Improvement of field fluorometry estimates of chlorophyll a concentration in a cyanobacteria-rich eutrophic lake

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Abstract

Instrumented buoys are used to monitor water quality, yet there remains a need to evaluate whether in vivo fluorometric measures of chlorophyll a (Chl a) produce accurate estimates of phytoplankton abundance. Here, 6 years (2014–2019) of in vitro measurements of Chl a by spectrophotometry were compared with coeval estimates from buoy-based fluorescence measurements in eutrophic Buffalo Pound Lake, Saskatchewan, Canada. Analysis revealed that fluorometric and in vitro estimates of Chl a differed both in terms of absolute concentration and patterns of relative change through time. Three models were developed to improve agreement between metrics of Chl a concentration, including two based on Chl a and phycocyanin (PC) fluorescence and one based on multiple linear regressions with measured environmental conditions. All models were examined in terms of two performance metrics: accuracy (lowest error) and reliability (% fit within confidence intervals). The model based on PC fluorescence was most accurate (error = 35%), whereas that using environmental factors was most reliable (89% within 3σ of mean). Models were also evaluated on their ability to produce spatial maps of Chl a using remotely sensed imagery. Here, newly developed models significantly improved system performance with a 30% decrease in Chl a errors and a twofold increase in the range of reconstructed Chl a values. Superiority of the PC model likely reflected high cyanobacterial abundance, as well as the excitation–emission wavelength configuration of fluorometers. Our findings suggest that a PC fluorometer, used alone or in combination with environmental measurements, performs better than a single-excitation-band Chl a fluorometer in estimating Chl a content in highly eutrophic waters.

Several methods exist to measure Chlorophyll a (Chl a) concentrations as an indicator of harmful algal blooms (HABs), ranging from precise laboratory techniques (in vitro, chl_a hereafter) such as high-performance liquid chromatography (HPLC), spectrophotometry (Lorenzen 1967), and fluorometry (Pinckney et al. 1994), to very fast field methods including in vivo fluorometry (Bittig et al. 2019), in vivo spectrophotometry (Davis et al. 1997; Roesler and Barnard 2013), and remote sensing (Pahleven et al. 2020). Although accurate, laboratory methods are less suitable for real-time monitoring or long-term observations due to logistic and economic constraints (Gregor and Maršálek 2004). Given this limitation, as well as the current absence of daily high-spatial-resolution satellite observations for small inland waters (Haig et al. 2022), in vivo fluorometry has been promoted as an important option for real-time monitoring of HABs in small water bodies (Richardson et al. 2010; Poxleitner et al. 2016; Silva et al. 2016; Wang et al. 2016; Karpowicz and Ejsmont-Karabin 2017). However, to date, few studies have critically evaluated the capabilities or limitations of buoy-based instrument platforms in estimating changes in freshwater Chl a concentrations (Bertone et al. 2018; Boss et al. 2018; Chaffin et al. 2018).

In vivo fluorometric Chl a measurements are obtained by sensors that are usually mounted on buoys and illuminate a known volume of water with an excitation irradiance, usually blue wavelengths (Roesler et al. 2017). This light stimulates the Chl a within phytoplankton to fluoresce at red wavelengths, which is quantified as a relative fluorescence unit before being converted to Chl a concentration as:

$$\text{Chl} = (F - F_0)/\Phi_e \int_{400}^{750} E(\lambda) \times a_{\text{Chl}}(\lambda) d\lambda.$$

(1)

Here, F is the intensity of measured fluorescence (in vivo fluorescence hereafter), $F_0$ is background fluorescence due to

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humic and other nonchlorophyllous constituents that fluo-
resce 400–750 nm, \( E \) is available spectral scalar irradiance
(\( \mu \text{mol photons m}^{-2} \text{ s}^{-1} \text{ nm}^{-1} \)), \( a'_{\text{Chl}} \) is spectral Chl \( a \)-specific
absorption coefficient (\( \text{m}^2 \text{ mg}^{-1} \)), and \( \Phi_F \) is fluorescence quantum yield (photons fluoresced/photons absorbed). Assuming
all fluoresced light is detected and \( E \) is known, only \( a'_{\text{Chl}} \) and
\( \Phi_F \) need to be known to estimate Chl \( a \) levels (Roesler et al. 2017). While \( a'_{\text{Chl}} \) and \( \Phi_F \) are known and constant for extracted Chl \( a \), values may vary for different phytoplankton,
depending on factors such as cell packaging, pigment quota,
accessory pigment composition, light history, growth phase,
and phytoplankton species composition (Morel and Bri-
caud 1981; Falkowski and Kolber 1995). Assuming \( a'_{\text{Chl}} \) and \( \Phi_F \)
are constant and that the relationship between \( F \) and Chl \( a \) is
linear, fluorometer manufacturers calibrate \( F \) with solutions
with known pigment content to estimate Chl \( a \) levels. We
refer to this in vivo Chl \( a \) concentration as factory-calibrated
Chl \( a \) (chl\text{FC}), hereafter.

Studies using multi-band fluorescence probes report a high
 correlation \( (r = 0.97, p < 0.05) \) between chl\text{FC} and spectropho-
tometric estimates (chl\text{M}) in productive rivers and reservoirs
(Gregor and Marsálek 2004; Catherine et al. 2012). Despite
strong linear relations between Chl \( a \) estimators, chl\text{FC} under-
estimates chl\text{M} by up to 50 \( \mu \text{g L}^{-1} \) and cannot retrieve Chl
\( a \) values above 50 \( \mu \text{g L}^{-1} \), especially when colonial cyanobacteria are present (Gregor and Marsálek 2004). Research
employing different fluorometers (Kaylor et al. 2018), analyti-
cal methods (e.g., HPLC vs. fluorometry), and phytoplankton
assemblages have produced evidence of similar biases
(Ostrowska et al. 2015; Wang et al. 2016).

Variations in phytoplankton type and growth phase, com-
munity composition, and water characteristics can also cause

\[ \text{chlFC} \]

\[ \text{chlM} \]

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**Fig. 1.** The map and location of the Buffalo Pound Lake (BPL), Saskatchewan, Canada. (a) Position of the Qu’Appelle River watershed within Canada. (b) Location of BPL within Qu’Appelle River watershed. (c) A Landsat-8 image of BPL, overlaid by a bathymetric map. The extent indicator represents the sampling area in the lake (d) a zoom view of the sampling area in BPL overlaid by sampling stations.
Table 1. Buffalo Pound Lake characteristics and water quality parameters in Sta. 1, averaged on late May to early September in 2014–2019, except pCO₂ from Finlay et al. (2019).

| Characteristics | Values |
|-----------------|--------|
| Altitude (m)    | 501    |
| Mean depth (m)  | 3      |
| Maximal depth (m) | 5.8  |
| Length (km)     | 35     |
| Average width (km) | 0.9  |
| Surface (km²)   | 30     |
| Volume (m³)     | 89 × 10⁶ |

| Water quality | Mean values |
|---------------|-------------|
| Water temperature (°C) | 19 |
| Conductivity (μS cm⁻¹) | 730 |
| DO (μg L⁻¹) | 9.5 |
| pH | 8.5 |
| Turbidity (NTU) | 9.1 |
| Total P (μg L⁻¹) | 86 |
| Chl a (μg L⁻¹) | 31 |
| pCO₂ (μatm) | 683 |

Substantial deviation of factory-calibrated Chl a and Φ values from those independently measured, resulting in poor Chl a retrieval (Choo et al. 2018; Bertone et al. 2019; Garrido et al. 2019). For instance, although in vivo fluorometry accurately assessed relative taxonomic composition in marine assemblages, phytoplankton biomass was overestimated by ~1.2–3.4-fold (Richardson et al. 2010). Such overestimation becomes crucial when Chl a data are used to train and test models using remotely sensed reflectance to estimate bloom abundance and cyanobacterial prevalence. As a result, many studies calibrate fluorescence measurements with coeval spectrophotometric or chromatographic measures of in situ Chl a, instead of using factory-calibrated Chl a (Mueller et al. 2003; Roesler et al. 2017; Bertone et al. 2018).

Chl a estimates based on in vivo fluorometry are often less accurate than those derived from in vitro methods. Apart from biofouling, which can be reduced by regular sensor maintenance (Davis et al. 1997; Manov et al. 2004), in vivo fluorescence can be reduced by nonphotochemical quenching (NPQ) at high irradiance (Huot and Babin 2010). Although various approaches have been proposed to compensate this signal contamination (Xing et al. 2018; Carberry et al. 2019; Scott et al. 2020), none of them completely remove the NPQ effect even when additional coeval water parameters are measured (Boss and Haëntjens 2016; Wojtasiewicz et al. 2018). In addition, the fluorescence of colored dissolved organic matter (CDOM) may interfere with that of Chl a, resulting in the overestimation of Chl a (Gregor and Maršálek 2004; Xing et al. 2017). Finally, water column turbidity due to abundant phytoplankton and inorganic particles can interfere with in vivo fluorometry (Choo et al. 2019). In contrast, fluorometric estimates of phytoplankton abundance in cyanobacteria-rich systems may be improved (e.g., up to R² = 0.87) through the use of in vivo phycocyanin (PC) fluorometers that use an orange excitation wavelength instead of blue light to quantify pigment concentrations (Seppälä et al. 2007; Rome et al. 2021). Despite its promise, most studies of field fluorometry are limited to laboratory-cultured populations or oligotrophic/mesotrophic waters with relatively low Chl a content, such as open oceans and coastal marine areas (Roesler 2016; Roesler et al. 2017; Wojtasiewicz et al. 2018).

In this paper, estimates of in vivo Chl a were compared to in vitro pigment measures in a shallow eutrophic polymeric lake subject to long-term monitoring by limnological

Table 2. Details of data used in this study.

| Parameter          | Name    | Station | Measurement method               | Available years | Sampling cycle | Depth (m) |
|--------------------|---------|---------|----------------------------------|-----------------|----------------|-----------|
| Chl a fluorescence | φchl   | 1       | Field fluorometry                | 2014–2019       | 10 min         | 0.8       |
| PC fluorescence    | φPC    | 1       | Field fluorometry                | 2014–2019       | 10 min         | 0.8       |
| PC fluorescence    | φPC_deep | 1     | Field fluorometry                | 2014–2018       | 10 min         | 2.8       |
| Chl a concentration| chlC   | 1       | Factory-calibration of field fluorometry | 2014–2019   | 10 min         | 0.8       |
| Chl a concentration| chlM   | 1       | Lab spectrophotometry            | 2017–2019       | Once/week      | 0.8       |
| Env. parameters    | —      | 1       | Field sensors                    | 2014–2019       | 10 min         | 0.8, 2.8  |
| RGB photos         | —      | 1       | Deployed camera                  | 2014–2019       | 30 min         | 0         |
| Phytoplankton biomass and taxonomy | — | 1   | Visual microscopy               | 2017–2018       | –Once/week     | 0.1       |
| Chl a concentration| chlM   | 2       | Lab spectrophotometry            | 2014–2018       | Once/week      | 3*        |
| Phytoplankton biomass and taxonomy | — | 2   | Visual microscopy               | 2014–2018       | –Once/week     | 3*        |
| Chl a concentration| chlM   | 3       | Lab HPLC                        | 2014–2019       | Twice/month    | 0–1 integrated |
| Chl a concentration| chlM   | 4       | Lab spectrophotometry            | 2015–2019       | Once/month     | 1         |

Env., environmental.

*Sample from drinking water intake which will include water derived from the entire water column.
sampling and remote-sensing technologies. Here, we take advantage of the rich dataset of lake parameters to apply machine-learning (ML) models to improve Chl a estimates. By assessing field measurements of Chl a, we seek to improve estimates of planktonic Chl a concentration needed to train and test remote-sensing algorithms. This study aims to: (i) identify potential flaws and issues associated with the use of factory-calibrated Chl a (chlFC); (ii) develop and assess three locally tuned models, namely, chlFC, chlFP, and chlMLP based on Chl a fluorescence (Fchl), PC fluorescence (FPC), and multiple linear regression (MLR) of environmental algae-derived factors, to approximate Chl a concentration; and (iii) evaluate the performance of newly proposed models in recovering accurate Chl a estimates in two real-world applications; monitoring Chl a time series and mapping Chl a by remote sensing. We show that, at least for a cyanobacteria-dominated eutrophic lake, Chl a was most accurately estimated when based on models using PC fluorescence or environmental predictors.

### Materials and procedures

#### Study site

Buffalo Pound Lake (BPL) is a long (~ 29 km), narrow (<1 km), shallow (<4 m) lake located in the Qu’Appelle River watershed, Saskatchewan, Canada (Fig. 1 and Table 1). Currently, the basin exhibits highly eutrophic waters, with summer blooms during June–September and peak surface populations of phytoplankton during July–August (Kehoe et al. 2019). Continuous monitoring for over 25 years shows that cyanobacteria are the predominant taxon during summer; however, other phytoplankton species may be abundant at the same time (Hammer 1983; Vogt et al. 2018; Swarbrick et al. 2019).

Several attributes make BPL suitable for the comparison of fluorometric, optical, and remotely sensed estimates of Chl a. First, the lake is an important freshwater resource, as it serves as drinking water reservoir for one-quarter of the provincial population, including nearby cities of Regina and Moose Jaw. Second, the lake has multi-decadal records of monitoring, including Chl a measurement and estimates of HAB abundance (Kehoe et al. 2015; Finlay et al. 2019). Third, lake size, elongate shape, and landscape orientation parallel to the direction of prevailing winds mean that the water column is polymeric, experiencing frequent mixing periods and limited vertical stratification (Dröscher et al. 2008). Finally, the lake has had an instrumented buoy present at a standard location annually from May to September (starting in 2014) that is equipped with in vivo sensors for both Chl a and PC.

### Data

Pigment data originated from different sources collected concomitantly during 2014–2019. Fchl, FPC, and chlFP were collected from an instrument platform buoyed in the center of BPL (Sta. 1 in Fig. 1). In contrast, in vitro Chl a concentration was measured from samples at four stations obtained at regular intervals. Moreover, environmental parameters were recorded by buoy sensors and were supplemented by sampling the water column adjacent to the buoy (see below). Table 2 lists the details of in situ measurements.

Two YSI-6600 multiprobes (YSI, Yellow Springs, Ohio) were deployed on the buoy at 0.8- and 2.8-m depth to measure fluorescence at Sta. 1. The shallow multiprobe recorded Fchl and FPC via YSI 6025 and YSI 6131 sensors, respectively, while the deep probe gauged only PC fluorescence (FPCdeep). At both depths, the PC fluorometers used an excitation wavelength of 590 ± 20 nm and measured fluorescence at 640 ± 40 nm, whereas the Chl a fluorometer used excitation and emission wavelength ranges of 470 ± 20 and 640 ± 40 nm, respectively. To eliminate fluorometer drift and convert relative fluorescence outputs to units of Chl a concentration (µg L⁻¹), a two-point calibration process (beginning and middle of sampling
season) was used for the Chl a fluorometer based on a standard solution of rhodamine provided by the sensor manufacturer. As a result, buoy multiprobes provided in vivo estimates of Chl a (chlFC), in addition to Chl a fluorescence (Fchl).

Samples for estimation of in vitro Chl a were collected from four locations; at the exact location of the buoy (Sta. 1), at ~100 m adjacent to the buoy (Stas. 2 and 4) and the site of a 28-yr monitoring program (Haig et al. 2020) ~2 km distant.

**Fig. 3.** Factory-calibrated Chl a concentration from YSI Chl a fluorometer (chlFC, green line), and in vitro Chl a concentration (chlM, circles), shows that chlFC significantly underestimates Chl a concentration in BPL. Stations are color-coded 1–4.

**Fig. 4.** Scatter plots of the fluorescence of (a) Chl a and (b) PC, vs. in vitro Chl a concentration (chlM). The blue and red colors represent the observations and equations when the ratio of cyanobacteria biomass to total phytoplankton biomass is larger and smaller than 0.5, respectively. The black color equations were developed for all observations regardless of algae or cyanobacteria are dominant. F and B stands for fluorescence and biomass, respectively.
(Station 3). Samples from Stas. 1 and 4 were collected from 0.8- to 1-m depth on Whatman GF/F filters, frozen, and later extracted following Wintermans and DeMots (1965) in cold 95% ethanol for 24 h before analysis using a ultraviolet–visible spectrophotometer (Shimadzu UV-1601-PC). Sta. 2 samples are from the water treatment plant intake which is at approximately 3-m depth, but which collects water from the entire water column. Samples from the intake were filtered onto a 0.45-μm pore membrane filter, extracted in 90% acetone and analyzed via spectrophotometry (APHA 2012). At Sta. 3, phytoplankton were collected on GF/C filters (nominal pore size 1.2 μm) following Swarbrick et al. (2019). Briefly, surface water (~ 0.5-m depth) and depth-integrated samples were filtered through GF/C filters and frozen (~ 10°C) until analysis for Chl a (μg L⁻¹) by standard trichromatic assays (Jeffrey and Humphrey 1975) and biomarker pigments (nmoles pigment L⁻¹) by HPLC (Leavitt and Hodgson 2001). Carotenoids, chlorophylls, and their derivatives were isolated and quantified using a Hewlett Packard model 1100 HPLC system calibrated with authentic standards.

Environmental data included estimates of phytoplankton species density (cells or colonies mL⁻¹) and biomass (μg mL⁻¹) collected in Stas. 1 and 2 and enumerated following Findlay and Kling (2001). In addition, turbidity (YSI 6560, NexSens T-Node FR), wind speed and direction (Vaisala WXT536), flux of photosynthetically active radiation (PAR) in the surface layer and water column (LiCor LI-192), and dissolved CO₂ (Vaisala GMP222) and O₂ (YSI 6150 ROX) concentrations were recorded by the buoy sensors at Sta. 1. Furthermore, two cameras, one mounted on the buoy and the other on the shore facing toward the buoy, regularly took RGB photographs of the water surface to detect surface-bloom events.

Data processing procedure

Coeval estimates of in vivo and in vitro Chl a were processed separately (Fig. 2). Samples from all stations were paired by collection date. We then developed and validated Chl a retrieval models based on paired measurements.

Data inspection and correction of fluorescence data

Raw fluorescence data were adjusted for effects of biofouling, sensor input noise (high impulse values), and NPQ before fluorescence measurements were converted to Chl a concentrations (Supporting Information Fig. S1). Potential biofouling was identified by comparing surface- and deep-sensor values with estimates of phytoplankton density and photographic evidence of surface blooms. Affected values were removed from further analysis, but accounted for only ~1% of Fchl and FPC observations, usually toward the end of the sampling season. As seen elsewhere (Sackmann et al. 2008; Wojtasiewicz et al. 2018), fluorescence of both PC and Chl a declined ~10% during most days as PAR increased (Supporting Information Fig. S1). However, as such a pattern may also reflect diel vertical movements of phytoplankton, no correction was made for the decline. Instead, NPQ effects were reduced by averaging fluorescence data over 24 h (Carberry et al. 2019). This procedure produced similar values to a second protocol, in which daytime extremes were replaced by night-time values (Sackmann et al. 2008; Roesler 2016), while also preserving daily variation in water column Chl a. Finally, high impulse values were defined by fluorescence observations that surpass 3σ of daily averaged fluorescence and were removed from the data set.

Model development

For data training, we paired Fchl and FPC observations with concomitant chlM measurements from Stas. 1 to 4. The comparison of coeval chlM measurements at the four sites revealed few significant differences. Consequently, Chl a values from all stations were used for model development, with the exception of periods in which surface scum were recorded by the buoy. For these periods, we only used the samples in Sta. 1 (the exact location of the fluorometers). To compensate for differences in sampling depth, location, and methods between chlM measurements, all in vitro values were averaged by day to produce 155 pairs of coeval Fchl/FPC and chlM measurements over 6 years of observations. We also extracted and tested pairs of Fchl / FPC and chlM sampled using other strategies (only one location; only adjacent; unaveraged); however, these protocols reduced the number of paired samples to one-half to two-thirds (to 56–77) and did not improve the accuracy of models, so were discontinued.

Model calibration and validation were carried out using a fivefold cross-validation approach (Hawkins et al. 2003), using 80% of pairs (n = 124) as training set and the remaining 20% (n = 31) as a test set. Moreover, because Fchl, FPC, and chlM measurements exhibited log-normal distributions, all data were subject to a log₁₀-transformation to achieve normal distribution of residuals. Finally, linear regression models were developed to predict chlM from Fchl and chlM from Fchl and FPC, respectively.

MLR models were developed to retrieve Chl a from environmental parameters independent of those inferred from fluorometric measurements. Predictors included turbidity, dissolved CO₂ concentration, and normalized PAR at 0.8-m

| Parameters | Coefficient | Sum of squares | F-test | p value |
|------------|-------------|----------------|--------|---------|
| Turbidity  | 0.36        | 12.08          | 262.14 | 9 × 10⁻³⁵ |
| nPARwater | −0.38       | 2.99           | 64.94  | 2 × 10⁻¹³ |
| CO₂       | −0.26       | 1.18           | 25.65  | 10⁻⁶    |
| Month      | 1.74        | 1.82           | 39.44  | 3 × 10⁻⁹ |
| Residual   | —           | 3.9            | —      | —       |

Table 3. Multiple linear regression (MLR) model to predict chlM from measured environmental parameters using forward selection.
depth, parameters which were all correlated individually with Chl a (Supporting Information Fig. S2). We defined normalized PAR (nPARwater) (Eq. 2), as the fraction of incident PAR at the lake surface that was not absorbed or scattered when transmitted through the water column. For CO2, we used raw signal data, assuming it to have a good correlation with actual CO2 concentration (Wiik et al. 2018; Finlay et al. 2019). We also input the month of observation to account for seasonality and excluded other environmental parameters that did not have a significant individual correlations with Chl a.

\[
\text{nPAR}_{\text{water}} = \frac{\text{PAR}_{\text{water}}}{\text{PAR}_{\text{air}}}.
\]

**Model assessment**

Predicted Chl a (chlP) was assessed on the basis on accuracy and reliability. Accuracy was defined as agreement between chlP and chlM (closeness of fit), while reliability measured the probability that chlP fell into a confidence interval around chlM. Reliability also took into account variation in in vitro estimates of Chl a concentration arising from differences in laboratory protocols.

![Figure 5](image_url)
Table 4. Reliability, estimated as the proportion of retrieved Chl a values that within three confidence intervals (± σ, ± 2σ, and ± 3σ) of the mean of in vitro Chl a concentration, for each of four fluorescence models.

|            | chlFC | chlP | chlchlor | chlPC |
|------------|-------|------|----------|-------|
| ± σ        | 0.11  | 0.2  | 0.54     | 0.46  |
| ± 2σ       | 0.11  | 0.49 | 0.68     | 0.66  |
| ± 3σ       | 0.14  | 0.6  | 0.77     | 0.89  |

For estimation of prediction accuracy, we examine both linear and log$_{10}$-transformed metrics following Seegers et al. (2018). Performance metrics included root mean square error (RMSE), root mean square logarithmic error (RMSLE), median percentage error (MAPE), bias (as log$_{10}$-transformed residuals), and mean absolute error in log$_{10}$-space (MALE):

\[
\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{N}(P_i - M_i)^2}{n}},
\]

\[
\text{RMSLE} = \sqrt{\frac{\sum_{i=1}^{N}(\log_{10}(P_i) - \log_{10}(M_i))^2}{n}}^{1/2},
\]

\[
\text{MAPE} = 100 \times \text{median}([|P_i - M_i|/M_i]),
\]

\[
\text{Bias} = 10^y z = \frac{\sum_{i=1}^{n}(\log_{10}(P_i) - \log_{10}(M_i))/n}{},
\]

\[
\text{MALE} = 10^y y = \frac{\sum_{i=1}^{n}|\log_{10}(P_i) - \log_{10}(M_i)|/n}{}
\]

where $P_i$ and $M_i$ stand for predicted and in vitro measured Chl a, respectively. Using these metrics, a Bias of 1.5 implies that predicted Chl a are, on average, 50% larger than those measured (Bias = 1 is ideal), whereas a MALE of 1.5 indicates a relative measurement error of 50% (Seegers et al. 2018).

Reliability was computed as the percentage of predicted Chl a that fit into a confidence interval derived from in vitro measurements. Given considerable variability in coeval Chl a values from different laboratories, and that the true value of Chl a was unknown, we assumed that log$_{10}$-transformed Chl a was a random variable with a normal distribution whose mean and standard deviation are estimated by coeval in vitro Chl a measurements. Given these assumptions, the reliability of predicted Chl a was measured as the probability that predicted values fell into the range of $c = \mu \pm k\sigma$, where $\mu$ and $\sigma$ are mean and standard deviation, respectively; $k = 1, 2, 3$ and $c$ is a confidence interval. Again, all calculations for reliability were conducted using log$_{10}$-transformed Chl a data.

Finally, to assess the effect of model selection on the ability of remote-sensing techniques to estimate Chl a, we downloaded and processed Sentinel-2 images of BPL corresponding to sampling dates during 2017–2019, the years following Sentinel-2 insertion into orbit. We then paired coeval, co-located Chl a and reflectance observations for training and testing a support vector regression (SVR) model to estimate Chl a. Details of remote-sensing data and processing are provided by Chegoonian et al. (2021) and elaborated briefly in Supporting Information Appendix S1.

Assessment

Models accuracy

Comparison of factory-calibrated Chl a (chlFC) with in vitro chlM measurements showed that fluorometric estimates of Chl a from the buoy substantially underestimated values derived from laboratory analyses in all years (Fig. 3). The degree of underestimation was not affected by correction for biofouling or NPQ effects, even though both are distinguishable from effects of inherent model inaccuracy due to their unique temporal patterns (Supporting Information Fig. S1). In general, agreement between chlFC and in vitro measurements was best early in each year and became markedly worse after June in each year through the period of high summer biomass, often improving again in early fall.

Although both chlP and chlPC measurements were correlated ($p < 0.01$) to in vitro Chl a, predictions based on PC fluorescence explained a much greater proportion of variation in chlM than did those based in chlFC (Fig. 4). Specifically, chlPC models were highly correlated with variability in chlM ($R^2 = 0.87$), whereas models with sensor-derived Chl a fluorescence values explained only ~34% of variance in chlM over a nearly 200 μg L$^{-1}$ range. In contrast, MLR models using forward selection explained 82% of variance in measured chlM values. Significant predictors included turbidity, normalized PAR, dissolved CO₂, and sampling month (Table 3), with about half of sums of squares related to turbidity alone.

Comparison of modeled (chlFC, chlP, chlPC, chlPC) and measured chlM showed predictions using chlFC were more biased than were values derived from other fluorometric models (Fig. 5). On average, chlPC was ~2.5-fold lower than chlM resulting in a biased estimator (Bias = 0.42), particularly at Chl a < 20 μg L$^{-1}$ (Bias = 0.74). Furthermore, the relationship between variables was nonlinear above 20 μg L$^{-1}$ (Bias = 0.33), similar to patterns previously reported for a FluoroProbe fluorometer for Chl a values > 60 μg L$^{-1}$ (Gregor and Maršálek 2004). Moreover, only a weak positive correlation ($r = 0.55$, $n = 155$) was found between log$_{10}$-transformed chlFC and chlM, suggesting that chlFC values should be treated with caution even in analysis of relative proportions. The remaining models were unbiased (Bias ~ 1), although they differed in terms of prediction accuracy.

Although chlP improved Chl a retrieval compared to the chlFC model, it still predicted in situ Chl a with only an average relative error of ~90% of true values (MALE = 1.9). Instead, errors declined ~55% when models employed the PC fluorometer to estimate water column Chl a (Fig. 5). For example, all error metrics were reduced by ~50% for the chlPC model compared to that based on chlP, with improvement...
Fig. 6. Visualization of the reliability of \( \text{chl}_{\text{ML}} \), measured as the number of predicted Chl \( \alpha \) that fall into the confidence zone, computed by the standard deviation of coincident \( \text{chl}_M \). Values are log\(_{10}\)-transformed, and the numbers in the legend represent the station from which \( \text{chl}_M \) is acquired.

Fig. 7. Time series of \( \text{chl}^{\text{FC}} \), \( \text{chl}^{\text{ML}} \), and \( \text{chl}^{\text{PC}} \) to retrieve continuous long-term Chl \( \alpha \) for 2016–2019 in Sta. 1 in BPL. Coincident in vitro Chl \( \alpha \) in Stas. 1–4 (\( \text{chl}_M \)) is used to assess the validity of retrieval.
evident throughout the range of observed values even though chlPC underestimated Chl a when concentrations exceeded 120 μg L⁻¹ (Fig. 5b,c). Similarly, the chlML model (Fig. 5d) significantly (p < 0.01) outperformed both Chl a-based fluorescence models (chlFC, chlchlP) in all metrics. In fact, a model with turbidity as the sole predictor (Supporting Information Fig. S3) still outperformed chlchlP (MALE = 1.66 vs. MALE = 1.9). In general, chlML exhibited similar performance relative to that of chlPC (MALE = 1.46 vs. MALE = 1.35) and did not underestimate extremely high Chl a values. Instead, the chlML model tended to overestimate Chl a when chlM was < 10 μg L⁻¹.

Models reliability
Analysis of model reliability revealed that predictions from the chlPC model fell within ±2σ distance (95% confidence interval of mean chlM on 66% (23/35) of observations with ~46% and ~89% falling within ±σ and ±3σ, respectively (Table 4). Overall, reliability of chlPC and chlML models outperformed those based on Chl a fluorescence (chlFC, chlchlP) irrespective of the confidence interval selected (Table 4). Compared to other models, chlPC exhibited the highest accuracy (Fig. 5), whereas chlML outperforms it in terms of reliability with 89% of observations within ±3σ. For demonstration purposes, a visual presentation of reliability for chlML with associated chlM measurements is depicted in Fig. 6.

Models performance in retrieving Chl a time series
Estimation of in situ Chl a using continuous buoy measurements at Sta. 1 and the proposed models revealed common seasonal patterns of Chl a concentration, but high variability
in absolute concentrations among years during 2016–2019 (Fig. 7). Overall, phytoplankton phenology was marked by the onset of elevated concentrations around mid-July, with more intense blooms (100 μg Chl a L$^{-1}$) in mid-August, and a slow decline in Chl a during September and October. For most models, peak water column Chl a occurred earlier and was lower in 2018, whereas other summers were similar to each other. Visual inspection suggested that peak in vitro Chl a values were more poorly approximated by models during 2017 than in other years.

Agreement with in vitro Chl a concentrations was much better for models based on PC fluorescence (chl$^{\text{PC}}$) and MLR (chl$^{\text{ML}}$) than for those based on Chl a fluorescence (chl$^{\text{FL}}$) (Fig. 7). For example, chl$^{\text{FL}}$ did not accurately capture values above ~70 μg Chl a L$^{-1}$, whereas chl$^{\text{PC}}$ estimated Chl a well to about 120 μg L$^{-1}$ and chl$^{\text{ML}}$ retrieved values up to 150 μg L$^{-1}$. Although chl$^{\text{PC}}$ and chl$^{\text{ML}}$ often performed equally well in long-term monitoring of Chl a, in vivo Chl a tended to underestimate in vitro values in some late-summer instances (e.g., mid-August of 2016 and 2017). Visual analysis of photographs from those dates showed that fluorometric models underestimated Chl a when severe surface blooms were evident (Supporting Information Fig. S4).

Models importance in retrieving remote Chl a

To examine the effect of the proposed models on estimation of remote-sensing Chl a concentration, we populated a recently developed remote-sensing Chl a algorithm, SVR (Chegoonian et al., 2021), with chlorophyll values estimated from chl$^{\text{PC}}$, chl$^{\text{FL}}$, chl$^{\text{PC}}$, and chl$^{\text{ML}}$ models (Supporting Information Appendix S1). These trained SVR models were then validated using unseen in vitro Chl a. When SVR models were applied to a common Sentinel-2 image of BPL, substantial differences were noted in the ability of remote sensing to capture spatial variation in Chl a concentrations (Fig. 8). In particular, the model trained by chl$^{\text{PC}}$ values showed poor relative and absolute performance (Slope = 0.1, MALE = 2.01), whereas use of chl$^{\text{FL}}$ increased model strength (Slope = 0.83) but not accuracy (MALE = 2.12). In contrast, both chl$^{\text{PC}}$ and chl$^{\text{ML}}$ significantly improved estimates of remotely sensed Chl a by increasing accuracy 30% (lowering MALE) compared to chl$^{\text{FL}}$. Furthermore, compared to chl$^{\text{PC}}$, heatmaps of Chl a based on other models detected consistent spatial patterns, although they differed in the maximum Chl a returned (Fig. 8).

Discussion

Fluorescence estimates of Chl a concentrations from instrumented buoys have been used widely to study the characteristics of surface blooms (Seppälä et al. 2007; Groetsch et al. 2014), temporal variability in water productivity (Serôdio et al. 2001; Frankenbach et al. 2020), importance of cyanobacteria in phytoplankton (Catherine et al. 2012; Zamyadi et al. 2016), and landscape patterns of water quality change (Hamilton et al. 2015; Boss et al. 2018). Often it is assumed that factory-presets of buoy fluorometers allow accurate estimation of absolute or relative Chl a concentrations over a wide range of lake production. Here we find that on-board fluorometry lacks both accuracy and reliability to estimate in situ Chl a relative to predictions based on models calibrated with 6 years of in vitro monitoring (Fig. 5; Table 4), as seen elsewhere (Gregor and Maršílek 2004; Catherine et al. 2012; Roesler et al. 2017; Piermattei et al. 2018). Unexpectedly, models based on fluorescence of PC were more accurate than those derived from Chl a fluorescence, or MLR models based on non-pigmented parameters (turbidity, CO2 concentration, transparency, and month). Although all models improved recovery of peak Chl a values by buoys, PC and MLR models were more reliable than those based on Chl a. When applied to remotely sensed reflectance, PC and MLR models also captured a wider range of spatial variability than did other approaches (Fig. 8), suggesting that they are more suitable to map transient blooms of cyanobacteria in highly eutrophic lakes.

Considerations to employ field fluorometers to retrieve Chl a

Weak correspondence between factory-calibrated fluorometric Chl a (chl$^{\text{FC}}$) and ground-truthed in situ values (chl$^{\text{FL}}$) have been reported in other studies and by instrument manufacturers (Gregor and Maršílek 2004; Catherine et al. 2012; even though chl$^{\text{FC}}$ can be reliable in limited applications (Ferreira et al. 2012; Chaffin et al. 2018) where $a^{\text{Chl}}_0$ and $\Phi_F$ (see Eq. 1) are constant and similar to manufacturer gains (Roesler et al. 2017). While in vivo fluorometric methods are not expected to replace in vitro methods in terms of absolute accuracy, they should maintain a reasonable relative accuracy to be useful in ecosystem monitoring applications. Instead, our analyses in a eutrophic lake demonstrated that chl$^{\text{FC}}$ does not capture even relative changes in laboratory-determined Chl a time series (Figs. 3, 5a), in contrast to other reports of high correlation between chl$^{\text{FC}}$ and chl$^{\text{FL}}$ (Catherine et al. 2012). This difference may relate to the type of fluorometer employed (Roesler et al. 2017), their excitation and emission characteristics (Catherine et al. 2012), or the composition of phytoplankton in the lake (Escoffier et al. 2015).

Regardless of the source of disagreement, the low relative accuracy of chl$^{\text{FC}}$ suggests that this metric cannot be used routinely to evaluate spatial or temporal variation in phytoplankton abundance. Our findings suggest that accuracy of chl$^{\text{FC}}$ estimations declined at higher Chl a values, making this parameter particularly unsuitable for early warning detection of HABs without correction and calibration (Roesler et al. 2017). We suggest that chl$^{\text{FC}}$ values must be increased by at least 150% to achieve comparable means to those derived from modeled and in vitro determinations of Chl a. Similar
conclusions were reached by Roesler et al. (2017) who proposed a twofold modification for all fluorometric Chl a observations for different sensors (excitation 470 nm, emission 695 nm).

Unlike models using chlFC and chlFl, those based on fluorescence of PC (chlPC) were capable of accurately estimating in situ Chl a concentrations, both in terms of absolute amount and relative variation during series trends or spatial patterns. Although the superior performance of the PC sensor is partly dependent on both the instrument type and lake parameters, we expect that this conclusion may be generalized to other mesotrophic or eutrophic lakes with abundant colonial cyanobacteria, given the wide range of Chl a values observed in BPL (2–200 µg L\(^{-1}\)). However, these results should be treated with caution in systems dominated by CDOM or nonalgal particles as CDOM fluorescence may largely interfere with that of Chl a and PC, thereby leading to overestimation of phytoplankton (Stedmon and Markager 2005; Goldman et al. 2013; Xiaoling et al. 2019), while nonalgal particles can cause underestimation of phytoplankton by absorbing both excitation and emission lights (Brient et al. 2008). The importance of phytoplankton composition to the performance of the PC-based analysis has also been recognized in other cyanobacteria-rich waterbodies (Bowling et al. 2016), including the Baltic Sea (Seppälä et al. 2007) where differences between the accuracy of Chl- and PC-based fluorometric sensors (\(R^2 = 39\%\) and 76%, respectively) were similar to those recorded in the present study (\(R^2 = 34\%\) and 87%).

Improved information on the mechanisms regulating site-specific variation in the relation between in vivo and in vitro Chl a concentrations is essential for the upscaling of lake analyses to broader geographic landscapes using satellite imagery. Presently, Chl a retrieval using remote-sensing technologies relies mostly on ML algorithms that are trained and tested using in situ Chl a measurements (Pahlevan et al. 2020). The competency of these algorithms is highly dependent on the quality of in situ Chl a estimates and is often limited by the low data availability or temporal coherence of observations. We note herein that in vivo Chl a measures were usually improved after our correction and calibration process; however, there remained some circumstances in which corrected Chl a still underestimated in situ concentrations (Fig. 7). Specifically, corrected Chl a values underestimated in vitro Chl a when concentrations were > 100 µg L\(^{-1}\) and positively buoyant colonial cyanobacteria (e.g., *Microcystis*, *Anabaena*, *Aphanizomenon* spp.) are abundant in the surface waters (Hayes et al. 2019; Swarbrick et al. 2019). Such extreme surface blooms tend to occur in hypereutrophic waters, particularly during calm intervals which follow prolonged periods of strong winds and turbulent mixing (Huisman et al. 2005). Under these conditions, transmission of photons is reduced by high densities of cyanobacteria, leading to underestimates of fluorescence but not in vitro estimates of pigment concentration based on chemical extraction. When possible, training of remote-sensing algorithms should not use in vivo Chl a estimates from buoys collected during these surface bloom intervals to avoid bias in analysis of temporal and spatial variability.

Effectiveness of PC vs. Chl a fluorometers in retrieving Chl a

PC-based fluorescence models were both more accurate and more reliable than those developed using Chl a fluorometry with both extended range (up to 120 µg Chl a L\(^{-1}\)) and more linear relationships with in vitro Chl a at lower concentrations (Fig. 5). Although reported recently (Rome et al. 2021), these findings were unexpected because Chl a fluorometers are designed to be sensitive to the total biomass of Chl a from phytoplankton, including both algae and cyanobacteria, whereas PC-based fluorometers are expected to be sensitive mainly to cyanobacteria and secondarily to cryptophytes.

Several characteristics of our study may have predisposed PC models to outperform those based on Chl a. First, cyanobacteria were the predominant taxon in BPL from June to September in all years (Vogt et al. 2018; Hayes et al. 2019; Swarbrick et al. 2019). As seen in Seppälä et al. (2007), a strong linear relationship between PC fluorescence and Chl a concentration is expected in instances where cyanobacteria compose over 50% of community biomass (Fig. 4b). In BPL, cyanobacteria usually comprise 40–100% of total phytoplankton biomass after late June based on both direct taxonomic counts 2015–2018 (Supporting Information Fig. S5) and 25 years of HPLC analysis of in situ biomarker carotenoid pigments (Vogt et al. 2018; Swarbrick et al. 2019). Given that phytoplankton biomass is generally low during June due to invertebrate grazing (Dröscher et al. 2009), and that cyanobacteria are rare at this time relative to cryptophytes and green algae (McGowan et al. 2005), we anticipate that correspondence between modeled Chl a (based on PC fluorescence) and in situ Chl a may be weaker in spring and early summer (Fig. 4b).

Second, cyanobacteria fluorescence originates in both Chl a and PC due to the role of the latter as an accessory pigment in these prokaryotes. PC plays a major role in harvesting light, but transfers most of energy to Chl a and fluoresces residual energy at ~ 650 nm (Simis et al. 2012). The transferred light stimulates Chl a to fluorescence in around 680 nm in direct proportion to Chl a concentration (Johnsen and Sakshaug 2007). Therefore, the PC fluorometer, whose emission filter is wide enough to pass both Chl a and PC fluorescence (640 ± 40 nm), senses both PC and Chl a fluorescence of cyanobacteria.

Third, in vivo Chl a fluorometer commonly used in freshwater instrument buoys can be insensitive to the presence of cyanobacteria if the prokaryotes do not possess short-wavelength forms of phycoerythrin (Raateoja et al. 2004; Suggett et al. 2004; Johnsen and Sakshaug 2007). As cyanobacteria predominate at very high Chl a levels (Vogt et al. 2018; Hayes et al. 2019), such insensitivity may disproportionately underestimate high Chl a values (Fig. 5b). In support of this
hypothesis, we note that *Anabaena* spp. and *Chlorella vulgaris* were the most abundant cyanobacteria and algae, respectively, based on cell enumerations at Sta. 2 during 2014–2018, and that the excitation–emission matrices of these taxa show that only Chl *a* from eukaryotes contributes strongly to Chl *a* fluorescence in the range of detection (Region 1 in Supporting Information Fig. S6). These sensors are often insensitive to the fluorescence of the cyanobacteria (Supporting Information Fig. S6b) relative to that from other phytoplankton (Supporting Information Fig. S6a).

Fourth, the PC fluorometer stimulates fluorescence from algae which lack PC, possibly by initiating energy transfer from accessory pigments to Chl *a* (e.g., Chl *b*, carotenoids). Region 2 in Supporting Information Fig. S6 confirms that there is fluorescence from *C. vulgaris* in the spectral range of the PC fluorometer. It has been shown that fluorometers with an emission band above 660 nm are affected by overlapping fluorescence signals of Chl *a* fluorescence (Simis et al. 2012). Given this sensitivity of the PC fluorometer to detect Chl *a* fluorescence, this system appears also capable of estimating lower Chl *a* values during intervals when cyanobacteria are normally rare (May to early June) (McGowan et al. 2005; Hayes et al. 2019; Swarbrick et al. 2019). This observation may also explain the significant relationship ($R^2 = 0.52$) between PC fluorescence and Chl *a* when eukaryotic phytoplankton (algae) are dominant in BPL (Fig. 4b).

Fifth, wide variation in the Chl *a* content and fluorescence characteristics among different algal taxa or growth phases may have contributed to the poor performance of the Chl *a* fluorometer even during intervals when cyanobacteria are abundant (Fig. 4a). According to Eq. 1, different algal taxa yield different $a_{Chl}^*$ and $\Phi_F$ values, causing nonlinearity in the relationship of fluorescence and Chl *a*. While a calibration slope varies as a function of growth irradiance or growth phase, the largest source of slope variability is due to variations in accessory pigmentation (Proctor and Roesler 2010). In the case of BPL, there are at least eight common accessory pigments present in the water column at any given time, with substantial seasonal variation in the predominant compound (McGowan et al. 2005; Swarbrick et al. 2019). This finding might also explain why a multiple linear model using both Chl *a* and PC fluorescence did not improve PC models (Supporting Information Fig. S7), in contrast to findings elsewhere (Rome et al. 2021).

**Performance of environmental MLR models**

Here, we demonstrated a reasonable ability of MLR to retrieve in vitro Chl *a* based on environmental factors alone. Although turbidity occasionally enters predictive models elsewhere (Rome et al. 2021), to the best of our knowledge, ours is the first model with turbidity as the main parameter that exhibits performance comparable to that of fluorescence-based models. This finding is consistent with the observation that phytoplankton abundance controls turbidity measures in regional lakes and streams (Dröscher et al. 2009; Bergbusch et al. 2021), although we recognize that this finding may not apply when nonalgal turbidity is considerable. As turbidity probes are inexpensive, robust, and easy to calibrate relative to fluorometric sensors (Rome et al. 2021), the strong performance of the MLR model should be compared to other locations to evaluate the suitability in monitoring phytoplankton blooms. If validated, this approach for the calibration of previous long records of turbidity measurements may be useful in expanding the spatial extent of Chl *a* retrieval for time series development in other eutrophic lakes dominated by phytoplankton.

Our MLR model includes several in situ factors as in vitro Chl *a* predictors. Apart from statistical justifications for these parameters (strong correlation and significant improvement of the model performance; Supporting Information Fig. S2 and Table 3), inclusion of the predictors was consistent with relationships with Chl *a* known from previous studies. For example, turbidity may have been included because it is mainly biological in nature in BPL and is known to increase with cyanobacterial density. Similarly, the negative correlation between light transmission (normalized PAR) and Chl *a* is consistent with the effects of light absorbance by Chl *a*, as well as physical shading by phytoplankton biomass. Inclusion of sampling month as a predictor is consistent with the strong seasonal succession of phytoplankton seen in these lakes (McGowan et al. 2005; Dröscher et al. 2008; Kehoe et al. 2019; Swarbrick et al. 2019). Finally, a negative relationship between Chl *a* and CO$_2$ concentration is expected as the diel cycles of CO$_2$ concentration are controlled in part by changes in pH and photosynthesis, both of which are strongly affected by the abundance of cyanobacteria (Wiik et al. 2018; Finlay et al. 2019).

**Comments and recommendations**

Analysis of long-term, coeval in vivo and in vitro Chl *a* measurements in a eutrophic lake demonstrated that factory-calibrated in vivo Chl *a* (chl$^{FC}$) of a single-excitation-band fluorometer should be corrected and tuned in a site-specific manner to provide more accurate and reliable estimates of phytoplankton abundance. Here, we introduced three different models to predict Chl *a* from in vivo measurements; two single-linear models based on raw fluorescence from Chl *a* and PC fluorometers (called chl$_{hl}^{FC}$ and chl$_{pc}^{FC}$, respectively) and one MLR model (called chl$^{FL}$), trained by environmental factors, such as turbidity and CO$_2$ concentration. These models were assessed and compared based on both individual metrics and their performance in real applications.

In terms of the ability to recover in vitro Chl *a* concentrations ("accuracy"), the model based on PC fluorescence (chl$_{pc}^{FC}$) compensated the high bias in factory-calibrated Chl *a* (chl$^{FC}$) and reduced average relative error from ~150% to ~35% when gauged by MALE among other metrics. In terms of reliability
of recovered values (ability to capture range of variation), chl$_{P/ML}$ model maintained ~90% reliability to return Chl $a$ values within ±3σ of mean in vitro Chl $a$ (chl$_{IV}$), whereas chl$_{PC}^c$ and chl$_{P/ML}$ returned ~77% and ~60%.

Application of in vivo estimates of Chl $a$ concentration to train algorithms used in remote sensing showed that only fluorescence-corrected models were capable of delineating the spatial variation in Chl $a$ within BPL. Specifically, models based on PC fluorescence and environmental factors significantly improved lake monitoring by increasing the range of Chl $a$ retrieval to up to 150 μg L$^{-1}$, whereas chl$_{P}$ could not retrieve values >70 Chl $a$ μg L$^{-1}$. Further use of chl$_{PC}^c$ instead of chl$_{P}^c$ also decreased the error of Chl $a$ retrieval using Sentinel-2 images by ~35%. For a system that is designed to monitor HABs in a highly eutrophic lake, system saturation at only moderate Chl $a$ levels is problematic.

We conclude that several features of the PC fluorometer make it more suitable to develop remote-sensing protocols for lakes subject to intense surface blooms of cyanobacteria. Given that uncertainties in Chl $a$ training data are an important source of inaccuracy in remote retrieval of Chl $a$, the methods proposed here may be considered as a preprocess step to improve consistency between in vivo and in vitro measurements of Chl $a$ before feeding into a remote-sensing model. Although the presented models (calibration coefficients) might be site-specific and should be recalibrated locally, the application of this technique to other well-monitored lakes will be an important next step to evaluate the generality of our findings, and evaluate the potential for calibrated, remotely sensed Chl $a$ values to estimate the spatial extent and timing of HABs in marine and freshwaters.

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Conflict of Interest
None declared.