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Evaluating Chlorophyll Fluorescence Sensors as a Proxy for Chlorophyll *a* Concentrations in the Illinois River, Illinois, Water Years 2018–24



Scientific Investigations Report 2026–5052

U.S. Department of the Interior
U.S. Geological Survey

Cover. Photograph showing surface algae in the Illinois River, taken at the U.S. Geological Survey streamgage Illinois River at Henry (05558300) on September 17, 2024, by Colin Peake, U.S. Geological Survey. Background photograph showing a micrograph of chlorophyll concentrated in structures called chloroplasts by Kristian Peters, licensed under the Creative Commons Attribution-Share Alike 3.0 Unported license.

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U.S. Geological Survey, Reston, Virginia: 2026

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Suggested citation:
Kamrath, B.J.W., and Peake, C.S., 2026, Evaluating chlorophyll fluorescence sensors as a proxy for chlorophyll *a* concentrations in the Illinois River, Illinois, water years 2018–24: U.S. Geological Survey Scientific Investigations Report 2026–5052, 13 p., https://doi.org/10.3133/sir20265052.

Associated data for this publication:
U.S. Geological Survey, 2026, USGS water data for the Nation: U.S. Geological Survey National Water Information System database, https://doi.org/10.5066/F7P55KJN.

ISSN 2328-0328 (online)

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Conversion Factors

U.S. customary units to International System of Units

Multiply	By	To obtain
	Length	
foot (ft)	0.3048	meter (m)

International System of Units to U.S. customary units

Multiply	By	To obtain
	Length	
nanometer (nm)	3.937×10^{-8}	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
meter (m)	1.094	yard (yd)
	Volume	
milliliter (mL)	0.3381	ounce, fluid (fl. oz)

Supplemental Information

A water year is the 12-month period from October 1 through September 30 of the following year and is designated by the calendar year in which it ends.

Concentrations of chemical constituents in water are in micrograms per liter (µg/L).

Abbreviations

±	plus or minus
Chl	chlorophyll *a*
CI	confidence interval
fChl	chlorophyll fluorescence
n	number of samples
NWIS	National Water Information System
r	correlation coefficient
RFU	relative fluorescence unit
USGS	U.S. Geological Survey

Evaluating Chlorophyll Fluorescence Sensors as a Proxy for Chlorophyll *a* Concentrations in the Illinois River, Illinois, Water Years 2018–24

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Abstract

The U.S. Geological Survey deployed instream chlorophyll fluorescence sensors on the Illinois River in Illinois to better understand the formation, dynamics, and persistence of riverine algal blooms. At five sensor locations across four U.S. Geological Survey streamgages, chlorophyll fluorescence (fChl) sensors were set to measure instantaneous fChl in micrograms per liter as chlorophyll at 15-minute intervals. These sensors estimate fChl based on the fluorescence response of algal pigments but do not directly measure chlorophyll *a* (Chl) concentrations or algal biomass. To evaluate the reliability of fChl as a proxy for Chl, fChl measurements were compared to laboratory-measured Chl concentrations. The Chl concentrations were determined from water-quality samples collected at the sensor locations and analyzed using the standard chromatographic-fluorometric method with acetone extraction. A total of 209 paired fChl and Chl measurements were analyzed using Pearson's correlation and model II regression. Statistically significant, positive correlations (Pearson's correlation coefficient values between 0.68 and 0.95) were detected at all sensor locations, indicating that fChl generally tracked with Chl. However, model II regression detected proportional and fixed bias at three of the five locations and proportional bias at another location. At locations with proportional biases, model II regression slopes were less than 1, indicating that fChl increasingly underestimated Chl as concentrations increased; however, paired measurements at high Chl concentrations were sparse. Substantial variability in bias was also observed across sensor locations with slopes ranging from 0.43 to 0.93. Overall, the results indicated that fChl correlated well with Chl in the Illinois River, but that fChl may not reliably represent Chl concentrations without adjustment. For applications requiring accurate Chl estimates, a specific surrogate model for each senor location could be beneficial. These findings underscore the importance of field validation, consistent sensor placement, and careful interpretation of fChl data, particularly when reported in micrograms per liter.

Plain Language Summary

In the Illinois River Basin in Illinois, the U.S. Geological Survey deployed instream fluorescence sensors to collect high-frequency chlorophyll fluorescence data. Fluorescence sensors measure a relative fluorescence response from chlorophyll in near real time and at high frequencies but do not directly measure chlorophyll *a* or algal biomass. Although not interchangeable, chlorophyll fluorescence, in micrograms per liter as chlorophyll, can serve as a useful proxy for chlorophyll *a* concentrations in the Illinois River if used with caution. The study described in this report may be used to provide a reference for future researchers and practitioners to justify their use, disuse, or adjustment of chlorophyll fluorescence as chlorophyll as a proxy for chlorophyll *a*.

Introduction

Large inputs of treated wastewater and agricultural runoff produce high nutrient concentrations and the potential for algal blooms throughout the Illinois River Basin in Illinois (Panno and others, 2008; Illinois Environmental Protection Agency and Illinois Department of Public Health, 2018; McIsaac and others, 2023; fig. 1). Because of the abundance of nutrients from point and nonpoint sources, the Illinois River Basin provides a unique opportunity to study how changes in land use, climate, wastewater treatment technology, and agricultural practices affect water quality (Van Metre and others, 2020). For this reason, the Illinois River Basin was selected to be a U.S. Geological Survey (USGS) Integrated Water Science basin (Miller and others, 2021). Integrated Water Science basins are priority basins across the United States targeted for intensive monitoring, research, and modeling to improve understanding of water quality and availability. As part of the Integrated Water Science program, enhanced monitoring was implemented through the USGS Next Generation Water Observing System program, which supports the collection of

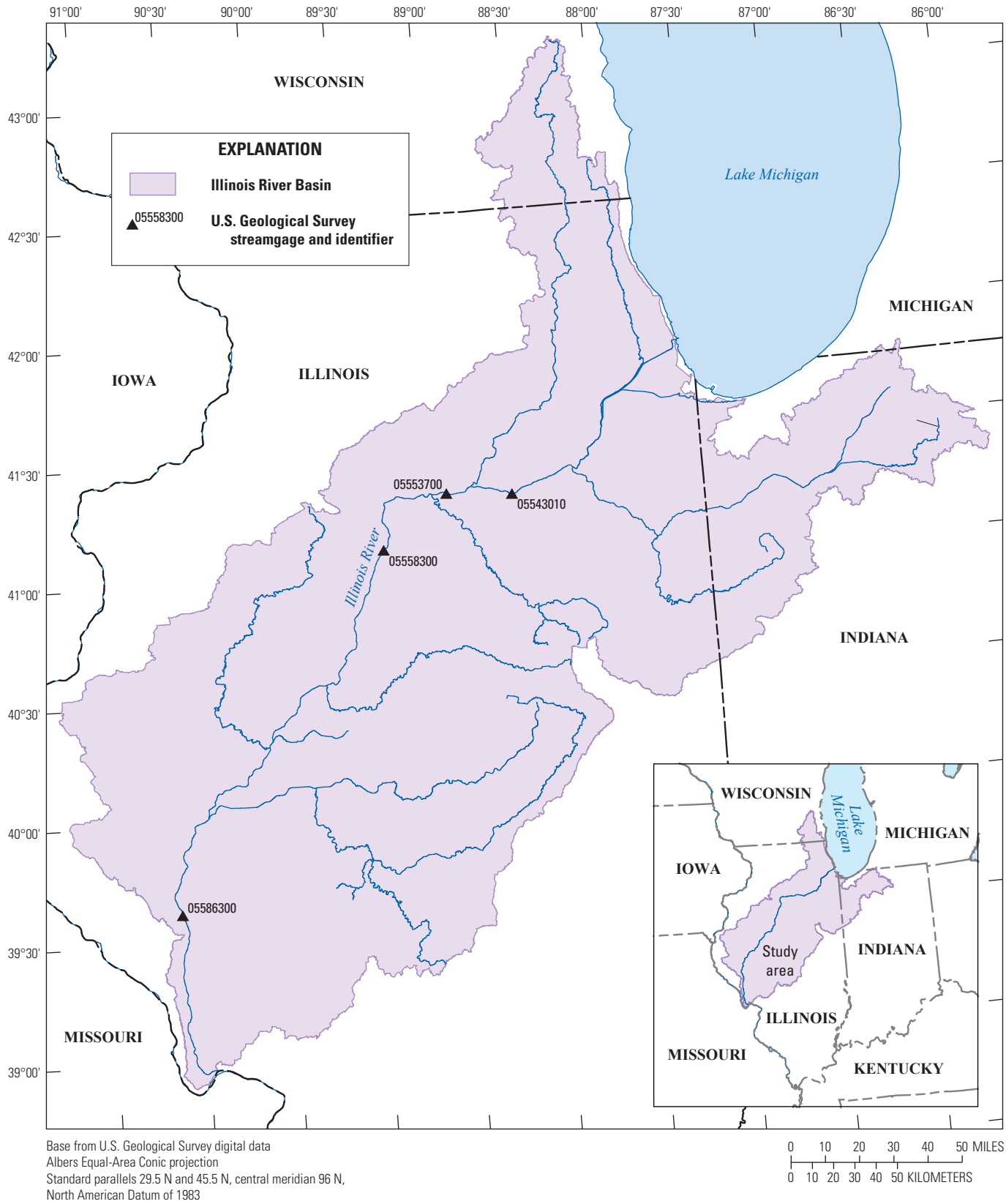


Figure 1. Map showing U.S. Geological Survey streamgages in the Illinois River Basin used in the study.

real-time water-quantity and quality data using state-of-the-art measurement technologies aimed at increasing spatial and temporal data coverage (Eberts and others, 2019).

As part of the USGS Next Generation Water Observing System program, USGS scientists and collaborators installed water-quality sensors in the Illinois River to obtain high-frequency measurements of multiple water-quality parameters. One of these parameters is chlorophyll fluorescence (fChl), which may allow for high-frequency estimates of algal abundance and growth—key measures in understanding the formation and persistence of algal blooms (Stauffer and others, 2019; Foster and others, 2022; Schmadel and others, 2024). However, fChl sensors do not measure algal biomass or even chlorophyll *a* (Chl) directly. Rather, they measure the relative fluorescence response of algal pigments within a sensing volume of water. Chlorophyll fluorescence should therefore be considered a potential proxy for laboratory-measured Chl concentrations, which themselves are a proxy for algal biomass (Foster and others, 2022). Measurements of fChl can be reported in relative fluorescence units (RFUs) or in micrograms per liter as chlorophyll. In the Illinois River, fChl was reported in micrograms per liter as chlorophyll. The reporting unit of micrograms per liter as chlorophyll is estimated using manufacturer-derived relations built between sensor outputs, in RFUs, and extracted pigments from laboratory-grown blue-green algae (YSI Incorporated, 2024).

Chlorophyll fluorescence can be affected by multiple biological and environmental factors, which can complicate its use as a proxy for algal biomass. Chlorophyll fluorescence not only responds to changes in relative fluorescence but can also respond to changes in algal health and algal community composition that alter the proportion of pigment to algal biomass and other environmental factors such as temperature and turbidity (Marra, 1997; Bertone and others, 2018; Foster and others, 2022). Additionally, fChl can change in response to nonphotochemical quenching, where algae pigment concentrations change but algal biomass does not (Foster and others, 2022). Because of the potential for different pigment to biomass proportions, changes in fChl do not always reflect a change in algal biomass or its proxy, Chl. Furthermore, the relation of fluorescence to chlorophyll used to convert fluorescence in RFUs to chlorophyll in micrograms per liter may not hold true for all algal communities in a water body. Previous studies evaluating instream fChl in lakes and reservoirs have determined fChl to have a strong positive correlation with Chl but variable levels of accuracy and precision (Gregor and Maršálek, 2004; Izydorczyk and others, 2009; Rolland and others, 2010; Catherine and others, 2012). As a result, fChl needs to be field validated in the Illinois River to determine if these measurements provide an accurate and precise estimate of Chl.

This study investigated the validity of using fChl, reported in micrograms per liter as chlorophyll, as a locally reasonable proxy for Chl in the Illinois River. We evaluated the relation between fChl and Chl by comparing concurrent measurements from five sensor locations at four USGS streamgages on the Illinois River. Although the spatial scale of this report is limited to the Illinois River, methods, insights, implications, and conclusions from this work may be applicable to other studies using high-frequency fluorescence sensors.

Purpose and Scope

This report presents the results from a field verification study of fChl using instream fluorescence sensors in the Illinois River. Field verification was completed by comparing paired measurements of fChl and Chl at five sensor locations across four USGS streamgages during water years 2018 to 2024. A water year is the 12-month period from October 1 through September 30 of the following year and is designated by the calendar year in which it ends. Pearson's correlation and model II regression methods were used to evaluate association and bias between fChl and Chl in the Illinois River. The causes of differences in agreement among sensor locations were not investigated. Rather, this report simply quantifies the relation between fChl and Chl in the Illinois River.

Methods

Chlorophyll fluorescence, in micrograms per liter as chlorophyll, was measured continuously at 15-minute intervals using YSI EXO2 multiparameter sondes equipped with EXO total algae PC smart sensors at five sensor locations across four USGS streamgages on the Illinois River. Discrete water-quality samples were collected approximately every 6 weeks and analyzed for Chl concentrations. The fChl and Chl data were retrieved from the USGS National Water Information System (NWIS) database and paired by date and time using a three-step matching procedure, which yielded 209 paired measurements. Paired values were then evaluated using Pearson's correlation to assess association and model II regression to assess fixed and proportional bias between the two measurements.

Study Sites

Four USGS streamgages on the Illinois River were equipped with fluorescence sensors measuring chlorophyll fluorescence between water years 2018 and 2024 (table 1; fig. 1). Moving upstream from the confluence of the Illinois and Mississippi Rivers, the streamgages include Illinois River at Florence (05586300; hereafter referred to as "Florence"), Illinois River at Henry (05558300; hereafter referred to as "Henry"), Illinois River at Starved Rock (05553700; hereafter referred to as "Starved Rock"), and Illinois River at Seneca (05543010; hereafter referred to as "Seneca") (U.S. Geological Survey, 2026). The Henry and Starved Rock sensor locations

Table 1. Descriptions of study sites within the Illinois River Basin.

[Streamgage data from U.S. Geological Survey (2026); dates are given in year-month-day format; USGS, U.S. Geological Survey; ID, identifier; IL, Illinois; Starved Rock BN, Starved Rock bullnose; Starved Rock SHD, Starved Rock Lock and Dam structure]

USGS streamgage ID	USGS streamgage name	Shortened name	Sensor location	Sampling start date	Sensor start date
05586300	Illinois River at Florence, IL	Florence	Florence	2017–11–30	2016–10–01
05558300	Illinois River at Henry, IL	Henry	Henry	2018–06–26	2018–06–06
05543010	Illinois River at Seneca, IL	Seneca	Seneca	2017–11–15	2017–10–01
05553700	Illinois River at Starved Rock, IL	Starved Rock	Starved Rock BN	2021–10–07	2021–09–23
05553700	Illinois River at Starved Rock, IL	Starved Rock	Starved Rock SHD	2018–11–14	2018–06–07

were moved during the period of interest. At Henry, data from the second location were not included in this effort because there were only five concurrent fChl and Chl measurements since the location shift in May 2023. At Starved Rock, the sensor was initially near a shed closer to the center of the Starved Rock Lock and Dam structure (hereafter referred to as "Starved Rock SHD"). The sensor was moved about 300 feet northeast to the bullnose of the lock (hereafter referred to as "Starved Rock BN") on September 23, 2021. A new sensor was used at the new location. In the analysis, Starved Rock data were split by the two sensor locations, Starved Rock SHD and Starved Rock BN, to accommodate this change. Overall, a total of five sensor locations across the four USGS streamgages were included in this study.

Continuous Measurements of Chlorophyll Fluorescence as Chlorophyll

At each site, fChl, in micrograms per liter as chlorophyll, was measured every 15 minutes using YSI EXO 2 multiparameter sondes (YSI Incorporated, Yellow Springs, Ohio) equipped with an EXO total algae PC smart sensor. According to manufacturer specifications, chlorophyll excitation is set at 470 plus or minus (±) 15 nanometers, and emission is set at 685±20 nanometers (YSI Incorporated, 2024). The sondes were deployed year-round, although occasional data gaps resulted from environmental conditions, equipment malfunctions, routine site visit activities, or operator errors.

Operation and maintenance of the sondes and sensors followed documented USGS protocols (Wagner and others, 2006; Foster and others, 2022). During routine site visits, sondes were removed from the water, cleaned, checked for fouling using an additional recently calibrated field water-quality sonde, and checked for calibration drift using a solution with a known concentration of rhodamine water tracer (WT). Data were analyzed after the site visit. Analysis included deleting erroneous data obtained during the site visit and, when appropriate, applying fouling and calibration drift corrections. Fouling corrections adjust measured values to account for physical blockages accumulating on the sensor between routine site visits and are determined by comparing sensor readings before and after cleaning. Calibration drift corrections adjust measured values to account for gradual deviations from calibration standards during deployment and are determined using the readings taken within calibration standards during site visits. Generally, data corrections are applied when the sum of the absolute values of the computed fouling and drift corrections are greater than 15 percent of the measured value. The fChl data with applied corrections were reviewed and approved by USGS personnel. Approved fChl data were stored in the USGS NWIS database using the parameter code 32316 ("chlorophyll fluorescence [fChl], water, in situ, concentration estimated from reference material, micrograms per liter as chlorophyll"; U.S. Geological Survey, 2026).

Discrete Chlorophyll *a* Data Collection and Analysis

Discrete water-quality samples were collected by USGS field crews about every 6 weeks during scheduled maintenance and routine sampling site visits. Along with samples collected during site visits, additional water-quality samples were collected at all streamgages during the spring and summer months as part of other USGS sampling efforts on the Illinois River. Samples were collected and processed using nationally consistent field protocols as outlined in the USGS field manual for collecting water-quality data (U.S. Geological Survey, variously dated). When the sample is collected, the sample bottle is submerged near and to the approximate depth of the sensor. Samples were either processed on site or placed on wet ice and brought back to the laboratory for processing. In accordance with chapter A7, section 7.4 of the field manual, samples were processed for Chl by filtering 50–200 milliliters of the sample through a 47-millimeter glass fiber filter, folding and placing the filter in aluminum foil to protect from light, and freezing the filter until it could be shipped on dry ice to the USGS National Water Quality Laboratory for analysis (Berkman and Canova, 2007). Each sample was

analyzed by the USGS National Water Quality Laboratory for Chl concentration, in micrograms per liter, using U.S. Environmental Protection Agency method 445.0 (Arar and Collins, 1997). Results from laboratory analysis were reviewed and quality assured by USGS personnel and stored in the USGS NWIS database with the parameter code 70953 "chlorophyll a, phytoplankton, chromatographic-fluorometric method, micrograms per liter" (U.S. Geological Survey, 2026).

Data Retrieval and Preparation

Data were obtained using the readNWISuv and readWQPdata functions for fChl and Chl, respectively, from the dataRetrieval R package for water year 2018 through 2024 (DeCicco and others, 2025). The Chl data were then filtered to method identifiers associated with samples collected at the sensor (that is, single vertical, point, or grab samples) to ensure that the sampled water was spatially representative of the water measured by the sensor. One sample collected at Henry had a Chl concentration of 840 micrograms per liter (µg/L), which was greater than three standard deviations above the mean (standard-score greater than 3). This measurement was removed from further analysis. There were a total of 250 Chl measurements collected at the sensor across all sensor locations.

Once the datasets were compiled, fChl and Chl data were merged by date and time into a single paired dataset. Because samples were not always collected exactly on the 15-minute interval, the merging process was completed in three steps: (1) exact matches, (2) forward matches, and (3) backward matches. Chl samples were first matched to fChl measurements taken at the exact same date and time, which accounted for 107 paired measurements. Next, Chl samples were paired with the nearest fChl measurement forward in time, within 15 minutes of the sample being taken, which accounted for 68 paired measurements. Forward matches were preferred because samples were often collected after sensor cleaning during routine site visits, and by selecting the fChl measurement forward in time, the sensor would have more time to equilibrate to the environmental conditions. For the remaining Chl samples, the nearest fChl measurement backward in time, but within 15 minutes, was used, which accounted for 34 paired measurements. Samples without a fChl measurement within ±15 minutes (41 in total) were excluded from further analysis. The final paired dataset included 209 paired measurements with between 18 and 81 measurements per sensor location (table 2).

Data Analysis

The analytical framework was structured around Pearson's correlation and model II regression. Following the recommendations of Ludbrook (1997, 2002, and 2010), the analysis prioritized detection of systematic bias over measures of association. R statistical software (R Core Team, 2024), RStudio (Posit Team, 2025), and the tidyverse (Wickham and others, 2019) were used for data processing and analysis. Unless noted otherwise, the base R stats package was used for statistical analyses.

Pearson's Correlation

Pearson's *r* was calculated to assess the strength of the linear relation between fChl and Chl at each sensor location. Although correlation is a measure of association rather than bias, it was included to characterize the overall linear relationship between the two methods before evaluating bias through model II regression analysis. A strong positive correlation between fChl and Chl indicates that the two values covary or move together (that is, when one value increases, the other increases) but it does not ensure agreement between the values. For example, a systematic positive bias in fChl relative to Chl would yield a strong correlation despite poor agreement between fChl and Chl.

Model II Regression Analysis

Bias between fChl and Chl was evaluated using model II linear regression, also known as ordinary least products regression, standard major axis regression, reduced major axis regression, or line of organic correlation (Helsel and others, 2020). Model II linear regression was selected because both the *x* variable (in this case, Chl) and the *y* variable (fChl) were subject to measurement error (Ludbrook, 1997, 2010). Following the method-comparison convention, the reference method (Chl) was assigned as the *x* variable and the new method (fChl) was assigned the *y* variable, which frames the resulting bias in terms of how fChl deviates from Chl. Regression models were fitted using the R package smatr (Warton and others, 2012) and used to identify the two forms of systematic measurement biases: fixed and proportional bias (Ludbrook, 2012). A fixed bias occurs when one method provides values consistently higher or lower than the other method, regardless of the measured value. A proportional bias occurs when one method gives values that are higher or lower than those from the other method in an amount proportional to the measured values. When a proportional bias is present, interpretation of the intercept is confounded because the apparent fixed bias shifts depending on the range of measured values. Therefore, the presence of a proportional bias was evaluated before interpreting any fixed bias. For each fitted model, the 95-percent confidence intervals (CIs) were calculated for the slope and the intercept parameters. A proportional bias was indicated when the 95-percent CI for the slope did not include 1. A fixed bias was indicated when the 95-percent CI for the intercept did not include 0.

Table 2. Summary statistics of sensor-measured chlorophyll fluorescence and laboratory-measured chlorophyll *a* concentration taken concurrently for the five senor locations used in this study.

[USGS, U.S. Geological Survey; ID, identifier; *n*, number of measurements; min, minimum; max, maximum; fChl, chlorophyll fluorescence; µg/L, microgram per liter; Chl, chlorophyll *a*; Starved Rock BN, Starved Rock bullnose; Starved Rock SHD, Starved Rock Lock and Dam structure]

Sensor location	USGS streamgage ID[1]	*n*	Min		Median		Mean		Max	
			fChl (µg/L)	Chl (µg/L)	fChl (µg/L)	Chl (µg/L)	fChl (µg/L)	Chl (µg/L)	fChl (µg/L)	Chl (µg/L)
Florence	05586300	81	4.9	0.8	17.6	17.2	19.9	19.6	43.0	56.7
Henry	05558300	33	4.6	2.9	19.0	18.6	20.1	20.2	44.4	72.5
Seneca	05543010	33	1.4	1.6	4.6	5.3	5.6	7.3	13.7	16.3
Starved Rock BN	05553700	44	2.2	1.5	11.4	20.6	12.5	22.7	25.8	65.9
Starved Rock SHD	05553700	18	4.9	4.4	17.8	18.6	19.8	20.6	52.1	54.1
All locations		209	1.4	0.8	14.4	16.1	16.1	18.5	52.1	72.5

[1]Streamgage identifiers from U.S. Geological Survey (2026).

Comparison of Sensor-Measured Chlorophyll Fluorescence as Chlorophyll and Laboratory-Measured Chlorophyll *a* Concentrations in the Illinois River

The following sections present the results of comparing fChl and Chl at the five sensor locations. In all comparisons, Chl was treated as the reference value against which each fChl measurement was evaluated. Pearson's *r* values were used to evaluate the association between fChl and Chl. Model II regression analysis was used to evaluate bias and summarize overall agreement between the two measurements. All analyses were completed by sensor location and pooled across all sensor locations.

A total of 209 paired measurements were analyzed, ranging from 18 at Starved Rock SHD to 81 at Florence (table 2). Across all sensor locations, fChl and Chl had similar ranges (1.4–52.1 µg/L and 0.8–72.5 µg/L, respectively). However, there were differences between sensor locations. For instance, Seneca had the lowest mean fChl and Chl at 5.6 and 7.3 µg/L, respectively, while Starved Rock BN had the highest mean Chl at 22.7 µg/L, and Henry had the highest mean fChl at 20.1 µg/L. At most sensor locations, maximum Chl values were substantially higher than median values, indicating that relatively few paired measurements captured high-concentration conditions (table 2).

Association and Bias

Statistically significant positive correlations between fChl and Chl were detected for each sensor location with Pearson's *r* ranging from 0.68 at Henry to 0.95 at Starved Rock SHD (table 3; fig. 2). Thus, the two measurements covaried, rising and falling together. Because correlation does not indicate agreement, model II regression was used to characterize how fChl deviated from Chl. Model II regression slopes ranged from 0.43 to 0.93 across sensor locations, indicating that fChl measured progressively lower than Chl as Chl increased (fig. 2). No inverse relation between fChl and Chl was detected at any sensor location.

Model II regression analysis detected both proportional and fixed bias at Florence, Henry, and Starved Rock BN (table 3; fig. 2). Because proportional bias was present at these locations, the intercept should not be interpreted as a standalone fixed offset. At Henry, the slope of 0.62 (95-percent CI of 0.48–0.81) and the intercept of 7.58 (95-percent CI of 3.37–11.8) indicated that fChl measured higher than Chl at low concentrations and lower at high concentrations. Florence demonstrated a similar but less pronounced pattern, with a slope of 0.82 (95-percent CI of 0.71–0.94) and an intercept of 3.86 (95-percent CI of 1.26–6.46). Starved Rock BN had the most severe proportional bias of any sensor location, with a slope of 0.43 (95-percent CI of 0.35–0.52) and an intercept of 2.80 (95-percent CI of 0.50–5.09). While the practical implications of the results were similar across these three sensor locations, there was clear variation in the magnitude of the biases present.

The remaining two sensor locations differed from this pattern. At Seneca, only a proportional bias was detected: the slope was 0.66 (95-percent CI of 0.52–0.83), whereas the intercept was not significantly different from zero at 0.76 (95-percent CI of −0.60–2.13). Because the intercept was near zero, fChl measured lower than Chl primarily at moderate to high concentrations. At Starved Rock SHD, neither a fixed nor a proportional bias was detected; the slope was 0.93 (95-percent CI of 0.79–1.10) and the intercept was 0.60 (95-percent CI of −3.14–4.33). However, Starved Rock SHD had the smallest sample size (number of samples [*n*] =18), and therefore the absence of detectable bias should be interpreted with caution.

Table 3. Detection of fixed and proportional bias between paired laboratory-measured chlorophyll *a* concentration (in micrograms per liter) from single vertical samples and sensor-measured chlorophyll fluorescence (reported in micrograms per liter as chlorophyll) for sensor locations on the Illinois River.

[USGS, U.S. Geological Survey; ID, identifier; CI, confidence interval; Starved Rock BN, Starved Rock bullnose; Starved Rock SHD, Starved Rock Lock and Dam structure]

Sensor location	USGS streamgage ID[1]	Pearson's correlation coefficient[2]	Linear regression		Bias	
			Intercept (95-percent CI)	Slope (95-percent CI)	Fixed	Proportional
Florence	05586300	0.78	3.86 (1.26–6.46)	0.82 (0.71–0.94)	Yes	Yes
Henry	05558300	0.68	7.58 (3.37–11.8)	0.62 (0.48–0.81)	Yes	Yes
Seneca	05543010	0.77	0.76 (−0.60–2.13)	0.66 (0.52–0.83)	No	Yes
Starved Rock BN	05553700	0.78	2.80 (0.50–5.09)	0.43 (0.35–0.52)	Yes	Yes
Starved Rock SHD	05553700	0.95	0.60 (−3.14–4.33)	0.93 (0.79–1.10)	No	No
All locations		0.73	2.17 (0.53–3.81)	0.75 (0.69–0.83)	Yes	Yes

[1]Streamgage identifiers from U.S. Geological Survey (2026).

[2]Values in this column indicate statistical significance at a probability of less than 0.001.

When all sensor locations were pooled, both proportional and fixed biases were detected with a slope of 0.75 (95-percent CI of 0.69–0.83) and an intercept of 2.17 (95-percent CI of 0.53–3.81). The pooled results reflect those of Henry, Florence, and Starved Rock BN with fChl generally exceeding Chl at low concentrations and falling below Chl at high concentrations.

Discussion

At all sensor locations, fChl was significantly and positively correlated with Chl (Pearson's *r* ranging from 0.68 at Henry to 0.95 at Starved Rock SHD; table 3). The positive linear correlations indicated that, regardless of location or sensor, fChl generally increased with increasing Chl. No evidence of inverse relations between fChl and Chl was detected, indicating that despite variability across sensor locations, fChl did not systematically trend in the opposite direction of Chl. The significant positive linear correlation between fChl and Chl in the Illinois River also matched the results of previous comparison studies between fluorescence-based and extraction-based chlorophyll measurements. Gregor and Maršálek (2004) reported high correlation (Pearson's r =0.91) between a submersible fluorescence probe and extracted Chl in rivers in the Czech Republic, and Prestigiacomo and others (2022) found that fluorometer-estimated Chl was highly correlated with extracted Chl (r greater than 0.8) in samples taken from New York State lakes. Rolland and others (2010) similarly found strong correlations between spectrofluorometric measurements and extracted Chl during a 2-year survey in the Marne Reservoir, France.

Despite the strong correlations, regression analysis revealed the presence of fixed and proportional bias at three of the five sensor locations (Henry, Florence, and Starved Rock BN) and in the pooled data across all locations (table 3). At Henry, the slope of 0.62 and intercept of 7.58 indicated that fChl measured higher than Chl at low concentrations and lower than Chl at high concentrations. Starved Rock BN had a most severe proportional bias (slope of 0.43), meaning that for every 1 µg/L increase in Chl, fChl increased by only 0.43 µg/L. At Seneca, only a proportional bias was detected (slope of 0.66, intercept not significant different from zero) and at Starved Rock SHD, neither bias was detected (slope of 0.93, intercept of 0.60); however, this result should be interpreted with caution given the small sample size (n=18).

Across sensor locations, the consistent pattern of model II regression slopes less than 1 (ranging from 0.43 to 0.93) indicated that fChl measured progressively lower than Chl with increasing Chl concentration. This concentration-dependent divergence between fChl and Chl has practical implications because the largest errors in fChl occurred when accurate measurements likely mattered the most (that is, during conditions most relevant to algal bloom detection and management). However, relatively few paired measurements were collected during high-Chl conditions at any sensor location, as reflected by the maximum Chl values being substantially higher than the median values (table 2). As a result, the size of this deviation at the upper end of the observed Chl concentration range carries greater uncertainty than at moderate concentrations, where more measurements occurred. Overall, the results of the regression analyses support the conclusion that fChl is a proxy for Chl rather than a direct substitute (Foster and others, 2022) and that a 1:1 relation between fChl and Chl should not be expected in the Illinois River. Instead, fChl tended to measure higher than Chl at low Chl concentrations and lower than Chl at high Chl concentrations, with the magnitude of the biases varying across sensor locations.

Substantial variability in all measures of agreement was observed across streamgages and sensor locations (tables 2 and 3). This variation indicated that not only the streamgage but also

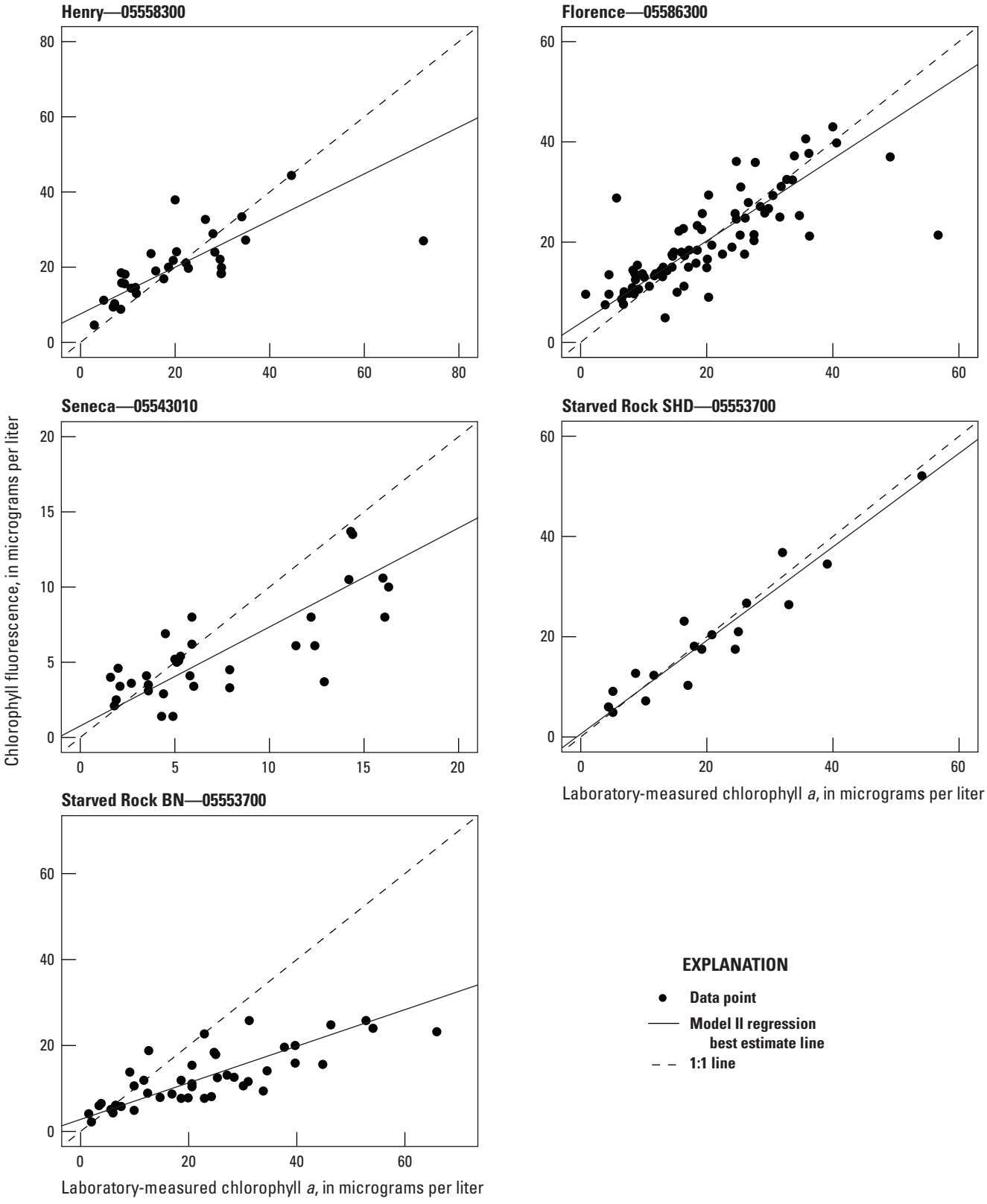


Figure 2. Graphs showing paired laboratory-measured chlorophyll *a* concentrations, in micrograms per liter, from single vertical samples and sensor-measured chlorophyll fluorescence, reported in micrograms per liter as chlorophyll, for each of the Illinois River sensor locations used in the study (Henry, U.S. Geological Survey [USGS] streamgage Illinois River at Henry [05558300]; Florence, USGS streamgage Illinois River at Florence [05586300]; Seneca, USGS streamgage Illinois River at Seneca [05543010]; and Starved Rock Lock and Dam structure [Starved Rock SHD] and Starved Rock bullnose [Starved Rock BN], USGS streamgage Illinois River at Starved Rock [05553700], Illinois).

the sensor location at the streamgage may affect the fChl:Chl relation. Starved Rock provides a clear example of sensor location variation. When the Starved Rock sensor was relocated about 300 feet from the SHD location to the BN location in fall 2021, the fChl:Chl relation changed substantially despite no substantial difference in Chl concentrations between the two locations (table 2). At Starved Rock SHD, neither fixed nor proportional bias was detected, and the Pearson's *r* was 0.95. At Starved Rock BN, both fixed and proportional biases were present, and the Pearson's *r* declined to 0.78. This change cannot be attributed to sensor location alone because a new sensor was installed at the BN location; therefore, the observed differences may reflect the individual sensor units, the location, or a combination of both. We do not attempt to determine the cause(s) of sensor location variation in the fChl:Chl relation, but the results demonstrate that changes in streamgage location, sensor location, the individual sensor, or a combination of these can substantially affect the fChl:Chl relation and should be considered in data interpretation.

Practical Use Cases

The results of the study indicate that fChl is best suited for applications focused on relative changes in Chl concentrations or the temporal dynamics of the system rather than on precise estimates of chlorophyll magnitude. For example, fChl could be used to determine whether Chl concentrations are relatively high or low, or to characterize the timing and duration of algal growth events, but fChl should not be relied upon to determine the exact Chl concentration at a given point in time. One potential application well suited to the strengths of fChl would be in a river health warning system for algal blooms, in which threshold fChl values indicate if the likelihood of bloom conditions was low or high. In such a system, agreement between fChl and Chl would be less critical than the presence of a strong positive correlation. The significant positive correlations observed at all sensor locations support this general application, if warning thresholds account for the direction and magnitude of bias.

Conversely, if fChl is to be used in a context that requires accurate and precise estimates of Chl, then a surrogate regression would likely be needed to adjust fChl to better represent Chl. Whereas the bias analysis treated Chl as the reference measurement, a predictive surrogate model reverses this orientation by regressing Chl on fChl so that a more accurate Chl time series can be developed from fChl. The strong correlations observed in this study support the use of these surrogate models to predict Chl using fChl; however, the variability in fChl:Chl relations across sensor locations indicates that these models should be developed on a per-location and potentially per-sensor basis.

From a site management perspective, the results (especially those from the two Starved Rock locations) indicated that sensor placement should be carefully considered during installation. Furthermore, relocations or sensor changes should be avoided if possible, and if not possible, these changes should be well documented because any change in location or sensor may invalidate previously developed surrogate models.

This study also raises considerations regarding the reporting of fChl values. Using units of micrograms per liter as chlorophyll instead of the more abstract RFUs recommended by the sensor manufacturer (YSI Incorporated, 2024) provides a greater measure of interpretability for end users of the data; however, reporting fChl in RFUs more clearly communicates that fChl is a proxy for Chl. Reporting fChl in micrograms per liter as chlorophyll introduces a high risk of misinterpretation because fChl may be assumed to directly represent Chl. For example, a change in fChl from 10 to 20 µg/L could be mistakenly interpreted as a change in Chl from 10 to 20 µg/L, whereas a change in fChl from 10 to 20 RFUs is more likely understood as a relative change. Although RFUs are also a proxy, they avoid the false accuracy implied by micrograms per liter. Therefore, unless a strong and well-documented relation between fChl and Chl has been established, reporting in RFUs may be more appropriate; nevertheless, the use of micrograms per liter can still be justified in public-facing datasets if accompanied by clear disclaimers that fChl does not equal Chl. In the Illinois River Basin, the strong correlations observed support continued reporting of fChl in micrograms per liter if users are made aware of its limitations and the potential for misinterpretation.

Limitations

Several limitations should be considered when interpreting these results. First, all paired measurements were treated equally in analysis regardless of season, hydrologic condition, time of day, or turbidity. Because algal community composition in temperate rivers can shift seasonally and because nonphotochemical quenching is light dependent (Foster and others, 2022), the fChl:Chl relation may vary across these conditions. Second, sample sizes varied substantially among sensor locations, ranging from 18 paired measurements at Starved Rock SHD to 81 at Florence (table 2). As noted above, the absence of detectable bias at Starved Rock SHD may partly reflect low statistical power rather than the absence of bias. Likewise, the comparatively small sample sizes at Henry (n=33) and Seneca (n=33) limit the precision of bias estimates at those locations. Third, discrete samples were collected as single vertical grabs near the sensor, following documented USGS protocols. Although this approach provides a spatially representative sample of the water measured by the sensor, it does not capture potential differences in Chl concentration throughout the river cross section. Thus, the ability of the sensor to represent Chl concentrations for the entire river cross section was not evaluated.

Summary

This report presents the results of a field validation study comparing chlorophyll fluorescence (fChl), reported in micrograms per liter as chlorophyll and measured by fChl sensors, and laboratory-measured chlorophyll *a* (Chl) concentrations at five sensor locations across four U.S. Geological Survey streamgages in Illinois on the Illinois River (Florence [Illinois River at Florence; 05586300], Henry [Illinois River at Henry; 05558300], Seneca [Illinois River at Seneca; 05543010], Starved Rock bullnose [hereafter referred to as "Starved Rock BN"; a Illinois River at Starved Rock; 05553700], and Starved Rock Lock and Dam structure [hereafter referred to as "Starved Rock SHD"; also 05553700]. A total of 209 paired fChl and Chl measurements were analyzed using Pearson's correlations and model II regression to assess the association and bias between the two measurements. Strong positive correlations (minimum Pearson's correlation coefficient of 0.68) were observed at all sensor locations, indicating that fChl generally tracked with Chl. However, model II regression detected both fixed and proportional biases at Henry, Florence, and Starved Rock BN and a proportional bias at Seneca. At Starved Rock SHD, neither bias was detected, though the small sample size (number of samples =18) limits confidence in this result. The model II regression slopes at all sensor locations were less than or equal to 1 (ranging from 0.43 to 0.93), indicating that fChl generally underestimated Chl to an increasing degree at higher concentrations; however, this pattern should be interpreted with caution because paired measurements during high-Chl conditions were limited across all sensor locations.

Overall, the results indicated fChl reported in micrograms per liter is best interpreted as an approximation of Chl and not a direct substitute. Although not interchangeable, fChl can serve as a useful proxy for Chl in the Illinois River if used with caution. For applications that require accurate and precise estimates of Chl, a location-specific and potentially a sensor-specific surrogate regression model would likely be more appropriate. On the broader scale, the results indicated that planning future sensor placements would be beneficial to avoid changing the fChl:Chl relation. Finally, this study may be used to provide a reference for future researchers and practitioners to justify their use, disuse, or adjustment of fChl as a proxy for Chl.

Acknowledgments

The U.S. Geological Survey (USGS) Next Generation Water Observing System and Integrated Water Availability Assessments supported the fieldwork, compilation of the data, and writing of this report. The authors of this report would like to thank the USGS staff whose work in maintaining USGS streamgages and collecting chlorophyll data made this report possible. The authors also express gratitude to the following contributors: Jenny Murphy (USGS), for help conceptualizing the report; Heather Krempa (USGS), for report supervision; Amanda Booth and Brianna Stanley (USGS), for providing insightful and thorough peer reviews; and USGS staff in the Science Publishing Network, for their editorial support and assistance with developing figures.

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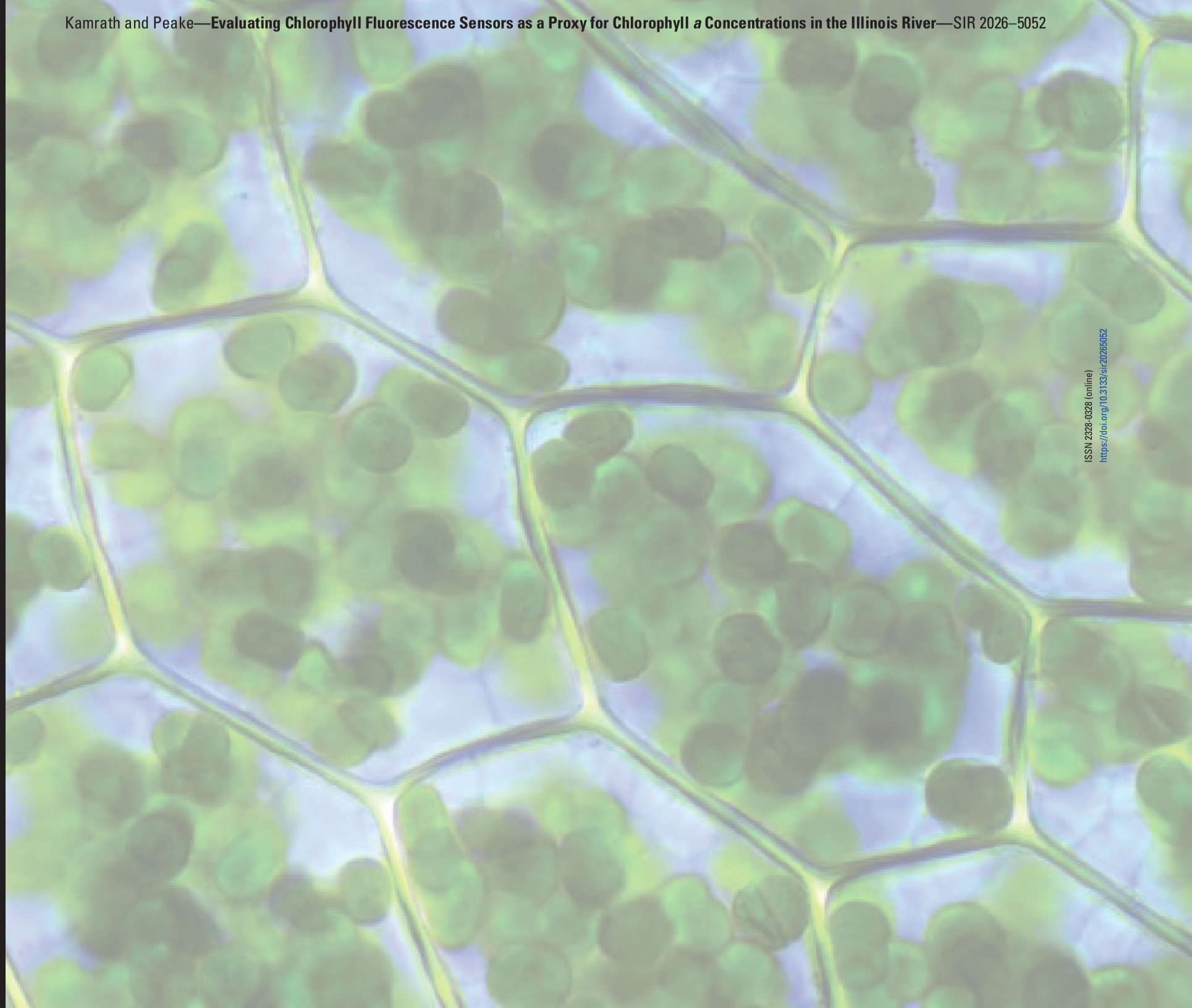
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ISSN 2328-0328 (online)
https://doi.org/10.3133/sir20265052