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Dear Dr. Hamdhani,

Thank you for submitting your manuscript:

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**Response to Reviewer Comments**

1. The rationale behind this study is not very clear. The authors mentioned that there are other in-vivo fluorometers available and their performances vary based on a number of factors: “Hand-held fluorometers to measure in vivo chlorophyll-a concentrations in the field vary greatly in their sensitivity, points of calibrations, and the number of excitation channels (Cremella et al., 2018). Some of them are equipped with integrated turbidity sensors and data loggers, while others lack these features. Publications about the efficiency of hand-held fluorometers suggest that their measurement methods are instrument-specific and vary greatly among brands and models due to variations in measured emission band-widths.” So, why specifically, The FluoroSense, hand-held fluorometer manufactured by Turner Designs was selected for this study needs to be explained clearly. A study based on this particular model is not making a lot of sense where clearly this type of instrument's performances vary greatly by different models.

   H: This is a good suggestion, but we think that this specific handheld fluorometer is a newly available tool in the market to measure chlorophyll-a in water and intended as an early warning device, this is the reason we interested to study for knowing its performance by comparing to a traditional method for those who potentially will use this handheld fluorometer.

2. The results of this study should have been found in the manual of this specific fluorometer. This study in its existing form doesn’t provide any additional scientific findings, or at least it is not clear from the current manuscript.

   H: We appreciate this suggestion, however based on the feedback from reviewer #2 and #3, we think that our study still provide some additional scientific findings, since in the manual of this handheld fluorometer does not provide information regarding potential effect of ambient light and turbidity, as well as comparison to a traditional method.

3. More robust study was needed to compare the results with the actual field condition. The sampling was done only once (October 2019) and then the samples were spiked with kaolinite clay mineral powder to vary the turbidity concentration. But in real field conditions there might be many other interferences based on the seasonal changes. If this study wants to propose a calibration equation that can be used widely, more field-based applications are needed to validate the equation.

   H: We appreciate this suggestion. We agree that in real field conditions there might be many other interferences based on the seasonal changes. Our study is a preliminary study of the specific handheld fluorometer, so at the end of the manuscript we have emphasized to suggest more extensive study to understand its performance better (including under seasonal changes)

4. The results suggest that the probe is not sensitive to ambient light and performed well at low chlorophyll-a concentrations (< 25 µg/L) across a range of turbidity levels (50 to 70 NTU). However, performance was lower when chlorophyll-a concentrations are > 25 µg/L and turbidity levels are <50 NTU. How this corresponds to different algal bloom conditions in real world scenarios need to be described. What is a typical chlorophyll-a concentration and turbidity condition of a eutrophic system (or systems) should be mentioned here. Then only the efficiency of this proposed calibration equation can be verified.

H: This is a good suggestion; we have added information regarding what Trophic status of a water body should match. We added the following statement:

"This result also suggests that in term of trophic category, this probe could work well at oligotrophic and mesotrophic water bodies (Istvánovics, 2010)"

5. Pricewise how this equipment compares with its competitive models? Also, same comparison is needed in terms of the efficiency.

H: We have added the following statement:

"Additionally, the FluoroSense is much cheaper than the equipment required by EPA's standard protocol (it costs only about $ 1,000.00 compared to almost $ 15,000.00 for standard fluorometer)"

6. Please provide the coordinates of both the lakes.

H: We have provided the coordinates of both lakes

7. Line 92: It should be EPA method 445.

H: We have made a correction as suggested

8. How many replicates were used for each analytical measurement?

H: We have made a clarification by stating: "There were 15 replicates for each treatment"

9. Table 1 should have standard deviation for each dataset.

H: We cannot use Standard Deviation because there were no multiple samples compared

10. Table 2 should have standard deviation for turbidity numbers.

H: We have added the Standard Deviation for turbidity in the table 2

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*Article*

# Performance of a Handheld Chlorophyll-*a* Fluorometer: Potential Use for Rapid Algae Monitoring

**Hamdhani Hamdhani *[1,2], Drew Eppehimer [1], David Walker [3] and Michael Bogan [1]**

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**Abstract:** Chlorophyll-*a* measurements are an important factor in water quality monitoring of surface waters, especially for determining trophic status and ecosystem management. However, collection of field samples for extractive analysis in a laboratory may not fully represent field conditions. Handheld fluorometers that can measure chlorophyll-*a* in situ are available, but their performance in waters with a variety of potential light interfering substances has not yet been tested. We tested a hand-held fluorometer for sensitivity to ambient light and turbidity, and compared these findings with EPA Method 445.0 using water samples obtained from two urban lakes in Tucson Arizona, USA. Our results suggest that the probe is not sensitive to ambient light, and performed well at low chlorophyll-*a* concentrations (< 25 µg/L) across a range of turbidity levels (50 to 70 NTU). However, performance was lower when chlorophyll-*a* concentrations are > 25 µg/L and turbidity levels are <50 NTU. To account for this discrepancy, we developed a calibration equation to use for this hand-held fluorometer when field monitoring for potential harmful algal blooms in water bodies.

**Keywords:** water quality; turbidity; ecosystem management; biomonitoring; freshwater

## 1. Introduction

In many water bodies, excess nutrient loading has contributed to proliferation of primary producers (e.g., phytoplankton) and resulted in eutrophication, which can directly reduce aquatic biodiversity (Elliott et al., 2006; Paerl and Huisman, 2009; Paerl and Otten, 2013; Zhu et al., 2017). However, harmful algal blooms can be controlled successfully with regular water quality monitoring to help guide appropriate remediation measures (Ibelings et al., 2014). Algae monitoring programs are necessary for tracking both aquatic and public health risks, and should include algal biomass estimation (Ibelings et al., 2014; Hollister and Kreakie, 2015).

Accurate measurement of chlorophyll-*a* is an important component of ambient monitoring programs in water bodies. The United States Environmental Protection Agency (EPA) has standards for measuring algae, but they rely upon an extractive analysis of chlorophyll-*a* concentration to estimate algal abundance (Arar and Collins, 1997). This extractive analysis (EPA Method 445.0) is time-consuming and involves collection, and potential preservation of field collected samples. An in vivo fluorometric method for the measurement of chlorophyll-*a* was proposed in the mid 1970s (Loftus and Seliger, 1975; Porter et al., 1977) but due to the expense of electronic miniaturization, never gained wide spread acceptance or use at the time. More recently, handheld probes have been devel-

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oped and refined for in vivo fluorometric measurement of chlorophyll-*a*. This methodology offers real-time measurement of chlorophyll-*a* without the need to collect samples for an extractive process (Salonen et al., 1999; Ghadouani and Smith 2005; Lu et al., 2020).

Hand-held fluorometers to measure in vivo chlorophyll-*a* concentrations in the field vary greatly in their sensitivity, points of calibrations, and the number of excitation channels (Cremella et al., 2018). Some of them are equipped with integrated turbidity sensors and data loggers, while others lack these features. Publications about the efficiency of hand-held fluorometers suggest that their measurement methods are instrument-specific and vary greatly among brands and models due to variations in measured emission bandwidths (Cremella et al., 2018).

The use of in vivo probes for field measurements also raises a number of questions concerning their precision in different environmental conditions. Many hand-held fluorometers available are sold without guidelines for their performance, including the potential need to measure other parameters (e.g., turbidity) to improve accuracy (Zamyadi et al., 2012). Previous studies demonstrate that in a natural aquatic environment, ambient light and turbidity could interfere with measurements of hand-held fluorometers (e.g., Vincent, 1981; Strass, 1990; Laney, 2010; Leeuw et al., 2013; Cui and Lv, 2014). For example, most hand-held fluorometers use dedicated light sources to illuminate a small volume of water that is passing through the measuring chamber. This technique may need to be shielded from ambient light to ensure the accuracy of the sensor's reading (Leeuw et al., 2013). Additionally, water turbidity can interfere with the transmission of the excitation wavelength and the cells' response to the probe. Although, some manufacturers recognize these limitations and recommend correction factors, many hand-held fluorometers require frequent validation with more robust methods (i.e., EPA Method 445.0) for better accuracy (Zamyadi et al. 2012; Leeuw et al., 2013).

We evaluated the performance of a newly available hand-held fluorometer (see Method section) under differing scenarios. Our goals were to: (1) explore its performance under different concentrations and assemblages of algae, (2) test its sensitivity to ambient light, (3) investigate the impact of turbidity on measurements, and (4) compare its measurements with those produced by EPA method 445.0 (Arar and Collins, 1997). If this hand-held fluorometer produces accurate measurements of chlorophyll-*a* concentrations across a wide range of environmental conditions, it could become a user-friendly and cost-effective tool in the monitoring of chlorophyll-*a* and harmful algal blooms.

## 2. Methods

### *2.1. Apparatus description*

The FluoroSense™ hand-held fluorometer (hereafter referred to as FluoroSense), manufactured by Turner Designs (Sunnyvale, California), detects the fluorescence of in vivo chlorophyll-*a* using excitation light from the fluorometer to excite chlorophyll-*a* within algal cells, causing them to fluoresce. The fluorescence is then detected and converted to a digital value and can then be correlated to a known concentration of chlorophyll-*a* in µg/L. Use of the FluoroSense probe is simple: submerge the probe into water and press the button for an immediate result. FluoroSense is factory-calibrated and capable of detecting algae concentrations within the range of 0-199 µg/L in vivo chlorophyll-*a*, with 1µg/L resolution. FluoroSense also is equipped with a shade cap intended to prevent ambient light interference during field measurements. The manufacturer claims that this instrument is intended as an early warning device to determine whether additional testing is required in a body of water. Additionally, the FluoroSense is much cheaper than the equipment required by EPA's standard protocol (it costs only about $ 1,000.00 compared to almost $ 15,000.00 for standard fluorometer).

The TD–700™ fluorometer, also manufactured by Turner Designs, is used in extractive chlorophyll-*a* quantification. Chlorophyll-*a* filtering and dissolving in acetone is required in this method before results can be read according to EPA Method 445 for in vitro

determination of chlorophyll-*a* in freshwater algae by fluorescence (Arar and Collins, 1997). Chlorophyll-containing phytoplankton in a measured volume of sample water are concentrated by filtering at low vacuum through a glass fiber filter. Chlorophyll-*a* is water-insoluble, but can be easily dissolved in organic solvents such as acetone. In 90% acetone, the pigments can be extracted from the phytoplankton with the aid of a mechanical tissue grinder to ensure thorough extraction of the chlorophyll-*a* (Arar and Collins, 1997).

### *2.2. Sampling and Laboratory Experiment*

We tested for differences in measurements between the FluoroSense probe and the TD-700 fluorometer under different environmental conditions using water samples from two urban, man-made lakes in Tucson, Arizona, USA (Figure 1). Lakeside Lake (5.7-hectare surface area at 32°11'10.1"N 110°48'58.8"W) and Silverbell Lake (5.3-hectare surface area at 32°17'05.0"N 111°01'55.0"W) both receive moderate recreational and fishing use and are fed by groundwater pumped to the surface via wells. The well that supports Silverbell Lake is influenced by treated wastewater recharge in the nearby effluent-dependent Santa Cruz River (Eppehimer et al., 2020) and Lakeside Lake also receives episodic runoff from Atterbury Wash, an ephemeral urban stream.


**Figure 1.** A) FluoroSense™ Handheld Fluorometer and B) TD-700™ Fluorometer (*source: Turner Designs*).


**Figure 2.** Water samples for analyses were collected from Lakeside Lake (A) and Silverbell Lake (B) in Tucson, Arizona (USA).

In each lake, we collected a 15-L composite water sample on October 5, 2019. Each composite sample consisted of five, 3-L grabs collected from different portions of the lake accessible from the shore. The composite samples were combined into a 5-gallon plastic water container, transported to the laboratory at the University of Arizona, and analyzed within 24 h. In the laboratory, each composite water sample was transferred into, and homogenized using, a mixing bucket. One L of homogenized water from each lake was

used for taxonomic analyses of algae. This taxonomic subsample was then transferred to a 1-L glass beaker and stirred with a magnetic stir bar for 1 min prior to pipetting out 1 mL of sample. Phytoplankton samples were read using an Olympus BH2 phase-contrast microscope and Sedgewick-Rafter (S-R) counting chamber (Wehr et al., 2015). The S-R cell was 10 $cm^2$. Both strip and field counts were performed and units/$cm^2$ calculated (Stevenson and Bahls,1999).

We tested the performance of the FluoroSense probe on water from both lakes at three different algal concentrations, under light and dark conditions, and under four different levels of added turbidity. We obtained 600 mL subsamples from the homogenized samples collected from each lake. These subsamples were subjected to the following treatments. To achieve three algal concentrations, we used (1) the original water collected from each lake, (2) a dilution with one-third distilled water, and (3) a dilution with two-thirds distilled water. Hereafter, these treatments are called *high*, *medium*, and *low* concentrations of algae, respectively. We selected these treatments after testing the undiluted samples to be sure that all treatment concentrations would be within the detection range (0-199 µg/L) reported by the FluoroSense manufacturer. Next, four turbidity treatments were created from undiluted subsamples (i.e., ambient algal concentrations) from each lake by adding kaolinite clay mineral powder. We added 0 (ambient turbidity), 0.01, 0.02, and 0.03 g of kaolinite clay (Cremella et al., 2018; Chang et al., 2011) into the same algal concentration treatment subsamples to create the four added turbidity treatments (*none*, *low*, *medium*, and *high*, respectively). There were 15 replicates for each treatment. The exact turbidity NTU in each replicate and treatment after clay powder addition was measured with a Sper Scientific 860040 Turbidity Meter (Sper Scientific).

After all treatment replicates were created, we used the FluoroSense to measure chlorophyll-*a* under the two different light treatments for all three algal concentration treatments and all four turbidity treatments. Dark treatment measurements were made in a completely dark chamber, while light treatment measurements were made under bright light (5500 Lux) generated from two LED lamps positioned at 45º angles, which simulated the intensity of mid-day outdoor light at the two lakes we sampled. Finally, we examined how well the FluoroSense probe compared to the EPA Method 445.0 approach under these varying algal concentrations and turbidities. For measurements using the TD-700, we filtered 20 mL subsamples from each treatment, and then extracted filtered algae in 10 mL of acetone for each subsample, as recommended in the TD-700 user's manual. Finally, we compared measurements of chlorophyll-a using the FluoroSense probe and the TD-700 for the same replicate samples from each lake under the three different algal concentrations and the four different added turbidity treatments.

Prior to testing, all instruments were calibrated using manufacturer-recommended procedures. Rhodamine dye 100µg/L was used for the FlouroSense and chlorophyll-*a* Solid Secondary Standard (P/N 7000-994) was used for the TD-700. Additionally, the TD-700, was zero-adjusted using acetone. Finally, the turbidity probe was calibrated using two points: the zero point was calibrated with 0 NTU solution and the second point was calibrated using a 100 NTU solution provided by the manufacturer.

### 2.3. Statistical Analyses

The FluoroSense readings under three different concentrations (with no added turbidity) in ~~in~~ the dark and light treatments were compared using paired t-tests using Stata Version 15.1 (StataCorp, 2017). Histogram analysis indicated that there were no outliers in the dataset and all variables were approximately normally distributed (data not shown). We used $\alpha$=0.05 as a threshold to identify statistical significance. To investigate the performance of the FluoroSense probe under different turbidity treatments across both lakes, an ANOVA ~~multiple regression~~ was run in R version 3.5.1 (R Core Team, 2019). The FluoroSense readings and turbidity measurements were included as independent variables for predicting the dependent TD-700 values, including the interaction between them.

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~~This approach aimed to determine if the hand-held fluorometer could reliably predict the measurements produced by EPA Method 445.0.~~ Light treatments were ~~not~~ included in the ~~regression model~~ ANOVA. ~~because they were not found to affect the probe's chlorophyll-a measurements (see below~~, and ~~).~~ ~~l~~Lake identity (Lakeside vs Silverbell) was ~~initially~~ also considered as a ~~model~~ factor to account for potential differences in probe performance in waters from differing algal assemblages. ~~We used ANOVA with light, turbidity, chlorophyll *a* concentrations, and lake identity.~~ Based on the paired t-test and the ANOVA results (see Results), we used a simplified Linear Regression model (run in R version 3.5.1) to provide calibration equation. This simplified equation removed light because it was not found to affect the probe's chlorophyll-a measurements~~,~~ W~~w~~e also removed lake identity~~,~~ so this equation could apply to a variety of environments. ~~However, lake identity was excluded after we found that the responses to light, turbidity, and chlorophyll *a* concentrations were not statistically different between the two lakes~~. This approach aimed to determine if the hand-held fluorometer could reliably predict the measurements produced by EPA Method 445.0.

## 3. Results

### *3.1. Algal Taxa and Concentrations in Lakeside and Silverbell Lakes*

Lakeside Lake supported a more diverse algal assemblage, with 8 genera in 4 phyla at relatively low to moderate concentrations, including two cyanobacteria taxa (Table 1). In contrast, we only detected a single algal taxon in Silverbell Lake, the cyanobacteria *Microcystis*, and it was found in relatively high concentrations. Chlorophyll-*a* readings (µg/L) under both measurement approaches (FluoroSense and TD-700) were roughly twice as high in subsamples from Silverbell Lake when compared to those from Lakeside Lake (Table 1, Figure 3). ANOVA results suggested that these different algal assemblages impacted the FluoroSense performance (Table S1). However, a detailed investigation of these impacts was beyond the scope of this study.

**Table 1.** Algal taxa identified in 1L samples collected from Lakeside and Silverbell Lakes, including the concentrations of individual taxa reported in units/mL.

| Phylum | Genus | Quantity (units/mL) |
|---|---|---|
| | Lakeside Lake | |
| Chlorophyta | *Dictyosphaerium* | 3,400 |
| Chlorophyta | *Chlamydomonas* | 3,000 |
| Chlorophyta | *Scenedesmus* | 2,800 |
| Pyrrophyta | *Gymnodinium* | 2,200 |
| Chrysophyta | *Fragilaria* | 2,000 |
| Chrysophyta | *Cymbella* | 800 |
| Cyanobacteria | *Microcystis* | 400 |
| Cyanobacteria | *Oscillatoria* | 200 |
| | Silverbell Lake | |
| Cyanobacteria | *Microcystis* | 32,600 |

**Table 2.** Paired t-test results for FluoroSense readings under light and dark conditions at three levels of algal concentration dilutions and three turbidity treatments. The mean, standard deviation (Std. Dev.), and minimum (min) and maximum (max) FluoroSense chlorophyll-a readings (µg/L) are provided for each dilution series (n= 15 for each series), and exact turbidity measurements (NTU) are provided for the three turbidity treatments as well. Significant t-test results are highlighted in bold with an asterisk.

| Sample origin | Algal concentration | Turbidity added | Turbidity | | Light treatment | | | | Dark treatment | | | | t test (p-value) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | (NTU) | Std. Dev. | Mean | Std. Dev. | Min | Max | Mean | Std. Dev. | Min | Max | |
| **Lakeside Lake** | High | None | 21.48 | 0.18 | 71.00 | 3.08 | 67 | 74 | 66.20 | 3.27 | 62 | 71 | >0.05 |
| | | Low | 45.39 | 1.61 | 66.00 | 2.45 | 63 | 69 | 63.60 | 2.97 | 59 | 67 | >0.05 |
| | | Medium | 61.00 | 1.58 | 63.80 | 1.79 | 62 | 66 | 62.40 | 3.65 | 58 | 67 | >0.05 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | High | 82.20 | 2.86 | 51.20 | 1.30 | 49 | 52 | 54.00 | 2.74 | 50 | 57 | >0.05 |
| | Medium | None | 16.65 | 0.74 | 47.00 | 2.55 | 44 | 50 | 50.60 | 5.86 | 43 | 57 | >0.05 |
| | | Low | 42.64 | 0.48 | 44.00 | 3.55 | 40 | 48 | 44.80 | 3.56 | 39 | 48 | >0.05 |
| | | Medium | 72.00 | 1.87 | 42.00 | 2.24 | 39 | 45 | 40.40 | 1.67 | 39 | 43 | >0.05 |
| | | High | 90.80 | 1.30 | 34.60 | 1.52 | 33 | 37 | 33.20 | 1.30 | 32 | 35 | >0.05 |
| | Low | None | 12.13 | 1.19 | 34.40 | 2.07 | 31 | 36 | 32.60 | 2.70 | 28 | 35 | <0.05* |
| | | Low | 34.00 | 0.38 | 29.20 | 0.84 | 28 | 30 | 28.20 | 1.10 | 27 | 30 | >0.05 |
| | | Medium | 52.80 | 0.84 | 30.20 | 1.64 | 28 | 32 | 28.40 | 1.52 | 27 | 30 | >0.05 |
| | | High | 71.00 | 0.71 | 25.00 | 2.00 | 23 | 27 | 26.20 | 1.10 | 25 | 27 | >0.05 |
| **Silverbell Lake** | High | None | 12.28 | 0.46 | 151.80 | 8.24 | 139 | 170 | 148.60 | 7.24 | 133 | 156 | >0.05 |
| | | Low | 34.03 | 0.46 | 136.07 | 7.37 | 122 | 150 | 136.67 | 6.86 | 126 | 148 | >0.05 |
| | | Medium | 56.60 | 0.55 | 121.67 | 5.84 | 114 | 134 | 125.13 | 7.38 | 112 | 138 | >0.05 |
| | | High | 89.00 | 1.00 | 112.73 | 3.71 | 105 | 118 | 111.33 | 5.02 | 104 | 124 | >0.05 |
| | Medium | None | 4.408 | 0.08 | 91.93 | 7.31 | 78 | 102 | 88.80 | 5.28 | 77 | 100 | >0.05 |
| | | Low | 35.68 | 1.05 | 83.47 | 3.58 | 78 | 90 | 82.40 | 5.05 | 74 | 89 | >0.05 |
| | | Medium | 61.40 | 0.55 | 75.73 | 4.25 | 71 | 85 | 72.20 | 6.06 | 66 | 85 | >0.05 |
| | | High | 97.80 | 1.48 | 64.67 | 6.22 | 55 | 76 | 62.67 | 4.43 | 55 | 70 | >0.05 |
| | Low | None | 3.08 | 0.12 | 51.53 | 6.00 | 41 | 63 | 47.33 | 5.42 | 39 | 61 | >0.05 |
| | | Low | 46.16 | 0.32 | 43.07 | 2.76 | 39 | 48 | 43.47 | 4.75 | 37 | 50 | >0.05 |
| | | Medium | 95.00 | 1.58 | 33.93 | 4.18 | 29 | 43 | 31.73 | 3.73 | 26 | 41 | <0.05* |
| | | High | 118.00 | 1.00 | 34.47 | 2.29 | 30 | 38 | 33.27 | 2.60 | 30 | 38 | >0.05 |

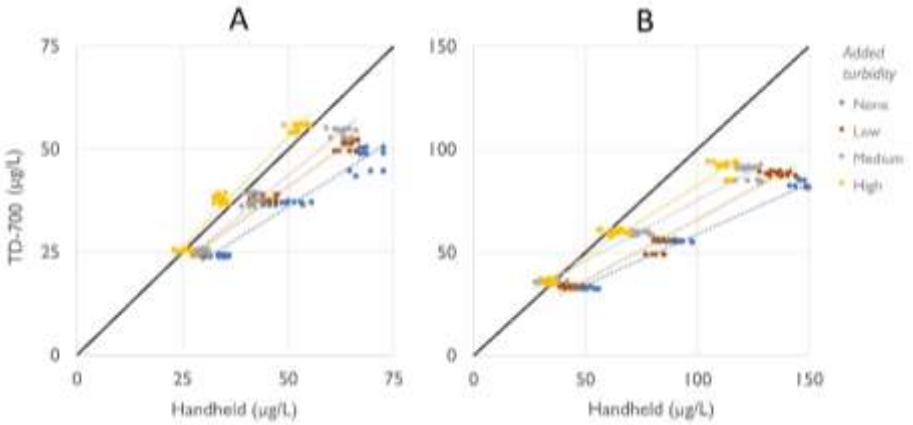


**Figure 3.** Comparison between extracted total chlorophyll-a using TD-700 versus chlorophyll-a estimated from in vivo measurements using the FluoroSense probe across different added turbidity levels [none (blue), low (brown), medium (grey), and high (yellow)] and three different sample dilutions from Lakeside and Silverbell Lake water samples (panels A and B, respectively). For all panels, the solid black line illustrates a 1:1 relation between the two measurement techniques.

### 3.2. Sensitivity to Ambient Light

FluoroSense chlorophyll-*a* measurements generally were not affected by light across the wide range of dilutions and turbidity treatments that we tested (Table 2~~1~~; Table S1). Only two of the 24 treatment combinations resulted in significant differences between light and dark conditions. Both of these significant results occurred under the low algal concentration treatments of Lakeside and Silverbell Lakes, with one occurring under no added turbidity and the other occurring under the medium turbidity treatment.

### 3.3. Sensitivity to Turbidity

Linear regression illustrated that chlorophyll-*a* estimations between the two methods were closest for the high turbidity treatment (i.e., fell closest to the 1:1 line) but grew farther apart with decreasing turbidity (Figure 3). These results suggest the FluoroSense probe overestimates chlorophyll-*a* concentrations in low turbidity situations. Additionally, measurements between the two methods were closer to the 1:1 line at lower chlorophyll-*a* concentrations than at higher concentrations. This pattern occurred in subsamples from both lakes, but was especially pronounced in samples from Silverbell Lake, which had much higher ambient algal densities of *Microcystis* (see Table 1) and higher concentrations of chlorophyll-*a* (Figure 3). Both the Fluorosense reading and turbidity level (NTU) were significant, ~~factors~~ had the largest F values in the ANOVA (Table S1), and were included in the final regression model (Table 3). Overall, our testing within the range of 25-150 µg/L chlorophyll-*a* across subsamples from both lakes resulted in a final model with the following Equation (1):

$$TD\text{-}700\ Chl\text{-}a = 1.7962 + (0.5897 * FluoroSense\ Chl\text{-}a) + (0.1862 * Turbidity) \quad (1)$$

Where *TD-700 Chl*-a is the predicted chlorophyll-a concentration in µg/L using EPA Method 445.0, *FluoroSense Chl-a* is the chlorophyll-a reading using the Fluorosense in µg/L, and the *Turbidity* is the ambient known turbidity in NTU. This model performed well, explaining~~ed~~ 94% of the variation in TD-700 readings across all samples from Lakeside and Silverbell Lakes. Observed vs expected residuals matched closely across the range of tested concentrations (Figure S1).~~.~~

**Table 3.** Multiple linear regression model for predicting TD-700 readings using FluoroSense readings and turbidity measurements across three algal concentration and turbidity treatments from Lakeside and Silverbell Lakes ($R^2 = 0.94$).

| | Coefficients | Std. Error | t value | p-value |
|---|---|---|---|---|
| Intercept | 1.796 | 0.563 | 3.189 | 0.0015 |
| FluoroSense reading | 0.590 | 0.005 | 107.204 | < 2e-16 |
| Turbidity | 0.186 | 0.006 | 30.565 | < 2e-16 |

## 4. Discussion

Studies examining the performances of low-cost portable fluorometers suggest that sensitivity to light during daytime deployment can be a primary limitation, with detectors easily becoming saturated by ambient light (Rovati and Docchio, 1999; Leeuw et al., 2013). As a result, two methods were suggested to reduce the light sensitivity of probes: (1) modulate the light source and apply a high frequency filter as part of detection circuit and (2) create a flow through system that excludes ambient light (Leeuw et al., 2013). The FluoroSense takes the latter approach, with a cap at the bottom tip of the unit that aims to prevent ambient light penetration. Our results indicate that the FluoroSense cap does block ambient light and that the probe can confidently be used for daytime field measurements, even in the bright conditions.

Turbidity can introduce errors into the measurements of fluorescence probes, leading to overestimating (e.g., Leeuw et al., 2013; Cui and Lv, 2014; Cremella et al., 2018) or underestimating of the actual fluorescence readings (e.g., Brient et al., 2008; Zamyadi et al. 2012). These errors likely arise due to the light scattering, so the optical configuration of the fluorescence probe may cause different responses to turbidity (Zamyadi et al. 2016). In our study, the added inorganic mineral turbidity treatments most likely reduced the FluoroSense's signal, leading to decreased estimations in chlorophyll-*a* values. Interestingly, this pattern almost seemed to correct for the probe's tendency to overestimate chlorophyll-*a*, such that the high turbidity treatments (~70 NTU) were closest to the 1:1 line, especially at lower ambient concentrations of algae (~25 µg/L of chlorophyll-*a*) (Figure 3). Whether this tendency to overestimate values is intentional to the design of FluoroSense is unknown, but it results in a probe that works better under some of the higher turbidity

situations that could be encountered in the field. Although we did not test the probe's performance on samples from lotic ecosystems, mean chlorophyll-*a* values in temperate streams tend to be low (~27 µg/L), even during the high productivity summer period (Van Nieuwenhuyse and Jones, 1996). Our finding of better performance under higher turbidities (50-70 NTU) and lower algal concentrations (<25 µg/L) suggests that the Fluorosense probe could work well in streams where these conditions are frequently encountered. This result also suggests that in term of trophic category, this probe could work well at oligotrophic and mesotrophic water bodies (Istvánovics, 2010)

We tested the effect of turbidity emanating from inorganic fine kaolinite clay. Different grain sizes of suspended sediment causing turbidity may affect the performance of hand-held probes differently than what was quantified in this study. One study revealed that smaller particle sizes result in higher reductions of florescence intensities when compared to measurements made in samples with the same mass of sediment, but larger particle sizes (Brient et al., 2008). Optical interference in fluorometer readings may also originate from dissolved organic compounds of different colors. For example, tannins from leaves emit florescence in a wide spectrum of wavelengths (Hudson et al., 2007; Cremella et al., 2018), and could interfere with probe measurements. More research is needed to understand the responses of the FluoroSense probe to colored dissolved organic compounds or sediments of different origin and grain size than what we examined in this study.

One concern about hand-held fluorometers is how well they perform across a range of sampling locations that vary widely in algae concentrations and taxonomic composition. Although we only tested water from two lakes in this study, algal assemblages and concentrations differed markedly between them, with one supporting a diverse assemblage (8 genera) –at lower concentrations (14,800 units/mL) and the other supporting only cyanobacteria at higher concentrations (32,600 units/mL) (Table 1). ~~Despite~~ Although there were differences in the FlouroSense's performance between these two lakes~~these differences~~, our results suggest that ~~FluoroSense's~~ its measurements, and its response to different light, turbidity, and algal dilution treatments, were ~~similar~~ reliable ~~between~~ across the two study lakes. The FlouroSense probe may overestimate chlorophyll in cyanobacteria, and further research is needed to identify ~~the complexities of the~~the exact relationships between algal assemblages and the readings of handheld fluorometers. Additionally, FluoroSense worked well in Silverbell Lake, which was dominated by the potentially harmful cyanobacteria (*Microcystis*). This taxon is a management concern due to its wide range of potential adverse health effects (e.g., Pip and Bowman, 2014; Yuan et al., 2014), so it is important that the probe works well to estimate concentrations of cyanobacteria.

## 5. Conclusions

Our testing of the hand-held FluoroSense probe showed that, as an in situ instrument, it is not sensitive to ambient light, but that it overestimates chlorophyll-*a* concentrations at lower inorganic turbidity levels and higher ambient algal concentrations. However, our regression model was able to adjust for these limitations within the range tested (25–150 µg/L). In these situations, FluoroSense can be used as a fast, simple, and easy method in monitoring algal biomass for determining trophic status and ecosystem management. Future studies evaluating FluoroSense or other hand-held fluorometers should address how they are affected by organic turbidity and colored dissolved organic matter, and also test their performance in measuring very low chlorophyll-*a* concentrations that were not assessed in our study.

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