2016 Study of Golden Brown Algae on the Bottom of Torch Lake, Lake Bellaire, and Clam Lake

R. Jan Stevenson, Professor

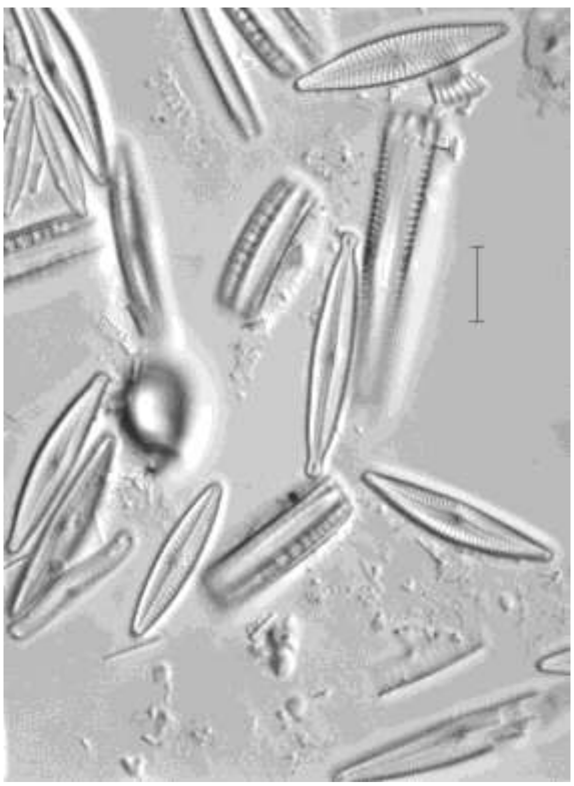
Department of Integrative Biology and

Center for Water Sciences

Michigan State University

East Lansing, MI 48824

June 20, 2017



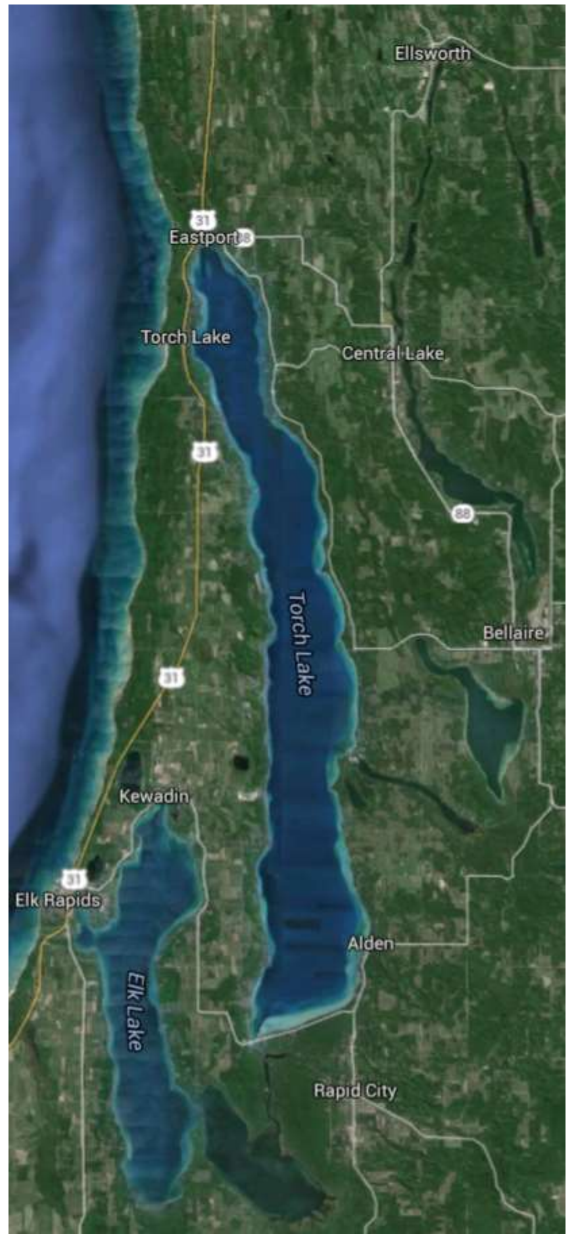


Image Credits: Stevenson's image of common benthic diatoms in Torch lake (scale bar = 10 µm). Map of Torch Lake, Lake Bellaire, Clam Lake and surrounding watershed by Google®.

Summary

Golden brown algae were studied for a second year on the bottom of Torch, Bellaire, and Clam Lakes during the 2016 summer. The goals of the 2016 summer study were to determine if the apparent amount of golden brown algae was related to season, numbers of algal cells in the sand, species of algae in the sand, or nutrients in the surface water or groundwater. Volunteers for the Three Lakes Association sampled four locations in Torch Lake, two in Lake Bellaire, and one in Clam Lake. They also sampled water chemistry and the algae in sand at each of these locations once per month during June, July, August, and September. Water chemistry samples were analyzed by the University of Michigan Biological Station. Algal samples were analyzed by Michigan State University.

Analyses of these data showed that the darkness and thickness of golden brown algae, which were quantified with a scuzziness score by volunteers during sampling, was greater during July and August than June and September.

The number of algae in sand was not related well to the scuzziness score for golden brown algae on sand. Densities of algae in sand were almost always high when scuzziness score was high, but densities of algae in sand could be high without high scuzziness score. Densities of algae can be high in sand with algae living among and on the sand grains. Scuzziness, or the appearance of the golden brown algae on top of the sand, is likely due to a change in where the algae grow in relationship to the sand.

Species composition was related to scuzziness during the summer of 2016, even though it was not during the summer of 2015. This may be due to the larger number of samples taken during early and mid-summer periods and the corresponding accumulation of algae during the summer and appearance of golden brown algae on sand during the 2016 summer study. Diatoms were much more abundant in sand than cyanobacteria or green algae. Diatoms were responsible for the golden brown color of algae on sand.

During the accumulation of algae and development of darker and thicker growths of diatoms on the surface of the sand from June to August, we observed a shift from diatom species that required relatively high nutrients to species that can survive and grow in low nutrient concentrations. Based on what we know about these species, the most likely nutrient limiting which species could occur later in the summer was phosphorus.

The development of scuzziness from June to July and August corresponded to an increase in phosphate concentrations in the water, but scuzziness was not related well to either phosphate or inorganic forms of nitrogen that diatoms use to grow. Indeed, as the phosphate in the water column increased from June to August, the shift from high to low phosphorus diatoms indicated phosphorus supply was actually decreasing for algae on the sands. This apparent contradiction can be explained by nutrient dynamics within algal mats. As density of algae on bottoms of aquatic habitats increases, the dissolved nutrient concentrations within mats can decrease because nutrient uptake within mats can exceed the supply from the water column, or potentially groundwater, which has to be delivered by diffusion or mixing.

I conclude, therefore, we have little evidence for nutrient pollution from local sources causing the recent appearance of golden brown algae in Torch Lake and Lake Bellaire. Different factors likely regulate golden brown algae in Clam Lake than the other two lakes. Nutrient pollution from septic tanks or agriculture remains a possibility for all these lakes, but little evidence from studies during summer 2015

and 2016 show relationships between scuzziness or algal species composition with changes in surface water or groundwater chemistry among sites. However, long-term records from the Tip of the Mitt Watershed Council hint that phosphorus concentrations are actually decreasing in Torch Lake, and maybe other lakes of northern Michigan. This decrease in P lake concentration may be related to atmospheric deposition of nitrogen increasing phosphorus use earlier in the spring-summer growing season for algae. Increased phosphorus limitation as a result of nitrogen deposition may be causing a shift in species composition from June to August and a shift from algae growing in the sand to algae growing on the sand surface, and thereby being more apparent as the coating of golden brown algae on sand. This presents a new hypothesis for why golden brown algae are developing in Torch Lake and Lake Bellaire. More research is needed to test this hypothesis, as well as others to determine whether golden brown algae can be managed.

Introduction

Many residents around Torch, Clam, and Bellaire Lakes are concerned about greater accumulation of golden brown algae (GBA) on the bottom of the lakes now than in the past. The accumulation has been reported to occur during summers and to be a relatively recent phenomenon. A research project on the ecology and cause of the GBA was started during summer 2015. That project was planned based on the observations that GBA accumulation is characteristically golden brown and seems to be accumulating on top of the sand bottom. Accumulations seem to be observed more in shallow than deep water, but they do extend to deeper water later in the summer. Basic algal ecology was reviewed with Three Lakes Association Water Quality Team to develop a set of alternative hypotheses, to select the most likely hypotheses, and then to test as many as possible starting in summer 2015.

Many alternative hypotheses were considered for GBA accumulating in recent years, which was assumed to be related to a change in the lakes during that time. One or more of these hypotheses could explain the recent accumulation of GBA.

1. Recent invasion of zebra and related mussels filtered particles in the water column, increased light to the lake bottoms, and stimulated growth of GBA.
2. Changes in light conditions caused by factors other than Dreissenid mussels stimulated growth of GBA.
3. Runoff from big storms in recent years has carried more nutrients to the lakes.
4. Disease killed herbivores of algae, and has allowed greater accumulation of GBA.
5. Non-native algal species invaded the lakes.
6. Climate change and warmer lake conditions changed species composition of algae, or other factors affecting algae, and caused GBA.
7. Groundwater contamination by septic tanks and agriculture increased nutrient supply to the lakes and stimulated GBA.
8. Public sensitivity to algal problems may have increased related to more publicity about algal problems in recent times, so observation and concern about GBA increased recently even though GBA has not changed.

Which of these factors, or other factors that directly or indirectly affect benthic algae, could have changed in the lakes to cause GBA development?

For a variety of reasons, which were reviewed in the report last year (Stevenson 2016), the 2015 study was designed to survey algal species composition and abundance, surface and ground water chemistry, and other habitat factors. These data helped us determine what the GBA was and whether it was related to any of the measured environmental factors. Plus, these data allowed us to start testing some of the hypotheses above.

Groundwater and surface water can be sources of current and past nutrient contamination to lakes, streams, and wetlands. Changes in algae on the bottom of lakes, at the interface of the groundwater and surface water, may be an early warning indicator of lake contamination by groundwater and the cause of the GBA that is affecting aesthetics.

In 2015 we found groundwater had higher phosphorus concentrations than surface water, but groundwater had lower nitrogen concentrations than surface water. Seasonal changes in groundwater were not observed. Little spatial difference was observed in groundwater chemistry, except for high ammonia concentrations at one site. Phosphorus and nitrogen concentrations in groundwater and surface water are likely sufficiently low that they independently and interactively control algal growth, but phosphorus was in relatively lower supply compared to nitrogen and thus probably limited algal growth more than nitrogen.

Cell densities of benthic algae in Torch Lake in 2015 were similar in areas of sand that have a visual coating of GBA and in areas with little visual coating of GBA. Species composition of benthic algae was dominated by diatoms. Diatom species composition did not differ among samples from dense GBA sand, sparse GBA sand, and rock habitats. Thus we hypothesized the appearance of GBA was likely due to a change in location of algae from below the top surface of the sand and closely attached to sand grains to a matrix of algae on top of the sand surface.

Diatom metrics used in ecological assessment of nutrient conditions were related to shifts in species composition among samples, but were not related to cell densities of algae in and on sand or depth of water, which is an indicator of distance from shore and the potential sources of groundwater contamination.

Limited areas of sampling, small numbers of samples, seasonal variation in the development of GBA, and extensive areas of dense GBA during late summer 2015 were the likely reasons for uncertainty in relationships among visually apparent algal density, measured algal density, diatom metrics of nutrients, and water chemistry. So for 2016, TLA decided to continue to address the relationships among visual GBA appearance, benthic algal abundance and species composition, and water chemistry. In addition, observations in 2015 supported the idea that GBA development has a strong seasonality. So sampling in 2016 was conducted during June, July, August and September at 7 locations in three lakes to evaluate seasonal changes in visual GBA appearance, benthic algal abundance and species composition, and water chemistry. Then I used these data from TLA to address the following hypotheses in the 2016 study:

1. Thickness and darkness of GBA color increase with algal abundance (cells/cm^2).
2. Thickness and darkness of GBA color vary with algal species composition.
3. Thickness and darkness of GBA color increase with diatom indicators of nutrient availability.
4. Thickness and darkness of GBA color, algal density and algal species composition vary with ground and surface water chemistry.

In addition, samples of algae from deep in sands were collected with the intent to characterize historic conditions in the lake. Sediments in lakes record a history of lake conditions in the chemistry and remains of organisms that are deposited, layer by layer and year after year. This study of historic conditions in lakes is called paleolimnology. Paleolimnological studies are usually done with sediments from the deepest parts of lakes, but that sampling was impractical. So a modified approach was used by collecting deep sand samples at the sampling locations for golden brown algae on the surface of sands.

Methods

Sampling and Sample Analysis

Algal and water chemistry samples were collected at 7 locations in three lakes on 4 dates. Four locations were in Torch Lake. Two locations were in Lake Bellaire. One location was in Clam Lake. Samples were collected in June, July, August, and September. When samples were collected, observations were taken to evaluate scuzziness, which was a visual assessment of the amount of GBA based on its thickness and the darkness of the golden brown color. The TLA water quality team developed this index of the amount of GBA. Scuzziness scores varied from 0-10, with 10 being the highest amount and dark color of GBA. Visual assessments of algal biomass can be very valuable for relating algal abundance and nuisance algal type to nutrient concentrations (Stevenson et al. 2006, 2012).

Water chemistry samples were collected from surface water, groundwater, and pore water in the sediment surface. Water chemistry was analyzed by Tim Veverica at The University of Michigan Biological Station. Veverica has reported on the seasonal changes in water chemistry patterns in oral and graphical reports. In this report, I will focus on the nutrient chemistry in the surface water and groundwater and its relationship to visual assessments of GBA thickness and darkness color, benthic algal cell abundance (cells/cm^2), and benthic algal species composition. Benthic refers to bottoms of aquatic habitats. So benthic algae live on the bottom of aquatic habitats, in contrast to phytoplankton or planktonic algae that live suspended in the water column. Soluble reactive phosphorus (SRP or phosphate), nitrate (NO_3), nitrite (NO_2), and ammonia (NH_4) concentrations were measured by Veverica. I calculated NO_x and dissolved inorganic nitrogen (DIN) concentration as:

$$NO_x = NO_3+NO_2 \text{ and}$$

$$DIN = NO_x+NH_4$$

NO_x and DIN characterize bioavailable nitrogen two ways, which is better than one way because uptake mechanisms for NO_x and NH_4 are different.

Algae were sampled from sand if sand habitats were available, otherwise algae on rocks were sampled. Algae in surface sand was collected by inserting a petri dish lid into the sand and capturing the sand-algal sample in the petri dish by sliding a spatula under the petri dish. This captures about a 1 cm deep core sample of sand and algae. Three petri dish samples were combined to make one benthic algal sample from sand per site per month. The area of sand sample collected was determined by the diameter of the petri dishes. Algae were scraped from rocks and the area sampled was measured for each rock. Algal samples were frozen and sent to Michigan State University (MSU).

Sands from deeper in sand were collected by shovel and scoop and frozen. One sample of algae from deep sand was collected per site per month.

Due to budgetary constraints, only half of the surface algal samples and most deep diatom samples were counted by Dr. Bo Liu at MSU. At MSU, algae were removed from the sand using a swirl and pour technique (Stevenson and Stoermer 1981). Algae for each sample were shaken rigorously to reduce clumping and then were mounted in Taft's syrup medium on microscope slides (Stevenson 1984). Using Taft's syrup medium enables observation of samples under oil immersion at 1000X, identification of diatoms, and determination of whether diatoms are live or dead by whether they have protoplasm in their glass cell walls or not. All these things are not possible in water or NAPHRAX mounts as used last year when we were learning the taxonomy of common diatoms in the lake, thereby justifying acid cleaning and mounting diatoms in NAPHRAX (a special resin medium) and assessing non-diatom algae in water mounts using a Palmer-Maloney counting chamber.

We did preliminary counts to determine counting protocols that would likely provide sufficient information to determine cell abundances and species composition of algae in samples with syrup slides. The protocol used was to count and identify 150 live diatom cells and all dead diatom valves (half a diatom cell wall) and all non-diatom cells encountered until 150 live diatom cells were identified and counted. We then used those protocols and made two "counts" from each sample so we could assess variability in density and species composition that could be attributed to counting. This also increased our sample size from 14 to 28 samples. For data analysis for this report, we did not distinguish between live and dead diatoms, but that capability exists in the data for future analyses of the data.

Diatoms in deep sediments were removed from sand by the swirl and pour technique (Stevenson and Stoermer 1981). Diatoms were then acid cleaned and mounted in NAPHRAX for identification and counting. Standard 600 valve counts of diatoms were made for almost all of the deep sediment counts. We used NAPHRAX for deep sediment samples because we assumed remains of non-diatom algae would be rare and all diatoms would be dead, and NAPHRAX provides higher detail of diatom cell walls for taxonomic identification.

Data Analysis

The sampling plan enabled analysis of our four hypotheses both indirectly and directly. By indirectly, I mean that we have the opportunity with the sampling design to determine whether scuzziness, algal density, and species composition varied with factors related to the sampling design, versus directly with the hypotheses that needed to be tested. The sampling design with 7 sites and 4 dates enabled testing effects of site, time of summer on GBA, and lake on water chemistry, scuzziness, algal abundance, and algal species composition. The value of the indirect approach is that we learn the ecology of the algae that may help us address the key hypotheses or develop new ones. So analysis of variance (ANOVA) tests were calculated to determine the probability that differences among sites or dates (on scuzziness, algal abundance, and algal species composition) could occur by chance. One-way ANOVA were run because the number of replicates was too low to evaluate interactions between site and date with a two-way ANOVA.

The direct approach for testing the four key hypotheses ignored the sampling design, which was however, intended to provide a range of conditions within and among lakes and among dates among which water chemistry, scuzziness, algal abundance, and algal species composition varied. Thus, we tested the relationships among water chemistry, scuzziness, algal abundance, and algal species composition by using regression analyses.

Standard figures were used to illustrate patterns of data and relationships to site and month. These figures had three panels. Panel A showed all data plotted independently. When there was only one data point per site and all four months had data, lines were used to connect data points for the same month across all sites to show both seasonal and spatial differences. This was the case for scuzziness score and water chemistry. No lines were used to connect data of different months for algal density and diatom metrics when two data points were plotted for the two counts from each sample for June and August. Panels B and C have box plots. Boxes are drawn to indicate the 25th and 75th percentiles of observations for that condition~~s~~ (site or month), with the median observation indicated by the solid line crossing the box. The so-called whiskers of the box indicate the range of data outside the boxes, excluding points considered statistical outliers. Statistical outliers are indicated by dots. Months of samples are indicated by colors of the symbols and lines. To make reading the report easier, and to provide as much data in graphical form as possible, I have placed the figures at the end of the text.

In these data analyses, water chemistry was SRP, NO_x, and DIN concentrations. Scuzziness was the scuzziness index for GBA developed by the TLA water quality team. Algal abundance was measured using cell abundances (cells/cm^2), which was determined by knowing; 1) the area of sand or rock sampled; 2) the volume of the algal sample; 3) the proportion of sample put on the microscope slide; and 4) the proportion of sample on the slide identified and counted (i.e. transect length and width scanned divided by the area of the coverglass with the algal subsample). Algal species composition was determined by using ordination and diatom metrics. Ordination measures algal species composition by using variables that are basically the result of a regression model predicting a sample location in species space. So, species are positively or negatively related to these variables. This variable enables using species data to produce a coordinate system to plot samples, for example, in a two-dimensional plane ("map"). More will be explained when ordination analyses are explained. The other method for measuring species composition is using variables, typically called metrics, that are weighted averages of species relative abundances and species traits. Non-diatom data were not used in metrics because non-diatom algae were relatively rare in the lake benthos and we know more about diatom species traits than non-diatom traits. Three species traits were used for diatoms: NLA total phosphorus optima, NLA total nitrogen optima, and nitrogen fixation capability. NLA stands for the USEPA's National Lakes Assessment. TP and TN optima were determined using data from the USEPA's National Lakes Assessment (Stevenson et al. 2013). In addition, weighted average metrics were developed with TLA data to use diatom species composition to infer scuzziness of assemblages and algal abundance (cells/cm^2) of assemblages. All data calculations and statistical analyses were coded and conducted with the statistical program R.

Results

Hypothesis: Thickness and darkness of GBA color increase with algal density (cells/cm^2).

Thickness and darkness of GBA color, indicated by scuzziness score, varied from 3 to 10 in the 3 lakes over the 4 months of sampling (Figure 1). Scuzziness score was higher at the Bellaire-Southworth and Torch-Gourley locations than other locations. Scuzziness score was higher in July and August than June and September. Scuzziness score was more consistently high in July than August, i.e. the two months had the same 75th percentiles but July had a higher 25th percentile than August.

Algal density varied from 57,793 to 2,765,558 cells/cm^2 in the 3 lakes over the 4 months of sampling (Figure 2). Algal density was lower at Torch locations HWH, Penoza, and Petty where rocks were sampled compared to Bellaire and Clam sites. Algal density was not statistically higher in August than June, but algal densities in sand were usually higher in August than June. Algal abundance was lower on rocks than sand in the 2016 samples, but not the 2015 samples. Because there was some question about the areas of rocks scraped, the data for algal abundance on rocks should be considered with caution.

Most benthic algae in the Three Lakes were diatoms (Figure 3). Proportions of diatoms of all cells in samples ranged from 0.87 to 1.00 (87 to 100%), with almost all the rest of the algae being cyanobacteria and green algae. No difference in the proportions of cells were observed among sites or months.

No consistent relationship was observed between scuzziness score and algal abundance (Figure 4). However, high scuzziness was only observed when algal abundance was high. So high cell abundance does not always produce high scuzziness, but high scuzziness only occurs with high cell abundance.

Hypothesis: Thickness and darkness of GBA color vary with algal species composition.

As stated in the methods, ordination analysis was used to evaluate relationships between scuzziness score and algal species composition. I'll use the example in Figure 5 to interpret this relationship and explain how ordination is used. The value of ordination is that 1) species are not assigned traits and 2) patterns in species abundances are not constrained by a-priori defined species traits. Again, repeating from the methods for clarity, ordination measures algal species composition by using variables that are basically regression models predicting a sample location in species space. So, species are positively or negatively related to these variables. These variables can be used as a coordinate system to plot samples, for example, in a two-dimensional plane. For example, we can think of Dimension 1 (Dim1) as an axis between east and west and Dimension 2 (Dim2) as an axis between north and south for a map of sample locations in species space. The distance between sample locations indicates the dissimilarity in species composition among samples, just like the distance between two locations on a map. The locations of samples in the ordination map are related to the relative abundances of species in samples, and species relationships to the ordination dimensions, which are sometimes called axes. Species relationships to the ordination axes can be thought of as some form of regression model (e.g. $Dim1 \approx \beta_1 Sp_1 + \beta_2 Sp_2 + \beta_3 Sp_3 \ldots + \beta_S Sp_S$), such that the location of a sample along a dimension is related to the relative abundances of species (Sp_i) and a weighting factor (β_i). As indicated in the tables on the left side of Figure 5, different species are positively or negatively related to the ordination dimensions. In those tables, the ordination dimensions are labeled NMDS1 and NMDS2 for the dimensions resulting from the ordination analysis called Non-metric Multidimensional Scaling. Note some species have high or low weights in a positive or negative direction along either ordination dimension, and this helps us determine what species are most abundant in samples. For example, all the Clam Lake samples (CH6r1, CH6r2, CH8r1, and CH8r2) are located higher along Dimension 1 than most other samples, and in the case of CH8r1 and CH8r2, lowest along Dimension 2. By looking at the species relationships to the ordination dimension in the tables, we can get a good idea of which species were relatively high or low in these samples. *Achnanthidium exiguum* (abbreviated Achexigu) with a species weight of 2.01 on dimension 1 (NMDS1) and a relative low weight (-0.467) on dimension 2 (NMDS2) should be relatively high in Clam Lake samples. *Amphipleura pellucida* (abbreviated Amppellu) with a relatively high species weight of 1.7 on dimension 2 (NMDS1) and a relatively intermediate weight (-0.133) on dimension 2 (NMDS2) should be relatively high in June Torch Lake samples from the Penoza site (TPn6r1, TPn6r2). Sample codes used in these samples start with the letter for the Lake, a short code for each site, a number for the month, and an r1 or r2 for replicate count 1 and 2 for that sample.

Ordination showed that Clam Lake samples had different diatom species composition than the other lakes (Figure 5). When I removed Clam Lake samples from the ordination, some separation of Torch Lake and Lake Bellaire samples was also evident (Figure 6). A statistical analysis called analysis of similarities (ANOSIM) confirmed the differences in diatom species composition among lakes. Ordination analysis also showed that species composition was very different in June and August (Figure 7), with ellipses drawn around center of ordination space regions around which samples were located. Based on size of ellipses and dispersion of samples in ordination space, variation in diatom species composition was greater in June than August.

Ordination also showed that scuzziness score was highly related to diatom species composition in Torch Lake and Lake Bellaire (Figure 8, Clam Lake was dropped for this analysis), with a high correlation between Dimension 1 of the NMDS analysis and scuzziness score. This was shown in Figure 8 by the contours from 4.6 to 6.0 on the scuzziness scale arranged from high to low values (from right to left) along Dimension 1 of the ordination map of samples. This also indicates that scuzziness probably increased from June to August because June and August samples are located positively and negatively, respectively, along Dimension 1.

Hypothesis: Thickness and darkness of GBA color increase with diatom indicators of nutrient availability.

The NLA diatom TP and TN metrics were higher at the Clam-Hoadley site than the Lake Bellaire and Torch-Gourley sites (Figures 9-10). The diatom TP and TN metrics were higher in June than August, which was particularly true for three Torch Lake Sites (HWH, Penoza, and Petty).

The proportion of N_2-fixing diatoms was highest at the Torch-Petty site, indicating the lowest nitrogen availability (Figure 11). N_2-fixing diatoms can have endosymbiotic cyanobacteria that can convert atmospheric N_2 into NH_4, which can be used in amino acid and protein synthesis. When these taxa are abundant, the usual sources of nitrogen for diatoms, NO_3 and NH_4, are likely low. N_2-fixing diatoms were somewhat higher in August than June.

Scuzziness score was negatively related to the diatom metric for total phosphorus (TP), but was relatively poorly related to diatom metrics for total nitrogen (TN) and nitrogen fixers (Figure 12). The negative relationship between scuzziness score and the NLA diatom TP metric was not a clear linear relationship; but it is shown by the observation that all samples with high scuzziness scores have low values of the diatom metric for TP, whereas samples with low scuzziness scores have both high and low values of the diatom metric for TN. This indicates samples from locations with high scuzziness scores had relatively higher proportions of diatom species adapted to low phosphorus conditions than samples with high scuzziness scores. The relatively poor relationship between diatom metrics of TN and N_2-fixers and better relationship with the diatom metric for TP indicated that changes in nitrogen concentration probably had relatively little effect compared to changes in phosphorus availability on scuzziness score.

Hypothesis: Thickness and darkness of GBA color, algal density and algal species composition vary with ground and surface water chemistry.

Surface water SRP concentration, measured as µg PO_4-P, was similar among sampling locations, but varied with month (Figure 13). Surface water SRP increased from June through August and then decreased in September. The higher range of SRP values at Torch-Penoza than other sites was an artifact of a couple unusually high measurements and was likely not a consistent difference.

Surface water nitrate-nitrite (NO_x) concentrations were consistently higher in Torch Lake than Lake Bellaire and Clam Lake (Figure 14). Surface water NO_x concentrations were also higher in June than July, August, and September.

Surface water DIN concentrations were higher in Clam Lake and Lake Bellaire than Torch Lake, which must be related to higher average ammonia concentrations causing different NOx and DIN patterns among lakes (Figure 15). Also, surface water DIN increased slightly from June to August.

Groundwater SRP concentrations were lower at Torch-Gourley than other sites (Figure 16). Ground water SRP was lower in September than June, July, and August.

Groundwater NO_x concentrations were highest at Bellaire-Southworth, and higher at Torch-Penoza and Torch-Petty than other sites (Figure 17). Groundwater NO_x concentrations were relatively constant during the summer with the upper quartiles of concentrations increasing slightly.

Groundwater DIN concentrations were highest at Torch-Gourley, which was due to very high NH_4 concentrations in groundwater at that site (Figure 18). Groundwater DIN concentrations did not change during the summer. The high DIN at Torch-Gourley could have increased P demand by bacteria in deep sediments, thereby causing the lower P concentrations at Torch-Gourley than other sites.

In general, surface and ground water nutrient concentrations were not related to scuzziness score, algal abundance, or the diatom TP, TN and N_2-fixer metrics (Figures 19-24). The only exception was the apparent negative relationship between surface water P chemistry and the diatom TP and TN metrics, but not the N_2-fixer metric (Figure 19C-D). This indicates that as surface water phosphate increased slightly during the summer, nutrient availability for algae decreased because relative abundances of low nutrient diatom species were increasing – as indicated by the decreasing diatom TP and TN metrics from June to August. There was no change in the relative abundance of N_2-fixing diatoms during the summer or with surface water TP, which indicates that N was not the limiting factor. The response of the TN metric may have been due to a poor assessment of species nitrogen traits in the NLA where nitrogen is seldom the limiting nutrient and regulating species composition. Thus, it is somewhat more likely that the change in diatom species composition from July to August was due to a decrease in P availability rather than N availability. The clearer negative relationship between scuzziness score and the diatom TP metric, versus the diatom TN metric, also indicates decreasing P availability may be related to development of GBA scuzziness.

Hypothesis: Benthic diatom assemblages differ in surface and deep sediments.

Ordination analysis was calculated with all surface and deep sediment diatom assemblages. Ordinations showed spatial separation between surface and deep sediments in ordination spaces (Figure 25).

Analysis of similarity also indicated diatom species composition differed between surface and deep sediments.

Diatom metrics were calculated for assemblages in surface and deep sediments (Figure 26). NLA diatom metrics for TN and TP and the N_2-fixer metric indicated that diatoms in surface sediments were exposed to lower nutrients than diatoms in deep sediments. Two new metrics were calculated based on data from the three lakes sampled. One was a diatom inference model for scuzziness and the other for algal density (cells/cm^2) in surface sediments. These indicators, interestingly, inferred that diatoms in deep sediments grew in conditions that were less like those that produced scuzziness than surface assemblages and diatoms in deep sediments grew in conditions that would support higher diatom densities than surface sediments.

These patterns in diatom-inferred conditions could be the result of historic changes in the Three Lakes, such that nutrient concentrations have been decreasing over time, and conditions supporting scuzziness have increased. Alternatively, they may be the result of simple seasonal changes in surface assemblages that are actively growing during summer seasons in which nutrients are lower than the rest of the year and more appropriate, for one reason or another, for development of scuzziness.

Discussion

Let's consider the results of testing of our four hypotheses with both direct and indirect analyses:

1. Thickness and darkness of GBA color increase with algal abundance (cells/cm^2).
2. Thickness and darkness of GBA color vary with algal species composition.
3. Thickness and darkness of GBA color increase with diatom indicators of nutrient availability.
4. Thickness and darkness of GBA color, algal density and algal species composition vary with ground and surface water chemistry.

For hypothesis 1, the results from 2015 and 2016 show that scuzziness of GBA was not related to cell abundance (cells/cm^2).

For hypothesis 2, results from 2016 indicated scuzziness of GBA was related to diatom species composition and diatom indicators of low, not high, P conditions. This result is not consistent with the hypothesis that groundwater nutrients were stimulating GBA growth. An ecological reason to explain these results was proposed last year, when I suggested that algae, mostly diatoms, are moving from within the surface sand matrix to the surface of the bottom sand matrix. The indications that changes in species composition and development of scuzziness of GBA is related to low P are also a key piece of information.

The most abundant diatom in samples in *Cymbella delicatula* (now considered renamed *Delicatula delicatula* by some taxonomists). This diatom forms mucilaginous stalks like the Cymbella in Figure 25. This is often a final stage of benthic algal succession (Figure 26). Benthic algal succession starts with diatoms accumulating on a surface and either lying flat on the surface or being apically attached. Over time, particularly as abundances of benthic diatoms increase, benthic diatoms take up the inorganic nutrients in interstitial waters of the benthic algal matrix faster than more nutrients can mix into those spaces from surface water (Stevenson and Glover 1986). This is also likely true for groundwater as well, because the same nutrient depletion and mixing mechanisms should operate for groundwater as with surface water. From studies of benthic diatom community development, we know that this decrease in nutrients affects benthic algae by reducing their growth rates as they become more nutrient limited. When benthic algae become nutrient limited, they no longer convert the carbohydrates produced from photosynthesis into amino acids, proteins, nucleic acids, and cell membranes because that synthesis is P or N limited. Instead they convert the carbohydrate products of photosynthesis into extracellular mucilages, like stalks, which have little N and P in them. Theoretically, the stalks, like trunks of trees, raise diatoms above other diatoms that do not have stalks and enable stalked diatoms to sequester more nutrients from overlying waters. Thus, nutrient limitation during summers increases the development of stalked diatoms that make the GBA more apparent (scuzzy) as diatoms move from among sand grains to the top surface of the sand.

How can this be related to a change in Torch Lake and Lake Bellaire? In discussions with Tim Veverica and review of surface water TP concentrations collected by the Tip Of The Mitt Watershed Council, we found that surface water P concentrations have been decreasing (Figure 27). This could be explained by changes in atmospheric nitrogen deposition caused by human activities. Nitrogen deposition has increased globally as a result of nitrogen fertilizers that contaminate the atmosphere, which then return to the ground and lake in rain (Figure 30). Nitrogen deposition in low nutrient waters, especially N-limited lakes, increases demand for phosphorus (Elser et al. 2009a, b). Thus, a global problem with atmospheric nitrogen pollution may be contributing to GBA scuzziness. Exactly how changes in global nitrogen pollution could affect GBA needs to be investigated. Past N deposition (e.g. 2000-2002) was higher when N fertilizer use was higher before problems with the Gulf of Mexico hypoxia were recognized (Figure 31). According to NADP (2016) deposition maps and past records, northern Michigan has had and continues to have elevated atmospheric N deposition. We should add the atmospheric N deposition hypothesis to our list of key hypotheses.

Some evidence still points to nutrient enrichment causing GBA, such as the higher GBA observations observed at the Bellaire-Southworth and Torch-Gourley sites throughout the summer, and these sites having indications of surface and groundwater enrichment. However, the similarity in diatom assemblages on rocks and sand during 2015 and 2016 indicates groundwater is not causing GBA. We would expect effects of groundwater to be greater at the sand-surface water interface, as it pushes up through interstitial spaces before dilution in surface water. Likely, groundwater has already mixed with surface water before it reaches algae on rocks. It is possible that boundary layer conditions constrain flow of groundwater so it flows through benthic algal assemblages on rocks before it moves into surface waters, but that does not seem likely.

Literature Cited

Elser, J., T. Anderson, J. S. Baron, A.K Bergstrom, M. Jansson, M. Kyle, K. R. Nydick, L. Steger, and D. O. Hessen. 2009a. Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. Science 326:835-837.

Elser, J., M. Kyle, L. Steger, K. R. Nydick, and J. S. Baron. 2009b. Nutrient availability and phytoplankton nutrient limitation across a gradient of atmospheric nitrogen deposition. Ecology 90:3062-3073.

NADP (National Atmospheric Deposition Program). 2016. Total Deposition 2015 Annual Map Summary. NADP Data Report 2016-02. Illinois State Water Survey, University of Illinois at Urbana-Champaign, IL.

Stevenson, R.J. and E.F. Stoermer. 1981. Quantitative differences between benthic algal communities along a depth gradient in Lake Michigan. Journal of Phycology 17(1):29-36.

Stevenson, R. J. 1984. Procedures for mounting algae in a syrup medium. Transactions of the American Microscopical Society. 103:320-321.

Stevenson, R.J., S.T. Rier, C.M. Riseng, R.E. Schultz, and M.J. Wiley. 2006. Comparing effects of nutrients on algal biomass in streams in 2 regions with different disturbance regimes and with applications for developing nutrient criteria. Hydrobiologia 561:149-165.

Stevenson, R.J., B.J. Bennett, D.N. Jordan, and R.D. French. 2012. Phosphorus regulates stream injury by filamentous green algae, thresholds, DO, and pH. Hydrobiologia 695:25-42.

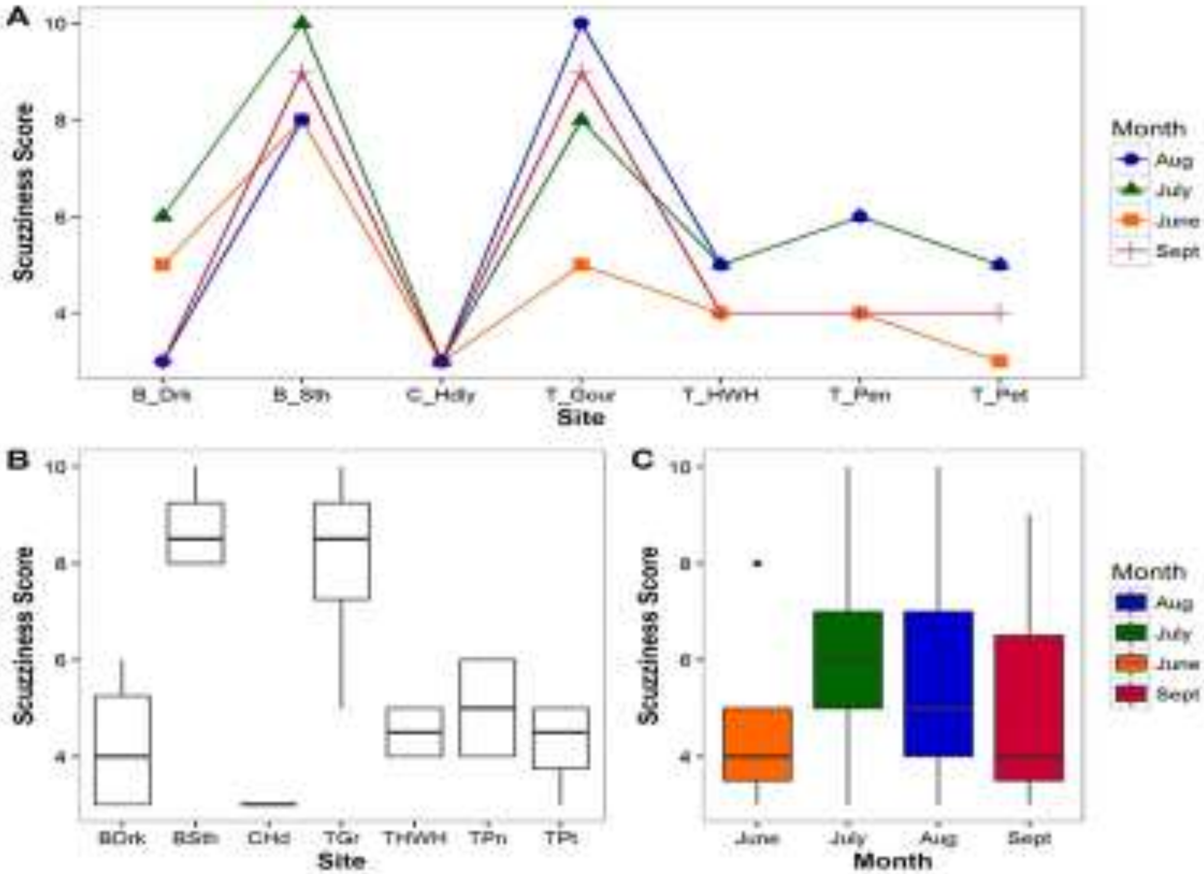


Figure 1. Scuzziness score relationship to site and month. In panel A, all scuzziness scores are plotted independently with one score per site and month. Scuzziness scores of the same month are connected by lines. In panels B and C, box plots are used to show the distribution of scuzziness scores by site and month.

Figure 2. Changes in abundance (cells/cm²) of all algae with site and month.

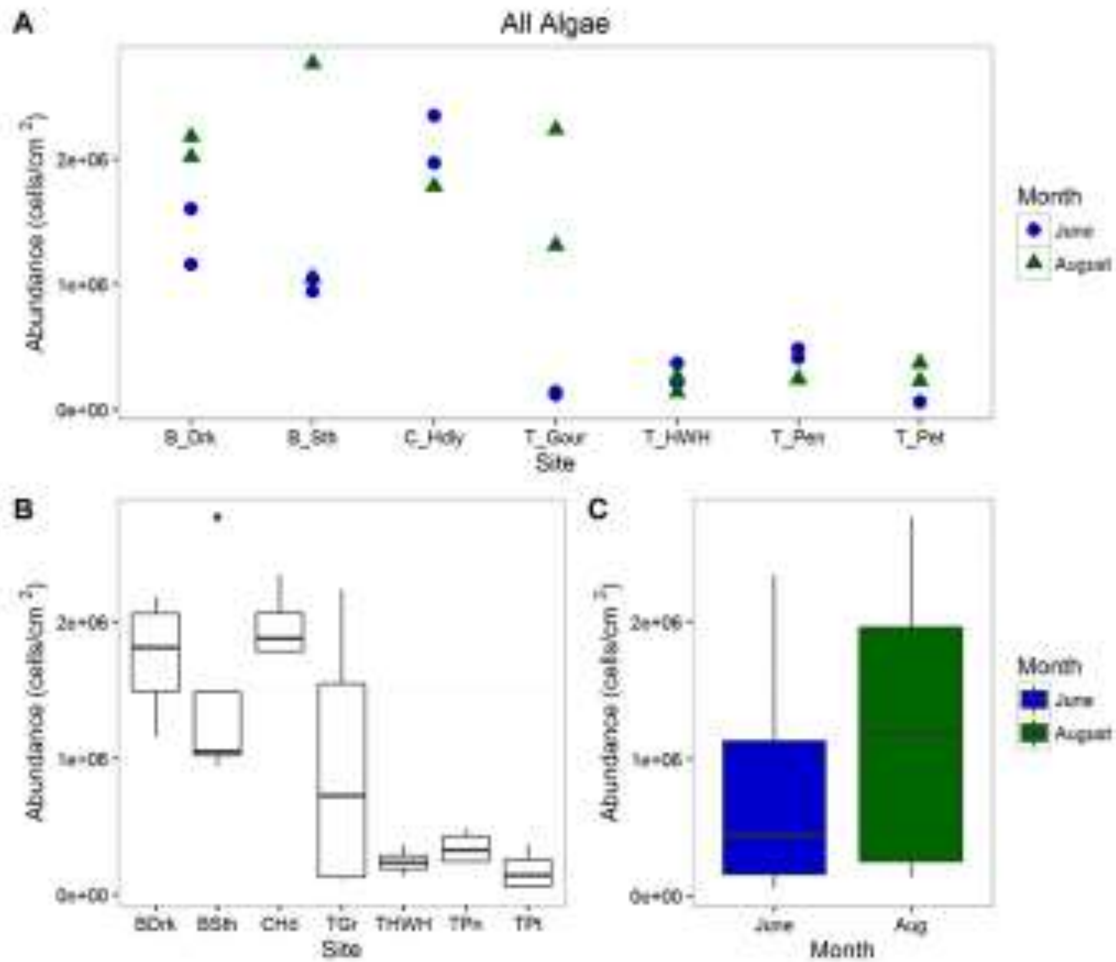


Figure 3. The proportion of diatom cells among all algal cells in samples.

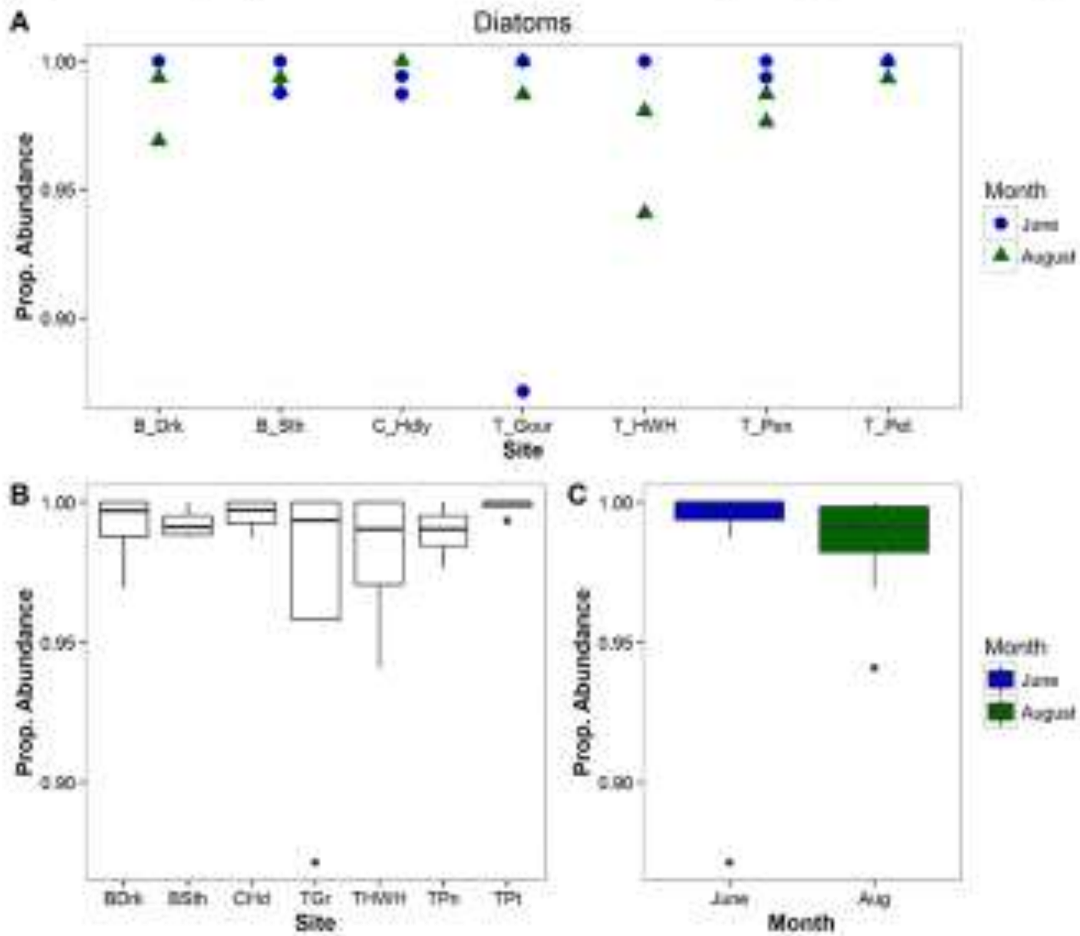


Figure 4. Changes in Scuzziness Score Are Not Related to Algal Density (cells/cm²).

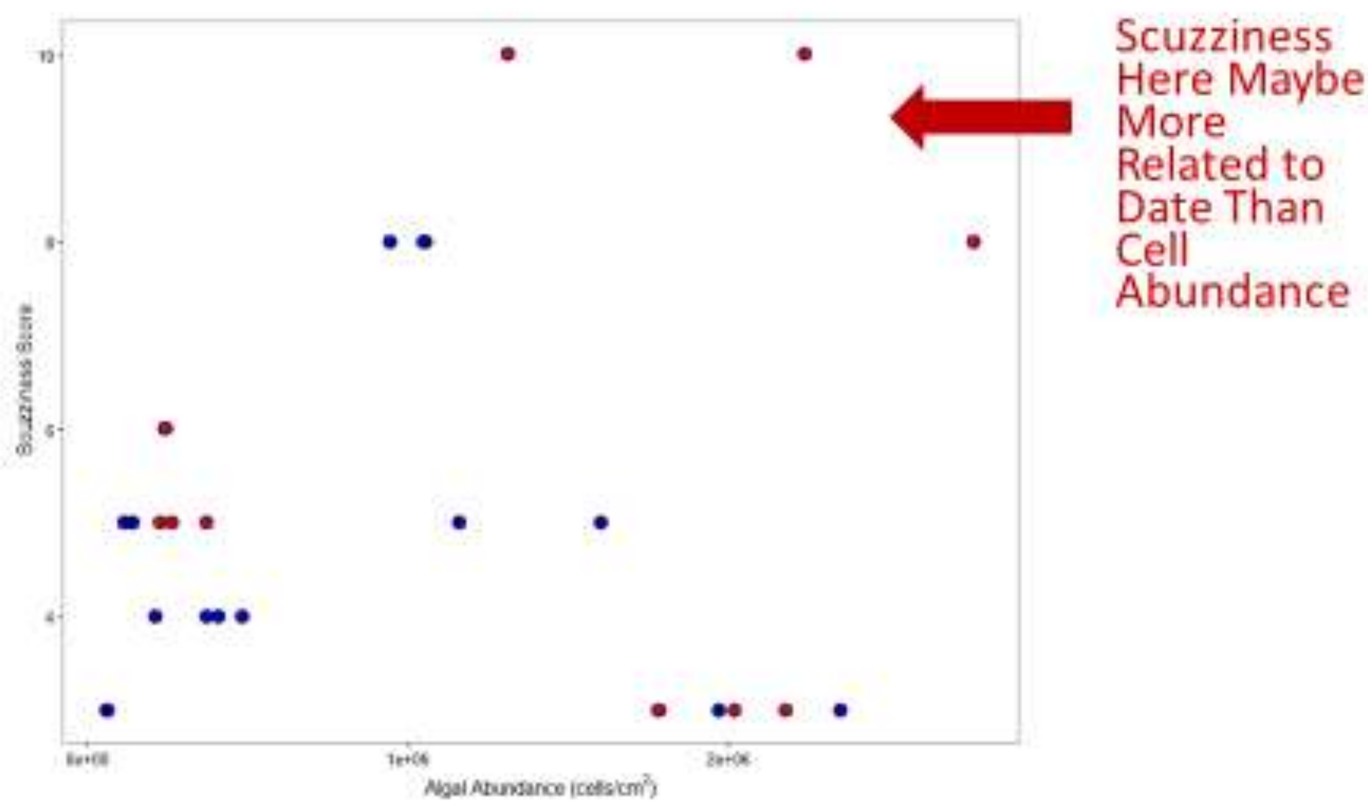


Figure 5. Variation in algal species composition in species space determined by Non-Metric Multidimensional Scaling. Sample codes used in these samples start with the letter for the Lake, a short code for each site, a number for the month, and an r1 or r2 for replicate count 1 and 2 for that sample.

	NMDS1	NMDS2
Achexigu	2.016417925	-0.46744342
Achminut	0.262782458	0.14934955
Achrivul	0.343972957	0.16100205
Achspp.	1.500287225	-0.09643049
Ampcopul	1.025950734	-0.32725286
Amphclsa	0.154935908	-0.09595606
Amppedic	1.411235318	-0.38753308
Amppellu	0.133345989	1.76585436
Ampthuma	-0.336455550	-0.57397270
Ampvenet	0.015252832	0.57204229
Anetuscu	0.813297769	-0.47203835
Aphspp.	NaN	NaN
Bacpared	-0.364491057	-0.61961377
Brabrebi	0.696436117	-0.22559875

****

Rhogibba	-0.661120667	-0.69082679
Scespp.	NaN	NaN
Selpupul	0.190247572	-0.34369231
Selstroe	-0.386370152	-0.14975779
Staancep	1.493305118	0.12681867
Staconst	1.761393361	-0.37640496
Staconvv	1.497658629	-0.24230826
Stamarcy	1.664261970	-0.13226039
Stapinna	0.927091709	-0.57100541
Staaxiva	0.293136003	-0.04804581
Syndelva	-0.364491057	-0.61961377
Synulna	-0.235367591	-0.34348800
Tabfenes	1.233361919	-0.11891756
UnkChlor	NaN	NaN
UnkCyano	NaN	NaN

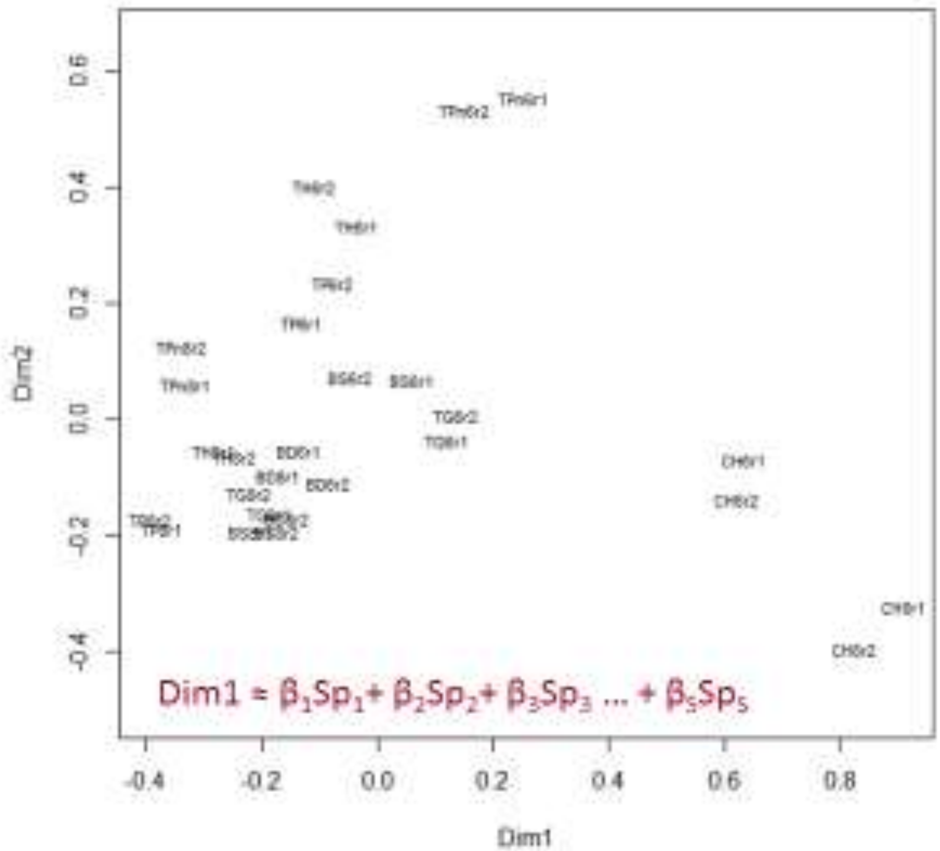


Figure 6. NMDS ordination indicated diatom (SS) species composition differs between Lakes Bellaire and Torch.

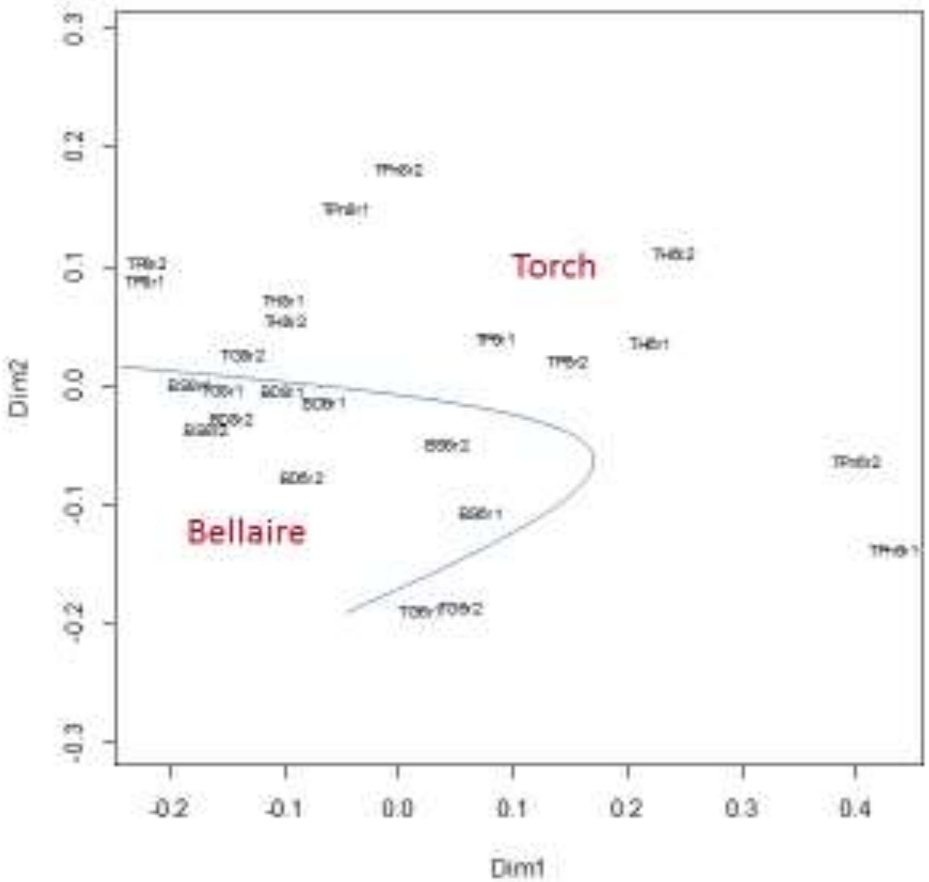


Figure 7. NMDS ordination indicates diatom (SS) species composition differs among dates (June and August).

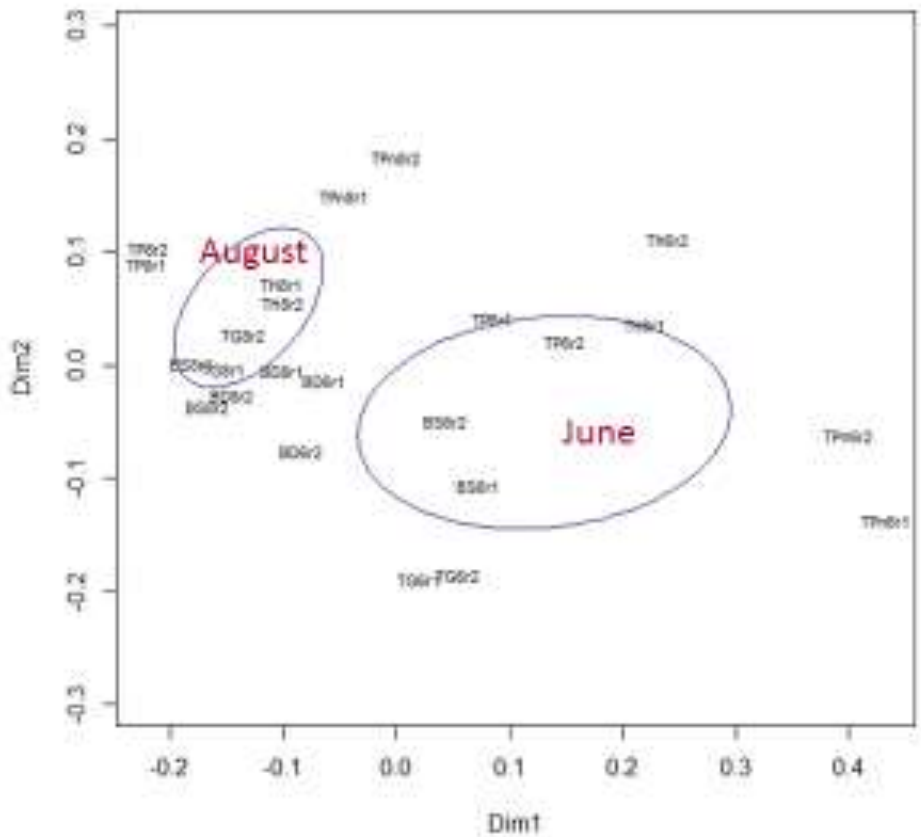


Figure 8. NMDS ordination indicates diatom species are related to scuzziness score.

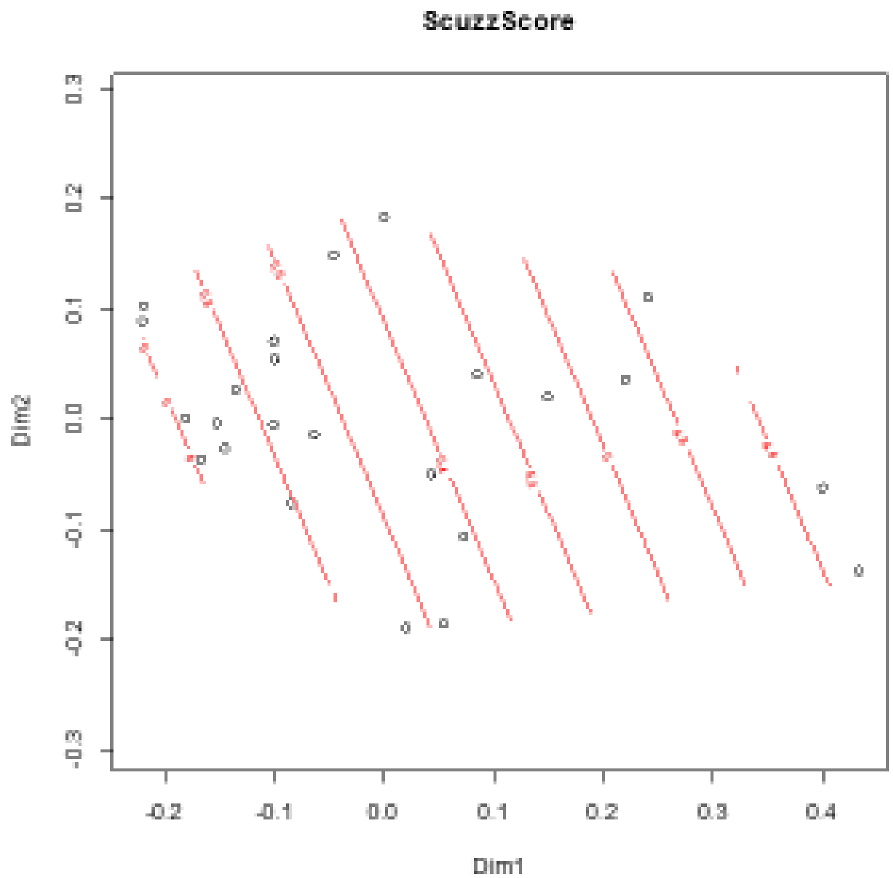


Figure 9. Changes in the NLA diatom TP metric vary with site and month.

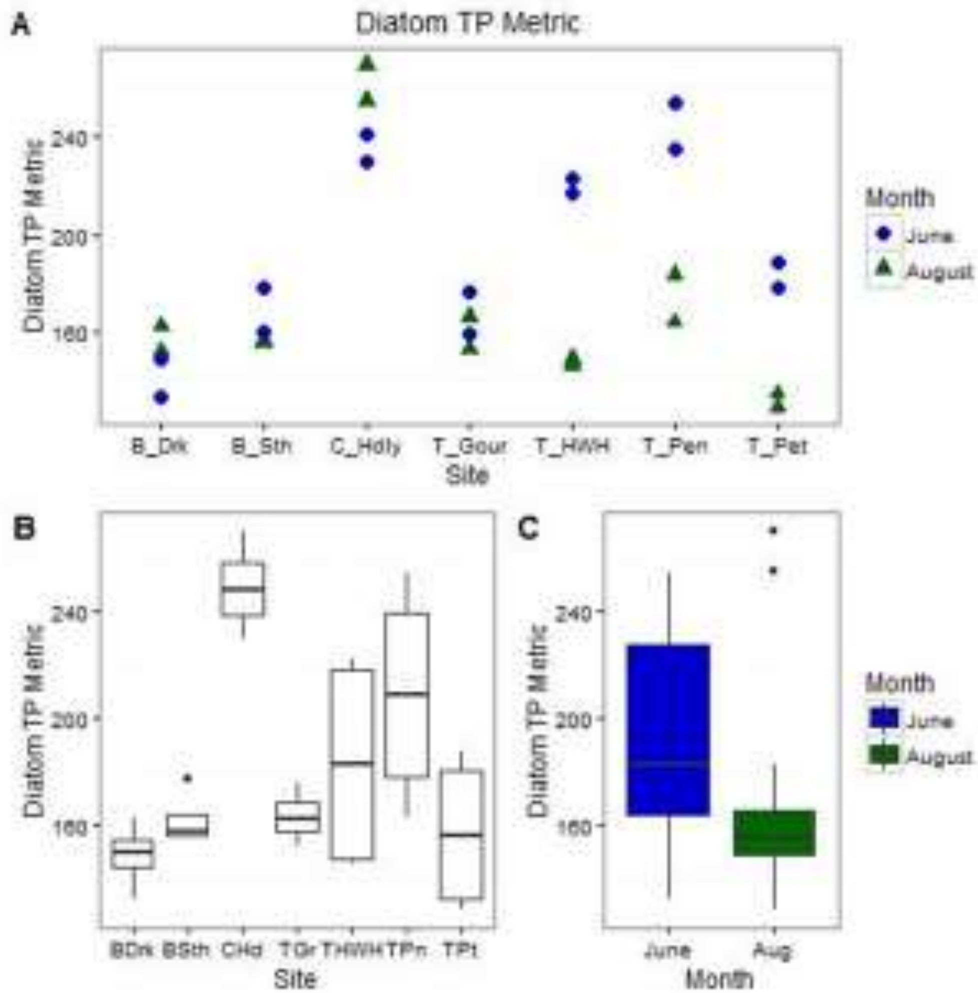


Figure 10. Changes in the NLA diatom TN metric with site and month.

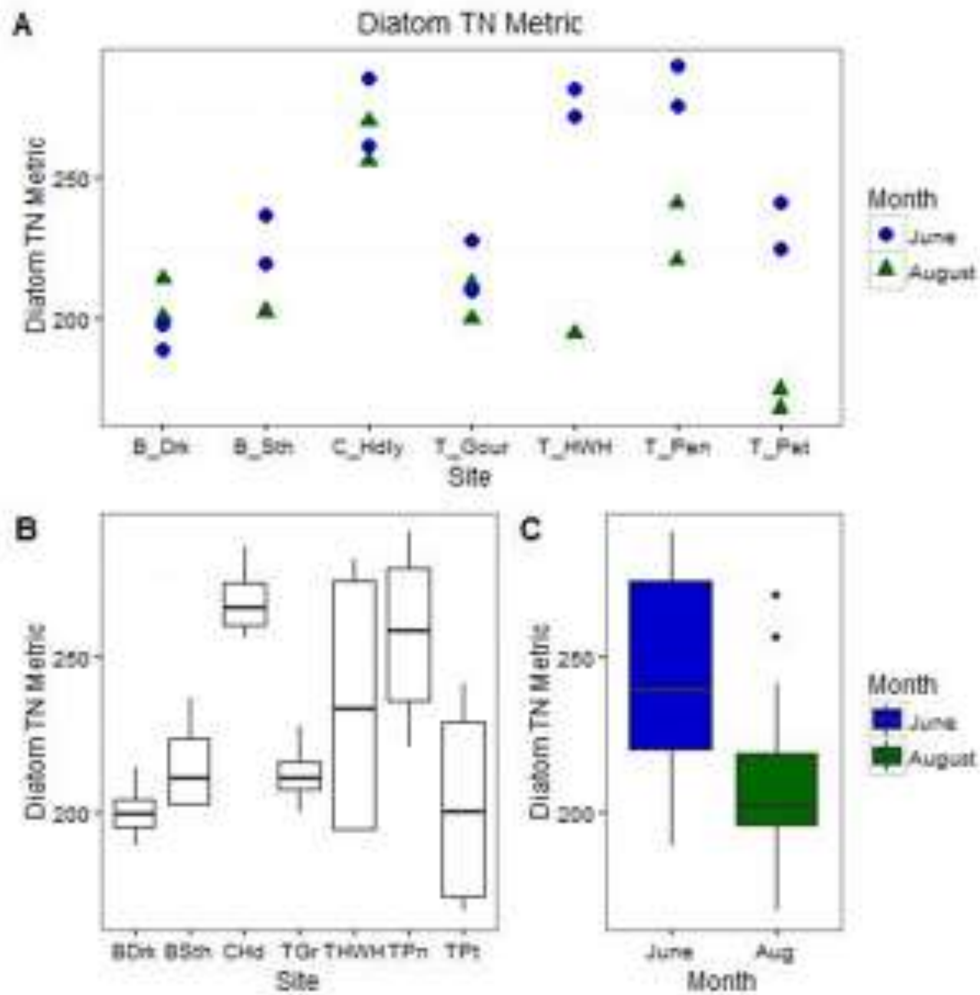
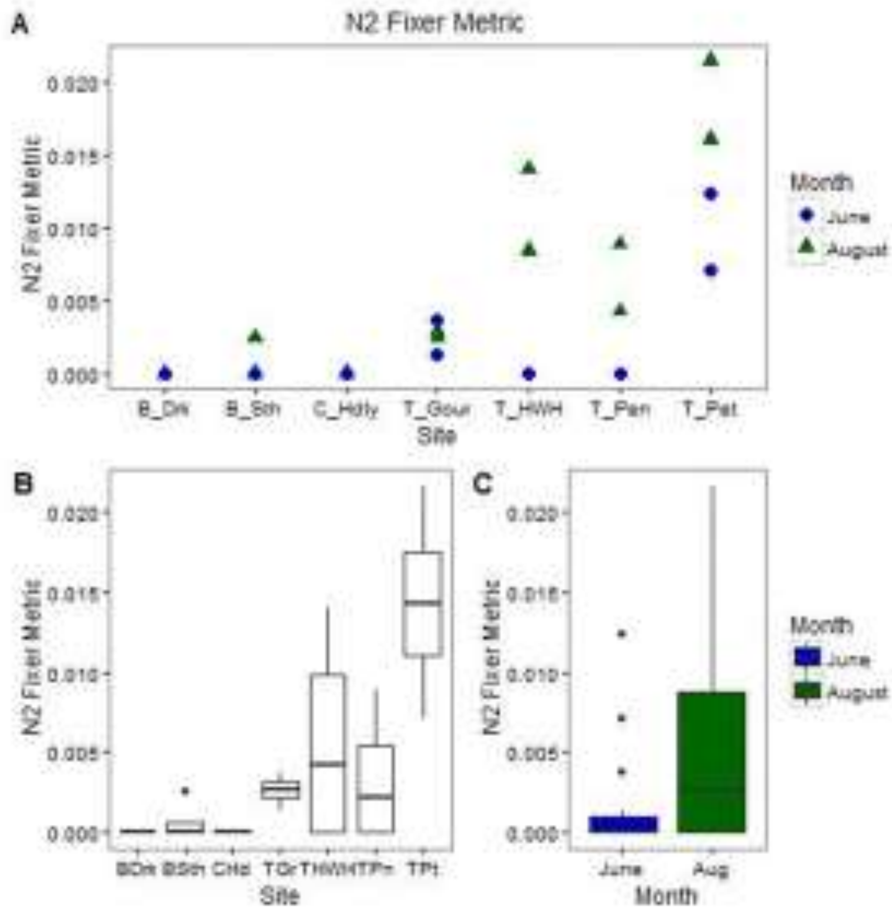


Figure 11. Changes in proportions of N_2 fixing diatoms (capable of containing cyanobacteria endosymbionts) with site and month.



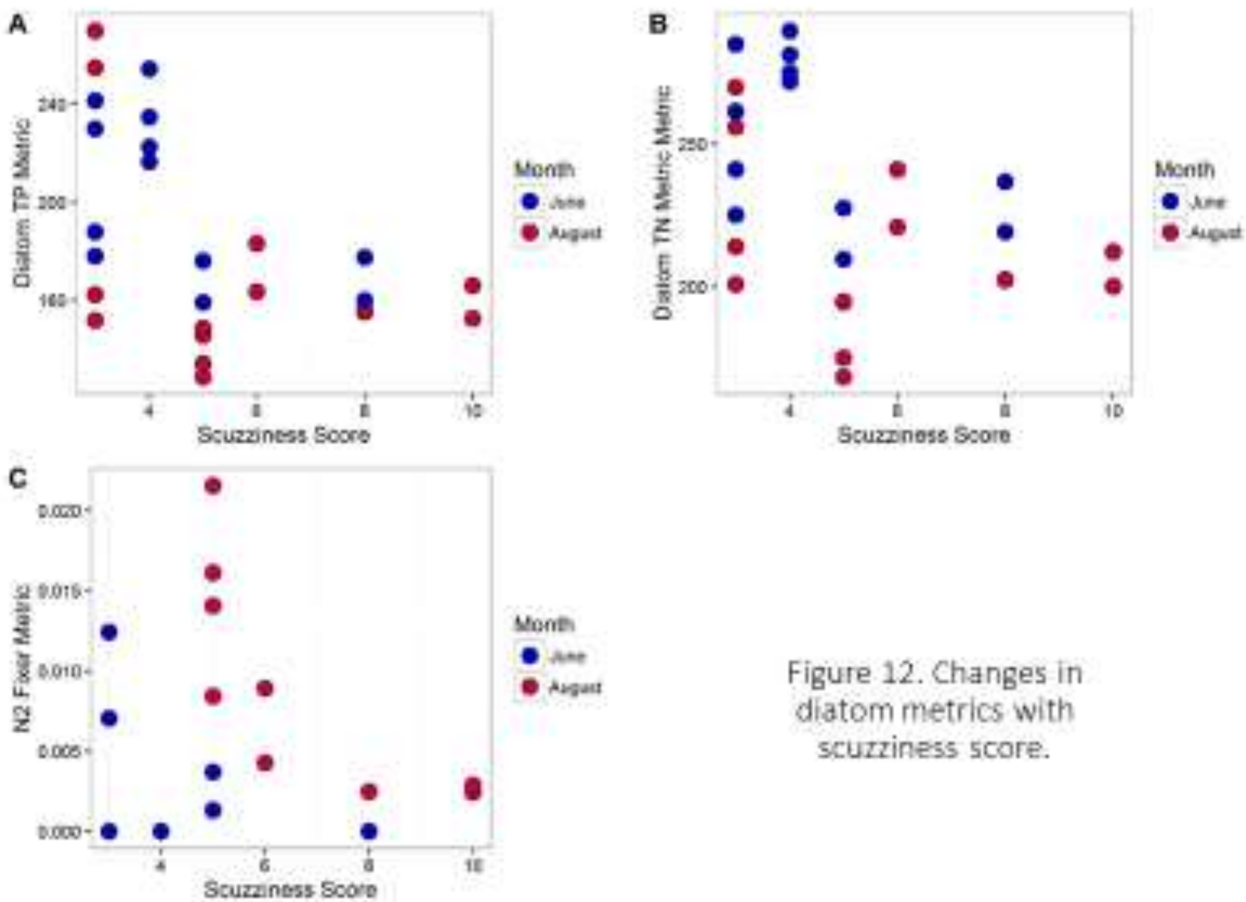


Figure 12. Changes in diatom metrics with scuzziness score.

Figure 13. Surface water (sw) soluble reactive phosphorus (PO_4-P) concentration with site and month.

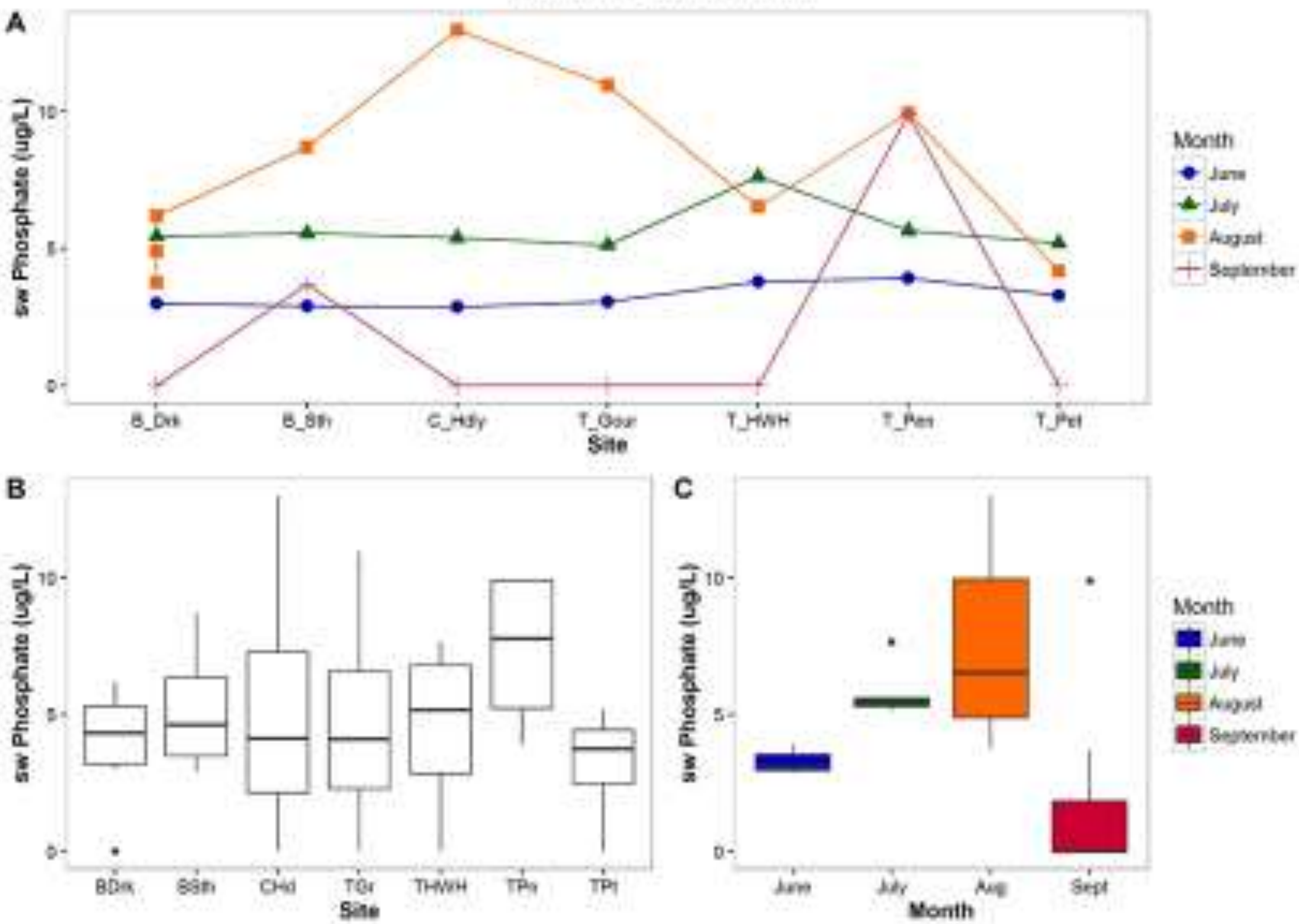


Figure 14. Changes in surface water (sw) No_x concentrations with site and month.

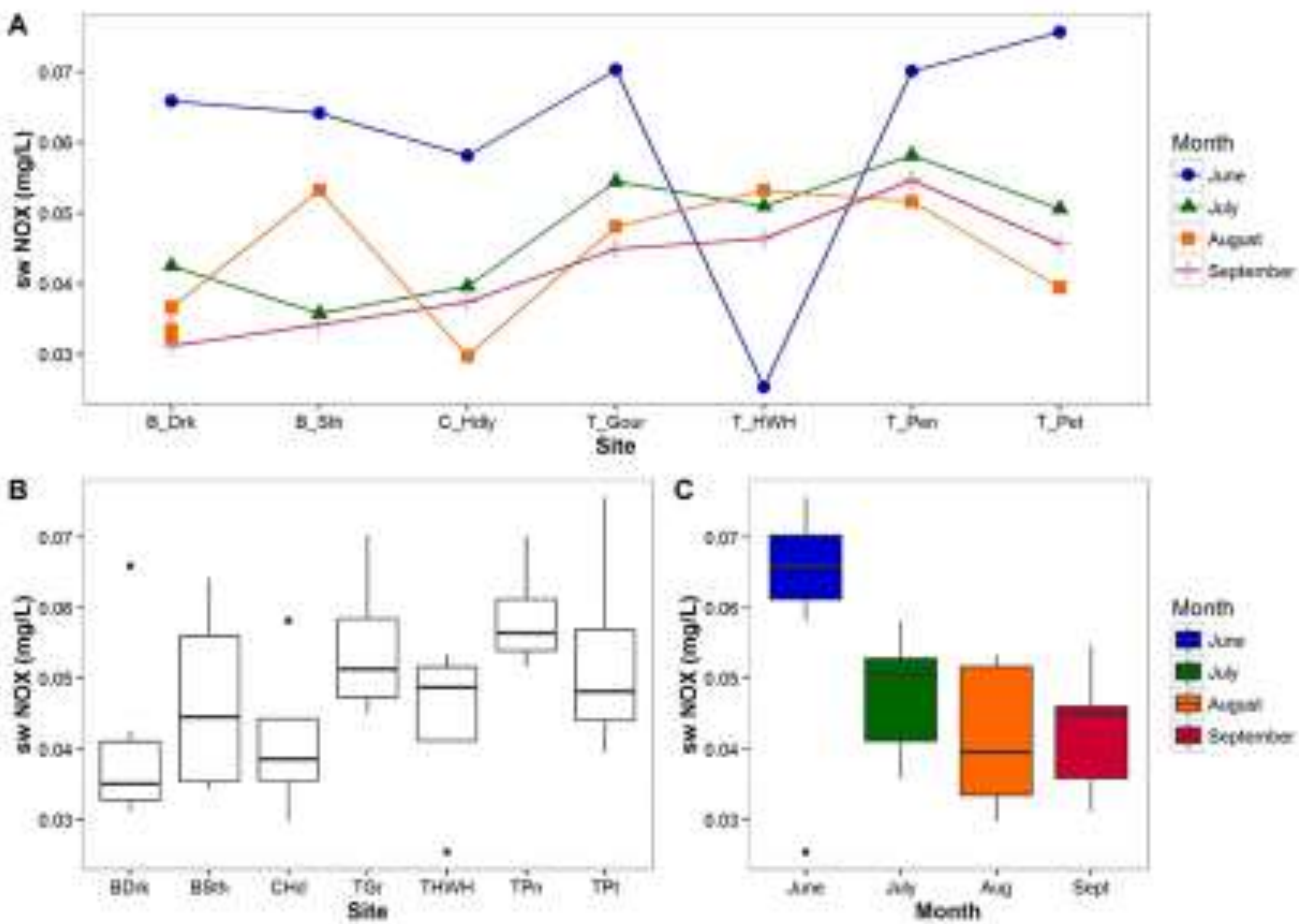


Figure 15. Surface water (sw) concentrations of dissolved inorganic nitrogen ($DIN=NO_3+NO_2+NH_4$) differences among sites and months.

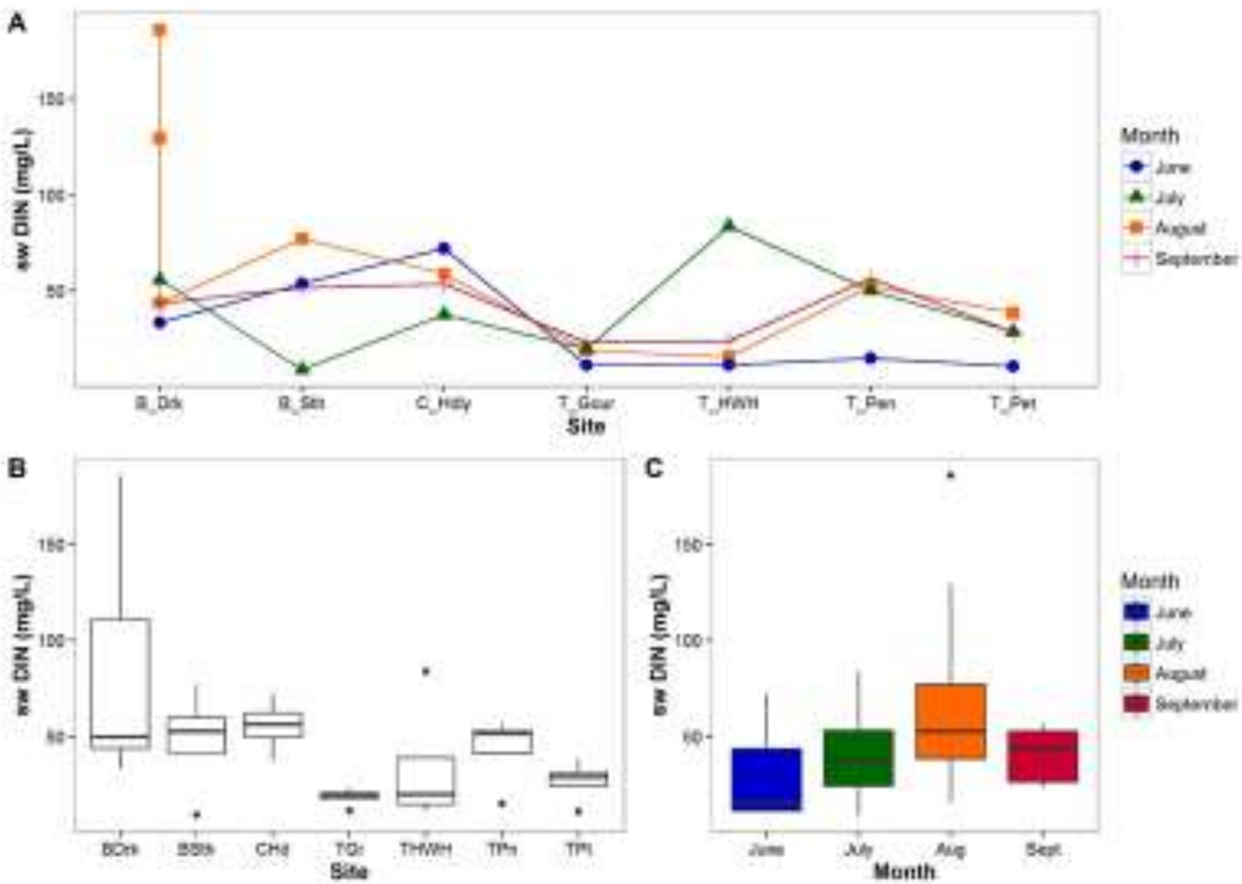


Figure 16. Groundwater concentrations of soluble reactive phosphorus (PO_4-P) differences among sites and month.

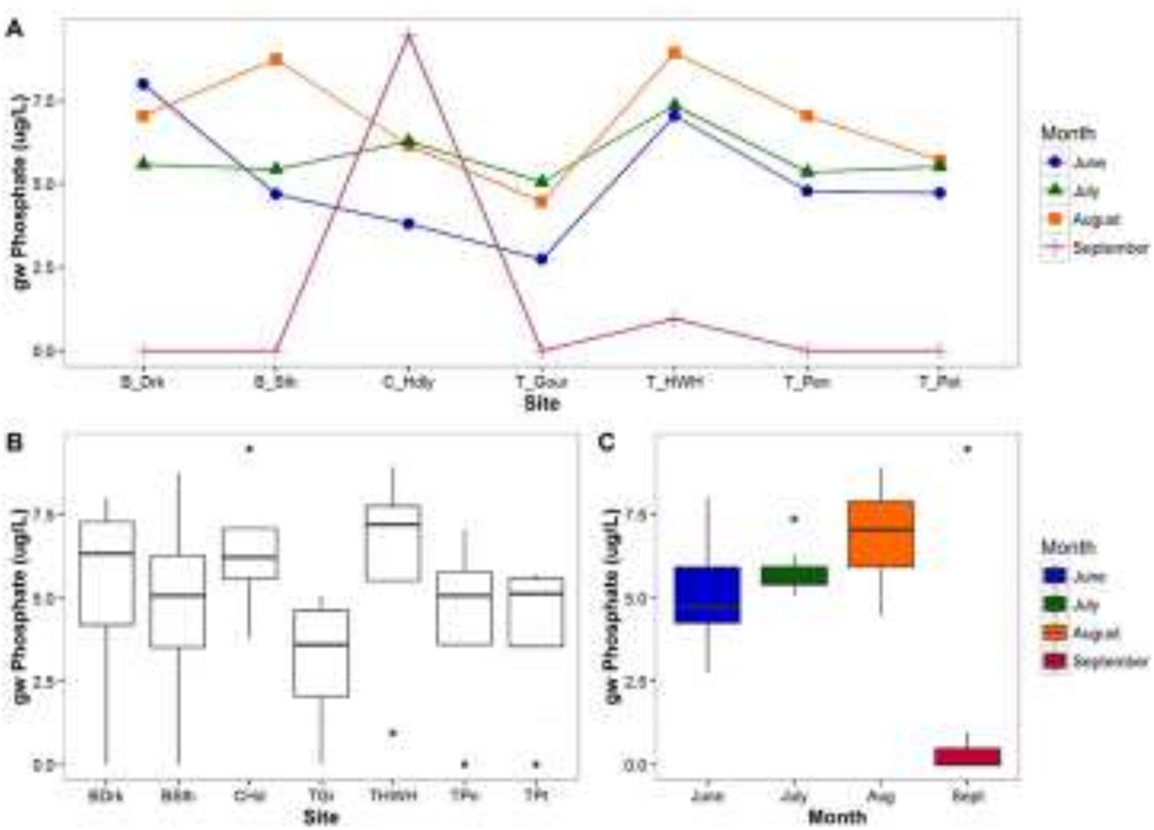


Figure 17. Groundwater No_x concentrations plotted by sites and months.

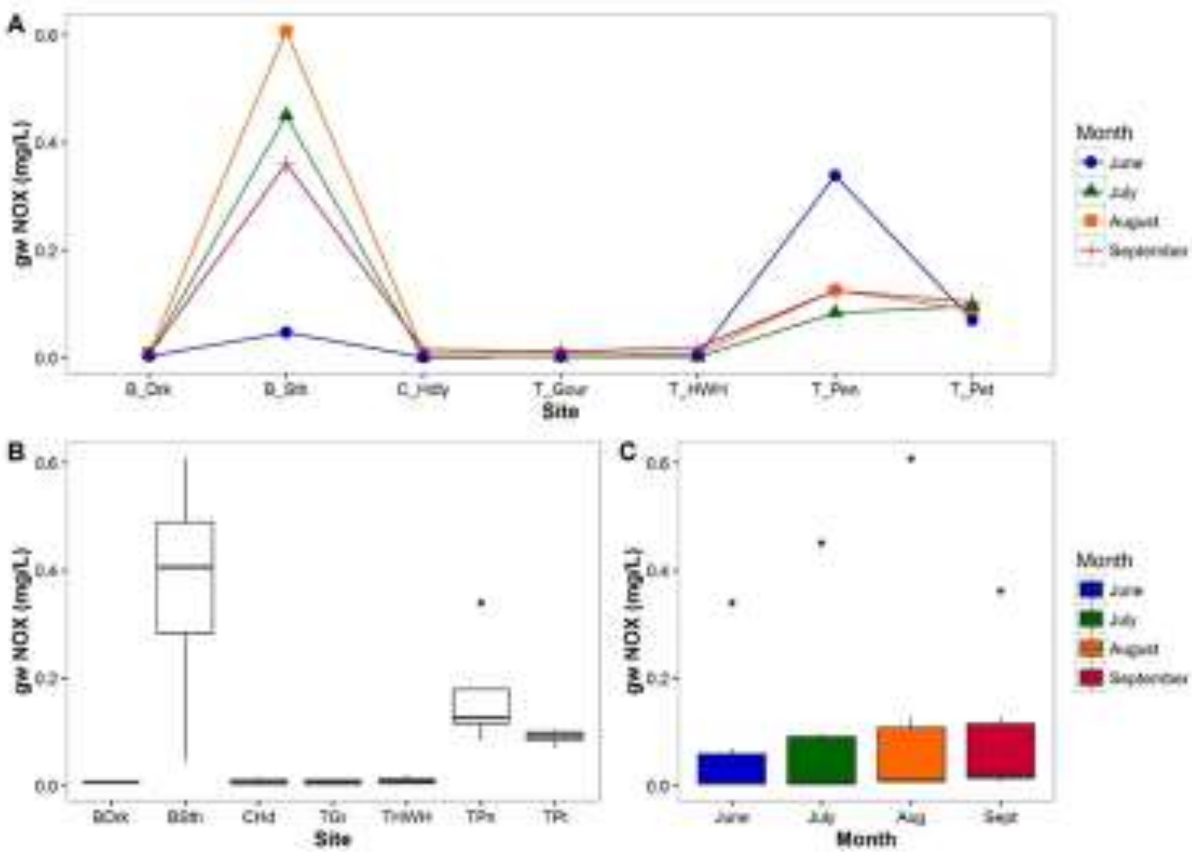


Figure 18. Groundwater concentrations of dissolved inorganic nitrogen ($DIN = NO_3 + NO_2 + NH_4$) plotted by site and month.

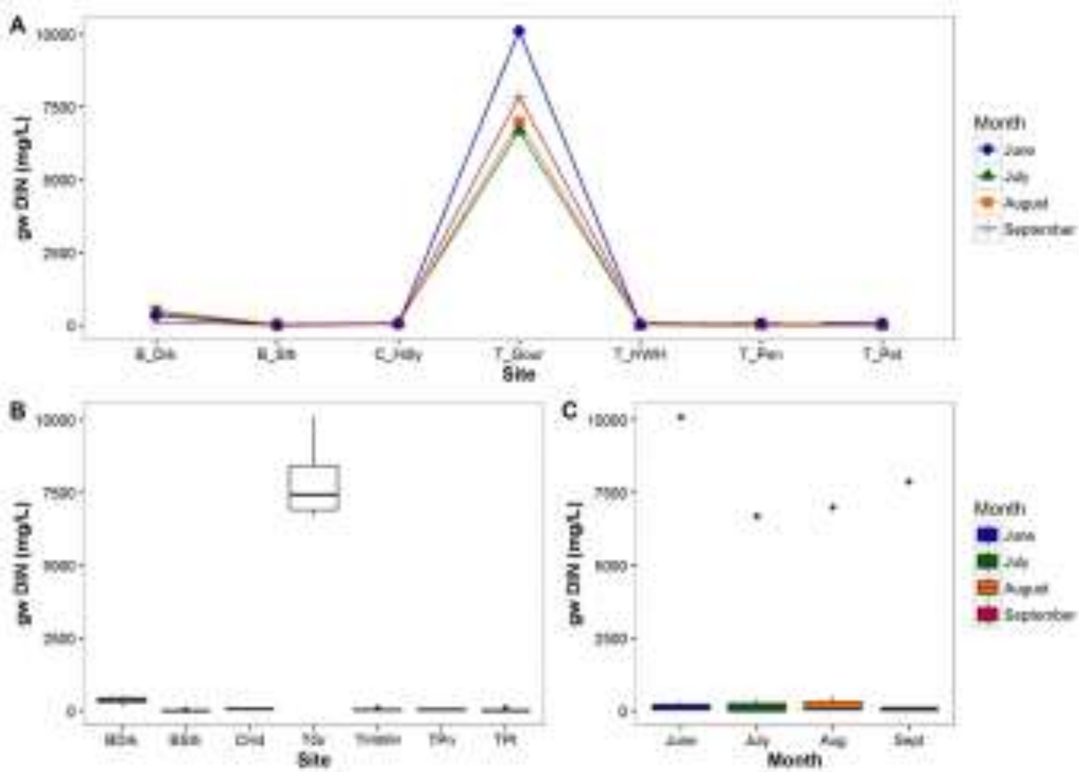


Figure 19. Relationships between surface water phosphorus (SRP, PO_4-P) with scuzziness score, algal abundance (cells/cm[2]), NLA diatom metrics for TP and TN, and proportion of diatoms capable of nitrogen fixation.

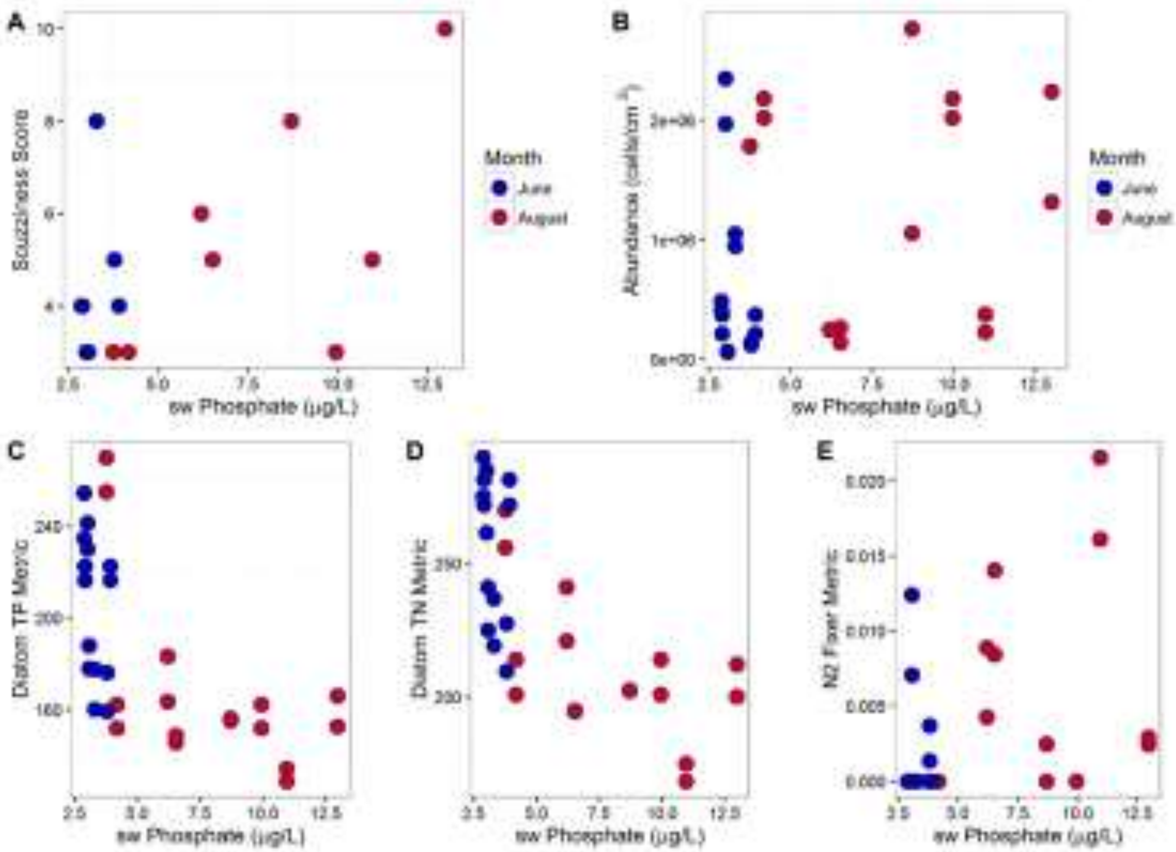


Figure 20. Relationships between surface water NO_x concentrations (log-transformed) with scuzziness score, algal abundance (cells/cm²), NLA diatom metrics for TP and TN, and proportion of diatoms capable of nitrogen fixation.

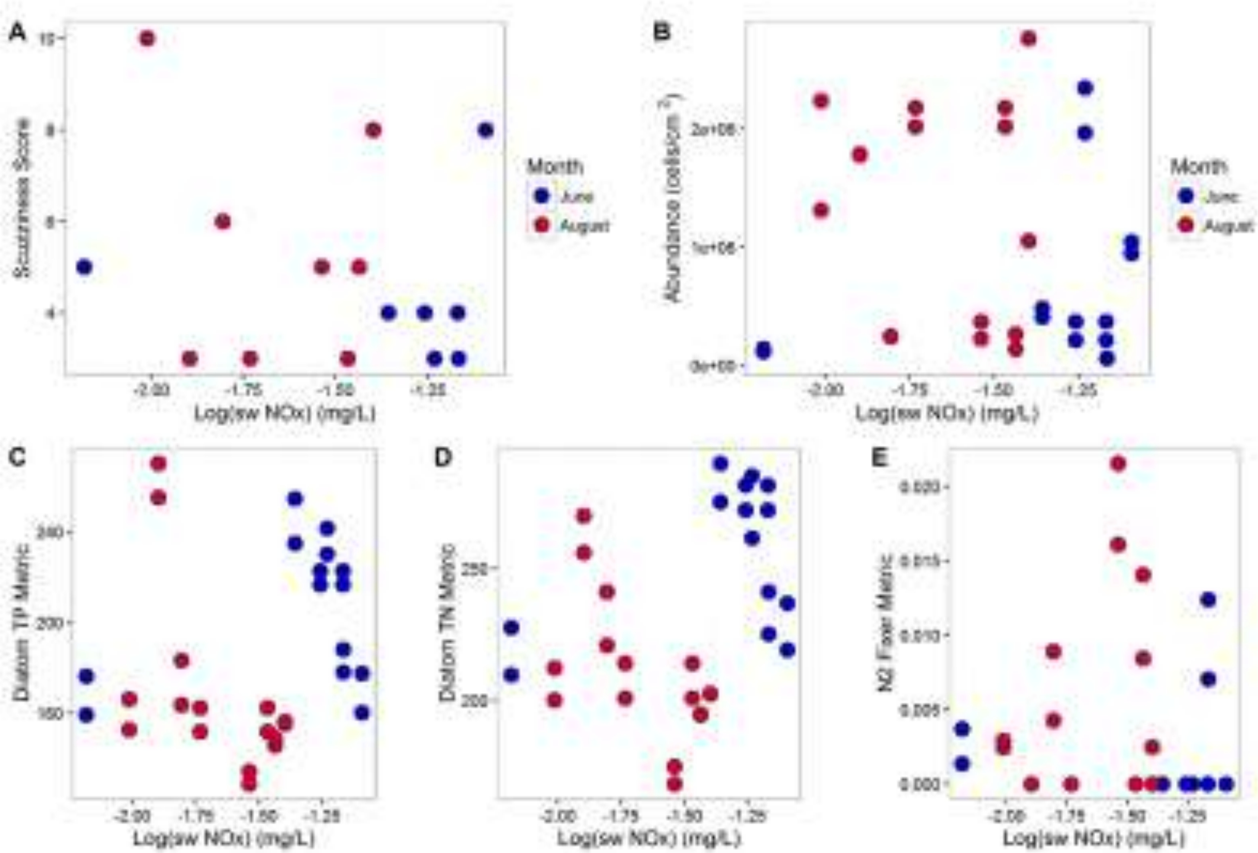


Figure 20. Relationships between surface water DIN concentrations (log-transformed) with scuzziness score, algal abundance (cells/cm^2), NLA diatom metrics for TP and TN, and proportion of diatoms capable of nitrogen fixation.

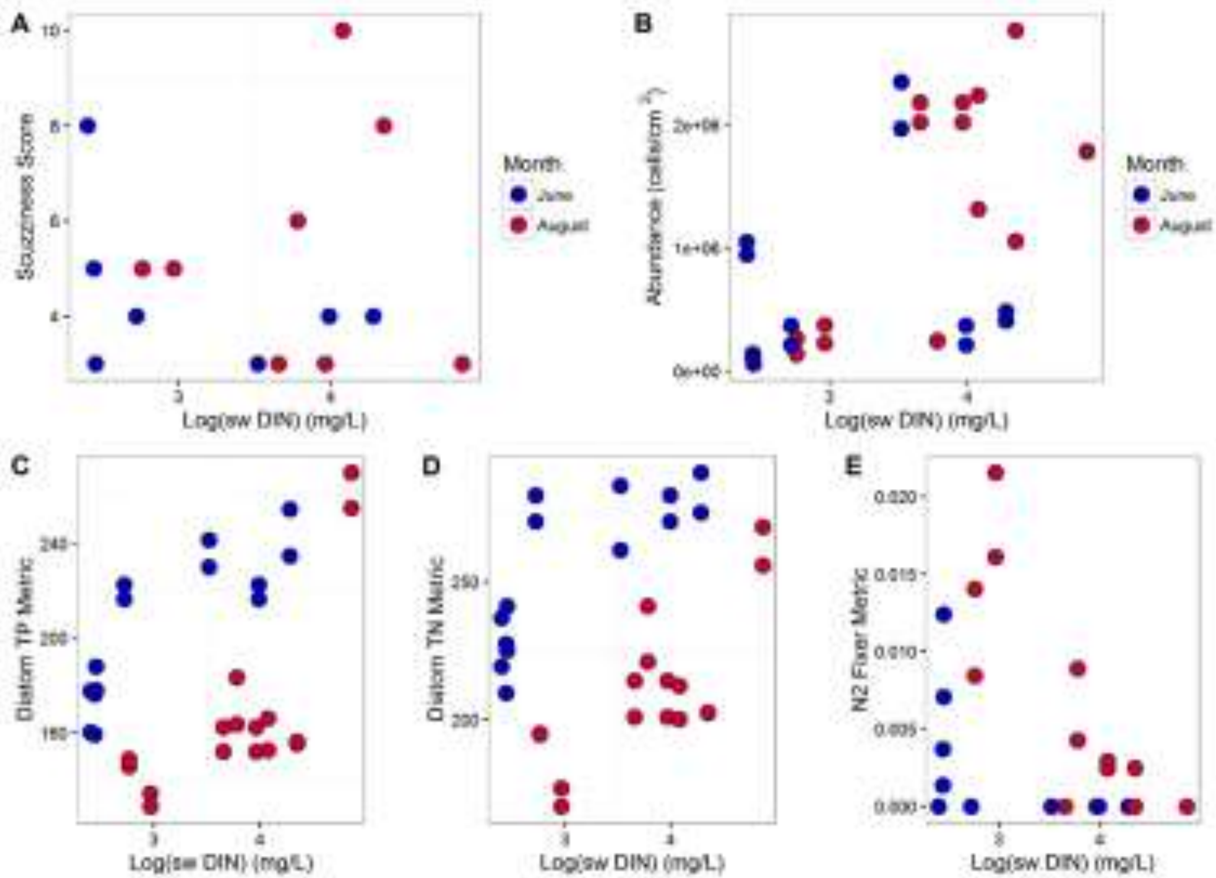


Figure 22. Relationships between groundwater phosphorus (SRP, PO_4-P) with scuzziness score, algal abundance (cells/cm^2), NLA diatom metrics for TP and TN, and proportion of diatoms capable of nitrogen fixation.

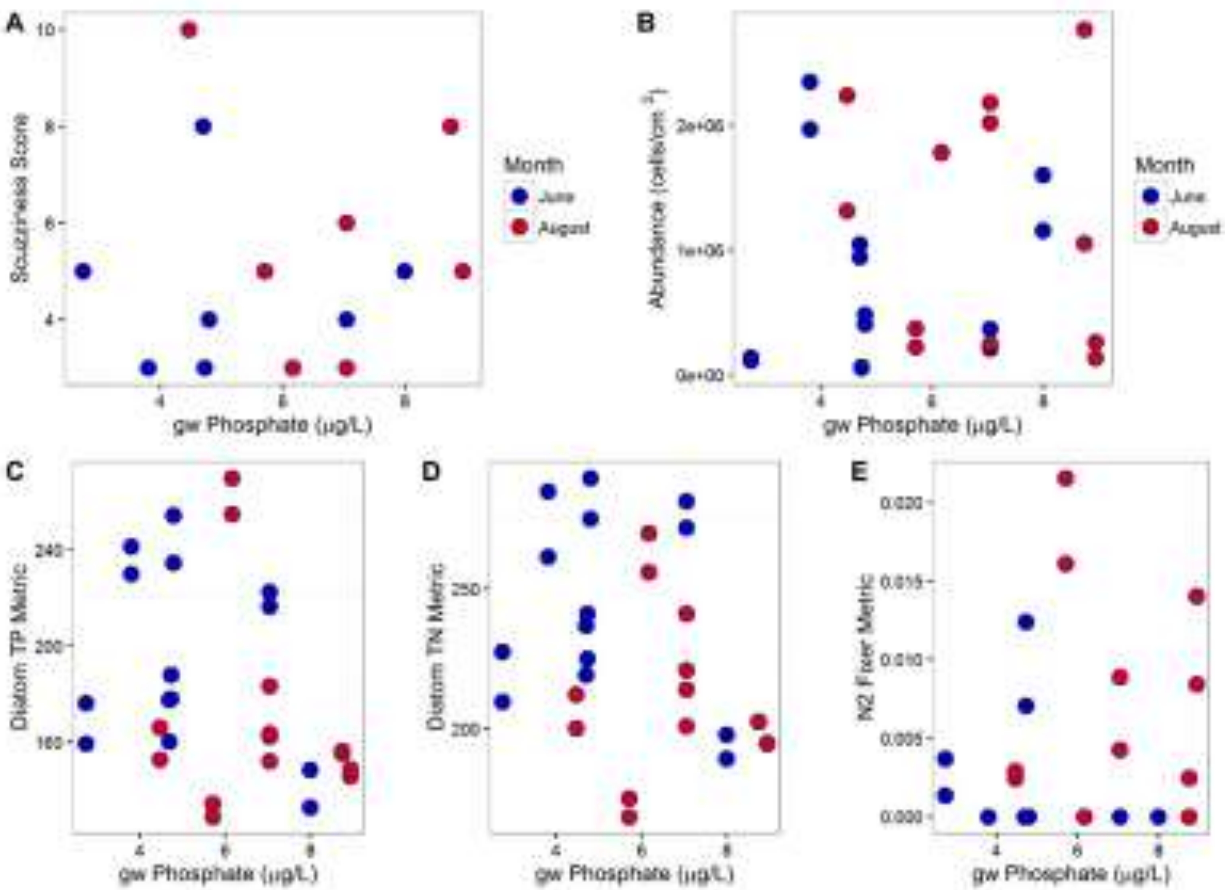


Figure 23. Relationships between groundwater NO_x concentrations (log-transformed) with scuzziness score, algal abundance (cells/cm^2), NLA diatom metrics for TP and TN, and proportion of diatoms capable of nitrogen fixation.

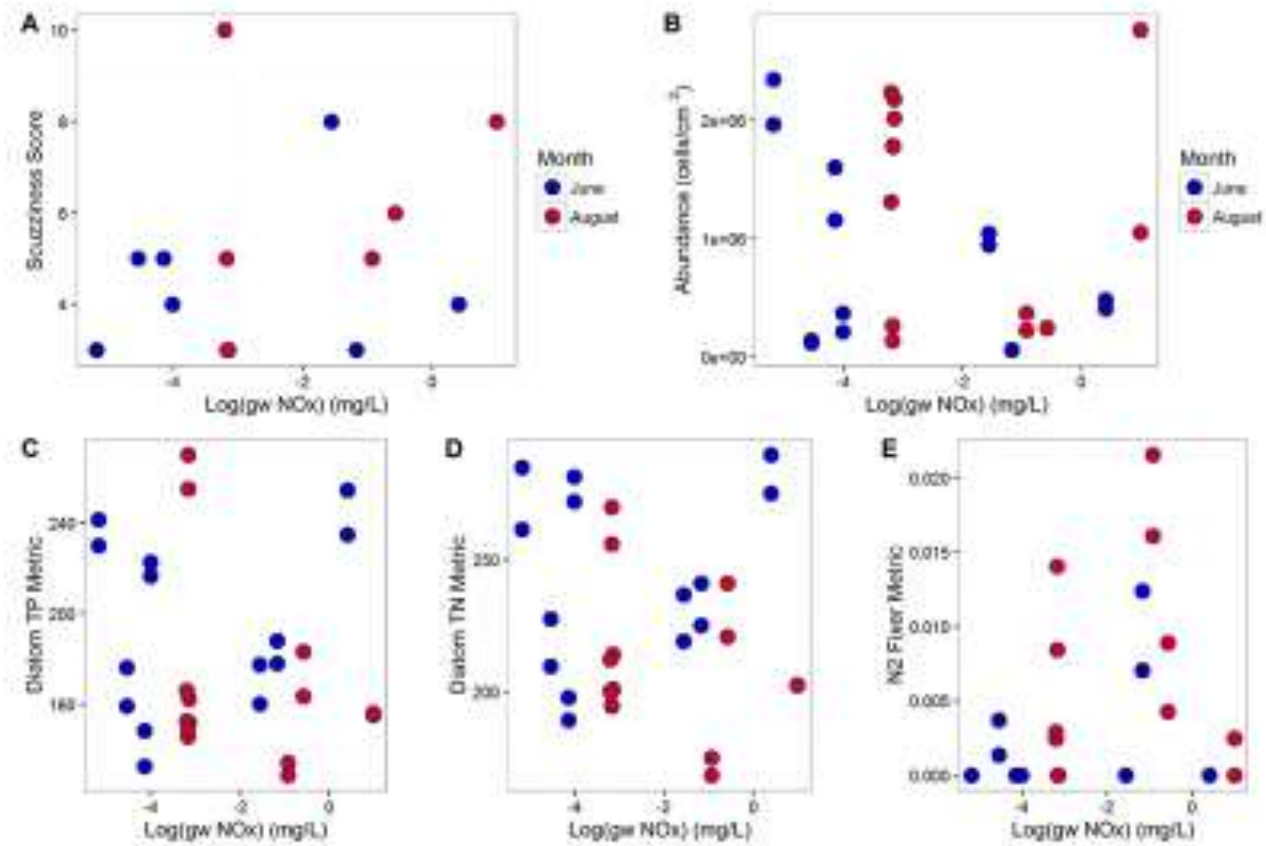


Figure 24. Relationships between surface water DIN concentrations (log-transformed) with scuzziness score, algal abundance (cells/cm²), NLA diatom metrics for TP and TN, and proportion of diatoms capable of nitrogen fixation.

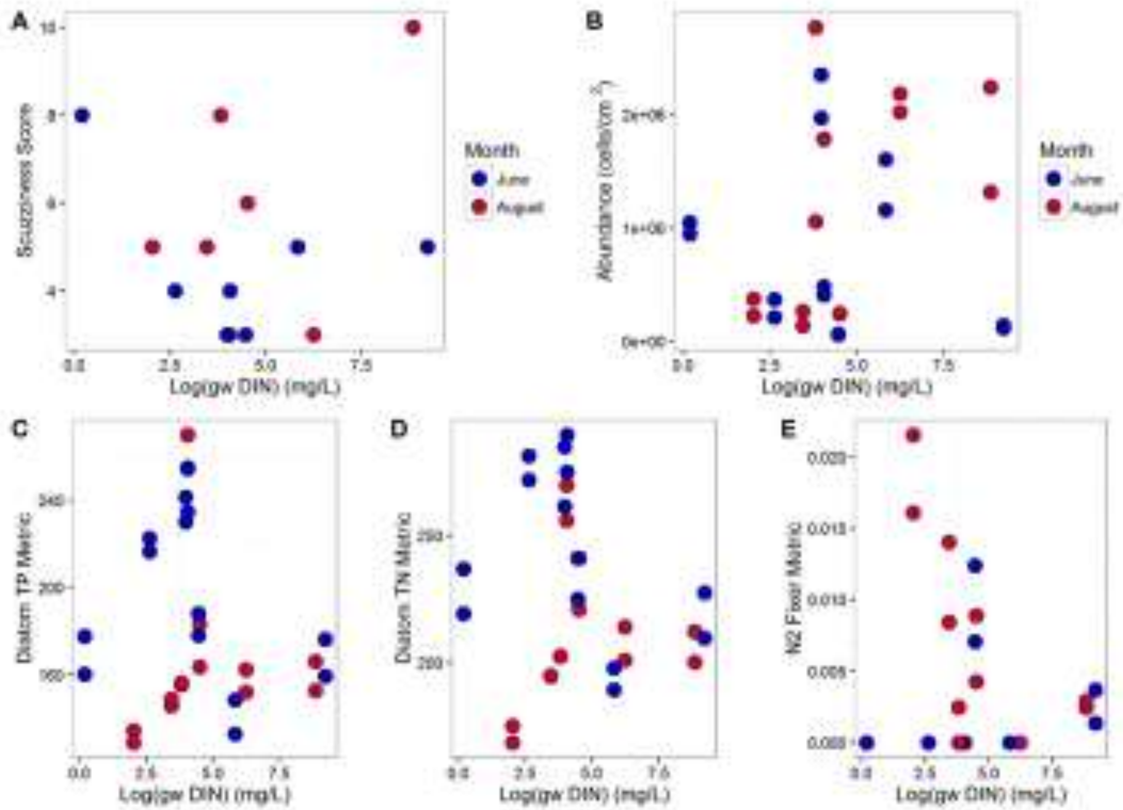


Figure 25. Variation in species composition among surface and deep sediment (_d) diatom assemblages illustrated by ordination.

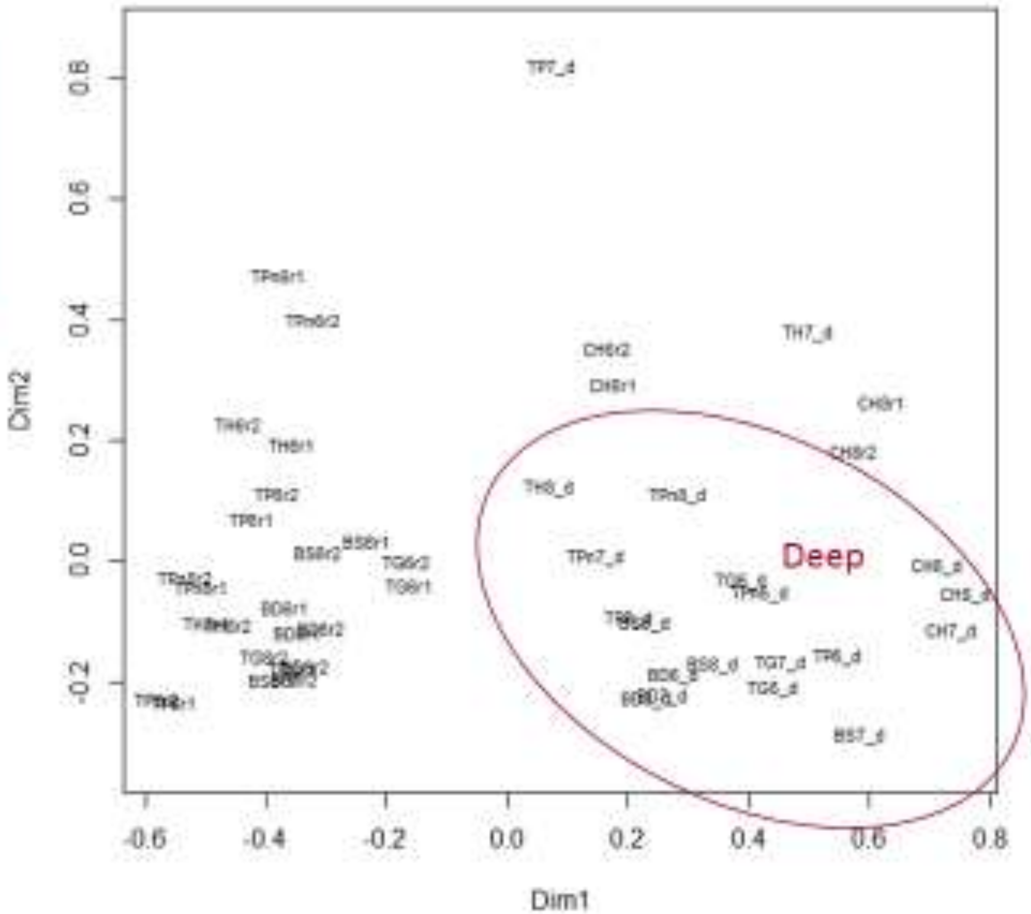


Figure 26. Differences between surface and deep sediments in NLA diatom metrics, N_2 fixers, and two Three Lakes diatom metrics for scuzziness and algal density.

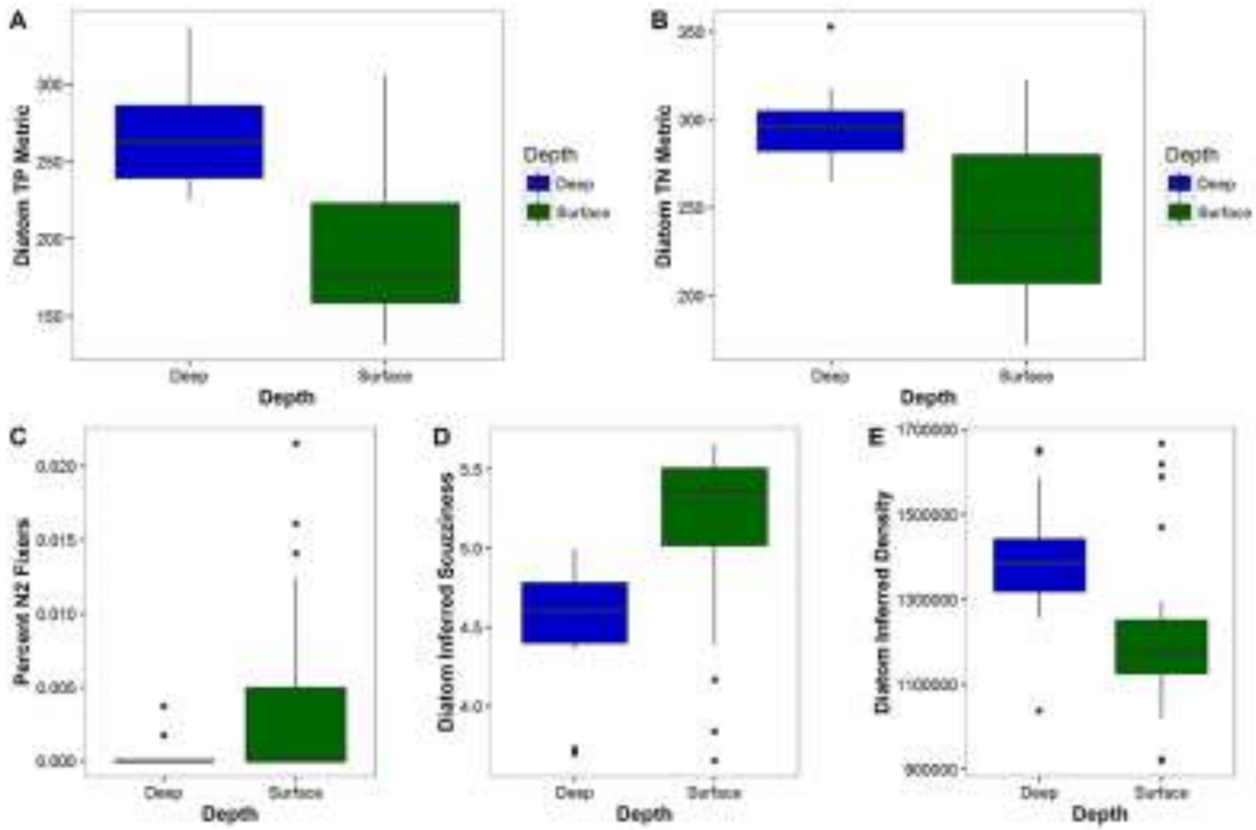


Figure 27. Diatoms in the genus *Cymbella* with mucilaginous stalks.

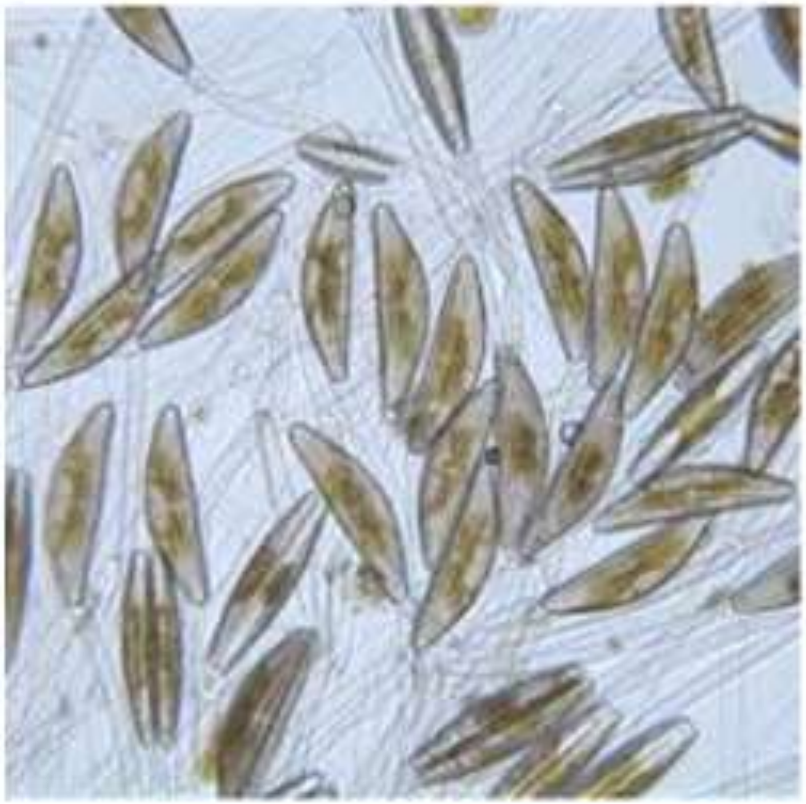
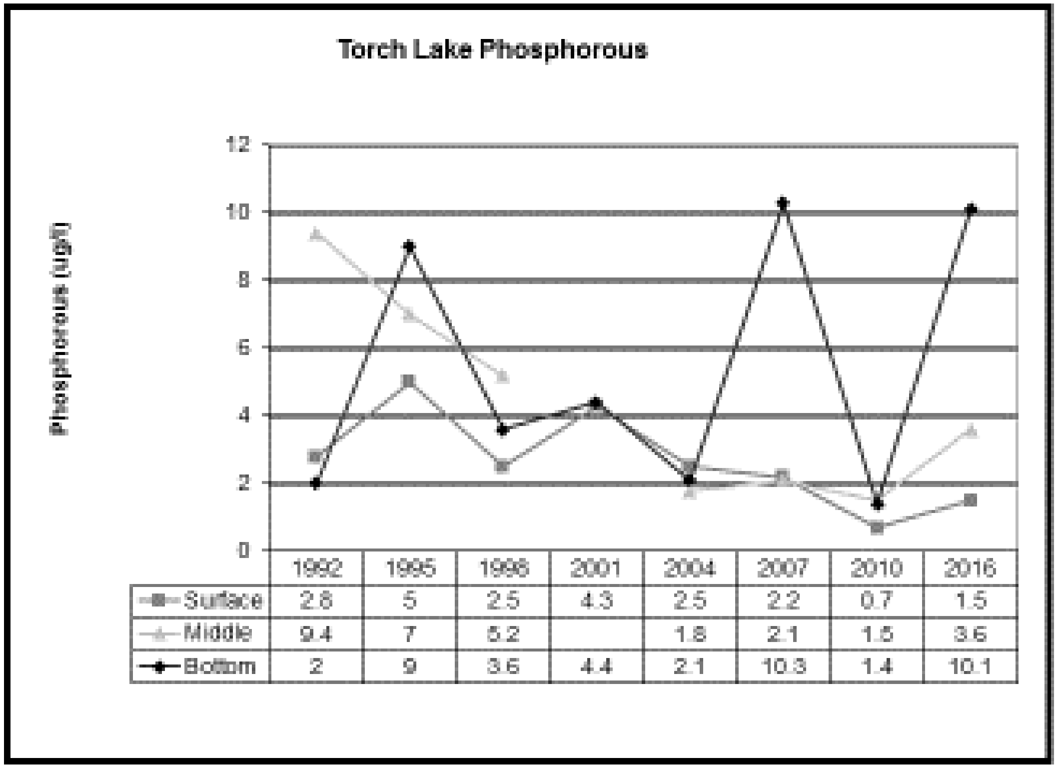


Figure 28. Idealized scenario of diatom succession on substrata with early succession stages of flatly and apically attached diatoms in early successional stages and stalked diatoms and filamntous green algae in late stages of succession.

Figure 29. History of total phosphorus concentrations in water collected from the surface, mid-depths, and deep water of Torch Lake by Tip of the Mitt Watershed Council (courtesy of Tim Veverica).



	1992	1995	1998	2001	2004	2007	2010	2016
Surface	2.8	5	2.5	4.3	2.5	2.2	0.7	1.5
Middle	9.4	7	5.2		1.8	2.1	1.6	3.6
Bottom	2	9	3.6	4.4	2.1	10.3	1.4	10.1

Figure 30. Figure 31. Annual total nitrogen deposition from atmospheric sources for 2000-2002 in kg N/ha (NADP 2016).

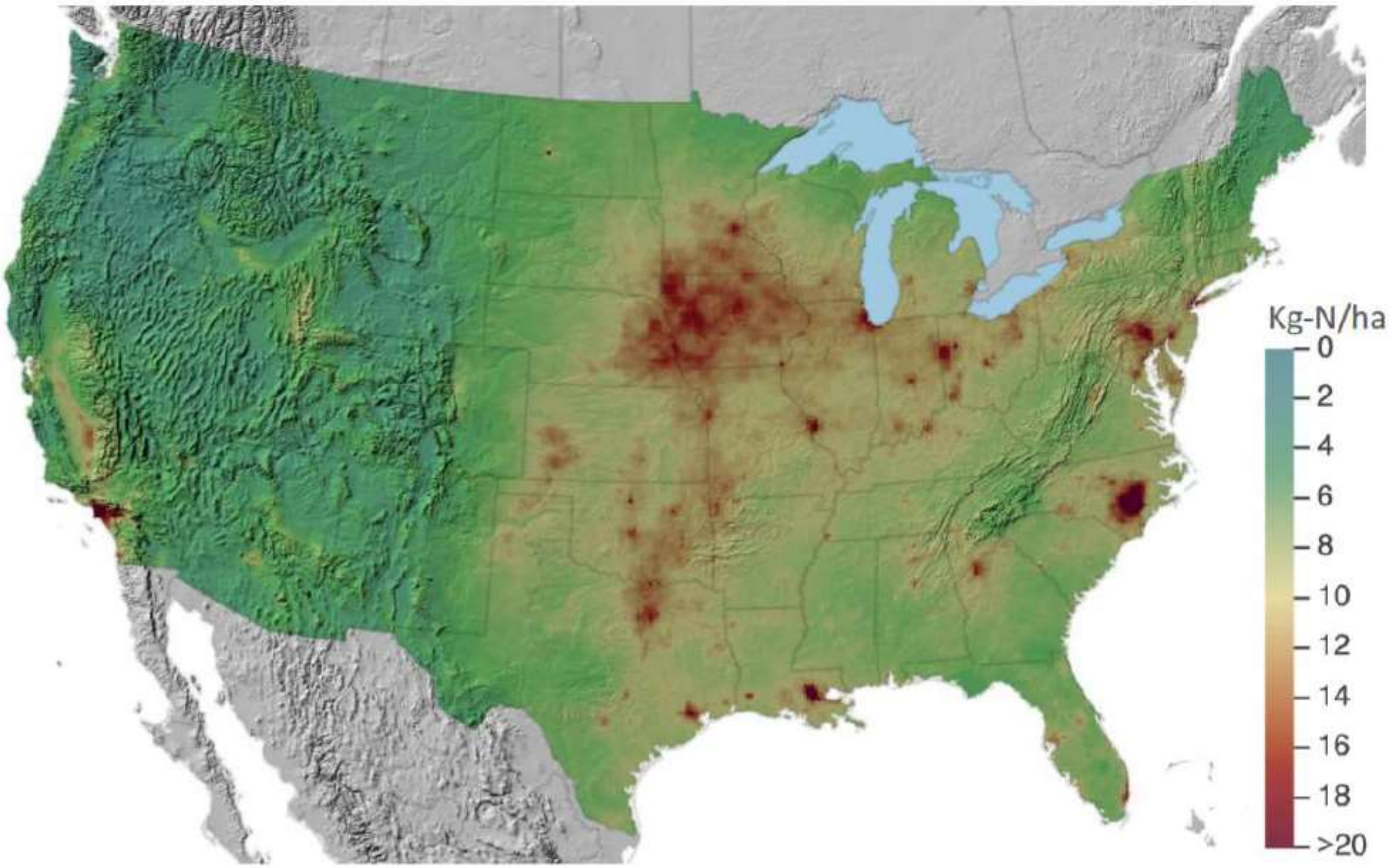


Figure 31. Annual total nitrogen deposition from atmospheric sources for 2000-2002 in kg N/ha (NADP 2016).