IN SITU CHLOROPHYLL MEASUREMENTS WITH FLUOROMETRY: IN-LAB VALIDATION AND USE IN LAKE VERTICAL PROFILING

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ABSTRACT

In situ chlorophyll measurements with fluorometry: In-lab validation and use in lake vertical profiling

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In situ chlorophyll sensors are beneficial for monitoring of long-term impacts of algal blooms and accessing water quality issues in bodies of water. However, more research is needed to validate their efficacy and understand how environmental conditions can influence sensor measurements. I assessed the performance of an *in situ* chlorophyll sensor under controlled environmental conditions and used the same sensor to collect vertical phytoplankton patterns in south-central Ontario boreal lakes. The performance of the sensor was assessed by examining the precision of chlorophyll measurements and determining the suitable timing length that would produce precise results. In general, the sensor was relatively insensitive to conditions under lower algal concentrations and the decent of the sensor should be slowed for vertical lake profiling in lakes with higher algal biomass. Most variation resulted from the movement of particle bound algal cells. We described chlorophyll profile characteristics including surface chlorophyll levels and chlorophyll peak depth and width and investigated the relationships of these features with environmental controls. The lakes showed a typical chlorophyll profile of low phytoplankton biomass lakes. Our results showed that dissolved organic carbon was a strong predictor of epilimnetic biomass while light attenuation and dissolved organic carbon were both strong predictors of peak depth. Light attenuation and surface area were small but significant predictors of peak width. We acknowledged that any uncertainties in sensor chlorophyll readings were not an issue in our lakes due to the overall low chlorophyll biomass.

Keywords: limnology, *in situ* profiling, chlorophyll, chlorophyll fluorescence, in lab validation, vertical chlorophyll profile, algae, phytoplankton biomass, lakes, water, water quality

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AUTHOR CONTRIBUTIONS

Melanie Annan - Primary author; r script and analysis

Paul Frost - Editor

Nolan Pearce - Editor; r script and analysis

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General introduction

Phytoplankton are communities of floating primary producers in lake ecosystems. In oceans and lakes, phytoplankton account for ~50% of global oxygen production (Chen et al. 2021) on an annual basis. This makes them a critical part of food webs as they are a source of energy and nutrients to higher trophic levels (Williamson et al. 1996; Wilkinson et al. 2014; Moriarty et al. 2021; Tonin et al. 2022). Additionally, phytoplankton are indicators of aquatic ecosystem health and water quality (Gregor et al. 2005; Liu & Georgakakos 2021). Its thus very important to understand the spatial and temporal patterns of phytoplankton abundance and their relationship to environmental conditions.

Phytoplankton are not randomly distributed in the water column (Marshall & Peters, 1989; Cullen et al., 2015; Zhao et al. 2019). The depth-related distribution in the water column can be assessed by estimating the fluorescence of chlorophyll *a*, an index of algal biomass (Paerl et al 1976; Uehlinger 1985). In general, phytoplankton biomass distribution is shaped by physical, chemical and biological processes (Zhao et al. 2019) including light penetration (Hamilton et al. 2010; Leach et al. 2018), zooplankton grazing (Moeller et al. 2019; Tonin et al. 2019), turbidity and DOC concentrations (Abbott et al. 1984; Tedford et al. 2019; Senar et al. 2021), mixing patterns (Carrick et al. 1993; Klausmeier & Litchman 2001; Mellard et al. 2011), resuspension of nutrients (Abbott et al. 1984; Senar et al 2021) and lake morphometry (Fee 1979; Fee et al. 1996). Chlorophyll distribution in the water column is known to vary across time. This includes seasonal mixing patterns since gradients change monthly due to changes in nutrient content (Scofield et al. 2017), temperature fluctuations (Reinl et al. 2020) and windspeed variation (Rusak et al. 2018).

Chlorophyll *a* is an important photosynthetic pigment in algae and plays a role as an indicator of algal biomass (Paerl et al 1976; Uehlinger 1985). Chlorophyll pigments possess the ability to fluoresce (absorb light at one wavelength and emit light at a longer wavelength) allowing such pigments to be detected by monitoring their fluorescence via a fluorometer (Lorenzen 1966). *In situ* fluorescence sensors were first introduced in the 1960s (Lorenzen 1966) and their popularity has seen an increase over the decades as a tool for phytoplankton biomonitoring (Gregor et al. 2005; Rolland et al. 2010; Catherine et al. 2012; Garrido et al. 2019; Rousso et al. 2021). These instruments have aided researchers with understanding spatial and temporal patterns of phytoplankton distribution (Uehlinger 1985; Xing et al 2017). Additionally, these *in situ* instruments offer an efficient approach to estimating chlorophyll fluorescence (chl F) compared to standard ethanol extraction techniques coupled with spectrofluorometry (Richards & Thompson, 1952; Strickland & Parsons 1965) and fluorometry (Holm-Hansen et al. 1965; Yentsch and Menzel 1963), which are time-consuming and require considerable laboratory preparation (Gregor et al. 2005; Cullen 1982). Furthermore, *in situ* sensors produce data in real time so it can be readily assessable. This has major benefits for accessing water quality monitoring, addressing concerns of algal blooms and aquatic research related to vertical phytoplankton pattern dynamics.

Testing the performance of phytoplankton detection tools such as *in situ* profilers can allow researchers to use the best tools in the field. Researchers can also determine if the tool is applicable in certain biomonitoring situations. *In situ* profilers have been validated in their use for assessing water quality issues in water bodies (i.e., lakes, oceans and reservoirs) (Gregor et al. 2005; Catherine et al. 2012; Garrido et al. 2019; Rolland et

al. 2020; Zamyadi et al. 2020; Liu & Georgakakos 2021; Rousso et al. 2021). However, *in situ* profiler data should continue to be validated. Profilers are employed for routine detection of toxic blooms including cyanobacteria blooms which poses a risk of water safety and human health (Leboulanger et al. 2002; Zamyadi et al. 2020; Liu & Georgakakos 2021) and additionally assessing water quality, for example eutrophication, caused by an excess of nutrients by human activities (Devlin et al. 2020 and land use (Kraemer et al. 2020). In both cases of algal blooms and eutrophication, *in situ* monitoring techniques can be used to forecast any water quality issues that may arise from these. Another instance where the deployment of *in situ* profilers is useful is for the characterization of spatial and temporal distribution of phytoplankton (Uehlinger 1985; Xing et al. 2017). This requires a verified high-resolution detector to take a vertical trasect on the water column.

A distinguishing part of phytoplankton profile in stratified water columns is a biomass peak of phytoplankton generally residing in the metalimnion (Fee et al. 1976; Leach et al. 2018). The term deep chlorophyll maximum, DCM (Fee et al. 1976; Hamilton et al. 2010; Leach et al. 2018), subsurface chlorophyll maximum layer, SCML (Cullen 2015), meroplanktonic algal maximum, MAM (Carrick et al 1993) and deep chlorophyll layer, DCL (Scofield 2017) has been widely used to term these peaks in phytoplankton biomass. This peak refers to the largest biomass peak (indicated by a spike in chl F) in the water column although multiple peaks can also occur due to species niche preferences (Selmeczy et al. 2016). Peaks form by balancing the light, nutrients and mixing patterns processes (Abbott et al. 1982; Camacho 2006; Mellard et al. 2011). Peak depth and width are common interests in describing depth related patterns of

phytoplankton (Varela et al 1992; Klausmeier & Litchman 2001; Leach et al. 2019; Loften et al. 2020). Peak depth has been strongly related to light attenuation while peak width has been linked to lake size (Klausmeier & Litchman 2001; Leach et al 2018). Lakes found to have high turbidity due to high dissolved organic matter have been found to contain no distinct peaks (Tonin et al. 2020). It is important to study phytoplankton peaks since they serve as an important food source for zooplankton (Moriarty et al. 2021; Tonin et al. 2022). Additionally, theses phytoplankton peaks are where most of the lakes' primary production occurs in the water column which is approximately 60% (Weston et al. 2005).

This research focused on estimating biomass using an *in situ* fluorescence sensor attached to a probe with emphasis on describing phytoplankton patterns in south-central boreal lakes of Canada. The sensor data consists of thousands of points that need to be validated by external experiments which test the performance of the sensor. This research has two objectives:

1) Estimating chlorophyll fluorescence using a sensor across a variety of conditions under controlled lab techniques.
2) Describing vertical patterns of phytoplankton biomass in south-central boreal lakes.

Previous studies addressed the performance of these probes by comparing chl F values obtained from a fluorescence sensor to values obtained from standard ethanol extraction using a specific type of sensor, Fluoroprobe® (Gregor & Maršálek 2004; Gregor et al. 2005; Rolland et al. 2010; Catherine et al. 2012; Garrido et al. 2019; Prestigiacomo et al. 2022). However, it is important to assess the precision of a variety of

widely available sensors in a series of conditions since light, temperature and turbulence can affect the fluorescence of the algal cells in turn altering the probe readings (Falkowski & LaRoche 1991; Finenko et al. 2003; Hodges et al. 2018; Girdner et al. 2020). Testing the size of the sampling environment may provide insights on proper device calibration when choosing a container for calibrating the sensor. We added to the understanding of *in situ* fluorescence devices by assessing the performance of a device (Turner® Cyclops 7F; Turner Designs Inc. USA) by demonstrating its precision in producing chl F values of a sample under a variety of conditions including container size, light condition, water temperature and turbulent mixing and determining a reliable time sampling length. In addition, chl F sensor measurements were compared to values obtained from standard ethanol extraction. The goal of the research was to demonstrate that the chlorophyll values obtained in our study lakes by an *in situ* sensor are reliable.

We deployed the same instrument to determine vertical patterns of phytoplankton in lakes within the Kawartha Highlands Provincial Park and surrounding area which were sampled in late summer of 2020 and 2021. Current phytoplankton distribution research is focused on biomass peak characteristics such as how peak depth relates to other metrics such as light attenuation and nutrients (Leach et al. 2018; Loften et al. 2020; Scofield et al. 2020) and seasonal variation of peak depth, concentration and width (Scofield et al. 2017; Reinl et al. 2019). My research focused on characterizing averages and variation of biomass in each thermal layer across lakes. I additionally described the complexing features of the phytoplankton profiles including the variation in the structure of chlorophyll peaks across lakes. This includes peak depth, maximum peak concentration, peak width, number of peaks and which of the thermal layers the peak resides in. Finally,

I considered the relationships between chl F and peak characteristics, and external metrics: light attenuation (measured by K_d), dissolved organic carbon (DOC), total phosphorus (TP) and lake morphometric properties: maximum lake depth, surface area.

Chapter 1: How do environmental conditions affect fluorescence-based measurements of algal biomass?

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Abstract

In situ fluorescence probes are increasingly being used to quantify phytoplankton biomass in aquatic ecosystems by measuring chlorophyll fluorescence. Despite this growing use, there remains uncertainty about the ability of the chlorophyll sensor to quantify algal biomass under different environmental conditions. We test the performance of a commercially available fluorometer with a series of laboratory experiments that varied environmental conditions. We performed four experiments to test the ability of the fluorometer to provide precise chl F measurements by altering: container size, light condition, temperature, and mixing condition. We determined an ideal sampling time of the sensor across a range of chlorophyll concentrations and experimental treatments. We next examined whether there were interactive effects between treatment and nominal algal biomass on the probe's estimates of algal biomass. In addition, we compared fluorometric chlorophyll estimates to those based on dissolved chlorophyll extracted in ethanol. Certain environmental conditions had small but significant effects on chlorophyll estimates by the fluorometer. These effects were likely due to the measurement of particle bound chlorophyll, which may be sensitive to algal sinking and turbulent mixing. We also found higher chlorophyll concentrations required longer sampling periods of up to 30 seconds to produce reliable estimates of chlorophyll biomass. There was strong correlation between *in situ* fluorescence and extracted chlorophyll measurements showing the fluorometer is responsive to increasing algal biomass. Our results indicate that

microenvironmental turbulence should be further studied due to its potential to affect *in situ* fluorescence-based estimates of particle bound algal biomass. While fluorometric sensors continue to be promising tools to estimate *in situ* concentrations of algal biomass, our experiments show that there needs to be further examination of their capabilities and constraints under ambient environmental conditions.

Introduction

The monitoring of phytoplankton in rivers, lakes, and oceans is an important part of detecting the development and presence of algal blooms (Leboulanger et al. 2002; Ghadouani & Smith 2005; Zamyadi et al. 2012; Liu & Georgakakos 2021), assessing water quality (Rolland et al. 2010; Devlin et al. 2020; Kraemer et al. 2020), and mapping spatial distributions of phytoplankton (Uehlinger 1985; Xing et al. 2017). In addition, measurements of algal biomass are central to studying ecological interactions in aquatic food webs (Williamson et al. 1996; Wilkinson et al. 2014; Moeller et al. 2019; Moriarty et al. 2021). Our ability to track algal biomass in space and time has dramatically expanded due to recent improvements in *in situ* sensors. Widespread use of *in situ* platforms promises to transform research and monitoring of algal populations provided that their measurements are reliable, precise, and comparable to other standard methods of measuring algal biomass.

Submersible probes are being increasingly used to measure phytoplankton biomass *in situ* by measuring the fluorescence created by chlorophyll-containing particles (Catherine et al. 2012; Liu & Georgakakos 2021; Moriarty et al. 2021; Garrido et al. 2019). This approach is similar to the standard method of estimating phytoplankton

biomass based on the measurement of the fluorescence of extracted chlorophyll a (Ghadouani & Smith 2005; Garrido et al 2019). The standard method extracts chlorophyll a from algae and cyanobacteria collected on filters and then measures the fluorescence of dissolved pigments with a spectrophotometer (Richards & Thompson, 1952; Strickland & Parsons 1965). Fluorometric measurements of extracted chlorophyll a require considerable laboratory preparation due to the filtration, extraction, and measurement of each sample (Lorenzen 1966; Uehlinger 1985). Chlorophyll can also be measured on whole, unprocessed water using fluorometers that emit light at a specified wavelength and detects the fluorescence emitted by chlorophyll-containing particles (Lorenzen 1966; Paerl et al 1976). Submersible fluorometers use this principle to measure chlorophyll in the field. The primary advantage of this approach is the speed and efficiency of taking real-time measurements, which allows for more detailed characterization of spatial and temporal patterns of algal biomass beyond that possible using standard methods of extracted chlorophyll estimation (Uehlinger 1985; Rolland et al. 2010; Catherine et al. 2012; Garrido et al. 2019).

There are potential constraints and complications to the use of *in situ* chlorophyll sensors. One is that ambient conditions may affect fluorescence of particle-based algal cells and yield widely varying chlorophyll estimates at a given algal biomass (Falkowski & LaRoche 1991; Finenko et al. 2003; Girdner et al. 2020). For example, chlorophyll fluorescence can vary with the past light exposure of the algal cell (Beutler et al. 2003; Rousso et al. 2021) and with water temperature (Hodges et al. 2018). As light and temperature both vary extensively within and among lakes and rivers (Finenko et al. 2003), sensor-based chlorophyll readings may provide inaccurate estimates of algal

biomass in these ecosystems. In addition, the measurement of particle bound chlorophyll could be highly variable due to the movement of particles past the sensor. These complications and limitations have yet to be assessed on *in situ* chlorophyll sensors under well controlled laboratory conditions. A better understanding of the limitations is needed given the potential value of wide-scale application of this technique for water quality research and monitoring.

We assessed the performance of a fluorescence-based chlorophyll sensor with measurements of chlorophyll fluorescence under controlled laboratory conditions. We used a commercially available sensor (Turner® Cyclops-7F submersible sensor, Turner Designs, USA) that has been previously deployed for field studies (Devlin et al. 2020; Peipoch & Ensign 2022). Despite these field applications, there are no previously published laboratory-based assessments of this or similar sensors. We quantified variability in chlorophyll measurements by rapidly measuring fluorescence over minute-long intervals across concentration gradients of algae. We also tested the effects of container size to mimic lab calibration settings and water temperature, light intensity and turbulence to mimic environmental conditions on chlorophyll measurements by the submersible sensor. Our results provide much needed information on the usefulness and limitations of this approach for field-based studies of algal monitoring in aquatic ecosystems.

Methods

In situ fluorescence probe. We used a Turner® Cyclops-7F submersible fluorescence-based chlorophyll sensor (Turner Designs Inc., USA) attached to a RBR

maestro^3® profiler (RBR Ltd., Canada) with a measurement rate set at a frequency of 8 measurements per second (1 reading every 0.125s). This sensor measures chlorophyll fluorescence using an LED light (blue excitation wavelength at 460 nm) and can be used across a wide range of chlorophyll concentrations (0 – 500 μg/L; Turner Designs Inc., USA). Additionally, the manufacturer provides the range of operating temperature as between -2 and 50 °C (Turner Designs Inc., USA).

Algal source and measurement. We used a laboratory grown alga, *Scenedesmus obliquus,* as our source of chlorophyll. Algae were grown under a 150W growth light and at 20 °C in 2 L Erlenmeyer flasks of COMBO media (Kilham et al. 1998) that was diluted daily. We collected samples of this algae before adding known quantities to experimental containers. To do so, we estimated the dry mass of the concentrated algae by weighing small samples that had been collected on duplicate pre-weighed GFF filters of the algae (0.8 mm pore size, 25 mm diameter) and dried for 24 hours at 60 °C. In addition to dry mass, we filtered algae onto duplicate GFF filters (0.8 mm pore size, 25 mm diameter) and stored these at -20 °C. We extracted chlorophyll from these filters in 20 ml of 95% ethanol under dark and cold conditions (~4 °C) for an additional 24 hours (Marker 1994). We subsequently measured extracted chlorophyll using a spectrofluorometer (Cary Eclipse® Fluorescence Spectrophotometer; Agilent Technologies, USA) calibrated with known standards.

Experimental procedure. For all measurements, we submerged the chlorophyll sensor into a container containing a nominal concentration of algae. Unless specified otherwise, we used a 20 L plastic container filled with tap water held at room temperature under fluorescent lighting. Prior to the start of each experiment, we added an aliquot of

algae to raise the algae concentration to a desired level. After adding and thoroughly mixing the algae, we submerged the chlorophyll sensor and recorded chl F readings for a duration of at least 1 minute in order to obtain a sufficient amount of data. This measurement was repeated in the same container with sequential additions of algae to produce a biomass concentration gradient. In total, the experiments were completed using six algal dry mass concentrations across the range of 0 mg C L^{-1} to 0.6 mg C L^{-1}. Assuming a C:Chl ratio in the whole algal cell of 30:1, this encompassed an approximate range of 0 to 20 μg chl L^{-1}. Three repeat measurements were completed for each algal biomass concentration by lowering the profiler in the same container for at least 60 seconds and removing it after each reading. All readings were taken more than 30 seconds after the algae were added to the sampling container and the water was lightly mixed to homogenize the added algae. For consistency, we removed the first 10 seconds of each set of readings and trimmed these readings to 50 seconds.

Experimental treatments. In order to test the performance of the chlorophyll sensor, we manipulated various aspects of this standard experimental set-up. We repeated sequential biomass measurements in containers of four different sizes: 100 ml beaker, 1 L beaker, 20 L small bucket, and 60 L large bucket. We also compared chlorophyll measurements under two different light conditions: full light exposure using a 150 W incandescent light bulb set up >1 m away from the water surface and a dark container with no light and covered with a black towel. In a third experiment, the temperature of the water in the 20 L bucket was set up at one of four different temperatures: 6 °C, 12 °C, 20 °C and 25 °C while maintaining the same gradient of chlorophyll. Finally, we compared chlorophyll readings under two different mixing treatments: no mixing except

at the beginning of each addition of algae and mixing via a submerged aquarium circulation pump to produce a constant and high level of turbulent mixing. For this last experiment, we performed an additional experiment where we extended our measurement period to 30 minutes to provide more opportunity to detect the movement of algal particles in turbulent (mixed) vs ambient (unmixed) conditions overtime. For this last experiment, we used six algal dry mass concentrations (0 mg C L^{-1} – 0.6 mg C L^{-1}) and obtained four 50-second chl F measurements for the span of the 30-minute period staring at the 0-minute mark, 10-minute mark, 20-minute mark and 30-minute mark.

Effects of treatments on chlorophyll readings. In order to determine the performance and precision of the sensor, we compared triplicate replicate chlorophyll fluorescence, chl F, means from each 50 second sampling time between algal concentrations and treatment types. A two-way ANCOVA was used to determine if there was a significant interaction effect between treatment and algal concentration on chl F measurements. Additionally, a Tukey post hoc test using the R package *emmeans* was performed to determine the effect sizes between treatments at each concentration level to determine differences among treatment combinations when we found a significant interaction term.

Reliable time sampling length. Using the data from the experiments described above, we assessed the sampling time length needed to produce a reliable estimate of chl F. For each experimental treatment and replicate, we first calculated the overall mean chl F of the 50 second sampling time. We then randomly resampled (100 permutations) 1 to 50 second-long continuous (forwards and backwards) subsets of this entire dataset of chl F readings and calculated each sampling time's grand chl F mean and corresponding 95%

confidence interval from the resampled data. The grand mean and 95% confidence interval from 1 to 50 seconds was then mean centered to the overall mean and visualized as a time series. We assumed that a threshold of ± 1 μg L^{-1} chl F relative to the overall mean was a reasonable measure of accuracy since this was normal variation seen in lakes from an ecological standpoint. Given this criterion, we then determined the mean sampling time length where the upper and lower 95% confidence intervals of the resampled dataset intersected ± 1 μg L^{-1} chl F along the mean centered time series (Figure 1). This time length was used as the sampling time needed to obtain an accurate measurement of chl F. In cases where the minimum resampled time length was found to be less than 1 second, the suitable time length for that treatment replicate was assigned a value of 1 second. Additionally, a two-way ANOVA followed by a post-hoc test was used to determine if there was a significant interaction effect between treatment type and algal concentration on the time sampling lengths.

In vivo chlorophyll fluorescence vs extracted chlorophyll. We also compared the average fluorescence of chlorophyll obtained by the sensor and that from the chlorophyll extract measurements. Sensor based fluorometric measurements were calculated for each algal concentration by averaging all measurements from each of the 3 replicates. Extracted chlorophyll fluorometric measurements were calculated based on the measurements of the filtered samples of the source algae with the spectrofluorometer and the volume of algal slurry added to the 20 L bucket. A simple linear regression was performed between sensor-based and extracted chlorophyll measurements to determine their comparability.

Results

Effects of treatments on chlorophyll readings. Chlorophyll fluorescence (chl F) estimates based on fluorescence measurement differed among our experimental treatments. These effects of the different treatments varied, to some extent, among the six concentrations of algal biomass that we used in the experiments (Figure 2). We found a significant interaction between algal biomass level and container size on the chl F means (Table 1; Figure 2a). Smaller containers (100 ml, 1L) had slightly higher chl F readings at lower algal levels and lower chl F readings at higher algal levels (figure 2a). Conversely, we found larger container sizes (20 L, 60 L) had lower chl F readings at lower algal levels and higher chl F readings at higher algal levels (Figure 2a). We did not find an interaction between irradiance and algal level (Table 1; Figure 2b). While there was slightly more chlorophyll measured at high algal levels, there was no significant effect of light on these measurements. We found a significant interaction between algal level and water temperature on chl F means (Table 1; Figure 2c). Generally, the 6 °C and 12 °C temperature treatments produced slightly higher chl F readings at lower algal levels and produced significantly higher chl F readings than the 20 °C and 25 °C treatments at higher algal levels (Figure 2c). We found a significant interaction between algal level and turbulent mixing on chl F means (Table 1; Figure 2d). The fluorometer produced significantly higher chl F readings under the mixed treatment compared to unmixed treatment across all algal levels (Figure 2d).

Reliable time sampling length. There was considerable variability of the suitable time sampling frequency between all replicates for all concentration and treatments (Figure 3). We found that samples with lower algal concentrations (~<0.06 mg C L^{-1})

required less than 1 second for estimating chlorophyll concentration within a desired range while higher algal biomass generally required a longer sampling time of at least 30 seconds (Figure 3). There was no interaction between treatment and algal biomass on suitable time sampling frequency for container size (Table 2; Figure 3a) and no interaction between dark treatments compared to light treatments (Table 2; Figure 3b). There was no significant interaction between temperature and algal biomass on suitable time lengths (Table 2; Figure 3c). The suitable time sampling length was slightly shorter for the mixed treatment compared to the unmixed treatment (Table 2; Figure 3d). After conducting a post hoc test, we determined that it would take the mixed treatment a significantly shorter amount of time to achieve a reliable chl F estimate compared to the unmixed treatment at higher algal concentrations.

Algal particle loss. There appeared to be considerable differences in chlorophyll measurements between mixed and unmixed conditions over longer time periods (Figure 4). The fluorometer produced higher chl F readings on average under the mixed treatment compared to unmixed treatment (Figure 4). The size and nature of these differences varied with the time duration (Figure 4). We estimated that the chl F loss for unmixed treatments was higher than the chl F loss for the mixed treatments with the highest drop in chl F occurring in the first 10 minutes.

In vivo chlorophyll fluorescence vs extracted chlorophyll. We found chlorophyll estimates produced by the submersible sensor were positively correlated with those based on chlorophyll extracted in ethanol ($R^2 = 0.99$, $p < 0.001$, Figure 5). While we found a strong relationship, chl F readings produced by the sensor were systematically higher than the chlorophyll values based on fluorometric readings of ethanol extracts (Figure 5).

Discussion

In situ fluorometers have become an important tool for monitoring phytoplankton abundance and water quality over the past decades. Compared to standard methods of estimating phytoplankton biomass, these sensors can be deployed broadly across space, measure rapidly through time, and can produce large quantities of data. As *in situ* fluorometers are being widely deployed and incorporated into lake ecosystem monitoring, there is a need to better assess their effectiveness at measuring algal biomass. While there has been previous work on the validation of fluorescence-based probes (Gregor & Maršálek 2004; Gregor et al. 2005; Rolland et al. 2010; Catherine et al. 2012; Roesler et al. 2017; Garrido et al. 2019; Prestigiacomo et al. 2022), this has largely been confined to comparing *in situ* measurements to extracted chlorophyll estimates. Here, we expand on this work by evaluating the performance of a sensor with a series of controlled experiments manipulating environmental conditions. We found that there were significant effects of turbulent mixing, minor but significant effects of container size and temperature on chl F readings. We also found that reliable estimates of chl F could be measured over short time periods at lower algal concentrations but that this minimum timeframe increased with algal concentrations. Similarly, like previous research (Roesler et al. 2017; Simonazzi et al. 2022) the difference between in situ and extracted chlorophyll measurements increased with greater concentrations. These disparities are likely of little concern for data collected from low productivity oligotrophic-mesotrophic ecosystems (Chl F mean ≤ 2.5 µg L^{-1}; Vollenweider & Kerekes 1982), but additional care should be taken in when monitoring eutrophic systems to ensure the highest quality data is collected.

We found chl F readings to vary among container size. Chl F readings were somewhat higher at lower algal concentrations and lower at higher algal concentrations in smaller container sizes. We suspect that container size can alter the microenvironmental differences in light or water mixing which can alter chl F readings. Regardless, future work testing the efficacy of *in situ* sensors should account for the effects that container size on fluorescence based chlorophyll estimates particularly when choosing a container for sensor calibration.

We found that average chl F readings were higher at lower temperatures and this effect was more evident at higher concentrations. This is an indication that sensor-based readings may be subject to error when deployed across seasons or among lakes with higher chlorophyll content. Nonetheless, we found no significant difference between temperature treatments at lower algal concentrations (0 – 0.12 mg C L^{-1}) indicating that reasonable estimations can be made in lower productive mesotrophic-oligotrophic lakes (0 – 4 µg L^{-1}). While temperature can affect algal cellular content of photosynthetic pigment proteins (Hodges et al. 2018), our short-term experiments would have limited the effect of physiological acclimation by the alga. Future research should be considered in acclimating the algae at varying temperature levels prior to testing.

We also found that chl F readings were significantly higher in the mixed treatment compared to the unmixed treatment at higher algal biomass concentrations. This likely reflects short-term settling of algal particles in the unmixed containers that reduces the chl F readings. Our results illustrate the need to consider the particle bound nature of sensor chl F readings and microenvironmental conditions while making these measurements. Microenvironmental turbulence should be further studied *in situ* due to its

potential to affect *in situ* fluorescence-based estimates of particle bound algal biomass. Caution should be taken when interpreting chl F readings taken from turbulent natural water bodies, particularly in eutrophic waters, as these measurements could appear to be higher and not representative of the system.

One environmental condition that we found that did not affect chl F readings was ambient light. There was no interaction between light condition and algal concentration on chl F readings in our experiments. Previous research was shown that chlorophyll content of algal cells can be modified by the intensity of solar radiation which inhibits fluorescence through light harvesting mechanisms (Falkowski & LaRoche 1991; Beutler et al. 2003; Finenko et al. 2003; Rousso et al. 2021). Our experimental duration was likely not long enough for the algae to physiologically acclimate to the varying light condition and that light intensity does not interfere with the sensor. Future work should consider longer-term acclimation of phytoplankton to different intensities of light and the effects of solar radiation on fluorescence-based chl measurements.

One benefits of *in situ* sensors is the rapid acquisition of data but there is a trade-off between measurement speed and quality of the results. While lower biomass concentrations ($\sim< 0.06$ mg C L^{-1}) required ~1 second of sampling time to achieve precise chl F readings, higher concentrations of biomass were found to require 10 to 30 seconds of sampling time to obtain a result within a desired range of +/- 1 ug/L of the true concentration. These estimates of sampling duration were highly variable among our repeated sampling events, but the source of the sampling error was not readily apparent. Neither container size, light nor temperature had an significant effect on sampling duration and there were minimal differences created mixing condition. The need for

longer sampling times to reliably measure higher algal concentrations is likely due to the variability created by measuring particle bound chlorophyll. As algal particles move past the sensor unevenly in space and time, this likely yields considerable fluctuations in measured fluorescence. Practically, this indicates the need to carefully consider the rate that probes are lowered and the uncertainty that accompanies relatively fast rates of descent when taking *in situ* depth profiles especially in water bodies with higher biomass and in turbulent waters. In addition, the physical micro-environment surrounding the sensor and how it effects detection and quantification of algal cells should be better understood as this appears to affect the amount of measurement error in fluorometric-based data.

Accuracy of sensor based measurements is very important when interpreting these data for monitoring and management. Our data shows that the sensor readily responses to increases in chlorophyll. Like previous research (Gregor & Maršálek 2004; Gregor et al. 2005; Rolland et al. 2010; Catherine et al. 2012; Garrido et al. 2019), we demonstrated that there is a strong relationship between extracted chlorophyll and sensor chl F measurements. However, the fluorescence-based chlorophyll measurements were always higher than those measured from extraction techniques which was consistent with other studies (Roesler et al. 2017; Simonazzi et al. 2022); although, the relative difference was not consistent across all concentration levels. At lower concentrations, the sensor produced around 37% higher chlorophyll estimates than those based on measurements of dissolved chlorophyll in ethanol extracts while at higher concentrations this difference increased to around 67%. This difference likely results from different fluorescence chlorophyll calibration standard curves between the two platforms (*in situ* sensor and

spectrofluorometer). The standard curve of the submersible sensor is unknown, and the device is calibrated using rhodamine dyes instead of known concentrations of a fresh algae culture; thus, limiting our ability to directly compare the fluorescence measurements. These discrepancies also may not be entirely unexpected given that the *in situ* sensor is measuring something (i.e., particle bound chl) different than standard ethanol extraction technique (i.e., non-particle bound chl) (Gregor & Maršálek 2004; Gregor et al. 2005). In addition, it would also be worthwhile to better understand the sources of experimental error and its effects on the estimation of algal biomass within lake ecosystems.

Fluorometric sensors remain an appealing and promising avenue to estimate *in situ* concentrations of algal biomass. We found that readings by an *in situ* fluorometer were sensitive to microenvironmental conditions that strongly connected to the movement of water, and hence particle bound chlorophyll, at smaller spatial scales. Our experiments demonstrated that when sampling chl at higher concentration, more time is required to produce reliable chl estimates. This amplifies the need to consider the sampling rate of which the device is lowered when collecting *in situ* measurements. The sensor was sensitive and responsive to increasing algal biomass, but its estimates scaled differently that those based on the standard extraction method demonstrating the need to understanding the differences between particle bound vs extracted algae estimates of chl F. Additionally, there exists the limitation of the unknown calibration standard curve of the sensor. Altogether our results show the potential value and utility of these sensors; however, testing and deployment need to carefully consider the sensitivity of this platform to ambient conditions.

Table 1. Summary of ANCOVA statistics for slopes (sensor chl F x dry mass concentration) which includes treatments (container size, light, temperature, and mixing).

Experiment	**DF**	***F***	***p***
Container Size			
Treatment	3	0.635	0.6
Concentration	5	126	<0.001
Treatment x Concentration	15	4.92	<0.001
Light			
Treatment	1	0.002	>0.9
Concentration	5	127	<0.001
Treatment x Concentration	5	0.207	>0.9
Temperature			
Treatment	3	0.26	0.9
Concentration	5	212.86	<0.001
Treatment x Concentration	15	3.24	0.001
Mixing			
Treatment	1	<0.001	>0.9
Concentration	5	389	<0.001
Treatment x Concentration	5	3.10	0.03

Table 2. Summary of ANOVA statistics for the suitable time sampling length based on three replicate measurements which includes treatments (container size, light, temperature, and mixing), concentration level and interaction effect between each treatment and concentration level.

Experiment	**DF**	***F***	***p***
Container Size			
Treatment	3	1.41	0.3
Concentration	5	8.41	<0.001
Treatment x Concentration	15	0.832	0.6
Light			
Treatment	1	0.070	0.8
Concentration	5	8.76	<0.001
Treatment x Concentration	5	2.73	0.04
Temperature			
Treatment	3	2.73	0.05
Concentration	5	38.3	<0.001
Treatment x Concentration	15	1.11	0.4
Mixing			
Treatment	1	6.38	0.02
Concentration	5	14.5	<0.001
Treatment x Concentration	5	3.12	0.03

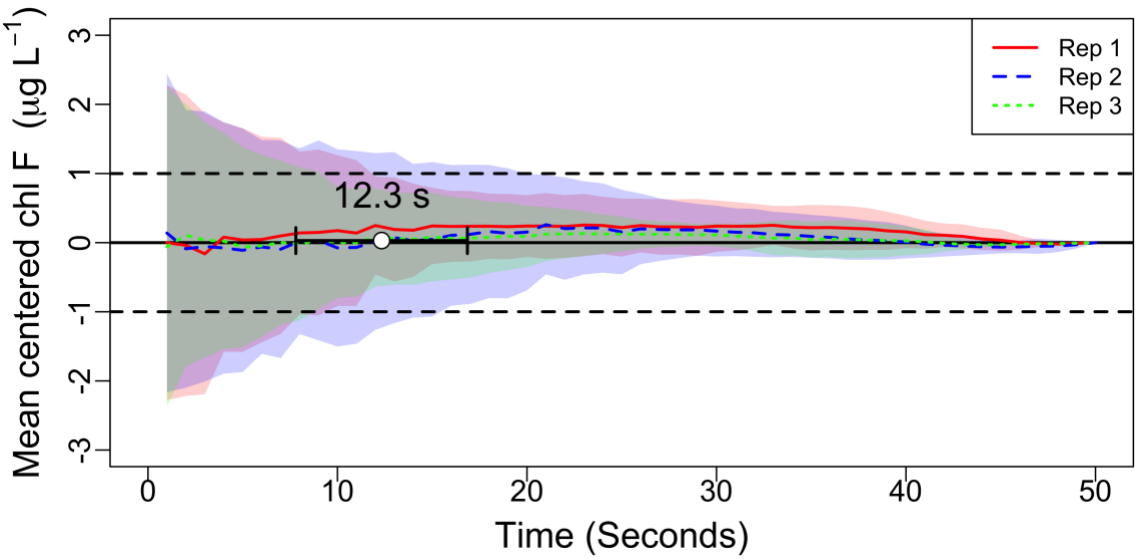

Figure 1. Example of centered mean chlorophyll fluorescence (chl F) measurements [µg/L] for the reliable time sampling length (+/- 1 SD). Coloured lines = chl F data, dotted black lines = threshold of accuracy [+/- 1 µg/L], coloured polygons = 95% confidence intervals. These readings were taken from data resampled from 3 replicate 50-second chlorophyll measurements of the 60 L container at the highest concentration level [dry mass = 0.387 mg C L^{-1}].

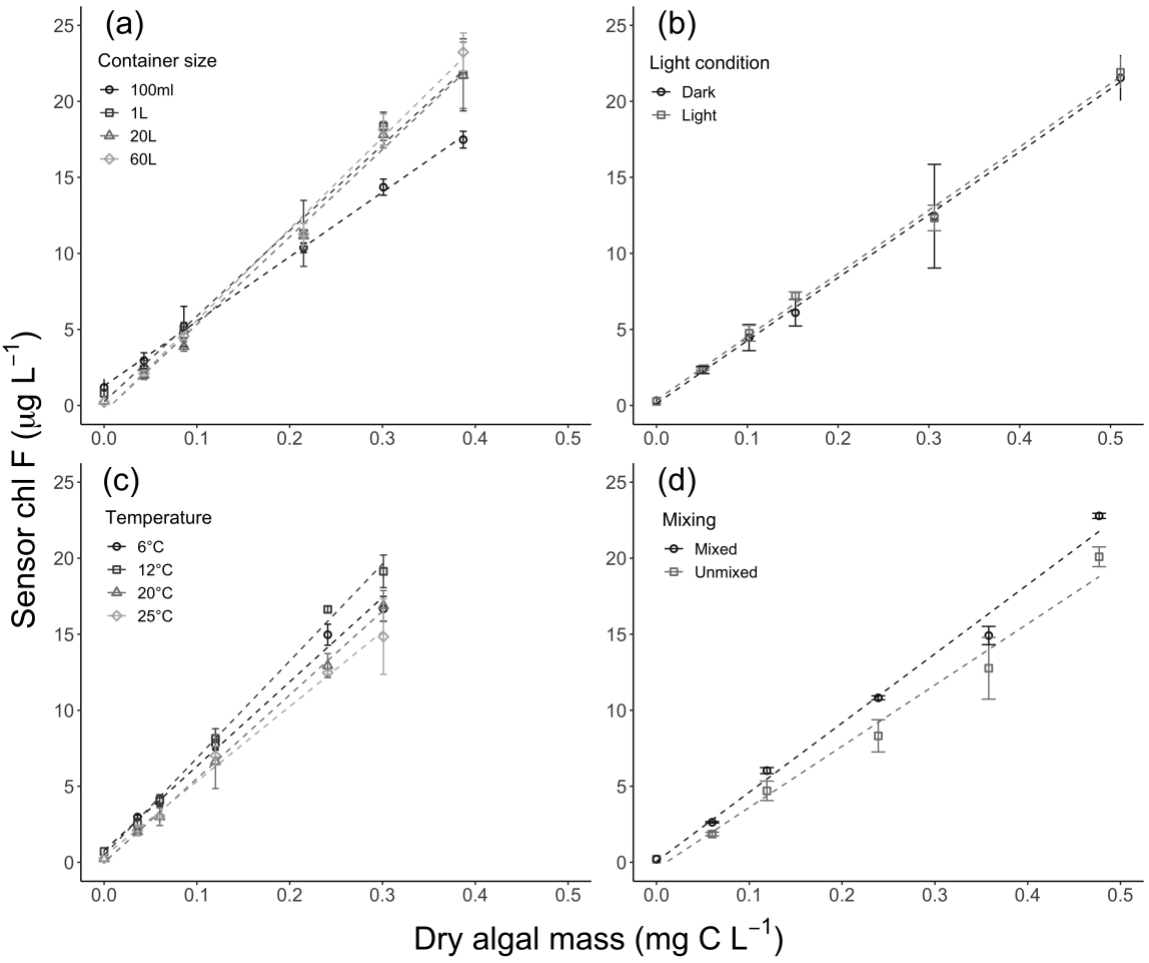

Figure 2. Relationship between chlorophyll fluorescence (chl F) values determined by sensor [μg L^{-1}] (+/- SD for the mean of each replicate) and dry mass calculations [mg C L^{-1}] across **A)** container size (100 ml, 1 L, 20 L, 60 L), **B)** light condition (dark, light), **C)** temperature (6 °C, 12 °C, 20 °C, 25 °C) and **D)** mixing condition (unmixed, mixed).

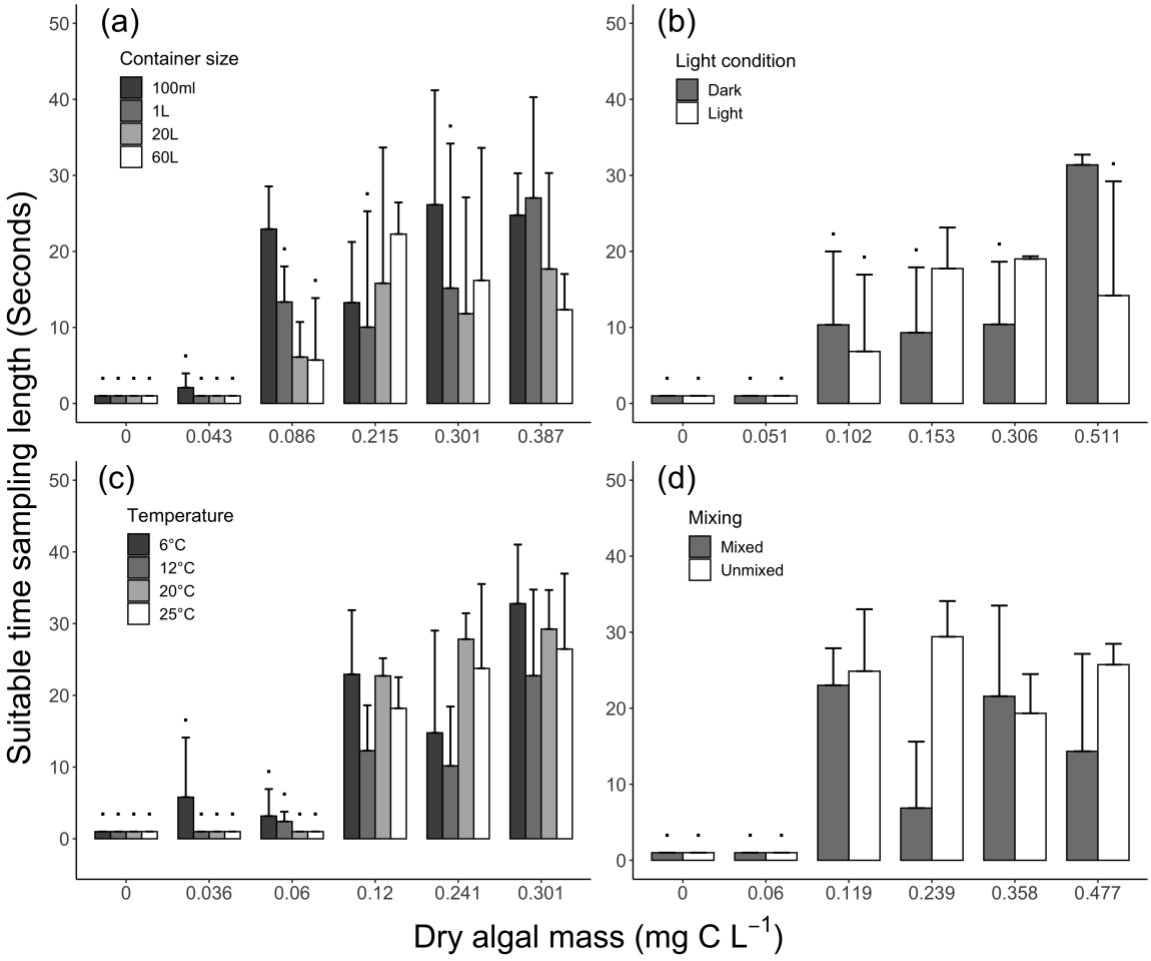

Figure 3. Suitable time sampling length for chlorophyll sampling [seconds] (+ SD for the mean of each replicate) compared across treatment types: **A)** container size (100 ml, 1 L, 20 L, 60 L), **B)** light condition (dark, light), **C)** temperature (6 °C, 12 °C, 20 °C, 25 °C) and **D)** mixing condition (unmixed, mixed). "·" over a bar represents experiments that had at least one replicate below the detection limit.

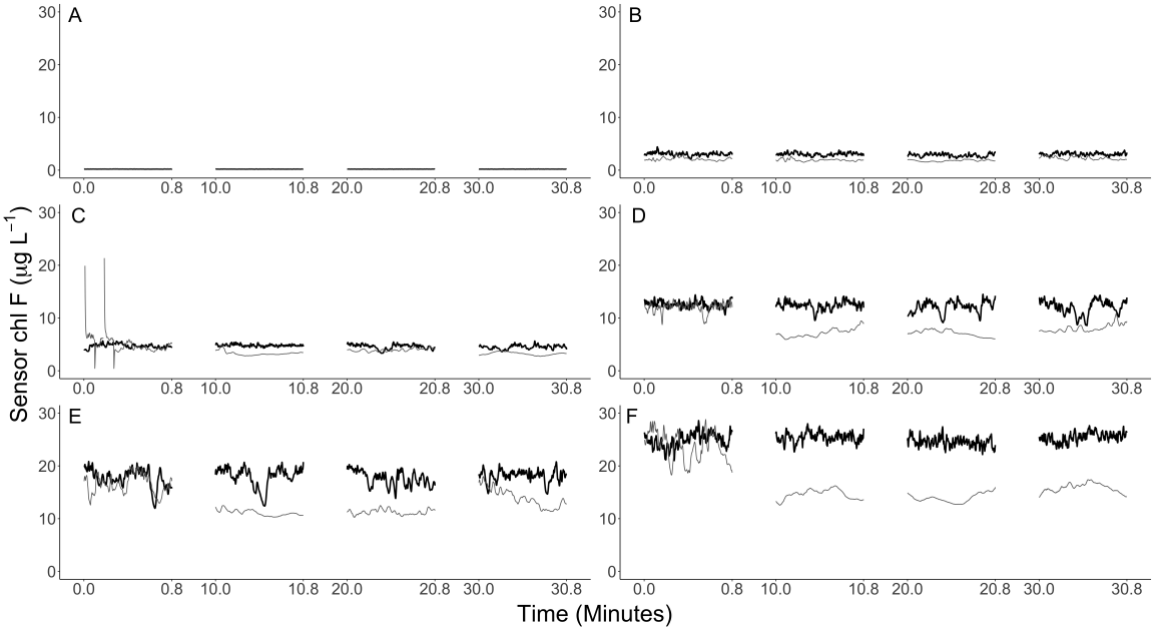

Figure 4. Chlorophyll fluorescence (chl F) measured by the sensor [mg L^{-1}] in mixed conditions (black) and unmixed conditions (grey) for a timeframe of four 50-second sampling intervals every 10 minutes for 30 minutes. Concentration levels include **A)** 0 mg C L^{-1}, **B)** 0.062 mg C L^{-1}, **C)** 0.124 mg C L^{-1}, **D)** 0.247 mg C L^{-1}, **E)** 0.371 mg C L^{-1} and **F)** 0.494 mg C L^{-1}.

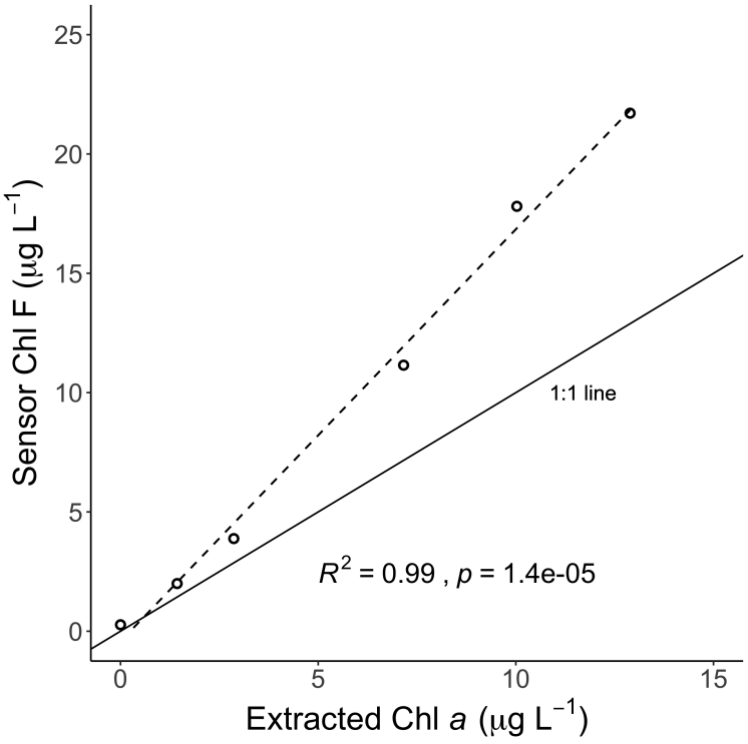

Figure 5. Relationship between chlorophyll fluorescence (chl F) values determined by sensor [μg L^{-1}] and extracted chlorophyll *a* (chl *a*) determined by spectrofluorometer [μg L^{-1}] for measurements taken in a 20 L bucket at room temperature, ambient light, and no turbulence. 1:1 line represents a relationship where sensor chl F equals extracted chl *a*.

Chapter 2: Vertical profiles of phytoplankton biomass in boreal lakes of southern Ontario

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Abstract

The vertical distribution of phytoplankton in the water column can vary widely among lakes. We used a multi-channel profiler to describe vertical chlorophyll profiles of small, forested boreal lakes in Central Ontario to better understand depth-related patterns of lake phytoplankton and their environmental correlates. Based on the chlorophyll profiles collected from 29 lakes in proximity of the Kawartha Highlands Provincial Park, we assessed the main features of chlorophyll profiles within and among thermal layers: mean chlorophyll content, standard deviation of chlorophyll content, peak depth, peak concentration, peak width, number of peaks and location of chlorophyll peaks. Finally, we described relationships between abiotic factors: namely light attenuation (measured by K_d), dissolved organic carbon (DOC), total phosphorus (TP) and lake size parameters (maximum depth, and surface area) with epilimnetic chlorophyll content and the depth, chlorophyll concentration and width of the highest peak. We found Kawartha Highland lakes generally had low epilimnetic chlorophyll biomass (mean chl F ~2 μg L^{-1}) with higher biomass observed in the metalimnion. Moreover, vertical profiles from more than 80% of lakes had a single chlorophyll peak and 2 or 3 were peaks found in 2 – 4 lakes per year. Average epilimnetic chlorophyll was positively related to DOC while peak depth was inversely related with K_d. Weak correlations were also found between the epilimnetic standard deviation of chlorophyll measurements and K_d and DOC, and with peak width and K_d, maximum lake depth and lake surface area. There appeared to be no relationship

between characteristics of vertical chlorophyll profiles with TP. Our study demonstrates the importance of assessing depth-related chlorophyll patterns as an essential part of long-term monitoring of lake ecosystem water quality.

Introduction

The vertical distribution of phytoplankton in the water column of lakes varies widely among lakes (Fee et al. 1976; Abbott et al. 1984; Marshall & Peters 1989, Rusak et al. 2018). One well known example of non-homogenous algal distribution in the water column is the deep chlorophyll maximum (DCM) where a layer of elevated algal biomass is found in the metalimnion or upper hypolimnion) reflecting the depth-related differences in water column mixing, light and nutrients (Fee 1976). Epilimnetic waters may show little depth-related variability in algal biomass due to relatively high levels of turbulent mixing, especially under windy conditions (Carrick et al. 1993; Mellard et al. 2011). Surface waters may also contain lower levels of biomass because of sinking phytoplankton and relatively limited nutrient inputs over the summer growing season (Camacho 2006; Mellard et al. 2011). Deeper in the water column (i.e., below the epilimnion) where there is less turbulent mixing, there could be greater variability and more depth-specific layers of biomass. While the flux of solar radiation decreases exponentially with depth, nutrient concentrations can be higher below the epilimnion (Bergström & Karlsson 2018; Hazuková et al. 2021; Senar et al. 2021). These conditions can produce peaks of algal biomass deeper in the water column where growth exceeds losses to sedimentation or grazing (Camacho 2006; Mellard et al. 2011).

The vertical distribution of algal biomass can be quite variable among lakes. In particular, the number, location and size (i.e., maximum chlorophyll and peak width) of chlorophyll peaks differs among lakes due to differences in lake nutrients and transparency (Fee et al. 1976; Leach et al. 2018; Loften et al. 2020). Nutrient concentrations widely range among lake ecosystems due to differences in external inputs and internal cycling (Wetzel 2001). For example, DOC concentrations vary among lakes reflecting differences in catchment type (Xenopoulos et al. 2003) and within lake processing (Waiser and Roberts 2000), which affects the light environment (Bukaveckas & Robbins-Forbes 2000). Differences in light and nutrients, and their unequal distribution with depth, contribute to the development of depth-related patterns in algal biomass that vary substantially even among lakes in the same region (e.g., Tonin et al. 2020; Senar et al. 2021). With recent improvements in high resolution chlorophyll fluorescence profiling (Rolland et al. 2010; Catherine et al. 2012; Roesler et al. 2017; Garrido et al. 2019), we have a greater ability to describe and understand these depth-related features of algal biomass and their variability among lakes.

We assessed the vertical distribution of phytoplankton biomass using *in situ* chlorophyll fluorescence (chl F) profiles collected from 29 lakes located in south central Ontario, Canada. We used the data to describe features of the chlorophyll profiles within and among the lakes' thermal layers (epilimnion, metalimnion, and hypolimnion; Figure 1). In addition, we determined the depth, size and number of biomass peaks in the water column. We further determined how features of chl F profiles relate to lake thermal structure, light and nutrient environments. This work provides a comprehensive

description of vertical patterns of phytoplankton biomass in the water column of boreal lakes.

Methods

Sampling area description and field sampling. We sampled 29 lakes in the general region of the Kawartha Highlands Provincial Park in south-central Ontario, Canada (Table 1). Lake sampling took place during morning to afternoon over a duration of about 3 weeks in late July and August of two years, 2020 and 2021. At this time, lakes would have reached full stratification. For this study, we included lakes that varied in morphometric characteristics (i.e., lake surface area, perimeter and maximum depth; Table 1) but that have similar epilimnetic chemistry (i.e., dissolved organic carbon, total phosphorus; Table 1). Lakes in this region are dimictic and exhibit thermal stratification during late summer. We sampled 26 lakes in 2020 and 29 lakes in 2021; however, one lake was removed from the dataset in 2020 and three lakes were removed from the dataset in 2021 due to sampling at an insufficient depth creating a sample of 25 lakes and 26 lakes for 2020 and 2021 respectively.

Each sampling event was conducted at the deepest location of each lake. Prior to chlorophyll-profiling, we measured depth profiles of solar irradiance on the sunny side of the boat. With these data, we calculated the diffuse light attenuation coefficient (K_d) for each lake calculated as the slope of natural logarithm of irradiance and lake depth. We used this K_d to estimate the depth to 1% irradiance as an indicator of photic zone depth. After completing irradiance profiles, we used a multi-channel profiler (RBR maestro^3; RBR Ltd. Canada) that collects high-frequency measurements (8 hertz) of depth and

temperature (both RBR sensors) and chlorophyll fluorescence (Turner® Cyclops -7F sensor; Turner Designs Inc., USA) as it is lowered through the water column. For each profile, we initially acclimated the profiler in the water by holding it just under the lake's surface for more than one minute. We then slowly lowered the profiler to the bottom of the lake over a duration of five to ten minutes depending on the depth of the lake. After collecting a water-column chl F depth profile for each lake, we sampled lake water at 1 m with a Van Dorn sampler to determine epilimnetic dissolved organic carbon (DOC), and epilimnetic total phosphorus (TP).

Water processing. TP was determined using the molybdate blue analysis method derived from APHA (2005) using unfiltered water samples digested with potassium persulfate in an autoclave at 121°C for 30 min. Subsequently, TP was estimated based on absorbance measurements by a spectrophotometer at 885 nm (Cary® 50 Bio UV-Visible Spectrophotometer; Agilent Technologies, USA). DOC concentrations were measured using unfiltered water samples with a TOC analyzer using the wet oxidation method (Shimadzu® TOC-V WP Total Organic Carbon Analyzer and ASI-V automatic sampler; Shimadzu Corporation, Japan).

Profile processing. After downloading profile data from the RBR profiler, we cleaned each profile by removing measurements taken after the profiler reached the bottom of the lake. We did this identifying and removing data below the depth where turbidity began to rapidly increase due to the disturbance of bottom sediments. We next averaged data across 5 cm depth intervals to reduce the quantity of measurements and to account for potential differences in measurement response times among the sensors. Additionally, a running average calculated for the temperature data using a window width

of 7 observations. Using trimmed and smoothed temperature profile data, we calculated depth of thermal layers (epilimnion, metalimnion, and hypolimnion; Figure 1) using the *rLakeAnalyzer* package in R (Albers et al. 2018).

Using chl F concentration averaged across the 5 cm depth intervals, we calculated the mean, standard deviation (SD), minimum and maximum chl F concentration ($\mu g\ L^{-1}$) within the epilimnion, metalimnion, hypolimnion and full water column for each lake. We next identified biomass peaks within each profile that met the following criteria which was created based on our sample of lakes: a) the chl F value was greater than 1.3 times than the average chl F of the full water column, b) this value exceeded 1.0 $\mu g\ L^{-1}$, and c) this maximum value was located more than 10% of the total depth below the surface and above the lake's bottom. We further restricted this analysis of biomass peaks to only include lakes in which the photic zone did not extend to the bottom of water column. A chl F value higher than 1.3 times than the average chl F of the full water column could exclude many lakes from our study with obvious peaks while a lower value may include lakes with slight fluctuations in chl F which are not to be considered a peak. A value smaller than 1.0 $\mu g\ L^{-1}$ was considered too small to be a peak for our sample of lakes. We next calculated the average, standard deviation, minimum and maximum values for each of the following: depth of the highest chl F peak, chl F at the highest peak and width of the highest peak. We also determined the number of peaks and thermal layer that the highest peak resides in (epilimnion, metalimnion or hypolimnion). Peak depth was determined as the depth of highest value in chl F in the profile (Figure 1). Peak width (Figure 1) was determined by calculating a fitted curve to the smoothed 5 cm data using the same methods used in Leach et al. (2018) and Loften et al. (2020).

Statistical analysis. We used simple linear regression (single predictor models) to determine the strength of the relationship between the variables (mean epilimnetic chl F, standard deviation of epilimnetic chl F, depth of highest peak, concentration of highest peak, width of highest peak) and water column metrics (K_d, DOC and TP) as well as morphometric characteristics (maximum lake depth and lake surface area). The data was log transformed where necessary before analysis to correct for nonlinearity.

Results

The mean chlorophyll concentration based on fluorometric chlorophyll averaged across all lakes varied between the epilimnion, metalimnion, and hypolimnion (Figure 2). The average value for the epilimnion chl F of each lake was quite low (Mean chl F = 2.07 µg L^{-1}, Figure 2a) and within the range of oligotrophic lakes (Vollenweider & Kerekes 1982). All lakes had average chlorophyll in the epilimnion less than 3 μ µg L^{-1} except for 3 lakes (Eels, Bottle and Pencil sampled in 2021; Figure 2a). We found more chlorophyll in the metalimnion with a higher average value (Mean chl F = 2.85 µg L^{-1}) than in the epilimnion (Figure 2b). Chlorophyll variation (measured by SD) in the epilimnion was also lower than what was found in the metalimnion (Figure 2b). There was also less chl F (Mean chl F = 1.86 µg L^{-1}) and little variance in chl F in hypolimnetic waters (Figure 2d). All lakes had average chlorophyll in the hypolimnion less than 3 µg L^{-1} except for 2 lakes (Crab and Wolf; Figure 2c). As with the epilimnion, hypolimnetic variation (measured by SD) in chl F was also lower than in the metalimnion (Figure 2f). The average value for full water column chl F was found to be low (Mean chl F = 2.06 µg L^{-1}) and within the range of oligotrophic lakes (Vollenweider & Kerekes 1982).

The average depth of the primary chl F peak was 6 m with considerable lake to lake variation (range of 2 – 12 m; Figure 3a). When combining both years, 75% of lakes had a peak chlorophyll concentration of less than 10 μg L^{-1} with a 25% of lakes having peak chlorophyll concentration between than 10 and 60 μg L^{-1} (Figure 3b). Peak width was generally less than 10 m (90% of lakes) with a few exceptions (Buzzard and Chandos sampled in 2020, Beaver and Jack sampled in 2021; Figure 3c).

In both years (2020 and 2021), we found more than 80% of lakes sampled exhibited at least one chl F peak (Table 2). Most of the profiles in our study had only one peak and a small number (2-3 lakes per year) had a smaller distinct secondary peak which met our criteria (Table 2). We only found one lake (Crystal in 2021) to have 3 peaks (Table 2). While in most cases of double peaks, one peak had a higher concentration than the other, there were 2 lakes (Loon Call and Salmon in year 2021) in our dataset where the chlorophyll concentrations of the 2 peaks were of similar magnitude. The depth of the higher peak usually occurred in the metalimnion just below the bottom of the mixed layer (Table 2). Metalimnetic peaks were identified in 17 of 22 lakes in 2020 and 12 of 21 lakes in 2021 (Table 2). We found a limited number of lakes had epilimnetic peaks with only 1 lake in 2020 and 5 lakes in 2021 (Table 2).

We examined the relationships between mean and standard deviation (SD) of epilimnetic chl F, chl F concentration of the highest peak, depth of the highest peak and width of the highest peak with K_d, epilimnetic DOC and epilimnetic TP. For mean values of epilimnetic chl F, we found a weaker relationship with K_d (Table 3; Figure 4a) and the strongest relationship with epilimnetic DOC (Table 3; Figure 4b), and no relationship with TP (Table 3). Epilimnetic SD of chl F was weakly correlated with K_d but showed no

relationship with DOC and TP (Table 3). Peak depth in the water column had a strong negative relationship with K_d (Table 3; Figure 4c) and epilimnetic DOC (Table 3; Figure 4d) but again no relationship with TP (Table 3). Highest chl F concentration at the peak weakly correlated with K_d but no relationship with DOC and TP (Table 3). While there was a weak negative relationship between peak width and K_d (Table 3; Figure 4e), there was no relationship found with DOC and TP (Table 3).

Additionally, we examined the relationships between chlorophyll and peak characteristics listed above with lake morphometric characteristics including maximum lake depth and surface area (Table 3). For the mean and SD of epilimnetic chl F, we found no relationships with either morphometric feature (Table 3). For peak depth, we found a weak positive relationship with maximum depth and no relationship with lake surface area (Table 3). The chlorophyll concentration at the peak demonstrated no significant relationships with any lake morphometric characters (Table 3). Lastly, peak width demonstrated a weak positive relationship with surface area (Table 3; Figure 4f).

Discussion

The characterization of phytoplankton biomass within and among lakes remains one of primary means of assessing water quality and estimating lake productivity. This characterization usually involves measuring algal chlorophyll fluorescence given its strong correlation with phytoplankton biomass (Wetzel and Likens 1992). *In situ* measurements of chlorophyll based on fluorescence are increasingly being used to better capture spatial patterns within lakes (Rolland et al. 2010; Catherine et al. 2012; Garrido et al. 2019) and also temporal patterns. Much of the past work has focused on the depth and

magnitude of biomass peaks found beneath the epilimnion (Hamilton et al. 2010, Leach et al. 2018; Scofield et al. 2020, Loften et al. 2020). Here we expand on these descriptions and show that there is considerable complexity to the vertical distribution of phytoplankton biomass in south-central Ontario boreal lakes.

We found the chlorophyll profiles of lakes in this study to generally resemble those previously reported for low nutrient, temperate lakes (Marshall & Peters 1989). There was low chl F in the epilimnion with little variation. This low variation would be expected given that this surface layer is subject to turbulent mixing produced by surface wind and heating/cooling (Mellard et al. 2011). We found more chlorophyll below the mixed layer in many lakes sometimes concentrated in relatively narrow layers. These deeper biomass layers have been previously reported in oligotrophic lakes where light and nutrients are sufficient to fuel algal growth (Fee 1976). The presence of one or more peaks is also consistent with less turbulent mixing in the metalimnion (e.g., Mellard et al. 2011, Selmeczy et al. 2016), which allows for greater structure (i.e., layers of concentrated chl) to develop. These deeper peaks may be short-lived since peak characteristics can vary due to changes in environmental stimuli (Scofield et al. 2017; Reinl et al. 2020). For example, peak depth can change monthly in response to differences in nutrients in the metalimnion (Scofield et al. 2017) and thermocline depth (Reinl et al. 2020) while peak width can change in response to surface temperature and thermocline depth (Reinl et al. 2020).

We found that peak depth was negatively correlated with both K_d and DOC respectively. Phytoplankton can grow deeper in the water column in lakes having more light penetration, which would lead to deeper peaks (Fee et al. 1976; Leach et al. 2018).

Consistent with this, we did not find any deep chlorophyll layers in higher DOC lakes, where solar radiation is largely restricted to the mixed layer (Tonin et al. 2020; Senar et al. 2021). While metalimnetic waters are colder, lower DOC lakes would have more solar radiation, which lead to a photosynthesis/respiration (P/R) ratio of more than one and allow primary producers to persist in these deeper waters (Wetzel 2001). Our results are further confirmation that even relatively small differences in DOC can alter the subsurface light environment and modify depth-related patterns in algal populations.

It was expected that the amount of total phosphorus would affect epilimnetic algal biomass (Wetzel 2001). However, we found no correlation between epilimnetic chl F and TP concentrations among the lakes included in our study. This could be due to the unproductive state of most of the lakes, as seen by the relatively low chlorophyll and TP in the epilimnion. Given the low concentration for these two variables, it is likely that other gain/loss processes (e.g., grazing) would affect algal biomass and create variability not accounted for by phosphorus (Mazumder 1994). Our data indicates that these lakes are strongly P-limited but the small differences (SD = 3.75 μg L^{-1}) in P availability among our lakes was insufficient to produce measurable changes in chl.

We found that width of the peak was more associated with light and lake size than DOC and nutrients. A significant negative association was found between layer width and K_d and a significant positive association with surface area. Peak layer width may reflect short term changes in gain-loss processes controlling phytoplankton biomass. As most of the peaks were located below the epilimnion, turbulent mixing should be generally low and unable to account for distributing phytoplankton across depths. Instead, phytoplankton sinking and/or buoyancy coupled to growth and death processes may

account for increases or losses of biomass at a particular depth (Loften et al. 2020). With more available light, the habitable depths that support phytoplankton growth should be wider, which could account partly for the wider biomass peaks. However, in the absence of mechanistic studies of phytoplankton populations in these deep-water communities, there is very high uncertainty about why the width of biomass peak layers varies with light and lake size.

Our results showed that chlorophyll profiles in Kawartha Highland lakes are similar to previously studied oligotrophic temperate lakes. Generally, these lakes have relatively low algal biomass in well-mixed epilimnions and complicated patterns of chlorophyll (i.e., one or more layers of variable thickness) in the metalimnion. This manifests itself as a diversity of profiles structures with the location and number of peaks varying among lakes. These patterns of phytoplankton vertical distribution are further indication that gain and loss processes on phytoplankton populations are not evenly distributed across the water column. Fully understanding these patterns and their in-lake controls will require more frequent profiling through time and direct study of growth and loss processes.

Table 1. Summary of data of the sampled lakes including sampling dates, locations of lakes, maximum depth (m), surface area (ha), total phosphorus (TP, μg L^{-1}) and dissolved organic carbon (DOC, mg L^{-1}) averaged from 2015 – 2021.

Lake	Dates sampled	Location (Lat, Long)	Max depth (m)	Surface area (ha)	TP	DOC
Anstruther	August 17, 2020 August 3, 2021	44.7360, -78.2313	39.14	639.5	4.78	4.59
Beaver	August 10, 2020 August 12, 2021	44.7419, -78.2864	19.29	154.9	6.51	5.86
Big Cedar	August 3, 2020 August 4, 2021	44.6073, -78.1728	18.86	219.3	6.43	4.21
Bottle	August 26, 2020 August 9, 2021	44.7636, -78.2809	24.03	151.1	9.26	8.62
Buzzard	August 24, 2020 August 11, 2021	44.6750, -78.2007	39.65	89.75	9.26	3.38
Catchacoma	August 10, 2020 August 18, 2021	44.7458, -78.3259	48.30	707.4	5.53	5.94
Chandos	August 14, 2020 August 10, 2021	44.8082, -77.9611	45.95	1651	7.34	4.28
Crab	August 16, 2021	44.7246, -78.1894	6.65	68.33	6.94	4.85
Crystal	August 7, 2020 August 18, 2021	44.7578, - 78.4796	35.07	487.4	7.60	5.37
Eels	August 14, 2020 August 17, 2021	44.8923, -78.1509	25.63	936.0	5.82	4.95
Gold	August 10, 2020 August 5, 2021	44.7223, -78.2708	38.89	331.9	8.60	4.75
Jack	August 5, 2020 August 10, 2021	44.6899, -78.0501	39.8	1344	5.31	4.71
Kasshabog	August 5, 2020 August 10, 2021	44.6342, -77.9651	23.99	997.9	5.49	4.21
Little Turtle	August 13, 2021	44.6198, -78.2090	28.25	14.88	5.08	3.96
Long	August 19, 2020 August 11, 2021	44.6908, -78.1648	22.52	96.68	6.85	4.05

Lake	Dates sampled	Location (Lat, Long)	Max depth (m)	Surface area (ha)	TP	DOC
Loon Call	August 17, 2020 August 3, 2021	44.7465, -78.1375	16.64	90.29	5.23	5.36
Loucks	August 19, 2020 August 11, 2021	44.6821, -78.2202	19.47	36.87	4.62	4.71
Lower Stoney	August 12, 2020 August 13, 2021	44.5533, -78.1351	----	----	14.6	5.48
Mississauga	August 10, 2020 August 5, 2021	44.7160, -78.3237	48.33	672.4	5.83	4.95
N. Rathbun	August 22, 2020 August 17, 2021	44.7949, -78.2135	12.87	38.51	10.6	5.93
Pencil	July 30, 2020 August 5, 2021	44.8113, -78.3487	22.55	90.81	7.06	8.39
Picard	August 27, 2020 August 18, 2021	44.7781, -78.3906	34.99	75.01	6.23	5.21
Raccoon	August 3, 2020 August 3, 2021	44.6060, -78.1978	16.09	50.40	5.12	6.00
Rathbun	August 22, 2020 August 17, 2021	44.7753, -78.2047	41.71	115.2	7.63	5.14
Salmon	July 30, 2020 August 5, 2021	44.8252, -78.4452	34.42	174.6	5.89	3.80
Sucker	August 26, 2020 August 9, 2021	44.7650, -78.2554	20.07	155.0	5.33	4.90
Upper Stoney	August 12, 2020 August 13, 2021	44.5723, -78.0644	32.58	2739	8.09	5.27
Wolf	August 17, 2020 August 16, 2021	44.7446, -78.1703	8.76	138.4	6.11	4.03
Wollaston	August 10, 2021	44.8363, -77.8418	32.79	361.6	----	----

Information obtained from Frost et al. 2021.

Table 2. Summary of peak statistics which includes the number of chlorophyll peaks in a single lake and location of the major chlorophyll peak for lakes that exhibited at least one chlorophyll peak. Data is shown for 25 lakes included the 2020 sample and 26 lakes included the 2021 sample.

	Fraction of lakes	
Number of peaks	**2020**	**2021**
0	3/25	5/26
1	20/25	17/26
2	2/25	3/26
3	0/25	1/26
Location of peak		
Epilimnion	1/22	5/21
Metalimnion	17/22	12/21
Hypolimnion	4/22	4/21

Table 3. Summary of regression statistics for single predictor linear models. Predictors include lake metrics (K_d, dissolved organic carbon [DOC], total phosphorus [TP], maximum depth [max depth], surface area) and response variables include lake properties (mean epilimnetic chlorophyll fluorescence [chl F], standard deviation [SD] of epilimnetic chlorophyll fluorescence [chl F], peak depth, peak chlorophyll [chl F] concentration, peak width).

Model	R^2	*F*	*p*	Slope
Log mean epilimnetic chl F [μg L^{-1}]				
K_d [m^{-1}]	0.17	8.83	0.005	1.01
Log DOC [mg L^{-1}]	0.59	69.39	7e-11	1.50
Log TP [μg L^{-1}]	0.02	0.85	0.4	0.15
Log Max depth [m]	0.02	0.86	0.4	-0.13
Log Surface area [ha]	0.00	0.11	0.7	-0.02
Log SD epilimnetic chl F [μg L^{-1}]				
K_d [m^{-1}]	0.20	10.65	0.002	2.22
Log DOC [mg L^{-1}]	0.10	5.3	0.03	1.16
Log TP [μg L^{-1}]	0.06	2.94	0.03	0.53
Log Max depth [m]	0.04	1.94	0.2	-0.35
Log Surface area [ha]	0.01	0.56	0.5	-0.08
Log peak depth [m]				
K_d [m^{-1}]	0.47	32.21	2e-06	-1.64
Log DOC [mg L^{-1}]	0.48	36.43	4e-07	-1.58
Log TP [μg L^{-1}]	0.00	0.09	0.8	0.05
Log Max depth [m]	0.19	8.9	0.005	0.51
Log Surface area [ha]	0.03	0.99	0.3	0.06
Log peak chl F [mg L^{-1}]				
K_d [m^{-1}]	0.20	9.19	0.004	2.63
Log DOC [mg L^{-1}]	0.13	6.15	0.02	1.96
Log TP [μg L^{-1}]	0.01	0.59	0.4	-0.3

Log Max depth [m]	0.06	2.49	0.1	-0.69
Log Surface area [ha]	0.06	2.42	0.1	-0.21
Log peak width [m]				
K_d [m^{-1}]	0.26	13.22	0.0008	-2.95
Log DOC [mg L^{-1}]	0.10	4.44	0.04	-1.66
Log TP [μg L^{-1}]	0.08	3.45	0.07	0.69
Log Max depth [m]	0.11	4.85	0.03	0.91
Log Surface area [ha]	0.21	10.35	0.003	0.38

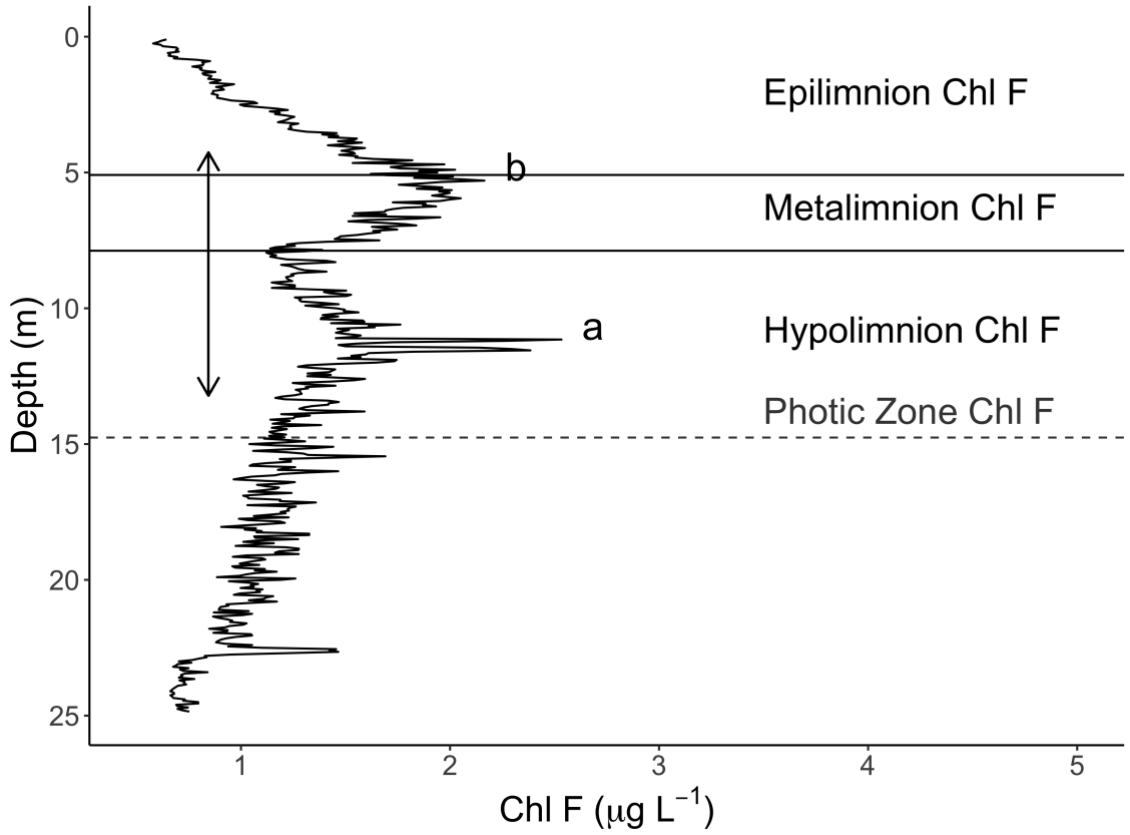

Figure 1. Depth profile of chlorophyll fluorescence (Chl F) [μg L^{-1}] for Salmon Lake with profile characteristics of interest (thermal layer divisions, photic zone division, major and minor peaks). Black horizontal lines represent thermal layer divisions. Grey dashed horizontal line represents bottom of the photic zone. Arrow represents peak width. "a" represents primary peak, "b" represents secondary peak.

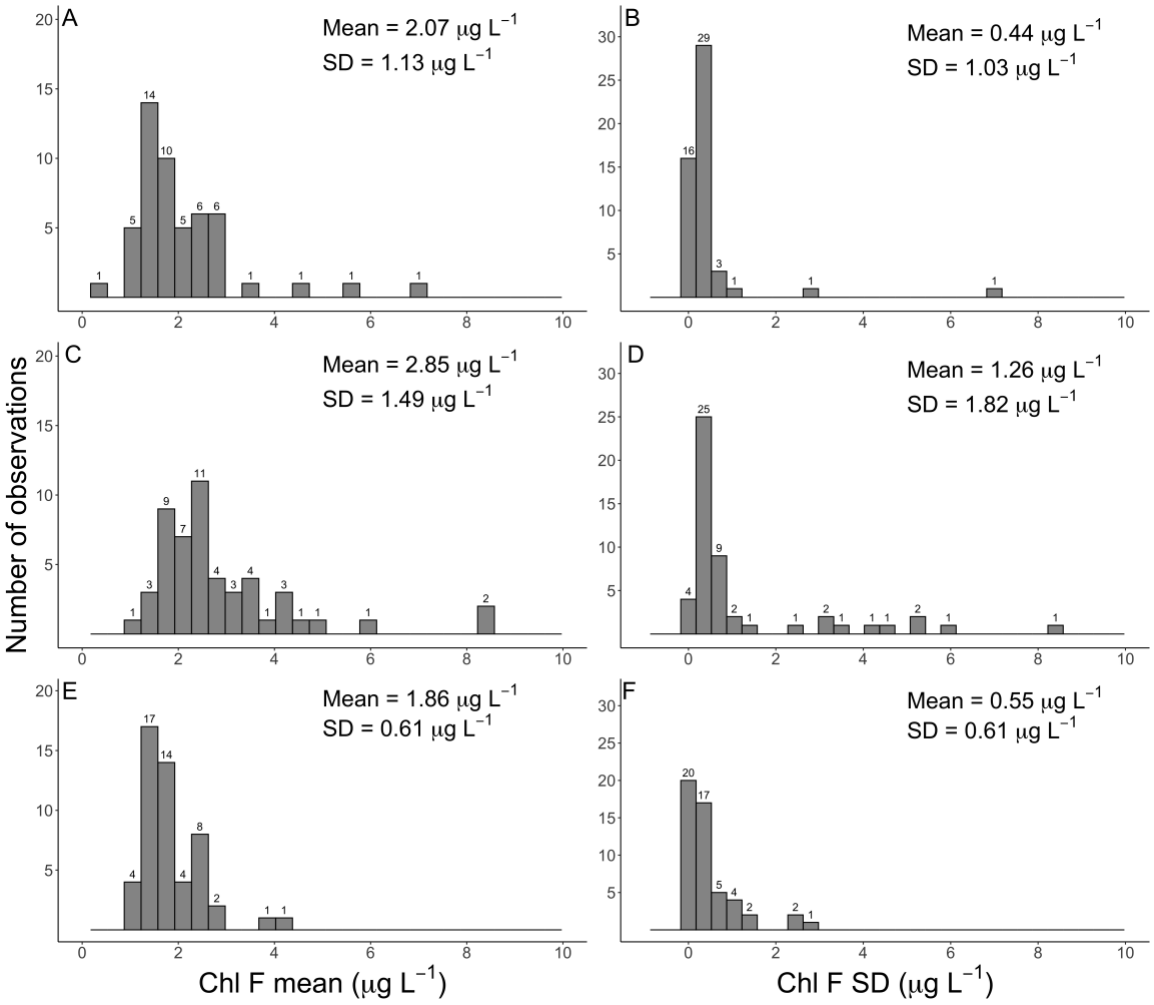

Figure 2. Mean chlorophyll (Chl F mean) [μg L^{-1}] and chlorophyll variation (Chl F SD) [μg L^{-1}] across all lakes for each thermal layer categories: **A)** epilimnion mean, **B)** epilimnion SD, **C)** metalimnion mean, **D)** metalimnion SD, **E)** hypolimnion mean, **F)** hypolimnion SD. Mean = averaged chl F mean and averaged chl F SD, SD = standard deviation of mean chl F and standard deviation of SD chl F. Includes data for both years.

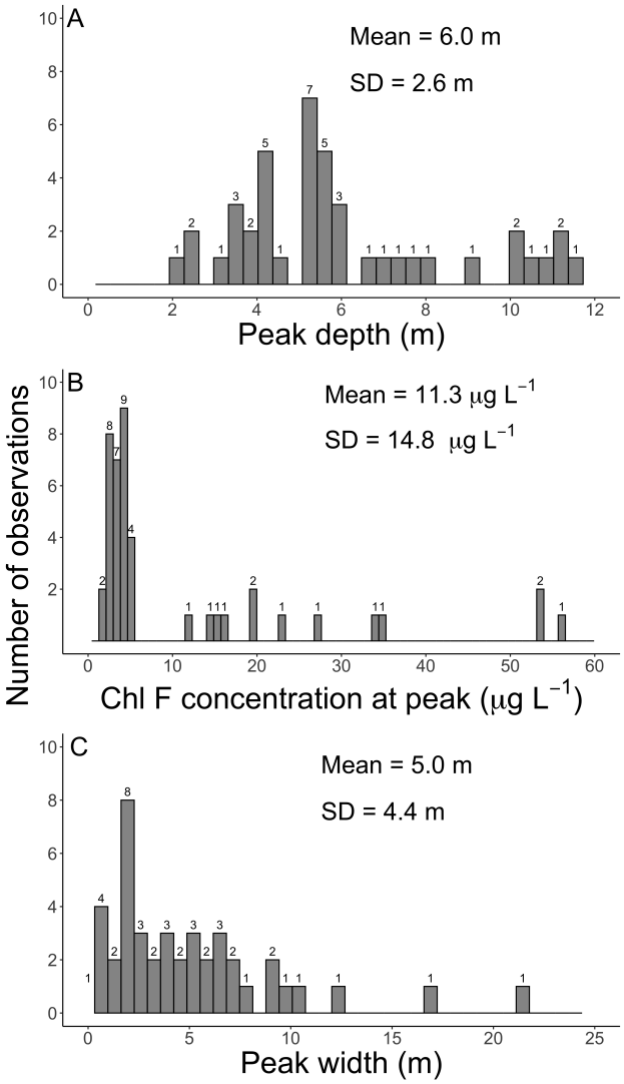

Figure 3. Peak statistics for **A)** peak depth [m], **B)** chlorophyll (Chl F) concentration at peak [μg L^{-1}] and **C)** peak width taken across lakes with at least 1 peak. Includes data for both years. Includes data for both years. Note that this data includes lakes that met peak criteria (see "Methods" section).

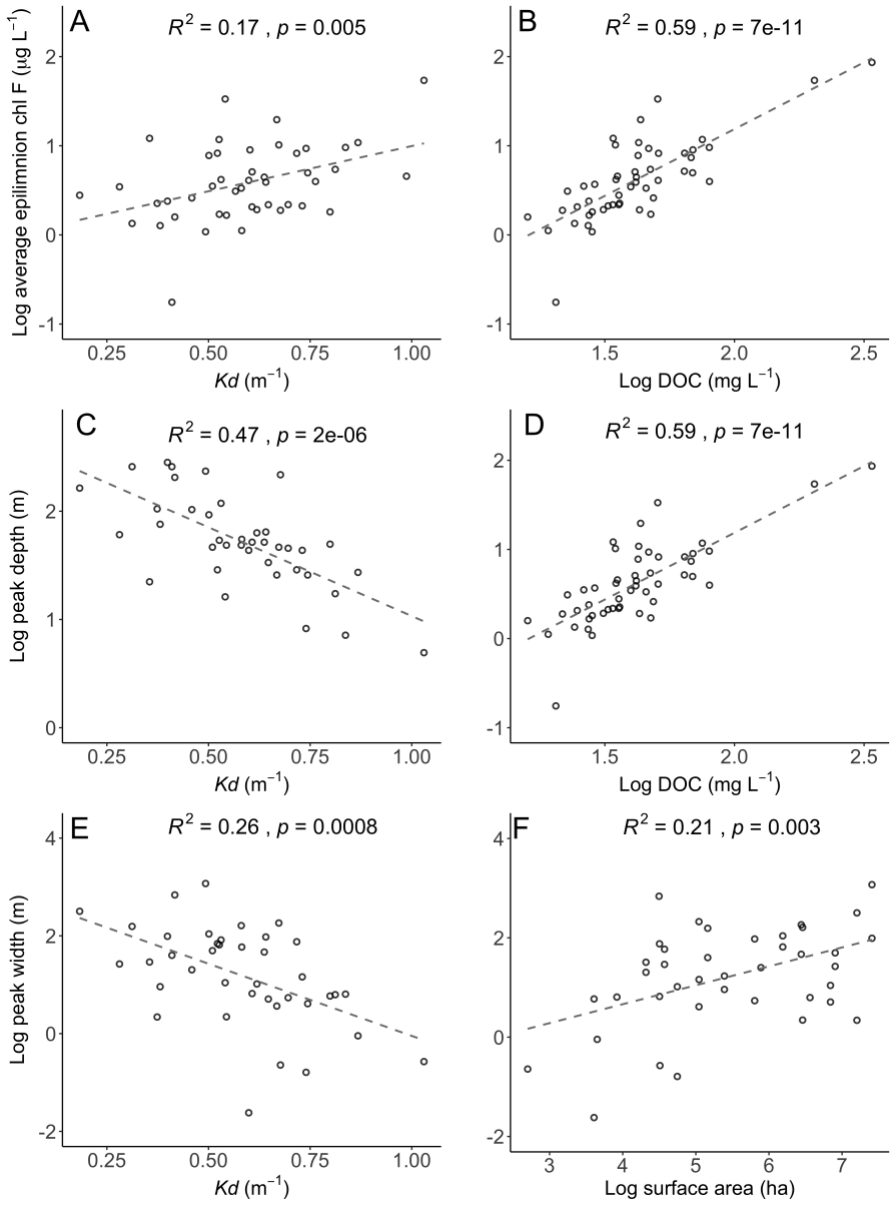

Figure 4. Relationship between lake characteristics and lake metrics for: Mean epilimnion chlorophyll fluorescence (chl F) [μg L^{-1}] as a function of light attenuation [K_d] **(A)** and as a function of dissolved organic carbon [DOC] **(B)**, peak depth [m] as a function of light attenuation [K_d] **(C)** and as a function of dissolved organic carbon [DOC] **(D)** and peak width [m] as a function of light attenuation [K_d] **(E)** and as a function of surface area (ha) **[F]**. Includes data for both years.

General conclusion

We demonstrated through a series of experiments that the *in situ* chlorophyll fluorescence (chl F) sensor is an effective tool which can be applied to collect precise measurements and assess the depth related patterns of phytoplankton in oligotrophic lakes. We concluded that there were some slight discrepancies in chlorophyll measurements under different conditions (i.e. container size, light, temperature). However, we found variation between mixed and unmixed treatments reflecting the differences of short-term settling of particles which should be considered while making these measurements.

We deployed the same sensor to access vertical chlorophyll profiles to observe the vertical patterns of phytoplankton in the southern boreal Kawartha lakes. The measurements from the sensor showed that the lakes are typical of temperate oligotrophic lakes of the area (≤ 2.5 $\mu g\ L^{-1}$). In addition, we provided insights on phytoplankton biomass and variation as well as chlorophyll characteristics in relation to lake metrics (i.e. light attenuation, dissolved organic carbon) and lake morphometry characteristics. However, we determined that the chlorophyll profile data obtained from these lakes have uncertainty based on the results from the experiments. This can be due to the lack of consideration of the speed of sampling which reflects the time period necessary to produce reliable results. This issue could have created uncertainty in the chl F values collected from a few of the higher chlorophyll lakes we sampled where chl F mean was greater than 2 $\mu g\ L^{-1}$ (0.06 mg C L^{-1}). There was also a limitation of the unknown manufacturer standard curve of which the device was calibrated which may account for discrepancies between fluorescence based measurements obtained from the sensor and

measurement obtained from extracted pigments. However, these differences between fluorometric chl F and extracted chlorophyll would not have been an issue as most of the lakes in our sample had lower chlorophyll biomass. Acknowledgement of trophic state/phytoplankton biomass of the lake is important since we determined that high concentrations of chlorophyll required a longer sampling time and the effect of light, temperature and mixing on time sampling length at lower concentrations is not significant. Thus, the aquatic environment should be considered before sampling.

The sensor is applicable in many biomonitoring situations involving water quality assessment (i.e., routine monitoring, bloom detection, characterizing spatial water column, etc.). *In situ* chl F sensors should continue to be studied in further detail to understand their response to different ambient/environmental conditions. Future research should continue to include acclimating the algae at varying light and temperature levels prior to testing the *in situ* sensor performance and accounting for the effects that container size and settling of particles has on fluorescence based chl estimates. Overall, *in situ* sensors are a promising tool to access chlorophyll and account for phytoplankton biomass patterns in lakes.

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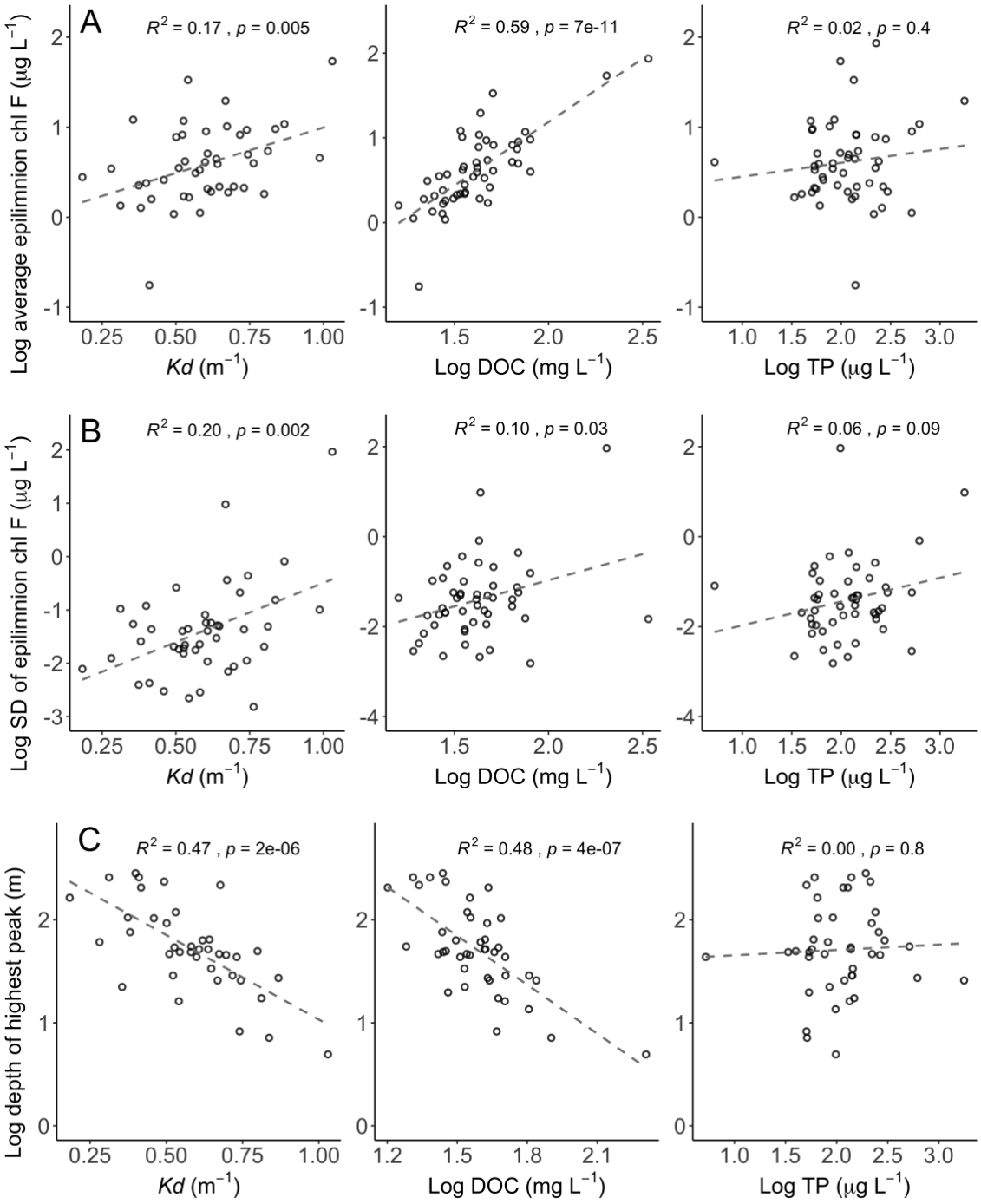
APPENDIX

Relationships between lake metrics and lake/peak characteristics.



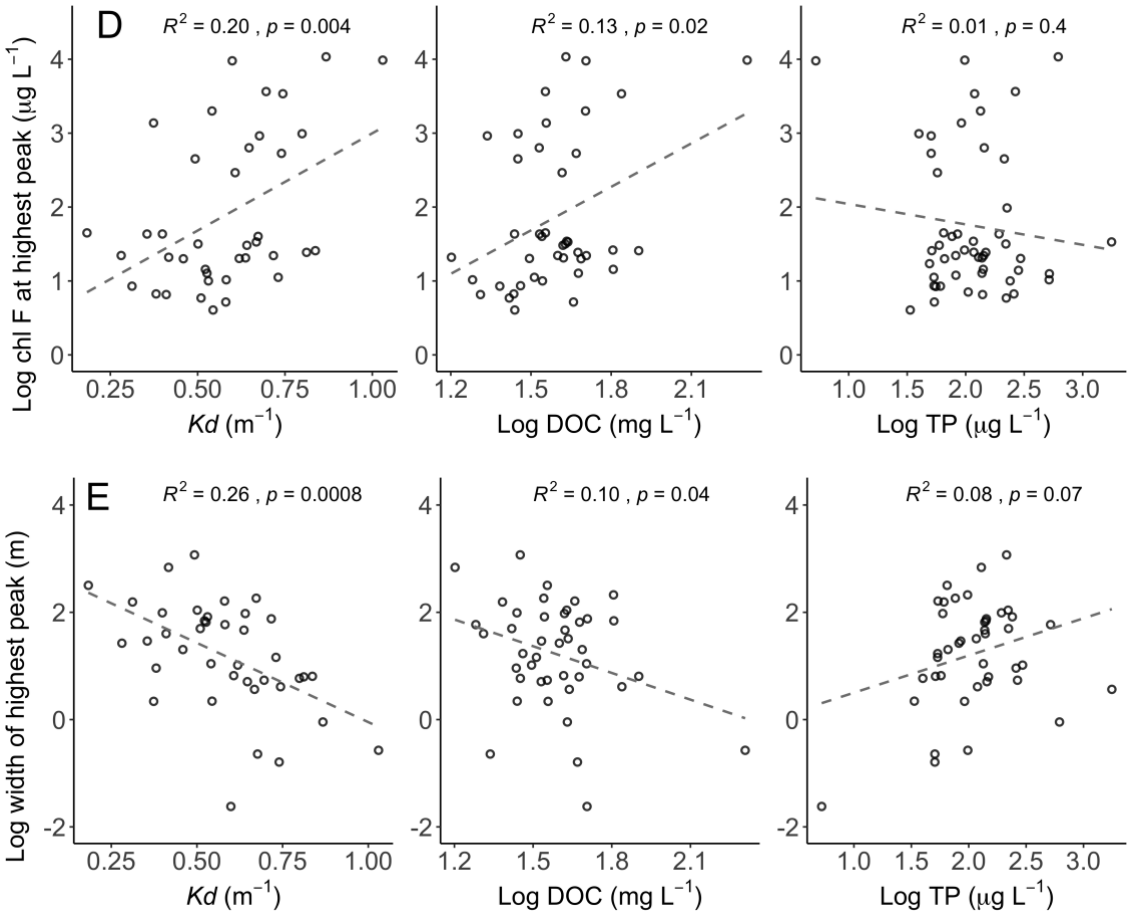
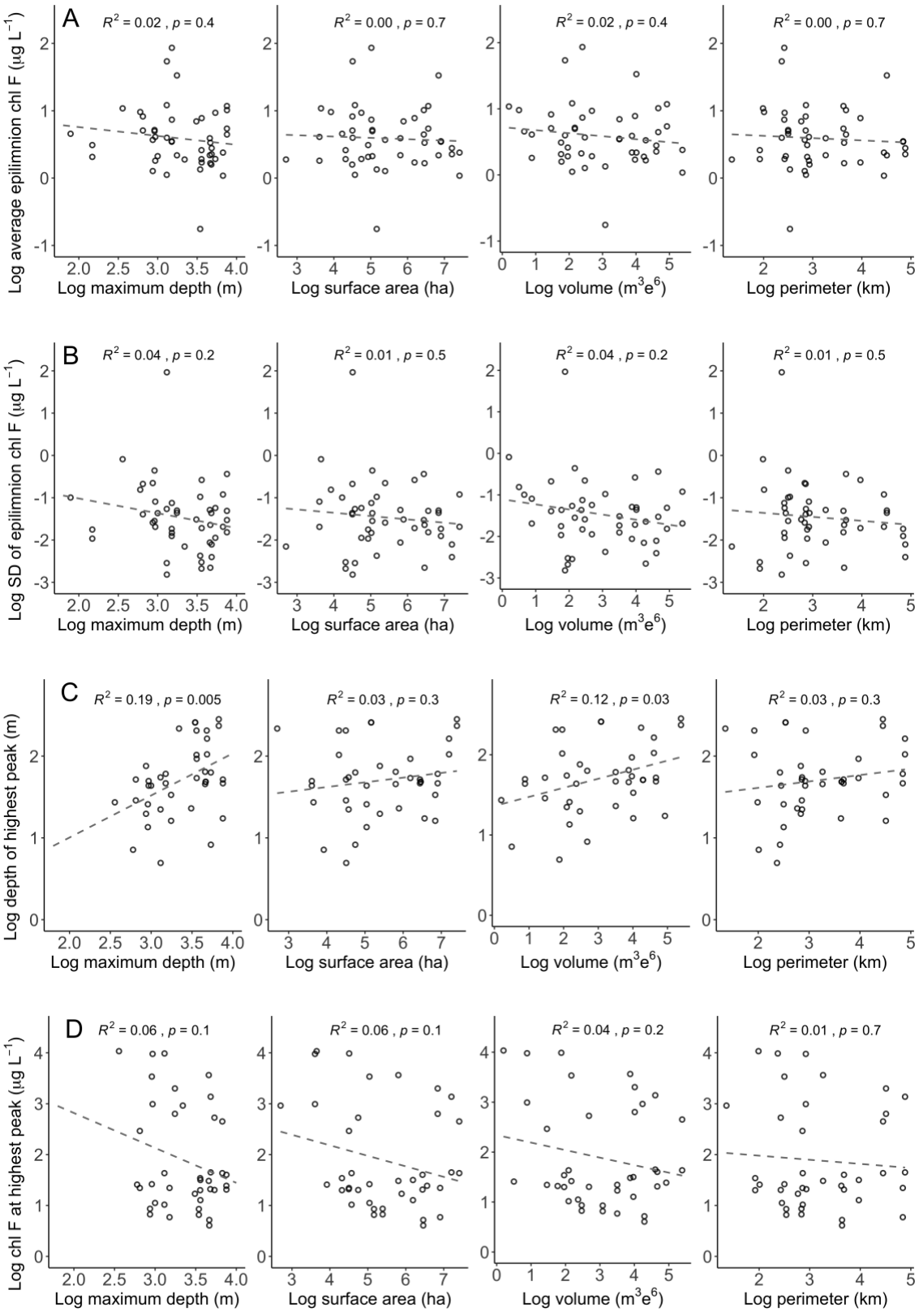


Figure 1. Mean epilimnion zone chlorophyll (chl F) [μg L^{-1}] **(A)**, standard deviation (SD) of epilimnion zone chlorophyll (chl F) **(B)**, maximum chlorophyll (chl F) at the highest peak **(C)**, peak depth [m] **(D)** and width of the highest peak [m] **(E)** as a function of diffuse light attenuation [K_d m^{-1}], dissolved organic carbon [DOC mg L^{-1}] and total phosphorus [TP μg L^{-1}] for lakes sampled in 2020 and 2021.

Relationships between lake morphometric properties and lake/peak characteristics.



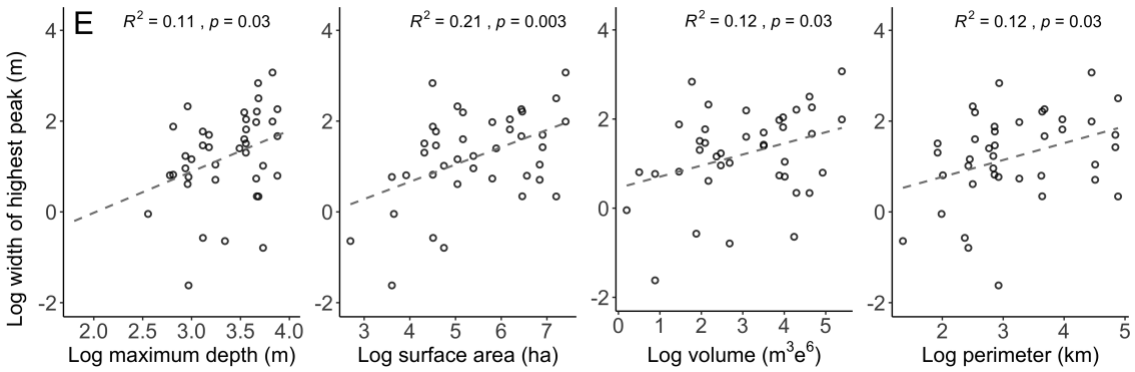


Figure 2. Mean epilimnion zone chlorophyll (chl F) [μg L^{-1}] **(A)**, standard deviation (SD) of epilimnion zone chlorophyll (chl F) **(B)**, maximum chlorophyll (chl F) at the highest peak **(C)**, peak depth [m] **(D)** and width of the highest peak [m] **(E)** as a function of maximum lake depth [m], lake surface area [ha], lake volume [m^3e^6] and lake perimeter [km] for lakes sampled in 2020 and 2021. Note that lake volume and perimeter were not included my study.