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A Survey of the Taxonomy of the Cyanobacteria from Northeast Florida, Descriptions of Novel Taxa, and an Investigation into the Factors Which Influence the Epibenthic Cyanobacterial Community

Holly Stephanie Stocks
University of North Florida

A SURVEY OF THE TAXONOMY OF THE CYANOBACTERIA FROM NORTHEAST FLORIDA, DESCRIPTIONS OF NOVEL TAXA, AND AN INVESTIGATION INTO THE FACTORS WHICH INFLUENCE THE EPIBENTHIC CYANOBACTERIAL COMMUNITY

By

Holly Stephanie Stocks

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ABSTRACT

Cyanobacteria are important components of the aquatic system, valued for their oxygen production, nitrogen fixation, and as the base of many aquatic food webs. This study investigated several aspects of cyanobacteria such as the diversity and response to nutrient enrichments. A survey of Northeast Florida was conducted between the years of 2010 and 2012; a total of 145 taxa were identified in freshwater habitats, such as springs, lakes, rivers, and retention ponds. While surveying the St. Johns River in Jacksonville, Florida, a novel Stigonematalean taxon was isolated and cultured. Subsequent morphological and genetic analyses indicate that this taxon is related to Fischerella, Nostochopsis, and Westelliopsis, though with poor bootstrap support. Thus, a new genus and species (*Reptodigitus chapmanii gen. et sp. nov.*) is proposed. Cyanobacterial community shifts are increasingly being employed as an indicator of ecosystem health. The last part of this study is an experimental manipulation of nutrients and subsequent community analyses. Chlorophyll *a*, total number of cells, and Dmax were significantly different between control groups and nutrient enriched groups. Phosphate was not strongly correlated to species richness, chlorophyll *a*, evenness, total number of cells, species richness, or diversity in either the control or the nutrient enriched groups. Nitrogen displayed similar results, though it was slightly more strongly correlated to evenness and diversity in the nutrient enriched group than the control group. The results of the survey and nutrient enrichment experiment are important parts of the investigation into how cyanobacterial communities respond to changes in nutrient concentrations, which can then be used to devise a standard metric against which water management agencies can compare to determine the health of a given aquatic system.

INTRODUCTION

Algal relationships

Algal lineages are divided, and related, by the photosynthetic pigments used and the systems that employ them. Cyanobacteria are unique among prokaryotes in that they produce chlorophyll a and release oxygen from photosynthesis. These oxygenic photosynthesizers first appeared ca. 2.7 billion years ago and gave rise to an oxygen-rich atmosphere, allowing the switch from anaerobic to aerobic respiration, and fostering the rise and diversification of eukaryotic algae (Eigenbrode & Freeman 2006).

Eukaryotic algae originated from early heterotrophic eukaryotic organisms that most likely engulfed cyanobacterial cells and formed an endosymbiotic relationship; these cyanobacterial endosymbionts eventually evolved into plastids. Primary plastids, those that arose from ingested cyanobacterial cells, possess two envelope membranes; this feature characterizes red and green algae, and glaucophytes. Secondary plastids arose from phagocytosis on a eukaryote containing a primary plastid; likewise, tertiary plastids evolved from phagocytosis on a eukaryote containing a secondary plastid. Based on these relationships, algal lineages are interrelated.

Red algae appeared about 1.2 billion years ago and form a monophyletic group. The red algae plastids are unique in that Type ID rubisco, primarily found in proteobacteria, is used as opposed to Type IB rubisco, which occurs in green algae and land plants, and is thought to have been present in the first cyanobacterial endosymbionts. This difference can be explained through horizontal gene transfer from Type ID rubisco-containing proteobacteria into early red algal plastids (Delwich & Palmer 1996, Rice & Palmer 2006). The red appearance of these algae is due to the pigment phycoerythrin, which is very efficient at harvesting light in the blue and green

spectrums. Along with phycocyanin and allophycocyanin, the five types of phycoerythrin are able to utilize wavelengths otherwise inaccessible to chlorophyll, and transfer the light energy directly to chlorophyll a.

Green algae have generally been regarded as a close relative of red algae due to the presence of primary plastids with similar genetic content. Although, the debate continues over primary plastids and whether the similar gene content between red and green plastids is due to a single evolutionary event in a common ancestor or convergent evolution (Stiller, Reel, & Johnson 2003, Keeling 2004). Evidence for convergent evolution comes from the lack of thylakoid-bound phycobilisomes and phycobilin accessory pigments, which are found both in cyanobacteria and the plastids of red algae. The major accessory pigments employed by green algae are chlorophyll b, lutein, and beta-carotene, which confer the various green colors for which these algae are named.

Dinoflagellates emerged around 200 million years ago and are generally considered heterotrophic phagotrophs, however, recently discovered plastid-bearing protists suggest the possibility that plastidless dinoflagellates formed from plastid-bearing ancestors (Taylor 2004, Moore *et al*. 2008). About half of all dinoflagellates contain plastids acquired from different photosynthetic eukaryotes, including cryptomonads, haptophytes, green algae, and diatoms (i.e. phagotrophy or kelptotrophy). Most plastid-bearing dinoflagellates utilize a unique xanthophyll pigment, peridinin, contained within golden-brown plastids. There are two basic hypotheses concerning the evolution of these plastids; either they descended from red algal plastids via secondary endosymbiosis, or via tertiary endosymbiosis with plastid-bearing stramenopiles (Bodyl & Moszczynski 2006, Delwiche 2007). Evidence for the tertiary endosymbiosis hypothesis is in the presence of chlorophyll *c*, contained within the golden-brown plastids; this

accessory pigment is present in photosynthetic stramenopiles, but not in red algae (Bodyl & Moszczynski 2006). The peridinin pigment absorbs light in the blue-green range, which is inaccessible to chlorophyll alone and present in aquatic habitats.

Diatoms have been found in deposits formed up to 180 million years ago. The major pigments found are beta-carotene and the xanthophyll fucoxanthin, which give diatoms and other photosynthetic stramenopiles a golden-brown or brown color; chlorophylls *a* and *c* are also present in diatoms. The photosynthetic stramenopiles are thought to have evolved from a common ancestor containing plastids obtained from a secondary red algal endosymbiont (Guillou *et al.* 1999, Karpov, Sogin, & Silberman 2001, Kuhn, Medlin, & Eller 2004). Xanthophylls not only absorb light wavelengths inaccessible to chlorophyll, they also provide protection to the photosystem from high levels of light intensity.

Cyanobacteria

Cyanobacteria, also known as blue-green algae, are among the most ubiquitous organisms on Earth. Representatives can be found in almost every ecosystem, from aquatic to terrestrial, from equatorial to polar (Birkemoe & Liengen, 2000; Novis *et al*. 2007). Cyanobacteria play a critical role in biogeochemical cycles accounting for ca. 30-40% of global oxygen production, while the genus *Trichodesmium* alone is responsible for ca. 42% of the global nitrogen fixation (Berman-Frank *et al*. 2005, Latysheva *et al.* 2012). While cyanobacteria form the basis of many aquatic foodwebs, other species are toxic to animals and deleterious to other members of the phytoplankton community. Furthermore, numerous species may form large blooms, rendering lakes and reservoirs unusable by man (Berg *et al*. 1987, Falconer 1998).

The terms cyanobacteria and blue-green algae originated from the organism's ability to produce phycocyanin, a phycobilin pigment, which in high concentrations result in a bluish tint. Many other pigments are produced by these photooxygenic prokaryotes, including chlorophyll *a* and *b*, which give a greenish tint. Cells may range in color from brown, red, orange, violet, blue, green, and any color in between depending on environmental conditions and the presence of accessory pigments (Graham *et al.* 2008). The term 'blue-green algae' is used almost exclusively in water management, whereas in research, the term 'cyanobacteria' is preferred; Stanier *et al.* (1978) suggests using the term 'cyanobacteria' exclusively (*sensu* Whitton & Potts 2000).

The morphology of cyanobacteria is possibly as diverse as the colors. Species are either unicellular (e.g., Chroococcales) or filamentous (e.g., Oscillatoriales), and may be solitary or form colonies. Many species form a mucilaginous sheath composed of exopolysaccharides, in which case the cells are termed trichomes; those species without sheaths are described as filaments (Graham & Wilcox 2008). Many species form gas vacuoles to aid in buoyancy. Heterocytes, which are differentiated cells in which nitrogen fixation occurs, are formed by species adapted to living in nitrogen depleted conditions (Anagnostidis & Komarek 1999). In less than optimum conditions, some cyanobacterial taxa may produce akinetes, which are dormant cells used for overwintering or surviving sub-optimal conditions. Filamentous species may also form hormogonia, which are modified filaments associated with dispersal and reproduction via production of necridial cells (Hernandez-Muniz & Stevens 1986). This large array of morphological features may stem from possessing multiple copies of the genome, which can also explain the occurrence of multiple phenotypes produced by clonal isolates of one filament (Swingley *et al.* 2008).

Cyanobacteria form the base of many aquatic food webs and are sensitive to environmental parameters such as light levels, nutrient concentrations and ratios, and dissolved oxygen levels (Case *et al.* 2008). As a result, phytoplankton in general and cyanobacteria specifically have been considered useful indicator species for monitoring of aquatic systems (Burford 1997, Perona *et al.* 1998, Douterelo *et al.* 2004, Case *et al.* 2008, Romo 2008, Leigh *et al.* 2010, Maske & Sangolkar 2010, Katsiapi *et al.* 2011). Several studies have observed dramatic shifts in cyanobacterial species composition following increased nutrient levels (Perona *et al.* 1998, Douterelo *et al.* 2003).

Nutrient loading in freshwater has been shown to exacerbate harmful cyanobacterial blooms (Paerl *et al*. 2011). To date, it is unclear which nutrient (nitrogen or phosphorus) is responsible, and it may differ depending on local conditions and which species are present. For example, some classical research has pointed out that phosphorus is the limiting nutrient in the Laurentian Great Lakes (Vollenweider 1975, Schindler 1977), while others suggest nitrogen (e.g., Moore *et al*. 2002), and still others suggest the N:P ratio is ultimately responsible, rather than individual nutrient levels (e.g., Huisman & Weissing 2001). Cyanobacteria have been shown to be highly competitive for ammonium in nitrogen-limited conditions, but not competitive for nitrate (Whitton & Potts 2000). Cyanobacterial diversity and abundance is greatest at mid- to high pH (e.g., 7-12), although some picocyanobacteria and filamentous species have been found in pH as low as 4.0. Many of these species are heterocystous, suggesting a competitive advantage to being able to fix nitrogen (Steinberg *et al.* 1998). Hakanson *et al*. (2007) compiled chlorophyll *a*, cyanobacteria community analyses, salinity, total phosphorous, and total nitrogen data from over 500 freshwater and coastal ecosystems in an attempt to create a model that accurately predicts cyanobacterial biomass. They determined that

the relationship between cyanobacterial or chlorophyll *a* concentrations and the ratio of total nitrogen to total phosphorus was a complex one and in need of much more data to be reliably predictable.

Cyanobacteria as indicator species

In a world of increasing anthropogenic eutrophication of freshwater ecosystems, monitoring the health of economically and recreationally important lakes and streams becomes vital to their continued use. Phytoplankton community assessment using species diversity, biomass, and abundance has been suggested as a tool for management of eutrophic waters (Burford 1997, Douterelo *et al.* 2004, Case *et al.* 2008, Romo *et al.* 2008, Leigh *et al.* 2010). For example, following excessive nutrient increases in tropical shrimp culture ponds in Brazil, Case *et al.* (2008) observed a dominance shift from diatoms and copepods to cyanobacteria, protozoa, and rotifers. Species composition shifts from diatoms to cyanobacteria and flagellates were found to coincide with decreased silicate concentration, increased ammonia concentrations, a higher ratio of total ammonia to total dissolved inorganic nitrogen, as well as a higher ratio of dissolved inorganic nitrogen to orthophosphate (Burford 1997).

For example, shifts in cyanobacterial species following nutrient alterations have been observed in Lake Albufera de Valencia, Spain which has been eutrophic since the 1970's (Romo *et al.* 2008). Due to conservation pressures, 30% of the nutrients entering the lake have been diverted elsewhere, which coincided with a trend towards fewer filamentous cyanobacteria and increased coccoid species. The filamentous *Planktothrix agardhii* in particular seemed to be a good indicator species of nutrient enrichment in the lake (Romo *et al.* 2008). Perona *et al.* (1998) observed a trend of decreasing species richness, abundance, and diversity, as nutrients,

mainly soluble reactive phosphate, increased downstream in the Alberche River, Spain. Although a decrease in species richness was shown to follow an increase in the soluble reactive phosphate and a decrease in the dissolved inorganic nitrogen to soluble reactive phosphate ratio, no such relationship was found with dissolved inorganic nitrogen alone (Perona *et al.* 1998). Decreases in both heterocystous and non-heterocystous species were observed; however, the reduced species richness was largely due to marked decreases in heterocystous species (Perona *et al.* 1998). Douterelo *et al.* (2003) noted changes in cyanobacterial species richness and diversity upstream and downstream from sewage effluent discharges. Particularly, they observed a shift from Nostocales species in lower nutrient loads to Oscillatoriales species in higher nutrient loads (Douterelo *et al.* 2003). They concluded that polluted sampling sites had low species richness, with large population sizes, while unpolluted sites had high species richness, but species typically exhibited lower abundance. In order to use cyanobacterial community composition to determine the level of eutrophication, a study of how the community composition and nutrient concentrations is vital.

Taxonomy

The cyanobacteria are amongst the most taxonomically challenging, yet species rich, lineages of microbes (Perkerson *et al.* 2011). Originally classified based solely on morphology, wholesale revisions of the cyanobacteria were proposed by the International Code of Botanical Nomenclature, which relied on a series of papers published from 1886 to 1892 as a starting point for taxonomy. Stanier *et al*. (1978) proposed that the cyanobacteria be placed under the International Code of Nomenclature of Bacteria. However, two of the major requirements (the need for axenic cultures and 16S DNA:DNA hybridization values) have proven nearly

impossible to satisfy and thus the majority of researchers continue to use the International Code of Botanical Nomenclature.

Two major attempts have been made to change the overall cyanobacterial classification system. One challenge in untangling the systematics of the cyanobacteria is the extensive phenotypic plasticity evidenced in some lineages. For example, heterocystous species only produce heterocysts in nitrogen limited conditions, while other morphological features may be altered in phosphorous limited conditions. Therefore, a cultured species may have a different phenotype from a field specimen, due to the environmental conditions in which it was cultured (Casamatta *et al.* 2003). Hypothesizing that the vast biodiversity of the cyanobacteria was really only the result of a few taxa that exhibit a tremendous amount of phenotypic plasticity, Drouet and Daily (1956) used only a few simple morphological characteristics for taxonomy, resulting in the total number of proposed species of the day being reduced from over 2000 to just 62. This system was quickly replaced, save for two generic names used extensively in research. Komárek and Anagnostidis (1985, 1999, 2005, etc.) proposed the other major revision to the classification system, suggesting many name changes, especially in the *Oscillatoriales*. Komarek and Anagnostidis advocated a system of smaller, monophyletic genera identifiable using morphological, genetic or ecological apomorphies (Johansen & Casamatta 2005). It is their revision which forms the basis of modern cyanobacterial systematics.

Morphological plasticity

Yet another obstacle in the road to identification is the tendency of cyanobacteria to display differential morphologies determined by environmental factors (e.g., Casamatta & Vis 2004). This morphological plasticity can occur due to temperature and light fluctuations, as well

as changes in nutrient concentrations, specifically nitrogen and phosphate (Zapomelova *et al.* 2008a, Zapomelova *et al.* 2008b, Bonilla *et al.* 2012). Temperature has been shown to significantly alter vegetative cell morphology, heterocyst morphology and trichome coiling in *Anabaena* sp.; vegetative cell morphology was also affected by differential phosphorous concentrations (Zapomelova *et al*. 2008a). In another study, Zapomelova *et al.* (2008b) found significant effects of growth conditions on the occurrence of heterocysts, branching of the trichomes, formation of necridial cells, and trichome coiling in multiple strains, including *Nostoc*, *Scytonema*, and *Tolypothrix* species. Differential responses of planktonic and soil strains to nitrogen concentration were observed as well; the frequency of heterocysts in all soil strains was significantly greater in nitrogen-limited medium, while only one of the planktonic strains showed sensitivity. It was suggested that two of the planktonic strains used probably did not reach nitrogen limitation in the given medium treatments (Zapomelova *et al.* 2008b).

In order to effectively research the ecology of cyanobacteria, multiple facets need to be examined more closely, such as the choice of strain, how the strains are stored, and nutrient concentration of the culture medium used. In this study, preserved field specimens were compared to cultured samples in an attempt to verify the species identity. One goal of this study is to survey the cyanobacterial species present in relatively natural freshwater streams, lakes, and rivers of North Florida. A second goal was to experimentally determine the changes in community composition over time in nutrient-enriched waters. The experimental results and the survey were then used to relate community composition to nutrient concentrations in order to provide a baseline for future studies of the effects of nutrient addition on species composition. A final goal was to name any taxa new to science discovered during the course of this project.

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Chapter 1

A survey of freshwater cyanobacteria from Northeast Florida.

INTRODUCTION

Cyanobacteria are photosynthetic prokaryotes that form much of the basis of aquatic food webs (Lance *et al*. 2006). Species respond differentially to environmental parameters, such as temperature, dissolved oxygen, and light, and therefore the natural fluctuation of these parameters can create a continuum of community compositions. With knowledge of the cyanobacterial community, it is possible to monitor aquatic habitats and accurately identify a system that is changing trophic state. As such, cyanobacteria are increasingly being employed as a means of assessing the health of aquatic ecosystems (Znachor *et al.* 2006, Fristachi et al. 2008, Wood *et al.* 2010).

Numerous biological indicators of ecosystem health have been proposed and are currently employed: diatoms, fish, invertebrates, phytoplankton, plants etc., all with variable success (e.g., Kelly 1998, Soto-Galera *et al.* 1999, Oertli 2008, Romo *et al.* 2008). Algae have several characteristics which make them excellent candidates for indicator species: they are ubiquitous, present year round, have rapid generation times, are sessile and are relatively easy to identify to broad taxonomic group (e.g., Reynolds 1984, Cattaneo 1987, Carrick, Lowe, & Rotenberry 1988, Lowe & Pan 1996, Kelly 1998).

Increased nutrient loading and eutrophication has been shown to often elicit a concurrent increase in cyanobacteria in numerous freshwater habitats (e.g., Douterelo *et al.* 2003). Likewise, a decrease in nutrient concentrations may lead to decreased cyanobacterial dominance in the phytoplankton community (e.g., Romo *et al.* 2008). As nutrient concentrations increased

downstream of the Alberche River, in Spain, cyanobacterial species richness, abundance, diversity, and total biomass decreased (Perona *et al.* 1998). Douterelo *et al.* (2003) also observed a decrease in species richness following sewage effluent discharge, but an increase in the overall cyanobacterial abundance. These contradicting results may stem from a difference in factors other than nutrient loads, such as light, dissolved oxygen, or biotic interactions (e.g., Havens *et al.* 1998, Smith & Lester 2007, Case *et al.* 2008).

Many studies that have investigated changes in cyanobacterial populations due to nutrient flux established reference populations before a nutrient-altering event occurred (i.e. diverting or adding sewage) (Douterelo *et al.* 2003, Case *et al*. 2008, Romo *et al.* 2008). Overall, these studies have shown dominance shifts from diatoms and copepods to cyanobacteria, protozoa, and rotifers following an excessive nutrient increase, while nutrient decreases produced communities dominated by cyanobacterial with variable or shifting compositions (Douterelo *et al.* 2003, Case *et al.* 2008, Romo *et al*. 2008). As a management tool, knowledge of cyanobacterial populations in recreational waters can be valuable, but only with continued sampling and comparison can they provide accurate representations of a nutrient flux. This survey provided a snapshot to which future samples can be compared.

One of the chief impediments to employing cyanobacteria as a surrogate for ecosystem health is the lack of a baseline assessment of the natural community. In this survey, springs and retention ponds make up the majority of the sampled sites due to their ubiquity and ease of sampling, followed by lakes and rivers.

METHODS AND MATERIALS

Site descriptions

The sites selected for sampling represent varied freshwater aquatic habitats from north Florida, including retention ponds, rivers, lakes, streams, and springs (Appendix 1, Figure 1). The Suwannee River is a blackwater river that flows through four of the nine parks sampled and is characterized by limestone bluffs, a sandy to muddy bottom, and aqueduct fed-springs that line the river. The Santa Fe River is a lake fed, slow moving river with dark water that flows into the Suwannee River. The Ichetucknee River is a tributary of the Santa Fe River, and is fed by crystal clear springwater. The springs have differing levels of anthropogenic influence, depending on size, flow, and frequency of visitors, while the Suwannee River is exposed to farm runoff near the headwaters. Lake Rosalie is bordered by Lake Kissimmee State park on one side and partially bordered on the other side by Catfish Creek Preserve. Some of these sites reside within state parks, and as such, are relatively untouched by anthropogenic forces. State parks have restricted access, and typically possess buffer areas of terrestrial plants which ameliorate excess nutrient runoff, and thus these sites experience reduced anthropogenic influence (Rossi *et al*. 2010). In contrast, retention ponds generally have very few terrestrial plants, unrestricted access, and are often in areas that experience very high levels of herbicide and fertilization (i.e., commercially tended lawns or golf courses). The retention ponds sampled were chosen haphazardly within the study area.

Sample collection and processing

Sampling season ranged from March to September, and was conducted during the years 2010 through 2012. Cyanobacterial samples were collected from sediments, surface scrapings of rocks and vegetation, floating algal mats, and the directly from the water column. For sediment samples, one liter of sediment was obtained using a PVC pipe and negative pressure (*sensu*

Round 1953). Planktonic species were sampled using a three minute plankton net tow. Floating mats (metaphyton) were sampled from the edge, one sample per mat, where available. Any submerged vegetation or rocks were scraped with a microscope slide and placed into whirlpacks. The total number of samples from each site varied depending on the different habitats available in each sample site (e.g. epilithic, episammic, epiphytic, etc.). Subsamples of each field sample were preserved in 2.5% glutaraldehyde and used in identification. Culturing took place on 1.5% nutrient (BG11/Z8 media) agar plates, with a natural light cycle (for NE Florida, 14:11 l:d in the summer, 10:14 l:d in the winter). To obtain single species colonies for genetic analysis, each sample was plated upon return to the lab and allowed to grow for a period of up to two weeks. Following this growth period, plates were subcultured onto multiple new plates and allowed to grow for up to another two weeks. This was repeated until a single species colony has formed on one agar plate. Cyanobacteria were identified using a light microscope at 40 and 100x magnification, and identified using standard taxonomic keys (e.g., Anagnostidis & Komarek 1999, Komarek & Anagnostidis 2005, Hindak 2008), with taxonomy updated as necessary (Komarek & Hauer 2013). Sediment collected was poured into large petri dishes and left to settle for 24h. Afterwards, excess water was siphoned off. A piece of water-permeable cloth was placed on top of the sediment and three glass cover slips were placed on top of the cloth. The petri dishes were left to sit for another 24-48h to allow time for cyanobacteria to migrate up onto the cover slips; after which the cover slips were placed on slides and examined under the microscope (400 cyanobacterial cell count per sample).

RESULTS AND DISCUSSION

A total of 145 cyanobacterial species were identified from the 25 sampling sites. The majority of species belong to the order Oscillatoriales (60%), followed by Chroococcales, Nostocales, and Stigonematales (27%, 10%, and 3%, respectively) (Appendix 2). No single species was present at all 25 sites, although *Oscillatoria limosa* and *Phormidium formosum* were present in ca. 50% of the sites. Filamentous taxa belonging to Oscillatoriales were present in all sites, while Chroococcalean and Nostocalean species were present in most sites. Most of the total species identified (69%) were found in only one or two sites, including all representative Stigonematalean taxa. Overall, retention ponds had the greatest species richness, with as many as 26 species, but on average contained 14 species. In contrast, samples from sites located within state parks or otherwise buffered (i.e. Lake Oneida) had an average of 6.4 species and a maximum of 13 species. In a previous epiphyte survey (Dunn, Dobberfuhl, & Casamatta 2008), numerous sites in the St. Johns River were sampled and found to have as many as 34 species and an average of 17.4 (Appendix 2).

A strong positive relationship between prevalence of taxa belonging to Oscillatoriales and high nutrient concentrations has repeatedly been established, particularly with soluble reactive phosphate (Douterelo *et al.* 2003, Kruskopf & Plessis 2005, Pulina *et al.* 2011, Salmaso 2011). A similar relationship has been found between Chroococcalean taxa and increased nitrogen and phosphate concentrations, as well as with Nostocalean taxa and low nutrient loads (Douterelo *et al.* 2003, Rejmankova & Komarkova 2005, Pulina *et al.* 2011). In nitrogen-limited environments, species that form heterocytes (Nostocales) have a competitive edge over those that do not; *Oscillatoria simplicissima*, for example, display shortened filaments and increased hormogonia production in nitrogen-depleted conditions (Kruskopf & Plessis 2005).

Due to the individual species' preferences for environmental parameters and rapid generation times, the cyanobacterial community displays great spatial and temporal heterogeneity. While many agree that increased cyanobacterial prevalence in relation to eutrophication and high organic pollution is the general rule, cyanobacterial blooms also occur in oligotrophic systems, and have been implicated in the acceleration of eutrophication of oligotrophic, nutrient-limited lakes (Douterelo *et al.* 2003, Carey *et al.* 2008, Romo *et al.* 2008).

Current surveys of algae are often lacking cyanobacteria; previous algal surveys such as the Dillard series and A Manual of Freshwater Algae focus on algae other than cyanobacteria (Whitford & Schumacher 1985, Dillard 1993, Dillard 2007, Hasler *et al.* 2012). Existing cyanobacterial surveys are spread across the world, and many are focused on extreme conditions (i.e., arid deserts, rocks, soils, or thermal vents) (Tilden 1910, Daily 1942, Castenholtz 1969, Skulberg & Skulberg 1985, Budel & Wessels 1991, Budel *et al.* 1994). In the United States, southern Massachusetts, eastern California, and western Nevada have been surveyed; worldwide, Venezuela, Chile, Norway, Iceland, and other European countries have been surveyed (Drouet 1938, Daily 1942, Drouet 1943, Castenholtz 1969, Skulberg & Skulberg 1985, Budel & Wessels 1991, Budel *et al.* 1994).

Very little attention has been paid to the cyanobacterial community in the past; recently however, in the interest of water management, cyanobacteria and other possible indicator species have increasingly been investigated for use in monitoring aquatic systems (Kelly 1998, Soto-Galera *et al.* 1999, Znachor *et al.* 2006, Fristachi *et al.* 2008, Oertli 2008, Romo *et al.* 2008, Wood *et al.* 2010). This study focused on compiling the cyanophycean taxa that live in the North Florida region from a variety of habitats, including springs, rivers, lakes, and retention ponds (Appendix 1, Figure 1).

Figure 1. Map of Florida depicting sampling sites chosen for study.

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Chapter 2

Reptodigitus chapmanii gen. nov.: a unique Stigeonematalean (Cyanobacteria) genus based on a polyphasic approach.

INTRODUCTION

Cyanobacteria are a morphologically and ecologically diverse group of photoxygenic prokaryotes found across the planet (Graham 2008). Traditionally classified based on morphological features, phylogenetic relationships of this clade have recently undergone major revisions (e.g., Anagnostidis and Komarek 1999, Komarek and Anagnostidis 2005). Not surprisingly, many morphological features traditionally employed as phylogenetic characters have been shown to be plastic, thus necessitating the use of molecular methods and a polyphasic approach employing characters such as ecology, life history, chemotaxonomy, 16S-23S ITS secondary folding structures, etc. (Casamatta *et al*. 2005, Rehakova *et al*. 2007, Perkerson *et al*. 2011, Engene *et al*. 2012). Further, many cyanobacterial taxa are difficult to identify due to lack of clear morphological apomorphies and generic placements that have been shown to be overly broad. As such, molecular methods (e.g., the 16S rDNA gene sequence and the 16S-23S ITS folding patterns) are increasingly being employed for phylogenetic assessments (e.g., Finsinger *et al*. 2008, Kastovsky and Johansen 2008, Casamatta *et al*. 2012).

The Stigonematales is a monophyletic lineage of cyanobacteria whose members produce specialized calls and exhibit cell division in multiple planes (Stanier *et al*. 1971). The Stigonematales is amongst the least studied cyanobacteria, possessing great genetic and ecological variability (Kastovsky & Johansen 2008). This group also displays true branching of filaments, which is the highest level of morphological differentiation and complexity in

cyanobacteria (Hoffmann and Castenholz 2001, Gugger and Hoffmann 2004). Members of the Stigonematales proliferate by various means, including hormogonia, hormocysts, trichome breakage, and akinetes, depending on the genus.

One of the most problematic clusters of organisms in the Stigonematales is the clade including the genera *Hapalosiphon*, *Westiellopsis*, and *Fischerella*. Unfortunately, many of these taxa have been described from very restricted habitats and thus their true genetic and ecological diversity remains unknown. A novel strain of this lineage was isolated as an epiphyte on eelgrass (*Vallisneria alternifolia* L.) from the St. Johns River (Jacksonville, FL, USA). Superficially resembling *Hapalosiphon*, this strain possesses some unique morphological characters, 16S rRNA gene sequence and 16S-23S ITS secondary folding patterns. Based on a polyphasic approach, this paper proposes the erection of a new genus and species within the Stigonematales, *Reptodigitus chapmanii*.

MATERIALS AND METHODS

Isolation and Culture

The cyanobacterial strain used in this study were collected, isolated and identified from the St. Johns River in Jacksonville Florida, United States. Strains were initially grown in liquid Z-8 media (Carmichael, 1986) and incubated at 20^{o} C under fluorescent light (200 $\mu E/s^{-1}/cm^{-2}$) with a 16:8 hr light/dark photoperiod. Growing cultures were then transferred to both Z-8 agar plates and slants and were maintained in the above conditions.

Morphological Characterization

All isolates were examined using a high-resolution Olympus BX-52 photomicroscope equipped with Nomarski DIC optics to study cellular features. Morphology was examined using

a stereomicroscope. Morphological characteristics such as sheath type, type of branching, cell and trichome dimensions, presence of peripheral thylakoids, constrictions at crosswalls, meristematic zones, and shape of end cells were noted.

Molecular Methods

Total genomic DNA was extracted from cultures using the CTAB method as modified by Cullings (1992) for the isolation and purification of DNA from mucilaginous organisms (Doyle and Doyle, 1987). DNA pellets were re-suspended in 50 μL of TE buffer and the resulting genomic DNA was checked using 1% agarose/ethidium bromide gels. Extracted DNA samples were stored at -20^0 C.

PCR primers were modified from Wilmotte *et al*. (1993) and Nübel *et al*. (1997). The relative locations and position of these primers with respect to the 16S rRNA and 23S rRNA genes are shown in Boyer *et al*. (2001 and 2002). All PCR reactions were performed in a total volume of 100 μL containing 10.0 μL of 10 X *Taq* polymerase buffer (Promega Corp., Madison, WI); 0.5 μL primer mixture (1.2 μL primer 1 or 6, 1.2 μL primer 2, 7.6 μL dH_2O); 0.5 μL of a stock solution of dNTPs [(10 mM in each dNTP); dATP, dCTP, dGTP, and dTTP]; 0.5 μL (Promega) *Taq* polymerase; 1.0 μL of extracted genomic DNA (50 ng), and the appropriate amount of dH_2O to bring the volume to 100μL. Thermal cycling was conducted using an Thermolyne's Amplitron and Temptronic thermalcyclers (Barnstead International, Dubuque, IA) using the following parameters: 94^o C for 60 s, 55^o for 45 s, and 72^o C for 4 minutes repeated for 35 cycles (primer pair 1 and 2), and 94^o C for 60 s, 55^o for 45 s, and 72^o C for 2 minutes repeated for 20 cycles (primer pair 2 and 6). After amplification, a 7-minute/72^o extension step was included for primer pair 1 and 2, whereas primer pair 2 and 6 received no such extension. PCR products were analyzed on 1% agarose/ethidium bromide gels in 1X TBE buffer.

Cloning

Amplified PCR products were cloned using the TOPO^{TM} TA cloning kit (Invitrogen Corp., Carlsbad, CA) under manufacturer specifications. Two replicate plasmid samples were isolated from each cloning plate and sequenced by Cleveland Genomics (Cleveland, OH). Automated sequencing was performed using universal infrared (IR) primers M13IR forward and reverse.

Data Analysis

The 16S rRNA gene and associated 16S-23S ITS region were sequenced following the molecular protocols detailed in Boyer *et al.* 2001, Boyer *et al.* 2002, Flechtner *et al*. 2002, Casamatta *et al.* 2005. Outgroup taxa sequence data were obtained from GenBank (http://www.ncbi.nlm.nih.gov) and other sequenced taxa (accession numbers in Figure 2). Maximum Parsimony trees were generated using a heuristic search constrained by random sequence addition (1000), steepest descent, and tree-bisection branch swapping using PAUP v.4.02b (Swofford, 1998). Bootstrap values were obtained from 1000 replicates with one random sequence addition to jumble the data. A Maximum Likelihood tree employing the General Time Reversible model with corrected invariable sites (I) and Gamma distribution shape parameters (G) obtained using Modeltest v3.06 (Posada and Crandall, 1998) was constructed with 100 rounds (each with its own random addition) of ML analysis, and bootstrap resampled (100 rounds due to computational constraints) using PAUP v.4.02b .

Secondary structure of the 16S-23S ITS was determined using Mfold version 2.3 (Zuker *et al.* 2003). Structures were determined by folding and identifying each conserved helix separately first, and then constraining the sequence to produce the entire structure. Apart from the folding temperature, which was set at 20º C, default conditions were in all cases used.

RESULTS

Phylogenetic analysis

In order to assess the phylogenetic placement of the proposed taxon, maximum parsimony, Bayesian analyses, and distance analyses of 75 stigonematalean OTUs yielded a tree that showed a poorly supported cluster sister to *Nostochopsis* (Figure 2). It should be noted, though, that our strain is poorly supported and distant from the highly supported cluster containing *Nostochopsis* (Figure 2). This clade is modestly associated with a cluster containing *Westiellopsis*, *Hapalosiphon*, and *Fischerella*.

Analysis of secondary structure of 16S-23S ITS motifs

ITS regions were amplified, and the D1-D1' helix was folded for use in phylogenetic analyses (Figure 3). The D1-D1' helices for all four taxa (*R. chapmanii* and three sister taxa) were highly similar, all consisting of 70 nt, with a 6 nt unilateral bulge (side loop) that has a highly conserved sequence (CAUCCC) found in three of the four taxa. The exception to this was *Nostochopsis* HA4207-MV1 (UCAUCC) (Figure 4). The loop at the apex of the helices consisted of 7 nt of two separate sequences; *Nostochopsis* and *Reptodigitus* displayed the same sequence (GUAAAAG), while *F. muscicola* and *Westiellopsis* displayed a sequence different by only two nucleotide substitutions (GUUGAAG). A bilateral bulge was also present just below the apex, ranging from 5-9 nt, with variable sequences. The basal pairings in each of the four taxa were also highly conserved (GACCU-AGGUC) (Fig. 5).

The Box-B helix ranged from 35-38 nt, each with variable sequences (Figure 5). The loop at the apex of the helices ranged from 10-15 nt (Figure 5). A highly conserved bilateral

bulge was also present in all four taxa, as well as highly conserved basal pairings (AGCA-UGCU).

The V3 helix was highly variable between all taxa sampled (Figure 6). Ranging from 41-66nt, and containing from 1-4 bilateral bulges, strains exhibited a highly variable loop at the apex of the helix, which ranged from 4-11 nt (Figure 6). Basal pairings were conserved in three of the four taxa (UUCA-UGAG), again, *Nostochopsis* was the odd taxon out (GUAA-UUAC).

DISCUSSION

Many Stigonematalean taxa are highly phenotypically plastic, which may account for their presence in a variety of environments, such as subaerial, aquatic, hot springs, cryptoendolith, in *Sphagnum* bogs, and in caves (Gugger & Hoffmann 2004; Finsinger *et al*. 2008; Soe *et al*. 2011). Stigonematalean taxa are separated from other cyanobacteria by the presence of true branching, which is characterized by branch-point cells that contact with three different adjoining cells (Stanier *et al*. 1971). Golubic *et al*. 1996 classified three major types of branches displayed in Stigonematales: 'T', 'V', and 'Y'. T-branches are formed by an almost perpendicular branching, caused by a change in the plane of division from transverse to longitudinal. V-branches arise when a dichotomous bifurcation originates from a change in the division plate at, or near to, the trichome tip. Y-branches are formed by meristematic growth that displaces an intercalary branch-point cell. Many forms of multiplication are employed by Stigonematales, including hormogonia, akinetes, hormocysts, and trichome breakage (Gugger and Hoffmann 2004). Our new taxon exhibits two types of branching, 'T' and 'V' (Figures 7 and 8), which has not been observed in sister taxa. While *R. chapmanii* superficially resembles *Hapalosiphon*, the branching pattern and growth is very different. Thus, we are confident that

this morphological autapomorphy warrants the erection of a new genus as advocated by others (e.g., Johansen and Casamatta 2005, Komarek and Mares 2012, Lamprinou *et al*. 2012)

An analysis of the 16S rRNA sequence places *Reptodigitus* as sister to *Fischerella* and *Nostochopsis*. While *Reptodigitus* clearly falls within the Stigonematales morphologically and genetically, poor bootstrap support indicates that many of the sister taxa in this lineage may not have been identified or sequenced thus far. The 16S-23S ITS secondary folding patterns suggest that *R. chapmanii* is related, but distantly, to the poorly defined genera *Fischeralla, Westiellopsis*, and *Nostochopsis* (e.g., Kastovsky & Johansen 2008). For instance, the D1-D1' and Box-B helices were both fairly well conserved among the aforementioned taxa, possessing similar structures (i.e. side loops, apex loops, bulges, etc.), numbers of nucleotides, and basal pairings. The V3 helices, however, were highly variable between the four taxa, varying greatly in structure and number of nucleotides. Both the ITS and 16S rRNA sequence analyses suggest erecting a new genus to include our new strain. With further sampling and sequencing of Stigonematales the phylogenetic relationships can be more accurately determined.

Recent trends in cyanobacterial systematics have advocated the erection of smaller, clearly defined genera based on a polyphasic approach employing clear, stable apomorphies (e.g., Johansen and Casamatta 2005, Hoffman *et al*. 2005, Komarek 2010). In order to actualize this approach, we are employing the phylogenetic taxonomy concept of Mishler and Theriot (2000), which seeks to erect monophyletic genera with clear apomorphies employing morphological, ecological and/or molecular characters (e.g., Komarek *et al*. 2009, Perkerson *et al*. 2011, Casamatta *et al*. 2012, Engene *et al*. 2012). Given the unique mode of growth, 16S rDNA gene sequence and unique 16S-23S ITS secondary folding patterns we are unable to

assign this new taxon to any currently described genus. Thus, the erection of a new genus to encompass our new taxon is proposed.

Figure 2. Bayesian analyses of 16S rRNA gene sequence from 76 taxa. Node support is indicated as bootstrap support from parsimony analysis/Bayesian posterior probabilities/bootstrap support from distance analysis; "-" means support <0.50 or 50%.

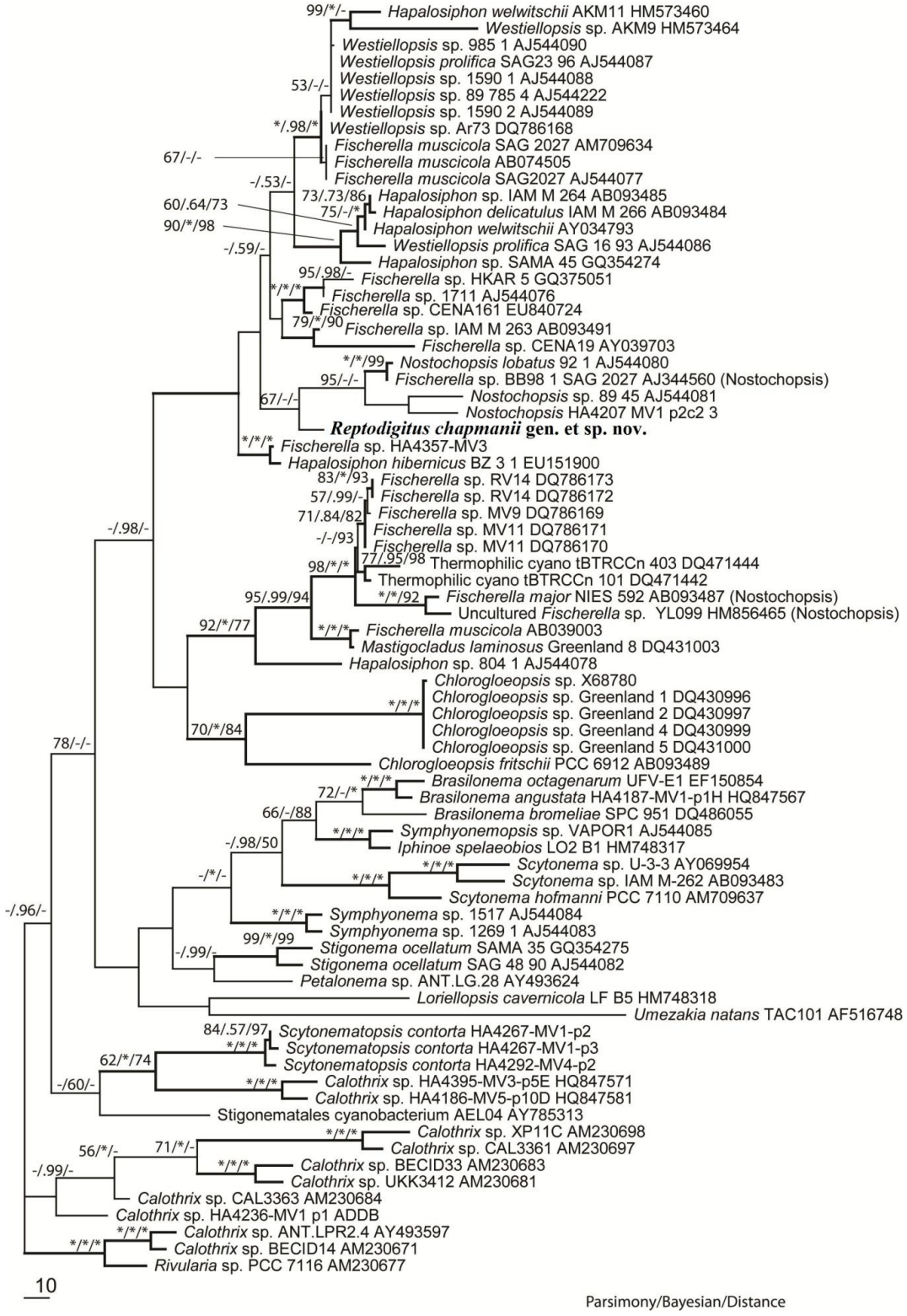

Figure 3. D1', BoxB, and V3 transcript secondary-structure helices sequenced from representative cyanobacterial taxa. (A-D) D1'. (E-H) BoxB. (I-J) V3.

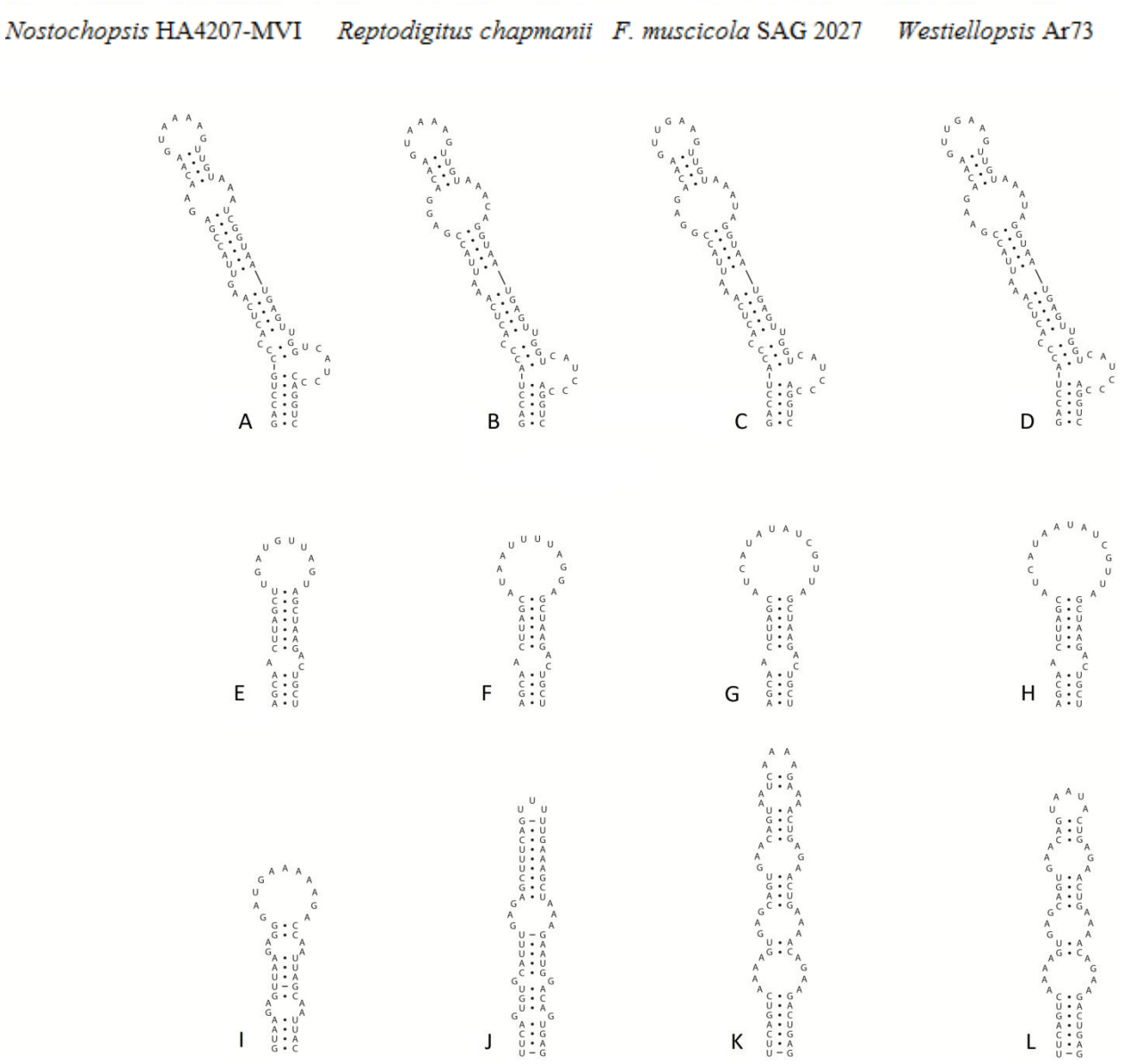

Figure 4. D1' transcript secondary-structure helices sequenced from representative cyanobacterian taxa. (A) *Nostochopsis* HA4207-MV1. (B) *Reptodigitus chapmanii* (C) *F. muscicola* SAG 2027. (D) *Westiellopsis* Ar73.

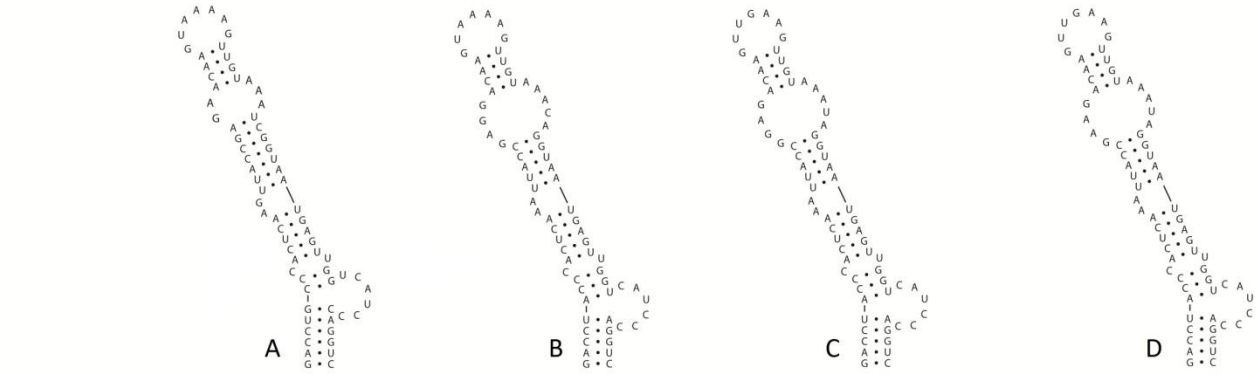

Figure 5. BoxB transcript secondary-structure helices sequenced from representative cyanobacterial taxa. (A) *Nostochopsis* HA4207-MV1. (B) *Reptodigitus chapmanii* (C) *F. muscicola* SAG 2027. (D) *Westiellopsis* Ar73.

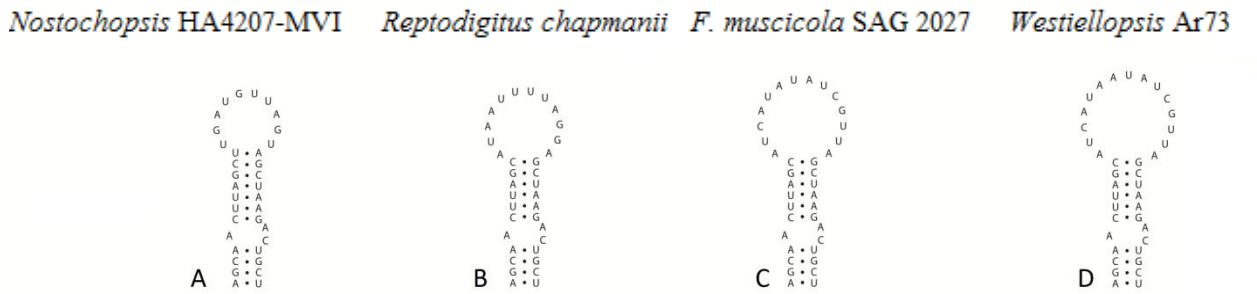

Figure 6. V3 transcript secondary-structure helices sequenced from representative cyanobacterial taxa. (A) *Nostochopsis* HA4207-MV1. (B) *Reptodigitus chapmanii* (C) *F. muscicola* SAG 2027. (D) *Westiellopsis* Ar73.

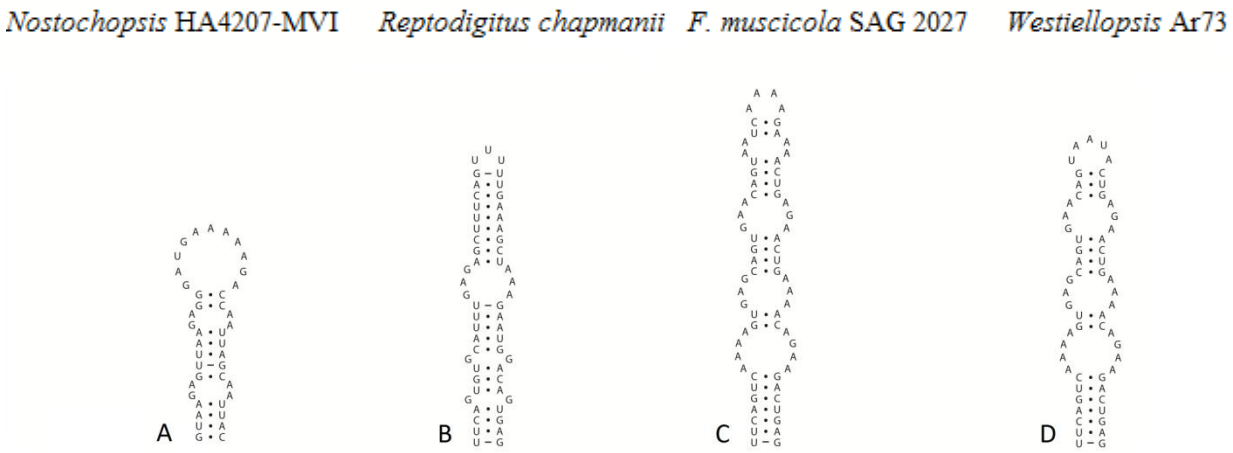

Figure 7. V-branching of *R. chapmanii* taken at 1000x magnification. Arrow points to branch-point cell.

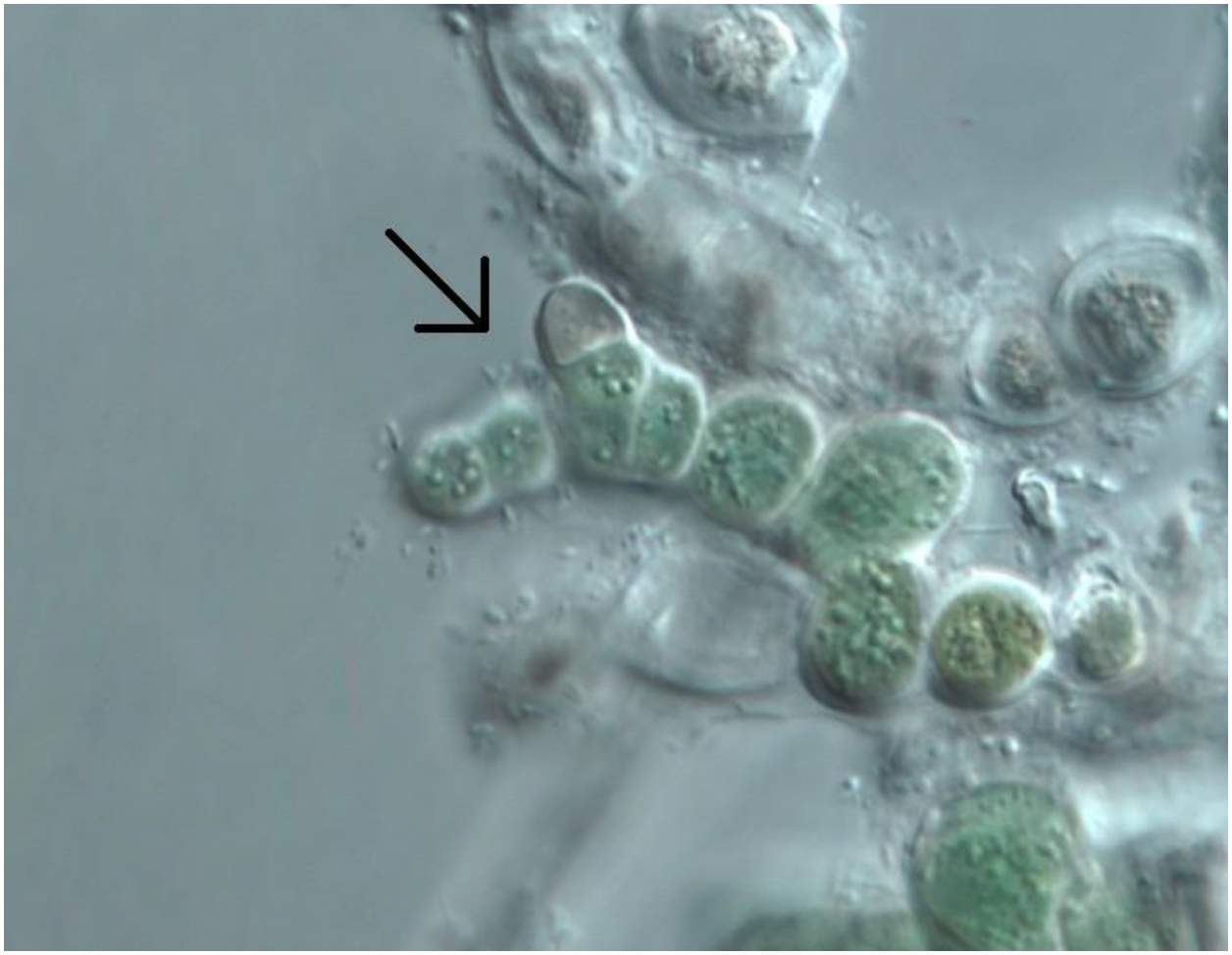

Figure 8. T-branching of *R. chapmanii* taken at 1000x magnification. Arrow points to branch-point cell.

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Chapter 3

An investigation into the factors which influence the epibenthic cyanobacterial community.

INTRODUCTION

Cyanobacteria play a major role in biogeochemical cycles of nitrogen and oxygen (Berman-Frank *et al*. 2005, Latysheva *et al.* 2012). Cyanobacteria are responsible for 30—40% of global oxygen production and are important components of aquatic ecosystems planetwide (Eigenbrode & Freeman 2006). Yet cyanobacteria are most often known for dominance in eutrophic systems as bloom-forming, often toxic species (Berg *et al*. 1987, Falconer 1998, Case *et al.* 2008).

Cultural eutrophication of economically and recreationally important freshwaters leads to many problems including reduced aesthetic value, light attenuation, mechanical issues from increased biomass and potentially toxic algal blooms (Paerl *et al*. 2011). To date, it is unclear which nutrient (nitrogen or phosphorus) is responsible for cyanobacterial blooms; classic and modern research has shown conflicting results with some researchers indicating nitrogen, others phosphorus, and still others suggest the ratio of nitrogen to phosphorus itself is the key (Vollenweider 1975, Schindler 1977, Huisman & Weissing 2001, Moore *et al*. 2002). For example, Hakanson *et al.* (2007) determined the relationship between cyanobacterial or chlorophyll *a* concentrations and the ratio of total nitrogen to total phosphorus was complex and in need of much more data to be reliably predicted. In general, though, it is agreed that polluted sampling sites have low species richness and excessive algal abundance, while unpolluted sites have high species richness and individual taxa in low abundance (Perona *et al.* 1998, Douterelo *et al.* 2003).

As the impact of cultural eutrophication becomes more apparent, researchers have turned to cyanobacteria as indicators of ecosystem health (Znachor *et al.* 2006, Fristachi et al. 2008, Wood *et al*. 2010). The cyanobacterial community displays great spatial and temporal heterogeneity, due to individual species' preference for environmental parameters and rapid generation times. Generally, individual cyanobacterial orders, such as Oscillatoriales and Nostocales, are dominant in differing nutrient concentrations. Oscillatorialles has a strong positive relationship with high nutrient concentrations, particularly soluble reactive phosphate; a similar relationship has been found between Chroococcales and increased nitrogen and phosphate concentrations (Douterelo *et al.* 2003, Kruskopf & Plessis 2005, Pulina *et al.* 2011, Salmaso 2011). Conversely, taxa belonging to the Nostocales are negatively related to nutrient concentrations (Douterelo *et al.* 2003, Rejmankova & Komarkova 2005). It is noted that these are general relationships, and orders may contain species that display great variability with nutrient concentrations.

Biological indicators of ecosystem health, such as diatoms, fish, invertebrates, phytoplankton, etc., have been explored and met with variable success (e.g., Kelly 1998, Soto-Galera *et al.* 1999, Oertli 2008, Romo *et al.* 2008). However, inherent limitations to using these organisms include seasonal or otherwise migratory species, taxonomic difficulties, and a lack of species appearances with ecological parameters. Several characteristics of algae make them useful as a bioindicator: their ubiquity, year-round presence, rapid generation times, sessile nature, and ease of identification to broad taxonomic group (e.g., Reynolds 1984, Cattaneo 1987, Carrick, Lowe, & Rotenberry 1988, Lowe & Pan 1996, Kelly 1998).

Some studies have suggested that the epipelic communities are not useful indicators of trophic state, in part because a large amount of planktic species can be found in the upper littoral

sediment layers (Poulickova *et al*. 2004, Kelly 2006, Poulickova *et al.* 2008b). Methods to separate the planktic species from the epipelic species were created by Round (1953). The methods used in this study are an adaptation of Round (1953), where negative pressure is used to collect the top few centimeters of sediment. The sediment is then transferred to a container and, after a period of time, excess water is siphoned off. A piece of water-permeable cloth is placed on top of the sediments and cover slips are placed on top of the cloth. This operates on the principle that phototaxic epipelic cyanobacteria will migrate and adhere to the cover slips, which can then directly be used in microscopy. The epipelic community is generally difficult to separate from the total microalgal community, although it has been agreed that using water permeable cloths to collect epipelic algae is an efficient means of separation (Round 1981, Spears *et al.* 2010, Poulickova *et al.* 2008a). These methods are a popular means of assessing the microalgal community (Lysakova *et al.* 2007, Hasler *et al.* 2008, Mann *et al*. 2008, Spackova *et al.* 2009).

Another step to understanding the relationship between nutrient concentrations and cyanobacterial community dynamics is an experimental manipulation of nutrients in enclosed systems. Knowledge of how the cyanobacterial community responds to environmental changes, especially anthropogenic ones, can be a powerful tool in management of recreational and residential waters (Steffensen *et al*. 1999). With full development of this tool, it should be possible to monitor aquatic habitats and accurately identify a system that is changing trophic state. This study is one more resource that may be used to develop a reliable method of observation of cyanobacterial communities and correlation with nutrient levels, thus enabling more effective management of aquatic systems.

METHODS AND MATERIALS

Sediment collection and processing

Sediment was collected from five ponds around UNF campus, Jacksonville, Duval County, Florida (Table 1). Ca. 9.5 l of sediment was collected with a shovel from each pond (total of 47 l) and formed the bulk of the mesocosms. One liter of the top layer (no deeper than 2.5 cm.) of sediment from each pond (total of 5 l) was collected using PVC pipe (1.27 cm diameter) and negative pressure (*sensu* Round 1953). The bulk sediments from each pond were mixed together and distributed into 20, 19 l buckets. Similarly, the top layer sediments from each pond were mixed and distributed evenly amongst the same buckets. To maintain a natural light cycle for Florida, buckets were kept in the UNF greenhouse at a constant 22°C. Ca. 20 cm of DI water was also maintained on each bucket. Water samples from each pond, as well as weekly from each treatment, were analyzed for measurements of nitrogen (NO_3-N) and phosphate (PO_4^{2-}) (LaMotte Smart2 colorimeter), and chlorophyll *a* (Turner Designs colorimeter) (Tables 1 and Appendix 3, respectively).

Nutrient additions and sample processing

In the nutrient enriched treatments, ca. 12 cm lengths of PVC pipe with 1 cm holes drilled into them were filled with 50 g of OsmocoteTM slow release fertilizer and suspended in the water approximately 10 cm above the sediment (Figure 9) (Bucolo, Sullivan, & Zimba 2008, Heck *et al.* 2000). Previous laboratory experiments on OsmocoteTM dissolution rates show that the fertilizer releases an initial burst of nutrients, followed by a relatively constant release rate; the laboratory and *in situ* dissolution estimates agreed within ±10% (Heck *et al.* 2000).

To determine the community composition of each treatment, as well as each pond individually, 50 ml of the top layer sediment, obtained by a pipette, was poured into petri dishes

and left to settle for 24 h. Afterwards, excess water was siphoned off. A piece of water-permeable cloth was placed on top of the sediment and three glass cover slips were placed on top of the cloth. The petri dishes were left to sit for another 24 h to allow time for cyanobacteria to migrate up onto the cover slips; after which the cover slips were placed on slides and examined under the microscope (400 cell counts per sample *sensu* Lund, Kipling, & LeCren 1958). The data were then analyzed for species richness, evenness, Simpson's diversity, and percent heterocytes.

Statistics

The 20 mesocosms were arranged in a randomized block design to eliminate any light or temperature gradients in the greenhouse. Means were compared using paired independent *t*-tests for nitrogen, phosphate, species richness, evenness, Simpson's diversity, chlorophyll *a*, total number of cells, and Dmax (the maximum diversity possible), between the control and the nutrient enriched treatments. In the case of nitrogen and phosphate, variances violated homogeneity requirements of ANOVA and were analyzed using the Wilcoxin Signed Rank Test. Correlations and linear regressions were performed on the following variables, nitrogen, phosphate, species richness, evenness, Simpson's diversity, and chlorophyll *a*. The following values were correlated with nitrogen and phosphate, respectively, species richness, Simpson's diversity, and evenness, among the treatments.

RESULTS

Mean chlorophyll *a* levels were significantly higher for the nutrient enriched treatment compared to the control group ($t = -5.183$, $df = 17$, $p<0.001$). Additionally, the mean number of cells per sample were also significantly higher for the nutrient addition treatment compared to

controls ($t = -2.937$, $df = 18$, $p<0.01$). Conversely, mean Dmax was significantly lower in the nutrient enriched treatment group compared to the control ($t = 2.849$, $df = 17$, $p<0.05$). Species richness, evenness, and diversity were not affected by nutrient addition ($t = 0.869, 0.429, 0.549$, $df = 17, 17, 17$, $p = 1$, respectively). For the chlorophyll *a* data, one outlier was discovered (>3sd from the mean) and was removed from the dataset and a t-test was performed (Table 2). No significant correlations between nitrogen or phosphate and chlorophyll *a*, species richness, total number of taxa, Simpson's diversity, Dmax, or evenness were found (Table 3). However, the nutrient enriched group showed slightly stronger correlations between nitrogen and Simpson's diversity, evenness, and species richness (Table 4). Likewise, linear regressions did not show a strong correlation between phosphate and species richness, total number of algal cells, or Simpson's diversity in either treatment (Appendix 4a-f). Linear regressions of nitrogen showed similar results, although nitrogen and evenness, and nitrogen and Simpson's diversity were more strongly correlated in the nutrient enriched group than in the control group ($r^2 = 0.52$ and $r^2 = 0.53$, respectively) (Appendix 4a-f). Two *Anabaena* species were found with heterocytes; overall, these two species increased in abundance in the control group, while the average number of heterocytes per filament increased greatly in the second to last week (Figures 10 and 11). Two replicates in the control group contributed the most to the increase in species abundance and heterocytes; individuals in these replicates had as many as ten heterocytes, while most filaments contained between one and four heterocytes.

DISCUSSION

This study both contradicts and supports previous experiments. For example, Perona *et al.* (1998) observed a decrease in species richness, abundance, and diversity as soluble reactive

phosphate increased, and found no relationship with nitrogen. This study found a similar relationship between phosphate and species richness, and a very slight negative relationship between phosphate and diversity in both treatments. Species richness, diversity, and evenness all displayed positive relationships with nitrogen, however previous research found no relationship (Perona *et al.* 1998, Douterelo *et al.* 2003). This may be due in part to nitrogen and phosphate levels between treatments (i.e., nitrogen levels in the nutrient enriched treatments averaged over 250x the levels in the control treatment, while phosphate levels in the nutrient enriched treatments averaged 2.5x those of the control group, thus the increase in phosphate may not have been enough to elicit a response). Chlorophyll *a* levels were highly significantly different between treatments. The nutrient enriched treatment averaged about double the chlorophyll *a* levels of the control; this generally agrees with other studies that have used chlorophyll *a* as a proxy for algal biomass (Barica 1993, Cano et al. 2008, Boyer et al. 2009). Pseudo-bloom conditions in two replicates of the control treatment are responsible for the majority of the increase in species abundance and concurrent increase in number of heterocytes. *Anabaena* spp. were recorded in all control replicates and the majority of the nutrient enriched treatments, though abundances varied among treatments and species.

Lake productivity in oligotrophic waters has been found to be heavily impacted by sediment associated communities (Libouriussen & Jeppesen 2003, Casco et al 2009). Even so, some authors are skeptical as to the capacity of sediment microalgae to indicate trophic level (Poulickova *et al.* 2004, Poulickova *et al.* 2008b, Kelly 2006). Lake sediments are known nutrient sinks, with a greater concentration of nutrients and less variability than in the water column, particularly for phosphorus (Rooney & Kalff 2003, Sondergaard *et al.* 2003, Casco *et al.* 2009). Indeed, in Lacombe Lake, total phosphorus concentrations in sediments were as much as

10 times the concentrations in the water column; variation in sediment chlorophyll *a* and total phosphorus concentrations in water were not significantly correlated (Casco *et al*. 2009). Nutrients in the water column may be sequestered by sediment-dwelling microalgae for growth, thus decreasing the water column nutrient concentrations and trapping those nutrients within the sediments (Wetzel 2001, Dodds 2003). Conversely, phytoplanktic *Gloeotrichia echinulata* blooms may move nutrients from sediments into the water, which could accelerate eutrophication in oligotrophic lakes (Carey et al. 2008).

The true diversity of epipelic microalgae is largely unknown and, with the continued discoveries of cryptic diversity, difficult to elucidate (Poulickova *et al.* 2013). Increasing use of molecular methods coupled with morphological means of identification has led to the discovery of many new species (e.g., Hasler et al. 2012). For example, analysis of three epipelic species of *Sellaphora* revealed species complexes that contained many morphologically similar, yet reproductively isolated and genetically different species (Mann *et al.* 2008, Evans *et al.* 2009). Similarly, the cyanobacterium *Microcoleus vaginatus* displays cryptic diversity between strains from desert soil crusts and strains from the epipelon, whereas *Phormidium autumnale* is morphologically and genetically similar between strains from Europe and strains from the Arctic and Antarctic (Strunecky *et al.* 2010, Hasler *et al.* 2012, Poulickova *et al.* 2013).

It is important to keep in mind that a general model for prediction of nutrient concentrations by cyanobacterial communities is inherently simple and as such, should be adapted to the individual environment of interest. For example, lakes may experience alternative states of equilibrium, where temporal and spatial variability, such as in the total phosphorus to chlorophyll *a* ratio, are heavily regulated by growth cycles of macrophytes (Casco *et al.* 2009). Sediment quality also appears to influence cyanobacterial distribution (Hasler *et al.* 2008).

Creating the model for bioindication of trophic level is only the first step; much more difficult is to apply the model to environments while taking into account the various adaptations to the model each environment necessitates. In the grand scheme of things, the true diversity of cyanobacteria is unknown, as well as exactly how the community responds to environmental flux. In order to elucidate this information, there is a strong need for more surveys, more experiments, and more genetic sequencing.

Table 1. Environmental parameters for the five UNF campus ponds from which sediment was taken.

Pond	T°C	DO	Chl *a*	Nitrogen	Phosphate	pH	GPS
Building 52	16.2	4.6	7.3	1.6	2.94	8	30°15'33.31"N 81°30'17.34"W
The Village	16.8	5.4	8	0.21	0.06	8	30°16'0.76"N 81°30'32.08"W
Lot 14	16.5	10.7	6.6	0.21	0.04	7	30°16'9.77"N 81°30'47.56"W
Lot 18	17.6	9.1	5.4	0.16	0.1	7	30°16'50.47"N 81°30'38.31"W
Engineering building	15.3	8.5	6.7	0.26	0.1	7	30°16'20.29"N 81°30'26.43"W

Table 2. Means and standard errors from t-test for treatment differences. C = control, NE = nutrient enrichment, s = species richness, N = total number of algal cells, Ds = Simpson's diversity, Dmax = maximum value of Simpson's diversity, Chl *a* = chlorophyll *a*; * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

Treatment	**s**	**N**	**Evenness**	**Ds**	**Dmax**	**Chl *a***
C	14.34 ± .47	666.24 ± 40.51	0.55 ± 0.03	0.52 ± 0.03	0.92 ± 0.003	15.05 ± 1.84
NE	13.79 ± .44	917.56 ± 75.36**	0.54 ± 0.18	0.50 ± 0.02	0.91 ± 0.002*	40.26 ± 4.72***

Table 3. Correlation table for the control group showing correlation coefficients. s = species richness, N = total number of algal cells, Ds = Simpson's diversity, Dmax = highest possible value for Simpson's diversity.

	Nitro.	**Phos.**	**Chl *a***	**s**	**N**	**Ds**	**Dmax**	**Evenness**
Nitro.	1							
Phos.	-0.08502	1						
Chl *a*	-0.17451	-0.05607	1					
s	0.379189	-0.06034	-0.16869	1				
N	0.256826	-0.08424	-0.19105	-0.03428	1			
Ds	0.316648	0.006877	0.023479	0.731285	-0.32632	1		
Dmax	0.373377	-0.0218	-0.08405	0.927929	-0.10573	0.734631	1	
Evenness	0.314513	0.012259	0.032431	0.711352	-0.33175	0.999448	0.721198	1

Table 4. Correlation table for the nutrient enriched group showing correlation coefficients. s = species richness, N = total number of algal cells, Ds = Simpson's diversity, Dmax = highest possible value for Simpson's diversity.

	Nitro.	**Phos.**	**Chl *a***	**s**	**N**	**Ds**	**Dmax**	**Evenness**
Nitro.	1							
Phos.	-0.01467	1						
Chl *a*	-0.00269	0.107712	1					
s	0.664569	-0.35935	-0.14851	1				
N	0.224368	-0.30129	-0.25932	0.56873	1			
Ds	0.732473	-0.06773	-0.10192	0.832372	0.265864	1		
Dmax	0.643106	-0.35365	-0.06027	0.931075	0.478169	0.794076	1	
Evenness	0.727431	-0.03237	-0.09503	0.80355	0.238651	0.998454	0.765426	1

Figure 9. Nutrient enriched treatments received a PVC pipe (1.27 cm x 20 cm) filled with 50 g of Osmocote brand plant fertilizer and capped using parafilm.

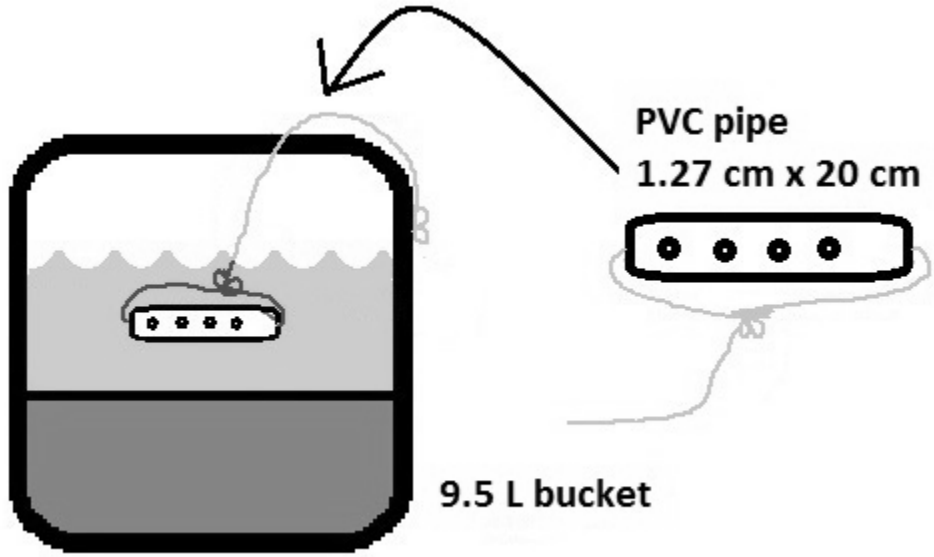


Figure 10. Filament count of two *Anabaena* species over a 24 d period in the control and nutrient enriched treatments. C = control, NE = nutrient enriched.

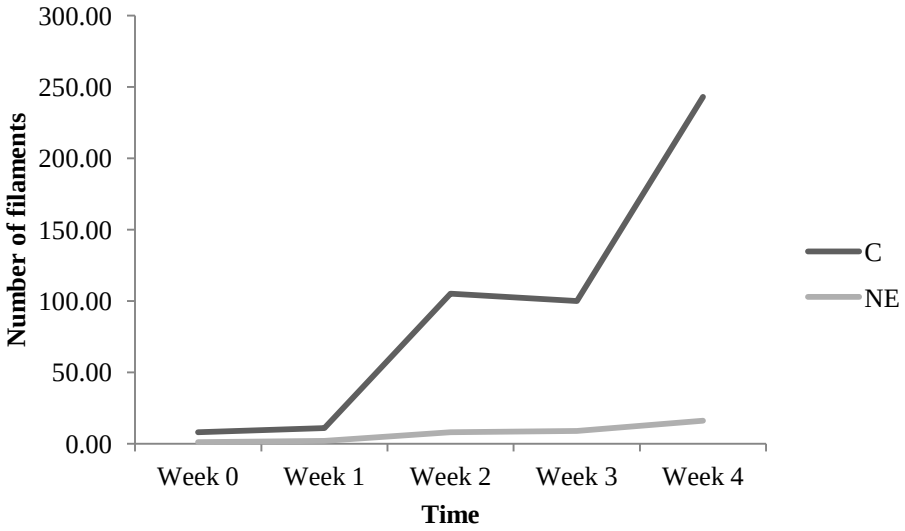
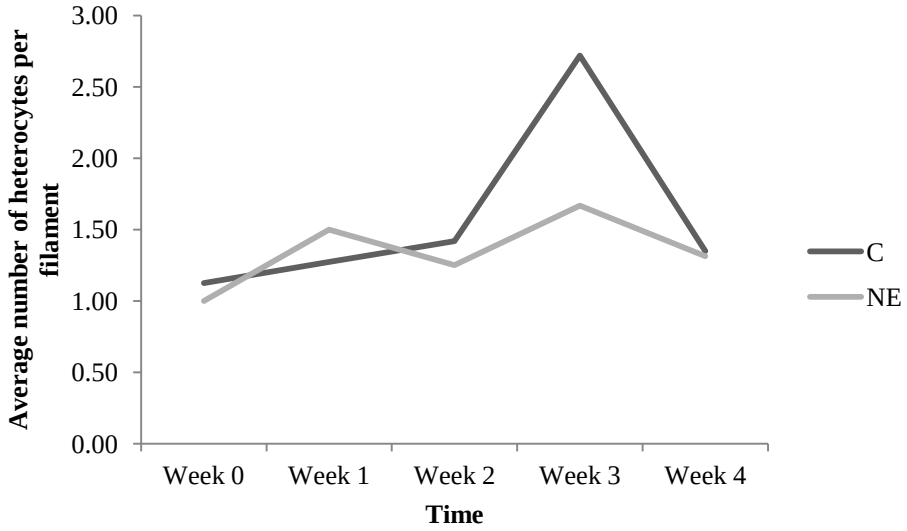


Figure 11. Average number of heterocytes per filament of two *Anabaena* species over a 24 d period.



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Appendix 1. Description of sites, including specific habitats sampled, county, and GPS coordinates. Sampling sites are located in the northern half of Florida, USA, and are comprised of springs, lakes, retention ponds, and rivers.

Site	Type	Habitat(s) sampled	County	GPS
Ocean pond	Lake	Benthos	Baker	30°14'14.53"N 82°25'38.74"W
Commador Point	Retention pond	Benthos	Clay	30° 6'32.55"N 81°42'59.04"W
Fiddler's Ridge	Retention pond	Benthos	Clay	30° 6'31.26"N 81°42'52.27"W
Forest Park	Retention pond	Benthos	Clay	30° 6'45.76"N 81°43'9.83"W
Water Park Pond	Retention pond	Benthos	Clay	30° 6'22.65"N 81°42'48.25"W
Tennis Court	Retention pond	Benthos	Clay	30° 6'20.19"N 81°42'32.11"W
O'Leno State Park	Spring	Benthos, epiphyton, metaphyton	Columbia	29°55'31.43"N 82°34'49.93"W
Ichetucknee State Park	River	Benthon, epilithon, epiphyton, metaphyton	Columbia	29°58'5.65"N 82°45'57.57"W
Jarbo Park	Retention pond	Benthos	Duval	30°18'55.71"N 81°23'56.94"W
Selva Marina Country Club	Retention pond	Benthos	Duval	30°20'25.55"N 81°24'18.61"W

Jack Russel Park	Stagnant channel	Benthos	Duval	30°19'50.58"N 81°24'9.52"W
Huguenot Park	Retention pond	Benthos	Duval	30°16'21.36"N 81°23'16.36"W
Sunshine Park	Retention pond	Benthos	Duval	30°15'57.10"N 81°23'43.73"W
Crossroad Church	Retention pond	Benthos	Duval	30°15'31.02"N 81°32'24.95"W
Crossroad Church 2	Retention pond	Benthos	Duval	30°15'29.13"N 81°32'27.41"W
Merrill Lynch	Retention pond	Benthos	Duval	30°15'37.03"N 81°33'1.36"W
Premier Park	Retention pond	Benthos	Duval	30°15'11.27"N 81°33'20.50"W
Aventine	Retention pond	Benthos	Duval	30°15'25.23"N 81°32'18.06"W
Lake Oneida	Lake	Benthos	Duval	30°15'57.05"N 81°30'48.15"W
Fanning Springs State Park	Spring	Epilithon, epiphyton, plankton	Levy	29°35'13.89"N 82°56'8.23"W
Madison Blue Springs State Park	Spring	Epiphyton, plankton	Madison	30°28'39.71"N 83°14'39.81"W
Lake Rosalie	Lake	Epilithon, epiphyton, plankton	Polk	27°56'42.28"N 81°23'0.36"W
Kissimmee Canal	Slow-flowing canal	Benthos, epiphyton, metaphyton	Polk	27°56'38.71"N 81°22'28.10"W

Suwannee State Park	River	Benthos, epilithon, epiphyton, plankton	Suwannee	30°23'4.81"N 83°10'12.04"W
Manatee Springs State Park	Spring	Epilithon, epiphyton, metaphyton, plankton	Volusia	29°29'59.80"N 82°58'12.00"W

Appendix 2. Taxa found in each habitat type. SP = State Parks, RP = retention ponds, SJR = St. Johns River.

Taxa	SP	RP	SJR
Chroococcales (total of 40 taxa)			
Aphanocapsa cf. *delicatissima*		X	X
Aphanocapsa holsatica			X
Aphanocapsa incerta		X	
Aphanocapsa sp.		X	X
Aphanothece microscopica		X	
Aphanothece sp.			X
Aphanothece stagnina			X
Chamaesiphon sp.		X	
Chamaesiphon minutus		X	
Chondrocystis deromochroa		X	
Chroococcidiopsis sp.		X	
Chroococcus limneticus		X	X
Chroococcus cf. *minutus*		X	X
Chroococcus prescottii	X		
Chroococcus obliteratus		X	
Chroococcus sp.			X
Chroococcus turgidus		X	X
Coelomoron pusillum		X	
Coelomoron sp.		X	
Coelosphaerium aerugineum		X	
Coelosphaerium kuetzingianum			X
Cyanogranis ferruginea		X	
Cyanothece aeruginosa	X		
Dactylococcopsis sp.			X
Eucapsis minor		X	
Eucapsis parallelepipedon		X	
Gomphosphaeria lacustris			X

Gomphosphaeria sp.			X
Mantellum sp.		X	
Merismopedia elegans		X	X
Merismopedia glauca	X	X	
Merismopedia punctata	X	X	
Merismopedia spp.			X
Merismopedia tenuissima		X	X
Microcystis aeruginosa			X
Synechococcus sp.		X	
Synechocystis aquatilis		X	
Synechocystis sp.		X	
Microcystis sp.		X	
Xenococcus sp.	X		
Total number	5	27	17

Oscillatoriales (total of 87 taxa)

Arthrospira jenneri		X	
Coleofasciculus chthonoplastes		X	
Geitlerinema acutissimum		X	
Geitlerinema cf. *acus*	X		
Geitlerinema amphibium	X	X	
Geitlerinema carotinosum	X		
Geitlerinema lemmermannii		X	
Geitlerinema splendidum	X	X	
Geitlerinema pseudacutissimum		X	
Geitlerinema spp.	X	X	
Geitlerinema unigranulatum		X	
Heteroleibleinia sp.	X		
Homeothrix sp.			X
Homeothrix stagnalis			X
Jaaginema spp.	X	X	

Johannesbaptista sp.		X	
Johansenia constricta	X	X	
Johansenia pseudoconstricta	X	X	
Komvophoron hindaki	X	X	
Komvophoron schmidlei		X	
Komvophoron minutum	X	X	
Komvophoron spp.	X	X	
Leibleinia epiphytica		X	
Leibleinia cf. *epiphytica*		X	
Leptolyngbya boryana			X
Leptolyngbya halophila		X	
Leptolyngbya lagerheimii			X
Leptolyngbya cf. *protospira*		X	
Leptolyngbya spp.	X	X	X
Leptolyngbya subtilis			X
Leptolyngbya tenuis			X
Limnothrix cf. *mirabilis*		X	
Lyngbya birgeii			X
Lyngbya confervoides		X	
Lyngbya contorta			X
Lyngbya hieronymussi		X	
Lyngbya maior		X	
Lyngbya cf. *martensiana*		X	
Lyngbya meneghiniana		X	
Lyngbya salina		X	
Lyngbya semiplena		X	
Lyngbya sordida		X	
Lyngbya spp.			X
Microcoleus spp.		X	
Microcoleus vaginatus		X	
Oscillatoria anguina			X

Oscillatoria curviceps		X	
Oscillatoria froelichii		X	
Oscillatoria limosa	X	X	
Oscillatoria lloydiana		X	
Oscillatoria margaritifera		X	
Oscillatoria minata		X	
Oscillatoria nigro-viridis		X	
Oscillatoria princeps		X	
Oscillatoria sancta	X		
Oscillatoria simplicissima		X	
Oscillatoria spp.	X		X
Phormidium animale		X	
Phormidium autumnale		X	
Phormidium chalybeum		X	
Phormidium chlorinum		X	
Phormidium aerugineo-caruleum		X	
Phormidium formosum		X	
Phormidium subfuscum		X	
Phormidium terebriforme		X	
Phormidium tergestinum	X	X	
Phormidium spp.	X	X	X
Planktolyngbya contorta			X
Planktolyngbya limnetica			X
Planktothrix agardhii		X	
Pseudanabaena catenata	X	X	
Pseudanabaena galeata	X	X	X
Pseudanabaena galeata Fe3+	X	X	
Pseudanabaena limnetica	X	X	X
Pseudanabaena lonchoides		X	
Pseudanabaena minima		X	
Pseudanabaena spp.	X	X	X

Schixothrix calcicola			X
Spirulina labyrinthiformis		X	
Spirulina major		X	
Spirulina cf. *major*		X	
Spirulina spp.	X		
Spirulina subsalsa		X	
Spirulina cf. *subsalsa*	X		
Synechocystis aquatilis		X	
Synechocystis sp.		X	
Tychonema bornetii		X	
Total Number	25	67	19

Nostocales (total of 14 taxa)

Anabaeba fuscovaginata	X		
Anabaena planctonica		X	
Anabaena spp.	X	X	X
Calothrix fusca		X	
Calothrix epiphytica			X
Calothrix spp.		X	X
Cylindrospermopsis raciborskii		X	
Cylindrospermum sp.			X
Nostoc carneum		X	
Nostoc spp.		X	
Scytonema coactile		X	
Scytonema crispum		X	
Tolypothrix spp.		X	X
Trichormus variabilis	X	X	
Total number	3	11	5

Stigonematales (total of 4 taxa)

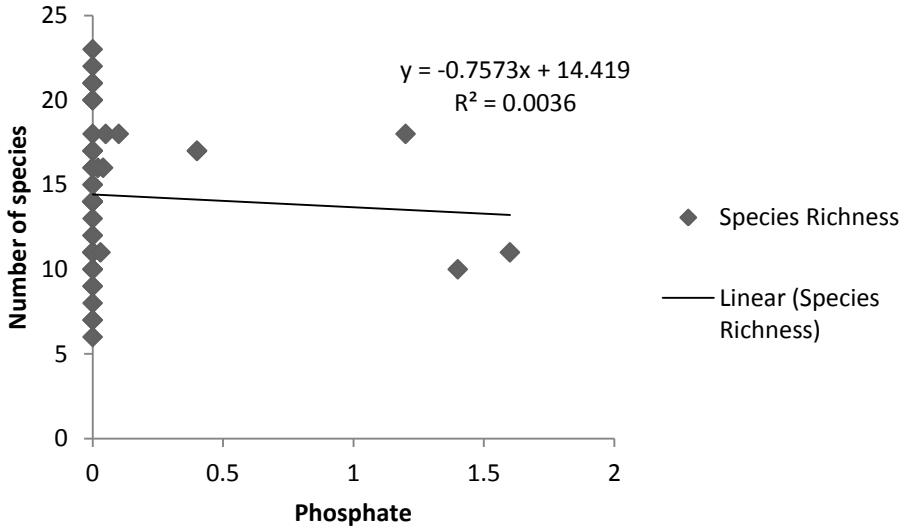
Fischerella spp.		X	

Hapalosiphon spp.		X	
Reptodigitus chapmanii		X	
Stigonema spp.	X		X
Total number	1	3	1
Synechococcales (total of 1 taxon)			
Synechococcus sp.			X
Total number	0	0	1
Total number of taxa	34	108	43

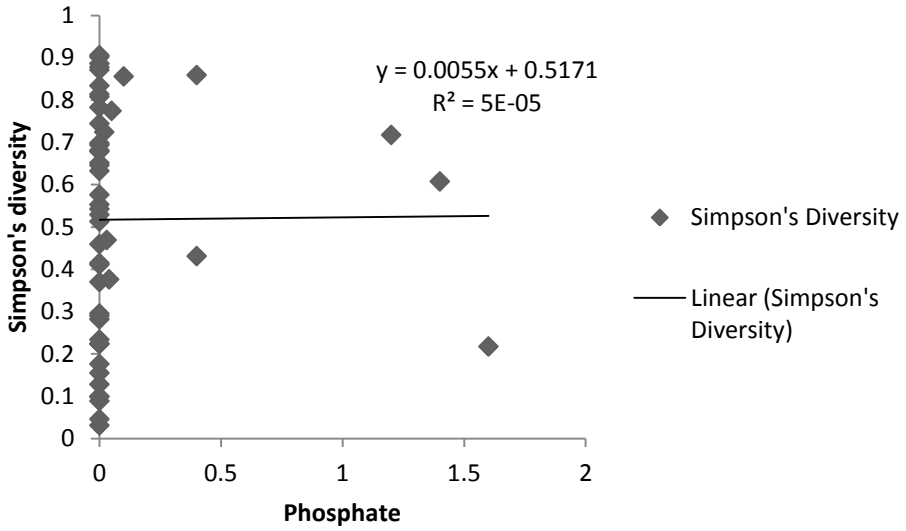
Appendix 3. Weekly values for nitrogen (N), phosphate (P), and chlorophyll *a*.

	Week 0			Week 1			Week 2			Week 3			Week 4		
Treatment	**N**	**P**	**Chl *a***	**N**	**P**	**Chl *a***	**N**	**P**	**Chl *a***	**N**	**P**	**Chl *a***	**N**	**P**	**Chl *a***
C	0	0	18	0	1.4	11	0.1	0	18	0.4	0.4	7.6	0.1	0	8.6
C	0	0	17	0.1	0	114	0.3	0	8.3	0.2	0.4	5.5	0.2	0.1	7.8
C	0	0	9.4	0.1	0	11	0.3	0	7.1	0.1	0	8.6	0.2	0	12
C	0	0	13	0.1	0	22	0.3	0	8.2	0.1	0	7.2	0.2	0.1	11
C	0	0	17	0	0	23	0.3	0	13	0.1	1.2	8.8	0.2	0	15
C	0	0	11	0	1.6	19	0.4	0	11	0	0	12	0.1	0	13
C	0	0	15	0	0	20	0.4	0	11	0.1	0	9.9	0.1	0	11
C	0	0	15	0	0	34	0.1	0	9.7	0.1	0	13	0.2	0	15
C	0	0	11	0	0	20	0.2	0	15	0.2	0	10	0.1	0	12
C	0	0	10	0	0	20	0.3	0	9	0.1	0	11	0	0	12
NE	0	0	10	15	1.4	107	9.4	0	430	28	0.2	15	22	0	15
NE	0	0	17	17	3.1	82	22	0	24	30	1.2	29	28	0	15
NE	0	0	11	16	3.2	78	30	0	76	23	0.9	17	20	0.1	17
NE	0	0	9.4	14	2.4	60	20	0.6	136	24	2.3	7.1	46	0.1	19
NE	0	0	11	19	4.2	49	30	1.4	86	22	0.6	30	35	0	17
NE	0	0	13	17	0.5	37	22	0.4	65	27	0	39	37	0	25
NE	0	0	11	15	2	50	30	1.1	15	23	1.1	24	44	0	28
NE	0	0	12	15	2	88	27	0	9.9	21	0.2	28	35	0	33
NE	0	0	9.2	13	0.8	48	30	0	49	20	0.4	37	44	0	26
NE	0	0	15	22	2.6	62	30	0.3	206	20	0.8	49	35	0	43

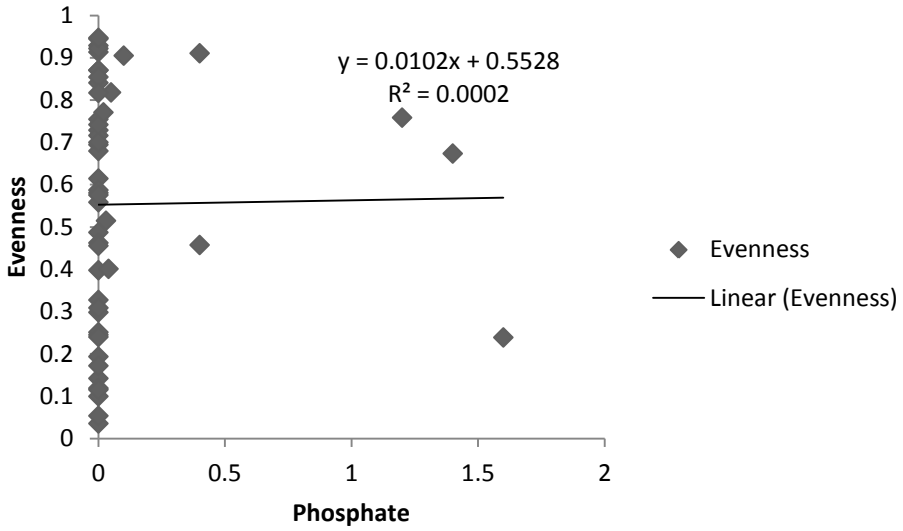
Appendix 4a. Linear regression for phosphate and species richness in the control group.



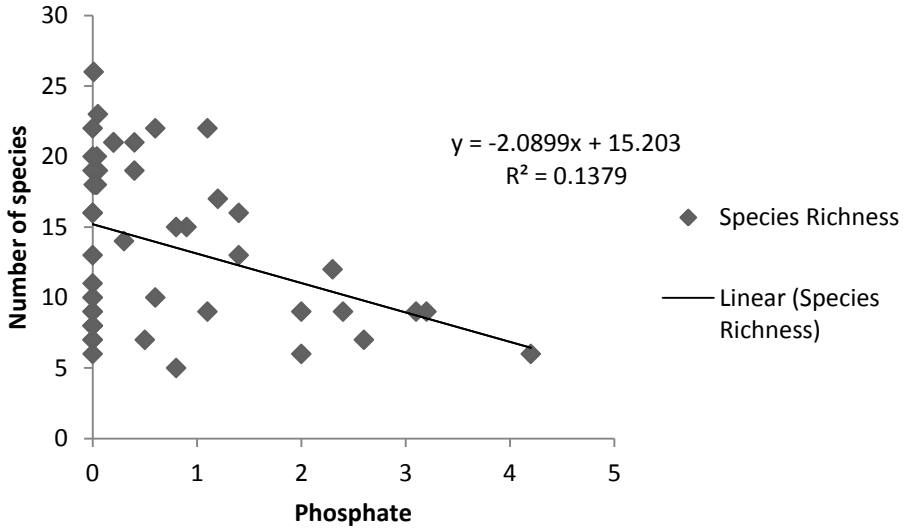
Appendix 4b. Linear regression for phosphate and Simpson's diversity in the control group.



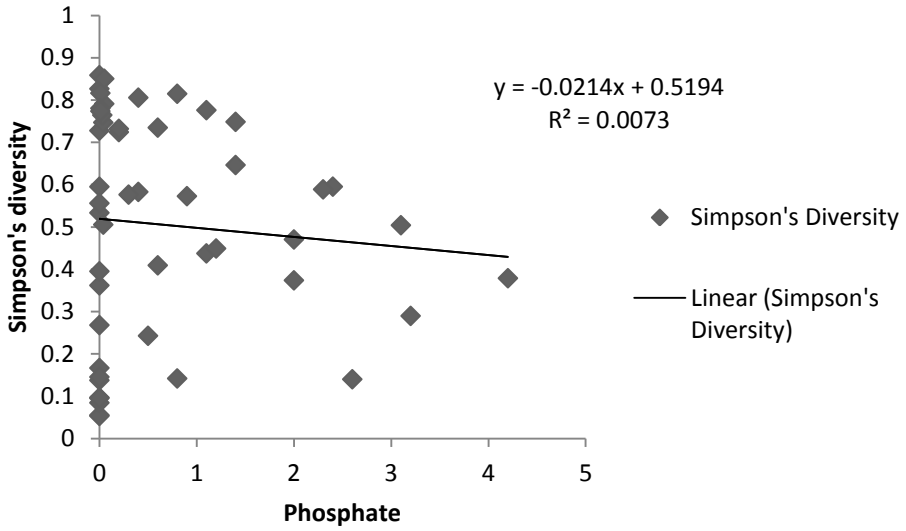
Appendix 4c. Linear regression for phosphate and evenness in the control group.



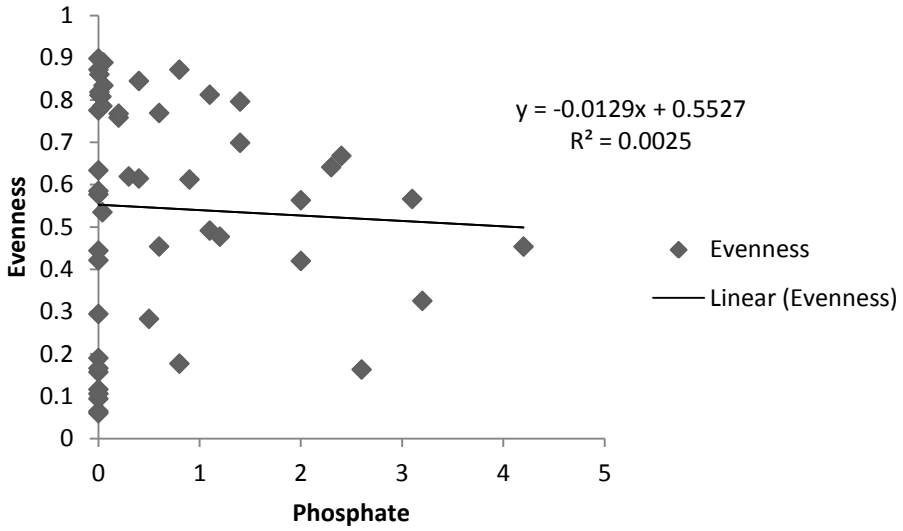
Appendix 4d. Linear regression for phosphate and species diversity in the nutrient enriched group.



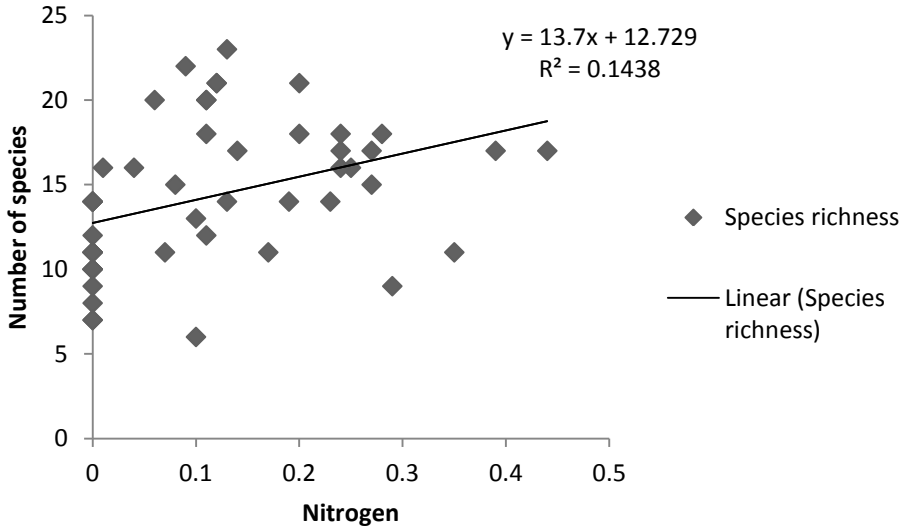
Appendix 4e. Linear regression for phosphate and Simpson's diversity in the nutrient enriched group.



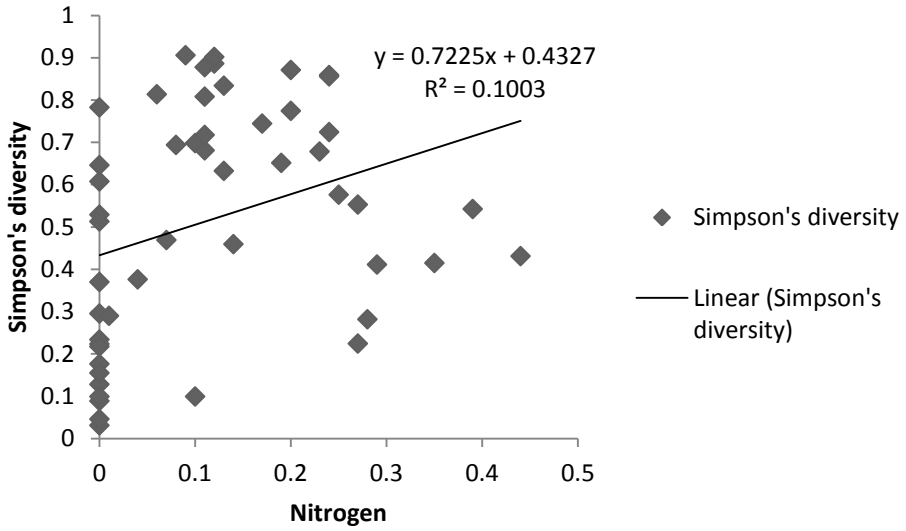
Appendix 4f. Linear regression for phosphate and evenness in the nutrient enriched group.



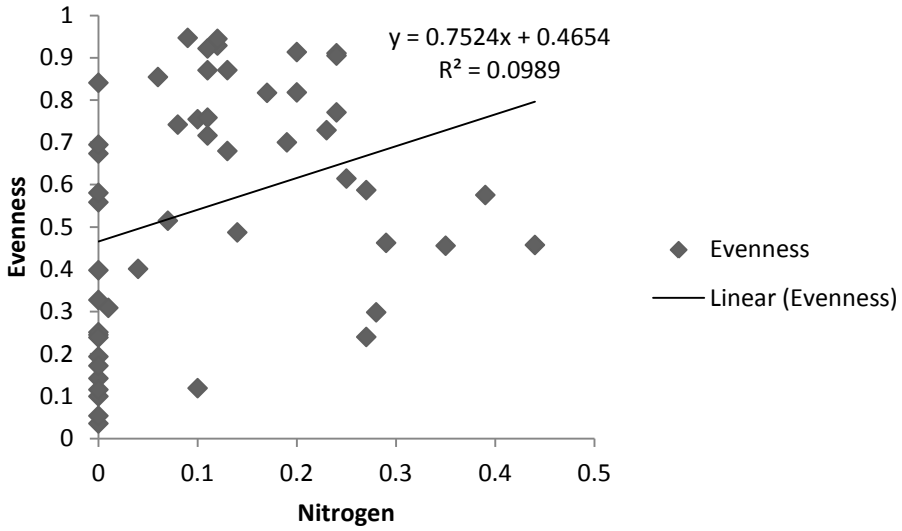
Appendix 5a. Linear regression for nitrogen and species richness for the control group.



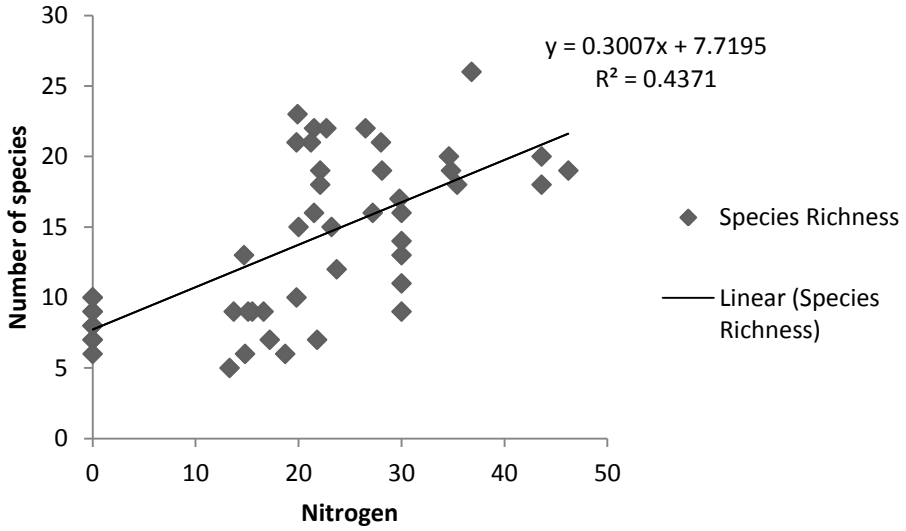
Appendix 5b. Linear regression for nitrogen and Simpson's diversity in the control group.



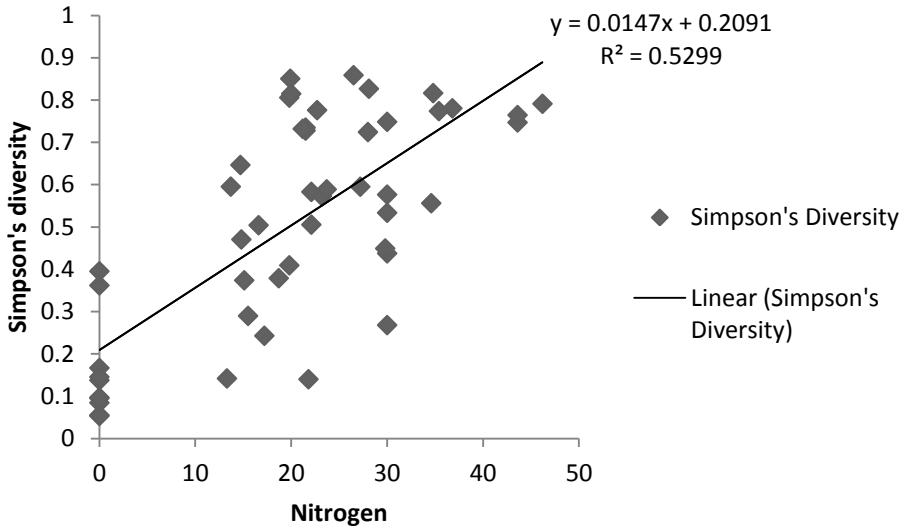
Appendix 5c. Linear regression for nitrogen and evenness in the control group.



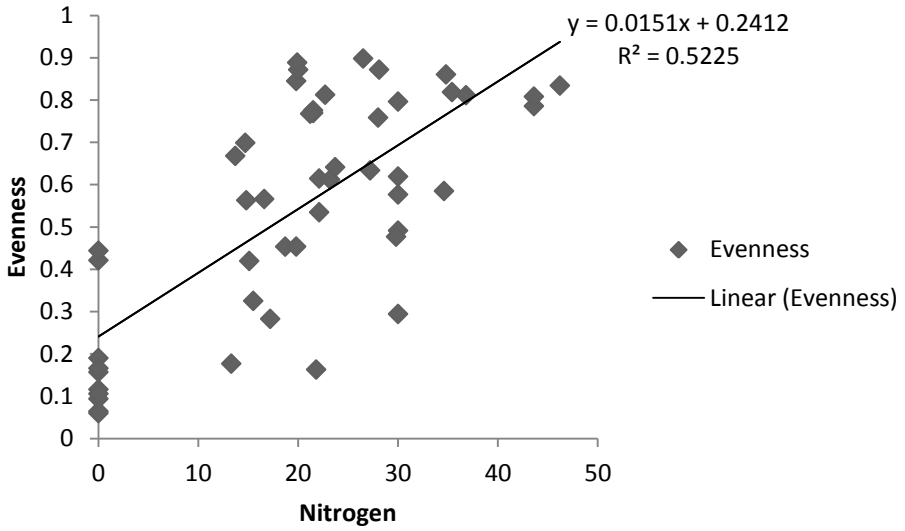
Appendix 5d. Linear regression for nitrogen and species richness in the nutrient enriched group.



Appendix 5e. Linear regression for nitrogen and Simpson's diversity in the nutrient enriched group.



Appendix 5f. Linear regression for nitrogen and evenness in the nutrient enriched group.



Curriculum Vitae
Holly Stocks

Masters Student
Department of Biological Sciences
University of North Florida

Education:

M.S. Biology – In progress
University of North Florida, Jacksonville, Florida
Thesis: Chapter 1 Survey of the α-level taxonomy of the cyanobacteria from North Florida

B.S. Biology – 2009
Western Carolina University, Cullowhee, North Carolina

Professional Experience:

- Graduate Teaching Assistant, University of North Florida, 2011
- Microbial Biology Lab, University of North Florida, 2012, 2013

Organizational Memberships:

Phycological Society of America

Teaching Experience:

General Biology I, University of North Florida

Talks/Presentations:

H.S. Stocks, S.A. Verhulst, and D.A. Casamatta. Preliminary Survey of Filamentous Cyanobacteria from Suwannee River State Park. Southeastern Phycological Colloquy, October 2011.

H.S. Stocks. S.A. Verhulst, and D.A. Casamatta. Leptolyngbya ferruginosa sp. nov., a novel siderophoric cyanobacterium isolated from an iron-depositing hot spring. Phycological Society of America, July 2012.

A.P. Omran, N.A. Pennington, **H.S. Stocks**, S.A.Verhulst, C.I. Ross and D.A. Casamatta. Putative roles of microcystins isolated from Microcystis aeruginosa (Cyanobacteria) on

heterotrophic bacterioplankton isolated from the St. Johns River (FL). Phycological Society of America, Seattle, Washington, July 2011.

S.A. Verhulst S. F. Eastman A.P. Omran **H.S. Stocks** D.A. Casamatta. Response of the epiphytic algal community to eutrophic conditions in the Guana Tolomato Matanzas National Estuarine Research Reserve. Phycological Society of America, Seattle, Washington, July 2011.

Papers in Prep:

H.S. Stocks, M. Vaccarino, J.R. Johansen, & D.A. Casamatta. *Reptodigitus chapmanii* gen. nov.: a unique Stigeonematalean (Cyanobacteria) genus based on a polyphasic approach.

H.S. Stocks & D.A. Casamatta. A survey of the freshwater cyanobacteria from Northeast Florida.

H.S. Stocks & D.A. Casamatta. An investigation into the factors which influence the epibenthic cyanobacterial community.