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DIATOM COMMUNITY COMPOSITION FROM LOW HUMAN IMPACT AREAS IN SOUTHEAST U.S.

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ABSTRACT

Algal assemblages were evaluated at two Southeastern U.S. streams with reduced human impact, Upper Three Runs Creek and Tobbler Creek. The two sites were collected using standard protocols for stream samplings. The algal community was dominated by diatoms. Community attributes were found to be similar between the two sites, while indicator species varied as determined by Trophic Diatom Index values and the percentage of tolerant taxa to pollutants. Low pH tolerant Eunotia species were dominant in Upper Three Runs Creek while pH neutral, mesotrophic species were dominant in Tobbler Creek. This is the first report on primary producers from both sites and a taxonomic evaluation of the more common taxa is given for future reference.

Key words: Trophic Diatom Index, Shannon Biodiversity Index, diatoms, reference site, morphological characteristics

INTRODUCTION

Locations with low anthropogenic influence are becoming scarce and the opportunities to study them are diminishing. Pristine environments, or reference sites, play an integral role in assessing environmental conditions. Reference sites help determine the importance of an ecological parameter since the value of each parameter varies among different environments (1). For the purposes of environmental policy and management, reference sites are essential to understanding the natural variability of biological associations and their dependence on abiotic conditions (1). Reference sites provide a model to serve as the basis for comparison for a range of variables, including both physical-chemical and biological characteristics of the system. The use of biological characteristics, in contrast to the use of chemical and physical conditions, has become central to assessment of environmental conditions. Chemical and physical factors vary at relatively short spatial and temporal scales, making measurements of them impractical for ecosystem monitoring (2). Biological indicators, in contrast, respond to variations in the physical and chemical conditions, but at longer spatial and temporal scales.

Reference sites have been developed across the United States to use as comparison in studies; as yet, however, there are no well-established reference sites for the Southeast United States. The region has unique climate, hydrology, and soils. For example, the area is very humid due to precipitation rates greater than 120 cm/yr. and evapotranspiration rates greater than 65% (3). Late winter/early spring flooding is common throughout the area, with 71% of the annual floods occurring from January through April (4). Droughts are also common in the Southeast (5).

Algae and other aquatic organisms such as fish and macroinvertebrates are commonly used indicators in studies aimed at evaluating reference sites. Diatoms (Bacilliariophyta), in particular, are often used to assess environmental conditions. Diatoms are considered among the best biological indictor because of their importance in ecosystems as primary producers, their utility as indicators of environmental conditions, and their ease of use (2). They also are preferred over other algal groups because they are readily identified at the species and subspecies levels on the basis of distinctive morphological features. Diatoms have direct and sensitive species-specific responses to many physical, chemical, and biological changes in rivers and streams (6). Data on diatom assemblages in ecosystems with minimal human alteration can provide insights on diatom species characteristics, population and community interaction under low nutrient conditions.

Indices that use diatom communities are more useful than physical and chemical measurements to assess water quality of rivers, particularly inorganic and organic pollutants. Multiple indices have been created and are being used to assess water pollution around the world. These indices are based on the diatom assemblages and sensitivities of particular regions. However, regions vary from one another on many environmental characteristics. Variation in results have been seen when diatom indices based on one region, like the Trophic Diatom Index (7), are used on another (6, 8, and 9).

The Trophic Diatom Index (TDI), developed by Kelly and Whitton (7) for the European Framework Directive (WFD) in Europe on major rivers, serves as an example of this discrepancy. This variation in results has been attributed to species physiological responses, varying species competitive abilities, and the possibility that some taxa have one or more ecotypes that are identical or vary similar morphologically but differ in their physiological requirements (6). Variation in taxonomic identification of species also can prevent the direct use of indicators from one area to another (10 and 11).

Many indices, like the Trophic Diatom Index, use community composition, rather than diversity calculations only, to assess environmental conditions. Indices based on algal community diversity cannot be the only tool when assessing conditions based on algae. The Shannon Diversity Index shows variable relationships with pollution (2) which has been attributed to, but not limited to, different effects of pollution on evenness (10). Also, algal community composition has been seen to have a bigger impact on higher trophic levels than algal diversity (12). Diatom communities from two streams in the Southeast with current low anthropogenic impact for at least thirty years were assessed in this study with attention to community composition as well as diversity. It was hypothesized the two streams will have similar community attributes and TDI values.

MATERIALS AND METHODS

Site descriptions

Upper Three Runs Creek is located on the 310 square-mile property of the U.S. Department of Energy's Savannah River Site near Aiken, South Carolina. The area contains five reactors which were used for the production of raw nuclear materials (13). By the early 1990's, all five reactors were shut down and environmental clean-up processes were implemented; these continue today. Dense pine forest surrounds the buildings on the property. The stream had a sandy bottom with vegetation on the banks and fallen trees across it. Diatom taxonomy and community composition has been conducted; however, no indicators have been identified.

Tobbler Creek is a part of a yearlong study on aquatic environments (wetlands, pond, and creek) at Andalusia Farm located in Baldwin County, Georgia. Monthly samples from February 2011 were incorporated during data analysis (14). Flannery O'Connor, a well-known southern writer, lived on Andalusia Farm for thirteen years in the latter part of her life. The property has been on the National Register of Historic Places since 1980 and has largely been unaltered for three decades. The land has reverted back to a pine forest. Tobbler Creek had no vegetation on the banks and had a rocky bottom with various sized rocks. The algal community has not been previously assessed.

Sampling

Sampling of Upper Three Runs Creek (33.370750, -81.627738) was conducted during the month of October, 2011, following standard protocol (15). The creek is located above the nuclear reactor facilities. A composite sample of vegetation and woody debris scrapings was taken, as well as a sand sample taken with a 5.3 cm diameter petri dish. Samples were not combined in order to differentiate the communities associated with each habitat. The two samples were processed in the same manner following standard protocol (15). Dissolved oxygen, pH, temperature, nitrate nitrogen, and total phosphorus were measured from January, 2010, to December, 2010, at site U3R-1A.

Sampling of Tobbler Creek (33.121218, -83.268171) was conducted from February 2011 to March 2011 and from May 2011 to July 2011. Samples consisted of scrapings from small stones in three different locations. Tobbler Creek was subjected to drought during the July 2011 sampling, so these samples were not included in this paper. Dissolved oxygen and temperature were measured during each sampling, except for the July sample. pH data was provided by the GCSU Andalusia Research Project (Manoylov and Mutiti, personal communication). Nitrate nitrogen and total phosphorus were measured from January 2011 to March 2011. Sampling of each site consisted of collection of algae from habitats traditionally reported as preferred algal habitats.

Physicochemical characteristics

EPA Aggregate Nutrient Ecoregion's Reference Condition standards for Ecoregion XII (Alabama, Florida, Mississippi, and Georgia) for streams and rivers were used to assess nutrient levels at both sites. Standards for total phosphate and nitrate nitrogen were 0.04 mg/L and 0.90 mg/L, respectively.

Algal Community Structure

All samples were preserved within an hour of collection with formaldehyde (3% final concentration). Samples were then siphoned down to 40 mL and separated into 3 vials each; 20mL for soft algae and 10mL each for diatoms and archival samples. At least 100 natural algal units were enumerated in each soft sample to determine if diatoms were the dominant algal group. Enumeration of soft samples was performed using a Palmer-Maloney counting chamber (17). Natural counting units were defined as one unit for each colony, filament, diatom frustule (regardless if colonial or filamentous) or unicellular algae. A minimum number of algal units were enumerated in each sample because a large colony might under represent the number of algal taxa within complex photosynthetic communities if only individual cells were counted. Observations for algal identification to the lowest taxonomic level possible were conducted at 400X using a Jenalumar (AusJena) microscope equipped with differential interference contrast (DIC); images were captured with a ProgRes C3 digital camera. Taxonomy followed Wehr and Sheath (18). Collected material was archived and deposited as part of the algal collection of the Georgia College and State University Natural History Museum.

To determine diatom relative abundances, 30 mL of 70% nitric acid was added to each of the diatom samples in a beaker. Potassium dichromate and heat were added to the mixture to catalyze the digestion of the sample. Samples were let to digest for one day then rinsed daily for eight days with distilled water to remove oxidation byproducts. Processed subsamples were evaporated onto cover slips and mounted on microscope slides with Naphrax® (Brunel Microscopes Ltd., Chippenham, Wiltshire, UK) mounting medium to make permanent slides. Transects were examined under oil immersion at 1000X on a Jenalumar (AusJena) microscope equipped with DIC and ProgRes C3 digital camera. 600 valves were enumerated from each sample (14). Identification was based on Krammer and Lange-Bertalot (19, 20, 21 and 22), Patrick and Reimer (23), and Hofmann *et al*. (24). Permanent slides were archived and deposited as part of the algal collection of the GCSU Natural History Museum.

Indices

Species richness, species diversity, and species evenness were calculated for each sample. Species richness (SR) describes the number of species, or variety of forms, documented from each sample. Relative abundance of each diatom species was calculated as a proportion of the total. The Shannon Diversity Index (H') considers species richness recorded and proportion of each species out of total abundance documented (25). Maximum diversity (H'_{max}) was calculated as the natural logarithm of the documented total species richness number [H'_{max}=ln(SR)]. Species evenness (J') was calculated as a proportion of the Shannon diversity and Maximum diversity recorded ($J'=H'/H'_{max}$ 26).

Calculating TDI requires the additional calculation of the percentage of taxa present in the sample that are tolerant to organic pollution. The TDI values and the percentage of tolerant taxa infer evidence of inorganic and organic pollution, respectively, based on taxa tolerances (7). Samples with a value and percentage that are less than 20 are considered significantly free of that pollutant. Samples with a value and percentage between 21-40 are considered to have some evidence of that pollutant. A value and percentage between 41-60 indicates that the pollutant likely contributes significantly to eutrophication, and samples with numbers higher than 61 are considered heavily contaminated.

Taxonomic evaluations

Additional taxonomic evaluation was conducted on dominant species. Species were determined to be dominant if their abundance in a count was greater than 10%. Slides were scanned on a Leica DMB2000 microscope for 15 specimens of each dominant species and photographs were taken with a Leica DFC265 digital camera. Measurements of length and width were recorded along with striae density/10 µm for each specimen. The data were then averaged for each dominant species and then compared to literature (23 and 24).

RESULTS

Average temperature for the Upper Three Runs Creek was 15.27 ± 1.45°C with a range of 8.33°C to 21.60°C and for Tobbler Creek, 18.86 ± 2.70°C with a range of 11.98°C to 25.64°C (Table I). Each site showed typical seasonal change in temperature. Dissolved oxygen for Upper Three Runs Creek had an average of 6.55 ± 0.37 mg/L with Tobbler Creek having an average of 7.60 ± 1.89 mg/L (Table I). Average pH for Upper Three Runs Creek was 5.58 ± 0.13; however, pH readings for Tobbler Creek were assumed neutral based on other studies (Manoylov and Mutiti, Table I). Nitrate nitrogen for Upper Three Runs Creek ranged from 0.025 mg/L to 0.41 mg/L , average of 0.22 ± 0.04 mg/L, while Tobbler Creek ranged from 0.00 mg/L to 1.03 mg/L, average of 0.58 ± 0.30 mg/L (Table I). Upper Three Runs Creek total phosphorus ranged from 0.01 mg/L to 0.38 mg/L, average of 0.08 ± 0.03 mg/L and Tobbler Creek ranged from 0.12 mg/L to 0.69 mg/L, average of 0.36 ± 0.17 mg/L (Table I).

Table I: Characteristics of Upper Three Runs Creek and Tobbler Creek (mean ± SE, Upper Three Runs Creek: n=12, Tobbler Creek: nutrients: n=3, physical: n = 5)

	Aggregate Nutrient Ecoregion XII Reference Conditions	Upper Three Runs Creek	Tobbler Creek
Nitrate Nitrogen (mg/L)	0.90	0.22 ± 0.04	0.58 ± 0.30
Total Phosphorus (mg/L)	0.04	0.08 ± 0.03	0.36 ± 0.17
D.O. (mg/L)		6.55 ± 0.37	7.60 ± 1.89
pH		5.58 ± 0.13	7.00*
Temperature (°C)		15.27 ± 1.45	18.86 ± 2.70
*long term measurement, Andalusia Farm			

Dominant taxa
Tobbler Creek

Achnanthidium minutissimum (Kützing) Czarnecki (Plate 1: Figs. 1-11, slides – RCM0153, RCM0160, RCM0167, RCM0171, and RCM0176)

Size Range: Length, 8.5-17.5 µm - Avg. – 13.7 µm. Breadth, 2.5-3 µm - Avg. – 2.7 µm. Striae 25-31 in 10 µm. – Avg. - 27 µm

Description: Average breadth measurement of this taxon fell around the median of both Patrick and Reimer (23) and Hofmann *et al*. (24) and the average length fell around the middle of the Hofmann et al. (24) descriptions. However, using Patrick and Reimer (23) descriptions, the taxon's average length fell on the lower end of their length range. This could be attributed to the possibility that the species population at Andalusia Farm reproduces at a faster rate, resulting in smaller forms (27). Specimens had a linear-lanceolate shape with slightly drawn-out (Plate 1: Figs. 5 & 7) or slightly capitate ends (Plate 1: Figs. 4 &10). Striae are radiate with central striae interrupted by a fascia (Plate 1: Figs. 1-11). Psuedoraphe-valves had finer striae than raphe-bearing valves.

Ecology: *A. minutissimum* is a widespread taxon in the U.S., can be found within a very wide pH range of 6.5-9.0 (23). This can help explain *A. minutissimum* being the most dominant species in Tobbler Creek (abundance of 31.81 percent). *A. minutissimum* is periphytic and can tolerate small amounts of salt and is found in low nutrient to high nutrient environments (27), is a good initial colonizer on different habitats, and is present in both lotic and lentic environments with temperatures greater than 15°C (28). Average temperature for Tobbler Creek was 18.86°C.

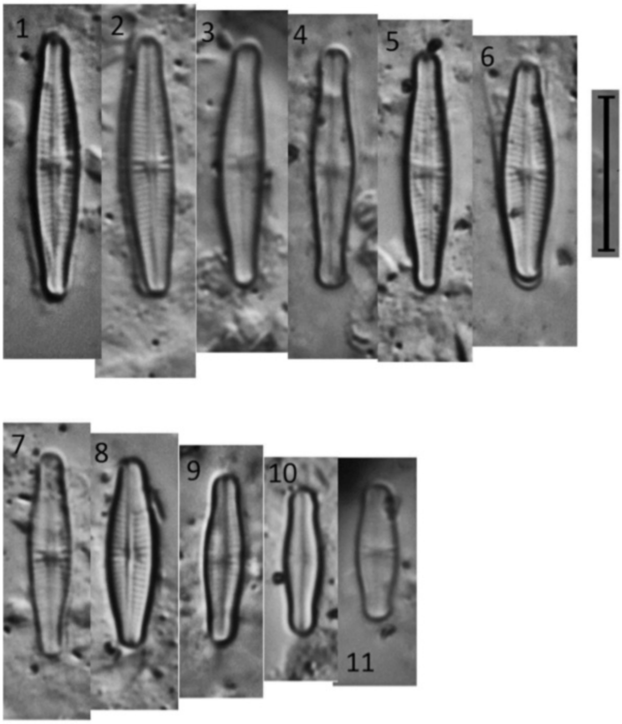

Plate 1: Figs. 1-11. LM. *A. minutissimum*. Valve views showing the size diminution series of *A. minutissimum* from Tobbler Creek. Scale bar = 10 µm.

Gomphonema parvulum **(Kützing) Kützing *sensu lato*** (Plate 2: Figs 1-11, slides - RCM0153, RCM0160, RCM0167, RCM0171, and RCM0176)

Size Range: Length, 15-28 µm - Avg. – 19.6 µm. Breadth, 3.5-5.5 µm - Avg. – 4.7 µm. Striae – 10-14 in 10 µm. – Avg. - 12.4 µm

Description: Representatives of *G. parvulum* from Tobbler Creek had length, width, and striae density as described by Hofmann *et al*. (24) and Patrick and Reimer (23), however, varying morphologies were observed as is common in *G. parvulum*. Varying morphologies can occur due to *Gomphonema* species displaying Janus cells (29). Janus cells are cells that have noticeably different valves that are so morphological different, they are often mistakenly considered separate varieties, species, or even genera. Within the project, *G. parvulum* represented a species complex (19) for analytical purposes. Three groups were formed based on shape; Morph 1 – Plate 2: Figs. 1-5, 7, 8, 10, and 11, Morph 2 – Plate 2: Fig. 9, and Morph 3 – Plate 3: Fig. 6. Morph 1 consisted of forms with a clavate-lanceolate shape with narrow, capitate-rostrate ends. Morph 2 forms had the same clavate-lanceolate shape as Morph 1 forms but more rounded, rostrate ends. Morph 3 had an elliptical shape with more distinct capitate-rostrate ends. All groups had striae that converged towards the center and become more perpendicular near apices. Single median striae were observed on both sides of the raphe, with one shortened and the opposite of regular length ending with an isolated punctum.

Morph 1 (Plate 2: Figs. 1-5, 8, 10, and 11) specimen fell within *Gomphonema parvulum* while Morph 2 (Plate 2: Fig. 9) fell within the bound of *Gomphonema aquamineralis* Krammer descriptions (29). No similar forms or descriptions were found in NAWQA (2001) for Morph 3 (Plate 2: Fig. 6), so *Gomphonema parvulum* variety 1 was given to it. For this project, morphological differences were recognized, but formal descriptions of the morphs were not given so we used *sensu lato*.

Ecology: Periphytic, generally found in lotic environments with an optimum range for pH of 7.8-8.8, but has been found in ranges of 4.2-9.0. Can tolerate small amounts of salt and can be found in moderately polluted environments (considered an indicator species), particularly waters containing sanitary or farm wastes (23).

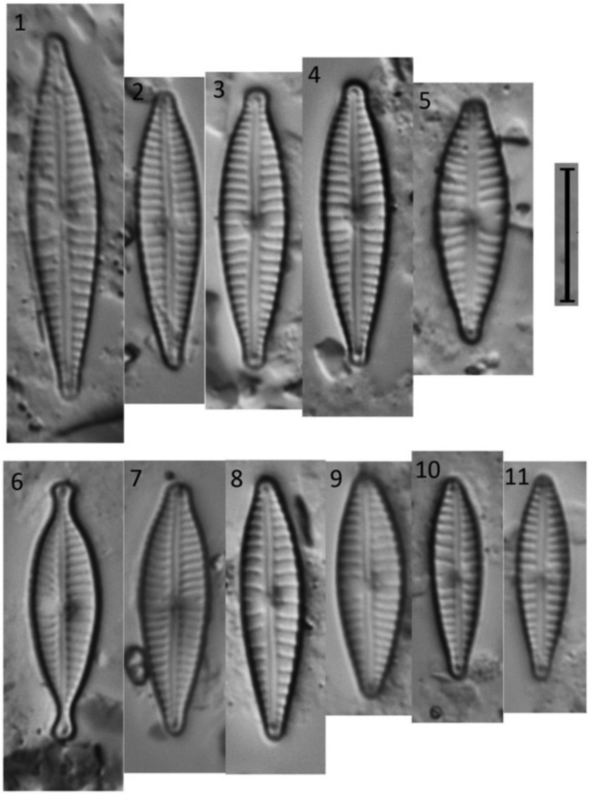

Plate 2: Figs 1-11. LM. *G. parvulum*. Valve views showing the size diminution series of *G. parvulum* from Tobbler Creek. Scale bar = 10 µm.

Upper Three Runs Creek

Eunotia sp. 2 (Plate 3: Figs. 1-8, slides – RCM0216 and RCM0217)

Size Range: Length, 12.5-20.5 µm - Avg. – 18 µm. Breadth, 3.5-4.5 µm - Avg. – 4 µm. Striae – 12-18 in 10 µm - Avg. – 15.1 µm

Description: Terminal nodules were distinct and near the apices of the valve. Striae were fine and parallel. Valves were elongate, dorsal side was slightly bent or convex with ventral side straight or slightly concave (Plate 3: Figs. 1-8). Some specimens fell within the length, width, and striae density of *Eunotia siolii* Hustedt and resembled valve view (Plate 3: Figs. 1-8) but some specimens were measured below width and length of *E. siolii* and had finer striae. Valve view also resembled *Eunotia rhomboidea* Hustedt (Plate 3: Figs. 9 & 10) but *E. rhomboidea* has coarser striae and rhomboidal girdle view (Plate 3: Fig. 11) which eliminated it as a description. No taxa were found in literature that had similar morphology, length, width, and striae density.

Ecology: Species are able to withstand slightly acidic water.

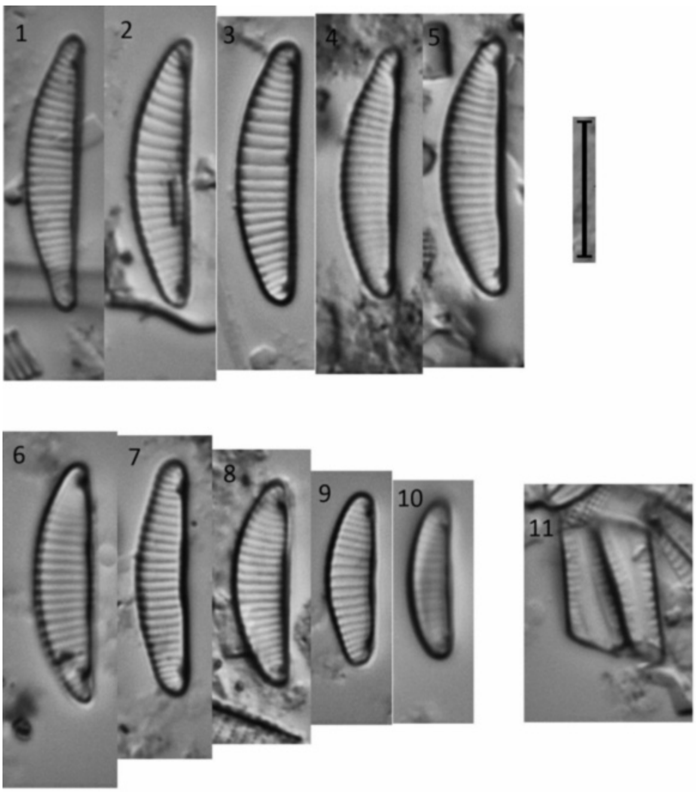

Plate 3: Figs. 1-11. LM. *Eunotia sp. 2* and *E. rhomboidea*. Figs. 1-8: Valve views showing the size diminution series of *Eunotia sp. 2* from Upper Three Runs Creek. Figs. 9 & 10: Valve views of *E. rhomboidea* from Upper Three Runs Creek. Fig. 11: Girdle view of *E. rhomboidea*. Scale bar = 10 µm.

***Eunotia carolina* Patrick** (Plate 4: Figs. 1-10, slides – RCM0216 and RCM0217)

Size Range: Length, 28-52 µm - Avg. – 37.9 µm. Breadth, 6-8 µm - Avg. – 7 µm. Striae – 11-14 in 10 µm – Avg. – 12.2 µm

Description: Patrick's (32) type locality for this species is near the mouth of Upper Three Runs Creek, which is where these samples were taken. Populations had a straight ventral margin of the valve with the dorsal valve being convex at the center with narrowing at the ends. Striae were parallel and became coarser and slightly radiate towards the end. Terminal nodules of species were distinct (Plate 4: Figs. 1-10). Length of species is 28-52µm, breadth is 6-8µm. Center striae are 11-14 per 10µm. Morphology of the population fits into the Patrick (32) description of *E. carolina*. This species shares similar characteristics with *E. pectinalis* and *E. sudetica*, but more narrowly protracted ends separate it (32).

Ecology: Distribution of this species is only known from its type locality in streams, with an exception with Camburn and Charles (33) that is a publication on lakes and we did not incorporate it since this study is on creeks, so all ecological data for this species can be taken from Upper Three Runs Creek's environment. Patrick (32) describes the water from Upper Three Runs Creek as dystrophic which might not be accurate anymore since the water was low in nutrients but was slightly acidic (Table I).

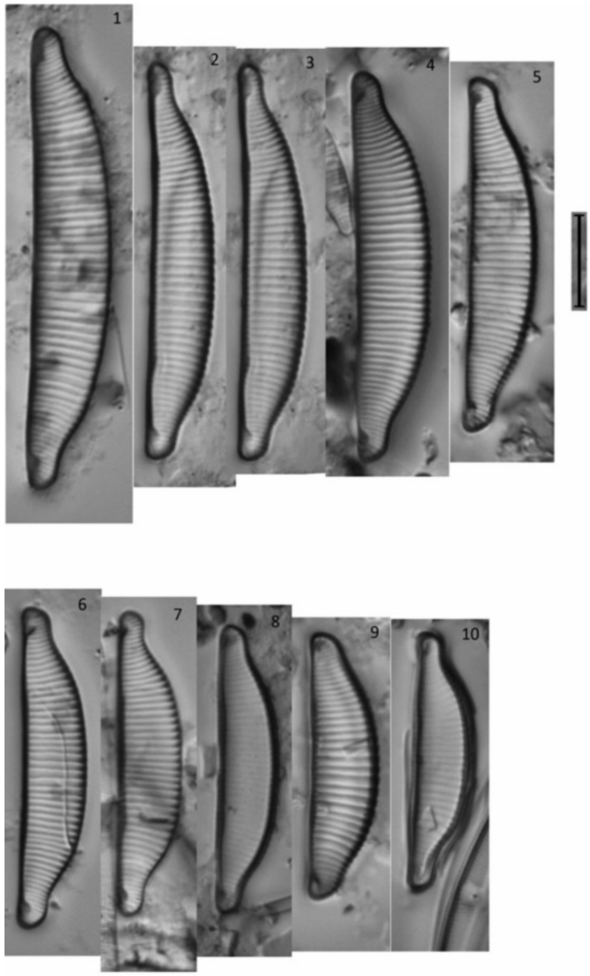

Plate 4: Figs. 1-10. LM. *E. carolina*. Valve views showing the size diminution series of *E. carolina* from Upper Three Runs Creek. Scale bar = 10 µm.

***Luticola goeppertiana* (Bleisch) D.G. Mann** (Plate 5: Figs 1-10, slides - RCM0216 and RCM0217)

Size Range: Length, 20-33 µm - Avg. – 26.1 µm. Breadth, 6-8 µm - Avg. – 7.0 µm. Striae – 17-20 in 10 µm – Avg. – 18.1 µm

Description: Valves are lanceolate with rounded ends. Striae are punctate and radiate, and central area is expanded with a distinct stigma. Central raphe ends are slightly deflected away from the stigma.

Ecology: Species are able to withstand slightly acidic conditions and are often aerophilic. They can be found in fresh, brackish, and alkaline water (23).

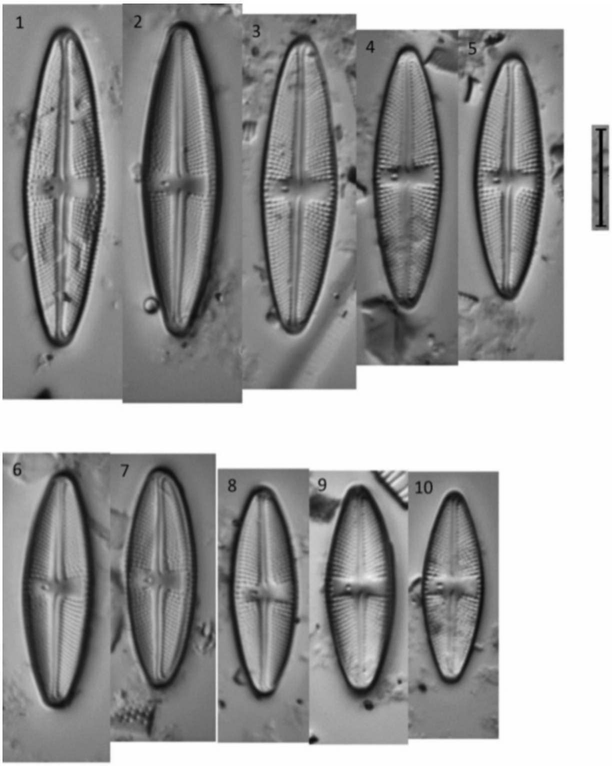

Plate 5: Figs 1-10. LM. *L. goeppertiana*. Valve views showing the size diminution series of *L. goeppertiana* from Upper Three Runs Creek. Scale bar = 10 µm.

Tabellaria flocculosa **(Roth) Kützing** (Plate 6: Figs. 1-10, slides – RCM0216 and RCM0217)

Size Range: Length, 30-72 µm - Avg. – 40.8 µm. Breadth, 5-7 µm - Avg. – 6.2 µm. Striae – 13-18 in 10 µm – Avg. – 15.7

Description: Sizes of *T. flocculosa* from Upper Three Runs Creek fell within the size ranges provided by both Patrick and Reimer (23) and Hofmann *et al*. (24). Patrick and Reimer (23) distinguish this species by the median swelling usually being greater than apical swelling and its often asymmetrical appearance. The Upper Three Runs Creek population shows a swollen central area wider than the swollen spices and taxa are slightly asymmetric (Plate 6: Figs. 1-10). Figures 2-5, 7 and 10 (Plate 6) have parallel striae that are regularly spaced while figures 1, 6, 8 and 9 (Plate 6) have irregularly spaced striae facing in different directions.

Koppen's (31) classifications of the varieties of *T. flocculosa* exposed divergences in grouping the SRS population. The differences were based on inflation widths of the median and apices area and on the expansion of the pseudoraphe in the center. SRS specimens' median inflation is wider than their apical inflation and striae continue in the central area. Koppen descriptions imply that specimens with equal inflation widths do not have widening at the central area (*T. flocculosa* var. *linearis*) and specimens with greater median inflations do have widening at the central area (*T. flocculosa* III, IIIp, and IV).

Wisconsin's Department of Natural Resources attempted to improve Koppen (31) descriptions of *Tabellaria flocculosa* (Roth) Kützing. WDNR modified descriptions separates the SRS population into two of Koppen's groups – *T. flocculosa* III and *T. flocculosa* III or IIIp. Figures 6-10 fall within *T. flocculosa* III which is described as having gradual widening of the median inflation with no distinctly capitate ends and lengths of less than 37µ. Figures 1-5 fall within T. flocculosa III or IIIp which has the same shape description as above but have lengths between 37µm and less than 83µm. Figures 1, 4, 5, and 10 have widths greater than 6.5 which gives the only discrepancy that each of these descriptions have widths are less than 6.5µ. For the purpose of this paper, we recognize morphological differences, but we do not have enough data to address it so we used one name *sensu lata*. These variations in the classification of *T. flocculosa* are beyond the scope of this paper, but are presented to put perspective on identification discrepancies.

Ecology: *T. flocculosa* has a wide tolerance for different types of waters (23). Specimens with shorter frustules are more often found in the acidic water of bogs and ponds, while specimens with longer frustules are more likely to be found in oligotrophic to mesotrophic water. The average pH for Upper Three Runs Creek is 5.5 which, when using Patrick and Reimer (23) ecological description and Hofmann *et al*. (24) size description, can be expected since the average length is on the lower end of the size range. *T. flocculosa* is generally found in standing water systems but can be found in flowing systems. It has a pH range of 6.6-8.0 but is considered alkaliphilous (occurs around 7, best development over 7), and can be found in non-polluted to moderately polluted environments.

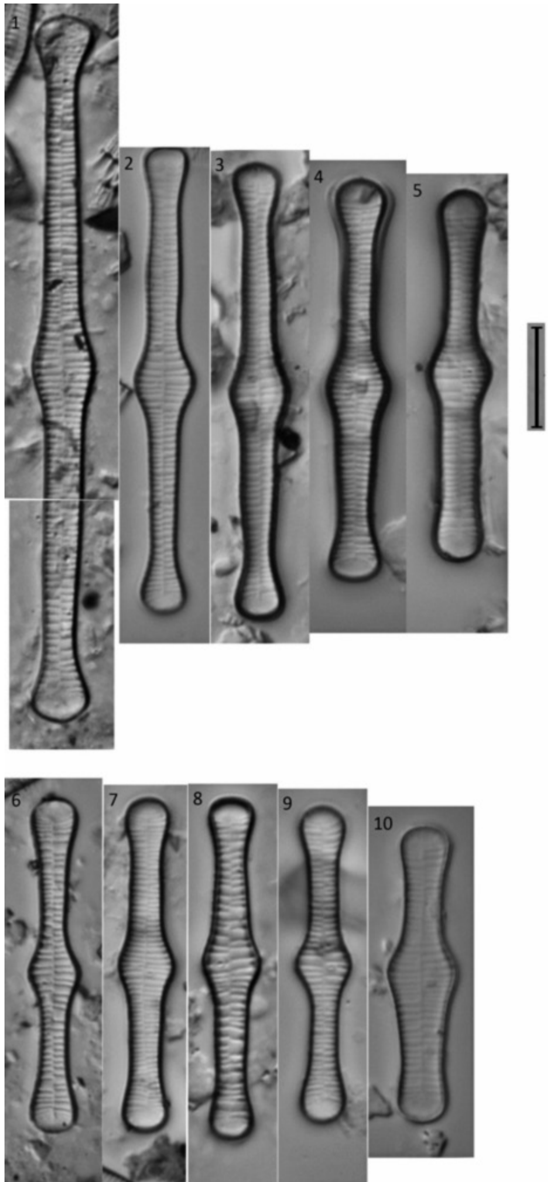

Plate 6: Figs. 1-10. LM. *T. flocculosa*. Valve views showing the size diminution series of *T. flocculosa* from Upper Three Runs Creek. Scale bar = 10 µm.

Gomphonema parvulum* (Kützing) Kützing *sensu stricto (Plate 7: Figs. 1-11, slides – RCM0216 and RCM0217)

Size Range: Length, 14-24.5 µm - Avg. – 18.2 µm. Breadth, 4.5-6 µm - Avg. – 4.8 µm. Striae – 13-17 in 10 µm. – Avg. – 15.7

Description: Specimens of *G. parvulum* from Upper Three Runs Creek are of average size when using Hofmann *et al.* (24) descriptions. Population specimen had a clavate-lanceolate shape with narrow, capitate-rostrate ends. Striae were evenly spaced and convergent towards the center while perpendicular to raphe at near apices. Single median striae is found on each side of the central zone with one shortened and the opposite of regular length with isolated punctum (Plate 7: Figs. 1-11). Striae density for Upper Three Runs Creek specimen (Plate 7: Figs. 1-11) was finer than Tobbler Creek specimen (Plate 2: Figs. 1-11). Length and breadth were relatively the same at each site for *G. parvulum*, but morphologies were different. However, based on morphology, Upper Three Runs Creek specimen would fall into Morph 1 (Plate 2, Figs. 1-5, 8, 10, 11) of Tobbler Creek so we gave it the name *Gomphonema parvulum sensu stricto*.

Ecology: Periphytic, generally found in lotic environments with an optimum range for pH of 7.8-8.8, but has been found in ranges of 4.2-9.0. Can tolerate small amounts of salt and can be found in moderately polluted environments (considered an indicator species).

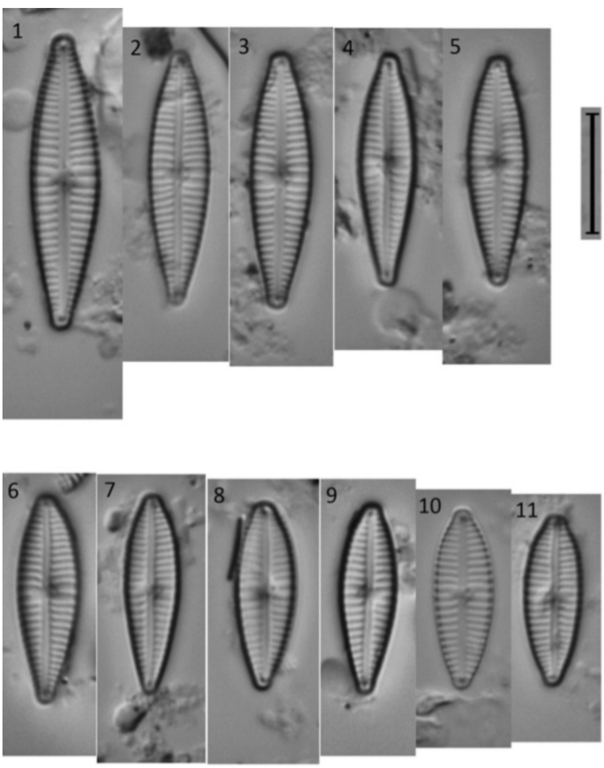

Plate 7: Figs. 1-11. LM. *G. parvulum*. Valve views showing the size diminution series of *G. parvulum* from Upper Three Runs Creek. Scale bar = 10 µm.

Species information summary

Both sites were dominated by pennate diatoms (Table II). Upper Three Runs Creek was dominated by Eunotia species while Nitzschia was the most abundant genus in Tobbler Creek. *Eunotia* sp. 2 and *Tabellaria flocculosa* had the highest abundance in the Upper Three Runs Creek, while *Achnanthidium minutissimum* had the highest abundance in Tobbler Creek (Table II). *Gomphonema parvulum* was the only dominate taxa found in both sites (Table II).

Table II: Relative abundances of taxa from each sample (x – 5-10%, xx - <5%,) (Species must be >10% at least in one sample)

	Upper 3 Runs Creek		**Tobbler Creek**				
Taxa	**Veg. & Sand Scraping**		**2/18/11**	**3/29/11**	**5/9/11**	**6/3/11**	**7/21/11**
Achnanthidium minutissimum (Kützing) Czarnecki			0.21	0.12	0.25	0.22	0.32
Eunotia sp. 2	0.33	0.28					
Eunotia carolina Patrick	xx	0.15					
Gomphonema parvulum (Kützing) Kützing *sensu stricto*	0.14	xx					
Gomphonema parvulum (Kützing) Kützing *sensu lata*			0.24	0.19	0.14	0.17	0.14
Luticola goeppertiana (Bleisch) Mann	0.13	xx					
Nitzschia palea var. debilis (Kützing) Grunow				x	0.12	0.10	xx
Tabellaria flocculosa (Roth) Kützing	0.15	0.28					

Maximum diversities for Upper Three Runs Creek and Tobbler Creek were all high; however, their actually diversity values for each sample were low (Table III). The highest value (3.32) and closest to its maximum diversity (3.74) was the August sample from Tobbler Creek which had an evenness of 0.86 (Table III).

Table III: Community attributes assessed for two Southeast streams [Species Richness (SR), Shannon index (H'), maximum diversity (H'max), and evenness (J')]

	Upper 3 Runs Creek		Tobbler Creek				
	Veg. & Sand Scraping		2/18/11	3/29/11	5/9/11	6/3/11	7/21/11
SR	23	27	24	32	33	32	33
H'	2.23	2.20	2.26	2.89	2.65	2.73	2.68
H'_{max}	3.14	3.30	3.18	3.47	3.50	3.47	3.50
J'	0.71	0.67	0.71	0.83	0.76	0.79	0.77

All samples' TDI values and their percentages of tolerant taxa were all above 21, except for the Upper Three Runs Creek's sand sample (Table IV). Trophic diatom index values were lower in Upper Three Runs Creek than in Tobbler Creek, as well as the proportion of taxa tolerant to organic pollution (Table IV). Both samples were under the proportion of taxa (40%) where organic pollution is likely to contribute significantly to eutrophication; however, Tobbler Creek's proportions were all above 41% (Table IV). TDI values and percentage of tolerant taxa that are below 20 indicate a site of being free of inorganic pollutants while values above 21 indicate increasing levels of inorganic pollutants (7). Upper Three Runs Creek sand sample was the only sample to be below 20 with a value of 13.76 (Table IV). The vegetation and scraping sample from Upper Three Runs Creek had a low TDI value (37.62) in relation to all the Tobbler Creek samples, which were all above 50 (Table IV).

Table IV: Each samples Trophic Diatom Index value and proportion of taxa tolerant to organic pollutions

	TDI	Percentage of Taxa Tolerant to Organic Pollution
Upper 3 Runs Creek		
Scrapings	37.62	21
Sand	13.76	6
Tobbler Creek		
2/18/2011	50.88	30
3/29/2011	52.78	40
5/9/2011	52.80	47
6/3/2011	58.20	51
7/21/2011	51.34	41
8/29/2011	51.09	44

DISCUSSION

Only some chemical measurements corresponded with the expectation that both streams are pristine due low human impacts for several decades. Nutrients were mostly below EPA standard for Upper Three Runs Creek, but phosphorous was double the amount considered low for the region. Nitrate nitrogen was within margin of error similar to the EPA limitation at Tobbler Creek but phosphorus was orders of magnitude higher, potentially due to leaching from sediments.

Specie richness, Shannon index values, maximum diversity index, and evenness, for each site were relatively similar. Diatom species have varying levels of sensitivity to environmental factors. Assessing water quality based on diatom biodiversity is not sufficient for understanding of a stream's integrity. The Trophic Diatom Index makes inferences on the level of inorganic pollution in a stream, and calculating the proportion of taxa tolerant to organic pollution. Taxa used to calculate proportions were *Gomphonema parvulum, Navicula gregaria, Navicula lanceolata*, small *Navicula* (<12µm) and *Sellaphora* spp., and *Nitzschia* spp. as given by Kelly and Whitton (7). Only the diatom association collected from the sand habitat at the Upper Three Runs Creek had TDI value of less than 20 with a very small proportion of taxa tolerant to organic pollution. The rest of the TDI values were higher and classified sites with moderate to high pollution as indicated by proportion of taxa tolerant to organic pollution. Upper Three Runs Creek and Tobbler Creek had significantly different values for both of the pollution indicating indices while having similar biodiversity values. Since TDI values are based on qualitative data rather than quantitative data, the difference between the two sites is community composition. The community composition variation is primarily due to different acidity levels of the two streams. Upper Three Runs Creek was more acidic compared to the neutral Tobbler Creek.

Most variation in the results of indices can be traced back to misidentification of a species (11). Tobbler Creek and Upper Three Runs Creek differed in diatom composition with *G. parvulum* (*sensu lato*) considered to be the only common dominant species. Documented high morphological diversity in the diatom, especially in representatives of *Eunotia* and *Gomphonema* requires further investigation that beyond the scope of this study. Similar to the *Gomphonema parvulum* community in Tobbler Creek, Morales (34) found a need to use *G. parvulum sensu lato* for populations from the Savannah River. In his study large percent *Gomphonema* species was reported as new to science. The Southeastern U.S. remain largely unexplored in terms of algal biodiversity. Also, taxonomic descriptions are reported and many taxa required consideration of current taxonomy, both can be challenging and time consuming. Taxa like *Tabellaria flocculosa* and representatives of the genus *Eunotia* from Upper Three Runs Creek were morphological very diverse and need more taxonomic work, while population of *Gomphonema parvulum* from the same location presented a close fit to *G. parvulum sensu stricto* (19 and 34).

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