ASSESSING AN IN-SITU TOTAL ALGAE FLUOROMETER FOR PERFORMANCE IN FRESHWATER ESTUARIES

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INTRODUCTION

Every living thing needs water. Whether it be for humans or wildlife, drinking or bathing, water is a basic need for living organisms. It should come as no surprise then that there is an ever increasing demand to understand this vital resource and monitor its health. Freshwater systems are of particular interest, as they are often directly important to societal function. Historically, freshwater resource monitoring has required going out into the field, grabbing a bottle of water, and bringing it back to a lab to run tests on it. This general method is referred to as *ex-situ* monitoring, meaning "from the site." However, new methods for monitoring water quality at the site (or *in-situ*) have begun to rapidly develop in recent years.

The monitoring of water quality *in situ* has become more complex and more widely distributed with the emergence of new, less expensive sensor technologies. While grab sample, single time point measurements still remain relevant due to their accuracy and consistency with prior methods, scientists, agencies, and long-term research sites hope to augment these water quality monitoring strategies with the advanced sensor technologies that are becoming more accessible.

In-situ sensors offer some key advantages that water quality monitors find appealing, the biggest advantage being the reduction in time, money, and labor per measurement. Grabbing water samples for analysis requires time to go out into the field, obtain the sample, and do a full analysis in the lab. Auto samplers can be installed at high frequency sampling sites, but these setups are expensive and still require human hands for

lab analysis. Meanwhile, *in-situ* sensors can be left in the field for weeks and sometimes months without human intervention ("YSI", 2015). Some sensors can also be arranged to transmit live feed of field parameters via satellite hookup. These added conveniences make *in*-situ sensors an attractive option for high-frequency sampling, low accessibility, resource-strapped monitoring sites.

Old Woman Creek (OWC) is one of the few intact freshwater estuary systems remaining on Lake Erie. Since its designation as a National Estuarine Research Reserve (NERR) in 1980, OWC has been monitoring baseline conditions of its estuary ecosystem via the System-Wide Monitoring Program (SWMP). SWMP is part of a coordinated effort at 28 other NERRs, all monitoring a standard set of water quality parameters to inform long-term patterns in water quality in natural estuaries across the United States ("OWC: Research", 2020). One of these long-term datasets is that of algal biomass, measured every two weeks at OWC using grab samples from each of the four SWMP sites located in the estuary and its watershed. The algal biomass is estimated using an acidified spectrophotometric method for measuring extracted chlorophyll-*a* concentration as a proxy for total algal biomass (Arar, 1997).

Estimating total algal biomass is important because phytoplankton form the base of the food web in Old Woman Creek and many other freshwater systems (Hernendorf et al., 2006). This makes monitoring total algal biomass a key priority, as changes to this vital food source can have rippling effects throughout the entire food web. Additionally, monitoring algal biomass can help inform us of freshwater systems' responses to human activity. For example, algae of all kinds have been shown to respond to changing nutrient

availability. Agricultural land use often results in excess nutrients being added to stream systems, causing eutrophication and algal blooms in the streams themselves and the downstream systems like Old Woman Creek's estuary (Hernendorf et al., 2006). These connections make monitoring the algal community an important mid-step in understanding the impact of human activity on freshwater ecosystems and food webs.

In addition to grab sample spectrophotometry, all SWMP sites are equipped with a YSI EXO-2 water quality monitoring data sonde. The EXO-2 is comprised of a main body with multiple slots for YSI probes that measure specific parameters ("EXO2", 2020). The sondes at SWMP sites currently measure pH, temperature, turbidity, conductivity, and dissolved oxygen concentration (DO), with some NERRs also adding probes for monitoring salinity and fluorescent dissolved organic matter (fDOM). These parameters are measured every 15 minutes and either downloaded manually every two weeks or transmitted directly to the research station and uploaded to the SWMP website ("OWC: Research", 2020).

In recent years, the NERRs have been considering the addition of a total algal biomass probe to the EXO-2 units. The probes use fluorometry to estimate chlorophyll-*a* concentration, beaming an LED light into the water and reading the fluorescence signal that returns ("EXO Total Algae", 2020). This would offer a quicker approach to measuring algal biomass and allow for much greater data frequency at the SWMP sites compared to the classic grab sample spectrophotometry method.

However, before the NERRs allocate funding to purchase and maintain the probes, the sensor driven data need to be validated against the existing spectrophotometry method for comparability. YSI has conducted extensive work that shows the probes function well in a lab setting, but there is still potential for site-specific environmental parameters to impact the algal measurements.

A preliminary study across several NERRs, including OWC, developed correlational models comparing the measurements from the *ex-situ* extracted chlorophyll-*a* method with the *in-situ* EXO-2 total algal probe measurements. Interestingly, the NERRs in marine estuaries had exceptionally better performance than the two freshwater NERRs when comparing spectrophotometry chlorophyll-*a* measurements to fluorometry probe chlorophyll-*a* measurements ("NOAA", 2020). Interferences from environmental parameters, specifically temperature, turbidity, and colored dissolved organic matter (CDOM) were cited from previous studies of similar sensors as potential reasons for the difference in comparison models.

I set out with the goal of understanding the reasons for the lack of performance of the YSI Total Algae probe at OWC. Additionally, I wanted to develop a strong comparison model to relate historical chlorophyll-*a* measurements from spectrophotometry with new measurements from the YSI probes, much like the NERRs were already seeing with marine estuary results. I hypothesized that temperature, turbidity, and CDOM all had interference effects on the probes in OWC. In order to develop this overall correction model, I attempted to identify which of these environmental parameters were impacting the performance of the YSI Total Algae probes

at Old Woman Creek and develop individual correctional coefficients for the effect of each interfering parameter (Fig 2).

METHODS

Field Methods

To assess the performance of the YSI Total Algae probe in Old Woman Creek, I used both field and laboratory based approaches. In the field, I analyzed the longterm record of paired data points for which spectrophotometry and fluorometry probe methods were used simultaneously during the 2016-2018 monitoring season. During this field test, a YSI Total Algae probe was added to an EXO-1 data sonde, along with temperature, turbidity, and fDOM probes. fDOM sensors have been shown to act as a strong proxy for measuring CDOM, and work on the same fluorometric measurement principle as the total algae sensors (Norelli & Smith, 2014). This EXO-1 was added to the regular SWMP schedule of collecting grab samples for spectrophotometry every two weeks until the estuary freezes over, around November. However, to get a wider sample of the algal community at OWC, grab samples and sonde data were collected at five sites, as opposed to the original four (Fig 3).

During each sampling event, the EXO-1 was fully submerged for 5 minutes at the site location and measurements were recorded every minute. Sonde readings were averaged over the five minute time interval of each collection to produce a single sonde reading to pair with its in-lab extracted chlorophyll-*a* reading. Grab samples (1L) were collected at the midpoint of this time window as close to the sonde as possible. The grab samples were returned to the lab, where they were filtered through 0.45 µm glass fiber

filters. The filters were then placed in buffered acetone solution and left in the freezer for one week to allow for full chlorophyll-*a* extraction before absorbance was read on the spectrophotometer (665 nm).

Laboratory Methods

I assessed the possible interferences from environmental conditions using a series of laboratory experiments where I manipulated temperature, turbidity, and CDOM. My procedure takes much guidance from Watras et al. (2011 and 2017), who developed a temperature correction model for fDOM and phycocyanin sensors respectively and Norelli & Smith (2014) at North Inlet-Winyah Bay NERR, assessing interferences of temperature, turbidity, salinity, and chlorophyll-*a* concentration on fDOM sensors.

To develop a correction for temperature interference, I conducted two tests under controlled conditions in the lab setting. In the first test, a 10L sample of deionized (DI) water was placed in a 20L HDPE carboy and chilled to ~5º C. The carboy was then removed, covered with opaque plastic to prevent incident light from interfering with the fluorometers, and placed on a heated magnetic stirrer. The EXO-1 was placed into the carboy so that the sensor faces sat at mid-depth in the water. The stirrer was set to a constant, low-speed setting to keep the sample homogenized. The EXO-1 was set to take readings of all parameters every 15 seconds as the water heated up from 5º C to 25º C over 4 hours, the general range of temperatures historically recorded in the estuary. This DI sample allowed us to see the impact on fluorescence with no source of chlorophyll-*a* present. The same chilling and heating was repeated with an unfiltered 10L sample from

the lower estuary site in OWC (Fig 3). This live sample represents the effect of temperature on the probe's readings in the estuary when all other factors are held constant, allowing for a simple linear relationship to be drawn between temperature and chlorophyll-*a*.

To develop a correction for turbidity interference, 10L of DI and live samples were again collected, placed in the carboy, and homogenized continuously with a stirring plate. However, the temperature was held constant at 20°C while sediment was added in serial amounts to develop the turbidity range of values. The sediment added was collected from the same site as the water samples, dried, sieved, and combusted in a muffle furnace at 500º C for 6 hours to remove all organic compounds that may have an interaction with the sensor (i.e. CDOM). This inorganic sediment was serially added to the samples in 0.5 g doses, to develop the more common turbidity range seen in the estuary of 0-100 FNU, before raising the additions to 5 g each to develop the 100-600 FNU range seem during and following storm events. After each addition, the sediment was allowed to homogenize for 5 minutes and after which turbidity and chlorophyll-*a* measurements were made. Again, a live sample was used to determine the relationship between turbidity and chlorophyll-*a* with algae present.

To develop a correction for CDOM interference, I followed an analogous procedure to turbidity, replacing serial additions of sediment with 5 ml serial additions of a CDOM-rich solution and measuring fDOM as a proxy for CDOM. The solution was created by collecting partially decayed leaves from the forest surrounding the estuary, placing them in a large cylinder with DI water, and allowing the leaves to leach CDOM

compounds into the water. This CDOM-rich water was filtered through a 0.45 µm glass fiber filter to create the final CDOM addition solution. My results indicate that the filtering procedure did not remove small particles and colloids, causing turbidity to increase along with fDOM. Thus I was not able to develop a simple linear relationship between CDOM fluorescence and chlorophyll-*a* readings.

Statistical Analysis

I used simple linear regression to determine the effect of temperature and turbidity on chlorophyll-*a* measurements and extracted the resulting slope terms from the live sample tests as potential correction coefficients. The live sample slope terms, rather than the DI sample slope terms, were used to best simulate and correct for interferences actually present within the estuary. *In-situ* sensor readings of chlorophyll-*a* (µg/L) from the 2016-2018 dataset were then corrected to account for the potential effects of temperature and turbidity using equation 1. To find the most parsimonious correction model, I also calculated corrected chlorophyll-*a* concentrations that accounted for just temperature or turbidity alone, as well as the natural log of temperature alone.

To test different correction models, I conducted simple linear regression between Chl_corr and field-collected chlorophyll-*a* measured via spectrophotometry from the same location and time. I then compared models with different predictor variables (i.e., Chl_corr, raw sensor chlorophyll-*a*) using Akaike Information Criterion (AIC) tests. The models were applied to two separate datasets for which I had complete data for

spectrophotometry chlorophyll-*a*, fluorometry sensor chlorophyll-*a*, temperature and turbidity. The first dataset was the full dataset, which included 163 samples across all sites of the estuary, during all times of the 3 years of sampling. The second dataset was the best-case dataset. This dataset is a subset of the full dataset, focused on constraining the 2016-2018 samples used to those most similar to the live samples used for the individual parameter lab tests. All 22 samples of the best-case dataset come from the same site (Fig 3), season (summer), and spectrophotometry chlorophyll-*a* range (<60 µg/L) as the live samples used for the lab tests. This best-case dataset was included to assess the sensitivity of the correction models to changes in estuary location, time of year, and total algal biomass.

RESULTS

Laboratory Results

In the laboratory studies, I was able to develop correction models for temperature and turbidity in both DI and live sample tests. Controlled increases in turbidity and temperature caused linear changes in apparent chlorophyll-*a*, with potential for a logarithmic change in the case of temperature (Fig 4C). DI sample correction slopes were much smaller than live sample correction slopes. Apparent chlorophyll-*a* decreased by ~0.5 µg/L over the full range of temperature values in the DI sample test, while chlorophyll-*a* decreased by ~15 µg/L over the same temperature range (Fig 4A & C). Apparent chlorophyll-*a* increased by ~2.5 µg/L over the full range of turbidity values in the DI sample test, while apparent chlorophyll-*a* decreased by ~20 µg/L over the same turbidity range (Fig 4B & D). The live sample logarithmic temperature model followed a similar pattern to its linear counterpart, explaining slightly more variation (r^2=0.71) than the live sample linear temperature correction model (r^2=0.69)(Fig 4C). It is important to note that due to the laboratory test design, there are substantially more data points for the temperature tests compared to the turbidity tests (Fig 4).

Statistical Analysis Results

I created multiple correction models to compare against the uncorrected linear relationship between *ex-situ* spectrophotometry chlorophyll-*a* and *in-situ* fluorometry

sensor chlorophyll-*a* as well as test the relative sensitivity of these correction models to inclusion of turbidity and temperature.

Despite testing multiple models on two different datasets, no correction model did a significantly better job of explaining variation when compared to the uncorrected base relationship of the dataset used (Table 1). In the case of the full dataset, the turbidity only correction model did a slightly better job of explaining variability, but this was only slightly better than the uncorrected model (ΔAIC=0.5) (Table 1, Fig 5A & B). Similar results were observed for the temperature only correction model, which was the lowest AIC value model for the best-case dataset, but was only slightly lower than the uncorrected model (ΔAIC=0.5) (Table 1, Fig 5C & D). Overall, the uncorrected and corrected sensor and spectrophotometry chlorophyll-*a* measurements were in stronger agreement for the best-case dataset.

However, all models, corrected and uncorrected, of the best-case dataset showed significant improvement in variation explained ($r^2 \approx 0.66$) compared to the full dataset models ($r^2 \approx 0.42$) (Table 1). It is important to note that sample size differed greatly between datasets, as the best-case dataset (n=22) is a subset of data from the full dataset (n=162).

DISCUSSION

There is still much to be understood about the field behavior of *in-situ* fluorometers, no matter what they are measuring. YSI has done extensive work to produce the most accurate instruments possible, but even they admit that calibration to specific field sites and constant consideration of environmental interferences are a must ("YSI", 2015). My research highlights a few key areas of focus for future probe assessments and deployments at freshwater NERRs and beyond.

Incident Light Refraction

The individual correction models point out a concern of this particular sensor assessment; the interference from environmental parameters, like temperature and turbidity, behave differently in DI water compared to wetland water. In the case of turbidity, the relationship was actually flipped from positive to negative when going from DI to live samples (Fig 4B & D). This turbidity result was most likely due to incident light refraction, where the fluorometer's excitation light simply has something additional to bounce off of, producing a false positive measurement when the refracted light hits the photodetector.

Total algae fluorometers work by emitting an excitation light signal from a source on the sensor. The excitation wavelength of light reacts to the chlorophyll-*a* molecule, causing it to fluoresce and emit light in a small range of different wavelengths. A

photodetector, equipped with an optical filter to cancel out false signals from excitation light, detects these fluorescence wavelengths and uses the intensity of the fluoresced light to calculate a concentration of the chlorophyll-*a* molecule ("EXO Total Algae", 2020). Unfortunately, simple refraction of light off of other particles in the water can also alter the wavelength of the excitation light (Beeson, 2000). The photodetector can then detect the refracted light if it happens to fall in its specified signal range, bypassing the filter, and producing a false signal of chlorophyll-*a*.

In my lab assessment, incident light refraction likely caused a false positive signal as inorganic sediment was added throughout the turbidity tests. Incident light refraction has been recognized in previous studies as an effect, but it is often ignored as it usually has only a minor or undetectable impact on the sensor's performance (Watras et al., 2017). Incident refraction should always be considered as an effect, but in the case of my assessment, the impact on sensor readings is far outweighed by the attenuation effect in normal live samples (Fig 4B & D).

Concentration Effect

Chlorophyll-*a* measurements also showed different relationships to changes in temperature between the DI and live sample tests. While both relationships were negative, the attenuation effect of temperature increase was much greater in the live sample compared to the DI sample (Fig 4A & C). This result points to another compounding effect to be considered in correcting sensor measurements: chlorophyll-*a* concentration. Previous studies have found that the attenuation effect from increased

temperature is stronger at higher concentrations of chlorophyll-*a*. Watras et al. (2017) found, using multiple dilutions of lake water, that the negative relationship between temperature and chlorophyll-*a* measurements was increasingly more negative in direct proportion to chlorophyll-*a* concentration.

The concentration effect observed in the results of Watras et al. (2017) also offers a possible explanation for the lack of improvement seen in this assessment's correction models (Table 1). In this study, as the degree of negativity in the temperature to chlorophyll-*a* measurement relationship increased, so too did the individual linear models' slope values (Watras et al. 2017). Ideally, a correction model for the effect of temperature would account for the concentration effect by applying the proper slope-based correctional coefficient to each chlorophyll-*a* measurement based on its concentration. Unfortunately, a limitation of my assessment is the use of a single correctional coefficient based on a single, live, high chlorophyll-*a* concentration sample from the estuary. This likely led to the overcorrection of chlorophyll-*a* readings in the moderate to low chlorophyll-*a* concentration range (Fig 5D). Perhaps appropriate consideration of the differences of temperature effect and potentially turbidity effect at differing chlorophyll-*a* concentrations, such as analyzing the effects using percent dilutions of a live sample, would improve my correction models and others.

Model Applicability

Observing the results of the simple linear regression and AIC tests, I found a large difference in the correction model quality between the full 2016-2018 dataset and the

best-case dataset. Constraining the full dataset to a more specific range of chlorophyll-*a* values, season of the year, and location in the estuary helped to improve upon the models much more than the interference corrections themselves (Table 1). This improvement makes sense for a few reasons, but it also brings attention to the balance of model specificity and applicability.

By reducing the chlorophyll-*a* range to any spectrophotometry measurement less than 60 µg/L in the best-case dataset, I eliminated a large source of unexplained variation in the high chlorophyll-*a* concentration measurements (Fig 5A & B). Why these higher concentration measurements show more variation between the *ex-situ* spectrophotometric and *in-situ* fluorometric methods is still unclear. However, estuary chlorophyll-*a* concentrations rarely reach higher than 60 µg/L (SWMP, 2019), making the best-case models, corrected or not, more useful for comparing the vast majority of fluorometry sensor measurements to spectrophotometric lab measurements.

By including only summer measurements of chlorophyll-*a* taken at the same site as the live samples were obtained, I created a temporal and spatial limitation for the best-case dataset. Algae are known to vary spatially throughout the estuary due to the variety of environmental conditions present at OWC (Hernendorf et al., 2006). For example, the algal class *Bacillariophyceae* (diatoms) is found almost exclusively with the phytoplankton of the lower estuary, and is rarely observed in the more stream-like environments farther from Lake Erie (Hernendorf et al., 2006). By reducing the dataset to include only the chlorophyll-*a* measurements taken at the same site as the live samples, I removed any differences in the algal communities of the various sites, as well as any

differences in the interference effects the sensor might experience across sites. The same concept applies to temporal changes as well. Imai et al. (2009) found that the algal community dominance of two *Microcyctis* species over one another was heavily dependent on water temperature, with community dominance shifting throughout the water year. By reducing the dataset to only include measurements obtained during the season in which the live samples were taken, I helped to remove some of the variation in algal communities and the interference effects the sensor might experience throughout the year.

On a smaller temporal scale, algal community composition, along with the environmental parameters that impact it, often changes significantly in freshwater systems during and after storm events. Storms introduce additional, often colder, water, disturb and add sediments to the system, as well as flush out existing phytoplankton. These effects can have large impacts on temperature, turbidity, and algal community composition respectively, and these storm-driven effects on water quality have been observed at Old Woman Creek ("SWMP", 2019). Storm events and their impacts also act quickly. As part of SWMP, grab samples are collected at a higher frequency by auto-samplers when the forecast calls for heavy rain. A similar approach of increasing reading frequency may be useful for researchers or monitors looking to understand the role of storms in their freshwater systems using *in-situ* sensors. Along with this comes an additional need for assessment of sensors under storm conditions (i.e., fluctuating temperature, high turbidity).

With algal biomass varying both spatially and temporally in these estuary systems, researchers and water quality monitors must be clear in their goals and specific in the questions they seek to answer. The NERRs set out with the goal of determining whether the deployment of total algae sensors would be a worthwhile venture, but I showed that assessing "worth" depends on what specific questions they want to answer. If Old Woman Creek wants to get a whole-ecosystem understanding of total algal biomass dynamics, many more total algae sensors than the existing SWMP sites would likely be required, simply due to the high level of heterogeneity in the system. However, if they seek to answer questions about a smaller spatial scale near the sensor site, or get a rougher average value for the system as a whole, deployment at just SWMP sites may be enough to provide the necessary insight. Other researchers and monitors must also be cognizant of the scale and type of questions they seek to answer using these total algae sensors.

Colony Effect

Throughout the live sample lab tests and field sensor data collections, I observed a considerable amount of variability in fluorometry sensor chlorophyll-*a* concentration readings (Fig 4 & 5). While trends were still evident in most cases, higher frequency measurements, such as that of the lab temperature correction tests, revealed a concerning level of chlorophyll-*a* reading variability in very short time intervals. This high variability in readings can make model creation difficult and increases the chance of inaccurate readings when using lower measurement frequencies. One potential explanation for this high variability in chlorophyll-*a* readings is colony effect. Colony

effect in this instance is defined as the impact of algal clumping behavior or colonial morphology on chlorophyll-*a* sensor readings.

Algae is a broadly defined term for a wide range of photosynthetic organisms, often, but not necessarily related to aquatic systems. Algae can take many forms, from large, attached, multicellular seaweed to tiny, motile, unicellular microalgae. However, the *in-situ* total algae probe is designed specifically to measure free-floating algae in the water column ("EXO Total Algae, 2020). The mechanics of fluorometry measurement require a well-homogenized sample to get consistent, low variability measurements. Unfortunately, many algae exhibit clumping behavior or grow colonially. *Microcystis*, a free-floating unicellular algae, has been shown to shift morphologies and exhibit colonial growth behavior under environmental pressures such as temperature decrease and resource competition (Xiao et al., 2018). *Spirogyra*, a very common filamentous algae, grows in long strands and often forms slimy mats using mucilage to stick to other *Spirogyra* (Ehrenberg et al., 1820). Some diatoms have also been shown to excrete polysaccharides that help them stick to surfaces and each other (Drum, 1969). All of these algal groups commonly occur in freshwater estuaries, with diatoms being the most dominant at Old Woman Creek (Hernendorf et al., 2006).

All forms of free-floating clumped or colonial algae have the potential to produce colony effect as they move through the water and past the *in-situ* fluorometer. Rather than a homogenized water body of unicellular algae giving a consistent reading of chlorophyll-*a* concentration, clumps and colonies of algae produce a more dichotomous signal. The resulting sensor measurements read more like a measurement of algal

presence, with very high values occurring as clumps or colonies pass over the sensor, and very low values occurring when the clump or colony is absent from the probe face (Chaffin et al., 2018).

The presence of clumps and colonies of algae matters less in the case of grab sample spectrophotometry methods due to the spatial dependency of colony effect. The 1 L volume of water obtained for most algal grab samples at OWC is large enough to capture an inclusive sample of the algal community, with the vacuum filtration process that follows ensuring all algae are included in the chlorophyll-*a* extraction process. The *in-situ* fluorometer, by comparison, is sampling a small optical area in front of the probe face, with the volume of sample taken dependent upon the signal light penetration ("YSI", 2015). Additionally, most algal grab samples are done by hand, with standard procedures in place to avoid collecting oversized algal mats and ensuring proper homogenization before testing (Hernendorf et al., 2006).

Averaging chlorophyll-*a* sensor results can assist in reducing colony effect, but this approach is still vulnerable to random chance when sampling with longer intervals, such as the 15-minute intervals used for the EXO sensors at Old Woman Creek. Without higher frequency sampling, researchers and water quality monitors run the risk of either missing large masses of algae or sampling only abnormally large masses of algae by coincidence.

To compound the issue of colony effect, algal communities change under varying environmental conditions such as temperature, sunlight availability, and hydrology (Imai

et al., 2009). The OWC estuary is also host to a wide variety of algal groups, including the previously mentioned *Microcystis* and *Spirogyra*, which peak during the summer months (Hernendorf et al., 2006). Additionally, colonialism of diatoms, the dominant algae in OWC, has been shown to be related to the environmental randomness, with colonial diatoms prevailing in more unpredictable environments (Passy, 2002). All of these independent variables could also cause the overall colony effect in the estuary to significantly change over time, impacting sensor performance to different degrees throughout the year.

CONCLUSION

Moving forward, a greater focus needs to be placed on the inherent variability of total algal sensors due to colony effect as well as the importance of correcting for interferences by environmental parameters such as temperature and turbidity. Chlorophyll-*a* sensor assessments should also consider dedicating the extra time and resources necessary to conduct live sample tests across multiple chlorophyll-*a* concentrations (e.g., Watras et al. 2017).

CDOM should be tested, and I recommend the use of a pre-developed standard, such as Suwannee River NOM standard to avoid issues related to CDOM standard development. Special attention should also be paid to the known attenuation effects of temperature and turbidity on fDOM sensors, as there could be interaction effects occurring between fDOM and temperature and/or turbidity readings *in-situ* (Norelli & Smith, 2014).

Researchers and water quality monitors must also think ahead, and determine precisely what type of questions they seek to answer using these sensors, and at what scale. In the specific case of Old Woman Creek, deployment of total algae sensors across the reserve's 4 SWMP sites could offer a useful glimpse into the spatial dynamics of algal biomass across the estuary's 4 sites. However, any use of the sensor data alone for estimating total algal biomass of the entire reserve should be done with caution and consideration of sensor limitations, environmental randomness, and model applicability. Total algae fluorometry sensors offer a revolutionary new way to measure algal biomass

via chlorophyll-*a* concentration, but limitations of this new technology must be considered before, during, and after deployment.

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FIGURES, EQUATIONS, AND TABLES

Figure 1) Pictured on the left is an EXO-2 sonde with various sensor probes attached at the bottom. On the right is a picture of EXO-2 field deployment at Old Woman Creek NERR. The EXO-2 is attached to an established pole with a vertical track for easy deployment and maintenance access.

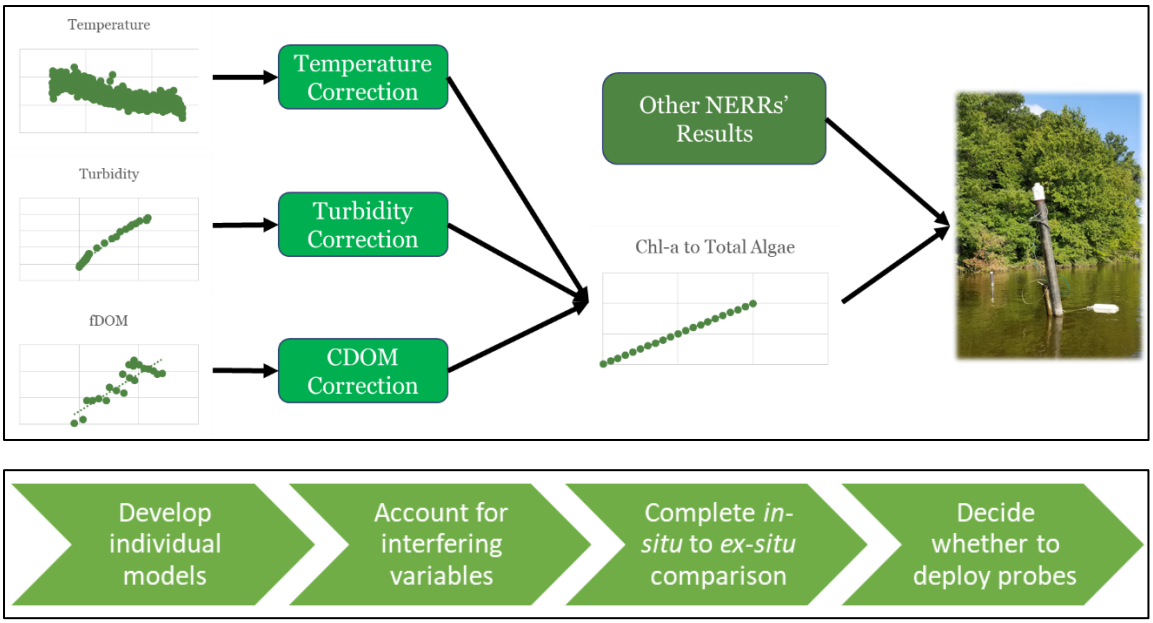

Figure 2) Diagram depicting the process for total algal sensor assessment at Old Woman Creek. Note that the CDOM correction process was removed due to issues with standard development.

Figure 3) A map depicting algal sampling sites for the 2016-2018 paired spectrophotometry and fluorometry probe measurements. The sites include stream, estuary, and Lake Erie environments. Samples collected for the lab methods were obtained from the red dot estuary site with a black center.

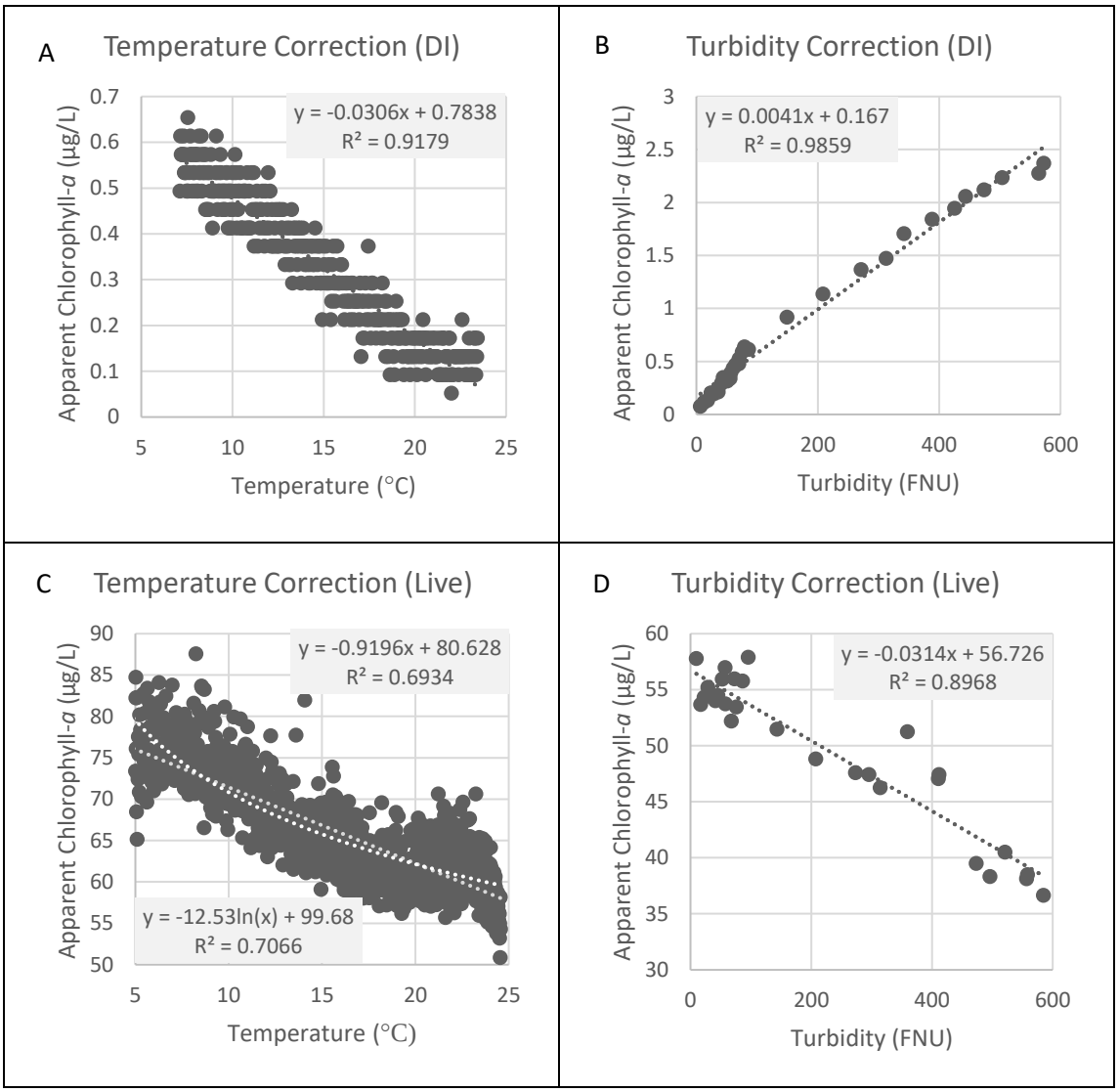

Figure 4) Individual correction models for temperature and turbidity using deionized (DI) water and live samples. The live sample turbidity correction model was significant only across the entirety of the turbidity range, with high variability occurring in the estuary's common turbidity range of 0-100 FNU (r^2=0.02). Figure 3C also displays a logarithmic correction model and trendline. "Apparent chlorophyll-*a*" refers to the sensor reading raw measurement, apparent because it is not known how inaccurate the values are.

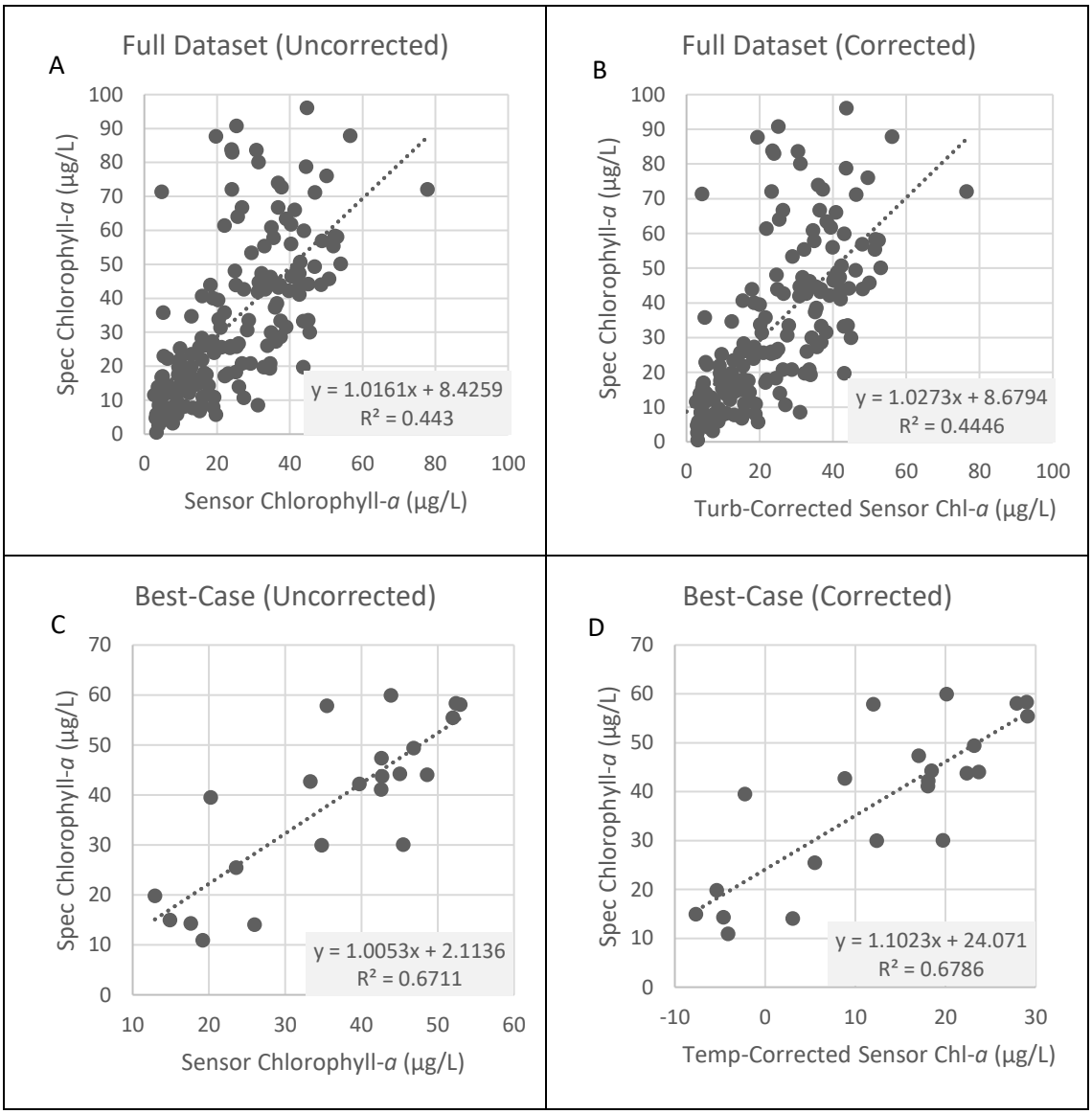

Figure 5) Uncorrected and best corrected models (determined by AIC, Table 1) comparing sensor and lab-derived chlorophyll-*a* measurements from the full and best-case datasets. The best correction models were a turbidity only model for the full dataset and a temperature only model for the best-case dataset. In both cases, the lowest AIC value models only slightly improved upon the uncorrected relationship.

$$Chl\_Corr = SensChl + Temp(\beta_{temp}) + Turb(\beta_{turb})$$

Equation 1) This equation displays the general form correction model for YSI sensor chlorophyll-*a* measurements. *Chl_corr* represents the corrected estimate of chlorophyll-*a* accounting for the effects of turbidity and temperature. *Ch_sensl* is the raw sensor-measured value of chlorophyll-*a* concentration. Model slopes (β) are from the linear model between chlorophyll-*a* and the environmental variables of temperature (Temp) and turbidity (Turb).

Table 1) A table depicting the correction models tested via simple linear regression and AIC. Variation explained (r^2) and AIC values are included for each model and dataset. Chl_{spec} represents the spectrophotometric measurement of chlorophyll-*a* concentration and acts as the "true" target of the correction models. Chl_{sens} represents the paired sensor-measured value of chlorophyll-*a* concentration. *Temp*, *LogTemp*, and *Turb* are the paired sensor temperature, natural log of temperature, and turbidity values, respectively. β_{temp}, $\beta_{logtemp}$, and β_{turb} were derived from the slope of the temperature, natural log of temperature, and turbidity models generated during the lab incubations. ΔAIC is in relation to the uncorrected model of each dataset.

Full Dataset Correction Models	**r^2 Value**	**AIC Value**	**ΔAIC**
$Chl_{spec} \sim Chl_{sens} + Turb(\beta_{turb})$	0.441	1384.2	-0.5
$Chl_{spec} \sim Chl_{sens}$	0.440	1384.7	0
$Chl_{spec} \sim Chl_{sens} + LogTemp(\beta_{logtemp})$	0.420	1390.3	+5.6
$Chl_{spec} \sim Chl_{sens} + Temp(\beta_{temp}) + Turb(\beta_{turb})$	0.404	1394.5	+9.8
$Chl_{spec} \sim Chl_{sens} + Temp(\beta_{temp})$	0.402	1395.1	+10.4
Best-Case Dataset Correction Models	**r^2 Value**	**AIC Value**	**ΔAIC**
$Chl_{spec} \sim Chl_{sens} + Temp(\beta_{temp})$	0.663	164.5	-0.5
$Chl_{spec} \sim Chl_{sens} + LogTemp(\beta_{logtemp})$	0.662	164.5	-0.5
$Chl_{spec} \sim Chl_{sens} + Temp(\beta_{temp}) + Turb(\beta_{turb})$	0.661	164.6	-0.4
$Chl_{spec} \sim Chl_{sens}$	0.655	165.0	0
$Chl_{spec} \sim Chl_{sens} + Turb(\beta_{turb})$	0.653	165.1	+0.1