NOVEL METHODS FOR DETECTION AND MANAGEMENT OF THE EPIPHYTIC CYANOBACTERIUM (ORDER STIGONEMATALES) ON *HYDRILLA VERTICILLATA*

by

JAMIE RYAN MORGAN

(Under the Direction of Susan Bennett Wilde)

ABSTRACT

Invasive submerged aquatic vegetation (SAV) such as *Hydrilla verticillata* and influxes of excessive nutrients drastically alter the composition of primary producers in aquatic systems, creating unique challenges for managers. Avian vacuolar myelinopathy (AVM) is an often-lethal disease affecting waterbirds and raptors linked to a neurotoxin-producing epiphytic cyanobacterium (Order Stigonematales; stig) that grows primarily on invasive SAV. Managers have difficulty reducing loss of avian wildlife to AVM due to the difficulty of detecting stig in systems prior to an outbreak; additionally, the only current management option is the complete removal of SAV. We developed proximal hyperspectral remote sensing methods to rapidly detect the presence of stig on SAV. Additionally, we evaluated the use of algaecides as a management strategy to reduce the presence of stig. Of the algaecides tested, none proved to be effective at reducing the presence or density of stig on hydrilla leaflets in field or laboratory trials.

INDEX WORDS: Avian Vacuolar Myelinopathy, AVM, *Hydrilla verticillata*, Stigonematales, hyperspectral remote sensing, lake management, wildlife management

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Freshwater ecosystems are among the most diverse and dynamic systems on Earth. Basic factors such as flow, nutrient levels, and physical habitat availability determine species assemblages. These broad factors in turn depend on the combination of more specific factors such as watershed use, amount of anthropogenic influence, weather, flow regulations, and presence of impoundments/reservoirs. The connectivity of all these factors create systems that can easily be altered and most of these alterations degrade natural habitats. Abell et al. (2009) termed six major threats to freshwater species and habitats: habitat degradation, water pollution, flow modification, species invasion, overexploitation, and climate change. For the purpose of this study, we will focus on species invasion and water pollution as the two most influential threats to freshwater ecosystems.

Invasive species are defined as a species introduced outside of its native range that has established a reproducing population causing undesirable effects to the environment, economy, or human health. The intentional introduction of freshwater organisms for food, sport, and aesthetics has occurred throughout history (Keller & Lodge 2009). However, the introduction sites are usually poorly contained and these species invade other areas during storm events, through pet trade, and as aquatic hitchhikers on boats. Aquatic plants are among the most invasive aquatic species as they are prevalent and readily available in the water garden industry, have multiple means of reproduction, and can easily be transported by boat. Once these aquatic plants invade a particular area of a reservoir, they are quickly spread throughout the system via

wind, boaters, and wildlife. Invasive submerged aquatic vegetation (SAV) have detrimental economical and ecological effects on aquatic systems as they can hinder flow and navigability, decrease property values, lower dissolved oxygen, and alter water clarity and chemistry (Pimentel et al. 2000; Gettys et al. 2009).

Water pollution is generally classified as originating from one of two major types of sources. Point source pollution refers to situations where the pollutant can be traced to a single identifiable source such as a drainage pipe; whereas nonpoint source refers to broader pollution from watershed runoff which contains excess nutrients, pesticides, oil, and any other compound that may degrade habitat (Abell et al. 2009). Nonpoint pollution, primarily influxes of phosphorus and nitrogen, is more common and can be traced back to anthropogenic activities throughout a watershed (Carpenter et al. 1998). Nutrient levels determine a system's trophic state and its assemblage of primary producers. Oligotrophic systems support low phytoplankton assemblages dominated by diatoms and mesotrophic systems support diverse phytoplankton and SAV communities. Although eutrophic systems are able to support a greater abundance of organisms, excess nutrients can become detrimental as overproduction of SAV causes various water quality problems. At the hypereutrophic level, cyanobacteria dominate as primary producers and can produce various toxins in addition to creating anoxic water conditions overnight or when dense blooms senesce (Carmichael 1992).

Excess nutrients coupled with an invasive submerged plant species often create systems choked out by monotypic vegetation, creating ecological issues such as reduced species diversity, increased detritus buildup, and poor water quality–mainly a reduction in dissolved oxygen and dramatic fluctuations of pH in poorly buffered systems (Gettys et al. 2009). Dense SAV infestations also reduce flow and promote conditions favored by mosquito larvae.

Cyanobacteria, particularly epiphytic species found on SAV, benefit from copious available substrate (Cattaneo et al. 1998). The literature lacks substantial evidence of the composition and issues associated with epiphytic communities found on invasive SAV as it is relatively new in many systems; however, researchers have discovered novel consequences of invasive SAV as it has become more consistent in established systems and continues to become more prevalent.

A previously undescribed cyanobacterium, order Stigonematales (stig), grows epiphytically on SAV and field and laboratory evidence indicate that it produces a neurotoxin linked to a neurologic disease primarily affecting waterbirds and their avian predators (Wilde et al. 2005). Avian vacuolar myelinopathy (AVM) was first documented during the winters of 1994-1995 after high mortalities of bald eagles (*Haliaeetus leucocephalus*) and American coots (*Fulica americana*) occurred on DeGray Lake, Arkansas (Thomas et al. 1998). This neurologic disease, confirmed by the vacuolation of white matter of the brain observed during necropsies, has been documented in Southeastern reservoirs from Texas to North Carolina during fall and winter (Thomas et al. 1998; Rocke et al. 2002; Fischer et al. 2006). Each reservoir affected by AVM supports dense SAV populations, including the federal noxious weeds Brazilian elodea (*Egeria densa*), Eurasian watermilfoil (*Myriophyllium spicatum*), and hydrilla (*Hydrilla verticillata*) (Wilde et al. 2005; Wilde et al. 2013). Examination of epiphytic algal assemblages found on the SAV of AVM-positive reservoirs revealed the neurotoxic-cyanobacteria stig was present at all sites (Wilde et al. 2005).

The AVM-neurotoxin enters the food web as waterfowl ingest leaflets of SAV containing colonies of stig (Birrenkott et al. 2004; Wiley et al. 2007; Haynie 2008). Clinical signs of affected birds include ataxia, difficulty flying and swimming, general uncoordination, and death, making them easy prey for avian predators (Thomas et al. 1998). The AVM toxin moves further

up the food chain as a secondary intoxication occurs in predatory raptors after consumption of infected prey (Fischer et al. 2003). Laboratory feeding trials indicate that transfer is caused by the raptors' consumption of the gastrointestinal contents of the waterfowl that contain stig-positive hydrilla and not through the bioaccumulation of toxins within the flesh of waterfowl (Lewis-Weis et al. 2004). Over 150 documented bald eagle mortalities and thousands of American coot mortalities have been documented since the discovery of AVM in 1994. These numbers are likely underestimated, as birds have to be recovered within days of mortality for diagnosis prior to the breakdown of tissues.

In all previous cases, the presence of AVM and stig in a reservoir has become apparent only after a high mortality event. Currently, the only method of detecting stig in a reservoir prior to an AVM outbreak is through the examination of SAV with an epifluorescent microscope. Colonies of stig appear bright red under fluorescent illumination using a rhodamine filter set and can be identified from other epiphytic species of algae and cyanobacteria. The identification process is slow and expensive, as it requires that SAV samples be harvested from the field and taken back to the laboratory for analysis by a trained phycologist. This complex methodology limits the investigation of stig to a few local sites per year. Even thorough investigations of known AVM reservoirs with the current method do not fully represent the prevalence of stig as researchers are limited to the small sample size of leaflets collected within hectares of SAV. Additionally, calculation of prevalence and density of stig is only a semi-quantitative visual estimation. The ability to macroscopically identify stig (or other toxin-producing epiphytic cyanobacteria) on SAV would allow for rapid, precise, early detection of stig in a reservoir prior to an outbreak of AVM.

Remote sensing techniques offer rapid, relatively easy, and inexpensive collection of large-scale environmental data (Tueller 1982; Everitt et al. 1992; 2012). However, the application of remote sensing is often limited by its spatial resolution and coarse bandwidths (Turner et al. 2003). Hyperspectral remote sensing is capable of collecting multi-channel reflectance data of a target, be it vegetation, water, soil, throughout the electromagnetic spectrum at resolutions <1 nm. Such fine scale resolution in and beyond the visible spectrum provides the ability to discern specific spectral attributes associated with the reflectance of targets; for example, vegetation spectra are easily discerned from those of freshwater or barren land. Furthermore, combinations of these specific attributes are used to develop spectral profiles of targets and allow accurate predictions of the species of the target based solely on its spectrum. These techniques are more developed for terrestrial targets, as reflectance values are lower in aquatic targets due to the high absorption of water. Limited research has been conducted on the hyperspectral remote sensing of phytoplankton and SAV, although researchers have developed methods to distinguish different species of SAV from one another based on reflectance data (Blanco et al. 2012; Everitt et al. 1999; 2000; 2007; 2012). More complex uses of hyperspectral remote sensing in the SAV community have yet to be fully investigated.

The few studies on hyperspectral sensing of aquatic autotrophs have looked at phytoplankton and SAV independently, but no one has investigated the possibility of remotely detecting epiphytic cyanobacteria found on SAV. Unlike plants and green algae, whose primary photosynthetic pigments are chlorophylls, cyanobacteria contain a distinctive pigment known as phycocyanin. Phycocyanin absorption at 620 nm ($Absorbance_{max}$ = 620 nm) can be exploited to spectrally distinguish cyanobacteria from green algae and plants and could potentially be used to remotely detect stig on SAV (Richardson 1996). Researchers have been fairly successful in their

ability to detect and estimate cyanobacteria levels in water through the use of the phycocyanin absorption maxima at 620 nm (Dekker 1993; Schalles & Yacobi 2000; Simis et al. 2005). However, these estimates are often skewed by the intrusion of the chlorophyll absorption (A_{max}= 675 nm). Band ratios of reflectance values outside of the interference area between 620-675 nm, namely reflectance at 600 and 700 nm, can more accurately estimate cyanobacteria values through phycocyanin values (Mishra et al. 2009). It is possible that these algorithms used to predict densities of planktonic cyanobacteria can accurately detect and predict densities of stig on SAV. Rapid macroscopic detection and quantification of stig would allow managers to tailor treatment plans to target areas of SAV identified as "stig hotspots" and prevent loss of valuable wildlife.

The only current management strategy used to reduce the occurrence of AVM is the removal of the SAV substrate on which stig is found in the year following an outbreak (Haynie et al. 2013). Reduction of problematic SAV has been achieved through the use of herbivorous fish such as grass carp (*Ctenopharyngodon idella*), mechanical removal, and herbicides (Gettys et al. 2009). However, there are ecological and economical disadvantages associated with each of these strategies (Madsen 2000). Grass carp are an effective long-term management option for some species of SAV but the stocking of non-native fish species can be controversial and some states only permit the stocking of sterile-triploid fish. Mechanical removal of SAV is often inefficient as it is resource intensive and can potentially increase the range of SAV in a system due to the ability of many species of SAV to reproduce through fragmentation. Herbicides offer effective acute and long-term management, but are often expensive. In addition, although all aquatic herbicides are approved and regulated by the Environmental Protection Agency, legacy effects of the widespread use of chemicals have yet to be determined. Regardless of method,

complete removal of SAV is rarely achieved as multiple applications are required to maintain reduction and may not always be an option for managers due to budgets or stakeholder constraints.

Some managers may feel that the complete removal of SAV is unwarranted, as anglers and hunters of waterfowl often do not support full removal of SAV as it provides useful habitat for fish and wildlife (Henderson et al. 2003). In the summer of 2012, high densities of stig were found growing on native Southern naiad (*Najas guadalupensis*) collected from a central Georgia wildlife refuge (Wilde, unpublished data). Although naiad is often non-problematic, it is structurally similar to hydrilla and provides excellent substrate for epiphytic cyanobacteria. Managers valued the native SAV of the property for its attraction to migratory birds, while efforts were made to eliminate invasive SAV. Removal of all SAV from ponds was deemed unwarranted as it would decrease the use of the property by waterfowl. Thus, the need for new AVM-management options beyond complete removal of SAV exists for both economic and ecological reasons.

There is dire need for more AVM-management options beyond complete removal of SAV for both economic and ecological reasons. Treatments designed to reduce densities of stig on SAV or prevent the release of its toxins would allow managers to reduce the occurrence of AVM mortalities on reservoirs where removal of SAV is not an option. The use of chemical algaecides, namely copper compounds, to effectively treat problematic planktonic and mat-forming cyanobacteria is a widely accepted management strategy (Gettys et al. 2009; Jancula et al. 2011). However, the effectiveness of current approved algaecides against stig has not been examined. Although some algaecides, like herbicides, require repeated applications, they are often effective as acute control options as they are designed attack the cells directly. Treatment

of the source of toxins, stig itself, through algaecides could offer an effective acute management option. Reduction in stig through treatment in late fall, prior to the arrival of migratory waterfowl, could reduce the presence of AVM-toxins; therefore, greatly reducing the number of sick birds while avoiding the removal of valuable resources.

Goal & Objectives

The overall goal of this study was to develop methods to better detect, quantify, and manage stig found on SAV of reservoirs. The study objectives are listed below:

1. Determine the plausibility of using proximal hyperspectral remote sensing to detect epiphytic cyanobacteria of SAV.
2. Evaluate the efficacy of three algaecides at reducing stig when applied to plots of hydrilla in an AVM-positive reservoir.
3. Evaluate the efficacy of three algaecides at reducing moderate to high densities of stig on individual hydrilla leaflets from an AVM-positive reservoir.

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CHAPTER 2

DETECTION AND QUANTIFICATION OF EPIPHYTIC CYANOBACTERIA ON *HYDRILLA VERTICILLATA* BY PROXIMAL HYPERSPECTRAL REMOTE SENSING[1]

[1] Morgan, J.M., B. Bartelme, S.B. Wilde, D. Mishra. To be submitted to *Lake and Reservoir Management*

Abstract

Avian vacuolar myelinopathy (AVM) is an often-lethal disease affecting waterbirds and raptors and is linked to a neurotoxin-producing epiphytic cyanobacterium (Order Stigonematales; stig). Stig is primarily found epiphytically on the leaflets of *Hydrilla verticillata* and other structurally similar species of submerged aquatic vegetation (SAV). In all previous cases, the presence of AVM and stig in a reservoir becomes apparent only after a high mortality event. Currently, the only method of detecting stig in a reservoir prior to an AVM outbreak is through the examination of SAV with an epifluorescent microscope, as colonies of stig are easily discerned from other epiphytic cyanobacteria and algae under fluorescent illumination with a rhodamine filter. Remote sensing techniques offer rapid, relatively easy, and inexpensive collection of large-scale environmental data. Proximal hyperspectral remote sensing is capable of collecting high-resolution (< 1 nm) reflectance data, providing the ability to discern spectral attributes associated with targets such as the presence and quantity of photosynthetic pigments. We provide the framework on hyperspectral remote sensing techniques as a novel method for remote, macroscopic detection of stig on SAV through the exploitation of phycocyanin's absorbance max at 620 nm.

Introduction

Avian Vacuolar Myelinopathy (AVM) is a neurologic disease that primarily affects waterbirds and their avian predators on reservoirs throughout the southern United States. The disease was formally described in 1994 on DeGray Lake, Arkansas when 29 bald eagles (*Haliaeetus leucocephalus*) were found dead or dying. Affected birds have difficulty flying, swimming, and are generally uncoordinated, making them easy prey for avian predators (Thomas et al. 1998). Only one consistent finding was noted during necropsies of affected birds: unique

lesion presenting as diffuse, spongy degeneration of the white matter of the central nervous system (Thomas et al. 1998). This disease has been documented only in Southern reservoirs from Texas to North Carolina during fall and winter (Rocke et al. 2002; Fischer et al. 2006). Each reservoir affected by AVM supports dense submerged aquatic vegetation (SAV) populations, including the federal noxious weeds Brazilian elodea (*Egeria densa*), Eurasian watermilfoil (*Myriophyllium spicatum*), and hydrilla (*Hydrilla verticillata*) (Wilde et al. 2005; Wilde et al. 2013).

A previously undescribed cyanobacterium, order Stigonematales (stig), grows epiphytically on SAV and is believed to produce a neurotoxin that causes AVM (Wilde et al. 2005). Movement of the AVM toxin through this aquatic-based food web has been well documented. Laboratory and field studies showed that disease transmission is dietary (Rocke et al. 2002; 2005). Waterbirds ingest the toxin when feeding on aquatic plants with stig and become symptomatic within 5 days (Birrenkott et al. 2004; Wiley et al. 2007). Predatory birds become affected after consuming moribund or dead waterbirds from AVM reservoirs containing stig-positive hydrilla (Fischer et al. 2003; Wilde et al. 2005). There have been over 150 documented bald eagle and thousands of American coot mortalities since the discovery of AVM in 1994. These numbers are likely underestimates, as birds have to be recovered within 48 hours of death for diagnosis prior to the breakdown of tissues.

In all previous cases, the presence of AVM and stig in a reservoir has become apparent only after a high mortality event. Currently, the only method of detecting stig in a reservoir prior to an AVM outbreak is through the examination of SAV with an epifluorescent microscope. Colonies of stig appear bright red under fluorescent illumination using a rhodamine filter set and can be identified from other epiphytic species of algae and cyanobacteria (Figure 1.1). The

identification process is slow and expensive, as it requires that SAV samples be harvested from the field and taken back to the laboratory for analysis by a trained phycologist. This complex methodology limits the investigation of stig to a few local sites per year. Even thorough investigations of known AVM reservoirs with the current method do not fully represent the prevalence of stig as researchers are limited to the small sample size of leaflets collected within hectares of SAV. Additionally, calculation of prevalence and density of stig is only a semi-quantitative visual estimation. The ability to macroscopically identify stig (or other toxin-producing epiphytic cyanobacteria) on SAV would allow for rapid, precise, early detection of stig in a reservoir prior to an outbreak of AVM.

This complex methodology limits the investigation of stig to a few local sites per year. Even thorough investigations of known AVM reservoirs with the current method do not fully represent the prevalence of stig as researchers are limited to the small sample size of leaflets collected within hectares of SAV. Additionally, calculation of prevalence and density of stig is only a semi-quantitative visual estimation. The ability to macroscopically identify stig (or other toxin-producing epiphytic cyanobacteria) on SAV would allow for rapid, precise, early detection of stig in a reservoir prior to an outbreak of AVM.

Remote sensing techniques offer rapid, relatively easy, and inexpensive collection of large-scale environmental data (Tueller 1982; Everitt et al. 1992; 2012). However, the application of remote sensing is often limited by its spatial resolution and coarse bandwidths (Turner et al. 2003). Hyperspectral remote sensing is capable of collecting multi-channel reflectance data of a target, be it vegetation, water, soil, throughout the electromagnetic spectrum at resolutions <1 nm. Such fine scale resolution in and beyond the visible spectrum provides the ability to discern specific spectral attributes associated with the reflectance of targets; for

example, vegetation spectra are easily discerned from those of freshwater or barren land. Furthermore, combinations of these specific attributes are used to develop spectral profiles of targets and allow accurate predictions of the species of the target based solely on its spectrum. These techniques are more developed for terrestrial targets, as reflectance values are lower in aquatic targets due to the high absorption of water. Limited research has been conducted on the hyperspectral remote sensing of phytoplankton and SAV, although researchers have developed methods to distinguish different species of SAV from one another based on reflectance data (Blanco et al. 2012; Everitt et al. 1999; 2000; 2007; 2012). More complex uses of hyperspectral remote sensing in the SAV community have yet to be fully investigated.

The few studies on hyperspectral sensing of aquatic autotrophs have looked at phytoplankton and SAV independently, but no one has investigated the possibility of remotely detecting epiphytic cyanobacteria found on SAV. Unlike plants and green algae, whose primary photosynthetic pigments are chlorophylls, cyanobacteria contain a distinctive pigment known as phycocyanin. Phycocyanin absorption at 620 nm ($Absorbance_{max}$ = 620 nm) can be exploited to spectrally distinguish cyanobacteria from green algae and plants and could potentially be used to remotely detect stig on SAV (Richardson 1996). Researchers have been fairly successful in their ability to detect and estimate cyanobacteria levels in water through the use of the phycocyanin absorption maxima at 620 nm (Dekker 1993; Schalles & Yacobi 2000; Simis et al. 2005). However, these estimates are often skewed by the intrusion of the chlorophyll absorption (A_{max}= 675 nm). Band ratios of reflectance values outside of the interference area between 620-675 nm, namely reflectance at 600 and 700 nm, can more accurately estimate cyanobacteria values through phycocyanin values (Mishra et al. 2009). It is possible that these algorithms used to

predict densities of planktonic cyanobacteria can accurately detect and predict densities of stig on SAV.

The overall goal of this study was to explore the plausibility of using proximal hyperspectral remote sensing techniques to detect epiphytic cyanobacteria (ultimately stig) on SAV and develop the framework for a novel method for remote, macroscopic detection of AVM in reservoirs. The objectives of this study were to 1) assess the advantages and disadvantages of available sensors and 2) develop a field collection protocol for hyperspectral remote sensing data of hydrilla in AVM positive reservoirs.

Materials & Methods

Study Sites

We collected hydrilla from 12 sites at Lake Tohopekaliga, FL (April 2013) and 30 sites at Long Branch Reservoir, GA (September and November 2013). Lake Tohopekaliga (Toho) is a 7,612 ha natural freshwater lake located in Osceola County, Florida. Typical of many Floridian lakes, Toho is shallow with an average depth of 2.2 m and supports a variety of aquatic vegetation but hydrilla is most prominent. The warm Floridian climate allows SAV to grow throughout the year. During the summer of 2012, colonies of the neurotoxin-producing stig were identified on hydrilla at Toho and toxicity was confirmed in a chicken feeding trial during 2012 (Wilde lab, unpublished data). The second reservoir, Long Branch, is a 112 ha man-made water supply reservoir located southeast of Atlanta, GA (Henry County) in the Ocmulgee River Basin. Managers observed isolated SAV in Long Branch during 2008, but the SAV was not identified as hydrilla until October 2010. Southeastern Cooperative Wildlife Disease Study (SCWDS) pathologists confirmed AVM in a bald eagle recovered from the adjacent Upper Towaliga

Reservoir in 2010 and the disease has since been diagnosed in wild birds annually (SCWDS unpublished data).

Equipment & Data Collection

There were two field portable hyperspectral remote sensors available for use in the lab at the time of this study: the JAZ (Ocean Optics Inc., Dunedin, FL) and the GER 1500 (Spectra Vista Corp., Poughkeepsie, NY). The JAZ unit was operated from a field laptop and collects reflectance data via a submersible fiber optic cable (Figure 1.2). The GER operates more like a traditional camera as data is collected and stored internally with the push of a button on the top of the unit (Figure 1.3). Both units required calibration by scanning a lambertian fluoropolymer reflective panel that reflects ~99% of down-welling sunlight prior to data collection. One calibration was sufficient for a day's sampling as long as cloud coverage was consistent; recalibration was conducted if changes occurred in cloud coverage. Use of the JAZ's submersible fiber optic cable allowed hydrilla to be scanned from above the water, below the water, after harvest in the field and another in a dark room; the GER is not submersible and data was collected only of the water and harvested hydrilla (Table 1.1). The GER also required a single reference scan of the sky immediately following a scan set of a target to collect real time irradiance as a means of correcting the data for any changes in cloud coverage; this allows the data to be presented as remote sensing reflectance.

Sampling sites at each reservoir were chosen haphazardly and the boat was anchored upon arrival to minimize variation in the area sampled. A set of three replicate scans was collected of hydrilla from above the water. If data were collected with the JAZ, another scan set of the same area was collected with the sensor under the water surface. The patch of hydrilla was harvested from the lake by a rake, placed on a black plastic lid, and scanned on the boat. After all

the field scans had been collected, the hydrilla was placed in a labeled plastic bag and stored on ice for transport back to the laboratory. All of the samples collected from Lake Toho were scanned on the black plastic lid under two 500-watt halogen light bulbs in a dark room with the JAZ for comparison to the data collected in the field; the dark room and halogen light bulbs provide a controlled environment for a more accurate reading of reflectance and often provide clearer spectra. Reflectance data was smoothed with an algorithm and plotted as typical spectra in Microsoft Excel 2011.

Stig presence and density (% stig coverage) was estimated for each site by traditional epifluorescence microscopy. Five to ten leaflets were haphazardly selected from each sample bag of harvested hydrilla, mounted on glass slides, and viewed at 100X under epifluorescent illumination (Nikon Eclipse Ti, Nikon Corp., Melville, NY). All estimates were conducted by the same user to reduce variability. The average density calculated for the leaflets was assumed to be representative of the sample scanned and was assigned as its stig density.

Results and Discussion

Spectral data collected by the JAZ and GER were similar but data collection and processing is much more simplified with the GER. The most notable difference was the ability to operate the GER without a laptop, as field conditions (sun and heat) caused overheating and it was difficult to maintain laptop battery life. We concluded that the GER holds the most potential for future development of this detection tool and its application in the field of aquatic plant management. The simple point and shoot collection method of the GER allows data to be collected with limited experience and data downloaded from the GER (.acii files) can be easily imported and analyzed with common spreadsheet software such as Microsoft Excel.

Qualitative visual assessment of the reflectance spectra of hydrilla revealed variation among the four scanning perspectives collected with the JAZ (Figure 1.4). Reflectance spectra collected in the field were more variable than those collected in the controlled laboratory dark room. Although the spectra were less rigid, the phycocyanin absorption of interest was not more apparent when reflectance data was collected in a controlled environment. We have concluded scanning harvested hydrilla on the boat will not provide any less information than in the controlled laboratory, and is more applicable to the goal of developing a field detection method. Reflectance spectra were lowest for scans collected below the water surface as the water absorbed much of the available light; scanning hydrilla underwater did not produce reflectance data of use for this particular study. The absorptions of phycocyanin (~ 620 nm) and chlorophyll-a (~675 nm) were detectable from above the water but the magnitude of their absorptions changed with the height of hydrilla in the water column (Figure 1.5). Traditional SAV spectra, such as those reported by Everitt et al. (2012), were apparent in the scans of the water collected above the surface. Spectra of sites with hydrilla at the surface are comparable to those of harvested hydrilla. As the depth of water between the surface and hydrilla bed increased, the spectra shifted to what is traditionally seen for water.

Although photosynthetic pigments were identified in spectra collected of the submerged and harvested hydrilla, their absorptions were more easily identified in the spectra of harvested hydrilla. Additionally, the harvest of the hydrilla eliminates the possibility that phycocyanin from ambient planktonic cyanobacteria may appear in the spectra. Large densities of planktonic cyanobacteria typically do not occur in areas of dense SAV, but we did not evaluate the phytoplankton assemblage of the water at our sample sites. Future studies should examine and account for the potential of high levels of phycocyanin within the water column. For the purpose

of this study, strong phycocyanin absorptions around 620 nm were assumed to be from the presence of stig on hydrilla. The spectra of hydrilla vacant of stig will lack this absorption and remain relatively flat between 600-675 nm; similar to the hydrilla spectra presented in Everitt et al. (2012). Additionally, a "false" reflectance peak around 650 nm occurs as a result of the absorption on both sides, phycocyanin at 620 nm and chlorophyll at 675 nm.

Differences between the two sampling dates are apparent as the spectra from samples collected on 5 November 2013 have an overall lower reflectance than those collected on 26 September 2013 (Figure 1.6) This is likely due to the difference in growth states of hydrilla as it is more healthy and bright green in the beginning of fall and begins to turn darker (more brown) as it traps sediment and begins to senesce in the late fall. The absorption of both phycocyanin and chlorophyll-a pigments are less defined in the samples collected on 5 November 2013. However, this is typically the time of peak stig densities and toxicity as stig seems to thrive during the senescence of hydrilla. We hypothesize that stig absorbs the newly available nutrients released its degrading hydrilla substrate, causing it to thrive and peak just after the highest point of hydrilla growth. Stig has not been shown to occur without its SAV substrate, however, and densities decline with the hydrilla biomass (Figure 1.7).

Microscopy revealed a variety of stig densities in the samples collected from Long Branch (Table 1.2). The majority of samples (12 of 30) were identified to contain a low density of 2 – 14% stig coverage; only six samples contained high densities of stig and only two samples contained < 2% stig coverage (Table 1.2). Visual analysis of spectra based on stig classification did not reveal any apparent correlations between stig density and phycocyanin absorption (Figure 1.8). It is possible that our calculation of stig densities were not representative of the entire area scanned. The examination of all hydrilla biomass contributing to the reflectance data is needed

for an accurate calculation of the amount of stig within the sample. The subjective measure of stig density through visual assessment provides dubious measures of stig density at best; however attempts to reduce variation were addressed by having the same person evaluate all samples. A more quantitative method of measuring stig density is required before attempts can be made at estimating stig density through hyperspectral sensing. Computer programs capable of photographic analysis through pixel counts could be used to consistently and objectively measure the stig coverage. Additionally, future studies should investigate the phycocyanin levels of stig-positive hydrilla determined through quantitative methodology such as high-performance liquid chromatography (HPLC).

Considering all of the information gathered from our initial attempts of detecting stig on SAV, we propose the following protocol be used for future data collection. Future data should be collected on hydrilla above the water and after harvest on a black surface at the boat with a GER 1500. Three scans should be collected at each perspective followed by a reference scan of the sky as previously described. Hydrilla of known AVM reservoirs such as Long Branch should be surveyed at multiple predetermined GPS locations in a single day trip to reduce any temporal or down welling light variations associated with multiple sampling days. Data should only be collected on clear, sunny days with minimal cloud coverage to provide useful spectra for analysis. Sampling should begin in mid October just as hydrilla reaches peak biomass to provide higher stig densities without the interference of lower pigment values associated with senesced hydrilla. Sites should be revisited on a weekly basis, weather permitting, until the hydrilla loses the majority of its leaflets and begins to sink in late November. Reflectance data should be processed and smoothed in Microsoft Excel. Stig density of 15-20 leaflets of each sample should be calculated using traditional microscopy methods and phycocyanin should be quantified by

HPLC. Phytoplankton assemblages at each of the sites should be examined for planktonic cyanobacteria to determine potential sources of phycocyanin.

This study marks the first attempt to detect epiphytic cyanobacteria on SAV with hyperspectral remote sensing techniques. We have begun the process of developing a tool to macroscopically detect stig, and in turn, AVM in reservoirs. The use of this protocol in future studies will lead to the development of an accurate means to detect stig on SAV. A more confident quantification of stig densities would allow algorithms and predictive models such as those used to estimate planktonic cyanobacterial densities to be applied for potential estimations of stig densities (Dekker 1993; Schalles & Yacobi 2000; Simis et al. 2005; Mishra et al. 2009). The presence of stig and its association with AVM presents a unique challenge to managers of southeastern reservoirs. A more accessible means of detection would allow for a more widespread investigation of the occurrence of stig and AVM, potentially exposing the existence of the disease in reservoirs outside of the current range. Additionally, early detection of stig would allow managers to tailor treatment plans to target areas of SAV identified as "stig hotspots" and prevent loss of valuable wildlife.

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Table 1.1: Description of the four scanning perspectives and equipment used to collect spectral data for the detection of epiphytic cyanobacteria on hydrilla at Lake Tohopekaliga, Florida and Long Branch Reservoir, Georgia.

Scanning Perspective	Equipment	Target	Distance From Target (cm)	Approximate Scan Diameter (cm)
Above water	JAZ, GER 1500	Water	50	22
Below water	JAZ	Hydrilla	15	7
Vegetation	JAZ, GER 1500	Hydrilla	15	7
Lab	JAZ	Hydrilla	15	7

Table 1.2: Results of microscopy analysis of presence and average densities of the epiphytic cyanobacterium in Order Stigonematales (stig) area coverage of hydrilla collected from the Long Branch reservoir in Georgia.

Sample	Date (2013)	Stig Coverage (%)	Stig Classification*
1	26-Sep	40	High
2	26-Sep	38	Medium
3	26-Sep	20	Medium
4	26-Sep	6	Low
5	26-Sep	17	Medium
6	26-Sep	26	Medium
7	26-Sep	3	Low
8	26-Sep	25	Medium
9	26-Sep	3	Low
10	26-Sep	14	Low
11	26-Sep	29	Medium
12	26-Sep	6	Low
13	26-Sep	52	High
14	26-Sep	43	High
15	26-Sep	27	Medium
16	5-Nov	9	Low
17	5-Nov	17	Medium
18	5-Nov	3	Low
19	5-Nov	50	High
20	5-Nov	6	Low
21	5-Nov	25	Medium
22	5-Nov	40	High
23	5-Nov	32	Medium
24	5-Nov	55	High
25	5-Nov	3	Low
26	5-Nov	11	Low
27	5-Nov	3	Low
28	5-Nov	9	Low
29	5-Nov	1	None
30	5-Nov	1	None

*High – averages >40%; Medium – 15-39%; Low – 2-14%; None – <2%.

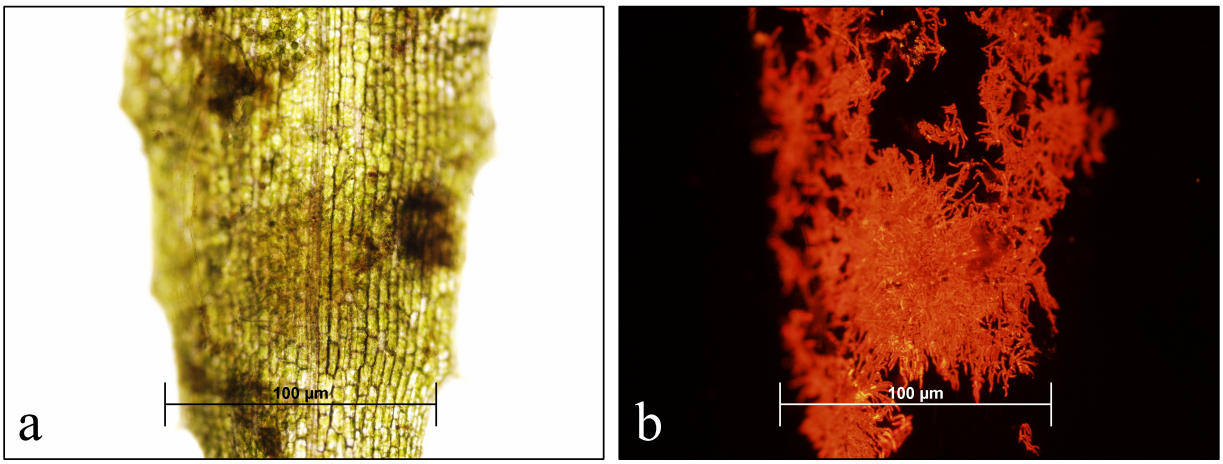

Figure 1.1: (a) A *Hydrilla verticillata* leaflet containing the epiphytic cyanobacterium in Order Stigonematales (stig) under light microscopy. (b) Colonies of stig appear bright orange under epifluorescence with a rhodamine red filter.

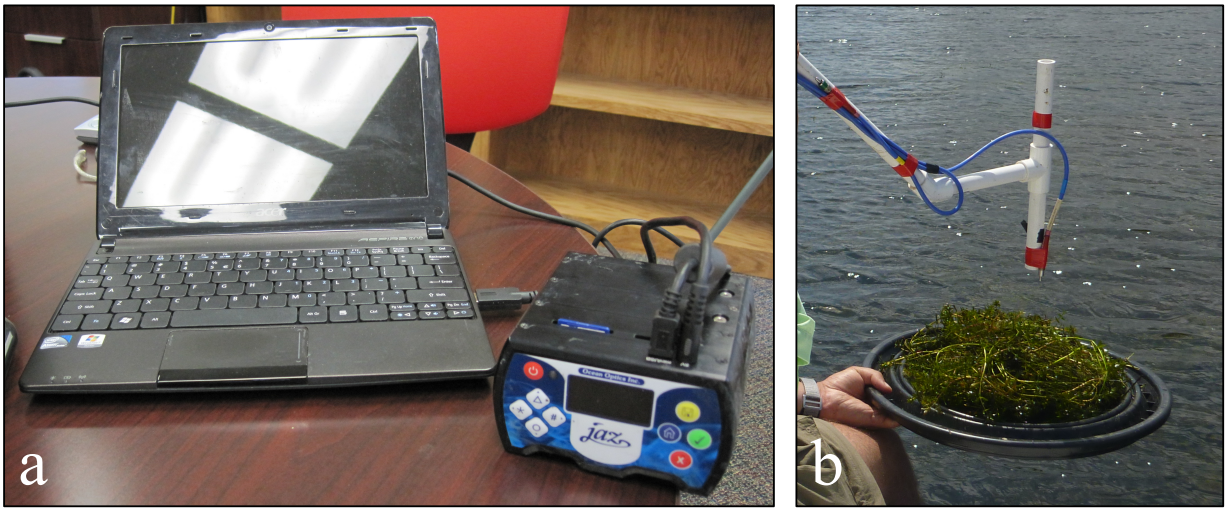

Figure 1.2: (a) Ocean Optics JAZ spectrophotometer connected to a field laptop. (b) Collection of the reflectance values of harvested hydrilla with the JAZ's submersible fiber optic sensor and frame.

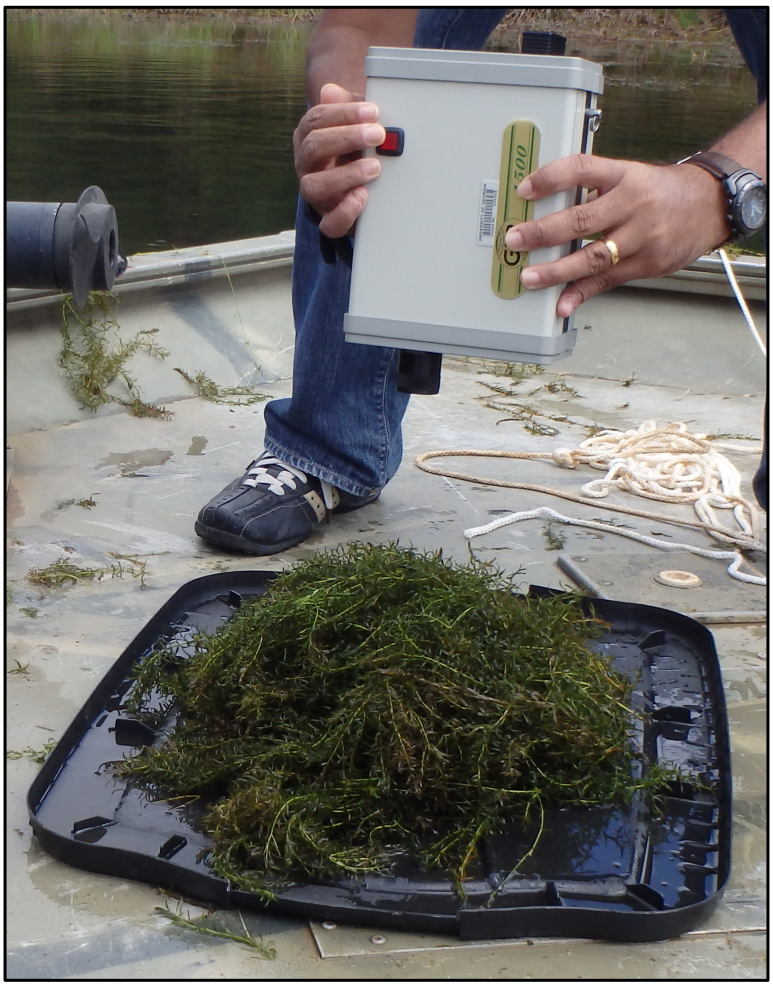

Figure 1.3: Collection of the reflectance values of harvested hydrilla with the Spectra Vista Corporation GER 1500.

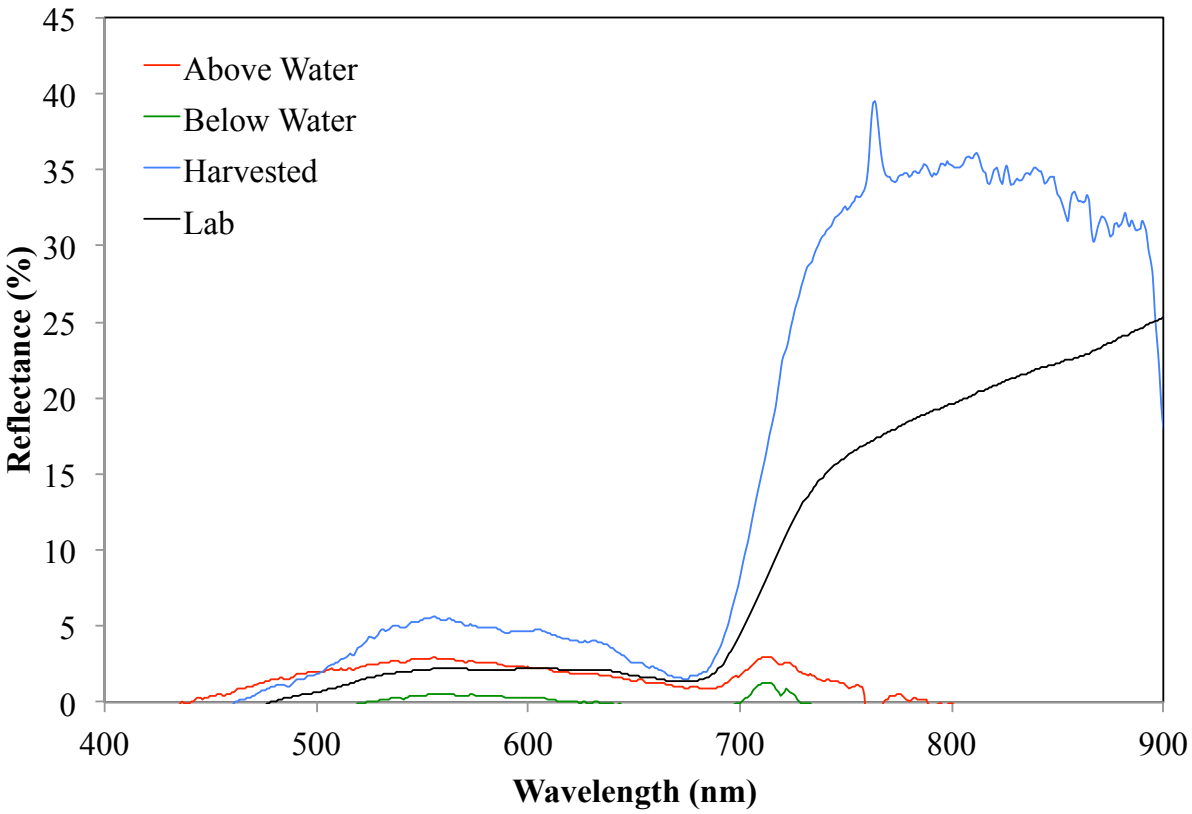

Figure 1.4: General variations between average reflectance values of hydrilla at all Lake Tohopekaliga, FL (18 April 2013) sites collected with the four scanning perspectives of the Ocean Optics JAZ. Above and below water scans were collected of submerged vegetation; vegetation and lab scans were of harvested hydrilla.

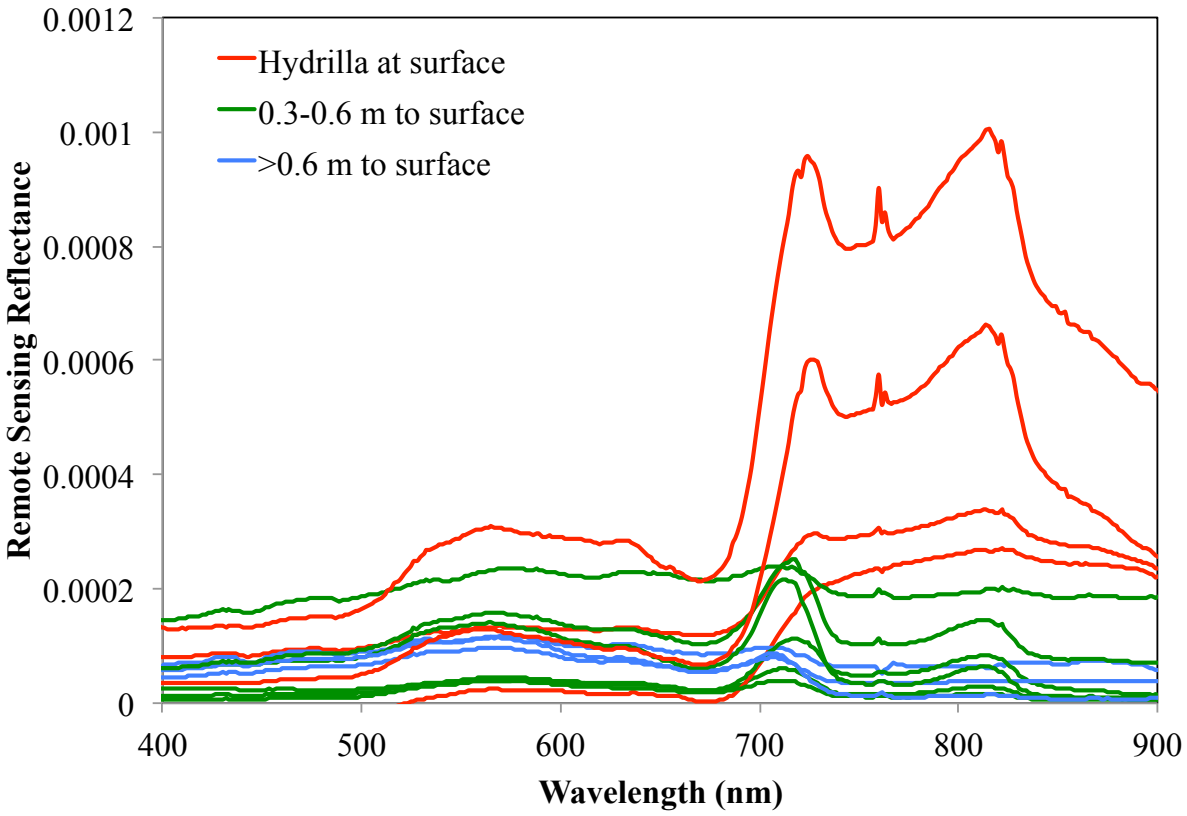

Figure 1.5: Remote sensing reflectance spectra of 15 hydrilla sites (each line represents an individual sample) at Long Branch reservoir, GA collected on 26 September 2013 by the SVC GER 1500. The data are categorized by height of hydrilla beds in regards to the total water column to demonstrate that a traditional vegetation spectrum is seen at sites where hydrilla has reached the surface.

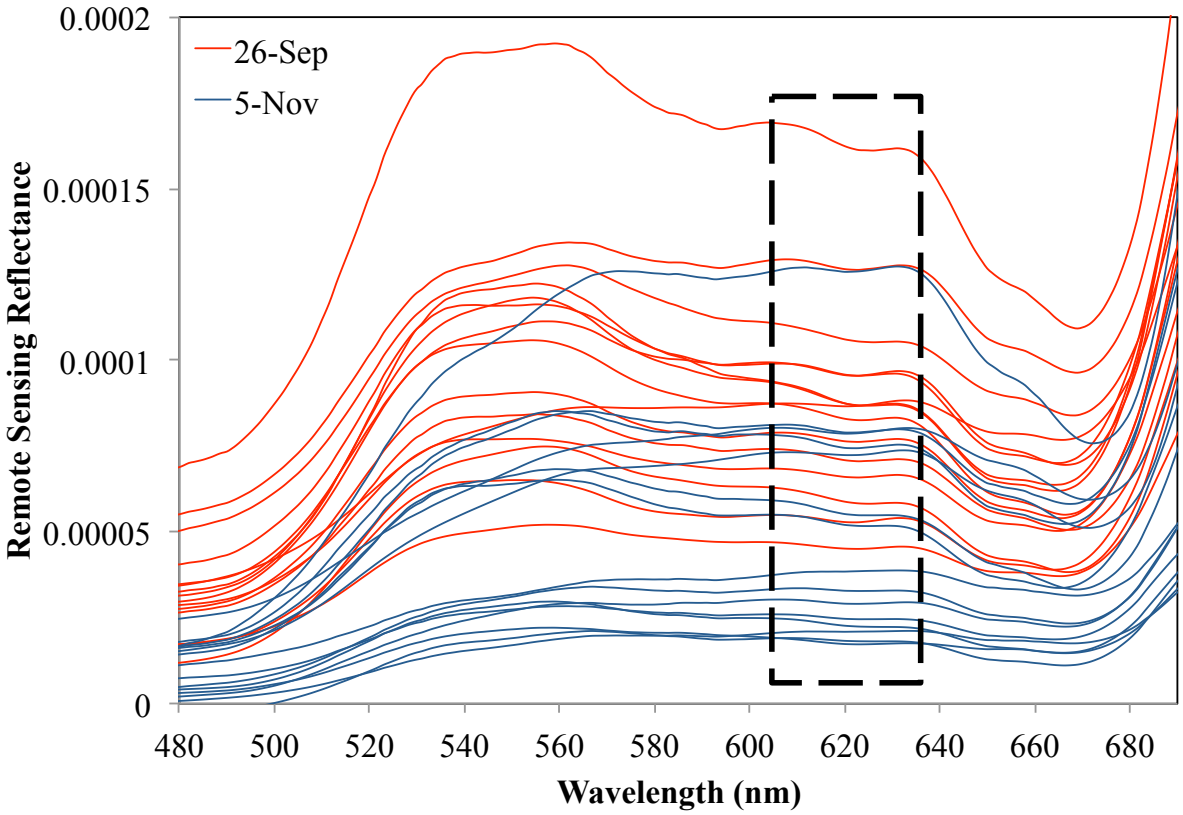

Figure 1.6: Remote sensing reflectance spectra of 30 harvested hydrilla samples (each line represents an individual sample) at Long Branch reservoir, GA collected on 26 September 2013 and 5 November 2013 by the SVC GER 1500. The data are categorized by sampling date to demonstrate temporal differences in the spectra. The 620 nm absorption of phycocyanin (outlined by the dotted line) is more distinct in samples collected on 26 September 2013.

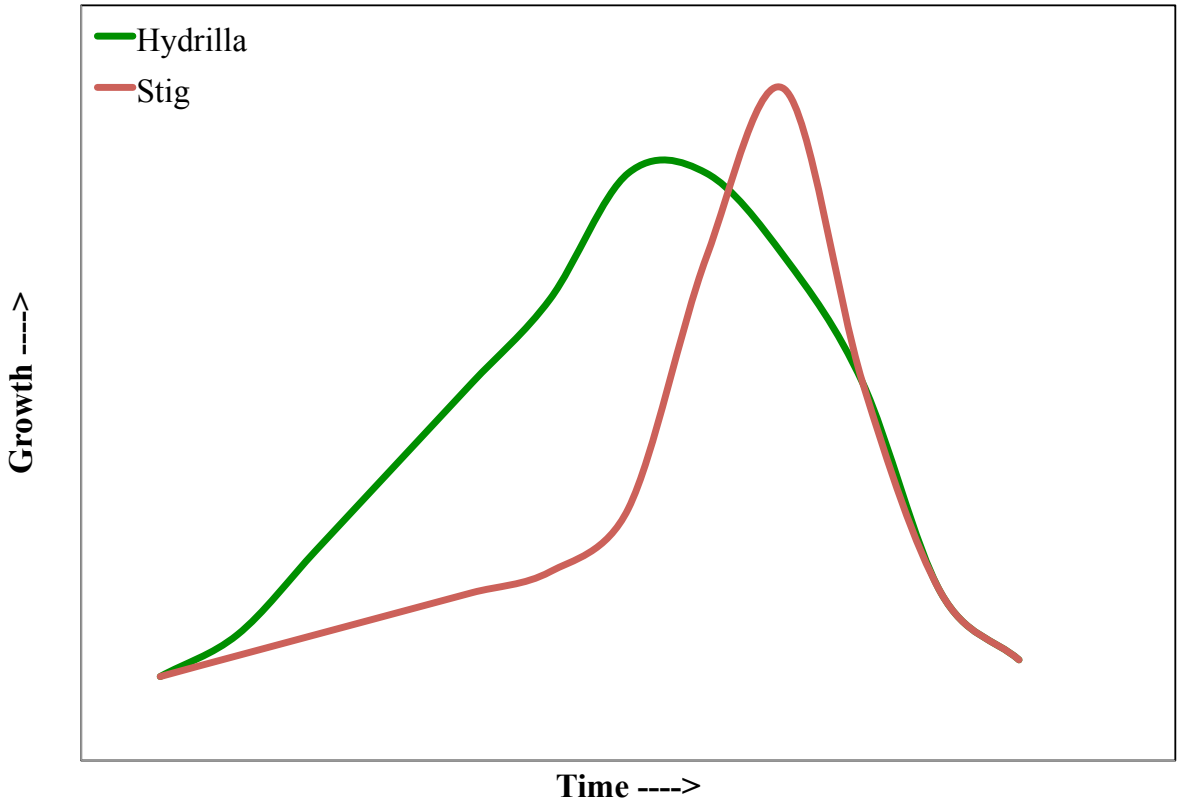

Figure 1.7: Proposed conceptual model of the epiphytic cyanobacterium in Order Stigonematales (stig) and hydrilla growth over time. Densities of stig remain low as hydrilla grows throughout the summer and begin to increase rapidly as hydrilla begins to senesce; however, as stig has not been documented free in the water column, both densities decline rapidly as the hydrilla sinks down the water column.

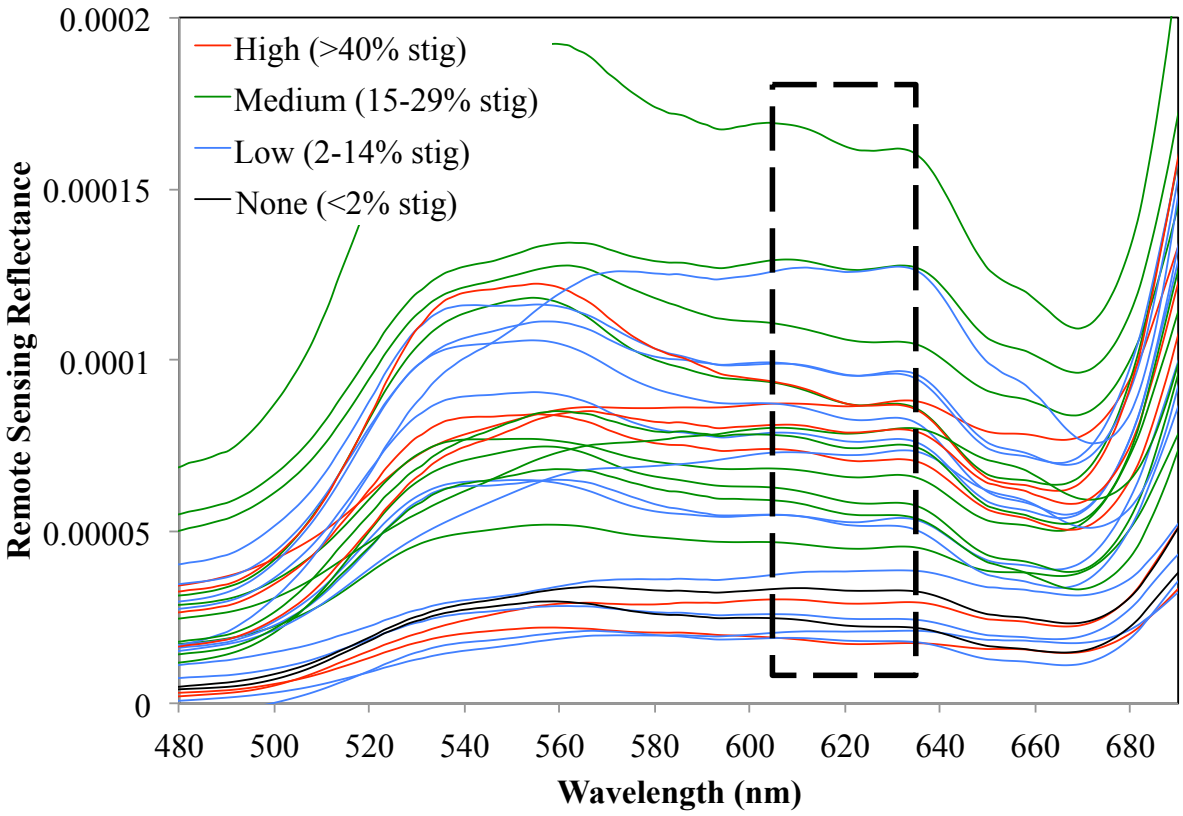

Figure 1.8: Remote sensing reflectance spectra of 30 harvested hydrilla samples (each line represents an individual sample) at Long Branch reservoir, GA collected on 26 September 2013 and 5 November 2013 by the SVC GER 1500. The data are categorized by the average density of the epiphytic cyanobacterium in Order Stigonematales (stig) on 10 hydrilla leaflets collected from the sample identified by microscopy. There was not a visual correlation between the magnitude of phycocyanin's 620 nm absorption (outlined by the dotted line) and the density of stig identified from the sample.

CHAPTER 3

EFFICACY OF THREE ALGAECIDES ON A NOVEL EPIPHYTIC CYANOBACTERIUM (ORDER STIGONEMATALES) ASSOCIATED WITH AVIAN VACUOLAR MYELINOPATHY[2]

[2] Morgan, J.R., R.S. Haynie, W.M. Bishop, S.B. Wilde. To be submitted to *Journal of Aquatic Plant Management*

Abstract

Avian vacuolar myelinopathy (AVM), an often lethal disease affecting waterbirds and raptors, has been linked to an epiphytic cyanobacterium (Order Stigonematales; stig) which grows primarily on nonindigenous submerged aquatic vegetation (SAV). The putative toxin produced by this species remains uncharacterized and human health risks have not been fully evaluated. Controlling SAV that provides substrate for stig is currently the only management against AVM. Directly targeting stig is a potential alternative to aggressive SAV control. We conducted field and laboratory trials to evaluate algae management strategies for systems affected or potentially affected by AVM. In a field study conducted in a reservoir, replicated plots of *Hydrilla verticillata* with the toxin producing stig in a Piedmont GA reservoir were treated with one of three commercial algaecides, Captain XTR™ (liquid copper ethanolamine complex), SeClear™ (liquid copper sulfate pentahydrate), and Pak27™ (sodium carbonate peroxyhydrate), at the maximum rate for planktonic algae. Hydrilla leaflets from untreated areas of this reservoir were collected and treated individually with the same algaecides in a laboratory trial. In the field, the only significant difference ($p=0.0422$) between treatments occurred at 0 days after treatment, prior to application of algaecides; no significant difference was found between treatments at any time point after application. In the laboratory, the same analysis failed to show a significant treatment effect ($p=0.1711$) on the percent area leaf coverage of stig. The application of Captain XTR, SeClear, and Pak27 at maximum rate for planktonic algae was not effective at reducing the presence of stig on hydrilla. Targeted algaecide treatments may not be a viable alternative to aggressive SAV control when selecting an effective management tool for aquatic systems affected by AVM.

Introduction

A previously undescribed cyanobacterium, order Stigonematales (hereafter, stig), grows epiphytically on submerged aquatic vegetation (SAV). Field and laboratory evidence indicate that it produces a toxin linked to a neurologic disease primarily affecting waterbirds and their avian predators (Wilde et al. 2005). Avian vacuolar myelinopathy (AVM) was first documented during the winters of 1994-1995 after high mortalities of bald eagles (*Haliaeetus leucocephalus*) and American coots (*Fulica americana*) occurred on DeGray Lake, Arkansas (Thomas et al. 1998). Researchers found no association with toxicants or infectious agents (Rocke et al. 2005; Larsen et al. 2003). Necropsies of affected birds revealed diffuse, spongy degeneration of the white matter of the central nervous system (Thomas et al. 1998). This disease has been documented only in Southern reservoirs from Texas to North Carolina during fall and winter (Rocke et al. 2002; Fischer et al. 2006). Each reservoir affected by AVM supports dense SAV, including the federal noxious weeds Brazilian elodea (*Egeria densa*), Eurasian watermilfoil (*Myriophyllium spicatum*), and hydrilla (*Hydrilla verticillata*) (Wilde et al. 2005; Wilde et al. 2013). Examination of epiphytic algal assemblages in SAV samples from affected reservoirs revealed that stig was prevalent at all sites. The cyanobacterial colonies on the surface of the plant leaflets are conspicuous when viewed under epifluorescent illumination with a rhodamine filter set (Wilde et al. 2005).

The AVM-neurotoxin enters the food web as waterfowl ingest leaflets of SAV containing colonies of stig (Birrenkott et al. 2004; Wiley et al. 2007; Haynie et al. 2013). Clinical signs include ataxia, difficulty flying and swimming, general lack of coordination, and death; making affected birds easy prey for avian predators (Thomas et al. 1998). Secondary intoxication occurs in predatory raptors after consumption of affected prey (Fischer et al. 2003). Laboratory feeding

trials indicate that disease transfer is caused by the raptors' consumption of the gastrointestinal contents of the waterfowl that contain stig-positive hydrilla and not through the bioaccumulation of toxins within the flesh of waterfowl (Lewis-Weis et al. 2004).

Over 150 documented bald eagle mortalities and thousands of American coot mortalities have been documented since the discovery of AVM in 1994 (Thomas et al. 1998; Fischer et al. 2006; Augspurger 2008). However, these numbers are likely not a true reflection of mortalities associated with AVM because histology is the sole diagnostic method and birds must be recovered and tissues processed within 48 hours post mortem. This sample collection and processing timeline is improbable in the rural heavily wooded areas surrounding many Southeastern reservoirs. To promote the continued recovery of the bald eagle and ensure wild avifauna health, a management strategy is needed to reduce the prevalence of AVM associated with Southeastern reservoirs. Controlling nonnative SAV, which provides substrate for the toxin-producing stig, may restrict wild avifauna exposure and therefore reduce disease prevalence. Sites affected by AVM that have implemented aggressive SAV control using chemical and biological methods have seen a reduction in disease prevalence (Wilde lab, unpublished data). This suggests that the putative toxin is either absent or no longer bioavailable in the absence of vegetative substrate.

Effective management of problematic SAV includes biological, mechanical, and chemical strategies tailored for target species and specific management goals (Gettys et al. 2009). However, complete removal of SAV is rarely achieved and may not always be an option for managers due to budgets or stakeholder opinions. Anglers and waterfowl hunters often do not support full removal of SAV, as it is perceived as useful fish and wildlife habitat (Henderson et al. 2003). Treatments designed to reduce densities of stig on SAV or prevent the release of its

toxins would allow managers to reduce the occurrence of AVM mortalities on reservoirs where complete removal of SAV is not an option. The use of chemical algaecides, namely copper and peroxide compounds, to effectively treat problematic planktonic and mat-forming cyanobacteria is a widely accepted and cost-efficient management strategy (Gettys et al. 2009; Jancula et al. 2011). Effectiveness of current approved algaecides against stig, however, has not been examined. Reducing the number of stig colonies on SAV in late fall, prior to the arrival of migratory waterfowl, would theoretically result in a reduction in the putative toxin. This scenario would also leave the SAV intact, which provides a food source for the waterfowl and habitat for other aquatic species.

The goal of this study was to evaluate three algaecides, Captain XTR™, Pak27™, and SeClear™ against stig colonies 1) in hydrilla plots within AVM-positive reservoir and 2) on individual hydrilla leaflets from the same reservoir with moderate to high stig densities under controlled laboratory settings.

Materials and Methods

Study Site

Upper Towaliga Reservoir (UTR; 7,404 ha) is a water supply reservoir located southeast of Atlanta, GA (Henry County, 33° 20.834'N 84° 12.882'W) in the Ocmulgee River Basin. It was constructed and filled in the 1990s and, at full pool, is 219.5 m above sea level. Upper Towaliga Reservoir is a typical Piedmont reservoir with predominantly clay substrate and several shallow coves with a maximum depth of 24 m. Managers observed isolated SAV in UTR during 2008, but the SAV was not identified as hydrilla until October 2010. Southeastern Cooperative Wildlife Disease Study (SCWDS) pathologists confirmed AVM in a moribund bald eagle recovered from UTR in 2010 and the disease has since been diagnosed in wild birds annually

(SCWDS unpublished data). Approximately 5,000 (15 fish/vegetated-ha) triploid grass carp (*Ctenopharyngodon idella*) were stocked in UTR for SAV-control during April 2011.

Field application of algaecide

We drove rebar poles into the sediment at four points (42-m apart) in three separate coves, each containing healthy hydrilla, to identify the center of twelve 0.4 ha plots (Figure 2.1). Following a random complete block design, each plot within a cove was randomly assigned one of four treatments (Table 2.1). Prior to the application (0 days after treatment; DAT), we used a rake to collect hydrilla samples from the center of each plot for microscopic analysis of stig density prior to treatment. We applied the three algaecides from an airboat via weighted spray hoses on 2 October 2012 and collected samples, in an identical manner, four times after treatment (7, 14, 28, 56 DAT).

We haphazardly selected ten leaflets from each sample bag and mounted them on glass slides. We viewed leaflets at 100X under epifluorescent illumination to evaluate stig presence (Nikon Eclipse Ti, Nikon Corp., Melville, NY) and estimate density for each plot; densities were estimated by the same person every time to reduce variability between users. For example, if stig was found on 2 of 10 leaflets, we assigned the plot a density of 20% for that sampling date. We conducted a repeated measures analysis of variance (ANOVA; α=0.05) to assess the effect of algaecides on the density of stig (in each 0.4 ha plot) over time. A profile summary was used to examine any significant effects that occurred among time periods and effects found to be significant were analyzed by a Tukey's test. We conducted all statistical analyses with SAS (version 9.3; SAS Institute, Cary, NC, USA).

Laboratory application of algaecide

We collected and screened hydrilla from an untreated area of UTR on 22 November 2012 following the protocols detailed above. We selected 108 leaflets with ≥ 30% stig coverage and placed them, individually, in a 12-well plate containing autoclave-sterilized UTR site water. We assigned treatments (Captain XTR, SeClear, Pak27, and deionized water) following a randomized complete block design using three blocks with nine replicates of each treatment within each block for a total of 27 replicates for each treatment. We prepared batch volumes of algaecides to ensure chemicals were mixed to appropriate label rates (Table 2.1). Each algaecide was delivered in a 1-mL carrier of deionized water with a 5-mL polypropylene syringe and 1-mL of deionized water was delivered to the controls. We placed all plates in an incubator (25 °C, 12L:12D photoperiod) to control for extraneous environmental variation and maintain leaflet health. Water quality of the wells was not measured, but the pH of our deionized water was within the ranges recommended by the algaecides' labels. We estimated percent stig coverage prior to treatment (0 DAT), 3 DAT, and 7 DAT. Estimates of percentages higher than the most recent evaluation were ignored and the previous value was assigned for that date; only decrease in percent area leaf coverage of stig was of interest for this study. Additionally, we did not expect stig colonies to increase over a 7-day period as previous cultures in our lab have shown slow growth. We conducted a repeated measures ANOVA (α=0.05) to assess the effect of algaecides on stig over times and significant effects were analyzed by a Tukey's post-hoc test.

Results and Discussion

Field application of algaecide

Algaecide treatment in the field resulted in significant treatment (p=0.0422) and block (p=0.0210) effects on stig density (Table 2.2). However, Tukey's revealed that the only

significant difference between treatments occurred at 0 DAT, prior to application of algaecides; no significant difference was found between treatments at any time point after application. Tukey's also revealed site A had significantly lower stig densities than the other two sites at 7 and 28 DAT (Figure 2.1). Hydrilla at site A exhibited fewer leaflets in comparison to other sites throughout the trial, likely due to an unknown variation in environmental conditions. Time had a significant effect ($p=0.0004$) but there was not a time × treatment effect ($p=0.0508$; Table 2.2). None of the algaecides significantly decreased stig density and densities of all plots decreased at a similar rate between 28 and 56 DAT (Figure 2.2). Reduction in stig densities at 56 DAT could be attributed to a reduction in substrate as the hydrilla was senescing and had collapsed down in the water column. The senescence of hydrilla alters water quality (pH, DO, light availability, etc) and potentially reduces the microhabitat favored by stig. As the favorable habitat created within hydrilla beds during the early fall promotes the growth of stig, its senescence could close the window of optimal growth. Alternatively, stig could benefit from the senescing hydrilla taking up recently released nutrients as its leaflets begin to decompose. We have had a difficult time culturing stig in the laboratory and future research on optimal growth conditions would offer insight to its seasonal prevalence within hydrilla beds.

We hypothesized that if any treatment were effective, significant differences in stig density would occur within 7-14 DAT as the mode of action for these algaecides is contact. Mastin et al. (2002) documented a >78% decrease in chlorophyll-a concentrations 7 days after application of an elemental copper algaecide to a cyanobacteria bloom in a reservoir and Barrington et al. (2011) found peroxide compounds to reduce cyanobacterial biomass by 57% within 48 hours. Given the fast action of these algaecides and the fact that this reservoir experiences low flows, contact time and retention of compounds was sufficient. Application of

chemicals with a weighted hose allows for a more even application within the water column but cells located at the hose depth receive the most effective treatment; we believe our application method provided adequate opportunity for contact to stig colonies. Stig colonies are densest at roughly 0.5 m below the canopy of hydrilla beds as leaflets are more prevalent in the upper 2 m of canopy (Herrin 2012). As light penetration of a hydrilla bed decreases with depth, greater epiphytic algal biomass occurs in this upper region (Gosselain et al. 2005).

This is the first study to attempt chemical control of epiphytic cyanobacteria or algae found on SAV; prior investigations of algaecide efficacy have all focused on planktonic and filamentous species. We hypothesize that we failed to apply an effective rate to reach the critical burden necessary for control (Murray-Gulde et al. 2002). The maximum label rate was likely insufficient for treatment because the biomass of SAV and associated epiphytic fauna within the plots is high and there are many potential cells for treatments to infiltrate. All three chemicals are non-selective contact compounds, meaning they would act on the first vegetative cells encountered; there were more vegetative cells in the treated plots than would be found in the same volume of a planktonic bloom. Also, the presence of a thick mucilaginous sheath around stig colonies offers additional protection against deleterious compounds (Figure 2.3). A combination of peroxide and copper treatments may be more effective at controlling stig, as experiments on similar sheathed species such as *Lyngbya wollei* have shown that initial treatment with peroxide compounds degrade the sheath and allow for better penetration of a secondary algaecide compound (Herrin et al 2011). Lastly, it is possible that our assessment of treatment was not sufficient to detect subtle changes in stig densities. Because of the complex methodology of detection via microscopy, time and effort limit assessment to a few leaflets within each plot. Another method of detection and quantification would allow for an increase in sample size and

overall assessment and may provide greater resolution of stig density, thereby increasing power to detect differences.

Lab application of algaecide

Algaecide treatment in the lab showed no significant treatment effect ($p=0.1711$) on the percent area leaf coverage; blocking was also not significant and was excluded from the analysis. Time had a significant effect ($p<0.0001$) but there was not a time × treatment effect ($p=0.1204$; Table 2.3). Again when the data is displayed graphically, it is apparent that none of the algaecides had a significant effect on the percent area leaf coverage and that each treatment declined over time at a similar rate with the exception of the control (Figure 2.4). As we were mainly interested in the decrease of stig, our protocol prevented us from recording an increase in percent area leaf coverage over time. A few leaflets of the control group experienced increases in stig coverage, however, following protocol, were recorded as having no change. If increases of stig coverage were measured we could have potentially found significance between the algaecides and controls. Since this occurred in only a few replicates it is unlikely this would have significantly increased the average used in analysis.

The refinement of the trial to a single leaflet instead of a plot of hydrilla should have allowed ample contact for the algaecides; however, exposure was limited to 7 days to prevent deterioration of the leaflets. Results of the laboratory trial reiterate stig's resistance to the toxicity of copper and peroxide compounds. The issues of sheath-penetration and dilution of contact rates by non-target cells described above were reduced but still exist in the laboratory trials. Microscopic assessment of leaflets allowed us to collect better visual assessment of the stig colonies and mucilaginous sheath. Damage to either the cells or sheath would have been apparent under magnification; however, none of the colonies appeared damaged or stressed at

any point of evaluation. Visual assessment of the sheaths of stig treated by peroxide (Pak27) did not show substantial degradation, but closer examination at a higher microscopic power, such as electron microscopy, was not conducted.

Application of Captain XTR, Pak27, and SeClear at current label rates failed to effectively reduce stig density on hydrilla in both the field and laboratory. Until additional research on the use of algaecides to reduce epiphytic cyanobacteria, namely stig, is conducted, we encourage managers to remove invasive SAV whenever possible. In addition to paradigmatic issues associated with invasive SAV, the potential of an AVM outbreak demands attention and should be prioritized by managers, especially at sites highly frequented by waterbirds. In the summer of 2012, high densities of stig were found growing on native Southern naiad (*Najas guadalupensis*) collected from a central Georgia wildlife refuge (Wilde, unpublished data). Although naiad is often non-problematic, it is structurally similar to hydrilla and provides excellent substrate for stig. Managers valued the native SAV of the property for its attraction to migratory birds, while efforts were made to eliminate invasive SAV. Removal of all SAV from ponds was undesirable as it would decrease the use of the property by waterfowl.

Regardless of the method, for both economic and ecological reasons there is a dire need for more AVM-management options beyond complete removal of SAV. Managers of the majority of AVM-positive sites employ methods to control SAV and hope that reduction of AVM is consequently achieved; meanwhile thousands of birds are affected by AVM each year. Future research on treatment of the source of toxins, stig itself, is needed to offer an effective acute management option. Reduction in stig through treatment in late fall, prior to the arrival of migratory waterfowl, could reduce the presence of AVM-toxins; therefore, greatly reducing the number of sick birds while avoiding the removal of valuable resources.

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Table 2.1: Product description and application rates of algaecides applied in field and laboratory studies examining efficacy against the epiphytic cyanobacterium in Order Stigonematales.

Tradename®	**Product**	**Rate**	**ppm Active Ingredient**
Captain XTR®	liquid copper ethanolamine complex	3.0 gal/ac ft	1.0 ppm Cu
SeClear®	liquid copper sulfate pentahydrate	6.4 gal/ac ft	1.0 ppm Cu
Pak27®	sodium carbonate peroxyhydrate	100 lb/ac ft	10.2 ppm H_2O_2

Table 2.2: Results of the repeated measures analysis of variance used to examine (a) the efficacy of algaecides (Captain XTR, SeClear, and Pak27) against the epiphytic cyanobacterium in Order Stigonematales found on *Hydrilla verticillata* leaflets when applied to 0.4 plots within a reservoir and (b) effects of time and time × treatment interactions.

A. Between-subjects				
Source	df	MS	*F*	*P* > *F*
Treatment	3	4406.11	5.17	0.0422
Block	2	6721.67	7.88	0.0210
Error	6	852.48		
B. Within-subject				
Source	df	MS	*F*	*P* > *F*
Time	4	3140	12.59	<0.0001
Time × Treatment	12	542.22	2.17	0.0508
Time × Block	8	548.75	2.20	0.0646
Error (time)	24	249.31		

Table 2.3: Results of the repeated measures analysis of variance used to examine (a) the efficacy of algaecides (Captain XTR, SeClear, and Pak27) against the epiphytic cyanobacterium in Order Stigonematales when applied to individual *Hydrilla verticillata* leaflets in the laboratory and (b) effects of time and time × treatment interactions.

A. Between-subjects				
Source	df	MS	*F*	*P* > *F*
Treatment	3	1362.84	1.70	0.1711
Error	104	800.45		
B. Within-subject				
Source	df	MS	*F*	*P* > *F*
Time	2	936.65	34.40	<0.0001
Time × Treatment	6	46.53	1.71	0.1204
Error (time)	208	27.23		

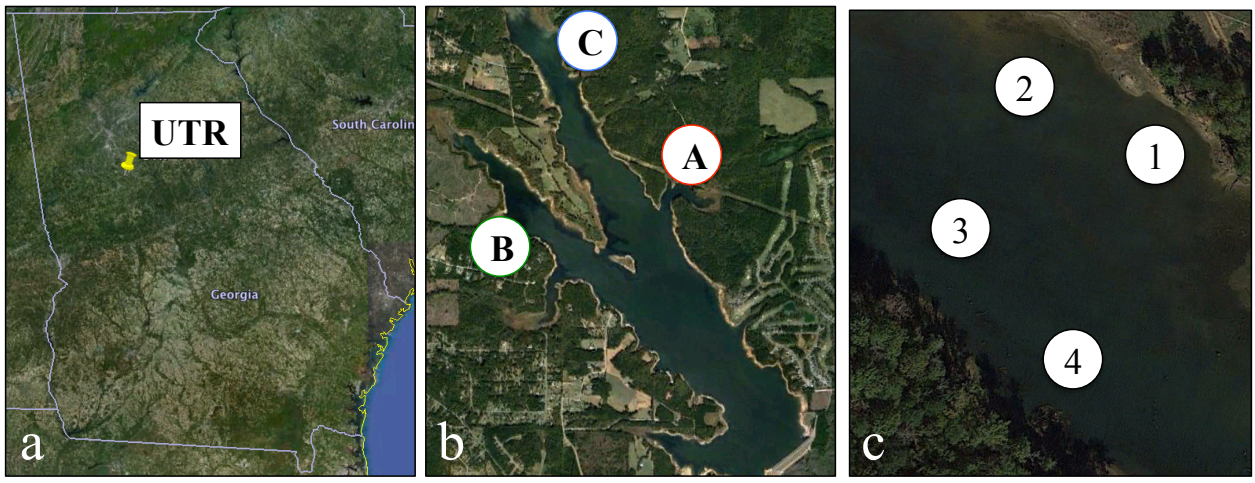

Figure 2.1: (a) Upper Towaliga Reservoir (UTR) was the study site for the field application of algaecides and the collection site of the hydrilla leaflets used in the laboratory application of algaecides. (b) Location of the coves A, B, and C used in the field application of algaecides within UTR. (c) Example of the 0.4 ha plots within cove A; arrangement of plots was similar in other coves.

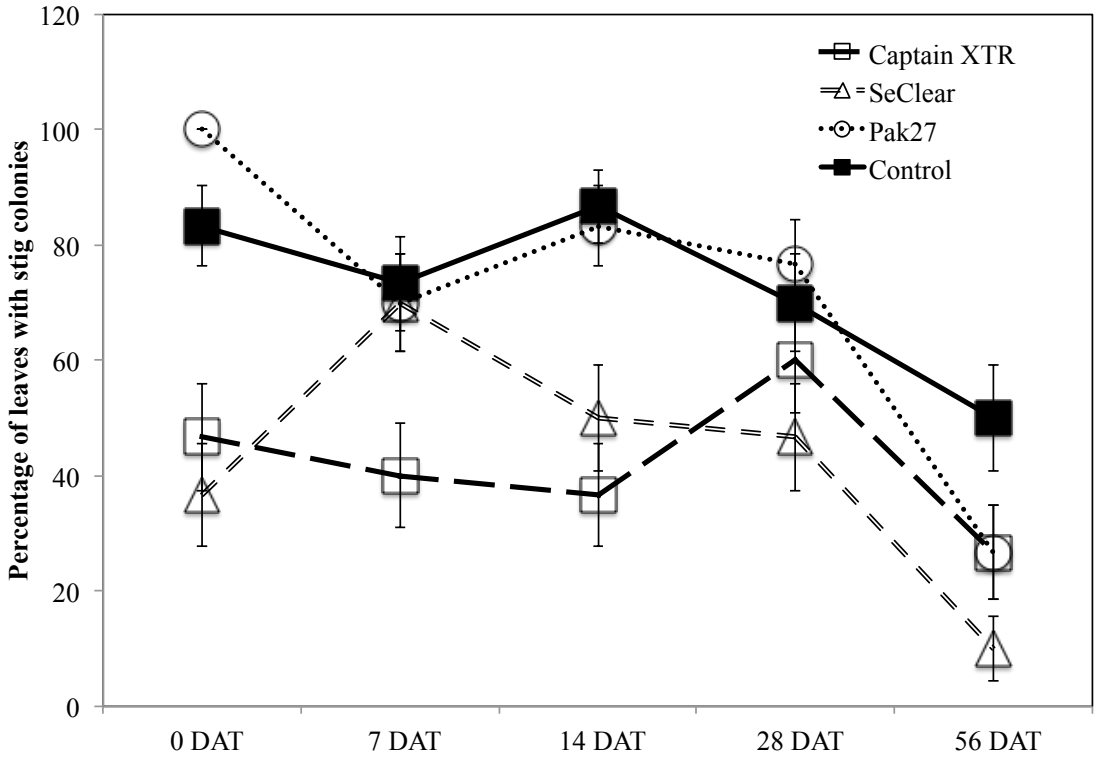

Figure 2.2: Average percentage of ten leaflets containing the epiphytic cyanobacterium in Order Stigonematales collected from each 0.4 ha plot after field application of maximum label rates of Captain XTR, SeClear, and Pak27 at 0, 7, 14, 28, and 56 days after treatment (DAT). Error bars at each point represent standard error.

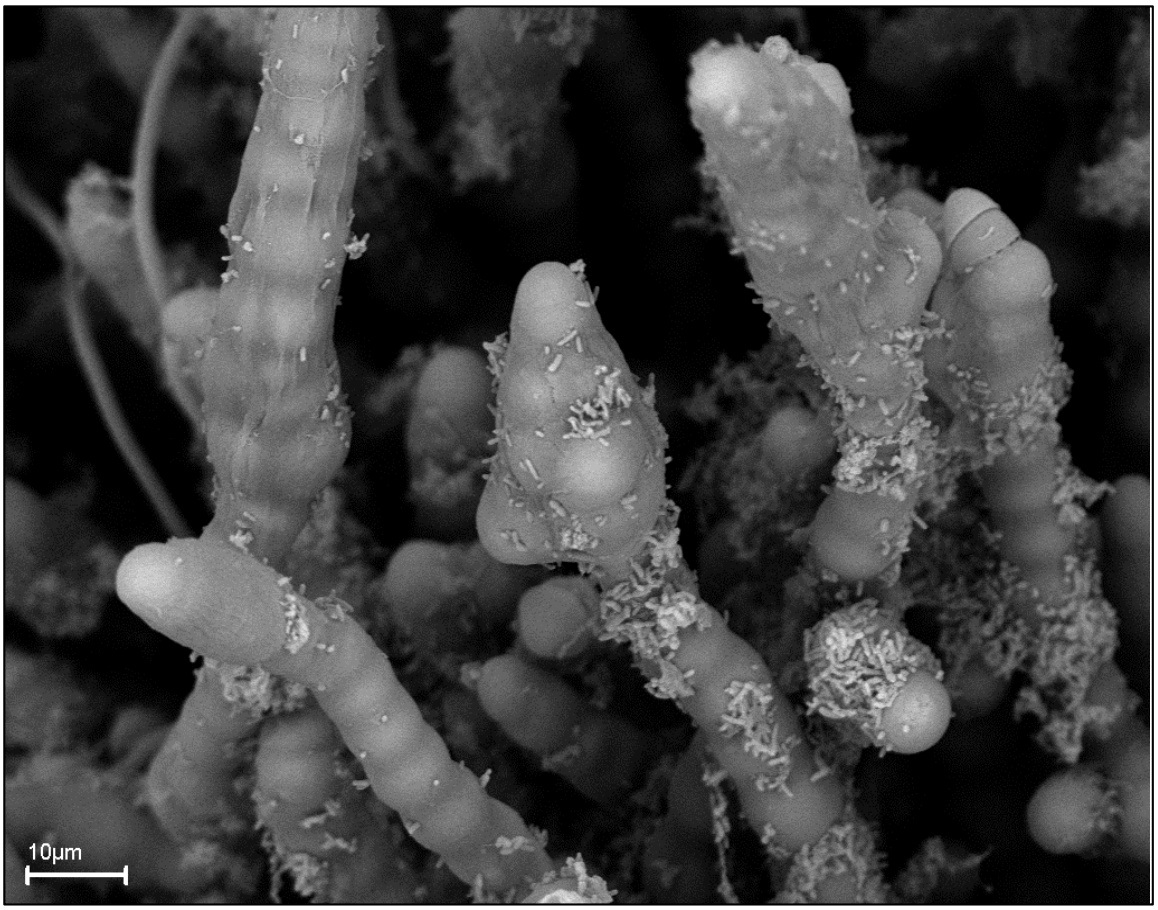

Figure 2.3: Electron micrograph indicating the thick mucilaginous sheath of the epiphytic cyanobacterium in Order Stigonematales.

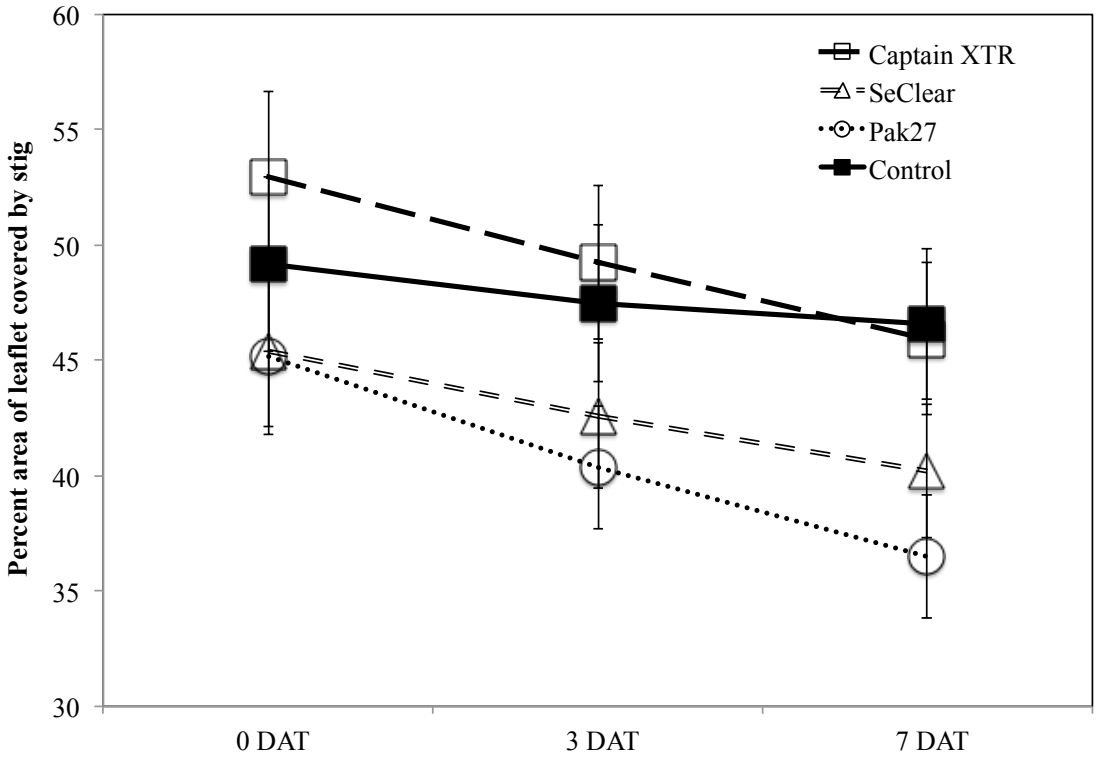

Figure 2.4: Average percent coverage area of the epiphytic cyanobacterium in Order Stigonematales on 27 hydrilla leaflets collected from Upper Towaliga Reservoir, GA after laboratory application of maximum label rates of Captain XTR, SeClear, and Pak27 at 0, 3, and 7 days after treatment (DAT). Error bars at each point represent standard error.

CHAPTER 4

CONCLUSIONS

The purpose of this study was to develop methods to better detect, quantify, and manage the epiphytic cyanobacterium (Order Stigonematales; stig) associated with avian vacuolar myelinopathy (AVM) found on submerged aquatic vegetation (SAV) of reservoirs. The presence and spread of invasive SAV such as hydrilla pose great ecological threats to reservoirs. In addition to traditional problems associated with the widespread establish of invasive SAV, the threat of stig offers managers a unique challenge. Loss of avian wildlife would have grave ecological and economical effects. Stig has the potential to remove numbers of wildlife from regional and, in the case of migratory waterfowl, global populations. Without the ability to properly detect and manage against this new threat, these populations will undoubtedly decline.

Although over 150-documented bald eagle (*Haliaeetus leucocephalus*) mortalities and thousands of American coot (*Fulica americana*) mortalities have been documented since the discovery of AVM in 1994, these numbers are likely not a true reflection of mortalities associated with AVM (Thomas et al. 1998; Fischer et al. 2006; Augspurger 2008). We believe the risk of AVM is likely greater than current research has shown. Currently, the disease has been documented in five orders of avifauna: Anseriformes, Charadiriiformes, Falconiformes, Gruiformes, and Strigiformes (Fischer et al. 2002; Augspurger et al. 2003). Because toxin-transfer has been shown to occur after ingestion of the gastrointestinal contents of affected waterfowl, it is likely that AVM occurs after ingestion of stig material (Lewis-Weis et al. 2004). It is possible that any organism that consumes stig-positive hydrilla directly or indirectly can

contract AVM. Limited research on the susceptibility of invertebrates, fish, and mammals to AVM has been conducted, but more in-depth studies are needed to determine the biological extent of AVM.

The range of stig may expand beyond the southeastern United States and could occur everywhere that its SAV counterpart has been established. Hydrilla occurs in the majority of southeastern and southwestern states and is expected to expand with global climate change (U.S. EPA 2008). The expansion of AVM outbreaks would ultimately lead to a greater loss of avian predators such as the highly regarded bald eagle. This study provides framework for a new detection tool through the use of hyperspectral remote sensing. Phycocyanin, indicative of cyanobacteria, was detectable in scans collected above the water as well as in harvested hydrilla. In the future, scans of the submerged and harvested hydrilla should be collected with the GER 1500 only on clear days. We believe this method can be tailored to accurately detect the presence of stig on SAV with future research and a more quantitative measure of stig density. This will allow for a more widespread investigation of the prevalence, geographical range, a more accurate estimate of avian loss from stig. Additionally, rapid detection methods would allow managers to assess their risk of AVM and preemptively manage against stig. Detection of stig beyond the southeastern United States would likely increase the awareness and expand research in all aspects of AVM.

Currently the only proven management plan is complete eradication of its SAV substrate. Eradication of SAV from a reservoir is often expensive and extremely difficult to maintain as long term treatment strategies are a necessity. A management strategy that targets stig directly could provide a short-term management option to reduce loss of wildlife from AVM. Algaecides are extremely effective short-term management options once a treatment plan has been

established. Although we found maximum label rates of three algaecides to be ineffective at reducing the presence of stig, there are numerous other compounds and strategies that should be explored. The ability to test the efficacy of these compounds in controlled mesocosm experiments would allow researchers to tailor application rates and strategies. We have currently failed to maintain a culture of stig-positive hydrilla in the laboratory, but advances in the culture and growth of axenic stig colonies provide promise of inoculating SAV and establishing a culture of toxic stig-positive hydrilla.

Avian vacuolar myelinopathy and its establishment of a lethal link between SAV and wild avifauna offer a unique challenge to biologists. The expansion of invasive SAV to new reservoirs in and beyond the Southeastern US could lead to an increase in the occurrence of AVM. Managers currently lack the methodology to rapidly detect stig in reservoirs and the resources needed to reduce the loss of wildlife to AVM. Although this study has provided some framework on the development of new detection and management methods, future research is needed to determine effective strategies and tailor management plans to preserve aquatic and avian resources.

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