THE USE OF 23S RIBOTYPING TO DETECT HARMFUL AND NUISANCE PHYTOPLANKTON IN A LARGE, SUBTROPICAL RESERVOIR

DURING AN EXTENDED DROUGHT PERIOD

by

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DEDICATION

This thesis is dedicated to Kevin, Bart and C. Hoover.

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LIST OF ABBREVIATIONS

Abbreviation

Description

- 2-MIB: 2-methylisoborneol
- HAB: Harmful Algal Bloom
- LCRA: Lower Colorado River Authority
- PCR: Polymerase Chain Reaction
- TSI: Trophic State Index

ABSTRACT

Inland subtropical water bodies are highly susceptible to freshwater harmful algal blooms (HABs). Still, there remains a lack of studies on this subject and the conditions encouraging blooms in this climate. Central Texas, USA, went through an extended drought from 2011-2015 – a phenomenon common in the subtropics. Lake Buchanan, a large inland reservoir, experienced rapid shifts in the phytoplankton community during this period as the lake transitioned to more eutrophic conditions, and serves as an excellent model for subtropical lakes due to its location and size. Samples were taken bimonthly and included measuring water quality parameters, nutrients and phytoplankton, along with the identification of living and preserved phytoplankton to assess the impacts of the transition. The phytoplankton community was evaluated by cell counts and DNA barcoding using 23S ribotyping to verify the presence and abundance of different strains. Abiotic and biotic factors were evaluated to determine which variables contributed to the formation of HABs. DNA sequencing analysis confirmed the presence of known bloom-forming cyanobacteria. Overall, this study shows that the saxitoxinproducers Planktothrix, Aphanizomenon, and Cylindrospermopsis thrived in drought conditions (p < 0.001) whereas *Limnothrix* and *Pseudanabaena* did not. The diatoms Fragilaria and Lindavia increased in terms of community dominance after the end of the drought. Following the drought period, Aphanizomenon ovalisporum, Phormidium tenue, and *Planktothrix* sp. were present along with additional potentially harmful yet rarely studied species. These results suggest that drought-induced eutrophication lead to the

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dominance of harmful cyanobacteria in Lake Buchanan. Thus, subtropical reservoirs should be monitored closely during extended drought periods, as the risks associated with eutrophication and HABs are predicted to be higher.

I. INTRODUCTION

Incidences of eutrophication are increasing worldwide (Chislock et al. 2003; Sinha et al. 2017), and there are fewer studies performed in subtropical areas in comparison to other climates (Boynton et al. 1996; Jeppesen et al. 2005). The need for eutrophication and harmful algal bloom (HAB) studies in a variety of regions is increasing as the world is exhibiting rapid human population growth (Yang et al. 2008; Zahid et al. 2016). Additionally, phytoplankton blooms are often due to unique environmental factors, with certain species favoring specific conditions. Therefore it is difficult to make assumptions on which inland water bodies are at risk for certain types of blooms, and further research is required.

Among the largest concerns associated with eutrophication are the abundance of cyanobacteria and the conditions that encourage their growth (Sayers et al. 2015). The dominance of cyanobacteria in the phytoplankton community is common in subtropical reservoirs due to their affinity for the conditions representative of the climate (Ni et al. 2012; O'Farrell et al. 2012; Zhang et al. 2016). However, cyanobacterial dominance in a phytoplankton community does not always indicate toxin-producing species are abundant and susceptible to bloom formation (Morais da Rosa et al. 2017), thus toxin contamination events can be difficult to predict in lakes that do not exhibit seasonal cyanobacterial blooms. Cyanobacterial dominance may also inhibit other bloom-forming phytoplankton growth, such as the icthyotoxic golden algae, or Prymnesiophytes (Neisch et al. 2012). This further indicates the significance of local phytoplankton monitoring programs to determine abundant taxa and assess whether reservoirs are at risk. Although eutrophic conditions are

commonly associated with cyanobacterial blooms, not much is known about the role of increasingly eutrophic conditions for Prymnesiophyte blooms or other lesserstudied freshwater taxa. Additional supporting research for conditions favoring HABforming genera in inland waters is required to make further conclusions and help solidify possible mitigation strategies and the factors that influence presence, abundance, and toxin production.

As a larger reservoir located in a region of central Texas that is not highly developed, Lake Buchanan serves as an excellent model for eutrophication studies in subtropical climates, as the development occurring in the area is not directly on the reservoir. In fact, the closest major city to the Lake Buchanan reservoir is approximately 70 miles southeast. Data from the past 7 years via the Lower Colorado River Authority (LCRA) shows that this reservoir, previously classified as mesotrophic, is now considered eutrophic after an extended drought period based on measurements of Chlorophyll-a, Secchi depth and total P (TCEQ 2018). In nearly every documented case in the subtropics (Havens et al. 2003; Chen & Xie 2005; McCarthy et al. 2007), this results in a reservoir dominated by cyanobacteria. This is an undesirable result of eutrophication, as dominant cyanobacteria species are capable of HABs detrimental to both the aquatic and terrestrial community, and may even result in the release of aerosols hazardous to human health (May et al. 2018). A detailed analysis of the phytoplankton communities for Lake Buchanan has yet to be performed, including all of the reservoir systems in the watershed. These lakes are the uppermost reservoirs in the Colorado River Highland Lakes chain, which are valued as a recreation destination and source of drinking water (Lin et al. 2015). Researchers

have found that in lake systems experiencing drought, phytoplankton communities can see a dramatic shift in composition and structure (Brasil et al. 2015), and they have the tendency to shift towards cyanobacterial dominance in the phytoplankton community (Reynolds et al. 2012; Lehman et al. 2017).

The purpose of this study was to analyze the phytoplankton community of the Lake Buchanan reservoir through cell counts supported by genetic identification of critical species using ribotyping with multiple primers. In doing so, this will strengthen the understanding for monitoring programs in terms of prediction and decision-making in regard to the eutrophication and HABs within the ecosystem. The occurrence of a severe drought during the study period and the breaking of the drought provided a dramatic change in the environment, which can be noted in standard water quality parameters and chemical measurements. From these changes in water chemistry, resulting shifts in major taxa can additionally be used as bioindicators for drought conditions. It is possible that these drought conditions may magnify the potential for success for certain taxa over others. In addition to these benefits for future studies in freshwater HABs, the utilization of multiple primers in ribotyping will assist in metagenomic community analyses by determining a more favorable primer to use for success and simplicity. As the field of metagenomics increases in popularity and usefulness, rapid and simplified methodologies to assess phytoplankton biodiversity will be highly valuable to the field and for implementation across phytoplankton monitoring programs.

Despite quantifying cellular biovolume via inverted microscopy for species identification, accurate data of toxin-producing species does not exist and cannot exist

based on this method alone. Although a genus can be considered toxic, such as *Anabaena* or *Planktothrix*, not all species are recognized as HAB-formers. It is virtually impossible to determine whether an abundant species is capable of toxin production unless symptoms of the toxins have been reported in nearby populations after the bloom has already occurred, an assay for identification of the particular toxin from field samples is completed, or field samples have their DNA sequenced (Aranda-Rodriguez et al. 2015).

The first method of analysis for toxin symptoms provides insufficient details in bloom prevention, as the bloom will have already occurred and caused its damage. The second assay method is also somewhat inefficient due to the presence of some harmful toxins being undetectable via chemical assay unless the harmful bloom has already occurred. This method can also be quite expensive if researchers are unaware of which specific toxin they are testing for and will have to run multiple assays for different toxins. Testing a field sample for DNA identification is most efficient when determining whether or not a study area is prone to HAB formation, however, should be used with cell counts of field samples to determine abundance of potentially harmful cells. With DNA confirmation, morphology can be linked to an exact species.

DNA barcoding has not been used to identify subtropical phytoplankton at the community level. This information, along with cell counts for verification of cellular abundance and nutrient analysis, will provide insights for increased efficiency of phytoplankton monitoring programs and yield additional information on the role of phytoplankton in subtropical biogeochemistry. This study will expand the publicly

accessible National Center for Biotechnology Information GenBank of DNA sequences for freshwater phytoplankton for future identification purposes via similar methodologies. This will assist future research in metagenomic community analyses as well, as researchers may have a clearer picture of previously unidentified community members, or constructing phylogenetic trees with an emphasis on new species.

II. MATERIALS & METHODS

2.1 Study site

Lake Buchanan is located at 30.8 °N, 98.4 °W in the Burnet and Llano counties of Texas, USA. It was built in 1938 and contains one of the longest multiplearch dams in the world. Although it is responsible for providing water resources for many surrounding communities, Lake Buchanan is considered to be located in a less developed region of Texas, decreasing the likelihood of point-source pollution (LCRA 2017). Lake Buchanan consists of 22,335 surface acres and has a maximum depth of 40 meters. Considering its dimensions, it is the largest of the Highland Lakes of central Texas, which are monitored by the LCRA.

Despite the exceptional health of its connecting rivers upstream pre- and postdrought, the uppermost section of Lake Buchanan is of concern to the LCRA due to its high chlorophyll measurements, presumably due to reduced inflow in the area (LCRA 2017). Following the conclusion of the drought in 2015, Lake Buchanan had become increasingly eutrophic using the Carlson's TSA classification standards based on chlorophyll-a, Secchi depth and total P measurements (Carlson 1977).

2.2 Water quality and chemical data

 Cl^{-} , $SO_4^{2^-}$, turbidity, the amount of days since a significant rainfall event, dissolved oxygen, dissolved oxygen saturation, pH, specific conductance, temperature, Secchi depth, alkalinity, Chlorophyll-a, NH₃, PO₄³⁻, the sum of NO₃⁻ and NO₂⁻, and Kjeldahl N were all measured and accounted for in this study. Total N to total P ratio was also calculated and included for analysis of phytoplankton abundance patterns, as some studies have expressed that this ratio may have an impact on cyanobacteria populations (Smith et al. 1995; Molot & Dillon 2011; Winder et al. 2009).

All data was collected in situ simultaneously with the phytoplankton samples. Dissolved oxygen, pH, specific conductance, and temperature were collected with an EXO1 multiparameter 4-port water quality Sonde calibrated one day before the sample collection date. Measurements were taken from the Sonde suspended 0.3 m from the surface. Turbidity was measured using a Hatch 2100Q Portable Turbidimeter. The days since significant rainfall event parameter was established using rain gauges courtesy of the LCRA, considering ¹/₄ of an inch to be significant. Chlorophyll-a was measured using fluorometric analysis, with samples being preserved at 4°C before filtration (Arar & Collins 1997).

Common inorganic anions were measured from field samples using mass spectroscopy. Field samples were maintained at 4°C for a holding time of 48 hours prior to analysis. 2 mL H₂SO₄ was added at the time of collection to the water samples that would be used for Kjeldahl N, ammonia, and total P. All field samples were immediately placed on ice as a preservative measure. Approximately 2 days following collection, 100 μ l of sample is loaded onto an ion chromatograph. The target anion separates and is measured by the machine via guard column (Dionex Ion Pac AG14 P/N 046134), analytical column (Dionex Ion Pac AS15 Analytical Columns P/N 046126), suppressor device (Dionex Anion Self Regenerating Supressor ASRS P/N 43187) and a detector for conductivity (Dionex ED40 Electrochemical Detector with Detection Stabilizers). A chromatograph was

generated with retention time in minutes on the x axis and signal in μ S on the y axis. Peaks were analyzed according to the anion in question. The eluent solution used consisted of 7.2 mL 0.5 M Na₂CO₃ and 6.8 mL 0.5 M NaHCO₃ in 2 L deionized water. Analysis was carried out via the Dionex Chromeleon computer program. Quality control verification of accurate measurements was completed by the LCRA Environmental Laboratory Services in Austin, TX with laboratory protocol adapted from Pfaff 1993.

2.3 Sample collection

Phytoplankton samples were collected at 2 m from the surface using a clear PVC pipe with a plunger screwed tightly to the bottom of the tube, which was vertically inserted into the water column (LCRA 2014). Subsamples were then transferred from the lake by carrying the apparatus up to the surface and emptying the plunger into 125 mL glass bottles. 3 mL Lugol's solution was added per sample to preserve phytoplankton in situ for an accurate representation of the community. Samples were transported in an ice chest to a -20°C freezer, where they were kept anywhere between 3 hours and 5 years before analysis via inverted microscopy. Water samples specific for chemical analysis were taken at 0.3 m at the time of phytoplankton collection. Samples were taken during even numbered months (i. e. February, April, May...) of the year during the first week (Table 2.1).

2.4 Phytoplankton enumeration and quantification

Samples were enumerated at 400X magnification on an Olympus IX73 inverted microscope and quantified via Olympus cellSens using an Olympus Th4-100 halogen powered light source. Individual cells were identified to the genus level and counted to estimate community composition using the methods described in Utermöhl 1958 from 10 fields. 110 mLs of the preserved field sample was settled in a HydroBios Utermöhl-style Combined Plate Chamber (Product # 435 025) of the same volume for 24 h prior to decanting. Width and height measurements were taken in µm to calculate biovolume using the following formula (Figure 2.1), adapted from Olsen et al. 2016.

Biovolume calculations were based on the shape of the cell, using Sun & Liu 2003 as a reference for more complex cells such as *Pediastrum* and *Ceratium*. Biovolume and cells per mL were multiplied for an estimate of genus-specific cell abundance in the sample. The sum of relative abundances of the same group (i.e., diatoms) was taken to obtain a percentage estimate for the sample to determine an approximate phytoplankton community composition. Special attention was directed towards possible toxin-producing genera, such as icthyotoxic *Prymnesium* and hepatotoxic cyanobacteria *Microcystis* and *Cylindrospermopsis*.

The common groups Pyrrhophyta, Cryptophyta, Euglenophyta, Chrysophyta, Chlorophyta, Cyanophyta, Bacillariophyta, Prymnesiophyta, Synurophyta and Xanthophyta were used to outline community composition by determining the relative abundance percentage. Due to a much lower abundance in comparison to the other groups, Xanthophyta, Synurophyta, Prymnesiophyta, Euglenophyta and Chrysophyta were grouped and categorized as "Others" in the analysis. Biomass percentages were compared with chemical data that was simultaneously taken from the same sampling site. Parameters were selected for comparison primarily due to

consistency across sampling times, but the ability to detail drought conditions, potential for blooms and describing possible pollution events were also taken into consideration.

2.5 Cloning phytoplankton from field samples

Two samples were collected from Lake Buchanan for the purpose of DNA extraction: 1 sample for June 2017 and 1 sample for September 2017 to align with variation in community composition occurring between Summer and Fall months. Normally, the turnover for Lake Buchanan is between mid September and mid October, resulting in a dramatic increase in cyanobacterial density to be anticipated in the September sample. It was expected that the June sample would yield primarily chlorophytes and diatoms, with little diversity in cyanobacteria.

For individual cultures, samples were obtained similarly to the preserved samples with the exception of additive preservation dye. Samples were kept at room temperature and loosely capped. For culturing purposes, cells were isolated from 2 mL aliquots of live sample in 12-well plates under 400X magnification via specialized 1 mL Pasteur pipettes, a method modified from Rippka 1988. Aliquots were created 24 h after sample collection and maintained for approximately 168 h before discarding.

Isolated cells were pipetted into 96-well plates and maintained at 12 h light/dark cycles at 23°C. About 30 μ l of media was added to each well and refilled as appropriate for the duration of the experiment. The type of culture media added to each well depended on the cell's classification: Colonial cyanobacteria and eukaryotic

cells excluding diatoms were grown in MB3N medium (Karampudi & Chowdhury 2011) filamentous cyanobacteria were grown in Spirulina medium (Aiba & Ogawa 1977) and diatoms were grown in WC+ medium modified from Guillard & Lorenzen 1972 by adding 100 μ M sodium metasilicate, 11 nM Na₃VO₄, and 31 nM H₂SeO₃. Once cultures were visibly pigmented, additional aliquots were made in 500 μ l microcentrifuge tubes with removable caps composed of approximately 25 μ l culture and 100 μ l respective culture medium. Aliquots were kept at 12 h light/dark cycles at 23°C until the water appeared a light to deep green or brown color, loosely capped in a clear 80-well microcentrifuge tube rack.

Although effective, this culturing methodology did not eliminate the possibility of environmental bacterial contamination from *Pseudomonas aeruginosa* and *Acetinobacter pittii*, two planktonic species of freshwater bacteria that are highly abundant in Lake Buchanan. Due to difficulties detecting via inverted microscopy, the pipetting method often collected these bacterial specimen to then be cultured in liquid media with the target phytoplankton isolate. Although generally harmless due to their inability to amplify with selected primers (Table 2.2), these non-photosynthetic bacteria had higher growth rates than the photosynthetic cells under culture conditions. Their abundance interfered with the quality of the DNA sequences for photosynthetic organisms, and successfully amplified with the 23S primers. Samples that were low quality or did not yield results in the PCR reaction despite the presence of visible cells prior to extraction were plated on agar-infused MB3N media solidified on sterile polystyrene petri dishes using sterile wooden sticks to transfer cells from liquid cultures.

DNA was extracted from algal colonies by using a colony PCR method. A sterile wooden stick was used to transfer an individual algal colony of about 2mm diameter to 200 μ L PCR tubes with 100 μ l TE (10 mM Tris, 1 mM EDTA, pH 8) with 1% Triton then vortexed for 15 sec. The tubes were then transferred to the thermocycler and maintained at 99°C for 10 min followed by a 4°C holding time of about 10 min. Samples were removed from the thermocycler and had 100 μ l 24:1 chloroform:isoamyl alcohol added and was inverted with a micropipette for approximately 3 min estimating 100 inversions per min. Samples were then centrifuged at 14,000 rpm for 5 min. 1 μ l DNA supernatant was added to PCR reactions, which were adjusted accordingly. Samples were stored at -20°C when not in use.

2.6 Sequencing rDNA of independent cultures

gDNA was extracted from environmental phytoplankton culture aliquots following the same protocol for the duration of the experiment using the Gene Clean Turbo kit from MP Biomedicals #111102200, an extraction protocol modified from Fawley & Fawley 2004. 1-2mL aliquots were centrifuged using an Eppendorf 5415 R at 1 min at 14,000 rpm. Supernatant was discarded and 200 µL lysis buffer (1M NaCl, 70 mM Tris, 30 mM Na₂EDTA, 10 mL H₂O, 2 mL 5M NaCl) was added after decanting media. The solution was then vortexed for even distribution of components and centrifuged again at 14,000 rpm for 1 min. The supernatant was again discarded and 200 µl lysis buffer was added along with acid washed 300 µm diameter Sigma G-8772 glass beads to fill conical section of the microcentrifuge tube, 25 µl 10%

dodecyl trimethyl ammonium bromide (DTAB), and 200 µl 24:1

chloroform: isoamylalcohol. The aliquots were placed in a BioSpec Mini-Beadbeater-96, homogenized for 40 sec then centrifuged at 5,000 rpm for 2 min. With a clear top layer now visibly distinct from the colored lower layer, 100 µl of the aqueous phase was removed and added to a new microcentrifuge tube along with 500 µl Gene Clean salt solution. The new 600 μ l solution in the microcentrifuge tube was transferred to a Gene Clean Turbo cartridge and centrifuged for 5 sec at 14,000 rpm. After emptying the catch tube, 500 µl Gene Clean Wash solution (10 mM Tris-HCl pH 7.5, 80%) EtOH) was added to the filter apparatus and centrifuged for 5 sec at 14,000 rpm. The catch tube was then emptied and centrifuged again at 14,000 rpm for 4 min, and the filter apparatus was transferred to a new microcentrifuge tube of the same size. 30 µl sterile ddH₂O was added directly to the white filter and incubated at room temperature for 11 min, then centrifuged at 14,000 rpm for 1 min. A NanoDrop ND-1000 Spectrophotometer was used for verification that the DNA extraction had performed well and was therefore acceptable for utilization in PCR. 2 µl extracted gDNA solution were added to the NanoDrop and checked for 260/280 ratios of around 1.8 to assess nucleic acid purity. Concentrations of >10 ng/µl were required for continuation to the PCR step.

PCR protocol remained the same for extracted DNA, but the primers used varied depending on whether or not the culture was determined to be eukaryotic or prokaryotic based on morphological analysis via inverted microscopy. Depending on the NanoDrop reading for each sample, 2 μ l gDNA/39.9 μ l NF H₂O was used for samples with concentrations >25 ng/ μ l and 4 μ l gDNA/37.9 μ l NF H₂O was used for

samples with concentrations <25 ng/µl. For every sample, 5 µl Invitrogen 10X PCR buffer (200 mM Tris HCl (pH 8.4), 500 mM KCl), 1 µl New England BioLabs dNTPs (solution 10 uM each of dATP, dCTP, dGTP and dTTP), 1.5 µl Invitrogen MgCl₂ (50 mM), 1 µl 25 µM forward primer, 1 µl 25 µM reverse primer and Invitrogen Platinum Taq DNA polymerase were used in the reaction. Reactions were all performed in 200 µl thin-walled tubes on a ThermoFisher SimpliAmp Thermal Cycler. Cyano ITS cycles were run at 94°C for 2 min 1x, 94°C for 1 min, 57°C for 1 min, and 72°C for 4 min 35x, 72°C 10 min 1x, and maintained at 4°C until removed. 18S samples were run at 95°C 1x for 3 min, 94°C for 45 sec, 55°C for 1 min, and 72°C for 3 min 30x, 72°C for 5 min 1x and maintained at 4°C until removal. Samples amplified with the ITS2 primer prior to acquiring the 18S primers were run at 94°C for 2 min 1x, 94°C 30 s, 53°C 1 min, and 72°C 1 min 5x, 94°C 30 sec, 55°C 1 min, and 72°C 1 min for 25x, 72°C 10 min 1x and kept at 4°C until samples were removed.

Gel electrophoresis was used for verification of successful PCR prior to sequencing. Results were viewed on 2% agarose gels (Fisher Scientific BP160-500 Molecular Biology Grade Agarose) in 1X TAE buffer (40 mM Tris, 20 mM acetic acid, and 1 mM EDTA). Gels ran at 67 V for 1.5 h and stained with 3.75 µl EtBr for 25 min. Wells consisted of a combination of 2 µl 6X loading dye (0.25% w/v bromophenol blue, 40% w/v sucrose) and 10 µl PCR sample. 5 µl Thermo Scientific GeneRuler 1 kb Plus DNA Ladder was used to improve accuracy when analyzing fragment size. Gels were placed in a 20 mL 1X TAE, 3.5 µl EtBr solution on a Precision Reciprocal Shaking Bath Model 50 at 22.5 rpm for 25 min. Gels were viewed under UVP PhotoDoc-It Imaging System High Performance Ultraviolet

Transilluminator after staining.

DNA was purified using the GeneJET FFPE DNA Purification Kit #K0881. A 1:1 mixture of binding buffer and sample was created, then transferred to a GeneJET Purification Column and centrifuged for 30 m at 14,000 rpm. The column was then washed with 700 μ l wash buffer and centrifuged for 1 min, emptied, and centrifuged again at 1 min, 14,000 rpm. The column was then transferred to a 1.5 ml microfuge catch tube and 50 μ l elution buffer was added directly to the white filter, centrifuged for 1 min then sequenced, with the remaining purified DNA sample being stored at -20°C to be reused should sequencing errors have occurred. All Sanger sequencing was performed at the UT Austin Core Sequencing Facility (Austin, TX) using a mix of 11.7 μ l autoclaved nuclease-free water, 20 ng/ μ l purified DNA sample and 0.2 μ l respective primer. Consensus sequences were aligned, edited and exported using Geneious 11.0.4 and examined using the BLAST nucleotide alignment tool with >97% accuracy (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

III. RESULTS

3.1 Changes in water chemistry

During drought years, it is anticipated that the water will have evaporative losses, contributing to elevated specific conductance (Ignatius & Rassmussen 2016). This trend appeared in data collected for Lake Buchanan, along with a decrease in total annual rainfall and inflows from 2013 to early 2015 (Figure 3.1). Despite a lack of noticeable changes in dissolved oxygen and pH during the study period, Secchi depth and chlorophyll-a exhibited a negative correlation. Precipitation and inflow data further supported the hypothesis that drought chemistry was influencing reservoir dynamics during the first 3 years of the study period. On average, pH of Lake Buchanan was 8.26, and the dissolved oxygen saturation was 97.92.

From 2013-2014 (Figure 3.2), diazotrophs were dominant, and there was a decline in N. Beversdorf et al. 2013 describes a trend in which declining N concentrations can select for diazotrophs, which may have contributed to the trends observed in Figure 3.2. Additionally (Figure 3.3), Cl⁻ and SO_4^{2-} were highest when diazotrophs were dominant (Table 3.1).

3.1.1 Phytoplankton abundance patterns

Significant changes in the phytoplankton community occurred as drought conditions subsided. Towards the end of 2015, cyanobacterial biovolume decreased, which was likely as a result of increased inflows thus concluding a hydrologic drought in Lake Buchanan. The amount of cyanobacteria in the community decreased, and the dominant genera changed from the notoriously hepatotoxic Planktothrix and Cylindrospermopsis to nuisance genera Limnothrix and
Pseudanabaena (Table 3.1). Although still present in the community,
Cylindrospermopsis decreased in abundance following increases in inflow.
Cryptophytes became more abundant after the drought period, but dinoflagellates
remained consistent members of the community independent of drought conditions
(Figure 3.4).

3.1.2 Conditions favored by cyanobacteria

In general, cyanobacteria as a group seemed to favor higher levels of $SO_4^{2^-}$ >40 mg/L, Cl⁻>20 mg/L and specific conductance >500 µS/cm in this study (Figure 3.3; Figure 3.4). The major genera of cyanobacteria that were capable of producing saxitoxins included *Cylindrospermopsis*, *Planktothrix*, and *Aphanizomenon*. When pooling their total biovolume, separating between drought years and non-drought years and performing a Welch two-tailed T test, the difference in means was statistically significant, where *t* = 4.14, df = 21.66, and p = 0.0004. These results suggest filamentous freshwater saxitoxin-producing cyanobacteria may, in fact, thrive in conditions associated with droughts in subtropical climates.

Major shifts within the cyanobacterial community occurred following a *Lindavia ocellatea* bloom in February 2015. An anomaly in the cyanobacterial community occurred within a brief period mid-2016 where *Anabaena* was the dominant cyanobacteria, a trend not yet observed in Lake Buchanan. *Anabaena* likely favor different conditions than *Planktothrix* and *Cylindrospermopsis*.

Morphologically distinct, *Anabaena* is notably diazotrophic and has different nutrient requirements from other filamentous cyanobacteria.

3.1.3 Conditions favored by Prymnesiophytes

Although Prymnesiophytes were not considered a major component of the phytoplankton community and were grouped in with other small community percentages, their relative abundance increased following the conclusion of the drought period. They were not observed in the reservoir until 2015, and experienced a slight increase from 1 to 6 cells/mL in 2017. Despite this, it cannot currently be expected that Prymnesiophytes will cause HABs in this reservoir. The extremely low cell counts and the fact that Prymnesiophytes are notorious for higher salinity conditions representative of the more arid western Texas reservoirs resulted in this conclusion. Still, due to Lake Buchanan's close proximity to West Texas with frequent *Prymnesium parvum* blooms, the possibility for blooms in the future should not be ruled out entirely. The reservoir also tested positive for *P. parvum* using qPCR from a sampling site near the dam in December 2004 (Southard et al. 2010), corresponding with Manning & La Claire (2010)'s assertion that HABs from *P. parvum* occur in winter months in central Texas.

3.2 Shifts in phytoplankton group dominance

During drought years, filamentous cyanobacteria were overwhelmingly dominant whereas diatoms and chlorophytes were lower, yet still detectable. In 2017, diatoms were the dominant phytoplankton group, with cyanobacteria showing anticipated seasonal increases in population density (Figure 3.5). These shifts in community composition suggest a much healthier reservoir in comparison with the beginning of the study period.

The major species of diatoms in these phytoplankton communities were *Fragilaria crotonensis* and *Lindavia ocellatea*. Although certain species may have tolerance for elevated ion concentrations and specific conductance, this does not always mean they will thrive under these conditions. In fact, the success of diatoms in hydrological drought conditions may be hindered by an overabundance of cyanobacteria in the system.

3.3 Morphological Index of Algae in Lake Buchanan for 2017

In general, potentially toxic cyanobacteria were lower in abundance than usual for the live sampling period considering Lake Buchanan's biovolume data for the study period. However, the cyanobacteria frequently noted in preserved cell counts were still present and considered abundant although not enough to consider a bloom event to be occurring or even pending. Additionally, some isolates did not grow under culture conditions and were therefore discarded, suggesting isolated cells were unhealthy or culture conditions were not adequate for certain strains. In some instances, isolates were cultured in bulk yet received poor base pair yields upon sequencing and were no longer viable for another extraction attempt. As a result, many frequently counted genera were not identified to the species level via Sanger sequencing. Excluding *Anabaena*, all collected living strains of potentially harmful cyanobacteria were successfully cultured, sequenced and identified. For more information on inadequate culture conditions for *Anabaena* isolates, see Appendix A.

3.3.1 June

The majority of successfully sequenced isolated phytoplankton strains were limited to chlorophytes for the month of June. While a high abundance of *Fragilaria* in comparison to other phytoplankton was noted for this month, the strain failed to successfully grow out to an acceptable amount for DNA extraction. This is largely due to the choice of growth media for the strain. MB3N had a lack of silica and was therefore likely an inappropriate choice for rapidly culturing freshwater diatoms in general. Following this instance, WC+ media was used to grow diatoms for the remainder of the experiment. For additional information on optimal growing conditions of isolated strains, see Appendix B.

Previously unidentified picoplankton were successfully isolated, cultured, and identified as *Parachlorella kessleri* or *Hafniomonas*. Some species of *Micractinium* were misidentified in cell counts as either *Chlamydomonas* or *Chloromonas* due to the lack of spines present when preserved with Lugol's solution. Some species of chlorophytes such as *Crucigenia* were misidentified by BLAST, most likely due to the lack of sequenced samples for less common genera. In these instances, new sequences were submitted to BLAST as "*Crucigenia* sp.", or "*Closterium* sp." in terms of certainty of given genera. This methodology was much more challenging using chlorophytes with less distinct physical features, thus those isolates were left only partially categorized.

Pseudanabaena sp., *Pseudanabaena minima* and *Pseudanabaena catenata* were the most abundant cyanobacteria in this sample, which is confirmed by

preserved cell-count data. Given this information, it is plausible that some species of *Pseudanabaena* were previously misidentified in the cell counts as *Limnothrix* due to their morphological similarities, especially considering the densities of filaments and distortion from the preservation process contributing to a challenging identification process. *Pseudanabaena* is widely accepted as a genus that produces 2-MIB (Wang et al. 2011), and some strains have confirmed production of microcystin (Sivonen & Börner 2008). *Phormidium tenue* was also sequenced and is known to produce 2-MIB (Oikawa & Ishibashi 2004) and possibly microcystins (Teneva et al. 2005). For specific sequences and additional species, see Appendix A. For micrographs of abundant phytoplankton, see Figures 3.6-3.9.

3.3.2 September

As September marks the beginning of fall when seasonal increases in cyanobacteria are anticipated, this month consisted of more cyanobacterial sequences than in June. The most abundant species was *Limnothrix planktonica*, a hardy filamentous cyanobacterium that has not been studied to a great extent in terms of secondary metabolite production or culture conditions. *L. planktonica* was dominant in the sample and was often difficult to separate from the other isolates for culturing purposes. *L. planktonica* was not noted in the June sample, suggesting a lower density within the community despite its probable presence. Although not previously noted in literature, *L. planktonica* exhibited a pungent, earthy odor when cultured and is certainly capable of producing nuisance blooms due to their rapid growth abilities in culture and calculated biovolume in field samples.

Achnanthidium, a pennate diatom shown in Figure 3.3, was also more abundant in this sample than previously noted in Lake Buchanan. Molecular confirmation via 23S ribotyping revealed that this unidentified, smaller pennate diatom was a close relative of *Phaeodactylum tricornutum*, a euryhaline pennate diatom similar in morphological structure that lacks a frustule. It is easily distinguishable from *P. tricornutum*, but was not identifiable beyond the genus level and was therefore left classified as *Achnanthidium* sp. in BLAST. For previous years, this diatom was observed at much lower biovolumes and not considered a significant part of the diatom community until August 2017, one month before DNA samples were taken.

Aphanizomenon ovalisporum, a filamentous cyanobacterium widely recognized for producing cylindrospermopsin, was also successfully sequenced via 23S and CyanoITS amplification. In culture conditions, this strain did not possess heterocysts. *A. ovalisporum* did not appear to be nearly as abundant as *Achnanthidium* sp. and *L. planktonica*, and was difficult to culture in liquid media. However, when transferred to plated MB3N agar media, the strain grew to rapidly fill the agar plate in a matter of 48 h. It is also notable that this species has been frequently documented in mesotrophic and oligotrophic lakes, rather than eutrophic-hypereutrophic (Quesada et al. 2005).

IV. DISCUSSION

The occurrence and duration of droughts are increasing worldwide as a result of climate change (Sheffield et al. 2011). Considering that the primary causes of modern eutrophication is linked to human development, it is important to think of the issues that arise and the causes of said issues. Cyanobacterial dominance is commonly associated with drought periods (Costa et al. 2015). As a result, cyanobacteria are a major concern in eutrophic freshwaters. In more arid regions where natural sources of freshwater are scarce, reservoirs are important water sources and therefore must be maintained at healthy, usable states for neighboring cities. High abundances of cyanobacteria are correlated with unhealthy reservoirs and are characteristic of blooms, which can yield toxin production deeming the reservoir unusable.

In this study, it was demonstrated that seasonality does not seem to impact cyanobacterial abundance patterns during droughts. Instead, hydrologic drought conditions and eutrophication seem to be the major factors driving cyanobacterial growth – most notably taxa that have previously exhibited the ability to produce HABs. As a result, mitigation strategies for drought-induced eutrophication and requirements for reduction in nutrient output in nearby areas are necessary. The inflow data in this study (Figure 3.1) further supports the idea that mitigation strategies for cyanobacterial blooms which implement flushing are both ideal and successful (Paerl 2014).

As expected during drought periods (Bouvy et al. 2003), the ions SO_4^{2-} and Cl⁻ displayed a significant decrease from 2013 to 2017. As shown by the T-test,

saxitoxin-producing cyanobacteria correlated positively with drought conditions. It is not certain which factors contributed specifically to the success of these cyanobacteria, but it may be that the relationship between these ions and harmful cyanobacteria should be explored further to reach a more discreet conclusion on this phenomenon, which was previously suggested by Wetzel (2001).

4.1 Genetics studies and harmful cyanobacteria

This study helped show that blooms can be anticipated in drought conditions in subtropical reservoirs and specific attention should be directed towards filamentous species. Such as was demonstrated with *L. planktonica*, filaments could be hardy and outcompete other phytoplankton, quickly becoming the dominant taxa in the reservoir. In the case of *L. planktonica* and *P. minima*, much is still not known regarding the toxic potential of these species. Therefore, further investigation is required for these types of studies where genetic analysis using primers targeting certain toxin-producing genes may yield beneficial information on lesser-studied cyanobacteria. This study also suggested *L. planktonica* likely possesses the genes associated with either 2-MIB or geosmin production in cyanobacteria.

Genetic analysis is an important component of reservoir studies especially for filamentous cyanobacteria as many species with different ecological functions have highly similar morphological characteristics. Cyanobacteria pose a significant threat to society (O'Neil et al. 2012), and should continue to be monitored for prevention of mammalian illness and death associated with bloom events, especially in eutrophic waters. Based on this study, the quickest and most accurate way to obtain identities

and determine abundances of the cyanobacterial community is to implement a metagenomic community analysis using 23S ribotyping of the whole planktonic microbial community at a given time while collecting a preserved sample for relative abundance calculations. 23S ribotyping will successfully amplify the whole phytoplankton community, along with some bacterial strains, in a given sample using metagenomics (Johnson & Martiny 2015).

4.2 Additional notes on DNA barcoding of phytoplankton communities

This experiment demonstrated that *Pseudomonas* grows symbiotically within certain chlorophytes. Additionally, the genetic library for many chlorophytes was significantly limited in terms of the other phytoplankton that were successfully barcoded in this experiment. Oftentimes, the e value and percent matches (see Appendix B) suggested that successfully sequenced isolates were not given a true match in the BLAST database. As chlorophytes are not associated with harmful or nuisance secondary metabolite production, studies for genetic sequencing of this group are not as common as for other freshwater groups, such as the cyanobacteria or prymnesiophytes.

4.3 Conclusions

As a result of this study, further evidence was provided that drought conditions exacerbate the growth of cyanobacteria. Most notably, filamentous genera capable of producing saxitoxin were sequenced with the most success by amplifying the 23S gene, and displayed the most significant trends when correlated with cell
count data in drought conditions. Additional details were obtained regarding phytoplankton blooms and community dynamics in subtropical reservoirs, leaving Texas monitoring groups and others studying subtropical reservoirs with increased knowledge of how phytoplankton will react during droughts, and the trends that can be anticipated in community structure. This information can be used to make more accurate predictions for community dominance in subtropical reservoirs given certain environmental conditions. In conclusion, monitoring programs should be alert when drought conditions are evident, and the phytoplankton community should be analyzed with a decrease in Secchi depth, increase in specific conductance, Kjeldahl N or Cl⁻ levels. **Table 1** Sampling period - shows the sampling period and frequency per monthacross years. Live phytoplankton samples were taken from Lake Buchanan for Juneand September 2017 to account for general abundance during summer and fall.

| Sampling Period | | | |
|-----------------|------------------------|--|--|
| January | | | |
| February | 2014, 2015, 2016 | | |
| March | | | |
| April | 2013, 2015, 2016, 2017 | | |
| May | | | |
| June | 2013, 2015, 2016, 2017 | | |
| July | | | |
| August | 2013, 2015, 2016, 2017 | | |
| September | 2016 | | |
| October | 2014, 2015, 2016, 2017 | | |
| November | | | |
| December | 2014, 2015, 2016 | | |

Table 2 Primers used in this experiment – A list of the primers used in this experiment, their sequences and the group that they were used for. All primers were synthesized by Integrated DNA Technologies (IDT, Coralville, IA, USA), and were maintained in -20°C when not in use. Following the protocol described in Boyer et al. 2001, the Cyano ITS F5 primer was not used unless the Cyano ITS F2 primer yielded multiple bands via gel electrophoresis.

| Target Group | Region Amplified | Primer Sequence (5'-3') |
|-------------------|-------------------------|------------------------------|
| Eukaryotic algae | 18S/F | aacctggttgatcctgccagt |
| Eukaryotic algae | 18S/R | tgatccttctgcaggttcaccta |
| Prokaryotic algae | Cyano ITS/R1 | ctctgtgtgcctaggtatcc |
| Prokaryotic algae | Cyano ITS/F2 | gggggattttccgcaatggg |
| Prokaryotic algae | Cyano ITS/F5 | tgtacacaccggcccgtc |
| Eukaryotic algae | ITS2/F | gggatccgtttccgtaggtgaacctgc |
| Eukaryotic algae | ITS2/R | gggatccatatgcttaagttcagcgggt |
| Algae | 23S/F | aggggtaaagcactgtttcg |
| Algae | 23S/R | ccttctcccgaagttacg |

Table 3 Dominant genera. Genera that composed the majority of their respectivegroup at a given time are expressed as percentages of biovolume. Significance wasdetermined by a group consisting of >5% of the overall phytoplankton community,and a certain genus comprising >30% of the total genera identified for each group.

| Month | Cyanophyta | Chlorophyta | Bacillariophyta | Dinophyta | Cryptophyta |
|-------|---------------------|-------------|-----------------|--------------------|-------------|
| & | | | | | |
| Year | | | | | |
| Apr. | Cylindrospermopsis | Pediastrum | Lindafvia(50%) | Ceratium | |
| 2013 | (63%) | (63%) | Fragilaria | (62%) | |
| | | | (41%) | Durinskia (38%) | |
| June | Planktothrix (82%) | Pediastrum | | | |
| 2013 | | (81%) | | | |
| Aug. | Cylindrospermopsis | Cosmarium | | Durinskia | |
| 2013 | (75%) | (61%) | | (33%) | |
| | | | | Ceratium | |
| | | | | (67%) | |
| Dec. | Planktothrix (99%) | | | | |
| 2013 | | | | | |
| Feb. | Planktothrix (100%) | | | | |
| 2014 | | | | | |

| Oct. 2014 | Planktothrix (37%) | Cosmarium (44%) Mougeotia | Fragilaria (40%) Lindavia | Durinskia (46%) | |
|--------------|---|---------------------------------|--|---|----------------------|
| | | (33%) | (60%) | | |
| Dec. 2014 | Planktothrix (70%) | Cosmarium (60%) | Lindavia(7 1%) | Ceratium (100%) | Cryptomonas (100%) |
| Feb. 2015 | Planktothrix (99%) | Cosmarium (40%) | Lindavia(7 8%) | | |
| Apr. 2015 | | Oocystis (30%) | Lindavia (99%) | | |
| June 2015 | Planktothrix (78%) | Cosmarium (32%) | Lindavia (50%) Fragilaria (50%) | Durinskia (53%) Ceratium (47%) | |
| Aug. 2015 | Cylindrospermopsis (42%) Limnothrix (33%) | Cosmarium (39%) | Lindavia (40%) Fragilaria (60%) | Durinskia (88%) | |
| Oct. 2015 | Limnothrix (96%) | | | | |
| Dec. 2015 | Limnothrix (56%) Planktothrix (43%) | Cosmarium (48%) | | | |
| Feb. 2016 | | Staurastrum (51%) | Lindavia (96%) | Ceratium (100%) | |
| Apr. 2016 | Anabaena (93%) | Cosmarium (39%) | Fragilaria (34%) Lindavia (66%) | | |
| June 2016 | Anabaena (88%) | Mougeotia (40%) | Lindavia (95%) | Ceratium (88%) | |
| Aug. 2016 | Limonthrix (55%) | Cosmarium (54%) | Fragilaria (71%) | | |
| Sep. 2016 | Cylindrospermopsis (47%) Limnothrix (39%) | Cosmarium (57%) | Fragilaria (81%) | Durinskia (83%) | Cryptomonas (100%) |
| Oct. 2016 | Limnothrix (35%) | Cosmarium (48%) | Fragilaria (87%) | Durinskia (100%) | Cryptomonas (97%) |
| Dec. 2016 | Limnothrix (55%) | Cosmarium (48%) | Fragilaria (48%) Lindavia (52%) | | Cryptomonas (99%) |
| Feb. 2017 | | Cosmarium (49%) | Lindavia (87%) | Ceratium (100%) | Cryptomonas (76%) |
| Apr. 2017 | | Cosmarium (46%) | Lindavia (80%) | | Cryptomonas (96%) |
| June 2017 | Anabaena (42%) Pseudanabaena (36%) | Cosmarium (71%) | Lindavia (89%) | Durinskia (100%) | |
| Aug. 2017 | Cylindrospermopsis (39%) Limnothrix (49%) | Cosmarium (48%) | Acnanthes (94%) | Ceratium (75%) | |

Table 3. Continued

$$\frac{\frac{C}{F}*D}{v_s}*v_c$$

Figure 1. Cell abundance calculation - displays the formula used to calculate approximate cell abundances for individual taxa preserved in field samples for this study. Here, C represents the number of cells counted per sample, F represents the number of fields counted per slide, and D represents a conversion factor found by dividing the chamber area by the field area. The value V_s represents the volume of the sample that was settled in the chamber, and V_c is the average volume for the cell.



Figure 2. Water quality data. This data was taken from the same sampling site as the phytoplankton samples were collected during the study period. The drought period in central Texas had begun to conclude in 2015.



Figure 3. Nutrient data. Total Kjeldahl Nitrogen (TKN), PO_4^{3-} , $NO_3^- + NO_2^-$, and NH₃ concentrations (mg/L) from samples taken simultaneously with phytoplankton and water quality data.



Figure 4. Additional ion data. Cl^- and SO_4^{2-} concentrations (mg/L) from samples taken concurrently with nutrients, water quality, and phytoplankton.





is expressed as the percentage of the total community that a specific group composed at a given time. Fall seasons in the Highland Lakes are traditionally when cyanobacteria are most abundant (LCRA 2016). From August – October, higher volumes of cyanobacteria are not to be considered an anomaly.



Figure 6. Biovolume (μm^3) during the sampling period for diatoms and cyanobacteria, the two major phytoplankton groups observed during this study.



Figure 7. Micrographs of cyanobacteria. A shows *Pseudanabaena minima* taken from a live sample for September 2017. B and C both show *Limnothrix planktonica*, also from the September 2017 live sample, with B outlining the lack of segments and C showcasing the rounded ends. D shows *Anabaena* sp. from April 2016, preserved in Lugol's dye. E shows *Microcystis* sp. also from April 2016. F shows *Pseudanabaena catenata* from a live September 2017 sample.



Figure 8. Micrographs of diatoms. A shows *Lindavia ocellatea* preserved in Lugol's from the February 2015 bloom in Lake Buchanan. B shows *Achnanthidium* sp. from a live September 2017 sample. C shows *Aulecoseira sp*. from a preserved April 2016 sample. D shows *Fragilaria crotonensis* from a live September 2017 sample. E shows preserved *Cymbella sp*. from April 2016.



Figure 9. Micrographs of dinoflagellates. Each image shows *Durinskia baltica* at a different part of its life cycle. A shows *D. baltica* from the September 2017 live sample in cyst form, B shows *D. baltica* from an April 2016 preserved sample, and C shows *D. baltica* from June 2016 previously identified as *Katodinium sp*.



Figure 10. Micrographs of chlorophytes. Starting from the far left and going clockwise, the image shows *Chodatella sp.*, *Phacotus lenticularis*, *Hafniomonas sp.*, *Tetracystis pulchra*, and *Cosmarium quinarium*. Excluding *T. pulchra*, which was taken from a culture of clonal isolates from June 2017, all photos were taken from preserved samples in 2% Lugol's solution.

APPENDIX SECTION

| Species | Agar Growth | Liquid | Ideal Media | Colony Notes |
|-----------------|-------------|--------|-------------|-----------------|
| | | Growth | | |
| Anabaena sp. | No | No | Unknown | N/A |
| Aphanocapsa | Yes | No | BG-11 | Teal specks |
| sp. | | | | scattered |
| | | | | across plate |
| Limnothrix | Yes | Yes | MB3N | In-agar swirls, |
| planktonica | | | | deep green, |
| | | | | rapid growth |
| Crucigenia sp. | Yes | Yes | MB3N | Uniform, |
| | | | | green |
| Closterium sp. | Yes | Yes | MB3N | Circular, light |
| | | | | green |
| Achnanthidium | Yes | No | MB3N | Dark brown, |
| sp. | | | | round |
| Durinskia | Yes | No | MB3N | In-agar, light |
| baltica | | | | brown regions |
| Aphanizomenon | Yes | No | Spirulina | In-agar, deep |
| ovalisporum | | | | green |
| Fragliaria | No | Yes | WC+ | N/A |
| crotonensis | | | | |
| Aulacoseria sp. | No | No | Unknown | N/A |
| Ceratium | No | No | Unknown | N/A |
| furcoides | | | | |
| Ochromonas | No | Yes | WC+ | N/A |
| sp. | | | | |
| Synechocystis | Yes | Yes | BG-11 | Small, |
| sp. | | | | speckled, |
| | | | | scattered, blue |
| Trachelomonas | No | No | Unknown | N/A |
| sp. | | | | |

Name: This is used to denote the name of the closest known matching sequence for a given organism. It is important to consider that many strains may be either undiscovered or have yet to be sequenced. In these instances, E values may be > 0.0 and identity percentages may be < 95%. Micrographs were used during this experiment to confirm identities revealed via BLAST, since it is common to receive multiple results with perfect values.

Group: The group that the organism was placed under during the community assemblage portion of this project. For more information on grouping, see section 2.4 Phytoplankton Enumeration and Quantification.

Sample: The month and year in which the sample that a given organism was isolated from was taken during.

E value: The expected value for the identity of a given sequence being statistically significant, where the closer to 0.0 the value is, the more likely that the organism name and sequence were matched appropriately in BLAST.

Percent Identity: The presumed closeness of the new sequence to an existing sequence for a given organism. The closer to 100%, the more likely the organism has been sequenced and was assigned appropriately by BLAST.

CyanoITS

Name: *Pseudanabaena sp*. Group: Cyanophyte Sample: June 2017 E value: 0.0 Percent Identity: 97% Raw Sequence: GCAAGCATGCGAATGCAAGNNAATCTCATAAACCCGGTCTTAGTTCAGATTGCAGGNTGCAANTCNNNGCATGAA GGCGGAATCGNTAGTAATCGCAGGTCAGCATACTGCGGTGAATACGTTCCCGGNCNTNGTACACACCGCCCGTCA CACCATGGAAGCTGGTCACGCCNGAAGTCGTTATCTCAACCCGCAAGGGAGGGAGGCGCNTAAGGCAGGCNGG TGACTGGGGTGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGCGGCTGGATCACCTCCTTATAGGGAGGACCTAT TTATGTCTAGACTGAACCAATACAGAATTAGGTCAGACATAAGTCATCCCAAGGTCGTTCGAGATTTATTGGAAA GCTTTCAAACTAAGTCAGGTTCTAAATTTGGCTAACATAAAGGGCCATTAGCTCAGTTGGTTAGAGCGCACCCCTG ATAAGGGTGAGGTCACTGGTTCGTGTCCAGTATGGCCCACTTGAGAATCTAACAACTAGAAGCCAAAAGCTGAAA GTGATAGACAACTCTGGGGATATAGCTCAGTTGGTAGAGCGCCTNCTTTGCACGCAGGAAGTCAGGAGTTCGAAT CTCCTTATCTCCNCCAAACTACTAAAACCAACAAAAGCAAAAGCAAAGGCAAGAGTTTAGCAACTCATCTAGTCNAGGTAA A

Name: *Pseudanabaena catenata* Group: Cyanophyte Sample: June 2017 E value: 0.0 Percent Identity: 99%

Raw Sequence:

Name: *Chlorella sp*. Group: Chlorophyte Sample: June 2017 E value: 0.0 Percent Identity: 97%

Raw Sequence:

CTATGGGTTGTAAACTTCTTTTCTCAGAGAAGAAGAAATTTTGACGGTATCTGAGGAATAAGCATCGGCTAACTCTGTG CCAGCAGCCGCGGTAAGACAGAGGATGCAAGCGTTATCCGGAATGATTGGGCGTAAAGCGTCTGTAGGTGGCTTA AAAAGTCTCCTGTCAAAGATCAGGGCTTAACCCTGGGCCGGCAGGAGAAACTCTTAGGCTAGAGTTTGGTAGGGG CAGAGGGAATTCCCGGTGGAGCGGTGAAATGCGTAGAGATCGGGAGGAACACCAAAGGCGAAAGCACTCTGCTG GGCCACAACTGACACTGAGAGAGGAGAGAGGGAGGGAGCAAAAGGGATTAGATACCCCTGTAGTCCTCGCCGTAA ACGATGGATACTAGATGTTGGATAGGTTAAAGCATTCAGTATCGTAGCTAACGCGTGAAGTATCCCGCCTGGGGA GTATGCTCGCAAGAGTGAAACTCAAAGGAATTGACGGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA TGCAACGCGAAGAACCTTACCAGGACTTGACATGCCNACTTTTTCCCTGAAAGGGGAAGTTTCCGAGTGGACACA GGTGGTGCATGGCTGTCGTCAGCTCGTGTCTTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTTTG AATTGCTATTATTAGGAAATTCAAAAGACTGCCGGTGACAAGCCGGAGGAAGGTGANGATGACGTCAAGTCAGC ATGCCCCTTACGTCCNGGGCGACACACGTGCTACAATGGCCGGGACAAAGAGATGCNAACCCGCGAGGGCTAGC CAACCTCAAAAAACCCGGTCTCAGTTCGGATNGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATCGCTAGTAATC GCAGGTCAGCCATACTGCGGTGAATACGTTCCCGGGCNTTTGTACACACCGCCCGTCACACCATGGGAGCTGGCT ATGCCCAAAGTCGTTACCCCANCCTTTTGGAGGGGGGCGCCTAAGGCAGGGCTAGTGACTGGGGTGAAGTCGTAA CAAGGTAGCCGTACTGGAAGGTGCGGCTGGAACNCCTCCTTNAAAAGGATATAAAACATCCCTTTCCAWTTCCAA AAAAAATTCAATTTGGTTTTTGGAAAATRGAAAAGGGATCTTTCTTACAGATAAAAACTCATTTTTTGCTGTGCATGA GTCACTAGCTTGCTTTTAATGTACCTCTATTGACAAAACCAAAACGGTTTGTCAAACTCTGTGTTGGGAAAAAGC AACGGGCTATTAGCTCAGTTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGCTGGTTCAAATCCAGCATAGCC CACCACTTACCAAAAAACACACAGTTTTTGGTACGAGACACAAGTAAGGGGGGTATAGCTCAGTTGGTAGAGCGCTGC CCTTGCAAGGCAGATGTCAGCGGTTCGAGTCCGCTTATCTCCACCAGTGATCAACACAGAACCAAAATTTCTGG ATTTTGGTTACAGGAAAAAAAGCAAGTTAGTGGCTCATGCACGAGTAAAA

Name: *Phormidium tenue* Group: Cyanophyte Sample: June 2017 E value: 4e-125

Percent Identity: 91%

Raw Sequence:

AGGCTNGNTTAATAANNCTGNTGNCAAAGCCCNAGGCTCAACCTTGGATCGGCAATGGAAACTGTTAGACTAGAN AGAGATAGGGGCAGGAGGAATTCCNNGTGNNNCGGTGAAATGCGTNNANATCTGGAAGAACACCANTGGCNAA AGCGTCCTGCTGGATCTCAACTGACGCTGAAGTACGAAAGCTAGGGGAGCGAATGGGATTAGATACCCCNNTANT CCTAGCCGTAAACGATGGACACTAGGTGTTGGCCGTATCNNCCCGGTCAGTGCCGTANCTAACGCGNTNNNNGTC CCGCCTGGNNANNNCGGT

Name: *Limnothrix planktonica*

Group: Cyanophyte Sample: September 2017

E value: 0.0

Percent Identity: 99%

Raw Sequence:

GANGGANGAAGGCCTGTGGGTTGTAAACCTCTTTTCTCAGGGAAGAAGATCTGACGGTACCTGAGGAATCAGCAT CGGCTAACTCCGTGCCAGCAGCCGCGGTAAGACGGAGGATGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGT CCGCAGGCGGTCTCGTAAGTCTGTCTTTAAAGCGTGGAGCTTAACTCCATAAAGGGGATGGAAACTGCGAGACTA GAGGTAGGTAGGGGTAGAAGGAATTCCCAGTGTAGCGGTGAAATGCGTAGATATTGGGAAGAACACCAGCAGCG AGTCCTAGCCGTAAACGATGGACACTAGGTGTTGCACGTATCGACCCGTGCAGTGCCGTAGCCAACGCGTTAAGT GTCCCGCCTGGGGAGTACGCTCGCAAGAGTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTA TGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATCCTGCGAATCCTGGCGAAAGTCGGGAG TGCCTTCGGGAGCGCAGAGACAGGTGGTGCCATGGCTGTCGTCAGCTCGTGTGGGAGATGTTGGGTTAAGTCCCG CAACGAGCGCAACCCACGTCCTTAGTTGCCAGCATTTAGTTGGGGAACTCTAGGGAGACCGCCGGTGACAAACCGG AGGAAGGTGTGGATGACGTCAAGTCATCATGCCCCTTACGTCTTGGGCTACACACGTACTACAATGGTCGGGACA GAGGGCTGCAAGCTCGCGAGAGCAAGCTAATCTCGTAAACCCGGCCTCAGTTCAGATTGCAGGCTGCAACTCGCC TGCATGAAGGAGGAATCGCTAGTAATCGCAGGTCAGCATACTGCGGTGAATACGTTCCCGGGCCTTGTACACACC GCCCGTCACACCATGGGAGTTGGTTTTGCCCGAAGTCATTACCCTAACCGCAAGGAGGGGGATGCCTAAGGCAGG GCTGATGACTGGGGTGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGTGGCTGGATCACCTCCTTTCAGGGAGA CCTTACCCACTCAATTCCGAGAGCAATCAGCTAATAGGAATGAGTTTGGTCAACCTAGGTCGTTCGAGGAATTTGT GTGGCTCTCAAACTTGTCTGGGTTTGCTTCTAAGAAGAAGGAAAACGAGGGCTATTAGCTCAGGTGGTTAGAGCG CACCCCTGATAAGGGTGAGGTCCCTGGTTCGAGTCCAGGATGGCCCACCTTCGAAAGGGTCAAAGAGATTTGTCT CTTTAAATCGGTTCTCTGAAAGAGAATCAAAAGTTTTGCCTTCCAAGAAGAAATAACGTGAGTTCAGCAACTTCTA GCGATTCAGAAGACTGCTGAACTAATGTTCAGCCAGAACCTTGAAAAACTGCATAGCAACAAAAGCCAAAGCAAG GTAGTCGAGANTCAACAGCG

Name: Planktothrix sp.

Group: Cyanophyte

Sample: September 2017

E value: 0.0

Percent Identity: 99%

Raw Sequence:

GGANGAAGGCCTGTGGGTTGTAAACCTCTTTTCTCAGGGAAGAAGATCTGACGGTACCTGAGGAATCAGCATCGG ${\tt CTAACTCCGTGCCAGCAGCCGCGGGAAGACGGAGGATGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGTCCG}$ CAGGCGGTCTCGTAAGTCTGTCTTTAAAGCGTGGAGCTTAACTCCATAAAGGGGATGGAAACTGCGAGACTAGAG GTAGGTAGGGGTAGAAGGAATTCCCAGTGTAGCGGTGAAATGCGTAGATATTGGGAAGAACACCAGCAGCGAAG GCGTTCTACTGGACCAAAACCTGACGCTCATGGACGAAAGCTAGGGGAGCGAAAGGGATTAGATACCCCTGTAGTC CTAGCCGTAAACGATGGACACTAGGTGTTGCACGTATCGACCCGTGCAGTGCCGTAGCCAACGCGTTAAGTGTCC CGCCTGGGGAGTACGCTCGCAAGAGTGAAACTCAAAGGAATTGACGGGGGGCCCGCACAAGCGGTGGAGTATGTG GTTTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATCCTGCGAATCCTGGCGAAAGTCGGGAGTGCC TTCGGGAGCGCAGAGACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC GAGCGCAACCCACGTCCTTAGTTGCCAGCATTTAGTTGGGGACTCTAGGGAGACCGCCGGTGACAAACCGGAGGA AGGTGTGGATGACGTCAAGTCATCATGCCCCTTACGTCTTGGGCTACACACGTACTACAATGGTCGGGACAGAGG GCTGCAAGCTCGCGAGAGCAAGCTAATCTCGTAAACCCGGCCTCAGTTCAGATTGCAGGCTGCAACTCGCCTGCA TGAAGGAGGAATCGCTAGTAATCGCAGGTCAGCATACTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCC GTCACACCATGGGAGTTGGTTTTGCCCGAAGTCATTACCCTAACCGCAAGGAGGGGGGATGCCTAAGGCAGGGCTG ATGACTGGGGTGAAGTCGTAACAAGGTAGCCGTACCGGAAGGTGTGGCTGGATCACCTCCTTTCAGGGAGACCTT ACCCACTCAATTCCGAGAGCAATCAGCTAATAGGAATGAGTTTGGTCAACCTAGGTCGTTCGAGGAATTTGTGTG GCTCTCAAACTTGTCTGGGTTTGCTTCTAAGAAGAAGGAAACGAGGGCTATTAGCTCAGGTGGTTAGAGCGCAC CCCTGATAAGGGTGAGGTCCCTGGTTCGAGTCCAGGATGGCCCACCTTCGAAACGCTCAAAGAGATTTGTCTCTTT AAATCGGTTCTCTGAAAGAGAATCAAAAGTTTTGCCTTCCAAGAAGAAATAACGTGAGTTCAGCAACTTCTAGCG

ATTCAGAAGACTGCTGAACTAATGTTCAGCCAGAACCTTGAAAACTGCATAGCAACAAAANCCAAAGCAAGGNA GTCGAGA

Name: Aphanizomenon ovalisporum

Group: Cyanophyte Sample: September 2017 E value: 0.0

Percent Identity: 86%

Raw Sequence:

GTTTCGGTGCGGNNTGCGAGAGCGGTACCAAATYGAGWCRWACTTCTGAATACKKGGTAAAGCACWSATTCCCA GCNTCAGACGGWGGGGGATAAGCACTKCWTCGTCAAGASGGRAGCAGCCCAGRCCACCAGCTAAGGTCCCCAAA TCGACATTAAGTGGTAAAGGAGGTGGGAGTGCACAGACAACCAGGAGGGTTGCCTAGAAGCAGCCATCCTTAAA AGAGTGCGTAATAGCTCACTGGTCAAGCGCTCCTGCGCCGAAAATGAACGGGACTAAATGTCGTACCGAAGCTGT GGAATTCGAAAGAATTGGTAGGGGAGCGTTCTGCCATAGGGAGAAGCATCAGCGGAAGCAGGGTGTGGACGAAGC AGAAGTGAGAAATTGGTAGGGGAGCGTTCTGCCATAGGGAGAAGCATCAGCGGAAGCAGGGGGAGGCGAGGCGAAGC GGAAGTCACGGAAGCTTCGGCTAGGGGACCTAAGGGCGAGGCCGAAGCCGAAGCCAGGGTGGGACGAAGC GGAAGGTCGTCCACGGAGGGTTAGTCGGGACCTAAGGGCGAGGCCGAGGGCGAAGCCCTAAGGGTCCTCC GGAAGGCTCGTCCACGGAGGGTAGTCGGGACCTAAGGGCGGGGGCGAGGCGAAGCCCTAATGGANNNTTNGGG AGGAGTCTACGGACTCTGCGTAGTGAAACAGGAACAGGGAAAACTAGCCTGTTCCAGTGTGAAAGSSAGTAC CCGTAAANCCCGAAACCGAMACAGKTAGGGAGRWTGAGAAWASCAMGGGGCGCGAGGTAACTCTCTCTAAGGA ACTCGGCAAAATGGCCCCGTAACTTCNGGGA

ITS2

Name: *Scenedesmus armatus* Group: Chlorophyte Sample: June 2017 E value: 1e-94

Percent Identity: 99%

Raw Sequence:

Name: *Desmodesmus intermedius*

Group: Chlorophyte Sample: June 2017

E value: 0.0

Percent identity: 99%

Raw Sequence:

Name: *Chlamydomonas sp*. Group: Chlorophyte Sample: June 2017 E value: 0.0 Percent identity: 96%

Raw Sequence:

Name: Pediastrum boryanum

Group: Chlorophyte Sample: June 2017 E value: 0.0

Percent Identity: 100%

Raw Sequence:

Name: Chlamydomonas sp.

Group: Chlorophyte

Sample: June 2017

E value: 0.0

Percent Identity: 97%

Raw Sequence:

Name: *Micractinium sp.* Group: Chlorophyte Sample: June 2017 E value: 0.0 Percent Identity: 96% Raw Sequence: GGCGGGCGTCCCTCCGGGGGTTGGGGCTCTGCCCCTCCGCTGGGCCGCTGGAAATTCGTATCCAACTCAACC CACCCCAAACCTCAACTTACTCTGAAGCTGTCTGGTGGATGCGCTCCGGCGCCCCACTCTAACCAAAGACAACTCT CAACAACGGATATCTTGGCTCCCGTATCGATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAATTGCAGAATT ${\tt CCGTGAACCATCGAATCTTTGAACGCAAATTGCGCCCGAGGCTTCGGCCAAGGGCATGTCTGCCTCAGCGTCGGC}$ TTACCCCCTCGCCCTCCCCCTCCTATGGAGTGGGCGGTGCGGATCTGGCCCTCCCGGCTCTGCTNCCNATCTAGGT CGCCCGAACGCNNCAACCTTTNATTTAAAGATTAGCCAAAATTTCCCCNNGGGGGGCCNNCCNTNTNNNANANNA ATTTNNGTTGNNNNNTTTCNGCNACGCGCAAGAACGGTTTGTGATATCCGNGNTCTGGTGACGTGGNTGNNGTCT GNAAAAAGAGTTGGTAGCTCTNGATNCGGCCAACCAGGGGCTGGGGGGTCTGCCCGGTTTTTTTGTGGGCCCGCTGG AAAATTACANCCAAANNAAAACGCCCCAAANANCATCCTNTGATCNTTTNGNCGGGNGCAGACGCTCCGGGGAC CCAATCCTCNCNAAGACGAATCTNGGNTATGGANATATNGGATCCGATCTTCANGAAGATCCNTTTNAATTGCAA TAGAAATTGTGAATTGCTCAATTCCATNNAACATTAAATCTGTGCAGACANATTGCANTGCAGGCTCAGGGAAGC GNCNAGCNCGCCNNTCTGTCTGTTTACCNCNTCCCCNTCCNCCTGANACNGAGTGGGGGGGGAAATACGNNCCT NNAGGGTTTGCCACCTNNCCCCGNGGCTCCGGTGNNNCCGANAGACCCAGGCTCGACGGTGCCNCATTTTTGNAC GGTAAAGCATCGGNANGNAACGTCCGANNACCCAACTGGCCCTGGCCCTATNTCACNCTCCNNGCGGCCNATTAN GAGTTGCCNGNAAGCTNGANCNCGNCGNCCGACAGCTNANANTTTGCNCATGATGTTGGCANTGCTACCCGCNTN AANNTGTCNCATTNGATCCCAAGNATGGCTTCATTCACTCCGGTTCCCANCGATCAAGGCGAGTTACATGATCCCC CATGTTGTGCAAAAAAGCGGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCANAAGTAANNNGGCCNCNNTGTNNT CACTCANGGTTATGGCAGCNCTGCNTAATTCTCTTACTGNCATGCCNNCCNAAGAAGCTTTTCNGTGACTGGNNG AGTACTCNACCAAGTCATTCTGAAAANNAGTGTATGCGGCNACCGAATTGGTCTGCCCGGCGNCANACGGNAAA ATACCGCGCCCCNTAACAAAAACTTTAAANNGNNTCNNCATGGNAAA

Name: Micractinium sp.

Group: Chlorophyte

Sample: June 2017

E value: 0.0

Percent Identity: 86%

Raw Sequence:

Name: Coelastrum microporum

Group: Chlorophyte Sample: June 2017 E value: 0.0

Percent Identity: 99%

Raw Sequence:

Name: *Micractinium sp*.

Group: Chlorophyte Sample: June 2017 E value: 0.0 Percent Identity: 89%

Raw Sequence:

Name: Coelastrella striolata

Group: Chlorophyte

Sample: June 2017

E value: 1e-82

Percent Identity: 82%

Raw Sequence:

Name: *Parachlorella kessleri* Group: Chlorophyte Sample: June 2017 E value: 0.0 Percent Identity: 100%

Raw Sequence:

Name: *Didymogenes palatina* Group: Chlorophyte Sample: June 2017 E value: 0.0

Percent Identity: 86%

Raw Sequence:

Name: Desmodesmus communis

Group: Chlorophyte Sample: June 2017 E value: 0.0 Percent Identity: 99%

Raw Sequence:

TGGGATCCGTTCCGTAGGTGAACCTGCGGAAGGATCATTGAATATGCAAACCACAACACGCACTCTCTACTTGTGT ACCGACGTTAAGTTACACACGCAAGTGTGTGTGCTTACTAACTTACACACCAATGACAACCAATGATTAAACCAAAC TCTGAAGTTTTGGCTGCTGTTAACCGGCAGTTTTAACCAAGAACAACTCTCAACAACGGATATCTTGGCTCTCGCA ACGATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAATTGCAGAATTCCGTGAACCATCGAATCTTTGAACGC ATATTGCGCTCGACTCCTCGGAGAAGAGCATGTCCGCCTCAGCGTCGGCTTTTCACCCTCACCCTCTTACTTTTCAA GTAAGCCTGCCGTGTTTGCTCAAACCGGCAATAGGGGTGGATCTGGCTCTGGCTCTCCCAACTGATTTCTAACCGGTTGGGT TGGCTGAAGCACAGAGGCTTAAACTGGGACCCGTATCGGGCTCAACTGGATAGGTAGCAACACCCTCGGGTGCCT ACACGAAGCTGTCTGAGGACCTGGTTAGGAGCCAAGCAGGAAACACGCTTAACCGCGTGTATCATGGGTTCGA CCTGAGCTCGGGCAAGGCTACCCGCTGAACTTA

Name: *Micractinium sp*. Group: Chlorophyte Sample: June 2017 E value: 0.0 Percent Identity: 89%

Raw Sequence:

Name: *Parachlorella kessleri* Group: Chlorophyte Sample: June 2017 E value: 0.0 Percent Identity: 99%

Raw Sequence:

Name: *Tetracystis pulchra*

Group: Chlorophyte

Sample: June 2017

E value: 1e-112

Percent Identity: 82%

Raw Sequence:

Name: *Desmodesmus spinosus* Group: Chlorophyte

Sample: June 2017 E value: 0.0

Percent Identity: 100%

Raw Sequence:

Name: Chlamydomonas debaryana

Group: Chlorophyte

Sample: June 2017

E value: 2e-156

Percent Identity: 84%

AGCTGTGCTTGTCGACCCAAACCAGGAACTTTGGCCTCGTGCCGAAGCAAACCCCTTATTTTCTCGACCTGAGCTC AGGCAAGATTACCCGCTGAA

Name: Closterium sp. Group: Chlorophyte Sample: June 2017 E value: 0.0

Percent Identity: 94%

Raw Sequence:

TGCATGTCTAAGTATACAAAGTTTACTTTGAAACTGCGAACGGCTCATTAACCNGNNNNNNTTATTTGATAGTTT TCTCTACTGGGATAACCGTAGTAATTCTAGAGCTAATACCTGCGCAACGTCCCGACTTCACAGAAGGGATGCGTTT ATTAGATCGAAACCAACACGGGGGCAACCCNCTTTAAGGTGATTCACAATAAGCAAGCAAATCGCACCGGCGCAA GCTAGGCGATGAGTCATTCAAGTTTCTGCCCTATCAGCTGCGATGGTACGGTATTGGCCTACCATGGCTTTGACGG CGCGAAAATTACCCAATCCCAACTCGGGGAGGTAGTGACAAGAAATACTTTGGCGGAGCCCTTTGGGTTCTGCCG TGGAATGAGCACNATTTAAATCCCTTAGCGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATT CCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCTTTTGCGTTTGGAGGGTTCGNTTACTGTGAAAAAATCAAANCGNTCAAAGCANGCTTTATGCNNTGAATGTCTCAGCATGNAATANTAATCNA ANNCNTCNNCTTATNTTGNTGGNTTACNAGATGAANGNNNGNTTNAATANNANCAGTCGGGNANATCCNAACTT NGNNAGTCANANGTGAAATTTCTTGNANTTTCCANGACNAACT

Name: Micractinium sp.

Group: Chlorophyte

Sample: June 2017

E value: 0.0

Percent Identity: 96%

Raw Sequence:

GCGTTCGGGCGGNGGGTGACCGCACGCACCGAATTCCTGCTGGGCCGCCAGCAAAGTCCCCTCGGGCCACGGCAG GCGGTGTAACCGAGGTTACCTACCAAGCCATTGCCCTACAAACGGGGTCCATGCTCAAGCCTCTACACTTCAGCC GACCCGGAGCCACCTAGATNGGNAGCAGAGCCGGGAGGGCCAGATCCGCACCGCCCACTCCATAGGAGGGGGAG GGCGAGGGGGTAAGCCGACGCTGAGGCAGACATGCCCTTGGCCGAAGCCTCGGGCGCAATTTGCGTTCAAAGATT CGATGGTTCACGGAATTCTGCAATTCACACTACGTATCGCATTTCGCTGCGTTCTTCATCGATACGGGAGCCAAGA TATCCGTTGTTGAGAGTTGTCTTTGGTTAGAGTGGGGCGCCGGAGCGCATCCACCAGACAGCTTCAGAGTNNNNN NAGGTTTGGGGTGGGTTGAGTTGGATACGAATTTCCAGCGGCCCANCCGAGGGAGGGGCAGAGCCCCAACCCCC CACCAAGCCACCGGGGGGGGGCGTTTGGNTACCAGAGTGGATTCGATCGATCGATCAATGATCCTTCCGCAGGTTCACC TACGGAAACGGATCCCA

Name: Actinastrum hantzchii

Group: Chlorophyte Sample: June 2017 E value: 0.0 Percent Identity: 97%

Raw Sequence:

ATAAACTĜCTTTATACTGTGAAACTGCNAATGGCTCATTAAATCAGTTATAGTTTATTTGATGGTACCTACTACTC AGGCCGACCGGGCTCTGCCCGACTCGCGGTGAATCATGATAACTTCACGAATCGCATGGCCTTGTGCCGGCGATG TTTCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTGGTAACGGGTGACGGAGG ACCCAATCCTGACACAGGGAGGTAGTGACAATAAATAACAATACTGGGCCTTTTCAGGTCTGGTAATTGGAATGA GTACAATCTAAACCCCTTAACGAGGATCAATTGGAGGGCAAGTCTGGTGCCAGCANCCGCGGTAATTCCNGCTCC NATAGCGNATATTTAAGTTGCTGCANTTAAAAAGCTCGTANTTGGATTTCNGGTGGGGNCTGCCGGTCCGCCGTTT CGGTGTGCACTGGCAGGGCCCACCTTGTTGCCGGGGACGGGCTCCTGGGCTTCACTGTCCGGGACTCGGAGTCGG NGCTGTTACTTTGAGTAAATTANAGTGNTCNAAGCANGCCTACNCTCT

Name: Chlamydomonas globosa Group: Chlorophyte

Sample: June 2017 E value: 0.0

Percent Identity: 99%

Raw Sequence:

TGCNTGTCTAAGTATAAACTGCTTATACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATAGTTTATTTGATGG TACCTACTCGGATAACCGTAGTAATTCTAGAGCTAATACGTGCGCACAACCCGACTTCTGGAAGGGTCGTATT TATTAGATAAAAGGCCAGCCGGGCTCTGCCCGACCTGCGGTGAATCATGATAACTTCACGAATCGTATGGCCTCG TGCCGACGATGTTTCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTGGTAACG ATTGGAATGAGTACAATCTAAATCCCTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAA TCCGCCTCTGGTGTGCACTGCTCCGCCCACCTTCCTGCCGGGGACGGGCTCCTGGGCTTCACTGTCTGGGACTCG GAGTCGGCGAGGTTACTTTGAGTAAATTAGAGTGTTCAAAGCAGGNCTACGCTCTGAATACATTAGCATGGAATA ACACGATAGGACTCTGGCCTATCTGTTGGTCTGTGGGACCGGAGTAATGATTAAGAGGGGGTAGTCGGGGGGCATTC GTATTCCGTTGTCAGAGGTGAAATTCTTGGATTTACGGAAGACGAACATNTGCGAAAGCATTTGCCAAGGATACT TTCATTGATCAAGAACGAAAGTTGGGGGGCTCGAAGACGATTAGATACCGTCGTAGTCTCMACCATAAACGATGCC GACTAGGGATTGGCAGATGTTYTTTTGATGACTCTGCCAGCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGG GGAGTATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCACGGCGTGGAGCCTGCGGCTTAA AGCCTGCTAAATAGTCAGCATCGCACCTGCGGTGCGCAGACTTCTTAGAGGGACTATTGGCGTTTAGCCAATGGA AGTATGAGGCGATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGACGCGACCAAC GAGCCTATCCTTGGCCGAGAGGCCCGGGTAATCTTGTAAACCGCGTCGTGATGGGGATAGATTATTGCAATTATTA GTCTTCAACGAGGAATGCCTAGTAAGCGCGAGTCATCAGCTCGCGTTGATTACGTCCCTGCCCTTTGTACACACCG AGAAGTTCATTAANCCCTCCCACC

Name: *Staurodesmus validus* Group: Chlorophyte Sample: September 2017 E value: 1e-64 Percent Identity: 95%

Raw Sequence:

<u>18S</u>

Name: *Bicosoeca petiolata* Group: Chrysophyte Sample: June 2017 E value: 0.0 Percent Identity: 96%

Raw Sequence:

TGCATGTCTAAGTATAAATAACTCTATACTGTGAAACTGCGAATGGCTCATTATATCAGTTATAGTTTATTTGATA GTCCCTTACTACTTGGATAACCGTAGTAACTCTAGAGCTAATACATGCGCCAAGTCCTGACTGCGGGGCAACTGCGG GAAGGATGTATTTATTAGATCCAACCAACCCACGGGGGCAACTCCGCGGTACCTGGTGAATCATGATAACTGAGC GAATCGCATGGCCTCGTGCCGGCGATGGTTCATTCAAGTTTCTGCCCTATCAGCTTTCGATGGTAGGGTATTGGCC TACCATGGCATTAACGGGTAACGGAGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATC CCAATGGCTTCGTAATTGGAATGAGTGCAAGCCAAATCTCTGCACGAGGAACAATTGGAGGGCAAGTCTGGTGCC AGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGTTCGTAGTTGAATGTCTG AACCCGTTCCGTCGGCACCCCTGCATCAATTACTGTGAACAAATTAGAGTGTTCAAAGCGGGCAGGACTGCTTGA GACAGTTGGGGGTATTCATATTTGAATGTCAGAGGTGAAATTCTTGGATTTTTCAAAGATGAACAACTGCGAAAG CATTTACCAAGGATGCTTTCATTAATCAAGATCGAAAGTCAGGGGATCGAAGAGGATTAGATACCCTCGTAGTCT TGACCATAAACTATGCCGACTCAGGATTGGCGGTGCACTTCAACGTCGCCGCCAGCACTGTATGAAAAATCAAAG GGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTTACCAGGTCCGGACATAGTAAGGATTGACAGATTGAT AGCTCTTTCTTGATTCTATGGGTGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGGTTAATTCCGAT AACGAACGAGACCTCCGCCTGCTAAATAGTCACGTGGTGCCTCGCGCACCCACGGCGTACTGACTTCTTAGAGGG ACTATGCACGGTAAGTGCATGGAAGTTGGAGGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACG CGCGCTACACTGACTGGTTCAGAGAGTTCTCCCTGCGCTCCGAAAGGCGTGGGTAATCTGTGAATGCCAGTCGTG ATGGGGATAGATTCCTGCAATGACAATCTTCAACGAGGAATTCCTAGTAAGCGCAAGTCAACAACTTGCACTGAT TACGTCCCTGCCCTTTGTACACACCGCCCGTCGCACCTACCGATTGAATGGTCCGGTGAAACTCTCGGAGCGTGGC

Name: *Pseudopediastrum alternans*

Group: Chlorophyte Sample: June 2017 E value: 1e-134 Percent Identity: 86%

Raw Sequence:

GATCCGTTTCCGTAGGTGAACCTGCGGAAGGATCATTGAATTATCAATACCACAATGTGAACCATTCGTCCATGTA ACTCCGCTCAGTTCAGTTCACGCTGAACTGGCGCCTAAACCAAACTCTGAAGCTATGATTGCTATTCATTTGGCAA TCCTAACAAAGACAACTCTCAACAACGGATATCTTGGCTCTCGCAACGATGAAGAACGCAGCGAAATGCGATACG TAGTGTGAATTGCAGAATTCCGTGAACCATCGAATCTTTGAACGCATATTGCGGCCGCGGCGTTCGGCCAAAGCCAT GTCTGCCTCAGCGTCGTTTTGATACCTCACACCCCTATCCTTTTGGGTAGCGTGCAATCAGCTGCGGCTGGTTGCCG GGCGTGGATCTGGCTTCCCCGGACTGCAAAGCCCGGGTTGGCTGAAGTTGAGAGGCTAGAACACAGACCCATTAT TGGGCTTCAACTGGATAGKKWGAACGCWWSSTTGCTGKMCCGCYGTGMWGAGCCWTTCWATKGGCGRGSTAGC YGCCYWCSGCAGTTAGGGGGCSCAGTGCGTGCGTGTCCTTTTCACTCCGCTCAGTTCACGCTGAACTGGCGC CTAAACCAAACTCTGAAGCTATGATTGCTATTCATTTGGCAATCCTAACAAGACAACTCTCAACAACGGATATCT TGGCTCTCGCAACGATGAAGAACGCAGCGAAATGCGATACGTAGTGTGAATTGCAGAATTCCGTGAACCATCGAA TCTTTGAACGCATATTGCGGCCGCGGCTTCGGCCAAAGCCATGTCTGCCTCAGCGTCGTTTTGATACCTCACACCC CTATCCTTTTGGGTAGCGTGCAATCAGCTGCGGCTGGTTGCCGGGCGTGGATCTGGCTTCCCCGGACTGCAAAGCCCGGGTTGGCTGAAGTTGAGAGGCTAGAACACAGACCCATTATTGGGCTTCAACTGGATAGGTAGAACGCAAGCTT GCTTGCACTACACGAAGTTGTTGCCTAGGGACCGTGTTGGCGGCCAGCAGGATAAACTTTCAACTTCGACCTGAG CTCAGGCAAGATTACCCGCTGAACTTAAGCAAATGGATCCCA

Name: *Neodesmus danubialis*

Group: Chlorophyte Sample: June 2017 E value: 2e-112 Percent Identity: 96%

Raw Sequence:

Name: *Coelastrella sp*. Group: Chlorophyte Sample: June 2017 E value: 1e-83

Percent Identity: 78%

Raw Sequence:

Name: Desmodesmus intermedius

Group: Chlorophyte Sample: June 2017 E value: 0.0 Percent Identity: 99%

Raw Sequence:

Name: *Tetradesmus dimorphus* Group: Chlorophyte Sample: June 2017 E value: 0.0 Percent Identity: 99%

Raw Sequence:

Name: Cosmarium bioculatum

Group: Chlorophyte Sample: June 2017 E value: 0.0

Percent Identity: 97%

Raw Sequence:

Name: *Chlorella vulgaris* Group: Chlorophyte Sample: June 2017 E value: 0.0 Percent Identity 99%

Raw Sequence:

AGGAATAAGCATCGGCTAACTCTGTGCCAGCAGCCGCGGGTAAGACAGAGGATGCAAGCGTTATCCGGAATGATTG GGCGTAAAGCGTCTGTAGGTGGCTTAAAAAGTCTCCTGTCAAAGATCAGGGCTTAACCCTGGGCCGGCAGGAGAA ACTCTTAGGCTAGAGTTTGGTAGGGGCAGAGGGAATTCCCCGGTGGAGCGGTGAAATGCGTAGAGATCGGGAGGG AGATACCCCTGTAGTCCTCGCCGTAAACGATGGATACTAGATGTTGGATAGGTTAAAGCATTCAGTATCGTAGCTA ACGCGTGAAGTATCCCGCCTGGGGAGTATGCTCGCAAGAGTGAAACTCAAAGGAATTGACGGGGGCCCGCACAA GCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCAKGACTTGACATGCCACTTTTTCCCTGAA AGGGGAAGTTCCAGAGTGGACACAGGTGGTGGCATGGCTGTCGTCAGCTCGTGTCTTGAGATGTTGGGTTAAGTCC CGCAACGAGCGCMACCCTTGTTTTGAATTGCCATTAATGGGAAATTCAAAAGACTGCCGGTGACAAGCCGAGGAA GGNGAGGATGACGTCAAGTCAGCATGCCCCTTACGTCCTGGGCGACACGTGCTACAATGGGCCGGGACAAAG AGATGCAAACCCGCGAGGGCTAGCCAACCTCAAAAACCCGGTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGC ATGAAGTCGGAATCGCTAGTAATCGCAGGTCAGCCATACTGCGGTGAATACGTTCCCGGGCNNGTACACNCCGCC CGTCACACCATGGGAGCTGGCTATGCCCAAAGTCGTTACCCCAACCGTTTGGAGGGGGACGCCTAAGGCAGGGCT AGTGACTGGGGTGAAGTCGTAACAAGGTNNCCGTACTGGAAGGTGCGGCTGGAACACCTCCTTTTAAAGGATATA ACAAATTTCTCAACTCGGTGGAAAGAATTCATTCTTTCCAACAAGTTGAGAATATATTGGTTTTTTTGCTGTGCATG AGTCACTAGCTTGCTTTTAAAATTTCCTCTGTTGACAAATTAGTTTTGTTTAAAAACACTCTGTGTCGGGAAAAAG CAACGGGCTATTAGCTCAGTTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGCTGGTTCAAATCCAGCATAGC CCACCCGTGCCAAAAAACATCTTAGAACCTATAAGATTTTTGGTAGCGGTTCTGATGAAACACGAGTGAGGGGGG GTATAGCTCAGTTGGTAGAGCGCTGCCCTTGCAAGGCAGATGTCAGCGGTTCGAGTCCGCTTATCTCCACCAGTAA TCAACAAGTCTGGTAAAAAAGGCAAGTTAGTGACTCATGCACGAGTAGAAAAACTCTCATGAATCAACACTAAAT TTTAA

Name: *Phacotus lenticularis* Group: Chlorophyte Sample: June 2017

E value: 0.0

Percent Identity: 99%

Raw Sequence:

GTCTAAGTATAAACTGCTTATACTGTGAAACTGCGAATGGCTCATTAAATCAGTTATAGTTTATTTGATGGTACTT ATAAAAGGCCAGCCGGGTTCTCCCGACTTGTGGTGAATCATGATAACTTCACGAATCGCATGGCCTTGTGCCGGC GATGTTTCAATTCAAATTTCTGCCCTATCAAGTATCGTATGTAGGATAGAGGCCTACATAGCTTATGACGGGTGACG TGAGTACAATCTAAATCCCTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGC TCTGGTGTGTACTGCTATGGCTCACNCTTTCTGACGGGGACCCGCTCTTGGGCTTNACTGTCCGGGACGGGGAATC ATAGGACTCTGGCCTATCTTGTTGGTCTGTAGGNCTGGAGTAATGATTAAGAGGGACAGTCGGGGGGCATTCGTATT TCATTGTCAGAGGTGAAATTCTTGGANTTATGAAAGACGAACTACTGCGAAAGCATTTGCCAAGGATGTTTCATTA ATCAAGAACGAAAGTGGGGGGGCTCGAAGACGATTAGATACCGTCGTAGTNTCAACCANTAAACGATGCCGACTA GGGATTGGCAGGTGTTTTATTGATGACCCTGCCCAGCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAG TATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGCGTGGAGCNTGCGGCTTAATTTGA GCTAAATAGTCACAGTTACTTTCTGTAGCTGCAAGACTTCTTAGAGGGACTATTGCCGTTAGGCAATGGAAGTGTG AGGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGCATTCAACGAGCCT ATCCTTGACAGAAATGTCCGGGTAATCTTTGAAACTGCATCGTGATGGGGATAGATTATTGCAATTATTAGTCTTC ANCGAGGAATGCCTAGTAAGCGCGAGTCATCAGCTCGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTC GCTCCTACCGATTGGGTGTGCTGGTGAAGTGTTCGGATTGGCTTTAGCTGGGGGCAACTTCGGCTCTTGCTGAGAA GAATATTAAACCCTCCCACCTAG

Name: *Phacotus lenticularis* Group: Chlorophyte

Sample: June 2017 E value: 0.0

Percent Identity: 96%

Raw Sequence:

Name: Desmodesmus bicellularis

Group: Chlorophyte

Sample: June 2017

E value: 0.0

Percent Identity: 99%

Raw Sequence:

Name: *Coelastrum proboscideum* Group: Chlorophyte Sample: June 2017 E value: 0.0

Percent Identity: 96%

Raw Sequence:

Name: Grasiella emersonii

Group: Chlorophyte

Sample: June 2017

E value: 0.0

Percent Identity: 99%

Raw Sequence:

CTAAGTATAAACTGCTTATACTGNGAAACTGCGAATGGCTCATTAAATCAGTTATAGTTTATTTGGTGGTACCTTA CTACTCGGATAACCGTAGTAATTCTAGAGCTAATACGTGCGTAAATCCCGACTTCTGGAAGGGACGTATATATTAG ATAAAAGGCCGACCGAGCTTTGCTCGACCCGCGGTGAATCATGATATCTTCACGAAGCGCATGGCCTTGCGCCGG CGCTGTTCCATTCAAATTTCTGCCCTATCAACTTTCGATGGTAGGATAGAGGCCTACCATGGTGGTAACGGGTGAC ATGAGTACAATCTAAATCCCTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTNAATTCCA CTATGGTGAGTACTGCTATGGCCCTCCTTTCTGTCNGGGACGGGCTTCTGGGCTTCACTGTCCGGGACTCGGAGTCGACGTGGTTACTTTGAGTAAATTAGAGTGTTCAAAGCAGGCTTACGCCCTGAATACTTTAGCATGGAATAACACG ATAGGACTCTGGCCTATCTTGTTGGTCTGTAGGACCGGAGTAATGATTAAGAGGGACAGTCGGGGGGCATTCGTAT TTCATTGTCAGAGGTGAAATTCTTGGATTTATGAAAGACGAACTACTGCGAAAGCATTTGCCAAGGATGTTTTCAT TAATCAAGAACGAAAGTTGGGGGGCTCGAAGACGATTAGATACCGTCGTAGTCTCAACCATAAACGATGCCGACTA GGGATTGGCGAATGTTTTTTTTAATGACTTCGCCAGCACCTTATGAGAAATCAAAGTTTTTGGGTTCCGGGGGGGAGT ATGGTCGCAAGGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGAC CTAAATAGTCTCATTCGCTTTTTGCGGATGGCTGACTTCTTAGAGGGACTATTGGCGTTTAGTCAATGGAAGTATG AGGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGATGCATTCAACAAGCCT ATCCTTGACCGAAAGGTCCGGGTAATCTTTGAAACTGCATCGTGATGGGGATAGATTATTGCAATTATTAGTCTTC AACGAGGAATGCCTAGTAAGCGCAAGTCATCAGCTTGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTC GCTCCTACCGATTGGGTGTGCTGGTGAAGTGTTCGGATTGGCAGCTTAGGGTGGCAACACCTCAGGTCTGCCGAG AAGTTCATTAANCCCTCCCACCNAG

Name: *Closterium sp*. Group: Chlorophyte Sample: June 2017 E value: 0.0

Percent Identity: 100%

Raw Sequence:

TGTCTAAGTATACAAAGTTTACTTTGAAACTGCGAACGGCTCATTAACCAGTTATAGTTTATTTGATAGTTTTCTCT ACTGGGATAACCGTAGTAATTCTAGAGCTAATACCTGCGCAACGTCCCGACTTCACAGAAGGGATGCGTTTATTA GGCGATGAGTCATTCAAGTTTCTGCCCTATCAGCTGCGATGGTACGGTATTGGCCTACCATGGCTTTGACGGGTAA AAAATTACCCAATCCCAACTCGGGGAGGTAGTGACAAGAAATACTTTGGCGGAGCCCTTTGGGTTCTGCCGTGGA ATGAGCACAATTTAAATCCCTTAGCGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAG CTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGGATTTCTTTTGCGTTTGGAGGGTTTGGGATTGCGTCTGCCTTCTTGGTAGGCGCGCCCTTCCTCTTGCGTGAAATACTTGCCGTTGGTTTACTGACGGCATCGTT ATCGTCTTATTTGGTTTACAAGATGAAGGAAGGAATGTTTAATAGGATCAGTCGGGGGATATCCGAACTTGGAAGTC AGAGGTGAAATTCTTGGATTTTCCAAAGTCGAACTACTGCGAAAGCATTTATCAANGGATGTTTTCATTAATCAAG AACGAAAGTTTGGGGATCGAAGATGATTAGATACCATCGTAGTCTAAACCATAAACTATGCCGATTCGGGATCGT AGGTTGAAACTTAAAGGANATTGACGGAAGGGCACCATCAGGCGTGGAGCCTGCGGCTCAATTTGACTCAACTCG ACAGGTCTGTGATGCCCTTAGATGTCCTGCGCCGCACGCGCGCCACAATGACCAGTTCAATCGGTACTTCCTTGGT CGAGAGGCCTGGGTAATCCTAGAACTCTGGTCGTGATGGGGGCTAGATTTTTGCAATTATTAATCTTCAACGAGGAA TGCCTAGTAAACGCAAGTCATCATCTTGCATTGAATACATCCCTGCCCTTTGTACACACCGCCCGTCGCATCTACC GATGAATTTTGCGGTGAAGTCTTGGGAGTTCTTTAGGTTTGCCTTCACGGGTAGGCTTAGAGGACGAACTTGCATA AACCTTAACATTAGAGGAA

23S

Name: *Chlorella variabilis* Group: Chlorophyte Sample: June 2017 E value: 0.0 Percent Identity: 95%

Raw Sequence:

GTTTNGNTNCGGGCTGNGNNANNGGTACCAANTCGTGGCAAACTCTGAATACTAGATATGCTATTTCTGGGCCAG TGAGACAGTGGGGGGATAAGCTTCATTGTCAAGAGGGGAAACAGCCCAGATCACTAGGTAAGGCCCCCAAAATGATC GTTAAGTGACAAAGGAGGTGAGAATGCAGAAACAACCAGGAGGTTTGCTTAGAAGCAGCCACCCTTTAAAGAGT GCGTAATAGCTCACTGGTGGAGCGTTCTTGCGCGAAAACATGTCCGGGGACTAAATGATCTGCCGAAGCTGTGGGAT ATTTCCAAGAGAAATATCGGTAGGGGAGCGTTCTGCTATAGGGTGAAGCATTGATGTAAGTCAATGTGGACGAAG CAGAAGTGAGAAATATCGGTAGGGGAGCGTTCTGCTATAGGGTGAAGCATTGATGTAAGTCAATGTGGACGAAG CAGAAGTGAGAATGTCGGCTTGAGTAACGCAAACATTGGTGAGAATCCAATGCCCCGAAAACCTAAGGATTCCTC CACTAGGTTCGTCCATGGAGGGTGAGTCAGGACCTAAGGCGAGGCCGAAAAGCCTAGGCCAAAACCTAAGGATTCCTC CACTAGGTTCGTCCATGGAGGGTGAGTCAGGACCTAAGGCGAGGCCGAAAAGGCGTAGTCGATGGCAAAACAGGTT AATATTCCTGTACTACTTTATGTCTGGTACCGAGGGACGGAGGAGGAGGAGGAGGAGGAGGCGAAAATTGGCCGAGATTGGGTTTAACTTTAAGA TAAAGATTAATTCCCTATCANACTTCCAAGAAAAACTAACCTTGAGTGGAGAAGTGATACGTCTTTATCTTTAAGA TAAAGATTAATTCCCTATCANACTTCCAAGAAAAGCTCGGAACTACTCTAAAACATAAAGTACCTGTACCGCAAANTGACC CGCACAGGTGGGTTGGTAGAGGTATACCNAGGGGGCGCGAGAACTCTCTCTAAAGAACCTGGAAACTCGGCAAAANTGACC CCGTA

Name: *Pseudanabaena sp.* Group: Cyanophyte Sample: June 2017 E value: 0.0 Percent Identity: 97%

Raw Sequence:

Name: *Durinskia baltica* Group: Dinoflagellate Sample: September 2017 E value: 0.0

Percent Identity: 98%

Raw Sequence:

AAATCGTAGCANTGTTTCGTACTTCGGGAGAAGGGGWAAAGCACTGTTTCNGTRAGCTTCGGGAGAAGGGGTAA ASCACTGTTTCGAKRAMWTCAGSMSARGGCNCCAGTTAAGGCCCCTAAATAATTACTANAGTGATAAAGGAGGGT GGGAGTGCAGAAACAATCAGGAGGTTTGCTTAGAAGCAGCAGCAATCCTTTAAAGAGTGCGTAATAGCTCACTGATCG AGTAAACCTGCGCCGAAAATGTACGGGACTAAGTAATTTGCCGAAACTGTGCGATATATTTGAAATATATCGGTA GGGGAGCGTTCTGTTGTAGGTTGAAGTATTAGCGGAAGCGGATATGGACGAAGCAGAAGTGAGGAATGTCGGCTTG AGTAACGAAAATATAGGTGAGAATCCTATACCCCGAAAACCCAAGGTTTCCTCCGGAAGGCTCGTCCGCGGGAGGG TAAGTCAGGACCTAAGGCGAGAGCTGAAAAGCGTAGTCGATGGATAACGGGTTAATNTNCCCGTACCATTATTTAT TGATAACGAAGGGACGGAGAAGGCTAAACTGGCCGGATATTGGTTACCGGKTTAAGCGTTCGAGMNTGTTKAGAA ACTTCGGGAGAARRSGTWAAGMRYTGWKWCGTRACTTCGRNNTGMKAMGGCRKTRAAGCACTGTWTNGTCAAA CTTCGGGAGAAGGGSTAAAGCACTGTTTCGTAACTTCGGGATAAAGCACTGTTYCGTAACTTCGRGAC AAGTGGGTANNAAGAGNACACTGAKSGGMGCGAGATAACTCTCTCTAAGGNACTCNNCAAANTGACTCCNTAAC TTCNGGAGAA

Name: *Phormidium sp*.

Group: Cyanophyte Sample: September 2017

E value: 1e-73

Percent Identity: 88%

Raw Sequence:

Name: *Phaeodactylum tricornutum* Group: Diatom Sample: September 2017 E value: 0.0

Percent Identity: 92%

Raw Sequence:

GTGAGACTGNGGGGGATAAGCTCCATNGTCAAGAGGGAAACAGCCCAGAGCNCCAGTTAAGGCCCCTAAATNAT TGCTAAGTGATAAAGGAGGTGGGAGTGCNGNNNCAATCAGGAGGTTTGNTNNGAAGCAGCAATCCTTTAAAGAG TGCGTAATAGCTCANTGATCGAGTAAACNTGNGCNGAAAATGTACGGGANTAAGTAATTNGCCGAAACTGTGCG ATATATGAAATATATCGGNANNGGAGCGTTCTGTTGTAGGTTGAAGTATTAGCGTAAGCGGATATGGACGAAGCA GAAGTGAGAATGTNNGCNTGAGTAACGAAAATATAGGTGAGAATCCTATACCCCGAAAACCCAAGGTTTCCTCCG NNNNNNNNNNNAAGCACTGTTTCGTANCTTCGGGAGAAGGGGTAAAGCACTGTTTCGTAACTTCGGGAGA AGGGGTAAAGCACTGTTTCGTAACTTCGGGAGAAGGGGTAAAGCACTGTTTCGTAACTTCGGGAGAAGGGGTAAA GCACTGTTTCGTAACTTCGGGAGAAGGGGTAAAGCACTGTTTCGTAACTTCGGGAGAAGGGGTAAAGCACTGTTT CGTAACTTCGGGAGAAGGGGTAAAGCACWANYCGCAAYCCKTTNNNNGNGCGTAATANCTCACTGATCNAGTN NNCCTGCNCCNAAAATGTACNGNACTANNTNATTTNCCNAANCTGTGCNATATATGAAATATNTCNNNNNGNGAG ${\tt CGNTCTGTTGTANNNNAANTATTANCNTNNGCNGANNTGGACGAAGCANAANTGAGAATGTCGGCTTGNGTAA}$ CGAAAATATAGGTGAGAATCCTATACCCCGNANNNNNAGGTTTCCTCCNGAAGGNNNNNNNNCNGAGGGTAAN TCANGNCCTAANGNNAGGCTGAAAANCGTAGNCGATGGACAANNNGTNNNNANTCNNNNNCCNTACTTTGNTGA TATCNNNGGACGGANAANGNTAANCTGGCCNGATATNGGNTTACCNNNTTAAACNNNCNNNATGATNAGAAACN NNNNANACGTNTTGANTNNANGNNTGNNTANGAGATGNTACNGNGTCNANNCANTCTATGTNATACTNNNNAN ANNNTTGNNCNNNCANNNACAANANNGNCNNNNCCATNNNCNNANNNNNG

Name: *Choricystis parasitica* Group: Chlorophyte Sample: September 2017 E value: 0.0

Percent Identity: 93%

Raw Sequence:

Name: *Kirchneriella aperta*

Group: Chlorophyte Sample: September 2017 E value: 0.0 Percent Identity: 90%

Raw Sequence:

AGCNCTGTTTCGTACTTCGGGAGAAGGGGTAAAGCACTGTTTCGTAACTTCGGGAGAAGGGGTAAAGCACTGTTT CGTAACTTCNGGAGAAGGGGNTCACCNNCTNNNGCCCCTNNNTGGCCGCTNNNNGGAAAAGGATGTGAAAATGC TGAAACAACCAGGAGGTTTGCTTAGAAGCAGCACCCTTTAAAGAGTGCGTAATAGCTCACTGGTAAAGCGTTCT TGCGCCGATAATGCCCGGGACTAAGCGGCCGCCGCGAAGCTGTGGGGATTTTTTTCAACGTTTTCGAAGGCGACTTCG GAATCGACGCGAAAAGAATCGGTAGGGGAGCGTTCGACATGGGGGTGAAGCGATGACGTGAGGTCTTCGTGGACGG TGTCGAAGTGAGAAATGTTGGCTTGAGTAACGAAAACATTGGTGAGAATCCAATGCCCCGAAAACCTAAGGGTTCC TCCACAAGGTTCGTCCATGGGGGGGGGAGCCAGGACCTAAGGCGAGGCCGAACGGCGTAGTCGATGGCAAACAGG TTAATATTCCTGTACTCNTTGTTGTGGGGACCGANGGACGAAGAAGGCTCACTTGTAGCTGGATGGCAAACAGG GGAAGCGTTCNAGGCGTCGATAGGTCGAATAAAAACGACTNNTAGAGCTGANACGTGATCCCATTCCCGGAACT GGAAGCGTTCNAGGCGTCAACGATGTCATACTTCCGAGAAAAGCNTCGCACACTATTAACACAACNACGACCTGT ACCAGAAACCGACACNNTAGGTAAGTCGAGAANACTCNNGGCGCNNNAGAACTCTCT

Name: *Brachteacoccus minor* Group: Chlorophyte
Sample: September 2017 E value: 0.0

Percent Identity: 84%

Raw Sequence:

Name: *Chlorotetraedron incus* Group: Chlorophyte Sample: September 2017 E value: 8e-91 Percent Identity: 94%

Raw Sequence:

AGTGCTTTACCCCTNTCTCCCGAAGTTACGAAACAGTGCTTTACCCCTTCTCCCGAAGTTACGAAACAGTGCTTTA CCCCTTCTCCCGAAGTTACGAAACAGTGCTTTACCCCTTCTCCCGAAGTTACGAAACAGTGCTTTACCCCTTCTCCC GAAGTTACGAAACAGTGCTTTACCCCTTCTCCCGAAGTTANCGAAACAGTGCTTTAYSCCTTCTCCCGAAGTTMMK AAACAGWSCCNNTTTASCYTTCTTCGTCCCKAAGTTACNGAAACTCWGYACAGGAATATYAACCNGTTTTCCATC GANTACGCCGCTCGGCCTQCCTNACGTCCNGACTCACCCCCCACGGACGAACCTTGTAGAGGAACCCTTAGGTT TTCGGGGCATTGGATTCTCACCAATGTTTTCGTTACTCAAGCCGACATTCTCACTTCCGCATCGTCCACAAAAACTT ACGTTTCAGCTTCACCAATGCTGAATCCACGCCCCCTACCGATCGTTCAATTCTCTTTTCCTTCTCCTCTACCATACC CAAAGGTATGGTGGAGTTCGTAATTCCACGCCGCCCTCTACCCCAGAGGGGGAGGGGACAGAAATGGCGAGAA AAAGAAATATACAAATCCCATAGCTTCGGCAGGCTGCNTTAGTCCCGGCCATTGTCGGCGAAGAACGCTTTACC AGTGAGCTATTACGCACTCTTTTCAAGGAAATAGCTGCTTCTAANCNAACCTNCCSCTTGTTYCRAAGTTACSWMA CAGTGCTTTWCCMCTTMTCCCCGAAGTTAGGGCCGAAACAGNTGCTYTASCCCTTCTCCCCGAAGTTACGAAACAG TGCTTTACCCCTTCTCCCGAAGTTACGAAACAGTGCCTTTACCCCTTCTCCCCGAAGTTACGAAACAG TGCTTTACCCCTTCTCCCGAAGTTACGAAACAGTGCCTTTACCCCTTCTCCCCGAAGTTACGAAACAG

Name: Oscillatoria sp. Group: Cyanophyte Sample: September 2017 E value: 9e-130 Percent Identity: 90%

Raw Sequence:

TGTTTNGGNGCGGNNNNGNGAGAGCGNTACCAAATCGAGACNAANCYNTGAATACTNGGNGCACTCTTTCGYAC TTCRGGMGAWGGGGKAWAGCACTGTTTCGTAACTTCGKSARRAGGGGTAMAGCMCTGTTTCGTAACTTCSRGAK AAGGGGTAAAKCMCYRWWTCGWCAYTTCGGGAGAARGGGNNAGGTGGGAGTGCNCAGWCMACCAGGAGGGTT GCCTAGAAGCAGCCATCCTTAAAAGAGTGCGTAATAGCTCACTGGTCAAGCGCTCCTGCGCCGAAAATGAACGGG ACTAAATGTCGTACCGAAGCTGTGGAATTCGAAAGAATTGGTAGGGGAGCGTTCTGCCATAGGGAGAAGCATCAG CGGAAGCAGGTGTGGACGAAGCAGAAGTGAGAATGTCGGCTTGAGTAGCGCAAATTAGTGTGAGAAACCATAC CCCGAAACCCTAAGGGTTCCTCCGGAAGGCTCGTCCACGGAGGGTTAGTCGGGACCTAAGGGCAGAGCCGAGGG CGTANTNTATGGATACAGGGTGAATATTCCCTGACTACTNNAGCGCTGGAGCCTAAGGCGAGGCCGAGAGG CGTANTNTATGGATACAGGGTGAATATTCCCTGACTACTNNAGCGCTGGAGCTGAACANNGGGACGCATNNGWA AACYAGCTRTKYCSTAAYTGGNTCGGGAGNNGRGTYWAMGCACTGYTKCGTAACTTCGGRAGATAGGGNNGTWA AAGMACNTMCCAAGGGGCGCGAGGTAACTCTCTCTCAANGAACTCNNCNAAANGGGCCCCGTAACTTC

Name: *Pseudochlorella pringsheimii* Group: Chlorophyte Sample: September 2017 E value: 1e-135

Percent Identity: 87%

Raw Sequence:

AGNNNNCNNANACNTCCNNGNNNNNNGGTNTGNNNNNNCCNCCCNNGAATNNNNGNGGANNAGNNNCNNNG GNAGCNNTGNNNNNNGAAAATGTCNNGGANNAANNGNCCNTCNGNNGNTGTGNNATNNNNGNGANNNAATAT NNCNNNNGNNATNCNNACNNTGNTGNGNNTNNNNNCCCNGNNANCNTNACGATTCCTCCANNGGGTTNGTCCA TGGAGGGTGAGTCNGNNCNTAAGGNGAGGCNGAAAGNNGTAGTNGAGGGCAAACAGGTNNNTNNTCNNGTNCT AGTTCNTANTNNGNNCNGNGGGNCGGNNNNNNCNNNANNGNNNNNCNNNGNTTNNTANNTTCGGGNNAAGN NNNAAAGCACTGTTTNGTAACTTCNNNNNAAGGGGTAAAGCACTGTTTCGTAACTTCGGGAGAAGGGGTAAAGC ACTGTTTCGTAACTTCGGGAGAAGGGGTAAAGCACTGTTTCGTAACTTCGGGAGAAGGGGTAAAGCACTGTTTCG TAACTTCGGGAGAAGGGGTAAAGCACNGNTCGAAACTGCANNNNCAACCNGGAGGTTTGCTTAGAAGCAGCCAC CCTTTAAAGAGTGCGTAATAGCTCANTGGTGGAGCGTTCTTGCNCCNAAAATGTCCGGGACTAAATGACCTGCCN AANCTGTGGGATATTTTGAGAAAAAATATCNGTAGGGGAGCGTTCTGCTGTAGGGTGAANCNNNGATGGAAGTC ACTGNGGACNAANCGGAAGTGANAATGTCNGCTTGAGTAACGCANACNTTGGTGAGAATCCAATGCCCCGAANA CCTAANGATTCCTCCNCTAGGTTNNTCCNNGGAGGGTGAGTCANGACCNNNNGCGAGGCNNANNNGCNTNNTCN NATTGGATTTNNGTGGAAACTNTCTNCNTGANNNNNGGNAGAAGANNNNANNAACCNTGANTGGANAANNNATA CNTTTTCNTCNTTTGNNNGAANNTNNNNNNNNNNNNACTTCNNNNAAAAGCTCGTACTACTNNAGAATAANA ATTACCTGTNCCGTNNACCNNCNNNNGNGGGTNGGNNANANTNNNCNAGGGNNNCNNNANAACTCTCTCTNANG NANNNNCNAAANNACCCCNNNNCNTNNGGANAA

Name: Crucigenia sp.

Group: Chlorophyta

Sample: September 2017

E value: 0.0

Percent Identity: 100%

Raw Sequence:

Name: *Dicloster acuatus*

Group: Chlorophyta Sample: September 2017 E value: 0.0 Percent Identity: 95%

Raw Sequence:

Name: *Choricystis parasitica* Group: Chlorophyta Sample: September 2017 E value: 0.0

Percent Identity: 96%

Raw Sequence:

Name: Spinoclosterium cuspidatum

Group: Chlorophyta Sample: September 2017 E value: 0.0 Percent Identity: 96%

Raw Sequence:

CTAAMTKMSSGMKAAGTGGCAAAGGAGGTGAGAGGTGAGAGTGCAAAGACAGCCAGGAGGTTTGCCTAGAAGCAGCCACC CTTGAAAGAGTGCGTAATAGCTCACTGATCGAGCGCTCTTGCGCCGAAGATGAACGGGACTAAGCGGGTCTGCCGA AGCTGTGGGATGTCTATCAAAGACATCGGTAGGGGAGCGTTCCGCATTAGGGAGAAGCATCAGTGAAAAACAGGT GTGGACGAAGCGGAAGTGAGAATGTCGGCTTGAGTAACGCAAACATTGGTGAGAATCCAATGCCCCGAAAACCC AAGGGTTCCTCCCGCAAGGCTCGTCCACGGAGGGTGAGTCAGGACCTAAGATCAAGCCGAACGGCGCAGTCGATG GACAACAGGTCAAGATTCCTGTACTGCCCTTGCTGGCAAAACATAGGGACGGAGGAGGCGAAGCCGAAGCCGAAC AATGGTNNNCATCGGTTCAAGGCCAAAAMMCTGAGTACTAGGCACCTWCGGGTCCGAAGTRGCTRAWGCCAC GCTYYCAAGAAAAGCTCTTMSGSCGATACRGGTMAARMAMWKTTSKTWWYTTNACRRGAGAAGSAAGGYAAG TGRGCACCTGTTYCCGWAACTTCGRSACARGKGGKWARGYASWGTTTMRTAMCTWCGGGMGCGAGGGGTAMAR CWCTCTCTAAGGAACTCGGCAAGATGCCCCGTAACTTCNNGGAGAA

Name: *Pedinomonas tuberculata*

Group: Chrysophyta Sample: September 2017

E value: 1e-92

Percent Identity: 82%

Raw Sequence:

Name: *Hafniomonas sp.* Group: Chlorophyta Sample: September 2017 E value: 2e-106 Percent Identity: 84% Raw Sequence: ACCTTATGGTGATGCGGTAGAGGAGCGTTCTGTAAGCCTGTGAAGGTGAGTTGAGAAGCTTGCTGGAGGGTATCAG AAGTGCGAATGCTGACATGAGTAACGACAATGCGAGGTGAAAAACTCGCACGCCGAAAGACCAAGGTTTCCTGCG CAACGTTAATCGACGCAGGGTTAGTCGGTCCCTAAGGCGAGGCTGAAAAGCGTAGTCGATGGAAAACAGGTTAAT ATTCCTGTACTTCCAGTTATTGCGATGGANGGACGGANAAGGTTANGCCNCCTGGCGTTGGTTGTCCAGGTTTANG GWGRWRGGGTAAAGCNTSTTTNGYAACTCCGGGATTTSAAGGGSTAAAGCACTGTTTMGTAACCTTCGGGRGAARGGGTAA GGGGTAAAGCACTGWTTCGTWRCTTCGGGAGAAGGGSTAAAGCACTGTTTCGNTAACTTCGGGRGAARGGGTAA AGCACTGWTTCGTAACTTCGGGAGANARGGG

Name: *Synechocystis sp.* Group: Cyanophyta Sample: September 2017 E value: 0.0

Percent Identity: 95%

Raw Sequence:

Name: Cyanobacterium sp.

Group: Cyanophyta Sample: September 2017 E value: 0.0

Percent Identity: 99%

Raw Sequence:

Name: *Pseudochlorella pringsheimii* Group: Chlorophyta Sample: September 2017 E value: 0.0 Percent identity: 98%

Raw Sequence:

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