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Ryan Alan Dunbeck
University Of South Dakota

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PHYTOPLANKTON AND CHLOROPHYLL DYNAMICS ACROSS VARIOUS AQUATIC SYSTEM TYPES IN THE MIDDLE MISSOURI RIVER BASIN

By

Ryan Dunbeck

B.S. University of Idaho, 2019

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of Master of Science

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The members of the Committee appointed to examine
the Thesis of Ryan Dunbeck
find it satisfactory and recommend that it be accepted.

DocuSigned by:

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Chairperson

DocuSigned by:

Mark Dixon

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ABSTRACT

Much like the response to naturally occurring physical, chemical, and biological variables controlling phytoplankton dynamics, anthropogenic modification to those variables may have profound implications on phytoplankton density and community structure in aquatic systems. We theorized that extensive land use and river channel modifications would result in (1) an increase in basin-wide phytoplankton density in the Middle Missouri River Basin (MMRB), and (2) a shift in community structure within and downstream of reservoirs filled after 1950 by examining data collected from 2020 and 2021 across the Middle Missouri River Basin to data collected in 1950. Our results suggest that system-wide increases in algal cell density were uncommon, yet variable with only two systems showing increases with high confidence and one showing a decrease. Some systems like the Missouri River reservoir sites and the James River showed an interannual shift in dominant phytoplankton genera, while other systems shared at least one dominant genus in 1950, 2020, and 2021. Our results suggest that despite modifications to land and water use, changes in phytoplankton density and community structure are clear but not consistent across the MMRB. Chlorophyll has been used extensively in ecological monitoring as a proxy for phytoplankton density or biovolume due to the relative simplicity of processing samples. We regressed the predictor variable of total chlorophyll and response variable of algal cell density as well as the predictor variable of $\log_{10}$ transformed Secchi depth (Secchi) and the response variables of either total chlorophyll or algal cell density from 161 samples across nine rivers of the MMRB. A positive relationship was observed between chlorophyll and algal cell density, while an inverse relationship was observed between Secchi and either chlorophyll or algal cell density. These findings suggest that using chlorophyll as a proxy for algal cell enumeration may provide an option to monitor phytoplankton dynamics in rivers. High suspended sediment loads may have confounded the relationship between Secchi and either chlorophyll or algal cell density. Chlorophyll determined by *in* vivo fluorescence provides a good proxy to rapidly monitor phytoplankton dynamics in lowland rivers.

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Thesis Advisor ______________________

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Dr. Jeff Wesner

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Equally, I would like to express my love and gratitude to (1) my wife, Whitney, who not only threatened me with a life of eternal misery if I didn't go to grad school but was extremely supportive of the effort from halfway across the country; (2) my mother who always wanted to know what I was doing, but I didn't really know so I had very short answers; (3) my mother-in-law who mostly asked questions about my thesis efforts for other folks that I also didn't have the answers for; (4) and Whitney's Grandfather, Coye and Uncle, John who provided years of incredible emotional and financial support.

And finally, to the Missouri River Basin and Upper Great Plains/Midwestern US. I likely wouldn't have spent much time there if I hadn't attended grad school in southeastern South Dakota, but to where I now have a strong visceral connection. I took more with me than understanding the phytoplankton dynamics of your waters; I acquired a sense of connection to the biodiversity in the Basin and now appreciate the beauty of your "muddy" waters.

Table of Contents

List of Tables

List of Figures

Chapter 1: Response of phytoplankton density and community structure to physical and chemical changes in the waters of the Middle Missouri River Basin

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Abstract

Much like the response to naturally occurring physical, chemical, and biological variables controlling phytoplankton dynamics, anthropogenic modification to those variables may have profound implications on phytoplankton density and community structure in aquatic systems. The Missouri River Basin has undergone considerable change in both its land and water use over the last 70 years, from an increased percentage of land in cultivation to extensive impoundment projects. We theorized that extensive land use and river channel modifications would result in (1) an increase in basin-wide phytoplankton density, and (2) a shift in community structure within and downstream of reservoirs filled after 1950 by examining data collected from 2020 and 2021 across the Middle Missouri River Basin to data collected in 1950. Our results suggest that system-wide increases in algal cell density were uncommon, with only two systems showing increases with high confidence and one showing a decrease. The relationships between discharge and algal cell density varied between distinct positive or inverse trends across systems, yet the low relative basin-wide discharge in 2021 resulted in remarkable increases in algal cell density in some systems. Some systems like the Missouri River reservoir sites and the James River showed a complete shift in dominant phytoplankton genera, while others (Big Sioux and Vermillion rivers and Lake Mitchell) shared at least one dominant genus from 1950, 2020, and 2021. Our results suggest that despite changes in land and water use designed to suit human needs, changes in phytoplankton density and community structure are clear but not consistent across the MMRB.

However, more research is needed to determine how phytoplankton dynamics in the MMRB may be affected by a changing climate or introduced planktivores.

Introduction

Shifts within the phytoplankton community composition in running waters are controlled by the interaction between hydrology, water residence time, light availability, nutrients, temperature, and herbivory (Bukaveckas et al., 2011; Lucas, Thompson, & Brown, 2009; Heiko Wagner, Fanesi, & Wilhelm, 2016; Winder & Cloern, 2010). However, human modifications to watersheds via dam construction also changes the hydrological regime, which may reduce phytoplankton taxonomic diversity and cause a shift in community structure from that normally found in rivers (da Silva, Pelicice, & Rodrigues, 2020; Okuku, Tole, Kiteresi, & Bouillon, 2016). In addition, changes in land-use patterns and introduced species have become entwined in the complex formula controlling phytoplankton dynamics in rivers (Bussi et al., 2016; DeBoer, Anderson, & Casper, 2018; Fukushima et al., 1999; Yang et al., 2022). These controls set forth by human need may in turn produce bottom-up countereffects on the food web, affecting not only direct consumers like microcrustaceans, macroinvertebrates, and fish, but the upper trophic levels as well (Hillebrand & Shurin, 2005).

The Missouri River Basin (MRB) drains 1,371,010 km^2 in ten U.S. states and two Canadian provinces. Recognized as the longest river in North America, the Missouri River flows roughly 3760 kilometers from its headwaters in western Montana to the confluence with the Mississippi River near St. Louis, Missouri. The MRB can be generally separated into the upper, middle, and lower subbasins as described by Kalkhoff (2013), and Pegg and Pierce (2022) (Kalkhoff, 2013). Anthropogenic influences in the MRB are multiple and range from water regulation, organic and inorganic pollution, and introduced species. Between 1933 and 1964 six hydroelectric dams were constructed on the Missouri River resulting in the largest reservoir system in the United States. Originally designed for flood control, irrigation, domestic water supply, hydroelectric production, and barge navigation, dams on the Missouri River have resulted in a heavily regulated system with fewer floods but higher baseflows than before dam construction (U.S.G.S., 2022). Dams have also been constructed on several tributaries in the MRB, which have altered their flow regimes into the mainstem. Flow regulation has triggered extensive channel incision not only in the mainstem but in tributaries upstream of their confluence (Heine & Lant, 2009); transformed aquatic and terrestrial biological systems (DeLonay et al., 2016; Johnson, Volke, Scott, & Dixon, 2015); and along with bank stabilization efforts, has decreased the sediment load an estimated 70 to 80 percent since the early 1930s while greatly increasing the photic depth in the mainstem (Blevins, 2007). Furthermore, increased cropland conversion and modifications to farming practices have produced measurable change in land use type across the MRB. For example, the percentage of agricultural coverage generally declined from 1950 through the late 1960s, although from 1970 to 2014 total cropland coverage increased by 30% (Ahiablame, Sheshukov, Rahmani, & Moriasi, 2017). Increased farming activities have also modified nutrient budgets in the MRB, where sources for watershed nitrogen deposition (i.e., atmospheric deposition, land development, synthetic fertilizer, manure, or point sources) vary by region, but are influenced largely by manure and fertilizer, while phosphorus contributions are influenced by manure, fertilizer, and stream channel erosion (Brown, Sprague, & Dupree, 2011; Robertson & Saad, 2021). The increasingly common practice of agricultural drain tile installation may also increase the rate of nitrogen, phosphorus, and agrochemicals entering waterways and influence baseflows within watersheds (Ahiablame et al., 2017; Domagalski et al., 2008; Miller, Tesoriero, Hood, Terziotti, & Wolock, 2017; Smith et al., 2015). Finally, along with other introduced species, planktivorous taxa such as bigheaded carps (*Hypophthalmichthys* spp.) and zebra mussels (*Dreissena* spp.) have become widespread in the MRB (Benson et al., 2019; U.S.G.S., 2022) and are considered of ecological concern (DeBoer et al., 2018; Hayer, Breeggemann, Klumb, Graeb, & Bertrand, 2014; Vanderbush, Longhenry, Lucchesi, & Barnes, 2021; Wang, Chapman, Xu, Wang, & Gu, 2018; Wanner & Klumb, 2009).

These anthropogenic influences may alter the density, abundance, community structure, biodiversity, and longitudinal variation of suspended primary producers in an aquatic system. In the Missouri River, historical estimates of phytoplankton density from spring to fall of 1945 in the recently channelized lower section suggested a highly turbid system with low productivity averaging ~67 algal cells L^{-1} across the seven month study (Berner, 1951). In 1950, Damann (1951) recorded between 40 and 160 algal cells mL^{-1} during summer months in that same reach of the Missouri River. Some decades later, from 1974 to 1977, a range of phytoplankton density was recorded in the middle to lower mainstem with values from fewer than 1000 to greater than 25,000 units L^{-1} (Reetz, 1982). Furthermore, changes in density and dominant taxa became evident in the reservoirs and inter-reservoir reaches of the Missouri River. In the tail waters of Lake Francis Case and in Lewis and Clark Lake (the two lowermost reservoirs), phytoplankton assemblages progressively increased in density through downstream reservoirs and were dominated by *Bacillariophyta* during spring and summer, together with a markedly low biovolume relative to natural lakes (Cowell, 1970). Likewise, Martin et al. (1980) documented a broad longitudinal increase in phytoplankton density through the lower four reservoirs during June and July, as well as a longitudinal shift from flagellates to pennate diatom dominance. More recently, in the channelized lower Missouri River, it was found that while phytoplankton biovolume was similar in the main channel, chutes, and newly constructed backwaters, microalgal diversity was significantly greater in backwater sites (Dzialowski, Bonneau, & Gemeinhardt, 2013). These results from past studies provide further evidence of the potential for the anthropogenic modification of waterways to alter phytoplankton dynamics in the MRB.

Considering the extent to which phytoplankton communities have been studied in the mainstem in response to anthropogenic modifications beginning in the mid-20th century, comparatively little is known about phytoplankton dynamics in the tributaries of the MRB. Here, we revisit the observations made by Damann (1951) in the first phytoplankton survey of the MRB in the 1950's; however we focused on both the mainstem Missouri River and its tributaries in the Middle Missouri River Basin (MMRB). Kenneth E. Damann was tasked by the former Federal Security Agency and Environmental Health Center in 1950 with completing an extensive survey of the density and taxonomical assemblage of phytoplankton across the MRB focusing on tastes and odors in drinking water. Although the focus of the study was on public health, the phytoplankton counts, and taxonomical records are invaluable sources of data to evaluate changes in the sestonic primary productivity and dominant genera in the MRB over the last several decades. We tested the hypotheses that extensive land use and river channel modifications would result in (1) an increase in basin-wide phytoplankton density, and (2) a shift in community structure within and downstream of reservoirs filled after 1950 by examining data collected from 2020 to 2021 across the MMRB to data collected by Damann (1951) in 1950 (K. E. Damann, 1951).

Methods

Study area

The Missouri River flows generally from northwest to southeast across the MMRB and contains the five lower dams in the reservoir system. Construction on three of those dams (Fort Randall 1946-1953, Garrison 1947-1953, Oahe 1948-1958) had begun before 1950; however, no dams were closed before 1950. In addition, both the Jamestown Dam, which impounds the James River in North Dakota and the East Vermillion Lake Dam, which impounds the east fork of the Vermillion River in South Dakota were constructed after 1950. Consequently, Damann (1951) likely encountered relatively free-flowing systems in the MMRB in 1950 during surveys of phytoplankton in these rivers. Further downstream on the Missouri River are 62- and 95-kilometer reaches of relatively free-flowing and meandering river sections above and below Gavins Point Dam, respectively. These reaches are now controlled by upstream dams and are subsequently incised and have lost floodplain connectivity compared to 1950. Finally, a channelized portion (roughly 190 river kms) from Sioux City, Iowa downstream to Omaha, Nebraska completes the mainstem lower reach of the Missouri River in the MMRB.

There was considerable interannual variation in basin-wide discharge between the study years (Supplemental F1). Discharge data, however, were limited in some systems for 1950. Basin-wide discharge ranged from ~9% below, ~4% above, and ~18% below the cumulative daily median in 1950, 2020, and 2021, respectively (U.S.G.S., 2019)(Oltman, 1951). Specifically, discharge in the Missouri River at Sioux City during the sampling period averaged 1039 m^3/s in 1950, 1179 m^3/s in 2020, and 848 m^3/s in 2021 (U.S.G.S., 2019).

Site selection

Damann (1951), sampled phytoplankton at a total of 168 sites from the mainstem and tributaries of the MRB throughout the summer of 1950. We sampled 31 sites representing nine systems within the MMRB during 2020 and 2021 (Figure 1). Each site was within one day's driving distance from Vermillion, SD. In 1950, 29 of the 31 sites were flowing rivers with two sites located in a reservoir (Lake Mitchell). In 2020, 23 of the 31 sites were still flowing rivers, with the remainder becoming reservoirs following dam construction in the 1950s. In addition, of the two sites classified as "Lake Mitchell", one (site 20) is semi-lotic and the other (site 19) is distinctly lentic. Lake Mitchell (filled in 1929), which feeds its tailwaters into the James River ~0.5 km downstream of site 18, was the only reservoir established before 1950 within our study extent. All waterbodies referred to hereafter will be defined as "systems" for uniformity. Site location descriptions (i.e., highway bridges, towns, river miles, etc.) from Damann (1951) were used to create waypoints in Google Earth to determine driving routes and sampling accessibility.

Damann (1951) sampled the 31 sites in the MMRB in July 1950. Consequently, taking into consideration the sampling duration from Damann (1951) and the potential for climate change induced variation (i.e., earlier start to growing season) in the Midwest and Great Plains (Xu, Liu, Williams, Yin, & Wu, 2016) since the summer of 1950, we sampled approximately one to two weeks earlier than dates in Damann (1951). Each of the 31 study sites was sampled on the same Julian day for the 2020 and 2021 seasons. Most sites consisted of a single sample on each date. However, to estimate inter-sample variation, we sampled in triplicate from 1-2 sites within each river system.

To estimate potential seasonal variations in phytoplankton blooms, five sites were repeatedly sampled in 2020 approximately one week apart throughout July. In 2021, we expanded the sampling duration from May to August, which both preceded and followed the single sampling event from Damann (1951). Triplicate samples were taken from repeat sites once monthly.

Sample design

We followed applicable methods from Damann (1951) to direct our field sampling. Between one and three surface water samples were collected in 125-mL brown plastic bottles at approximately 0.4 m after allowing river water to clear of disturbed sediment caused by wading. Samples were fixed with Lugol's iodine before being placed on ice. Although not a direct component of the survey by Damann (1951), we compiled historical and current discharge data on the Missouri River by averaging discharge at the nearest upstream and downstream United States Geological Survey (USGS) or United States Army Corps of Engineers (USACE) gauging stations on the mainstem if no gauging station was located directly at that site. The same protocol was performed for tributary sites. Average distances from sample site to gauging station were 11.4 and 23.6 river kilometers for the mainstem and tributaries, respectively. Sites with no corresponding gauging station were omitted from that part of the analysis.

Laboratory analysis

Phytoplankton were identified and enumerated using the Direct Count Method (DCM) following Damann (1950 and 1951), which was modified from methods described in Baylis (1922). Comparisons of mean count accuracy between the DCM and the Foerst Centrifuge method described in Damann (1950) yielded relatively similar results when counting units of phytoplankton mL^{-1}. Over a three-month study analyzing 119 samples, the DCM deviated 6% from the Foerst centrifuge sample mean mL^{-1} (Damann, 1950). Similar to other widely implemented protocols like the Utermöhl technique (H. Utermöhl, 1958; v. H. Utermöhl, 1931), the DCM uses unconcentrated samples for identification and enumeration. This method is particularly effective for the sediment-laden water encountered in the MMRB and has a relatively quick (~15 minutes) algal sedimentation time between samples.

We identified phytoplankton to genus using taxonomic texts (Spaulding et al., 2021; Wehr, Sheath, & Kociolek, 2015) at magnifications from 200 to 630x using brightfield, and phase contrast microscopy (Leica DMLB microscope, Leica Microsystems, Wetzlar, Germany). Sample bottles were gently inverted ten times to create a homogenous solution before transferring a 1 mL subsample into a gridded Sedgewick-Rafter counting chamber and assuming random distribution in the chamber. Enumeration was completed as 200 1 mm^3 fields in four randomly chosen rows as described in Damann (1950) as the recommended number of fields for combining timely enumeration with a high degree of accuracy for "a rather low population density". After counting was complete in two 50 mm^3 rows, the sample was discarded, and the cell was again filled with a fresh subsample of the sample to repeat the counting process. Enumerated values were then extrapolated to obtain a value for phytoplankton density mL^{-1}. Attempts were made to identify all clearly visible phytoplankton units and only those units with visible chloroplasts were counted. However, if units were unidentifiable caused by poor positioning, damage, obscuring detritus, or taxonomic uncertainty (~4% of all samples) those units were grouped in corresponding unresolved divisions (e.g., Unresolved Chlorophyta, Unresolved Bacillariophyta, etc.).

To allow comparisons with the taxonomy of 1950 while separating the genera to current taxonomical guidelines during counting and identification, we grouped the genera *Desmodesmus* back into *Scenedesmus* and *Cyclostephanos* back into *Stephanodiscus* for analysis. Additionally, we considered several taxonomic changes in the literature after 1950 between *Fragilaria*, *Synedra*, and *Ulnaria*, which were frequently observed at some sites. However, because taxonomic changes have been overseen by reassigning generic classification amongst these three genera without further dividing for reclassification (as seen in *Scenedesmus* and *Stephanodiscus*), we preserved current generic classification for these three taxa. Moreover, we followed the descriptions in Damann (1951) for what defined a single phytoplankton unit. Unicellular and colonial taxa such as discrete cells or single cells within the colony (*e.g.*, *Actinastrum, Scenedesmus*) were counted as one unit per cell observed in the colony. Using a calibrated ocular micrometer, colonial genera forming irregular masses or tightly bound clusters (*e.g.*, *Microcystis, or Coelastrum*) were counted as one unit per 20 µm in diameter. In addition, we counted filamentous taxa (*i.e., Aphanizomenon* and *Aulacoseria*) as one aerial unit per 100 µm in length irrespective of filament diameter.

After recognizing the lengthy duration required for identification and enumeration of plankton-dense samples frequently encountered in 2021, we revised our counting methods. In 2021 we continued counting 200 mm^3 for sites below 40 µg/L total chlorophyll (Supplemental T1) and reduced counting to 25 mm^3 for sites above 40 µg/L total chlorophyll. It is expressed in the DCM, as well as in other counting methods (Lund, Kipling, & Le Cren, 1958; H. Utermöhl, 1958) that the reliability of algal cell density estimates in a sample increases with increased unit counts. However, high precision around the mean is still expected when counting fewer fields in samples with high density. Moreover, our focus was to identify site-specific density and longitudinal trends of dominant taxa. This revised approach allowed us to obtain reasonable estimates for sites with abundant phytoplankton, while being conscientious of the time allotted to counting related sites and still allowing for detailed enumeration at low density sites. The same individual was responsible for identification and enumeration throughout the study.

Statistical analysis

To estimate system-specific phytoplankton densities, we modeled the relationship between the response variable of phytoplankton density and the predictor variable of system-year, which included data from 1950, 2020, and 2021, using a generalized linear model with a log link, Gamma likelihood, and site number as the random intercept (R Core Team, 2021). Prior values were chosen using prior predictive checks that ensured that they covered a wide but biologically reasonable range of values (Wesner & Pomeranz, 2021).

Bayesian inference was used to fit the model using Hamiltonian Monte Carlo methods in rstan (Carpenter et al., 2017) using the brms package in R (Bürkner, 2017). In the model, we ran four chains with 1000 iterations, where 500 of those iterations were discarded during sampling warmup. We checked convergence by confirming that r-hat values were <1.1. Model fit was evaluated with posterior predictive checking to ensure that the model could simulate data that resembled the original data (Gabry, Simpson, Vehtari, Betancourt, & Gelman, 2019).

Results

Phytoplankton density

Contrary to our hypothesis, and despite the increase in agricultural land use in the MMRB causing an increase potential for nutrients entering adjacent waterways, we observed a variable response across systems, but no consistent increase in phytoplankton densities since 1950 (Figure 2). More specifically, there was a >99% probability that current phytoplankton density was higher in the Missouri River compared to 1950, with an average increase of 2505 units mL^{-1} (95% credible interval (CrI) = 1,180 to 5,334). Similarly, an average increase from 1950 values was also estimated in the Platte River (site 27) of 211,183 units mL^{-1} (95% Crl = 31,615 to 1,487,773). In contrast, we estimated a decrease in phytoplankton in the Bad River (site 35) of 10,140 units mL^{1} (95% Crl = -46,818 to -1,615). Enumerated phytoplankton density values in 1950 across all sites in the MMRB ranged from 100 (site 28) to 86,960 units mL^{-1} (site 13) with a mean of 23,027 units mL^{-1}, in 2020 from 230 (site 19) to 235,770 (site 27) with a mean of 25,948 units mL^{-1}, and in 2021 from 135 (site 36) to 221,780 (site 27) with a mean of 58,061 units mL^{-1}.

Sites that were repeatedly sampled after (2020) and both before and after (2021) their primary sampling date showed some indication of temporal variation (Supplemental F2). Most notably, the phytoplankton in the Big Sioux River more than quadrupled in density during July 2020, while in 2021 it displayed large fluctuations in density from May to August. In the Vermillion River, we observed a substantial algal cell density increase in 2021 from the beginning of June to the middle of July. The Missouri and James rivers showed little intra-annual fluctuation.

Longitudinal change

Longitudinal trends in phytoplankton density were variable with distinct influences from dams closed after 1950 (Figure 3). In the Missouri River, we observed a similar density between years at the upper five sites in the reservoir system with an average of 324 units mL^{-1} in 1950, 912 units mL^{-1} in 2020 and 341 units mL^{-1} in 2021. Downstream of the reservoirs, however, a considerable increase in density was found in 2020 and 2021 relative to 1950. At site 14 (~8 km downstream of Gavins Point Dam tailwaters) we recorded an 8- to >31-fold increase in 2020 and

2021, respectively. In addition, we recorded a high variability in phytoplankton density in the Vermillion River both above and below artificially impounded East Vermillion Lake. Comparing 1950 and 2020, algal density trends in the Vermillion fluctuated longitudinally. Nevertheless, average system-wide counts were similar (27,952 in 1950 and 22,983 in 2020). Conversely, a remarkable system-wide increase in algal density was recorded in 2021 (average of 130,323), exceeding all values from 1950 and 2020, while decreasing longitudinally. In the James River a distinctive trend emerged in 2020 and 2021 where we detected a ~27-fold decrease in phytoplankton density relative to 1950 from the tail waters of Jamestown Dam, but a substantial increase in the longitudinal phytoplankton density profile was observed farther downstream (two-to three-fold at furthest three sites downstream) in 2020 and 2021 compared to 1950. In contrast to the Missouri, Vermillion and James rivers, the Big Sioux River is not directly influenced by impoundment. However, like the Vermillion River, an increase in algal density was recorded at most sites in 2021 compared to 2020 and 1950, with system-wide averages of 58,256-, 15227-, and 136,728 units mL^{-1} in 1950, 2020, and 2021, respectively.

Community shifts

We observed distinct changes between the relative abundance of representative phytoplankton divisions between 2020 and 2021 (Figure 4). Data from 1950 were only recorded as dominant genera without a complete taxonomic breakdown of counts per genus and are subsequently not included in this figure. Broadly, an interannual shift from *Bacillariophyceae* to *Chlorophyta* dominance or codominance was evident from 2020 to 2021. In 2021, a greater diversity of phytoplankton was recorded at numerous sites. More specifically, while not a substantial proportion of phytoplankton at most sites, *Cyanophyta* also were more prevalent at several sites in 2021 and composed >99% of the enumerated sample (predominantly *Aphanizomenon*) in Lake Mitchell.

We identified 90 phytoplankton genera across the MMRB and observed changes since 1950 in the dominant genera in some systems (Table 1). It is unclear why the "dominant" genera from 1950 range from one to five taxa. We chose the top three genera proportionally represented from enumerated samples as "dominant" to maintain consistency. Specifically, at Missouri River reservoir sites (33, 34, 36, 37, and-38) and tributary sites sometimes flooded by reservoir water levels (35 and 39) there was evidence of taxonomical shifts from the biraphid pennate diatom genus *Navicula* in 1950, to araphid colonial diatoms like *Asterionella* and *Fragilaria* in 2020 commonly proliferating in lakes and reservoirs, to a mix of dominant taxa at those sites in 2021. At James River sites, the once dominant filamentous cyanobacteria *Aphanizomenon* were replaced with relatively small representatives of centric diatom taxa like *Cyclotella* and *Stephanodiscus* in 2020, to colonial cyanobacteria *Microcystis* and *Merismopedia*, and chlorophytes *Actinastrum* and *Crucigenia* in 2021. Changes were also observed in the Platte, Bad, Grand, and Heart rivers and in the Missouri River sites downstream of the dams. Nevertheless, we did not observe striking changes in dominant genera in all systems. Of the dominant genera recorded in all years for the Big Sioux River, Vermilion River, and Lake Mitchell, at least one dominant genus was shared per system for each year.

Discussion

The most important single result of this study is that despite the increase in agricultural land use and channel modification from dam construction and channelization in the MMRB, an increase in average phytoplankton density was recorded in only two systems (Missouri and Platte rivers), and a decrease in phytoplankton density was recorded in the Bad River. In contrast, some of the tributaries in the MMRB remain largely free flowing lowland systems that are both turbid and turbulent where phytoplankton communities develop regardless of naturally caused adverse conditions but may be influenced by chemical rather than physical controls.

We did not record a distinct longitudinal or temporal increase in algal productivity across the reservoir sites in this study (sites 33-38). However, the effect of dams on longitudinal phytoplankton density and dominant taxa is evident in the MMRB. Based on findings from Beaver et al. (2013) phytoplankton productivity within the Missouri River reservoir system is expected to increase from upstream to downstream, given the downstream increase in trophic status from mesotrophic in Lake Oahe and Lake Sharpe, to meso-eutrophic in Lake Francis Case, and eutrophic in Lewis and Clarke Lake. Sites in what are now Lake Francis Case and Lewis and Clarke Lake were not sampled in 1950, only sites within Lake Oahe and Lake Sharpe were sampled, with one site (33) in the tail waters of Fort Randall Dam and none in Lewis and Clarke Lake. Consequently, the lack of representative longitudinal sampling in the mainstem Missouri River reservoirs may mask the trends of broad longitudinal increases in suspended algal productivity recorded in other studies (Beaver et al., 2013; Neel, Nicholson, & Hirsch, 1963). Because of the parameters controlling riverine productivity and variability in geology, depth and quantity or quality of newly submerged senescing vegetation, there is considerable variability in phytoplankton responses in recently filled reservoirs with significant increases (Okuku et al., 2016) or decreases in density (da Silva et al., 2020). From 1952-1957 (within the timeframe for groundbreaking, construction, and/or completion of the lower four Missouri River dams) Neel et al. (1963) recorded considerably increased average phytoplankton productivity (from 21 to 1029 units per mL) during July in Lake Francis Case between one and two years after dam closure in

1952. By 1955, those initial increases subsided, while still artificially sustaining enhanced phytoplankton yield compared to pre-dam values (Neel et al., 1963). Indeed, Neel et al. (1963) noted that "no noteworthy authentic autochthonous mainstem growth was noted in the middle Missouri River until some impoundment was realized in Ft. Randall Reservoir" (since renamed Lake Francis Case). This suggests that despite relatively low productivity when compared to other systems in this study, the mainstem reservoir system artificially increased productivity compared to the historical river and has permanently altered downstream phytoplankton dynamics. We observed similar phytoplankton densities in 2020 and 2021 to those recorded by Neel et al. (1963) in the two years following dam closure in Lake Francis Case. Given the duration since impoundment, it is likely that the reservoir system has reached an equilibrium state while still undergoing relatively small interannual fluctuations in phytoplankton productivity, likely controlled by water residence time affected by fluctuations in yearly or seasonal precipitation (Beaver et al., 2013).

Several studies have demonstrated predictable changes to phytoplankton communities (*i.e.*, decreased taxonomic diversity and evenness, decreased algal abundance, and community structure shifts) in reservoirs after dam construction (da Silva et al., 2020; Haruna Alhassan, 2015; Okuku et al., 2016; Znachor et al., 2020). For instance, Neel et al. (1963) reported a similar trend early on after some impoundment occurred from the filling of reservoirs behind Garrison, Fort Randall and Gavins Point dams in the Missouri River where from 1952-1957 benthic microalgae taxa frequently entrained by lotic conditions were no longer dominant by 1954 and several sites showed seasonal displacement of dominant *Bacillariophyta* (notably pennate diatoms) by codominant *Cyanophyta, Chlorophyta, Euglenophyta,* and *Ochrophyta* during summer months. We observed a similar shift from 1950 to 2020-2021, despite considerable time elapsed since the findings by Neel et al (1963), from a system dominated by biraphid diatoms commonly scoured from the benthos and entrained in the phytoplankton of running water (*Navicula*) recorded by Damann (1951), to frequently occurring colonial and motile lentic taxa (*Fragilaria, Asterionella, Tabellaria, Cryptomonas, Chlamydomonas,* and mixotrophic *Dinobryon*). High-head hydroelectric dams increase retention time relative to low head navigation dams (Charles Joseph Vörösmarty, Wasson, & Richey, 1997). The reservoirs produced by hydroelectric dams in the middle Missouri River produce varying algal residence times depending on the intensity of the water year, ranging from over 600 days during drought in Lake Oahe to under two days during flood in Lewis and Clark Lake (Beaver et al., 2013). Consequently, increased water residence time, low turbulence, and increased photic depth in reservoirs favored the production of common lentic taxa found in our survey (Abirhire et al., 2015; Beaver et al., 2013).

Phytoplankton density downstream of Gavins Point Dam increased longitudinally in 2020 and 2021, reaching values from ~8 to 31 to ~370 to 490 times those recorded in 1950 at Yankton, South Dakota (site 14) and Nebraska City, Nebraska (site 28), respectively. Historically described as the "Big Muddy", the Missouri River is responsible for ~75% of the Mississippi Basin natural sediment flux (Meade, 1996). Nevertheless, sediment capture by Missouri River reservoirs has reduced downstream sediment transport ~47% of historical values (Charles J Vörösmarty et al., 2003) causing a considerable increase in photic depth in the reach of the mainstem downstream of Gavins Point Dam. Despite highly productive tributaries providing phytoplanktonic inoculum to the mainstem in 1950 the shallow photic depth resulting from naturally high historical sediment loads may have prevented sustained longitudinal productivity at least as far downstream as Kansas City, Missouri, the furthest downstream site sampled in Damann (1951); and in the lower mainstem according to Berner (1951). This lack of a pre-dam longitudinal pattern in algal productivity contrasts with the longitudinal increase below Gavins Point Dam that we observed in 2020 and 2021. Although variable, longitudinal increases in algal productivity in lowland rivers during summer months enhanced by tributary inputs have also been recorded in the Ohio River (Wehr & Thorp, 1997), San Joaquin River (Leland, 2003), Tisza River (Istvánovics, Honti, Vörös, & Kozma, 2010), and Missouri River (Bukaveckas et al., 2011). In our study Secchi depth decreased from 52 and 75 cm at site 14 (~ 8 km downstream of Gavins Point Dam) to 10 and 16 cm at site 28 (~376 km downstream Gavins Point Dam) in 2020 and 2021, respectively (Supplemental T1). Secchi disk data were not available for sites from Damann (1951), although Galtsoff (1924) observed that a Secchi depth of ~2 cm in the lower Missouri River was common during summer months. In addition, the increased river depth and faster flow due to channelization (Galat, Robinson, & Hesse, 1996) in the lower reaches of the mainstem likely increased mixing depth of suspended algae, which may further strengthen the light limitation experienced by phytoplankton in an increasingly turbid system (Dokulil, 1994; Ochs, Pongruktham, & Zimba, 2013). Nevertheless, intermittent exposure to high light intensities may temporarily reduce light limitation to some phytoplankton taxa in rivers with relatively low light availability (Mitrovic, Howden, Bowling, & Buckney, 2003; H Wagner, Jakob, & Wilhelm, 2006). Specifically, commonly described riverine taxa (i.e., *Scenedesmus* and small centric diatoms) grew better under intermittent light conditions and small-scale turbulent mixing than at a fixed depth and no turbulence during *in situ* experiments (Köhler, 1997). Representatives of those groups (i.e., *Scenedesmus, Pediastrum, Cyclotella, and Stephanodiscus*) were dominant in the more turbid lower mainstem section of this study in 2020 and 2021. From 2004-2006 the average algal carbon composition of particulate organic carbon (mg L^{-1}) for the Missouri River was 52% (Bukaveckas et al., 2011), which suggests, due to historically low concentrations of phytoplankton yet high turbidity in the mainstem, downstream turbidity may now be partly influenced by relatively high algal density and less so by abiotic suspended sediment as observed historically.

The most striking change in the tributaries of the MMRB occurred in the Platte River (site 27). Phytoplankton density increased almost 100-fold since 1950 and the community structure has shifted during the sampling period from diatom and green algae to entirely green algae, with *Scenedesmus* comprising ~46 and 50% of the density, in 2020 and 2021, respectively. There are several possible explanations for these changes. Given the distance upstream to the nearest tributary reservoir (~226 river kms), it is unlikely that reservoir inputs influenced the relatively high algal density present in the Platte River. Instead, this increase in algal productivity may be explained by the expansion of agriculture made possible by center pivot irrigation invented in Nebraska in 1948, which allowed for efficient irrigation of previously marginal lands in the Platte River subbasin, thus, increasing the potential for agricultural nutrient inputs entering the Platte River. Several authors have suggested a positive correlation between nutrient loads and algal productivity in lotic systems (Basu & Pick, 1995; Bukaveckas et al., 2011; Chételat, Pick, & Hamilton, 2006; Cowell & Dawes, 2008; Sabater et al., 2008).

Conclusion

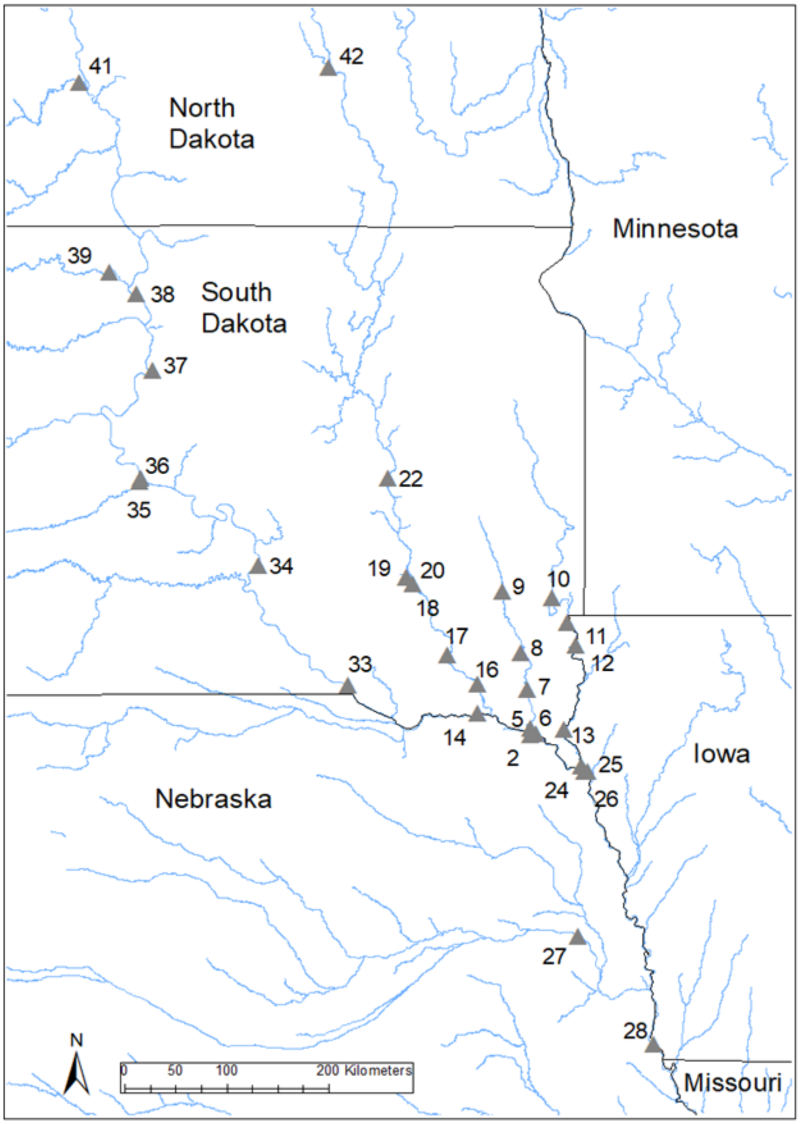
Our results indicate that, since 1950, changes in both phytoplankton density and community structure are clear but not consistent across the MMRB. The algal community and level of primary productivity have been transformed in impounded waters and downstream reaches deprived of sediment. The increase in toxin and bloom-forming *Cyanophyta* presence in both lotic and lentic systems during the low water year (2021) should be of concern when considering the predictions of how climate change may affect precipitation across the MMRB, increasing the intensity and duration of regional flood and drought (Wuebbles, Kunkel, Wehner, & Zobel, 2014). We did not address biotic controls of phytoplankton, but the increasing distribution of introduced planktivorous taxa such as bigheaded carps (*Hypophthalmichthys* spp.) across much of the U.S.–including in the Missouri River downstream of dams and tributaries like the Big Sioux, James, Platte, and Vermillion rivers– and zebra or quagga mussels (*Dreissena* spp.) found in some Missouri River reservoirs as well as smaller reservoirs suggests their capacity to reduce phytoplankton density and alter community structure, which may impact the survival of native planktivores (DeBoer et al., 2018; Fishman, Adlerstein, Vanderploeg, Fahnenstiel, & Scavia, 2010; Jack & Thorp, 2000; Ma et al., 2010). It is unclear whether these introduced species have already changed local aquatic food webs in the MMRB through bottom-up controls. Therefore, future studies on populations of introduced planktivores in the MMRB may help elucidate the effect these species have on the phytoplankton dynamics already changed through the influence of dams and increased agricultural production.

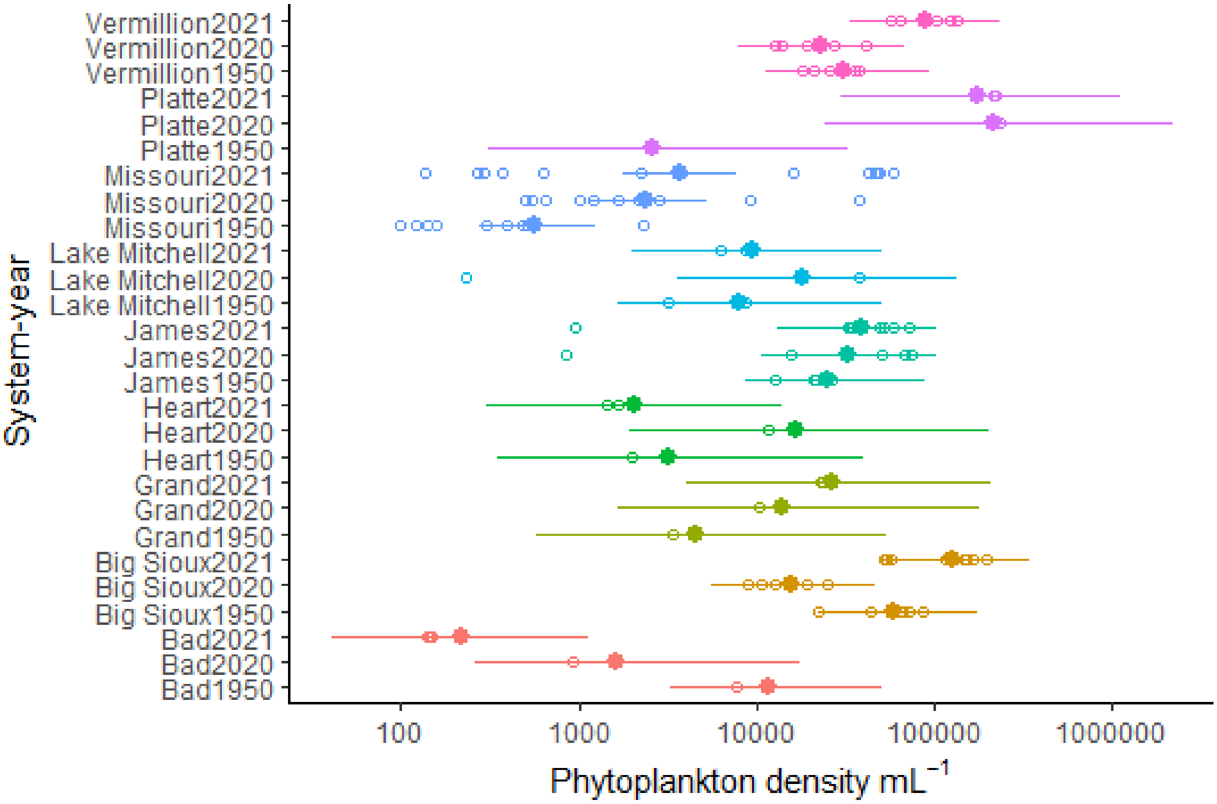
Tables

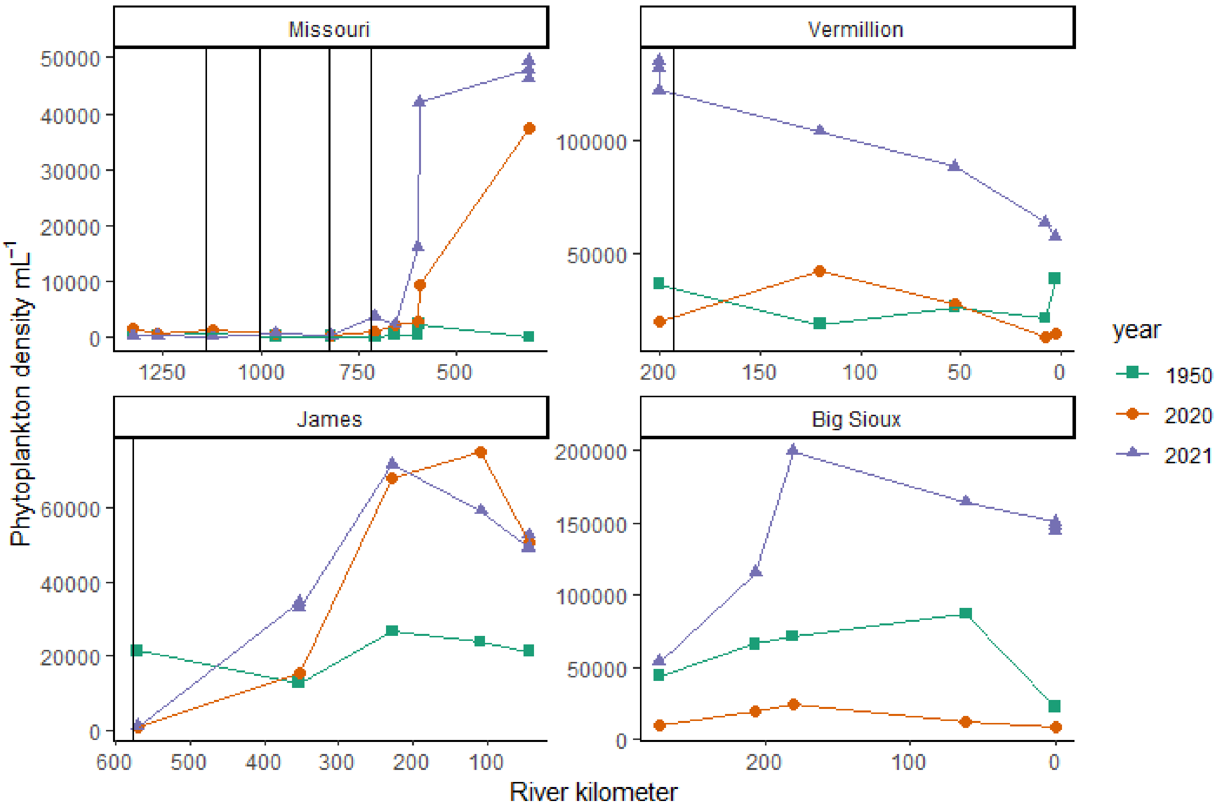
			Dominant genera	
Site number	**System**	**1950**	**2020**	**2021**
2	Missouri	*Aphanizomenon*	*Scenedesmus* *Actinastrum* *Stephanodiscus*	*Scenedesmus* *Tetrastrum* *Stephanodiscus*
5	Vermillion	*Nitzchia* *Cyclotella* *Actinastrum* *Monas*	*Cyclotella* *Stephanodiscus* *Actinastrum*	*Cyclotella* *Stephanodiscus* *Nitzchia*
6	Vermillion	*Nitzchia* *Cyclotella* *Actinastrum* *Monas*	*Cyclotella* *Actinastrum* *Nitzchia*	*Scenedesmus* *Nitzchia* *Cyclotella*
7	Vermillion	*Navicula* *Nitzchia* *Cyclotella* *Actinastrum* *Monas*	*Cyclotella* *Nitzchia* *Stephanodiscus*	*Microcystis* *Cyclotella* *Scenedesmus*
8	Vermillion	*Scenedesmus* *Nitzchia* *Cyclotella* *Actinastrum* *Monas*	*Cyclotella* *Nitzchia* *Stephanodiscus*	*Cyclotella* *Microcystis* *Scenedesmus*
9	Vermillion	*Cyclotella* *Nitzchia* *Actinastrum*	*Cyclotella* *Nitzchia* *Scenedesmus*	*Cyclotella* *Stephanodiscus* *Scenedesmus*
10	Big Sioux	*Cyclotella* *Actinastrum*	*Cyclotella* *Nitzchia* *Navicula*	*Melosira* *Scenedesmus* *Actinastrum*
11	Big Sioux	*Cyclotella* *Actinastrum*	*Cyclotella* *Scenedesmus* *Nitzchia*	*Melosira* *Scenedesmus* *Cyclotella*
12	Big Sioux	*Cyclotella* *Actinastrum*	*Cyclotella* *Scenedesmus* *Nitzchia*	*Melosira* *Cyclotella* *Scenedesmus*
13	Big Sioux	*Actinastrum* *Cyclotella* *Stephanodiscus*	*Cyclotella* *Scenedesmus* *Nitzchia*	*Cyclotella* *Stephanodiscus* *Scenedesmus*
14	Missouri	*Monas*	*Scenedesmus* *Actinastrum* *Microcystis*	*Scenedesmus* *Stephanodiscus* *Ankistrodesmus*
16	James	*Aphanizomenon* *Actinastrum* *Cyclotella*	*Cyclotella* *Actinastrum* *Stephanodiscus*	*Microcystis* *Crucigenia* *Merismopedia*
17	James	*Aphanizomenon* *Actinastrum* *Cyclotella*	*Cyclotella* *Stephanodiscus* *Ankistrodesmus*	*Actinastrum* *Microcystis* *Scenedesmus*
18	James	*Aphanizomenon* *Actinastrum* *Cyclotella*	*Cyclotella* *Stephanodiscus* *Ankistrodesmus*	*Nitzchia* *Merismopedia* *Crucigenia*
19	Lake Mitchell	*Aphanizomenon*	*Aphanizomenon* *Cyclotella*	*Aphanizomenon* *Microcystis*

			Euglena	
20	Lake Mitchell	*Aphanizomenon*	*Cyclotella* *Scenedesmus* *Nitzchia*	*Aphanizomenon* *Microcystis* *Kirchneriella*
22	James	*Aphanizomenon* *Actinastrum* *Cyclotella*	*Cyclotella* *Melosira* *Stephanodiscus*	*Scenedesmus* *Spermatozopsis* *Microcystis*
24	Missouri	*Navicula*	*Scenedesmus* *Cyclotella* *Nitzchia*	*Scenedesmus* *Ankistrodesmus* *Stephanodiscus*
25	Big Sioux	*Actinastrum* *Cyclotella*	*Scenedesmus* *Cyclotella* *Actinastrum*	*Cyclotella* *Stephanodiscus* *Scenedesmus*
26	Missouri	*Cyclotella* *Stephanodiscus*	*Cyclotella* *Scenedesmus* *Stephanodiscus*	*Scenedesmus* *Cyclotella* *Stephanodiscus*
27	Platte	*Navicula* *Actinastrum*	*Scenedesmus* *Actinastrum* *Pediastrum*	*Scenedesmus* *Actinastrum* *Dictyosphaerium*
28	Missouri	*Navicula* *Trachelomonas*	*Scenedesmus* *Cyclotella* *Stephanodiscus*	*Scenedesmus* *Pediastrum* *Microcystis*
33	Missouri	*Navicula* *Sphaerocystis*	*Chlamydomonas* *Oscillatoria* *Carteria*	*Cryptomonas* *Microcystis* *Scenedesmus*
34	Missouri	*Navicula* *Cyclotella* *Monas*	*Nitzchia* *Navicula* *Stephanodiscus*	*Nitzchia* *Actinastrum* *Cryptomonas*
35	Bad	*Synedra* *Actinastrum*	*Asterionella* *Scenedesmus* *Dinobryon*	*Coelastrum* *Surirella* *Monoraphidium*
36	Missouri	*Synedra* *Navicula*	*Fragilaria* *Asterionella* *Navicula*	*Dinobryon* *Coelastrum* *Cryptomonas*
37	Missouri	*Navicula* *Actinastrum*	*Fragilaria* *Asterionella* *Dinobryon*	*Cryptomonas* *Aphanizomenon* *Carteria*
38	Missouri	*Navicula*	*Fragilaria* *Tabularia* *Tabellaria*	*Microcystis* *Oocystis* *Dinobryon*
39	Grand	*Synedra* *Navicula*	*Aphanizomenon* *Scenedesmus* *Chlamydomonas*	*Actinastrum* *Microcystis* *Nitzchia*
41	Heart	*Synedra*	*Scenedesmus* *Ankistrodesmus* *Cyclotella*	*Scenedesmus* *Closteriopsis* *Nitzchia*
42	James	*Aphanizomenon* *Actinastrum* *Synedra*	*Pediastrum* *Scenedesmus* *Schroederia*	*Fragilaria* *Aulacoseria* *Chlamydomonas*

Figures


41
42
North Dakota
Minnesota
39
38
South Dakota
37
36
35
22
34
19
20
18
9
10
11
12
17
8
33
16
7
14
5
6
2
13
25
24
26
Iowa
Nebraska
27
28
N
0
50
100
200 Kilometers
Missouri


System-year
Vermillion2021
Vermillion2020
Vermillion1950
Platte2021
Platte2020
Platte1950
Missouri2021
Missouri2020
Missouri1950
Lake Mitchell2021
Lake Mitchell2020
Lake Mitchell1950
James2021
James2020
James1950
Heart2021
Heart2020
Heart1950
Grand2021
Grand2020
Grand1950
Big Sioux2021
Big Sioux2020
Big Sioux1950
Bad2021
Bad2020
Bad1950
100
1000
10000
100000
1000000
Phytoplankton density mL^{-1}


Missouri
Vermillion
James
Big Sioux
Phytoplankton density mL^{-1}
River kilometer
year
1950
2020
2021
50000
40000
30000
20000
10000
0
1250
1000
750
500
100000
50000
200
150
100
50
0
60000
40000
20000
0
600
500
400
300
200
100
200000
150000
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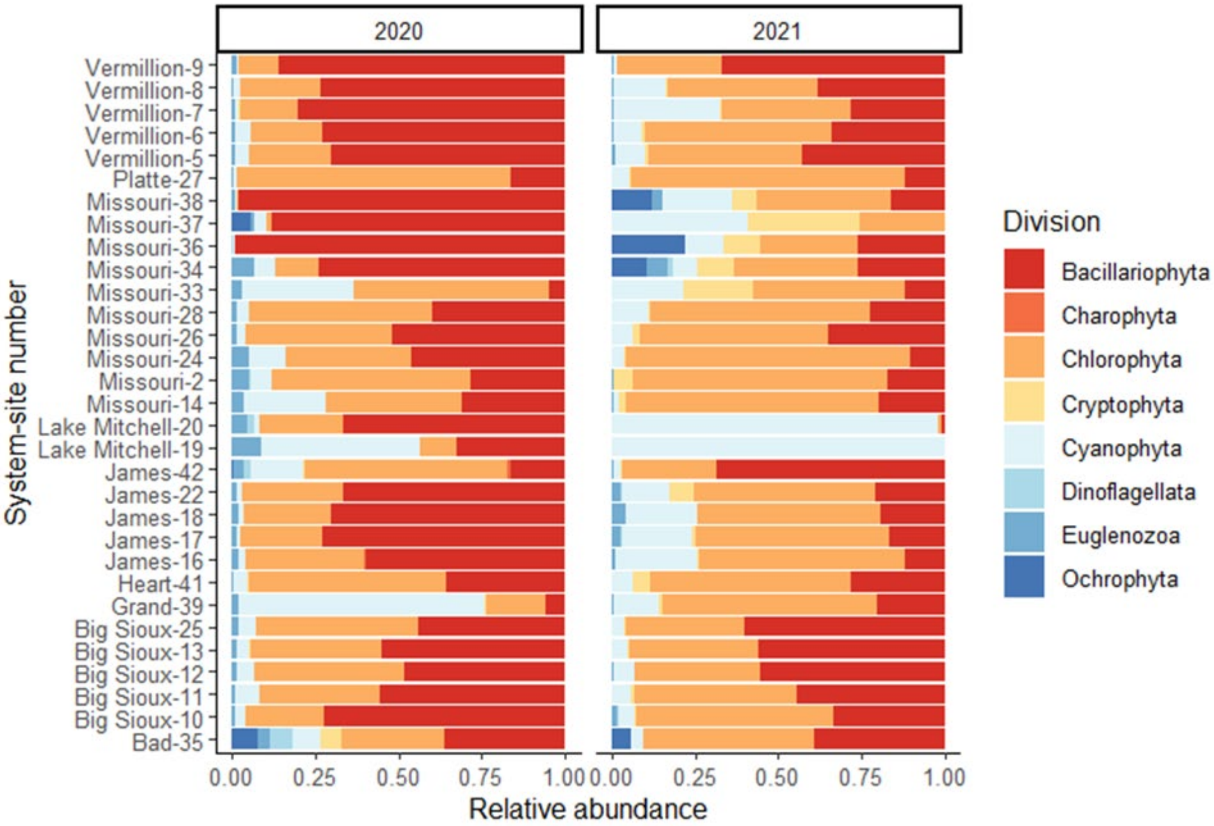
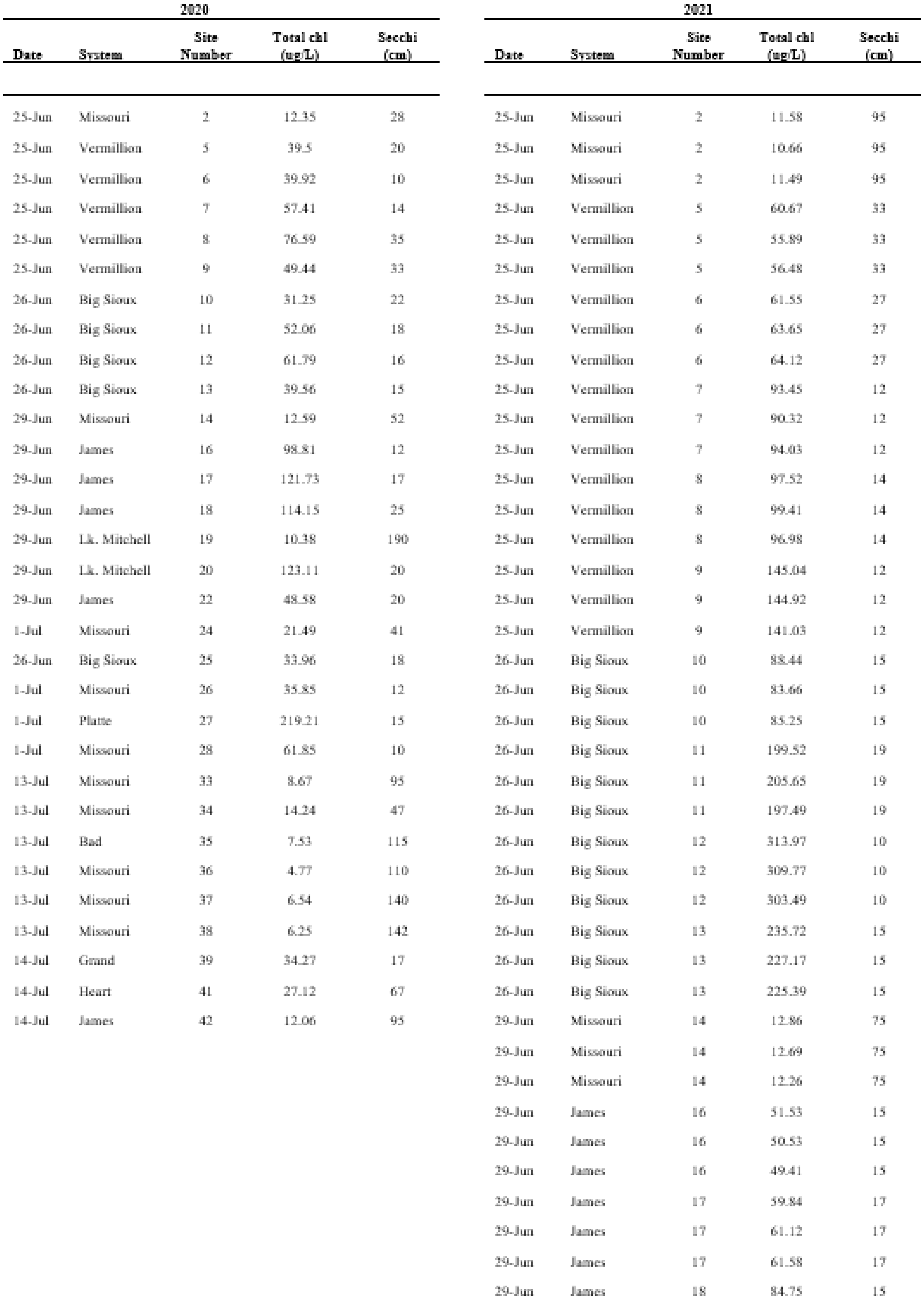
50000
0
200
100
0


2020
2021
Vermillion-9
Vermillion-8
Vermillion-7
Vermillion-6
Vermillion-5
Platte-27
Missouri-38
Missouri-37
Missouri-36
Missouri-34
Missouri-33
Missouri-28
Missouri-26
Missouri-24
Missouri-2
Missouri-14
Lake Mitchell-20
Lake Mitchell-19
James-42
James-22
James-18
James-17
James-16
Heart-41
Grand-39
Big Sioux-25
Big Sioux-13
Big Sioux-12
Big Sioux-11
Big Sioux-10
Bad-35
System-site number
0.00
0.25
0.50
0.75
1.00
Relative abundance
Division
Bacillariophyta
Charophyta
Chlorophyta
Cryptophyta
Cyanophyta
Dinoflagellata
Euglenozoa
Ochrophyta

Supplemental Materials



2020

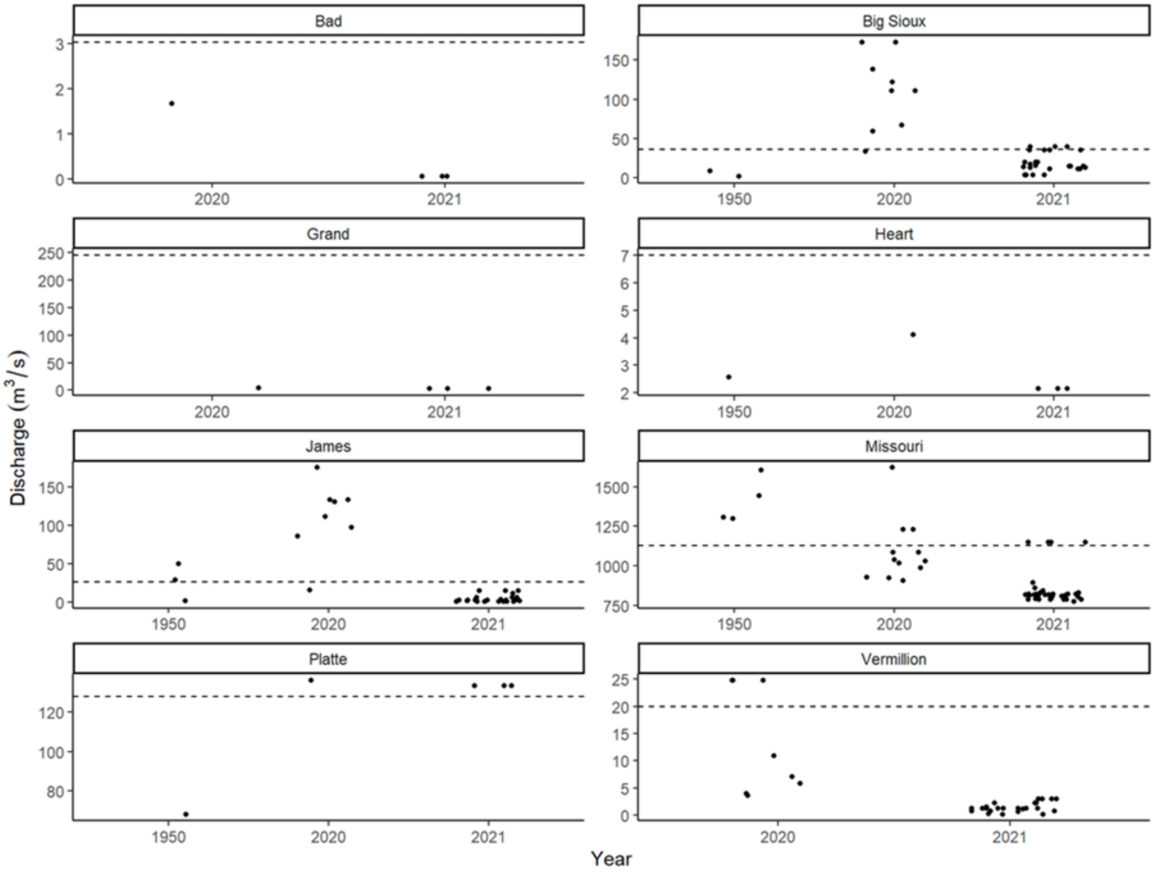
Date	System	Site Number	Total chl (ug/L)	Secchi (cm)
25-Jun	Missouri	2	12.35	28
25-Jun	Vermillion	5	39.5	20
25-Jun	Vermillion	6	39.92	10
25-Jun	Vermillion	7	57.41	14
25-Jun	Vermillion	8	76.59	35
25-Jun	Vermillion	9	49.44	33
26-Jun	Big Sioux	10	31.25	22
26-Jun	Big Sioux	11	52.06	18
26-Jun	Big Sioux	12	61.79	16
26-Jun	Big Sioux	13	39.56	15
29-Jun	Missouri	14	12.59	52
29-Jun	James	16	98.81	12
29-Jun	James	17	121.73	17
29-Jun	James	18	114.15	25
29-Jun	Lk. Mitchell	19	10.38	190
29-Jun	Lk. Mitchell	20	123.11	20
29-Jun	James	22	48.58	20
1-Jul	Missouri	24	21.49	41
26-Jun	Big Sioux	25	33.96	18
1-Jul	Missouri	26	35.85	12
1-Jul	Platte	27	219.21	15
1-Jul	Missouri	28	61.85	10
13-Jul	Missouri	33	8.67	95
13-Jul	Missouri	34	14.24	47
13-Jul	Bad	35	7.53	115
13-Jul	Missouri	36	4.77	110
13-Jul	Missouri	37	6.54	140
13-Jul	Missouri	38	6.25	142
14-Jul	Grand	39	34.27	17
14-Jul	Heart	41	27.12	67
14-Jul	James	42	12.06	95

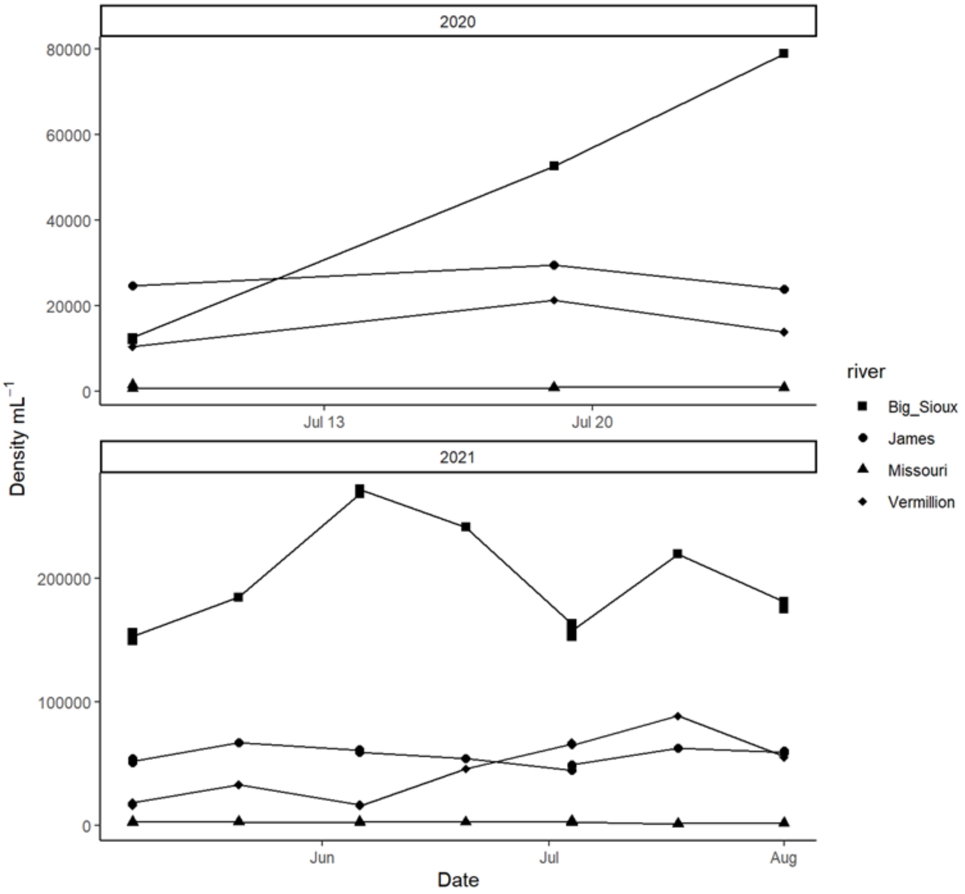
2021

Date	System	Site Number	Total chl (ug/L)	Secchi (cm)
25-Jun	Missouri	2	11.58	95
25-Jun	Missouri	2	10.66	95
25-Jun	Missouri	2	11.49	95
25-Jun	Vermillion	5	60.67	33
25-Jun	Vermillion	5	55.89	33
25-Jun	Vermillion	5	56.48	33
25-Jun	Vermillion	6	61.55	27
25-Jun	Vermillion	6	63.65	27
25-Jun	Vermillion	6	64.12	27
25-Jun	Vermillion	7	93.45	12
25-Jun	Vermillion	7	90.32	12
25-Jun	Vermillion	7	94.03	12
25-Jun	Vermillion	8	97.52	14
25-Jun	Vermillion	8	99.41	14
25-Jun	Vermillion	8	96.98	14
25-Jun	Vermillion	9	145.04	12
25-Jun	Vermillion	9	144.92	12
25-Jun	Vermillion	9	141.03	12
26-Jun	Big Sioux	10	88.44	15
26-Jun	Big Sioux	10	83.66	15
26-Jun	Big Sioux	10	85.25	15
26-Jun	Big Sioux	11	199.52	19
26-Jun	Big Sioux	11	205.65	19
26-Jun	Big Sioux	11	197.49	19
26-Jun	Big Sioux	12	313.97	10
26-Jun	Big Sioux	12	309.77	10
26-Jun	Big Sioux	12	303.49	10
26-Jun	Big Sioux	13	235.72	15
26-Jun	Big Sioux	13	227.17	15
26-Jun	Big Sioux	13	225.39	15
29-Jun	Missouri	14	12.86	75
29-Jun	Missouri	14	12.69	75
29-Jun	Missouri	14	12.26	75
29-Jun	James	16	51.53	15
29-Jun	James	16	50.53	15
29-Jun	James	16	49.41	15
29-Jun	James	17	59.84	17
29-Jun	James	17	61.12	17
29-Jun	James	17	61.58	17
29-Jun	James	18	84.75	15

29-Jun	James	18	86.66	15
29-Jun	James	18	81.54	15
29-Jun	Lk. Mitchell	19	8.38	45
29-Jun	Lk. Mitchell	19	8.57	45
29-Jun	Lk. Mitchell	19	8.44	45
29-Jun	Lk. Mitchell	20	9.13	15
29-Jun	Lk. Mitchell	20	10.56	15
29-Jun	Lk. Mitchell	20	10.68	15
29-Jun	James	22	52.08	18
29-Jun	James	22	52.3	18
29-Jun	James	22	50.41	18
1-Jul	Missouri	24	19.44	80
1-Jul	Missouri	24	19.61	80
1-Jul	Missouri	24	19.26	80
26-Jun	Big Sioux	25	170.57	18
26-Jun	Big Sioux	25	168.8	18
26-Jun	Big Sioux	25	174.93	18
1-Jul	Missouri	26	41.27	30
1-Jul	Missouri	26	40.62	30
1-Jul	Missouri	26	39.62	30
1-Jul	Platte	27	182.42	17
1-Jul	Platte	27	169.62	17
1-Jul	Platte	27	171.86	17
1-Jul	Missouri	28	63.44	16
1-Jul	Missouri	28	65.74	16
1-Jul	Missouri	28	62.02	16
13-Jul	Missouri	33	6.97	70
13-Jul	Missouri	33	6.9	70
13-Jul	Missouri	33	6.74	70
13-Jul	Missouri	34	10.75	47
13-Jul	Missouri	34	10.52	47
13-Jul	Missouri	34	10.7	47
13-Jul	Bad	35	4.25	92
13-Jul	Bad	35	4.14	92
13-Jul	Bad	35	3.86	92
13-Jul	Missouri	36	3.16	185
13-Jul	Missouri	36	3.04	185
13-Jul	Missouri	36	2.76	185
13-Jul	Missouri	37	3.33	135
13-Jul	Missouri	37	3.48	135
13-Jul	Missouri	37	3.18	135
13-Jul	Missouri	38	4.57	120
13-Jul	Missouri	38	4.58	120
13-Jul	Missouri	38	4.53	120

14-Jul	Grand	39	30.95	22
14-Jul	Grand	39	29.72	22
14-Jul	Grand	39	29.3	22
14-Jul	Heart	41	7.94	105
14-Jul	Heart	41	7.75	105
14-Jul	Heart	41	8.23	105
14-Jul	James	42	11.98	95
14-Jul	James	42	13.88	95
14-Jul	James	42	13.77	95


Bad
3
2
1
0
2020
2021
Big Sioux
150
100
50
0
1950
2020
2021
Grand
250
200
150
100
50
0
2020
2021
Heart
7
6
5
4
3
2
1950
2020
2021
James
150
100
50
0
1950
2020
2021
Missouri
1500
1250
1000
750
1950
2020
2021
Platte
120
100
80
1950
2020
2021
Vermillion
25
20
15
10
5
0
2020
2021
Discharge (m^3/s)
Year


2020
80000
60000
40000
20000
0
Jul 13
Jul 20
2021
200000
100000
0
Jun
Jul
Aug
Date
Density mL^{-1}
river
■ Big_Sioux
● James
▲ Missouri
◆ Vermillion

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Chapter 2: Using fluorometric *in vivo* chlorophyll determination as a proxy for phytoplankton density in rivers of the Middle Missouri River Basin

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Abstract

Chlorophyll has been used extensively in ecological monitoring as a proxy for phytoplankton density or biovolume due to the relative simplicity of processing samples. However, because several variables control the chlorophyll concentration in algal cells, the reliability of using chlorophyll solely to replace enumeration or biovolume estimates of phytoplankton has come under some scrutiny. We regressed the predictor variable of total chlorophyll and response variable of algal cell density as well as the predictor variable of $\log_{10}$ transformed Secchi depth (Secchi) and the response variables of either total chlorophyll or algal cell density from 161 samples across nine rivers of the Middle Missouri River Basin. A positive relationship was observed between chlorophyll and algal cell density, while a negative relationship was observed between Secchi and either chlorophyll or algal cell density. These findings suggest that measuring chlorophyll in lieu of algal cell enumeration and identification may provide an option to monitor phytoplankton dynamics in rivers. The regressions between either Secchi and chlorophyll or algal cell density suggested less predictability than the relationship between chlorophyll and algal cell density, likely due to high concentrations of suspended sediment at sample sites. Chlorophyll determined by *in* vivo fluorescence provides a good proxy to rapidly monitor phytoplankton dynamics in lowland rivers.

Introduction

Chlorophyll measurements have been used extensively in environmental monitoring as a comparative variable or proxy to estimate phytoplankton biovolume or cell densities (Fee, 1976; Houser, Bierman, Burdis, & Soeken-Gittinger, 2010; Marshall & Peters, 1989; Turner, Milan, Swenson, & Lee, 2022; Yacobi, Gitelson, & Mayo, 1995). Although the approaches vary (e.g., photometry, *in vivo* fluorescence, ultra-high-performance liquid chromatography, etc.), the relative ease of using chlorophyll measurements to rapidly obtain results for multi-sample studies illustrates the appeal of these accepted methods to reveal ecological changes in the trophic state of aquatic systems.

Several studies, conducted mostly in lakes and reservoirs, have focused on the relationship between algal cell counts and the photosynthetic chemical constituents found in phytoplankton, finding that variables like temperature (Geider, 1987; Lürling, Mello, Van Oosterhout, de Senerpont Domis, & Marinho, 2018), community structure (Canfield Jr et al., 2019; Kasprzak, Padisák, Koschel, Krienitz, & Gervais, 2008; Watson, Ridal, & Boyer, 2008), and waterbody trophic status (Kalchev, Beshkova, Boumbarova, Tsvetkova, & Sais, 1996; Kasprzak et al., 2008) may influence the shape and exponent of slope coefficients, and thus the predictive strength of those relationships. Consequently, the question of whether chlorophyll is a reliable proxy for biovolume or algal cell count has been debated due to the variability observed in those regressed associations (Canfield Jr et al., 2019; He, Wang, & Xu, 2022; Huot et al., 2007; Kasprzak et al., 2008). Irrespective of potential variability when using chlorophyll as a proxy for cell density or biovolume, positive relationships are commonly observed using both chlorophyll-biovolume or chlorophyll-cell density (Branco & Senna, 1996; Canfield Jr et al., 2019; Canfield Jr, Linda, & Hodgson, 1985; Cowell & Dawes, 2008; Desortová, 1981; Ietswaart, Breebaart, Van Zanten, & Bijkerk, 1999; Kalchev et al., 1996; Kivrak & Hasan, 2005; Xue-qin, Qiang, Ming-rui, Chun-yan, & Wen-hui, 2012). In some cases, however, using taxonomical methods to categorize phytoplankton community structure, along with estimating algal cell density or biovolume may be preferred for determining changes in algal community dynamics (da Silva, Pelicice, & Rodrigues, 2020; Fukushima et al., 1999) or monitor cyanobacterial blooms (Ewerts, Swanepoel, & Du Preez, 2013; Watson et al., 2008).

Similar to what is observed in lakes and reservoirs, phytoplankton dominate the basal food web in low gradient, lowland rivers with high suspended sediment loads, where algal periphyton may only form in a very shallow littoral zone (Descy & Gosselain, 1994). Although continually subject to downstream advection, the phytoplankton communities in rivers with relatively long residence times and abundant nutrient loads also exhibit seasonal bloom events when algal growth exceeds loss during times with relatively low water velocity (Lucas, Thompson, & Brown, 2009; Waylett, Hutchins, Johnson, Bowes, & Loewenthal, 2013). Lowland rivers may also share similarities with shallow lakes in the taxonomic representation of phytoplankton, at least to genus level (Reynolds, Descy, & Padisák, 1994). Despite sharing some chemical and biological similarities with lakes, there are comparatively fewer studies in rivers comparing direct relationship between chlorophyll and algal cell density (Cowell & Dawes, 2008; Sabater et al., 2008). From a management perspective, employing a reliable proxy for algal density in rivers is important to either complement later analyses using identification and enumeration of phytoplankton or as a standalone mechanism to make rapid environmental assessments.

Here, we use data collected from rivers across the Middle Missouri River Basin (MMRB) to test the hypothesis that chlorophyll is a good predictor of algal cell densities. We also investigate whether chlorophyll or algal cell density is more closely correlated to Secchi depth (Secchi).

Methods

Site selection

During 2020 and 2021, we collected 161 samples at 23 sites located along nine rivers in the MMRB (Figure 1) as delineated by Pegg and Pierce (2002) and Kalkhoff (2013) during 2020 and 2021.Sample site locations were entirely lotic, and were obtained using geographical descriptions (i.e., highway bridges, towns, river miles, etc.) from Damann (1951). The sampling duration for 2020 was from June to July and for 2021 from May to August in 2022.

Sample design

Surface water samples were collected in 125-mL brown plastic bottles at approximately 0.4 m depth after allowing river water to clear of any disturbed sediment caused by wading. Secchi was also measured at all sample locations using a standard 0.2 m diameter Secchi disk with two white and black opposing quadrants. During each sampling event, we determined total chlorophyll µg/L (chlorophyll) by measuring chlorophyll-*a*, *b*, c_1, c_2, and *d* though *in* vivo fluorescence using a factory-calibrated portable fluorimeter (AquaFlash, Turner Designs, San Jose, California, USA). The fluorimeter, which uses multiple turnover Pulse Amplitude Modulated Fluorometry consisting of varying light intensities, was checked weekly for instrument drift by fluorescing a solution of 200 ppb Rhodamine (Turner Designs, San Jose, California, USA). Calibration passed during all trials, suggesting satisfactory instrument performance. Finally, samples were fixed with Lugol’s iodine before being placed on ice.

Laboratory analysis

Phytoplankton were identified and enumerated using the Direct Count Method (DCM) described in Damann (1950 and 1951) based on methods from Baylis (1922). Comparisons of mean count accuracy between the DCM and the Foerst Centrifuge method described in Damann (1950) yielded relatively similar results when counting units of phytoplankton mL^{-1}. Over a three-month study analyzing 119 samples, the DCM deviated 6% from the Foerst centrifuge sample mean mL^{-1} (Damann, 1950). Like other widely implemented protocols such as the Utermöhl method (H. Utermöhl, 1958; v. H. Utermöhl, 1931), the DCM uses unconcentrated samples for identification and enumeration. This protocol was found to be particularly effective for the sediment-laden water encountered in the MMRB and has a relatively quick (~15 minutes) settling time between samples.

We identified phytoplankton to genus using taxonomic keys in (Spaulding et al., 2021; Wehr, Sheath, & Kociolek, 2015) at magnifications from 200 to 630x using brightfield and phase contrast microscopy (Leica DMLB microscope, Leica Microsystems, Wetzlar, Germany). Sample bottles were gently inverted ten times to homogenously distribute algal cells before transferring a 1 mL subsample into a gridded Sedgewick-Rafter counting chamber and assuming random distribution in the chamber. We enumerated algal cells in 200 total 1 mm^3 fields in four randomly chosen 50 mm^3 rows, the recommended number of fields for combining timely enumeration with a high degree of accuracy as described in Damann (1950) for "a rather low population density". After counting was complete in two of the 50 mm^3 rows, the sample was discarded, and the cell was again filled with a 1 ml aliquot sub-sample to repeat the counting process of 50 mm^3 twice more. Enumerated values were then extrapolated to obtain phytoplankton density mL^{-1}. We attempted to identify all clearly visible phytoplankton units and counted only those units with visible chloroplasts. However, if units were unidentifiable caused by poor positioning, damage, obscuring detritus, or taxonomic uncertainty (~4% of all samples) those units were grouped in corresponding unresolved divisions (e.g., Unresolved Chlorophyta, Unresolved Bacillariophyta, etc.) while still included in the total count.

We followed the descriptions from Damann (1951) to define a single phytoplankton unit. Unicellular and colonial taxa such as discrete cells or single cells within the colony (*e.g.*, *Actinastrum, Scenedesmus*) were counted as one unit per cell in the colony. Using a calibrated ocular micrometer, colonial genera forming irregular masses or tightly bound clusters (*e.g.*, *Microcystis, or Coelastrum*) were counted as one unit per 20 µm in diameter. In addition, we counted filamentous taxa (*i.e., Aphanizomenon* and *Aulacoseria*) as one unit per 100 µm in length, irrespective of filament diameter.

Given the duration required to identify and enumerate plankton-dense samples frequently encountered in 2020 we revised our counting methods in 2021 for samples with ≤ 40 µg/L total chlorophyll by reducing counting to 25 mm^3 for sites >40 µg/L total chlorophyll. It is expressed in the DCM, as well as in other counting methods (Lund, Kipling, & Le Cren, 1958; H. Utermöhl, 1958) that the reliability of cell density estimates in a sample increases with increased unit counts. However, high precision around the mean is still expected when counting fewer fields in samples with relatively high density. This revised approach allowed us to obtain reasonable estimates for both lower relatively higher density sites, while being conscientious of the time allotted to enumerating related sites. The same individual was responsible for identifying and enumerating all samples.

Statistical analysis

We evaluated the relationship between the predictor variable of standardized (z-score) total chlorophyll µL^{-1} and the response variable of algal cell density $mL^{-1}/1 \times 10^6$ using a generalized linear model with a Gaussian likelihood and random intercepts and slopes by river using R (R Core Team, 2021). In addition, we evaluated the relationship between the predictor variable of $\log_{10}$ transformed, standardized (z-score) Secchi (cm) and either standardized (z-score) total chlorophyll µL^{-1} or algal cell density $mL^{-1}/1 \times 10^6$ using a Gamma likelihood for both analyses. For all models, prior values were chosen using prior predictive checks that ensured coverage of a wide but biologically reasonable range of values (Wesner & Pomeranz, 2021).

Bayesian inference was used to fit all three models using Hamiltonian Monte Carlo methods in *rstan* (Carpenter et al., 2017) using the *brms* package (Bürkner, 2017). In each model, we ran four chains with 2000 iterations, where the first 1000 of those iterations were discarded during sampling warmup. Model convergence was checked by confirming that r-hat values were <1.1. We checked the model fit using posterior predictive checks to ensure that the models could simulate data that resembled the original data (Gabry, Simpson, Vehtari, Betancourt, & Gelman, 2019).

Results

Chlorophyll values ranged from 7 to 329 µg L^{-1} and algal cell density ranged from 700 to 272,000 cells mL^{-1} (Fig. 2). Chlorophyll was positively and directly related to algal cell density ($R^2 = 0.92$; $y = 0.06 + 0.07*x$).

Secchi ranged from 7 to 125 cm. Chlorophyll ($R^2 = 0.36$; $y = 0.97 – 1.15*x$; Fig. 3) or algal cell density ($R^2 = 0.43$; $y = 2.64 – 1.82*x$; Fig. 4) varied inversely as a function of Secchi.

Discussion

Our findings support our hypothesis that chlorophyll determined by *in* vivo fluorescence provides a good proxy to rapidly monitor phytoplankton dynamics in rivers throughout the MMRB. Thus, avoiding the time-consuming process of manually enumerating and identifying phytoplankton cells which requires specialist knowledge. The positive relationship between chlorophyll and algal cell density is similar to that reported by Canfield Jr et al. (2019), who described a positive relationship between chlorophyll-*a* and phytoplankton biovolume in lakes and reservoirs using a data set from Denmark, Florida, and the National Lakes Assessment from the US Environmental Protection Agency.

Other forms of chlorophyll in aquatic systems (i.e., macrophytes and terrestrial plants) may confound the relationship when using chlorophyll as a proxy for algal cell density (Papageorgiou, Tsimilli-Michael, & Stamatakis, 2007). He et al. (2022) described revised methods used in lake environments evaluating biogenic n-heptadecane (C_{17})–only produced in cyanobacteria and green algae–as a compensatory proxy to resolve the over predictive effects that macrophytes and terrestrial plants exert on the relationship between chlorophyll-*a* values and algal cell density. In rivers of the MMRB, macrophytes are sparsely distributed and terrestrial inputs are largely in the form of senesced material, inferring that these sources of chlorophyll likely have no measurable impact on phytoplanktonic chlorophyll analysis (Bukaveckas et al., 2011). Consequently, the potential for non-planktonic, autochthonous inputs to skew phytoplanktonic chlorophyll findings is likely low in MMRB rivers. This suggests that the chlorophyll readings are likely dominated by phytoplankton, not macrophytes, reinforcing the observed relationship between chlorophyll and algal cell density .

Most of the summertime chlorophyll values in the rivers of the MMRB matched or exceeded chlorophyll-*a* concentrations reported from several other lowland rivers globally (Bukaveckas et al., 2011; Engel et al., 2019; Ha, Jang, & Joo, 2002; Houser et al., 2010; Istvánovics, Honti, Vörös, & Kozma, 2010; Sabater et al., 2008) and generally paralleled values observed in eutrophic or hypertrophic lakes and reservoirs (Barone & Flores, 1994; C. D. Brown, Canfield Jr, Bachmann, & Hoyer, 1998; He et al., 2022; Vijverberg & Boersma, 1997). The similarity of our findings were likely influenced by relatively high nutrient load inputs from pervasive agricultural practices (J. B. Brown, Sprague, & Dupree, 2011) and relatively long phytoplankton residence times due to low flows at most sites. Dodds et al. (1998) classifies eutrophic conditions in streams as >30 µg L^{-1} chlorophyll-*a*, which would classify most (104 of 161) samples in this study as eutrophic. If using a classification system for lakes that includes the hypertrophic category (56-155+ µg L^{-1} chlorophyll-*a*, (R. E. Carlson & Simpson, 1996), 65 of 161 samples would be classified as hypereutrophic. It should be noted that we determined total chlorophyll *in situ* (including chlorophyll-*a*, *b*, c_1, c_2, and *d*) using *in vivo* fluorescence, not solely chlorophyll-*a* in a laboratory setting as many other studies' methods have used. The chlorophyll pigment composition in phytoplankton varies depending on the environment and taxonomy (Schagerl, Pichler, & Donabaum, 2003). While chlorophyll-*a* is the dominant chlorophyll pigment in most phytoplankton without chlorophyll-related accessory pigments (e.g., Cyanophyta), chlorophyll-*b* is the accessory pigment in Chlorophyta, Charophyta, and Euglenophyta, and chlorophyll-*c* compounds are accessory pigments in Bacillariophyta, Cryptophyta, Pyrrhophyta, and Chrysophyta (Dring & Dring, 1992; Kuczynska, Jemiola-Rzeminska, & Strzalka, 2015). Notwithstanding these differences, chlorophyll values in this study were only slightly greater than other studies reporting on chlorophyll-*a* values from rivers in the MMRB (Beaver et al., 2013; Bukaveckas et al., 2011; Havel et al., 2009). Specifically, in the Missouri River, Buckaveckas et al. (2011) recorded average summertime chlorophyll-*a* values from ~5 to 65 µg L^{-1}; our chlorophyll values ranged from ~8 to 67 µg L^{-1} in that same reach of the Missouri River. Regardless of the accessory chlorophyll pigments determined in phytoplankton taxa of the MMRB, chlorophyll-*a* is the dominant chlorophyll pigment in the taxa identified in the MMRB, and although the inclusion of all chlorophylls may positively skew our findings when compared to other chlorophyll-*a* values, we should be justified in comparing our chlorophyll values with chlorophyll-*a* values.

Because chlorophyll or algal density data are not always available or attainable, we also postulated whether the relatively uncomplicated measurement of Secchi could be a reasonable proxy to determine changes in phytoplankton standing crop in lowland rivers. Using Secchi as a trophic indicator to detect changes in phytoplankton standing crop or chlorophyll frequently exhibits an inverse relationship in lakes and reservoirs when regressed with chlorophyll-*a* (Canfield Jr et al., 2019; Canfield Jr et al., 2016; Kivrak & Hasan, 2005) unless waters comprise high concentrations of suspended solids or colored dissolved organic matter (Brezonik et al., 2019; R. Carlson, 2007; Lind, 1986; Zou et al., 2020). Our results using Secchi regressed with chlorophyll ($R^2 = 0.36$) or algal density ($R^2 = 0.43$) yielded inverse relationships yet had unfavorable R^2 values for use as a good predictor for chlorophyll of algal cell density. Similarly, Cowell and Dawes (2008) observed an inverse relationship between Secchi and both algal biovolume and chlorophyll-*a*; however, these findings were from a lowland, spring-fed river with little suspended matter as is rarely observed in rivers in the MMRB. It is likely that the prevalence of relatively dense concentrations of inorganic suspended sediment inputs at most sites likely confounded the degree of predictability between Secchi and chlorophyll or algal cell density. The greatest Secchi measurements were consistently recorded at two sites below Gavins Point dam in the Missouri River, where given the short distance below a reservoir, sediment concentrations were not representative of other sites in this study (unpublished data; personal observations). Consequently, in temperate, lowland rivers these relationships may be less defined. Although the relationship of Secchi with chlorophyll or algal cell density may have been partially confounded with inorganic suspended matter, irradiance in the eutrophic rivers of the MMRB, and other eutrophic or hypertrophic lowland rivers is controlled–at least in part–by algal density (Bukaveckas et al., 2011), similar to observations from many eutrophic lakes and reservoirs (Kokociński, Dziga, Antosiak, & Soininen, 2021; Schanz, 1994).

Caveats

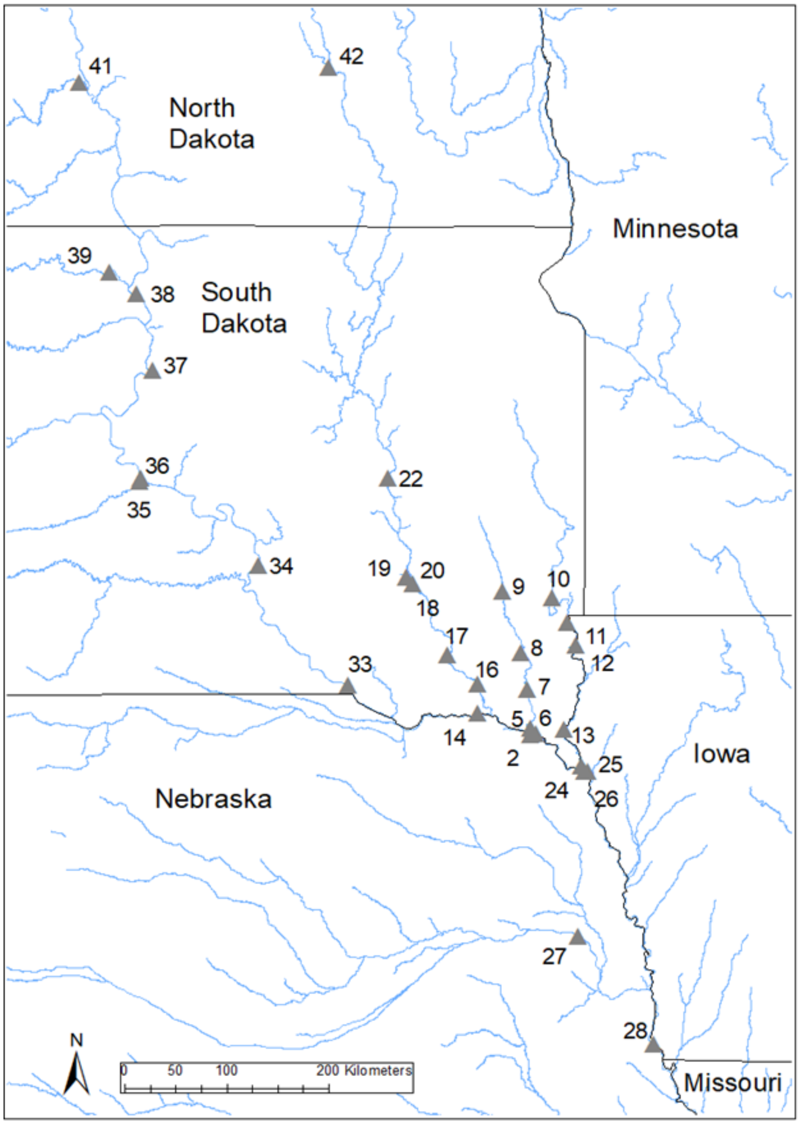
Our sample sites were restricted to a narrow (40.7° to 46.9°, with most sites between about 42.4° to 43.8°) latitudinal gradient which could limit the usefulness of the relationships outside these latitudes. However, including chlorophyll data from a larger latitudinal gradient may weaken the relationship between chlorophyll and density or biovolume (Canfield Jr et al., 2019). In addition, because we only sampled during summer months, it is unclear whether the relationship between chlorophyll and algal cell density observed in the MMRB remains evident during other seasons. Within an algal genus, there may be considerable variability between individual algal cell morphology and volumes, and within genera which if unaccounted for may skew relationships observed between algal cell densities and chlorophyll, especially during blooms dominated by a single genus. Thus, if encountering predominantly large or small phytoplankton in a sample without concurrently evaluating algal biovolume, the degree of predictability in the relationship between chlorophyll and algal cell density may be confounded. Although there were occasional bloom events where more than half of enumerated algal cells were composed of one genus or size range (unpublished data), a broad morphological and taxonomic distribution was generally observed. We believe that the broad size range and morphological variance encountered in samples from the MMRB reduces the potential for biases in relation toward one algal size spectra when using chlorophyll solely as a proxy for algal cell density without necessitating concurrent evaluation of algal biovolume. Consequently, because of the generally broad distribution of phytoplankton morphology and size encountered in samples we did not address whether the different levels of chlorophylls (*a, b,* c_1*,* c_2*, d*) expressed in a dominant genus or division of phytoplankton affected the chlorophyll to algal cell density relationship. In lowland temperate rivers subject to cultural eutrophication, phytoplankton standing crop is influenced largely by physical processes (*i.e.,* water residence time, temperature, day length, irradiance) and less by nutrient variability, which during the growing season are generally in excess (Bowes et al., 2012; Ietswaart et al., 1999; Miltner, 2018; Soballe & Kimmel, 1987; Waylett et al., 2013). Given the intensive and widescale agricultural practices across the MMRB, and the relatively high recorded phytoplankton density and chlorophyll values, it is unlikely that rivers were nutrient limited. Thus, addressing available nutrients and their relationship with chlorophyll or algal cell density was out of the scope for this study.

Conclusion

In vivo chlorophyll determination is useful for rapid monitoring of broad temporal and longitudinal changes in the algal standing crop of river systems. This cost-effective and time-saving approach may be especially beneficial in understudied rivers where chlorophyll concentrations are not well documented, like some of the rivers in this study. Chlorophyll or chlorophyll-*a* remain commonly used as proxies for characterizing sestonic algae in rivers without considering cell density or biovolume, (Bukaveckas et al., 2011; Houser et al., 2010;

Miltner, 2018; Turner et al., 2022) despite the numerous variables that may confound the relationship between chlorophyll and biovolume or algal cell density (e.g., temperature, community structure composition, waterbody trophic status, light availability). Considering the environmental influences like warming waters due to climate change and the persistence of cultural eutrophication and its propensity to rapidly alter lotic, algal standing crop, the capacity to use a proxy with known variability for rapidly detecting these changes remains an important tool for riverine ecological monitoring.

Figures


North Dakota
South Dakota
Minnesota
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Nebraska
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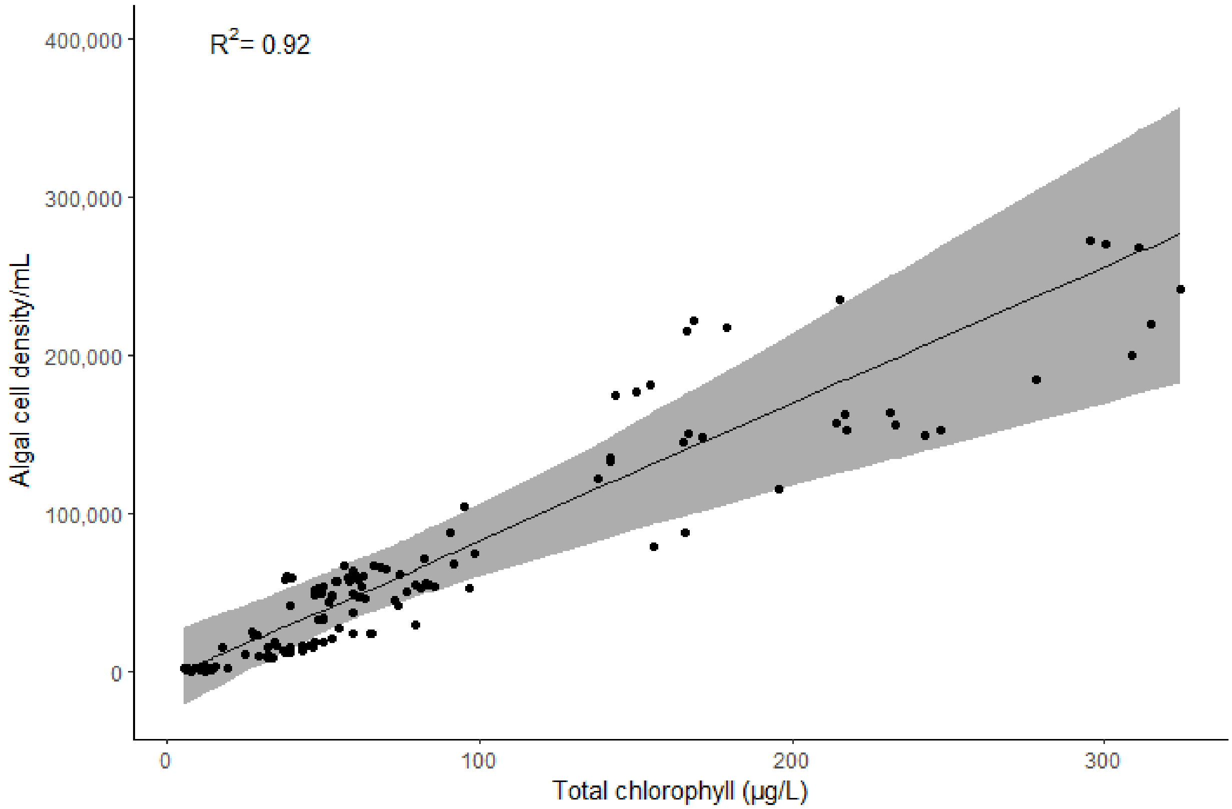
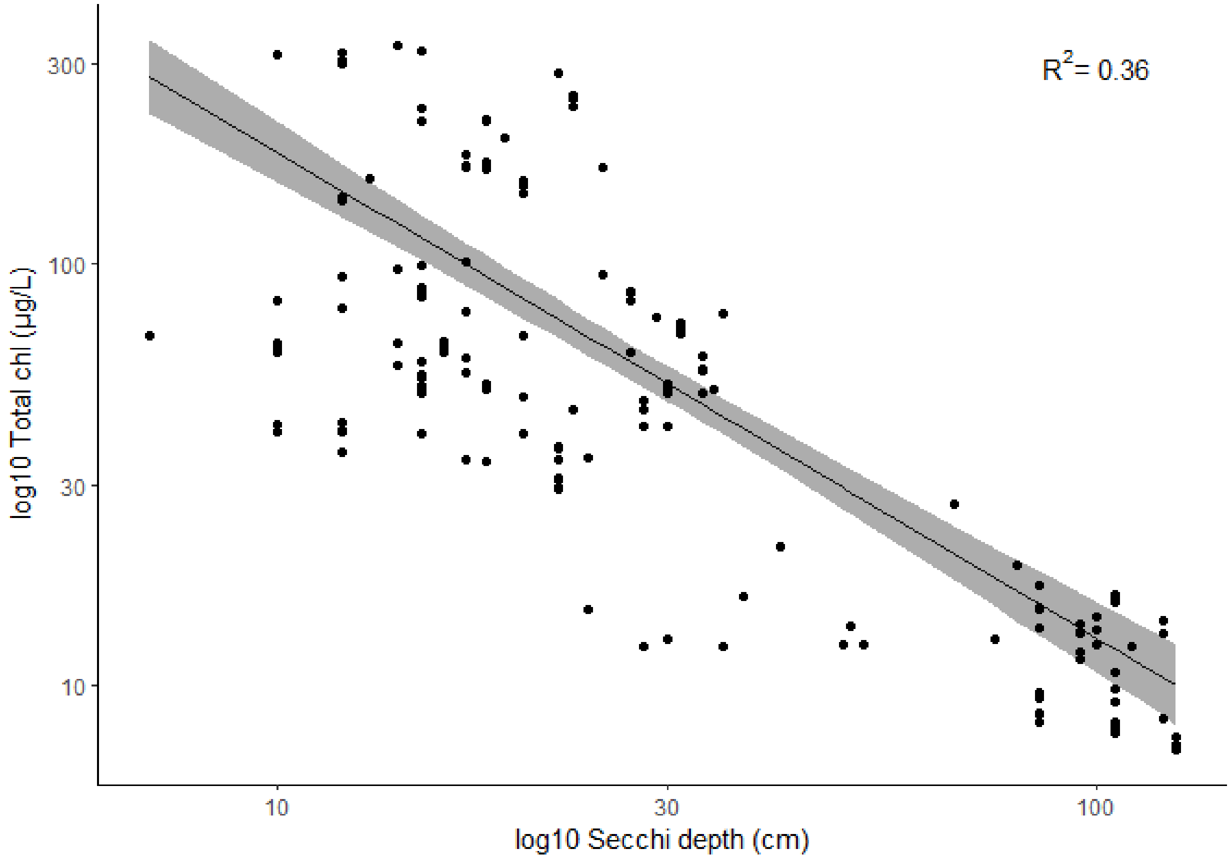
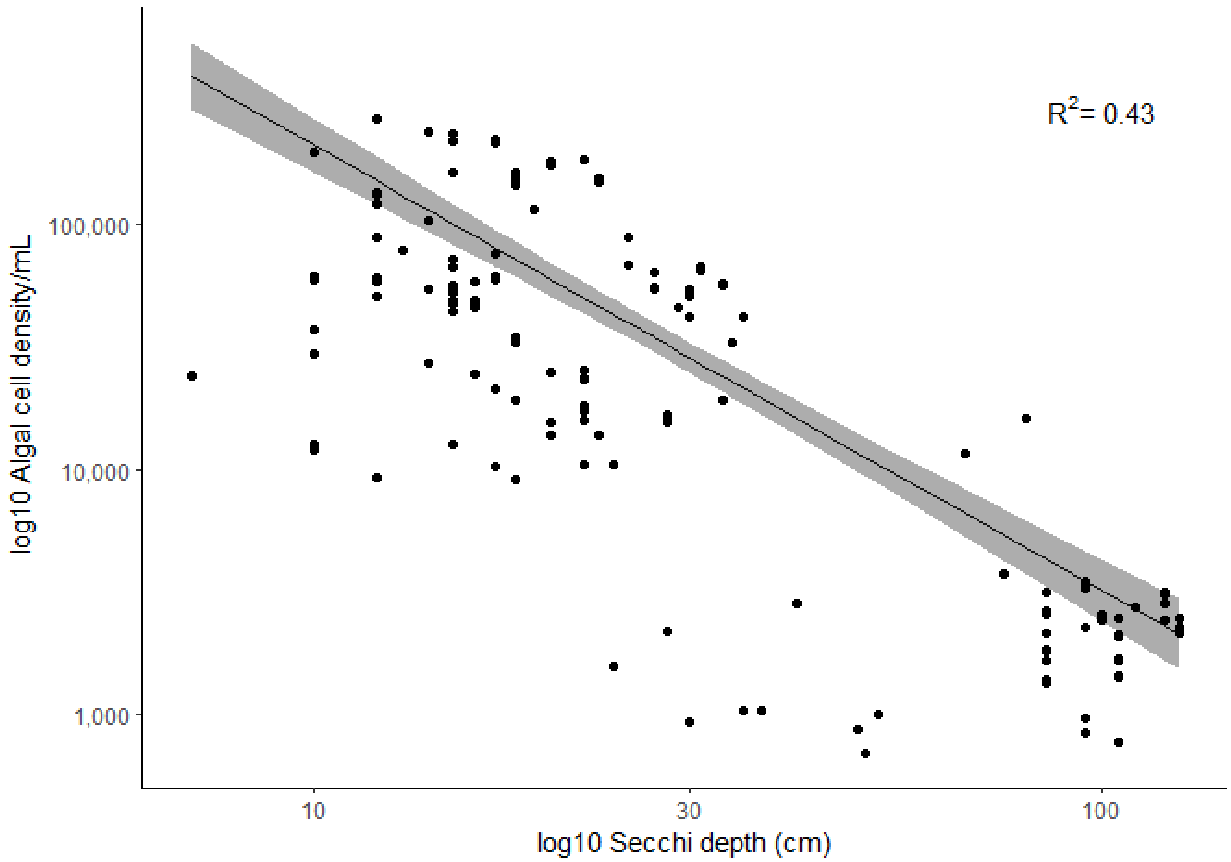
0 50 100 200 Kilometers


R² = 0.92
Algal cell density/mL
400,000
300,000
200,000
100,000
0
0
100
200
300
Total chlorophyll (µg/L)


R^2= 0.36
log10 Total chl (µg/L)
300
100
30
10
log10 Secchi depth (cm)
10
30
100


R^2= 0.43
log10 Algal cell density/mL
100,000
10,000
1,000
10
30
100
log10 Secchi depth (cm)

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