Diatom Voucher Flora and Comparison of Collection Methods for Biodiversity Hotspot Upper Three Runs Creek

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Diatom Voucher Flora and Comparison of Collection Methods for Biodiversity
Hotspot Upper Three Runs Creek

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Diatom Voucher Flora and Comparison of Collection Methods for Bio

Submitted by Katherine Margo Johnson in partial fulfillment of the requirements for the degree of M.S. Biology.

Accepted on behalf of the Faculty of the Department of Biological and Env Department and the College of Arts & Science by the Thesis Committee:

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PREFACE

This thesis has been written in journal format and conforms to the style appropriate to my discipline. Chapter II of this manuscript will be submitted for publication in *Botany Letters*, a peer reviewed interdisciplinary scientific journal, and therefore reflects the required formatting for this publication. This thesis does not contain a list of tables or a list of figures for Chapter II since these are not included in the submission directions for contributors to this journal. Figures and tables follow the text of the manuscript as required by *Botany Letters* and this thesis committee.
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General Introduction

Water quality monitoring through biological assessments is important for collecting and analyzing data concerning chemical changes and nutrient enrichment. Diatoms have been found to indicate changes in water quality better than other biota (fish and macroinvertebrates) currently used in biological assessments. Under the Endangered Species Act of 1973, endangered algal species and their habitats are protected. Therefore, understanding diatom biodiversity could facilitate the protection of aquatic ecosystems and conservation of surface water resources.

Changes in the algal community composition are potentially determined by habitat modification, increased recreational activities, introduced species and regional agricultural activities. For this reason, algal biodiversity is of great importance. Given algae are biological indicators, understanding algal species richness in surface waters could yield valuable baseline data. Currently, little remains known about the algal species present in the Iowa Great Lakes or southeastern United States. With the phenomenon of a ‘shifting baseline syndrome’ observed in other scientific studies, it is crucial to collect and document current data in a manner that can be verified for future surface water resource management and habitat conservation.

To better understand diatom communities and condition gradients, the U.S. Geological Survey and other North American institutions have created “voucher flora” consisting of light micrographs of samples with corresponding names associated with each diatom and project. In this study, we provide a baseline voucher flora of dominant and subdominant species found at these sites. These data along with long-term monitoring of algae and physicochemical parameters could facilitate future conservation efforts and the mitigation of anthropogenic impacts to these resources.
1 ABSTRACT

Surface freshwater is a scarce resource. Due to the scarcity and necessity of this resource, it is imperative that its quality is routinely monitored. One way of monitoring water quality is through biological assessments, which include examining algal assemblages. Regionally, little remains known of algal taxa in the southeastern United States. In the past, the Academy of Natural Sciences of Philadelphia (ANSP) conducted biological assessments along the Savannah River finding *Gomphonema parvulum* (Kützing) Kützing 1849 to be a dominant taxon rendering assessments inconclusive. Recent studies have provided evidence that species complexes and semi-cryptic taxa have been identified within the *Gomphonema* Ehrenberg 1832 genus and as *G. parvulum*. These studies have also shown that misidentifications have been made largely due to difficulty in using traditional light microscopy and biometric techniques (length, width, striae density in 10 μm). For an understanding of current water quality along the Savannah River, this study sought to investigate and resolve complexes found in a high quality habitat within the genus *Gomphonema*, as well as other common taxa found within the *Eunotia* Ehrenberg 1837, *and Tabellaria* Ehrenberg ex Kützing 1844 genera. We report the morphological diversity found among these taxa. We also provide a table with the biometric techniques (length, width, striae density in 10 μm, raphe form, central area characteristics, striae orientation, valve and poles shape) and supporting literature that we used to resolve *G. parvulum* complex representatives. In this study archives from past surveys located in the ANSP Diatom Herbarium were used to aid in resolving species complexes. Once resolved, we applied our species concepts to algal enumeration of current sampling conducted near a past Georgia College & State University (GCSU) research and reference site, Upper Three Runs Creek (UTRC). We used diatometer and composite sampling methods at our study site. These methods were chosen to compare present
results with 1956 past ANSP study results and assess differences between composite and diatometer collection methodologies. Diatometer sampling was conducted over an 18-day period from March 23rd to April 9th in order to account for optimal diatometer colonization time. Composite sampling was conducted at the end of this time period upon diatometer retrieval. We calculated species richness, species relative abundances, species evenness, and diversity indices: Shannon-Wiener, Sorenson index, and Jaccard similarity index. Based on the current taxonomic approach of samples collected in 1956 and in 2018, high biodiversity at the site has been maintained with high values of community indices. Diatom species specific ecological indication demonstrate that UTRC is a nutrient rich environment and according to 2018 physicochemical data, UTRC is an acidic environment with high dissolved oxygen levels. Microbial heterogeneity of single location was confirmed in this study and sampling methodology is important for monitoring and ecological inferences.

2 INTRODUCTION

Currently, 2.5% of the global water is freshwater, of which only 1.2% is surface water (Shiklomanov 1993). Due to the scarcity and necessity of this resource, it is imperative that the quality of freshwater resources is routinely monitored, measured, and evaluated. Monitoring water resources through biological assessments that include diatoms is important for collecting and analyzing data concerning water quality. In the past, the Academy of Natural Sciences of Philadelphia (ANSP) conducted water quality surveys along the Savannah River from the 1950s to early 2000s using diatoms as biological indicators (Patrick Center for Environmental Research 1996, Patrick Center for Environmental Research 2001). Diatoms are an ubiquitous group of algae, often found in environments that other trophic levels of life are not and, due to their silica cell wall, diatoms make excellent organisms for taxonomic study. During the Savannah River
surveys, ANSP also collected biological samples from Upper Three Runs Creek (UTRC). Upper Three Runs Creek is a tributary of the Savannah River, and is known as a southeastern biodiversity hotspot (Voelz & McArthur 2000). As a headwater stream that is protected by the U.S. Department of Energy (DOE), UTRC receives minimal land use disturbance and is considered a high quality habitat that is used as a control site to monitor other anthropogenic impacts (nuclear waste) on the Savannah River Site (SRS).

The ANSP reports and archives are important because little remains known about diatom species present in the southeastern United States. What is known has been largely contributed by late phycological pioneer Dr. Ruth Patrick in these reports and detailed in her monographs from 1966 (Patrick & Reimer 1966, Patrick & Reimer 1975). However, information from research conducted throughout Europe remains a primary literature source (Krammer & Lange-Bertalot 1988, Krammer & Lange-Bertalot 1991, Levkov et al. 2016). Potentially, this has led to assigning published names to southeastern diatom species, which could change perspectives on freshwater conditions in these areas. A 2007 study compared North American lists to European taxa assignments and found that regional and national metrics performed better in the U.S. than those with European assignments (Potapova & Charles 2007).

In surveys conducted along the Savannah River by ANSP, *Gomphonema parvulum* was found to be one of the dominant taxa leading to inconclusive results (Bouchard *et al.* 2001). Recent discoveries using molecular testing demonstrated semi-cryptic species of the *G. parvulum* complex (Kermarrec *et al.* 2013, Abarca *et al.* 2014, Ponader *et al.* 2017). Researchers from Georgia College & State University (GCSU) conducted past studies, which sampled UTRC and found *Gomphonema* taxa, including *G. parvulum*, to be highly variable (Moseley & Manoylov 2012). Due to challenges in the identification of this species complex with light microscopy
recent DNA sequencing questions the cosmopolitan nature of this species. Consequently, it may be possible that representatives of *Gomphonema* have been misidentified as *Gomphonema parvulum*. Therefore, original abundances of this species in past Savannah River water quality assessments may have been overestimated, which could change our understanding of the UTRC and the Savannah River’s ecology and water quality. Given the Savannah River is a southeastern surface water resource that provides potable water to surrounding areas, resolving the assessment of semi-cryptic species for continual monitoring is critical for understanding diatom diversity, local aquatic ecology, and water quality.

In this study, UTRC is investigated from the taxonomic perspective in order to aid in resolving complexes found within the genera *Gomphonema, Eunotia,* and *Tabellaria*. Due to the taxonomic nature of this study, and an effort to identify all valves (half frustules) to species level, we only sampled one area along UTRC, and do not make assumptions for the creek as a whole in our findings. We chose the previously mentioned species complexes after finding high abundances and morphological variability of members of these taxa in preliminary site assessments and because past taxonomy most likely “lumped” members of these taxa in species complexes. In this project we report the high morphological diversity found among these taxa, while referring to information from current molecular research of these taxonomic groups, as well as documenting other species and operational taxonomic units (OTUs) found at this site. In order to evaluate past biodiversity and morphological variability of possible species complexes from UTRC, archived material from 1956 composite samples and laboratory resources located in the ANSP Diatom Herbarium were used. However, the taxonomic analyses and algal enumeration from these archives was conducted in this GCSU study and not by the ANSP. From samples collected during this study we created a voucher flora for our site. This voucher flora
will aid in current biological surveys to improve assessments along the Savannah River and throughout the southeast. The objectives of this study include: 1) determining ecological indication from the diatom species collected at this site as well as physicochemical parameters such as pH, temperature, dissolved oxygen, and conductivity; 2) assessing biodiversity at a site along UTRC and creating a voucher flora; 3) comparing sampling methods/treatments (diatometers vs. composite samples) using biodiversity and similarity metrics, and 4) determining if separating taxa within species complexes or leaving them “lumped” or combined changes results of present and possibly past assessments of UTRC.

2.1 U.S. environmental policy, potable water, and site history and background

*Clean Water Act 33 U.S.C. §1251 et seq. (1972)*

Nutrient loading due to human activities has led to degradation of freshwater ecosystems and surface water resources (Smith *et al.* 1999). These ecosystems are necessary for providing potable water, productive fisheries, and safe recreational areas. The mediation of nutrient enrichment impacts has cost the United States approximately $2.2 billion annually (Dodds *et al.* 2009). Due to the scarcity and necessity of freshwater resources, it is imperative that the water quality of these systems be protected. Given the consequences of eutrophication, mainly hypoxia, fish kills, and harmful algal blooms (HABs), the Harmful Algal Bloom and Hypoxia Research and Control Amendments Act (S. 1254) was signed into law in June 2014 (U.S. EPA 2017). Although this legislation focuses on the northern Gulf of Mexico and authorizing a taskforce to research and mitigate both economic and ecological impacts of HABs and hypoxia, these issues occur throughout the United States. According to the U.S. Environmental Protection Agency (EPA), HABs are a problem in every state, having major impacts on ecosystems, human health and the economy (U.S. EPA 2018). Agriculture, industry, and urbanization have been
linked to the increase in nutrient pollution in aquatic ecosystems (Paerl and Huisman 2008; Chislock et al. 2013). The Clean Water Act (CWA) 33 U.S.C. §1251 et seq. (1972) regulates point source discharges of pollutants into navigable waters through National Pollutant Discharge Elimination System (NPDES) permits. These point sources include municipal wastewater and stormwater. However, the regulation of non-point source pollution, like those carrying nutrients, remains difficult and under Section 404(f)(1) of the CWA. These non-point sources generally come from farming, agricultural, and silvicultural activities, which are exempt from permitting requirements. Since permitting is not required for these activities, it is pertinent that water resources are routinely monitored in order to control, prevent or remediate impaired aquatic systems. Water quality monitoring through biological assessments is important for understanding the response biological communities have to nutrient enrichment and other stress factors. Biological assessments yield insight to ecosystem stressors over a period of time, whereas, physicochemical data provide real-time information (U.S. EPA 2002). Diatoms have been found to indicate nutrient enrichment better than other biota (fish and macroinvertebrates) currently used in biological assessments (Hausmann et al. 2016; Otto 2016). Therefore, understanding diatom biodiversity would provide a more complete understanding of the eutrophication of an ecosystem and consequently its protection.

In our study, we document physicochemical parameters at our sampling location, which has been preserved for the last 50 years. We provide a table of what the dominant (>1 % relative abundance) diatom species of our study indicate. We document sample type, habitat requirements from past literature, and take abundances into consideration when assessing our site along UTRC.
Water quality standards and why the Savannah River is important

Savannah River is one of the major rivers in the southeast and is approximately 483 km (300 mi.) long (Georgia EPD 2001). The Savannah River basin has a drainage area of 25,900 km$^2$ (10,000 square miles), which drains over the States of Georgia, South Carolina, and North Carolina. The Georgia Environmental Protection Division (EPD) has classified much of the Savannah River as “Drinking Water Supplies,” which is defined as: those waters approved as a source for public drinking water systems permitted or to be permitted by the Environmental Protection Division (Georgia EPD 2015). Although this surface water resource provides potable water to an estimated 1.4 million people, Georgia EPD has not yet designated specific water quality standards for the Savannah River. However, Georgia EPD has set specific criteria or water quality standards for different water classifications. These standards include ranges and/or limits for bacteria, dissolved oxygen, pH, and temperature. Currently, there are no nutrient criteria for drinking water, and only a limited number of counties have nutrient standards defined for lakes. Waters that do not meet the State of Georgia’s standards and criteria are placed on the 303 (d) list of impaired waters. Under the Clean Water Act 1972, every state is required to send a 303 (d) list to the EPA every two years. These lists name impaired waters and pollutants, if known, as well as the development of Total Maximum Daily Loads (TMDL). According to Georgia’s recent 303 (d) list, most of the Savannah River sites are supporting their designated use. Only one reach of the river from Brier Creek to Ebenezer Creek is impaired due to “Trophic-Weighted Residue Value of mercury in fish tissue exceeding the EPD human health standard of 0.3 mg/kg” (Georgia EPD 2016).

The Savannah River Site NPDES permit compliance and past biological assessments
The U.S. Department of Energy’s (DOE) Savannah River Site (SRS) is located along the Savannah River in South Carolina. Our site along Upper Three Runs is located within a protected area of the SRS complex, managed by the UGA Savannah River Ecology Lab (SREL). The SRS was originally constructed in the 1950s for the production of plutonium and tritium for nuclear weapons during the Cold War (Savannah River Nuclear Solutions, LLC 2017a). Today the site is responsible for environmental cleanup, disposing nuclear waste, and developing energy and defense technologies. The SRS covers an area of approximately 777 km² (300 square miles), which encompasses three counties: Aiken, Allendale, and Barnwell. In 1972, the SRS was designated as a National Environmental Research Park and is located in a variety of habitats (wetlands, hardwood stands, and riparian ecosystems). Upper Three Runs Creek is a tributary of the Savannah River, and is known as a southeastern biodiversity hotspot (Voelz & McArthur 2000). This site is designated by the SRS to receive minimal anthropogenic impacts and serve as a control site in scientific studies.

As sanctioned by the Clean Water Act, the SRS conducts an environmental monitoring program in accordance with NPDES permits. This program collects data on nonradiological industrial wastewater and stormwater discharges into surface waters. The SRS reports findings monthly to the South Carolina Department of Health and Environmental Control, which issues NPDES permits to the SRS. In 2017, SRS received five Notices of Violation (NOVs). Three of these notices were for noncompliance of the Clean Air Act (CAA) and two were for noncompliance of the CWA (Savannah River Nuclear Solutions, LLC 2017b).

Past monitoring programs along the Savannah River and Upper Three Runs Creek, include those conducted by the ANSP and GCSU. From the early 1950s to early 2000s ANSP conducted biological surveys for Westinghouse Savannah River Company to monitor and
evaluate potential impacts from SRS and Plant Vogtle (Bouchard et al. 2001). Although no physicochemical data were collected, ANSP did conduct algal surveys using composite samples in the 1950s and later switched to diatometers. In a synthesis of data given in the ANSP 2000 report, upstream sites were shown to have higher water quality when algal biodiversity was used as a proxy and algal biodiversity was also shown to decrease over time regardless of seasonality (Bouchard et al. 2001).

The Endangered Species Act (ESA) of 1973 (ESA; 16 U.S.C. § 1531 et seq.) and algal conservation

The purpose of the ESA 1973 is:

to provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved, to provide a program for the conservation of such endangered species and threatened species, and to take such steps as may be appropriate to achieve the purposes of the treaties and conventions set forth in subsection (a) of this section.

In this legislation, the term “endangered species” is defined as:

any species which is in danger of extinction throughout all or a significant portion of its range other than a species of the Class Insecta determined by the Secretary to constitute a pest whose protection under the provisions of this Act would present an overwhelming and overriding risk to man.
This Act protects algal species and their habitats, however, not many algal species are protected in the U.S. and most of the protected species that are protected are marine macroalgae. Currently, in the State of Georgia, there are no algal species listed as endangered. Despite the fact that most conservation efforts do not target the protection of algae, the importance of algae to human welfare is not disputed. Algae are used economically in the food, textile, and pharmaceutical industries. Algae produce 50% of all oxygen on the planet (Chapman 2013), and diatoms, which are the best indicators of changes in water quality, are used in biological assessments. Therefore, it is important to develop conservation initiatives that focus on the protection of algal species. Brodie et al. point out that habitat destruction and degradation are major threats to algae (2009). Anthropogenic impacts, including land use and increased nutrient loading to surface waters, have led to economic and human health consequences. When biodiversity declines, ecosystem functioning and stability also decline (Naeem et al. 2009). Consequently, it remains imperative to protect algal biodiversity in order to preserve ecosystem functioning and ecosystem services, such as water quality.

Challenges to algal conservation consist of determining the biogeography of algal species. It was once thought that the ubiquitous nature of microscopic algae meant that microalgal ranges would remain undetermined. However, recent studies have shown biogeography of microalgae through advanced technologies such as genome sequencing (Abarca et al. 2014; Kermarrec et al. 2013). Biogeography is necessary, not only due to anthropocentric urgency, given our dependence on biodiversity, but also due to its scientific importance for long term monitoring and making predictions about changes in dynamics of ecological communities. These predictions could help prioritize areas of concern for conservation, facilitate the
understanding of population dynamics, and shed light on how to restore already degraded systems. In an editorial featured in *Conservation Biology* in 2000 the author writes:

“Phylogenetic reconstruction, currently the dominating focus of systematics, obviously is worth doing, but more scientifically important and far more urgent for human welfare is the description and mapping of the world biota.” Although given 20 years ago, Edward O. Wilson’s insight expressed in “On the Future of Conservation Biology,” remains relevant today. If more conservation efforts focused on the biogeography of microalgae, it would be possible to protect their habitats and improve water quality under U.S. ESA 1973 legislation. However, given time constraints on complex conservation issues it is vital that conservationists recognize, as Balmford and Crowling point out in a *Conservation Biology* editorial, “that conservation is primarily not about biology but about people and the choices they make” (2006). This excerpt from “Fusion or Failure? The Future of Conservation Biology” makes clear that conservationists must rethink how they tackle today’s problems in order to provide solutions to them.

Acknowledging the integration of a human dimension when developing conservation efforts would undoubtedly minimize the time gap between science and action, research and implementation. An example of this time gap phenomenon was seen in legislation surrounding acid rain, which took over 30 years from the time when impacts from acid rain were acknowledged by policy makers (Likens 2010). In the State of Georgia, public participation of citizens can ensure that government agencies, like Georgia Environmental Protection Division (EPD), carry out policies that protect their watershed (Thompson 2001).
2.2 Assessment metrics in past studies

Comparing regional, national, and European assignment of biological indicators

In 2007 Potapova and Charles conducted a study, investigating the metrics used for algal assessments measuring eutrophication. Given that diatoms have traditionally been used as biological indicators of water quality, the accuracy of metrics associated with them is crucial for precise evaluation of water quality. In the 2007 study, metrics included assigning relative abundance percentages to categories of low and high total phosphorous (TP) and low and high total nitrogen (TN) and then calculating the ratio of high nutrient indicators to low nutrient indicators. Because a large amount of work in this field has been done in Europe, American diatomists have used European metrics in the past to infer regional and national water quality. However, in this 2007 study, Potapova and Charles demonstrated the importance of using metrics designed for the original site study.

This 2007 study evaluated and compared the U.S. Geological Survey National Water-Quality Assessment (NAWQA) regional list of indicators, the NAWQA national list of indicators, and 1994 Van Dam et al. taxa assignment to nutrients. By using Van Dam et al. (1994) assignments the authors were able to compare North American lists to European taxa assignment. Results from their study demonstrated that metrics created for U.S. rivers were better than those created for Europe when assessing water quality because European indicators of low nutrient waters weakly correlated with U.S. river nutrient concentrations. This study also found that combining regional and national metrics was better at assessing water quality than using only national criteria. For this reason, the authors conclude that future water quality assessments should: 1) consider environmental gradients of species distributions, 2) use datasets
with similar taxa as the river under investigation when applying metrics, 3) agree on taxonomic identifications, and 4) collect data such as water chemistry during conditions of algal growth.

Because there are remaining challenges in identifying species within the *Gomphonema* genus, it has been difficult for taxonomists to agree on species identification and sometimes nomenclature. Thus, investigating these complexes may refine assessments. By investigating species complexes found in the southeastern U.S. we hope to facilitate agreement on taxonomic identifications with species that are difficult to identify within the genus *Gomphonema*. Due to the need of continuous research on U.S. southeastern diatoms, European literary resources as well as American were used in this GCSU study. The taxonomic approach used for resolving species complexes is provided in this GCSU study’s voucher flora via size series and descriptive tables. The voucher flora from this study can be applied to future studies by other diatomists and used to compare findings.

**Reference conditions and diatoms as indicators for watershed disturbance**

A study conducted in 2008 investigated traits and species composition of benthic diatoms in order to evaluate the accuracy of regional and western U.S. indicators of stressors of environmental conditions (Stevenson *et al.* 2008). These conditions included percent watershed disturbance, total phosphorous, total nitrogen, pH, conductivity, percent fine sediments, and percent embeddedness. Methods for this study calculated traits for 242 diatom taxa. The authors used a subset of 1203 of the streams and rivers sampled by the U.S. Environmental Protection Agency (EPA) Environmental Monitoring and Assessment Program Western Pilot Survey (EMAP-West) to perform a Principal Components Analysis (PCA). This analysis was used to pinpoint environmental conditions that affect diatom assemblages. Traits, or characteristics related to the fitness of species were evaluated according to weighted average inference models.
Results showed that indicators reliably demonstrated the environmental gradient of watershed disturbance (urban or agricultural land use). Although, diatom indicators were developed to assess tolerances to varying water qualities based on nutrient and water chemistry criteria, this study showed these indicators to more strongly correlate to stressors such as conductivity, watershed disturbance, and multivariate indicators. The authors concluded that because water chemistry and land use varied greatly in streams, incorporating reference conditions of a narrower environmental gradient could develop a more accurate assessment.

In this GCSU study, UTRC was chosen as a study site for the biodiversity it comprises and as a reference condition with minimal land use disturbance for the Savannah River. By incorporating physicochemical data into analyses for this study, a narrower environmental gradient could be provided for representatives of the genera *Gomphonema*, *Tabellaria*, and *Eunotia*. Understanding reference conditions for species within these genera may provide insight to conditions of the Savannah River. In this GCSU study physicochemical parameters such as pH, temperature, dissolved oxygen, and conductivity were collected. Due to constraints on project resources, nutrient assessment at this UTRC site was not conducted. However, taxonomic identifications from this reference site provided probable inferences about the type of nutrient environment found at this UTRC site and therefore, rendered nutrient assessment not necessary for the objectives of this study.

2.3 **Past research on Upper Three Runs Creek and the Savannah River**

*Upper Three Runs Creek as a reference site*

A baseline study assessing algal assemblages was conducted at two southeastern sites, Tobbler Creek and UTRC in 2012 (Moseley & Manoylov). The sites for this study were chosen
because they were in areas of low anthropogenic impacts. Although similarities were found between the algal biodiversity at both sites, indicator species for pollution differed. Biological and chemical sampling occurred and followed American Public Health Association (APHA) standard protocol (APHA 2005). However, sampling did not occur at the same time or same months in some cases. The following dominant species were found: *Gomphonema parvulum* (Kützing) Kützing sensu lato, *Gomphonema parvulum* (Kützing) Kützing sensu stricto, *Eunotia carolina* R.M. Patrick 1958, *Luticola goeppertiana* (Bleisch) D.G. Mann ex J. Rarick, S. Wu, S.S. Lee & Edlund 2017, *Achnanthidium minutissimum* (Kützing) Czarnecki 1994, and *Tabellaria flocculosa* (Roth) Kützing 1844. The species found belonging to the *Eunotia* or *Gomphonema* genera exhibited high morphological diversity. The 2012 GCSU study calculated Trophic Diatom Indices (TDI) for each site using information from Kelly & Whitton (1995) for *Gomphonema parvulum, Navicula gregaria, Navicula lanceolata, small Navicula (<12μm) and Sellaphora* spp., and *Nitzschia* spp. to evaluate proportions. The authors concluded that acidity had a role to play in community composition, and in turn TDI values. They found that all samples except a sand sample at UTRC scored values indicating moderate to high levels of organic pollution. The authors concluded that variation in indices results are likely due to species misidentification as *G. parvulum sensu lato*, which was found to be the only dominant species that was common and that was also highly variable in its morphology. Besse-Lototskaya *et al.* (2006) also explained that identification is a large contributing factor in uncertainty and variation in biological assessments.

Given that species complexes within *G. parvulum sensu lato* are challenging to identify and may lead to misidentifications, assessment of organic pollution at UTRC should be reassessed taking into account morphological differences of this species. By exploring variation
in this genus, identification uncertainty could be minimized, which could improve biological assessments using diatoms. In our study, we document *G. parvulum sensu lato* morphological variability and provide a matrix of the differences and similarities of our separations according to biometrics of valve length, width, stria density in 10 μm, raphe form, central area, striae orientation, and valve and pole shape. We document previous literature with similar observations where applicable.

*Savannah River algal communities and challenges in recent techniques*

Manoylov *et al.* conducted a southeastern baseline study along the Savannah River in 2016. This study investigated algal communities in Savannah River mud flats near the Savannah River Port. The authors were interested in this area because it experiences continued anthropogenic alterations and algae provide biological indicators for aquatic ecosystem health and changes. Manoylov *et al.* (2016) believed that studying algal community changes in this area would bring insight to changes in water quality. Investigating algal composition in estuarine mudflats also provided a gradient of study of many interactions that may yield communities that are not representative of distinctive habitats. After sampling five times along the Savannah River estuary in December 2011, following APHA protocol, this study found high diversity of diatom species. However, this study also found that less than 20% of the community was considered active or alive. The authors used presence of chloroplasts to determine whether or not an algal cell was active or alive. Difficulties in algal taxonomy lead the authors to use molecular identification of species. Using molecular identification, the authors found the following diatom taxa to show 95% or greater similarities: *Pleurosira laevis* (Ehrenberg) Compère 1982, *Cymbella aspera* (Ehrenberg) Cleve 1894, *Odontella* C. Agardh 1832 (*Biddulphia*) sp. Another interesting finding from this study showed that some diatom species are resistant to
mechanical lysis and some species are difficult to culture. These results may make the study of some algal species difficult, since DNA sequencing techniques are not successful in distinguishing some species that light microscopy (LM) methods could not.

The 2016 study demonstrates that traditional techniques of counting processed and cleaned cells may impact estimates if live cells are not estimated before nitric acid digestion. In the current 2018 GCSU study, diatometer slides we observed prior to processing to ensure slides were dominated by live diatom colonization. Taking challenges of mechanical lysis into consideration, taxonomic techniques via light microscopy (LM) and scanning electron microscopy (SEM) were conducted prior to diatom culturing and molecular techniques involving polymerase chain reaction (PCR) methods to amplify diatom DNA.

2.4 Recent molecular research

Diatom phylogeny and evolution with genome sequencing

In Algal Taxonomy a Road to Nowhere, De Clerck et al. (2013) point out that future phycological work will incorporate more molecular techniques in the naming and identification of algal species. Especially, given the levels of crypticism in algal species (De Clerck et al. 2013). In 2008 a study was conducted by Bowler et al., which revealed the complete genome sequence for the pennate diatom *Phaeodactylum tricornutum* Bohlin 1898, and compared it to that of a centric diatom species *Thalassiosira pseudonana* Hasle & Heimdal 1970. The authors were interested in investigating evolutionary divergence, as well as gaining some insight into the functionality of these genes. This study found that close to 40% of the genes were not shared by these two species, which may represent two different lineages. The authors demonstrated that this dissimilarity provides evidence for fast divergence in diatoms. By comparing these diatom genomes to those of bacteria the authors also found that more than 300 genes (5%) are
transferred between bacteria and diatoms. The authors concluded that the presence of these shared genes yields evidence for the origin from secondary endosymbiosis as explained in Bhattacharya et al. 2007. The authors explained that other studies have shown that diatoms kept genes from both partners (e.g., a bacterium and eukaryote), which allowed them to photosynthesize and produce nitrogen (Armburst et al. 2004, Allen et al. 2006). Bowler et al. (2008) explained that due to a lack of sampling of genome sequences, analyses in their study might have been biased. However, molecular phylogenies are supported. They explained that they believe this gene transfer between bacteria and diatoms to have been a usual event in marine habitats, which has played a major role in diatom evolution.

Understanding diatom evolution is essential given that diatoms are responsible for about one-fifth of global primary production (Falkowski et al. 1998, Field et al. 1998). Therefore, the results reported in Bowler et al. 2008 study may reveal the basis for the relatively short rate of diversification in diatoms, which could explain why diatoms have so swiftly dominated current marine ecosystems.

This 2018 study explores questions for *Gomphonema* species considering the short diversification of pennate diatoms. This short diversification of pennate diatoms may provide insight into the variability of species and cryptic species within this genus. This study documents this variation and *Gomphonema parvulum* was cultured to sequence its DNA. We compare and hope to contribute our findings to GenBank sequence database.

*Testing molecular techniques when determining diatom DNA*

Diatoms have posed challenges in molecular work, mainly because of the little genetic information found in their cells and the nature of their silica cell wall. In a 2011 study, Nguyen et al. found it necessary to use additional techniques for the identification of diatom species with
high morphological diversity. However, they found that some molecular tools are not adequate in determining DNA from rare species that do not contribute large amounts of genetic information in environmental samples. Also, because many available techniques are complicated, their use in evaluating environmental samples has proved inadequate. This study compared the efficacy of six different kits for extracting DNA and developed a new technique for adequately extracting DNA representative of species that were not abundant in samples. The six kits included were: Sigma, Invitek, Mobioplant, Mobiosoil, Promega, and Nucleospin. To test these methods, DNA was extracted from the following pennate diatom strains living in cultures: *Nitzschia frustulum*, *Neidium affine*, and *Entomoneis paludosa*. The authors found that the kits that used real-time PCR and standard PCR did the best. However, all yielded minimal diatom DNA. Therefore, the authors developed, FoRWARD (FilteR–WAsh–Resuspend–Dry), a new protocol. They developed this procedure to avoid the need for extracting DNA, since the DNA purification step caused many of the kits to be inadequate. Nguyen *et al.* (2011) addressed issues of quantitative assessments of algal cells. They explained that a data set of single copy genes specific to diatoms would be necessary for conducting quantitative evaluations. Because this is a fairly new technique when evaluating algal communities, this type of data set is currently under development. Presently, only two species of diatoms have complete genomes available: *T. pseudonana* and *P. tricornutum* (Armburst *et al.* 2004, Bowler *et al.* 2008).

Like other studies, this 2011 Nguyen *et al.* study makes evident the issues associated with molecular testing for some diatom species. Given these challenges, it is important that LM and SEM techniques are used as primary techniques when conducting algal assessments. Therefore, using taxonomic approaches first to distinguish species from species complexes is necessary. For this reason, we created a trait matrix coupled with LM images of *Gomphonema parvulum sensu*
lato representatives documenting this complex’s diversity at our UTRC site. We used a fine level taxonomic approach before moving forward with monoculture and molecular techniques. Given the success with diatom molecular work in Nguyen et al. 2011, PCR methods were used with the monocultures prepared in this GCSU study in collaboration with University of Virginia’s College at Wise (UVA Wise).

Molecular research with the Gomphonema parvulum complex

In 2013, Kermarrec et al. conducted DNA sequencing on representatives of the Gomphonema parvulum complex due to its morphological diversity and challenges in identification with light microscopy. The objective of that study was to investigate whether or not G. parvulum exhibited biogeography. Through sequencing the authors were able to separate representatives into four different clades (A, B, C, D), suggesting semi-cryptic species. The study used pyrosequencing to evaluate and confirm biogeography. Clades A and B were found to be significantly different for length and valve width. Clades A and B were different than Clades C and D in striae density. In their phylogenetic tree, the authors also found that G. exilissimum and G. lagenula strains were included with the G. parvulum cluster. Therefore, the authors questioned the taxonomy that separates them and concluded that DNA sequencing can overcome challenges in taxonomy. However, the authors also showed that in order to evaluate biogeography further, more extensive studies of G. parvulum would need to be conducted in tropical islands of the Indian Ocean and in south-west Africa to confirm endemism and rule out ecotypes.

For preliminary work for this thesis, archived slides from past algal research conducted at UTRC by GCSU were assessed for Gomphonema representatives. Surprisingly, the “Clade B” description and micrographs from the Kermarrec et al. 2013 paper match those found from a past
GCSU research site. Therefore, this Kermarrec et al. 2013 paper is considered in this GCSU study while separating taxa traditionally included *G. parvulum* species complex.

**Cosmopolitan nature of *Gomphonema parvulum***

It has been accepted and widely known that *G. parvulum* is ubiquitous and considered cosmopolitan (Stenger-Kovács *et al.* 2007, Abarca *et al.* 2014). In 2014, Abarca *et al.* conducted a study investigating *G. parvulum* (Kützing) Kützing *sensu lato* to determine if there were biogeographical patterns of *G. parvulum* based on morphological and molecular differences, since it has been found to be highly variable (Wallace 1950, Abarca *et al.* 2014). Methods for this study included taking 21 cultures from the following sites: Faroe Islands, Sweden, Germany, Mexico and Korea. Whether intended or not, the authors highlighted the many challenges in studying this species: 1) this species has been mentioned in other studies as a complex containing cryptic species (Kermarrec *et al.* 2013, Rose & Cox 2014), so in the past, this species may have been misidentified by non-expert taxonomists (Besse-Lototskaya *et al.* 2011) making *G. parvulum* seem more ubiquitous than it is, 2) some studies have implied that environmental factors have had an impact on morphological features, so it is possible that representatives of this group may have been misidentified as other species and vice versa, 3) similar to other *Gomphonema* species, but not as frequently observed, *G. parvulum* has been known to produce a Janus cell, two valves of the same frustule or cell that are morphologically different (McBride & Edgar 1998, Moseley & Manoylov 2012, Andrejić *et al.* 2019), and 4) according to Abarca *et al.* (2014), for 200 years taxonomists have used *G. parvulum* as a “collective name”, or known to others as *G. parvulum sensu lato*. Abarca *et al.* (2014) argued that combining molecular-phylogenetic analyses with taxonomic approaches might yield more accurate results given past studies (Wortley & Scotland 2006, Moniz & Kaczmarska 2010). Consequently, Abarca *et al.* (2014) assessed a combination
of methods such as, micropipette isolation (DNA), PCR amplification, DNA sequencing, and frustule cleaning with hydrogen peroxide at 80 °C. Statistical analyses that included Mantel tests with 999 permutations were performed to analyze potential relationships between geographical and genetic distances.

Abarca et al. (2014) found that *Gomphonema parvulum sensu stricto* (Clade 1) was the most genetically and morphologically diverse. However, because there was less than 1% difference among the 11 strains that were tested, “Clade 1” remained a separate group. The authors also found that cultures of *Gomphonema saprophilum* (Lange-Bertalot & E. Reichardt) Abraca, R. Jahn, J. Zimmermann & Enke 2014, (Clade 2) were genetically and morphologically similar (1% - 1.5%). *Gomphonema lagenula* Kützing 1844 (Clade 3) was a clade with marker distances of 1.2 - 2% difference. *Gomphonema narodoense* R. Jahn, Abarca, J. Zimmermann & Enke (Clade 4) was not distinct morphologically, but genetically distinct with 1.2 - 2.3% differences. In conclusion, the Abarca et al. (2014) study found four distinct taxa supporting the idea of biogeography and not supporting the idea that *G. parvulum* is cosmopolitan. The authors also concluded that their findings did not support the idea that only environmental factors, like water quality, select for certain species. If this were so, they argue, *G. lagenula* and *G. saprophilum* would be the same species since they are both found in human impacted waters and are morphologically similar.

This 2014 Abarca et al. study and the 2013 Kermarrec et al. study establish biogeography of microalgae in their studies. Given algal species are protected under U.S. environmental policy, establishing biogeography of diatom species and identifying species ranges could aid in the protection of the freshwater resources in which they are found. In this GCSU study new species...
that are found at our site are documented and described in an attempt to contribute to surface water resource conservation and water quality protection.

*New species of Gomphonema and Gomphonema as a morphological group*

In 2017 a study was published describing two new species found within the genus *Gomphonema* (Ponader *et al.* 2017). The two new species are *Gomphonema caperatum* Ponader & Potapova 2017 and *Gomphonema obstipum* Potapova, Ponader & Desianti 2017. The authors also described similar taxa and compared differences. Similar taxa include: *Gomphonema amerhombicum* E. Reichardt 2007, and *Gomphonema stoermeri* Kociolek & J.C. Kingston, nom. illeg. 1999. The authors reported a species, *Gomphonema incognitum* E. Reichardt, Jüttner & E.J. Cox 2004, which documented its occurrence in North America for the first time. This genus, *Gomphonema*, is widespread with 338 recorded in North America, and more than 1700 globally (Kociolek 2005, Fourtanier & Kociolek 2011). This genus is also notorious for species complexes and the high morphological variability found in these complexes. Many of the species that are difficult to identify or are misidentified in this genus have narrow cells with wide axial areas (Ponader *et al.* 2017). The authors explained that it was necessary to use scanning electron microscopy (SEM) to observe some morphological features that were in question when describing these new species. These species were found while conducting diatom enumerations at the Academy of Natural Sciences of Philadelphia (ANSP). All materials for this 2017 Ponader *et al.* study can be found in the Diatom Herbarium of ANSP.

Ponader *et al.* (2017) showed that because these species were found in well-studied areas of North America, much is still unknown about diatom diversity. They also provided a table showing how many species belonging to this genus have variations in features, yet still overlap in traditional taxonomic measurements such as, length to width ratio and stria density. Therefore,
they argued that *Gomphonema* has become a morphological group, of which the phylogeny of *Gomphonema* species is impossible to determine. In conclusion, Ponader *et al.* (2017) explained that in order to understand phylogenetic relationships, testing at the molecular level with these species is necessary.

Because the Ponader *et al.* (2017) study described variations of this taxonomic group that still overlap in traditional taxonomic measurements such as, length to width ratio and stria density, the current GCSU study investigated additional measurements for assessing this group. The *Gomphonema parvulum* diversity plate and trait matrix provided in this GCSU study will aid in resolving taxa from UTRC from this *G. parvulum sensu lato* species complex.

3 METHODS

3.1 Study sites

Field observations were conducted at UTRC, located at (Lat. 33.393067, Long. -81.610719). This study site was located upstream of a previous 2012 GCSU study site (Lat. 33.370750, Long. -81.627738) due to bridge construction. This site is located between a range of 50 m to 155 m above sea levels (Fig. 1). UTRC has a 255-km² basin and is a tributary, which drains into the Savannah River. Georgia and South Carolina border the Savannah River, which is 483 km long and comprises a basin of 25,900 km². This site is also designated by SRS to receive as minimal anthropogenic impacts as possible. Located in a protected area and designated as a biodiversity hotspot, UTRC, provides a unique habitat for studying diatom taxa (Voelz & McArthur 2000).
3.2 Sampling

Diatoms were collected with diatometers, plastic housing devices attached to floats, which hold a total of seven glass slides each. Diatometers were deployed on both right and left sides of the creek for a period of 18 days from March 23rd to April 9th 2018; following methodologies specified in past ANSP reports. Because of the differences in widths of the Savannah River and UTRC, only two diatometers were used. However, triplicate samples were taken from each diatometer for analysis and the remaining four slides were archived in the GCSU Manoylov Phycology Lab. These devices are commonly used in algal monitoring studies to standardize substrate area of algal colonization. Because these devices select for adnate species, triplicate composite samples were also taken at this site at the time of diatometer retrieval. Sampling followed 2005 APHA standard methods for examination of water and wastewater and currently used 1999 EPA periphyton protocols. Composite sampling consisted of collecting triplicate samples of at least 250 mL of a representative sample of site vegetation, woody debris, silt and sand. Half of each triplicate was processed with nitric acid and 125 mL were preserved and archived in the GCSU Manoylov Phycology Lab. Physicochemical data were collected at the time of diatometer deployment and retrieval. These data were collected with a YSI 556 MPS (Multi-probe System) that measured pH, temperature (°C), dissolved oxygen (%., mg/L), and conductivity (mS/cm²) simultaneously. Measurements were recorded once the instrument readings stabilized.

3.3 Laboratory methods

Processing

After collection, archived composite samples were preserved with formaldehyde of 3% final concentration. Before preservation, 30 mL of 70% nitric acid was added to samples for heat and
potassium dichromate digestion. Samples were digested for one day and then decanted daily for eight days with deionized water or until sample yielded a pH of 7. After decanting, excess water was siphoned and samples were concentrated in 20 mL vials. From these vials, an optimum amount of suspension from each slide was mixed with at least 1 mL of deionized water and distributed on 22 x 22 mm coverslips to dry. After drying, Naphrax® (Brunel Microscopes Ltd., Chippenham, Wiltshire, UK) mounting medium was used to make permanent slides. These methods follow those detailed in Moseley & Manoylo (2012). Diatometer samples were prepared in a similar manner, except all processing was conducted within a 50 mL falcon tube so as not to lose frustules from scraping slides before nitric acid digestion. Slides from diatometers were selected using Microsoft Excel 2010 random generator. After selected, slides were placed in tubes. Twenty-seven to 30 mL of hydrogen peroxide 30% concentration were added to the tubes. Tubes were then placed in a 200 °C water bath for 2-3 hours before flipping slides within tubes to ensure entire immersion into the hydrogen peroxide. After 2-3 hours, 20-27 mL of 70% nitric acid was added to the tubes while in water bath. After 2-3 hours, deionized water was used to rinse excess frustules stuck to slides into tubes. Falcon tubes were then placed in LEC Model K centrifuge at 2,550 rpms for 8 minutes. After tubes were centrifuged, excess water was siphoned off and replaced with deionized water. This centrifuge and decanting process was repeated 6-8 times or until suspension was of a neutral pH. This process was developed through personal communication with diatomist and Senior Scientist, Dr. Mark Edlund, of the Science Museum of Minnesota.

Enumeration

Before processing, diatometer slides were viewed with Light Microscopy at 400X to verify that live diatoms dominated slide colonization. After processing, algal enumeration was
conducted on three permanent slides from the left diatometer, three from the right diatometer, one from each of the triplicate composite samples and two archived slides from past ANSP bioassessments of UTRC. Slides from past ANSP studies on UTRC were dated for May 1956, which coincides with the spring season of this GCSU study’s sampling period. Algal enumeration was conducted by identifying and counting between 400 - 415 units, or valves (400 - 415 cells) with at least 60% of the valve intact along a transect. Although EPA periphyton standard protocol recommends counting to 600 valves, due to the high biodiversity found at this site, we counted only to at least 400. Valves were identified under oil immersion at 1000X on a Leica CTR5000 equipped with DIC and Leica DFC450C digital camera. After algal enumeration was conducted, raw data of triplicates and past slides were combined for each method/treatment (2018 composite sampling, 1956 composite sampling, left diatometer, and right diatometer). After triplicate data were combined, each treatment under separated taxonomic identification was assessed for and followed the 10 valves of 10 species rule according to EPA periphyton protocols (Stevenson & Bahls 1999). We used Adobe Photoshop® to create diatom voucher flora plates documenting the species found at this site for each method and Gomphonema, Eunotia, and Tabellaria morphological variability within species complexes.

**Culturing and sequencing**

Live samples from a composite triplicate were viewed at 400X with a Palmer Maloney counting slide. We washed samples with living Gomphonema parvulum frustules with diluted Bold Modified Basal Freshwater Nutrient Solution (50X) into Bacto ™ Agar prepared agar plates. Bold nutrient solution was prepared at 20 mL/ L with deionized water. For agar plate preparation we modified an agar recipe found in Algal Culturing Techniques (2005). Modification was performed to allow for diatom motility and an environment of optimum
nutrients to promote growth. For agar preparation we dissolved 25 g of Bacto™ Agar in 950 mL of deionized water. We warmed mix to 100 °C, but did not boil, for 30 minutes while stirring. We then added 20 mL of pre-diluted Bold nutrient solution to flask and filled the remaining 30 mL of flask with deionized water before pouring plates. Before washing live samples into plates, plates were stored overnight at 4 °C. After live samples were added to plates, cultures were fed with diluted Bold nutrient solution, by covering the surface of the agar. Algae were fed once every 15 days or when agar exhibited desiccation. Due to blue-green algal contamination and attempts to achieve a monoculture, G. parvulum colonies were transferred to new plates every 2-3 months for 9 months. After 9 months, we noted a decreased in colony size and sent live monoculture colonies to UVA Wise for molecular analysis. Because diatoms yield a small amount of DNA for molecular analysis (Manoylov et al. 2016), collaborators at UVA Wise used PCR techniques to amplify chloroplast genes. For results and further information please see Appendix A.

3.4 Data and statistical analyses

To assess biodiversity and compare similarity at this UTRC site and between methods (diatometer, composite, and separating vs. combining taxa), the following were calculated: species richness, Shannon-Wiener diversity indices, Pielou’s evenness, species relative abundances, Jaccard similarity indices and distances, and Sorenson index. Equations for each are as follows:

Shannon-Wiener Diversity Index:

$$H' = -\sum p_i \log(b)p_i$$, where $p_i$ is the proportional abundance of species $i$ and $b$ is the base of the logarithm” (Hill 1963 In Oksanen 2013)

Pielou’s evenness:
R project code for reference, “$J = H' / \log(S)$, where $H'$ is Shannon-Wiener diversity, and $S$ is the number of species found in a sample” (Hill 1963 In Oksanen 2013)

Jaccard Similarity Index:

$$S_{i,j} = c/(a+b+c),$$

where $c$ is the number of species found in both samples, $a$ is the number of species found only in sample a, and $b$ is the number of species found only in sample b. We report Jaccard distance as 1 - $S$ (Jaccard 1901).

Sorensen–Dice similarity coefficient:

$$CC = 2C/ (A+B),$$

where $C$ is the number of species both samples have in common, $A$ is the number of species only found in sample A, and $B$ is the number of species only found in sample B (Sorensen 1948).

Relative abundance:

$$RA\% = (S/T) * 100,$$

where $S$ is species abundance found in sample and $T$ is total abundance per treatment

Analyses and dendrographs were conducted in R: A language and environment for statistical programming, with Vegan: Community Ecology Package, version 2.5-6 (R Development Core Team, 2018; Oksanen et al. 2019). Jaccard dendrographs were generated using complete cluster analyses. Box and whisker plots comparing species richness across treatments, standard deviation and confidence intervals calculations were generated in Systat 13. For ecological information, literature was compiled for species or operational taxonomic units (OTUs) found to make up > 1% relative abundance (RA) for our study. Percent relative abundance for UTRC for 2018 was calculated for separated taxa by combining all composite and diatometer triplicate observations. Percent relative abundance for UTRC for 1956 was calculated for separated taxa by combining observations from 1956 ANSP slides.
4 RESULTS

4.1 Physicochemical results

Physicochemical parameters taken during this GCSU study’s sampling period were as follows: temperature ranged from (13.5 - 14.45 °C), dissolved oxygen ranged from (99.1% - 106.4%), pH ranged from (5.28 - 6.46) and conductivity was 13.0 mS/cm. These readings show that our site was acidic and high in dissolved oxygen during our sampling period. Depths for these readings were recorded at 1.4 and 1.8 meters. Parameter readings during the warmer months varied from those taken during this sampling time and can be found in Table 1. Means for all readings were as follows: temperature (17.68 ± 4.29), DO % (86.83 ± 23.63), DO mg/L (8.44 ± 2.84), pH (5.12 ± 1.01), and conductivity mS/cm (10.75 ± 7.22).

4.2 Biodiversity

Overall, a total number of 4,464 valves (half frustules) were counted for this study (817 across 1,956 samples and 3,647 across 2018 samples) belonging to a total number of 297 species/OTUs for separated taxa designations and 259 species /OTUs for combined taxa designations. Voucher floras for this study documenting taxa and OTUs are found on Plates 1 – 6. A novel species, Gomphonema “marrii,” currently under description from this study can be found in Appendix B. We found that for our separated taxa designations, of the 3,647 counts, there were a total 218 species found in our 2018 samples (n= 9), and of the 817 counts in the 1956 samples (n=2) there was a total of 155 species found. For taxa separated from sensu lato species complexes, we found that 1956 composite sample had the highest species richness (n=153) followed by the left diatometer (n=129), 2018 composite sample (n=111), and right diatometer sample (n=67). Taxa combined or “lumped” together under past taxonomy followed similar trends as species richness findings (Table 2 and Table 3).
Overall, for separated taxa, treatments showed high biodiversity with Shannon-Wiener diversity indices ranging from (3.242 - 4.342) with the 1956 composite sample exhibiting the highest diversity and the 2018 samples exhibiting the lowest, however still high. Pielou’s evenness followed the same trends as Shannon-Wiener diversity exhibiting fairly high-to-high evenness with ranges 0.688 - 0.863.

Combined taxa indices, yielded lower biodiversity results than those calculated from separated taxa. Shannon-Wiener diversity indices remained high for 1956 composite samples (4.09) and left diatometer samples (3.138) and much lower for 2018 composite samples (2.320) and right diatometer samples (2.217). Pielou’s evenness followed the same trends as Shannon-Wiener diversity, however exhibiting moderate to high evenness with ranges (0.517 - 0.833).

For separated taxa, species richness means for 1956 composite samples (mean +/- sd = 99.5 ± 7.78) were greater than those for left (75.33 ± 4.73) and right (43.67 ± 2.08) diatometers and 2018 composite samples (52.67 ± 19.86), and this difference was significant (ANOVA, df= 3, p = 0.004). For combined taxa similar trends were found: species richness means for 1956 composite samples (87.5 ± 6.36) were greater than those for left (58.0 ± 5.0) and right (26.33 ± 2.08) diatometers and 2018 composite samples (40 ± 20.22), and this difference was significant (ANOVA, df= 3, p = 0.003). See Fig. 2 a & b for a box and whiskers plot of species richness data by collection method type.

4.3 Similarity

Jaccard and Sorenson indices for separated taxa followed similar trends with the shortest distance (greatest similarity) found between the right and left diatometers (0.658, 0.51) respectively and the least similarity found between the 1956 composite samples and the right diatometer samples (0.817, 0.309) respectively. For combined data, these sampling method
pairings followed the same tendency with greatest similarity found between the right and left diatometers and the least between the 1956 composite samples and the right diatometer. However, these indices were somewhat lower than those of the separated taxa (Table 4). Method pairings with 2018 samples did not follow the same order in most to least similar as did those of separated taxa indices. Jaccard distance (dissimilarity) indices ranges from (0.728-0.866) and Sorenson indices ranges from (0.236-0.428) for combined taxa.

Samples analyzed by separating taxa clustered by Jaccard similarity reflected indices found in Table 4 and Figure 3 for right and left diatometers intersecting at a distance of (0.658). However, using the complete linkage cluster analysis right and left diatometer samples were found equally distant from new composite samples. This hierarchical cluster analysis also showed the 2018 samples more similar to each other than to the 1956 samples. These findings differ from indices calculated from individual treatment pairs in Table 4.

Samples analyzed by combining taxa clustered by Jaccard similarity reflected indices found in Figure 4 and Table 4 for right and left diatometers intersecting at a distance of (0.728). However, using the complete linkage cluster analysis right and left diatometer samples were not found equally distant from new composite samples. This hierarchical cluster analysis also showed the 2018 composite samples more similar to 1956 composite samples, intersecting at (0.822). In these findings similarity differs from indices calculated from individual treatment pairs in Table 4.

4.4 Relative abundance and biological indication

_Gomphonema parvulum sensu lato, G. parvulum Morphotype 2, and Gomphonema parvulum “protracted off-center”_ were found to be > 1% relative abundance for 2018 and 1956 samples (Table 5). In this study we found 10 taxa that were most likely combined into
*Gomphonema parvulum sensu lato* in past studies, three of which have been raised to species level (*Gomphonema exilissimum* (Grunow) Lange-Bertalot & E. Reichardt 1996, *G. lagenula*, and *Gomphonema confusum* Levkov, Mitic-Kopanja & E. Reichardt 2016), two that are recognized as a morphotypes (*G. parvulum* Morphotype 2 and *G. parvulum* Morphotype 3) and five separated in our analysis after documenting delineations (Plate 1: Figs. 1-48, Plate 2: Fig. 2, and Table 6).

Other taxa that we found to be > 1% relative abundance include: *Eunotia incisa* W. Smith ex W. Gregory 1854, *Eunotia rhomboidea* Hustedt 1950, *Eunotia* spp. girdle, *Fragilaria* Lyngbye 1819 spp. girdle, *Fragilariforma* D.M. Williams & Round 1988 spp. girdle, *Fragilariforma virescens* var. 1, *Frustulia crassiretia* (Brébisson ex W. Smith) Lange-Bertalot & Krammer 1996, *Frustulia saxonia* Rabenhorst 1853, *Gomphonema* spp. girdle, *Gomphonema preliciae* Levkov, Mitic-Kopanja & E. Reichardt 2016, *Luticola* D.G. Mann 1990 spp. girdle, *L. goepertiana*, *Navicula leptostriata* Jørgensen 1948, *Navicula notha* J.H. Wallace 1960, Past “*Navicula minima* group”, *Nitzschia* Hassall 1845 nom. cons. spp. girdle, *Nitzschia recta* Hantzsch ex Rabenhorst 1862, *Pinnularia* Ehrenberg 1843, nom. et typ. cons. spp. girdle, *Tabellaria flocculosa* “intermediate”, and *Tabellaria* spp. girdle (Table 5). Dominant taxa only found in past 1956 samples include *F. saxonia* and *G. preliciae, N. notha* was only found in composite samples. Taxa only found in 2018 samples include: *G. parvulum* “protracted off-center” and *F. virescens* var. 1. Taxa or OTUs that were found abundant in both 2018 and 1956 samples had a higher relative abundance for 2018 samples, except for *Gomphonema* spp. girdle. The OTU with highest relative abundance in both 2018 and 1956 samples were *Eunotia* spp. girdle with 31.5 % for 2018 and 10% for 1956. Percent relative abundance calculated for combined taxa were higher and in most cases, and over double the percentage for separated
values: *E. incisa* (2018: 4.8%, 1956: 2.3%), *G. parvulum* (2018: 16.2%, 1956: 3.4%), and *T. flocculosa* “intermediate” (2018: 2.6%, 1956: n/a). In all cases relative abundance was generally low. Of the 24 taxa, or OTUs, that were > 1% relative abundance for 2018 and 1956 samples, literature reporting their ecological indication was found for 23 (Table 5). Overall, the dominant species found indicate an acidic environment that is highly oxygenated and high in nutrients. These data are supported by physicochemical data that were collected at our study site (Table 1).

5 DISCUSSION

This 2018 GCSU study is taxonomic in nature. In an effort to identify all valves (half frustules) to species level, only one area along UTRC was sampled. Therefore, we do not make assumptions for UTRC as a whole, but rather for this study, in our findings or conclusions. For this UTRC site, species richness, biodiversity, and evenness were high as well as for separated versus combined taxa. These results were expected since this study site is part of a headwater stream located in a protected area with minimal anthropogenic land use and agricultural impacts. Values for combined taxa were somewhat lower than those for separated taxa. Because species richness values become much lower when taxa are combined, this difference is expected as final index results are affected. 1956 composite values were higher than 2018 composite values, reflecting a decrease in biodiversity over time. This trend in decreasing diatom diversity over time was also noted in past ANSP reports (Bouchard et al. 2001). Although only two slides representing composite sampling from 1956 were analyzed (due to availability and accessibility and season of sampling), it remains doubtful that analysis of a third triplicate would yield results with a different trend; data found in the lower quartile for composite samples are still relatively higher than observations found in 2018 sampling methods. Results from an ANOVA test show that there is a significant difference across sampling methods. However, this may be due to the
higher biodiversity present in 1956 or the variability found in new composite samples. Also worth noting, the 1956 composite sampling site was most likely much further downstream from this study according to ANSP report, which could account for differences between 2018 and 1956 samples (ASNP 1956). We did not sample here due to accessibility issues, and because the exact location of the 1956 site remains unclear.

When comparing all sampling methods, the left diatometer species richness and diversity parameter indices were higher than other 2018 methods, both for combined and separated treatments. This result is interesting because slides assessed for live diatom colonization prior to diatom enumeration were more heavily covered with diatoms. Right diatometer samples exhibited lower species richness and evenness indices. When comparing relative abundance indices from these samples, although right diatometer species richness is much lower than that of other 2018 samples, more species with higher abundances were found on them. Therefore, right diatometer slides had a higher biomass, but they did not exhibit higher diversity. Differences between diatometer methods could be attributed to differences in the amount of light exposure between banks, changes in stream flow or discharge, or depth. During field observations, it was noted that a small deep pool was located slightly downstream of left diatometer. Surrounding vegetation could also play a role in the difference found between diatometer methods. Vegetation on or near the left bank was different than that of the right bank, with tall pine and cypress trees on the left bank and grasses and smaller trees or shrubs on the right bank.

In assessing similarity for separated taxa, the right and left diatometers were found the most similar sharing about half to over half of their species in common. The 1956 composite samples and right diatometers were the least similar with only 31% overlap. Given percentages of species overlap, we conclude that using all sampling methods is important and one should not
be chosen over the other when assessing species richness or investigating microbial biogeography and especially pertaining to environmental policy initiatives. These differences in percentage of shared species can be explained by biodiversity and evenness indices for these sample types. Similarity values for combined data followed similar trends however, there were slight differences in ranks across pairs that were not the most or least similar between combined and separated data. These differences are depicted in the Jaccard dendrographs presented. Because dendrographs do not strictly match table values for pairs that were not the most or least similar, it is concluded that these differences could be largely due to the binary structure of data for these tests and the complete linkage clustering method that was used. Because the focus and interest in this study is to investigate differences at the species level, the similarity metrics (Jaccard and Sorenson methods) used calculated values based on presence-absence and not abundances and after finding the maximum mathematical distances between points of two clusters. Because the right and left bank diatometers are almost the same “distance” from 2018 composite samples for separated data, they appear more similar to the 2018 composite sample than 1956 composite samples. However, for combined data, 2018 composite samples appear more similar to 1956 composite samples than both the right and left diatometers. For these data, the right and left bank diatometers are not almost the same distance from the 2018 composite samples. Here, the software chose the sample method with the greatest distance before combining them and creating a new matrix. When comparing similarities with presence-absence data, these results show that splitting or lumping species complexes changes hierarchical clustering analyses even if they follow the same trends otherwise.

In analyzing relative abundances for 2018, the most abundant taxa belonged to the genus *Eunotia*, more specifically *Eunotia* girdle views. *G. parvulum* was also dominant in 2018
samples (7.2% separated vs. combined 16.2%). Although this taxon was not the most dominant, the objective of capturing high morphological diversity of this taxon was met. Because percentages were also found to increase by over double, the importance of splitting taxa in *G. parvulum* species complexes for future studies remains pertinent. In Europe, about 180 *Gomphonema* taxa are known and documented. In the southeastern U.S., the total of *Gomphonema* taxa are still being estimated. As recent studies have pointed out, continued study of this genus remains relevant as it is known to produce Janus cells, exhibit high variability, semi cryptic taxa, and biogeography (Abarca *et al.* 2014, Abarca *et al.* 2020, Kermarrec 2013, Levkov 2016, McBride & Edgar 1998). More species complexes were encountered in this study including members of the *Tabellaria* and *Eunotia* genera. Because members of these genera and the *Gomphonema* genus account for 79.8% of 2018 total counts, it is critical to distinguish morphotypes from species within these species complexes to gain better insight about diatom biodiversity at this site.

In this GCSU study, as shown in voucher flora Plates 1-2 and Table 6, 10 taxa were found that were most likely combined in past assessments into the *G. parvulum* species complex. Since the 1956 ANSP study, three of the 10 *Gomphonema* OTUs found in this study have been raised to species level, two are recognized as morphotypes, and five more were separated in this 2018 GCSU study. North American and European taxonomic and molecular literature were used for these separations. Originally it was intended to incorporate species level separations described in Abarca *et al.* 2014 and Kermarrec *et al.* 2013. However, upon further inspection, we found the uniformity and delineation of the population micrographs and corresponding descriptions unclear from a taxonomic perspective. These studies also differed in separations and conclusions about results surrounding taxa such as *G. exilissimum* and *G. lagenula.* However,
both molecular studies either highlight the importance of continued morphotype identification or acknowledge the contribution of taxonomic varieties in water quality indication. Therefore, we chose a more conservative approach and separated *Gomphonema parvulum sensu lato* representatives based on observed differences within the population found in this GCSU study. For the purposes of water policy and understanding habitat water quality, it is recommended to split taxa and document morphotypes or ecotypes. Because diatoms reproduce asexually and rely on available conditions from their surrounding environment for cell wall production, documenting species morphotypes or ecotypes could answer questions about water quality that molecular studies may not.

In 1956 the ANSP reports a total of 84 taxa at UTRC. In this GCSU study’s diatom enumeration of 1956 composite slides, 153 taxa (split), and 136 (lumped) are reported. However, species per slide and treatment (split or lumped) range from 83-105. In the comparison to past ANSP studies along the Savannah River, which found an overwhelming dominance (75%) of *G. parvulum*, we found *G. parvulum* to account for only 7.2% of 2018 total counted units not including morphotypes/ ecotypes and 16.2% including morphotypes/ ecotypes for this headwater stream. Therefore, past dominant species of the Savannah River are not dominant today at UTRC regardless of lumping or splitting morphotype representatives of *G. parvulum* species complex. For 1956 UTRC studies, the ANSP found *Navicula mutica* Kützing 1844 to be the dominant taxon accounting for 15 % relative abundance at the site (ANSP 1956). In our study, the taxon identified to species level that was the most dominant for 2018 samples was *E. rhomboidea* accounting for 8.3% for separated taxa methods. For combined taxa methods, *G. parvulum sensu lato* (16.2%) was the most abundant taxon identified to species level. When applying our techniques of species complex separations, we found *L. goeppertiana* (5.4%) to be the most
dominant taxon identified to species level. This result differs from that of the ANSP 1956 report. However, we were unable to determine if standard periphyton protocols were used in 1956 when collecting composite samples or during diatom enumeration. Information outlined in the reports document the use of forceps, spoons, knives and 17 mL vials, but whether or not habitats were sampled based on the percentage of site make-up is unclear. It also remains unclear how many valves were counted per sample, although it is suspected to be in the thousands. In the 1956 report, the ANSP documents that members of the genera Eunotia, Tabellaria, Pinnularia, and Achnanthes were common in all samples. With the exception of Achnanthes representatives, findings from this GCSU study are the same as those of the ANSP in 1956 with respect to the common genera found.

According to diatom species ecological indication, UTRC is a nutrient rich environment and according to 2018 physicochemical data, UTRC is an acidic environment with high dissolved oxygen levels. These findings are the same as those in 1956, as the ANSP reports that dominant genera represent “cool, slightly acidic streams,” and that indicate that the “river has a high nutrient level” (ASNP 1956). G. parvulum sensu stricto is frequently reported from eutrophic waters, while G. exilissimum is found in oligotrophic habitats. Both species are documented here at UTRC, which is considered a reference site that has been protected for at least 50 years. Therefore, nutrient levels reflected by the bioindicators found in this study are more likely contributed by a natural source (e.g., vegetation falling into the system) than anthropogenic land use or agriculture. However, continued evaluation of the G. parvulum complex is necessary in order to understand the ecological preferences of representatives.

During field observations, it was noted that UTRC is high in tannins, which could explain the acidity found at this site. Physical aspects of this site, include a high sediment load with a
mostly sandy bottom and some grass-like and woody vegetation. Sandy bottom creeks are typical for the coastal plain, which plays a role in driving algal communities as well. Other references that document coastal plains diatoms from the southeastern U.S. include the Gaiser & Johansen 2000 study. Some of the dominant taxa in their study were also found in this 2018 study.

Although this GCSU study did not find trend differences in biodiversity indices between lumping and splitting techniques, disregarding fine level taxonomy is not suggested or recommended. Investigation at the genus level or coarser levels of identification may yield insights about the physical aspects of this site. However, species level investigation gives unique insight about dominance, community, and ecosystem health as chemical factors play a role in reproduction and frustule development. Species level identification is also necessary for mapping microbial biogeography and establishing species ranges for habitat protection under environmental policy. In this study, an undescribed species with a moderate population size was discovered, which we are currently describing. Documentation of this species and its range could contribute to the protection of its habitat and therefore, that of a surface water resource that drains into potable water resources.

6 LITERATURE CITED


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**Software**


**Figure 1.** Map (top) of the United States depicting the location of the SRS from 2004 Savannah River Site Environmental Report [www.srs.gov](http://www.srs.gov). Map (bottom) showing the sampling location of past GCSU studies (Lat. 33.370750, Long. -81.627738) and this 2018 study (Lat. 33.393067, Long. -81.610719) on UTRC, which is located on SRS, ©2020 Google
Table 1. Physicochemical data for 2018 Upper Three Runs Creek study site at the time of deployment and retrieval of diatometers. Composite samples taken during diatometer retrieval.

*denotes unreliable readings around sonde servicing

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Depth (cm)</th>
<th>Temperature (°C)</th>
<th>DO (%)</th>
<th>DO (mg/L)</th>
<th>pH</th>
<th>Conductivity (mS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23 March deployment</td>
<td>140</td>
<td>13.5</td>
<td>106.4*</td>
<td>11.09</td>
<td>6.46</td>
<td>13</td>
</tr>
<tr>
<td>9 April Retrieval</td>
<td>177</td>
<td>14.45</td>
<td>99.1</td>
<td>10.12</td>
<td>5.28</td>
<td>*</td>
</tr>
<tr>
<td>15 Aug. deployment</td>
<td>177</td>
<td>21.33</td>
<td>88.7</td>
<td>7.85</td>
<td>4.17</td>
<td>15</td>
</tr>
<tr>
<td>30 Aug. Retrieval</td>
<td>177</td>
<td>21.43</td>
<td>53.1</td>
<td>4.69</td>
<td>4.55</td>
<td>15</td>
</tr>
</tbody>
</table>
Table 2. Biodiversity and species richness for 2018 and 1956 samples (*Gomphonema*, *Eunotia*, and *Tabellaria* taxa separated from *sensu lato* species complexes) where *d* indicates diatometer sample and *c* indicates composite sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>Species richness</th>
<th>SWDI (H’)</th>
<th>Pielou’s evenness (J’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2018 c</td>
<td>111</td>
<td>3.242</td>
<td>0.688</td>
</tr>
<tr>
<td>Right d</td>
<td>67</td>
<td>3.263</td>
<td>0.776</td>
</tr>
<tr>
<td>Left d</td>
<td>129</td>
<td>3.661</td>
<td>0.753</td>
</tr>
<tr>
<td>1956 c</td>
<td>153</td>
<td>4.342</td>
<td>0.863</td>
</tr>
</tbody>
</table>
Table 3. Biodiversity and species richness for 2018 and 1956 samples (*Gomphonema*, *Eunotia*, and *Tabellaria* taxa combined to *sensu lato* species complexes) where *d* indicates diatometer sample and *c* indicates composite sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>Species richness</th>
<th>SWDI (H’)</th>
<th>Pielou’s evenness (J’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2018 c</td>
<td>89</td>
<td>2.320</td>
<td>0.517</td>
</tr>
<tr>
<td>Right d</td>
<td>42</td>
<td>2.217</td>
<td>0.593</td>
</tr>
<tr>
<td>Left d</td>
<td>103</td>
<td>3.138</td>
<td>0.677</td>
</tr>
<tr>
<td>1956 c</td>
<td>136</td>
<td>4.09</td>
<td>0.833</td>
</tr>
</tbody>
</table>
**Figure 2 a & b.** Box-plots of species richness per treatment (2018 composite, right diatometer, left diatometer, and 1956 composite) using data for combined taxa unit counts vs. separated taxa unit counts for *Gomphonema*, *Eunotia*, and *Tabellaria* species complexes.
Figure 3. Jaccard dendrogram clustering treatments (2018 composite, 1956 composite, right diatometer, and left diatometer) with separated taxa by Jaccard distance using complete linkage clustering analysis.
Figure 4. Jaccard dendrograph clustering treatments (2018 composite, 1956 composite, right diatometer, and left diatometer) with combined taxa by Jaccard distance using complete linkage clustering analysis.
Table 4. Jaccard and Sorenson similarity indices for 2018 and 1956 samples (*Gomphonema*, *Eunotia*, and *Tabellaria* taxa separated from *sensu lato* species complexes compared to combined), where *d* indicates diatometer sample and *c* indicates composite sample

<table>
<thead>
<tr>
<th>Sample pair</th>
<th>Taxa separated</th>
<th></th>
<th></th>
<th>Taxa combined</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jaccard similarity</td>
<td>Jaccard distance</td>
<td>Sorenson</td>
<td>Jaccard similarity</td>
<td>Jaccard distance</td>
<td>Sorenson</td>
</tr>
<tr>
<td>2018 c &amp; Right d</td>
<td>0.219</td>
<td>0.781</td>
<td>0.360</td>
<td>0.149</td>
<td>0.851</td>
<td>0.260</td>
</tr>
<tr>
<td>2018 c &amp; Left d</td>
<td>0.218</td>
<td>0.782</td>
<td>0.358</td>
<td>0.164</td>
<td>0.836</td>
<td>0.281</td>
</tr>
<tr>
<td>Right d &amp; Left d</td>
<td>0.347</td>
<td>0.658</td>
<td>0.510</td>
<td>0.272</td>
<td>0.728</td>
<td>0.428</td>
</tr>
<tr>
<td>2018 c &amp; 1956 c</td>
<td>0.211</td>
<td>0.789</td>
<td>0.348</td>
<td>0.178</td>
<td>0.822</td>
<td>0.302</td>
</tr>
<tr>
<td>1956 c &amp; Left d</td>
<td>0.236</td>
<td>0.763</td>
<td>0.383</td>
<td>0.201</td>
<td>0.799</td>
<td>0.335</td>
</tr>
<tr>
<td>1956 c &amp; Right d</td>
<td>0.182</td>
<td>0.817</td>
<td>0.309</td>
<td>0.134</td>
<td>0.866</td>
<td>0.236</td>
</tr>
</tbody>
</table>
Table 5. List of diatom taxa and operational taxonomic units (OTUs) found at > 1% relative abundance (RA) identified from 2018 samples and 1956 archived slides collected from Upper Three Runs Creek, South Carolina. In sample type, R= right diatometer, L= left diatometer, NC= 2018 composite, and OC= 1956 composite. (*) denotes relative abundance with taxa not split from species complexes for *Gomphonema parvulum* sensu lato, *Eunotia incisa* sensu lato, and *Tabellaria flocculosa* sensu lato. In RA, (-) represents taxa not found in sample year or scored < 1% relative abundance

<table>
<thead>
<tr>
<th>Scientific Name or OUT</th>
<th>2018 Sample type</th>
<th>2018 %RA</th>
<th>1956 %RA</th>
<th>Ecological indication</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eunotia</em> spp. girdle</td>
<td>X</td>
<td>X</td>
<td>A</td>
<td>31.5</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Genus ecology: found in soft-water lakes, oligotrophic, areas with low light, low turbulence, narrow temperature gradient; acidophilic</td>
<td>Lowe In: Stevenson et al. 1996; Furey et al. 2011</td>
</tr>
<tr>
<td><em>Eunotia incisa</em></td>
<td>X</td>
<td>X</td>
<td>A</td>
<td>3.7, 4.8*</td>
<td>2.3*</td>
</tr>
<tr>
<td>Plate 5, Figs. 22-31</td>
<td></td>
<td></td>
<td></td>
<td>Acidobionic; optima: pH 5.87, TP 9.85, DOC 9.21, acidophilic; Epiphytic on bryophytes in streams and observed on wet walls; common and abundant in peat bogs, acidic waters, and low electric conductance, most frequent taxon in Holarctic zone; acidophilic, high (100% saturation) oxygen requirements, tolerating small concentrations of organic nitrogen, oligosaprobous, oligotraphentic, mainly occurring in water, but sometimes in wet places out of water bodies</td>
<td>Planas In: Stevenson et al. 1996; Barinova et al. 2006; Furey et al. 2011; Horst Lange-Bertalot et al. 2011; Van Dam et al. 1994</td>
</tr>
<tr>
<td>Species</td>
<td>Optima pH</td>
<td>pH Range</td>
<td>TP</td>
<td>DOC</td>
<td>Notes</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-----------</td>
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<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td><em>Eunotia rhomboidea</em></td>
<td>4.89</td>
<td>4.7-5.0</td>
<td>7.48</td>
<td>6.95</td>
<td>acidophilic; epiphytic on bryophytes in streams; cosmopolitan, found in oligotrophic to dystrophic waters, and in mineral poor and acidic environments; also found in waters with high nutrient concentrations; acidophilic, high (100% saturation) oxygen requirements, can tolerate small concentrations of organic nitrogen, oligosaprobic, oligotraphentic, and mainly found in water, but regularly found in wet places.</td>
</tr>
<tr>
<td><em>Fragilaria spp.</em> girdle</td>
<td>-</td>
<td></td>
<td>OC</td>
<td>-</td>
<td>Genus ecology: dominate headwater reaches, nutrient rich environments, and settle rapidly.</td>
</tr>
<tr>
<td><em>Fragilariforma spp.</em> girdle</td>
<td>X</td>
<td></td>
<td>A</td>
<td>9.8</td>
<td>Acidophilic, and occurring in environments high in tannic acid.</td>
</tr>
<tr>
<td><em>Fragilariforma virescens var. 1</em></td>
<td>X</td>
<td>-</td>
<td>NC, R, L</td>
<td>1.1</td>
<td>pH indifferent, oligosaprobic, current (flow) indifferent, found in streams and ditches, and seasons spring and fall.</td>
</tr>
<tr>
<td><em>Frustulia crassinervia</em></td>
<td>X</td>
<td></td>
<td>R, L, OC</td>
<td>1.8</td>
<td>Acidophilic; genus found in soft-water lakes; acidophilic; acidobiontic, high (100% saturation) oxygen requirements, tolerating small concentration of organic nitrogen, oligosaprobic, oligotraphentic,</td>
</tr>
<tr>
<td>Species</td>
<td>Genus ecology</td>
<td>Species ecology</td>
<td>References</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>------------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Frustulia saxonica</strong></td>
<td>Genus ecology: found in soft-water lakes; Species ecology: pH optimum 5.49;</td>
<td>Acidophilic; acidobiontic; high (100% saturation) oxygen requirements, tolerating small concentrations of organic nitrogen, oligosaprobic, oligotraphentic, mainly found in water, but regularly observed in wet places</td>
<td>Lowe In: Stevenson et al. 1996; Barinova et al. 2006; Van Dam et al. 1994</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mainly in water regularly in wet places</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gomphonema spp. girdle</strong></td>
<td>Genus ecology: found in areas of high light, high turbulence, broad temperature ranges, found in nutrient rich environments, and oligotrophic hard water</td>
<td></td>
<td>Lowe In: Stevenson et al. 1996</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gomphonema parvulum</strong></td>
<td>Found in eutrophic and moderately polluted waters</td>
<td></td>
<td>Levkov et al. 2016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphotype 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gomphonema parvulum</strong> “protracted off-center”</td>
<td>Acidophilic, and occurring in environments high in tannic acid</td>
<td></td>
<td>This 2018 study</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gomphonema parvulum sensu</strong></td>
<td>Optimums: pH 6.66, DOC 5.86; pH indifferent, mesosaprobic, thriving in</td>
<td></td>
<td>Barinova et al. 2006;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mainly in water regularly in wet places</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
Plate 1, Figs. 1-15

*Gomphonema preliciae*

Epiphytic on macrophytes, found in shallow areas, and in eutrophic to hyper-eutrophic environments

Levkov *et al.* 2016

*Luticola spp.*

Acidophilic, and occurring in environments high in tannic acid

This 2018 study

*Luticola goeppertiana*

Found in eutrophic and eusaprobic environments; circumnuetral, found in fresh-brackish water, needs periods of high organic nitrogen concentrations, low (above 30% saturation) oxygen requirements, mainly occurring in water, but regularly observed in moist and wet places; found in “electrolyte-rich running waters” and rivers with high industrial pollution

Levkov *et al.* 2013; Van Dam *et al.* 1994; Cantonati *et al.* 2017

*Navicula leptostriata*

Genus ecology: found in eulittoral environments and areas of low light, can

Peterson; Lowe; Tuchman In:
Plate 4, Fig. 16

Survive in ground water, and resettle rapidly from suspension;
Species ecology: optima: pH 6.26, TP 13.07, DOC 15.48, acidophilic;
acidophilic, high (100% saturation) oxygen requirements, tolerates small concentrations of organic nitrogen, oligosaprobous, oligo-mesotraphentic, mainly in water, and regularly observed in wet places

Stevenson et al. 1996; Barinova et al. 2006; Van Dam et al. 1994

Navicula notha
Plate 4, Fig. 15

Genus ecology: found in eulittoral environments and areas of low light, can survive in ground water, and resettle rapidly from suspension;
Species ecology: Alkaliphilous, found in eutrophic environments, halobion indifferent, current (flow) indifferent, observed in lakes and ponds, periphytic

Peterson; Lowe; Tuchman In: Stevenson et al. 1996; Lowe 1974

Past “Navicula minima group”
(Navicula difficillima/Sellaphora difficillima/Adlafia spp.)

Alkaphilous, found in fresh-brackish waters, needs periods of high concentrations of organic nitrogen. Low (above 30% saturation) oxygen requirements, eutraphentic, mainly found in water, but regularly observed in wet places

Van Dam et al. 1994

Nitzschia spp. girdle

Diurnal migration, found in low light and nutrient rich environments, remain in suspension longer instead of settling immediately

Goldsborough and Robinson; Hill Peterson In: Stevenson et al.
<table>
<thead>
<tr>
<th>Organism</th>
<th>Genus</th>
<th>X</th>
<th>X</th>
<th>NC, L, OC</th>
<th>A</th>
<th>Sensu lato ecology: optima: pH 5.22, TP 7.45, DOC 8.83 acidophilic; acidophilous, mesotrophic-oligotrophic-dystrophic, halophilous, found in ponds, periphytic, and tychoplanktonic, seasons spring and fall; acidophilous, fresh water species, tolerating very small concentrations of organic nitrogen, high (about 100% sat) oxygen requirements mesosaprobous, mesotraphentic, mainly occurring in water bodies, and sometimes observed in wet places; oligotrophic, found in areas with high light, high turbulence, and broad temperature changes</th>
<th>Referenced by: Barinova et al. 2006; Lowe 1974; Van Dam et al. 1994; Lowe In: Stevenson et al. 1996</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nitzschia recta</em></td>
<td></td>
<td>X</td>
<td>X</td>
<td>NC, L, OC</td>
<td>-</td>
<td>Alkaphilous, found in fresh-brackish water, can tolerate high concentrations of organic nitrogen, fairly high (above 75% saturation) oxygen requirements mesosaprobous, oligo-eutrphentic, never to rarely observed outside of water bodies</td>
<td>Referenced by: Van Dam et al. 1994</td>
</tr>
<tr>
<td><em>Pinnularia</em> spp.</td>
<td></td>
<td>-</td>
<td>X</td>
<td>OC</td>
<td>-</td>
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<tr>
<td><em>Tabellaria flocculosa</em></td>
<td></td>
<td>X</td>
<td>X</td>
<td>A</td>
<td>1.3, 2.6*</td>
<td>Genus ecology: found in soft-water lakes</td>
<td>Referenced by: Lowe In: Stevenson et al. 1996</td>
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<td>“intermediate”</td>
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<tr>
<td><em>Tabellaria</em> spp. girdle</td>
<td></td>
<td>X</td>
<td>X</td>
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Table 6. Morphological features distinguishing taxa originally belonging to *Gomphonema parvulum sensu lato* species complex for 2018 Upper Three Runs Creek study

<table>
<thead>
<tr>
<th>Taxon/OTU</th>
<th>Length (μm)</th>
<th>Width (μm)</th>
<th>Striae density (in 10 μm)</th>
<th>Raphe</th>
<th>Central area</th>
<th>Striae orientation</th>
<th>Valve and Pole shape</th>
<th>Previous literature</th>
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<tr>
<td><em>Gomphonema parvulum sensu lato</em></td>
<td>12.6-31</td>
<td>4.5-5.7</td>
<td>14-20</td>
<td>Narrow</td>
<td>Small-moderate depending on area opposite isolated punctum</td>
<td>Not radial across transapical axis, parallel or curved towards isolate puncta</td>
<td>Ends rostrate, valves elliptic-lanceolate</td>
<td>Patrick &amp; Reimer (1975)</td>
</tr>
<tr>
<td><strong>Plate 1, Figs. 1-15</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Levkov <em>et al.</em> (2016)</td>
</tr>
<tr>
<td><em>G. parvulum</em> morphotype 2</td>
<td>12.4-18.9</td>
<td>4.5-5.2</td>
<td>17-20</td>
<td>Similar to <em>G. parvulum</em></td>
<td>Similar to <em>G. exilissimum</em></td>
<td>Similar to <em>G. exilissimum</em></td>
<td>Sub-capitate headpole, valve</td>
<td>Levkov <em>et al.</em> (2016)</td>
</tr>
<tr>
<td>Plate 1, Figs.</td>
<td>Plate 1, Figs.</td>
<td>Plate 1, Figs.</td>
<td>Plate 1, Figs.</td>
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<td>19-21</td>
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<td>22-28</td>
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<tr>
<td><strong>G. parvulum</strong></td>
<td><strong>G. parvulum</strong></td>
<td><strong>G. parvulum</strong></td>
<td><strong>G. parvulum</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>“off-center and protracted”</td>
<td>“intermedio”</td>
<td>“radial – rhombic”</td>
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<tr>
<td>16-21</td>
<td>16-18</td>
<td>15-20</td>
<td>15-20</td>
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<tr>
<td>18.8-24.6</td>
<td>18.3-19.1</td>
<td>15.1-20.5</td>
<td>5.3-5.4</td>
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<td>4.5-5.6</td>
<td>5.3-5.4</td>
<td>3.5</td>
<td>3.5</td>
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<tr>
<td>Asymmetrica l along the apical axis, also bisecting foot poles</td>
<td>Similar to <strong>G. parvulum</strong> sensu lato</td>
<td>Similar to <strong>G. parvulum</strong> sensu lato</td>
<td>Similar to <strong>G. parvulum</strong> sensu lato</td>
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<td>Small</td>
<td>Small</td>
<td>Small</td>
<td>Small</td>
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<tr>
<td>Variable-weakly radiate, curved near isolate</td>
<td>Not radial across transapical axis</td>
<td>Radial across transapical axis</td>
<td>Radial across transapical axis</td>
<td></td>
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<tr>
<td>Ends protracted and headpoles weakly capitate, valves lanceolate</td>
<td>Not applicable</td>
<td>Ends weakly protracted and headpoles weakly capitate, valves lanceolate-rhombic</td>
<td>Not applicable</td>
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<tr>
<td>Not applicable</td>
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<td>Not applicable</td>
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<tr>
<td><strong>G. lagena</strong></td>
<td><strong>16-20.1</strong></td>
<td><strong>4.6-5.7</strong></td>
<td><strong>17-18</strong></td>
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<tr>
<td><strong>G. parvulum</strong></td>
<td><strong>19.3-23.2</strong></td>
<td><strong>4.9-5.7</strong></td>
<td><strong>16-17</strong></td>
<td></td>
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<tr>
<td><strong>“rhombic”</strong></td>
<td><strong>23.2</strong></td>
<td><strong>G. parvulum</strong></td>
<td><strong>sensu lato</strong></td>
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<tr>
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<td><strong>39-41</strong></td>
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<tr>
<td><strong>G. parvulum</strong></td>
<td><strong>23.2-34.6</strong></td>
<td><strong>3.3-10.6</strong></td>
<td><strong>14-18</strong></td>
<td></td>
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<tr>
<td><strong>“Cox”</strong></td>
<td><strong>(10)-</strong></td>
<td><strong>(21)</strong></td>
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<td><strong>Plate 1, Figs.</strong></td>
<td><strong>42-45</strong></td>
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<tr>
<td><strong>Valves</strong></td>
<td><strong>Small</strong></td>
<td><strong>Not radial</strong></td>
<td><strong>Valves rhombic</strong></td>
<td><strong>Not applicable</strong></td>
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<tr>
<td><strong>across</strong></td>
<td><strong>throughout</strong></td>
<td><strong>application</strong></td>
<td><strong>Not</strong></td>
<td><strong>applicable</strong></td>
<td></td>
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<tr>
<td><strong>transapical</strong></td>
<td><strong>Foot poles acutely</strong></td>
<td><strong>Foot poles broader and</strong></td>
<td><strong>Rose &amp; Cox</strong></td>
<td><strong>(2014)</strong></td>
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<tr>
<td><strong>axis</strong></td>
<td><strong>rounded and head</strong></td>
<td><strong>more obtuse,</strong></td>
<td><strong>valves lanceolate</strong></td>
<td><strong>not</strong></td>
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</tbody>
</table>

- **G. lagena**: Straight and filiform, asymmetric, depending on area opposite isolated punctum. Ends distinctly capitate, valves slightly narrow lanceolate to elliptic. Patrick & Reimer (1975).
- **G. parvulum**: Similar to G. parvulum sensu lato. Small to moderate, rectangular depending on cell size and area opposite isolated punctum. Slightly radiate throughout. Foot poles acutely rounded and head poles broader and more obtuse, valves lanceolate. Rose & Cox (2014).
<table>
<thead>
<tr>
<th><strong>G. confusum</strong></th>
<th>14.8-22.3</th>
<th>4.5-5.1</th>
<th>15-18</th>
<th>Similar to <strong>G. parvulum sensu lato</strong></th>
<th>Small, rectangular depending on area opposite isolated punctum</th>
<th>Radiate to parallel across transapical axis</th>
<th>Ends acutely rounded and protracted, valves lanceolate to rhombic-lanceolate</th>
</tr>
</thead>
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<tr>
<td><strong>Plate 1, Figs.</strong></td>
<td><strong>46-48</strong></td>
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</table>
PLATE 1

Scale bars = 10 μm

Figures 1-15 *Gomphonema parvulum sensu lato*

Figures 16-18 *Gomphonema exilissimum*

Figures 19-21 *Gomphonema parvulum* Morphotype 2*

Figure 22-28 *Gomphonema parvulum* “protracted and off- center ”*

Figures 29-31 *Gomphonema parvulum* “intermedio”

Figure 32-34 *Gomphonema parvulum* “radial – rhombic”

Figures 35-38 *Gomphonema lagenula*

Figure 39-41 *Gomphonema parvulum* “rhombic”

Figures 42-45 *Gomphonema parvulum* “Cox”

Figures 46-48 *Gomphonema confusum*

* Denotes taxon or OTU found at > 1% relative abundance (RA) identified from 2018 samples and 1956 archived slides collected from Upper Three Runs Creek, South Carolina.
PLATE 1
PLATE 2

Scale bars = 10 μm

Figure 1  *Gomphonema* “marrii”

Figure 2  *Gomphonema parvulum* Morphotype 3

Figure 3  *Gomphonema* spp. girdle*

Figure 4-6  *Fragilariforma virescens* var. 1*

Figures 7-9  *Fragilariforma virescens* var. 2

Figure 10  *Pinnularia* sp.1

Figures 11-12  *Fragilariforma* spp. girdle*

Figure 13  *Fragilariforma* “coarse”

Figure 14  *Fragilaria* sp.1

Figure 15  *Fragilaria exigua*

Figure 16  *Achnanthidium subhudsonis* var. *kraeuselii*

Figures 17-18  *Fragilariforma* cf. *floridana*

Figure 19  *Fragilariforma bicapitata*

Figure 20  *Fragilariforma virescens* small

Figures 21-22  *Fragilariforma polygonota*

Figures 23-24  *Psammothidium altaicum*

Figures 25-26  *Psammothidium helveticum*

Figure 27  *Psammothidium bioretii*

Figure 28-29  *Psammothidium subatomoides*

* Denotes taxon or OTU found at > 1% relative abundance (RA) identified from 2018 samples and 1956 archived slides collected from Upper Three Runs Creek, South Carolina.
PLATE 2

Scale bars = 10 μm

Figures 30-31 Achnanthidium catenatum girdle

Figure 32 Achnanthidium minutissimum

Figure 33 Stauroneis smithii var. incisa

Figure 34 Lemnicola hungarica

Figures 35-37 Brachysira brebissonii

Figure 38 Cymbopleura naviculiformis

Figures 39-41 Neidium alpinum

Figure 42 Staurosirella martyi

Figure 43 Planothidium lanceolatum
PLATE 3

Scale bars = 10 μm

Figures 1-3  *Tabellaria quadriseptata*

Figures 4-8  *Tabellaria fenestrata*

Figures 9-12  *Tabellaria flocculosa* strain III

Figure 13  *Tabellaria* spp. girdle*

Figure 14  *Tabellaria flocculosa*

Figure 15  *Tabellaria flocculosa* girdle band

Figure 16  *Tabellaria* sp. transitional

Figure 17-25  *Tabellaria flocculosa* intermediate*

* Denotes taxon or OTU found at > 1% relative abundance (RA) identified from 2018 samples and 1956 archived slides collected from Upper Three Runs Creek, South Carolina.
PLATE 3
PLATE 4

Scale bars = 10 μm

Figure 1  Frustulia rhomboïdes
Figure 2  Pinnularia cf. mesogongyla
Figure 3  Nitzschia intermedia
Figures 4-5  Nitzschia recta*
Figure 6  Meridion alansmithii
Figure 7  Pinnularia obscura
Figure 8  Pinnularia metzeltinii Krammer
Figures 9-11  Luticola goeppertiana and girdle*
Figure 12  Frustulia spp. girdle
Figure 13  Frustulia crassinervia *
Figure 14  Neidium sacoense
Figure 15  Navicula notha*
Figure 16  Navicula leptostriata*
Figure 17  Navicula exilis
Figure 18  Navicula cf. cryptotenella
Figure 19  Pinnularia appendiculata
Figure 20-21  Geissleria kriegeri

* Denotes taxon or OTU found at > 1% relative abundance (RA) identified from 2018 samples and 1956 archived slides collected from Upper Three Runs Creek, South Carolina.
PLATE 5

Scale bars = 10 μm

Figures 1-9  *Eunotia rhomboidea* with girdle (figs. 8-9)*

Figures 10-13  *Eunotia mucophila*

Figure 14  *Eunotia schwabei*

Figures 15-16  *Eunotia pirla*

Figure 17  *Eunotia mucophila* var. 1

Figure 18  *Eunotia serra*

Figure 19  *Eunotia veneris*

Figures 20-21  *Eunotia subarcuatoides*

Figures 22-31  *Eunotia incisa* with girdle (fig. 31)*

Figures 32-34  *Eunotia cf. botulitropica*

*  Denotes taxon or OTU found at > 1% relative abundance (RA) identified from 2018 samples and 1956 archived slides collected from Upper Three Runs Creek, South Carolina.
PLATE 6

Scale bars = 10 μm

Figures 1-4  *Eunotia bilunaris*

Figures 5-7  *Eunotia* site 4

Figures 8-10  *Eunotia* site 2

Figures 11-13  *Eunotia* site 3

Figures 14-19  *Eunotia* site

Figures 20-23  *Eunotia boreotenuis*

Figure 24  *Eunotia* cf. *macroglossa*

Figures 25-26  *Eunotia carolina*

Figure 27  *Eunotia exigua*

Figure 28-29  *Eunotia* 238 new

Figure 28-29  *Eunotia pectinalis* var. *undulata*
List of Acronyms

ANSP: Academy of Natural Sciences of Philadelphia

APHA: American Public Health Association

CAA: Clean Air Act

CWA: Clean Water Act

DNA: Deoxyribonucleic Acid

EPA: Environmental Protection Agency

EPD: Environmental Protection Division

ESA: Endangered Species Act

GCSU: Georgia College & State University

HAB: Harmful Algal Bloom

LM: Light Microscopy

NAWQA: National Water-Quality Assessment

NOV: Notice of Violation

NPDES: National Pollutant Discharge Elimination System

OTU: Operational Taxonomic Unit

PCR: Polymerase Chain Reaction

SEM: Scanning Electron Microscopy

SREL: Savannah River Ecology Lab

SRS: Savannah River Site

TMDL: Total Maximum Daily Loads

TN: Total Nitrogen

TP: Total Phosphorous
UGA: University of Georgia

USDOE: United States Department of Energy

UTRC: Upper Three Runs Creek

UVA Wise: Virginia’s College at Wise
**Appendix A.** *Gomphonema parvulum* (Kützing) Kützing chloroplast sequencing from 2018-2019 cultures and live monoculture images for publication: K. Johnson¹, K. Manoylov¹, and B. Cahoon²

1 Department of Biological and Environmental Sciences, Georgia College & State University
2 Department of Natural Sciences UVA Wise.

**Table 1A.** Chloroplast gene marker barcode from 2019 PCR amplification

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<th>Sequence Details</th>
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Figures A-B. Images of live *G. parvulum* (Kützing) Kützing monoculture (September 2018 – May 2019) before chloroplast sequencing. Scale bar = 10 μm at 400X magnification
Appendix B. *Gomphonema* “marrii” description, LM and SEM images for publication K. Johnson\textsuperscript{1} & K. Manoylov\textsuperscript{1}

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Figure A. Light microscopy images of *Gomphonema* “marri” and size diminution series found in Upper Three Runs Creek 2018 study site. Images taken at 1000X magnification, scale bar = 10 μm
Figures B-E. Scanning Electron Microscope images of *Gomphonema* “marri” found in Upper Three Runs Creek 2018 study site. Fig B. isolated pore, external morphology of striae and raphe endings, Fig. C entire valve with moderate axial area, Fig. D headpole, and Fig. E footpole and apical porefield.
Figures F-G. Images of live *Gomphonema* “marrii” cells and mucilage stalks from epiphytic scraping of 2018 Upper Three Runs composite sample material

*Gomphonema* “marrii” (Figs. A-G)

**Description:** Valves heteropolar, rhombic, widest around center. Valve margins slightly undulate near headpole. Headpole apiculate. Footpole slightly cuneate to acutely rounded. Valve length 41.2-61.8 μm, width: 8-10 μm. Axial area moderate to small, almost linear, but widest around center and narrowing near apices. Central area variable, small to moderate, slightly
rectangular and wider on side opposite isolated punctum. Isolated “punctum” near the end and slightly “off-center” of shortened median stria. In SEM (Fig. B), punctum opening is round and small. Raphe is lateral to undulate and simple. Proximal raphe endings slightly expanded and weakly deflected away from isolated punctum. Distal raphe ends long and bent away from isolated punctum. At footpole, raphe bisects apical porefield, which is made up of porelli. Striae slightly radiate towards apices and parallel, but sometimes variable around central area, 10-12 in 10 μm. Striae are uniseriate with large areolae. Areolae not distinct in LM. In SEM, areolae are 3-5 in 2 μm, or 12-14 in 10 μm. Areolae are round to elongated with variable and sometimes overlapping “occlusions” in the valve surface around areolae. Depressions are round and found on the central and axial area. Living specimens (Figs. F-G.) have “H-shaped” to rhombic “H-shaped” chloroplasts.

**Distribution and ecology:** This population was found in a headwater stream, Upper Three Runs Creek, near Aiken, South Carolina. Upper Three Runs Creek has a slightly acidic to acidic environment, is high in tannic acid, organic debris and has a sandy and silty bottom. Live specimens of *G. “marrii”* were found growing together on mucilage stalks in epiphytic mats of filamentous green algae growing on aquatic macrophytes at this location (Figs. F-G). Upper Three Runs Creek is known for its high biodiversity and is partially located within the protected boundaries of the Savannah River Site.
CHAPTER II

Review of historical and current algal communities from hydrologically related lakes in agriculturally altered landscapes

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Abstract

The value of historic documents is rarely critically evaluated by ecologists. Newly discovered evidence of species level algal identification from 1915 in the Iowa Northeast region surrounding Lake Okoboji contributes to the evaluation of community composition. Although there exists uncertainty when referring to historical archives, such as unclear sampling methodology and proximity of location, they provide rare historical evidence of gamma diversity that contributes to a more complete understanding of the aquatic ecology of the region. In 2017, composite samples from the same region were collected following standard APHA collection protocol. Live algae were identified and documented to the lowest taxonomic level with light microscopy. Of the same sites sampled in the 1915 archives, a total of 76 algal taxa were documented with 84 % identified to species level. These communities were dominated by green (Charophyta) filamentous taxa. In the 2017 collection, species level identification was below 50%. When comparing the 2017 and 1915 collections, only one species was found at the same location 100 years later. However, several taxa remained in the same region, but at a different site. The difference of species level identification observed is striking, and may be due to the enormous increase and changes in available taxonomic keys or due to the lack of reproductive structures of filamentous algae collected in 2017. However, detailed descriptions of physiologically active algal communities, contribute to the understanding of the biodiversity of an area where land use has developed significantly towards agricultural activities over the past century.

Key words: Iowa algal community, algae, natural history, biodiversity, community ecology, Charophyta, historical archives
Introduction

In order to prevent algal blooms, aquatic ecosystem services rely on maintenance of high algal biodiversity (interspecific competition), low nutrient conditions, seasonal and life history changes, and grazer pressure (Tilman 1977, Cardinale et al. 2012). Long-term changes in the algal community composition are strongly influenced by human activities, which affect most water bodies (e.g., habitat modification, increase in recreational activities, introduction of invasive species, and nutrient loading from agricultural activities). Understanding algal biodiversity changes in the Iowa Great Lakes would provide valuable insight into potential changes in ecosystem function. Because algae are biological indicators, and this region is heavily influenced by industrial agriculture activities, understanding algal species richness in these lakes could yield valuable baseline data when assessing future ecological changes. Currently, little is known about the historical algal species that were present in Iowa’s great lakes. Past documentation of algae from this region often only provide hand drawn images, which are difficult to use for verifying species identifications. With the phenomenon of a ‘shifting baseline syndrome’ observed in other scientific studies, it is crucial to collect and document current data in a manner that can be verified for future surface water resource management and habitat conservation.

Recently, handwritten archives from Lakeside Laboratory dated 1915 were found documenting algal species collected from Dickinson County, Iowa. Critical evaluation of the document is valuable, as G. W. Prescott reported no studies of Iowa algae prior to 1920 (Prescott 1931). The
1915 archives document provides specific dates and locations for reference collections sampled by Lewis Hanford Tiffany (Ohio State University) during his visit to Iowa’s Lakeside Laboratory by the invitation of the then director, Dr. R. B. Wylie. Later field surveys from 1922 and 1923 were used for descriptions of new taxa found in Tiffany’s 1924 and 1926 publications. Tiffany (1926) reports 200 species and varieties of filamentous algae and describes seven as new to science. Given algae are biological indicators, understanding algal species richness collected from the same localities at the time Tiffany sampled Dickinson County lakes could yield insight to lake ontogeny when compared with the algal community collected in recent years (Wolfe et al. 2001). Considering the increase of agricultural activities in the State of Iowa, species found in Tiffany’s records are expected to differ from those found in these lakes today. Finally, species-specific ecology and distribution records from Iowa and the region remain sparse (Wehr et al. 2015; Guiry & Guiry 2018), notable exception is the Ambient Lake Monitoring program through Iowa State University. The later monitors 130 lakes in Iowa each summer for 17 years with phytoplankton identification mostly to genus (https://www.iowadnr.gov/Environmental-Protection/Water-Quality/Water-Monitoring/Ambient-Lake-Monitoring).

Since the documentation of these historic samples, land use surrounding Lakeside Laboratory has vastly changed. The native vegetation of Iowa has been reported as prairies and deciduous forest along stream ravines (Braun 1964). Over the past century, Dickinson County’s wetlands have been reduced and prairies have been converted to farmland for different agricultural purposes (Smith 1998). Land use changes would change lake chemistry (i.e., nutrient loading). There are semi-long term physical and chemical data available through the Iowa DNR. Because algae respond to physicochemical changes, these alterations in land use could have
impacted algal assemblages and species richness (Stevenson 2006; Bellinger and Sigee 2010; Stevenson 2014).

Using historical data presents certain challenges: 1) often the method of collection of these data remains unclear, 2) there may be uncertainty in the exact location of past sampling, 3) deciphering handwriting leaves room for subjectivity, and 4) historical information may be fragmented. However, the value that historical data provide cannot be overlooked (McClenachan et al. 2012; Zu Ermgassen et al. 2012; Thurstan et al. 2016). With the exception of extensive paleo research from cores (Heathcote 2013), and monitoring and recording of phytoplankton to genus level (http://publications.iowa.gov/22312/1/WFS-2016-4.pdf), there are no current natural history studies from the area, which use cell physiology and community composition. Without considering these data, the understanding of the ecology of these lakes remains incomplete. Evaluating a system using only current baselines may lead to inaccurate or imprecise assessments of degradation. This idea has been described in other papers as the ‘shifting baseline syndrome’ (Pauly 1995; Papworth et al. 2009). By overlooking the value of historical data, a more complete idea of the potential biodiversity that once was may be is lost. Consequently, to demonstrate the value of historical data, we glean and incorporate as much information from these records as possible in this study.

In this study, composite samples were collected from seven sites in surrounding lakes of Iowa Lakeside Laboratory and one preserve. These sites include: Big Spirit Lake, West Lake Okoboji, East Lake Okoboji, Center Lake, the Freda Haffner Kettlehole Preserve, Millers Bay, Upper Gar Lake, and Emerson Bay. Here, we present our findings from June and July 2017 as species richness with the intent to provide a baseline voucher flora of dominant and subdominant species found at these sites. These data along with long-term monitoring of algae and
physicochemical parameters could facilitate future conservation efforts and the mitigation of anthropogenic impacts to these resources. Our review of Tiffany’s 1915 records was conducted through current taxonomic perspective (i.e., all nomenclatural changes were considered).

**Materials and methods**

**Sampling and site descriptions**

Composite littoral zone samples, following APHA standard methods (2005) and EPA periphyton protocols, were taken of all eight sites (Big Spirit Lake, Upper Gar Lake, West Okoboji Lake, Millers Bay, Emerson Bay, Center Lake, East Okoboji Lake, and the Freda Haffner Preserve) during the months of June and July 2017 (Figure 1). All GPS coordinates for our sampling sites are located in Table 1. According to the Iowa Department of Natural Resources (2012, 2017), the descriptions of these sites are as follows (information for these sites came from data, descriptions and tables on the Iowa DNR website):

**Big Spirit Lake** - comprises the largest surface area of all eight sites (5,684 acres). This lake has a mean depth 5.18 meters, and a maximum depth of 7.32 meters. Big Spirit Lake and its surrounding watershed are located in the Des Moines Lobe ecoregion, which is characterized by bluestem prairie, mollisols, and plains. The elevation of this ecoregion ranges from 274.3- 457.2 meters above sea levels. Surrounding land use of this site consists of predominantly agriculture, followed by grasslands, forest and urban development.

**Upper Gar Lake** – This lake and its surrounding watershed are located in the Des Moines Lobe ecoregion. Upper Gar Lake comprises a surface area of 36.1 acres, a maximum depth of 1.5
meters, a mean depth of approximately 1 meter. The surrounding land use of this lake consists of mostly agriculture, followed by grassland, forest, and urban development.

West Okoboji Lake – This lake was sampled as composite samples from two specific sites (Millers Bay and Emerson Bay). Millers Bay is found on the southwestern side of the lake closest to Iowa Lakeside Laboratory. Emerson Bay is found east of Millers Bay and is open to the public for boat ramp access. West Okoboji Lake has a surface area of 3,847 acres, a mean depth of 11.6 meters, and a maximum depth of 40.8 meters. This lake’s surrounding land use is predominantly grassland, followed by agriculture, forest and urban areas. This lake is located 274.3-457.2 meters above sea levels and is also within the Des Moines Lobe ecoregion.

Center Lake – This lake has a surface area of 257 acres, a mean depth of 3.7 meters, and a maximum depth of 5.5 meters. Center lake is found in Des Moines Lobe ecoregion and its surrounding land is predominantly grassland, followed by urban development, agriculture, and forest cover.

East Okoboji Lake- This site is located at elevations of 274.3-457.2 meters above sea levels within the Des Moines Lobe ecoregion. The surface area of East Okoboji Lake is 1,835 acres with a mean depth of 3 meters and a maximum depth of 6.7 meters. This lake’s surrounding land use consists of mostly agriculture followed by grasslands, forest cover and urban areas.
“The Kettlehole” – This site is located in a glacial kettlehole found in the Freda Haffner Preserve. The land around this site consists of mainly native prairies, of which this State Preserve protects 110 acres.

**Taxonomic evaluations**

Live algal units were evaluated from samples within two days of sampling. Preservation changes the shape and color of chloroplasts. Therefore, after observation, the representative sample was preserved for further analyses and deposited in the Georgia College Natural History Museum Algae Collection.

Identification of algal units and micrographs were conducted at 100X – 1000X magnification using a Leica DM2000 microscope and Leica DFC295 camera (Leica Microsystems, Wetzlar, Germany). Per collected sample, at least two entire slides were scanned for algal taxa that represented at least 10% or more of the total estimated algal biomass (relative abundance of the taxa documented). Species richness was documented for each site. An opportunistic approach was taken for algal taxa micrography, as it was not conducted for all taxa present at sites, but rather only for those that represented at least 10% or more observed biomass and, which clearly displayed key identification characteristics. Species identification was conducted using morphometrics for all documented populations by measuring 15 cells where possible.

**Results**

*Tiffany’s archives*
 Archived handwritten records from 1915 were transcribed and analyzed for comparison to current observations. Specific dates, locations and habitats were meticulously documented (Figure 2). A compilation of 1915 taxa by site according to the sites sampled in this study presented a total of 76 unique taxonomic entities (Appendix A), 84% were identified at species or variety level. Algal communities from nine locations we documented. Center Lake had the highest number of algal species (n=55), followed by the Kettlehole (n=21), and Emerson Bay (n=18). Filamentous green algae were documented in all locations, Cyanobacteria were documented in all sites except East Lake Okoboji and the Kettlehole. All sites excluding Gar Lake and Big Spirit Lake were dominated by filamentous green algae. Big Spirit Lake was dominated by Cyanobacteria. Green filamentous algae made up the highest percentage of the taxa recorded in these archives (79%), followed by Cyanobacteria (10%), and yellow-green algae (7%). Taxa with an asterisk (*) were found in past archives and current samples from the same site. Taxa denoted by a cross (†) were found in current samples but in a different site from those originally documented. Only one species, *Melosira varians* C. Agardh, was found at the same site over 100 years later. However, five unique taxonomic entities from past archives were found in current samples at different sites: *Chaetophora elegans* (Roth) C. Agardh, *Cladophora glomerata* (L.) Kützing, *Gloeotrichia echinulata* P. G. Richter, *Hydrodictyon reticulatum* (L.) Lagerheim, *Microcystis aeruginosa* (Kützing) Kützing. Several genus level records of different algal groups *Spirogyra* spp., *Gomphonema* sp., *Oedogonium* spp. were documented also.

**Summer 2017 Collection**

Fifty-four unique taxonomic entities were documented in this study (Table 2), only 49% were identified to species and variety level. Overall, 35% of these species were green algae, 26%
were diatoms, 15% were Cyanobacteria, 9% were Euglenoids, and 7% and less were desmids, yellow-green algae, dinoflagellates and Glaucophytes. Center Lake had the highest species richness (n=13), followed by the Kettlehole (n= 11). The Kettlehole and Center Lake had the most species richness for desmids (n=3). However, the Kettlehole had the highest number of Euglenoid algal species recorded (n=5), and was the only site where Euglenoid genera were found to be dominant in the collected sample. Cyanobacteria were found in all sites except East Lake Okoboji and Upper Gar. Diatoms were found at all sites. Species richness for diatoms was highest at Center Lake (n=4), followed by East Lake Okoboji, West Lake Okoboji, and Spirit Lake (n=3). Filamentous green algae were found across all sites except the Kettlehole. Spirit Lake and West lake Okoboji had the highest species richness for green algae (n=4) followed by Center Lake and Emerson Bay (n=3). One dinoflagellate and one Glaucophyte species were documented at the Kettlehole. Spirit Lake and Emerson Bay comprised the only yellow-green algae found in these samples. Millers Bay had the highest evenness across algal groups. Due to the uncertainty of collection protocols, goals, and sampling effort of past surveys, we are unable to compare past and present data in a manner to infer ecological change of these habitats. However, notable differences that may reveal changes algal community are as follows: for Center Lake and Millers Bay, we were unable to document yellow-green algae. We found no Cyanobacteria in East Lake Okoboji. Diatoms were documented in our 2016 West Lake Okoboji sample, but were missing from past archives for this site. We did not find desmids in the 2016 Emerson Bay sample. However, they were documented in 1915. We did not find Cyanobacteria in our 2017 Upper Gar sample, although they were found in 1915. In the past archives, the species found in the Kettlehole were entirely composed of green algae. However, in current samples of the Kettlehole, no green algae were documented, potentially due to the higher
precipitation for 2017 (https://mesonet.agron.iastate.edu/ASOS/reports/mon_prec.php?year=2017). This site was the most diverse in species richness across algal groups with representatives of desmids, dinoflagellates, diatoms, glaucophytes, and Euglenoids. In Spirit Lake we found fewer Cyanobacteria species (n=1) and more green algae species (n=4) than those documented in 1915 (n=5) and (n=1) respectively. Fifty percent of sites were dominated by green algae species, 25% by diatoms and 12.5% by Euglenoid species.

*Common Taxa for the 2017 study,* ‘*’ indicates Taxon present in 1915 sample

*Cladophora glomerata*

Plate 4, D-F; Plate 5, C

References: Prescott, G.W., 1951. P. 138, Pl. 20, Figs. 8, 9; Pl. 21, Figs. 1, 2.

Documented from: Millers Bay, Emerson Bay, East and West Okoboji Lakes.

Cell sizes in our population vary with cylindrical cells in main and side branches gradually decreasing in diameter and length to with ratio. Filaments profusely branched and attached by rhizoids, dark green, branching pseudodichotomous with insertion of side branches generally oblique to horizontal; cells cylindrical; chloroplast filing the volume of the cell parietal net-like with numerous pyrenoids; main axis up to 300 µm in diameter; cells of side branches 21.9-35.9 µm wide, 1.5 to 10 times longer than wide (41.4- 100 µm in length).
**Closterium parvulum** Nägeli 1849

Plate 3, I-J

References: John, D.M. and Williamson, D.B., 2009. P. 38, Pl. 6, Fig. H.

Documented from: Freda Haffner Kettlehole Preserve, Dickinson County.

Cell sizes in current population fit available literature. Cells strongly curved, outer margin with arc of 102-158°, inner margin sometimes straight, attenuating to acutely rounded apices; girdle bands absent; chloroplasts with 5-6 longitudinal ridges and 3-6 axial pyrenoids; wall smooth, colorless; terminal vacuoles containing 2-8 ovoid crystals; cells 14.4-19.4 μm wide, 137.4-146.2 μm long.

**Cocconeis pediculus** Ehrenberg 1838

Plate 5, C


Documented from: East Okoboji Lake

Cell sizes in the current population vary with cells ranging in sizes of 12-22 μm in breadth and 18.5-31.7 μm in length.

**Cymbella mexicana** (Ehrenberg) Cleve 1894

Plate 2, J; Plate 6, E


Documented from: Emerson Bay
Cell sizes in the current population vary with cells ranging in sizes of 35.8-37.8 µm in breadth and 111.9-174.9 µm in length with striae 8 in 10 µm.

**Dolichospermum circinale** (Rabenhorst ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek Plate 1, A

References: Komárek, 2013. Page 703, Fig. 867;
Documented from: Center Lake

Trichomes coiled, usually solitary, embedded in diffluent colorless mucilage. Cells spherical, slightly bent, 10.4-12.4 µm wide, 7–11 µm long, filled with aerotopes. Heterocyst spherical, 10-11 µm in diameter. Akinete not observed. In collection form 1923, this taxon was reported as common in west Okoboji Lake (Smith 1926).

**Gloeotrichia echinulata** P.G.Richter 1894*

Plate 2, L; Plate 4, A-C

References: References: Prescott, G.W., 1951. P. 557-558, Pl. 134, Figs. 1, 2
Documented from: Millers Bay

Colonies spherical, free floating, yellow to brown, soft, bullate. Colonies visible macroscopically. Trichomes slightly bent, usually ending in a distinct hair, radiating form a common center. Cells 2.1-5.2 µm wide, 2.9-23.23 µm in length, barrel-shaped, becoming quadrate or cylindrical in the apical area, sometimes thinning to a hair. Heterocyst basal, more or
less spherical, 6-12 μm in diameter. Akinetes solitary, adjacent to heterocyst, cylindrical, straight or bent, with a thick smooth wall, 10-18 μm wide, 40-250 μm long. In collection form 1923, this taxon was reported as abundant in West Okoboji Lake and rare in Little Spirit and Spirit Lakes (Smith 1926).

**Hydrodictyon reticulatum** (L.) Lagerheim 1883*

Plate 8, A-B

References: Prescott, G.W., 1951. P. 219, Pl. 47, Fig.1

Documented from: Upper Gar Lake

Coenobia contain cylindrical cells, which form colonies of a mesh or a net-like appearance. Cell arrangement forms pentagonal shaped spaces with three to five cells connected by their edges throughout. Cell sizes in the population vary greatly with small cells ranging in sizes of 11.3-12.5 μm in diameter and 47.5-62.5 μm in length to large cells ranging from 32.5-37.5 in diameter and 230-315 μm in length. Chloroplast parietal with many pyrenoids.

**Klebsormidium cf mucosum** (J.B. Petersen) Lokhorst 1985

Plate 7, J-N


Documented from: Big Spirit Lake

Cell sizes in the population vary with cells ranging in sizes of 21.9-35.9 μm in diameter and 41.4-61.7 μm in length. Ours are slightly larger than what is in reference
Microcystis aeruginosa (Kützing) Kützing 1846 *

Plate 2, H-I


Documented from: Emerson Bay

Colonies microscopic, mucilaginous, irregular and clathrate with distinct holes, or composed of subcolonies, with irregularly and densely packed cells. Mucilage colorless. Cells spherical to slightly elongate, with numerous aerotopes 4.3-4.6 µm in diameter and 4.6-10.3 µm in length. In collection from 1923, this taxon was reported as rare in East Okoboji Lake, Welch Lake, and Pleasant Lake (Smith 1926).

Phacus acuminatus A. Stokes 1885

Plate 3, B


Documented from: Freda Haffner Kettlehole Preserve

Cells ovate to oval in outline with greatest width below middle, thin, shallow dorsal furrow extending half to ¾ cell length, short cauda at posterior end, shallow incision at anterior end; numerous parietal disc-shaped chloroplasts; 1-2 ring-like paramylon bodies; visible pellicle strips longitudinally striated, flagellum approximately length of cell. Cell size 22.8 µm wide and 24.5 µm long. Eye spot evident at the base of the flagellum.
**Spirogyra cf. borgeana** Transeau 1916

Plate 6, B


Documented from: West Okoboji Lake

Cell sizes in the population range in sizes of 27-27.3 µm in diameter and 70.8-72 µm in length. Size fits reports from North American populations, cell length about 2 times the diameter. Chloroplast in the form of spiral band spiraling around 5 times. Several pyrenoids in a linear row. Gametangia and zygospores were not observed.

**Woronichinia neageliana** (Unger) Elenkin 1933

Plate 1, M-N


Documented from: Center Lake

Spherical or irregular microscopic colony, with densely arranged cells at the periphery of the colony with numerous aerotopes, up to 96-165 µm in diameter and 155.2-177.7 µm in length. Cell obovoid/ellipsoid of 5.2-7.25 µm in diameter and 4.7-7.9 µm in length. This taxon was reported by Smith (1926) as *Coelosphaerium Naegelianum* Unger and was reported as rare in Center Lake, Upper Gar Lake and several sampling locations of East and West Okoboji Lakes.
Unique taxa

**Chaetophora elegans** (Roth) C. A. Agardh 1812

Plate 7, D-E


Documented from: Big Spirit Lake

Thallus globose, with irregular light or dark green mucilaginous masses. Attached by rhizoids. Colonies are continuous and intertwined, most often with uniseriate, dichotomous branching, which radiates from a common center. Uppermost branches of thallus more numerous and densely arranged than branching near center, ending infrequently with pointed cells. Cells 10-11.6 µm in diameter near center of thallus, 21.6 - 37.12 µm long in the main axis. Chloroplast a parietal band with 1-more pyrenoids.

**Draparnaldia acuta** (C.Agardh) Kützing 1845

Plate 5, A-B

References: Prescott, G.W., 1951. P. 119-120, Pl. 15, Figs. 5

Documented from: East Okoboji Lake

Filaments branched under a right angle from the main axis, attached by rhizoids and enclosed within a soft mucilaginous envelope. Primary branches bearing oppositely, alternatively or in whorls tufts of richly divided and smaller-celled secondary branches Lateral branching under sharp angles. Small branching terminal cells end in fine hair-like structure. Cells of primary
branches 37.8-47 µm in diameter and 59.4 – 84.4 µm in length, square-shaped without constrictions; Chloroplast a parietal band in the cells of the main axis with several pyrenoids; secondary branches alternatively divided and with a distinct main axis, 25.9-37 µm in diameter and 27.3- 38.8 µm in length. This taxon is attached and

*Glaucocystis* sp. Itzigsohn 1866

Plate 3, A &C


Documented from: Freda Haffner Kettlehole Preserve

Cell sizes vary 29.64 - 35.74 µm in length and 17.8 - 20.7 µm in width. Cells were observed solitary and in 8 cell colonies. The documented colony on Plate 3 measures an old mother cell with visible nodules was 73.71 in length and 54.72 in width. Important taxon from soft water habitats (Prescott 1951) showing round endosymbionts with bright blue coloring due to phycocyanin and allophycocyanin (Graham *et al.* 2009).

**Discussion**

Although all study lakes are currently mesotrophic to hypereutrophic (https://www.iowadnr.gov/Environmental-Protection/Water-Quality/Water-Monitoring/Ambient-Lake-Monitoring), it is not clear at what point and at what rate eutrophication occurred. The changes in the lakes (excluding Kettlehole) have been well documented with organic carbon increase (Heathcote 2013) and increase in biogenic silica from 2 to 8 times (Heathcote *et al.* 2014). Biogenic silica is a measure of the silica produced by algae such as diatoms, and is
positively correlated with increased primary production due to eutrophication (Schelske et al. 1983).

Only one diatom species was found at the same site over 100 years later. It is not surprising that this species was found at the same site today given that *Melosira varians* is known to be ubiquitous in North America as well as inhabiting low-flow areas (Cardinale 2011). Although only one species was present at the same site, eight unique taxonomic entities from past archives were found in current samples at different sites, making up 10.5% of all past taxa, representing Cyanobacteria, filamentous green algae, and diatom algal groups. L.H. Tiffany (1926) published descriptions on new *Oedogonium* species, he confirmed that he used the 1915 collections and targeted a group designated as ‘filamentous algae’. The author provides a little bit of clarity about single or repeated collection, and effectively describes a composite sample collection. Tiffany (1926) used 34 references most notable Collins (1909), Tiffany (1924), Transeau (1915) and West (1904, 1916) for identification to species. In the 2017 collection reproductive structures were rare. The Plankton Algae of the Okoboji Region by G.M. Smith (1926) is another valuable resource for what taxa were present in the area at that time. Today many phycologists use techniques outlined by the United States Environmental Protection Agency for biomass analyses and/or community composition.

In collections from summer of 1915, potentially toxin producing Cyanobacteria were documented in all sites except for the Kettlehole and Emerson Bay, where *Tolypothrix tenuis* Kützing was reported. This Cyanobacteria filament was not observed in high abundance. It is uncertain whether these algae were also documented in East Lake Okoboji. It remains unknown
what collectors meant by “Okoboji Lake” in past documentation as currently, West Lake Okoboji and East Lake Okoboji are clearly distinguished. Today Cyanobacteria genera were documented in only four sampling locations (Millers Bay, Emerson Bay, West Lake Okoboji, and Center Lake). It remains unclear what this difference in Cyanobacteria species richness signifies across sites over time, so conclusions about this change can only be speculative. Difficulties in using past archives arise when there are gaps in records and field sampling protocols are unknown or not standardized. It is also difficult to draw conclusions from one snapshot sampling event, even though the effort for finest level taxonomy was a valuable tradeoff. However, documenting the presence of these species to provide a baseline may prove helpful to future studies in understanding the ecology of Iowa lakes, especially when monitoring water quality. Significant increase in Cyanobacteria may not cause systems to collapse, however they may yield a lower gamma biodiversity, which may go unnoticed (Dayton et al. 1998). Another interesting difference is noted when investigating algae from Spirit Lake as it comprised the highest species richness of almost entirely Cyanobacteria in 1915. However, in 2017 samples a much higher species richness of green algae was documented in Spirit Lake. Having a detailed record for locations and algal species composition collected on specific dates during summer is an exception rather than a norm in phycological research.

Many of the documented taxa in 1915 like *Cladophora glomerata, Rhizoclonium hieroglyphicum* (C. Agardh) Kützing are considered nuisance growth today due to the fast production of large amount of biomass (Dodds and Gudder 1992; Higgins et al. 2005). These species were not found in the 2017 Spirit Lake sample, but were found in Millers Bay, Emerson Bay, East Lake Okoboji and West Lake Okoboji. In the past, *C. glomerata* was found in almost all sites. Representatives
of family *Zygnemataceae*, another filamentous green algae group producing large amount of biomass, require high light availability (Graham *et al.* 1995) and prefer standing water with minimal mechanical disturbances as they are holdfast challenged (Tiffany 1926). This trend could be due to the goals and type of collection as stated before. Another similar trend in past archives reflecting mostly representatives of filamentous green algae can be seen when observing Kettlehole samples. All past taxa at this site are recorded as green algae, whereas, in our 2017 sample the community is more diverse with mostly Euglenoid representatives, some desmids, a diatom, glaucophyte, and dinoflagellate representative. It is interesting that the Kettlehole preserve (recognized as a protected site) would comprise the more Euglenoid taxa than any of the other sites that were sampled given its formation occurred from glacier melting and there is no boat traffic as observed at other sites. Studies have shown that species of class *Euglenophyceae* are found in sewage water and have been used as biological indicators for pollution and acidic peat bogs (Munawar 1972). With high evaporation the Kettlehole preserve site gets shallower, and macrophyte vegetation might shade representatives of *Zygnemataceae* to the proliferation of Euglenoid representatives.

The changes in the hydrologically connected Great Lakes of Iowa (Tiffany 1926) mirror other areas with cultural and recreational importance. Thomson (1992) documented the significant transformation of Iowa prairies, forests, and wetland to farmland as valuable to restore. Physicochemical indicators such as relatively high pH and dissolved oxygen may explain the observed taxa for West Lake Okoboji in the 2017 study.
Overall, taxa documented in 1915 represented only four different algal groups: Charophyta, Cyanobacteria, Bacillariophyta, and Ochrophyta, dominated by green filamentous representatives of *Zygnematophyceae* and Chlorophyta. In contrast, current taxa sampled in 2017 reflect higher diversity across algal groups, where in addition to the four phyla documented in the 1915 study, there were representatives of *Dinophyceae*, *Euglenophyceae*, and *Glaucophyta*. Past collectors did not identify most collections to genus or species level when assessing desmids or diatoms. Therefore, it is unknown what the species richness for these groups would be, or if those species we observed in this group where in fact present at the time of collection in 1915. Conducting a long-term repeated measures study with composite samples at these sites would give a better understanding of possible changes in algal communities and if there is in fact a trend in a change towards higher algal diversity. Composite sampling would also be crucial in future studies as higher biodiversity may be found in niche partitioning (Hutchinson, 1965; Tilman 1977) and, still to be uncovered, molecular diversity as shown for a *Zygnematophyceae* community (Pichrtova *et al.* 2018). Globally, niche partitioning can be seen across biogeography. Diatoms have been used as biological indicators, which have shown biogeography in taxa distribution (Hohn 1969; Kermarrec *et al.* 2013; Potapova and Charles 2007; Abarca *et al.* 2014).

**Conclusions**

Our paper is one of the few where live algal species with variable physiological tolerances for abiotic conditions were used to compare 5 hydrologically related lakes in northwest Iowa. Algal species presence and abundance are used to compare overall health of the systems in which they thrive. Today’s algae are expected to respond to runoff nutrient enrichment from the surrounding agricultural areas.
Benthic and littoral algal community analyses of the Northwest region of Iowa has not been completed recently. Baseline data with micrographs of seven hydrologically related aquatic habitats and a nature preserve provide valuable insight to community composition and diversity in this region. Algal population characteristics and species richness can be used in the future. Phycologists finding hand written records, archiving activities of known experts is always exciting and important. This was an opportunistic current collections study with an attempt to achieve the finest level of taxonomic identification and relate current findings to algal community collections from approximately 100 years ago.

**Acknowledgements**

Thank you to the students of the 2017 Ecology and Systematics of Algae course at Iowa Lakeside Lab for their help in collecting the material. We are thankful to Dr. Mary Skopec and the Friends of Lakeside at Iowa Lakeside Laboratory for awarding a visiting research scholarship to Katie Johnson. This manuscript benefitted from discussions and review by Drs. Mindy Morales and Rosalina Stancheva. This work was supported by the Department of Biological and Environmental Sciences and the Graduate School at Georgia College and State University.

**References**


Prescott, G.W. 1931. *Iowa Algae*. The University of Iowa press.


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FIGURE 2  Images from Lewis Hanford Tiffany’s notebook of algal sampling and identification from the 1915


FIGURE 4  Algae found in Emerson’s Bay, Dickinson County, Iowa. A. Cladophora sp. Scale: 40 µm. B.-C. Cladophora sp. Scale: 20 µm. D. Cladophora sp. Scale: 40 µm, E. Spirogyra sp., F. Spirogyra sp., G. Spirogyra sp. H. Microcystis aeruginosa (Kützing) Kützing Scale: 20 µm, I. Microcystis aeruginosa (Kützing) Kützing , Scale: 20 µm, J. Cymbella mexicana (Ehrenb.) Cleve, K. Tribonema sp., L. Gleotrichia echinulata, M. Quadrigula chodatii (Tanner-Fülleman) G.M. Smith. Scale bars equal 10 µm if not otherwise indicated

FIGURE 5  Algae found in the Freda Haffner Kettlehole Preserve, Dickinson County, Iowa. A, C. Glaucocystis sp. B. Phacus acuminatus A. Stokes, D. Xanthidium uncinatum (Ralfs) Stastny,

Figure 6 Algae found in Millers Bay, Dickinson County, Iowa. A. *Gleotrichia echinulata*, B. *Gleotrichia echinulata* Scale: 40 µm, C. *Gleotrichia echinulata*, D-F *Cladophora glomerata*, Scale: 20 µm, G. *Gomphonema sp.*, H. *Chamaeosiphon incrustans*, I. *Chamaeosiphon incrustans*, J. *Encyonema sp.* K. *Stigeoclonium lubricum*. Scale bars equal 10 µm if not otherwise indicated

Figure 7 Algae found in East Okoboji Lake, Dickinson County, Iowa. A.-B. *Draparnaldia acuta* (C. Agardh) Kützing. Scale: 40 µm. C. *Cladophora glomerata* with epiphytic *Cocconeis pediculus and Gomphonema sp*. Scale: 20 µm

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Figure 9 Algae found in Spirit Lake, Dickinson County, Iowa. A-C. *Stephanodiscus reimeri* D-E. *Chaetophora elegans* D. Scale: 20 µm E. Scale: 40 µm F-G. *Aulacoseira sp*. H. *Micractinium sp*. I. *Gomphosphaeria sp.* J-N. *Characiopsis braunii* Scale: 20 µm. N. *Characiopsis braunii* epiphytic; O. *Aulacoseira sp*. P-Q. *Diatoma vulgaris*, R. *Pediastrum simplex var. duodenarum* Scale bars equal 10 µm if not otherwise indicated

Figure 10 Algae found in Upper Gar Lake, Dickinson County, Iowa. A. *Hydrodictyon reticulatum* Scale: 20 µm, B. *Hydrodictyon reticulatum* Scale: 40 µm, C. *Oedogonium sp*. D. *Hydrodictyon reticulatum*. Scale bars equal 10 µm if not otherwise indicated
TABLE 1. GPS coordinates of sampling locations for all eight sites for summer 2017

<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
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<tbody>
<tr>
<td>Freda Haffner Kettlehole Preserve</td>
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<tr>
<td>Center Lake</td>
<td>43.406908°</td>
<td>-95.140512°</td>
</tr>
<tr>
<td>Millers Bay</td>
<td>43.378333°</td>
<td>-95.181111°</td>
</tr>
<tr>
<td>East Lake Okoboji</td>
<td>43.376111°</td>
<td>-95.126111°</td>
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<tr>
<td>Big Spirit Lake</td>
<td>43.445810°</td>
<td>-95.102304°</td>
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<tr>
<td>West Lake Okoboji</td>
<td>43.379920°</td>
<td>-95.141378°</td>
</tr>
<tr>
<td>Upper Gar Lake</td>
<td>43.364444°</td>
<td>-95.125000°</td>
</tr>
<tr>
<td>Emerson Bay</td>
<td>43.356369°</td>
<td>-95.174774°</td>
</tr>
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</table>
FIGURE 2

Mixed floating algae, North and West Okoboji Lake (Swamp) Aug. 14
Hydrodictyon reticulatum
Oedogonium crispum
Tolypothrix tenera

North and East Okoboji Lake Aug. 14
Stigonemia tenera

North and West Okoboji Lake Aug. 14
Spirogyra stitites +
Oscillatoria princeps
Cylindrotheca geminata

50,5 Kettle 3 8-19-5
Oedogonium fenzelii
“ aeroporum
“ boricenum
“ granillum
“ crispin
“ densipenna
“ piaum vea granilia
Oblongus conciliatus f. nitidus
Bolbitius tenuis
Spirogyra thunbergii
Monostroma nunnucetum
Stigonemia denticulata
Chlorella incosta
FIGURE 4
FIGURE 6
Center Lake
Chaetophora elegans †
Cladophora glomerata †
Coleochaete scutata
Cosmarium sp.
Cylindrocapsa geminella var. minor
Desmids * †
Diatoms * †
Gloeotrichia echinulata †
Hydrodictyon reticulatum †
Lyngbya aestuarii
Melosira varians *
Microcystis aeruginosa †
Microcystis aeruginosa var. major
Mougeotia genuflexa f.
Nostoc caeruleum
Oedogonium crassiviriculum idioavdrosporum f.
Oedogonium cf. thalassiothrix var. hustedtii
Oedogonium crispum f.
Oedogonium oblongum f.
Oedogonium franklinianum f.
Oedogonium capilliforme
Oedogonium coutortula f.
Oedogonium fragile f.
Oedogonium exocostatum f.
Oedogonium dictyoosporum f.
Oedogonium macrosporum f.
Oedogonium iorvense f.
Oedogonium capilliforme debaryanum f.
Oedogonium supremum f.
Oedogonium cardiacum carbinicum f.
Ophiocytium cochleare
Ophiocytium parvulum
Pandorina morum
Pediastrum sp.
Pediastrum boryanum
Pediastrum duplex
Rhizoclonium hieroglyphicum
Rhizoclonium fontanum
Rhizoclonium bombicinum
Scenedesmus sp.
Spirogyra crassa f.

Kettlehole
Bulbochaete intermedia
Bulbochaete congen.
Chaetophora incrassata
Cylindrocapsa geminella var. minor
Mougeotia nummuloides
Oedogonium hystrix
Oedogonium verrucosum
Oedogonium crispum
Oedogonium crenulata-costatum
Oedogonium borisanum
Oedogonium oblongum
Oedogonium landsbroorglin
Oedogonium aerosporum
Oedogonium borisanum
Oedogonium gracillimum
Oedogonium decipens
Oedogonium pisanum var. gracilis
Oedogonium concatenum f. hutchinsiae
Spirogyra tenuissima
Stigeoclonium absecundum
Ulothrix zonata

Emerson Bay
Cladophora glomerata †
Cocconema sp.
Coleochaete scutata
Desmids †
Diatoms * †

Gar Lake
Anabaena circinalis
Cladophora glomerata †
Lyngbya estuarii
Rhizoclonium hookeri

Big Spirit Lake
Anabaena circinalis
Cladophora glomerata †
Diatoms * †
Gloeotrchiia echinulata †
Lyngbya estuarii
Myrocytis aeruginosa †
Scytonema crispum
<table>
<thead>
<tr>
<th>Location</th>
<th>Species</th>
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<tr>
<td><strong>West Okoboji Lake</strong></td>
<td>Cylindrocapsa geminella</td>
</tr>
<tr>
<td></td>
<td>Hydrodictyon reticulatum †</td>
</tr>
<tr>
<td></td>
<td>Oedogonium pringsheimii nordstedtii f.</td>
</tr>
<tr>
<td></td>
<td>Oedogonium crispum f.</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Pithophora varia</td>
</tr>
<tr>
<td></td>
<td>Rhizoclonium hieroglyphicum</td>
</tr>
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<td></td>
<td>Spirogyra strictica f.</td>
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<tr>
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<tr>
<td></td>
<td>Tolypothrix tenuis</td>
</tr>
<tr>
<td></td>
<td>Tribonema bombycinum</td>
</tr>
<tr>
<td><strong>Lyngbya estuarii</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Oedogonium spp. †</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Rhizoclonium hieroglyphicum</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Spirogyra strictica f. var. serobiculata</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Stigeoclonium subsecundium</strong></td>
<td></td>
</tr>
<tr>
<td><strong>East Okoboji Lake</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stigeoclonium tenue</td>
</tr>
</tbody>
</table>
Appendix B.

June and July 2017 monthly averages ± one standard deviation of the mean for dissolved oxygen (DO) and temperature (at depths 3m and 5m), specific conductance, and pH for Lake Okoboji. Time-series data provided by Iowa Lakeside Laboratory.

<table>
<thead>
<tr>
<th>Month</th>
<th>DO 3m (mg/L)</th>
<th>Temp 3m (°C)</th>
<th>DO Sat 3m (%)</th>
<th>DO 5m (mg/L)</th>
<th>Temp 5m (°C)</th>
<th>DO Sat 5m (%)</th>
<th>Sp Cond (µS/cm)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 2017</td>
<td>9.31 ± 0.62</td>
<td>20.37 ± 1.83</td>
<td>102.93 ± 4.69</td>
<td>9.33 ± 0.68</td>
<td>19.86 ± 2.16</td>
<td>102.14 ± 4.91</td>
<td>456.29 ± 2.13</td>
<td>8.41 ± 0.09</td>
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</tbody>
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