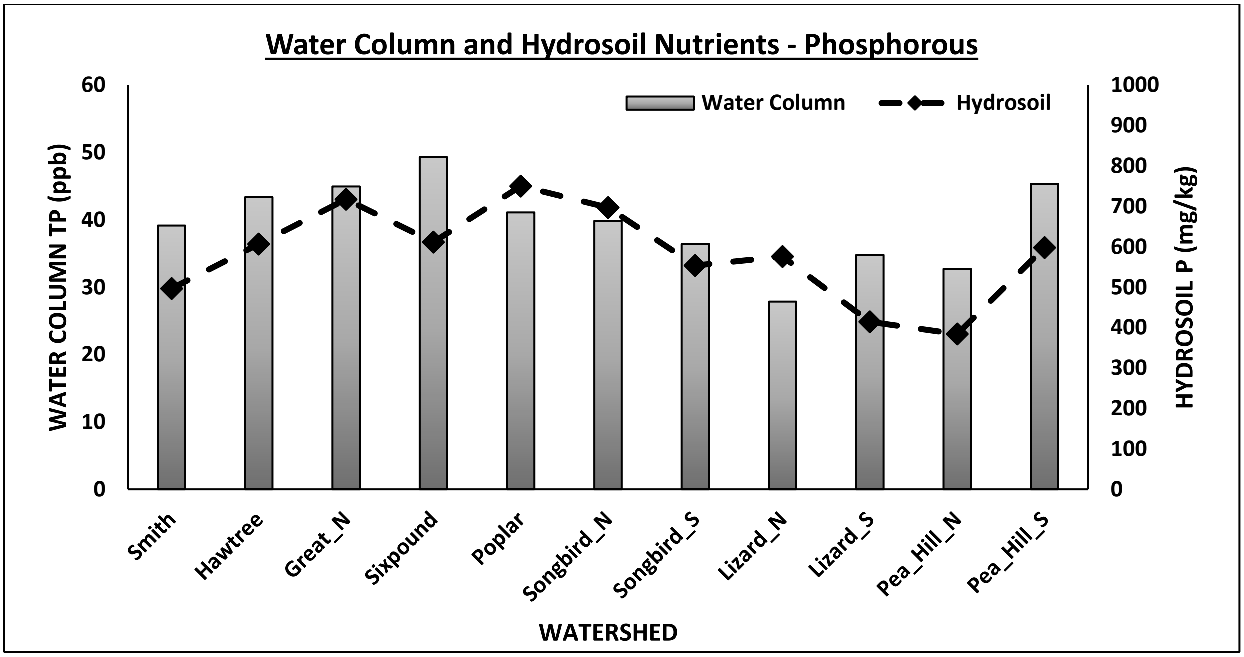

Figure 2.4.2. Hydrosoil phosphorous levels and average total phosphorous (TN) values reported for water column samples between 2019 and 2020 for Lake Gaston's associated watersheds. Watershed data is reported in order of most upstream to downstream locations and watersheds that encompass both the northern and southern shorelines are divided into two independent sub-watersheds (N, S).

2.5 FUTURE WATER QUALITY MONITORING RECOMMENDATIONS

Continued routine water quality and hydrosoil monitoring should be performed to identify potential changes in water chemistry and overall nutrient dynamics which could decrease overall water quality and drive growth for nuisance aquatic species within the lake.

OBJECTIVES:

Perform regular water quality monitoring with the goal of 1) characterizing basic water chemistry and nutrient parameters for Lake Gaston, 2) monitoring changes in nutrient dynamics in the hydrosoil, and 3) identifying any potential negative impacts to the water quality of Lake Gaston.

SCOPE:

- Collect water quality data twice per season (n = 8) at 18 water quality sites representing all major tributaries and associated watersheds.
    - Report on the following parameters from each sample: water temperature, pH, secchi depth, chlorophyll-a, conductivity, dissolved oxygen, total nitrogen, total phosphorous, total Kjeldahl nitrogen, ammonia, and orthophosphorus.
- Collect hydrosoil samples and alkalinity levels twice per year (summer / winter).
    - Report on the following parameters from each sample: total nitrogen, total phosphorous, carbon, nitrate, ammonia, iron, and copper.

3.1 Shoreline Management

To assist landowners in using best management practices for their lakefront properties, we developed the following brochure on shoreline management. This brochure highlights landscaping techniques that will help reduce the overall nutrient loading occurring from highly maintained lawns of lakefront properties. It describes the impacts that lawn debris and fertilizers can have on the aquatic ecosystem surrounding these types of properties and highlights the benefits of developing a well maintained buffer zone along the shoreline. This brochure also provides tips on how property owners can create these vegetated buffer zones, including identifying beneficial native plant species that would aid in the effectiveness of these areas. This brochure is intended to be used as educational material and distributed at outreach events.

Beneficial Native Species

Planting species native to North Carolina & Virginia in your yard provides food and cover to a wide range of native wildlife, and can attract beautiful birds and pollinators to your property.

Here are a few beneficial examples that would aid in the effectiveness of a vegetated buffer:

Milkweed (*Asclepias incarnata*)
Solidaster (*Solidaster luteus*)
Sage (*Salvia sp.*)
Obedient Plant (*Physostegia virginiana*)
Lupine (*Lupinus perennis*)
Lavender (*Lavandula angustifolia*)
Blueberry (*Vaccinium angustifolium*)
Striped Maple (*Acer pensylvanicum*)
Lilac (*Syringa sp.*)
Cinnamon Fern (*Osmunda cinnamomea*)
Big Bluestem (*Andropogon gerardii*)
Switchgrass (*Panicum virgatum*)

Here is a specific example of what a vegetated buffer could look like. Different variations are great for encouraging plant diversity.

Photo Courtesy: Jessica Baumann, NC State University Aquatic Plant Management Group

Get in Touch

North Carolina State University Aquatic Plant Management Group:
aquaticplants@ncsu.edu

Lake Gaston Weed Control Council:
info@LGWCC.org

NC STATE UNIVERSITY

LKGWCC
Lake Gaston Weed Control Council

References

Goatley, Michael et al. 2021. *Lawn Fertilization in Virginia.* Virginia State University Cooperative Extension.

Hardesty, Phoebe, and C. Kuhns. 1998. *A Guide to Creating Vegetated Buffers for Lakefront Properties.* Androscoggin Valley Soil & Water Conservation District.

McCarthy, Jillian. 2011. *New Hampshire Homeowner's Guide to Stormwater Management.* New Hampshire Department of Environmental Services, Watershed Assistance Section.

How Managing Your Shoreline Can Benefit Lake Gaston!

Photo Courtesy: Jessica Baumann, NC State University Aquatic Plant Mangement Group

NC COOPERATIVE EXTENSION · NC STATE UNIVERSITY · N.C. A&T STATE UNIVERSITY

Vegetated Buffers

Buffer zones provide a vegetated region of land between a water body and developed property. The goal of these zones is to limit the amount of debris and runoff added to the water from urban areas.

Effective buffer zones improve and/or maintain overall water quality by preventing nutrient loading and sedimentation. This decreases the chances of noxious algal blooms.

Create your own buffer

Vegetated buffers can look very different depending upon plant composition and personal taste. The height and width of your buffer does not have to be uniform across the entire shoreline, nor does the species planted.

Ways to create your own:

- Pick a section of your lawn along the shoreline and allow it to grow without intense mowing or trimming. (Occasional maintenance is ok!)
- Plant seeds or mature vegetation along the shoreline in a desired manner. Mulching around these plants can aid in their efficiency.

Lawn Care

When caring for your lawn, there are some key ways you can help keep the lake clean!

Debris such as twigs, leaves, and lawn clippings should ***NOT*** be disposed of in the lake. These can instead be composted or placed back on your lawn. Distributing lawn clippings throughout your yard will provide your grass with a natural fertilizer.

Why should we avoid adding nutrients to the lake?

Lawn debris and fertilizers contain nutrients such as nitrogen and phosphorus that can alter the natural balance of the lake's chemical composition. Having increased levels of these nutrients often results in a process known as eutrophication which can lead to algal blooms.

Undesirable algal blooms can cause:

- Negative impacts to human health
- Decreases in water quality
- Rapid decreases in oxygen levels that can be deadly to aquatic organisms
- Aesthetically unappealing growth

Best Fertilizer Practices

Fertilizers, when applied inappropriately, can lead to nutrient loading and negative impacts on water quality.

Here are some ways that you can ensure you are safely using fertilizers on your lakefront property:

- Ensure time of application follows instructions provided by fertilizer company, plants typically have a harder time absorbing nutrients in the winter months.
- Don't fertilize prior to major weather events, rain can wash fertilizers into the lake.
- Check that the type of fertilizer you are using is appropriate for your lawn/garden.

The reality of algal blooms:

Photo Courtesy: McCarthy, Jillian. 2011. *New Hampshire Homeowner's Guide to Stormwater Management*.

3.2 Novel Vegetated Fish Attractors for Habitat Enhancement

Introduction

Reservoirs are engineered to meet multiple objectives including flood control, water supply, and hydropower generation. These objectives require reservoirs to retain large amounts of water on historically terrestrial terrain, creating unnatural areas and unique habitat challenges. As a result, it is common to encounter barren littoral zones due to degradation of woody debris as well as limited native seed banks and unfavorable physical conditions for submersed aquatic vegetation (SAV) establishment and growth. The lack of habitat within reservoirs creates an environment that is deprived of the ecological services that a diverse community of native aquatic plant species and coarse woody habitat can provide.

Krogman and Miranda (2016) conducted a survey that focused on issues affecting available fish habitat within a reservoir environment. Fisheries biologists across the continental US identified 12 major factors that influence reservoir habitat degradation. These factors included issues with pollution and excessive nutrients, sedimentation and shallowness, and limited littoral structure. Native SAV plantings and artificial fish attractors have been used separately by reservoir managers to combat some of these major factors. Aquatic vegetation provides stabilization for both shoreline and lake-bottom sediment, helps control nutrient loading by increasing the buffer zone and recycling nutrients, and provides critical habitat for aquatic life including fish spawning and nursery areas. Fish attractors provide increased structure that concentrates fish, gives trophic advantages to both ambush predatory fish as well as their prey, and increases surface area for periphyton growth and aquatic invertebrate habitat.

While increasing aquatic habitat is important from an ecological perspective, some reservoir managers have to also consider the economic value of the reservoir when determining appropriate methods for habitat enhancement. Lakefront properties increase housing market values and vast open water provides recreational opportunities for both pleasure boaters and anglers. Fish attractors are beneficial for both these user groups by providing critical structure that concentrates fish for anglers in a manner that does not impact the aesthetic value of the waterfront homes. These structures also have the added advantage of providing instantaneous habitat that results in rapid responses from reservoir fish communities (Baumann et al. 2016). However, these structure are limited in the amount of habitat they can provide by the amount of structure that is deployed. Native SAV reestablishment projects will not produce the rapid response time like deployment of fish attractor structures, however the expansion of these reestablished native aquatic plant communities provide important ecological services and long-term benefits. Expansion of these aquatic communities are also beneficial for homeowners and anglers in the sediment stabilization and habitat that they provide. However, historical methods for SAV establishment include building bulky metal cages that are considered aesthetically unappealing by property owners. Balancing the ecological benefits with the economic benefits of individual designs can be a challenge for reservoir managers when trying to determine the most appropriate habitat enhancement design for their system.

The objective of this project was to address the habitat enhancement challenges faced by reservoir managers by developing a design that would maximize the benefits of both planted native aquatic vegetation and artificial fish attractors, while maintaining a low visual profile for waterfront homeowners. To accomplish this we developed an enhancement design that incorporated designs for both aquatic vegetation establishment and artificial fish attractors that have been proven effective in previous studies, hereafter refer to as the novel vegetated fish attractor design. The combination of these two habitat enhancement designs creates a complex fish habitat by integrating large, course, and instantaneous structure with the future benefits of established native aquatic vegetation.

Methods

We deployed novel vegetated fish attractors at a total of five locations within Lake Gaston during August of 2021 and 2022 (Figure 3.2.1). For the fish attractor design, we used a structure commonly referred to as the Georgia Structure (Figure 3.2.2). This cube shaped structure utilizes a combination of PVC pipes and corrugated drainage pipes to create large, solid, vertical surfaces that have been proven to concentrate fish (Baumann et al. 2016). The size of these cubes can be customized to the depth of deployment, but typically encompass a volume of approximately 3.3 cubic meters. At any size, this design has the advantage of providing a large open space that is protected by four high vertical walls. For the SAV reestablishment, we used submersed cage cubes (Figure 3.2.3) that have been proven effective in other reservoirs (Figure 3.2.4). We capitalized on the open spaces proved by the fish attractor design by incorporating the vegetation cage cubes into those spaces and deploying them within and near the outside edges of the structure (Figure 3.2.3). In contrast to classic shoreline vegetation establishment cages, submersed cubes are smaller in scale and provide 360° herbivore protection for submersed aquatic vegetation. Cubes are hand-cut out of PVC-coated wire fencing, and because of this, their size and shape can be highly customizable. Cubes that have a volume of 0.3 cubic meters have worked well in the other reservoir systems in which they have been deployed and therefore were utilized for this project. To maximize plant establishment, a burlap sack was positioned inside each vegetation cube and filled with soil. Native plant roots were affixed to the burlap using thin wire to ensure that they remain inside the cube structure and did not float out after installation. Based on work that has been completed at other reservoirs, we have found that *Vallisneria americana* (eel grass) can successfully grow within the cube set-up (Figure 3.2.3) and multiple individual clumps were planted within each cube. Eel grass was collected locally within Lake Gaston in order to maintain species population consistency. Each habitat enhancement site consisted of one Georgia fish attractor and five submersed vegetation cubes that were marked by a single NCWRC fish attractor buoys to reduce the level of navigational hazard.

Preliminary Results

Due to the required time needed for eel grass to establish within the submersed cubes, only preliminary data has been captured at this point in time (Figure 3.2.6). Sonar images of individual attractor locations established in 2021 show varying preliminary results from no visible growth to a plethora of eel grass growth (Figure 3.2.6). Continued monitoring of these sites will occur by capturing sonar images of eel grass growth within and adjacent to established habitat enhancement sites. If water clarity permits, visual observations will also be attempted to capture the level aquatic plant growth.

References

Baumann, J.R, N. C. Oakley, and B. J. McRae. 2016. Evaluating the Effectiveness of Artificial Fish Habitat Designs in Turbid Reservoirs Using Sonar Imagery. North American Journal of Fisheries Management 36: 1437-1444.

Krogman, R. M., and L. E. Miranda. 2016. Rating US reservoirs relative to fish habitat condition. Lake and Reservoir Management 32: 31 - 60.

Figures

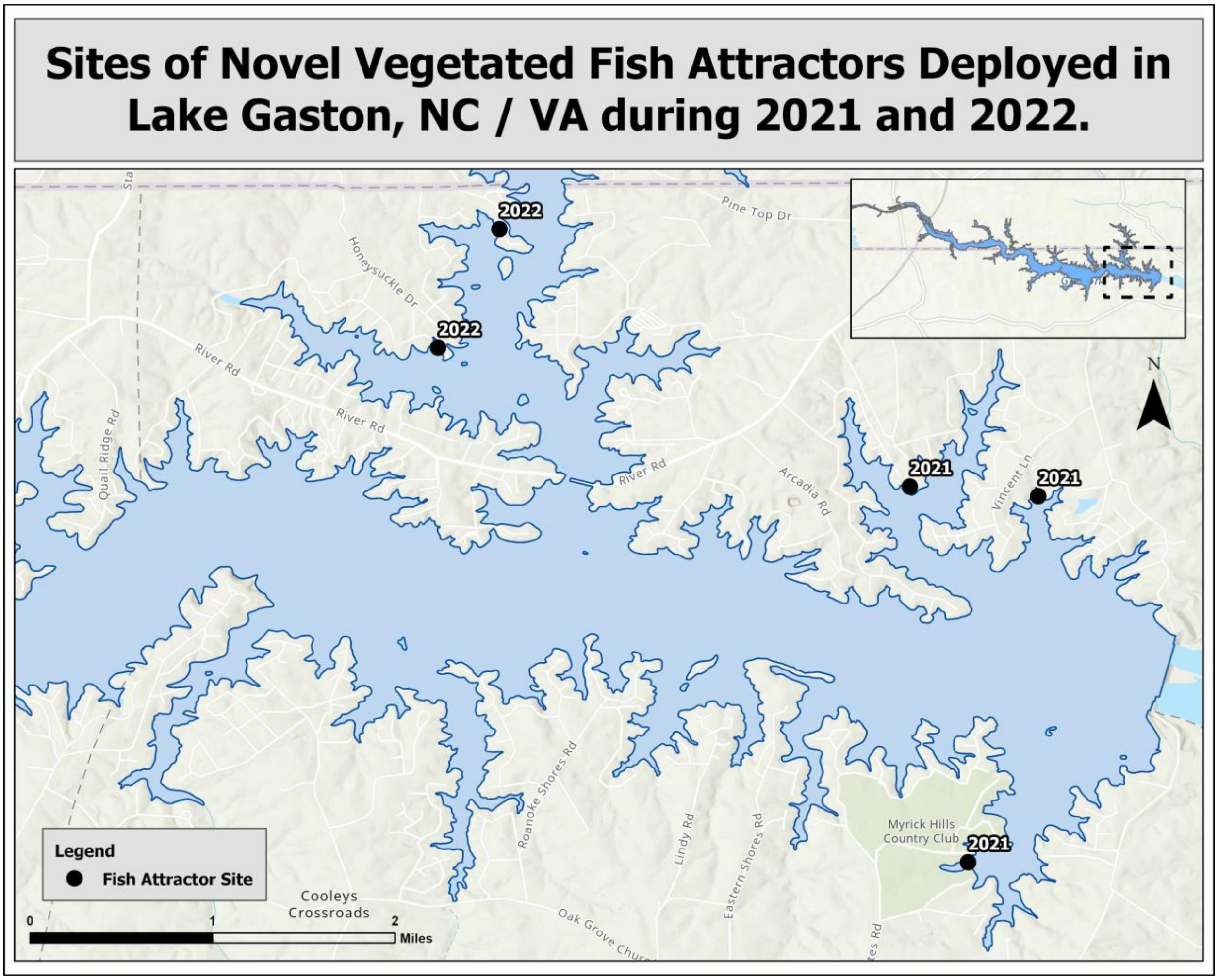

Figure 3.2.1. Map showing site locations of five novel vegetated fish attractors deployed in Lake Gaston during 2021 and 2022. Year of deployment is indicated at each site location.

Figure 3.2.2. Artificial fish attractors deployed into Lake Gaston during 2021 and 2022 as part of the novel vegetated fish attractor habitat enhancement design. These structure are commonly referred to as Georgia structure fish attractors.

Figure 3.2.3. Cage cube structures deployed into Lake Gaston during 2021 and 2022 as part of the novel vegetated fish attractor habitat enhancement design. Each cube housed multiple clumps of native eel grass that were individually planted within a soil filled burlap sack.

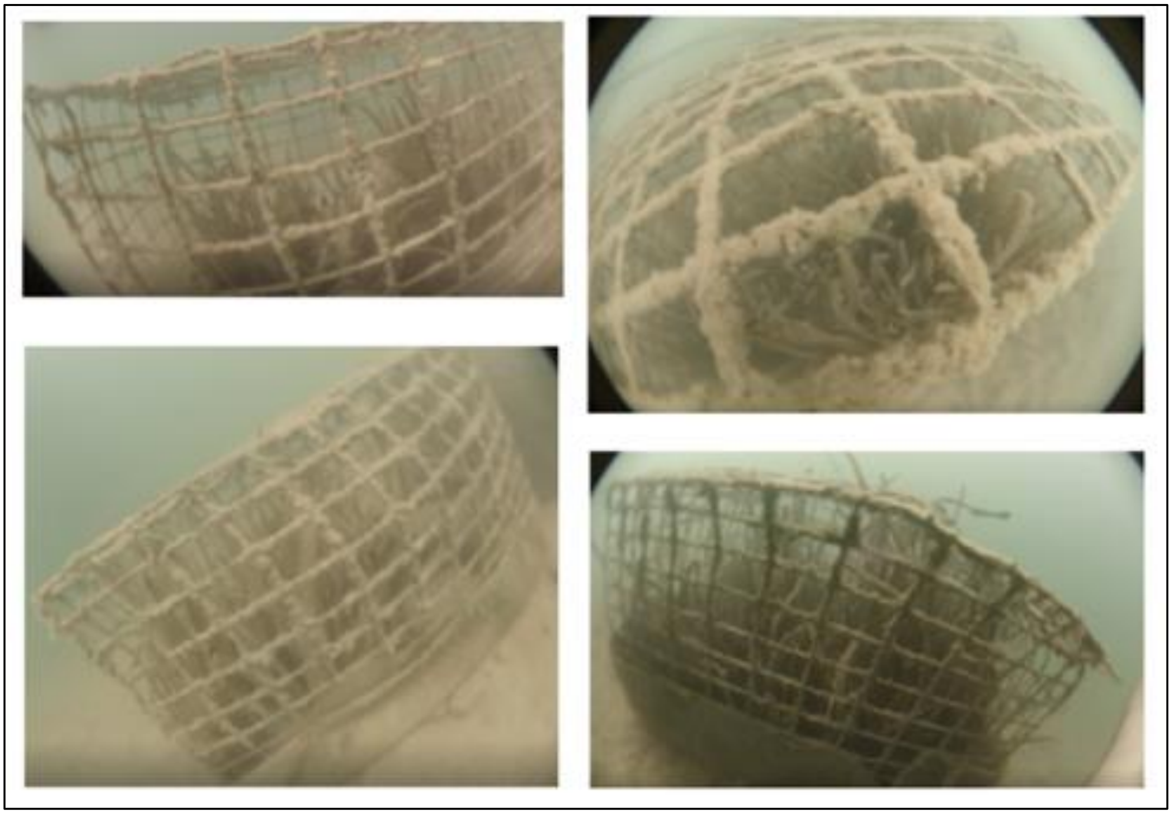

Figure 3.2.4. GoPro images showing examples of established submersed vegetation cage cubes at Philpott Reservoir (VA).

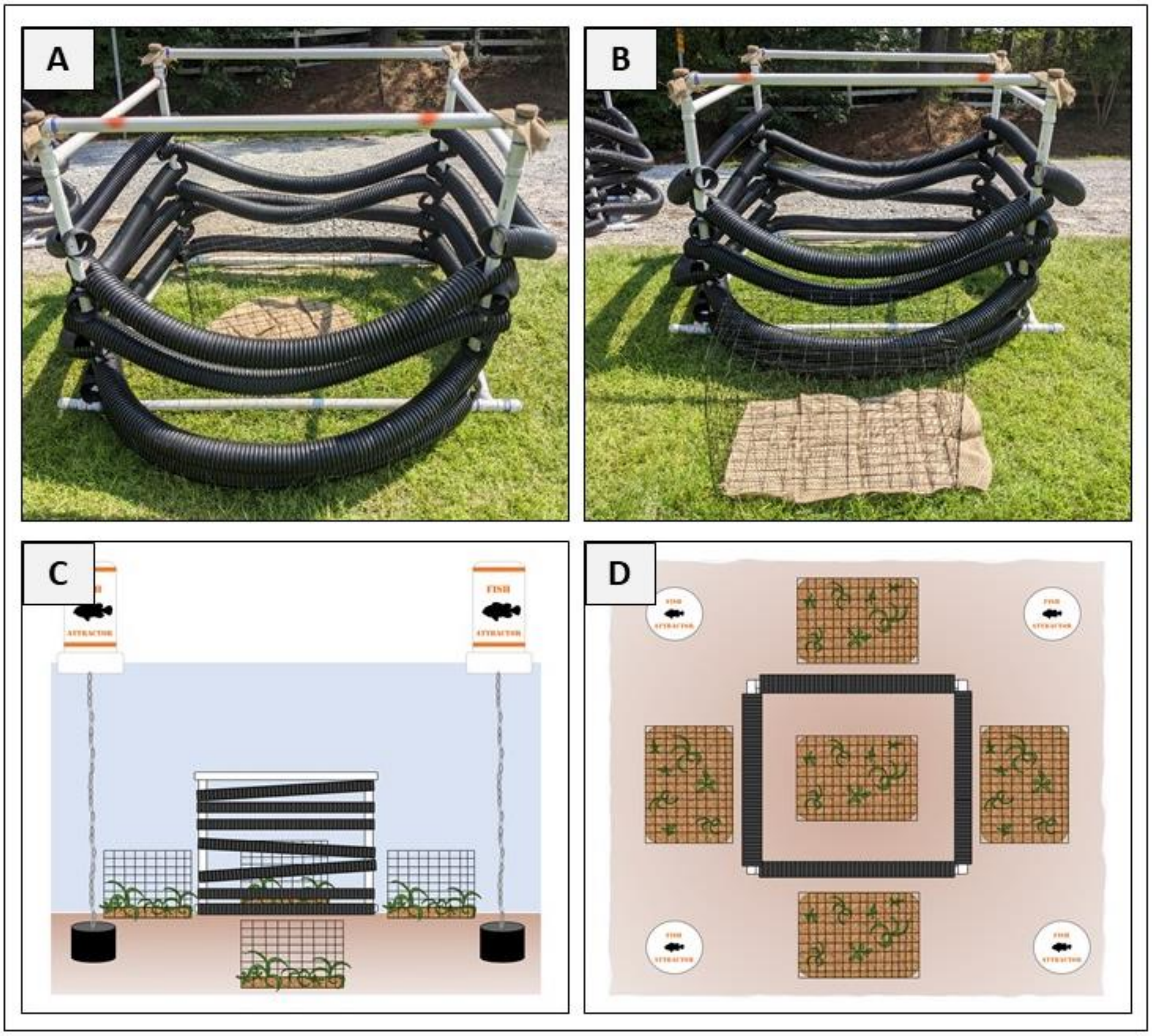

Figure 3.2.3. Images of the novel vegetated fish attractor habitat enhancement design deployed in Lake Gaston during 2021 and 2022. The top images show cube placement A) within and B) outside of the fish attractor. The bottom images show conceptual designs of the entire enhancement site from a C) side view and D) surface view.

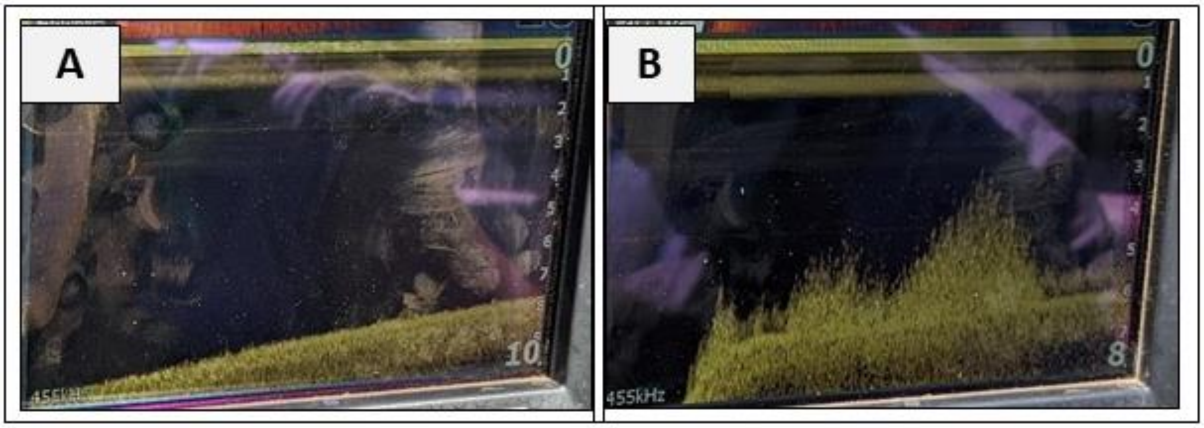

Figure 3.2.6. Sonar images of enhancement sites where novel integrated fish structures and submersed vegetation cubes were deployed in Lake Gaston during 2021. Images show varying levels of growth from A) no growth visibly detected to B) a plethora of eel grass growth visible.

4.1 Integrated Hydrilla Management

The goal of this research was to determine the effectiveness of ProcellaCOR (florpyrauxifen-benzyl) on hydrilla management in both mesocosm and field trial scenarios. The following report is adapted from a poster presented by Ph.D student Jen Beets at the National Meeting of the Aquatic Plant Management Society (see included copy of poster presentation). Due to the low level of hydrilla acreage currently found in Lake Gaston, field evaluation of ProcellaCOR efficacy was unable to be conducted.

Introduction

Hydrilla (*Hydrilla verticillata* L.f. Royle) is a submersed, rooted aquatic macrophyte native to Asia and was first documented in North Carolina in the 1980s. Since its introduction, many of North Carolina's Piedmont reservoirs have been infested with monoecious hydrilla. Chemical control with herbicides in combination with grass carp stocking is often the most efficacious and selective method for hydrilla control.

American waterwillow (*Justicia americana* [L.] Vahl) is the primary desirable emergent native plant species in most NC Piedmont reservoirs. This is due to the plant's hardy nature including persistent rhizomes and ability to withstand wave energy (Lewis 1980). Waterwillow typically grows in margins and shallow areas of freshwater systems and can spread quickly via fragmentation (Niering and Olmstead 1979).

Lyngbya wollei is a filamentous cyanobacteria that has been spreading rapidly in NC reservoirs and is most problematic due to formation of dense mats. Infestations of hydrilla go back as much as 30 years in some reservoirs whereas nuisance lyngbya mats are a relatively new problem seen only in the last decade. The longevity of hydrilla presence has allowed it to spread throughout these infested reservoirs, including areas previously dominated by waterwillow. As acreages of lyngbya and hydrilla increase in the, the two species are overlapping to a greater extent. Due to this increasing co-occurrence, identification of herbicide/algaecide treatments that can control both species can lead to more cost-effective management. Additionally, management programs are needed that will control hydrilla, while not significantly impacting waterwillow.

Objectives

- Identify hydrilla and lyngbya treatments that will not impact co-existing waterwillow stands
- Determine the efficacy of florpyrauxifen-benzyl on monoecious hydrilla

Methods

- Hydrilla/lyngbya trial run separately from florpyrauxifen-benzyl trial
- Hydrilla tubers collected from stock at NCSU and established for 4 weeks, then planted in 14-L mesocosms with established waterwillow
- 1-week establishment/acclimation period for co-planted waterwillow and hydrilla prior to treatment
- One treatment applied per mesocosm, with 4 replications per treatment
- CET achieved by removing plants from treated water and placing in non-treated mesocosm at target time interval
- All treatments performed via injection into water column
- Aboveground biomass harvested and dried 4 weeks after treatment
- Aboveground biomass analyzed in JMP Pro 14 using Dunnett's test to determine significant differences from non-treated control
- Biomass of florpyrauxifen-benzyl treatments compared to non-treated biomass to obtain % reduction
- Biomass calculations analyzed using ANOVA and Tukey HSD for mean separation

Results

Hydrilla/Lyngbya Treatments

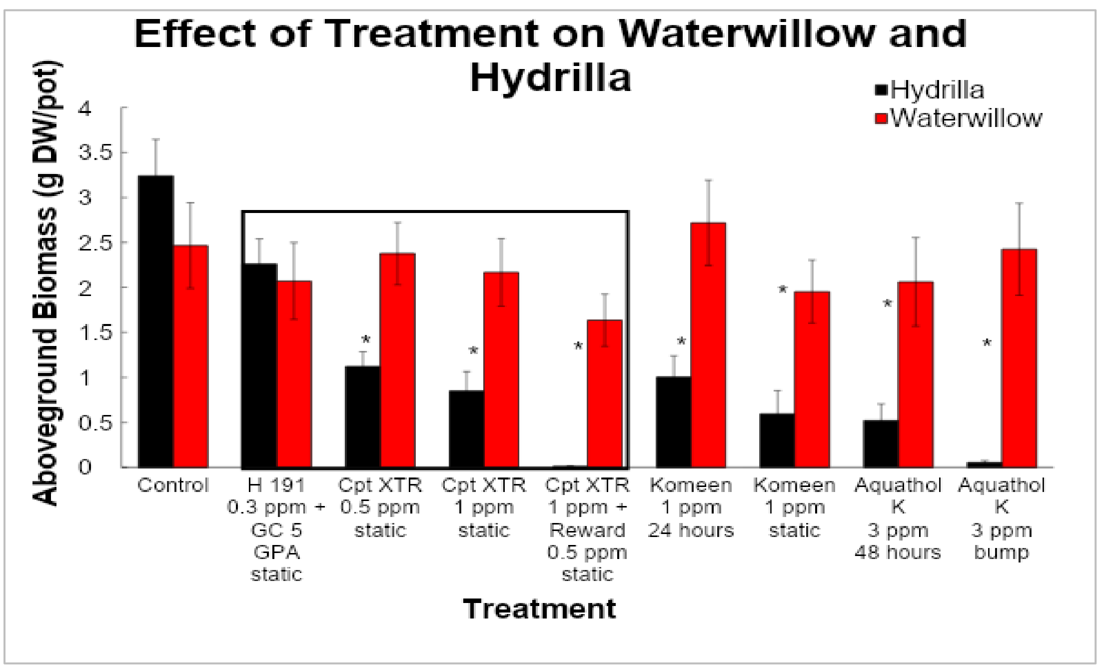

Figure 4.1.1: Mean plant dry biomass (±SE) for hydrilla and waterwillow 4 weeks after treatment in 14-L mesocosms. Two runs were pooled (n=8). Asterisks above bars indicate significance difference from non-treated controls at ($p < 0.001$).

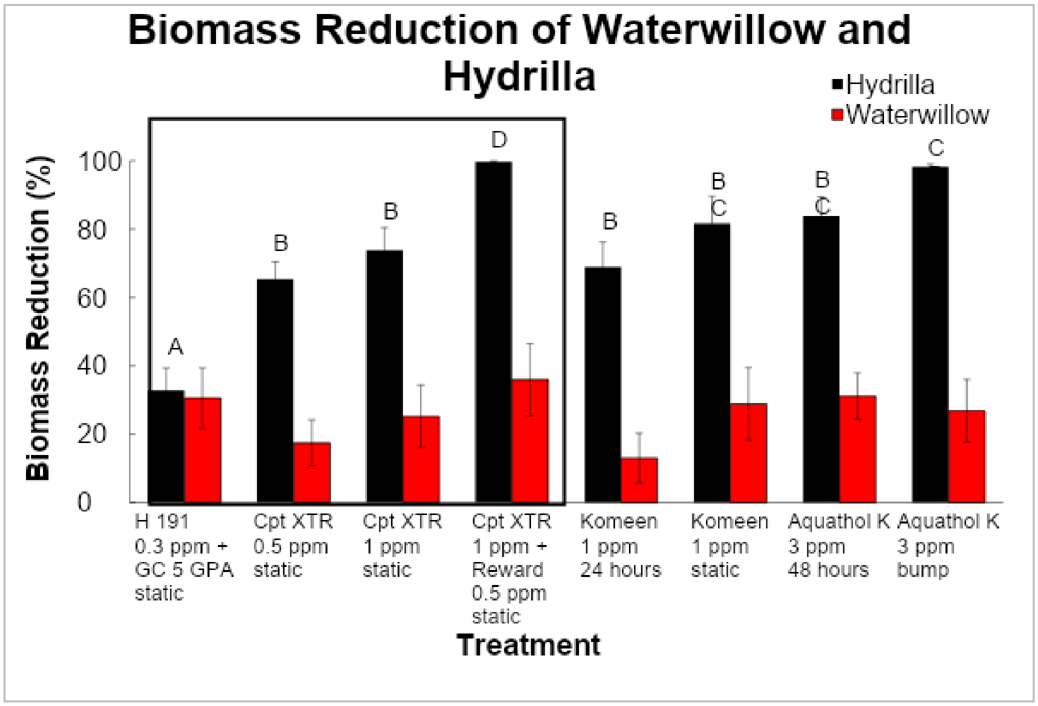

Figure 4.1.2: Mean reduction in dry biomass (±SE) for eight treatments 4 weeks after treatment. Two runs were pooled (n=8). Bars that share the same letter are not significantly different at (p = 0.05).

Florpyrauxifen-benzyl Treatments

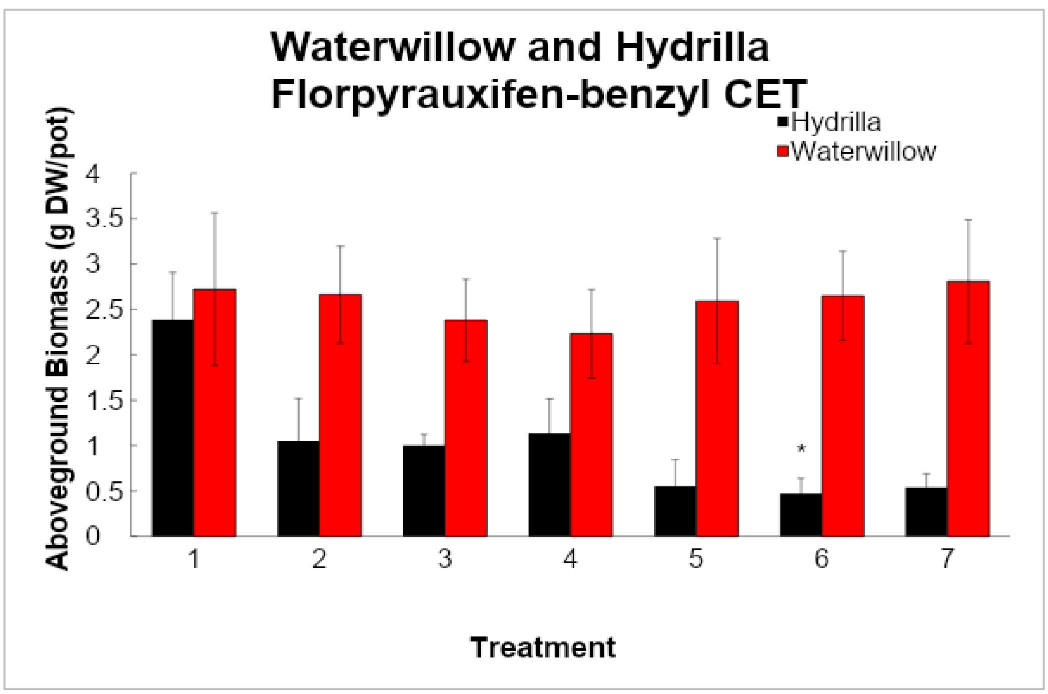

Figure 4.1.3: Mean plant dry biomass (±SE) for hydrilla and waterwillow 4 weeks after treatment in 14-L mesocosms (n=4). Asterisks above bars indicate significance difference from non-treated controls at ($p < 0.05$).

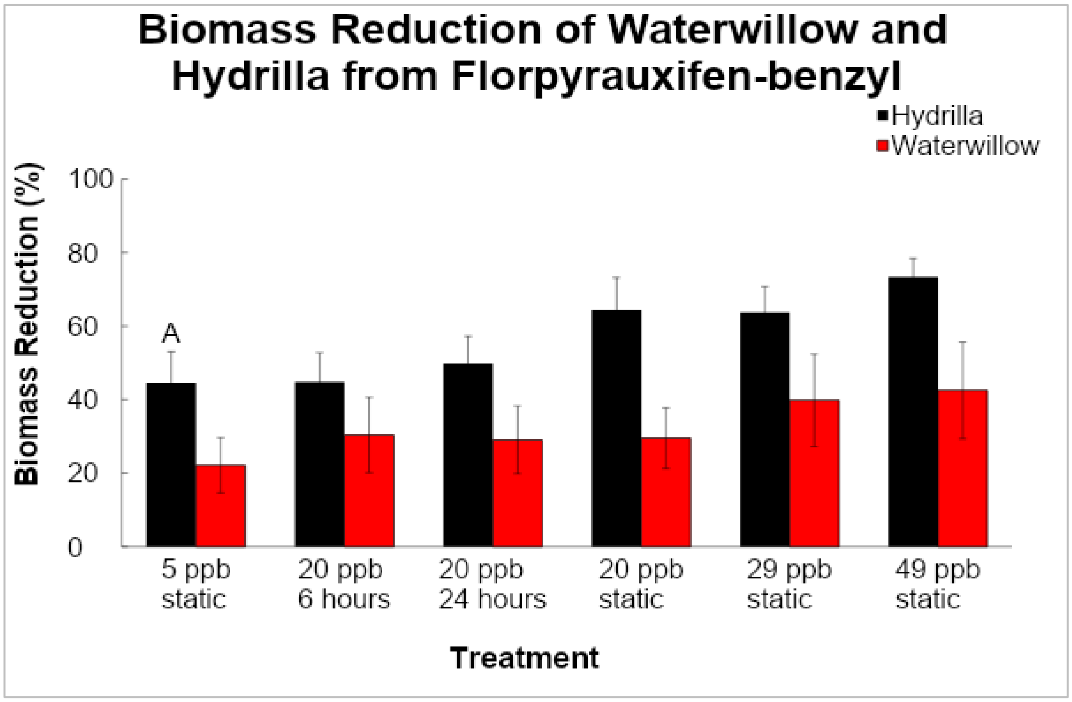

Figure 4.1.4: Mean reduction in dry biomass (±SE) for six CET scenarios 4 weeks after treatment. Three runs were pooled (n=12). Bars that share the same letter are not significantly different at (p = 0.05).

Mesocosm Images

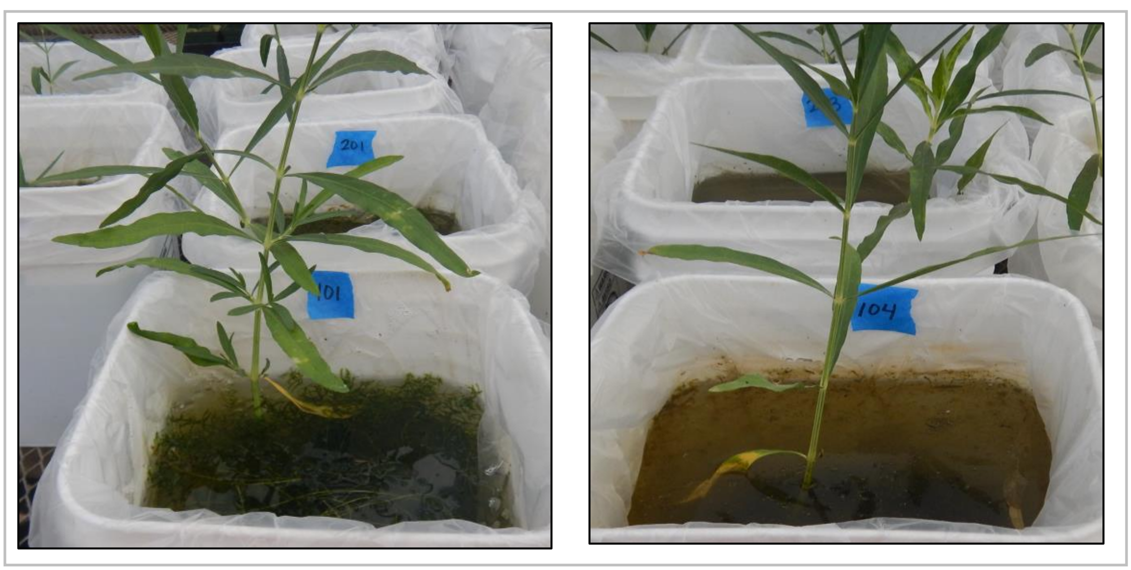

Figure 4.1.5: Non-treated plants (left) compared to plants treated with copper + diquat (right).

Figure 4.1.6: Visual symptomology of florpyrauxifen-benzyl on waterwillow 2 WAT.

Figure 4.1.7: Mesocosm setup 1 WAT with florpyrauxifen-benzyl.

Conclusions

- No treatment resulted in significant impact on waterwillow biomass
- Most treatments had minimal visual effects on waterwillow 4 WAT
  - Florpyrauxifen-benzyl symptomology persisted at least 2 WAT
- Waterwillow growth was greatly variable
- Copper (1ppm static), copper + diquat , and endothall consistently provide significant reductions in monoecious hydrilla biomass
  - Copper (Komeen) required more than 24 hour exposure for consistent efficacy
- 20-49 ppb static doses of florpyrauxifen benzyl suppress monoecious hydrilla
- Possibility of hydrilla and lyngbya management with copper + diquat
- Increased efficacy may be observed in longer trials
  - Other products such as fluridone known to require longer exposure times – fluridone 4 and 8 week data being analyzed
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References

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Acknowledgements

We would like to thank North Carolina Department of Environmental Quality and the Lake Gaston Weed Control Council for providing funding, NCSU for providing facilities, and the rest of the Richardson lab for technical support.

NC STATE UNIVERSITY

Non-Target Impacts of Hydrilla and Lyngbya Treatments on Waterwillow (*Justicia americana*)

Jens Beets[1] , Erika Haug[2], Emily Vulgamore[2], and Robert J. Richardson[2]

[1]North Carolina State University, Department of Fisheries, Wildlife and Conservation Biology; Raleigh NC, jbeets@ncsu.edu

[2]North Carolina State University, Department of Crop & Soil Sciences; Raleigh NC

Introduction

Hydrilla (*Hydrilla verticillata* L.f. Royle) is a submersed, rooted aquatic macrophyte native to Asia and was first documented in North Carolina in the 1980s. Since its introduction, many of North Carolina's Piedmont reservoirs have been infested with monoecious hydrilla. Chemical control with herbicides in combination with grass carp stocking is often the most efficacious and selective method for hydrilla control.

American waterwillow (*Justicia americana* [L.] Vahl) is the primary desirable emergent native plant species in most NC Piedmont reservoirs. This is due to the plant's hardy nature including persistent rhizomes and ability to withstand wave energy (Lewis 1980). Waterwillow typically grows in margins and shallow areas of freshwater systems and can spread quickly via fragmentation (Niering and Olmstead 1979).

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Objectives

- Identify hydrilla and lyngbya treatments that will not impact co-existing waterwillow stands
- Determine the efficacy of florpyrauxifen-benzyl on monoecious hydrilla

Methods

- Hydrilla/lyngbya trial run separately from florpyrauxifen-benzyl trial
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Hydrilla/Lyngbya Treatments

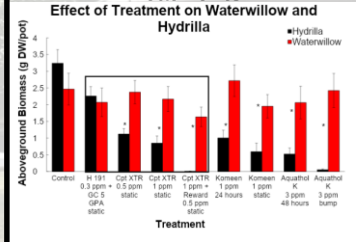

Figure 1: Mean plant dry biomass (±SE) for hydrilla and waterwillow 4 weeks after treatment in 14-L mesocosms. Two runs were pooled (n=8). Asterisks above bars indicate significance difference from non-treated controls at ($p < 0.001$).

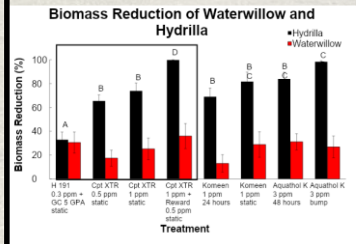

Figure 2: Mean reduction in dry biomass (±SE) for eight treatments 4 weeks after treatment. Two runs were pooled (n=8). Bars that share the same letter are not significantly different at ($p = 0.05$).

Florpyrauxifen-benzyl Treatments

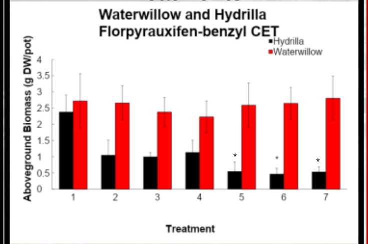

Figure 3: Mean plant dry biomass (±SE) for hydrilla and waterwillow 4 weeks after treatment in 14-L mesocosms (n=4). Asterisks above bars indicate significance difference from non-treated controls at ($p < 0.05$).

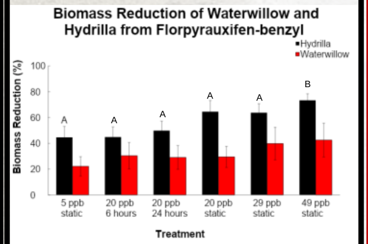

Figure 4: Mean reduction in dry biomass (±SE) for six CET scenarios 4 weeks after treatment. Three runs were pooled (n=12). Bars that share the same letter are not significantly different at ($p = 0.05$).

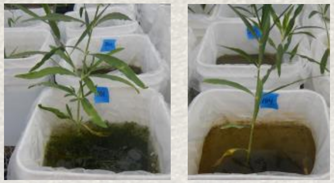

Figure 5: Non-treated plants (left) compared to plants treated with copper + diquat (right).

Figure 6: Visual symptomology of florpyrauxifen-benzyl on waterwillow 2 WAT.

Figure 7: Mesocosm setup 1 WAT with florpyrauxifen-benzyl.

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