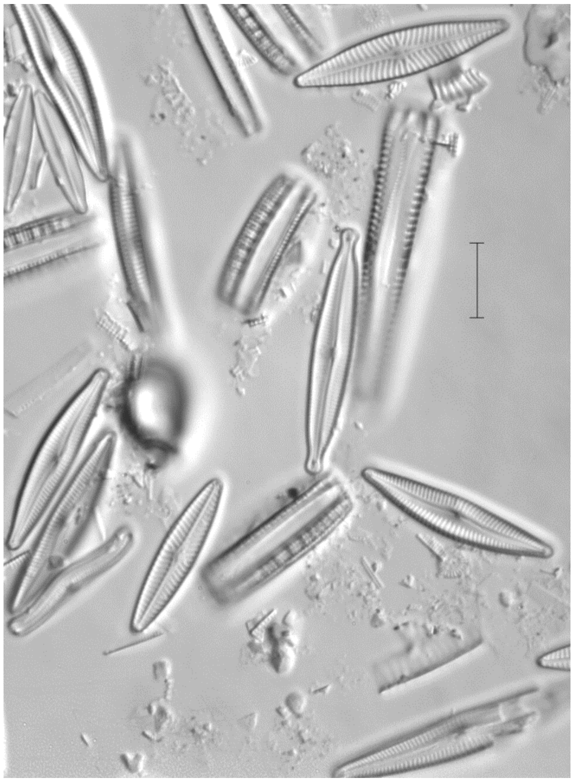
2017-2021 Studies of Golden Brown Algae on the Bottom of Torch Lake, Lake Bellaire, and Clam Lake

R. Jan Stevenson, Professor
Department of Integrative Biology
Michigan State University
East Lansing, MI 48824
October 25, 2021



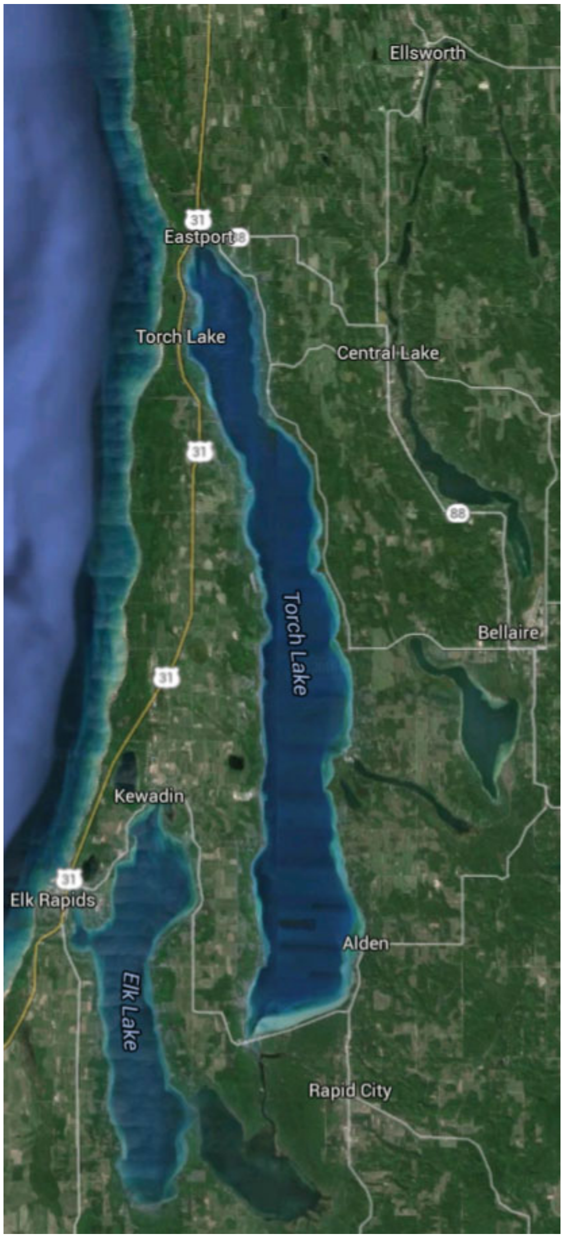


Image Credits: Stevenson's image of common benthic diatoms in Torch lake (scale bar = 10 µm). Map of Torch Lake, Lake Bellaire, Clam Lake and surrounding watershed by Google®.

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ACKNOWLEDGEMENTS

Acknowledgements usually go at the end of a report, but I wanted to put them at the beginning to make readers aware that many people put a lot of effort and funding into sampling and sample analyses that generated the data for this report. The Three Lakes Association supported all chemical sample analysis and benthic algal sample analysis and report writing from 2015-2018. Additional support was provided for my time by Michigan State University. The sampling team from Three Lakes Association was led by Becky Norris and commonly included Dean Branson, Rick Doornbos, Art Hoadley, Trish Narwold, and Fred Sittel. Tim Veverica headed the water chemistry analyses at the University of Michigan Biological Station for many years of the project. Rex Lowe and Pat Kociolek from the University of Michigan Biological Station collaborated on research during 2015. Brian Moore helped with set up of the *in situ* experiment. Sherry Martin from Michigan State University managed water chemistry assays during 2015. Bo Liu from Michigan State University identified and counted algae in samples from 2015-2017. Mariellyn Stevenson helped with field sampling on many occasions. Both the Tip of the Mitt Watershed Council and the MiCorps Cooperative Lakes Management Program at the Michigan Department of Environment, Great Lakes, and Energy provided long term water chemistry data. Finally, Becky Norris, Trish Narwold, Jeanie Williams, and Dean Branson reviewed a draft of this report.

EXECUTIVE SUMMARY

Golden brown algae (GBA) accumulating on the bottom of clearwater lakes in the northern lower peninsula of Michigan have been identified by people living around those lakes as a problem that has developed in the past 10 to 20 years.

In 2014, members of the Three Lakes Association (TLA) contacted three professors from Michigan State University and the University of Michigan Biological Station to help determine the causes. From initial meetings with TLA, we developed a set of hypotheses to test and thereby determine causes of GBA. The key hypotheses were: groundwater contamination by septic waste; change in nutrient runoff or atmospheric deposition; zebra mussels or other invasive non-native species; change in surface water temperature, clarity, or chemistry; and loss of animals eating algae on the bottom of the lake.

Starting during summer 2015 and continuing through summer 2020, algae on the bottoms of lakes and chemistry of water in the lakes, in porewaters among sand grains on the bottom of the lakes, and in groundwater have been sampled by TLA and assayed by professional laboratories to test our set of hypotheses. This effort has resulted in more than 100 algal samples and 1000 water samples with over 5000 chemistry assays. In addition, aerial photographs, satellite imagery and long-term datasets from state and regional partners have been analyzed.

Previous study has shown GBA is a matrix of algae, bacteria, and fungi growing on the bottom of the lake over sand and cobble, rather than among the sand grains as is common in sandy bottom lakes with low nutrients. The golden-brown color could come from a dominance of diatoms in ¼-½ inch thick mats, which is a type of algae with mostly golden brown rather than the green pigments used in photosynthesis by many algae and plants. Diatoms secrete mucilages outside their cells that can hold the mats of algae, bacteria, and fungi together. Cyanobacteria also have been observed in the GBA mats, but none of the cyanobacteria are known as toxin producing forms. Green algae, another common benthic algal group, were very rare in GBA samples. Based on literature reviews and discussions with colleagues, GBA is a different problem than the nuisance and harmful algal growth problems that have been studied in the past, so GBA could have different causes and require different method for management.

Aerial photography indicates that GBA was not widely distributed in Torch Lake in 2010 but was widely distributed in 2015. Aerial photography, confirmed by underwater video, shows GBA is widely distributed over most of the sand and cobble bottom of the nearshore shoal shallower than the drop-off. GBA cover of the bottom increases during the summer, but there is some spatial variability in GBA abundance and seasonal development around the lake.

Concentrations and ratios of phosphorus and nitrogen indicate phosphorus limits growth of algae more than nitrogen in Torch Lake and Lake Bellaire, the two lakes studied with GBA.

What has changed in lakes that could cause this change in algae on the bottom? One key factor could be decreasing phosphorus concentrations in surface waters for at least 20 years and perhaps as long as 70 years. These observations are based on strong evidence in histories of the lakes told by sediments deposited in them and by 20-30 year records from water samples from the lakes. Although groundwater entering the lake can have higher phosphorus than surface waters, there are few areas around the lake with evidence of humans contaminating groundwater by riparian septic systems, and there is no evidence of an increase in groundwater phosphorus over the last 15 years. Changes in light, temperature, and water chemistry (other than phosphorus concentrations) have not been sufficient or have not coincided with the timing of GBA development, where it occurs around the lakes, or the types of changes in algal species composition that we have observed.

Although our knowledge about the cause or causes of GBA is not sufficient to make decisions about large scale management or stop learning more about it with research, a plausible set of hypotheses can explain GBA development.

> Phosphorus concentration decreases in the mid-lake surface water may have limited species composition of algae to those species that live on the bottom of the lake and to diatoms and cyanobacteria that produce mucilages when limited by nutrients. This has been observed in other ecosystems. The importance of phosphorus in groundwater has increased as a source of this key limiting nutrient, because phosphorus has decreased in the surface water; even though groundwater is not contaminated with phosphorus at many locations where GBA occurs. Groundwater seeping into the lake through the sands on the bottom likely bathes benthic algae in low concentrations of phosphorus. These phosphorus concentrations are sufficient for GBA to grow slowly and steadily into thick mats that are relatively resistant to loss by animal consumption or by wave disturbance and currents because mucilages hold mats together.
>
> Although causes of decreased phosphorus concentrations in lake mid-lake surface waters are not well known, I hypothesize it is in part due to nitrogen deposition into oligotrophic lakes causing more efficient use of phosphorus by phytoplankton and then phosphorus settling out of the water column. The nitrogen revolution in fertilizer production with the Haber-Bosch process was extensive in the mid-1900s, which coincides with the evidence for early phosphorus decreases in lakes of the northern lower peninsula of Michigan. Zebra mussel invasion, which could also remove phosphorus from the water column and focus it on the bottom of lakes, did not occur until the late 1990s and into the early 2000s; but they may have been an important recent contributing factor to the surface water phosphorus decrease and thereby, widespread occurrence of GBA.

Long-term increases in chloride concentration and water temperature may be enough to shift benthic algal species composition and thereby, forms of algae on the bottom of the lake. In addition, interactions among multiple factors could be responsible for GBA development.

More research before management strategies for the lakes are changed. On-going efforts to manage additional nutrient loading into the lakes, especially via groundwater, are important so the GBA problem does not get worse. The question remains, “What has changed in Torch Lake, Lake Bellaire, and other northern Michigan lakes that caused golden brown algae to flourish on the bottom of the lakes?” Experiments, regional surveys, paleolimnology, and other approaches should be used to find causal relationships between changes in the lakes and development of GBA.

INTRODUCTION

Algae of oceans, lakes, streams, rivers, and wetlands are responsible for about a half of global carbon and oxygen cycling (Field, Behrenfeld, Randerson and Falkowski, 1998). Algae are also important bases of food webs in marine and freshwater ecosystems that support fisheries, many other ecosystem services, and as a result, human well-being. Despite the critical role that algae play in support of human well-being, and their potential as a source of renewable energy in the future, algae can be a nuisance or even hazardous for some of our uses of marine and freshwater ecosystems. Different kinds of algae cause different kinds of problems in freshwater and marine ecosystems. The most common problems in freshwater can be caused by: all algae contributing to dissolved oxygen depletion; cyanobacteria causing toxic phytoplankton blooms; filamentous green algae fouling streams or beaches and nearshore areas of lakes; and diatoms fouling beaches and nearshore areas, but typically to a lesser magnitude than filamentous green algae.

Table 1. List of hypotheses for golden brown algal mats in Torch Lake and Lake Bellaire.

1. Zebra Mussels & Relatives
2. Changing Light Conditions
3. Runoff from Big Storms
4. Disease Killed Grazers Allowing Algae to Grow
5. Non-native Invasive Algal Species
6. "Algae in the News and on Your Mind"
7. Climate Change & A Warmer Lake
8. Groundwater Contamination – Progressive
9. N Deposition Hypothesis
10. P Deposition
11. Increases in chloride
12. Nutrients from streams
13. Indirect and interactive effects of any factors.
14. Recent rise in lake levels

The Three Lakes Association (TLA) contacted several researchers from Michigan State University and the University of Michigan's Biological Station in 2014 and started discussions about a problem TLA called golden brown algae (GBA), which was growing on the bottoms of Torch Lake and Lake Bellaire. GBA is considered a nuisance for swimming, alters the aesthetics of clean sands on the bottoms of lakes, and may also affect other ecosystem structural and functional attributes, such as productivity, food webs, and biodiversity. In addition, GBA could be a sign the lake is changing more rapidly than the natural aging process would predict, thereby suggesting human causes of eutrophication and an early warning sign of potential groundwater contamination.

After some initial observations we developed a list of hypotheses for causes of GBA (Table 1) that included: groundwater contamination, zebra mussels, other invasive species, stormwater runoff of nutrients, and changes in water temperature and water level. This list of hypotheses was based on the algal ecology expertise of researchers and local stakeholder knowledge about what they have observed, recently and in the past, that could be the kinds of changes in the lakes that could cause GBA. So our approach involves basic steps in causal analysis: determine what has changed in the lake and determine if those changes could cause GBA.

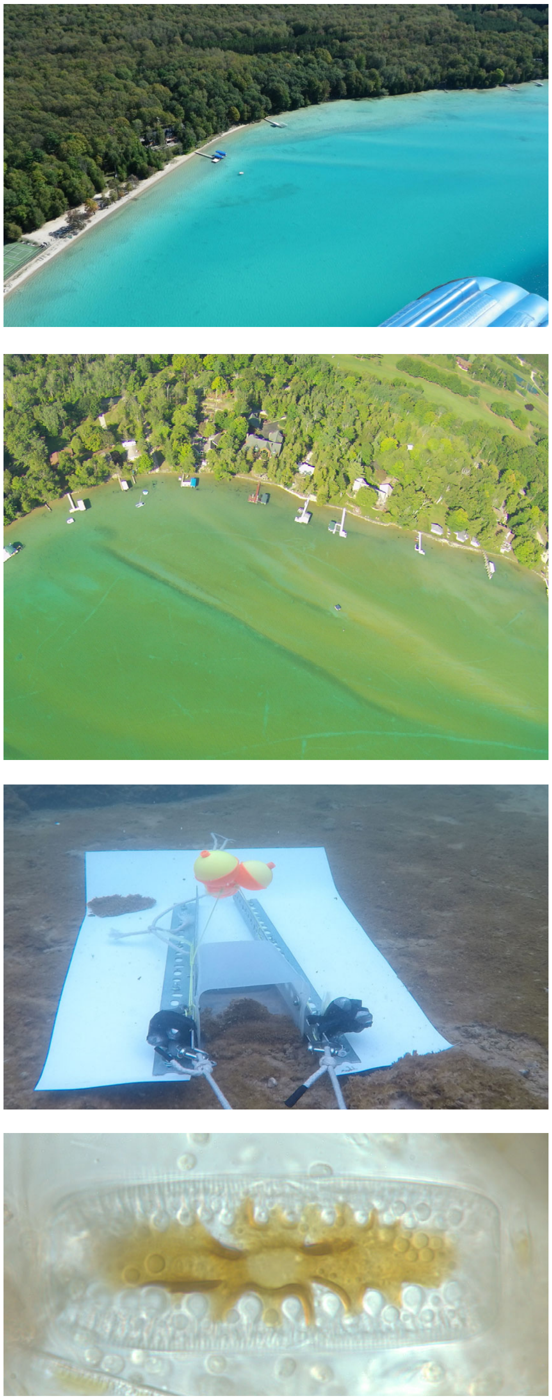
We initiated pilot studies in 2015 to determine the cause of GBA occurrence in Torch Lake, Lake Bellaire, and potentially Clam Lake. Every summer thereafter, research on the cause of GBA has continued, stepwise, focusing on hypotheses and adding new strategies, methods, and information based on what we learned the previous years. Early research resulted in four reports, two by me (Stevenson 2016, 2017) and researchers at the University of Michigan Biological Station (Lowe, 2016; Lowe and Kociolek, 2016). During that phase of research, our list of hypotheses grew as we learned more. The following report builds on those reports, an important groundwater study by Bretz et al. (2006), and it addresses results of research from 2017-2020 with data from 2015 and 2016 included in updated data analyses.

GBA was mostly found in Torch Lake and Lake Bellaire and determined to be mats of algae, bacteria, and fungi dominated by diatoms, which have golden brown pigments (Stevenson 2016, Fig. 1). The mats have great amounts of mucilage in them, much of which had been secreted by diatoms. The mucilages hold the mat together as can be seen in videos of the benthic algal sampler scooping up samples (YouTube Video Link: https://youtu.be/vQTnvGEx0No). Past work has found cyanobacteria in mats, usually in small amounts, and no evidence of cyanobacterial species that are known to produce toxins and thereby pose a health risk (Stevenson 2016, 2017).

Both phosphorus and nitrogen concentrations are low in the three lakes, and sufficiently low to limit algal growth; but phosphorus concentrations are so low in the lakes that phosphorus is the more limiting of the two nutrients (Stevenson 2016, 2017; Lowe and Kociolek, 2016). In addition, ratios of N:P concentration in nearshore surface water are so high, that phosphorus is in relatively low supply. Experiments by Sanchez, Lowe and Kociolek (Lowe, 2016) and Lowe and Kociolek (2016) also indicated phosphorus was the most likely nutrient affecting algal accumulation.

In these early years of study we also found phosphorus concentrations in lake floor groundwater sampled with piezometers were higher than both nearshore and mid-lake surface water concentrations, whereas nitrogen concentrations were lower in groundwater than

Figure 1. Illustrations of golden-brown algae in Torch Lake. From top to bottom: two aerial photos taken north of Sand Point in 2010 and 2020, respectively (note sand waves in same location); 1 cm thick mats of benthic algae remaining intact when sampled in container and laying on back of white sled; and a diatom from a Torch Lake mat sample with golden brown pigment (fucoxanthin) in its chloroplast.



these surface waters. We found little relationship between algal biomass measured by cell counts and visual assessments, which indicated algae were abundant below the sand surface at some sites. In addition, measures of algal biomass were not related to differences in groundwater or nearshore surface water chemistry (Stevenson 2017).

If phosphorus contamination of groundwater, runoff, or streams was causing GBA, we would expect to see spatial relationships between GBA and land use in riparian zones and stream locations. Visual observation by shore, boat, and Art Hoadley's aerial photographs have shown no consistent spatial relationship with tributaries, although a tendency of high GBA biomass north and south of the Clam River in Torch Lake has been evident. Gaulke (2021) with Environmental Consulting and Technology, Inc. also observed little relationship between riparian land use and benthic algae (a.k.a. crusts in their report), phytoplankton, or surface water chemistry.

Visual assessments of algal biomass in shallow nearshore zone by TLA volunteers have indicated an increase in GBA from June to a mid-summer peak in July and August, and then a decrease in GBA from August to September. Corresponding to this summer season change in GBA biomass was a shift in diatom species composition in mats from species requiring high nutrients to species tolerant of low nutrient concentrations (Stevenson 2017). The lack of correspondence of this change in species composition to changes in water column, pore water, or ground water concentrations indicated this change was due a decrease in mixing of surface water into the interstitial spaces in the GBA mat as biomass increased during the summer. Thus, the seasonal change in diatom species composition was likely regulated by changes in mat structure and perhaps secondarily related to exogenous factors like surface water or groundwater chemistry.

So, after the 2016 summer season analyses and my 2017 report about them, we were particularly interested in the seasonal development of GBA biomass, corresponding changes in species composition, and factors regulating those seasonal changes. At the time these 2017 studies were being designed (winter 2017), there were no indications of groundwater pollution causing GBA based on the complete analysis of 2015 and 2016 algal data and partial analysis of the 2015 and 2016 water chemistry data analyzed for my reports. So, what else could have changed in the lakes to cause GBA?

I became particularly interested in a new hypothesis that decreasing phosphorus in the lake of the northern lower peninsula of Michigan was causing GBA. Preliminary analyses of long-term data from Tip of the Mitt Watershed Council and Michigan's MiCorps showed phosphorus concentrations were decreasing in mid-lake deep basin surface waters, which would differentially favor the benthic versus planktonic habitat for algal growth because groundwater was a source of water with higher phosphorus than nearshore surface water (Bretz, Branson, Hannert, Roush and Endicott, 2006; Stevenson, 2017). In addition, algal mats were thick with mucilages, which are often produced by algae when they become nutrient limited (Kilroy and Bothwell, 2011). Mucilages can make benthic algal mats more resistant to physical disturbance (Hoagland, Rosowski, Gretz and Roemer, 1993). This hypothesis set up part of the rationale for the next three years of research in the lakes and my experiments.

Recently, a new paper was written and published in BioScience by Yvonne Vadeboncoeur and colleagues (Vadeboncoeur et al., 2021). The paper reviewed extensive literature and experience of the co-authors about worldwide proliferations of filamentous green algae on the bottoms of lakes. One of the primary causes of these nuisance algal problems is groundwater contamination and discharge into lakes. Unfortunately, problems with thick microalgal mats dominated by diatoms were not addressed in this paper. But the paper provides a wealth of new and synthesized ecological understanding of benthic algae in lakes.

Specific Purpose of This 2021 Report

This report provides an update on data analysis, literature review, and hypothesis testing to determine the cause of GBA. I review results of periphyton and water chemistry studies from 2017-2021, other data gathered earlier by TLA and me, and data reported by the Cooperative Lakes Monitoring Program (CLMP, Michigan's Department of Environmental Quality and now Department of Environment, Great Lakes, and Energy) and Tip of the Mitt Watershed Council (ToMWC). This report was written for those involved in the research and for the public that have a scientific background or basic knowledge of the lakes. The report may be difficult for many people to understand because of the scientific terminology, which is necessary to keep the report as accurate as possible and reasonably short. The TLA team plans to write a summary for the general public and the internet can serve as a glossary to support a broad audience of readers interested in the technical details.

Organization of Report

After this brief introduction, there is a methods section in which I describe the goals of successive years of sampling from 2015-2021, the sampling plan, sampling methods used, and methods used to analyze the data for addressing hypotheses. Then I present the results as they became available during the research. I present results of water chemistry first and then algae. Because the results are so long, I repeat some of the background information for why and how results were generated in the results section. I also discuss the results in the results section to explain why those results are important. Afterward I present a Discussion section in which I more thoroughly integrate the discussion of the results, and I present each of the hypotheses for GBA development that we have generated with explanations of the hypothesis and how it is related to GBA - plus an assessment of the evidence that either supports or contradicts the hypothesis. I also present a detailed explanation of the most likely explanation for GBA given current knowledge. Finally, I present some concluding remarks, and describe the ongoing work that I plan and recommend.

How to read this report

This is a relatively long and detailed, yet concise report. I have written the text so that it can be understood without looking at figures and tables. However, I do parenthetically reference figures and tables, which I did not put in the body of the report. If I put figures and tables in the body of the report, it would interrupt the text in sections that would often be as short as a paragraph with multiple pages of figures and tables separating sections of text. Thus, figures and tables can be found in this report after the Literature Cited section. I also provide two separate files for figures and tables so they can be easily accessed. Figures and tables are numbered consecutively through the document and in the figure and table files. For ease of reading if you do not have multiple computer screens, I suggest printing the report and opening the figure and table files on your computer so you can read the text and review the details of results in figures and tables.

METHODS

Common Sampling and Sample Analysis Methods

Water chemistry and benthic algae were most commonly sampled by trained TLA volunteers and me. I should note another important source of information is the collection of thousands of aerial photographs taken by a TLA volunteer and pilot, Art Hoadley.

Water chemistry

Water chemistry was usually sampled from three primary sources: 1) nearshore surface water with grab samples using acid cleaned polyethylene bottles; 2) benthic pore water samples by turkey baster insertion into sands and suction of water out of sands, draining pore water out of sands, and water in dialysis tubing placed in sand allowing for diffusion of ions into deionized water in the dialysis tubing; and 3) lake floor groundwater using lake floor piezometers inserted 1 ½ to 2 feet into the bottom of the lake. In addition, during one year we sampled household well water to determine chemistry of deep groundwaters, which was presumably water uncontaminated by riparian septic wastes. Also, on-shore seeps and lake floor groundwater were sampled during one year for comparison with lake floor samples.

Water chemistry samples were analyzed by staff at the University of Michigan Biological Station (UMBS) and supervised by Tim Veverica at UMBS for many of the summers. A long list of parameters were assayed over the 6 years of sampling and many were processed by Tim without compensation. The parameters assayed will be listed in the following section. Standard EPA-approved methods were used to assay samples, and in rare situations Tim experimented with new approaches. During one year, samples were also analyzed by Great Lakes Environmental Center, Inc. (GLEC), but those results and comparison to UMBS results are not presented in this report.

Benthic algae

Benthic algae on and among surface sands (epipsammic algae) were sampled by inserting a petri dish into sand, inserting a spatula under the petri dish to capture the sand in the petri dish, removing the sample from the lake, and then placing the sand into a Ziplock® or Whirl-Pak® bag. Rocks (epilithic algae) were sampled by scraping algae from the surface and placing scrapings into a Ziplock® or Whirl-Pak® bag. Benthic algal samples were frozen until they could be processed to avoid using toxic chemicals for preservation.

Species composition and abundance of benthic algae were determined by quantitative microscopic examination of samples and by measurements of the area sampled with petri dishes or surface of rocks scraped. Fully quantitative analyses of algae were completed in 2015 and 2016 showing that most benthic algae were diatoms. These samples were assayed with two counts of algae: 1) all algae were identified to lowest possible taxonomic level and cells were counted at 400X using a Palmer-Maloney counting chamber; and 2) acid-cleaning samples and mounting the glass frustules of diatoms in NAPHRAX to determine species-level identification of diatoms. Thereafter, most samples were only assayed for diatom species composition after acid cleaning samples. Exceptions are samples generated in experiments that are still to be assayed and future assays of 2020 samples that I collected at 3.3 m water depth from 16 stations around Torch Lake. All benthic algal samples were assayed at Michigan State University by Bo Liu for 2015-2016 samples or by me for samples thereafter. I harmonized taxonomy of all samples by reexamination of early samples to refine taxonomy of some difficult to distinguish taxa.

Consistent use of terms

I will consistently use the following terms to describe the source of water chemistry: mid-lake and nearshore surface water to distinguish surface water grab samples from mid-lake and nearshore samples because there may be a difference in some cases; benthic pore water for water collected from the interstitial spaces of surface sands (top 1-5 cm of sand); lake floor groundwater to refer to water sampled by lake floor piezometer; and well water to refer to water sampled from wells. I use these common terms for these different zones of sampling because often different methods were used to sample these zones. I use the term source to describe the zone from which water was sampled because, in part, we are sampling these 4 zones to determine the source of water at the sediment-water interface where the GBA is developing.

I will also use the term benthic algae to refer to algae occurring on the sand surface, attached to sand grains, or attached to rocks. Benthic algae are algae attached to or associated with surfaces in aquatic habitats, so this term contrasts with the more specific term of GBA which has been used by stakeholders to refer to the substantial accumulations of diatom dominated algal biomass on the surface of sands. As noted above, algae growing in mats on the sand surface or cells directly attached to sands by mucilages are referred to as epipsammic algae (epipsammon). Algae growing on rocks is referred to as epilithic algae (epilithon). Mats refers to algae with bacteria, fungi, and some very small invertebrates that form accumulations from ¼ -3/4 inch (1-20 mm) thick on the surface of sands and rocks. I usually reserve GBA to refer to the thick accumulations of what appear to be diatom-dominated benthic mats. So in this sense, all GBA are benthic algae, but not all benthic algae are GBA. I often use the term “we” to refer to the team of TLA volunteers and me.

Aerial photographs

From 2015 to present, Art Hoadley flew routes with his single engine float plane around Torch Lake and other northern Michigan shorelines to document nearshore conditions with aerial photography, and particularly the occurrence of benthic algae that looks like GBA. In addition, he took some photographs during 2010 and 2012 that will be valuable for estimating when GBA first developed in the lakes. Methods for using aerial photographs to estimate benthic algal biomass were developed and tested for this project and will be presented in the results of this report.

Gathering data from existing sources

Long-term water chemistry has been collected in the three lakes at deep-water locations by CLMP for almost 20 years and by ToMWC for almost 30 years. I downloaded the CLMP data for analyses and I got the ToMWC data from Tim Veverica, because the University of Michigan Biological Station has analyzed water chemistry for ToMWC for many years. Subsequently I got ToMWC data directly from their website with their permission.

Study Plans from 2015 to 2020

2015

Water chemistry and benthic algal samples were collected to determine what GBA was, to relate GBA to water chemistry, and to relate both GBA biomass and species composition to riparian land use. Samples were collected from Hayo-Went-Ha, Petty, and Gourley sites in Torch Lake with Hayo-Went-Ha assumed to have low riparian impact. Water chemistry and benthic algal samples were collected every two weeks from late June to September. Water samples included both filtered and unfiltered samples from piezometers and unfiltered surface water samples. The chemistry assays and calculations include: TP_ugL, NO3-N_mgL, NH4-N_mgL, dissolve nitrogen mgL (NO3+NH4), DN/TP ratios, and NH4/NO3 ratios. A report was produced for these results (Stevenson 2016).

2016

Water chemistry and benthic algal samples were collected to gather more data and increase our certainty for determining what GBA was, to relate GBA to water chemistry, and to relate both GBA biomass and species composition to riparian land use and types of water sources. Chemistry samples were collected monthly from multiple water sources (well, lake floor piezometer, benthic pore water collected by suction with a turkey baster, benthic sediment water drained from sampled surface sediments, and surface water. Samples were collected from 6 locations routinely: Gourley, Petty, Hayo-Went-Ha, and Penoza in Torch Lake and Drake and Southworth in Lake Bellaire. Samples were also collected from Hoadley's in Clam Lake, except for well water as there was no well on the property. The chemistry assays and calculations include: NO3_mgL, NH4-N_mgL, PO4-P_ugL; Cl_mgL; quinate_mgL, fluoride_mgL, lactate_mgL, acetate_mgL, propionate_mgL, formate_mgL, butyrate_mgL, nitrite_mgL, bromide_mgL, malonate_mgL, maleate_mgL, sulfate_mgL, oxalate_mgL, phosphate_ugL, citrate_mgL, A 545, boron. A report was produced for part of these results that were deemed most related to algal results (Stevenson 2017).

2017

Water chemistry and benthic algae were sampled in Torch Lake, Lake Bellaire, Elk Lake, and Lake Leelanau monthly from July to September. PO4-P concentrations were measured in samples from peepers on the sediment (sub-benthos) and buried a cm or so in the sediment (benthos), as well as a grab sample from nearshore surface water. Peepers, dialysis tubing filled with deionized water and protected in PVC tubes, were used to get more accurate spatially defined sampling of phosphate conditions than our routine pore water samples. Methods experiments were run to determine the amount of time needed for ions to diffuse into the dialysis tubing and reach equilibrium with surrounding nearshore surface water.

In addition, filtered (single) and unfiltered (triplicates) groundwater and filtered nearshore surface water (triplicates) was sampled during late July, August, and September at Petty, Gourley, and Hayo-Went-Ha sites. The chemistry assays and calculations included: TP_ugL; Fl_mgL; Cl_mgL; NO2_mgL; Br_mgL; NO3_mgL; NO3-N_ugL; SO4_mgL; PO4_mgL; PO4-P_ugL; NH4-N_ugL; and boron. In addition, BTH_ugL, DEET-ugL, caffeine_ugL, triclosan (ug/L), and Ace-K were assayed to provide evidence for contamination by human wastewater in samples from selected sites. Ace K is acesulfame potassium, an artificial sweetener), DEET is the chemical in insect repellents, BTH (benzothiazole) is a common chemical in consumer products (Liao, Kim and Kannan, 2018), and triclosan is an antibacterial compound found in soaps and cosmetics.

2018

Chemistries and algal species composition were assessed for a long summer season, May 15 to September 17, and at a relatively high frequency (every two weeks) to understand seasonal changes in water chemistry and benthic algae. We expected that more detailed seasonal sampling would help us understand GBA ecology and cause. Water chemistry samples were collected from an on-shore piezometer, on-shore seeps, lake floor piezometers, nearshore surface water, and benthic pore waters.

Chemical assays were done by both UMBS and GLEC for comparison, but we used UMBS data because they seemed to be more sensitive than GLEC data. Total and dissolved fractions of P and N were assayed.

Ace K was assessed from May to July from nearshore surface water, sub-benthic and benthic habitats, lake floor piezometers, and on-shore seeps.

In 2018, I ran the first two of three experiments. I ran one experiment in Torch Lake to determine the effect of groundwater on benthic algae. I ran a second experiment at my home to determine the importance of groundwater and nearshore surface water enrichment on benthic algal species composition. Details about these experiments are provide in the results section.

2019

Water and algae samples were collected biweekly from mid-June to late August from nearshore surface water, benthic pore water, benthic dialysis tube, and lake floor piezometers to determine if seasonal changes observed in 2018 (and earlier) were the same from year to year. Benthic algal samples were sampled at the same time. Benthic algal and water chemistry samples were collected at Petty, Drake, Southworth and Gourley sites. Late April water chemistry samples were collected from many tributaries. The chemistry assays and calculations include: fluoride_mgL; chloride_mgL; nitrite_mgL; sulfate_mgL; bromide_mgL; PO4-P _mgL; TP_ugL; TN_ugL; NH4-N_ugL; pH; NO2-N_ugL; and NO3-N_ugL.

2020

Water chemistry and benthic algal samples were collected to continue the analysis of summer trends, diurnal variation in water chemistry, and differences between nearshore and open water (offshore) surface water chemistry. We added diurnal variation in water chemistry to detect benthic algal regulation of nutrient flux out of sediments. We compared nearshore and mid-lake water chemistry to determine whether differences might occur that could be related to groundwater discharge and slow mixing of nearshore and mid-lake open waters. Water and benthic algal samples were collected biweekly from early June to mid-August at Gourley, Petty, Clam DNR, and Southworth. Integrated water samples were also collected at CLMP sites to compare to nearshore chemistry. Benthic water, nearshore surface water, and lake floor piezometers were sampled at the standard mid-day time. In addition, nearshore surface water and benthic water were sampled in the early morning and evening to evaluate diurnal variability. The chemistry assays and calculations include: TP_ugL; PO4-P_ugL; TN_ugL; NH4-N_ugL; fluoride_mgL; Cl_mgL; NO3-N_mgL; sulfate_mgL.

In 2020 I ran a third experiment at my house to determine the independent and interactive effects of nitrogen and phosphorus enrichment on benthic algal species composition.

Samples Assayed

All water chemistry samples have been assayed from 2015-2020. Benthic algal samples from the lakes from 2015 through 2018 have been analyzed. Only part of the benthic algal samples from my 2018 experiments have been analyzed. None of the samples from my 2020 experiment have been analyzed. This totals over 100 benthic algal samples and 1000 water chemistry samples being analyzed with between 1 and 15 parameters being analyzed for each water chemistry sample.

Aerial photographs

From 2015 to 2020 Art Hoadley took aerial photographs from his plane that he built, a Kitfox IV on Zenair straight floats without wheels. He flew routes around the shorelines of the three lakes, and other lakes as well. Photographs were usually taken as early in the season as conditions were safe for flying, which usually was May. He also flew routes and took aerial photographs later in the season when we expected GBA would be most developed (mid-August). During some seasons, aerial reconnaissance and photographs were done during other months as well. A few pictures were taken during 2010 and 2012. I analyzed the 2020 photographs in detail to compare to my underwater videos of benthic algae that I took when sampling algae around Torch Lake. Using that comparison, I tested methods for assaying benthic algal biomass with aerial photography. I also reviewed photographs during other years to determine consistency with the 2020 observations.

Data Analysis

Data compilations and analyses were conducted using R programs to provide a complete record of the steps of data aggregation and data analysis. All water chemistry data were compiled for 2015-2020, checked for accuracy and converted to common units of measurement.

To keep the reporting of results as clear and understandable as possible, I will **not** refer usually to the details of the statistics used or the specific probabilities of a pattern in results occurred by chance. The latter is referred to as the P value or chance of an observed pattern if results were actually just random. The details of those statistical methods and results will be presented in tables with those results.

Patterns in the data, e.g. changes over years at sites in the lakes or differences among water sample sources at sites, were evaluated with a diversity of statistical approaches for this report. When I report changes in conditions over time or differences among sites or sources of water among sites, I will only report those results that have a low probability of occurring by chance – unless I explicitly state another reason. Statistically speaking, that would be referring to a P value (attained significance) less than 0.05, meaning a 5% or 1 in 20 chance that the pattern could be observed by chance. Magnitudes of differences, as well as a low probability of the difference being observed by chance, are also important when reporting results. Usually, differences with large magnitudes have low probabilities of occurring by chance, but not if there is high variability in measurement methods or due to different factors than those considered in the analysis. These kinds of issues will be addressed if deemed important for testing and evaluating results and hypotheses in the report.

Long-term and summer season trends in water chemistry, Secchi depth, chlorophyll a, and diatom species relative abundances were characterized with parametric and non-parametric linear regression, polynomial regression when non-linear trends were suspected, and Kendall-Theiu-Seigal non-parametric regressions when data had many chemistry measures below detection limit. This latter problem was greatest for some CLMP TP measurements. Measurements of water chemistry that were below the minimum detection level were revised to be half of the value of the minimum detection level since I did not want to eliminate the information those determinations provided, and they were known to be someplace between the minimum level of detection and zero. Water chemistry data were often transformed by adding 1 to values and then transforming the X + 1 values with a log-based 2 function. This log2+1 transformation reduced skewness in data to provide more accurate characterizations of whether patterns in long-term or summer trends and differences among sites and among water sources could be observed by chance. 1 was added to values because the log of 0 cannot be calculated. This transformation is commonly used in statistical analyses.

Water chemistry data collected by ToMWC were collected from mid-lake deep basin sites at three depths. I used surface measurements or the surface and mid-depth sample results in most analyses to increase sample size and ability to detect statistical significance of trends. When there was a concern that the mid-depth sample might not be biologically relevant, as for example with TP concentration, I added an analysis of long-term trends with mid-lake surface water samples alone.

RESULTS – Water Chemistry

What has changed in the lakes that could cause development of GBA?

Long-term changes in water chemistry

We have two datasets, ToMWC and CLMP to evaluate long-term changes in water chemistry and Secchi depth in Torch, Clam, and Bellaire. CLMP starts in 2004 with sampling and results reported annually until 2019. Sampling was late in 2020 because of COVID, so I did not update my dataset to include those data. ToMWC sampling and results start in 1992 and occur every three years until 2019. I have analyzed both these datasets for long-term trends with regression analyses. Results are plotted in some cases with individual data points and also with lines representing linear regression relationships for measured conditions with the goal to show readers the different ways to think about patterns in the results.

TP Results: Analyses of TP results were challenging because many TP values were below the detection limit. For my statistical analysis, I changed water chemistry values from less than detection limit to half the detection limit. In one analysis, I used parametric linear regression and reported those results in Table 2. Skewness (many low values) in the TP data was reduced by transforming TP data with a log-base 2 + 1 function for the parametric linear regression.

Phosphorus concentrations are likely decreasing in the deep basins of Torch Lake and Lake Bellaire (Table 2, Figs. 2-6). Although interannual variability was high (illustrated in Figs. 2 & 4 with year-to-year changes connected by lines), a long-term trend of phosphorus was evident in both the ToMWC and CLMP datasets during both spring and summer (with long-term linear trends illustrated in Figs. 3, 5, 6). CLMP data over the last 16 years show phosphorus concentrations have steadily decreased. ToMWC data also show TP concentrations have decreased over the 1992-2019 time period when using samples from the top two sampling depths, even though there was some evidence for an increase in TP in Torch Lake and Lake Bellaire in 2016 and 2019 (Fig. 8). Linear trends in TP concentration were statistically significant in ToMWC TP data when using the top two depths to run the analysis. However, the non-linear trends in ToMWC data were also statistically significant (Table 2A), indicating two possible relationships: 1) a negative exponential decay such that TP decreased to a minimum concentration and stayed close to that level, where that minimum is partly defined by a minimum level of analytical detection; or 2) a decrease in TP with a subsequent increase in the latest years. With the data currently available, the difference between options 1 and 2 cannot be distinguished reliably. Note, the 2016 and 2019 increases in spring TP in ToMWC data (Fig. 6) were not observed in long-term CLMP data in Torch Lake, but a couple high TP concentrations were observed in Lake Bellaire and Clam Lake during the past 2015-2020 period. The high P during 2016 and 2019 in ToMWC data could have been related to weather disturbances or actually an end to the processes that caused the decrease in TP over the last 30-70 years.

I initially chose to analyze ToMWC data using the two samples from the shallowest depth to have a sample size as high as possible and based on the assumption that the top two depths would be most associated with phytoplankton communities interacting with nutrients. I ran a sensitivity analysis to evaluate those issues and found the P values for ToMWC TP models with just surface samples were lower than when two samples per year from the top two depths were used. P values for ToMWC TP models with all three depths (surface, mid-depth, and bottom) were sometimes as high as the initial models with the top two depths, and sometimes not (Table 2).

Even with the log2 plus 1 transformation, many low (i.e. below detection limit) values skewed the data. To further evaluate the likelihood that the negative relationship between TP could be observed by chance, I conducted nonparametric regression and a simple probability analysis. The nonparametric regression used the Seigal variation of the Kendall-Theil regression, which calculates the magnitude of change per year between all pairs of observations to determine the model slope as the median slope, which is the change per year in this case. This nonparametric regression analysis also showed a relatively high certainty for a decrease in TP over time and not likely due to random variation in the data (Table 3). I conducted numerous additional analyses of the long-term TP patterns in data from CLMP and ToMCW from Torch Lake, Lake Bellaire, and Clam Lake (e.g. Table 2A). In addition, I reviewed data for many other lakes. These analyses usually show the decrease in TP concentration over the period samples were collected. You might suspect that analytical techniques have been improved over this time period and perhaps that is why we see the decrease in TP concentrations. However, the methods for analysis of phosphorus are largely unchanged, and detection levels were as low back in the 1970s and 80s as are reported now for the lakes. More years of data will be required to determine whether there is now an increase in TP following the long period of TP decreasing as suggesting in the non-linear trends.

Decreasing phosphorus concentrations in lakes of northern lower peninsula of Michigan started in the mid-1900s, as suggested by review of the paleolimnological study by Fritz, Kingston and Engstrom (1993). They analyzed sediment cores of lakes to determine age of sediments and relative abundance of diatom species in the sediments that had accumulated during the last 290 years. Using the relative abundances of diatom species and known species optima for TP concentrations, they inferred changes in TP concentrations over that 290-year history for four lakes: Bellaire, Elk, Glen, and Intermediate. Their results (Fig. 7) show decreases in diatom inferred TP concentrations in three of the four lakes, starting about the middle of the 20th century. I wrote that this decrease in TP was suggested in this study because Fritz et al. determined the variability in diatom inferred TP was too great for high statistical certainty in their analyses and reported trend. However, when considering both the Fritz et al. (1993) results and the long-term decreases in TP found by in ToMWC and CLMP data, the certainty increases that TP concentrations have been decreasing in lakes of the northern lower peninsula of Michigan for 30 years and likely 70 years. In addition, this decrease in TP is being observed in many lakes across northern Michigan based on a review of ToMWC and CLMP data that is not presented in detail in this section of the report.

The reasons for this phosphorus decrease are not known, but Fritz et al. (1993) quote Dr. Eugene Stoermer's hypothesis that atmospheric nitrogen deposition from suspended fertilizers used in the Midwest could cause a decrease in TP in lakes. Nitrogen deposition is known to be a major source of contamination, and it likely peaked soon after 2000 because of new regulations (National Atmospheric Deposition Program, 2021). Dreissenid mussel filtration of algae from the water column of lakes has also been proposed for the oligotrophication of lakes (Cha, Stow and Bernhardt, 2013), but Dreissenid mussel invasion in the Great Lakes region did not occur until after 1988 (Nalepa and Fahnensteil, 1995) and then inland lakes afterward with the Elk River Chain of Lake around 1994 (Ackerman et al., 2009). The potential significance of long-term decreases in TP for GBA and likely causes of this change will be discussed more in the Discussion section of the report. This long-term decrease in TP provides one answer to our question about what has changed in the lakes that could cause GBA. Later I will discuss more about why a decrease in nutrients could cause an increase in algal biomass on sand.

Spring TN remained unchanged across the 1992-2019 ToWMC sampling period and at all three locations with a long-term record (Bellaire, Clam, and South Torch stations) based on the small changes observed in regression models and high year to year variability (Fig. 8, Table 2). Nitrate-N concentrations remained unchanged over that same period at the Bellaire and Clam Lake sites, but NO3-N concentrations decreased at the South Torch site (Fig. 9). As a result, the ratio of bioavailable nitrogen (as nitrate) to total nitrogen is decreasing over the 1992-2019 period, indicating an increase in nitrogen demand.

Chlorophyll a concentrations were very low in Torch Lake, below normal detection limits of 1 ug/L, and therefore no change over time could be detected in CLMP data (Fig. 10, Table 2). Chlorophyll a concentrations in Lake Bellaire and Clam Lake were higher than Torch Lake and showed a tendency to decrease over the 2004-2019 CLMP monitoring period; but both of these trends were not reliable because they had moderate chances of occurring by random variation in data because variation in data around the calculated regression line was relatively high compared to the rate of change in chlorophyll calculated over the 16 year time period.

Secchi depth transparency measured for the CLMP program increased at both sites in Torch Lake, decreased in Clam Lake, and was unchanged in Lake Bellaire during the 2004-2019 period (Fig. 11, Table 2). The increase in Secchi depth in Torch Lake is likely related to decreases in phytoplankton abundance or their metabolism causing calcium precipitation in the water column, both of which can decrease water transparency. Chlorophyll a data do not show an increase over this time period in Clam Lake, therefore the decrease in Secchi depth in Clam Lake could be related to increases in brown staining of water by dissolved organic carbon from upstream wetlands rather than an increase in phytoplankton abundance. The lack of change in Secchi depth over the monitoring period and the fact that phosphorus decreased in Lake Bellaire is difficult to explain without long-term chlorophyll a data to measure phytoplankton abundance.

pH in ToMWC records showed an increase resulting from a sudden change between 1997 and 2000. Before and after that date pH changes little (Fig. 12, Table 2). The reasons for this pH change are not known. Perhaps the method for collecting samples for pH or measurement of pH changed. It seems unlikely that there was a sudden change in environmental conditions, biological or physicochemical, that caused this change to occur in all three lakes at the same time. Review of other ToMWC data is warranted to see if this punctuated change occurred in all data or just data for these three lakes.

This punctuated change in pH is not observed in specific conductivity, which remains relatively unchanged in the ToMWC records from 1992-2019 in all three lakes (Fig. 13, Table 2). Often pH and conductivity are highly related in other studies. If they are here too, then the lack of a threshold in conductivity between 1997 and 2000 also indicates the pH threshold needs to be studied further.

Chloride concentrations increased greatly during the 1992-2019 ToMWC monitoring period for all three lakes (Fig. 14, Table 2). The magnitude of Cl concentration change was greater in Torch and Bellaire compared to Clam. Chloride concentration doubled in Torch and Bellaire, increasing over that time period from estimated lows near 4 and 6 mg/L to highs near 9 and 11 mg/L, respectively. Chloride concentration changes over this range in Lake Michigan have been raised as a cause of concern by E. F. Stoermer (personal communication). In a subsequent paleolimnological study to test this hypothesis, changes in diatom assemblages of Fonda Lake, Michigan were associated with the installation of a salt storage facility near (Tuchman, Stoermer and Carney, 1984). Although we do not see the high chloride species of Fonda Lake in abundance in Torch Lake and Lake Bellaire, further investigation of chloride preferences of diatoms and cyanobacteria and relationship to taxa in GBA would help evaluate the potential effects of chloride on GBA.

Comparing Lake floor groundwater Phosphorus in Bretz et al. (2006) versus 2015-2020

Another source of historic data (16 years ago) is the Bretz et al. (2006) study of groundwater P input into Torch Lake. Groundwater was sampled during 2005 from piezometers driven into the bottom of Torch Lake at 13 nearshore locations. Two locations had two piezometers, which were grouped by site in my analysis. Twelve of the 13 locations had total phosphorus measurements for the nearshore surface water. I compared TP concentrations of nearshore surface water and groundwater at sites measured by Bretz et al. (2006) and by TLA from 2015-2020 at four Torch Lake sites. Samples were collected from May to early October in both study periods.

Nearshore surface water and ground water concentrations of TP differed little in this comparison between 2005 and 2015-2020 sample periods. Nearshore surface water and groundwater water TP concentrations were, respectively, 1.0 and 1.4 ug/L higher in 2005 than 2015-2020 (Figs. 15-16). Therefore, we do not see an increase in TP concentrations that we would associate with groundwater contamination increasing over that 10-15 year time period, which we would expect if GBA appearance occurred as a result of increasing phosphorus contamination in groundwater. I would also not conclude with certainty that there was a decrease in TP concentrations over those time periods, even though the statistical probability (P values) of a decrease in TP concentration differences for nearshore surface water and groundwater, were respectively 0.10 and 0.001 according to analyses of variance and Scheffé multiple comparisons analysis. I describe these statistics in this case because I do not have a table where these results are explained.

So, how much credibility should we give these observed decreases in nearshore surface water and groundwater TP concentration over this 10-15 year period when GBA developed, and what could explain them? First, decreases in groundwater concentrations from 2005 to the 2015-2020 period could be due to the different sites selected during the two projects. Only the Hayo-Went-Ha site (site 4 in 2005) was sampled during both periods. Groundwater was only monitored at three sites in Torch Lake from 2015-2020 versus 13 sites in 2005. There was great variability among sites in 2005, with five sites in the 2005 study having groundwater TP concentrations averaging close to 30 ug/L and one site averaging 66 ug/L. While there was considerable interannual variability in the 2015-2020 period, the differences among the three sites were much less than among the 13 2015 sites. Thus, the likely reason for the observed decrease in P in the dataset from the 2005 pre-GBA period to the 2015-2020 GBA period is the sites selected for study.

However, I must note the coincidence that the 1.0 ug/L groundwater difference between 2005 and 2015-2020 is the same as calculated for mid-lake surface water with regression models using long-term CLMP and ToMWC data. Those models estimated a 0.10-0.15 ug TP/L decrease per year in mid-lake surface water. If the difference in TP from 2005 to the 2015-2020 period was not due to random error or site selection, then perhaps decreases in phosphorus in lawn fertilizers could have resulted in a decrease in phosphorus in nearshore lake floor groundwater where piezometers were located. We would not expect a lake wide decrease in groundwater P to result from a surface water decrease in P because groundwater in most areas of Torch Lake is being discharged into the lake, and surface water is not entering (recharging) the groundwater.

Chemistry Differences Among Water Sources in 2016

Goals, rationale, and approach

In 2016, water chemistry sampling focused on the question of differences in water chemistry in nearshore surface water, interstitial pore waters of surface sands, lake floor groundwater, and well water to understand the potential for contamination of lake floor groundwater entering the lake and how that affected the benthic environment at the sand-water interface where GBA grows. Elevated concentrations of PO4-P, NO3-N, NH4-N, or Cl in lake floor groundwater entering the lake (collected with piezometers) versus well water would indicate riparian contamination of water entering the lake. Differences in water chemistry from lake floor piezometers, benthic pore water, and nearshore surface water would help us understand how water chemistry is diluted or altered by benthic activity in ways that could affect the ecology of algae growing on the bottom of the lake.

Water chemistry was sampled in June, July, August, and September at six sites: Gourley, Petty, Hayo-Went-Ha, and Penoza sites on Torch Lake; and Drake and Southworth site on Lake Bellaire. Water chemistry was also collected at the Hoadley site on Clam Lake, but well sampling was not replicated there. Therefore, the Hoadley site data were not evaluated in this section of the results focusing on well water during the only year it was sampled. It will be included in some following analyses of spatial and temporal trends in water chemistry.

Sampling of these habitats is described best by this description by Dr. Becky Norris.

A. Benthic sediment: A sample - about a cup full - of benthic sediment was collected from 3 - 4 inches below the sediment surface. The collection jar was empty (filled with air) and lowered to the collection site inverted so as to minimize the chances of simply collecting lake water. The water component of the sample was analyzed for chemistries.

B. Lake floor groundwater: Piezometer well point collections from about 2 feet deep into the lake floor.
C. GW Human: Same as lake floor groundwater, a bigger volume intended to be analyzed for the anthropogenic markers.
D. Pore water: Turkey baster suction (a la Rex Lowe) of water from inch or two deep in the lake floor sediment. Might have been thought to be comparable to the benthic sediment samples.
E. Well water: Household well water taken from an outside hose bib.
F. Surface water (nearshore): Grab sample within several inches of the lake water surface.

In my analyses, pore water collected with a turkey baster was renamed benthic water. The benthic porewater collected with the benthic sediment sampling method and turkey baster methods were kept separate in my analyses to compare results and learn about sampling this habitat. GW Human was grouped with lake floor groundwater since they were both collected with the lake floor piezometer. Comparisons among water sample sources were evaluated statistically with analyses of variance and Tukey pairwise comparisons to determine differences in chemistry among water sources that were consistently sampled among sites.

Results and Discussion

No difference was observed in PO4-P concentrations among water sample sources (Fig. 17, Table 4-5). This is important because it shows that nearshore lake floor groundwater is not greater than well water, and therefore not likely contaminated by riparian activities at the sites we sampled. In addition, no difference was observed between lake floor piezometer, either measure of benthic pore water, and nearshore surface water collected right above the surface of the sand. These results differ from more spatially accurate characterizations of water source phosphorus concentrations with piezometers in 2017 and with larger datasets (which will be described in later sections). Well water, however, was not sampled from 2017-2020. Note: well water was resampled during summer 2021 and phosphorus was elevated compared to well water at 1 of 3 2021 sites (see later results section).

Nitrate-N concentrations did vary consistently among water sources, but they were higher in well water and lake floor piezometers than nearshore surface water at two (Penoza and Southworth) of the six sites (Fig. 18, Table 4-5). The elevated NO3 concentrations could be the result of deep and lake floor groundwater contamination by low-P, N-rich fertilizers or septic systems at these sites where P was not moving through the groundwater.

NH4-N was most variable of all the chemistries with occasional high values that were frequent enough that I did not treat them as outliers (Fig. 19, Table 4-5). Despite the high variability, there is a high certainty that NH4-H was elevated in subsurface ground water from piezometers and in benthic pore water compared to well and nearshore surface water at the Gourley site during summer 2016. Elevated NH4-N concentrations in piezometer samples at the Gourley site will be shown in other years in parts of the report below.

Chloride concentrations were higher in well and lake floor piezometer samples than in benthic pore water and nearshore surface water (Fig. 20, Table 4-5), even though high variability and several high Cl concentrations were observed in benthic pore water at the Gourley site. This is a general trend across all sites, but exceptions are evident in high Cl concentrations in benthic sediment and benthic water samples compared to other water sources at the Southworth site. The pattern at the Southworth site was also observed at the Gourley site, but variability was too high to reach conclusions about a reliable pattern. These exceptions are important to note to show that variability in the general trends can occur at specific sites – or be observed by chance. Higher chloride in benthic pore water than lake floor groundwater and nearshore surface water is difficult to explain without elevated Cl in piezometer samples, which we would expect for Cl transport to the lake from road salt and septic wastes.

Little consistent difference was observed across sites in SO4 concentration in different water sources (Fig. 21, Table 4-5). Across sites, well water tended to have slightly higher SO4 concentrations than other water sources. Differences were observed in concentrations among sites, such as the relatively high values at Hayo-Went-Ha. This could indicate different groundwater geology, but elevated SO4 in the nearshore surface water is unexpected because mixing would minimize this effect.

2017 Peeper Study

Goals, Rationale, and Approach

The goal of the 2017 water chemistry studies was to examine PO4-P concentrations in the habitat at the water-sand interface with more accurate sampling methods than had been used in our earlier sampling. PO4 was the target of this study because it had been determined in previous work to be the most limiting nutrient for algal growth in the three lakes of TLA. After considering a number of options, we decided to use a form of peeper, in which a dialysis tube is filled with deionized water, placed in the lake where ions diffuse into the deionized water of the dialysis tube until they reach equilibrium with the lake water, and then water chemistry of water inside the dialysis tubing is analyzed for target chemicals. Our peepers had the dialysis tubing placed in protective tubes of PVC pipe that had numerous holes drilled through the PVC to expose the dialysis tubing to surrounding water but protect the dialysis tubing from physical disturbance when anchoring the tubes in place. Preliminary studies were used to determine that 2 days was a sufficient incubation period for the water inside and outside dialysis tubing to become equilibrated. Dialysis tubing was placed in PVC tubes in two positions: laying on top of the sand and buried under approximately 1 cm of sand (Fig. 22). These water sources were referred to as benthos and sub-benthos, respectively. After 4 weeks incubation of the peepers and dialysis tubes, both dialysis tubes and a lake water grab sample were collected for PO4 analyses. Dialysis tubes were stored in Ziplock® bags and both grab samples and dialysis tube samples were frozen until analysis. During thawing, water in some of the dialysis tubes leaked out of the tubes into the Ziplock® bags and may have come in contact with biofilms on the outside of the dialysis tubing.

TLA members coordinated this project with members of lakes associations for Elk Lake and Lake Leelanau. As a result the peeper study was conducted in Torch Lake, Lake Bellaire, Elk Lake and Lake Leelanau. Peepers were placed and samples were collected in July, August, and September for all four lakes. Benthic algae were also collected at these times to associate with the water chemistry. The results for benthic algae will be discussed in the part of the results about benthic algae.

Results and Discussion

PO4-P concentration was higher in benthos and sub-benthos samples than nearshore surface water samples (Figs. 22-23, Tables 6-7). Benthos and sub-benthos PO4-P concentrations did not differ from each other, so those data were aggregated to test subsequent hypotheses. Benthos and sub-benthos PO4-P concentrations were highest in Lake Leelanau (Table 6-7) and decreased steadily in all four lakes during the summer with the fastest rate of decrease in Lake Leelanau. In contrast nearshore surface water PO4-P did not differ among lakes and stayed relatively constant during the summer (Tables 6-7).

The 2017 peeper study showed water just above the sands and in the surface of the sands had higher phosphorus concentrations than lake water. In addition, the study showed that water in this benthic habitat, just above and below the sand surface, could differ among lakes even though nearshore surface water concentrations did not differ. I considered how different sampling methods might affect measured water chemistry for the lake water grab sample and the peeper sampling for sub-benthos and benthos. I would have expected peepers to underestimate the concentration of PO4 in waters compared to a grab sample because there is greater reason to expect that PO4 concentrations in the dialysis tubing did not reach equilibrium with nearshore surface water than to expect they become over saturated with an ion by diffusion across the dialysis membrane. Contamination of peepers could increase PO4 concentrations on surface of peepers and increase local concentrations around the dialysis tubing. However, I would expect that concentration to increase over the summer. PO4 concentrations in peepers' dialysis tubing decreased over the summer. We might also expect that biofilms growing on peepers to have affected ions diffusing into peepers because of algal or microbial uptake of nutrients. Since PO4 concentrations in benthic and sub-benthic peepers did not differ, and biofilms on peepers above and below the sand surface surely would differ, I doubt the extended incubation period created a difference between ion concentrations inside and outside peepers. Therefore, I feel relatively certain in the conclusions above: 1) PO4 concentrations are higher at the sand-water interface and in interstitial waters of surface sands than in the water column; and 2) nutrient concentrations of the benthic habitat can differ among lakes even though surface chemistries do not. PO4-P concentrations in lake floor groundwater entering the lake is probably an important factor in benthic habitat conditions.

A third conclusion is that benthic and sub-benthic PO4-P concentrations decreased during the summer. This could be related to a decrease in lake floor groundwater loading in lakes as groundwater falls during the late summer months, which is likely a primary cause of seasonal decreases in lake levels. One plausible reason for the decreases in PO4-P concentrations with reduced flux rates is microbes on deeper sediments (not algae on surface sediments) have longer to take up PO4-P and remove it from the water column.

2018-2020 Summer Water Chemistry Pattern Characterization

Goals, Rationale, and Approach

As a result of seeing PO4 decreasing during the summer in the peeper study and observations that GBA develops and expands spatially during the summer, more thorough characterization of May-September water chemistry patterns was deemed important to understand their potential role in GBA development and expansion during the summer. Due to interannual variability and seasonal (i.e. summer-long) variability, water chemistry was sampled either biweekly or monthly for three years to accumulate sufficient information to determine seasonal patterns and how these would differ among sites and among water sample sources. Our plan was to relate these dynamic seasonal and spatial patterns in water chemistry to GBA development and differences in species composition of the GBA. Future benthic algal sample analyses will build on this extensive water chemistry dataset, including data from 2015-2017, and expand that understanding.

The water chemistry parameters measured varied from year to year. In this report, I focus on total phosphorus (TP), phosphate phosphorus (PO4-P), total nitrogen (TN), nitrate nitrogen (NO3-N), ammonia nitrogen (NH4-N), chloride (Cl), and sulfate (SO4) because they are different forms of the dominant nutrients that would affect GBA, are a tracer for human contamination from septic or road salt runoff (Cl), or an indicator of natural variation in groundwater sources that can interact with other chemicals (SO4). These parameters were also routinely measured so sufficient data is available to evaluate seasonal patterns and how they vary among years, sites, and water sample sources.

Statistical Approach

Skip this section and just read the summary of results and the section on differences among water source and site to avoid confusion from the statistics, which needs to be explained for credibility of the results and for more statistically inclined readers.

Given our primary goal of determining if average and seasonal changes in water chemistry vary among sites and water sources, the full data analysis is rather complex and difficult to interpret without ambiguity. Therefore, I have conducted a series of 6 types of statistical analyses on all the selected water chemistry patterns. Stepwise, I start with an analysis of all the data and then start aggregating and subsetting the data to clearly isolate effects of water source and site on water chemistry, including seasonal variability. The strength of this approach is clearer isolation of effects of water source and site. The weakness of this approach is the loss of statistical power to distinguish differences among water source and site because at different steps I aggregate data together for a variable that I know could affect water chemistry and I subset data that reduces sample size, which also affects ability to distinguish differences or effects.

I start with a full analysis of covariance with all the data for each water chemistry parameter to determine effects of water source, site, year of sampling, and summer day, which is the indicator for summer season temporal change. This analysis includes all possible interactions among water source, site, and year to account for all possible variation in the data, but these interactions can be difficult to interpret, especially when we are also looking at within summer season change. But this analysis provides “the big picture” of effects of our four major factors (water source, site, year of sampling, and summer day) which we have to keep in mind as we focus on water source, site, and whether there is consistent seasonal variability in patterns. The next 5 steps are:

2. Subset the data for each water source, site, and year to determine consistency across years in the effects of season, as indicated by the variable summer day.
3. Conduct another overview analysis of variance with the full dataset and the 3 factors, water source, site, and year. In this analysis all samples for a water source, site, and year combination are treated as replicates. This analysis produces three-way interactions among the three variables that are difficult to interpret.
4. Determine effects of water source and site with the full 2018-2020 dataset with analysis of variance and by assuming year and summer day have no effect so they can be used as replicates. As a result, we have a 2-way interaction term that is easy to interpret, but we also have the potential for high variability for each site and water source because within summer and interannual variability are not accounted for.
5. Determine effect of water source with analysis of variance by subsetting data for each site and by assuming year and summer day have no effect as they will be treated as replicates.

6. Determine effect of site with analysis of variance by subsetting data for each water source and by assuming year and summer day have no effect as they will be treated as replicates .

Results: The Step 1 Overview with All Parameters in the Analysis

All water chemistry parameters consistently differed among sites and water sample source, but some parameters did not differ consistently among years or across the summer period (Table 8). PO4-P and SO4 did not vary among years, whereas other parameters did. TN and NH4-N did not change in a consistent direction, increasing or decreasing, during the summer period.

Although there are exceptions to these patterns (as indicated by higher order interactive effects, Table 8), seasonal patterns (Sumr_Day in Table 8) for most water chemistry parameters did not vary for different water sample sources, sites, and years (e.g. Site:Sample_Type:Sumr_Day interaction in Table 8). However, differences in water chemistry parameters among sites often varied for water sample source, and differences among sites often varied among years and thereby produced higher order interactions.

Results: Step 2 for Summer Seasonal Patterns in Water Chemistry

Relatively few seasonal patterns in water chemistry were observed during the summer that were statistically significant, i.e. had a low chance of occurring by chance (Fig. 24, Table 9). Chloride had the most likely changes during the summer with 17 of 25 patterns (linear regression relationships) being statistically significant (having only a 1 in 20 chance of occurring by chance). Given analyses of 25 patterns, we would expect on average either 1 or 2 statistically significant relationships with summer day by chance. So, 17 significant relationships is certainly worth note. However, sometimes the changes during the summer were positive and sometimes negative, i.e. increasing or decreasing during the summer; and that varied from year to year at a specific site and for a water source type. Therefore, it is unlikely that summer changes in chloride are causing the increases in GBA during summers because GBA always increases over the summer.

Other parameters had a lower proportion of relationships being statistically significant compared to chloride. For TP, PO4-P, TN, NO3-N, NH4-N, and SO4 the ratio of statistically significant to all possible relationships were, respectively: 2 of 26, 10 of 25, 7 of 19, 7 of 25, 7 of 25, and 11 of 25. In all but TP, the number of statistically significant relationships was greater than the 1-2 that we would expect by chance. To investigate these further, I sorted relationships by water sample source and site to see if there were consistencies among years that would indicate patterns that were important. In all cases, whenever there was more than one statistically significant relationship at a site for a specific water source type, one year would have a positive change during the summer and the other year was negative. All three relationships for 2018, 2019, and 2020 were never statistically significant for any parameter at any site for any specific water source. Therefore, none of the summer changes in these parameters is likely driving seasonal change in the GBA.

The remaining likely determinants for season development and succession in diatom species composition of benthic algae are light, temperature, and wave disturbance, and changes in the micro-environment within mats as algae grow and mats become thicker during the summer. These factors and GBA will be discussed more completely in the Discussion section.

Differences among lake nearshore surface water and its groundwater and tributary sources

Step 3 with all factors in the analysis except summer day: This 3-factor analysis of variance uses the water chemistry results from 2018-2020 and determines effects of site, water source, and year as well as all interactions among those factors in which repeated sampling at sites and water sources within a year are considered replicate measurements (Table 10). The results from this relatively complex analysis of variance are still difficult to use for isolating effects of water source and site, but are important for showing effects of year because I will simplify analyses further and use samples for all years as replicates for each water source and site in subsequent analyses.

Site and sample type had significant effects on all water chemistry parameters (Table 10). In contrast, year only had significant effects on water chemistry for the dissolved nutrients PO4-P and NO3-N (Table 10), but they did have some significant interactions with other variables indicating effects of year varied with site and water source. Therefore, I will explore those interactions as I continue aggregating and subsetting the 2018-2020 water chemistry data to evaluate effects of specific water sources and sites on water chemistry.

Step 4 with water source and site as variables: At the next level of simplification to just two factors, the first step is to aggregate data for all years and summer days of sampling into groups by site and water sample source (Table 11). This approach answers the question, "Despite variability in water chemistry concentrations among the months and years sampled, do we see differences among sites, groups of sites, and water sample sources?" These results show distinct differences in water chemistry among lakes, sites in lakes, and water sample sources, despite the great variability in concentrations between the times sites were sampled. In addition, interactive effects between water source and site are highly certain for all chemicals except TP and PO4-P, which have interactive effects with relatively low certainty.

Summary for analysis steps 5 and 6: At most sites, TP and PO4-P concentrations were higher in lake floor groundwater sampled from lake floor piezometers than nearshore surface water sampled with grab samples of lake water. Depending on site, TN and NO3-N were not different or were lower in lake floor groundwater when compared to nearshore surface water. Depending on site, NH4-N, Cl, and SO4 can have been higher or lower in lake floor groundwater than nearshore surface water. These results indicated: 1) groundwater entering the lakes was a source of phosphorus, whether it was contaminated by human activities or not; and 2) groundwater entering the lakes likely diluted the concentrations of nitrogen in the lake. Other sources of nitrogen, such as atmospheric deposition, must explain the higher nitrogen concentrations in surface than ground water. Variation in NH4-N, Cl, and SO4 indicate differences among sites in groundwater conditions entering the lake. Tributaries may also be a source of P because spring TP in tributaries was greater than summer TP in lakes, but the difference in seasons sampled limits certainty of this conclusion. In the following paragraphs, I will go into some detail about differences in chemistries of water sources.

Steps 5 and 6 for phosphorus as TP and PO4-P: TP and PO4-P were low in the nearshore surface waters of all three lakes (Figs. 25-26; Table 12). Nearshore surface water phosphorus concentrations from 2018-2020 in both phosphorus forms were lowest in Torch Lake and highest in Lake Bellaire. Medians of nearshore surface water TP were 2.2-2.5 ug/L, 3.0 ug/L, and 4.1-5.1 ug/L at sites in Torch Lake, Clam Lake, and Lake Bellaire, respectively. Medians of nearshore surface water PO4-P were 1.9-2.4 ug/L, 2.7 ug/L, and 2.1-2.7 ug/L at sites in Torch Lake, Clam Lake, and Lake Bellaire, respectively. These phosphorus concentrations are very low relative to needs for algal growth (see discussion in Stevenson, 2016).

Concentrations of both forms of phosphorus were usually, but not always, higher in lake floor groundwater measured by lake floor piezometer than in nearshore surface waters (Figs. 27-28; Tables 12-14). Medians of TP concentrations from lake floor piezometers were 2.1-6.0 ug/L and 9.8-21.6 ug/L at sites in Torch Lake and Lake Bellaire, respectively. Medians of PO4-P concentrations from lake floor piezometers were 3.3-3.7ug/L and 5.8-8.6 ug/L at sites in Torch Lake and Lake Bellaire, respectively. Lake floor groundwater from piezometers was not sampled in Clam Lake. TP concentrations in lake floor groundwater were higher than in nearshore surface water in Torch Lake at the Gourley and Hayo-Went-Ha sites and in Lake Bellaire at the Drake site, but not at the Petty site in Torch Lake and the Southworth site in Lake Bellaire. PO4-P concentrations in lake floor groundwater were higher than in nearshore surface water in Torch Lake at the Gourley and Petty sites and in Lake Bellaire at the Drake and Southworth sites, but not at Hayo-Went-Ha. These results show lake floor groundwater is a source of phosphorus to the lakes and the magnitude of phosphorus loading likely varies spatially in lakes. Bretz et al. (2006) found the same results. The 2018-2020 sampling efforts show these conclusions hold for TP and PO4-P, whereas the Bretz et al. (2006) results only characterized TP. These results differ from analysis of 2016 chemistry alone when we did not observe differences in phosphorus between surface and ground water, likely because sample size was low and variability was too high in 2016 to detect differences.

Concentrations of both forms of phosphorus in benthic porewaters at the sand-water interface ranged between concentrations observed in nearshore surface water and lake floor groundwater sampled by lake floor piezometer (Figs. 27-28; Tables 12-14). When concentration differences between surface and lake floor groundwater concentrations were great, as for the Drake site, benthic porewater tended to be intermediate between surface and subsurface concentrations (Figs. 27). When concentration differences between surface and lake floor groundwater concentrations were relatively small, then benthic porewater had concentrations that tended to be more like nearshore surface water (TP at Southworth and PO4-P at Petty) or like lake floor groundwater (TP and PO4-P at Gourley). These benthic porewater PO4-P concentrations are important because they are likely sources of phosphorus for GBA – as are nearshore surface waters.

Concentrations of phosphorus in nearshore surface waters of the three lakes during summers were lower than in the tributaries in the spring (Figs. 27-28; Table 12). Median concentrations of TP in the watershed tributaries ranged from 7.9 to 29.4 ug/L. Median concentrations of PO4-P in the watershed tributaries ranged from 2.3-8.8 ug/L. The differences in nearshore surface water phosphorus between the three lakes and the tributaries was greater for TP than PO4-P. Therefore, tributary discharge could increase phosphorus concentrations in the lakes during summers when GBA develops if we assume that spring and summer phosphorus concentrations in tributaries are similar and if tributary loading is sufficient to affect P concentrations in the lakes. In addition, there was evidence of low levels of phosphorus pollution by humans in Spencer Creek and Grass Creek at Bellaire Highway because P was highest at those sites.

> *During the 2018-2020 sampling period we have two Clam River samples, 6 Clam Lake samples from the DNR boat launch, and more than 30 water samples from the Gourley and Petty site that give us comparisons of Torch Lake sites close to and far from Clam River discharge into Torch*

> *Lake. I am hesitant to make these comparisons, because the sample size from the Clam River is low, which reduces certainty in these comparisons, even though means may be significantly different statistically. However, I will make these comparisons because they are a model for the future, and readers could make these comparisons on their own if I do not do them. Additions of data from other sources and controlling for temporal variation in future analyses and reviews could help understand the water chemistry dynamics around the mouth of the Clam River. So, consider the following results with caution.*
>
> *Clam River discharge into Torch Lake is sufficient to affect local waters near the mouth of the Clam River. Clam River TP and PO4-P concentrations are higher than Clam Lake TP and PO4-P (Table 12-13), and the magnitude of the differences are greater for TP than PO4-P. This indicates a local source of P contamination between the Clam Lake DNR site and the Clam River at Dockside where sampling occurred. The greater difference for TP than PO4-P indicates that both inorganic and organic fractions of P have increased. The difference between TP and PO4-P is likely composed of both particulate P, which may be algae, and dissolved organic P, which is largely P associated with organic molecules in the water. The latter are certainly related to the brown color of the water indicating high dissolved organic carbon, which has been observed in previous research (Kintigh, Stillwell and Pedersen, 2012). TP and PO4-P are lower at the Gourley and Petty sites than the Clam River. In addition, TP but not PO4-P is higher at the Gourley site than the Petty site. So, the Clam River is increasing P in Torch Lake and more locally than farther away. Using the difference between TP and PO4-P again to determine organic P concentrations (both particulate and dissolved organic P), the concentration of organic P in the Clam River is 5.5 ug/L, 3.0 ug/L at the Gourley site, and 0.7 ug/L at the Petty site. The differences in organic P are probably due to both dilution with mixing of Clam River water into Torch Lake and breakdown of organic P by biological and abiotic processes (e.g. microbial and solar radiation).*

Steps 5 and 6 for nitrogen as TN, NO3-N, and NH4-N: Nitrogen concentrations in nearshore surface waters of the three lakes were not particularly low relative to other lakes and needs for algal growth (Figs. 27-29; Tables 12-14). In addition, nearshore surface water nitrogen concentrations varied relatively little among the three lakes and sites in them. Median nearshore surface water TN varied from 397-463 ug/L at all sites in the three lakes. Median nearshore surface water NO3-N concentration was 144-216 ug/L at sites in the three lakes, except for the low median in Clam Lake (67 ug NO3-N/L) and high median (711 ug NO3-N/L) at Hayo-Went-Ha in Torch Lake. The high median is likely an overestimate of NO3-N due to low numbers of samples at that site from 2018-2020 and because NO3-N concentrations measured in 2016 were not different at Hayo-Went-Ha versus other sites. NH4-N concentrations in nearshore surface waters did not vary among sites with medians ranging from 17-39 ug/L.

Nitrogen concentrations varied greatly among the tributary sampling sites but were seldom greater than concentrations of nearshore surface waters in the three lakes (Figs. 27-29, Tables 12-13). Medians of TN concentrations varied from 378-650 ug/L at most sites, with exceptions for low medians (209 and 317 ug/L) in Eastport Creek and Grass Creek at Bellaire Highway and a high median (977 ug/L) in Grass Creek at Honey Hollow. Medians of NO3-N concentrations varied from 192-484 ug/L at most sites, with exceptions noted at the same sites as for TN: low medians (45 and 147ug/L) in Eastport Creek and Grass Creek at Bellaire Highway and a high median (771 ug/L) in Grass Creek at Honey Hollow. NH4-N concentrations in the tributaries were consistently lower in all the tributaries than nearshore surface waters of the three lakes, likely because of naturally high nitrification (conversion of NH4-N to NO3-N) in the running water environments. There is little evidence of human contamination of the tributaries with nitrogen and tributary potential for polluting the three lakes with nitrogen.

Nitrogen in lake floor groundwater differed from nearshore surface water in ways that indicate spatial variability in groundwater conditions resulting from either natural or human factors. Median TN was measured in groundwater at only four sites. Medians of TN concentrations in lake floor groundwater were the same as nearshore surface water TN at the Gourley site, but were lower than nearshore surface water at Drake and Petty with groundwater medians were 300 and 150 ug/L lower than nearshore surface water at Drake and Petty sites, respectively. High variability in lake floor groundwater TN concentration at Southworth prevented meaningful comparison with nearshore surface water.

NO3-N concentration was measured in lake floor groundwater at five sites and was 83 ug/L higher than nearshore surface water at the Petty site, the same as nearshore surface water at the Southworth site, and really low (5-26 ug/L) at Drake, Gourley, and Hayo-Went-Ha sites. NH4-N was measured at the same five sites as NO3-N and had somewhat the opposite site-by-site relationship to nearshore surface water.

NH4-N concentrations in lake floor groundwater were lower than nearshore surface water at the Petty site, the same as nearshore surface water at the Southworth and Hayo-Went-Ha sites, and higher than nearshore surface water at the Drake and Gourley site. NH4-N concentrations in lake floor groundwater were particularly high at the Gourley site with a median 428 ug N/L, compared to Drake with 56 ug N/L and the three other sites ranging from 5-46 ug N/L. High NH4-N concentrations at the Gourley site indicate a source of nitrogen that is greater than other sites and low oxygen conditions, perhaps due to either natural or human sources of organic carbon contamination. Dissolved organic carbon from wetlands upstream from Clam Lake could be moving through lake floor groundwater and lowering oxygen concentrations. Alternatively, wastewater from septic systems may be rich in organic carbon and generating oxygen demand in lake floor groundwaters.

Nitrogen in benthic porewaters varied among sites and seemed related to groundwater nitrogen patterns among sites. TN in benthic porewaters were somewhat higher than nearshore surface water at the Southworth, Clam Lake, and Gourley sites, but did not differ from nearshore surface water at Drake and Petty sites. NO3-N concentrations in benthic porewaters were the same as nearshore surface water concentrations at all five sites. NH4-N in the benthic porewater was elevated at the Drake site as well as the Southworth, Clam Lake, and Gourley sites where TN was also somewhat higher in benthic porewater than nearshore surface water. Nitrogen in lake floor groundwater was not measured at the Clam Lake DNR site, so we cannot know if the elevated nitrogen there indicates the same source of nitrogen as the Gourley site; but it does seem worth noting and helping with understanding spatial variability in groundwater N and other ions among sites. The higher NH4-N at Drake and Southworth could be related to groundwater contamination by humans because we also see elevated TP and PO4-P in groundwater at those sites compared to nearshore surface water. Chemical comparisons of well water, lake floor groundwater (lake floor piezometer), benthic porewater, and nearshore surface water with 2016 samples also showed some evidence of groundwater N contamination at the Southworth site, but not the Drake site. It should also be noted that PO4-P concentrations did not vary among water sources in 2016 at the Drake or Southworth sites.

Steps 5 and 6 for Chloride: We have seen an increase in chloride concentrations in the three lakes over the last 20-30 years according to data from CLMP and ToMWC. During the 2018-2020 sampling of 6 locations in the three lakes, medians of chloride concentration varied from 8.8-11.7 mg/L (Figs. 30; Tables 12-14). Tributary chloride concentrations were typically much higher than nearshore surface waters of the three lakes. Median tributary Cl concentrations ranged from 34 to 153 mg/L at different sites, except for Grass Creek at Bellaire Highway and Spencer Creek that had some unusual values. Lake floor groundwater Cl concentrations were higher than nearshore surface water at two sites, Gourley and Drake, the same at Southworth and Hayo-Went-Ha, and lower than nearshore surface water at the Petty site. Median Cl concentrations at the Gourley and Drake sites were 138 and 26 ug/L, respectively. The lake floor groundwater Cl concentrations at the Petty site, with a median of 2.7 ug/L, were lower than in the nearshore surface water measured in 1992 by the ToMWC; therefore they may represent the background natural Cl concentration in regional waters without contamination from human wastewater and road salt. TLA's 2018-2020 Cl results indicate tributaries and some groundwater pathways are sources of Cl contamination in the three lakes.

Steps 5 and 6 for Sulfate: Sulfate concentration patterns among sites and water sources indicate groundwater processes vary among sites (Figs. 31; Tables 12-14). Medians of SO4 concentration in nearshore surface water did not vary a lot among sites, ranging from 6.5 to 9.2 mg/L; but medians of SO4 concentrations were consistently 1-2 mg/L lower at sites in Lake Bellaire than Torch Lake. Sulfate concentrations in lake floor groundwater were consistently 4-5 mg/L higher than in nearshore surface water, except for two sites. Sulfate concentrations were much higher, about 22 mg/L higher, in lake floor groundwater than nearshore surface water at Hayo-Went-Ha. Unusually high sulfate concentrations (compared to other sites) were observed in surface, ground, and well-water at Hayo-Went-Ha in the 2016 TLA samples. The reason for this abnormality is not known but could be natural variations in geology and mineral dissolution or contamination by humans.

Lake floor groundwater concentrations at the Gourley site were unusual. Sulfate concentrations were very low in the lake floor groundwater at the Gourley site. Here, the low sulfate concentrations may be due to low oxygen conditions and conversion of sulfate to sulfide. This corroborates the unusual groundwater conditions at the Gourley site that were previously described with high NH4-N and low NO3-N concentration.

Veverica's Detailed Water Chemistry Analysis of 2017 and 2018 Samples

Goals, Rationale, and Approach

Tim Veverica, in his data report to TLA dated January 29, 2018, analyzed concentrations of a long list of chemicals sampled and assayed in 2017 that were not in the usual list of chemicals analyzed during the TLA studies. His goal was a better understanding of potential differences among sites and water sources. These chemicals included quinate, fluoride, lactate, acetate, propionate, formate, butyrate, malonate, maleate, oxalate, and citrate. This set of chemicals was analyzed in all 2017 samples. In addition, Tim and colleagues at UMBS analyzed chemicals that would only be generated by human sources, such as: acesulfame potassium (Ace K) is an artificial sweetener; DEET (N, N-Diethyl-meta-toluamide) would be from insect repellents; BTH (benzothiazole) is a common chemical in consumer products; caffeine; and triclosan is the antibacterial compound found in soaps and cosmetics. These analyses of anthropogenic chemicals were only run on a subset of 2017 samples. In 2018, Veverica analyzed Ace K in 63 samples.

Results and Discussion

Ratios of the common:rare isotope ($\delta^{11}B$) of boron indicated groundwater at many sites was contaminated with septic wastes or detergents with high concentrations of the common isotope of boron. Many TLA samples had boron isotope ratios greater than 10 (Fig. 32), with some as high as 40-60 $\delta^{11}B$. Boron isotope ratios between -10 and 10 indicate natural background freshwater (Fig. 33a, Vengosh, Kolodny and Spivack, 1998). Higher boron isotope ratios (5-30) indicate contamination by domestic wastewater source, particularly boron rich detergents. Boron isotope ratios of 40 and above can indicate seawater brine aquifer sources of water.

Further analysis of water chemistry results indicated that high $\delta^{11}B$ does not necessarily show ancient brine aquifers. Boron ranged from close to 0 to 25 ug/L and B:Cl ratios were less than 0.005 in all water samples (Fig. 32). There were a group of water samples from the Gourley site, which had higher than average boron concentrations, but even they fell well below the range for fossil brines (Dotsika, Poutoukis, Michelot and Kloppmann, 2006). The B:Cl concentrations could have come from fossil brines, which have a wide range of possible B:Cl ratios.

I have just recently gotten matches for the sample numbers and sample sources to evaluate the 2017 analyses of Ace K, DEET, BTH, caffeine, and triclosan. I did get that done for this report.

The 63 samples collected during 2018 and analyzed for Ace K came from nearshore surface water, benthic pore water, and lake floor groundwater at four sites, an onshore piezometer at the Gourley site, and onshore seeps at three other sites: Southworth, Hayo-Went-Ha, and Petty. Samples were collected at Gourley and Southworth on six dates, biweekly from mid-May to mid-July. Samples were collected at Hayo-Went-Ha and Petty one time. I would expect higher Ace K in onshore seeps or lake floor groundwater than benthic pore water with lowest Ace K in nearshore surface water if we have a pathway of septic wastes from onshore via lake floor groundwater to benthic porewater and the lake. We did not observe that pattern consistently in the data (Fig. 34). Ace K was undetectable in 35 of the 63 samples which is indicated by a value of 1 in Fig. 34, which results from a transformation that I used to plot the Ace K data on a logarithmic scale and observe patterns due to high variability in high values of Ace K. Differences in Ace K among water sources and sites were not sufficiently consistent to draw conclusions about dominant pathways for human contaminants in benthic pore water and nearshore surface water. High values of Ace K were observed frequently in lake floor groundwater at all sites, but high values of Ace K were not observed consistently in onshore samples. Benthic pore water Ace K was consistently low, and nearshore surface water Ace K was low at the Gourley site. In contrast, nearshore surface water Ace K was occasionally high at Southworth. It is possible that onshore locations missed human waste pathways when Ace K was low, and lake floor groundwater was a better indicator of local contamination by septic waste. And it is possible that high Ace K in nearshore surface water at Southworth was due to a nearshore surface water source or just entrainment of a higher load of Ace K from groundwater; but it was not observed in benthic pore water which would be expected if groundwater was the source of nearshore surface water contamination. Thus, the variability in results makes it difficult to draw conclusions about septic waste contamination based on Ace K values but results certainly indicate human contamination from one source or another at some sites.

Any presence of anthropogenic chemicals in lake floor groundwater, presumably from septic waste contamination, without higher phosphorus concentrations in lake floor groundwater than well water (our indicator of natural background P) could be explained by adsorption of P to soils and glacial tills.

Inorganic phosphorus has long been regarded as safe to apply to soils if surface runoff of soil particles is prevented because inorganic phosphorus adsorbs onto minerals in soils and subsoil till material (Sharpley et al., 2013). It was generally believed that phosphorus does not move through the groundwater. However, a more recent understanding shows that phosphorus binding capacity of soils can become saturated, and phosphorus in the groundwater can be transported by groundwater pathways to surface water (Robinson, 2015). Thus, phosphorus movement through the groundwater from anthropogenic sources remains a concern for future contamination, if it is not already contaminating lake floor groundwater and not traveling by undetected pathways to surface water in Torch Lake and Lake Bellaire.

2020 Analysis of Diurnal Patterns in Water Chemistry

During the summer of 2020, TLA sampled water to test the hypothesis that benthic algae were impacting the flux in nutrients from the groundwater and porewater into the nearshore surface water. We know that algae are most metabolically active during the day with light availability and least metabolically active at night. This diurnal metabolic pattern results in diurnal variation in nearshore surface water nutrients in streams and wetlands such that nutrient concentrations are higher in the early morning than late afternoon. We expected the same kind of algal-nutrient interactions in the shallow nearshore zones of the lakes. We would expect no difference in concentrations of ions that are not regulated by algal metabolism, such as Cl or SO4. Therefore, nearshore surface water and benthic porewater were sampled early in the morning and late in the day at four sites (Petty, Gourley, Clam DNR, and Southworth) on three dates, one each in June, July, and August.

We did not observe diurnal patterns in water chemistry that indicated an effect of algal metabolism on groundwater flux or nutrient concentrations in the nearshore zone (Figs. 35-37 and Table 15). We did observe diurnal differences at 2 or 3 of the 4 sites in TP, TN, and PO4-P (aka SRP), but the differences were opposite of what we would expect as a result of algal metabolism. We observed higher nutrient concentrations in the late afternoon than the morning. Otherwise, we only saw haphazard differences for different chemistries, sites, and times that could be the result of random variation. Therefore, algae are not depleting nutrient supply during the day in shallow, nearshore surface water in ways that likely affect ecology of GBA.

2020 Analysis of Nearshore/Offshore Patterns in Water Chemistry

During the summer of 2020 with extra sampling of the mid-lake zone in Torch Lake, we were able to compare surface water chemistry in the shallow nearshore zone and offshore deep waters. We hypothesized that nearshore zones could have higher phosphorus concentrations than offshore zones because fluxes of phosphorus from the groundwater would mix with a smaller volume of water in shallower areas. We observed similar TP and PO4-P concentrations in early June in nearshore and offshore zones, but then a decrease in TP and PO4-P concentrations during the summer (marginally statistically significant) in the open water that was not observed in the nearshore zone (Fig. 38, Tables 16-17). At the same time, we observed an increase in nearshore TN concentrations from June to August and a similar increase in offshore TN concentrations. As TN increased, offshore nitrate did not change significantly. Nearshore nitrate and PO4-P did not change significantly during the summer. Nearshore and offshore NH4-N and SO4 did not differ or change significantly, although the magnitude of increase in SO4 predicted by the best-fit regression line was similar to an increase in Cl in the nearshore zone that was statistically significant. Offshore Cl did not change significantly and was similar to average nearshore

Cl. Offshore Fl decreased significantly from a value higher than nearshore to a similar concentration to what was observed throughout the summer in nearshore zone.

The different patterns of water chemistry in the nearshore/offshore zones of Torch Lake indicate an incomplete mixing over time scales long-enough for these patterns to develop. The relatively constant P concentration in nearshore waters as phosphorus decreases offshore indicates phosphorus is being loaded into the nearshore zone as particulate and dissolved phosphorus are decreasing offshore, likely because of algal uptake and sinking. The increase in TN could be due to an increase in algal accumulation in the water column with nitrogen loading during the summer. The rate of change in nitrate as TN increased in offshore waters indicated that the fraction of bioavailable N was decreasing during the summer, which is consistent with increasing algal biomass and increasing nutrient limitation during the summer. Additional data is needed to test these patterns and reevaluate their significance. Enhanced sampling was being conducted during summer 2021 to accomplish that, those data were evaluated and presented in results below as a 2021 addendum.

The increases in nearshore Cl and perhaps SO4 concentrations and decreases in offshore Fl concentrations are a bit more difficult to explain. One possibility is spring rains diluted Cl and SO4 concentrations in the nearshore subsurface groundwater and they then recover to background concentrations later in the summer. Decreases in Fl concentrations are not well understood but may be co-precipitated with other inorganic or biological particles associated with phytoplankton metabolism and sinking in the open lake.

RESULTS – Algae

2017 Benthic Diatom Study

Goals, Rationale, and Approach

In 2017 benthic algae were sampled two or three times, more or less monthly during the summer (July-September) when peepers were sampled in Torch Lake, Lake Bellaire, Elk Lake, and Lake Leelanau. Because of the dominance of diatoms in previous samples, I focused analyses on the differences in diatom species abundances among these four lakes to continue developing relationships between changes in species composition and changes in water chemistry that could test hypotheses about causes of GBA. Sampling during summer 2017 provided an opportunity to evaluate changes in species composition with changes in water chemistry among lakes, for benthic pore water, near-benthos nearshore surface water, as well as nearshore surface water, and for changes during the summer. Benthic algal sampling was conducted at the same time and location as water chemistry sampling with peepers. In Torch Lake, benthic algae and water chemistry were sampled at the Gourley site and also two or three times monthly from July to September at the Petty and Hayo-Went-Ha site.

Results and Discussion

Species composition differed substantially among lakes, with Lake Leelanau the most different, even though most of the dominant species were found in all lakes (Figs. 39-40). The three sites in Torch Lake had four species that had relative abundances greater than 10 percent of all diatoms observed during sample analysis: *Encyonema evergladianum*, *Delicata delicatula*, *Encyonopsis subminuta*, and *Fragilaria sp. 1*. The 10 percent benchmark for discussing taxa was for convenience and has no particular ecological significance. These taxa are listed in order of highest to relatively lowest relative abundances of the four taxa. To be clear, there are many rarer taxa in the samples. There is no consistency in changes in relative abundance of these taxa or others from July to September across all three Torch Lake sites. In Lake Bellaire and Elk Lake, we again have *Encyonema evergladianum* as most abundant, with *Delicata delicatula* again highly abundant in Lake Bellaire but not Elk Lake. In Elk Lake, *Encyonopsis subminuta* was again above 10% in August. In addition, *Achnanthidium caledonicum* was breaking across the 10% benchmark in both Lake Bellaire and Elk Lake in July. Lake Leelanau was different because it had highest relative abundances of *Cyclotella* species, mostly *Cyclotella commensis*, in the benthos with very little of the *Encyonema evergladianum*. *Encyonopsis subminuta* and *Achnanthidium caledonicum* again had relatively high relative abundances compared to rarer taxa. In addition, another *Achnanthidium* species, *Achnanthidium minutissimum*, became more abundant than 10% of all diatoms in September samples in Lake Leelanau. This taxon was also relatively common at other sites, but just not greater than 10% and thus not reported in previous sentences.

These results start to give a foundation for understanding the factors regulating diatom species composition in these lakes, but the following is largely speculation and only intended to provide examples of how species composition data can be used to understand the ecology of GBA. Clearly, species composition of benthic diatom samples is most different in Lake Leenanau when compared to the other three lakes. The evenness of relative abundances and abundances of Cyclotella spp., which are characteristic planktonic diatoms, indicates no species are growing fast enough to out compete planktonic taxa settling into the benthos and no single benthic taxon is really outcompeting others. This indicates plankton abundances are higher in Lake Leelanau than the other lakes and nutrient limitation is moderate because many species can grow at relatively the same rate. Even relative abundances of taxa indicate evenness in the accumulation processes, which are settling from plankton or reproduction of cells within the benthos. When nutrients are low or high, abundances of species are commonly uneven because in low nutrients, only a small number of taxa are able to grow in the low nutrient conditions and in high nutrients, often a small number of taxa are able to exploit high nutrient conditions and grow much faster than other species.

Another interpretation of the results could use the diatoms as in past reports (Stevenson, 2016; 2017), when environmental preferences for specific species were used to determine whether changes in species composition indicated an increase or decrease in nutrient availability. The problem with those past analyses and such an analysis at this point is we know little about the relative nutrient requirements of the dominant taxa in these lakes because they are rare in other lakes of the US and world. *Encyonema evergladianum*, as the name suggests, is found in the Everglades, and as one of the dominant species in the more natural areas of the Everglades; and the Everglades is an extremely low phosphorus habitat. But these relatively anecdotal, one-off kind of observations are not sufficient to develop a critical interpretation of the meaning of changes in diatom species composition among lakes and across seasons. That is why I initiated experimental studies using diatoms from Torch Lake in 2018, which I will explain more after I present results of sampling and diatom analyses of benthic algae in 2018.

2018-2020 Benthic Diatom Studies

2018 Nearshore Benthic Algal Species Composition

Goals, rationale, and approach. In 2018 benthic algae were sampled frequently during the summer to characterize the changes in diatom species composition during the summer, which is another approach to find clues about factors that were causing GBA. Since GBA develops greater thickness during the summer, perhaps that process and changes in nearshore surface water and benthic pore water chemistry can tell us something about factors regulating GBA. Benthic algae were sampled biweekly (10 times) at the Gourley site in Torch Lake and Southworth site in Lake Bellaire in 2018 from mid-May to September. Benthic algae were also sampled four times, monthly, from mid-June to mid-September at the Petty and Hayo-Went-Ha sites in Torch Lake.

Results and Discussion. With the higher frequency sampling, clear seasonal patterns were evident in diatom species composition (Fig. 41). *Encyonema evergladianum* was again, one of the most abundant diatoms, and it became relatively more abundant in benthic diatom samples as the summer progressed, with convincing evidence repeated in all four sites. Other than that similarity, other taxa played different roles in seasonal succession at each of the sites. At the Gourley site, early season assemblages were dominated by a group of taxa in the *Fragilaria vaucheria* species complex. By mid-June, *Achnanthidium caledonicum* became highly abundant, and then *Encyonema evergladianum* became most abundant for the rest of the summer. At the Petty and Hayo-Went-Ha sites, *Fragilaria perdelicatissima* was abundant early in season, with considerable abundances of *Achnanthidium caledonium* again, and at both sites. At the Petty site, *Delicata delicatula* had high relative abundances in all samples, and *Encyonema evergladianum* replaced *Fragilaria perdelicatissima* as the summer progressed. Worth note is the abundance of *Nitzschia amphibia* in mid-July and August at the Petty site because this taxon usually indicates high nutrients; but I caution against any over-interpretation until we know more about this taxon in northern Michigan lakes and its role in Torch Lake benthic algae. Hayo-Went-Ha differed from Petty by having more persistence of *Fragilaria perdelicatissima* during the summer and less of an increase in *Encyonema evergladianum*. In addition, *Delicata delicatula* and *Nitzchia amphibia* played secondary roles in benthic diatom assemblages at the Hayo-Went-Ha site during the latter part of the summer. Southworth had relatively high evenness in relative abundances of diatom taxa during the summer, with many taxa commonly having about 5-10% composition of assemblages. Although highly speculative, this could indicate relief from severe nutrient limitation and relatively even growth rates of many taxa as I described for Lake Leelanau in 2017. However, *Encyonema evergladianum* become highly abundant in September and a group of small diatoms in the genus *Adlafia* also increased in abundance late in the season at Southworth, but not other sites.

During 2019 and 2020, benthic algal samples have been collected by the TLA crews from mid-June to mid-August. Our philosophy was to collect benthic algal samples along with water chemistry samples, because they are easy to sample and store and might be valuable in the future. Decisions about processing these samples will be based on what we can learn from their analysis versus effort on other tasks.

Photomicrographs of some of the most abundant diatoms are in Figure 42. Kociolek, Lowe, Sanchez and Stepanek (Accepted) will illustrate many new diatom species from Torch Lake.

2018 Ex-situ Experiment to Identify Species Responses to Nutrient Manipulations

Goal: During the summer of 2018, I set up an experiment in my side-yard to determine the effect of nutrient enrichment from groundwater and surface water on growth rates of diatom species in GBA and thereby help interpret the ecological significance of changes in diatom species composition among lakes, locations in lake, and during the summer growing season.

Construction of water bath, microcosms, and water delivery system: I constructed a water bath with 2” x 4” boards as walls around a ¾” plywood base, over which a thick piece of polyethylene plastic sheet was placed that would hold water within the shallow box created with wood (Fig. 43a). The resulting box was 4 ft x 4 ft. I made microcosms in which I can manipulate groundwater and surface water with one 2.5” long piece of 3” inside-diameter PVC pipe, a 3” diameter PVC cap, and a 3.5” diameter circle of fine mesh screen (hops bag material purchased from Northern Brewery Supplies, Inc) that would support sand but allow water to pass through. One end of the 2.5” long piece of PVC pipe was covered with a 3.5” diameter circle of fine mesh screen and inserted into the PVC cap until it reached the mid-point of the cap, so the region below the screen served as a reservoir for groundwater. The area above the screen served as the bowl in which sand, water, and algae collected from Torch Lake were placed to simulate the surface of sand in a lake (Fig. 43). Silicon caulk around the 2.5” pipe at the lip of the cap was used to seal water in chambers and hold the PVC assembly together. To get water into this lower chamber, I drilled a 3/16” hole, inserted a 6” long piece of 3/16” outside-diameter plastic tubing (common aquarium hose for air pumps), and sealed the connection with silicone caulk both inside and outside the hole. I used a 50 mL syringe that is usually used for water filtration to inject known volumes of water into the groundwater chamber; that enabled manipulating nutrient conditions in the groundwater chamber and groundwater discharge through the sands. I constructed a water drip system from 8” long 3” diameter PVC pipe, a PVC cap, a multi-valve air manifold, and plastic hose to drip water into the surface water chamber of each microcosm after sand, water, and algae were initially placed in the microcosm (Fig.43b). 200 mL of water was placed in the surface water chamber. This water had been collected in carboys from the surface of Torch Lake at a mid-lake location. I replaced 50 mL of the 200 mL of surface water every 3 days to maintain treatment conditions according to common protocols of a semi-continuous batch culture. If no water was replaced, algae in the chamber would rapidly change nutrient concentrations. Replacing water during the experiment helps maintain targeted treatment concentrations. I withdrew 50 mL of water with a 50 mL syringe and added 50 mL of water using the water drip deliver assembly. Dripping water into the microcosm over 150 mL of water did not disturb the sand and benthic algae in the microcosm. I added 10 mL of new groundwater to the groundwater chamber every 3 days to simulate movement of groundwater into the porewaters of sands. This 10 mL volume was chosen by testing for appearance of dyed water leaking from the groundwater chamber through the sands with minimal mixing with surface water.

Initiation of the experiment and treatments: I collected benthic algae from two locations in Torch Lake, Becky’s Beach and the Gourley site where I expected differences in species composition that would help diversify the species for which I determine responses to nutrient enrichment. I also collected 80 liters of water from Torch Lake to be used in the experiment.

The groundwater chamber of microcosms was filled with water using the syringe and tube and the appropriate nutrient treatment solution. A challenge developed when first adding water to the groundwater chamber at the beginning of the experiment because air would get trapped in groundwater chamber when the fish mesh screen was wet. When the fine mesh screen was wet, it would not allow air to pass through it. To prevent air bubbles from forming between the groundwater and fine mesh screen, the screens were dried before groundwater was added and microcosms were tilted slightly to allow air to pass through the screen on upper side of the chamber where the screen remained dry until the final volume of water was added.

Sand and algae collected from Torch Lake were mixed, spread out in a cookie sheet with sides to hold water, sand, and algae. Then known amounts of the sand-algae-water mixture were spooned into microcosms as consistently as possible to create a 1 cm thick layer of sand and algae on the bottom of the surface water chamber of the microcosm. 150 mL of surface water with the appropriate nutrient treatment were dripped into the microcosms after sand and algae were added. Then the microcosm was shaken gently, back and forth (not up and down), to flatten out and homogenize the sand-algae mixture across the microcosm. Finally, 50 mL of the appropriate nutrient treatment solution was added to each microcosm. I did not add all 200 mL of water to microcosms before shaking and homogenization because water would have spilled out of the microcosms.

Nutrient treatments were designed to simulate the low nutrient enrichment conditions thought to be occurring in northern Michigan Lakes. Eight treatments were made by manipulating nutrients in the groundwater and surface water chambers independently. The four basic nutrient manipulation treatments were: 1) a control, which was just Torch Lake water from carboys that had been collected from the lake in both groundwater and surface water chambers; 2) surface water only enrichment with both nitrogen and phosphorus; 3) groundwater only enrichment with both N and P; and 4) both surface water and groundwater enrichment with both N and P. Nutrient enrichment targets were 5 ug PO4-P/L and 70 ug NO3-N/L for both ground and surface water. Concentrations of PO4-P, TP, NO3-N, and TN in carboys of water from Torch Lake averaged 3.1 (standard deviation, SD=0.22, N=6), 3.1 (SD=0.25, N=6), 176 (SD=19.4, N=6), and 461 (SD=4.34, N=6) ug/L. Concentrations of PO4-P, TP, NO3-N, and TN in carboy water enriched for dosing microcosms averaged 7.8 (SD=0.57, N=2), 10.8 (SD=2.33, N=2), 228 (SD=1.6, N=2), and 534 ug/L. (SD=9.1, N=2). Differences before and after dosing show nutrient enrichment targets were met.

These four treatments were used with algae from either Becky's Beach or the Gourley site, which made a total of eight treatments. I kept the two sources of algae and sand separate because the fine sediments at Becky's Beach were a concern for sample processing and analysis, and I hoped to better diversify the species observed in samples by keeping the two assemblages separate. Four replicates of each treatment were produced for a total of 32 microcosms.

As indicated before, 25% of water with or without appropriate nutrient amendments was replaced every 3 days. Water in the water bath was replaced in part with well water to prevent high water temperatures from developing. Three layers of shade cloth that reduced light by approximately 50% were placed over the water bath and microcosms to keep water cooler and to reduce light inhibition of algal metabolism from light level being too high. Water and algae samples were harvested on day 15 of the experiment.

Nitrogen and phosphorus concentrations in water samples were measured by the University of Michigan's Biological Station. Algae samples were acid cleaned, rinsed in deionized water, and mounted in NAPHRAX on microscope slides. Diatoms were identified to lowest possible taxonomic level and counted. Samples have only been partially analyzed at this time. Only the control and groundwater/surface water enriched (TB, for top-bottom) treatments for the Gourley site assemblages have been counted (8 samples). Other treatments will be counted in the future and more detailed counts will be targeted for species that were observed in Torch Lake and Lake Bellaire GBA. The rest of these samples and extended counts will be completed during fall 2021.

Results: Ten taxa occurred in sufficiently high abundances to evaluate their responses to nutrient enrichment. Proportional relative abundances of four of the 10 taxa were statistically different between treatments. *Encyonema evergladianum* and *Navicula wildii* had higher relative abundances in the control than TB treatments (Fig. 44). *Nitzschia amphibia* and *Cymbopleura subaequalis* had higher relative abundances in the TB than control treatment. Other taxa common in Torch Lake and Lake Bellaire GBA, such as *Delicata delicatula*, *Achnanthidium caledonicum*, *Encyonopsis subminuta*, and *Gomphonema sp. 1*, were not different in control and TB treatments.

Discussion: What we learn from species responses in experiments can be used to help understand the causes of GBA. For example, the great seasonal increases of *Encyonema evergladianum* could be possible because this taxon is tolerant to low nutrients as indicated by its highest relative abundance in the control treatment. I would also expect nutrient availability to decrease as benthic algae accumulate in dense mats (Fig. 1) on the sand because nutrient availability decreases inside these mats as benthic algal biomass increases (Stevenson and Glover, 1993). The decrease in nutrient availability in mats occurs because algae use up nutrients faster than nutrients can mix into mats and diffuse to cells, and the mixing of nutrients from surrounding waters into mats decreases as benthic algal biomass and mat thickness increase. Thus, *Encyonema evergladianum* would be tolerant to those conditions. *Encyonema evergladianum* was not reported in the Fritz et al. paper, indicating it may have increased in abundance greatly as phosphorus decreased in surface waters of Torch Lake and Lake Bellaire during the past 3-6 decades. *Navicula wildii* that was also more abundant in control than TB treatments and was also observed in significant abundances in some GBA samples from the lakes. The other taxa that did not respond to the change in nutrients among treatments or that required the nutrient enrichment of the TB treatment were early season or relatively rare taxa in GBA, indicating that nutrients were more abundant during the early season when GBA biomass is relatively low and nutrient availability is relatively high compared to later in the summer in the shallow waters where GBA was sampled.

2018-2019 In-situ Experiment

Goal: During August 2018, a second experiment was conducted in Torch Lake at the Gourley site to determine if connectivity to groundwater nutrient supply was needed for development and persistence of GBA mats.

Construction and deployment of microcosms: Microcosms were constructed of a 3.5" section of 4" diameter PVC, a 4" diameter PVC couplings, and a 5" discs of fine mesh screen (hops bag material from Northern Brewery Supply, Inc.) or a 5" disc of polypropylene plastic. A disc of either fine mesh screen or a sheet of poly-propylene plastic was placed over the PVC coupling and then the 3.5" pieces of PVC pipe were inserted into the PVC coupling to create a chamber in the PVC pipe with a bottom of either fine mesh screen or polypropylene plastic sheet. The fine mesh screen allowed water to pass through it when it was immersed, and the polypropylene sheet was a barrier to water passing through it (Fig. 45). Frames to hold four microcosms were made of 1" PVC tubing, T's, elbows, and caps. Before closing the frame with the cap, the pipe was filled with sand so it was strongly negatively buoyant and would help hold microcosms in place on the lake bottom.

Near shore where GBA mats were well developed, Brian Moore from UMBS and Bowling Green State University helped me fill the microcosms with 1 cm deep layer of a sand-algae mixture created by sampling algae in the nearshore zone of the Gourley site. The microcosms were then filled with lake surface water, covered by plastic sheeting held in place with rubber bands, and transported to a 2.3 m depth farther offshore for deployment. We expected that 2.3 m depth would be deep enough to avoid severe wave disturbance. The microcosms were lowered into the water, placed in a PVC tube frame, and the plastic covering the sand-algal mixture was removed (Fig. 45). Eight microcosms with fine mesh screen and eight with plastic sheet were placed during August 2018 with 2 of each type of microcosm in each frame that held four microcosms during August 2018. The microcosms were left in place over the winter until July 2019, when they were collected with the help of Becky Norris and Mariellyn Stevenson. When samples were collected, they were placed in quart Ziplock® bags and preserved with formaldehyde when back on shore.

Results and Discussion: When collecting samples, visual observations showed dense GBA mats in the microcosms. In addition, there was no difference between appearance of GBA outside the microcosms and inside the microcosms with fine mesh screen (i.e. the control to simulate natural conditions) or between mesocosm with plastic sheet preventing direct contact with sediment. I decided to leave the samples unprocessed because it was highly unlikely that species-specific treatment effects were created. We did learn, however, that direct contact with sediment, benthic porewater, and groundwater via direct transport through sands below the mats was not necessary for GBA biomass development. This leads us to an understanding for why the same taxa are growing on rocks and sands, as shown in early reports (Stevenson, 2016). It is likely that groundwater does not readily mix with surface water when it reaches the sand water interface, because of temperature differences and slow mixing with overlying nearshore surface water. Then P-rich groundwater, at least rich relative to surface water, could bathe benthic algae for at least a short time, often enough in quiet water conditions, that benthic algae get exposed to the higher phosphorus conditions.

We also learned that many kinds of algae are in the older benthic algal mats. Microscopic examination revealed filamentous and colonial cyanobacteria were common with many kinds of diatoms, including stalked diatoms (Fig. 1). There was also evidence of calcium carbonate deposition associated with mucilages in the mats, like the floating calcareous mats of the Everglades, which could contribute to added structural integrity of the GBA mats.

The 2020 Lake-wide Benthic Algal Survey of Torch Lake

Goal, rationale, and approach: After seeing Art Hoadley's aerial photographs with extensive GBA at many depths to the dropoff on the nearshore shoal, we needed to take samples from deeper locations to determine GBA composition for potentially most of the area of the bottom of Torch Lake. In 2020, in addition to the samples collected by TLA volunteers at the usual set of nearshore sites, I collected benthic algal samples at 16 locations around Torch Lake during June and again in August to determine if abundances and species composition of benthic algal mats varied around the lake and could be related to Clam River, topographic features, and land use around the lake. Samples were collected at 3.3 m depth at all locations, versus the 0.67-1.0 m that was practical for volunteers in the nearshore areas. 3.3 m was considered to be deeper than frequent wave disturbance and shallower than the drop-off and where light levels would be optimal for GBA development. Although water clarity is usually great in Torch Lake, during August 2020 water clarity was greatly reduced and may have been related to unusually heavy rains about that time.

Sampling sites were located relatively evenly around the lake and with consideration of local factors that could affect groundwater chemistry and discharge (Fig. 46, Table 18). Local factors were determined with satellite imagery and topographic maps. Land use and elevation of surrounding hills were used as variables in site selection because it is possible they could affect groundwater contamination and groundwater flow quantity, respectively. Land use was remarkably uniform around the lake, except it was very low north of Hayo-Went-Ha point (Site 3); so I would suspect that groundwater would have the least contamination there if contamination came from riparian septic systems. Some sites were located where high forested hills are close to the lake and could be sources of low nutrient groundwater (Sites 12 and 13). Riparian contamination of lake floor groundwater could be diluted if: 1) forested hills had little source of nutrients other than the rain and thereby also had low nutrient concentrations in groundwater and 2) groundwater flow into the lake was high from these hills because of the great difference in elevation of groundwater in the hills and the lake level. Lake floor groundwater from nearshore zones with lower elevations near the lake could have higher levels of contamination without regional sources of clean groundwater and high regional groundwater flows to dilute riparian contamination. Samples were collected along the south end of the lake (Site 9) where I would hypothesize that groundwater could be flowing out of Torch Lake toward the lower elevations of Lake Skegamog, as with surface water through Torch River. For the same reason, samples were collected offshore from a small wetland along the coast south of Torch Lake (Site 16) and at Becky's Beach (Site 1), which are shoreline areas closest to Grand Traverse Bay and where lake water could be leaking into groundwater that is flowing downslope toward Grand Traverse Bay. However, I must note comments from TLA members that groundwater upwelling at Becky's Beach has been noted in the past.

If benthic algal biomass varies around the lake and was associated with high groundwater nutrients being released into nearshore surface water in the nearshore zone, I would expect lower algal biomass: associated with low riparian land use; with high groundwater discharge arising from high shoreline hills if groundwater nutrients were diluted with rainfall dominated groundwater flow; and in groundwater recharge zones where water flows from the lake into the groundwater because lake water with relatively low P concentrations would be flowing from the lake, through benthic sands, and into the groundwater. I expected the effects of riparian zone land use and groundwater discharge from hills to be greatest in June because of higher spring than summer rains and decreasing groundwater discharge to the lake during the summer. These predictions are based on many assumptions. For example, the prediction of the effect of hills on water chemistry of groundwater discharge to the lake would differ depending upon whether land on the hills was developed by humans and groundwater in the hills was contaminated by human activity. However, these hypotheses about site conditions did provide a rationale for selecting these specific sampling locations.

Algal samples were collected with a scoop on a benthic sled that was drawn across the bottom of the lake with a pole (Fig. 47). The sled was attached to the end of the pole by a rope harness and was designed to slide across the surface of the sand. The scoop extended 1 cm below the sled, so when drawn across the bottom of the lake it scooped the top centimeter of sand, sediment, and algae into a container. The sled and scoop were then lifted into the boat and the scoop contents were transferred to a 1 gallon plastic bag. In August when the mat was well developed and firm, subsamples of just the mat were taken from the scoop samples to target the benthic algal analysis. All samples were preserved with formaldehyde after returning to shore. These samples will be processed during late fall and winter of 2021-2022. Preliminary reviews of these deeper samples show similar diatom species as in shallower collections from previous years. In addition, cyanobacteria were in greater abundance in some of the thicker mats than previous samples.

Algal biomass was determined using close-up underwater videos (https://youtu.be/vQTnvGEx0No) of sample collection to characterize algal color and mat thickness. Initial assessments were reviewed and compared visually to each other to increase accuracy of algal biomass ranking among sites.

Results and Discussion: Algal biomass varied substantially among sites in June and was both higher and less variable in August than in June (Fig. 48). Algal biomass was noticeably lower at Hayo-Went-Ha North, the Petty site, and near the high hills in the West side of Torch Lake. During August, algal biomass at the Petty site and near the high hills was more similar to other locations than during June. Algal biomass remained low at the site north of Hayo-Went-Ha point in August. Algal biomass patterns also indicated low GBA development at the site at the east end of the Sandbar where algal biomass was as low in August as in June and at Becky's Beach where algal biomass decreased from June to August.

First, these results show that benthic algal biomass is not the same at all locations around Torch Lake and groundwater conditions may regulate GBA based on the presumed differences in groundwater conditions among sites that were stated earlier. Although we do not know groundwater flow conditions at the sites with certainty, groundwater regulation of GBA is indicated by spatial variation in GBA around the lake and low biomass GBA conditions associated with: low landscape disturbance at Hayo-Went-Ha North; potential flow of lake water into the groundwater at the east end of the Sandbar at the south end of the lake and at north end of the lake at Becky's Beach; and near the high hills on the west side of the lake.

Analyses of Hoadley's Aerial Photographs for GBA Biomass

Goals and rationale: Art Hoadley has taken aerial photographs of the nearshore zone for the last seven years, 2015-2021. In addition, there are a few aerial photographs and useful satellite images from previous years. These photographs have the potential to answer many questions about the spatial and temporal variability in GBA, including when GBA first occurred in the lakes.

Developing and Testing the Method for Analyzing GBA from Aerial Photographs: I developed a method for characterizing the abundance of GBA using the photographs, which I tested by comparing the GBA characterized using Hoadley's aerial photographs and visual assessments made with the GoPro camera during my 2020 survey of GBA around Torch Lake. Visual assessments of algal biomass have provided valuable spatially integrated estimates of algal biomass in stream, lake, and wetland studies. We found highly informative relationships between cover of filamentous benthic algae on the bottoms of streams and nutrients which could not practically have been done by sampling rocks and measuring biomass on those rocks because spatial variability is so high (Stevenson and Bahls, 1999; Stevenson, Rier, Riseng, Schultz and Wiley, 2006). We found a threshold in response of naturally occurring floating calcareous algal biomass along a phosphorus gradient in the Everglades by using aerial photography, which would have been impractical by just sampling algae in the wetlands (Stevenson unpublished data). Secchi depth is widely used in lakes to characterized phytoplankton abundance. *Cladophora* surveys around lakes are used to detect septic tank leakage. Looking at Hoadley's aerial photographs, I felt that I could identify areas with high and low biomass of GBA based on color of the lake bottom. This method, if shown to be reliable, could then be used more extensively to address questions about GBA biomass over larger spatial and temporal scales.

I established a gradient of golden brown color ranging from white sand to dark golden brown, which I ranked from zero to 6 (Fig. 49a). I used a gradient of colors in the Excel palette of colors for highlighting cells in a spreadsheet to establish a standard to help provide consistency in characterizations of the color of the bottoms of the lake. I expected the gradient of colors to be related to thickness of GBA that I observed in GoPro videos taken when sampling during my 2020 GBA survey of Torch Lake.

During 2020, Hoadley flew a route around Torch Lake in May and August with more than 1000 images of the shallow water zone taken during each flight. I downloaded these photographs from Hoadley's Google Folders, with his permission, and then examined the photographs all the way around the lake from his starting point south of the Clam River to Alden, the southern sandbar, a route up the west shore to Eastport and back south along the east shore to Clam River. I took notes that could be used to characterize GBA around the entire shoreline. For my current goal, I did more intensive examinations of images that were located where I sampled in 2020, which I had established by GPS when I was sampling. I pinpointed exactly where I had sampled in Hoadley images based on where I had dropped my pin on a Google Earth map, that Google Earth image on my phone, and the matches between my Google Earth map and Hoadley's image matches. Shoreline features helped get in the general area of where I sampled, but I could more accurately identify locations by matching the remarkably detailed patterns in sand waves on the bottom of the lake and the shape of the drop-off boundary, such as curves, distance from shore, and apparent depth gradients. These bottom features were evident in both the Google Earth image on my phone and Hoadley's aerial photographs.

I chose among several aerial images with the location I sampled based on the angle from the camera to the sampling location, because slight deviations in that angle did affect apparent intensity of the GBA color. I routinely selected the richest GBA color because it seemed most likely to be a path with least glare and light reflection obscuring true color of the mats. I noted that there were some regions of images where angle of the light was sufficiently extreme that color of the lake bottom could be misinterpreted to have no GBA.

Next, I visualized a transect perpendicular from shore that would intersect my sampling location at 3.3 m depth offshore and characterized 6 conditions along that transect. The first condition recorded was the proportion of the width of the aerial photograph image transcended by the transect. This provided a rough characterization of the relative length of the transect. I usually had transects run for shore to the drop-off. If the drop-off was not distinct, then I chose an endpoint based on what appeared to be a change in depth at a relatively deep depth.

Then I broke the transect up into zones with different conditions and recorded for each zone: 1) percent of the transect covered by the zone; 2) richest color of benthic algae in the zone, 3) greyness of the sand because zones of black sand were observed and I think often benthic algae appeared black in deep water; 4) percent cover of benthic algae for when it was patchy or 100 percent if it was not patchy; and 5) texture such as grainy, smooth, wavy, or linear patterns in GBA along the shoreline. Characterizing greyness of sand seemed necessary as the black sand and grey to black color found in different areas complicated the characterization of the golden brown color, which was pervasive. Therefore, I again made a white-grey-almost black scale from 0 to 6 (Fig. 49a) and I used that to independently characterize both the golden brown and grey scale colors for each zone. In some cases the grey became so dark that golden brown color could not be assessed. In those cases, that zone of the transect was eliminated from the analysis. I was not able to see macroalgae, such as *Chara* or *Cladophora*, or submerged plants in images. Before moving on to the next transect, I also recorded the image number in the filename for later reference.

These data were loaded into a spreadsheet and analyzed with the R statistical program. With that program, I calculated weighted averages of golden brown color along the transect ($\Sigma_{i=1,Z}\ p_iG$) where weights were for all zones (Z) based on the percent of the transect (p_i) that was a specific golden color (G) for one characterization of GBA biomass. For the second measure of GBA biomass, I corrected for percent of the zone covered by GBA as well as weighted the characterization of GBA biomass for the transect based on proportion of the transect with GBA values.

No patches of green algae were observed around Torch Lake when reviewing images for benthic algal biomass characterizations with aerial photographs.

Results – Testing the Method: GBA biomass estimated with aerial photographs was related to GBA thickness observed with the GoPro videos taken when sampling benthic algae with the sampling sled (Fig. 49b-c, Table 19). Some error variance could be due to the discrepancy between obtaining aerial photographs in May and GoPro videos in June, as indicated by May estimates of GBA biomass by aerial photographs being lower than GoPro-predicted values based on the linear regression (i.e. June values falling below the regression line and August values usually above the regression line, Figs. 49b-c). In addition, the difference between my sampling at a point along the transect with the video and estimated GBA biomass for a whole transect with aerial photographs could be a source of error. Based on my experience looking at GBA in the lake and the photographs, plus this statistical relationship between color-based estimates of GBA from aerial photographs matching GoPro estimates, I decided to advance this approach and use it to evaluate spatial variability in GBA around Torch Lake and how that differed between May and August in 2020. I decided to use estimates of GBA without correcting for percent cover within zones because the relationship with that estimate of GBA and the GoPro estimate was better than the estimate with corrections for percent cover based on the amount of variation explained (Table 19).

Results - Patterns of GBA biomass around Torch Lake in 2020. GBA biomass varied among locations around Torch Lake, with low as well as high biomass at different sites (Fig. 50). Estimates of GBA biomass by aerial photography were usually lower in May than August, as with GoPro estimates of biomass. GBA biomass in May tended to be higher along the southeast shoreline from the Clam River, south to the sandbar, across the south end of the lake to the west shore near Torch River, and then north past Deepwater Point to a location south of the high hills on the west shore. Although these GBA values were relatively high, at most sites they averaged less than 2, which I think of as a benchmark to distinguish moderate from high GBA biomass. According to this benchmark, GBA biomass was high in June only at the Gourley site, the southwest corner of Torch Lake, and just north of Deepwater Point (near Penoza's). In August, most sites had GBA ranks greater than 2, except north of Hayo-Went-Ha, the sites near hills on the west side of Torch Lake, and the wetland near Torch Lake William B. Good Day Park in the northwest corner of the lake.

One additional observation from aerial photographs is important. Patterns in GBA color and presumed biomass varied with position on what appear to be sand waves in the bottom of the lake. These waves are relatively permanent features based on the consistency in their location in satellite imagery and from season to season in the aerial photographs. Benthic algal cover patterns vary along the rising edge, crest, and falling edge of these waves.

These observations again, as with the biomass estimates from GoPro videos when sampling around the lake, indicate groundwater affects GBA and in ways consistent with a priori predictions.

The GBA patterns on what are presumed to be sand waves (Fig. 51) were observed during a boat survey of Torch GBA in 2015. The Hoadley aerial photographs indicated how widespread and stable the sand waves are. More work needs to be done to determine what these features are on the bottom of lakes, but if they are waves of sand, then interactions with groundwater passing through those waves is one hypothesis for consistent patterns of GBA on the waves. An alternative hypothesis could be related to sand movement, disturbance of benthic algal communities and resulting changes in accumulation across the sand waves. However, the consistency of location in the sand waves from year to year indicates that the sand on the waves is not moving frequently with currents on the bottom of the lake.

Challenges remain with interpretation of the aerial photographs. In particular, distinguishing dark sands from heavy algal growth on rocks in deeper water that also appear dark grey is important to prevent underestimation of algal biomass along a transect. This was an issue at the Gourley site in August, when dark grey zones were observed at the deep end of the transect and could not be classified as GBA without further study. As a result, GBA at the Gourley site was likely underestimated in August.

Results – Patterns in GBA Biomass in 2010, 2012, and 2015. I reviewed Hoadley's aerial photographs from August and September 2010, May 2012, monthly from May to September in 2015, with less attention to detail from 2016 to 2019, and with great attention to detail in 2020. Basically, GBA was not evident in photographs during 2010 and 2012 but was quite abundant in 2015 and thereafter. 2015 was the first year of our studies, which must mean that stakeholders around the three lakes observed GBA during the summer of 2014, at least. Even though we do not have aerial images from the peak GBA months of July, August, or September in 2012, the lack of GBA in images in May 2012 indicated GBA throughout that summer was likely low. If these observations and assumptions are correct, GBA development was a rapid expansion over just a few years and probably became a noticeable nuisance during summer 2013 or 2014.

RESULTS – Addendum for 2021

Water Chemistry 2021

Goals, Rationale and Approach

We had three main goals for the water chemistry sampling during summer 2021 that were related to developing more certainty about previous results. We wanted to further evaluate my conclusion that: 1) lake floor groundwater was not extensively contaminated with nutrients; 2) there was evidence that water chemistry in mid-lake surface waters changed during the summer; and 3) nearshore surface water phosphorus concentrations were higher than mid-lake concentrations. Therefore, volunteers sampled well water, lake floor groundwater, and nearshore surface water multiple times during the summer at four locations: Gourley and Petty sites in Torch Lake; Drake site in Lake Bellaire; and the Hoadley site in Clam Lake. Volunteers sampled mid-lake at the southern Torch Lake site throughout the summer. Nearshore and mid-lake water chemistry was compared to address the third goal.

Results

Comparing water chemistry among water sources: As an overview of the results of sampling wells, lake floor piezometers and nearshore surface water, water chemistry varied substantially more among sites in lake floor groundwater and well water compared to nearshore surface water (Fig. 52). Phosphorus (both PO4-P and TP) was slightly lower in well water at the Gourley site than other sites. NOx-N and TN, but not NH4-N were higher in well water higher at the Gourley and Petty sites than the Drake site. Cl was higher at the Drake site than the Gourley and Petty site.

I compared lake floor groundwater and well water to determine if lake floor groundwater was contaminated. Compared to well water, lake floor groundwater had: higher PO4-P and TP concentrations only at the Gourley site; had lower NOx-N at the Gourley and Petty site; had higher NH4-N at the Drake and Gourley site; had higher TN at the Gourley; and had higher Cl at the Gourley, Drake, and Petty site.

Compared to lake floor groundwater, nearshore surface water: usually had lower PO4-P and TP concentrations and higher NOx-N concentrations at all sites; lower NH4-N at all sites except Petty; had lower TN at Gourley due to the unusually high NH4-N; and lower Cl at the Drake and Gourley site but higher Cl at the Hoadley site.

Surface water analyses. The spatial differences in surface water chemistry between nearshore and mid-lake locations and temporal differences during the summer at mid-lake were not observed in 2021 as in 2020. No difference in any water chemistry parameters was observed between the nearshore and mid-lake Torch Lake locations (Fig. 53). No changes were observed in water chemistry with the 12 samples over nearly a 90-day summer period, expect possibly for NO3-N (Fig. 54). NO3-N was consistently low during the midsummer period, compared to earlier and later in the summer, but this pattern had a reasonable likelihood of occurring by chance and was not observed in previous results.

Discussion

Many of the water chemistry differences between lake floor piezometer and nearshore surface water during 2021 were similar to those observed during prior years. Key among those observations were the unusual lake floor groundwater chemistry at the Gourley and Drake sites with high ammonia and chloride concentrations and low NOx concentrations. The evidence of phosphorus contamination of lake floor groundwater was limited, with only the Gourley site having higher phosphorus in lake floor groundwater than well water. That was not observed at the Gourley site in 2016 when well water was last sampled and only phosphate was assayed.

The higher phosphorus concentrations in nearshore than mid-lake surface waters that were observed in 2020 were not observed again in 2021, which reduces the likelihood that phosphorus concentrations in nearshore surface water are somewhat enriched with phosphorus from groundwater discharge and thereby supporting extensive growths of GBA. In addition, the season changes in some water chemistry concentrations in mid-lake surface water that were observed in 2020 were not observed in 2021.

Long-term Trends in TP in Lakes of Northern Lower Peninsula of Michigan

Goals, Rationale and Approach

The goal of this study was to determine the regional pattern in lake TP concentrations over the last 20-30 years. The rationale was that the list of potential causes of TP decreases in Bellaire, Clam and Torch would be narrowed if we knew that the lake TP decrease was regional or watershed specific. CLMP and ToMWC have long term datasets that I got permission to use to test the hypothesis that lake TP has been decreasing regionally in the northern lower peninsula of Michigan. I downloaded data for seven sites in four lakes in the CLMP dataset that had relatively long-term data (27 years). I downloaded data for 23 sites in 17 lakes from the ToMWC website and related lake TP to year with linear regression.

Results

Spring and summer TP concentrations from mid-lake surface waters in CLMP dataset did not decrease significantly with year in analyses using CLMP data (Table 20). However, in the CLMP data all seven estimates of change in summer TP with year were negative, and five of the seven estimates of spring TP changes with year were negative.

All except 3 of the 23 sites in the ToMWC data, which were the largest and smallest of the 17 lakes, had statistically significant negative changes in TP with year (Table 21).

Discussion

Changes in TP concentrations measured over the last 20-30 years indicate TP has decreased in mid-lake surface waters of many lakes in the northern lower peninsula of Michigan. The ToMWC data show this for most lakes, except the smallest and largest. The lack of statistically significant change in independent regressions for each lake and season with CLMP data could be related to interannual variability and challenges with sampling and measuring TP in low nutrient lakes. Also, sample numbers were smaller for CLMP data than ToMWC for many lakes. Combining spring and summer data into one analysis for each lake site and combining data for lakes could also help evaluate certainty of TP patterns in lakes evaluated with CLMP data. Another way to evaluate the CLMP data is to consider the probability that 7 out of 7 lake sites would have decreases in summer TP and 5 out of 7 lake sites would have decreases in summer TP. Given there are two possibilities for changes in TP, either increases or decreases, the probability of having 7 out of 7 and 5 out of 7 decreases can be determined as you would calculate the probability of getting 7 out of 7 or 5 out of 7 heads in a row if flipping a coin just 7 times. Those probabilities are 0.5^7 and 0.5^5, which are 0.007813 and 0.03215. Thus, there is very little chance, less that 1 in 125 times or 1 in 25 times that 7 out of 7 and 5 out of 7 changes would have been negative. Therefore, I conclude that there is strong evidence that TP concentrations have decreased in lakes of the northern lower peninsula of Michigan over the last 20 to 30 years. I plan to evaluate these datasets further to provide additional information to help us determine the cause of decrease in lake TP concentrations.

Physical and Biological Structure of the GBA Mats

Goal, Rationale, and Approach

The goal of this project was to evaluate the physical structure of algal mats in Torch Lake and link biological components to that physical structure. This effort would advance our understanding of GBA mat characteristics at a scale that we have not investigated rigorously and provide observations for future hypotheses to test.

On September 22, 2021, Dean Branson, Becky Norris and I collected six GBA samples with the benthic algal sampling sled at two locations in the northeastern corner of Torch Lake and at 10 feet depth. Mats were separated from the rest of the sand and calcareous material scooped into the sampling container on the sled when the sled was pulled from the water. The mats were placed carefully into Whirl-pak® bags without added water or preservative to protect the structural integrity of the mat so it would remain as close as possible to what it was at the bottom of the lake. Within the next 24 hours, each mat was removed from the Whirl-pak® bags and placed on a white plate filled with well water which did not contain water purification chemicals and had chemistry similar to Torch Lake (based on earlier comparisons for experiments). Each mat was examined macroscopically to determine physically delineated layers. The mat was also probed with forceps to examine the structural integrity of delineated layers and subsections. Then each physically delineated later of the mat was separated into the major layers and other macroscopically different sections of those major layers. These smallest sections of the mat were then examined with a light microscope with magnifications starting at 100X and finishing at 1000X. Each macroscopically evident section was examined independently, and then further divided after microscopic observation to get a closer and closer look (at higher and higher magnifications) at the physical structure of the mat and the biological components associated with that physical structure. The mat was observed at 100X and 200X on microscopes slides in a drop of water without a coverglass covering the specimens. Then specimens were covered by a coverglass and observed at 200X, 400X and 1000X. This process was repeated with the first two mat samples and most specimens separated from the mats but was not repeated in detail with the other four mat samples because the structures appeared very physically and biologically like the first mat samples. After all observations of samples were complete, subsamples from each of the mat layers and each of the samples were separated, placed in vials, and preserved for more detailed analysis in the future.

Results

All mats had the same basic three-layered physical structure (Fig. 55). The surface layer, closest to lake surface water, was 1-2 mm thick, highly colored and the most structurally strong. For example, many parts of it could be grasped with forceps and remain intact as it was removed from the water despite the drag on the mat layer by the surface tension of water. Underlying the colored surface layer was a jelly like gray layer that did not fall apart when underwater and probed with forceps, but it could not be lifted from the water without falling apart. Sections of this were sampled for microscopy by trapping it in a drop of water in the wedge created behind the tips of the forceps. The surface layer did not change shape when underwater and probed, whereas the jelly-like gray middle layer changed shape a little but remained intact. The third, bottom layer was gray matter like the middle layer, but it fell apart when probed. The third layer had sufficient structural integrity to separate with the top two layers when sampled but dissociated from them when placed in water on the plate.

The surface layer was composed of two major components when observed macroscopically, which was confirmed with similarity among repeated subsamples being examined microscopically. The first and somewhat more common component based on these qualitative assessments, were lobes of cyanobacteria with high numbers of loosely associated diatoms that were observed at 100X. The second structural feature were lobes of diatoms that were closely and tightly bound together.

Using higher magnifications ranging from 200-1000X, the cyanobacteria in the surface layer were identified as coccoid forms consistent with characteristics of genera in the order Pleurocapsales (Fig. 55). They created a leathery layer of cells overlain by dense mucilages that were colored golden brown. Many smaller motile diatoms were in this surface layer, and they rapidly moved into water surrounding the mat when placed in water on a microscope slide. Some moved around independently and some attached together in rafts of 100s of cells. These small motile and rafting diatoms appeared to be in the genera *Encyonema* and *Delicata*. They were also golden brown because of pigments in their chloroplasts.

The middle layer was mostly composed of diatoms in a gray matrix that appeared to be aggregates of calcium carbonate. Some very narrow filaments of cyanobacteria were observed in the gray matrix, but they were relatively rare compared to diatoms. The mucilages holding the middle layer together were fine and could not be discerned and therefore attributed to a likely source, such as algae, bacteria, or fungi. The living diatoms in the middle layer differed from those in the surface layer, being mostly large diatoms of the genus *Navicula* that were moving through the gray matrix or *Eunotia,* which were not moving through the gray matrix. Occasional colored pieces of debris of unknown origin were observed in the middle gray layer. The golden brown color of diatoms and reddish color of debris in the middle gray layer were not evident macroscopically.

The bottom, disaggregated layer was not examined microscopically. Macroscopically it appeared like the middle gray layer, but without significant structural integrity. As with the two top layers, subsamples were collected and preserved for detailed analysis at a later date.

Discussion

My earlier reports have emphasized diatom dominance in the benthic algal mats, because that is what we saw in counts when we examined all algae in 2015 samples. I have noted that mats sampled from deeper water have considerable amounts of cyanobacteria. St. Armand also observed cyanobacteria in benthic algal mats in TLPA study (Gaulke, 2021). Differences in the relative importance of cyanobacteria in mats could be due to several factors. First, the relative importance of cyanobacteria may have increased during the time GBA has been developing in the lakes over the last 5 years. TLA volunteers note that GBA was more loosely associated during early years of development. Second, the importance of cyanobacteria may increase with time during the summer, because cyanobacteria generally (but not always) perform better in warmer water temperatures. Third, cyanobacteria may be less disturbance resistant and more abundant in deep water habitats, than diatoms. Benthos in shallow nearshore areas may be scoured by ice early in the year and waves at other times of the year, so may be restricted to diatoms that may colonize more rapidly than cyanobacteria. Most of TLA's sampling of benthic algae has been in the wadeable nearshore zone, which is also where stakeholders interact most with the GBA. So interactive effects of depth, seasonal differences, and longer-term development of GBA in the lakes could be explaining differences in relative and absolute abundances of cyanobacteria and diatoms in benthic algae.

The layered structure of mats on the bottom of the lake indicated different functions for these layers that could help mats persist. The vertical organization of benthic algal mats has been observed and hypothesized for decades (Hoagland, Roermer and Rosowski, 1982; Johnson, Tuchman and Peterson, 1997). On the bottom of Torch Lake, the surface mat was more tightly aggregated, which would protect the mat from physical disturbance and many types of herbivores. The lower structure of the mat may function to remineralize phytoplankton that have settled onto the lake bottom as well as dying benthic algae. The tightly aggregated surface mat would also seal the nutrients generated by remineralization of organic matter by the lower layer into the mat. In the last 20 years, tests of hypotheses explaining that vertical structure have been possible with new analytical techniques and have documented specialized function and algal-bacterial interactions in different layers in benthic algal mats. Some evidence that these mechanisms are plausible will be detailed in the discussion below.

DISCUSSION

In this discussion, I will first address some key questions and present an explanation for the cause of GBA in Torch Lake that is consistent with the data we currently have. I will also discuss what this explanation for GBA means for management. This explanation is actually a set of hypotheses for how Torch Lake and Lake Bellaire have changed and how that change could cause GBA. I will discuss the strengths and weaknesses of this explanation. I want to emphasize that this is set of hypotheses for GBA that needs be tested in the future to increase the certainty of our understanding of the causes of GBA before any management actions are taken. I would not be satisfied with the current level of certainty that I have for this explanation if I were making final decisions about how to manage GBA. But I think this explanation is consistent with the results we have learned over the last seven years of research. Further research will increase certainty in this explanation or lead to other explanations. This explanation may also provide the reasons for GBA in Lake Bellaire and other northern oligotrophic lakes, but much more research is needed in those systems to extrapolate from the data presented in this report.

After I present this explanation, I will provide a full review of the set of hypotheses for GBA that we have considered and tested. That review will include an explanation of the hypothesis, how it could cause GBA, and the evidence that we have that supports or contradicts those hypotheses (Table 1). Some of those hypotheses are integrated, either in whole or in part, into explanation for GBA that is consistent with current results. I present the hypothesis that is consistent with the evidence first so you know what it is and you can compare it with other hypotheses as they are discussed.

An Explanation for GBA

What has changed in the lakes that could cause GBA?

The decrease in phosphorus concentration is one thing that we know has changed in Torch Lake and Lake Bellaire, and many northern Michigan lakes, according to ToMWC and CLMP monitoring data. In addition, paleolimnological research by Fritz et al. (1993) suggested phosphorus was decreasing in northern Michigan lakes as early as the mid-20th century. The cause for the decrease in phosphorus is likely more than Dreissenid mussel filtration of phytoplankton and climate change warming water which has been said to increase co-precipitation of calcium and phosphorus if we assume that phosphorus has been decreasing since the middle of the 20th century.

I think nitrogen deposition is an important possible cause for long-term phosphorus decreases in northern Michigan lakes. In causal analysis (Beyer 1989), the cause must precede the effect. Nitrogen deposition from upwind farms in the Midwest has been occurring for a sufficiently long time to explain the long-term decrease in phosphorus if that decrease does indeed extend back to the mid-1900s as indicated by Fritz et al. (1993). E. F. Stoermer, an esteemed algal ecologist retired from the University of Michigan, was quoted in the Fritz et al. paper as hypothesizing nitrogen deposition as the cause for the suspected decreasing phosphorus in northern Michigan lakes. The start of the phosphorus decrease in northern Michigan lakes in the mid-1990s follows the invention of the Haber-Bosch process of converting molecular hydrogen (H_2) and molecular nitrogen (N_2) to ammonia. That ammonia fueled a global revolution in agricultural productivity because it was a relatively inexpensive way to produce fertilizer.

The importance of nitrogen deposition on terrestrial and aquatic ecosystems was initially recognized in the mid to late 1990s (Stoddard, 1994; Vitousek et al., 1997), note after Stoermer's hypothesis in Fritz's 1993 paper. More detailed investigations of effects on lakes started soon thereafter with work led by

Bergstrom and Hessen in Scandinavian lakes where they found atmospheric N deposition could increase productivity of oligotrophic lakes (Bergström, Blomqvist and Jansson, 2005; Bergström and Jansson, 2006) and reduce phosphorus concentrations (Hessen, Andersen, Larsen, Skjelkvåle and de Wit, 2009). Bergstrom and others went so far as to suggest that the natural state of many oligotrophic lakes is nitrogen rather than phosphorus limited, and atmospheric nitrogen deposition has historically changed the primary limiting nutrient of many lakes from nitrogen to phosphorus. Significant work continued with some work in the US on alpine lakes of the Rocky Mountains showing nitrogen limited lakes were becoming more productive as a result of nitrogen deposition (Elser, Kyle, Steger, Nydick and Baron, 2009).

In Sweden, Liess, Drakare and Kahlert (2009) showed atmospheric nitrogen deposition could increase phosphorus limitation by benthic algae. They found higher in N:P ratios in benthic algae and grazers and changes in species composition in lakes with high versus low atmospheric nitrogen deposition rates.

Maps for NO3 and NH4 deposition (National Atmospheric Deposition Program, 2021) show deposition in the Torch Lake area is elevated for both forms of nitrogen compared to natural background observed in regions without extensive agriculture or urban activities. These maps show a clear spatial relationship with agricultural regions in the Midwest. The USGS National Atmospheric Deposition Program has maps dating back to 1985, which show that NO3 and NH4 deposition in the Torch Lake area have been and continue to be elevated above natural background, which can be observed in regions without extensive agriculture or urban activities (National Atmospheric Deposition Program, 2021).

Thus, nitrogen deposition is a plausible explanation for long-term decreases in phosphorus concentrations in Torch, Clam and Bellaire as well as other northern Michigan lakes. Nitrogen deposition may be increasing the efficiency of phytoplankton uptake of available phosphorus and settling more phosphorus out of the water column. If increased nitrogen availability shifted phytoplankton species to those that could use lower concentrations of phosphorus, then more soluble phosphorus could be transformed into particulate form as algae settled out of the water column. Paleolimnological study would be a good method for testing these interrelated hypotheses.

Another plausible explanation for phosphorus decreases during this longer period are the 1977 phosphate bans in detergents and phosphorus fertilizer regulation in 1994 legislation. These bans took place well after the mid-1900s when Fritz's data shows the potential start of phosphorus declines in lakes. So they are likely contributors, but not a complete explanation if we want to find causes back to the mid-1900s.

In addition, Dreissenid mussel filtration of phytoplankton could have decreased phosphorus in lake surface waters and played a key role in phosphorus depletion (Cha, Stow and Bernhardt, 2013; Li et al., 2021). Dreissenid mussels have been widely cited as an issue in the oligotrophication (decrease in productivity rather than eutrophication as an increase in productivity) of the Great Lakes and inland lakes as well. The additive effects of nitrogen deposition, phosphate bans, and Dreissenid mussel filtration are plausible explanations for decreases in lake phosphorus concentrations and may be sufficient for decreasing surface water phosphorus to the concentrations observed in the last decade. Perhaps it was the pulse in Dreissenid mussels that lowered phosphorus concentrations in lakes in recent years; but if that were the case, we would expect a rebound in phosphorus if we assume Dreissenid mussels have decreased from densities sufficient to impact phytoplankton.

How can a decrease in phosphorus in surface waters of a lake cause an increase in benthic algae?

Changes in water chemistry, including nutrient concentrations and nutrient ratios, can cause shifts in algal species composition. Phosphorus is the most limiting nutrient for algal growth in Torch Lake and

Lake Bellaire (Lowe and Kociolek, 2016; Stevenson, 2016; Stevenson, 2017). The decrease in phosphorus concentration and corresponding increase in N:P ratios could affect species composition and species metabolism in ways that could produce mats of cyanobacteria and diatoms that have substantial structural integrity as a result of high quantities of mucilage. Benthic algae produce more mucilages when their growth is limited by low phosphorus concentrations (Kilroy and Bothwell, 2011). Physiologically, we would expect that photosynthesis would not be reduced as much as cell growth, because: 1) sugars from photosynthesis are composed of carbon, hydrogen, and oxygen and 2) the proteins, nucleic acids, and phospholipids of membranes needed for cell reproduction have high quantities of nitrogen and phosphorus (as well as C, H, and O). Therefore, when cells cannot reproduce because of nitrogen or phosphorus limitation, the excess photosynthate could be excreted by cells to produce mucilages that protect algae as they grow slowly and accumulate over longer periods of time. Mucilages reduce algal susceptibility to physical disturbance (Hoagland, Rosowski, Gretz and Roemer, 1993; Underwood and Paterson, 2003) and potentially to grazing as well (Chick, Geddes and Trexler, 2008). Mucilages can also entrain nutrients near cells and increase nutrient cycling (Reynolds, 2007).

Everglades: The calcium rich, low phosphorus waters and diatom species composition in Torch Lake and Lake Bellaire have many similarities with conditions in hard water regions of the Everglades. Some of the key taxonomic similarities are *Encyonema evergladianum* and *Mastogloia lacustris* (Slate and Stevenson, 2007). Differences in species composition may also provide keys to the ecology of benthic algae in Torch Lake and Lake Bellaire. For example, *Delicata delicatula* is rare in the Everglades. Comparisons of the cyanobacteria species are challenging because taxonomy is not as well understood as diatoms.

Epiphytic and floating calcareous algal mats of the Everglades accumulate to high biomasses in low phosphorus conditions, as in Torch Lake and Lake Bellaire. In the Everglades, the mats are resistant to grazers and physical disturbance from wind and rain. The disturbance resistance of the calcareous mats of the Everglades is related to the mucilages binding mats together and the calcium carbonate depositions in mats. Calcium carbonate depositions are also evident in Torch Lake mats, particularly in the middle and lower layers of the mat. Photosynthesis by algae, CO2 uptake, and resulting alkalization of waters within the thick mucilaginous matrix localize calcium carbonate deposition within the mat. The role of calcium deposition in the Torch Lake benthic mats, where they were observed, is not known; but based on other similarities with Everglades mats, it is reasonable to hypothesize that calcium carbonate deposition in mats could increase mat resistance to disturbance. Another intriguing hypothesis is calcium deposition stores phosphorus in mats as calcium phosphate, but this hypothesis has not been tested rigorously.

The calcareous algal mats are a natural feature of the Everglades. Their sensitivity to phosphorus pollution was one of the key reasons for establishing a low, 10 ug TP/L management target for the Everglades (Stevenson, 2014). When total phosphorus concentrations exceeded 10 ug/L, the occurrence of calcareous algal mats decreased greatly, which was related to a loss of calcium deposition in mats and resulting loss caused by wind and grazer resistance. The loss of calcium carbonate in mats could have been due to many factors and potentially a combination of those factors as phosphorus increased: a great change in species composition of diatoms in mats at the 10 ug TP/L threshold and presumed changes in cyanobacteria species as well; increased algal growth rates and reduced mucilage deposition; and a change inorganic carbon uptake for photosynthesis that decreases the alkalization of water in mats that regulates calcium deposition.

The application of what we learned in the Everglades requires reversing the causal pathway because we understood how phosphorus increases negatively affected natural algal mats in the Everglades. If we reverse the Everglades causal pathway for benthic algae in Torch Lake and Lake Bellaire, I would predict a decrease in phosphorus caused a change in species composition, reduced algal growth rates, increased mucilage production, and potentially increased calcium deposition in benthic mats that increased their resilience to physical disturbance.

Didymosphaenia: Another important analog to the development of GBA in lakes with decreases in phosphorus concentration is the case of a benthic diatom in streams, *Didymosphenia*. *Didymosphenia* is becoming a nuisance problem around the world in habitats, that for one reason or another, have had reductions in phosphorus concentration to extremely low concentrations (Bothwell, Taylor and Kilroy, 2014). *Didymosphenia* produces dense mucilaginous mats with relatively few cells compared to all the mucilages that are produced. *Didymosphenia* is a stalk forming diatom, so it produces long branching stalks with cells at the ends, like leaves on a tree. The mats can be over an inch thick on rocks in streams. Some of the first blooms of *Didymosphaenia* were observed in forest streams of Vancouver Island, which it is now know had started an island-wide nitrogen forest fertilization program that started in the early 1990s.

A common feature of these mats is mucilages produced by the algae, which can be greater when algal reproduction is limited by low nutrient concentrations (Kilroy and Bothwell, 2011). When algal cell reproduction is limited by phosphorus and nitrogen, carbon fixed in carbohydrates by photosynthesis cannot be used in production of proteins, cell membranes and other cytoplasmic elements that require phosphorus and nitrogen. Those N and P-rich elements of the cytoplasm are needed for cell reproduction. The excess carbohydrates, almost entirely composed of carbon, oxygen, and hydrogen, are then stored as starches and oils in cells, depending upon the kind of algae. In addition, the excess carbon can be excreted by cells. Excreted carbohydrates often form mucilaginous structures that are hypothesized to help cells capture nutrients. One of these mechanisms is entrainment of dissolved and particulate organic material in benthic mats and trapping extracellular enzymes secreted by algae (and also by co-adapted bacteria and fungi) that breakdown those organic materials. Within-mat changes in pH can release phosphorus for cell uptake (Wood, Depree, Brown, McAllister and Hawes, 2015). Alkaline phosphatase plays a key role in mucilagenous matrices as an exoenzyme that remineralizes phosphate from organic compounds with phosphorus (Sharma, Inglett, Reddy and Ogram, 2005).

Some diatom species are adapted to grow in low nutrients, whereas others require higher nutrient concentrations to grow. Those adapted to grow in low nutrients are expected to have lower maximum growth rates because they adapt by allocating cellular resources for sequestering and uptake of nutrients in low concentrations. Even if these species grow slowly, they can grow over longer periods of time to form dense mats because the mats are resistant to disturbance.

Finally and importantly, the two richest sources of phosphorus remaining in the lakes was lake floor groundwater when surface water phosphorus was depleted and phytoplankton settling onto the lake bottom. Phosphorus concentrations were higher in the lake floor groundwater than benthic pore waters, and the benthic pore water concentrations were higher than nearshore surface water. Phosphorus concentrations have not decreased in the groundwater based on a comparison with 2005 sampling (Bretz et al. 2006). Thus, the interface between sand or rock and the water column became the most nutrient rich habitat with light for algae to grow after surface water phosphorus was depleted.

We have some evidence that nearshore surface waters had higher phosphorus concentrations than mid-lake, deep basin surface waters. This difference was not evident in sampling conducted in 2021 which was designed to compare nearshore and mid-lake surface water chemistry. In addition, the benthic algal mats can occur relatively far from shore and in some deeper waters, so it is likely that they have been exposed to the decreases in surface water phosphorus concentrations measured in the mid-lake, deep basin surface waters sampled by CLMP and ToMWC.

Why has GBA development seemed to occur so rapidly over the last decade?

One possibility for the recent, rapid occurrence of GBA after a potentially long period in phosphorus decreases is the rapid and relatively short-lived invasion and relatively high densities of Dreissenid mussels. Dreissenid mussel filtration of algae in the water column reduces water column phosphorus (Cha, Stow and Bernhardt, 2013). With the zebra mussel invasions, phosphorus concentrations may have decreased faster than other times in the last 50-80 years and produced a tipping point for shifts in algal species composition.

Alternatively, threshold responses by algae to nutrient gradients are common. Floating calcareous algal mats of the Everglades decrease dramatically in coverage of open water sloughs at TP concentrations above a 10 ug/L threshold (Stevenson, 2014). The filamentous green alga Cladophora increases in cover of stream bottoms dramatically about 25 ug/L TP because the Cladophora can outgrow grazing invertebrates (Stevenson, Bennett, Jordan and French, 2012). Perhaps a similar phosphorus threshold exists for GBA in Torch Lake and Lake Bellaire. As phosphorus decreased to the very low concentrations now observed, that threshold was crossed.

Another mechanism to explain the relatively rapid appearance of GBA given the slow steady decrease in phosphorus is founded in community development dynamics (Stevenson, 1984; Stevenson, 1986; Stevenson, 1996; Stevenson, 1997). Changes in species composition for any habitat can take time as either 1) rare taxa reproduce and replace function of more common taxa that can no longer survive in the habitat, or 2) until new species invade the habitat and replace function of those maladapted common taxa. So, there can be a lag between when environmental changes occur and when manifestations of biological community processes of adaptation to those changing conditions become apparent. Many of the accumulation processes are based on exponential growth of algae, so sudden changes can be generated by exponential accumulation processes that have been occurring over hundreds of generations by the organisms.

Whereas there is some record of *Delicata delicatula* as a relatively rare taxon in the paleolimnological records in sediments of northern Michigan lakes, *Encyonema evergladianum* has not been reported in sediment cores of northern Michigan lakes (Fritz et al. 1993) or the database of the 2007 National Lakes Assessment by the US EPA that sampled more than 1000 lakes nationwide (Stevenson, Zalack and Wolin, 2013). *Encyonema evergladianum* is highly associated with thick GBA mats and when nutrient concentrations are likely lowest (Mazzei and Gaiser, 2017). The 2018 experiment showed *Encyonema evergladianum* grew relatively better than other species of diatoms in the low nutrient control treatment. It likely took years for *Encyonema evergladianum* to accumulate or invade and accumulate to the high abundances needed to fill the high biomass niche of benthic diatoms in Torch Lake and Lake Bellaire. The same may also be true for the other coccoid and filamentous cyanobacteria in GBA mats that we know less about.

Why do we see variation in GBA around Torch Lake that may be related to groundwater discharge of phosphorus into the nearshore zone?

We had initially expected to see GBA variation around Torch Lake in areas where phosphorus loading was greatest. After all, nutrient enrichment is the usual cause of nuisance algae in waterbodies around the world (Vadeboncoeur et al., 2021). Those phosphorus sources could have been stream and river tributaries of the lakes, atmospheric deposition, or groundwater input (Bretz et al. 2006). Reviews of aerial photography by Hoadley only show elevated GBA biomass around the Clam River input to Torch Lake and not other smaller tributaries. Elevated GBA biomass is also located at many other locations around the lake that are not near the Clam River. In addition, groundwater conditions are highly abnormal around the mouth of the Clam River at the Gourley sampling site in ways that are likely not due to the Clam River itself. So, we should question whether a causal relationship between Clam River input to Torch Lake and elevated GBA biomass near the Clam River would apply to the rest of the shallow water nearshore zones around Torch Lake and Lake Bellaire. Even if Clam River discharge was elevating benthic algal biomass near Clam River, that would not explain the high benthic algal biomass around almost all of Torch Lake observed during the 2020 benthic algal survey and in aerial photographs.

I would expect atmospheric deposition of phosphorus to stimulate phytoplankton growth rather than stimulate benthic algal growth and not phytoplankton (Camarero and Catalan, 2012). If phosphorus is the most limiting nutrient for algal growth, and it is added via the atmosphere to the water column, then algal growth in the water column should be elevated, not decreasing as indicated by long-term trends in chlorophyll and Secchi depth.

Groundwater input from septic wastes has recently been associated with widespread problems with filamentous green algae in lakes (Vadeboncoeur et al. in press), but we do not have consistent or lake-wide evidence that groundwater contamination is the cause of the GBA problem around Torch Lake and Lake Bellaire. Groundwater contamination by phosphorus has been a major concern driving the TLA study of GBA because elevated GBA could indicate human contamination of groundwater and the potential for conditions to get worse. However, evidence of phosphorus contamination of the lake floor groundwater entering Torch Lake and Lake Bellaire and causing GBA is not consistent. The 2016 comparison of PO4-P in groundwater indicated no contamination from riparian sources. The high variability in TP among sites in both the Bretz et al. (2006) and TLA 2015-2020 studies could be related to challenges with particulate phosphorus variability generated during sampling groundwater with peizometers. In addition, groundwater TP did not increase from 2005 (Bretz et al. 2006) and the 2015-2020 sampling, which is the period when GBA became evident in the lakes. The 2021 sampling and comparison of sampling of well water and lake floor groundwater only showed elevated phosphorus concentrations at the Gourley site, which has unusual groundwater conditions.

Groundwater input of phosphorus has been related to proliferations of filamentous green algae in lakes, not benthic diatom problems (Vadeboncoeur et al., 2021). However, the spatial patterns in elevated GBA biomass at different locations around the lake and the local variations associated with waves of sand (Fig. 52) do suggest groundwater has a role in regulating GBA in some way. It is likely that phosphorus loading via groundwater at those locations, even though not elevated above natural background concentrations, is sufficient to stimulate GBA growth but not to support the benthic diatom assemblages of the past given the great decreases in water column phosphorus concentrations. Thus, new taxa could have become most dominant and changed benthic algal mat structure in ways that mucilage-thickened mats persist on the surface of sands. Although we did not see direct contact with groundwater being necessary for development of GBA in the 2018 *in-situ* experiment, peepers showed that groundwater and potentially remineralization processes in surface sands elevated phosphorus concentrations in waters just above the sand surface, which would wash over the upper surface of benthic algal mats. Thus, even if not contaminated by human sources, groundwater phosphorus could be stimulating GBA growth and generating the patterns in benthic algae on sand waves observed in aerial photographs and over extensive areas of the bottoms of Torch Lake and Lake Bellaire.

Stepwise Review of Alternative Hypotheses

In the following section, I will review the alternative hypotheses (Table 1), what we know and don't know that relates to those hypotheses for GBA, and both supporting and contradictory evidence for those hypotheses. I will discuss how what we don't know would be helpful to know in this review of hypotheses. Learning more is part of ongoing GBA research that I am doing and that TLA is conducting. Learning more will increase our certainty about causes of GBA and which if any management actions should be taken.

Many of the hypotheses below have guided research by TLA and me and have been integrated into the explanation of causes of GBA described above; which to remind and emphasize to readers, is a plausible model of interrelated processes causing GBA that is consistent with (i.e. not proven wrong by) what we know. In the review of the hypotheses below, I may repeat some information above and elaborate further on those points so that coverage of topics is complete.

At this point I want to emphasize the importance of causal analysis in environmental research. Causal analysis is a process of bringing multiple lines of evidence together to test models. Causal analysis was originally developed as part of human health diagnosis and has been adapted for use in diagnosis of causes of ecological problems (Beyers, 1998; Cormier and Suter II, 2008; Norris, Webb, Nichols, Stewardson and Harrison, 2012). Here I use multiple approaches including making observations of patterns and interrelationships in the natural systems, conducting experiments to isolate factors regulating ecological processes, and using what we know about algal ecology to make sure we have biological plausibility in our explanation, which is our model for how GBA developed and has persisted.

Whether nutrient enrichment, nutrient depletion, or some combination of the two are involved with GBA development is a key area of uncertainty in our understanding of GBA. Current evidence leans more toward nutrient depletion causing GBA at the lake-wide and regional scale. But I want to keep all options clearly in the discussion as new evidence emerges over the next couple years.

Zebra Mussels & Relatives (Quagga) caused GBA

Hypothesis 1: Dreissenid mussels could have caused GBA by enriching benthic habitats with nutrients because they filter phytoplankton out of the water column, excrete those algae in pseudofeces on the lake bottom, and nutrients in those pseudofeces stimulate growth of benthic rather than planktonic algae. This is an important mechanism for blooms of the filamentous green algae *Cladophora* in Great Lakes (Higgins et al., 2008) and other filamentous green algae in Saginaw Bay (Francoeur, Pillsbury and Lowe, 2015).

Support for hypothesis: Dreissenid mussels did affect benthic algae in some fairly low nutrient lakes, such as Lake Michigan and affect phytoplankton species composition in some low nutrient inland lakes (Raikow, Sarnelle, Wilson and Hamilton, 2004). In addition, Dreissenid invasions invaded the Elk Lake Chain of Lakes about 10 years before we suspect the development of GBA started (Ackerman et al., 2009). Of course, one of the basic tenets of causal analysis is the causing factor must occur before the effect.

Contradictions to the hypothesis: Dreissenid mussel density is not likely sufficient to produce pseudofeces to enrich the benthic habitat. Very few mussels are observed on the bottom of the lakes where GBA has been studied. Increases in water clarity have been responsible for increasing benthic algal productivity in some deeper and more turbid habitats, but light levels probably do not critically limit benthic algal productivity in GBA areas of Torch and Bellaire because waters have been so clear, historically, and GBA occurs on the sand shelf at such shallow depths that light levels do not reach critical levels affecting algal growth. Benthic algae grow very effectively at low light levels (Rier, Stevenson and LaLiberte, 2006).

Hypothesis 2: Die off of Dreissenid mussels left decomposing mussel remains in surface sands that elevated nutrients and stimulated growth of GBA. This is a second mechanism for Dreissenid mussels causing GBA.

Support for this hypothesis: Dreissenid invasion and die-off may have occurred just before the time GBA started developing. Decomposing organic material should elevate nutrient concentrations in benthic pore waters and at the sand-surface water interface.

Contradictions to the hypothesis: Nutrient concentrations in benthic porewater are not greater than lake floor groundwater. Therefore, there is no evidence for current benthic porewater conditions being enriched by Dreissenid mussels in the shallow water habitats where we have sampled. In addition, wave disturbance of shallow waters likely washed organic matter from surface sands. However, that may not be true in deeper waters where wave disturbance may not have disturbed surface sands and washed organic matter out of sands. To further test this hypothesis for deep water mats we need to estimate the relative contribution of past mussel detritus compared to other ongoing sources of detritus accumulating at deeper depths close to the dropoff in the sand shelf. This is relevant if deeper depths are more year-round refuges for benthic algae from which the benthic algae in deep zones of the sand shelf can disperse by currents and increase summer GBA development rates. Elements of this latter hypothesis could be explored with analyses of aerial photography and sampling GBA at depths greater than the 3.3 m we have sampled.

Hypothesis 3: Dreissenid mussels reduced phosphorus in lakes by filtering phytoplankton out of the water column.

Support for this hypothesis: Dreissenid invasion has reduced phosphorus in some lakes and increased Secchi depth by filtering phytoplankton out of the water column (Cha, Stow and Bernhardt, 2013). So this is a plausible mechanism contributing to decreases in phosphorus in surface waters of northern Michigan lakes.

Contradictions to the hypothesis: Decreases in phosphorus in some northern Michigan lakes likely started before Dreissenid mussels invaded northern Michigan lakes (Fritz et al. 1993). So Dreissenid mussel filtration is not likely the only mechanism for phosphorus reductions in northern Michigan lakes. If Dreissenid mussels densities have decreased, phosphorus concentrations in lakes should recover to pre-invasion levels if phosphorus loading rates have not changed.

Changing Light Conditions Related to Dreissenid Mussels or Other Factors

Hypothesis: Increasing light levels would increase ability of algae to grow in deeper water and presumably in thicker mats where self-shading of understory algae by overstory algae can be an issue if light gets low enough. I would hypothesize also that increasing light levels could also stimulate greater mucilage production and mat integrity if reproduction is limited by nutrients.

Support for this hypothesis: Water column transparency has increased and light now penetrates to deeper depths in Torch Lake according to long term increases in Secchi depth, but Secchi depth has not increased since 2014 in Lake Bellaire. Even though light levels are high, light penetration into the water column decreases greatly as angle of the sun decreases either earlier or later in the day than midday. This makes water clarity even more important during those times of day at deeper depths. I am not familiar with any research showing light stimulates mucilage production by algae when algae become nutrient limited, but this does seem likely based on first principles of algal physiology.

Contradictions to the hypothesis: Algae need relatively little light to reproduce effectively. High light levels can limit algal production because of photoinhibition. Light is certainly high enough for high algal performance in shallow water habitats that did not have GBA in the past. GBA occurs in Lake Bellaire despite the lack of change in Secchi depth. Light penetration to the drop-off is evident in almost all areas of Torch Lake in Hoadley's aerial photographs because you can see quite clearly the bottom of the lake in most areas. The usual benchmark for light limitation of algal accumulation is 1% of surface light levels, or about 20 um quanta m^{-2} s^{-1}. In experiments with benthic algae, my students and I have found algae reproduce very effectively at light levels even below 20 um quanta m^{-2} s^{-1} (Rier, Stevenson and LaLiberte, 2006). Therefore, nutrient limitation of reproduction could produce excess carbohydrates that could be used to produce mucilages even under relatively low light levels.

Runoff from Big Storms, including Nutrients from Streams and Rivers

Hypothesis: Runoff from lawn fertilizers, fields, and impervious surfaces increased nutrient concentrations and stimulated growth of GBA.

Supporting evidence: Runoff from lawn fertilizers, fields, and impervious surfaces can be important sources of nutrients to surface waters in many habitats, whether directly into the habitat or via wetlands, streams, and rivers discharging into the habitat. Bretz et al. (2006) suggest that about 1/3 of the phosphorus entering Torch Lake is from surface water sources. That suggests that runoff from lands throughout the three lakes watershed could be contributing nutrients or other contaminants (e.g. chloride discussed below) to the three lakes. Phosphorus concentration is higher in many of the tributaries entering Torch Lake than in Torch Lake. In particular, the Clam River probably provides the biggest load, even though phosphorus concentrations are not that much higher in the Clam River than Torch Lake. We do observe high benthic algal biomass north and south of the Clam River mouth in Torch Lake, even though water from Clam River is usually more turbid. That turbidity appears to be high concentrations of dissolved organic carbon, which is likely from wetlands upstream from Clam Lake (Kintigh, Stillwell and Pedersen, 2012).

Contradictions to the hypothesis: In Torch Lake, phosphorus concentrations are decreasing and nitrogen concentrations are generally unchanged. In addition, most water entering many waterbodies arrives via groundwater in regions of Michigan with deep glacial tills, not surface water runoff. Water percolates rapidly into sand-cobble tills until it reaches the lake floor groundwater, which then flows down slope to lakes, streams, or wetlands.

The concepts addressed for this hypothesis do not have to be limited to address local and direct runoff from riparian zones into lakes. These concepts also apply to runoff into tributaries and then tributaries into Torch Lake and Lake Bellaire. Whether runoff directly into the three lakes or into tributaries, I would expect to see plankton blooms, because high nutrient loading by runoff or tributaries would enrich surface waters and cause phytoplankton blooms, rather than benthic algal blooms. We do not see evidence of elevated plankton of a magnitude that approaches the problem with benthic algae. Even though we see high benthic algal biomasses north and south of the mouth of the Clam River as it enters

Torch Lake, we also see these high biomasses at many locations around Torch Lake and Lake Bellaire. Reviews of Hoadley's aerial photographs by Hoadley and my surveys found areas of high benthic algal biomass that are not associated with locations of tributaries entering Torch Lake. If we had runoff directly from riparian zones, I would expect higher biomass close to shore than farther from shore, except for very shallow water where wave disturbance is a persistent disturbance for benthic algal accumulation. I do not see that pattern in Hoadley's aerial photographs, but a more thorough analysis of aerial photography and algal sampling along depth gradients would address this hypothesis more completely.

Grazers Changed Allowing Algae on Surface of Sands to Grow

Hypothesis: Invasive species, disease, or disease cycles have caused loss of a key grazer that regulated GBA accumulation on surface sands in the past. Grazers, such as snails, mayfly larvae, and some fish, eat benthic algae on the bottoms of lakes, streams, and wetlands (Steinman, 1996). Considering benthic algal consumers more broadly, many insect larvae, crustaceans, and isopods indiscriminately collect and gather algae (a functional feeding group called collector/gathers) when also collecting and gathering loose organic particles with algae and bacteria on them. I suspect some planktonic animals (zooplankton), also collect and gather benthic algae (Karlsson and Säwström, 2009), which could limit their growth on the surface of sand, but not attached to sand grains or living among sand grains. Loss of these algal consumers (grazers and collector/gatherers, truly benthic or facultatively benthic) could allow benthic algae to accumulate on the surface of sands.

In some streams, caddisfly larvae live in cases that make them resistant to predators. In hydrologically stable streams, as many are in Michigan, their predator resistance enables accumulation of cased caddisflies to densities that eventually are limited by their food, which is benthic diatoms on rocks (Kohler and Wiley, 1992). These densities, 100s of animals per m^2, are commonly high enough to consume all visible accumulation of diatoms on rocks in the streams. Loss of cased caddisflies due to a disease cycle enables benthic diatoms to grow and accumulate to high densities in streams when disease depresses caddisfly larval densities.

In addition to diseases, changes in predator densities could affect algal consumers. For example, if densities of fish increased and increased consumption of algal consumers, the reduced number of algal consumers could allow slow growing algae to accumulate. Have there been changes in predators of algal grazers, particularly as non-native species, that have invaded the lakes, reduced densities of algal consumers, and allowed accumulation of benthic algae on the surface of sands? The invasion of gobies is one possibility, but gobies are reported to eat small fish and mussels rather than the snails, amphipods, and insect larvae that would consume bent invasion could upset benthic food webs in ways that allow benthic algae to accumulate (Diggins, Kaur, Chakraborti and DePinto, 2002; Brush, Fisk, Hussey, Johnson and Sprules, 2012).

Supporting evidence: The lack of knowledge here is a reason to keep this hypothesis in our minds, but it does not increase likelihood of importance. There is little known about benthic invertebrate densities in the three lakes and their regulation of benthic algal abundances (Ackerman et al., 2009). Also, we know little about changes in fish densities and their potential effect on benthic grazers and collector/gathers that would consume algae, except gobies have invaded the three lakes and could reduce invertebrate grazers of benthic algae.

Contradictions to the hypothesis: There is no evidence, even reports from the public, of high snail densities that would have historically consumed all the algae on the surface of sands and rocks in the three lakes. Reported herbivore densities are actually low. I do not know of reports of loss of grazers or collector/gathers for inland lakes from invasive species disturbance or disease, although loss of amphipods (*Diporeia* spp.) in the Great Lakes is associated with Dreissenid mussel invasion and could have occurred in Torch Lake and Lake Bellaire. Amphipods collect planktonic algae that settled onto the lake bottom. Dreissenid mussels filter plankton from the lake and thereby reduce the food source for *Diporeia* (Nalepa, Fanslow and Lang, 2009). Extensive bioturbation of benthic algae by gobies does not seem widespread, but more evaluation of this hypothesis is warranted. I would expect extensive bioturbation of benthic algae by this benthic fish while it is foraging for prey.

Another related issue for grazer/algal interactions that is relevant to benthic algal accumulations is grazers concentrating on areas where algal biomass is low rather than areas where benthic algae have accumulated to high densities. In other words, benthic algae can escape grazing pressure in patches, which could then spread across the rest of the habitat. Some grazers in streams will preferentially graze previously grazed areas versus thicker mats of algae surrounding those areas. Thus, GBA could be resistant to grazers and collector/gathers once the dense, mucilaginous mat starts to develop into thicker mats.

Bioturbation by Larger Animals Has Decreased

Hypothesis: Fish nesting or even benthic invertebrate movements can knock algae off substrata in benthic habitats. This is referred to as bioturbation. We see evidence of bioturbation around some underwater structures, such as logs, where no GBA occurs near the log. This is likely due to bioturbation or potentially consumption of the GBA by animals such as crayfish hiding under the logs from predators. Loss of an animal controlling benthic algae by bioturbation is an unlikely reason for the absence of GBA throughout the lakes in the past because bioturbation is not usually a lake-wide phenomenon. But I can imagine exceptions.

Non-native Invasive Algal Species

Hypothesis*: One or more key algal species invaded northern Michigan lakes and caused GBA*

Invasions of algae into habitats have been reported as environmental conditions change, either related to local watershed and water chemistry changes or climate change. This is most widely suspected for macroalgae, because their presence is easier to document than microalgae. The increased occurrence of *Didymosphenia*, the diatom with big mucilaginous stalks in streams around the world, was suspected to be related to invasion of altered habitats but is likely wrong (Bothwell, Taylor and Kilroy, 2014; Taylor and Bothwell, 2014). The filamentous cyanobacterium *Lyngbya* that has recently become a nuisance in Lake Erie has been called an invader (Hudon, Sève and Cattaneo, 2014).

The abundance of *Encyonema evergladianum* in GBA is curious, because I know it from extensive work in the Everglades where I see it abundant in low phosphorus, hard water conditions. The name of this diatom indicates the species was described from populations in the Everglades. The biogeographic distribution of *Encyonema evergladianum* is not well known because it can be hard to distinguish from other benthic diatoms. It has not been identified in many other lakes. It was not observed in any paleolimnological records of northern Michigan lakes by Fritz et al. (1993). It was not observed in surface or deeper sediment records diatoms in the 1000 lakes sampled for the 2007 National Lakes Assessment by the USEPA. It was considered endemic to tropical calcareous wetlands (Mazzei and Gaiser, 2017), but Loren Bahls (a highly experienced diatom taxonomist) reports it from Montana, North Dakota, South Dakota, and Wyoming (Bahls, 2009).

Again, *Encyonema evergladianum* is widely observed in the low phosphorus, hard water habitats of the Everglades, i.e. like Torch Lake and Lake Bellaire. *Encyonema evergladianum* does form mucilaginous stalks, according to my microscopic observations of live material. In addition, it creates rafts of cells attached together. All motile raphid diatoms secrete mucilages as they move, either across substrata or through mats. So, we might suspect *Encyonema evergladianum* as a potential invader and an "ecological engineer" changing the physical structure of benthic algal accumulations.

But *Encyonema evergladianum* is not the only mucilage producing diatom in the GBA mats and is likely not the dominant mucilage producer. Mucilage in GBA mats is important for the cohesiveness of the mats and their resistance to consumption, bioturbation, and wave disturbance. So, invasion of *Encyonema evergladianum* is not the likely cause of GBA development, even though decreases in surface water phosphorus concentration could have been the environmental trigger for *Encyonema evergladianum* increases in Torch Lake and Lake Bellaire. Decreases in surface water phosphorus could also been the trigger for other mucilage producing organisms, like other diatom species and the filamentous and colonial cyanobacteria observed in mats. Therefore, more thorough microscopic examination of benthic mats that have been carefully collected and stored without physical disturbance of mat structure is warranted so we can learn the roles of different algal species in formation of the mats.

"Algae in the News and on Your Mind"

Algae have been in the news as harmful and nuisance algal blooms caused by agricultural runoff and for their future application as a source of renewable energy. Shengpan Lin, one of my students, tallied the number of newspaper articles with algae in the story and found a dramatic increase in popular news stories about algae in the last decade. So, could the increased exposure to information and potential concerns about algae have made the public more aware of algae, more likely to observe and recognize it, and more likely to be concerned about it?

There are two lines of evidence that contradict this hypothesis. One is that GBA is so noticeable and so much of an aesthetic nuisance for vistas of the lake, wading, and swimming that people would have noticed it in the past if it was present. And many people have lived around the lakes for many years, so they would have noticed it in the past.

The second line of evidence is that significant GBA was not observed in Hoadley's aerial photographs during 2010 and 2012 but was quite abundant in 2015 and thereafter. More photography prior to 2015 would be helpful to increase certainty that GBA was not present prior to 2012, 2010, and earlier. Remote sensing images have not been very informative because quality of many images is poor. One image set from 2005 does show some GBA-like color on the sandbar in southern Torch Lake, but not elsewhere. Based on the evidence that we have, I estimate that GBA developed extensively in 2013-2014, but this is based on relatively little evidence.

Climate Change & A Warmer Lake

Hypothesis: Warmer lake temperatures associated with climate change could have caused a change in species composition of benthic algae, or their physiology, and thereby affected physical structure of the algal mats. This is a hypothesis to consider, even though we know little about the magnitude of temperature change needed to change benthic diatom species composition and physiology, and whether those changes would or could cause GBA. What we do know about climate change effects on algae is for planktonic algae and concerns for harmful algal blooms.

Supporting evidence for the hypothesis: There have been reports of increasing water temperatures in the Great Lakes related to climate change. In addition, extreme weather events could be enough to warm shallow nearshore waters where GBA occurs, versus the whole lake. We know relatively little about temperature effects on species composition and benthic algal ecology. Diatoms are relatively cold-water taxa, compared to green algae and cyanobacteria. Cyanobacteria typically grow better in warmer waters than other taxa (Paerl and Huisman, 2008). Thus, increases in nearshore water temperatures could have stimulated more cyanobacterial growth that has formed mats over the sands.

Evidence contradicting the hypothesis: Shengpan Lin, one of my graduate students with expertise in remote sensing (Lin, Novitski, Qi and Stevenson, 2018; Lin, Qi, Jones and Stevenson, 2018), provided an analysis of temperature changes in the surface water of Torch Lake and found great seasonal variability, as we would expect, but no long-term trends in summer maxima over the past 30 years (Stevenson 2016). This analysis explicitly addressed nearshore as well as offshore patterns. Further, his dissertation research studied lakes throughout Missouri and found temperature increases over the 1980-2010 period were relatively small (Lin, 2017). On the other hand, local experience by TLA volunteers suggests faster warming in shallower nearshore areas than in the past. How great have these temperature changes been? Are they sufficient to affect benthic algal species composition and physiology in ways that would cause mats? More records of summer water temperatures and algal response to those temperature ranges are needed to test this hypothesis and its relevance to conditions in Torch Lake and Lake Bellaire.

Groundwater Contamination

Hypothesis: Septic tanks and fertilizers have percolated down to shallow groundwater zones, moved to groundwater below lakes, and discharged into shallow nearshore waters to cause GBA.

Nutrient flux from the groundwater is the most likely cause of benthic algal proliferations without evidence of phytoplankton increases from surface water inputs (direct runoff or tributaries) or atmospheric deposition. Therefore, I suspected that groundwater contamination was a high priority to test when we first started. In addition, groundwater contamination is an important hypothesis to test because management options exist to control this problem and a timely confirmation of this hypothesis would be important to start controlling contamination as soon as possible.

Supporting evidence for the hypothesis: Benthic algae are proliferating, which we would expect from groundwater contamination by nutrients (Périllon, Pöschke, Lewandowski, Hupfer and Hilt, 2017; Vadeboncoeur et al., 2021). Phosphorus can move through the groundwater, contrary to past paradigms. Phosphorus movement through groundwater is based on factors such as phosphorus binding capacity of soils, phosphorus concentrations, phosphorus loading and saturation of binding sites, and the equilibrium created between phosphorus concentration and available phosphorus adsorption sites on soils (Robinson, 2015). Groundwater contamination is recognized as a widespread problem causing proliferations of filamentous green macroalgae on lake bottoms (Vadeboncoeur et al., 2021). Benthic algae such as *Cladophora* are often surveyed to find potential sources of septic contamination, and these localized sources of contamination have been observed around Torch Lake and Lake Bellaire (The Northwest Michigan Regional Planning and Development Commission, 1983; Conkle, Lunn, Menestrina, Bretz and Hannert, 2004; Blaney et al., 2010).

Nitrogen and phosphorus concentrations and N:P ratios indicate phosphorus is the most likely limiting nutrient for algal growth; and when comparing nearshore surface water and groundwater, groundwater had relatively high P concentrations and relatively low N concentrations. Since mid-lake surface water phosphorus concentrations have decreased, groundwater may be a more important source of P than in the past.

In addition, chloride and markers of human waste are evident in lake floor groundwater and interstitial waters. There is some evidence for P contamination of groundwater, but the spatial extent of that contamination is unclear. If we assume that background phosphorus concentrations in groundwater are in the range of 7-10 ug TP/L based on well water samples, then elevated and likely contaminated phosphorus concentrations were observed at about half of the sites in 2005 by Bretz et al. (2006). Although evidence for P contaminated groundwater was not observed in the 2016 TLA sampling comparing lake floor piezometer and well water, those measures were made with dissolved inorganic phosphorus rather than total phosphorus. In the 2021 comparison of groundwater from lake floor piezometers and wells, one of three sites had elevated phosphorus in both dissolved inorganic and total phosphorus forms.

Evidence contradicting the hypothesis: We would expect a change in groundwater phosphorus contamination to have occurred before GBA started to develop. However, evidence indicates groundwater contamination has not increased in concentration or spatial extent since 2005, which was the period when GBA developed in Torch Lake.

In addition, groundwater contamination has great spatial variability based on the Bretz et al. (2006) study. Although we do see spatial variability in GBA, we do not find high GBA biomass strictly in areas with high land use near the lake. In addition, we find GBA occurring throughout the lake, whereas we only expect elevated groundwater phosphorus concentrations at half the locations in Torch Lake.

Getting better evidence for the number of people in households over an extended history of time could help refine our estimates of where septic system loading could be higher, but these data are not now available.

GBA grows on rocks and hard surfaces in the water column, like docks; so not just on the sands through which the groundwater would flow most directly to the algae.

GBA biomass differences were not observed in experimental chambers that excluded or allowed direct connection to groundwater. One possible explanation for GBA on impenetrable substrata is dispersal of algae that can form mats in low phosphorus and resist disturbance. In addition, groundwater could wash over benthic algae at the sediment water interface just as it enters the lake. Currents in surface waters may move groundwater laterally as it slowly discharges from sands such that the groundwater would flow across the sediment water interface rather than mixing it with overlying surface water. This would maintain the relatively high P concentrations of groundwater in the boundary layer at the sediment water interface where it could wash over rock surfaces and over the tops of experimental chambers until that P-rich groundwater was mixed more thoroughly with surface water. I have observed this lack of mixing of waters in boundary layers in streams and experimental channels by releasing dye at the sediment water surface. The dye stays entrained in the boundary layer more than mixing with overlying waters for an extended period of time, with some dye persisting in the boundary layer and staying entrained with waters at the sediment water interface for remarkably long periods of time.

Increase in Chloride Concentrations

Hypothesis: Changes in chloride concentration could change algal species composition and cause GBA.

Evidence supporting the hypothesis: Chloride concentrations have steadily increased over the past 20 years in the three study lakes, and perhaps for longer. Changes in chloride concentration can cause changes in diatom species composition (Tuchman, Stoermer and Carney, 1984).

I will have to conduct further literature and database research on species preferences, but my sense is these chloride changes would not likely be responsible for the changes in species composition or algal physiology causing GBA and high amounts of mucilages. There is the question of biological plausibility and analogy, where decreasing nutrients does have a biological and evidence-based linkage to development of thick benthic algal mats. Chloride contamination does not.

Also, questions exist about species responses to changes in chloride concentrations specifically or the changes in conductivity and osmotic stress associated with higher ionic content of water. Since Torch Lake and Lake Bellaire waters have high conductivity, it may be less likely that changes in chloride concentration alone would cause shifts in diatom species composition.

Atmospheric Nitrogen Deposition

Hypothesis: Atmospheric nitrogen deposition has decreased phosphorus concentrations and N:P ratios in surface waters, which caused changes in benthic algal species composition and mucilage production to enable development of GBA.

Evidence supporting the hypothesis: Atmospheric nitrogen deposition has likely increased for almost a century because of human use of nitrogen fertilizers (Galloway, Leach, Bleeker and Erisman, 2013), and around 1970 the nitrogen from human sources started to exceed natural sources (Aber, Nadelhoffer, Steudler and Melillo, 1989). Around Torch Lake, nitrogen deposition was highest about the turn of the century (National Atmospheric Deposition Program, 2021). Even though it was higher then, it continues today at levels that are higher than before the green revolution and extensive and intensive use of nitrogen fertilizer in agriculture. Luscz, Kendall and Hyndman (2015) modeled and mapped the nitrogen deposition in the Torch Lake area to be around 14 kg/ha/yr. Atmospheric nitrogen pollution has caused lakes in some parts of the country to undergo substantial ecological shifts (Elser et al., 2009). Typically, these are low nitrogen lakes where addition of nitrogen releases algae from nutrient limitation. Researchers in Sweden have documented reduced phosphorus in lakes with nitrogen deposition (Hessen, Andersen, Larsen, Skjelkvåle and de Wit, 2009) and argue that many lakes may have been nitrogen limited before nitrogen deposition caused their change to phosphorus limitation (Bergström and Jansson, 2006). Liess, Drakare and Kahlert (2009) have shown nitrogen deposition can lead to greater phosphorus limitation for benthic algae and the food web.

Algal growth in Torch Lake and Lake Bellaire is regulated by low phosphorus more than low nitrogen, based on their concentrations in lake water and N:P ratios. According to classical limnological theory, addition of nitrogen to Torch Lake and Lake Bellaire, where algae are much more limited by phosphorus than nitrogen, should not release algae from nutrient limitation. But we also know that different algal species have different nutrient requirements. Therefore, it is possible that addition of nitrogen could enable a shift in species composition that are adapted to use lower phosphorus concentrations. That would enable uptake of phosphorus to lower phosphorus concentrations by algae, which would then settle out of the water column and thereby reduce phosphorus concentrations in the lake.

Phosphorus concentrations have decreased in larger lakes across the Tip of the Mitt (northern lower peninsula of Michigan). Perhaps Dreissenid mussel invasion, consumption of phytoplankton, and reduction in surface water phosphorus concentrations caused this region-wide decrease in P. Perhaps the phosphorus bans in detergents and fertilizers decreased P loadings. But as mentioned earlier, P decreases in Tip of the Mitt lakes likely started before phosphorus bans and Dreissenid mussel invasion (Fritz, Kingston and Engstrom, 1993).

As described in the section above for the explanation for GBA that is most consistent with the evidence, if an increase in nitrogen can decrease phosphorus in surface waters, it is plausible that a decrease in phosphorus in surface waters has caused changes in benthic algal species composition and metabolism in ways that produce persistent algal accumulations with the characteristics of GBA: high biomass, high amounts of mucilage, and high amounts of calcium carbonate in cohesive mats that are resistant to physical disturbance and may be resistant to grazing.

Evidence contradicting the hypothesis: There is no evidence contradicting the hypothesis that nitrogen deposition could deplete phosphorus in the surface waters of P-limited lakes. However, we do not have good evidence that the decrease in phosphorus concentration caused the development of GBA in Torch Lake and Lake Bellaire. We have analogous situations in the Everglades and with *Didymosphaenia* (see discussion above), but that is not the same set of species in the same environment as Torch Lake and Lake Bellaire. More research is needed to test this hypothesis.

Atmospheric Phosphorus Deposition

Hypothesis: An increase in atmospheric phosphorus has stimulated benthic algal growth.

Evidence supporting the hypothesis: I know little about changes in atmospheric phosphorus deposition in relationship to human activities. We do know estimates of phosphorus deposition rates based on models and maps by Luscz, Kendall and Hyndman (2015). Hamlin et al. (2020) also modeled nitrogen and phosphorus deposition for Michigan. He has provided SENSEmap estimates of atmospheric deposition to us for total nitrogen and total phosphorus for the greater Torch Lake region. Because there are no observational data points in this region for phosphorus; these maps provide interpolated estimates from Great Lakes region kriging. In the Pyrenees of Spain, lakes trophic status has been affected by phosphorus deposition (Camarero and Catalan, 2012).

Evidence contradicting the hypothesis: If atmospheric deposition of phosphorus has increased into lakes via precipitation, wet or dry, it would enter lakes via the water column and presumably increase phytoplankton abundances more than benthic algal abundances. Increases in phosphorus via atmospheric deposition is inconsistent with our observations that phosphorus concentration is decreasing in the lakes and Secchi depth has increased in Torch Lake. Nitrogen deposition on the other hand, would decrease the surface water phosphorus and likely not increase phytoplankton abundances greatly because phosphorus would still be highly limiting for algal growth. This is true especially over the 70 years that nitrogen deposition may have been occurring and reducing productivity in lakes. In addition, the mass ratios of N:P deposition rates (kg/ha/yr) based on models and maps by Luscz, Kendall and Hyndman (2015) are 14 to 0.065, way above the 7:1 mass ratio of these nutrients in algae. Thus, phosphorus is in very short supply for algal needs compared to nitrogen in atmospheric deposition.

Recent Rise in Lake Levels

Hypothesis: Increases in lake water levels connect nearshore lake surface water, lake floor groundwater, with riparian groundwaters that have not usually been connected. This could enhance movement of contaminated groundwater to lake floor groundwater and stimulate GBA. In addition, the high lake levels could have resulted from increased rainfall, which would move more phosphorus through groundwater and into the lake.

Evidence supporting the hypothesis: Lake levels in many northern Michigan waterbodies have increased in the last 5-10 years. The increase in Lake Michigan started, according to US Army Corp of Engineers water level records (https://www.lre.usace.army.mil/Missions/Great-Lakes-Information/Great-Lakes-Information-2/Water-Level-Data/) in 2014, but did not exceed average levels until 2017-2018 and peaked in summers of 2019 and 2020. This period of increase, from 2014-present does coincide with the period in which we expect GBA to have started and expanded in the lakes. I have not had time to review rainfall records in detail for the report, but that is a logical explanation for increases in lake levels.

Another line of evidence that could support one or more of this set of interrelated hypotheses is the variability that we see from year to year in lake floor groundwater conditions. Fundamentally, this indicates that interannual variability in rainfall and chemistry of groundwater and surface water are related and potentially important.

Evidence refuting the hypothesis: Over the past 50 years, there have been many periods when Lake Michigan water levels have increased as they have the last 7 years. Assuming similar increases also happened in the inland lakes, those increases in lake levels did not stimulate development of GBA. Also, movement of riparian groundwater to the lake is more rapid when lake levels are low than when they are high, because water flows downslope, even underground. One of the reasons that Vadeboncoeur et al. (2021) list as reasons for filamentous green algal proliferations in the benthic nearshore zone is low water levels because riparian groundwater flows farther downslope to the lake floor. On the other hand, there is the idea that lake levels increases could affect biogeochemistry of nearshore riparian groundwaters in ways that released more phosphorus into the groundwater. Although the latter is possible, I do not know that biogeochemical processes would cause that effect.

Related to this hypothesis is the idea that rising lake levels are due to increases in rainfall, and increased rainfall could flush more phosphorus through lake floor groundwaters to support development of benthic algae. This hypothesis is hard to address, because I would suspect that higher rainfall would dilute lake floor groundwater concentrations. This assumption/suspicion should be evaluated by a hydrologist with more experience than I have as an algal ecologist. In the survey of benthic algae around Torch Lake, I assumed high rainfall dominated groundwater would dilute high phosphorus in lake floor groundwater.

An alternative hypothesis is that an increase in phosphorus load with rainfall without an increase in phosphorus concentration would increase phosphorus availability to benthic algae. This gets into the nutrient load versus nutrient concentration debate about what affects the amount of algae in a waterbody. Nutrient load, the mass of nutrient per unit time that is added into a waterbody, determines nutrient concentration in the waterbody. But algae directly respond only to nutrient concentration and only indirectly to the load. So, load only matters if algae can sequester enough nutrients to decrease the concentration in the waters that surround their cells, and that concentration is then affected by nutrient load. From past research we know that nutrient concentrations in mats are depleted by algal uptake exceeding mixing of fresh high nutrient water from the surface waters. Past research has shown that higher water column nutrient concentrations and higher current velocities of water can increase nutrient concentrations for cells within thick benthic algal mats (Stevenson and Glover, 1993). However, load has not been shown to be important in these cases – just concentration. If load were important, we'd expect to see differences in algal species composition on rocks, which would not be exposed to as high nutrient enrichment from groundwaters as would benthic algae growing on sand. We saw no difference in benthic algal species growing on sand and rocks.

From the balance of evidence supporting and contradicting the water level, rainfall, and loading hypotheses, I deem these hypotheses of low likelihood for explaining GBA development in Torch Lake and Lake Bellaire.

Indirect and interactive effects of any one or more of these conditions in the lakes

The explanation for GBA development in the last decade in Torch Lake and Lake Bellaire that is not contradicted by evidence is: atmospheric nitrogen deposition reduced phosphorus in the surface water, changed algal species composition and metabolism, and enabled development of GBA at the sediment water interface. Our understanding could also involve other hypotheses.

More than one factor could be responsible for GBA development.

CONCLUSIONS AT THIS TIME AND NEXT STEPS

I do not understand the ecology of GBA and the factors regulating GBA characteristics enough to select one hypothesis over all others with high certainty, particularly with sufficiently high certainty to alter current pollution management policies to control GBA. GBA accumulation in lake surface waters seems to be a new problem, based on my conversations with colleagues in algal ecology and my review of the literature.

However, I do have an explanation for GBA that is not contradicted by the evidence and is biologically plausible. 1) We know that phosphorus has decreased over the last 30-70 years in many lakes of the northern lower peninsula of Michigan. 2) Decreasing phosphorus can plausibly cause algae to accumulate in thick mats. Analogous ecological effects of low phosphorus causing large accumulations of benthic algae occur in Everglades calcareous mats and stream blooms of *Didymosphaenia*. As I have indicated in my explanation of this hypothesis at the beginning of the discussion, multiple factors may have contributed to development of GBA in Torch Lake and Lake Bellaire, not just phosphorus decreases in surface waters.

Two other documented changes in the lakes could have caused GBA, increases in chloride concentrations and invasion of gobies that have reduced grazer densities. For the chloride hypothesis we do not have biological plausibility or analogous effects. For the goby hypothesis, we have modest biological plausibility but no analogous effects (places where gobies have caused similar problems). In addition, we have many other hypotheses for which we do not have documented changes in the lake or evidence with high certainty, such as warming nearshore water temperatures and groundwater contamination.

We need to know more about the characteristics of the benthic algal mats and factors that regulate them to confirm that decreases in phosphorus concentrations could cause it. Experiments that I have conducted and for which I have yet to analyze samples are a first step along that line of investigation. The experimental approach is a complement to the multiple relationships we are developing between environmental change and GBA with samples of algae and water chemistry collected in the three lakes over the last seven years. The experiments allow us to carefully manipulate environmental factors to isolate individual and interactive effects among factors, such as phosphorus concentration, chloride, disturbance resistance, water temperature, and grazing. If we see changes in benthic algal species composition, biomass, or mat integrity in experiments that correspond to changes we expect to have occurred in Torch Lake and Lake Bellaire, we have experimental confirmation of a cause or causes.

For field work, we need to gather more information to test and search for all plausible hypotheses:

- Invertebrate and fish surveys can help us determine if composition of fish and benthic invertebrates has changed to address the plausible loss of grazers as a cause for GBA development. Have the fish populations changed in the lake sufficiently to change the invertebrate populations?
- Paleolimnological study may be able to help us determine when the benthic algal and invertebrate taxa changed in lakes and what environmental factors covaried with that change.
- Expanding algal surveys to more lakes that have monitoring data can help us determine which lakes have GBA and which do not, and what environmental factors covary with the occurrence of GBA that could be causes.

- We need to know about mass balance of phosphorus and nitrogen entering the lake. Is groundwater contaminated? How extensively? More work on spatial variability in benthic algae within lakes with follow-up assessment of groundwater conditions will help us understand that relationship better.
- More extensive analyses of Hoadley's aerial photographs to evaluate seasonal and spatial patterns of GBA in lakes.
- Maintain monitoring programs to establish trends and certainty for what we know.

Establishing a certain understanding of the causes of novel environmental problems are challenging and should be done with great scientific rigor.

Understanding GBA ecology is important for management. For example, if the GBA problem is associated with decreasing phosphorus concentration due to atmospheric nitrogen deposition or Dreissenid mussels, or both, then managing water quality with sewers to divert septic wastes will not solve the problem. However, ongoing efforts to reduce fertilizer input into the lake, which is mostly nitrogen, could prevent exacerbation of the phosphorus decreases that may be due to excess nitrogen loading from atmospheric deposition.

My goal in this report was to inform stakeholders and other scientists with an update about what I have learned about the ecology of the golden brown algal mats on the bottoms of Torch Lake and Lake Bellaire with the help of the great effort and data production by Three Lakes Association. Future research is important for further advancing our understanding of GBA, the regional extent of GBA in lakes of the northern lower peninsula of Michigan, and factors affecting variability in GBA among lakes. During the next year, I will continue to process samples, analyze data, review literature, and talk to colleagues about likely causes of GBA. My goal is to increase certainty of our understanding of the environmental factors regulating GBA in ways that will help manage it.

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Table 2. Regression analysis results for long-term (2-3 decades) water chemistry parameters using data from the MiCorp's Cooperative Lakes Monitoring Program (CLMP) and from Tipp of the Mitt Watershed Council (ToMWC). Separate univariate regression models (Y=a+bX) were calculated to determine the annual change in each water chemistry parameter (X) separately for each lake, season samples were collected, and data source (CLMP and ToMWC). For TP by ToMWC which samples 3 depths, I initially chose to run the analysis with the top two depths to increase samples size in the analysis and selecting depths that were likely exposed to phytoplankton activity. In addition, I ran to additional analyses with ToMWC TP data to determine sensitivity of results to selecting depths to include in the analyses (1, either the surface and middle depth = Top 2; 2, Surface; or 3, All Depths). For each regression model (Mod #), the table includes coefficients for an intercept (a) and slope estimate (b) that describes the rate of change in the water chemistry parameter (Y) per year (X), a standard error (SE) for the model coefficients (a and b), and P that estimates the probability that the coefficient (either intercept a or slope b) is equal to zero. For TP, water chemistry values were transformed to reduce skew in the data distribution and improve statistical testing by adding 1 to the value and calculating the log based 2 of the resulting sum (i.e. Log2 + 1 transformation). The regression model statistics are reported in two rows for each model with one row for the intercept and the other row for the slope. The table is sorted with slope coefficients for all models first and then intercepts for all models because the slopes, or change per year, are the key parameters that are most important. For clarity, models are numbered (Mod #) to indicate the rows with the intercept and slope for the same regression models. A P Code is provided to help draw attention to key results and describe certainty in observed patterns in data. P Codes mean: . for P less than 0.10, which indicates an interesting result worth note, but not sufficiently reliable to meet standard criteria for statistical significance; * for P less than 0.05, which means the result is reliable with less than a 5 in 100 chance that a consistent change in results (slope for regression) or difference among conditions (analysis of variance) could occur by chance; ** for P less than 0.01, which indicates a very reliable result with less than a 1 in 100 chance of occurring by chance; *** for P less than 0.001, which indicates a highly reliable result with less than a 1 in 1000 chance of occurring by chance; and **** for P less than 0.0001, which indicates a very highly reliable result with less than a 1 in 10000 chance of occurring by chance.

Mod #	DepVar	factor	Site	Season	Depths	Program	Coefficient	SE	P	Pcode	Data Trans
1	TP_ugL	Slope	Bellaire	Spring		CLMP	-0.09766	0.051082	0.078182	.	Log2 + 1
2	TP_ugL	Slope	Bellaire	Spring	Top 2	ToMWC	-0.03475	0.013868	0.022669	*	Log2 + 1
3	TP_ugL	Slope	Bellaire	Spring	Surface	ToMWC	-0.03306	0.017007	0.087818	.	Log2 + 1
4	TP_ugL	Slope	Bellaire	Spring	All	ToMWC	-0.03004	0.011908	0.017856	*	Log2 + 1
5	TP_ugL	Slope	Bellaire	Summer		CLMP	-0.11948	0.033487	0.003088	**	Log2 + 1
6	TP_ugL	Slope	Clam	Spring		CLMP	-0.0855	0.049312	0.10854		Log2 + 1
7	TP_ugL	Slope	Clam	Spring	Top 2	ToMWC	-0.0548	0.012964	0.000844	***	Log2 + 1
8	TP_ugL	Slope	Clam	Spring	Surface	ToMWC	-0.05894	0.017631	0.010187	*	Log2 + 1
9	TP_ugL	Slope	Clam	Spring	All	ToMWC	-0.05496	0.01367	0.0005	***	Log2 + 1

10	TP_ugL	Slope	Clam	Summer		CLMP	-0.06045	0.042191	0.175542		Log2 + 1
11	TP_ugL	Slope	Torch N	Spring		CLMP	-0.09105	0.050603	0.097148	.	Log2 + 1
12	TP_ugL	Slope	Torch N	Summer		CLMP	-0.12482	0.040751	0.009846	**	Log2 + 1
13	TP_ugL	Slope	Torch S	Spring		CLMP	-0.15939	0.03867	0.001416	**	Log2 + 1
14	TP_ugL	Slope	Torch S	Spring	Top 2	ToMWC	-0.0462	0.01819	0.021155	*	Log2 + 1
15	TP_ugL	Slope	Torch S	Spring	Surface	ToMWC	-0.03167	0.023792	0.219861		Log2 + 1
16	TP_ugL	Slope	Torch S	Spring	All	ToMWC	-0.05289	0.026514	0.056248	.	Log2 + 1
17	TP_ugL	Slope	Torch S	Summer		CLMP	-0.12166	0.044948	0.017032	*	Log2 + 1
18	TN_ugL	Slope	Bellaire	Spring		ToMWC	-1.25594	4.563766	0.786686		None
19	TN_ugL	Slope	Clam	Spring		ToMWC	2.776592	3.683626	0.464424		None
20	TN_ugL	Slope	Torch S	Spring		ToMWC	3.23906	3.05368	0.304573		None
21	NO4N_TN	Slope	Bellaire	Spring		ToMWC	0.005016	0.003597	0.182201		None
22	NO4N_ugL	Slope	Bellaire	Spring		ToMWC	3.009573	1.721844	0.097522	.	None
23	NO4N_TN	Slope	Clam	Spring		ToMWC	0.003879	0.004719	0.427087		None
24	NO4N_ugL	Slope	Clam	Spring		ToMWC	1.886607	1.333589	0.179016		None
25	NO4N_TN	Slope	Torch S	Spring		ToMWC	-0.02101	0.00657	0.0056	**	None
26	NO4N_ugL	Slope	Torch S	Spring		ToMWC	-7.95438	1.895126	0.000541	***	None
27	Chla_ppb	Slope	Bellaire	Summer		CLMP	-0.03074	0.02087	0.162949		None
28	Chla_ppb	Slope	Clam	Summer		CLMP	-0.03735	0.02234	0.116713		None
29	Chla_ppb	Slope	Torch N	Summer		CLMP	-0.0106	0.007795	0.203556		None
30	Chla_ppb	Slope	Torch S	Summer		CLMP	-0.01116	0.006982	0.134075		None
31	Secchi_m	Slope	Bellaire	Spring		CLMP	0.006749	0.036938	0.857842		None
32	Secchi_m	Slope	Clam	Spring		CLMP	-0.15522	0.011401	1.82E-09	****	None
33	Secchi_m	Slope	Torch N	Spring		CLMP	0.09809	0.026507	0.003498	**	None
34	Secchi_m	Slope	Torch S	Spring		CLMP	0.156765	0.065718	0.032972	*	None
35	Cl_mgL	Slope	Bellaire	Spring		ToMWC	0.166639	0.021535	3.92E-07	****	None
36	Cl_mgL	Slope	Clam	Spring		ToMWC	0.099985	0.044991	0.042062	*	None
37	Cl_mgL	Slope	Torch S	Spring		ToMWC	0.175252	0.041583	0.000521	***	None
1	TP_ugL	Intercept	Bellaire	Spring		CLMP	198.4681	102.7636	0.075542	.	Log2 + 1
2	TP_ugL	Intercept	Bellaire	Spring	Top 2	ToMWC	72.30963	27.8168	0.0187	*	Log2 + 1
3	TP_ugL	Intercept	Bellaire	Spring	Surface	ToMWC	68.93796	34.1079	0.077917	.	Log2 + 1
4	TP_ugL	Intercept	Bellaire	Spring	All	ToMWC	62.79377	23.88391	0.013956	*	Log2 + 1

5	TP_ugL	Intercept	Bellaire	Summer		CLMP	241.9353	67.3599	0.002947	**	Log2 + 1
6	TP_ugL	Intercept	Clam	Spring		CLMP	174.0819	99.20222	0.104763		Log2 + 1
7	TP_ugL	Intercept	Clam	Spring	Top 2	ToMWC	112.495	25.992	0.000695	***	Log2 + 1
8	TP_ugL	Intercept	Clam	Spring	Surface	ToMWC	120.7314	35.36025	0.009164	**	Log2 + 1
9	TP_ugL	Intercept	Clam	Spring	All	ToMWC	112.5214	27.41013	0.000404	***	Log2 + 1
10	TP_ugL	Intercept	Clam	Summer		CLMP	124.007	84.85097	0.167635		Log2 + 1
11	TP_ugL	Intercept	Torch N	Spring		CLMP	184.4512	101.7997	0.095083	.	Log2 + 1
12	TP_ugL	Intercept	Torch N	Summer		CLMP	252.4416	81.98255	0.009552	**	Log2 + 1
13	TP_ugL	Intercept	Torch S	Spring		CLMP	322.0878	77.79246	0.00137	**	Log2 + 1
14	TP_ugL	Intercept	Torch S	Spring	Top 2	ToMWC	94.52626	36.48529	0.019039	*	Log2 + 1
15	TP_ugL	Intercept	Torch S	Spring	Surface	ToMWC	65.23902	47.71541	0.208724		Log2 + 1
16	TP_ugL	Intercept	Torch S	Spring	All	ToMWC	107.4899	53.17901	0.053266	.	Log2 + 1
17	TP_ugL	Intercept	Torch S	Summer		CLMP	246.0676	90.41375	0.016542	*	Log2 + 1
18	TN_ugL	Intercept	Bellaire	Spring		ToMWC	3126.092	9159.547	0.737324		None
19	TN_ugL	Intercept	Clam	Spring		ToMWC	-5005.67	7390.89	0.510105		None
20	TN_ugL	Intercept	Torch S	Spring		ToMWC	-6036.8	6128.781	0.33929		None
21	NO4N_TN	Intercept	Bellaire	Spring		ToMWC	-9.40072	7.218615	0.211253		None
22	NO4N_ugL	Intercept	Bellaire	Spring		ToMWC	-5648.58	3453.19	0.119256		None
23	NO4N_TN	Intercept	Clam	Spring		ToMWC	-7.16386	9.463121	0.463638		None
24	NO4N_ugL	Intercept	Clam	Spring		ToMWC	-3449.26	2672.286	0.217698		None
25	NO4N_TN	Intercept	Torch S	Spring		ToMWC	42.82796	13.18643	0.005043	**	None
26	NO4N_ugL	Intercept	Torch S	Spring		ToMWC	16240.57	3800.71	0.000458	***	None
27	Chla_ppb	Intercept	Bellaire	Summer		CLMP	63.29279	41.97951	0.153861		None
28	Chla_ppb	Intercept	Clam	Summer		CLMP	76.41044	44.9372	0.111156		None
29	Chla_ppb	Intercept	Torch N	Summer		CLMP	21.87601	15.68165	0.193222		None
30	Chla_ppb	Intercept	Torch S	Summer		CLMP	22.97942	14.04721	0.125835		None
31	Secchi_m	Intercept	Bellaire	Spring		CLMP	-9.43131	74.28344	0.900912		None
32	Secchi_m	Intercept	Clam	Spring		CLMP	317.0963	22.9338	1.49E-09	****	None
33	Secchi_m	Intercept	Torch N	Spring		CLMP	-188.529	53.30928	0.004661	**	None
34	Secchi_m	Intercept	Torch S	Spring		CLMP	-306.585	132.2156	0.037331	*	None
35	Cl_mgL	Intercept	Bellaire	Spring		ToMWC	-325.629	43.18883	5.64E-07	****	None
36	Cl_mgL	Intercept	Clam	Spring		ToMWC	-192.75	90.19489	0.049474	*	None

37	Cl_mgL	Intercept	Torch S	Spring	ToMWC	-344.705	83.3947	0.000624	***	None

Table 2A. Analyses of TP patterns in long-term data from ToMWC to determine whether non-linear trends are likely and the effect of sample size on model significance. Three types of models were calculated with linear (Y=a+bX) and polynomial regression (Y=a+bX+bX^2), with linear indicated by Year as a Model X and Year and Year-squared (Year2) for polynomial regression. The other model element varied was whether top and mid depth TP concentrations (Model Y) were considered independent (Top & Mid) or lumped by averaging (TopMidAve). Each type of three types of models were calculated separately for Lake Bellaire, Clam Lake, and Torch Lake. For each regression model (Mod #), the table includes coefficients for an intercept (a) and slope estimate (b) that describes the rate of change in the water chemistry parameter (Y) per year (X), a standard error (SE) for the model coefficients (a and b), and P that estimates the probability that the coefficient (either intercept a or slope b) is equal to zero. For TP, water chemistry values were transformed to reduce skew in the data distribution and improve statistical testing by adding 1 to the value and calculating the log based 2 of the resulting sum (i.e. Log2 + 1 transformation). The regression model statistics are reported in two rows for each model with one row for the intercept and the other row for the slope. The table is sorted with slope coefficients for all models first and then intercepts for all models because the slopes, or change per year, are the key parameters that are most important. For clarity, models are numbered (Mod #) to indicate the rows with the intercept and slope for the same regression models. A P Code is provided to help draw attention to key results and describe certainty in observed patterns in data. See the title for Table 2 for an explanation of the P code.

Mod #	DepVar	Site	Model Y	Model X(s)	factor	Coefficient	SE	P	P Code
1	TP_ugL	Bellaire	Top & Mid	Year and Year2	Intercept	16812.34	6417.152	0.018573725	*
1	TP_ugL	Bellaire	Top & Mid	Year and Year2	Slope	-16.7299	6.399921	0.018795082	*
1	TP_ugL	Bellaire	Top & Mid	Year and Year2	Slope2	0.004163	0.001596	0.019003571	*
2	TP_ugL	Clam	Top & Mid	Year and Year2	Intercept	23927.39	3579.16	1.50E-05	****
2	TP_ugL	Clam	Top & Mid	Year and Year2	Slope	-23.8034	3.569176	1.54E-05	****
2	TP_ugL	Clam	Top & Mid	Year and Year2	Slope2	0.00592	0.00089	1.58E-05	****
3	TP_ugL	Torch S	Top & Mid	Year and Year2	Intercept	22781.91	8294.917	0.014337131	*
3	TP_ugL	Torch S	Top & Mid	Year and Year2	Slope	-22.6728	8.272644	0.01450893	*
3	TP_ugL	Torch S	Top & Mid	Year and Year2	Slope2	0.005641	0.002063	0.01467616	*
4	TP_ugL	Bellaire	TopMidAve	Year	Intercept	70.18939	38.44032	0.105291356	
4	TP_ugL	Bellaire	TopMidAve	Year	Slope	-0.03371	0.019167	0.116706166	
5	TP_ugL	Clam	TopMidAve	Year	Intercept	104.8948	29.52692	0.007481942	**
5	TP_ugL	Clam	TopMidAve	Year	Slope	-0.05093	0.014723	0.008580861	**
6	TP_ugL	Torch S	TopMidAve	Year	Intercept	80.79405	34.67899	0.04817901	*
6	TP_ugL	Torch S	TopMidAve	Year	Slope	-0.03914	0.017292	0.0534234	.
7	TP_ugL	Bellaire	TopMidAve	Year and Year2	Intercept	17679.18	8580.108	0.078309073	.
7	TP_ugL	Bellaire	TopMidAve	Year and Year2	Slope	-17.5947	8.556689	0.078799595	.

Mod #	DepVar	Site	Model Y	Model X(s)	factor	Coefficient	SE	P	P Code
7	TP_ugL	Bellaire	TopMidAve	Year and Year2	Slope2	0.004378	0.002133	0.079258676	.
8	TP_ugL	Clam	TopMidAve	Year and Year2	Intercept	20907.64	2783.683	0.000136064	***
8	TP_ugL	Clam	TopMidAve	Year and Year2	Slope	-20.797	2.776085	0.000138291	***
8	TP_ugL	Clam	TopMidAve	Year and Year2	Slope2	0.005172	0.000692	0.000140445	***
9	TP_ugL	Torch S	TopMidAve	Year and Year2	Intercept	18194.98	7006.655	0.035589842	*
9	TP_ugL	Torch S	TopMidAve	Year and Year2	Slope	-18.104	6.987531	0.035897757	*
9	TP_ugL	Torch S	TopMidAve	Year and Year2	Slope2	0.004504	0.001742	0.036191633	*

Table 3. A non-parametric regression analysis for long-term (2-3 decades) changes in spring and summer total phosphorus (TP) concentrations at off-shore sites with CLMP data. Separate Kendall-Theiu-Seigal analyses were calculated for each season and site (model numbers 1-8). The Kendall-Theiu-Seigal analysis compares all pairs of data for a season and site to determine if the later year is either greater or less than the earlier year. The slopes in these analyses are the mean changes between years calculated by comparing these pairs of data. The estimated coefficients, both intercept and slope, are presented for each model with the P value (likelihood the coefficient deviates by chance from 0) and a P code associated with the coefficient. See the title for Table 2 for an explanation of the P code.

DepVar	factor	Site	Season	Estimate	MAD	V.value	P
TP_ugL	Intercept	Bellaire	Spring	629.0714	566.2473	109	0.005866
TP_ugL	Slope	Bellaire	Spring	-0.30952	0.2824	11	0.005866
TP_ugL	Intercept	Clam	Spring	507.5	743.1533	95	0.008342
TP_ugL	Slope	Clam	Spring	-0.25	0.37065	4	0.011238
TP_ugL	Intercept	Torch N	Spring	82.28846	202.8026	97	0.005718
TP_ugL	Slope	Torch N	Spring	-0.04006	0.100582	6	0.010757
TP_ugL	Intercept	Torch S	Spring	169.5	91.16755	105	0.001094
TP_ugL	Slope	Torch S	Spring	-0.08333	0.045302	0	0.001082
TP_ugL	Intercept	Bellaire	Summer	535.2222	674.4771	125	0.003472
TP_ugL	Slope	Bellaire	Summer	-0.26389	0.33535	8	0.005659
TP_ugL	Intercept	Clam	Summer	729.3173	166.5469	117	0.000305
TP_ugL	Slope	Clam	Summer	-0.36058	0.082511	2	0.001696
TP_ugL	Intercept	Torch N	Summer	632	557.8283	41	0.184858
TP_ugL	Slope	Torch N	Summer	-0.3125	0.277988	14	0.184573
TP_ugL	Intercept	Torch S	Summer	141.5	145.2021	136	0.000479
TP_ugL	Slope	Torch S	Summer	-0.06944	0.072071	0	0.001091

Table 4. Analysis of variance results testing the hypothesis that water chemistry concentrations during summer 2017 differed among groups of samples grouped by sample types (water sources), sites sampled. An interaction term was included in the analysis, between sample types and sites sampled, which evaluated differences related to specific sites and sample types. Five analyses are reported, one each for PO-P, NO3-N, NH4-N, Cl, and SO4. Only inorganic forms of nutrients were assayed during 2017 because dialysis tubing was impermeable to particulate matter. The key result is the probability (P) that differences in means among sample groups could have occurred by chance. The F ratio, sum of squares (Sum Sq), and mean square error are used to determine P and also reported in the table. If P is less than 0.05, then differences are usually considered reliable. Also reported are the degrees of freedom (DF). All water chemistry concentrations were transformed (recalculated) with a Log2 Plus 1 transformation. See the title for Table 2 for an explanation of the Log2 Plus 1 transformation and P code. See Table 5 for the probability that differences among specific sample types and sites could have occurred by random chance.

Analysis	Factor	Df	Sum Sq	Mean Sp	F	Pr(>F)	P Code
PO4-P	Sample_Type	4	13.07	3.268	1.905	0.115	
PO4-P	Site	5	16.77	3.355	1.955	0.091	.
PO4-P	Sample_Type:Site	20	33.89	1.695	0.988	0.483	
PO4-P	Residuals	109	187.01	1.716			
NO3-N	Sample_Type	4	33.62	8.4	8.573	4.64E-06	****
NO3-N	Site	5	165.57	33.11	33.779	< 2.00E-16	****
NO3-N	Sample_Type:Site	20	214.64	10.73	10.947	< 2.00E-16	****
NO3-N	Residuals	110	107.84	0.98			
NH4-N	Sample_Type	4	669.9	167.49	60.713	< 2.00E-16	****
NH4-N	Site	5	157.4	31.49	11.414	3.96E-08	****
NH4-N	Sample_Type:Site	20	255.1	12.75	4.623	7.41E-07	****
NH4-N	Residuals	72	198.6	2.76			
CL	Sample_Type	4	22.8	5.701	10.944	1.66E-07	****
CL	Site	5	14.23	2.845	5.462	0.000158	***
CL	Sample_Type:Site	20	86.95	4.347	8.346	2.86E-14	****
CL	Residuals	110	57.3	0.521			
SO4	Sample_Type	4	9.09	2.274	3.696	0.00731	**
SO4	Site	5	66.8	13.361	21.718	4.51E-15	****
SO4	Sample_Type:Site	20	71.33	3.567	5.797	5.11E-10	****
SO4	Residuals	110	67.67	0.615			

Table 5. Comparisons of average water chemistry concentrations for different water sources during summer 2017. The comparisons are Tukey contrasts between the water sources. ChemVar is the water chemistry parameter being analyzed. The difference is calculated between the average water summer 2017 chemistry concentrations for the two water sources and that water chemistry parameter. The P adj value is an adjusted probability for the difference between summer chemistry averages occurring by chance that is adjusted for the number of comparisons being made. The P adjustment requires bigger differences specific P values because repeated tests are more likely than one test to have significant differences (e.g. $P < 0.05$) by chance. The water source codes are: LFP is the lake floor piezometer; Well is well water; BS is benthic sediment sample (drained water from sediments); BW is the water extracted from sediments with a turkey baster; SW is nearshore surface water.

Comparison	ChemVar	Difference	P adj	PrCode
LFP vs. Well	PO4-P	-0.76329	0.140649	
BS vs. Well	PO4-P	-0.73988	0.304856	
BW vs. Well	PO4-P	-0.34805	0.918098	
SW vs. Well	PO4-P	-0.84837	0.156652	
BS vs. LFP	PO4-P	0.023412	0.999994	
BW vs. LFP	PO4-P	0.415247	0.792337	
SW vs. LFP	PO4-P	-0.08507	0.998856	
BW vs. BS	PO4-P	0.391835	0.882544	
SW vs. BS	PO4-P	-0.10848	0.998447	
SW vs. BW	PO4-P	-0.50032	0.737078	
LFP vs. Well	NO3-N	0.843232	0.007732	**
BS vs. Well	NO3-N	0.291057	0.846443	
BW vs. Well	NO3-N	1.373456	0.000264	***
SW vs. Well	NO3-N	1.305788	8.66E-05	****
BS vs. LFP	NO3-N	-0.55218	0.173503	
BW vs. LFP	NO3-N	0.530224	0.32217	
SW vs. LFP	NO3-N	0.462556	0.310145	
BW vs. BS	NO3-N	1.0824	0.00702	**
SW vs. BS	NO3-N	1.014731	0.004003	**
SW vs. BW	NO3-N	-0.06767	0.99948	
BS vs. LFP	NH4-N	6.498719	<1.0 E-06	****
BS vs. Well	NH4-N	8.722929	<1.0 E-06	****

Comparison	ChemVar	Difference	P adj	PrCode
BW vs. BS	NH4-N	-5.73534	<1.0 E-06	****
BW vs. Well	NH4-N	2.987593	0.000004	****
LFP vs. Well	NH4-N	2.22421	0.000146	***
SW vs. BS	NH4-N	-7.66708	<1.0 E-06	****
SW vs. BW	NH4-N	-1.93175	0.004227	**
BW vs. LFP	NH4-N	0.763383	0.614372	
SW vs. LFP	NH4-N	-1.16836	0.105447	
SW vs. Well	NH4-N	1.055846	0.175002	
LFP vs. Well	Cl	0.125415	0.956648	
BS vs. LFP	Cl	0.871581	4.03E-05	****
BS vs. Well	Cl	0.996996	5.19E-05	****
BW vs. LFP	Cl	0.854525	0.000502	***
BW vs. Well	Cl	0.97994	0.000376	***
SW vs. LFP	Cl	0.502853	0.038576	*
SW vs. Well	Cl	0.628268	0.021862	*
BW vs. BS	Cl	-0.01706	0.999993	
SW vs. BS	Cl	-0.36873	0.376073	
SW vs. BW	Cl	-0.35167	0.524713	
LFP vs. Well	SO4	-0.70119	0.004458	**
BS vs. Well	SO4	-0.63044	0.048608	*
BW vs. Well	SO4	-0.47013	0.328386	
SW vs. Well	SO4	-0.68469	0.021322	*
BS vs. LFP	SO4	0.070746	0.99628	
BW vs. LFP	SO4	0.231057	0.833028	
SW vs. LFP	SO4	0.016496	0.999987	
BW vs. BS	SO4	0.160311	0.967279	
SW vs. BS	SO4	-0.05425	0.999201	
SW vs. BW	SO4	-0.21456	0.904762	

Table 6. Results of 5 analyses of variance (AOV) of phosphorus concentrations in peeper study (2017) collected from Torch Lake, Lake Bellaire, Elk Lake, and Lake Leelanau. When lakes equal all, then the AOV includes 3 factors and their interactions: sample type (dialysis or nearshore surface water grab sample); month of sample; and lake. When lakes equal all, there are three 2-way interactions and 1 three way interaction. When lakes equal a specific lake, the AOV includes two factors and one one-way interaction: sample type and month of sample. The key result is the probability (P) that differences in means among sample groups could have occurred by chance. The F ratio, sum of squares (Sum Sq), and mean square error are used to determine P and also reported. If P is less than 0.05, then differences are usually considered reliable. Also reported are the degrees of freedom (DF). All water chemistry concentrations were transformed (recalculated) with a Log2 transformation. See the title for Table 2 for an explanation of the Log2 Plus 1 transformation and P code.

AOV factors	Model	Lake	Df	Sum Sq	Mean Sq	F	P	P Code
Sample_Type	1	All	2	216.129	108.0645	132.6595	3.60E-22	****
Month	1	All	2	123.5894	61.79468	75.85887	8.61E-17	****
Lake	1	All	3	25.08486	8.361622	10.26469	1.68E-05	****
Sample_Type:Month	1	All	4	12.84238	3.210594	3.94131	0.006814	**
Sample_Type:Lake	1	All	6	35.32968	5.88828	7.228426	9.11E-06	****
Month:Lake	1	All	6	23.31891	3.886485	4.771031	0.000528	***
Sample_Type:Month:Lake	1	All	12	19.85817	1.654847	2.031483	0.037645	*
Residuals	1	All	57	46.43224	0.814601	NA	NA	
Sample_Type	2	Torch	2	46.30107	23.15054	20.77481	6.46E-05	****
Month	2	Torch	2	22.9126	11.4563	10.28065	0.00179	**
Lake	2	Torch	4	3.221054	0.805263	0.722627	0.590759	
Sample_Type:Month	2	Torch	14	15.60098	1.114356	NA	NA	
Sample_Type:Lake	3	Bellaire	2	42.00661	21.00331	23.88907	2.17E-05	****
Month:Lake	3	Bellaire	2	45.92997	22.96498	26.12028	1.30E-05	****
Sample_Type:Month:Lake	3	Bellaire	4	10.38393	2.595981	2.952659	0.055239	.
Residuals	3	Bellaire	15	13.18802	0.879201	NA	NA	
Sample_Type	4	Elk	2	20.32933	10.16466	21.70112	5.13E-05	****
Month	4	Elk	2	44.16785	22.08393	47.14823	6.03E-07	****
Lake	4	Elk	4	2.957257	0.739314	1.578404	0.234541	
Sample_Type:Month	4	Elk	14	6.557509	0.468393	NA	NA	
Sample_Type:Lake	5	Leelanau	2	144.6656	72.3328	91.34804	9.25E-09	****
Month:Lake	5	Leelanau	2	32.42872	16.21436	20.47688	6.96E-05	****

AOV factors	Model	Lake	Df	Sum Sq	Mean Sq	F	P	P Code
Sample_Type:Month:Lake	5	Leelanau	4	15.84972	3.962431	5.004097	0.010239	*
Residuals	5	Leelanau	14	11.08572	0.791837	NA	NA	

Table 7. Comparisons of average PO4-P concentrations between different water sources sampled with dialysis tubing and between lakes during 2017. The comparisons are Tukey contrasts. The water sources were lake nearshore surface water grab samples, dialysis tubing on top of sand (benthos), and dialysis tubing buried about 2 cm into the sand (sub-benthos). The P adj value is an adjusted probability for the difference between summer chemistry averages occurring by chance that is adjusted for the number of comparisons being made. The P adjustment requires bigger differences specific P values because repeated tests are more likely than one test to have significant differences (e.g. P < 0.05) by chance. PO4-P concentrations were transformed (recalculated) with a Log2 transformation. See the title for Table 2 for an explanation of the P code.

Comparison variable	Comparison	Difference	P adj	PrCode
Water Source	Lake Water Grab vs. Benthos	-3.6074386	< 1.0E-06	****
Water Source	Sub vs. benthos vs. Benthos	-0.1875829	0.701751	
Water Source	Sub vs. benthos vs. Lake Water Grab	3.4198557	< 1.0E-06	****
Lake	Lake Leelanau vs. Lake Bellaire	1.4119724	9.1E-06	****
Lake	Lake Leelanau vs. Elk Lake	1.0268863	0.001628	**
Lake	Torch Lake vs. Lake Leelanau	-0.9252979	0.005265	**
Lake	Torch Lake vs. Lake Bellaire	0.4866745	0.262071	
Lake	Lake Bellaire vs. Elk Lake	-0.3850861	0.466748	
Lake	Torch Lake vs. Elk Lake	0.1015884	0.980887	

Table 8. Analyses of covariance for water chemistry to determine the important factors affecting concentrations. This is the first statistical step to determine effects of Site, Sample_Type, Year, and Sumr_Day on water chemistry. It provides the overall importance of factors. Subsequent steps will deal more with one variable at a time, but within the context of this more complete. Site, Sample_Type, and Year were included as categorical factors and summer day (Sumr_Day) was a continuous variable in the analysis. All possible interactions were included in the model. When an interaction is significant (P<0.05), that indicates that one factor either increases or decreases the effect of the other factor. Three-way (Site:Sample_Type:Year, Site:Sample_Type:Sumr-Day, or Smple_Type:Year:Sumr_Day) and four-way interactions (Site:Sample_Type:Year:Sumr_Day) are difficult to interpret but important to include in the analysis to determine total explainable variation. See the title for Table 2 for an explanation of the P code.

DepVar	Factor and Interaction	Df	Sum Sq	Mean Sq	F value	P	P Code
TP_ugL	Site	13	101.7111631	7.82393563	7.27590619	8.06E-13	****
TP_ugL	Sample_Type	2	101.7529621	50.8764811	47.3128258	3.68E-19	****
TP_ugL	Year	1	19.80390443	19.8039044	18.4167352	2.23E-05	****
TP_ugL	Sumr_Day	1	11.07459069	11.0745907	10.2988683	0.001438998	**
TP_ugL	Site:Sample_Type	8	19.67385576	2.45923197	2.28697448	0.021063798	*
TP_ugL	Site:Year	3	11.24786254	3.74928751	3.48666777	0.015902232	*
TP_ugL	Sample_Type:Year	2	27.63775076	13.8188754	12.850929	3.91E-06	****
TP_ugL	Site:Sumr_Day	5	10.05095957	2.01019191	1.86938754	0.098637329	.
TP_ugL	Sample_Type:Sumr_Day	2	3.460577379	1.73028869	1.6090902	0.201368321	
TP_ugL	Year:Sumr_Day	1	0.664027049	0.66402705	0.61751512	0.432439761	
TP_ugL	Site:Sample_Type:Year	5	38.48879656	7.69775931	7.15856792	1.98E-06	****
TP_ugL	Site:Sample_Type:Sumr_Day	8	10.35073378	1.29384172	1.20321427	0.295554978	
TP_ugL	Site:Year:Sumr_Day	3	3.02028476	1.00676159	0.93624273	0.423109222	
TP_ugL	Sample_Type:Year:Sumr_Day	2	11.39871487	5.69935744	5.30014458	0.005349289	**
TP_ugL	Site:Sample_Type:Year:Sumr_Day	5	14.33455681	2.86691136	2.66609787	0.02191304	*
TP_ugL	Residuals	398	427.9778078	1.07532113	NA	NA	
PO4P_ugL	Site	18	61.26205459	3.40344748	6.60228353	1.32E-14	****
PO4P_ugL	Sample_Type	2	23.36526878	11.6826344	22.662922	4.48E-10	****
PO4P_ugL	Year	1	0.000288388	0.00028839	0.00055944	0.981141007	
PO4P_ugL	Sumr_Day	1	63.82075593	63.8207559	123.80468	2.16E-25	****
PO4P_ugL	Site:Sample_Type	11	24.56691865	2.23335624	4.33244562	3.76E-06	****
PO4P_ugL	Site:Year	4	4.825337951	1.20633449	2.34014551	0.054466068	.

DepVar	Factor and Interaction	Df	Sum Sq	Mean Sq	F value	P	P Code
PO4P_ugL	Sample_Type:Year	2	13.72599042	6.86299521	13.3133949	2.47E-06	****
PO4P_ugL	Site:Sumr_Day	11	16.10754106	1.46432191	2.84061045	0.001339884	**
PO4P_ugL	Sample_Type:Sumr_Day	2	6.177167942	3.08858397	5.99148576	0.002717626	**
PO4P_ugL	Year:Sumr_Day	1	5.636968438	5.63696844	10.9350487	0.001024356	**
PO4P_ugL	Site:Sample_Type:Year	6	2.028969499	0.33816158	0.65599327	0.685304439	
PO4P_ugL	Site:Sample_Type:Sumr_Day	11	7.746097732	0.7041907	1.36604625	0.186107201	
PO4P_ugL	Site:Year:Sumr_Day	4	1.757298396	0.4393246	0.8522375	0.492735507	
PO4P_ugL	Sample_Type:Year:Sumr_Day	2	8.597614392	4.2988072	8.33917495	0.000280594	***
PO4P_ugL	Site:Sample_Type:Year:Sumr_Day	6	1.928293658	0.32138228	0.62344341	0.711571456	
PO4P_ugL	Residuals	422	217.5391028	0.5154955	NA	NA	
TN_ugL	Site	11	14.99179134	1.36289012	7.44658111	7.71E-11	****
TN_ugL	Sample_Type	2	14.25373857	7.12686928	38.9399038	3.26E-15	****
TN_ugL	Year	1	1.276589384	1.27658938	6.97504975	0.008855387	**
TN_ugL	Sumr_Day	1	0.127558636	0.12755864	0.69695694	0.404709651	
TN_ugL	Site:Sample_Type	7	13.0504448	1.86434926	10.1864617	4.82E-11	****
TN_ugL	Site:Year	2	1.385082771	0.69254139	3.78391883	0.024220077	*
TN_ugL	Sample_Type:Year	2	0.459001886	0.22950094	1.25395097	0.287397375	
TN_ugL	Site:Sumr_Day	4	3.991783586	0.9979459	5.45259295	0.000332478	***
TN_ugL	Sample_Type:Sumr_Day	2	1.860618822	0.93030941	5.08303962	0.006945962	**
TN_ugL	Year:Sumr_Day	1	0.720428913	0.72042891	3.93629116	0.048492286	*
TN_ugL	Site:Sample_Type:Year	3	0.213945433	0.07131514	0.38965284	0.760566527	
TN_ugL	Site:Sample_Type:Sumr_Day	7	4.063230483	0.5804615	3.17153493	0.00323165	**
TN_ugL	Site:Year:Sumr_Day	1	0.006002685	0.00600268	0.03279757	0.85645429	
TN_ugL	Sample_Type:Year:Sumr_Day	2	0.542148186	0.27107409	1.48109902	0.229635779	
TN_ugL	Site:Sample_Type:Year:Sumr_Day	1	0.006202075	0.00620207	0.033887	0.854116189	
TN_ugL	Residuals	221	40.44791997	0.18302226	NA	NA	
NO3N_ugL	Site	15	475.4057608	31.6937174	18.7903058	4.14E-39	****
NO3N_ugL	Sample_Type	2	429.3623006	214.68115	127.278363	8.94E-45	****
NO3N_ugL	Year	1	100.3891481	100.389148	59.5178775	7.50E-14	****
NO3N_ugL	Sumr_Day	1	11.03554578	11.0355458	6.54266197	0.010849142	*
NO3N_ugL	Site:Sample_Type	11	553.3564362	50.3051306	29.8244845	2.41E-47	****

DepVar	Factor and Interaction	Df	Sum Sq	Mean Sq	F value	P	P Code
NO3N_ugL	Site:Year	4	151.3629067	37.8407267	22.4346931	5.96E-17	****
NO3N_ugL	Sample_Type:Year	2	9.427218577	4.71360929	2.7945652	0.062173131	.
NO3N_ugL	Site:Sumr_Day	7	18.81339135	2.68762734	1.59341799	0.135125011	
NO3N_ugL	Sample_Type:Sumr_Day	2	0.129398274	0.06469914	0.03835828	0.962371137	
NO3N_ugL	Year:Sumr_Day	1	10.4696673	10.4696673	6.20716867	0.013072432	*
NO3N_ugL	Site:Sample_Type:Year	6	42.43860012	7.07310002	4.19344031	0.000403821	***
NO3N_ugL	Site:Sample_Type:Sumr_Day	11	16.11189144	1.4647174	0.86838939	0.571570746	
NO3N_ugL	Site:Year:Sumr_Day	4	59.39748101	14.8493703	8.80377028	7.41E-07	****
NO3N_ugL	Sample_Type:Year:Sumr_Day	2	2.262928764	1.13146438	0.67081313	0.511788871	
NO3N_ugL	Site:Sample_Type:Year:Sumr_Day	6	37.45635518	6.24272586	3.70113503	0.001325024	**
NO3N_ugL	Residuals	463	780.9447778	1.68670578	NA	NA	
NH4N_ugL	Site	15	737.8813847	49.1920923	21.1782104	8.17E-43	****
NH4N_ugL	Sample_Type	2	198.1371258	99.0685629	42.6510597	1.28E-17	****
NH4N_ugL	Year	1	175.7818263	175.781826	75.6777019	7.20E-17	****
NH4N_ugL	Sumr_Day	1	7.641156008	7.64115601	3.28967527	0.07041535	.
NH4N_ugL	Site:Sample_Type	11	1059.730095	96.3390996	41.4759694	1.07E-60	****
NH4N_ugL	Site:Year	4	91.56252574	22.8906314	9.85488897	1.23E-07	****
NH4N_ugL	Sample_Type:Year	2	74.970545	37.4852725	16.138183	1.75E-07	****
NH4N_ugL	Site:Sumr_Day	7	26.78510329	3.82644333	1.64736278	0.12033914	
NH4N_ugL	Sample_Type:Sumr_Day	2	0.491684903	0.24584245	0.10584025	0.899591828	
NH4N_ugL	Year:Sumr_Day	1	11.72657424	11.7265742	5.04853208	0.025154527	*
NH4N_ugL	Site:Sample_Type:Year	6	31.99269439	5.33211573	2.2955858	0.034181737	*
NH4N_ugL	Site:Sample_Type:Sumr_Day	11	30.81879668	2.80170879	1.20619342	0.280235646	
NH4N_ugL	Site:Year:Sumr_Day	4	14.30699011	3.57674753	1.53986358	0.189656635	
NH4N_ugL	Sample_Type:Year:Sumr_Day	2	1.867567051	0.93378353	0.40201307	0.669223754	
NH4N_ugL	Site:Sample_Type:Year:Sumr_Day	6	32.02992315	5.33832053	2.29825709	0.033982833	*
NH4N_ugL	Residuals	429	996.4679371	2.32276908	NA	NA	
Cl_mgL	Site	14	316.7266742	22.6233339	16.6437221	1.52E-30	****
Cl_mgL	Sample_Type	2	15.6164956	7.8082478	5.74443656	0.003539677	**
Cl_mgL	Year	1	65.93105038	65.9310504	48.5047025	1.88E-11	****
Cl_mgL	Sumr_Day	1	30.16422526	30.1642253	22.1914677	3.68E-06	****

DepVar	Factor and Interaction	Df	Sum Sq	Mean Sq	F value	P	P Code
Cl_mgL	Site:Sample_Type	11	160.6278712	14.6025337	10.7429133	7.35E-17	****
Cl_mgL	Site:Year	4	24.65603734	6.16400934	4.53478956	0.001413271	**
Cl_mgL	Sample_Type:Year	2	77.82470331	38.9123517	28.6273619	3.65E-12	****
Cl_mgL	Site:Sumr_Day	7	64.33799328	9.1911419	6.76181557	1.72E-07	****
Cl_mgL	Sample_Type:Sumr_Day	2	0.393752356	0.19687618	0.1448395	0.865217825	
Cl_mgL	Year:Sumr_Day	1	0.007003684	0.00700368	0.00515253	0.942820944	
Cl_mgL	Site:Sample_Type:Year	5	32.80684365	6.56136873	4.82712222	0.000285907	***
Cl_mgL	Site:Sample_Type:Sumr_Day	10	5.278624115	0.52786241	0.3883422	0.951397518	
Cl_mgL	Site:Year:Sumr_Day	4	31.06268276	7.76567069	5.71311308	0.000187804	***
Cl_mgL	Sample_Type:Year:Sumr_Day	2	9.163182107	4.58159105	3.37062294	0.035592854	*
Cl_mgL	Site:Sample_Type:Year:Sumr_Day	5	14.30341215	2.86068243	2.1045706	0.064617484	.
Cl_mgL	Residuals	320	434.9668184	1.35927131	NA	NA	
SO4_mgL	Site	14	124.9722357	8.92658827	30.5403382	1.05E-50	****
SO4_mgL	Sample_Type	2	4.300585366	2.15029268	7.35674859	0.000752108	***
SO4_mgL	Year	1	0.484542481	0.48454248	1.65775443	0.198837189	
SO4_mgL	Sumr_Day	1	2.544458293	2.54445829	8.70529864	0.003406951	**
SO4_mgL	Site:Sample_Type	11	135.1691335	12.288103	42.041014	1.21E-55	****
SO4_mgL	Site:Year	4	30.77628389	7.69407097	26.3235541	6.96E-19	****
SO4_mgL	Sample_Type:Year	2	6.431843411	3.21592171	11.0025615	2.39E-05	****
SO4_mgL	Site:Sumr_Day	7	5.39384263	0.77054895	2.636262	0.011611898	*
SO4_mgL	Sample_Type:Sumr_Day	2	0.353330847	0.17666542	0.60442149	0.547012998	
SO4_mgL	Year:Sumr_Day	1	0.638965138	0.63896514	2.18607723	0.14024723	
SO4_mgL	Site:Sample_Type:Year	5	11.40609071	2.28121814	7.80468095	6.01E-07	****
SO4_mgL	Site:Sample_Type:Sumr_Day	10	2.542857585	0.25428576	0.86998222	0.561717333	
SO4_mgL	Site:Year:Sumr_Day	4	3.05538	0.763845	2.61332593	0.035395476	*
SO4_mgL	Sample_Type:Year:Sumr_Day	2	3.235795094	1.61789755	5.53527694	0.004332192	**
SO4_mgL	Site:Sample_Type:Year:Sumr_Day	5	7.681933951	1.53638679	5.256406	0.000117877	***
SO4_mgL	Residuals	320	93.53230574	0.29228846	NA	NA	

Table 9. Regression analysis results to determine if water chemistry concentrations changed consistently during spring-summer season. Linear regression was used to relate water chemistry to summer day, a variable constructed to be 1 on May 1 and increase by one unit each day during the summer. Separate univariate regression models (Y=a+bX) were calculated to determine the relationship between summer day (X) and each water chemistry parameter (Y) separately for each site, year samples were collected, and water source. For each regression model, the table includes coefficients for an intercept (a) and slope estimate (b) that describes the rate of change in the water chemistry parameter (Y) per year (X), a standard error (SE) for the model coefficient, and P that estimates the probability that the coefficient (either intercept a or slope b) is equal to zero. All water chemistry concentrations were transformed (recalculated) with a Log2 Plus 1 transformation. See the title for Table 2 for an explanation of the Log2 Plus 1 transformation and P code.

Model	DepVar	Coefficient	Site	Year	WS	Estimate	SE	P	PrCode
1	TP_ugL	Slope	Cla	2020	BW	-0.02235	0.017941	0.280851	
2	TP_ugL	Slope	Cla	2020	SW	0.020505	0.01512	0.246552	
3	TP_ugL	Slope	Dra	2019	BW	0.005834	0.006319	0.366889	
4	TP_ugL	Slope	Dra	2019	LFP	0.004113	0.010544	0.702757	
5	TP_ugL	Slope	Dra	2019	SW	0.005737	0.003889	0.159563	
6	TP_ugL	Slope	Gou	2018	BW	0.002968	0.018048	0.874789	
7	TP_ugL	Slope	Gou	2018	BW	0.002968	0.018048	0.874789	
8	TP_ugL	Slope	Gou	2019	BW	-0.00752	0.00741	0.326397	
9	TP_ugL	Slope	Gou	2020	BW	0.016885	0.010223	0.142575	
10	TP_ugL	Slope	Gou	2018	LFP	-0.00606	0.01006	0.563776	
11	TP_ugL	Slope	Gou	2019	LFP	-0.02307	0.012281	0.082907	.
12	TP_ugL	Slope	Gou	2020	LFP	-0.06856	0.024779	0.069738	.
13	TP_ugL	Slope	Gou	2018	SW	-0.01973	0.009757	0.077791	.
14	TP_ugL	Slope	Gou	2019	SW	0.012813	0.006261	0.057493	.
15	TP_ugL	Slope	Gou	2020	SW	-0.01796	0.013546	0.221411	
16	TP_ugL	Slope	Pet	2019	BW	-0.00539	0.007069	0.456707	
17	TP_ugL	Slope	Pet	2020	BW	-0.02847	0.014231	0.073338	.
18	TP_ugL	Slope	Pet	2019	LFP	-0.02259	0.007625	0.011879	*
19	TP_ugL	Slope	Pet	2019	SW	-0.00283	0.005681	0.624654	
20	TP_ugL	Slope	Pet	2020	SW	0.013646	0.013922	0.350101	
21	TP_ugL	Slope	Sou	2018	BW	0.013407	0.014282	0.384101	
22	TP_ugL	Slope	Sou	2020	BW	-0.03087	0.017732	0.112304	

Model	DepVar	Coefficient	Site	Year	WS	Estimate	SE	P	PrCode
23	TP_ugL	Slope	Sou	2018	LFP	-0.024	0.009989	0.043017	*
24	TP_ugL	Slope	Sou	2020	LFP	0.005342	0.024693	0.839301	
25	TP_ugL	Slope	Sou	2018	SW	-0.01772	0.011799	0.171452	
26	TP_ugL	Slope	Sou	2020	SW	0.003043	0.019618	0.879817	
27	PO4P_ugL	Slope	Cla	2020	BW	-0.00196	0.006976	0.792953	
28	PO4P_ugL	Slope	Cla	2020	SW	0.008711	0.011927	0.505662	
29	PO4P_ugL	Slope	Dra	2019	BW	-0.00776	0.004725	0.116358	
30	PO4P_ugL	Slope	Dra	2019	LFP	-0.007	0.005246	0.205287	
31	PO4P_ugL	Slope	Dra	2019	SW	0.010313	0.005483	0.078308	.
32	PO4P_ugL	Slope	Gou	2018	BW	-0.01637	0.00615	0.044831	*
33	PO4P_ugL	Slope	Gou	2019	BW	-0.00141	0.006112	0.820981	
34	PO4P_ugL	Slope	Gou	2020	BW	-0.00372	0.010251	0.727713	
35	PO4P_ugL	Slope	Gou	2018	LFP	-0.01445	0.004891	0.021305	*
36	PO4P_ugL	Slope	Gou	2019	LFP	0.008915	0.002713	0.005907	**
37	PO4P_ugL	Slope	Gou	2020	LFP	-0.01088	0.011219	0.403706	
38	PO4P_ugL	Slope	Gou	2018	SW	-0.01769	0.004106	0.003528	**
39	PO4P_ugL	Slope	Gou	2019	SW	0.015346	0.002455	1.16E-05	****
40	PO4P_ugL	Slope	Gou	2020	SW	-0.01148	0.008387	0.208339	
41	PO4P_ugL	Slope	Pet	2019	BW	0.0047	0.003091	0.14791	
42	PO4P_ugL	Slope	Pet	2020	BW	-0.015	0.005571	0.022591	*
43	PO4P_ugL	Slope	Pet	2019	LFP	0.015155	0.002628	8.92E-05	****
44	PO4P_ugL	Slope	Pet	2019	SW	0.003807	0.002673	0.173611	
45	PO4P_ugL	Slope	Pet	2020	SW	-0.00011	0.00894	0.990607	
46	PO4P_ugL	Slope	Sou	2018	BW	-0.01223	0.01016	0.295137	
47	PO4P_ugL	Slope	Sou	2020	BW	-0.03047	0.007469	0.002218	**
48	PO4P_ugL	Slope	Sou	2018	LFP	-0.02031	0.00603	0.011944	*
49	PO4P_ugL	Slope	Sou	2020	LFP	0.008057	0.015431	0.629169	
50	PO4P_ugL	Slope	Sou	2018	SW	-0.01934	0.002729	0.000196	***
51	PO4P_ugL	Slope	Sou	2020	SW	-0.00577	0.007519	0.460474	
52	TN_ugL	Slope	Cla	2020	BW	0.003232	0.005434	0.583994	
53	TN_ugL	Slope	Cla	2020	SW	0.003128	0.004116	0.489533	

Model	DepVar	Coefficient	Site	Year	WS	Estimate	SE	P	PrCode
54	TN_ugL	Slope	Dra	2019	BW	-0.00943	0.003487	0.013663	*
55	TN_ugL	Slope	Dra	2019	LFP	-0.01441	0.008496	0.113726	
56	TN_ugL	Slope	Dra	2019	SW	-0.00996	0.005052	0.066149	.
57	TN_ugL	Slope	Gou	2019	BW	-0.00484	0.002793	0.103423	
58	TN_ugL	Slope	Gou	2020	BW	0.007332	0.001898	0.006184	**
59	TN_ugL	Slope	Gou	2019	LFP	0.020822	0.008524	0.029617	*
60	TN_ugL	Slope	Gou	2020	LFP	0.015025	0.005791	0.080755	.
61	TN_ugL	Slope	Gou	2019	SW	-0.00634	0.002831	0.039602	*
62	TN_ugL	Slope	Gou	2020	SW	0.003602	0.00185	0.087449	.
63	TN_ugL	Slope	Pet	2019	BW	-0.00478	0.003365	0.174842	
64	TN_ugL	Slope	Pet	2020	BW	0.004004	0.000829	0.00069	***
65	TN_ugL	Slope	Pet	2019	LFP	0.018094	0.005276	0.004994	**
66	TN_ugL	Slope	Pet	2019	SW	1.53E-05	0.004471	0.997317	
67	TN_ugL	Slope	Pet	2020	SW	0.004171	0.001968	0.060043	.
68	TN_ugL	Slope	Sou	2020	BW	0.003515	0.005242	0.517714	
69	TN_ugL	Slope	Sou	2020	LFP	-0.00981	0.02304	0.692048	
70	TN_ugL	Slope	Sou	2020	SW	0.00531	0.002008	0.024532	*
71	NO3N_ugL	Slope	Cla	2020	BW	-0.00089	0.005431	0.878081	
72	NO3N_ugL	Slope	Cla	2020	SW	0.046536	0.011927	0.017517	*
73	NO3N_ugL	Slope	Dra	2019	BW	-0.00976	0.013878	0.489921	
74	NO3N_ugL	Slope	Dra	2019	LFP	-0.08254	0.016284	0.000215	***
75	NO3N_ugL	Slope	Dra	2019	SW	0.002202	0.016968	0.89837	
76	NO3N_ugL	Slope	Gou	2018	BW	0.005168	0.011042	0.656254	
77	NO3N_ugL	Slope	Gou	2019	BW	-0.00643	0.002537	0.022861	*
78	NO3N_ugL	Slope	Gou	2020	BW	0.00782	0.013188	0.571849	
79	NO3N_ugL	Slope	Gou	2018	LFP	-0.03237	0.013235	0.044374	*
80	NO3N_ugL	Slope	Gou	2019	LFP	-0.02745	0.019022	0.17266	
81	NO3N_ugL	Slope	Gou	2020	LFP	-2.83E-19	1.81E-18	0.886093	
82	NO3N_ugL	Slope	Gou	2018	SW	-0.00671	0.008458	0.450713	
83	NO3N_ugL	Slope	Gou	2019	SW	-0.00424	0.00136	0.006598	**

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84	NO3N_ugL	Slope	Gou	2020	SW	0.012739	0.009759	0.228051	
85	NO3N_ugL	Slope	Pet	2019	BW	0.008602	0.015297	0.581685	
86	NO3N_ugL	Slope	Pet	2020	BW	0.010815	0.007706	0.19078	
87	NO3N_ugL	Slope	Pet	2019	LFP	0.022271	0.002005	1.14E-07	****
88	NO3N_ugL	Slope	Pet	2019	SW	-0.00388	0.000984	0.001172	**
89	NO3N_ugL	Slope	Pet	2020	SW	0.00377	0.004688	0.439981	
90	NO3N_ugL	Slope	Sou	2018	BW	-0.00014	0.011326	0.990598	
91	NO3N_ugL	Slope	Sou	2020	BW	0.015531	0.018101	0.41097	
92	NO3N_ugL	Slope	Sou	2018	LFP	-0.0104	0.025299	0.691782	
93	NO3N_ugL	Slope	Sou	2020	LFP	-0.01445	0.037529	0.719763	
94	NO3N_ugL	Slope	Sou	2018	SW	-0.01199	0.008178	0.180815	
95	NO3N_ugL	Slope	Sou	2020	SW	0.021687	0.013399	0.13662	
96	NH4N_ugL	Slope	Cla	2020	BW	-0.01835	0.010584	0.158002	
97	NH4N_ugL	Slope	Cla	2020	SW	-0.01561	0.010465	0.21017	
98	NH4N_ugL	Slope	Dra	2019	BW	-0.01634	0.008059	0.056205	.
99	NH4N_ugL	Slope	Dra	2019	LFP	0.013139	0.012397	0.308514	
100	NH4N_ugL	Slope	Dra	2019	SW	-0.01411	0.002973	0.000219	***
101	NH4N_ugL	Slope	Gou	2018	BW	0.036188	0.008216	0.006993	**
102	NH4N_ugL	Slope	Gou	2019	BW	-0.00322	0.011941	0.790953	
103	NH4N_ugL	Slope	Gou	2020	BW	0.012698	0.006485	0.09109	.
104	NH4N_ugL	Slope	Gou	2018	LFP	0.031823	0.016336	0.087252	.
105	NH4N_ugL	Slope	Gou	2019	LFP	-0.07398	0.02078	0.003489	**
106	NH4N_ugL	Slope	Gou	2020	LFP	-0.0162	0.034123	0.667455	
107	NH4N_ugL	Slope	Gou	2018	SW	0.02078	0.00908	0.051392	.
108	NH4N_ugL	Slope	Gou	2019	SW	-0.0011	0.002751	0.695721	
109	NH4N_ugL	Slope	Gou	2020	SW	-0.02744	0.015842	0.121513	
110	NH4N_ugL	Slope	Pet	2019	BW	-0.00787	0.005842	0.196853	
111	NH4N_ugL	Slope	Pet	2020	BW	-0.02108	0.006119	0.006287	**
112	NH4N_ugL	Slope	Pet	2019	LFP	-0.00947	0.019204	0.630966	
113	NH4N_ugL	Slope	Pet	2019	SW	0.043221	0.010946	0.00115	**
114	NH4N_ugL	Slope	Pet	2020	SW	-0.01436	0.004765	0.013021	*

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115	NH4N_ugL	Slope	Sou	2018	BW	0.033191	0.006422	0.00356	**
116	NH4N_ugL	Slope	Sou	2020	BW	-0.00176	0.0109	0.875081	
117	NH4N_ugL	Slope	Sou	2018	LFP	0.010178	0.018138	0.590047	
118	NH4N_ugL	Slope	Sou	2020	LFP	0.045657	0.024688	0.138093	
119	NH4N_ugL	Slope	Sou	2018	SW	0.020023	0.011184	0.111191	
120	NH4N_ugL	Slope	Sou	2020	SW	-0.00888	0.005551	0.140639	
121	Cl_mgL	Slope	Cla	2020	BW	0.014648	0.004888	0.040073	*
122	Cl_mgL	Slope	Cla	2020	SW	0.045966	0.006678	0.002335	**
123	Cl_mgL	Slope	Dra	2019	BW	-0.03367	0.011007	0.006197	**
124	Cl_mgL	Slope	Dra	2019	LFP	-0.06121	0.01721	0.00351	**
125	Cl_mgL	Slope	Dra	2019	SW	-0.03177	0.008878	0.002513	**
126	Cl_mgL	Slope	Gou	2018	BW	-0.00428	0.004915	0.475579	
127	Cl_mgL	Slope	Gou	2019	BW	-0.0242	0.0085	0.012243	*
128	Cl_mgL	Slope	Gou	2020	BW	0.016295	0.007843	0.076349	.
129	Cl_mgL	Slope	Gou	2018	LFP	0.002012	0.002438	0.433244	
130	Cl_mgL	Slope	Gou	2019	LFP	-0.05099	0.011806	0.000834	***
131	Cl_mgL	Slope	Gou	2020	LFP	0.023824	0.020722	0.333617	
132	Cl_mgL	Slope	Gou	2018	SW	-0.00135	0.001297	0.407293	
133	Cl_mgL	Slope	Gou	2019	SW	-0.05115	0.013117	0.001275	**
134	Cl_mgL	Slope	Gou	2020	SW	0.021334	0.007353	0.019841	*
135	Cl_mgL	Slope	Pet	2019	BW	-0.03498	0.00896	0.001263	**
136	Cl_mgL	Slope	Pet	2020	BW	0.015227	0.005237	0.015637	*
137	Cl_mgL	Slope	Pet	2019	LFP	-0.08437	0.021179	0.001814	**
138	Cl_mgL	Slope	Pet	2019	SW	-0.03703	0.011957	0.006925	**
139	Cl_mgL	Slope	Pet	2020	SW	0.012242	0.004299	0.017323	*
140	Cl_mgL	Slope	Sou	2018	BW	-0.00377	0.002398	0.257024	
141	Cl_mgL	Slope	Sou	2020	BW	0.021209	0.009437	0.048391	*
142	Cl_mgL	Slope	Sou	2018	LFP	0.012936	0.004847	0.028418	*
143	Cl_mgL	Slope	Sou	2020	LFP	0.017078	0.01012	0.166755	
144	Cl_mgL	Slope	Sou	2018	SW	0.000366	0.003547	0.9272	
145	Cl_mgL	Slope	Sou	2020	SW	0.032574	0.010578	0.011652	*

Model	DepVar	Coefficient	Site	Year	WS	Estimate	SE	P	PrCode
146	SO4_mgL	Slope	Cla	2020	BW	0.013281	0.002365	0.00494	**
147	SO4_mgL	Slope	Cla	2020	SW	0.037327	0.008769	0.013088	*
148	SO4_mgL	Slope	Dra	2019	BW	-0.00275	0.001137	0.025338	*
149	SO4_mgL	Slope	Dra	2019	LFP	-0.00326	0.00328	0.339089	
150	SO4_mgL	Slope	Dra	2019	SW	-0.00305	0.000777	0.001225	**
151	SO4_mgL	Slope	Gou	2018	BW	0.015038	0.006316	0.140237	
152	SO4_mgL	Slope	Gou	2019	BW	-0.00326	0.001285	0.022865	*
153	SO4_mgL	Slope	Gou	2020	BW	0.014957	0.008643	0.127143	
154	SO4_mgL	Slope	Gou	2018	LFP	-0.00672	0.011732	0.582646	
155	SO4_mgL	Slope	Gou	2019	LFP	-0.00047	0.00509	0.928331	
156	SO4_mgL	Slope	Gou	2020	LFP	0.019728	0.013421	0.237923	
157	SO4_mgL	Slope	Gou	2018	SW	0.044458	0.025042	0.217835	
158	SO4_mgL	Slope	Gou	2019	SW	-0.00238	0.001065	0.04038	*
159	SO4_mgL	Slope	Gou	2020	SW	0.014734	0.007473	0.084127	.
160	SO4_mgL	Slope	Pet	2019	BW	-0.00282	0.000865	0.00488	**
161	SO4_mgL	Slope	Pet	2020	BW	0.014142	0.005245	0.022455	*
162	SO4_mgL	Slope	Pet	2019	LFP	-0.00441	0.001717	0.024515	*
163	SO4_mgL	Slope	Pet	2019	SW	0.00713	0.006835	0.312412	
164	SO4_mgL	Slope	Pet	2020	SW	0.007744	0.00358	0.055785	.
165	SO4_mgL	Slope	Sou	2018	BW	-0.00099	0.006674	0.895383	
166	SO4_mgL	Slope	Sou	2020	BW	0.012177	0.004715	0.027292	*
167	SO4_mgL	Slope	Sou	2018	LFP	-0.00059	0.002521	0.820464	
168	SO4_mgL	Slope	Sou	2020	LFP	0.012467	0.011189	0.327594	
169	SO4_mgL	Slope	Sou	2018	SW	0.018371	0.007819	0.143244	
170	SO4_mgL	Slope	Sou	2020	SW	0.019326	0.006349	0.012384	*
1	TP_ugL	Intercept	Cla	2020	BW	4.53422	1.537028	0.04197	*
2	TP_ugL	Intercept	Cla	2020	SW	0.389944	1.295351	0.778389	
3	TP_ugL	Intercept	Dra	2019	BW	2.517341	0.419932	7.33E-06	****
4	TP_ugL	Intercept	Dra	2019	LFP	4.058401	0.741052	0.000106	***
5	TP_ugL	Intercept	Dra	2019	SW	1.909165	0.310149	1.38E-05	****
6	TP_ugL	Intercept	Gou	2018	BW	2.313186	1.292021	0.123591	

Model	DepVar	Coefficient	Site	Year	WS	Estimate	SE	P	PrCode
7	TP_ugL	Intercept	Gou	2018	BW	2.313186	1.292021	0.123591	
8	TP_ugL	Intercept	Gou	2019	BW	3.339989	0.571215	3.21E-05	****
9	TP_ugL	Intercept	Gou	2020	BW	0.717466	0.842161	0.422436	
10	TP_ugL	Intercept	Gou	2018	LFP	3.84968	0.876669	0.002314	**
11	TP_ugL	Intercept	Gou	2019	LFP	4.046834	0.86319	0.000424	***
12	TP_ugL	Intercept	Gou	2020	LFP	8.237454	1.94543	0.024107	*
13	TP_ugL	Intercept	Gou	2018	SW	4.688032	0.850301	0.000565	***
14	TP_ugL	Intercept	Gou	2019	SW	0.516657	0.499273	0.316142	
15	TP_ugL	Intercept	Gou	2020	SW	2.693211	1.116762	0.0424	*
16	TP_ugL	Intercept	Pet	2019	BW	2.587183	0.563709	0.000302	***
17	TP_ugL	Intercept	Pet	2020	BW	4.417913	1.174556	0.003714	**
18	TP_ugL	Intercept	Pet	2019	LFP	3.130554	0.537695	8.18E-05	****
19	TP_ugL	Intercept	Pet	2019	SW	1.835242	0.453008	0.000927	***
20	TP_ugL	Intercept	Pet	2020	SW	1.004478	1.149064	0.402531	
21	TP_ugL	Intercept	Sou	2018	BW	1.942298	1.022471	0.106227	
22	TP_ugL	Intercept	Sou	2020	BW	5.961302	1.463506	0.002238	**
23	TP_ugL	Intercept	Sou	2018	LFP	5.815339	0.870473	0.000156	***
24	TP_ugL	Intercept	Sou	2020	LFP	2.241362	1.957593	0.316078	
25	TP_ugL	Intercept	Sou	2018	SW	4.432611	1.028256	0.002578	**
26	TP_ugL	Intercept	Sou	2020	SW	2.436119	1.619197	0.163358	
27	PO4P_ugL	Intercept	Cla	2020	BW	1.988574	0.597689	0.029184	*
28	PO4P_ugL	Intercept	Cla	2020	SW	0.939939	1.021796	0.409686	
29	PO4P_ugL	Intercept	Dra	2019	BW	2.613801	0.313982	6.27E-08	****
30	PO4P_ugL	Intercept	Dra	2019	LFP	3.637819	0.368725	2.10E-07	****
31	PO4P_ugL	Intercept	Dra	2019	SW	0.923973	0.437246	0.05065	.
32	PO4P_ugL	Intercept	Gou	2018	BW	3.434319	0.465833	0.000722	***
33	PO4P_ugL	Intercept	Gou	2019	BW	2.400529	0.471162	0.000132	***
34	PO4P_ugL	Intercept	Gou	2020	BW	1.530306	0.844481	0.112863	
35	PO4P_ugL	Intercept	Gou	2018	LFP	3.538707	0.446838	9.72E-05	****
36	PO4P_ugL	Intercept	Gou	2019	LFP	1.565958	0.190701	1.68E-06	****
37	PO4P_ugL	Intercept	Gou	2020	LFP	2.509944	0.880825	0.065134	.

Model	DepVar	Coefficient	Site	Year	WS	Estimate	SE	P	PrCode
38	PO4P_ugL	Intercept	Gou	2018	SW	3.098153	0.375071	7.43E-05	****
39	PO4P_ugL	Intercept	Gou	2019	SW	0.527651	0.195751	0.015917	*
40	PO4P_ugL	Intercept	Gou	2020	SW	1.978182	0.69148	0.021126	*
41	PO4P_ugL	Intercept	Pet	2019	BW	1.524708	0.246499	1.31E-05	****
42	PO4P_ugL	Intercept	Pet	2020	BW	2.691362	0.459773	0.000161	***
43	PO4P_ugL	Intercept	Pet	2019	LFP	1.104456	0.185287	6.60E-05	****
44	PO4P_ugL	Intercept	Pet	2019	SW	1.564828	0.213197	1.66E-06	****
45	PO4P_ugL	Intercept	Pet	2020	SW	1.46427	0.737889	0.075319	.
46	PO4P_ugL	Intercept	Sou	2018	BW	3.199804	0.811829	0.016939	*
47	PO4P_ugL	Intercept	Sou	2020	BW	3.966103	0.616454	7.50E-05	****
48	PO4P_ugL	Intercept	Sou	2018	LFP	4.140212	0.550848	0.000135	***
49	PO4P_ugL	Intercept	Sou	2020	LFP	1.455056	1.223323	0.300048	
50	PO4P_ugL	Intercept	Sou	2018	SW	3.283766	0.24933	3.40E-06	****
51	PO4P_ugL	Intercept	Sou	2020	SW	1.753807	0.620614	0.017977	*
52	TN_ugL	Intercept	Cla	2020	BW	8.623624	0.46551	5.00E-05	****
53	TN_ugL	Intercept	Cla	2020	SW	8.368487	0.352597	1.87E-05	****
54	TN_ugL	Intercept	Dra	2019	BW	9.379267	0.231737	1.15E-20	****
55	TN_ugL	Intercept	Dra	2019	LFP	8.286663	0.597173	3.59E-09	****
56	TN_ugL	Intercept	Dra	2019	SW	9.305671	0.402877	1.03E-13	****
57	TN_ugL	Intercept	Gou	2019	BW	9.118466	0.215301	5.00E-17	****
58	TN_ugL	Intercept	Gou	2020	BW	8.380672	0.156346	2.06E-10	****
59	TN_ugL	Intercept	Gou	2019	LFP	7.074134	0.599133	2.54E-08	****
60	TN_ugL	Intercept	Gou	2020	LFP	8.084646	0.454655	0.000388	***
61	TN_ugL	Intercept	Gou	2019	SW	8.996349	0.225785	1.94E-17	****
62	TN_ugL	Intercept	Gou	2020	SW	8.357733	0.152541	1.37E-11	****
63	TN_ugL	Intercept	Pet	2019	BW	9.167999	0.268379	2.21E-16	****
64	TN_ugL	Intercept	Pet	2020	BW	8.560458	0.068396	2.60E-17	****
65	TN_ugL	Intercept	Pet	2019	LFP	7.143465	0.372066	2.25E-10	****
66	TN_ugL	Intercept	Pet	2019	SW	8.866685	0.356581	3.26E-14	****
67	TN_ugL	Intercept	Pet	2020	SW	8.455756	0.162393	1.65E-13	****
68	TN_ugL	Intercept	Sou	2020	BW	8.916244	0.432673	1.60E-09	****

Model	DepVar	Coefficient	Site	Year	WS	Estimate	SE	P	PrCode
69	TN_ugL	Intercept	Sou	2020	LFP	9.489876	1.826564	0.006536	**
70	TN_ugL	Intercept	Sou	2020	SW	8.373388	0.165725	2.23E-13	****
71	NO3N_ugL	Intercept	Cla	2020	BW	5.861317	0.465271	0.000229	***
72	NO3N_ugL	Intercept	Cla	2020	SW	1.584658	1.021826	0.195887	
73	NO3N_ugL	Intercept	Dra	2019	BW	8.046497	0.922259	2.98E-08	****
74	NO3N_ugL	Intercept	Dra	2019	LFP	8.469659	1.144517	5.19E-06	****
75	NO3N_ugL	Intercept	Dra	2019	SW	7.057936	1.353145	8.49E-05	****
76	NO3N_ugL	Intercept	Gou	2018	BW	8.925341	0.790492	2.89E-05	****
77	NO3N_ugL	Intercept	Gou	2019	BW	8.24763	0.195539	5.31E-17	****
78	NO3N_ugL	Intercept	Gou	2020	BW	5.522315	1.086431	0.001426	**
79	NO3N_ugL	Intercept	Gou	2018	LFP	6.519281	1.134585	0.000701	***
80	NO3N_ugL	Intercept	Gou	2019	LFP	4.504895	1.336978	0.005029	**
81	NO3N_ugL	Intercept	Gou	2020	LFP	1.807355	1.42E-16	1.08E-48	****
82	NO3N_ugL	Intercept	Gou	2018	SW	9.326357	0.737121	1.43E-06	****
83	NO3N_ugL	Intercept	Gou	2019	SW	8.093329	0.108471	8.95E-22	****
84	NO3N_ugL	Intercept	Gou	2020	SW	5.6233	0.804577	0.000114	***
85	NO3N_ugL	Intercept	Pet	2019	BW	6.831053	1.219828	3.99E-05	****
86	NO3N_ugL	Intercept	Pet	2020	BW	5.673888	0.636025	4.48E-06	****
87	NO3N_ugL	Intercept	Pet	2019	LFP	6.423695	0.141394	8.44E-15	****
88	NO3N_ugL	Intercept	Pet	2019	SW	8.166864	0.078448	4.38E-24	****
89	NO3N_ugL	Intercept	Pet	2020	SW	6.559267	0.38696	1.07E-08	****
90	NO3N_ugL	Intercept	Sou	2018	BW	9.145946	0.81085	2.90E-05	****
91	NO3N_ugL	Intercept	Sou	2020	BW	4.393754	1.493991	0.014761	*
92	NO3N_ugL	Intercept	Sou	2018	LFP	9.32559	2.204769	0.002877	**
93	NO3N_ugL	Intercept	Sou	2020	LFP	7.389628	2.975245	0.067941	.
94	NO3N_ugL	Intercept	Sou	2018	SW	9.583678	0.712726	8.97E-07	****
95	NO3N_ugL	Intercept	Sou	2020	SW	4.376503	1.105951	0.002699	**
96	NH4N_ugL	Intercept	Cla	2020	BW	6.601847	0.906725	0.001891	**
97	NH4N_ugL	Intercept	Cla	2020	SW	5.629062	0.896534	0.003285	**
98	NH4N_ugL	Intercept	Dra	2019	BW	6.243746	0.535568	2.26E-10	****
99	NH4N_ugL	Intercept	Dra	2019	LFP	4.786159	0.871349	0.000103	***

Model	DepVar	Coefficient	Site	Year	WS	Estimate	SE	P	PrCode
100	NH4N_ugL	Intercept	Dra	2019	SW	5.426748	0.237062	1.18E-13	****
101	NH4N_ugL	Intercept	Gou	2018	BW	2.241573	0.521782	0.007745	**
102	NH4N_ugL	Intercept	Gou	2019	BW	4.968227	0.920488	7.41E-05	****
103	NH4N_ugL	Intercept	Gou	2020	BW	4.138644	0.534264	0.000112	***
104	NH4N_ugL	Intercept	Gou	2018	LFP	6.116573	1.423622	0.002628	**
105	NH4N_ugL	Intercept	Gou	2019	LFP	14.83194	1.46053	1.50E-07	****
106	NH4N_ugL	Intercept	Gou	2020	LFP	8.383062	2.679058	0.052102	.
107	NH4N_ugL	Intercept	Gou	2018	SW	3.04006	0.791338	0.004935	**
108	NH4N_ugL	Intercept	Gou	2019	SW	4.553213	0.219389	5.40E-13	****
109	NH4N_ugL	Intercept	Gou	2020	SW	6.278986	1.306036	0.001342	**
110	NH4N_ugL	Intercept	Pet	2019	BW	5.409611	0.465901	3.31E-09	****
111	NH4N_ugL	Intercept	Pet	2020	BW	5.770479	0.505056	4.63E-07	****
112	NH4N_ugL	Intercept	Pet	2019	LFP	3.26372	1.354144	0.032902	*
113	NH4N_ugL	Intercept	Pet	2019	SW	1.716704	0.872865	0.066804	.
114	NH4N_ugL	Intercept	Pet	2020	SW	5.384974	0.393271	8.37E-08	****
115	NH4N_ugL	Intercept	Sou	2018	BW	2.437418	0.407851	0.001879	**
116	NH4N_ugL	Intercept	Sou	2020	BW	5.762211	0.899684	7.79E-05	****
117	NH4N_ugL	Intercept	Sou	2018	LFP	4.294664	1.580641	0.026369	*
118	NH4N_ugL	Intercept	Sou	2020	LFP	0.420918	1.957234	0.840242	
119	NH4N_ugL	Intercept	Sou	2018	SW	3.213005	0.974659	0.010915	*
120	NH4N_ugL	Intercept	Sou	2020	SW	5.081596	0.458138	6.10E-07	****
121	Cl_mgL	Intercept	Cla	2020	BW	1.871131	0.418769	0.01109	*
122	Cl_mgL	Intercept	Cla	2020	SW	-0.91174	0.572148	0.18626	
123	Cl_mgL	Intercept	Dra	2019	BW	6.735981	0.731447	1.24E-08	****
124	Cl_mgL	Intercept	Dra	2019	LFP	9.787636	1.209573	1.97E-06	****
125	Cl_mgL	Intercept	Dra	2019	SW	6.602522	0.707993	7.19E-08	****
126	Cl_mgL	Intercept	Gou	2018	BW	3.73022	0.192957	0.002665	**
127	Cl_mgL	Intercept	Gou	2019	BW	6.138714	0.655219	1.17E-07	****
128	Cl_mgL	Intercept	Gou	2020	BW	1.748077	0.646116	0.030395	*
129	Cl_mgL	Intercept	Gou	2018	LFP	7.340328	0.212484	5.39E-10	****
130	Cl_mgL	Intercept	Gou	2019	LFP	11.0257	0.829815	6.10E-09	****

Model	DepVar	Coefficient	Site	Year	WS	Estimate	SE	P	PrCode
131	Cl_mgL	Intercept	Gou	2020	LFP	4.642378	1.626956	0.064927	.
132	Cl_mgL	Intercept	Gou	2018	SW	3.471918	0.050895	0.000215	***
133	Cl_mgL	Intercept	Gou	2019	SW	8.478946	1.046048	4.68E-07	****
134	Cl_mgL	Intercept	Gou	2020	SW	1.620307	0.606171	0.02823	*
135	Cl_mgL	Intercept	Pet	2019	BW	6.935663	0.714504	4.15E-08	****
136	Cl_mgL	Intercept	Pet	2020	BW	1.883922	0.432277	0.001425	**
137	Cl_mgL	Intercept	Pet	2019	LFP	8.743994	1.493434	7.78E-05	****
138	Cl_mgL	Intercept	Pet	2019	SW	7.222655	0.9535	1.12E-06	****
139	Cl_mgL	Intercept	Pet	2020	SW	2.33165	0.354824	6.30E-05	****
140	Cl_mgL	Intercept	Sou	2018	BW	3.746071	0.094152	0.000631	***
141	Cl_mgL	Intercept	Sou	2020	BW	1.196897	0.778895	0.155391	
142	Cl_mgL	Intercept	Sou	2018	LFP	2.48487	0.422408	0.000369	***
143	Cl_mgL	Intercept	Sou	2020	LFP	3.066439	0.802265	0.018742	*
144	Cl_mgL	Intercept	Sou	2018	SW	3.506601	0.139247	0.001573	**
145	Cl_mgL	Intercept	Sou	2020	SW	0.52025	0.873085	0.564501	
146	SO4_mgL	Intercept	Cla	2020	BW	1.952735	0.202596	0.000648	***
147	SO4_mgL	Intercept	Cla	2020	SW	-0.21175	0.751251	0.79203	
148	SO4_mgL	Intercept	Dra	2019	BW	3.152064	0.075579	6.38E-21	****
149	SO4_mgL	Intercept	Dra	2019	LFP	3.771163	0.230527	4.71E-10	****
150	SO4_mgL	Intercept	Dra	2019	SW	3.178812	0.061992	3.53E-19	****
151	SO4_mgL	Intercept	Gou	2018	BW	2.814519	0.247951	0.007672	**
152	SO4_mgL	Intercept	Gou	2019	BW	3.433513	0.099041	9.78E-16	****
153	SO4_mgL	Intercept	Gou	2020	BW	1.842788	0.712024	0.036044	*
154	SO4_mgL	Intercept	Gou	2018	LFP	1.251404	1.022447	0.255799	
155	SO4_mgL	Intercept	Gou	2019	LFP	0.678404	0.357729	0.080347	.
156	SO4_mgL	Intercept	Gou	2020	LFP	0.943658	1.053698	0.436475	
157	SO4_mgL	Intercept	Gou	2018	SW	2.154701	0.983045	0.159722	
158	SO4_mgL	Intercept	Gou	2019	SW	3.377958	0.08493	1.99E-17	****
159	SO4_mgL	Intercept	Gou	2020	SW	2.131442	0.616103	0.008573	**
160	SO4_mgL	Intercept	Pet	2019	BW	3.425427	0.068982	5.88E-19	****
161	SO4_mgL	Intercept	Pet	2020	BW	1.9893	0.4329	0.000987	***

Model	DepVar	Coefficient	Site	Year	WS	Estimate	SE	P	PrCode
162	SO4_mgL	Intercept	Pet	2019	LFP	4.213279	0.121048	2.02E-13	****
163	SO4_mgL	Intercept	Pet	2019	SW	2.50153	0.545067	0.000302	***
164	SO4_mgL	Intercept	Pet	2020	SW	2.748615	0.295459	3.07E-06	****
165	SO4_mgL	Intercept	Sou	2018	BW	3.235525	0.261983	0.006493	**
166	SO4_mgL	Intercept	Sou	2020	BW	1.936806	0.389143	0.000556	***
167	SO4_mgL	Intercept	Sou	2018	LFP	3.632254	0.21972	1.81E-07	****
168	SO4_mgL	Intercept	Sou	2020	LFP	2.923626	0.887002	0.030044	*
169	SO4_mgL	Intercept	Sou	2018	SW	2.496908	0.306954	0.014779	*
170	SO4_mgL	Intercept	Sou	2020	SW	1.560224	0.524063	0.013875	*

Table 10. Analysis of variance of water chemistry concentrations measured from 2018-2020 to determine effects of site, water source (sample_type), year, as well as two and three-way interactions among those factors. Separate analyses were run for TP, PO4-P, TN, NO3-N, NH4-N, Cl, and SO4. The key result is the probability (P) that differences in means among sample groups could have occurred by chance. The F ratio, sum of squares (Sum Sq), and mean square error are used to determine P and also reported. If P is less than 0.05, then differences are usually considered reliable. Also reported are the degrees of freedom (DF). All water chemistry concentrations were transformed (recalculated) with a Log2 Plus 1 transformation for these analyses. See the title for Table 2 for an explanation of the Log2 Plus 1 transformation and P code.

Model	DepVar	Factors	Df	Sum Sq	Mean Sq	F	P	P Code
1	TP_ugL	Site	5	79.18296	15.83659	13.33038	9.65E-12	****
1	TP_ugL	Sample_Type	2	37.20544	18.60272	15.65875	3.40E-07	****
1	TP_ugL	Year	1	4.079784	4.079784	3.434139	0.064844	.
1	TP_ugL	Site:Sample_Type	8	30.63158	3.828948	3.222999	0.001566	**
1	TP_ugL	Site:Year	2	7.599914	3.799957	3.198596	0.042215	*
1	TP_ugL	Sample_Type:Year	2	5.844516	2.922258	2.459797	0.087174	.
1	TP_ugL	Site:Sample_Type:Year	4	10.63849	2.659622	2.238725	0.064877	.
1	TP_ugL	Residuals	300	356.4023	1.188008	NA	NA	
2	PO4P_ugL	Site	5	9.998972	1.999794	4.76952	0.00033	***
2	PO4P_ugL	Sample_Type	2	26.57566	13.28783	31.69155	3.41E-13	****
2	PO4P_ugL	Year	1	8.166127	8.166127	19.47626	1.43E-05	****
2	PO4P_ugL	Site:Sample_Type	8	9.74749	1.218436	2.905977	0.003914	**
2	PO4P_ugL	Site:Year	2	1.196248	0.598124	1.426529	0.241793	
2	PO4P_ugL	Sample_Type:Year	2	1.944533	0.972267	2.318861	0.100176	
2	PO4P_ugL	Site:Sample_Type:Year	4	0.988437	0.247109	0.589357	0.670593	
2	PO4P_ugL	Residuals	295	123.6894	0.419286	NA	NA	
3	TN_ugL	Site	4	8.142533	2.035633	9.273685	5.70E-07	****
3	TN_ugL	Sample_Type	2	14.25374	7.126869	32.46771	3.65E-13	****
3	TN_ugL	Year	1	1.276589	1.276589	5.815728	0.016658	*
3	TN_ugL	Site:Sample_Type	7	13.12487	1.874982	8.541811	2.60E-09	****
3	TN_ugL	Site:Year	2	1.436182	0.718091	3.27139	0.039704	*
3	TN_ugL	Sample_Type:Year	2	0.458571	0.229286	1.044551	0.353491	

Model	DepVar	Factors	Df	Sum Sq	Mean Sq	F	P	P Code
3	TN_ugL	Site:Sample_Type:Year	3	0.644661	0.214887	0.978956	0.403372	
3	TN_ugL	Residuals	233	51.14499	0.219506	NA	NA	
4	NO3N_ugL	Site	5	136.0203	27.20407	15.78545	8.32E-14	****
4	NO3N_ugL	Sample_Type	2	299.224	149.612	86.81398	1.71E-30	****
4	NO3N_ugL	Year	1	299.7474	299.7474	173.9317	1.17E-31	****
4	NO3N_ugL	Site:Sample_Type	8	383.664	47.958	27.82815	3.02E-32	****
4	NO3N_ugL	Site:Year	2	1.082364	0.541182	0.314027	0.730739	
4	NO3N_ugL	Sample_Type:Year	2	3.889818	1.944909	1.128555	0.324865	
4	NO3N_ugL	Site:Sample_Type:Year	4	4.533598	1.1334	0.657667	0.621885	
4	NO3N_ugL	Residuals	301	518.7323	1.723363	NA	NA	
5	NH4N_ugL	Site	5	136.0327	27.20654	16.62906	1.74E-14	****
5	NH4N_ugL	Sample_Type	2	86.84996	43.42498	26.54201	2.47E-11	****
5	NH4N_ugL	Year	1	0.383047	0.383047	0.234124	0.628838	
5	NH4N_ugL	Site:Sample_Type	8	403.2193	50.40242	30.80673	5.45E-35	****
5	NH4N_ugL	Site:Year	2	5.92048	2.96024	1.809344	0.165556	
5	NH4N_ugL	Sample_Type:Year	2	15.37714	7.68857	4.699372	0.009786	**
5	NH4N_ugL	Site:Sample_Type:Year	4	5.76704	1.44176	0.881226	0.475458	
5	NH4N_ugL	Residuals	298	487.5532	1.636085	NA	NA	
6	Cl_mgL	Site	5	167.6478	33.52956	20.43902	2.36E-17	****
6	Cl_mgL	Sample_Type	2	94.10654	47.05327	28.68284	4.81E-12	****
6	Cl_mgL	Year	1	3.983913	3.983913	2.428522	0.120291	
6	Cl_mgL	Site:Sample_Type	8	194.8543	24.35679	14.84747	4.63E-18	****
6	Cl_mgL	Site:Year	2	5.312765	2.656382	1.619283	0.199916	
6	Cl_mgL	Sample_Type:Year	2	10.89381	5.446905	3.320336	0.03759	*
6	Cl_mgL	Site:Sample_Type:Year	4	12.94534	3.236334	1.972811	0.098846	.
6	Cl_mgL	Residuals	276	452.7692	1.640468	NA	NA	
7	SO4_mgL	Site	5	51.76421	10.35284	35.78454	3.48E-28	****
7	SO4_mgL	Sample_Type	2	5.479706	2.739853	9.470285	0.000105	***
7	SO4_mgL	Year	1	0.200868	0.200868	0.694298	0.405427	
7	SO4_mgL	Site:Sample_Type	8	119.9392	14.9924	51.82115	1.09E-50	****
7	SO4_mgL	Site:Year	2	0.321832	0.160916	0.556205	0.574023	

Model	DepVar	Factors	Df	Sum Sq	Mean Sq	F	P	P Code
7	SO4_mgL	Sample_Type:Year	2	5.292644	2.646322	9.146996	0.000142	***
7	SO4_mgL	Site:Sample_Type:Year	4	2.329545	0.582386	2.013015	0.092851	.
7	SO4_mgL	Residuals	276	79.8497	0.289311	NA	NA	

Table 11. Analysis of variance of water chemistry concentrations measured from 2018-2020 to determine effects of site and water source (sample_type) as well as two-way interactions among those factors. Year in this analysis is treated as replicate samples. Separate analyses were run for TP, PO4-P, TN, NO3-N, NH4-N, Cl, and SO4. The key result is the probability (P) that differences in means among sample groups could have occurred by chance. The F ratio, sum of squares (Sum Sq), and mean square error are used to determine P and also reported. If P is less than 0.05, then differences are usually considered reliable. Also reported are the degrees of freedom (DF). All water chemistry concentrations were transformed (recalculated) with a Log2 Plus 1 transformation for these analyses. See the title for Table 2 for an explanation of the Log2 Plus 1 transformation and P code.

DepVar	Factors	Df	Sum Sq	Mean Sq	F	P	P Code
TP_ugL	Site	5	79.18296	15.83659	12.62467	3.61E-11	****
TP_ugL	Sample_Type	2	37.20544	18.60272	14.82978	7.08E-07	****
TP_ugL	Site:Sample_Type	8	27.58199	3.447749	2.748489	0.006054	**
TP_ugL	Residuals	309	387.6146	1.254416	NA	NA	
PO4P_ugL	Site	5	9.998972	1.999794	4.433857	0.000651	***
PO4P_ugL	Sample_Type	2	26.57566	13.28783	29.4612	2.02E-12	****
PO4P_ugL	Site:Sample_Type	8	8.619712	1.077464	2.388906	0.016454	*
PO4P_ugL	Residuals	304	137.1126	0.451028	NA	NA	
TN_ugL	Site	4	8.142533	2.035633	8.853955	1.10E-06	****
TN_ugL	Sample_Type	2	14.25374	7.126869	30.99821	1.05E-12	****
TN_ugL	Site:Sample_Type	7	12.677	1.811	7.876919	1.34E-08	****
TN_ugL	Residuals	241	55.40886	0.229912	NA	NA	
NO3N_ugL	Site	5	136.0203	27.20407	11.24174	5.65E-10	****
NO3N_ugL	Sample_Type	2	299.224	149.612	61.82531	2.54E-23	****
NO3N_ugL	Site:Sample_Type	8	461.4758	57.68447	23.8374	1.96E-28	****
NO3N_ugL	Residuals	310	750.1736	2.419915	NA	NA	
NH4N_ugL	Site	5	136.0327	27.20654	16.12299	4.07E-14	****
NH4N_ugL	Sample_Type	2	86.84996	43.42498	25.73427	4.65E-11	****
NH4N_ugL	Site:Sample_Type	8	400.1767	50.02209	29.64381	3.70E-34	****
NH4N_ugL	Residuals	307	518.0435	1.687438	NA	NA	
Cl_mgL	Site	5	167.6478	33.52956	19.4645	1.13E-16	****
Cl_mgL	Sample_Type	2	94.10654	47.05327	27.31525	1.40E-11	****
Cl_mgL	Site:Sample_Type	8	189.8181	23.72727	13.7741	6.78E-17	****

DepVar	Factors	Df	Sum Sq	Mean Sq	F	P	P Code
Cl_mgL	Residuals	285	490.9412	1.722601	NA	NA	
SO4_mgL	Site	5	51.76421	10.35284	33.35588	9.11E-27	****
SO4_mgL	Sample_Type	2	5.479706	2.739853	8.827548	0.000191	***
SO4_mgL	Site:Sample_Type	8	119.4769	14.93461	48.1179	1.23E-48	****
SO4_mgL	Residuals	285	88.45696	0.310375	NA	NA	

Table 12. Summary statistics for each water chemistry concentration for water source and site. Means, medians, standard deviations (SD), standard error (SE), and number of observations are the summary statistics listed. Surface water refers to nearshore surface water.

Var	Site	Waterbody	Water Source	Mean	Median	SD	SE	Number
TP_ugL	Drake	Bellaire	Benthic Water	7.231	6.104	4.325	0.922	22
TP_ugL	Southworth	Bellaire	Benthic Water	15.234	6.365	22.928	4.781	23
TP_ugL	Clam DNR	Clam	Benthic Water	7.589	3.977	8.235	3.362	6
TP_ugL	Gourley	Torch	Benthic Water	6.592	4.935	7.888	1.353	34
TP_ugL	Petty	Torch	Benthic Water	4.938	3.057	5.622	1.026	30
TP_ugL	Drake	Bellaire	Lake Floor Piezo	21.808	21.658	11.146	2.878	15
TP_ugL	Southworth	Bellaire	Lake Floor Piezo	19.105	9.807	31.527	7.233	19
TP_ugL	Gourley	Torch	Lake Floor Piezo	11.202	5.630	14.894	2.719	30
TP_ugL	Hayo-Went-Ha	Torch	Lake Floor Piezo	5.854	6.044	2.953	1.476	4
TP_ugL	Petty	Torch	Lake Floor Piezo	2.596	2.113	1.904	0.437	19
TP_ugL	Drake	Bellaire	Surface Water	4.285	4.139	1.560	0.368	18
TP_ugL	Southworth	Bellaire	Surface Water	11.071	5.063	16.473	3.295	25
TP_ugL	Clam DNR	Clam	Surface Water	3.986	2.999	3.006	1.227	6
TP_ugL	Gourley	Torch	Surface Water	5.167	2.530	9.272	1.504	38
TP_ugL	Hayo-Went-Ha	Torch	Surface Water	2.187	2.838	1.291	0.745	3
TP_ugL	Petty	Torch	Surface Water	3.176	2.218	3.220	0.560	33
TP_ugL	Clam River @ Dockside	Watershed	Surface Water	7.931	7.931	0.194	0.137	2
TP_ugL	Eastport Creek	Watershed	Surface Water	10.930	10.930	1.648	1.165	2
TP_ugL	Grass Cr @ Bellaire Hwy	Watershed	Surface Water	18.264	18.264	0.388	0.274	2
TP_ugL	Grass Cr @ Honey Hollow	Watershed	Surface Water	12.361	12.361	3.211	2.271	2
TP_ugL	McLachlan Creek	Watershed	Surface Water	9.131	9.131	1.066	0.754	2
TP_ugL	Spencer Cr @ Alden Harbor	Watershed	Surface Water	29.429	29.429	19.230	13.597	2
TP_ugL	Wilkinson Creek	Watershed	Surface Water	11.718	11.718	1.139	0.805	2
PO4P_ugL	Drake	Bellaire	Benthic Water	3.675	3.471	1.738	0.371	22
PO4P_ugL	Southworth	Bellaire	Benthic Water	3.470	2.723	3.367	0.735	21
PO4P_ugL	Clam DNR	Clam	Benthic Water	2.646	2.365	0.939	0.383	6
PO4P_ugL	Gourley	Torch	Benthic Water	3.667	3.234	2.666	0.464	33
PO4P_ugL	Petty	Torch	Benthic Water	2.482	2.608	0.956	0.174	30

Var	Site	Waterbody	Water Source	Mean	Median	SD	SE	Number
PO4P_ugL	Drake	Bellaire	Lake Floor Piezo	8.281	8.635	2.384	0.616	15
PO4P_ugL	Southworth	Bellaire	Lake Floor Piezo	5.058	5.763	2.760	0.651	18
PO4P_ugL	Gourley	Torch	Lake Floor Piezo	3.725	3.347	1.708	0.317	29
PO4P_ugL	Hayo-Went-Ha	Torch	Lake Floor Piezo	3.713	3.660	3.481	1.740	4
PO4P_ugL	Petty	Torch	Lake Floor Piezo	3.357	3.283	1.571	0.360	19
PO4P_ugL	Drake	Bellaire	Surface Water	2.504	2.735	1.122	0.264	18
PO4P_ugL	Southworth	Bellaire	Surface Water	2.275	2.112	1.591	0.325	24
PO4P_ugL	Clam DNR	Clam	Surface Water	2.409	2.711	1.325	0.541	6
PO4P_ugL	Gourley	Torch	Surface Water	2.152	2.435	1.319	0.217	37
PO4P_ugL	Hayo-Went-Ha	Torch	Surface Water	1.970	1.851	1.480	0.740	4
PO4P_ugL	Petty	Torch	Surface Water	2.400	2.347	1.261	0.216	34
PO4P_ugL	Clam River @ Dockside	Watershed	Surface Water	3.422	3.422	0.276	0.195	2
PO4P_ugL	Eastport Creek	Watershed	Surface Water	3.285	3.285	0.329	0.232	2
PO4P_ugL	Grass Cr @ Bellaire Hwy	Watershed	Surface Water	8.817	8.817	1.049	0.741	2
PO4P_ugL	Grass Cr @ Honey Hollow	Watershed	Surface Water	3.183	3.183	0.673	0.476	2
PO4P_ugL	McLachlan Creek	Watershed	Surface Water	4.445	4.445	0.113	0.080	2
PO4P_ugL	Spencer Cr @ Alden Harbor	Watershed	Surface Water	3.929	3.929	0.109	0.077	2
PO4P_ugL	Wilkinson Creek	Watershed	Surface Water	6.455	6.455	0.610	0.431	2
TN_ugL	Drake	Bellaire	Benthic Water	460.465	471.416	127.613	27.207	22
TN_ugL	Southworth	Bellaire	Benthic Water	565.993	522.702	243.542	62.882	15
TN_ugL	Clam DNR	Clam	Benthic Water	482.043	464.764	106.595	43.517	6
TN_ugL	Gourley	Torch	Benthic Water	462.290	445.507	93.387	18.315	26
TN_ugL	Petty	Torch	Benthic Water	465.008	448.581	100.843	18.411	30
TN_ugL	Drake	Bellaire	Lake Floor Piezo	174.052	159.109	76.565	19.769	15
TN_ugL	Southworth	Bellaire	Lake Floor Piezo	514.594	342.598	485.109	161.703	9
TN_ugL	Gourley	Torch	Lake Floor Piezo	456.134	407.655	223.997	50.087	20
TN_ugL	Petty	Torch	Lake Floor Piezo	340.439	296.549	129.896	33.539	15
TN_ugL	Drake	Bellaire	Surface Water	402.196	462.909	145.798	34.365	18
TN_ugL	Southworth	Bellaire	Surface Water	417.877	401.595	86.422	22.314	15
TN_ugL	Clam DNR	Clam	Surface Water	398.134	415.922	60.710	24.785	6
TN_ugL	Gourley	Torch	Surface Water	383.694	397.954	73.912	13.968	28

Var	Site	Waterbody	Water Source	Mean	Median	SD	SE	Number
TN_ugL	Petty	Torch	Surface Water	473.320	446.787	161.067	29.407	30
TN_ugL	Clam River @ Dockside	Watershed	Surface Water	473.952	473.952	17.891	12.650	2
TN_ugL	Eastport Creek	Watershed	Surface Water	209.378	209.378	12.181	8.613	2
TN_ugL	Grass Cr @ Bellaire Hwy	Watershed	Surface Water	317.091	317.091	23.781	16.816	2
TN_ugL	Grass Cr @ Honey Hollow	Watershed	Surface Water	977.637	977.637	0.387	0.274	2
TN_ugL	McLachlan Creek	Watershed	Surface Water	650.854	650.854	25.293	17.885	2
TN_ugL	Spencer Cr @ Alden Harbor	Watershed	Surface Water	558.485	558.485	72.747	51.440	2
TN_ugL	Wilkinson Creek	Watershed	Surface Water	378.282	378.282	18.532	13.104	2
NO3N_ugL	Drake	Bellaire	Benthic Water	212.032	198.792	79.830	17.020	22
NO3N_ugL	Southworth	Bellaire	Benthic Water	283.634	103.010	355.461	74.119	23
NO3N_ugL	Clam DNR	Clam	Benthic Water	55.157	54.442	11.073	4.520	6
NO3N_ugL	Gourley	Torch	Benthic Water	304.925	208.167	325.474	55.818	34
NO3N_ugL	Petty	Torch	Benthic Water	171.014	197.550	77.590	14.166	30
NO3N_ugL	Drake	Bellaire	Lake Floor Piezo	18.819	5.422	35.896	9.268	15
NO3N_ugL	Southworth	Bellaire	Lake Floor Piezo	1180.034	277.179	2834.055	650.177	19
NO3N_ugL	Gourley	Torch	Lake Floor Piezo	15.290	6.325	23.835	4.426	29
NO3N_ugL	Hayo-Went-Ha	Torch	Lake Floor Piezo	40.197	26.217	49.667	24.833	4
NO3N_ugL	Petty	Torch	Lake Floor Piezo	425.421	299.543	458.335	105.149	19
NO3N_ugL	Drake	Bellaire	Surface Water	191.538	183.205	69.966	16.491	18
NO3N_ugL	Southworth	Bellaire	Surface Water	260.715	144.102	287.901	57.580	25
NO3N_ugL	Clam DNR	Clam	Surface Water	55.308	66.528	34.841	14.224	6
NO3N_ugL	Gourley	Torch	Surface Water	281.129	199.018	267.413	43.380	38
NO3N_ugL	Hayo-Went-Ha	Torch	Surface Water	711.821	705.081	567.548	283.774	4
NO3N_ugL	Petty	Torch	Surface Water	251.339	216.751	251.598	43.149	34
NO3N_ugL	Clam River @ Dockside	Watershed	Surface Water	291.975	291.975	0.799	0.565	2
NO3N_ugL	Eastport Creek	Watershed	Surface Water	44.955	44.955	1.280	0.905	2
NO3N_ugL	Grass Cr @ Bellaire Hwy	Watershed	Surface Water	147.285	147.285	8.309	5.875	2
NO3N_ugL	Grass Cr @ Honey Hollow	Watershed	Surface Water	771.110	771.110	3.041	2.150	2
NO3N_ugL	McLachlan Creek	Watershed	Surface Water	484.440	484.440	15.811	11.180	2
NO3N_ugL	Spencer Cr @ Alden Harbor	Watershed	Surface Water	353.420	353.420	7.184	5.080	2
NO3N_ugL	Wilkinson Creek	Watershed	Surface Water	192.015	192.015	6.074	4.295	2

Var	Site	Waterbody	Water Source	Mean	Median	SD	SE	Number
NH4N_ugL	Drake	Bellaire	Benthic Water	48.252	37.112	57.682	12.298	22
NH4N_ugL	Southworth	Bellaire	Benthic Water	42.165	30.640	30.308	6.462	22
NH4N_ugL	Clam DNR	Clam	Benthic Water	36.949	29.724	19.375	7.910	6
NH4N_ugL	Gourley	Torch	Benthic Water	31.546	30.583	17.131	2.982	33
NH4N_ugL	Petty	Torch	Benthic Water	25.942	21.472	19.158	3.498	30
NH4N_ugL	Drake	Bellaire	Lake Floor Piezo	58.135	55.978	26.769	6.912	15
NH4N_ugL	Southworth	Bellaire	Lake Floor Piezo	54.775	9.986	101.458	23.276	19
NH4N_ugL	Gourley	Torch	Lake Floor Piezo	1654.260	428.842	3366.080	614.559	30
NH4N_ugL	Hayo-Went-Ha	Torch	Lake Floor Piezo	114.012	46.251	158.564	79.282	4
NH4N_ugL	Petty	Torch	Lake Floor Piezo	9.149	5.013	10.802	2.620	17
NH4N_ugL	Drake	Bellaire	Surface Water	20.646	19.106	7.550	1.780	18
NH4N_ugL	Southworth	Bellaire	Surface Water	28.678	20.145	27.410	5.482	25
NH4N_ugL	Clam DNR	Clam	Surface Water	21.425	17.154	11.981	4.891	6
NH4N_ugL	Gourley	Torch	Surface Water	25.156	20.461	15.782	2.560	38
NH4N_ugL	Hayo-Went-Ha	Torch	Surface Water	53.631	39.840	48.186	24.093	4
NH4N_ugL	Petty	Torch	Surface Water	51.118	18.262	85.146	14.602	34
NH4N_ugL	Clam River @ Dockside	Watershed	Surface Water	9.315	9.315	5.302	3.749	2
NH4N_ugL	Eastport Creek	Watershed	Surface Water	0.814	0.814	0.027	0.019	2
NH4N_ugL	Grass Cr @ Bellaire Hwy	Watershed	Surface Water	8.986	8.986	11.631	8.224	2
NH4N_ugL	Grass Cr @ Honey Hollow	Watershed	Surface Water	15.038	15.038	8.329	5.889	2
NH4N_ugL	McLachlan Creek	Watershed	Surface Water	7.296	7.296	2.735	1.934	2
NH4N_ugL	Spencer Cr @ Alden Harbor	Watershed	Surface Water	0.932	0.932	NA	NA	1
NH4N_ugL	Wilkinson Creek	Watershed	Surface Water	5.108	5.108	5.294	3.743	2
Cl_mgL	Drake	Bellaire	Benthic Water	41.476	14.189	49.334	10.518	22
Cl_mgL	Southworth	Bellaire	Benthic Water	9.010	9.640	3.438	0.789	19
Cl_mgL	Clam DNR	Clam	Benthic Water	7.781	7.800	2.587	1.056	6
Cl_mgL	Gourley	Torch	Benthic Water	18.727	11.481	20.880	3.812	30
Cl_mgL	Petty	Torch	Benthic Water	24.301	10.476	52.016	9.497	30
Cl_mgL	Drake	Bellaire	Lake Floor Piezo	142.748	26.209	314.084	81.096	15
Cl_mgL	Southworth	Bellaire	Lake Floor Piezo	19.551	17.710	13.849	3.177	19
Cl_mgL	Gourley	Torch	Lake Floor Piezo	240.617	138.055	319.630	58.356	30

Var	Site	Waterbody	Water Source	Mean	Median	SD	SE	Number
Cl_mgL	Hayo-Went-Ha	Torch	Lake Floor Piezo	7.840	7.845	0.106	0.053	4
Cl_mgL	Petty	Torch	Lake Floor Piezo	27.949	2.652	67.895	15.576	19
Cl_mgL	Drake	Bellaire	Surface Water	29.045	11.676	39.952	9.417	18
Cl_mgL	Southworth	Bellaire	Surface Water	10.186	9.921	4.790	1.099	19
Cl_mgL	Clam DNR	Clam	Surface Water	8.425	8.780	6.014	2.455	6
Cl_mgL	Gourley	Torch	Surface Water	53.566	10.700	151.122	26.715	32
Cl_mgL	Hayo-Went-Ha	Torch	Surface Water	9.192	9.192	NA	NA	1
Cl_mgL	Petty	Torch	Surface Water	30.967	10.636	63.689	11.439	31
Cl_mgL	Clam River @ Dockside	Watershed	Surface Water	71.211	71.211	0.221	0.156	2
Cl_mgL	Eastport Creek	Watershed	Surface Water	105.467	105.467	0.016	0.012	2
Cl_mgL	Grass Cr @ Bellaire Hwy	Watershed	Surface Water	28.723	28.723	40.479	28.623	2
Cl_mgL	Grass Cr @ Honey Hollow	Watershed	Surface Water	152.999	152.999	1.396	0.987	2
Cl_mgL	McLachlan Creek	Watershed	Surface Water	53.761	53.761	3.610	2.553	2
Cl_mgL	Spencer Cr @ Alden Harbor	Watershed	Surface Water	34.394	34.394	0.252	0.178	2
Cl_mgL	Wilkinson Creek	Watershed	Surface Water	28.847	28.847	40.654	28.747	2
SO4_mgL	Drake	Bellaire	Benthic Water	6.930	6.921	0.795	0.170	22
SO4_mgL	Southworth	Bellaire	Benthic Water	7.093	7.322	1.927	0.442	19
SO4_mgL	Clam DNR	Clam	Benthic Water	7.481	7.467	2.093	0.854	6
SO4_mgL	Gourley	Torch	Benthic Water	8.271	8.261	2.056	0.375	30
SO4_mgL	Petty	Torch	Benthic Water	8.208	8.132	1.670	0.305	30
SO4_mgL	Drake	Bellaire	Lake Floor Piezo	10.881	10.702	2.045	0.528	15
SO4_mgL	Southworth	Bellaire	Lake Floor Piezo	13.863	12.424	4.908	1.126	19
SO4_mgL	Gourley	Torch	Lake Floor Piezo	2.026	0.521	4.678	0.854	30
SO4_mgL	Hayo-Went-Ha	Torch	Lake Floor Piezo	31.108	31.285	0.730	0.365	4
SO4_mgL	Petty	Torch	Lake Floor Piezo	13.703	13.349	1.745	0.400	19
SO4_mgL	Drake	Bellaire	Surface Water	6.741	6.503	0.627	0.148	18
SO4_mgL	Southworth	Bellaire	Surface Water	8.029	7.202	3.296	0.756	19
SO4_mgL	Clam DNR	Clam	Surface Water	7.733	9.181	4.654	1.900	6
SO4_mgL	Gourley	Torch	Surface Water	9.627	8.370	5.735	1.014	32
SO4_mgL	Hayo-Went-Ha	Torch	Surface Water	8.877	8.877	NA	NA	1
SO4_mgL	Petty	Torch	Surface Water	8.485	8.278	2.217	0.398	31

Var	Site	Waterbody	Water Source	Mean	Median	SD	SE	Number
SO4_mgL	Clam River @ Dockside	Watershed	Surface Water	6.962	6.962	0.168	0.119	2
SO4_mgL	Eastport Creek	Watershed	Surface Water	4.449	4.449	0.018	0.013	2
SO4_mgL	Grass Cr @ Bellaire Hwy	Watershed	Surface Water	10.067	10.067	0.035	0.025	2
SO4_mgL	Grass Cr @ Honey Hollow	Watershed	Surface Water	5.284	5.284	0.141	0.100	2
SO4_mgL	McLachlan Creek	Watershed	Surface Water	6.054	6.054	0.323	0.228	2
SO4_mgL	Spencer Cr @ Alden Harbor	Watershed	Surface Water	8.111	8.111	0.089	0.063	2
SO4_mgL	Wilkinson Creek	Watershed	Surface Water	5.455	5.455	0.046	0.033	2

Table 13. Analysis of variance of water chemistry concentrations measured from 2018-2020 to determine effects of water source (sample_type) for each site sampled. Samples taken during different years were treated as replicates for each site and water source. Separate analyses were run for each water chemistry parameter and site and designated as successive model numbers for clarity. Analyses were run for TP, PO4-P, TN, NO3-N, NH4-N, Cl, and SO4 for the Gourley, Petty, Southworth, Drake, and Clam DNR sites. The key result is the probability (P) that differences in means among sample groups could have occurred by chance. The F ratio, sum of squares (Sum Sq), and mean square error are used to determine P and also reported here. If P is less than 0.05, then differences are usually considered reliable. Also reported are the degrees of freedom (DF). All water chemistry concentrations were transformed (recalculated) with a Log2 Plus 1 transformation for these analyses. See the title for Table 2 for an explanation of the Log2 Plus 1 transformation and P code.

Model	DepVar	Site	Factors	Df	Sum Sq	Mean Sq	F	P	P Code
1	TP_ugL	Gourley	Sample_Type	2	23.88862	11.94431	10.55669	9.69E-05	****
1	TP_ugL	Gourley	Residuals	71	80.33259	1.131445			
2	PO4P_ugL	Gourley	Sample_Type	2	4.735662	2.367831	5.258749	0.007418	**
2	PO4P_ugL	Gourley	Residuals	71	31.96882	0.450265			
3	TN_ugL	Gourley	Sample_Type	2	0.977115	0.488558	2.288915	0.10881	
3	TN_ugL	Gourley	Residuals	71	15.1546	0.213445			
4	NO3N_ugL	Gourley	Sample_Type	2	343.16	171.58	162.2467	3.32E-27	****
4	NO3N_ugL	Gourley	Residuals	71	75.08429	1.057525			
5	NH4N_ugL	Gourley	Sample_Type	2	308.4291	154.2145	73.98955	4.32E-18	****
5	NH4N_ugL	Gourley	Residuals	71	147.9835	2.084274			
6	Cl_mgL	Gourley	Sample_Type	2	156.2789	78.13944	37.70103	6.96E-12	****
6	Cl_mgL	Gourley	Residuals	71	147.1551	2.072608			
7	SO4_mgL	Gourley	Sample_Type	2	64.16206	32.08103	82.40168	3.12E-19	****
7	SO4_mgL	Gourley	Residuals	71	27.64207	0.389325			
8	TP_ugL	Petty	Sample_Type	2	3.789466	1.894733	2.271047	0.110652	
8	TP_ugL	Petty	Residuals	71	59.23525	0.834299			
9	PO4P_ugL	Petty	Sample_Type	2	1.992138	0.996069	4.184009	0.019154	*
9	PO4P_ugL	Petty	Residuals	71	16.90266	0.238066			
10	TN_ugL	Petty	Sample_Type	2	2.475867	1.237933	8.530657	0.000478	***
10	TN_ugL	Petty	Residuals	71	10.30322	0.145116			
11	NO3N_ugL	Petty	Sample_Type	2	6.77113	3.385565	3.549108	0.033956	*

Model	DepVar	Site	Factors	Df	Sum Sq	Mean Sq	F	P	P Code
11	NO3N_ugL	Petty	Residuals	71	67.72832	0.95392			
12	NH4N_ugL	Petty	Sample_Type	2	45.31194	22.65597	17.55328	6.39E-07	****
12	NH4N_ugL	Petty	Residuals	71	91.63951	1.290697			
13	Cl_mgL	Petty	Sample_Type	2	8.647131	4.323566	1.85641	0.163736	
13	Cl_mgL	Petty	Residuals	71	165.3585	2.328992			
14	SO4_mgL	Petty	Sample_Type	2	6.321756	3.160878	14.75548	4.38E-06	****
14	SO4_mgL	Petty	Residuals	71	15.20942	0.214217			
15	TP_ugL	Southworth	Sample_Type	2	4.628973	2.314486	1.156399	0.326028	
15	TP_ugL	Southworth	Residuals	36	72.05255	2.00146			
16	PO4P_ugL	Southworth	Sample_Type	2	4.179306	2.089653	3.285295	0.048918	*
16	PO4P_ugL	Southworth	Residuals	36	22.89825	0.636063			
17	TN_ugL	Southworth	Sample_Type	2	1.474783	0.737391	2.093125	0.138054	
17	TN_ugL	Southworth	Residuals	36	12.68252	0.352292			
18	NO3N_ugL	Southworth	Sample_Type	2	5.262226	2.631113	1.195004	0.314425	
18	NO3N_ugL	Southworth	Residuals	36	79.26339	2.201761			
19	NH4N_ugL	Southworth	Sample_Type	2	21.78022	10.89011	12.08569	9.65E-05	****
19	NH4N_ugL	Southworth	Residuals	36	32.43868	0.901075			
20	Cl_mgL	Southworth	Sample_Type	2	17.1021	8.551048	9.203213	0.000591	***
20	Cl_mgL	Southworth	Residuals	36	33.44894	0.929137			
21	SO4_mgL	Southworth	Sample_Type	2	8.239689	4.119844	13.01539	5.58E-05	****
21	SO4_mgL	Southworth	Residuals	36	11.39531	0.316536			
22	TP_ugL	Clam DNR	Sample_Type	1	1.13818	1.13818	1.083343	0.322461	
22	TP_ugL	Clam DNR	Residuals	10	10.50619	1.050619			
23	TP_ugL	Clam DNR	Sample_Type	1	1.13818	1.13818	1.083343	0.322461	
23	TP_ugL	Clam DNR	Residuals	10	10.50619	1.050619			
24	PO4P_ugL	Clam DNR	Sample_Type	1	0.087013	0.087013	0.306569	0.591957	
24	PO4P_ugL	Clam DNR	Residuals	10	2.83829	0.283829			
25	TN_ugL	Clam DNR	Sample_Type	1	0.208561	0.208561	3.007189	0.113557	
25	TN_ugL	Clam DNR	Residuals	10	0.693542	0.069354			
26	NO3N_ugL	Clam DNR	Sample_Type	1	0.415456	0.415456	0.432654	0.525541	
26	NO3N_ugL	Clam DNR	Residuals	10	9.602512	0.960251			

Model	DepVar	Site	Factors	Df	Sum Sq	Mean Sq	F	P	P Code
27	NH4N_ugL	Clam DNR	Sample_Type	1	1.673593	1.673593	3.391553	0.095346	.
27	NH4N_ugL	Clam DNR	Residuals	10	4.934591	0.493459			
28	Cl_mgL	Clam DNR	Sample_Type	1	0.125252	0.125252	0.143068	0.713155	
28	Cl_mgL	Clam DNR	Residuals	10	8.754711	0.875471			
29	SO4_mgL	Clam DNR	Sample_Type	1	0.102379	0.102379	0.160143	0.69744	
29	SO4_mgL	Clam DNR	Residuals	10	6.392945	0.639294			
30	TP_ugL	Drake	Sample_Type	2	34.29779	17.14889	42.2278	1.28E-11	****
30	TP_ugL	Drake	Residuals	52	21.11743	0.406104			
31	PO4P_ugL	Drake	Sample_Type	2	18.19838	9.099191	31.62772	1.03E-09	****
31	PO4P_ugL	Drake	Residuals	52	14.96023	0.287697			
32	TN_ugL	Drake	Sample_Type	2	21.08638	10.54319	34.07439	3.49E-10	****
32	TN_ugL	Drake	Residuals	52	16.08967	0.309417			
33	NO3N_ugL	Drake	Sample_Type	2	216.7003	108.3502	36.03742	1.51E-10	****
33	NO3N_ugL	Drake	Residuals	52	156.3433	3.006602			
34	NH4N_ugL	Drake	Sample_Type	2	15.11769	7.558847	11.39824	7.86E-05	****
34	NH4N_ugL	Drake	Residuals	52	34.48428	0.663159			
35	Cl_mgL	Drake	Sample_Type	2	17.8561	8.928052	4.212469	0.020159	*
35	Cl_mgL	Drake	Residuals	52	110.2106	2.119434			
36	SO4_mgL	Drake	Sample_Type	2	3.74228	1.87114	67.21762	3.82E-15	****
36	SO4_mgL	Drake	Residuals	52	1.447526	0.027837			

Table 14. Analysis of variance of water chemistry concentrations measured from 2018-2020 to determine effects of site sampled for each type of water source. Samples taken during different years were treated as replicates for each site and water source. Separate analyses were run for each water chemistry parameter and water source and designated as successive model numbers for clarity. Analyses were run for TP, PO4-P, TN, NO3-N, NH4-N, Cl, and SO4 for the nearshore surface water (SW), benthic pore water (BPW), and lake floor piezometer (LFP, also called lake floor groundwater). The key result is the probability (P) that differences in means among sample groups could have occurred by chance. The F ratio, sum of squares (Sum Sq), and mean square error are used to determine P and also reported. If P is less than 0.05, then differences are usually considered reliable. Also reported are the degrees of freedom (DF). All water chemistry concentrations were transformed (recalculated) with a Log2 Plus 1 transformation for these analyses. See the title for Table 2 for an explanation of the Log2 Plus 1 transformation and P code.

Model	DepVar	Water Source	Factors	Df	Sum Sq	Mean Sq	F	P	P Code
1	TP_ugL	SW	Site	12	58.88168	4.906807	4.131226	1.99E-05	****
1	TP_ugL	SW	Residuals	124	147.2793	1.187736			
2	TP_ugL	BPW	Site	4	15.8775	3.969376	3.501275	0.009896	**
2	TP_ugL	BPW	Residuals	110	124.7064	1.133694			
3	TP_ugL	LFG	Site	4	66.42904	16.60726	11.65967	1.52E-07	****
3	TP_ugL	LFG	Residuals	82	116.7954	1.424334			
4	PO4P_ugL	SW	Site	12	12.92961	1.077467	2.677228	0.003111	**
4	PO4P_ugL	SW	Residuals	124	49.90459	0.402456			
5	PO4P_ugL	BPW	Site	4	2.427693	0.606923	1.256663	0.291668	.
5	PO4P_ugL	BPW	Residuals	107	51.67715	0.482964			
6	PO4P_ugL	LFG	Site	4	14.19231	3.548076	7.963067	1.87E-05	****
6	PO4P_ugL	LFG	Residuals	80	35.64533	0.445567			
7	TN_ugL	SW	Site	11	8.102392	0.736581	5.295949	1.55E-06	****
7	TN_ugL	SW	Residuals	99	13.7693	0.139084			
8	TN_ugL	BPW	Site	4	0.771878	0.19297	1.499159	0.208732	.
8	TN_ugL	BPW	Residuals	94	12.09954	0.128718			
9	TN_ugL	LFG	Site	3	17.92125	5.97375	11.09796	8.42E-06	****
9	TN_ugL	LFG	Residuals	55	29.60512	0.538275			
10	NO3N_ugL	SW	Site	12	64.69326	5.391105	3.471862	0.000194	***
10	NO3N_ugL	SW	Residuals	126	195.6527	1.552799			
11	NO3N_ugL	BPW	Site	4	21.67081	5.417704	2.366049	0.057208	.

Model	DepVar	Water Source	Factors	Df	Sum Sq	Mean Sq	F	P	P Code
11	NO3N_ugL	BPW	Residuals	110	251.8745	2.289768			
12	NO3N_ugL	LFG	Site	4	541.2916	135.3229	36.21609	2.48E-17	****
12	NO3N_ugL	LFG	Residuals	81	302.6598	3.736541			
13	NH4N_ugL	SW	Site	12	68.77704	5.73142	4.854115	1.61E-06	****
13	NH4N_ugL	SW	Residuals	125	147.5918	1.180734			
14	NH4N_ugL	BPW	Site	4	8.05299	2.013247	2.243187	0.069097	.
14	NH4N_ugL	BPW	Residuals	108	96.92939	0.897494			
15	NH4N_ugL	LFG	Site	4	506.8839	126.721	35.85056	3.86E-17	****
15	NH4N_ugL	LFG	Residuals	80	282.776	3.5347			
16	Cl_mgL	SW	Site	12	77.64141	6.470118	3.20851	0.000583	***
16	Cl_mgL	SW	Residuals	108	217.7873	2.016549			
17	Cl_mgL	BPW	Site	4	26.13585	6.533962	5.092218	0.00088	***
17	Cl_mgL	BPW	Residuals	102	130.879	1.283127			
18	Cl_mgL	LFG	Site	4	307.4702	76.86756	35.98885	2.43E-17	****
18	Cl_mgL	LFG	Residuals	82	175.1415	2.135872			
19	SO4_mgL	SW	Site	12	4.207122	0.350593	1.166787	0.316138	.
19	SO4_mgL	SW	Residuals	108	32.4516	0.300478			
20	SO4_mgL	BPW	Site	4	0.878924	0.219731	1.583623	0.184337	.
20	SO4_mgL	BPW	Residuals	102	14.15271	0.138752			
21	SO4_mgL	LFG	Site	4	169.5915	42.39787	83.05492	4.90E-28	****
21	SO4_mgL	LFG	Residuals	82	41.85935	0.51048			

Table 15. Analysis of variance for effects of site on the decrease in water chemistry concentrations from early morning to late afternoon with separate analyses for nearshore surface water (SW) and benthic pore water BPW. Differences in water chemistry were calculated by subtracting afternoon concentrations from morning concentrations so a positive number is a measure of a decrease in concentration and a negative number is a measure of an increase. The key result is the probability (P) that differences in means among sample groups could have occurred by chance. All water chemistry concentrations were transformed (recalculated) with a Log2 Plus 1 transformation for these analyses. See the title for Table 2 for an explanation of the Log2 Plus 1 transformation and P code.

Model	Water Source	Parameter	Factor	Df Site	Sum Sq	Mean Sq	F	P	P Code
1	SW	TP	Site	3	1269.229	423.0763	5.658475	0.027511	*
1	SW	TP	Residuals	7	523.3803	74.76862			
2	SW	TN	Site	3	19898.91	6632.969	1.576713	0.27851	
2	SW	TN	Residuals	7	29447.83	4206.832			
3	SW	SRP	Site	3	3.520548	1.173516	0.312948	0.815817	
3	SW	SRP	Residuals	7	26.24916	3.74988			
4	SW	NH4	Site	3	257.5818	85.86061	0.404107	0.754813	
4	SW	NH4	Residuals	7	1487.288	212.4697			
5	SW	NO3N	Site	3	0.003443	0.001148	0.571121	0.651692	
5	SW	NO3N	Residuals	7	0.014065	0.002009			
6	SW	Chloride	Site	3	30.26056	10.08685	0.658054	0.603285	
6	SW	Chloride	Residuals	7	107.2981	15.3283			
7	SW	Sulfate	Site	3	39.74735	13.24912	0.927533	0.475957	
7	SW	Sulfate	Residuals	7	99.98981	14.28426			
8	SW	Fluoride	Site	3	0.001201	0.0004	1.089347	0.414239	
8	SW	Fluoride	Residuals	7	0.002573	0.000368			
9	BPW	TP	Site	3	985.3653	328.4551	0.699259	0.581632	
9	BPW	TP	Residuals	7	3288.033	469.719			
10	BPW	TN	Site	3	27554.71	9184.903	1.074249	0.419584	
10	BPW	TN	Residuals	7	59850.48	8550.068			
11	BPW	SRP	Site	3	3.988271	1.329424	1.627835	0.267671	
11	BPW	SRP	Residuals	7	5.716774	0.816682			
12	BPW	NH4	Site	3	739.3599	246.4533	0.556803	0.66002	
12	BPW	NH4	Residuals	7	3098.352	442.6218			

Model	Water Source	Parameter	Factor	Df Site	Sum Sq	Mean Sq	F	P	P Code
13	BPW	NO3N	Site	3	0.002371	0.00079	1.092203	0.413236	
13	BPW	NO3N	Residuals	7	0.005065	0.000724			
14	BPW	Chloride	Site	3	27.92031	9.306771	1.847071	0.22671	
14	BPW	Chloride	Residuals	7	35.27065	5.038664			
15	BPW	Sulfate	Site	3	25.70003	8.566678	1.671755	0.258773	
15	BPW	Sulfate	Residuals	7	35.87053	5.124361			
16	BPW	Fluoride	Site	3	0.000855	0.000285	2.752311	0.121915	
16	BPW	Fluoride	Residuals	7	0.000725	0.000104			

Table 16. Analysis of covariance and analysis of variance for differences in 2020 water chemistry concentrations between mid-lake deep water and nearshore shallow water (i.e. factor WaterDepth) while taking into account potential differences in changes during the summer (Sumr_Day). Summer day is the number of days after May 15. Separate analyses were run for each water chemistry parameter and designated as successive model numbers for clarity. Analyses were run for TP, PO4-P, TN, NO3-N, NH4-N, Cl, and fluoride. Units of measurement are indicated as ug/L or mg/L. The key result is the probability (P) that differences in means among sample groups could have occurred by chance. The F ratio, sum of squares (Sum Sq), and mean square error are used to determine P and also reported. If P is less than 0.05, then differences are usually considered reliable. Also reported are the degrees of freedom (DF). All water chemistry concentrations were transformed (recalculated) with a Log2 Plus 1 transformation for these analyses. See the title for Table 2 for an explanation of the Log2 Plus 1 transformation and P code.

Model	Var	Analysis	Factors	Df	Sum Sq	Mean Sq	F	P
1	TP_ugL	ANCOVA	WaterDepth	1	12.61589	12.61589	3.652305	0.06162
1	TP_ugL	ANCOVA	Sumr_Day	1	11.84724	11.84724	3.42978	0.069823
1	TP_ugL	ANCOVA	WaterDepth:Sumr_Day	1	4.593771	4.593771	1.329898	0.2542
1	TP_ugL	ANCOVA	Residuals	51	176.1656	3.454228		
2	TP_ugL	ANOVA	WaterDepth	1	12.61589	12.61589	3.62928	0.062308
2	TP_ugL	ANOVA	Sumr_Day	1	11.84724	11.84724	3.408158	0.07057
2	TP_ugL	ANOVA	Residuals	52	180.7594	3.476142		
3	SRP_ugL	ANCOVA	WaterDepth	1	2.843919	2.843919	1.928886	0.170916
3	SRP_ugL	ANCOVA	Sumr_Day	1	10.18417	10.18417	6.907404	0.011313
3	SRP_ugL	ANCOVA	WaterDepth:Sumr_Day	1	3.547418	3.547418	2.406034	0.127052
3	SRP_ugL	ANCOVA	Residuals	51	75.19359	1.474384		
4	SRP_ugL	ANOVA	WaterDepth	1	2.843919	2.843919	1.878104	0.176437
4	SRP_ugL	ANOVA	Sumr_Day	1	10.18417	10.18417	6.725551	0.012315
4	SRP_ugL	ANOVA	Residuals	52	78.741	1.51425		
5	TN_ugL	ANCOVA	WaterDepth	1	0.091434	0.091434	1.46328	0.231986
5	TN_ugL	ANCOVA	Sumr_Day	1	1.198832	1.198832	19.18576	5.91E-05
5	TN_ugL	ANCOVA	WaterDepth:Sumr_Day	1	0.008792	0.008792	0.140697	0.709145
5	TN_ugL	ANCOVA	Residuals	51	3.186762	0.062486		
6	TN_ugL	ANOVA	WaterDepth	1	0.091434	0.091434	1.487867	0.228051
6	TN_ugL	ANOVA	Sumr_Day	1	1.198832	1.198832	19.50813	5.10E-05
6	TN_ugL	ANOVA	Residuals	52	3.195553	0.061453		
7	NH4_ugL	ANCOVA	WaterDepth	1	2.090268	2.090268	1.00825	0.320063

Model	Var	Analysis	Factors	Df	Sum Sq	Mean Sq	F	P
7	NH4_ugL	ANCOVA	Sumr_Day	1	8.211583	8.211583	3.960893	0.051942
7	NH4_ugL	ANCOVA	WaterDepth:Sumr_Day	1	0.963675	0.963675	0.464833	0.498459
7	NH4_ugL	ANCOVA	Residuals	51	105.7314	2.073165		
8	NH4_ugL	ANOVA	WaterDepth	1	2.090268	2.090268	1.018734	0.317493
8	NH4_ugL	ANOVA	Sumr_Day	1	8.211583	8.211583	4.002081	0.050678
8	NH4_ugL	ANOVA	Residuals	52	106.6951	2.051828		
9	Fluoride_mgL	ANCOVA	WaterDepth	1	0.377818	0.377818	1.203235	0.277828
9	Fluoride_mgL	ANCOVA	Sumr_Day	1	0.308884	0.308884	0.983702	0.325969
9	Fluoride_mgL	ANCOVA	WaterDepth:Sumr_Day	1	0.501806	0.501806	1.598101	0.21192
9	Fluoride_mgL	ANCOVA	Residuals	51	16.01408	0.314002		
10	Fluoride_mgL	ANOVA	WaterDepth	1	0.377818	0.377818	1.189553	0.280448
10	Fluoride_mgL	ANOVA	Sumr_Day	1	0.308884	0.308884	0.972516	0.32862
10	Fluoride_mgL	ANOVA	Residuals	52	16.51588	0.317613		
11	Chloride_mgL	ANCOVA	WaterDepth	1	0.479671	0.479671	0.324185	0.571603
11	Chloride_mgL	ANCOVA	Sumr_Day	1	9.03587	9.03587	6.106883	0.016846
11	Chloride_mgL	ANCOVA	WaterDepth:Sumr_Day	1	1.280198	1.280198	0.865221	0.356664
11	Chloride_mgL	ANCOVA	Residuals	51	75.46065	1.479621		
12	Chloride_mgL	ANOVA	WaterDepth	1	0.479671	0.479671	0.325027	0.571057
12	Chloride_mgL	ANOVA	Sumr_Day	1	9.03587	9.03587	6.122753	0.016645
12	Chloride_mgL	ANOVA	Residuals	52	76.74085	1.475786		
13	Nitrate_mgL	ANCOVA	WaterDepth	1	5.820106	5.820106	1.461548	0.232259
13	Nitrate_mgL	ANCOVA	Sumr_Day	1	3.511573	3.511573	0.881828	0.352128
13	Nitrate_mgL	ANCOVA	WaterDepth:Sumr_Day	1	1.792519	1.792519	0.450138	0.505297
13	Nitrate_mgL	ANCOVA	Residuals	51	203.0897	3.982151		
14	Nitrate_mgL	ANOVA	WaterDepth	1	5.820106	5.820106	1.477168	0.229708
14	Nitrate_mgL	ANOVA	Sumr_Day	1	3.511573	3.511573	0.891252	0.349504
14	Nitrate_mgL	ANOVA	Residuals	52	204.8822	3.940043		
15	NO3N_mgL	ANCOVA	WaterDepth	1	4.483267	4.483267	1.585547	0.213696
15	NO3N_mgL	ANCOVA	Sumr_Day	1	3.06209	3.06209	1.082935	0.30295
15	NO3N_mgL	ANCOVA	WaterDepth:Sumr_Day	1	1.689748	1.689748	0.597595	0.443066
15	NO3N_mgL	ANCOVA	Residuals	51	144.2067	2.827583		

Model	Var	Analysis	Factors	Df	Sum Sq	Mean Sq	F	P
16	NO3N_mgL	ANOVA	WaterDepth	1	4.483267	4.483267	1.597913	0.211836
16	NO3N_mgL	ANOVA	Sumr_Day	1	3.06209	3.06209	1.091381	0.300998
16	NO3N_mgL	ANOVA	Residuals	52	145.8965	2.805702		

Table 17. Regression results for changes in 2020 water chemistry concentrations with summer day in the open lake and nearshore water depths. Separate univariate regression models (Y=a+bX) were calculated to determine the rate of change (slope) and predicted May 15 concentration (intercept) for each water chemistry parameter (X) separately for OpenLake and Nearshore locations. For each regression model, the table includes coefficients for an intercept (a) and slope estimate (b), a standard error (SE) for each of these model coefficients, and a P value that estimates the probability that the coefficient (either intercept a or slope b) is equal to zero. P codes (explained in title of Table 2) have been provided for slopes indicating rates of change during the summer. P codes were not provided for intercepts for clarity, but P values were. In this analysis I examined which parameters had significant changes during the summer and if parameters were different between nearshore and offshore. Note: A simple way to get a first approximation of openlake-nearshore differences between slopes or intercepts is to multiply the SE by 2, add and subtract it from the coefficient to create an confidence interval for the coefficient (sometimes called the 75% error bound), and then determine if the confidence intervals of either coefficient overlap the coefficient for the other water depth. For example, the SE for the TP intercept for the OpenLake, 1.36, is doubled to 2.72 and subtracted from that intercept, 4.27, it does not overlap the 2.47 intercept for nearshore TP. The same is true for error bound of the nearshore intercept. This indicates there is a high likelihood that TP was lower in the nearshore zone than offshore zone at the beginning of the summer but decreased more rapidly in the openlake than nearshore zone. Upper and lower bounds (UpBnd and LowBnd, respectively) for coefficients were calculated to facilitate these comparisons. The coefficient value to compare to those upper and lower error bounds is indicated in the Comparison column. A DiffCode is provided to indicate when the comparison coefficient values are outside the error bounds of the paired coefficients, which were paired by openlake-nearshore model for the same chemistry parameter.

Model	DepVar	WaterDepth	Coefficient	Estimate	SE	P	P Code	UpBnd	LowBnd	Comparison	DiffCode
1	TP_ugL	OpenLake	Intercept	4.269568	1.362781	0.02587		6.99513	1.544007	2.4658707	
1	TP_ugL	OpenLake	Slope	-0.05453	0.016378	0.020789	*	-0.02178	-0.08729	-0.014706	*
2	TP_ugL	Nearshore	Intercept	2.465871	0.883236	0.007608		4.232342	0.699399	4.2695685	
2	TP_ugL	Nearshore	Slope	-0.01471	0.010761	0.178388		0.006816	-0.03623	-0.0545326	*
3	SRP_ugL	OpenLake	Intercept	3.697274	1.147573	0.023418		5.99242	1.402129	1.5294633	
3	SRP_ugL	OpenLake	Slope	-0.04881	0.013792	0.016585	*	-0.02122	-0.07639	-0.0138078	*
4	SRP_ugL	Nearshore	Intercept	1.529463	0.572284	0.010379		2.674032	0.384894	3.6972743	*
4	SRP_ugL	Nearshore	Slope	-0.01381	0.006972	0.053664	.	0.000137	-0.02775	-0.0488059	*
5	TN_ugL	OpenLake	Intercept	8.374282	0.434788	6.96E-06		9.243858	7.504706	8.4065228	
5	TN_ugL	OpenLake	Slope	0.007401	0.005225	0.21585		0.017852	-0.00305	0.0056587	
6	TN_ugL	Nearshore	Intercept	8.406523	0.111762	8.35E-50		8.630047	8.182998	8.3742823	
6	TN_ugL	Nearshore	Slope	0.005659	0.001362	0.000139	***	0.008382	0.002935	0.007401	
7	NH4_ugL	OpenLake	Intercept	3.8508	0.470649	0.000444		4.792098	2.909502	5.8598108	*

Model	DepVar	WaterDepth	Coefficient	Estimate	SE	P	P Code	UpBnd	LowBnd	Comparison	DiffCode
7	NH4_ugL	OpenLake	Slope	0.001385	0.005656	0.816287		0.012698	-0.00993	-0.0168561	*
8	NH4_ugL	Nearshore	Intercept	5.859811	0.691027	5.86E-11		7.241865	4.477756	3.8507996	*
8	NH4_ugL	Nearshore	Slope	-0.01686	0.008419	0.051189	.	-1.8E-05	-0.03369	0.0013851	*
9	Fluoride_mgL	OpenLake	Intercept	-3.28254	0.395906	0.000417		-2.49073	-4.07435	-4.5944328	*
9	Fluoride_mgL	OpenLake	Slope	-0.01493	0.004758	0.02571	*	-0.00542	-0.02445	-0.0017704	*
10	Fluoride_mgL	Nearshore	Intercept	-4.59443	0.266534	1.06E-21		-4.06136	-5.1275	-3.2825418	*
10	Fluoride_mgL	Nearshore	Slope	-0.00177	0.003247	0.588261		0.004724	-0.00827	-0.0149334	*
11	Chloride_mgL	OpenLake	Intercept	3.303176	0.173835	7.44E-06		3.650847	2.955505	1.9379975	*
11	Chloride_mgL	OpenLake	Slope	-0.00317	0.002089	0.189279		0.001005	-0.00735	0.0178515	*
12	Chloride_mgL	Nearshore	Intercept	1.937997	0.584927	0.001803		3.107851	0.768144	3.303176	*
12	Chloride_mgL	Nearshore	Slope	0.017851	0.007126	0.015852	*	0.032104	0.003599	-0.0031731	*
13	Nitrate_mgL	OpenLake	Intercept	0.051741	0.235934	0.835085		0.523609	-0.42013	-2.8941263	*
13	Nitrate_mgL	OpenLake	Slope	-0.01269	0.002836	0.00654	**	-0.00702	-0.01836	0.012185	*
14	Nitrate_mgL	Nearshore	Intercept	-2.89413	0.959728	0.004167		-0.97467	-4.81358	0.0517413	*
14	Nitrate_mgL	Nearshore	Slope	0.012185	0.011693	0.302816		0.035571	-0.0112	-0.0126932	*
15	NO3N_mgL	OpenLake	Intercept	-2.0945	0.235934	0.000302		-1.62263	-2.56637	-4.8646115	*
15	NO3N_mgL	OpenLake	Slope	-0.01269	0.002836	0.00654	**	-0.00702	-0.01836	0.0114613	*
16	NO3N_mgL	Nearshore	Intercept	-4.86461	0.808614	2.74E-07		-3.24738	-6.48184	-2.0945026	*
16	NO3N_mgL	Nearshore	Slope	0.011461	0.009852	0.250675		0.031165	-0.00824	-0.0126932	*

Table 18. Latitude and longitude of sampling locations during the 2020 benthic algal survey of Torch Lake.

Site #	SiteName	Latitude	Longitude
1	Becky's Beach	45.0938	-85.3519
2	Near Deans	45.07342	-85.323
3	HWH North	45.05362	-85.322
4	Petty	44.98963	-85.2884
5	Gourley	44.94535	-85.283
6	South of Clam R	44.94238	-85.2876
6b	South of Clam R	44.93839	-85.2915
7	Alden	44.88416	-85.2784
8	Sand Bar East	44.86128	-85.2922
9	Near Torch River	44.8593	-85.3263
10	Deepwater Point	44.877	-85.3193
11	SouthofHills	44.92393	-85.3205
12	South Hill	44.94853	-85.3199
13	North Hill	44.96616	-85.3208
14	Sand Point	45.02045	-85.3277
15	HWH South	45.04627	-8.5E+07
16	Torch Lake (Town)	45.06902	-85.3536

Table 19. Regression results for the relationship between algal biomass estimated with aerial photography from Hoadley (Y) and close-up video with a GoPro camera on the lake bottom (X). Separate univariate regression models (Y=a+bX) were calculated with algal biomass estimates corrected for PC cover (PC_corr) or not percent cover corrected. For each regression model, the table includes coefficients for an intercept (a) and slope estimate (b), a standard error (SE) for each of these model coefficients, and a P value that estimates the probability that the coefficient (either intercept a or slope b) is equal to zero. P codes (explained in title of Table 2) have been provided for slopes indicating rates of change during the summer. P codes were not provided for intercepts for clarity, but P values were. Adjusted R squared values are provided for the proportion of variance in biomass estimated with aerial photograph images explained by GoPro video estimates of biomass.

PC_corr	Coefficient	Estimate	SE	t-value	P	P Code	Adjusted R-squared
no	Intercept	-0.04061	0.51375	-0.079	0.937537		
no	Slope	0.79298	0.20073	3.95	0.000458	***	0.3863
yes	Intercept	-0.04061	0.51375	-0.079	0.937537		
yes	Slope	0.79298	0.20073	3.95	0.000458	***	0.3274

Table 20. Regression results for changes in total phosphorus concentrations (P ug/L) with year for two seasons at seven sites in four lakes (three lakes had two sites sampled). Data were provided online by the CLMP data (MiCorps) and used with their permission. Separate univariate regression models (Y=a+bX) were calculated for each site and season to determine the annual rate of change (slope) in TP over the time period of sampling, which was as much as 27 years for some sites. For each regression model, the table includes coefficients for an intercept (a) and slope estimate (b), a standard error (SE) for each of these model coefficients, and a P value that estimates the probability that the coefficient (either intercept a or slope b) is equal to zero. P codes (explained in title of Table 2) have been provided for slopes indicating rates of change during the summer. P codes were not provided for intercepts for clarity, but P values were. N is the number of samples in the analysis, which is an indicator of the number of years of sampling prior to 2019, which was the last year of data included in the analysis.

Model	DepVar	Lake (& Site)	Season	Coefficient	Estimate	SE	P	P Code	N
1	P_ugL	Crystal	Spring Overturn	Slope	-0.0263602	0.011209	0.028055	*	24
2	P_ugL	Crystal	Late Summer	Slope	-0.0309115	0.016735	0.083303	.	18
3	P_ugL	HigginsNB	Spring Overturn	Slope	-0.0068107	0.013819	0.627487		22
4	P_ugL	HigginsNB	Late Summer	Slope	-0.0438273	0.018003	0.028895		16
5	P_ugL	HigginsSB	Spring Overturn	Slope	0.0279441	0.025404	0.292915		14
6	P_ugL	HigginsSB	Late Summer	Slope	-0.0312025	0.026816	0.265501		15
7	P_ugL	GlenBig	Spring Overturn	Slope	-0.0159232	0.019394	0.422356		20
8	P_ugL	GlenBig	Late Summer	Slope	-0.0327378	0.021022	0.137811		19
9	P_ugL	GlenLittle	Spring Overturn	Slope	-0.0261481	0.016467	0.130723		19
10	P_ugL	GlenLittle	Late Summer	Slope	-0.0169212	0.021626	0.444714		20
11	P_ugL	LeelanauN	Spring Overturn	Slope	0.028191	0.049835	0.611155		5
12	P_ugL	LeelanauN	Late Summer	Slope	-0.0785851	0.093163	0.431276		8
13	P_ugL	LeelanauS	Spring Overturn	Slope	0.0744214	0.074916	0.358875		8
14	P_ugL	LeelanauS	Late Summer	Slope	-0.0915276	0.093759	0.361486		9
1	P_ugL	Crystal	Spring Overturn	Intercept	55.542248	22.47686	0.021692		24
2	P_ugL	Crystal	Late Summer	Intercept	64.539819	33.62585	0.072955		18
3	P_ugL	HigginsNB	Spring Overturn	Intercept	16.208816	27.74202	0.565572		22
4	P_ugL	HigginsNB	Late Summer	Intercept	90.6381	36.20743	0.025305		16
5	P_ugL	HigginsSB	Spring Overturn	Intercept	-53.752424	51.12375	0.31378		14
6	P_ugL	HigginsSB	Late Summer	Intercept	65.264489	53.9531	0.247951		15
7	P_ugL	GlenBig	Spring Overturn	Intercept	34.458576	38.98303	0.388384		20
8	P_ugL	GlenBig	Late Summer	Intercept	68.13339	42.25378	0.125266		19

Model	DepVar	Lake (& Site)	Season	Coefficient	Estimate	SE	P	P Code	N
9	P_ugL	GlenLittle	Spring Overturn	Intercept	55.036724	33.09805	0.11467		19
10	P_ugL	GlenLittle	Late Summer	Intercept	37.14473	43.4685	0.404698		20
11	P_ugL	LeelanauN	Spring Overturn	Intercept	-54.699076	100.4377	0.623897		5
12	P_ugL	LeelanauN	Late Summer	Intercept	160.82029	187.6894	0.424427		8
13	P_ugL	LeelanauS	Spring Overturn	Intercept	-147.37263	150.9283	0.366556		8
14	P_ugL	LeelanauS	Late Summer	Intercept	187.09069	188.9241	0.355018		9

Table 21. Regression results for changes in total phosphorus concentrations (P ug/L) with year at 23 sites in 17 lakes. Data were provided online by Tip of the Mitt Watershed Council and used with their permission. Separate univariate regression models (Y=a+bX) were calculated for each site to determine the annual rate of change (slope) in TP over the time period of sampling, which was as much as 27 years for some sites. For each regression model, the table includes coefficients for an intercept (a) and slope estimate (b), a standard error (SE) for each of these model coefficients, and a P value that estimates the probability that the coefficient (either intercept a or slope b) is equal to zero. P codes (explained in title of Table 2) have been provided for slopes indicating rates of change during the summer. P codes were not provided for intercepts for clarity, but P values were. N is the number of samples in the analysis, which is an indicator of the number of years of sampling prior to 2019, which was the last year of data included in the analyses. Depths indicates that sample results for surface and middle depths were included in analyses.

Model	Location	Depths	DepVar	Coefficient	Estimate	SE	P	P code	N
1	Bass	SurfMid	TP_ugL	Slope	-0.0050289	0.014967	0.7415261		17
2	Bellaire	SurfMid	TP_ugL	Slope	-0.0347539	0.013868	0.0226694	*	19
3	Black	SurfMid	TP_ugL	Slope	-0.0219641	0.007711	0.009348	**	24
4	Burt	SurfMid	TP_ugL	Slope	-0.0263321	0.009863	0.0139979	*	24
5	Charlevoix, Main	SurfMid	TP_ugL	Slope	-0.0665901	0.01429	0.0001204	***	24
6	Charlevoix, South Arm	SurfMid	TP_ugL	Slope	-0.0806875	0.015653	3.63E-05	****	24
7	Clam	SurfMid	TP_ugL	Slope	-0.0548025	0.012964	0.0008445	***	16
8	Douglas	SurfMid	TP_ugL	Slope	-0.0291931	0.010826	0.0135151	*	23
9	Elk	SurfMid	TP_ugL	Slope	-0.0501007	0.02333	0.0464692	*	19
10	Ellsworth	SurfMid	TP_ugL	Slope	-0.0369368	0.015781	0.0309702	*	20
11	Intermediate	SurfMid	TP_ugL	Slope	-0.037473	0.017356	0.0463758	*	18
12	Larks	SurfMid	TP_ugL	Slope	-0.0514649	0.018337	0.0229635	*	10
13	Michigan, GTB	SurfMid	TP_ugL	Slope	0.03653797	0.03106	0.2556518		19
14	Michigan, Little Traverse Bay	SurfMid	TP_ugL	Slope	-0.0640902	0.011769	1.80E-05	****	24
15	Mullett	SurfMid	TP_ugL	Slope	-0.0579034	0.013865	0.0003925	***	24
16	Round	SurfMid	TP_ugL	Slope	-0.0217465	0.021551	0.3424805		10
17	Skegemog	SurfMid	TP_ugL	Slope	-0.0660592	0.015575	0.0011448	**	14
18	Torch, South	SurfMid	TP_ugL	Slope	-0.0490999	0.019695	0.0232811	*	19
19	Walloon, Foot	SurfMid	TP_ugL	Slope	-0.0306359	0.012967	0.0253303	*	30
20	Walloon, Mud Basin	SurfMid	TP_ugL	Slope	-0.052819	0.016576	0.0078268	**	14
21	Walloon, North Arm	SurfMid	TP_ugL	Slope	-0.0521036	0.01108	6.26E-05	****	30
22	Walloon, West Arm	SurfMid	TP_ugL	Slope	-0.0487584	0.010644	8.72E-05	****	30

Model	Location	Depths	DepVar	Coefficient	Estimate	SE	P	P code	N
23	Walloon, Wildwood Basin	SurfMid	TP_ugL	Slope	-0.0436327	0.010954	0.0004393	***	30
1	Bass	SurfMid	TP_ugL	Intercept	13.6283091	30.00476	0.6561845		17
2	Bellaire	SurfMid	TP_ugL	Intercept	72.3096304	27.8168	0.0186996		19
3	Black	SurfMid	TP_ugL	Intercept	46.9052771	15.44127	0.0060426		24
4	Burt	SurfMid	TP_ugL	Intercept	55.3314665	19.75096	0.0104011		24
5	Charlevoix, Main	SurfMid	TP_ugL	Intercept	135.470113	28.61562	0.0001005		24
6	Charlevoix, South Arm	SurfMid	TP_ugL	Intercept	163.731985	31.34508	3.07E-05		24
7	Clam	SurfMid	TP_ugL	Intercept	112.494951	25.992	0.0006948		16
8	Douglas	SurfMid	TP_ugL	Intercept	61.530924	21.67852	0.0098442		23
9	Elk	SurfMid	TP_ugL	Intercept	102.752686	46.79453	0.0422701		19
10	Ellsworth	SurfMid	TP_ugL	Intercept	77.2627971	31.64945	0.0251969		20
11	Intermediate	SurfMid	TP_ugL	Intercept	77.8051438	34.83408	0.0401379		18
12	Larks	SurfMid	TP_ugL	Intercept	106.312542	36.82502	0.0202934		10
13	Michigan, GTB	SurfMid	TP_ugL	Intercept	-70.83553	62.32224	0.2714738		19
14	Michigan, Little Traverse Bay	SurfMid	TP_ugL	Intercept	130.605297	23.5668	1.43E-05		24
15	Mullett	SurfMid	TP_ugL	Intercept	118.887071	27.76481	0.000303		24
16	Round	SurfMid	TP_ugL	Intercept	46.6781848	43.27886	0.3122335		10
17	Skegemog	SurfMid	TP_ugL	Intercept	134.813449	31.20291	0.0009952		14
18	Torch, South	SurfMid	TP_ugL	Intercept	100.31758	39.50436	0.0211661		19
19	Walloon, Foot	SurfMid	TP_ugL	Intercept	63.7463152	25.97673	0.0206091		30
20	Walloon, Mud Basin	SurfMid	TP_ugL	Intercept	109.290756	33.21985	0.0064602		14
21	Walloon, North Arm	SurfMid	TP_ugL	Intercept	107.177353	22.19679	4.43E-05		30
22	Walloon, West Arm	SurfMid	TP_ugL	Intercept	100.104818	21.32357	6.39E-05		30
23	Walloon, Wildwood Basin	SurfMid	TP_ugL	Intercept	89.7837556	21.94326	0.0003285		30

Figure 1. Golden brown algal mats at different spatial scales.

- See text.

Figure 2. Long-term records of spring TP (ug/L) collected by the MICorps Cooperative Lake Monitoring Program (CLMP) showing temporal variability without linear smoothing of the long term pattern. Separate lines are plotted for open lake samples from Lake Bellaire, Clam Lake, and the North and South Torch Lake sampling sites with color coding of data points and lines. Note the TP axis is plotted on a square root scale, but data are not square root transformed.

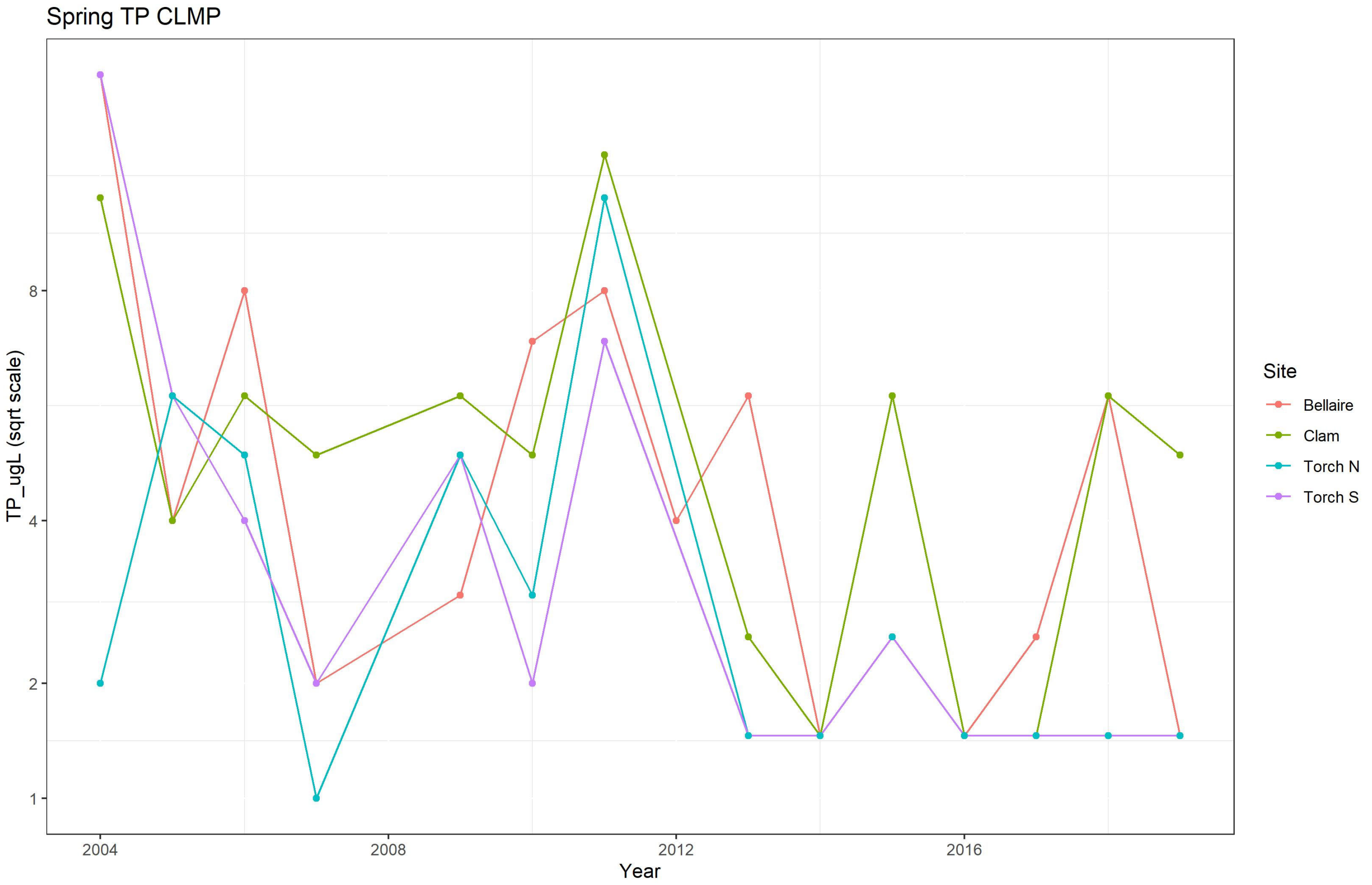

Figure 3. Long-term records of spring TP (ug/L) collected by the MICorps Cooperative Lake Monitoring Program (CLMP) showing longterm pattern with linear smoothing. Separate lines are plotted for open lake samples from Lake Bellaire, Clam Lake, and the North and South Torch Lake sampling sites with color coding of data points and lines. Note the TP axis is plotted on a square root scale, but data are not square root transformed.

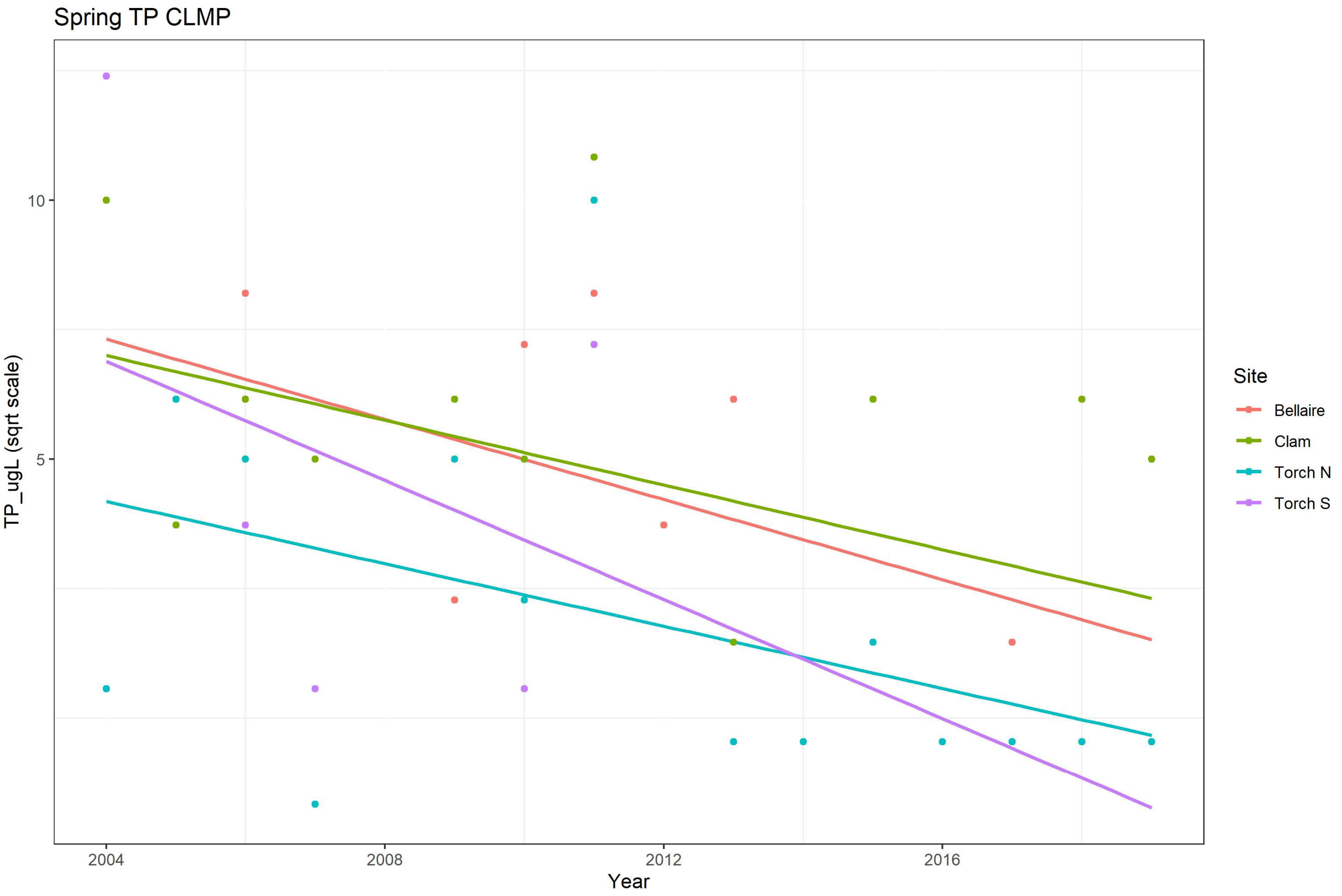

Figure 4. Long-term records of summer TP (ug/L) collected by the MICorps Cooperative Lake Monitoring Program (CLMP) showing temporal variability without linear smoothing of the longterm pattern. Separate lines are plotted for open lake samples from Lake Bellaire, Clam Lake, and the North and South Torch Lake sampling sites with color coding of data points and lines. Note the TP axis is plotted on a square root scale, but data are not square root transformed.

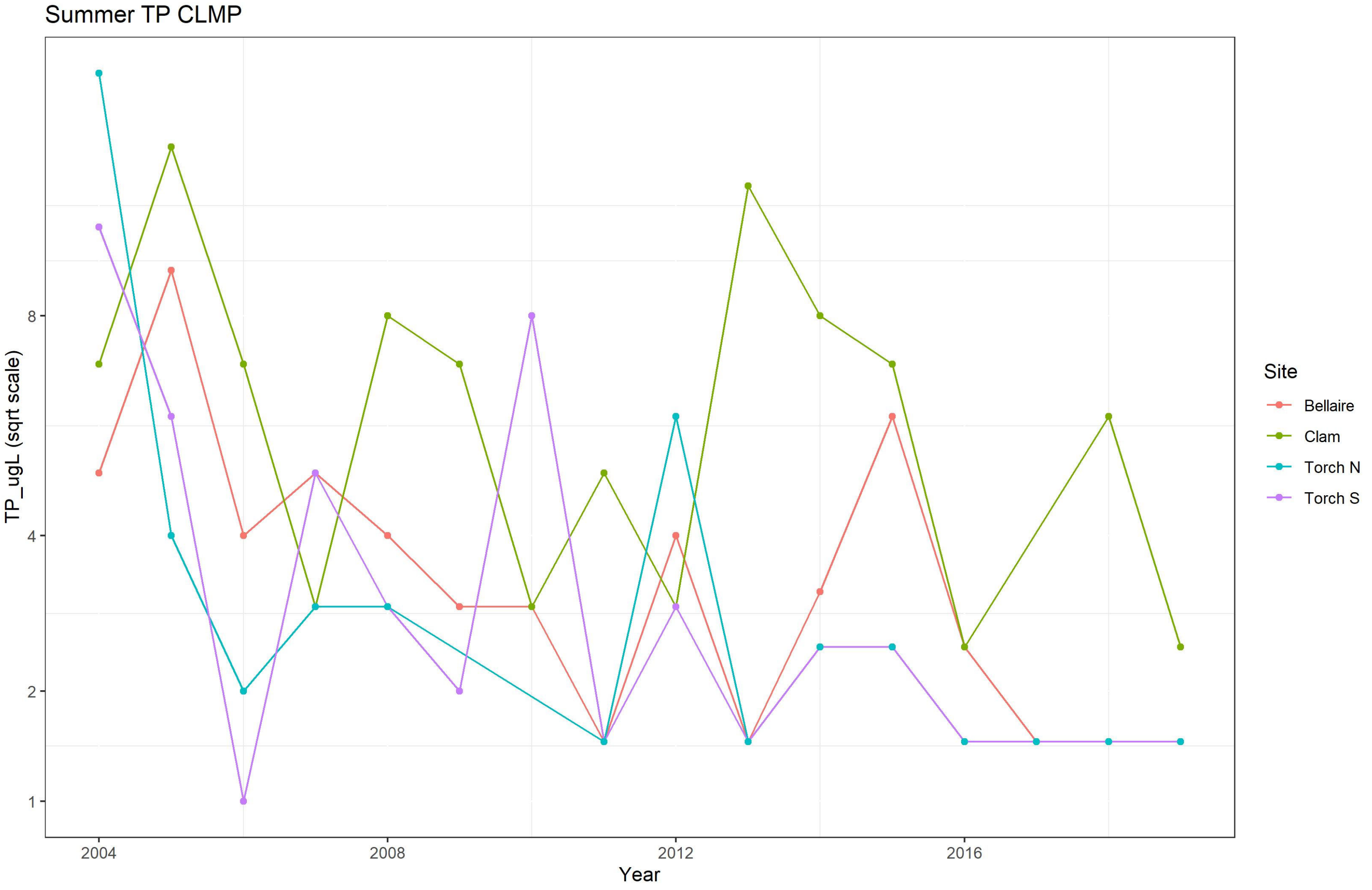

Figure 5. Long-term records of spring TP (ug/L) collected by the MICorps Cooperative Lake Monitoring Program (CLMP) showing longterm pattern with linear smoothing. Separate lines are plotted for open lake samples from Lake Bellaire, Clam Lake, and the North and South Torch Lake sampling sites with color coding of data points and lines. Note the TP axis is plotted on a square root scale, but data are not square root transformed.

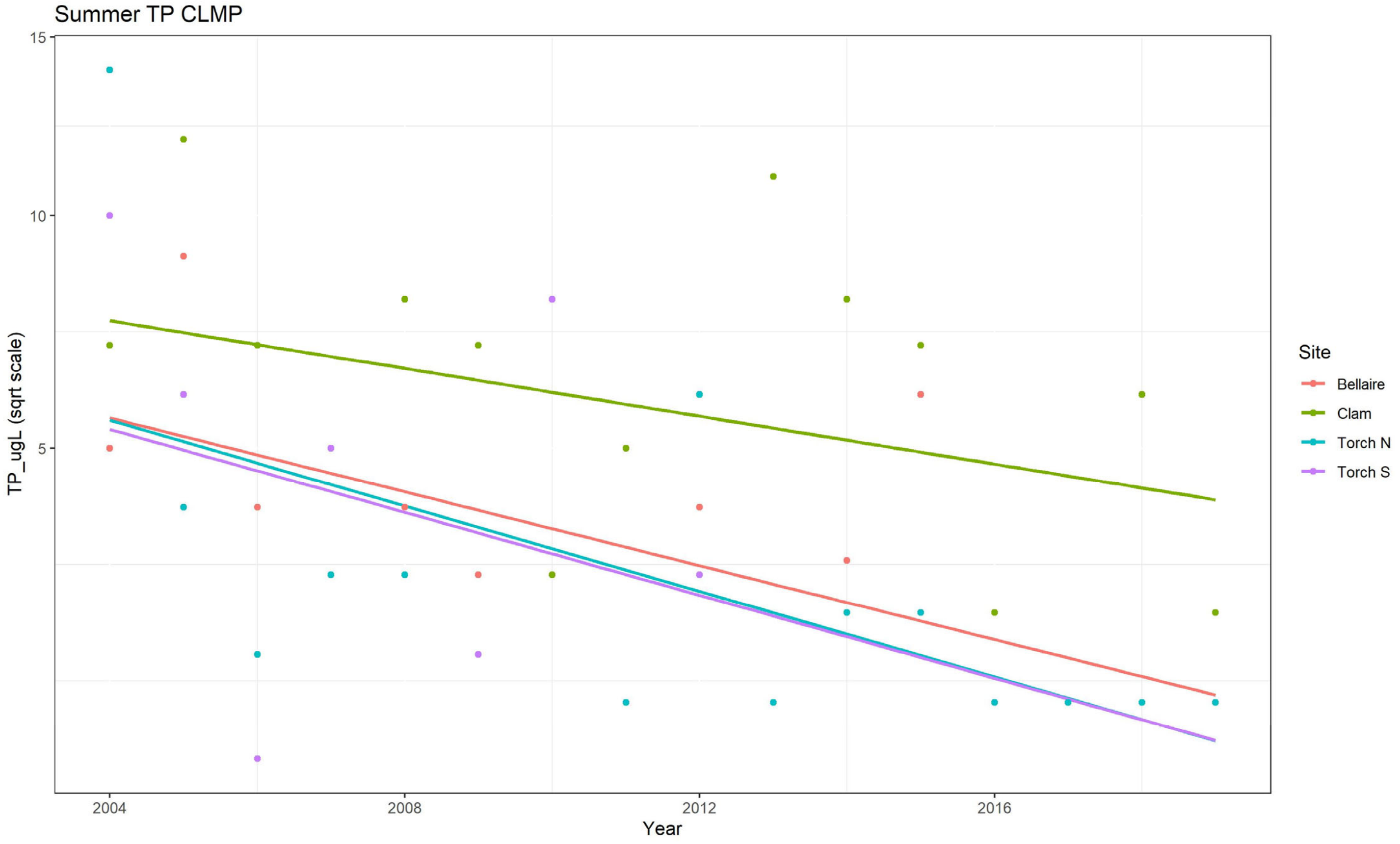

Figure 6. Long-term records of spring TP (ug/L) in surface and mid-depth water samples collected by the Tip of the Mitt Watershed Council (ToMWC) showing temporal variability with linear smoothing of the longterm patterns. Separate lines are plotted for open lake samples from Lake Bellaire, Clam Lake, and the South Torch Lake sampling sites with color coding of data points and lines. Note the TP axis is plotted on a square root scale, but data are not square root transformed. The hashed lines show potential non-linear patterns. Trend analysis is not possible for the North Torch Lake site because data were only recently collected.

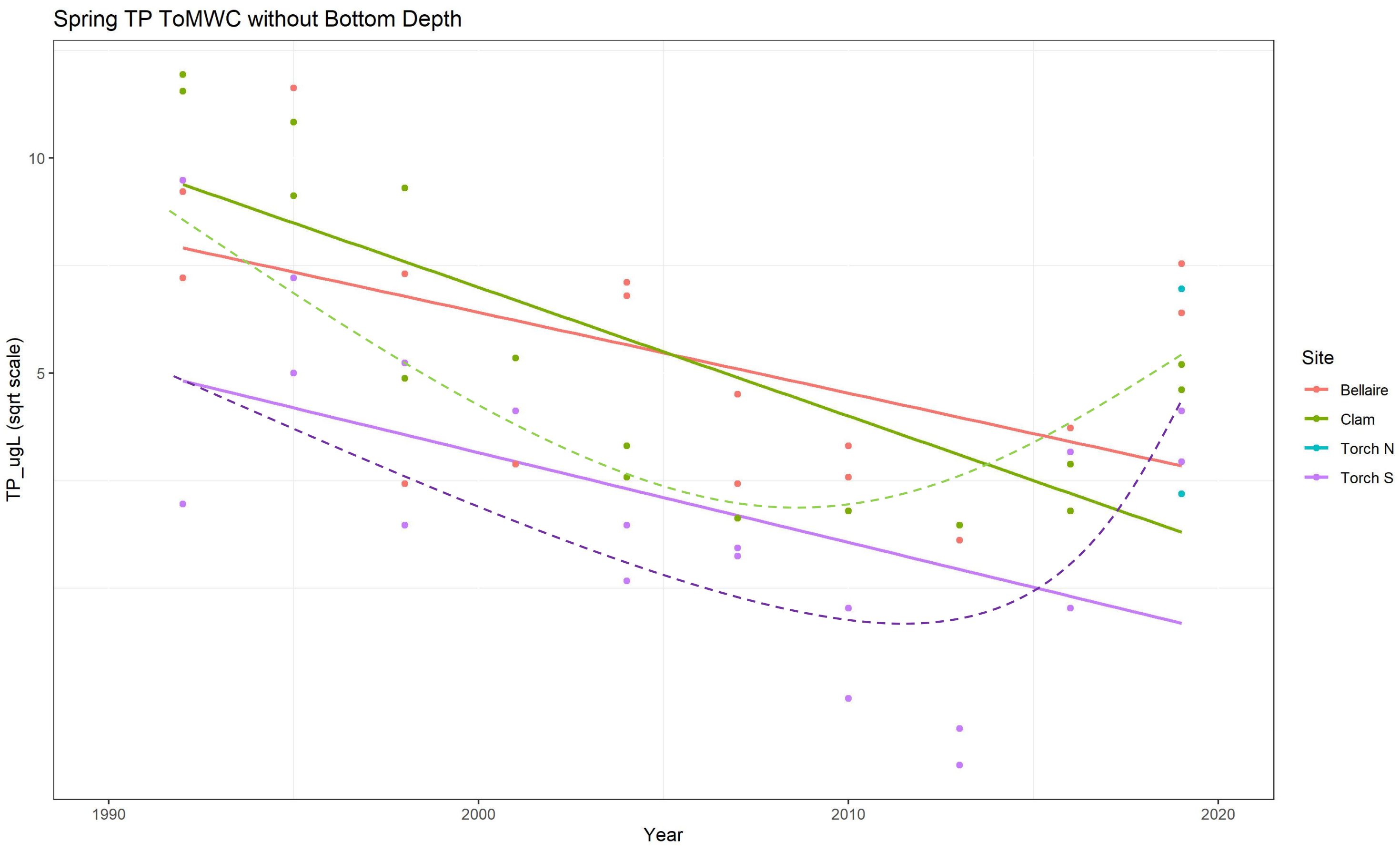

Figure 7. Changes in total phosphorus concentrations in four northern Michigan lakes during the last 300 years inferred from diatom species composition in sediment cores from the lakes and known total phosphorus preferences of the diatom species (Fritz et al. 1993). Results show a decrease in diatom-inferred TP in lakes Bellaire, Elk, and Glen starting about the middle of the 20th century. TP was transformed with a natural log plus 1 transformation for the diatom inferred TP.

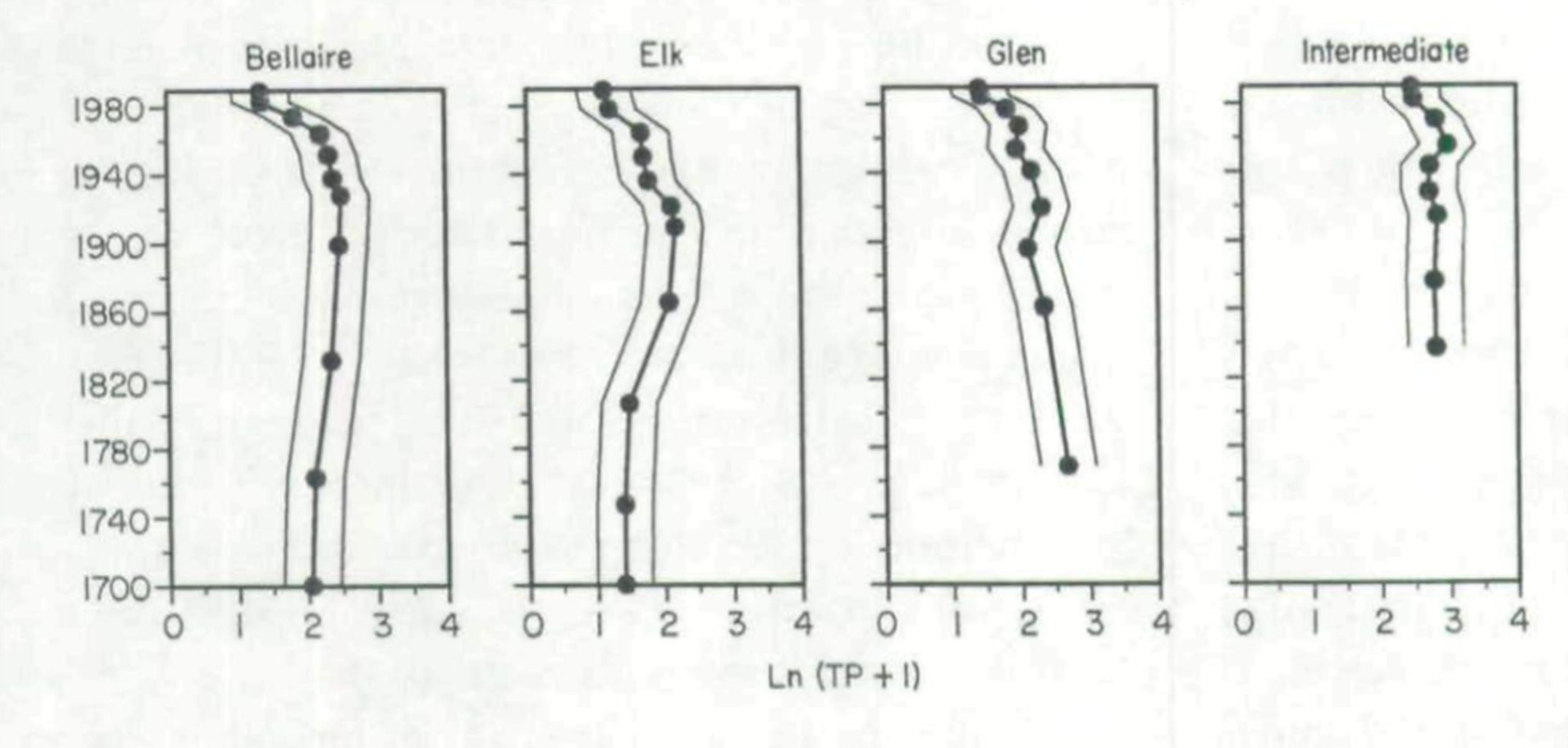

Figure 8. Long-term records of spring TN (ug/L) in surface and mid-depth water samples collected by the Tip of the Mitt Watershed Council (ToMWC) showing temporal variability with linear smoothing of the long-term patterns. Separate lines are plotted for open lake samples from Lake Bellaire, Clam Lake, and the South Torch Lake sampling sites with color coding of data points and lines. The TN axis is not log-transformed. Trend analysis is not possible for the North Torch Lake site because data were only recently collected.

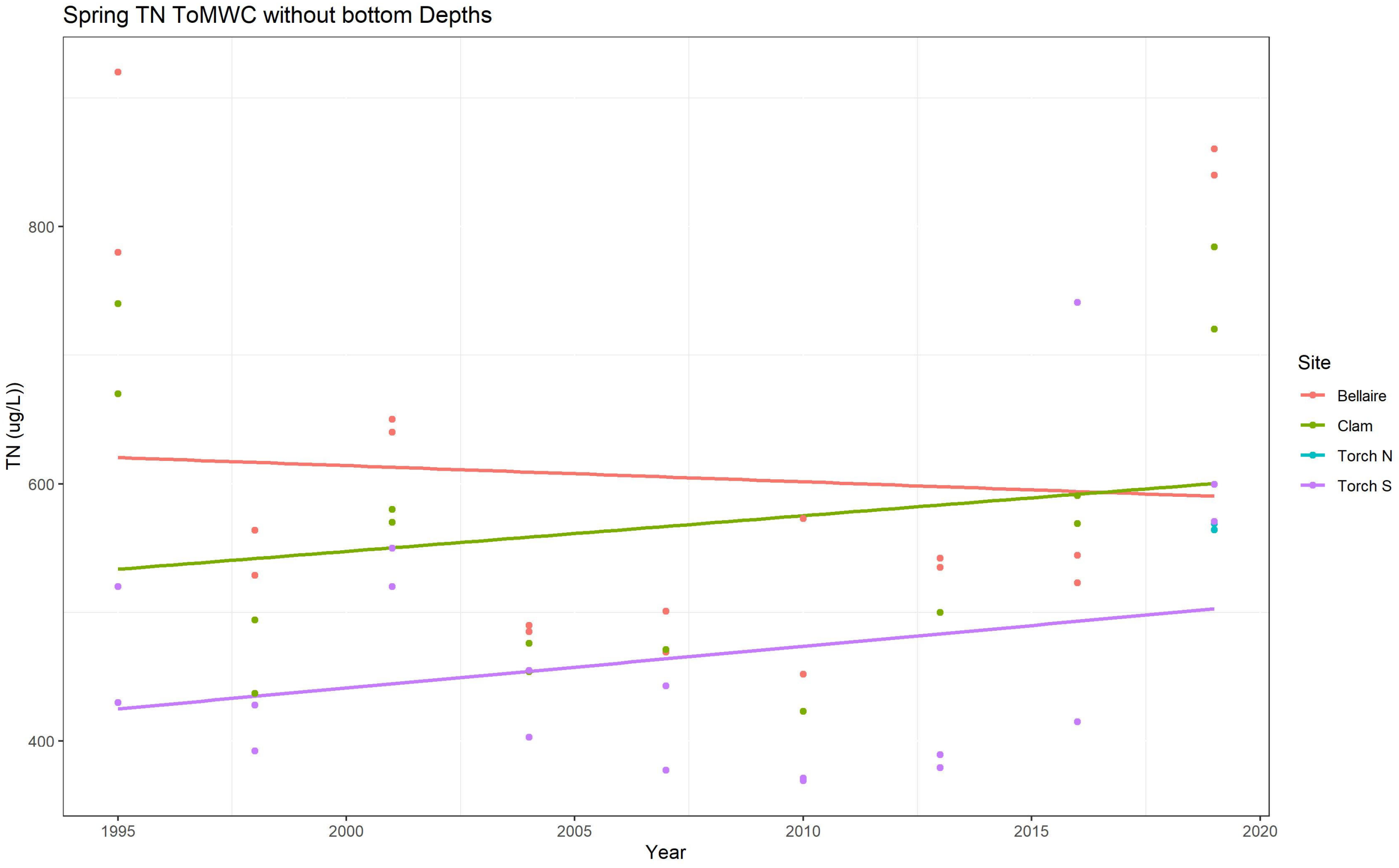

Figure 9. Long-term records of spring NO3-N (ug/L) in surface and mid-depth water samples collected by the Tip of the Mitt Watershed Council (ToMWC) showing temporal variability with linear smoothing of the long-term patterns. Separate lines are plotted for open lake samples from Lake Bellaire, Clam Lake, and the South Torch Lake sampling sites with color coding of data points and lines. Trend analysis is not possible for the North Torch Lake site because data were only recently collected.

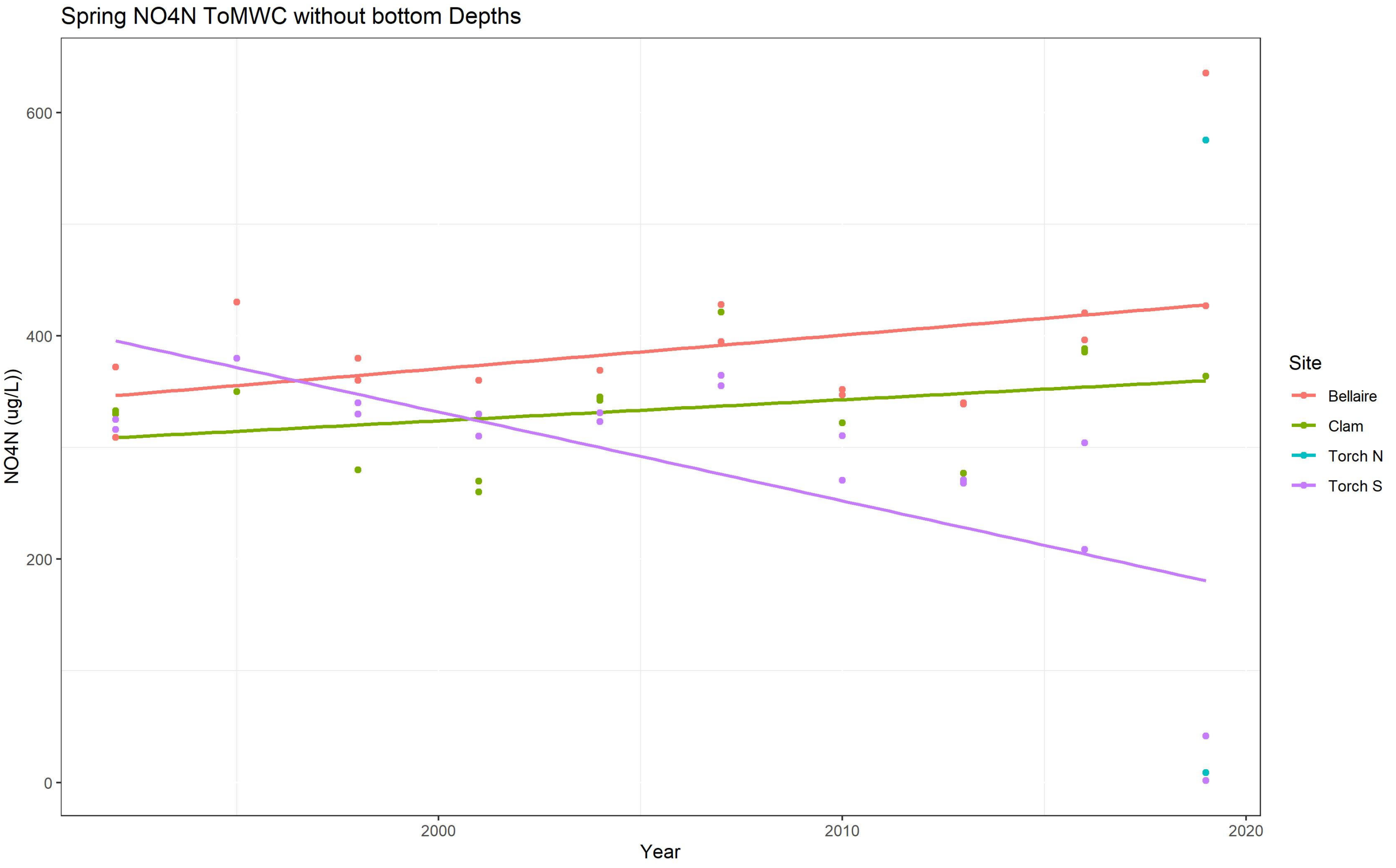

Figure 10. Long-term records of summer Chlorophyll a (ug/L) in surface water samples collected by the MICorps Cooperative Lake Management Program (CLMP) showing temporal variability with linear smoothing of the long-term patterns. Separate lines are plotted for open lake samples from Lake Bellaire, Clam Lake, and the South and North Torch Lake sampling sites with color coding of data points and lines.

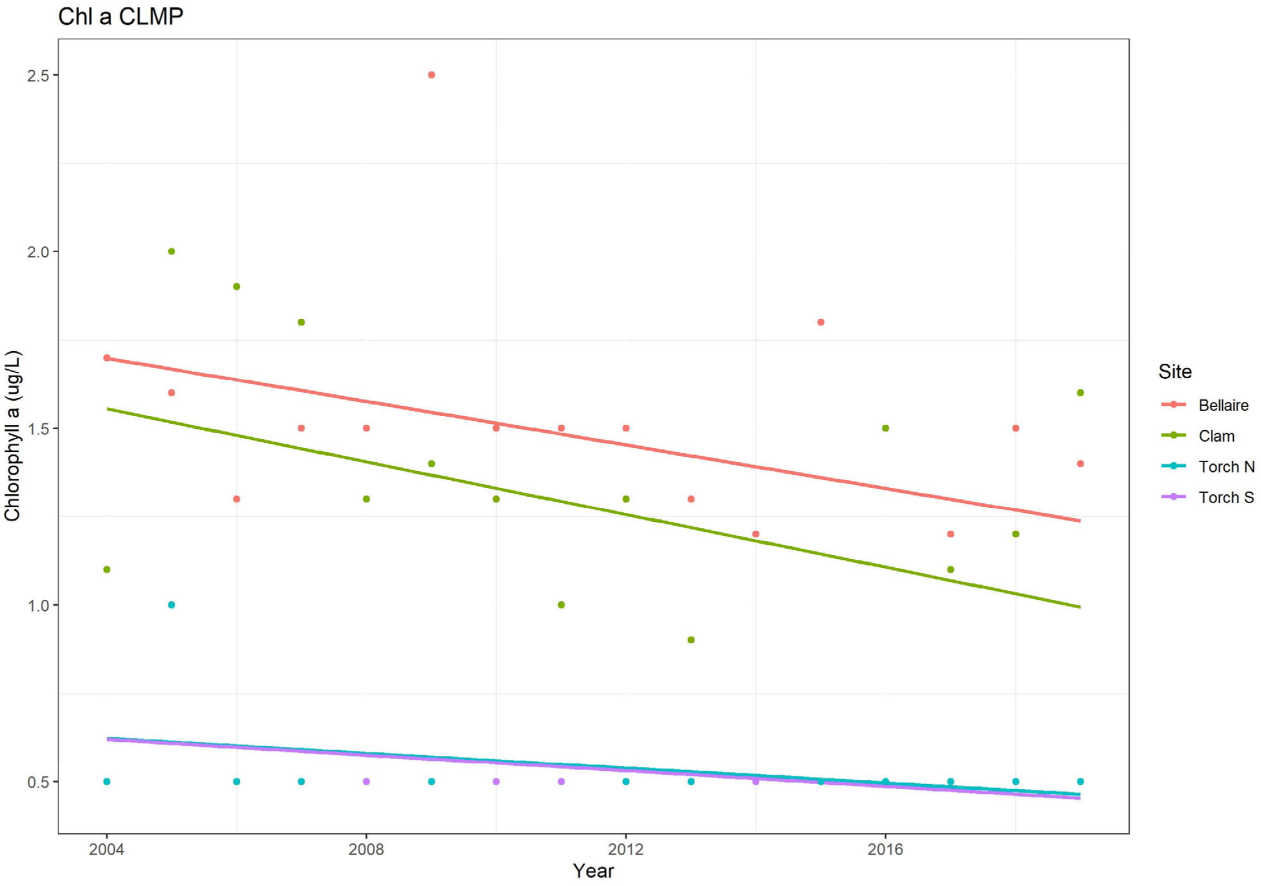

Figure 11. Long-term records of spring Secchi disk depth (m) measured by the MICorps Cooperative Lake Management Program (CLMP) showing temporal variability with linear smoothing of the long-term patterns. Separate lines are plotted for open lake samples from Lake Bellaire, Clam Lake, and the South and North Torch Lake sampling sites with color coding of data points and lines.

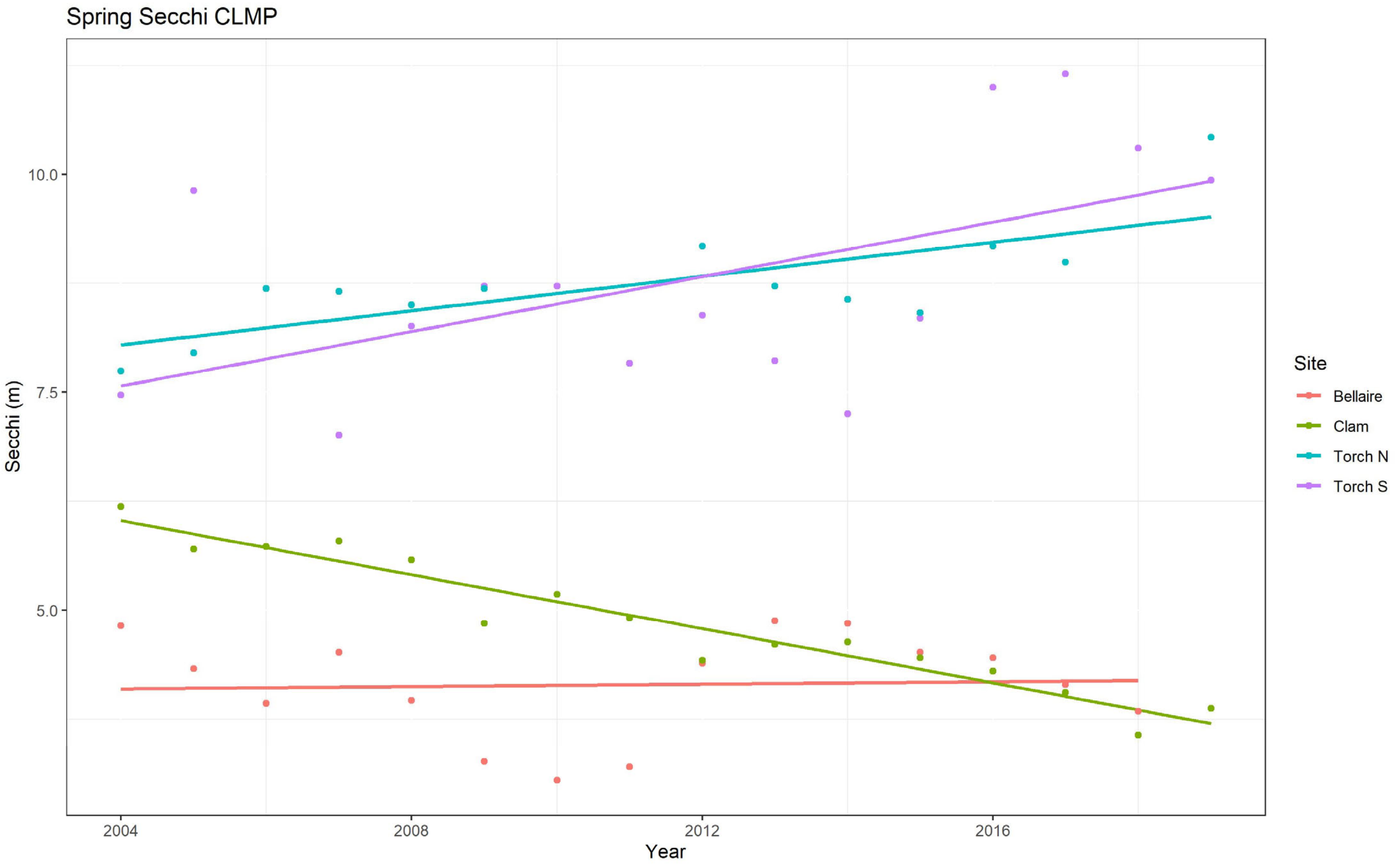

Figure 12. Long-term records of spring pH in surface and mid-depth water samples collected by the Tip of the Mitt Watershed Council (ToMWC) showing temporal variability with linear smoothing of the long-term patterns. Separate lines are plotted for open lake samples from Lake Bellaire, Clam Lake, and the South Torch Lake sampling sites with color coding of data points and lines. Trend analysis is not possible for the North Torch Lake site because data were only recently collected.

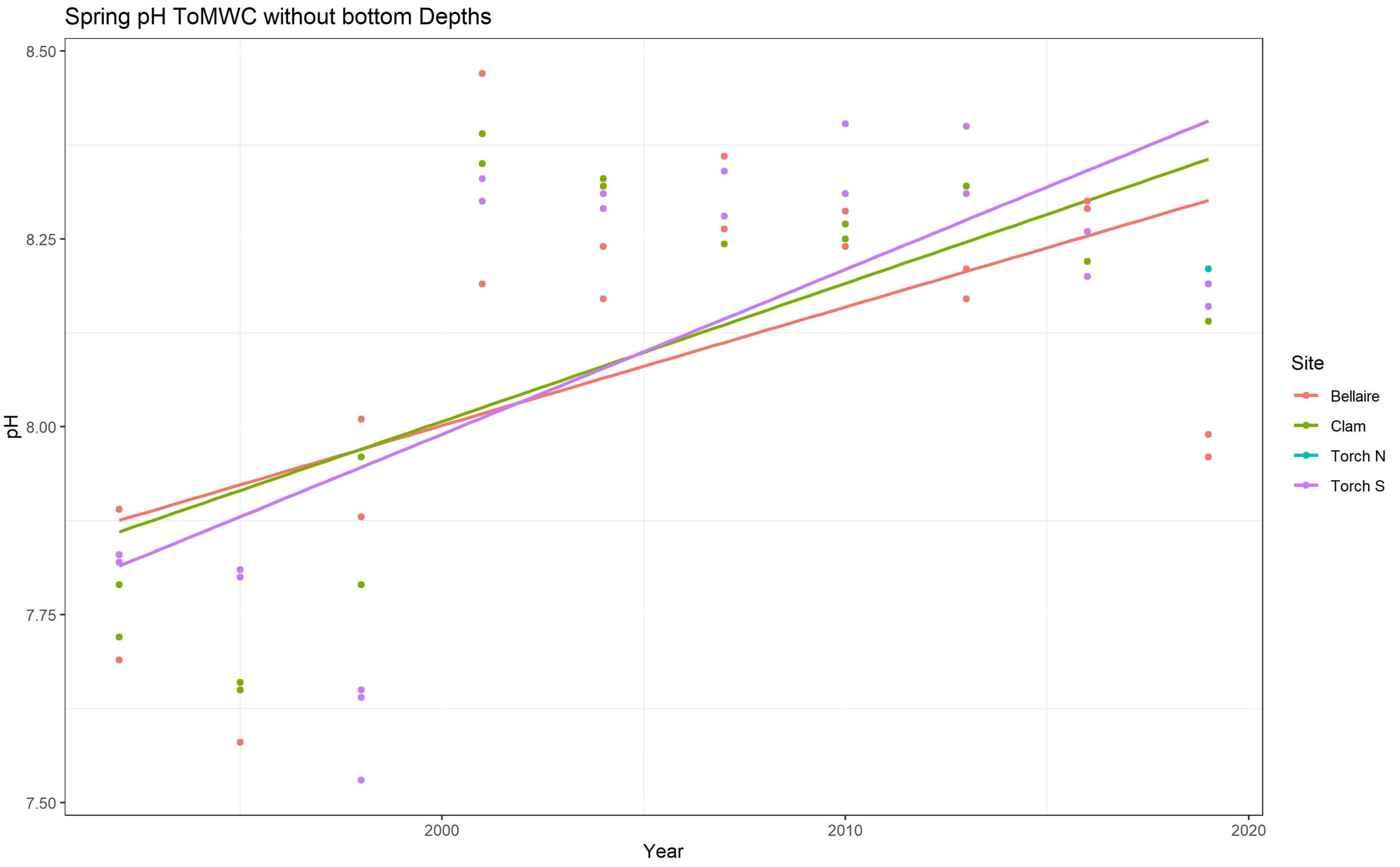

Figure 13. Long-term records of spring specific conductivity (uohms m^{-2} s^{-1}) in surface and mid-depth water samples collected by the Tip of the Mitt Watershed Council (ToMWC) showing temporal variability with linear smoothing of the long-term patterns. Separate lines are plotted for open lake samples from Lake Bellaire, Clam Lake, and the South Torch Lake sampling sites with color coding of data points and lines. Trend analysis is not possible for the North Torch Lake site because data were only recently collected.

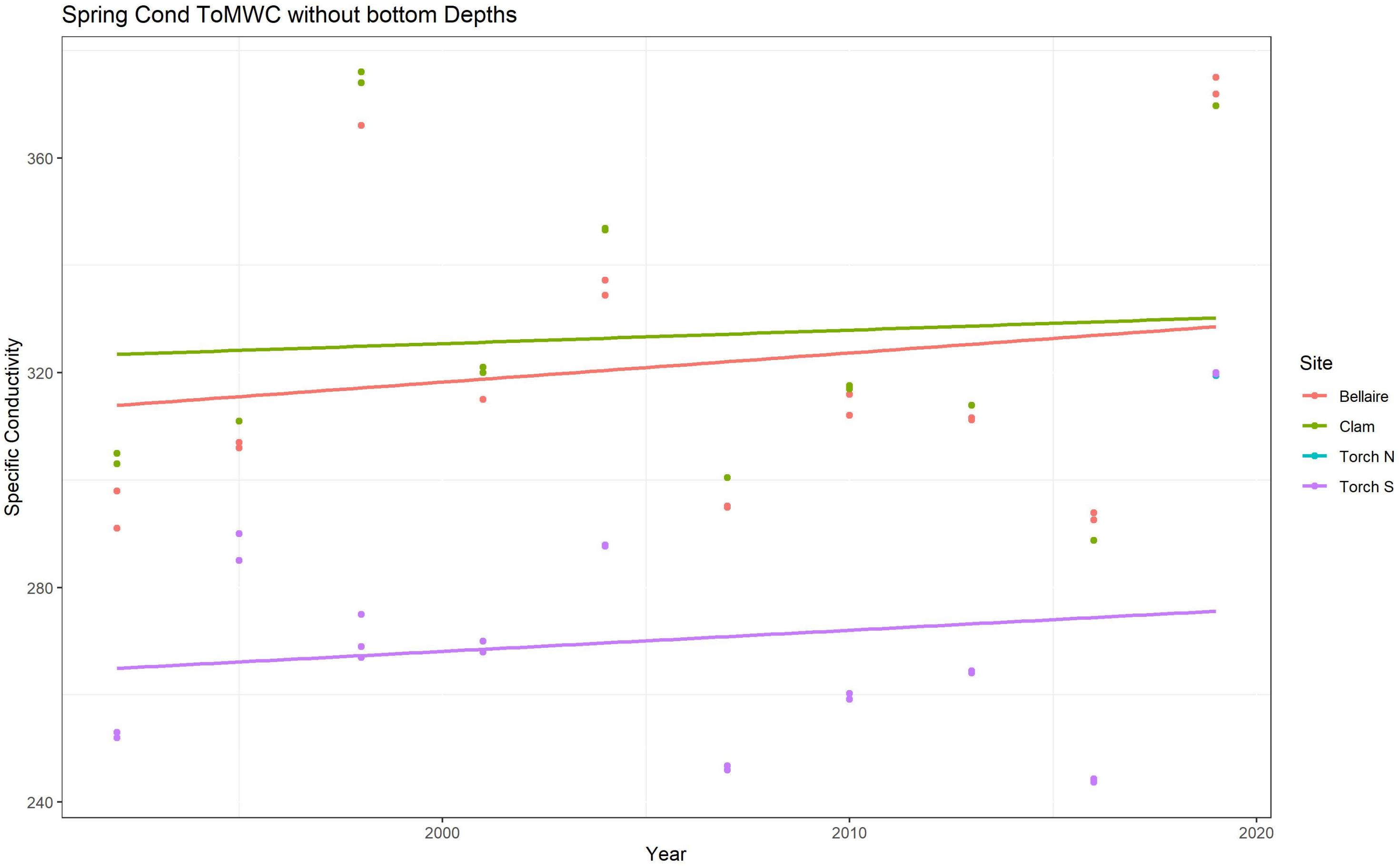

Figure 14. Long-term records of spring chloride concentrations (mg/L) in surface and mid-depth water samples collected by the Tip of the Mitt Watershed Council (ToMWC) showing temporal variability with linear smoothing of the long-term patterns. Separate lines are plotted for open lake samples from Lake Bellaire, Clam Lake, and the South Torch Lake sampling sites with color coding of data points and lines. Trend analysis is not possible for the North Torch Lake site because data were only recently collected.

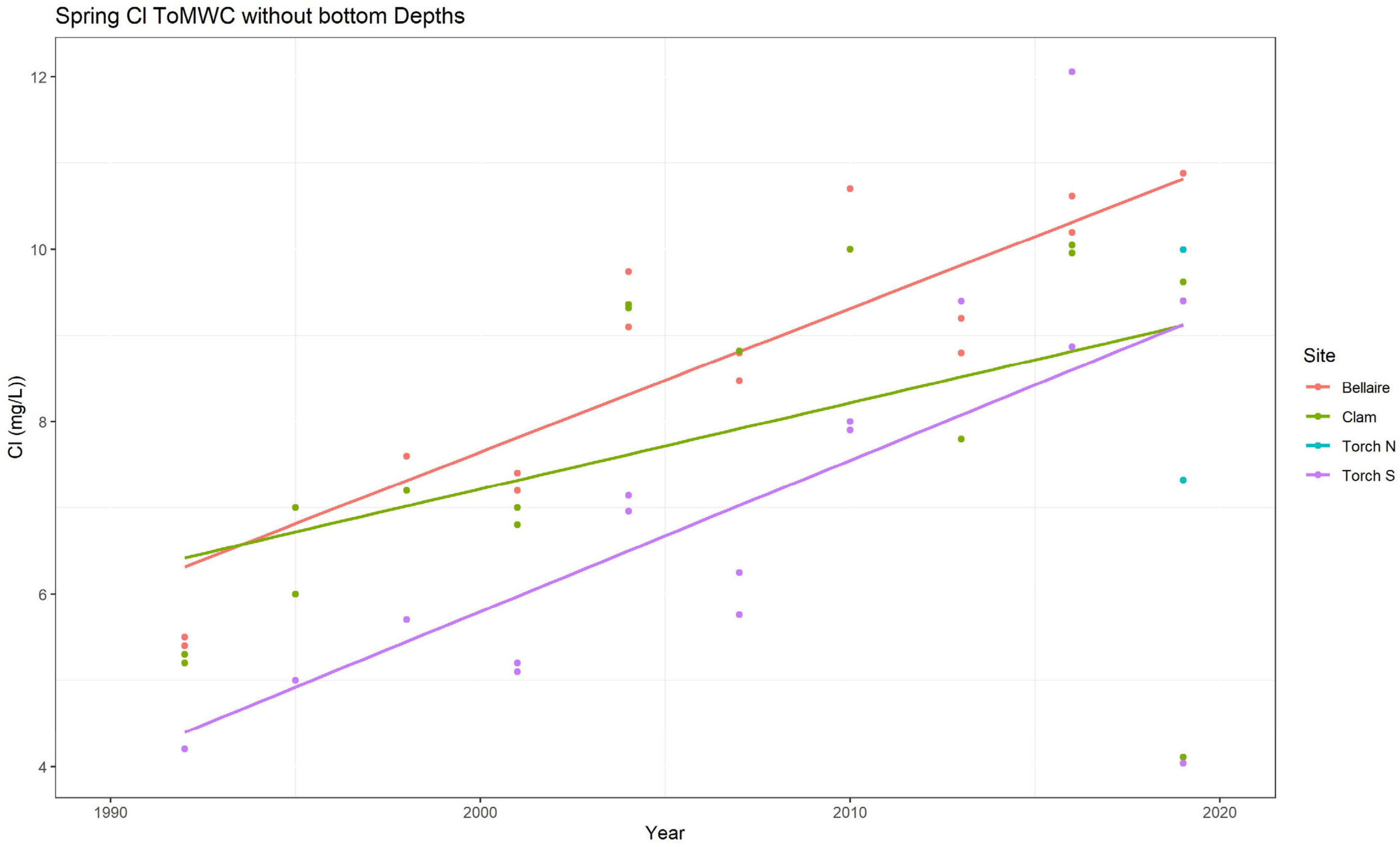

Figure 15. Differences in TP concentration (ug/L) in Torch Lake nearshore surface water from 2005 to 2015-2020 using 2005 nearshore surface water data from Bretz et al. (2006). Box plots illustrate variability and central tendency in TP concentrations at sites sampled with the midline in the box as median and upper and lower limits of the box being 75th and 25th percentiles of the data. The extended lines indicate the 95 percent confidence limits from the mean. The small closed circles are the measured TP concentrations at sites.

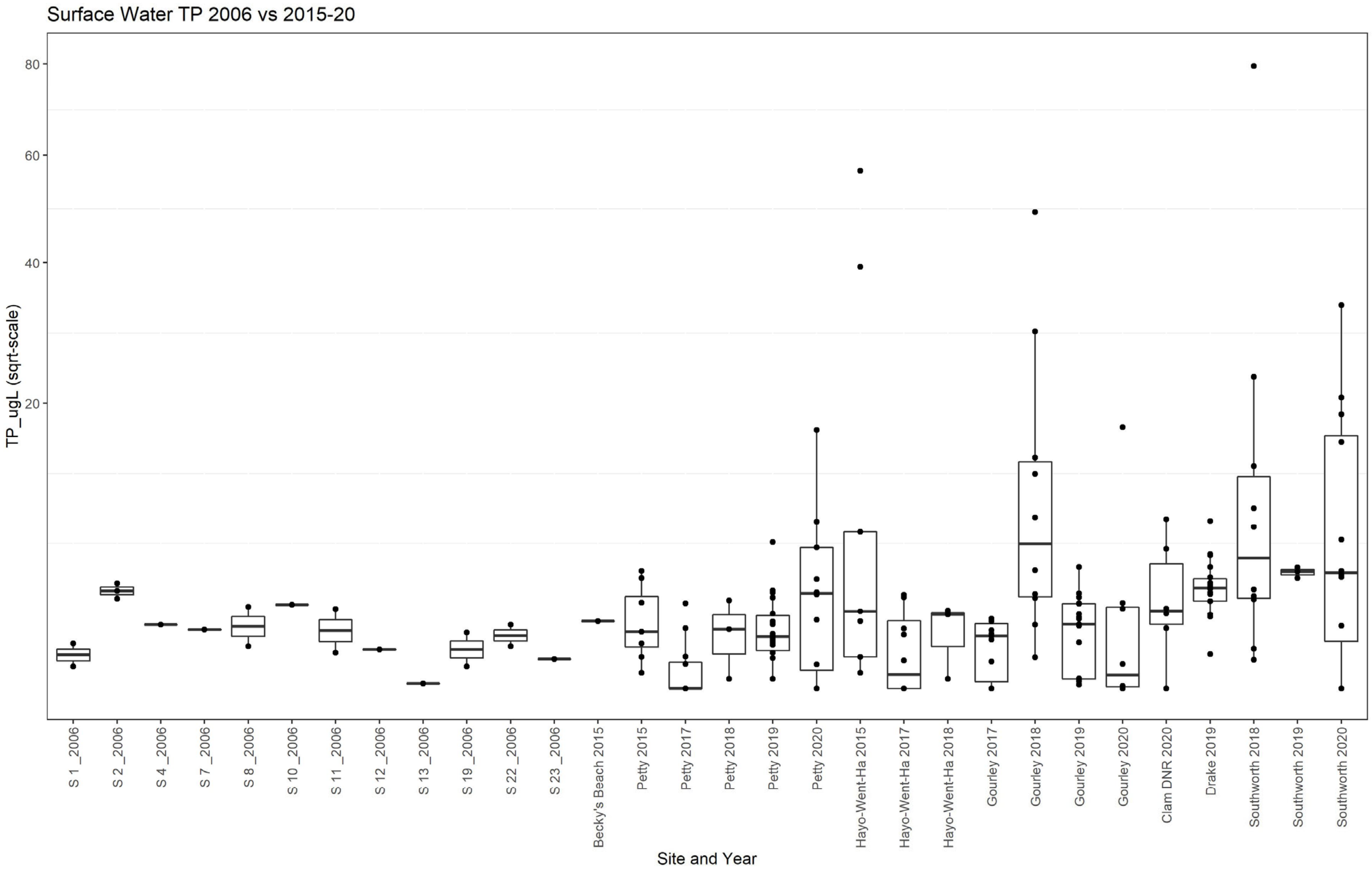

Figure 16. Differences in TP concentration (ug/L) in Torch Lake subsurface groundwater from 2005 to 2015-2020 sampled by lake floor piezometer with data from Bretz et al. (2006). Box plots illustrate variability and central tendency in TP concentrations at sites sampled with the midline in the box as median and upper and lower limits of the box being 75th and 25th percentiles of the data. The extended lines indicate the 95 percent confidence limits from the mean. The small closed circles are the measured TP concentrations at sites. The site labels include location of samples and year of sampling.

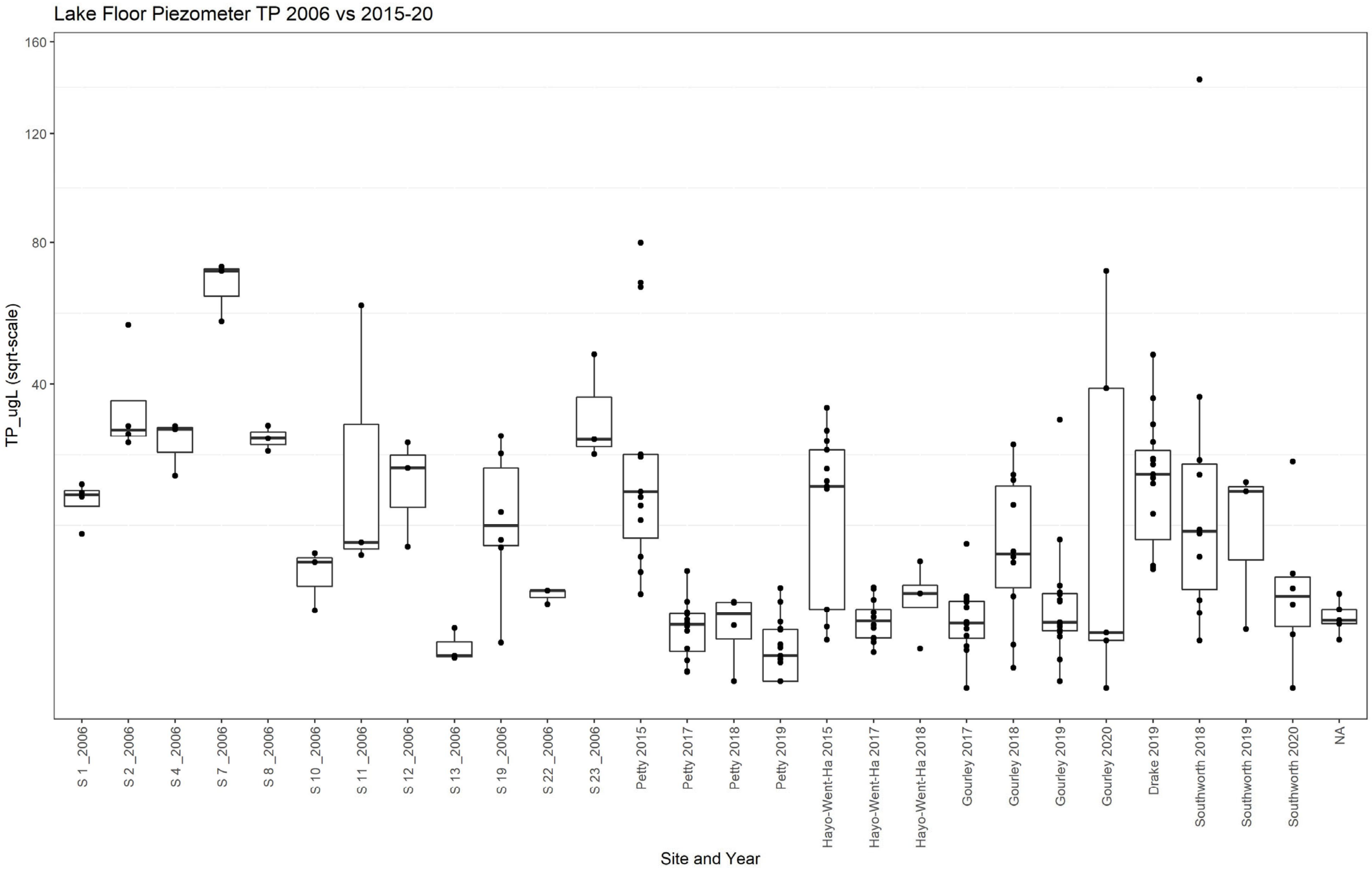

Figure 17. PO4-P concentrations in different water sources in 2016. Water sources are well water (well), lake floor piezometer (LFP), benthic pore water from sediment (BS) and benthic water by turkey baster (BW), and nearshore surface water (SW). Samples were collected throughout the summer at the Drake and Southworth sites in Lake Bellaire and the Gourley, Hayo-Went-Ha, Penoza, and Petty sites in Torch Lake. See box plot benchmarks in the caption for Fig. 15.

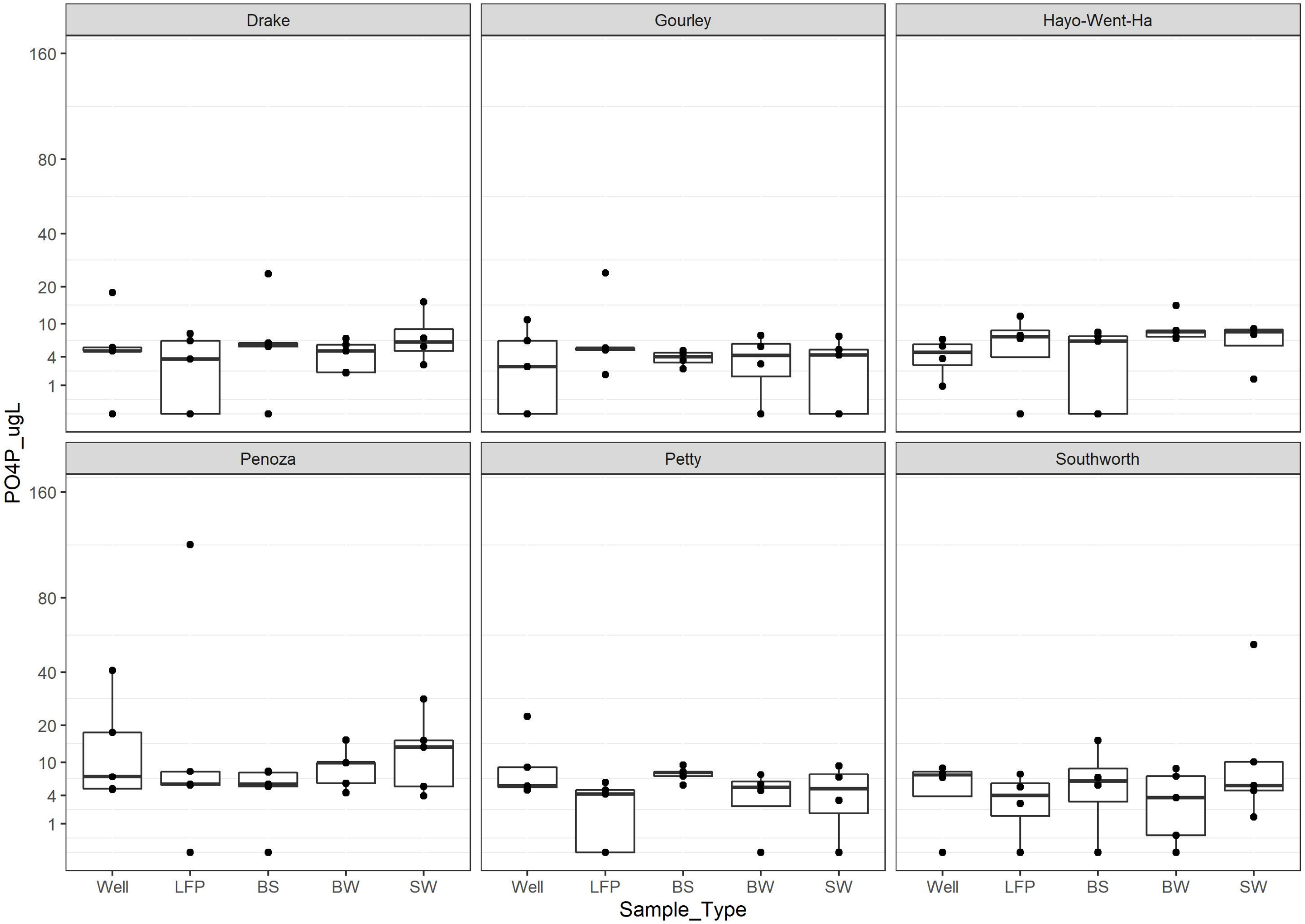

Figure 18. NO3-N concentrations in different water sources in 2016. Water sources and sites sampled are described in the caption for Fig. 21. See box plot benchmarks in the caption for Fig. 15.

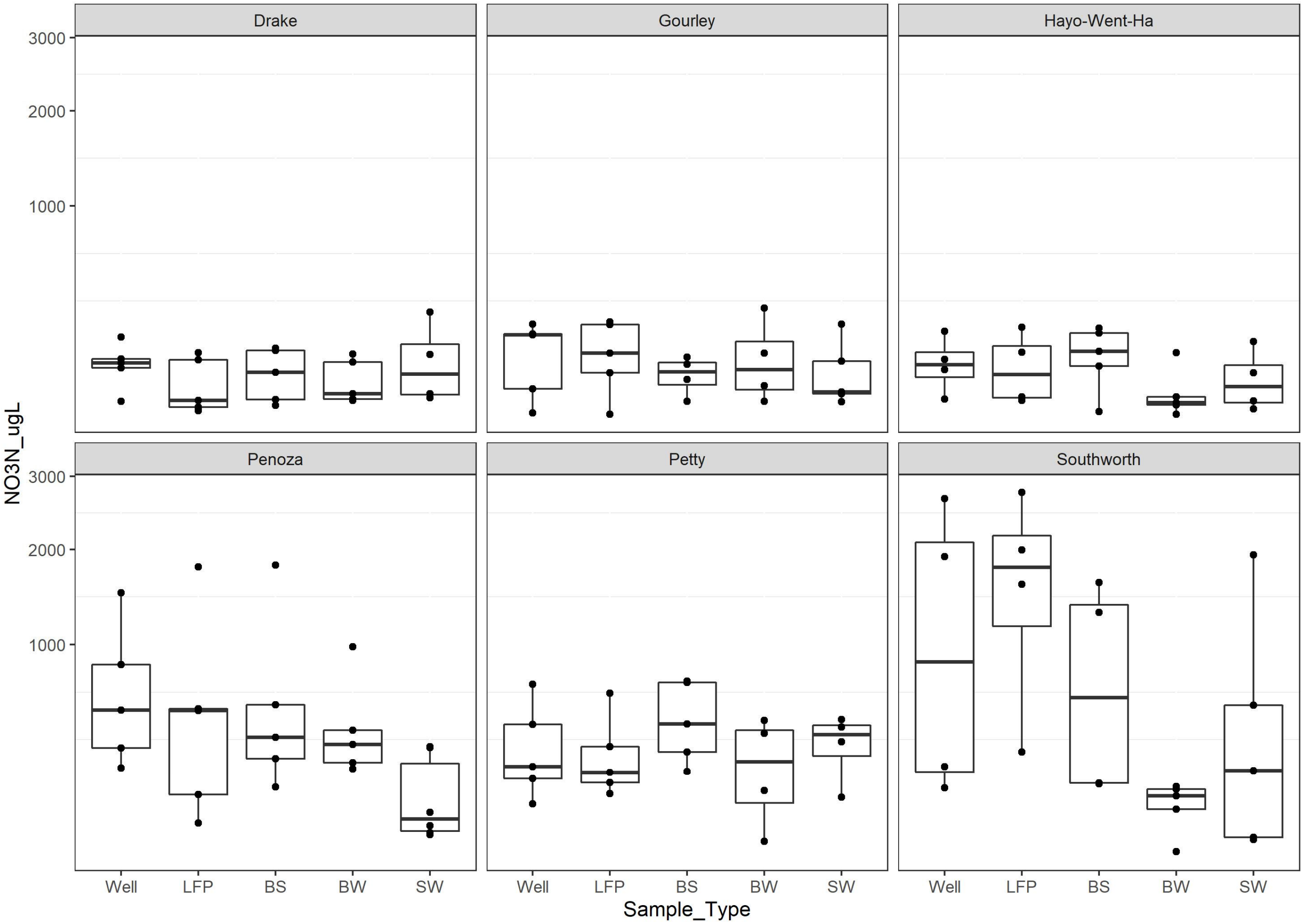

Figure 19. NH4-N concentrations in different water sources in 2016. Water sources and sites sampled are described in the caption for Fig. 21. See box plot benchmarks in the caption for Fig. 15.

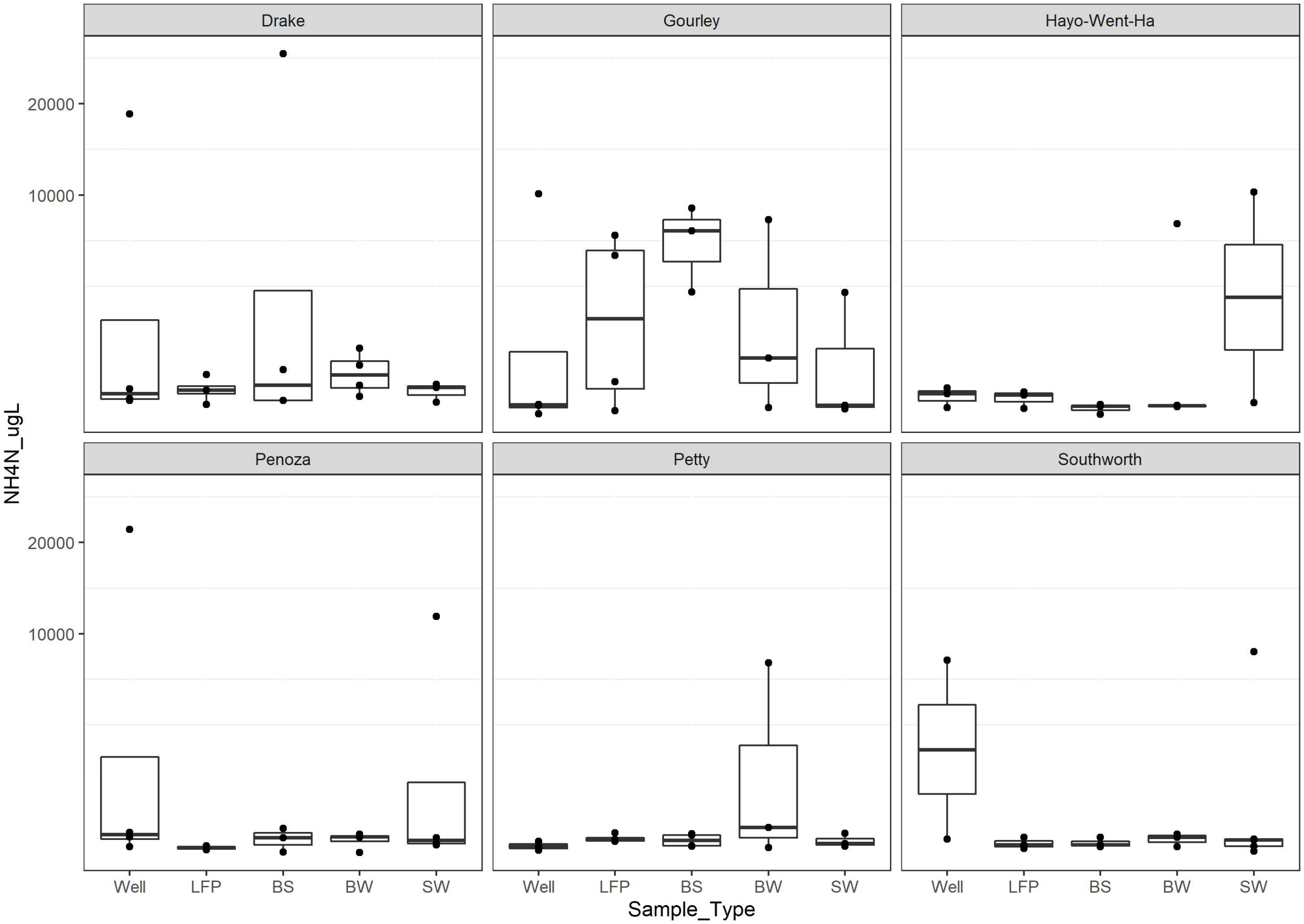

Figure 20. Cl concentrations in different water sources in 2016. Water sources and sites sampled are described in the caption for Fig. 21. See box plot benchmarks in the caption for Fig. 15.

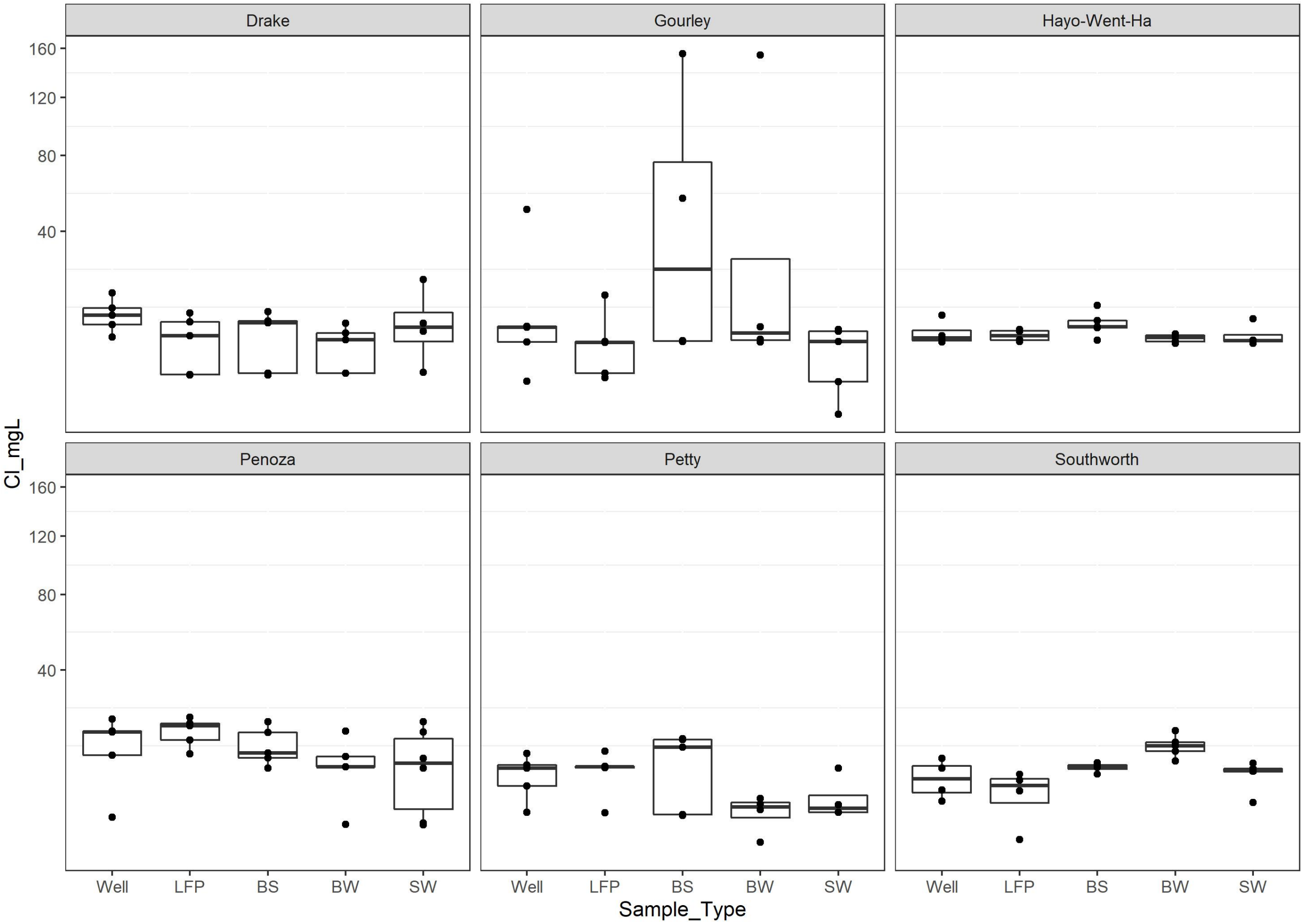

Figure 21. SO4 concentrations in different water sources in 2016. Water sources and sites sampled are described in the caption for Fig. 21. See box plot benchmarks in the caption for Fig. 15.

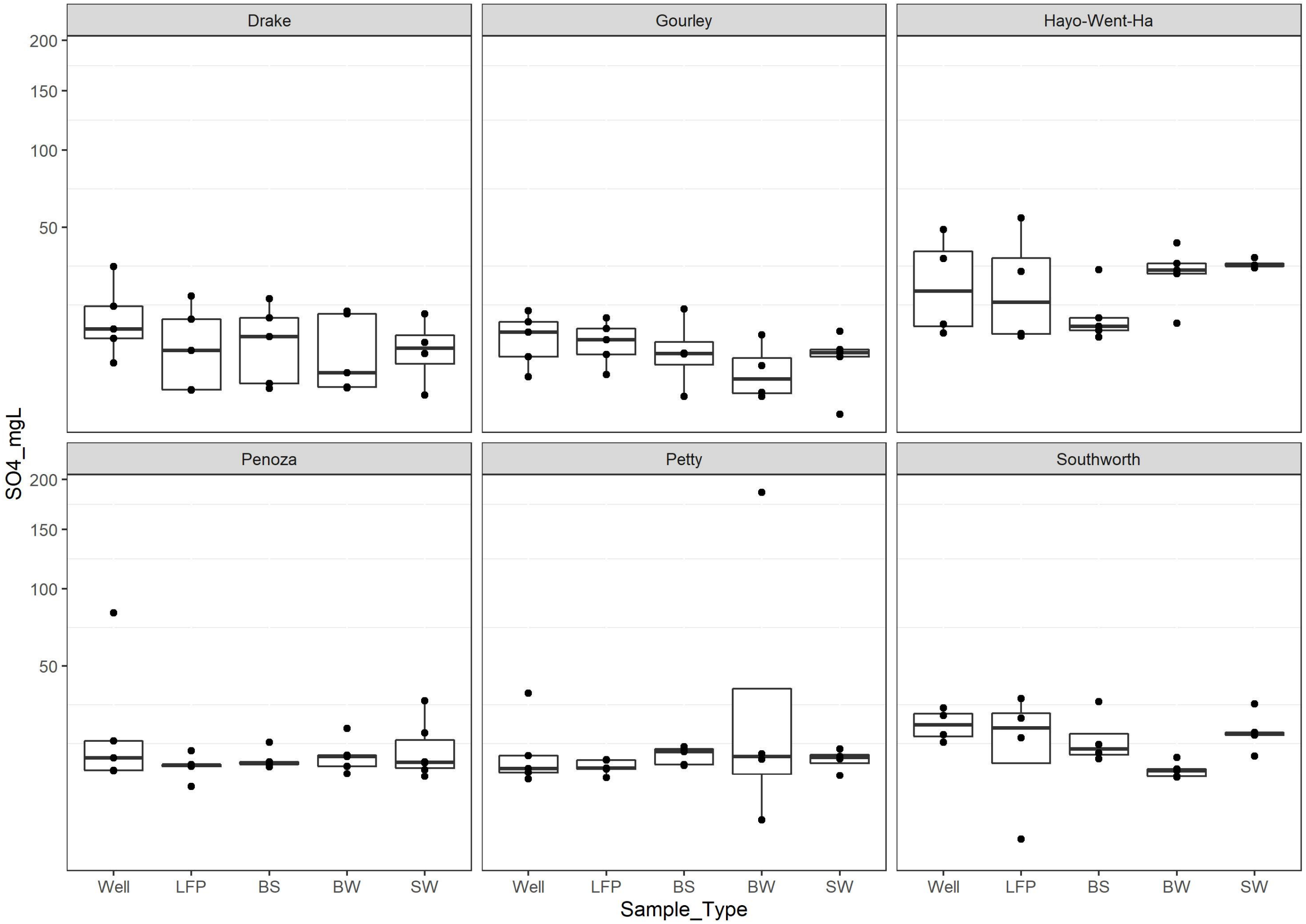

Figure 22. Illustration of peepers with dialysis tubing in PVC tubes, which were used to collect water samples at specific locations on the groundwater – surface water gradient. Concrete block on bottom of the image holds peepers in place for sampling water chemistry at the sand-water interface. The concrete block on sand is covering peepers buried just below to surface to sample water chemistry just below the sand surface.

Figure 23. Changes in PO4-P concentrations in different water sources during the 2017 summer sampled by nearshore surface water grab sample and benthic and sub-benthic peepers (dialysis tubing). Samples were collected in Torch Lake, Lake Bellaire, Elk Lake, and Lake Leelanau in July (6), August (7), and September (8). Linear trends in PO4-P concentration are illustrated and color coded for different water sources.

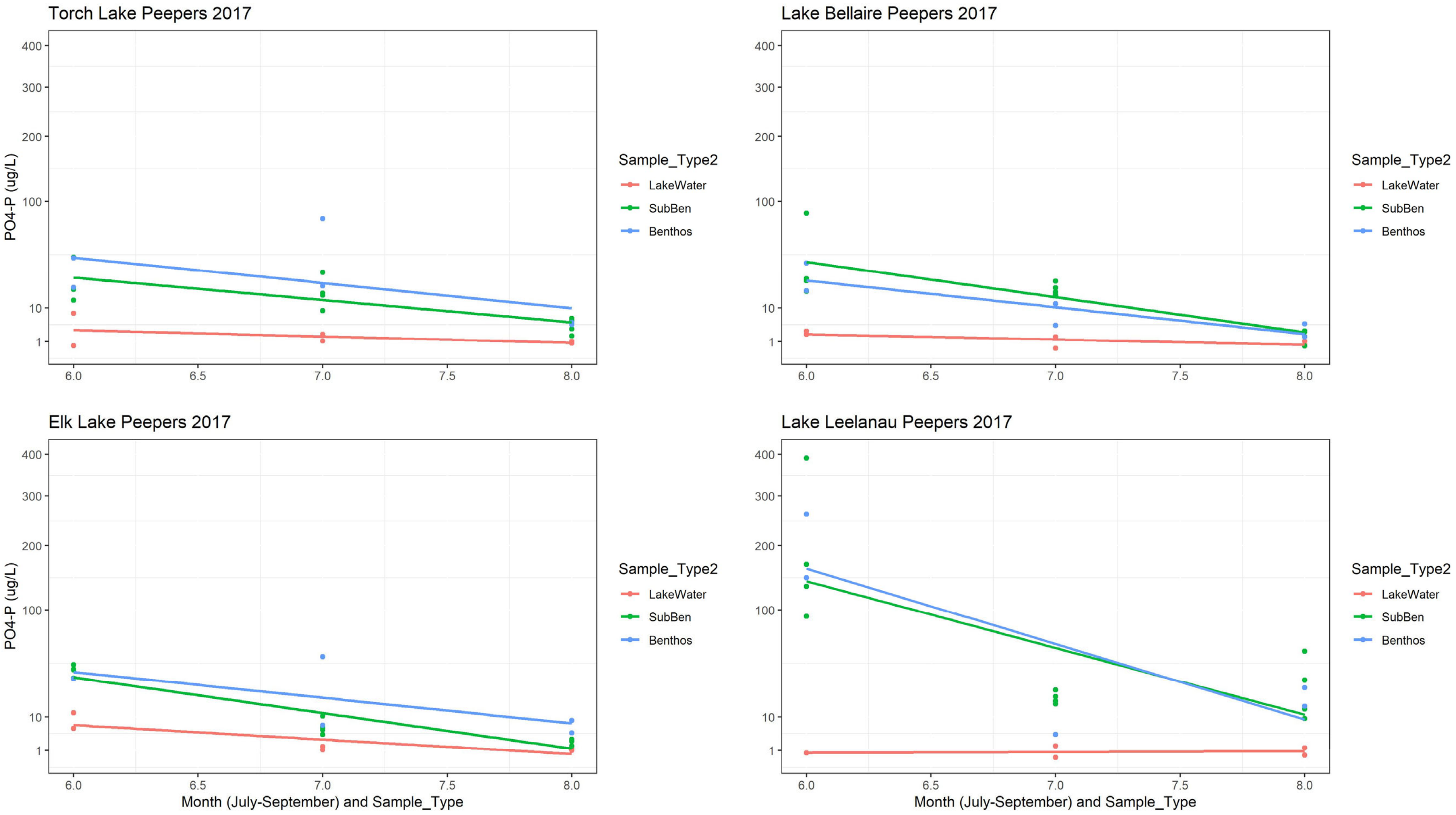

Figure 24. Seasonal patterns in TP concentrations (ug/L) for different water sources and years of sampling at the Gourley location. This illustration serves as an example of data being analyzed and variability in seasonal patterns among water sources and years. Patterns at other sites are not illustrated, but analyses of all seasonal patterns in water chemistry can be found in Table 9.

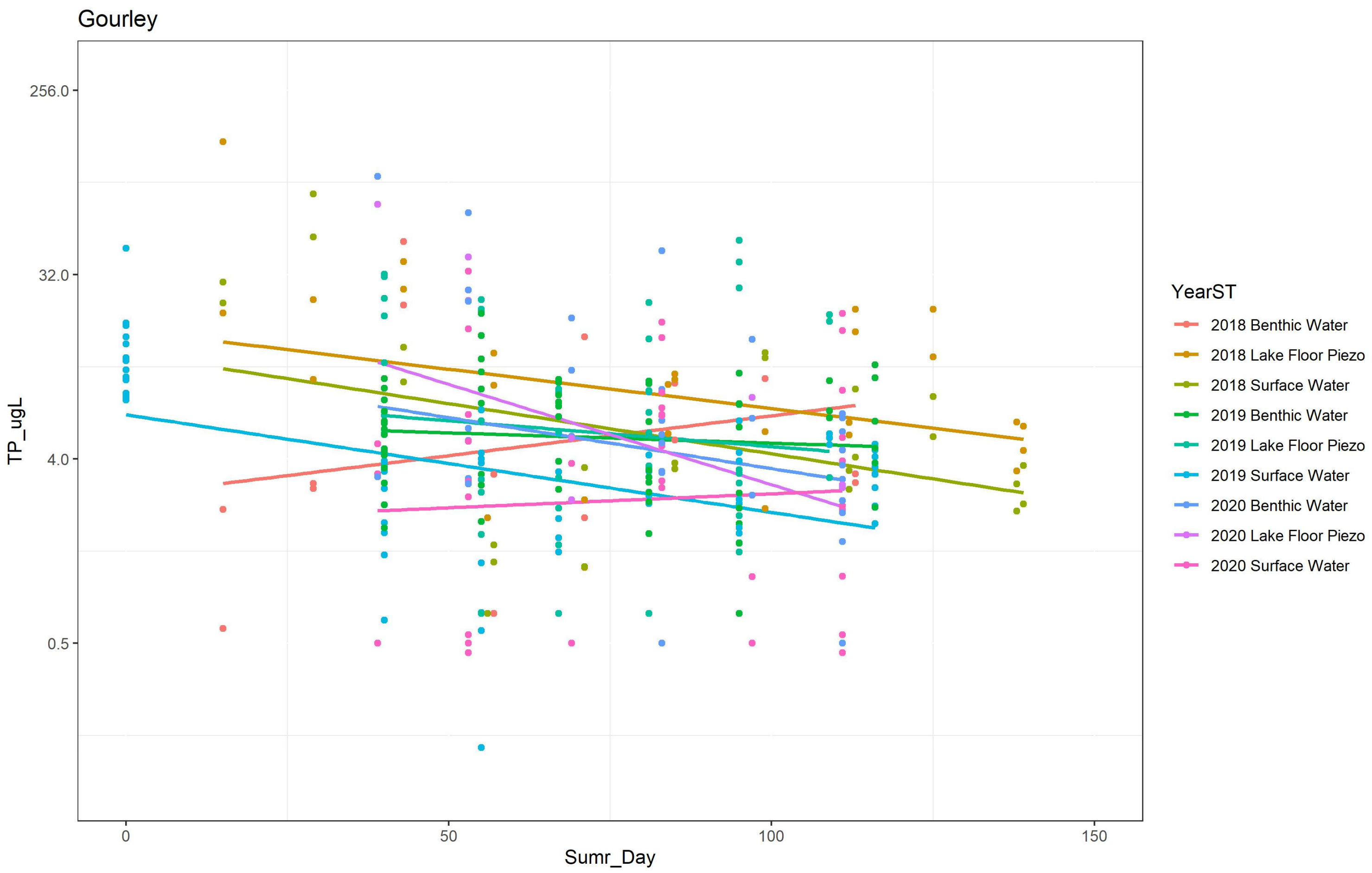

Figure 25. Differences in TP concentrations (ug/L) among sites and water sources sampled during summers from 2018-2020 with central tendency and variability shown by box plots. Data for all months and years of sampling are grouped by site and water source, with x-axis labels including code for lakes and streams (Bellaire, B; Clam, C; Torch, T; Watershed streams, W), specific sites (indicated by color and the legend on the figure), and water source (nearshore surface water, 1SW; benthic pore water, 2BW; and subsurface groundwater from lake floor piezometer, 3LP). See box plot benchmarks in the caption for Fig. 15.

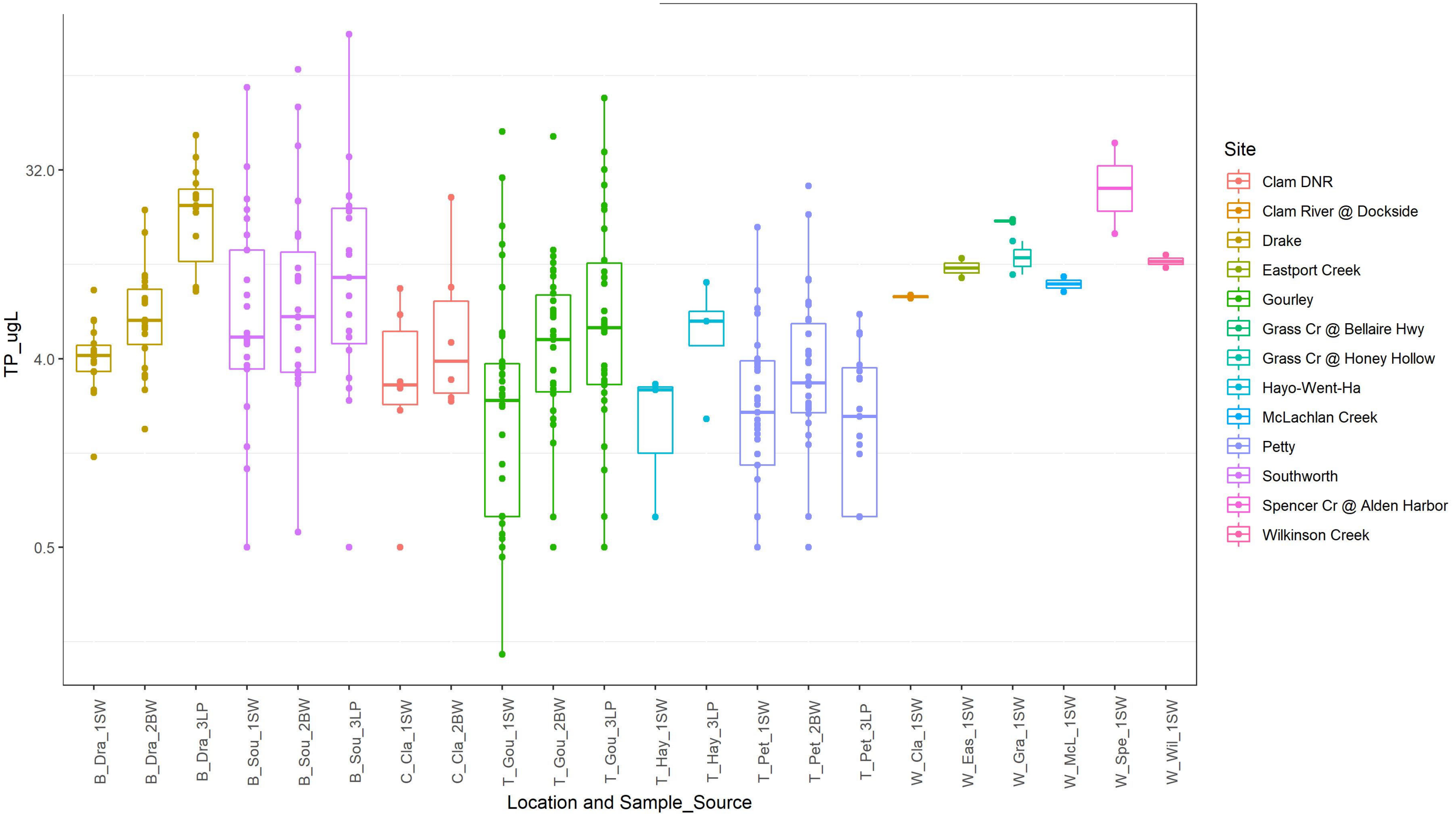

Figure 26. Differences in PO4-P concentrations (ug/L) among sites and water sources sampled during summers from 2018-2020 with central tendency and variability shown by box plots. Data for all months and years of sampling are grouped by site and water source, with x-axis labels including code for lakes and streams (Bellaire, B; Clam, C; Torch, T; Watershed streams, W), specific sites (indicated by color and the legend on the figure), and water source (nearshore surface water, 1SW; benthic pore water, 2BW; and subsurface groundwater from lake floor piezometer, 3LP). See box plot benchmarks in the caption for Fig. 15.

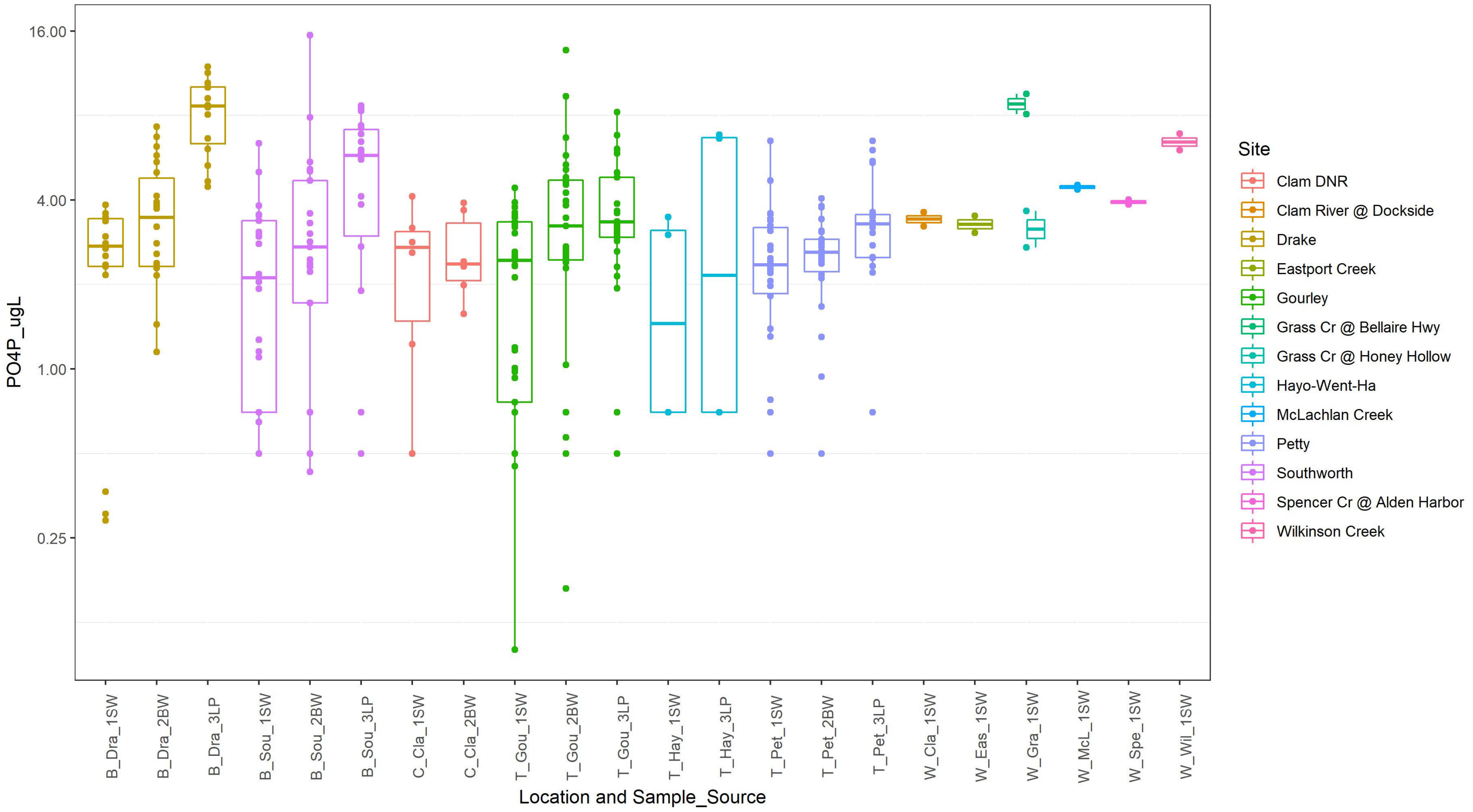

Figure 27. Differences in TN concentrations (ug/L) among sites and water sources sampled during summers from 2018-2020 with central tendency and variability shown by box plots. Data for all months and years of sampling are grouped by site and water source, with x-axis labels including code for lakes and streams (Bellaire, B; Clam, C; Torch, T; Watershed streams, W), specific sites (indicated by color and the legend on the figure), and water source (nearshore surface water, 1SW; benthic pore water, 2BW; and subsurface groundwater from lake floor piezometer, 3LP). See box plot benchmarks in the caption for Fig. 15.

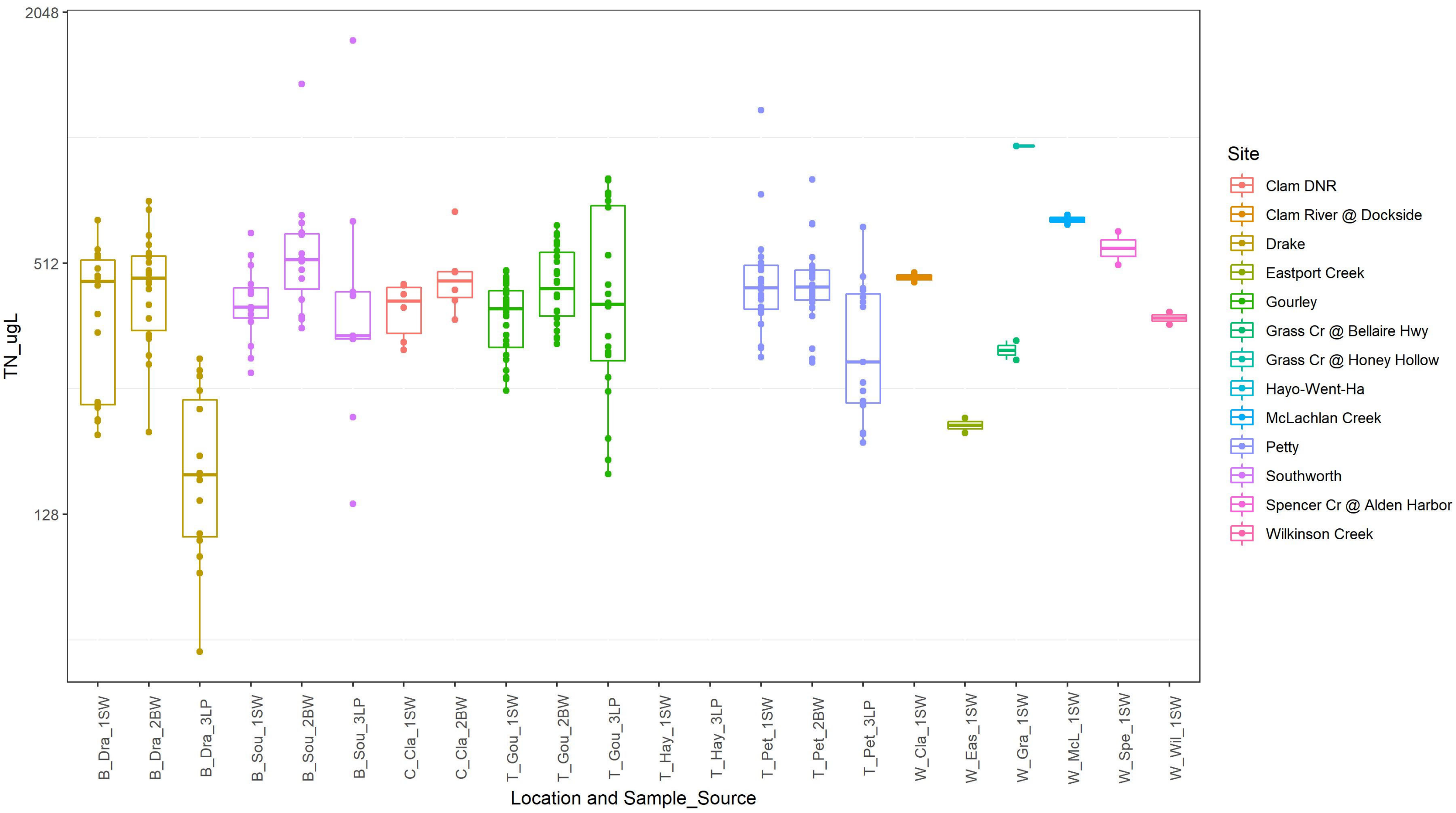

Figure 28. Differences in NO3-N concentrations (ug/L) among sites and water sources sampled during summers from 2018-2020 with central tendency and variability shown by box plots. Data for all months and years of sampling are grouped by site and water source, with x-axis labels including code for lakes and streams (Bellaire, B; Clam, C; Torch, T; Watershed streams, W), specific sites (indicated by color and the legend on the figure), and water source (nearshore surface water, 1SW; benthic pore water, 2BW; and subsurface groundwater from lake floor piezometer, 3LP). See box plot benchmarks in the caption for Fig. 15.

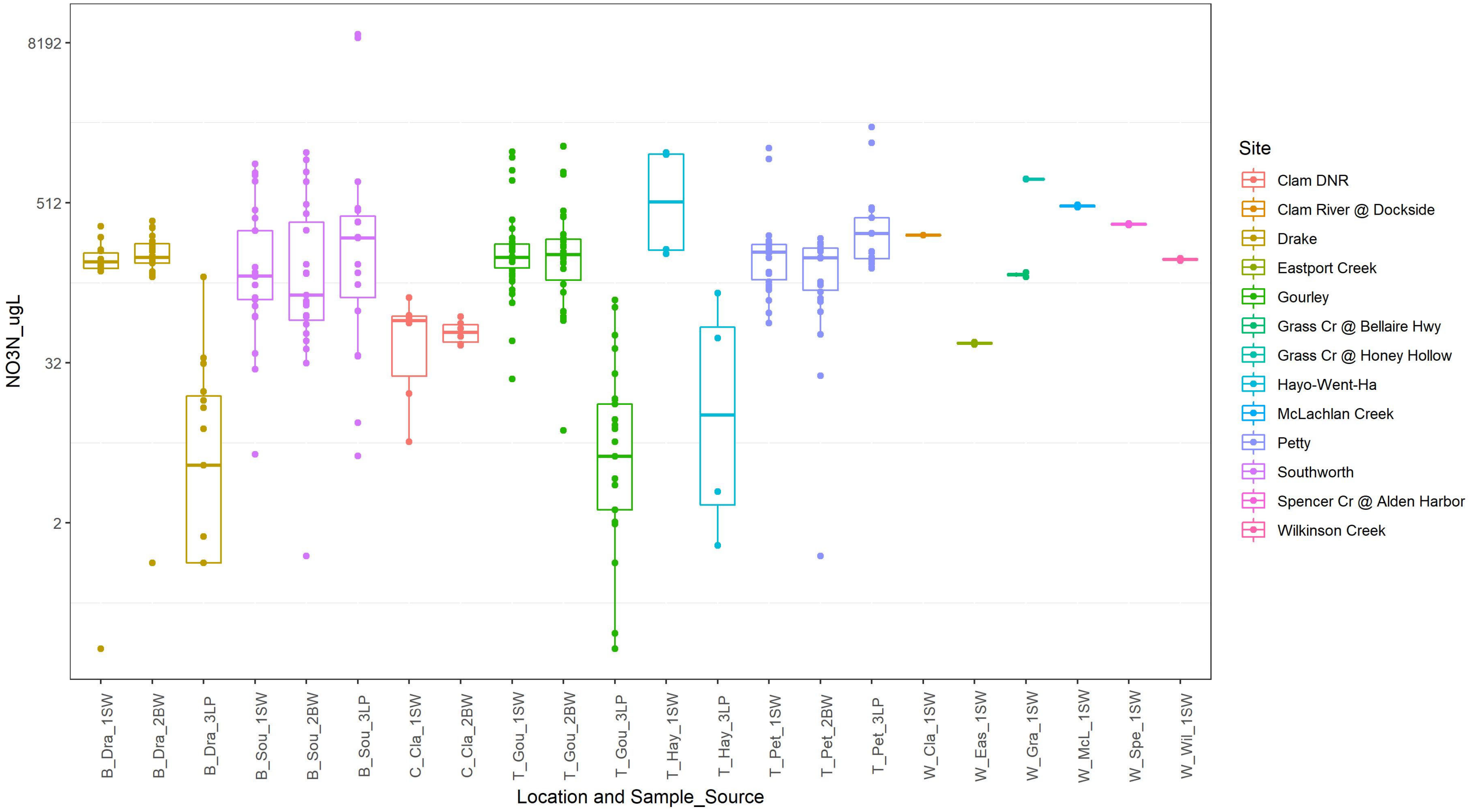

Figure 29. Differences in NH4-N concentrations (ug/L) among sites and water sources sampled during summers from 2018-2020 with central tendency and variability shown by box plots. Data for all months and years of sampling are grouped by site and water source, with x-axis labels including code for lakes and streams (Bellaire, B; Clam, C; Torch, T; Watershed streams, W), specific sites (indicated by color and the legend on the figure), and water source (nearshore surface water, 1SW; benthic pore water, 2BW; and subsurface groundwater from lake floor piezometer, 3LP). See box plot benchmarks in the caption for Fig. 15.

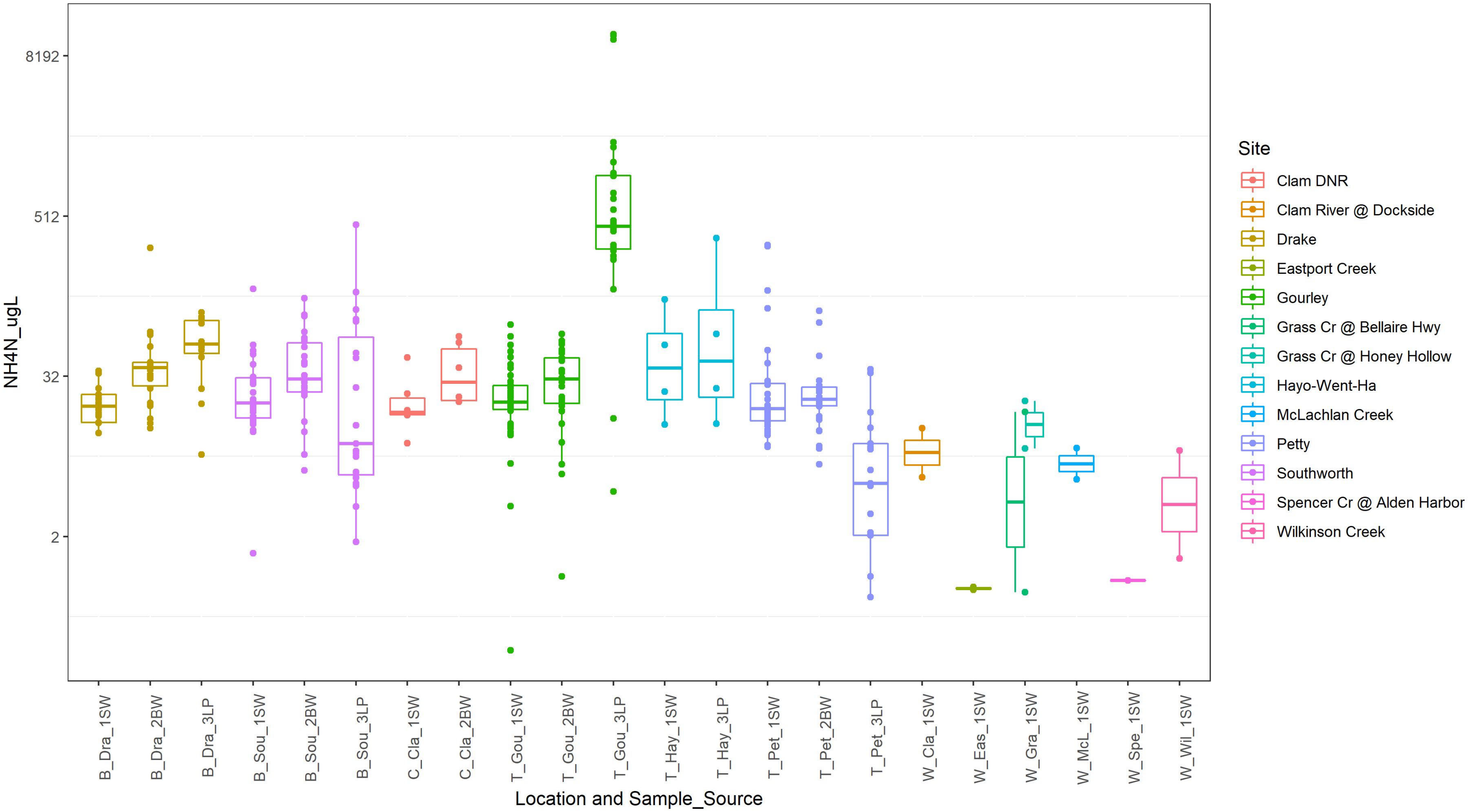

Figure 30. Differences in chloride (Cl) concentrations (mg/L) among sites and water sources sampled during summers from 2018-2020 with central tendency and variability shown by box plots. Data for all months and years of sampling are grouped by site and water source, with x-axis labels including code for lakes and streams (Bellaire, B; Clam, C; Torch, T; Watershed streams, W), specific sites (indicated by color and the legend on the figure), and water source (nearshore surface water, 1SW; benthic pore water, 2BW; and subsurface groundwater from lake floor piezometer, 3LP). See box plot benchmarks in the caption for Fig. 15.

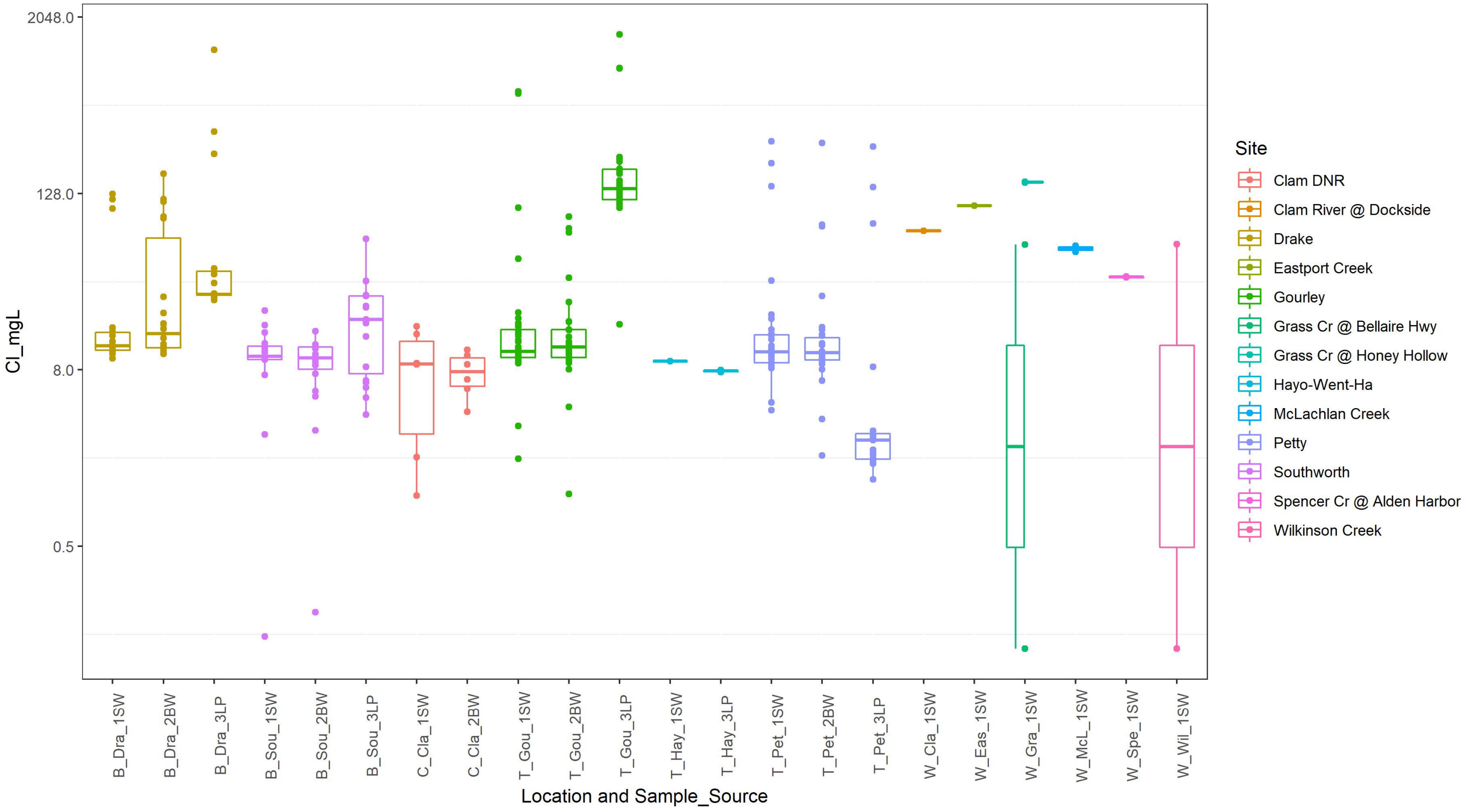

Figure 31. Differences in sulfate (SO4) concentrations (mg/L) among sites and water sources sampled during summers from 2018-2020 with central tendency and variability shown by box plots. Data for all months and years of sampling are grouped by site and water source, with x-axis labels including code for lakes and streams (Bellaire, B; Clam, C; Torch, T; Watershed streams, W), specific sites (indicated by color and the legend on the figure), and water source (nearshore surface water, 1SW; benthic pore water, 2BW; and subsurface groundwater from lake floor piezometer, 3LP). See box plot benchmarks in the caption for Fig. 15.

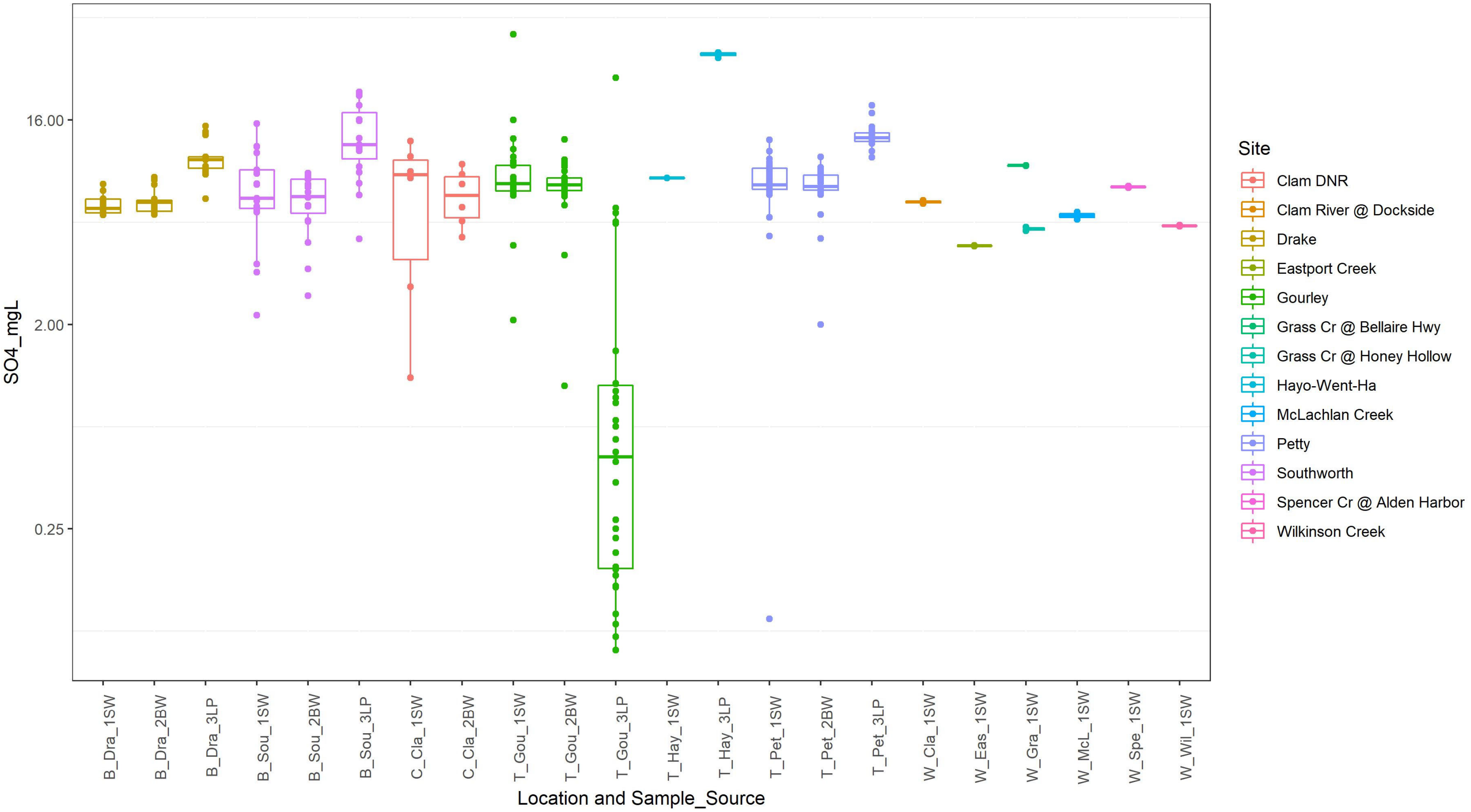

Figure 32. Boron concentration, boron stable isotope ratio (δ^{11}B), and boron:chloride (B:Cl) ratios of water sampled during summer 2017. a) on the right is a 3-dimensional plot of water samples as a function of B (ug/L), δ^{11}B, and B:Cl. b) on the right is a 2-dimensional plot that provides clearer resolution of B (ug/L) on the vertical axis and δ^{11}B on the horizontal axis. These figures were produced by Tim Veverica and reproduced with his permission.

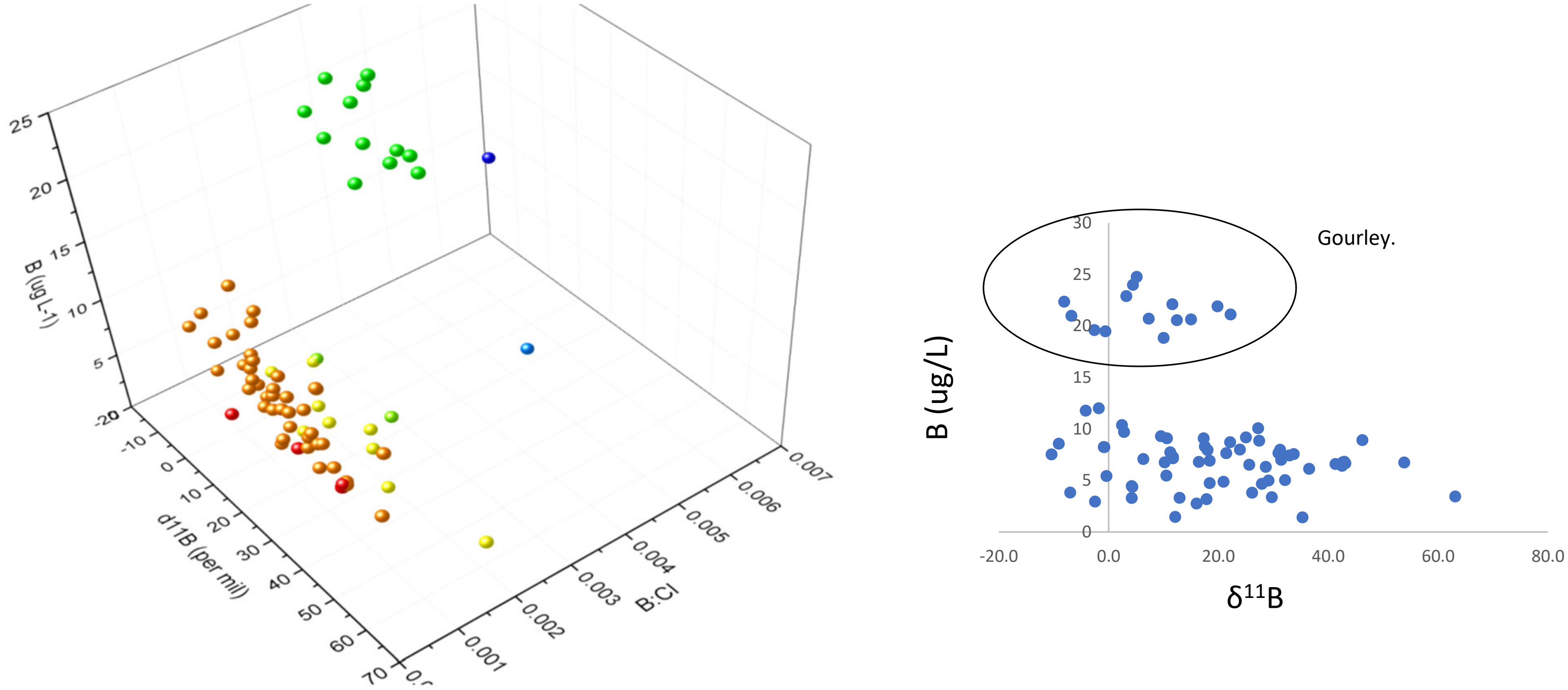

Figure 33. Information to interpret the significance of boron concentration, boron stable isotope ratio ($\delta^{11}B$), and boron:chloride (B:Cl) ratios in water. a) The figure on the left shows the $\delta^{11}B$ in waters from different sources (Vengosh et al., 1998) The table on the left shows B and B:Cl characteristics of water from different sources (Dotsika et al. 2006). Tim Veverica provided this figure and table.

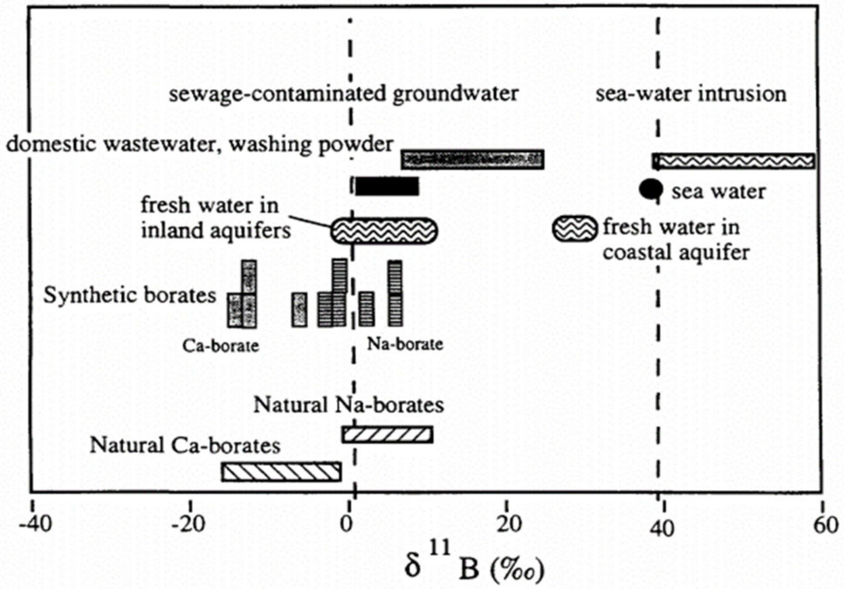

Fig. 1. $\delta^{11}B$ values and range. Note the large difference between sea water and fresh ground water; also ^{11}B depletion in Ca-borate versus Na-borate. Industrial products mimic the isotopic composition of their parent raw materials.

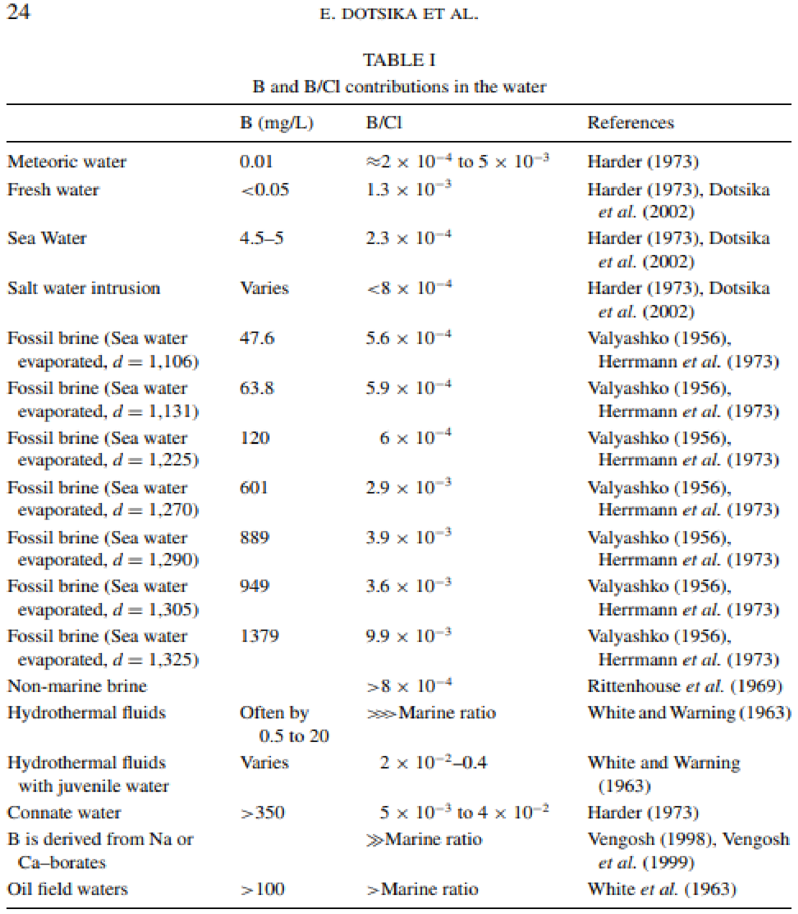
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TABLE I

B and B/Cl contributions in the water

	B (mg/L)	B/Cl	References
Meteoric water	0.01	$\approx 2 \times 10^{-4}$ to 5×10^{-3}	Harder (1973)
Fresh water	<0.05	1.3×10^{-3}	Harder (1973), Dotsika *et al.* (2002)
Sea Water	4.5–5	2.3×10^{-4}	Harder (1973), Dotsika *et al.* (2002)
Salt water intrusion	Varies	$<8 \times 10^{-4}$	Harder (1973), Dotsika *et al.* (2002)
Fossil brine (Sea water evaporated, $d = 1{,}106$)	47.6	5.6×10^{-4}	Valyashko (1956), Herrmann *et al.* (1973)
Fossil brine (Sea water evaporated, $d = 1{,}131$)	63.8	5.9×10^{-4}	Valyashko (1956), Herrmann *et al.* (1973)
Fossil brine (Sea water evaporated, $d = 1{,}225$)	120	6×10^{-4}	Valyashko (1956), Herrmann *et al.* (1973)
Fossil brine (Sea water evaporated, $d = 1{,}270$)	601	2.9×10^{-3}	Valyashko (1956), Herrmann *et al.* (1973)
Fossil brine (Sea water evaporated, $d = 1{,}290$)	889	3.9×10^{-3}	Valyashko (1956), Herrmann *et al.* (1973)
Fossil brine (Sea water evaporated, $d = 1{,}305$)	949	3.6×10^{-3}	Valyashko (1956), Herrmann *et al.* (1973)
Fossil brine (Sea water evaporated, $d = 1{,}325$)	1379	9.9×10^{-3}	Valyashko (1956), Herrmann *et al.* (1973)
Non-marine brine		$>8 \times 10^{-4}$	Rittenhouse *et al.* (1969)
Hydrothermal fluids	Often by 0.5 to 20	⋙Marine ratio	White and Warning (1963)
Hydrothermal fluids with juvenile water	Varies	2×10^{-2}–0.4	White and Warning (1963)
Connate water	>350	5×10^{-3} to 4×10^{-2}	Harder (1973)
B is derived from Na or Ca–borates		≫Marine ratio	Vengosh (1998), Vengosh *et al.* (1999)
Oil field waters	>100	>Marine ratio	White *et al.* (1963)

Figure 34. Acesulfame potassium concentrations (Ace K ug/L) in nearshore surface water (SW), benthic pore water (BPW), lake shore piezometer (LFP), and onshore (OS) at the Gourley (Gour), Southworth (South), Hayo-Went-Ha (HWH), and Petty sites during summer 2018. Onshore samples were collected from seeps or a piezometer. 1 was added to Ace K concentrations so they could be plotted on a log scale to spread out the range of low concentrations for illustration of differences among sites.

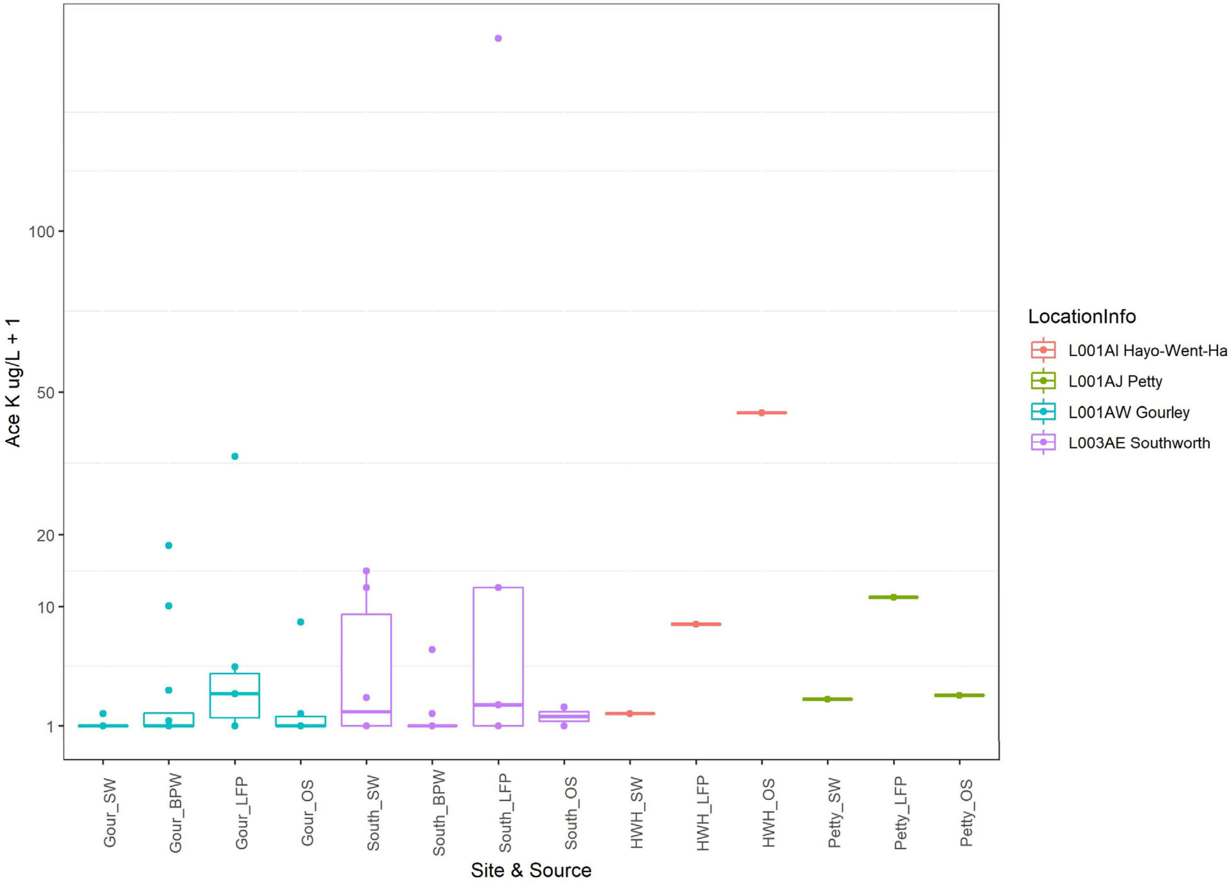
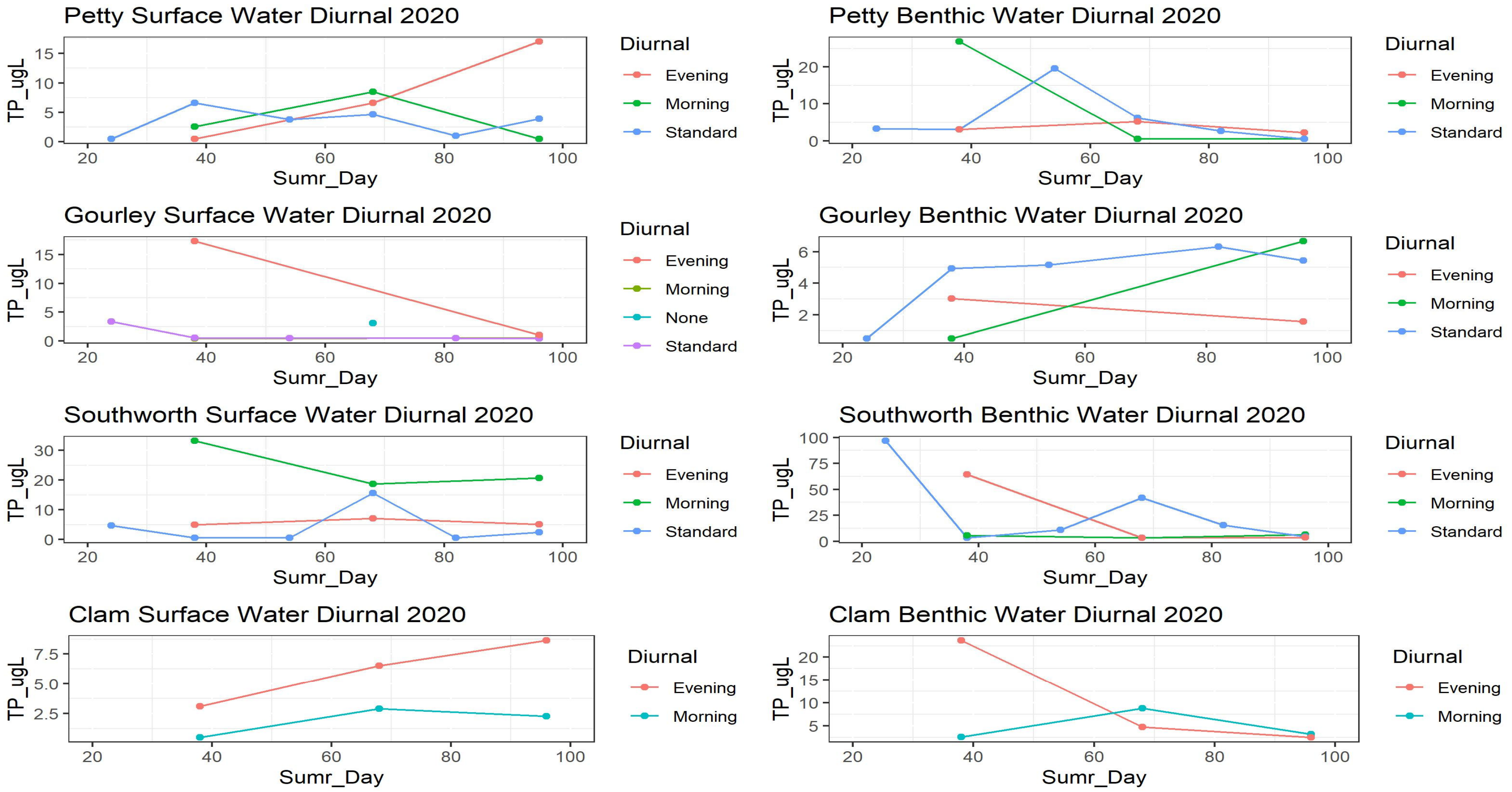


Figure 35. Total phosphorus concentrations sampled during summer 2020 at four locations (Petty, Gourley, Southworth, and Clam), from two water sources (nearshore surface water and benthic pore water), and at 3 times per day. Sampling early in the morning and in the evening was intended to determine the effect of biological activity at the sediment water interface on shallow nearshore water. The standard sampling time was the normal mid-day sampling time for the ongoing water chemistry survey.



Petty Surface Water Diurnal 2020

Petty Benthic Water Diurnal 2020

Gourley Surface Water Diurnal 2020

Gourley Benthic Water Diurnal 2020

Southworth Surface Water Diurnal 2020

Southworth Benthic Water Diurnal 2020

Clam Surface Water Diurnal 2020

Clam Benthic Water Diurnal 2020

Figure 36. PO4-P concentrations (here referenced as soluble reactive phosphorus, SRP) sampled during summer 2020 at four locations (Petty, Gourley, Southworth, and Clam), from two water sources (nearshore surface water and benthic pore water), and at 3 times per day. Sampling early in the morning and in the evening was intended to determine the effect of biological activity at the sediment water interface on shallow nearshore water. The standard sampling time was the normal mid-day sampling time for the ongoing water chemistry survey.

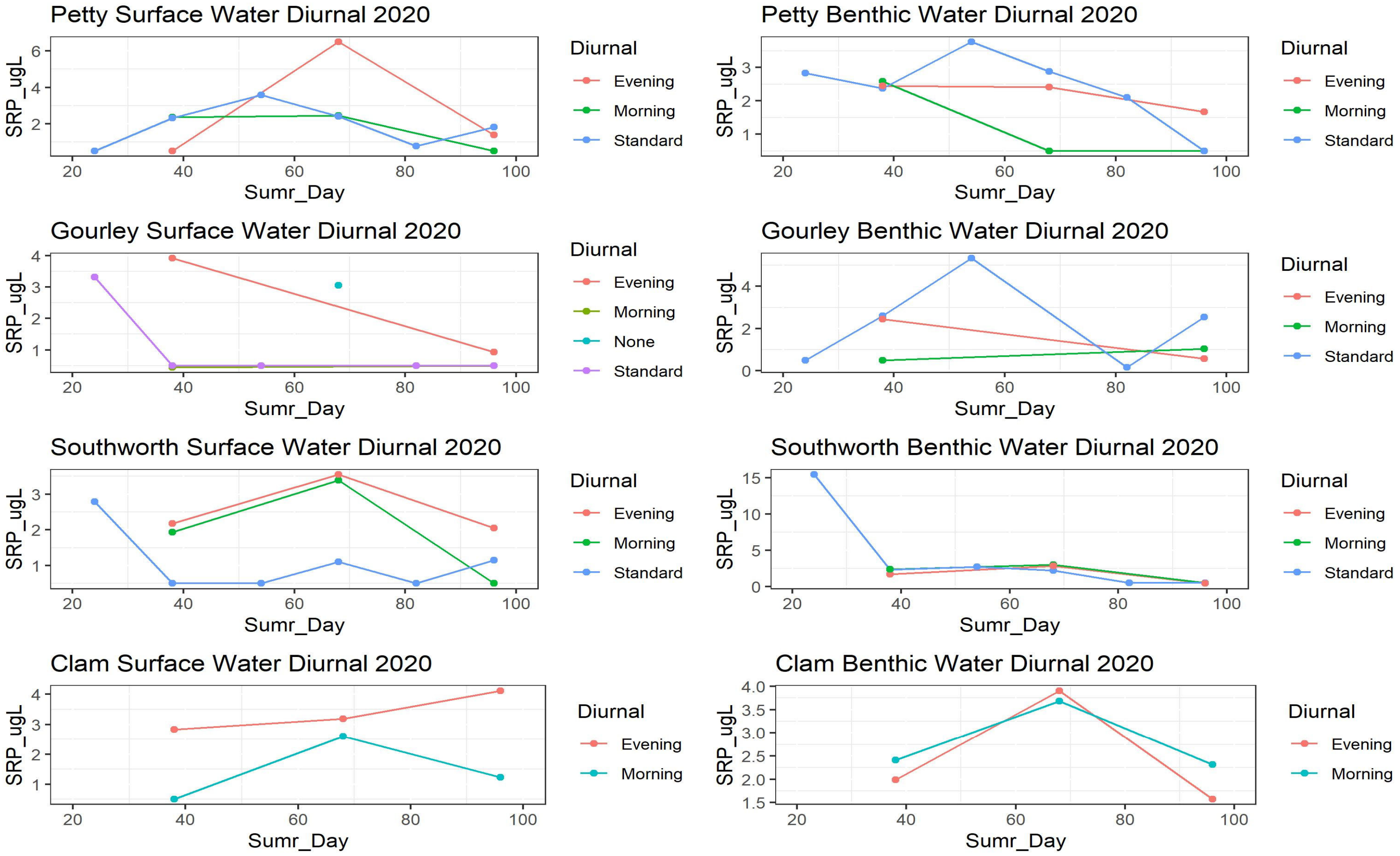


Figure 37. The change in water chemistry values from morning to night (indicated by the d preceding the chemical name and units of measurement) in nearshore surface water (eight panels on the left) and benthic pore water (eight panels on the right) at 4 sites during summer 2020. The horizontal line in graphs marks zero change. Box plots are described in Figure 15 and show central tendency and variation in chemistry changes for the multiple sampling times during the summer. A negative change, less than zero, indicates water chemistry concentrations decreased during the day, which expected for nutrients being depleted by photosynthesizing benthic algae.

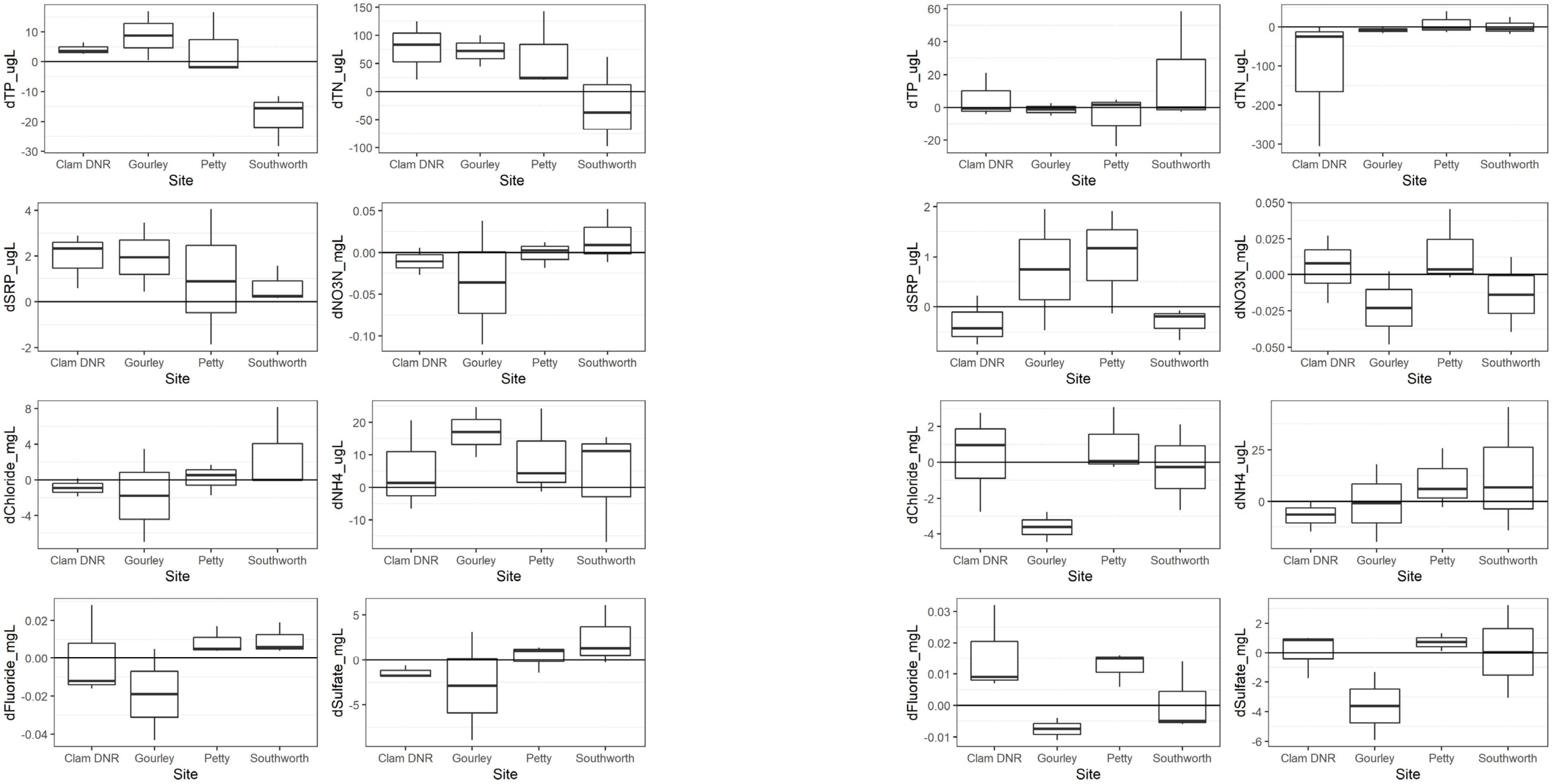


Figure 38. Changes in 8 water chemistry patterns during summer 2020 at two open lake sites (the North and South Torch Lake sampling sites) and three nearshore sites sampled multiple times per day. The lines (blue for open lake and red for nearshore) indicate the linear estimates of changes in water chemistry in relationship to summer day (number of days after May 15, Sumr_Day).

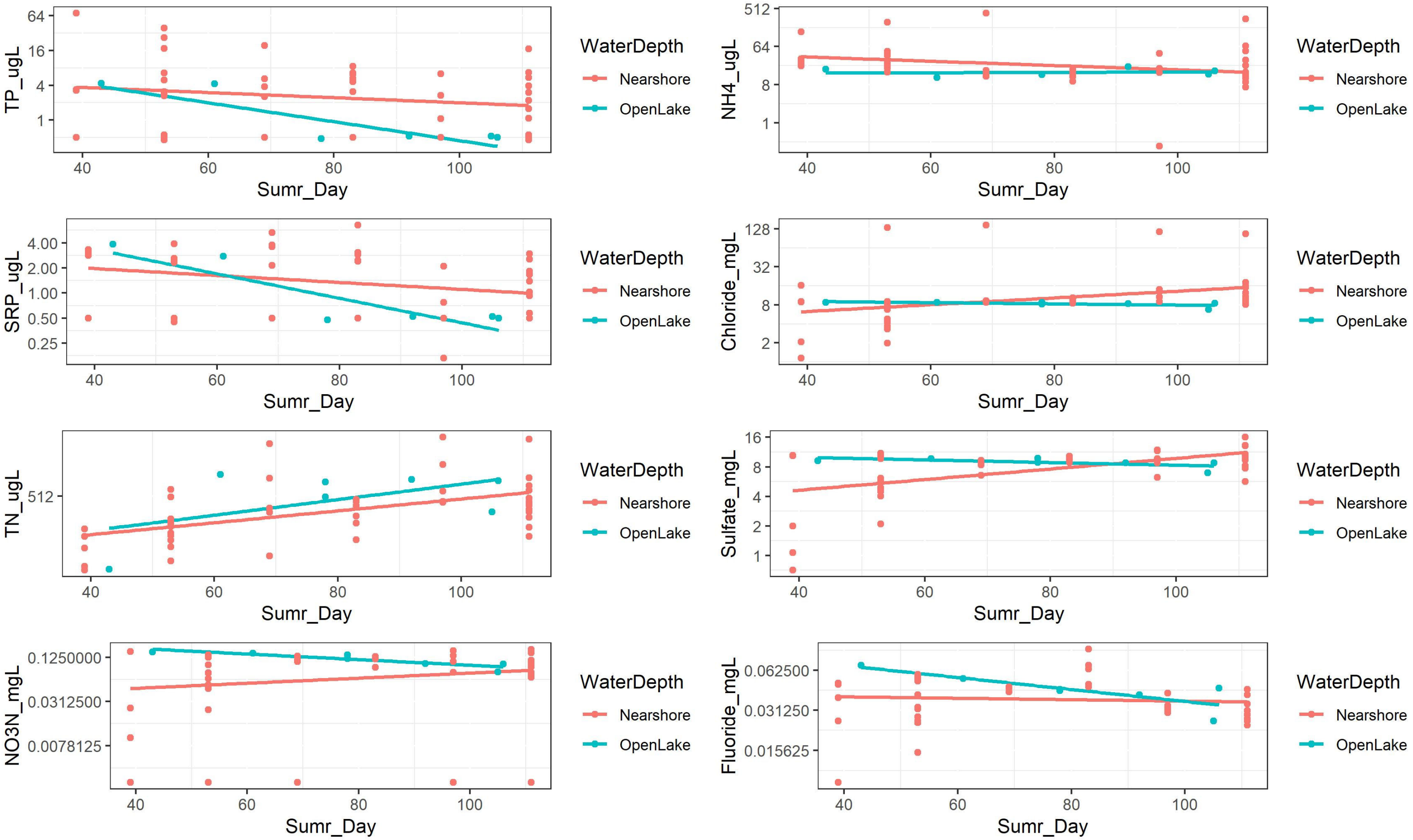


Figure 39. Changes in diatom species composition during the 2017 summer at three Torch Lake sites. Cumulative relative abundances (stacked bar charts) show the change in relative abundance of 15 diatom taxa: *Achnanthidium minutissimum, Achnanthidium caledonicum, Achnanthidium rosenstockii, Navicula cryptotenella* group, *Gomphonema* sp. 1*, Nitzschia amphibia, Amphora pediculus, Delicata delicatula, Encyonema evergladianum, Encyonopsis subminuta, Fragilaria* sp. 1*, Fragilaria vaucheriae group, Euccocconeis* spp., and Centrales. Codes for taxa in the legend constructed with the first 3 letters of the genus name and then as much of the species name as allowed for eight characters. The height of the color coded bar indicates the proportional (0-1) relative abundance of each taxon.

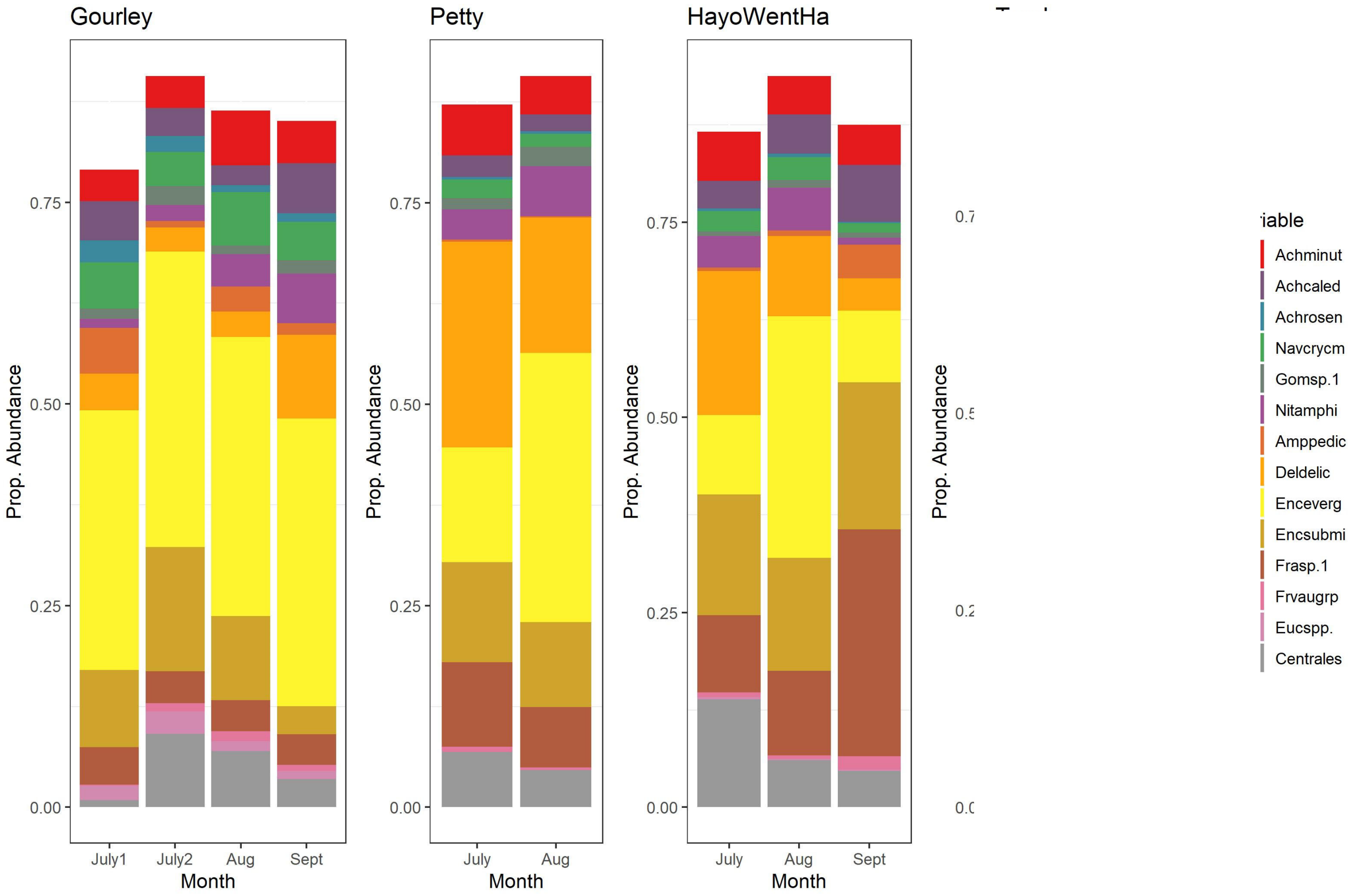


Figure 40. Changes in diatom species composition during the 2017 summer in Lake Bellaire, Elk Lake, and Lake Leelanau. Cumulative relative abundances (stacked bar charts) show the change in relative abundance of 14 diatom taxa. The code for taxa listed in the legend can be found in the caption of Fig. 43, as well as the list of possible species.

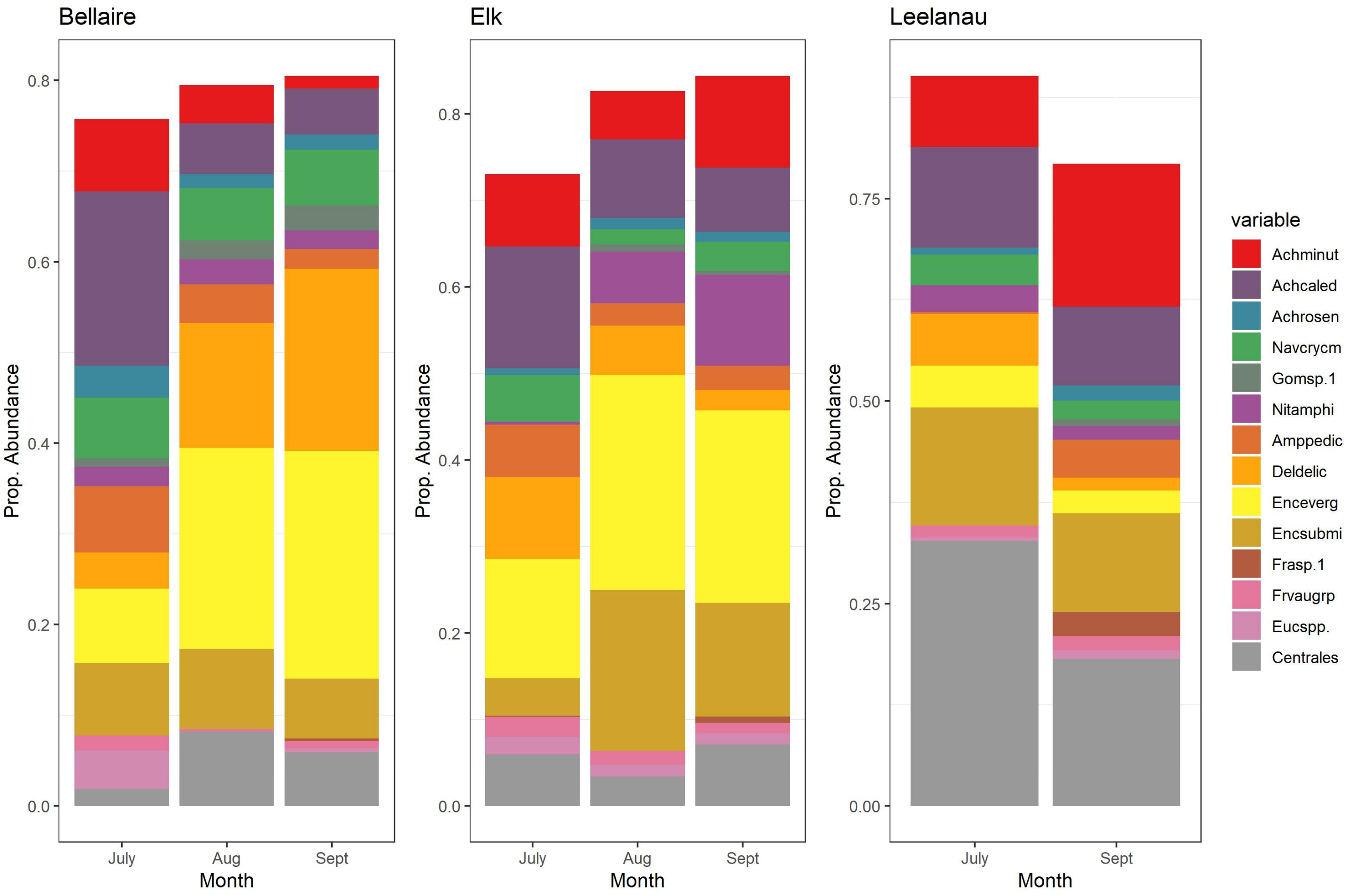
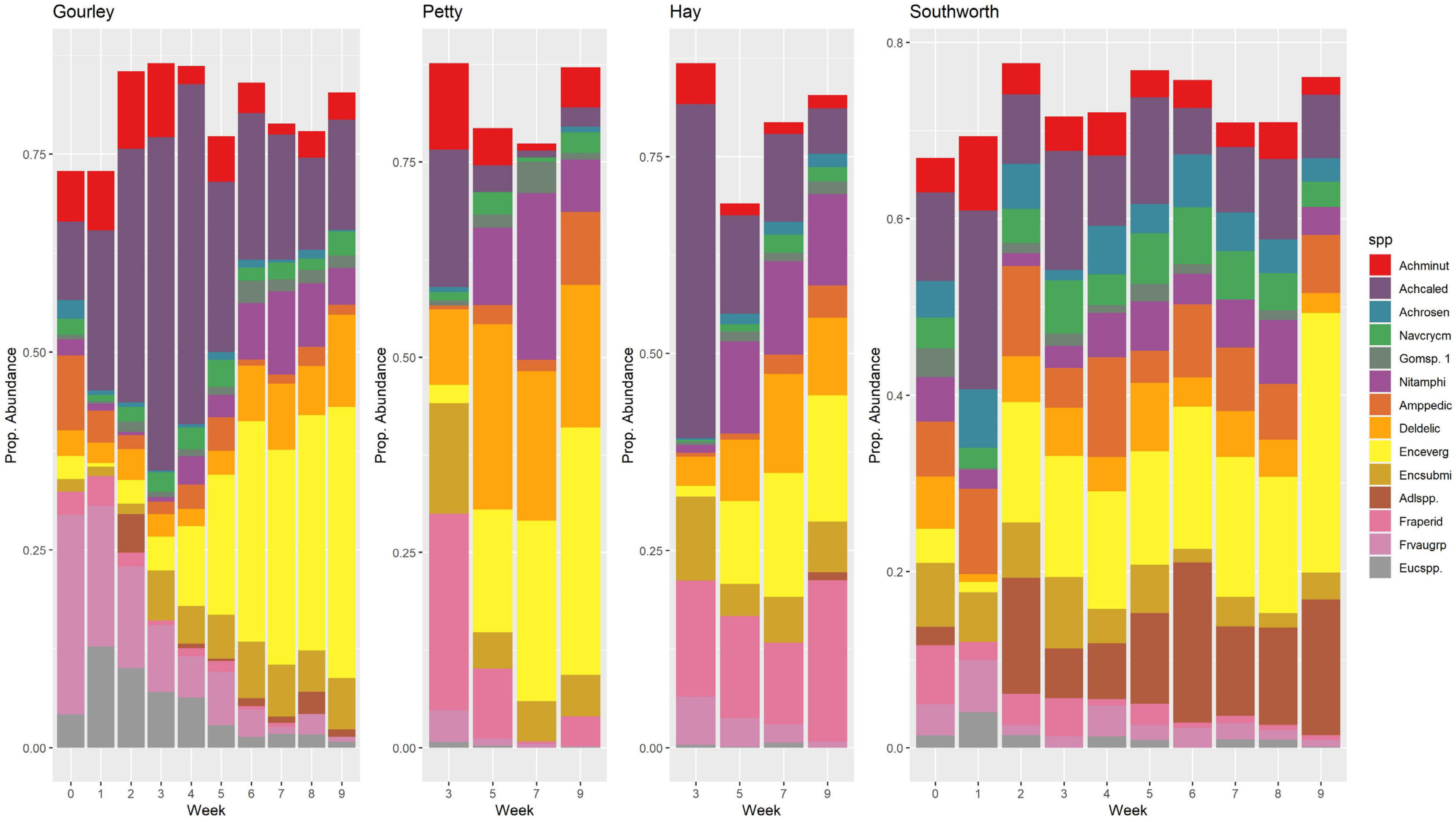


Figure 41. Changes in diatom species composition during the 2018 summer at four locations. Cumulative relative abundances (stacked bar charts) show the change in relative abundance of 14 diatom taxa. The code for taxa listed in the legend can be found in the caption of Fig. 43, as well as the list of possible species except for 2 new taxa: *Adlafia* spp. and *Fragilaria perdelicatissima*.



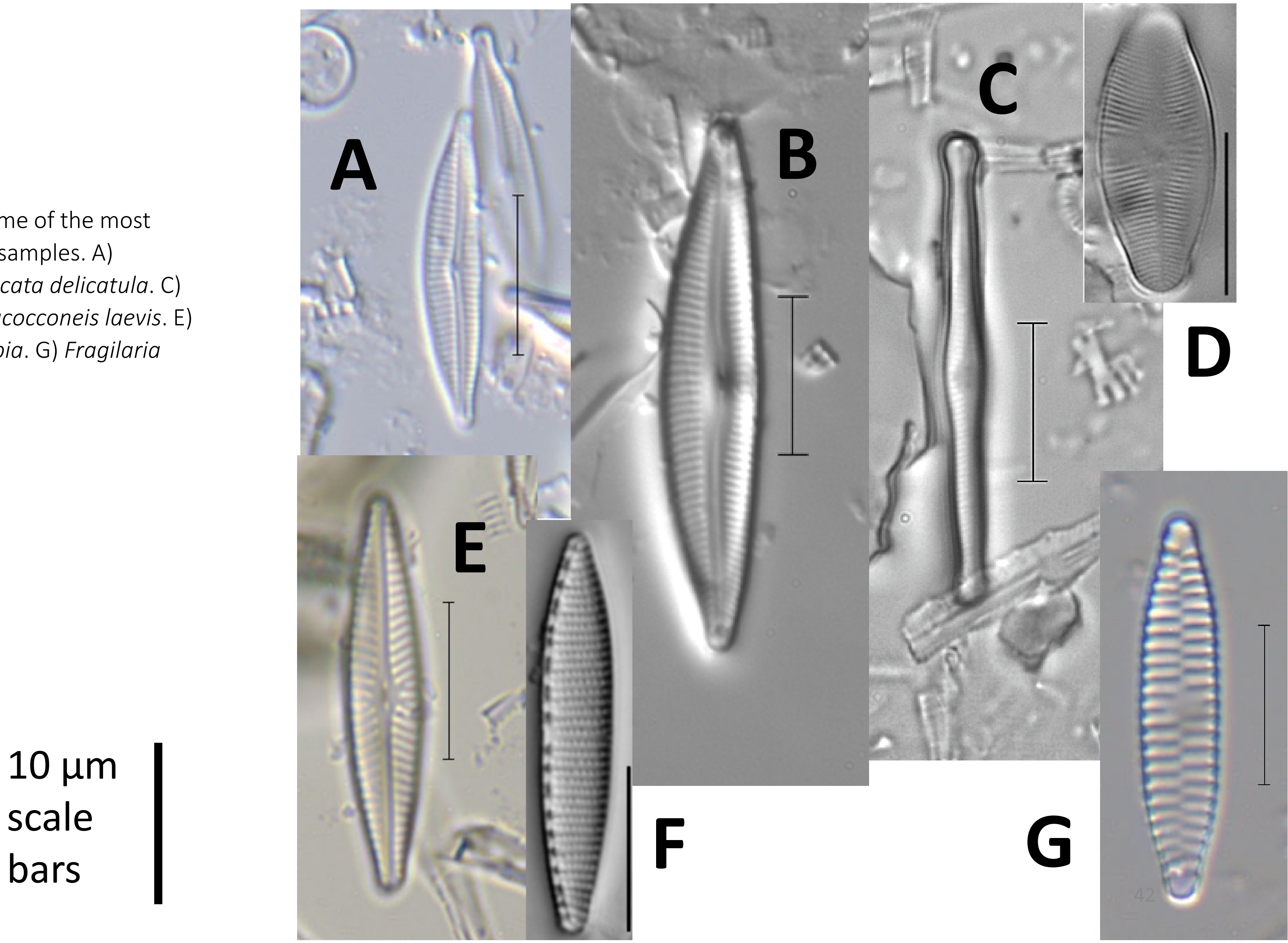


Figure 42. Photomicrographs of some of the most abundant diatoms in benthic algal samples. A) *Encyonema evergladianum*. B) *Delicata delicatula*. C) *Achnanthidium caledonicum*. D) *Eucocconeis laevis*. E) *Navicula wildii*. F) *Nitzschia amphibia*. G) *Fragilaria vaucheriae*.

Figure 43. The waterbath, microcosms, shade cover (image B), and water drip system (image B) used to determine diatom responses to nutrient enrichment via surface and groundwater.

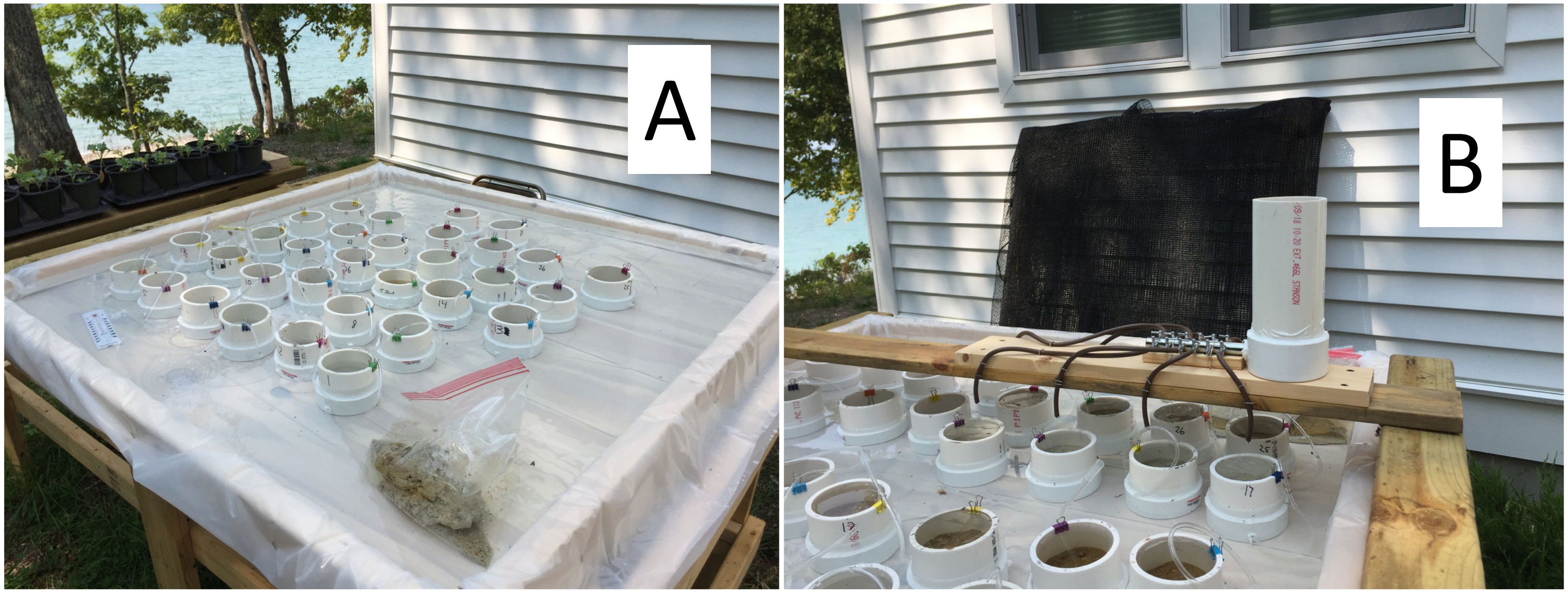


Figure 44. Responses of most common diatoms to top and bottom enrichment (TB) by both phosphate and nitrate. Box plots (explained in earlier figure caption) illustrate central tendency and variation of species proportional relative abundance in the two treatments. The greater the separation of boxes for the two treatments, the lower the likelihood that species relative abundances in the control and TB treatment were the same. The 10 species shown are: *Encyonema evergladianum, Delicata delicatula, Achnanthidium calidonicum, Nitzschia amphibia, Encyonopsis subminuta, Mastogloia lacustris, Gomphonema* sp. 1, *Amphora pediculus, Cymbopleura subaequalis*, and *Navicula wildii*.

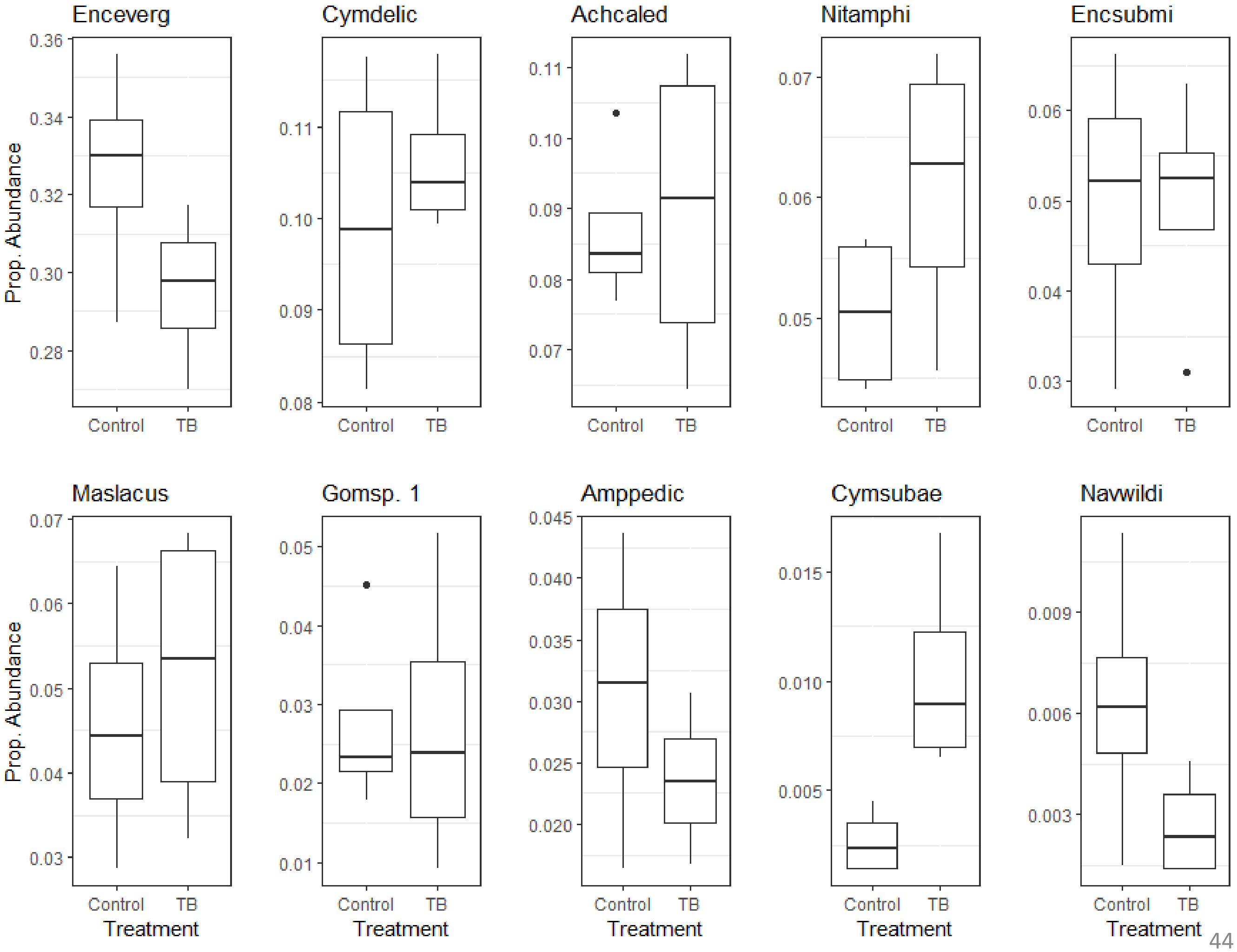
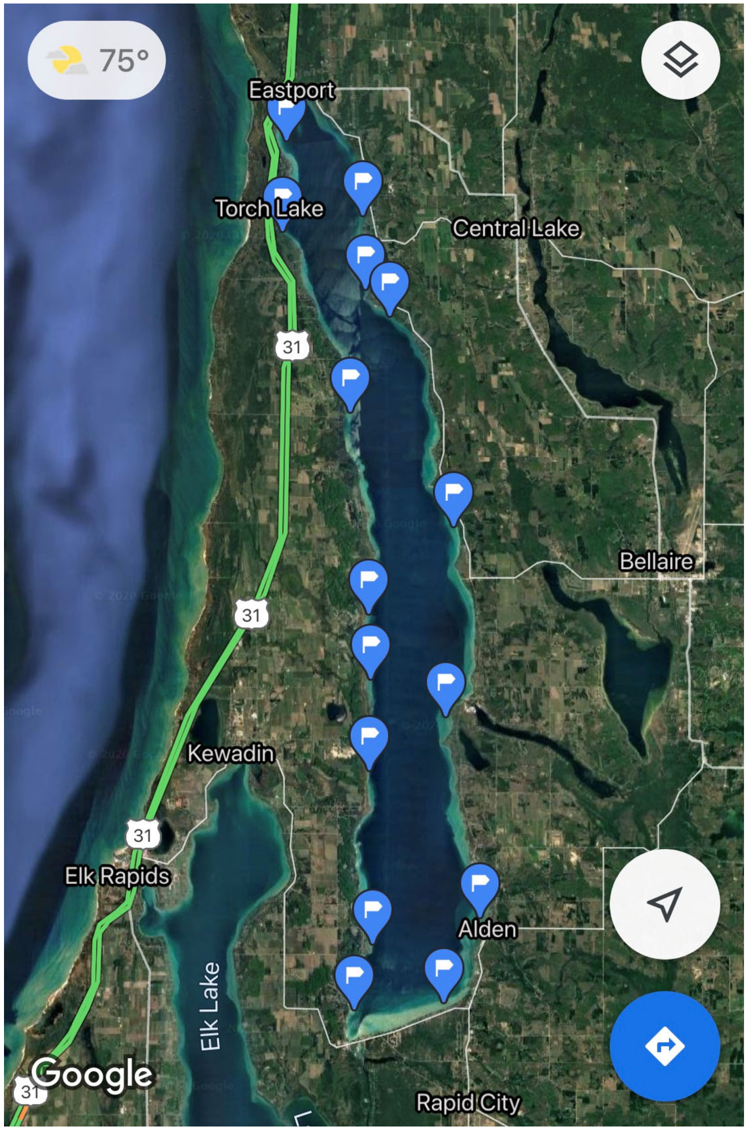


Figure 45. Photos of *in-situ* microcosms: Left image: microcosms with different membranes in the bottom. On the left is a polypropylene sheet and on the righ is a fine hops bag screen, as indicated by their transparency over the carpet. Right image: on the bottom of Torch Lake (courtesy of Fred Sittel).

Figure 46. Sixteen sampling locations around Torch Lake selected for benthic algal survey during summer 2020. Details about these locations can be found in the text.



I plan to upgrade the image with ArcGIS to have numbers and an outline of the lake.

Figure 47. The benthic algal sampling sled with scoop shown on the bottom of Torch Lake at a site known as Becky’s Beach.

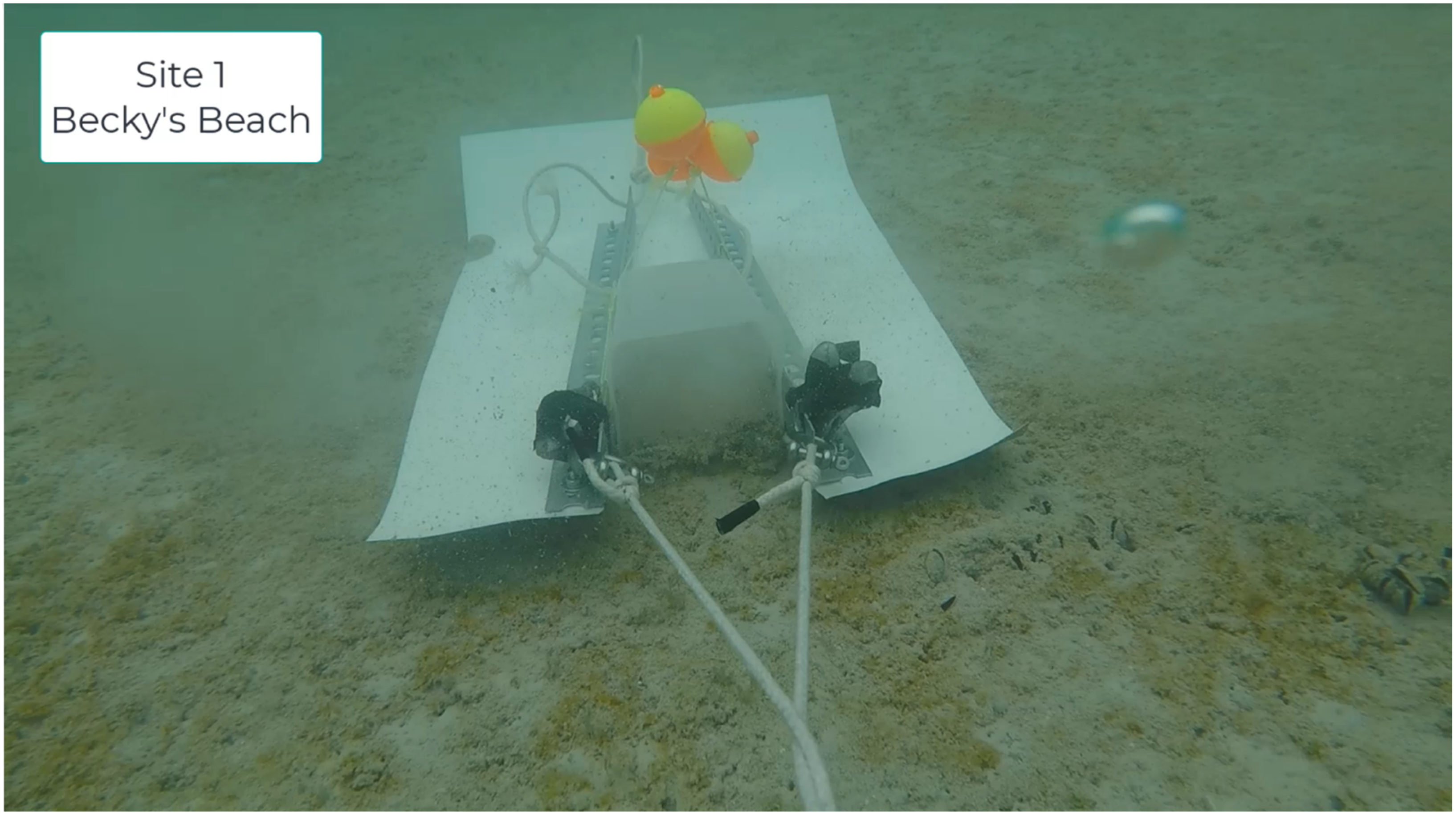


Figure 48. Benthic algal biomass estimated with GoPro video images from Torch Lake during June and August (Months 1 and 2 respectively) at the 16 sites surveyed during summer 2020.

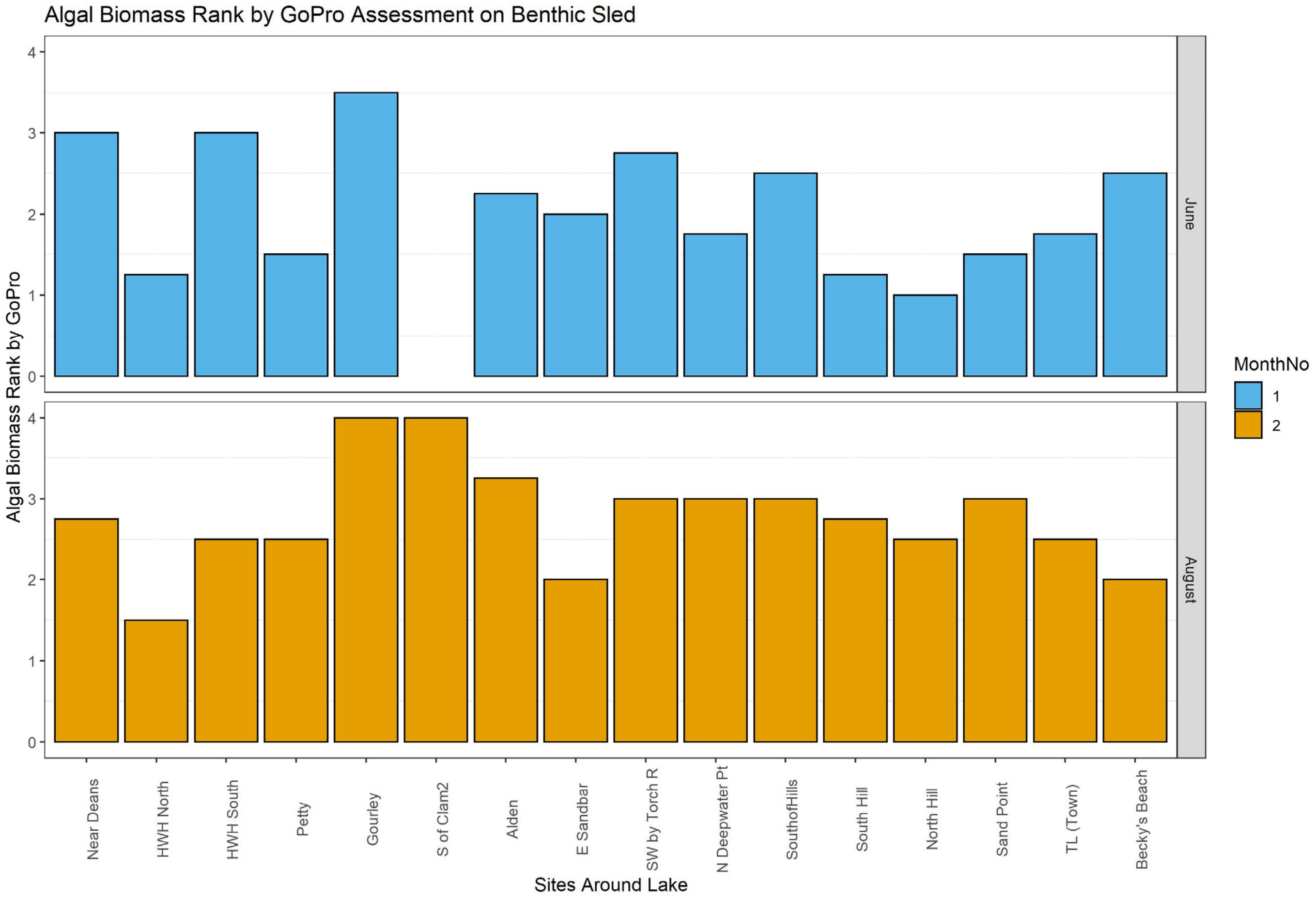


Figure 49. Mat biomass color chart (A) and relationships between algal biomass estimated with GoPro images taken when sampling the bottom of Torch Lake during June and August 2020 and estimated with the color chart and aerial photographs taken by Art Hoadley during May (color labeled June) and August 2020. The line in the figures indicates the least squares regression model relating aerial photograph and GoPro estimates of algal biomass. Figures B and C, respectively, have aerial photo estimates of algal biomass uncorrected and corrected for percent cover of the bottom.

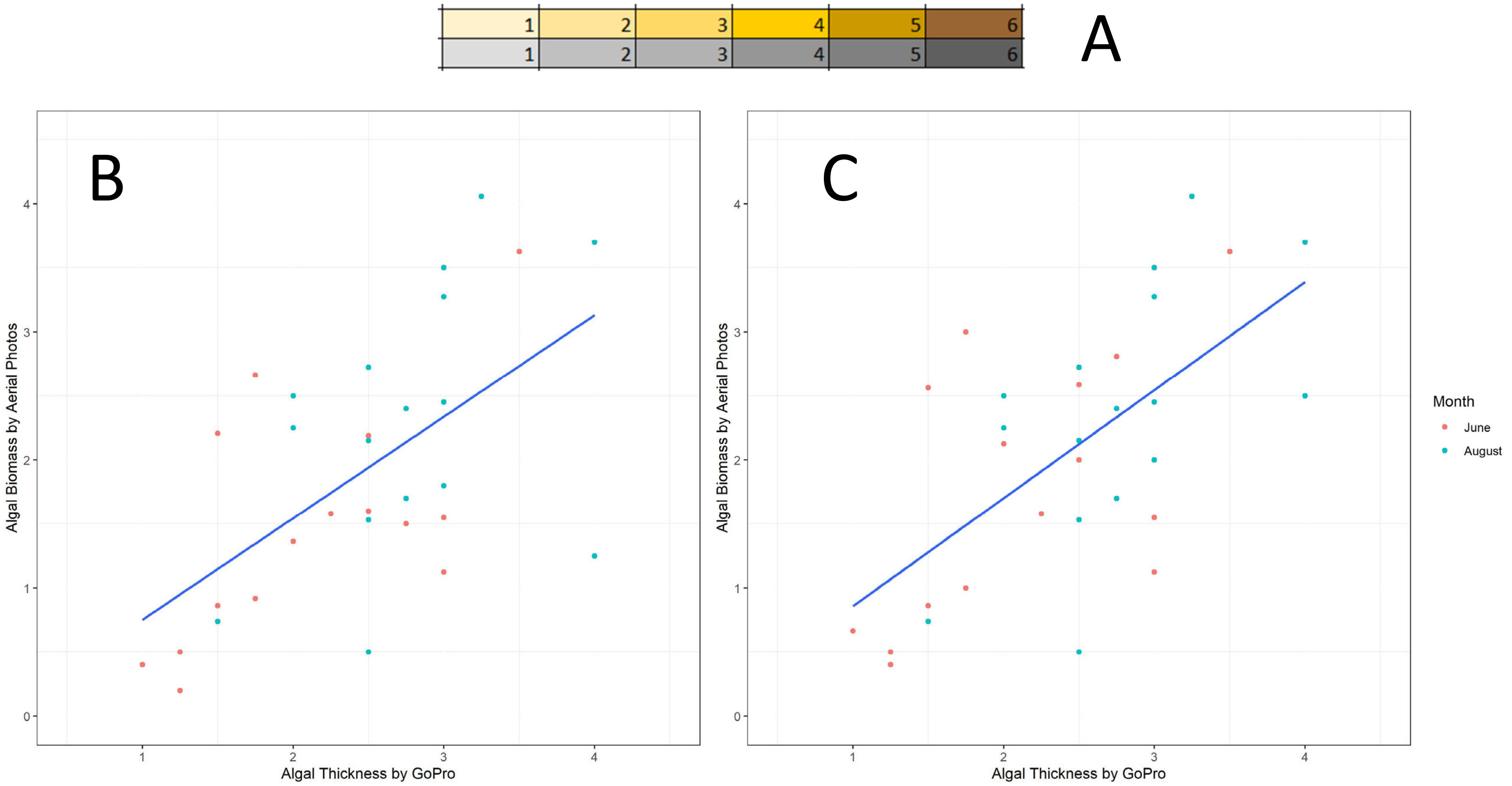


Figure 50. Benthic algal biomass estimated with aerial photography by Art Hoadley for Torch Lake during May and August (Months 1 and 2 respectively) at the 16 sites surveyed by boat and benthic algal sled during summer 2020.

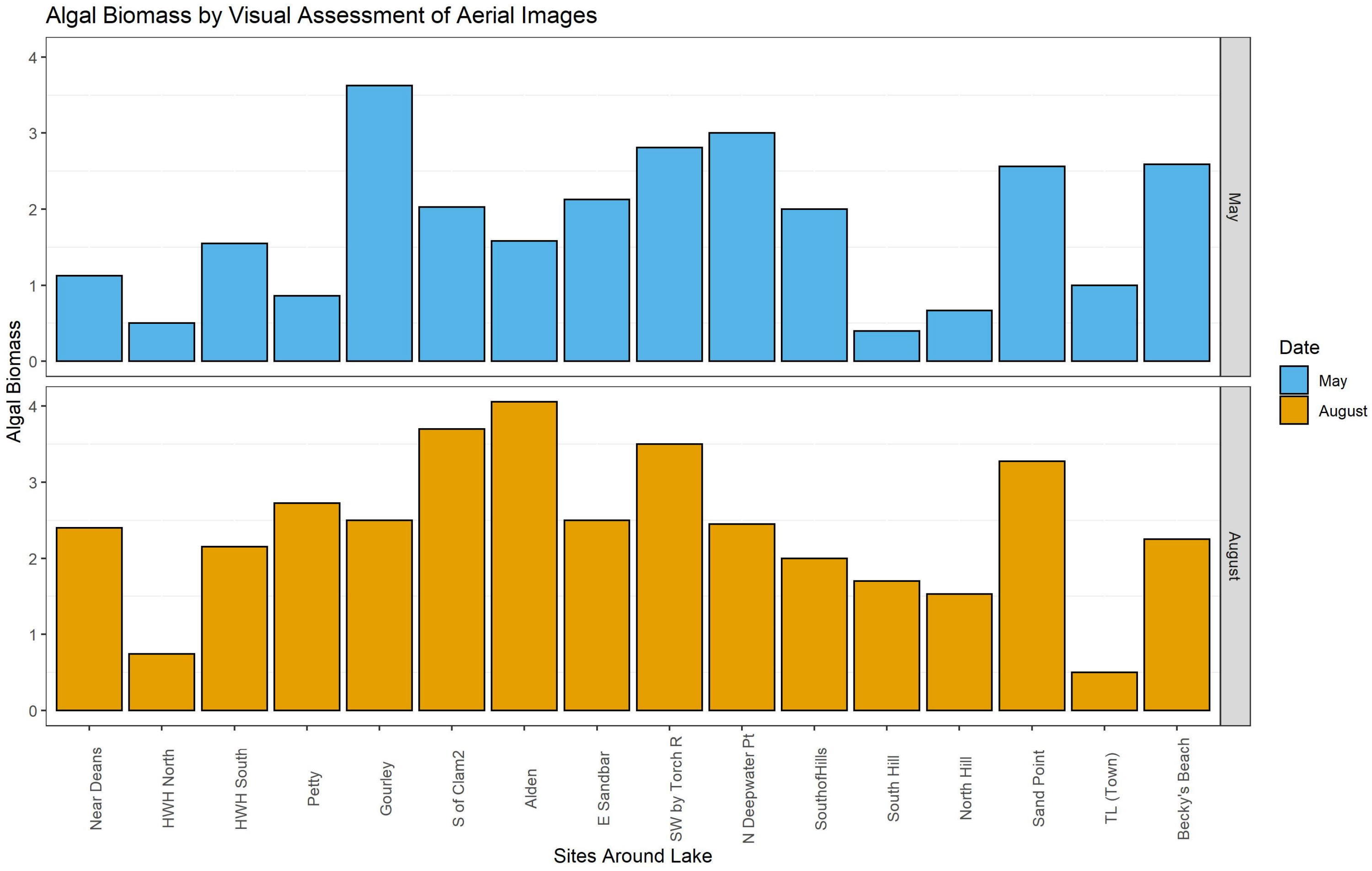


Figure 51. Aerial photographs by Art Hoadley during August 2020 showing waves of sand with corresponding patterns of benthic algae. Long lines in the benthic algae in the shallower sand lobe in the left photograph were likely made by boat propellers. These waves of sand are relatively stable and can be observed at the same location from year to year. Patterns of algae across the waves indicates sand waves structure local environmental conditions in some way that affects benthic algae, such as regulating groundwater transport or underlying water chemistry due to geochemical structure regulating the location of sand waves.

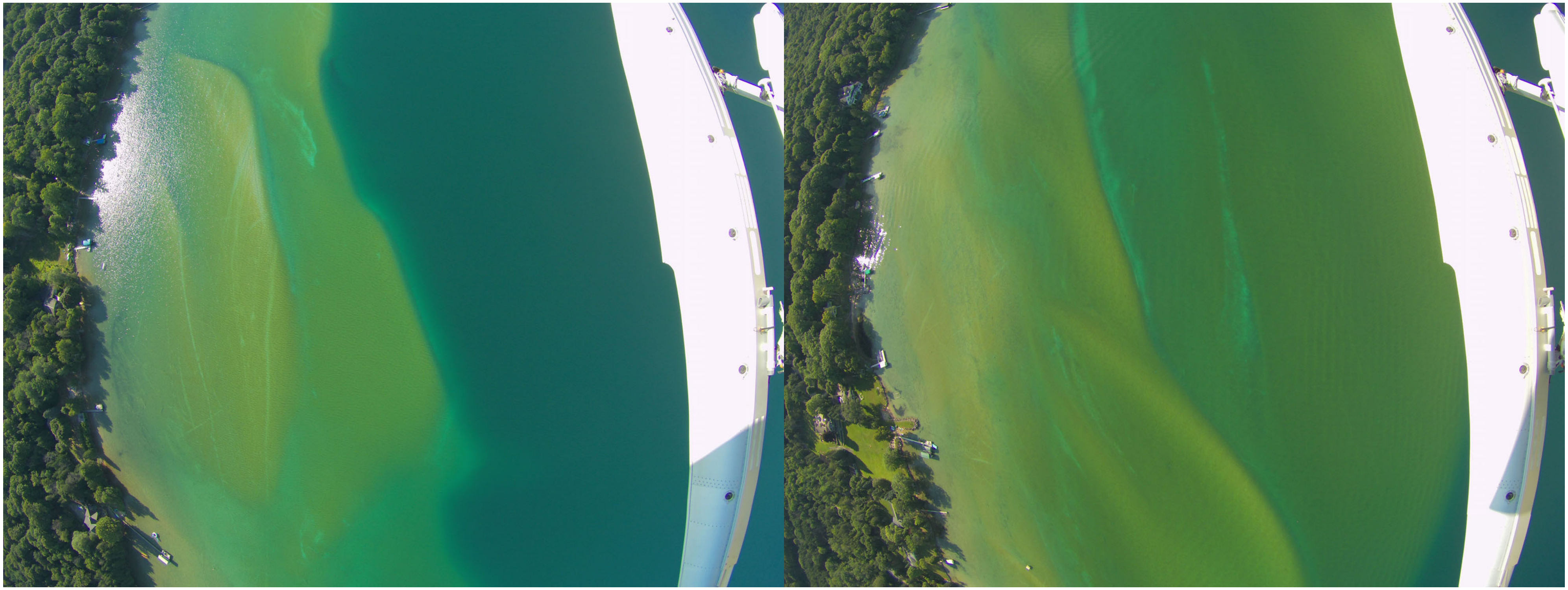


Figure 52. Differences in summer 2021 water chemistry in well water (WW), benthic pore water (BPW), and nearshore surface water (SW) at the Gourley and Petty sites in Torch Lake, the Drake site in Lake Bellaire, and the Hoadley site in Clam Lake.

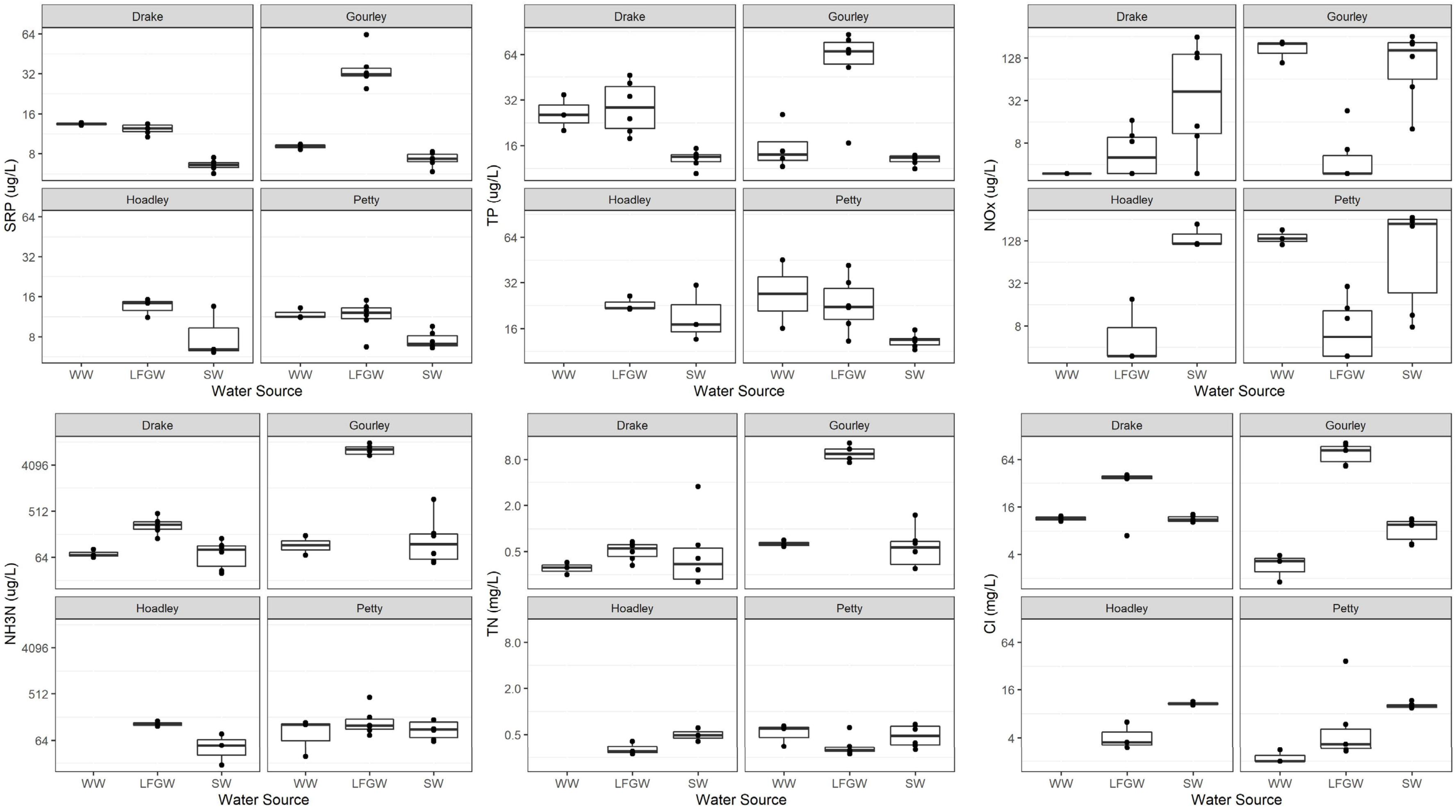


Figure 53. Differences in water chemistry at mid-lake and nearshore locations during summer 2021.

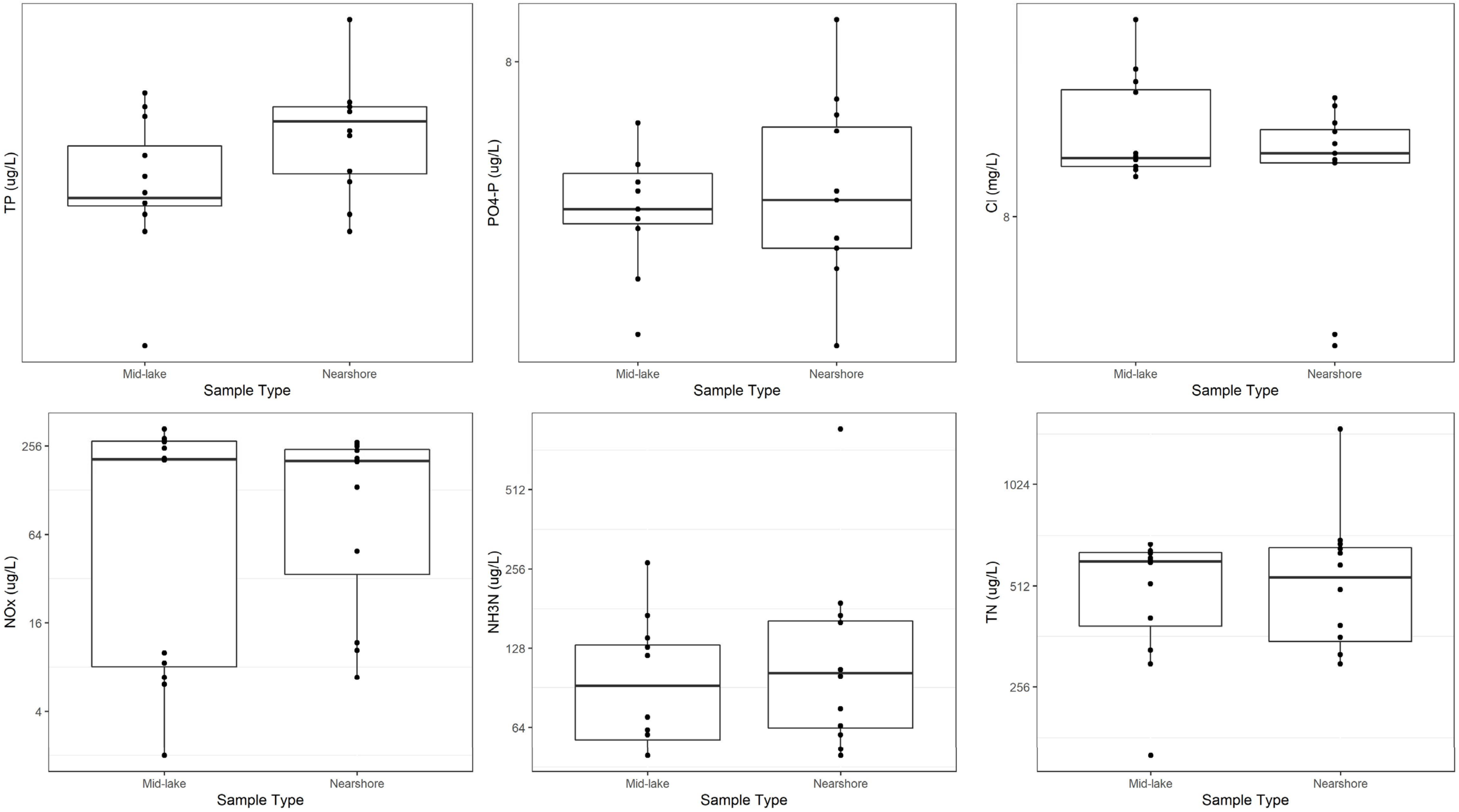


Figure 54. Changes in water chemistry with after May 15, the initial day of sampling, during summer 2021 in mid-lake surface water at southern mid-lake location in Torch Lake. Lines in the figures indicate the least squares regression pattern in the data. Statistical results indicate none of these patterns is statistically significant.

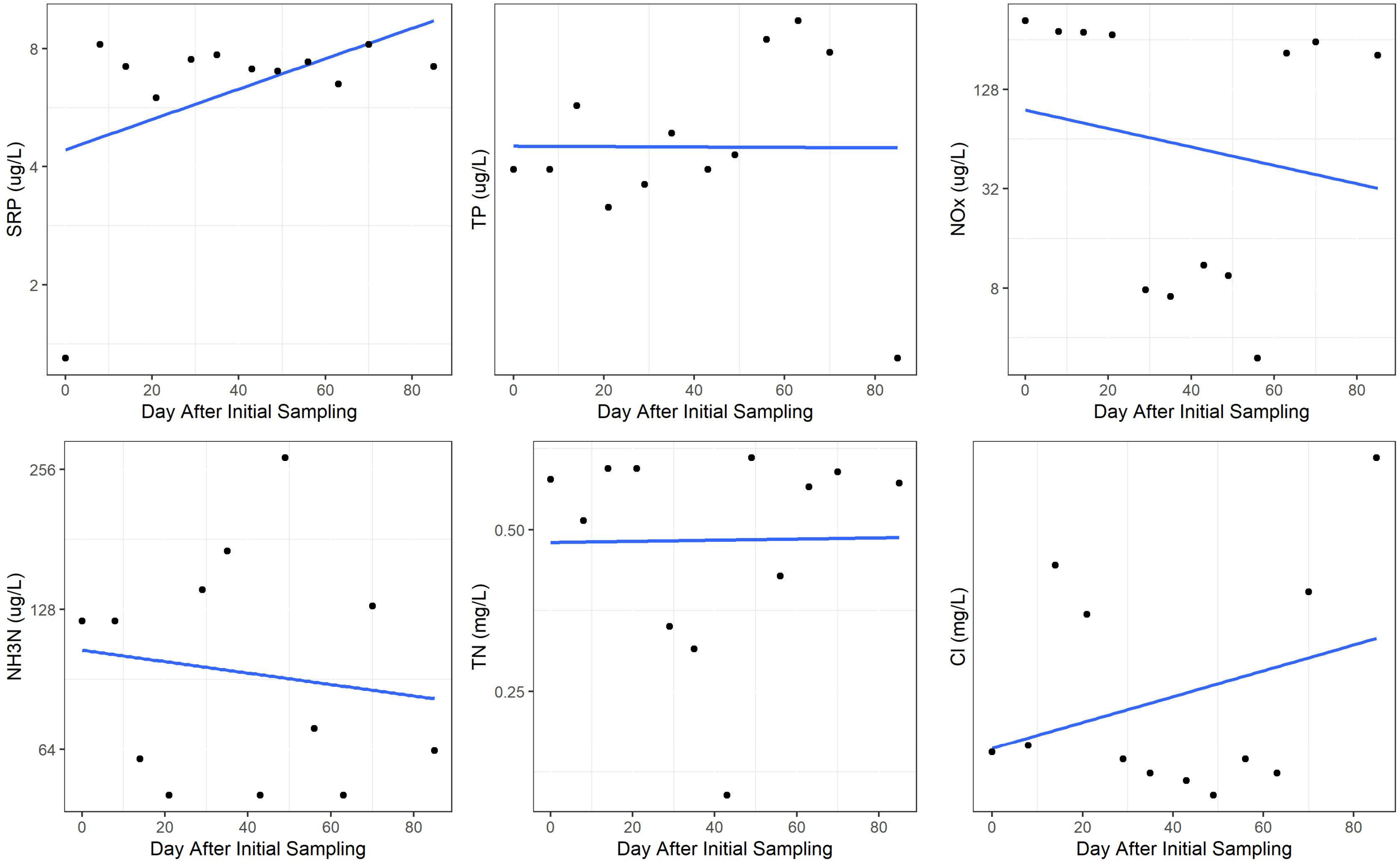


Figure 55. Golden brown algae mats. Top row from left to right: 1) macroscopic image of mat in water on white plate showing upper colored layer, jelly-like middle gray layer, and unconsolidated lower gray layer with insert showing strands connecting middle gray layer firmly to upper color layer; 2) small piece of colored upper layer with algae cyanobacteria and diatoms visible around the margin (200X); 3) 1000X image of cyanobacteria in upper colored layer overlain by stained mucilages; 4) raft of diatoms attached together from upper colored layer (200X). Lower row from left to right: 1) loose diatoms with many moving in water originating from colored upper layer in upper left background (200X); 2) jelly like gray matter in middle layer (100X); 3) closer look gray matter from middle layer with diatoms evident as golden brown dots (200X); 4) diatoms and cyanobacterial filaments in gray matter (400X).