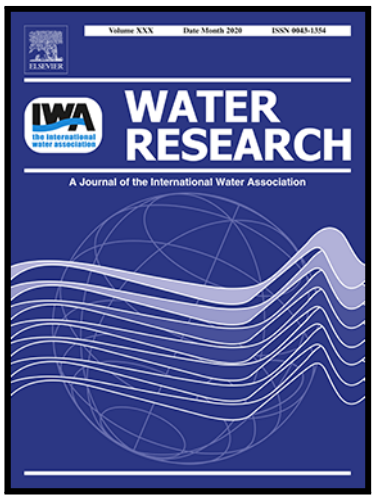

Using Cyanobacteria and Other Phytoplankton to Assess Trophic Conditions: A qPCR-Based, Multi-Year Study in Twelve Large Rivers across the United States

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Using Cyanobacteria and Other Phytoplankton to Assess Trophic Conditions: A qPCR-Based, Multi-Year Study in Twelve Large Rivers across the United States

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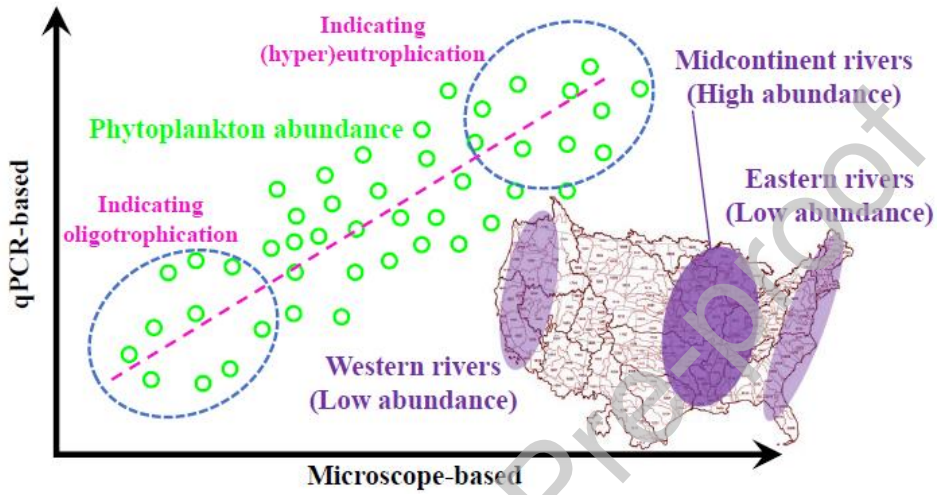
Tables

Highlights

- Assessed phytoplankton in 12 freshwater rivers across the U.S. using qPCR and microscopy
- qPCR- and microscope-based phytoplankton abundance had a significant positive correlation
- qPCR-based phytoplankton abundance was indicative of river trophic conditions
- qPCR is a promising numerical tool to quantify phytoplankton and assess river trophic status
- Higher phytoplankton abundance indicated the midcontinent river sites were more eutrophic

Graphical abstract

Tables



Abstract: Phytoplankton is the essential primary producer in fresh surface water ecosystems. However, excessive phytoplankton growth due to eutrophication significantly threatens ecologic, economic, and public health. Therefore, phytoplankton identification and quantification are essential to understanding the productivity and health of freshwater ecosystems, as well as the impacts of phytoplankton overgrowth (such as cyanobacterial blooms) on public health. Microscopy is the gold standard for phytoplankton assessment but is time-consuming, has low throughput, and requires rich experience in phytoplankton morphology. Quantitative polymerase chain reaction (qPCR) is accurate and straightforward with high throughput. In addition, qPCR does not require expertise in phytoplankton morphology. Therefore, qPCR can be a useful alternative tool for molecular identification and enumeration of

phytoplankton. Nonetheless, a comprehensive study is missing which evaluates and compares the feasibility of using qPCR and microscopy to assess phytoplankton in freshwater. This study focused on 1) comparing the performance of qPCR and microscopy in identifying and quantifying phytoplankton and 2) evaluating qPCR as a molecular tool to assess phytoplankton and indicate eutrophication. We assessed phytoplankton using both qPCR and microscopy in twelve large, freshwater rivers across the United States from early summer to late fall in 2017, 2018, and 2019. qPCR- and microscope-based phytoplankton abundance had a significant positive linear correlation (adjusted R^2 = 0.836, p-value < 0.001). Phytoplankton abundance had limited temporal variation within each sampling season and over the three years studied. The sampling sites in the midcontinent rivers had higher phytoplankton abundance than those in the eastern and western rivers. For instance, the concentration (geometric mean) of Bacillariophyta, Cyanobacteria, Chlorophyta, and Dinoflagellates at the sampling sites in the midcontinent rivers was approximately three times that at the sampling sites in the western rivers and approximately 18 times that at the sampling sites in the eastern rivers. Welch's analysis of variance indicated that phytoplankton abundance at the sampling sites in the midcontinent rivers was significantly higher than that at the sampling sites in the eastern rivers (p-value = 0.013) but was comparable to that at the sampling sites in the western rivers (p-value = 0.095). The higher phytoplankton abundance at the sampling sites in the midcontinent rivers was presumably because these rivers were more eutrophic. Indeed, low phytoplankton abundance occurred in oligotrophic or low trophic sites, whereas more eutrophic sites had greater phytoplankton abundance. This study demonstrated that qPCR-based phytoplankton abundance can be a useful numerical indicator of the trophic conditions and water quality in freshwater rivers.

Tables

Keywords: Microscopy; Freshwater bodies; Eutrophication; Spatiotemporal variation; Nutrients; Indicator

1. Introduction

Fresh surface water represents approximately 0.02% of the Earth's water but is the most important physical resource for society (Stets et al., 2020; Wilhelm et al., 2004). In fresh surface water, phytoplankton is a vital part of biogeochemical cycling and is the most critical primary producer. Phytoplankton photosynthesizes and provides food and energy to other organisms, ensuring normal and healthy ecosystem functioning and services (Dokulil and Qian, 2021; Li et al., 2022; Naselli-Flores and Padisák, 2022; Winder and Sommer, 2012). Eutrophication, the accumulation of nutrients (primarily nitrogen and phosphorus) and minerals in natural surface waterbodies due to natural and anthropogenic activities, threatens over 80% of rivers and lakes globally (Dodds, 2006; Smith et al., 2006; Sutcliffe and Jones, 1992; Wilhelm et al., 2004). In the United States (U.S.), total nitrogen (TN) and total phosphorus (TP) concentrations exceeded the reference median concentrations in 76% to 100% and 53% to 96%, respectively, of rivers in 14 ecoregions (Dodds et al., 2009). Over 90% of rivers in 12 of the 14 ecoregions had TN and TP levels higher than the reference median values. Overgrowth of phytoplankton, especially harmful or toxic Cyanobacteria, is the biological consequence of freshwater eutrophication (Duan et al., 2022; Lu et al., 2020; Wilhelm et al., 2004). Phytoplankton overgrowth deteriorates water quality, threatens ecosystems (e.g., aquatic organism mortality and biodiversity reduction), and can limit recreational opportunities. The overgrowth also poses public health risks and raises other issues by releasing harmful toxins (mainly cyanotoxins) and taste-and-odor compounds, causing the water to be unsafe and/or unpleasant for consumption and recreation (Chislock et al., 2013; Graham et al., 2010; Steffen et al., 2017; Wilhelm et al., 2004; Zhang et al., 2021b). In addition, the annual economic losses due to freshwater eutrophication are over 2.2 billion dollars in the U.S. (Dodds et al., 2009).

Tables

Phytoplankton overgrowth (e.g., algal and cyanobacterial blooms) is a consequence of eutrophication and poses substantial risks to the environment and public health. Therefore, evaluating phytoplankton is critical to understanding freshwater bodies' ecological conditions and trophic status (Aboim et al., 2020; Dembowska et al., 2018; Li et al., 2020; Sayers et al., 2021; Zhang et al., 2021c). Microscopy is the gold standard for assessing phytoplankton (Clementson et al., 2021; Ding et al., 2022; Dugdale et al., 2012; Hoang et al., 2018; Liu et al., 2019); however, microscopy is time-consuming, labor-intensive, and less efficient (Dunker, 2019; Malkassian et al., 2011; Rivas-Villar et al., 2021; Yu et al., 2021). Microscopy also requires rich experience in phytoplankton morphology and is difficult to operate for high-frequency (spatial and/or temporal) sample analysis. Moreover, microscopy is unable to identify cells with significant morphology changes (e.g., broken and shrunk cells), cannot discriminate cryptic species, cannot determine if a strain can produce toxins, and may be confounded by fixatives (Jeffrey and Vesk, 1997; Xiao et al., 2014). Furthermore, microscopic analysis of phytoplankton is prone to human error and bias (e.g., misidentification of species and missing picoplanktonic taxa) (Culverhouse, 2007; Luo et al., 2006; Palińska and Surosz, 2008).

To overcome the limitations of microscopy, researchers have developed alternative approaches to identify and quantify phytoplankton and study phytoplankton community structure. Those alternative methods include pigment-based approaches (often via high-performance liquid chromatography or spectrofluorometry) (Lee et al., 2020; Pan et al., 2020; Srichandan et al., 2020; Stoyneva-Gärtner et al., 2020; Yu et al., 2021), fatty-acid-based procedures (Cañavate et al., 2019; Canavate, 2019; Dijkman and Kromkamp,

2006; Galloway and Winder, 2015), analytical and imaging flow cytometry (Dunker, 2020; Latasa et al., 2021; Liu et al., 2021; Moorhouse et al., 2018; Read et al., 2014), and high-throughput sequencing or next-generation sequencing (dos Santos et al., 2021; Gong et al., 2020; Malashenkov et al., 2021; Yang et al., 2021b). These alternatives have advantages but also limitations. For instance, pigments (e.g., chlorophyll) are a widely used proxy for algal biomass and an indicator of trophic status (Chen and Chen, 2022; Dodds et al., 1998; Mineeva and Makarova, 2018). However, pigment-based approaches are insensitive and inaccurate (i.e., unable to differentiate phytoplankton species accurately), thereby providing little information on phytoplankton community composition (Catherine et al., 2012; Tian et al., 2020; Yu et al., 2021). Fatty-acid-based methods have similar limitations to the pigment-based approaches because they also lack sufficient reference libraries (Cañavate et al., 2019). Flow cytometry is less accurate, requires complicated staining, is bottlenecked by small sample volumes processed, and has a low taxonomic resolution (Dashkova et al., 2017; Dubelaar and Jonker, 2000; Markina, 2019). Even though high-throughput sequencing is much better at evaluating phytoplankton community diversity compared to quantitative polymerase chain reaction (qPCR), it is not as good at quantifying abundances, is expensive, could be disrupted by sequencing errors, and requires extensive bioinformatic expertise to interpret the data (Poretsky et al., 2014; Zhou et al., 2015).

Because of the shortcomings of microscopy and the alternative assays, we propose using qPCR to screen phytoplankton in surface waterbodies. qPCR is a rapid, accurate, sensitive, robust, reproducible, high-throughput, and cost-effective methodology (Galazzo et al., 2020; Smith and Osborn, 2009). qPCR instruments are more accessible than flow cytometers and high-throughput sequencing

platforms (Azat, 2021; Raso and Biassoni, 2014). qPCR also does not require knowledge of phytoplankton taxonomy or morphology but can provide information about both abundance and taxonomic identification of phytoplankton. In addition, unlike pigment-based approaches and flow cytometry, qPCR can be coupled with high throughput sequencing (Jian et al., 2020) to assess phytoplankton community with high accuracy, large dynamic range, and high sample processing capability (i.e., sequencing of millions of DNA molecules in parallel) (Churko et al., 2013; Reuter et al., 2015). Furthermore, because of the rapid innovation in qPCR instrumentation and protocols, we can upgrade and scale up qPCR to a high throughput assay (Pearson et al., 2021; Porter and Hajibabaei, 2018; Wilcox et al., 2020).

Most studies use qPCR to assess genes encoding cyanotoxins (e.g., microcystins, cylindrospermopsin, anatoxin-a, and saxitoxin) and Cyanobacteria (especially cyanotoxin-producing Cyanobacteria) (Almuhtaram et al., 2021; Christensen et al., 2019; Eldridge et al., 2017; Kulabhusan and Campbell, 2021; Otten et al., 2015; Pacheco et al., 2016; Sagova-Mareckova et al., 2021; Schweitzer-Natan et al., 2019; Tavakoli et al., 2021; Zupančič et al., 2021). However, the feasibility of qPCR in identifying and quantifying phytoplankton, especially non-Cyanobacterial phytoplankton, in freshwater rivers has not been fully evaluated in a comparative study with microscopy.

This study aimed to evaluate the feasibility of qPCR in assessing phytoplankton by monitoring phytoplankton in twelve large, freshwater rivers across the U.S. from early summer to late fall in 2017, 2018, and 2019. Because freshwater phytoplankton

community dynamics are driven by local environmental factors (Stomp et al., 2011), we assessed how environmental parameters, such as nutrients, shaped the phytoplankton community. We hypothesized that qPCR- and microscopy-based phytoplankton abundance would be positively correlated, supporting the use of qPCR as a numerical tool for phytoplankton assessment.

2. Materials and methods

2.1. Study sites and river water sample collection

This study included twelve large, inland and coastal freshwater rivers in the western (two), midcontinent (five), and eastern (five) regions across the U.S. (Table 1 and Figure 1). In this study, the western region refers to the region in the west of the Sierra Nevada and Cascade ranges, and the eastern region refers to the region in the east of the Appalachian range. The midcontinent region refers to the region between the western and eastern regions. We collected river water samples in 2017, 2018, and 2019 from one sampling site in each river according to the United States Geological Survey (USGS)'s National Water Quality Assessment (NAWQA) protocol (USGS, 2022a). We used isokinetic sampling techniques to ensure that the river samples are representative. The sampling spanned early summer through late fall each year. Since phytoplankton does not grow well and has low abundance during cold seasons (i.e., winter and spring), especially in large streams (Savoy et al., 2019), we did not sample during cold seasons. Two sets of river water samples were collected in parallel during each sampling event, defined as river water collection from a site on a sampling day. The first set was an isokinetic sample for physicochemical water quality parameter determination and microscopic analysis of phytoplankton. The second set was a near-surface grab sample collected at the centroid of a river for qPCR. In this study, we collected

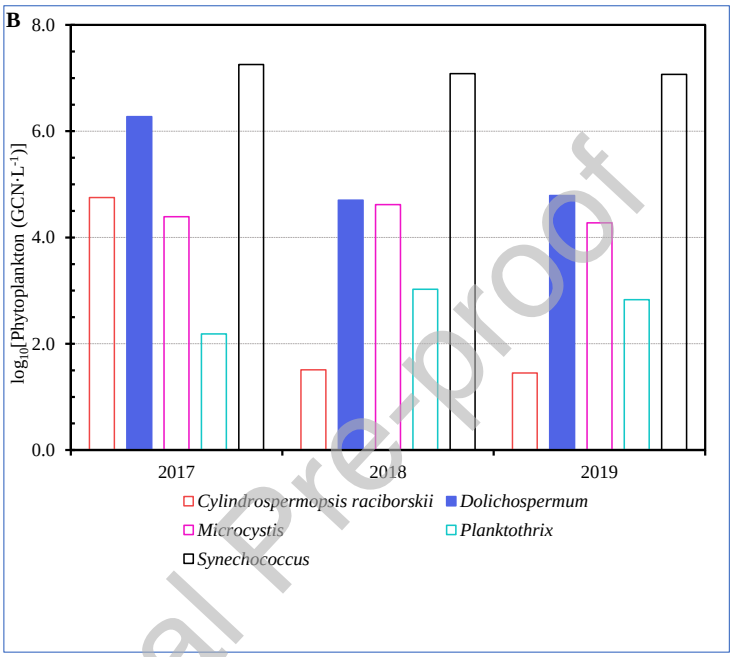
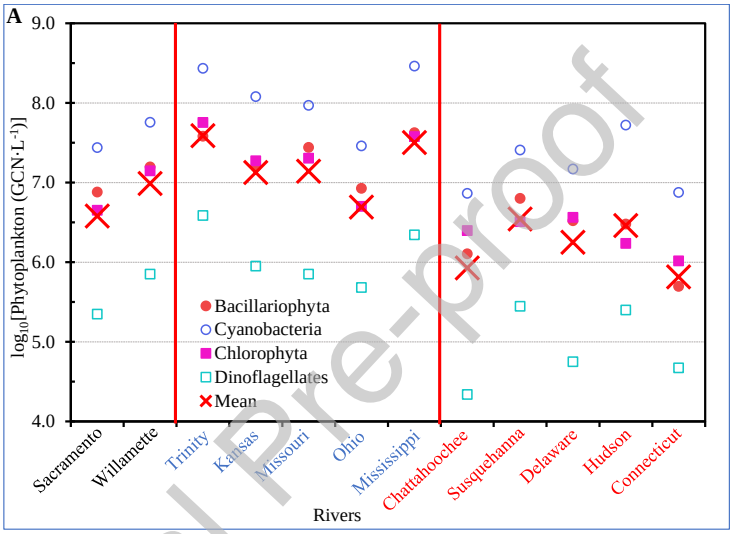


Figure 3. The geometric mean abundance (qPCR-based) of phytoplankton in 2017, 2018, and 2019 (sampling season: early summer to late fall) for all the sampling sites in the twelve rivers. One sampling site was selected from each river. **GCN**: Gene or genome copy number. (**A**) Four major phytoplankton groups. **Bacillariophyta** and **Cyanobacteria**: Divisions. **Chlorophyta**: Specific to three classes within the division Chlorophyta (Chlorophyceae, Trebouxiophyceae, and Klebsormidiophyceae). **Dinoflagellates**: Class. **Mean**: The geometric mean for the four major phytoplankton groups. (**B**) Four phytoplankton genera (Dolichospermum, Microcystis,

Planktothrix, and Synechococcus and a species (Cylindrospermopsis raciborskii) in Cyanobacteria.



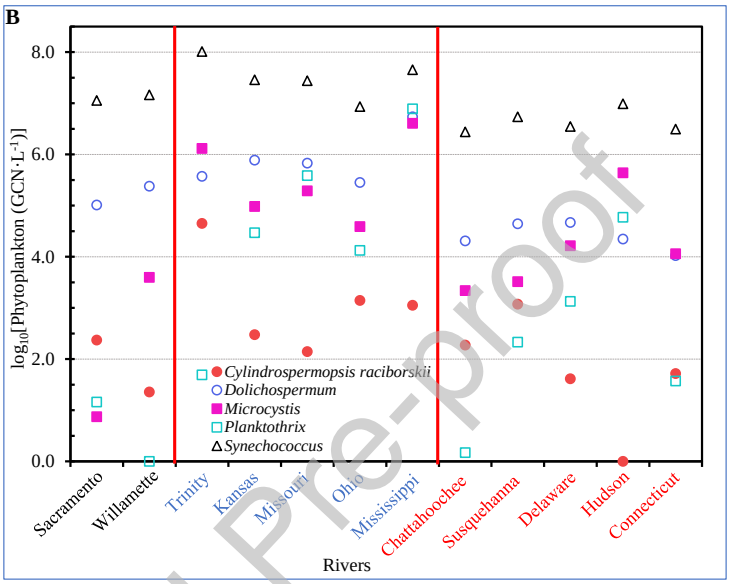


Figure 4. The geometric mean abundance (qPCR-based) of phytoplankton at the sampling sites in the twelve rivers (2017 to 2019, sampling season: early summer to late fall). One sampling site was selected from each river. **GCN**: Gene or genome copy number. (**A**) Major phytoplankton groups. **Bacillariophyta** and **Cyanobacteria**: Divisions. **Chlorophyta**: Specific to three classes within the division Chlorophyta (Chlorophyceae, Trebouxiophyceae, and Klebsormidiophyceae). **Dinoflagellates**: Class. **Mean**: The geometric mean for the four major phytoplankton groups. (**B**) Four genera (*Dolichospermum*, *Microcystis*, *Planktothrix*, and *Synechococcus*) and a species (*Cylindrospermopsis raciborskii*) in Cyanobacteria.

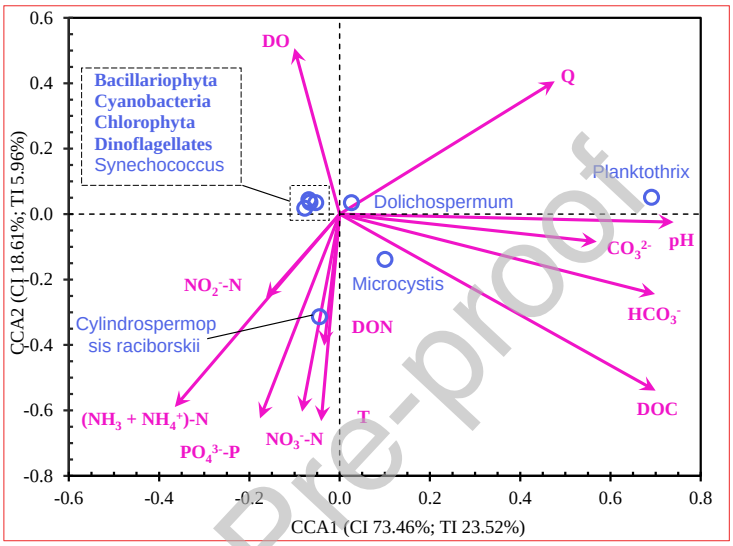
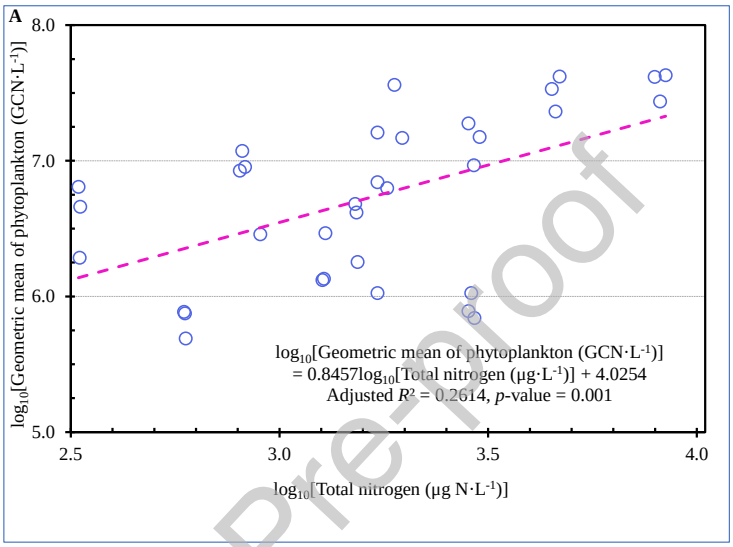


Figure 5. A constrained Canonical correspondence analysis (CCA) map (generated with XLSTAT) showing the linear associations among the qPCR-based abundance of eight phytoplankton groups and twelve critical water quality physicochemical parameters all measured at the sampling sites in the twelve rivers. One sampling site was selected from each river. The length of an arrow indicates the significance of the corresponding environmental variable, the direction of an arrow indicates the correlation between the corresponding environmental variable and an axis or a phytoplankton group, the angle between two arrows indicates the correlation between the two corresponding environmental variables, and the specific location of a phytoplankton group relative to an arrow indicates the environmental preference of the group (Palmer, 1993; Teer Braak, 1986). **Bacillariophyta** and **Cyanobacteria**: Divisions.

Chlorophyta: Specific to three classes within the division Chlorophyta (Chlorophyceae, Trebouxiophyceae, and Klebsormidiophyceae). **Dinoflagellates**: Class. Dolichospermum, Microcystis, Planktothrix, and Synechococcus Genera. Cylindrospermopsis raciborskii Species. Unit for phytoplankton abundance: $\log_{10}(GCN \cdot L^{-1})$. **GCN**: Gene or genome copy number. **T**: Water temperature (°C). **Q**: Discharge ($m^3 \cdot s^{-1}$). **DO**: Dissolved oxygen ($mg \cdot L^{-1}$). **DON**: Dissolved organic nitrogen (mg $N \cdot L^{-1}$). **(NH_3 + NH_4^+)-N**: Ammonia and ammonium nitrogen (mg $N \cdot L^{-1}$). **NO_2^--N**: Nitrite nitrogen (mg $N \cdot L^{-1}$). **NO_3^--N**: Nitrate nitrogen (mg $N \cdot L^{-1}$). **PO_4^{3-}-P**: Orthophosphate phosphorus (mg $P \cdot L^{-1}$). **DOC**: Dissolved organic carbon ($mg \cdot L^{-1}$). **CO_3^{2-}**: Dissolved carbonate ion ($mg \cdot L^{-1}$). **HCO_3^-**: Dissolved bicarbonate ion ($mg \cdot L^{-1}$). **CI**: Constrained inertia. **TI**: Total inertia.

Figures



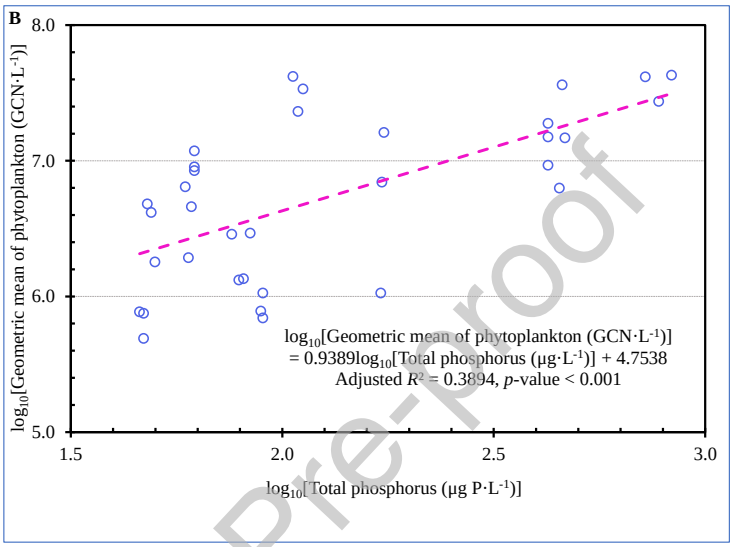


Figure 6. The linear correlations between the geometric mean abundance (qPCR-based) of four major phytoplankton groups (Bacillariophyta, Cyanobacteria, Chlorophyta, and Dinoflagellates) and (**A**) total nitrogen and (**B**) total phosphorus concentrations. The dashed lines: Simple linear regression lines. **Bacillariophyta** and **Cyanobacteria**: Divisions. **Chlorophyta**: Specific to three classes within the division Chlorophyta (Chlorophyceae, Trebouxiophyceae, and Klebsormidiophyceae). **Dinoflagellates**: Class. Adjusted *R*-squared's and *p*-values: SPSS-generated.