IT'S A SMALL WORLD AFTER ALL: INSIGHTS, INTERFERENCES, AND IMPLICATIONS OF *IN SITU* CHLOROPHYLL FLUORESCENCE MONITORING IN ESTUARIES

by

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Abstract

Concentrations of the photosynthetic pigment chlorophyll *a* are used as a proxy for phytoplankton biomass by estuarine scientists to study eutrophication, food web dynamics, and harmful algal blooms. Coastal managers use chlorophyll as an indicator of nutrient pollution and for assessments to meet Clean Water Act standards. Chlorophyll *a*, as measured in the laboratory by extraction from monthly discrete water samples, is a core component of the National Estuarine Research Reserve (NERR) System-Wide Monitoring Program (SWMP). Field-deployable sensors based on the excitation and emission spectra of *in situ* chlorophyll have not been incorporated into SWMP to date because past studies showed inconsistencies across reserves. Several studies have shown *in situ* chlorophyll fluorescence to be temperature sensitive as well as subject to spectral interference from fluorescent dissolved organic matter (fDOM) and turbidity. The project objectives included the assessment of sensor reliability across a range of environmental conditions, identifying interferences that may affect sensor output, and developing estuary-wide or conditional relationships between chlorophyll monitoring using the datasonde and extractive chlorophyll measurements. To achieve these objectives, validation of the sensor output through paired sampling with extractive analysis was conducted. Additional testing focused on identifying sensor interferences was also conducted including temperature, turbidity and fDOM. Results indicate *in situ* chlorophyll fluorescence correlates to extracted chlorophyll, but this relationship is influenced by the environmental interferences mentioned. Utilizing hierarchical regression modeling to incorporate data from interfering parameters improved the relation between sensor and extracted concentrations. Incorporating this sensor into NERR SWMP long-term water quality monitoring program as a surrogate for chlorophyll concentration will give coastal managers around the country increased insight into what drives the base of

estuarine food webs. Combined with satellite telemetry, these sensors provide near-real-time insight into phytoplankton dynamics, with the potential to provide early detection and rapid response to harmful algae blooms.

Introduction

Increases in anthropogenic influences are inducing multiple environmental stressors within our planet. These effects are altering environments more rapidly than our knowledge of the integrated underlying processes of nature (Vitousek et al., 1997). Human inhabitants and activities have dramatically increased in both area and density in the recent decades within coastal estuarine ecosystems, signifying the importance of monitoring the impact this increase in population has on the underlying mechanisms within the environment (Pinckney et al., 2001). Estuarine-coastal systems are increasingly vulnerable to anthropogenic impacts due to the inherently large amounts of population variability and rapid land-use change within these ecosystems (Sanger et al., 2015). The connectivity of the estuarine systems within the river-to-sea continuum emphasizes the importance of protecting these vulnerable areas and the synergistic ecosystem services they provide (Barbier et al., 2011).

Evaluating how increasing anthropogenic stressors manifest into ecological responses within an environment and understanding how varying influences are affecting ecosystem sensitivities are of chief importance (Pinckney et al., 2001). Increases in population, land-use change, and urban development frequently results in increased nutrient loading through both point and nonpoint sources, promoting amplified production of organic matter within these coastal estuarine ecosystems. The process of eutrophication, or the increase in the rate of supply of organic matter to an ecosystem, is considered a significant threat to the health of coastal areas (Nixon, 1995). Initially observed in freshwater systems, the prevalence of cultural eutrophication is now very apparent within coastal estuarine environments worldwide. Elevated nutrient concentrations, specifically nitrogen and phosphorus, contribute to anthropogenic pollution from

inefficient wastewater treatment facilities, failing septic systems, excess fertilizer runoff, and other sources (Cloern, 2001; Paerl et al., 2014; Glibert, 2020; Thrush et al., 2021). Amplified nutrient loading and availability can stimulate harmful algae blooms, potentially resulting in hypoxia and fish kills (Paerl et al., 2006; Boyer et al., 2009; Lapointe et al., 2015; Paerl et al., 2016). There is a need to understand how increases in nutrient loading and availability alter the biogeochemical processes and biological communities within these estuarine systems (Cloern, 2001). Understanding in what way human-induced ecological changes, such as declining water quality and decreased biodiversity, affect the functions of coastal ecosystems is a major priority (Paerl et al., 2006).

A National Estuarine Eutrophication Assessment was conducted in 1999 to provide a foundation for the development of a national approach to limit nutrient enrichment problems affecting coastal-estuarine systems in the United States. This assessment found nationwide, "82 of 139 estuaries, representing 67% of estuarine surface area, exhibit[ed] moderate to high expressions of at least one of the following symptoms: depleted dissolved oxygen, loss of submerged vegetation, and nuisance/toxic algal blooms". The Assessment further noted that more than half of the estuaries studied had impairments associated with eutrophication and most of these conditions were associated withed a high level of human influence, including wastewater treatment, agriculture, and urban runoff (Bricker et al., 1999). An update published in 2007 reported that estuarine conditions remained mostly the same, however authors noted that several estuaries had 'low' eutrophic conditions but 'moderate' or 'high' susceptibility to environmental stressors, emphasizing the importance of proactive management and practical monitoring. Forecasted conditions for 2020 were provided; 65% of estuaries analyzed were predicted to worsen, while improvements were projected for 20% (Bricker et al., 2008).

Estuarine scientists and coastal managers utilize concentrations of the photosynthetic pigment chlorophyll *a* as a proxy for phytoplankton biomass to study eutrophication (Nixon, 1995; Pickney et al., 2001; Kennish, 2004; Malone and Newton, 2020), ecosystem metabolism (Buzzelli et al., 2004), food web trophic dynamics (Alpine and Cloern, 1992), nutrient pollution (Paerl et al., 2006; Lapointe et al., 2015; Paerl et al., 2016), harmful algae blooms (Boyer et al., 2009), coastal acidification (Thrush et al., 2021), and for assessments relative to Clean Water Act standards (US EPA, 2009). Chlorophyll *a* is a light-harvesting pigment found in all photosynthetic organisms, essential for the assimilation of photic energy from the sun into chemical energy for biological processes. Photosynthetic plankton, or phytoplankton, are free-floating microorganisms forming the base of the food web in aquatic ecosystems (Winder and Sommer, 2012). Victor Hensen, a 19th-century German physiologist, was first to coin the term "plankton" and referred to these microscopic organisms as "the blood of the sea" (Smetacek et al., 2002). Oceanic phytoplankton account for approximately half of the global net primary production, but only account for a fraction of the total biomass, representing a major influence on the global carbon cycle (Falkowski et al., 1998). Phytoplankton readily respond to altered environmental conditions and nutrient regimes, making them ideal sentinel organisms for ecosystem health and function (Cloern et al., 2014). Monitoring estuarine-coastal ecosystems through tracking phytoplankton dynamics reveals the underlying synergistic relationship between biological communities and the biogeochemical functioning of estuaries (Cloern et al., 2016). Algae blooms are forecasted to increase in range, intensity, and duration as nutrient levels increase due to the co-occurrence of anthropogenic eutrophication and climatic alterations actively modifying biochemical and environmental conditions within coastal estuarine systems

(Glibert, 2020; Thrush et al., 2021). One of the most critical challenges for coastal resilience is monitoring and managing estuarine water quality.

The National Estuarine Research Reserve System (NERRS) conducts the only national, standardized water quality monitoring program in the United States, the System-Wide Monitoring Program (SWMP). The NERRS is comprised of 30 estuarine-coastal sites designated to protect and study natural ecosystems for generations to come. Established through the Coastal Zone Management Act of 1972, the reserves represent a partnership between the National Oceanic and Atmospheric Administration (NOAA) and the coastal states in which they reside. Each research reserve participates in SWMP, measuring short-term variability and long-term change in water quality indicators, determining how environmental change and ecosystem function vary spatially and temporally, and to what extent changes are attributable to anthropogenic activities (Kennish et al., 2004).

Each reserve maintains at least four long-term water quality monitoring stations at representative locations within the estuary designed to characterize gradients in environmental conditions. At these monitoring locations, an EXO2 water quality datasonde (Yellow Springs Instruments, Xylem, Inc.) are deployed to take readings of water temperature, specific conductivity, salinity, dissolved oxygen, pH, turbidity, and water depth at 15-minute intervals. Discrete water samples are collected monthly during ebb tides for analyses of nutrient and chlorophyll *a* concentrations at the same stations. Diel sampling is conducted at one station monthly to observe the impacts of tide and irradiance on nutrients and chlorophyll.

Currently, NERRs are required to measure extracted chlorophyll monthly (NERRS SWMP Plan, 2011), but monthly measurements are not frequent enough for tracking plankton dynamics, which fluctuate at much shorter timescales (Cloern and Jassby, 2010). Many studies

have utilized long-term SWMP chlorophyll data to characterize estuarine variability (Buzzelli et al., 2004; Apple et al., 2008; Dix et al., 2008; Dix et al., 2013; Jeppesen et al., 2018). In a recent survey of 1,036 stakeholders identified by NOAA and the NERRS Coastal Training Program, 404 respondents listed monthly chlorophyll *a* as a limitation in using SWMP data even though chlorophyll was identified as being one of the most important for addressing management needs (SWMP Needs Assessment, 2017). Relative contributions of "top-down" biotic mechanisms, such as trophic interactions, and "bottom-up" abiotic forces, such as environmental variability and nutrient fluxes, function in concert but at variable temporal frequencies (Alpine and Cloern, 1992). Understanding the temporal variability of phytoplankton abundance due to environmental drivers, such as rainfall-induced shifts in residence time, altered light and salinity regimes, changes in nutrient loading and availability, and potential shifts in grazer community structure gives insight into estuarine response and resilience to pulsed nutrient enrichment events (Boyer et al., 2009; Badylak et al., 2015).

Traditionally, chlorophyll measured via *in vitro* extraction involves collecting a water sample, filtering a known volume, steeping the filter in solvent to extract chlorophyll from the cells, measuring the amount of chlorophyll, and providing an estimate of the total amount of chlorophyll in a known volume (Arar and Collins, 1997). Field-deployable sensors based on the distinctive fluorescence excitation and emission spectra of *in situ*, or within the environment, chlorophyll was first developed through modification of a benchtop fluorometer. In this technique, an LED excitation light from a fluorometer passes through the untreated sample water and excites chlorophyll within living cells of algae present. The emitted fluorescence is measured using a detection filter located perpendicular to the excitation source (Lorenzen, 1966). Monitoring phytoplankton via *in situ* chlorophyll fluorescence (fCHLa) is a less expensive

alternative to frequent collection of grab samples for costly and labor-intensive laboratory analysis (Bertone et al., 2018). *In situ* fluorescence data supplies information on the relative distribution of chlorophyll concentrations and usually correlates well with a representative extracted chlorophyll sample. If discrete water samples are taken, then fCHLa data can be correlated to extracted chlorophyll data to estimate actual concentrations. Otherwise, data can be a relatively semi-quantitative measurement to identify trends and patterns in phytoplankton abundance and distribution (Bertone et al., 2019).

Recently, sensor technology has been developed that allows high frequency, *in situ* measurement of chlorophyll fluorescence on the YSI EXO datasondes used in SWMP. To date, *in situ* chlorophyll sensors have not been incorporated into SWMP because past studies on the previous generation of sensors showed results to be inconsistent across different environments and thus not reliable as a quantitative measure of chlorophyll concentrations. The confounding factors noted included light, tides, and water color (Lohrer et al., 2001). While *in situ* measurements are related to extracted measurements, there are variations in the natural environment that cause inconsistencies in the relationship therefore, it is still necessary to ground truth monitoring data (Boyer et al., 2009). Additionally, depending on how the chlorophyll is packaged within the cell, how the cell is oriented to the photodiode, and how much other material there is in the water to refract the blue light from the sensor or absorb some of the red light before it hits the photodiode, the estimate of chlorophyll will vary from what you would get if you extracted the pigments from that water volume (Choo et al., 2018; Choo et al., 2019; Rousso et al., 2022). This compounding variability is potentially unaccounted for when sensors are calibrated to rhodamine dye, the recommended calibration surrogate. Previous studies looking into the applicability of fCHLa sensors for cyanobacteria monitoring recommended a

comprehensive calibration approach beyond the manufacturer's recommendations, involving several context-specific experiments (Bertone et al., 2018). Furthermore, several existing studies have shown that the measurement of *in situ* chlorophyll fluorescence is temperature sensitive. Chlorophyll fluorescence intensity has been shown to decrease with increasing temperature due to quenching but is rarely considered (Watras et al., 2017). Chlorophyll fluorescence has also been shown to be subject to potential interference from turbidity (Downing et al., 2012) and the presence of fluorescent dissolved organic matter (fDOM) (Proctor and Roesler, 2010). Investigation into the comparative contributions of these interferences will provide insight into the relative significance of the other sensors deployed concurrently on the datasonde in data interpretation and analysis.

With the transition to YSI's EXO datasondes in SWMP, there is an increasing demand to reassess the latest generation of fluorescence sensors for *in situ* chlorophyll monitoring and evaluate their applicability for long-term deployment within these dynamic estuarine environments. Interest in general sensor accuracy resulted in a focused effort to empirically validate the sensor output through paired sampling with extractive analysis. Additional testing focused on identifying sensor interferences was also conducted. To assess the sensor for applicability within the entire NERR system, it was necessary to field test the technology through integrating collaborative and technical work amongst an array of stakeholders and end-users. This research was performed as part of a NERRS Science Collaborative Catalyst grant with data submissions from 12 reserves nationally. Many reserves were utilizing these sensors as a semi-qualitative measure of phytoplankton abundance and agreed to a proposed collaborative research investigation. The data included in this thesis was solely collected at GTM NERR.

The central research questions were as follows: Is there an empirical relationship between *in situ* chlorophyll fluorescence measurements using the YSI EXO Total Algae sensor and *in vitro* chlorophyll fluorescence measurements using traditional extractive analysis methods? Additionally, do temperature, turbidity, and fDOM influence sensor chlorophyll fluorescence data? The null hypothesis was that the sensor has no capability to predict the extracted biovolume of chlorophyll *a* within a water mass based upon *in situ* chlorophyll fluorescence. The primary alternative hypothesis was that the sensor does have the capability to predict the extracted biovolume of chlorophyll *a* within a water mass based upon *in situ* chlorophyll fluorescence. The secondary alternative hypothesis was that the sensor does provide predictive capabilities based on the chlorophyll fluorescence output, but this relationship can be improved through incorporating data from other sensors deployed simultaneously, such as temperature, turbidity, and fDOM. Research objectives included the assessment YSI EXO Total Algae sensor performance via field- and laboratory-based comparisons with extracted chlorophyll *a* concentrations across a range of environmental conditions, identifying possible sources of sensor interference that may have led to inconsistencies between sensor data and extracted measurements, and development of estuary or condition-specific relationships between the fCHLa sensor data and extracted chlorophyll concentrations.

Methods

Site description and sampling location

The Guana Tolomato Matanzas National Estuarine Research Reserve, GTM NERR, located in the Florida (USA) upper east coast drainage basin, includes over 24,281 hectares of publicly

owned forested uplands, tidal wetlands, estuarine lagoons, and nearshore seas. The Reserve is associated with the estuarine systems of the Guana and Tolomato Rivers to the north and the Matanzas River to the south. Tidal exchange with the Atlantic Ocean via two inlets is the major hydrological contributor within the estuaries, with scattered tidal creeks providing fresh water from terrestrial inputs. The estuary is considered well mixed (Phlips et al., 2004; Dix et al., 2008) and well flushed, with a residence time of approximately two weeks (Sheng et al., 2008). The climate of northeast Florida is classified as southern temperate, characteristic of the Gulf and Atlantic coastal plain of the southeastern United States. The average annual rainfall is approximately 52 inches (132 centimeters) per year, with the wet season extending from June through September. Seasonal variation in temperature within the Reserve follows that of rainfall with a summer period of high temperatures between June and September and a cooler period extending from December through March. The annual mean air temperature recorded at the GTM NERR SWMP weather station was 22.0 °C for 2020.

The primary sampling location for the project was at the GTM NERR Pellicer Creek (PC) SWMP station (29° 40.024′N, 81° 15.444′W), positioned at the end of a recreational dock within Faver-Dykes State Park and the Pellicer Creek Aquatic Preserve. Located within the Matanzas Basin, Pellicer Creek is one of the major freshwater tributaries into the southern reaches of the GTM estuary. Hydrology is determined by freshwater inputs from tidal branches and saltwater inputs from the Matanzas River, a lagoonal estuary connected to the Atlantic Ocean via the Matanzas Inlet - one of the last “natural” inlets on the east coast of Florida. Pellicer Creek is the border between Saint Johns County and Flagler County, extending eastward to the Matanzas River, which is part of the Atlantic Intracoastal Waterway. Pellicer Creek is a tidal, estuarine creek mostly bordered by publicly owned conservation land, including Faver-Dykes State Park,

Matanzas State Forest, Pellicer Creek Conservation Area, and the Princess Place Preserve. The average depth at this site is approximately 1.2 m with a tidal range of about 0.5 m; the bottom type is muddy sand. Salinity ranges from 0 to 35 psu.

Pellicer Creek was designated by the state of Florida as an Aquatic Preserve in 1970 to protect the waterbody's "near pristine condition" from development. The Aquatic Preserves were created to provide submerged lands which have "exceptional, biological, aesthetic, or scientific value for the benefit of future generations". Pellicer Creek was additionally signified as an "Outstanding Florida Water" in 1979 to provide additional protection due to its iconic natural attributes. In 1992, Pellicer Creek was further designated a "Special Water Outstanding Florida Water", recognized as having "exceptional recreational or ecological significance and that the environmental, social, and economic benefits of the designation outweigh the environmental, social, and economic costs" (Haydt and Frazel, 2003). Most of the adjacent area is forestland and wetlands, however much of this watershed is experiencing rapid land-use change. Expected increasing local development will likely affect timing, quantity, and quality of freshwater entering the creek (Kyzar et al., 2021).

Data Collection - EXO Total Algal & fDOM sensor calibration

YSI EXO2 datasondes were equipped with required SWMP sensors (temperature, conductivity/salinity, pH, dissolved oxygen, turbidity), plus total algae, and fDOM. Sensors were calibrated per NERR SWMP SOPs. A two-point calibration of the Total Algae sensor was done using Rhodamine WT dye and the calibration was performed in Relative Fluorescence Units (RFU) in addition to micrograms per liter (µg/L), as specified in the NERR SWMP SOP. The latest version of the Xylem YSI EXO User Manual (revision K) strongly recommends

calibrating/reporting in RFU. A two-point calibration of the fDOM sensor was performed using Quinine sulfate per the current YSI KorEXO SOP and was done using RFU in addition to quinine sulfate units (QSU).

Data Collection - comparisons between sensor fluorescence and extracted chlorophyll from discrete water samples

To capture a wide range of environmental conditions, sample collection was completed over various tidal cycles and repeated over multiple months and seasons. Two methodologies were employed for sample collection: field-based and laboratory-based. The field-based method ensured that water samples were collected following the discrete diel sampling methods from the SWMP. The laboratory-based method ensured the same water mass and plankton population was sampled and strived to minimize variabilities that may have affected the observed relationship between *in situ* and *in vitro* chlorophyll fluorescence.

For field-based sampling (n = 144), an automated water sampler was deployed immediately adjacent to the sensors while the EXO2 sonde was actively deployed at the Pellicer Creek water quality monitoring station. A 2" PVC tube was affixed to the datasonde housing, so the automated water sampler's suction tube intake was at the same position as datasonde sensors. The autosampler was programmed to sample hourly to align with timing of sensor readings, totaling 24 1-L samples per diel sampling. fCHLa data from the deployed SWMP datasonde was compared with extracted chlorophyll concentrations from samples collected simultaneously. Field-based sampling occurred at least quarterly from winter 2020 through fall 2021 (N = 6).

For laboratory-based sampling (n = 31), a large volume (approximately 20 L) of ambient sub-surface estuarine water was collected at the GTM NERR PC station using darkened carboys,

placed on ice, returned to the lab, and carefully transferred into a darkened mixing tank sitting atop a magnetic stir plate. A magnetic stir bar was placed in the mixing tank and was used to homogenize the sample. The revolutions per minute of the stir bar was set to avoid a vortex. EXO sensors were submerged into the tank and suspended in the water sample while the datasonde continuously recorded data at the highest possible sampling interval (2 sec). Following a 5-min acclimation period, sampling for extractive analysis began by withdrawing an aliquot (250 mL) for extracted chlorophyll determination. Three aliquots were collected over approximately 30 minutes. EXO fCHLa data averaged over the course of aliquot sampling was compared with the average chlorophyll concentrations in extracted samples.

Sampling for comparisons began in December 2020 and concluded in September 2021 (Table 1). Sampling efforts were focused on the Pellicer Creek monitoring station to avoid any variability potentially introduced by sampling multiple sites within the Reserve. The relationship between chlorophyll *a* concentration, as measured through extractive analysis, and chlorophyll fluorescence, as measured by the *in situ* YSI EXO Total Algae sensor, was investigated using the Pearson product-moment correlation coefficient.

Data Collection - extracted chlorophyll determination

Extractive chlorophyll analysis was performed via EPA method 445.0 (Arar and Collins, 1997) but without the use of grinding to achieve cell disruption and shortened extraction time. Filtration and extraction began within four hours of collection (or retrieval of samples in the case of field-based sampling). Water samples were stored on ice in the dark until filtration and were processed in a darkened lab area. Samples were filtered (250 mL) by hand pump on 47-mm diameter glass fiber filters with 0.7-µm pore size. Filters were placed in labeled screw top vials

and frozen at -4 °C or below for a minimum of 24 hr to fracture cells. For extraction, a known volume of 90% aqueous acetone (8 mL) was added to screw-top vials and samples were refrozen at or below -4 °C for 24-48 hours. Samples were vigorously shaken once during the steeping period. Once removed from the freezer for analysis, samples were inverted to ensure no settling had occurred. Prior to fluorometric analysis, samples were allowed to return to ambient temperature for approximately 30 min before drawing the supernatant into the sample cuvette. *In vitro* chlorophyll analysis was performed on a Turner Designs Trilogy® fluorometer using the chlorophyll *a* non-acidification module. The non-acidification optical kit is equipped with a blue mercury vapor lamp to reduce error from potentially interfering compounds, such as other accessory chlorophylls, pheopigments, and dissolved organic matter. This modified fluorometric module also features narrow-band interference filters to allow for specific excitation (436nm) and emission (680nm), which is highly selective for chlorophyll *a* and has the highest discrimination against chlorophyll *b* and other pheopigments (Welschmeyer, 1994). The benchtop fluorometer was calibrated with a liquid standard using a five-point serial dilution at the beginning of the data collection period. A solid standard was used to verify the fluorometer was reading less than 5% drift at the beginning of every batch run. The method detection limit was determined using seven replicate aliquots of low-level ambient water (Appendix). Quality control steps such as laboratory blanks and duplicates were performed to ensure quality and comparability throughout the experiment.

Data Collection - interference testing

Three potential sources of sensor interference were investigated: temperature, turbidity, and fDOM. All interference testing was conducted in a 4 L glass beaker wrapped in aluminum

foil due to experimental issues manipulating conditions in a large volume of ambient sample water within the darkened mixing tank used for laboratory-based sampling. The effect of temperature on fluorescence quenching was tested following the basic protocols of Watras et al. (2017), who assessed temperature compensation for *in situ* algal sensors manufactured by Turner Designs (Sunnyvale, CA). The temperature quench assessment was conducted using a natural water sample collected at the SWMP Pellicer Creek station. The effect of increasing temperature was assessed over the ambient temperature range observed within the reserves (≈ 4 – 30 °C). The relationship between fCHLa (RFU) and temperature was assessed for correlation.

The effect of turbidity on the attenuation of chlorophyll fluorescence was tested following the recommendation of previous research on *in situ* fluorescence sensors (Downing et al., 2012; Saraceno et al., 2017). These authors successfully assessed turbidity interference on fDOM sensors, which operate on similar optical principles as fluorescence-based total algae sensors. Turbidity interference assessments were conducted using natural water collected at the SWMP Pellicer Creek station and combusted marsh sediment provided by North-Inlet Winyah Bay NERR as a surrogate for suspended sediment. The effect of increasing turbidity on fCHLa data was evaluated over a range of turbidity up to approximately 1400 FNU by serially adding standardized source of turbidity to the water sample. Aliquots of sediment were added to the beaker of ambient water and the resulting influence on datasonde readings was measured for 5 min before another aliquot was added. Data from 5-min acclimation periods were averaged to provide a data point for each aliquot added. The relationship between fCHLa (RFU) and turbidity (FNU) was assessed for correlation.

The effects of interference from fDOM on chlorophyll fluorescence were tested in analogous manner to the turbidity interference assessment. Assessment of fDOM interference

was conducted using natural water samples collected at representative estuarine sites ideally low in dissolved organic matter, including the Pellicer Creek SWMP station and at a public recreational dock Tolomato River approximately 3 miles north of the Saint Augustine inlet. The effect of increasing fDOM on total algae sensor measurements was evaluated over a range of fDOM concentrations up to 200 QSU using a concentrated fDOM surrogate. The local fDOM surrogate was made by filtering ambient water from the SWMP Pellicer Creek station with a 0.2-μm filter to remove any chlorophyll-containing cells and concentrating this filtered ambient water to increase fDOM concentration within the surrogate. 5-L of filtered ambient water were concentrated into 1 L of fDOM surrogate through evaporation at 80 °C by means of a heated stir plate. Another concentrated fDOM surrogate was provided by North-Inlet Winyah Bay NERR to assess effects of varying carbon sources on sensor response. Crystalline humic acid brought into solution with 0.1 M NaOH, pH adjusted to 7, and diluted with deionized water (Mazzuoli et al., 2003), was also utilized as a commercially available standard reference surrogate to test the sensor response of a single fluorescent compound, as recommended in the literature (Downing et al., 2012; Loiselle et al., 2009). Aliquots of a surrogate (10 mL for natural surrogates, 5 mL for humic surrogate) were added to the beaker of ambient water and the resulting influence on fCHLa data was measured for 5-min before another aliquot was added. Data from 5-min acclimation periods were averaged to provide one data point for the each of the ten aliquots added. The relationship between fCHLa (RFU) and fDOM fluorescence (QSU) was assessed for potential correlation.

Data Analysis

The empirical relationship between *in situ* chlorophyll fluorescence in relative fluorescence units (RFU) from the EXO datasonde and *in vitro* chlorophyll concentrations in micrograms per liter (µg/L) from extractive analysis of discrete water samples was investigated. All data was compiled in Microsoft Excel and analyzed via SPSS. All assumptions of correlations and regressions were verified, including linearity, normality, homoscedasticity, independence, and multicollinearity. Initial correlations were performed using Pearson product-moment coefficient. Linear regression analysis between sensor and extraction data was conducted to determine the amount of variability within the extraction data predicted by the sensor. Significant correlations between sensor fluorescence and interfering parameters were investigated. Results from the interference experiments produced empirical relationships between chlorophyll fluorescence and sensor interferences, including temperature, turbidity, and fDOM. To determine the relative amount of chlorophyll fluorescence affected by the different interferences, the final sensor readings were compared to the ambient chlorophyll fluorescence data before manipulation began to determine a percentage of chlorophyll fluorescence amplified or attenuated during the experiment. A hierarchical regression analysis was performed to determine if the incorporation of other data collected simultaneously, specifically the interferences examined, increased the amount variation in the chlorophyll extraction data accounted for by the model. Data collected during paired sampling for the comparisons between sensor fluorescence and extracted chlorophyll from discrete water samples was used to incorporate into the hierarchical regression modeling. Results from interference testing were used to determine order of parameter addition into the hierarchical modelling, with the most significant interference introduced first and so on.

Results

Initial comparisons between in situ *sensor fluorescence and extracted chlorophyll from discrete water sampling efforts in SWMP*

YSI EXO TAL sensors have been deployed at the Pellicer Creek long-term water quality monitoring site since June 2019 as an optional data parameter collected by the SWMP. Initial observations of the data collected by the various methods showed usually decent agreement; however, there were some instances in which *in situ* fCHLa sensor data was not representative of extracted chlorophyll biomass, especially in fall and winter months (Figure 1). The mean, range, and standard deviation were compared across methods, discrete grabs (n = 24), the automated sampler (n = 106), and the sonde (n = 31,681) for data collected during 1/1/2020 – 12/31/2020. The mean of the sonde data ($\overline{x}$ = 14.65 µg/L) was greater than both discrete grab samples ($\overline{x}$ = 9.62 µg/L) and diel samples that were collected by the automated sampler ($\overline{x}$ = 8.17 µg/L). The range of the sonde data (55.03 µg/L) was likewise greater than both discrete grab samples (27.8 µg/L) and diel samples that were collected by the automated sampler (30.8 µg/L). The standard deviation (SD) of the sonde data (SD = 5.7 µg/L) was less than both discrete grab samples (SD = 8.7 µg/L) and diel samples collected by the automated sampler (SD = 7.1 µg/L).

Experimental comparisons between sensor fluorescence and extracted chlorophyll from discrete water samples

There was a strong, linear correlation ($r(173) = 0.783$, $p < 0.001$) between *in situ* chlorophyll fluorescence and extracted chlorophyll during the comparison tests (n=175). The direction of the relationship was positive; greater fCHLa was associated with greater extracted

chlorophyll concentrations. Simple linear regression analysis was used to test if chlorophyll fluorescence quantified by the datasonde significantly predicted chlorophyll concentration. The fitted regression model was:

Chlorophyll concentration (μg/L) = (2.066 * fCHLa) – 1.821

The overall regression was statistically significant ($R^2 = 0.613$, $F(1, 173) = 274.290$, $p < 0.001$). fCHLa significantly predicted extracted chlorophyll concentration ($\beta = 2.066$, $p < 0.001$).

Preliminary analyses were performed to ensure no violation of the assumptions of correlations and regressions, including linearity, the independence of residuals, homoscedasticity, normality, and the absence of outliers. Scatterplots showed that the assumption of linearity had been met (Figure 2). The calculated Durbin-Watson statistic of the regression showed that the independence of residuals assumption had been met, as the obtained value was close to 2 (1.534). The plot of standardized residuals against standardized predicted values showed no obvious signs of funneling, supporting the assumption of homoscedasticity had been met. The P-P plot for the model suggested that the assumption of normality of the residuals had been met. All Cook's Distance values were all less than 1 ($CD_{Max} = 0.589$), signifying individual data points were not disproportionately influencing the model. Linear regressions were also performed on data collected by each method individually (Figure 3). The observed relationship between *in situ* chlorophyll fluorescence and extracted chlorophyll concentrations was much tighter with the lab-based data (Figure 4; $n = 31$, $R^2 = 0.9278$) compared to the field-based data (Figure 5; $n = 144$, $R^2 = 0.0595$).

Interference testing

The relationship between chlorophyll fluorescence and potential interferences was investigated using Pearson product-moment correlation coefficient. There was a strong, linear correlation between fCHLa and all interferences tested; however, the direction of the relationship differed among interferences tested. Temperature ($r = -0.917$) showed a negative correlation with fCHLa data (Figure 6). The effect of increasing temperature on measured chlorophyll fluorescence resulted in a maximum of 17% attenuation in sensor fluorescence (Figure 7), which is comparable to other temperature quenching experiments in the literature (Watras et al., 2017). Turbidity ($r = -0.959$) also showed a negative correlation with fCHLa data (Figure 8). The effect of increasing turbidity on measured chlorophyll fluorescence resulted in a maximum of 55% attenuation in sensor fluorescence due to scattering (Figure 9). fDOM showed a strong positive correlation with fCHLa (Pellicer Creek source: $r = 1.0$, Humic source: $r = 0.987$, NIW source: $r = 0.913$). The relative effect of increasing fDOM on fCHLa resulted in a range of responses depending on the fDOM surrogate tested. Surrogate created using concentrated ambient water from Pellicer Creek, FL inflated measured fCHLa by a maximum of 79% at approximately 200 QSU (Figure 10, Figure 11). The surrogate created using concentrated ambient water from North Inlet-Winyah Bay, SC inflated measured fCHLa by a maximum of 169% at approximately 130 QSU (Figure 12, Figure 13). The commercially available humic acid surrogate inflated measured fCHLa by a maximum of 242% at approximately 155 QSU (Figure 14, Figure 15).

Model-building: Hierarchical linear regression

Hierarchical linear regression modeling was used to test if the addition of associated interfering parameters improved the relationship between fCHLa and extracted chlorophyll

concentrations. Analysis of collinearity statistics showed the absence of multicollinearity assumption had been met, as variance inflation factor (VIF) scores were well below 10 (VIF_{max} = 1.077), and tolerance scores above 0.2 (T_{min} = 0.928). The nested regression order was determined by most substantial effect on fCHLa data elicited during interference testing. The addition of fDOM data and turbidity data both improved the regression between fCHLa data and extractive measurements. The addition of temperature (R^2 = 0.686, F (4, 170) = 92.963, p < 0.001) slightly improved the regression, but was not a significant addition to the analysis (p = 0.224). The final fitted hierarchical regression model was:

Chlorophyll (µg/L) = (2.045 * fCHLa) + (0.263 * turbidity) – (0.033 * fDOM) + 0.159.

The overall regression was statistically significant (R^2 = 0.684, F (3, 171) = 123.105, p = < 0.001). It was found that fCHLa (β = 2.045, p < 0.001), turbidity (β = 0.263, p < 0.001), and fDOM (β = -0.033, p < 0.001) significantly predicted extracted chlorophyll concentration.

Model-implementation: Utilizing regression to predict chlorophyll concentrations

The final hierarchical regression equation, including turbidity and fDOM, was applied to 2021 data collected at the Pellicer Creek site to calculate forecasted chlorophyll concentrations from past fCHLa data (Figure 16, Figure 17). The resulting hindcast time series showed improved agreement between the two methods, providing a proof-of-concept that the data collected during the experiment can be applied to data collected for SWMP. Some of the corrected data points were negative during the winter months due to the large amount of fDOM influencing the regression equation results when applied to past time series data. Even though the beta coefficient for fDOM is small (0.033), a large amount of fDOM at this site (greater than 180

QSU during some timeframes) has a greater influence on the corrected data than turbidity, which is relatively muted in comparison to fDOM values.

Discussion

The methods of traditional *in vitro* extractive analysis and *in situ* fluorescence monitoring are not the same thing, nor is one intended to be a substitute for the other. Often how pigments behave *in situ* differs from when extracted into solvents. The datasonde manufacturer (Xylem/YSI) does provide some recommendations and clarifications regarding the interpretation of data coming from the EXO TAL sensor. The µg/L output of the sensor was developed based upon correlations between sensor output and extracted chlorophyll concentrations from laboratory-grown monocultures of blue-green cyanobacteria. The manufacturer even states that the user should not expect the µg/L sensor output to correlate with pigment extractions due in part to the plasticity of phytoplankton physiology and environmental inconsistencies of the natural world. Some corrections have been employed for the YSI EXO TAL sensors deployed for cyanobacteria monitoring in freshwater lakes (Bowling et al., 2016; Chegoonian et al., 2022) and single-cell cultures (Choo et al., 2018; Choo et al., 2019). Post-processing corrections have been developed for some sensor brands, but no tested relationships currently exist for the YSI EXO sensors deployed in estuarine environments. There is a need to know how to interpret *in situ* fCHLa sensor data considering historical and ongoing extraction data, as well as understand the influence of sensor interferences, to inform future management decisions.

The large amount of disagreement between extraction data and µg/L sensor data during the 2020 time series (Figure 1) can likely be attributed to the large amount of fDOM within the water

during the fall and winter months. Finds from correlation and regression analyses between fCHLa sensor data and extracted chlorophyll concentrations promoted rejection of the null hypothesis, that the EXO TAL sensor cannot predict the extracted biomass of chlorophyll *a*. The slope of the fitted regression line between sensor data and extracted chlorophyll concentrations was statistically different than zero, indicating the regression model does a better job at predicting extracted concentrations than intercepts alone. Results of the hierarchal regression further suggest that this regression modeling should be completed at various sites, as the variability of fDOM and turbidity influences on the regression will vary spatially, depending on site characteristics. Results from the individual regressions based upon collection method shows it's clear that the lab-based method was more effective at removing external variabilities that potentially influenced the field-based data. All efforts were made to time autosampler collections to align with the datasonde timestamps, but there are still some inherent uncertainties. Therefore, lab-based comparisons are recommended over field-based comparisons for future application of this research due to decreased ambiguity around water masses being sampled simultaneously.

Investigation into the effects of varying interferences on sensor response has shown the importance of other environmental parameters in data interpretation. The only interferences investigated here were parameters that could be quantified using the available datasonde sensors. Results from interference testing confirmed suspicions as to the presence of interferences referenced in the literature and helped to empirically quantify the degree of influence each interference had on the fCHLa sensor output. The results of the temperature interference testing shows there is still an effect of increasing temperature on chlorophyll fluorescence on the sensors measured output, but this is likely not a significant driver of differences between sensor and extraction data as the sensors are compensated to ambient temperature during calibrations and

likely do not experience the range of temperatures tested during a single deployment. The results from the interference testing suggest fDOM may be a more significant driver of sensor error in highly colored waters. Turbidity also has the potential to influence the sensor a significant amount as well, especially during heavy wind or storm events. Heavy precipitation associated with tropical systems has been shown to provide elevated inputs of terrestrial dissolved organic carbon into coastal ecosystems, sometimes persisting for 50 – 200 days (Asmala et al., 2021). These storm events also have the potential to resuspend sediment into the water column, increasing the turbidity of the water (Dix et al., 2008)**,** potentially introducing compounding interferences on the fluorescent sensors output (Saraceno et al., 2009). Variable sensor output responses to the addition of assorted fDOM surrogates suggest that determining the effect of local dissolved organic carbon is important when interpreting fCHLa data, as other research has also shown differences in optical properties between carbon sources (Oestreich et al., 2016; Cahyonugroho et al., 2022). Interestingly, the fDOM surrogate provided from North-Inlet Winyah Bay NERR resulted in what appears to be a saturation of sensor detection, as the last four additions of surrogate aliquots resulted in a decrease in fDOM as measured by the sensor (Figure 12). This response was not mirrored in the fCHLa data, as the YSI EXO TAL sensor output kept increasing with additional fDOM surrogate added.

Data collected during this experiment signify the importance of including both fDOM and turbidity parameters to explain variability in the fCHLa sensor data and aid in data interpretation, especially in Pellicer Creek. Results from the subsequent hierarchical regression analysis showed that fDOM and turbidity were both significant additions to the regression and increased the variability in the extraction data explained by the model. The interference testing conducted demonstrated that these interferences need to be considered when interpreting

chlorophyll fluorescence data, but not necessarily how to correct them. There is increasing research suggesting the importance of the inclusion of turbidity and fDOM data in the interpretation of fCHLa data from sensors deployed in environments where there are significant fluctuations in these parameters (Beutler et al., 2002; Courtois et al., 2017). For estuaries with large temperature ranges, including temperature in predictive models may be important. This research highlights the importance of including interference data to increase the predictive capabilities of the YSI EXO TAL sensor and that the potential for compounded interferences is of concern, requiring future research investigation.

The influence of temperature on both fCHLa and fDOM may introduce compounding errors on the sensor readings. YSI notes that the optical fluorescent sensors, TAL and fDOM, are compensated during deployment depending on the temperature-dependent calibration value given by supplementary tables in the manual. However, results from the temperature interference trial shows that the quenching of chlorophyll fluorescence is still present, even with the additional calibration adjustment to compensate (Figures 6, Figure 7). The fluorescence of dissolved organic matter has also been shown to be temperature-dependent as well (Watras et al., 2011), which was observed during the temperature interference trial (data not shown), so determining the relative contribution of temperature interference on the fDOM sensor output and relating that relationship to chlorophyll fluorescence sensor output is imperative for accurate data interpretation. Investigation into the appropriate stepwise corrective post-processing for the effect of temperature on the *in situ* fluorescence sensors is a future research priority. Additional research has shown the need for sensor compensation of turbidity and inner filter effects, suggesting dedicated experiments to establish site-specific corrections (Hoffmeister et al., 2020). There may be saturation thresholds in turbidity and fDOM that may deem the chlorophyll sensor

unreliable. Determining recommended thresholds for sensor applicability is also an avenue of research to be investigated.

Recommendations for prospective implementation of the *in situ* chlorophyll sensor are to initially compare fCHLa data to discrete extractive chlorophyll sampling, similar to the 2020 time series (Figure 1). If there is a lack of agreement between sensor data and extracted concentrations, then conducting laboratory-based comparisons is warranted. Paired sampling should be conducted for at least a year to capture as much environmental variability as possible with enough sampling size to conduct statistical analysis ($n \geq 30$). Initial correlation analysis will give insight into the strength of the relationship between sensor and extraction data, potentially promoting the inclusion of interference parameter data into the regression, depending on the characteristics of the site in question. Hierarchical regression analysis can be employed to create a predictive relationship between sensor data and extractions for the data collected during the sampling period. Developing a regression model specific to the site of interest has been shown to predict chlorophyll concentrations from theoretical extractions better than the stock chlorophyll biovolume conversation provided by the manufacturer (Figure 17). Continual pairwise sampling would be ideal for long-term, accurate interpretation of sensor data. Utilizing the regression equation for past or future data is cautiously advised, as the model is intrinsic to data collected concurrently.

Proactive ecosystem-based management is required to assess the issue of eutrophication within at-risk estuarine-coastal environments. As such, chlorophyll *a* is a parameter of principal significance to resource managers and regulatory agencies in defining numeric water quality standards and assessing impairments that could indicate ecosystem-scale changes (Cloern et al., 2016). Management and policy decisions are often based on existing short-term datasets or

models, or both. Long-term monitoring and generation of the associated databases are conducted for many purposes. Four major purposes include characterizing waters and identifying changes or trends in water quality over time, identifying specific existing or emerging water quality problems, gathering information to design specific pollution prevention or remediation programs, and determining whether management actions such as compliance with pollution regulations or implementation of effective pollution control actions are being met (Altenburger et al., 2019).

Chlorophyll concentration measurements compiled through extensive discrete sampling and extractive analysis give insight into tropic level changes that may be occurring at broad timescales. Chlorophyll fluorescence monitoring data compiled through *in situ* sensors can provide innate intuition into the temporal variability in primary production within estuaries. The implementation of high-frequency chlorophyll data has the advantage of capturing short-term variability in phytoplankton dynamics and the abiotic drivers which may drive primary production with good temporal resolution. Understanding the temporal variability in primary production of the system due to sporadic events, seasonality, and long-term changes, will give insight into the long-term resilience of the estuary (Cloern and Jassby, 2010). The significance of increased sampling frequency is the potential to understand event-scale divers of chlorophyll variability, such as high biomass algal blooms, that can potentially skew time-series collected using monthly sampling data.

"There is no vision to see, there is no time to learn, that land units with their natural occupants… are productive entities that under certain circumstances may be worth far more than anything man can put in their place and that once destroyed may never be reestablished" (Shaw and Fredine, 1956). Anthropogenic influences are accumulating in coastal ecosystems and the effect of exacerbated nutrient enhancement on estuarine water quality via cultural eutrophication

is becoming increasingly evident (Paerl, 2006). How these estuarine ecosystems respond to trophic level transformations due to evolving stressors, such as nutrient enrichment, land-use alteration, and climate change will define the resiliency of these systems long-term (Piehler et al., 2009; Malone and Newton 2020). Understanding how nutrient-regimes change with land-use change, and how shifts in phytoplankton community composition and nutrient utilization potentials are connected, is essential to future effective management (Cloern et al., 2020).

Residence time is a key indicator of an estuary's resiliency to eutrophication, as limited tidal exchange and excessive loading of nutrients have been shown time and time again to stimulate excessive growth of phytoplankton and intense algae blooms (Phlips et al., 2004; Dix et al., 2008; Paerl et al., 2014). Fortunately, the GTM estuary has relatively low residence time, due to tidal flushing through the two inlets of the estuary, compared to other estuarine systems that experience algae blooms more frequently, such as the Indian River Lagoon and Florida Bay (Phlips et al., 2004; Boyer et al., 2009; Dix et al., 2013; Gray et al., 2021). Pellicer Creek is more influenced by freshwater inflows than tidal exchange in contrast to other areas of the estuary (Sheng et al., 2008). Passage of tropical systems often results in increases in water depth due to increased storm-induced saline water intrusion and ensuing increased freshwater inflow into the creek due to high rainfall inputs in the watershed (Dix et al., 2008; Schafer et al., 2020, Phlips et al., 2020). Insight into the effects of rainfall-induced pulses of nutrients and DOM on phytoplankton dynamics within these dynamic estuarine systems is possible through the implementation of *in situ* chlorophyll monitoring. Often there are nutrients in and bound to terrestrial dissolved organic matter, which could "fuel the fire" within the estuary for an extended period following the rainfall event (Stedmon et al., 2006). Being able to track the temporal response of the estuary to sporadic environmental drivers, such as heavy rainfall, nor'easters, and

tropical systems, will increase the available knowledge provided by SWMP, a primary goal of long-term monitoring.

The primary end-users of this research are resource managers and regulatory agencies who can apply this technology to create hyperdense datasets to track both short-term pulse events, such as the influence of significant rainfall on the abundance of phytoplankton, and long-term trends, such as the increasing pervasiveness of eutrophic conditions, within estuarine-coastal ecosystems. Combined with telemetry, managers can have near-real-time insight into phytoplankton dynamics within a waterbody and can promptly react to potential algal blooms. Telemetry applications can provide near-real time alerts to changing conditions, promoting a rapid response to elevated chlorophyll levels. This rapid response can give insight into potential causes of algal blooms and can offer a method of mapping the spatial extent of these blooms using *in situ* sensors and a flow-through system installed on remotely operated vehicles or boats.

Data with low temporal resolution is often very difficult to interpret. Increasing data collection frequency can provide increased insight into estuarine dynamics through obtaining data that assists in the interpretation of short-term tendencies and long-term trends of primary productivity. Monitoring an estuary's response and resilience to short-term variabilities and long-term changes is the foundational objective of SWMP and implementing a sensor capable of detecting biological changes within the environment is of chief importance. The incorporation of sensors capable of monitoring *in situ* chlorophyll fluorescence would greatly enhance the value of SWMP, increasing the frequency of chlorophyll data collection from monthly to every 15 minutes. Until now, datasondes within the NERRS system were only able to detect fluctuations in abiotic parameters. With the inclusion of the total algae sensor, SWMP can provide a biotic proxy for ecosystem health and trophic level changes with much greater resolution than

traditional sampling approaches. The chlorophyll sensor will provide a long-term continuous baseline dataset of phytoplankton abundance instead of singular monthly timestamps (two discrete grab samples and diel sampling once per month compared to over 2900 monitoring timestamps per month). This project now provides a methodology for site-specific predictions specific to the sensor deployed and offers a reliable association between *in situ* chlorophyll data and traditional chlorophyll grab sampling.

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Figures and Tables

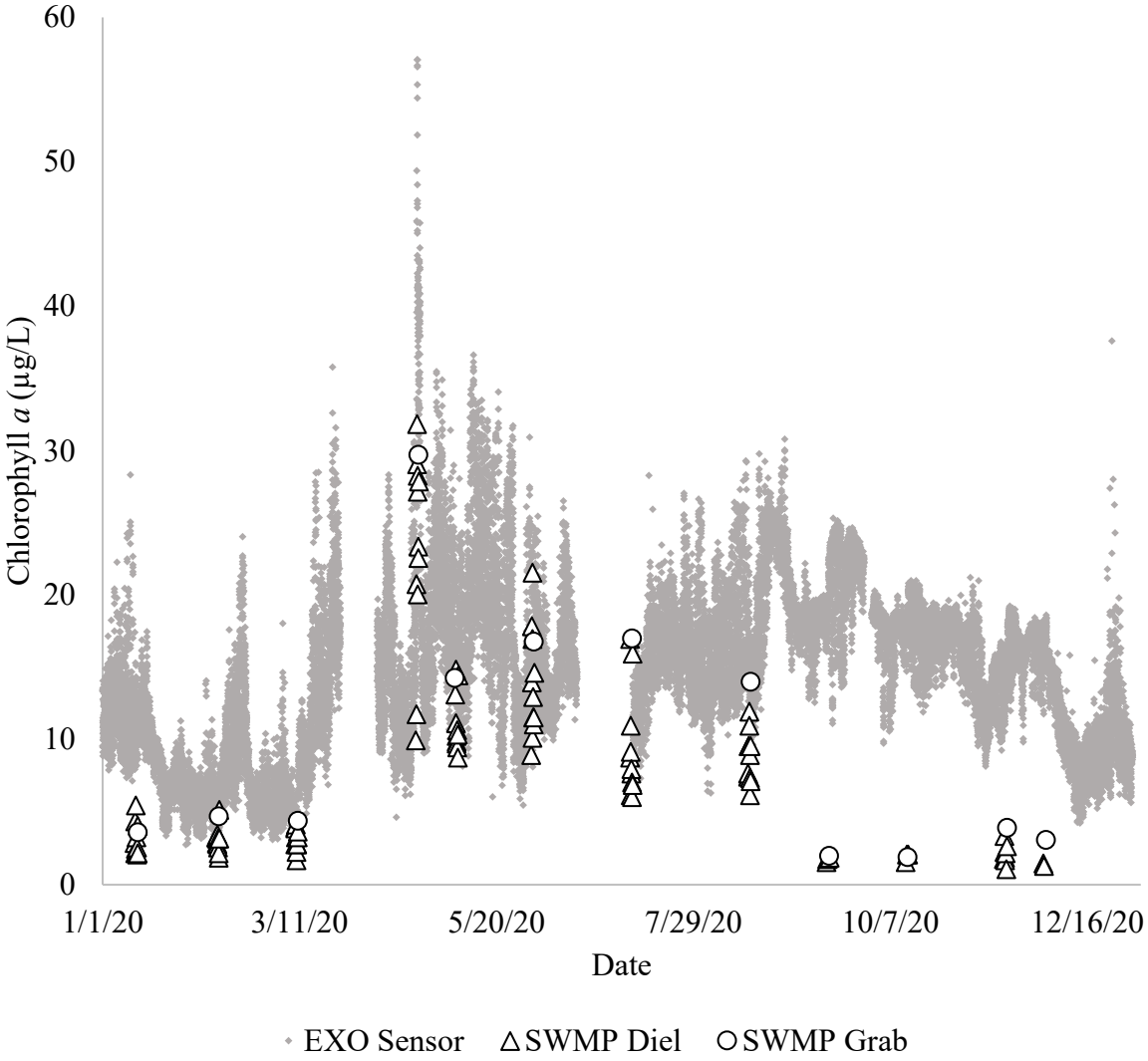

Figure 1 Time series of chlorophyll data collected at the Guana Tolomato Matanzas National Estuarine Research Reserve (GTM NERR) System-Wide Monitoring Program (SWMP) Pellicer Creek long-term monitoring site during 2020. Grey diamonds represent *in situ* YSI EXO chlorophyll fluorescence data, white triangles represent extracted chlorophyll concentrations from samples taken by an automated diel water sampler, and white circles represent extracted chlorophyll concentrations from discrete grab samples.

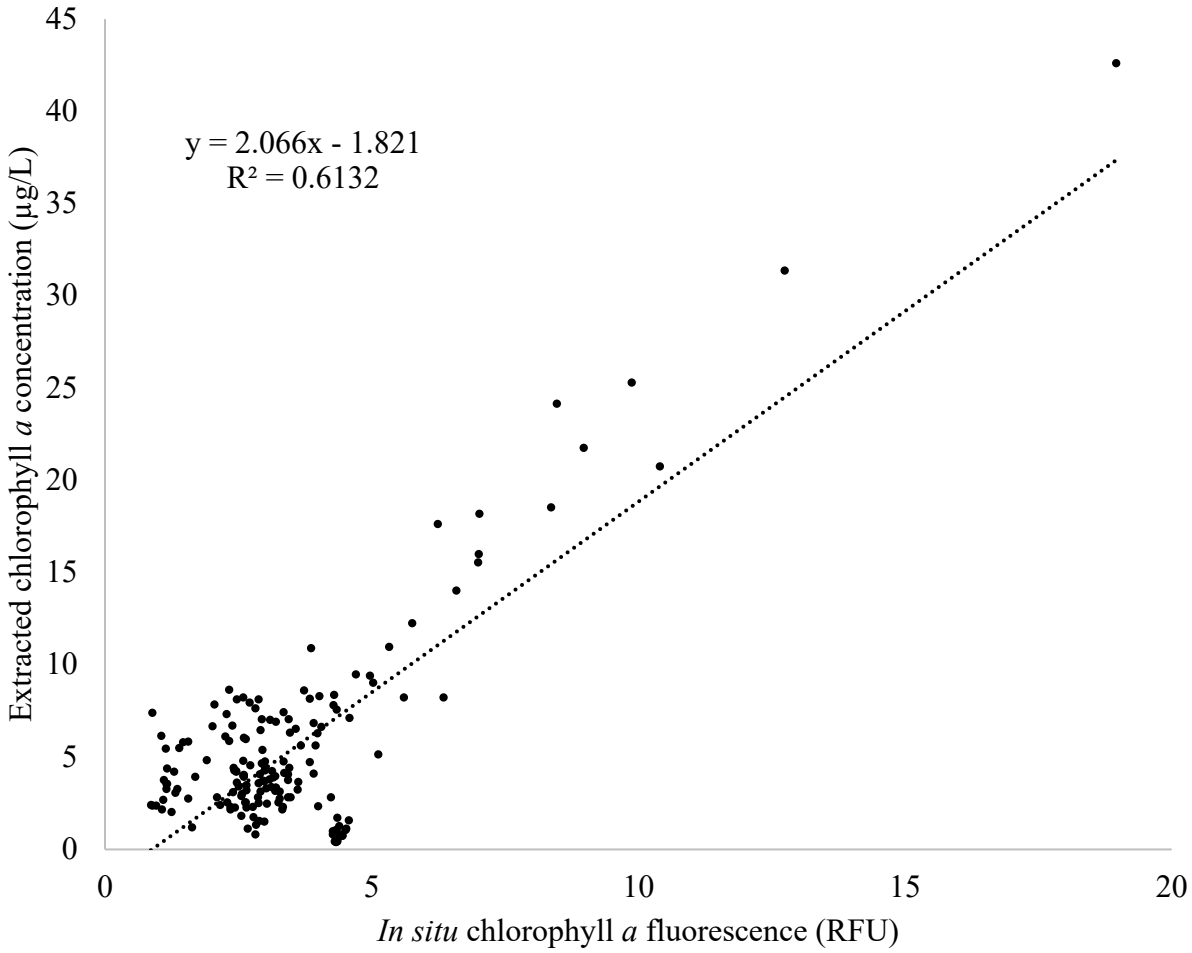

Figure 2 Linear regression of extracted chlorophyll concentrations on *in situ* chlorophyll fluorescence. Black dots represent all data collected (n = 175) using field- and lab-based methods.

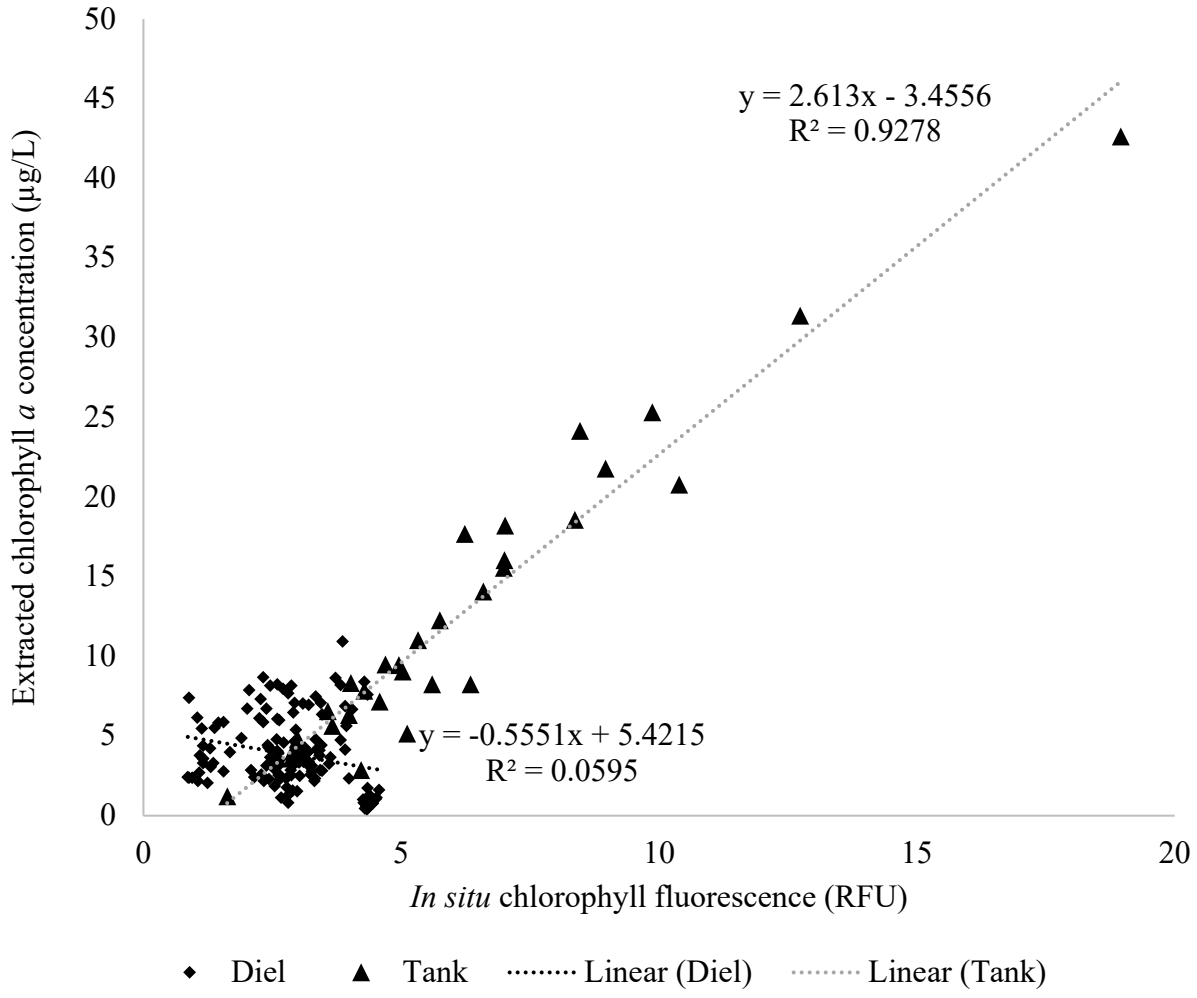

Figure 3 Linear regressions of extracted chlorophyll concentrations on *in situ* chlorophyll fluorescence of both methodologies. Data collected using field- (diamonds, n = 144, R^2 = 0.0595) and lab-based (triangles, n = 31, R^2 = 0.9278) methods.

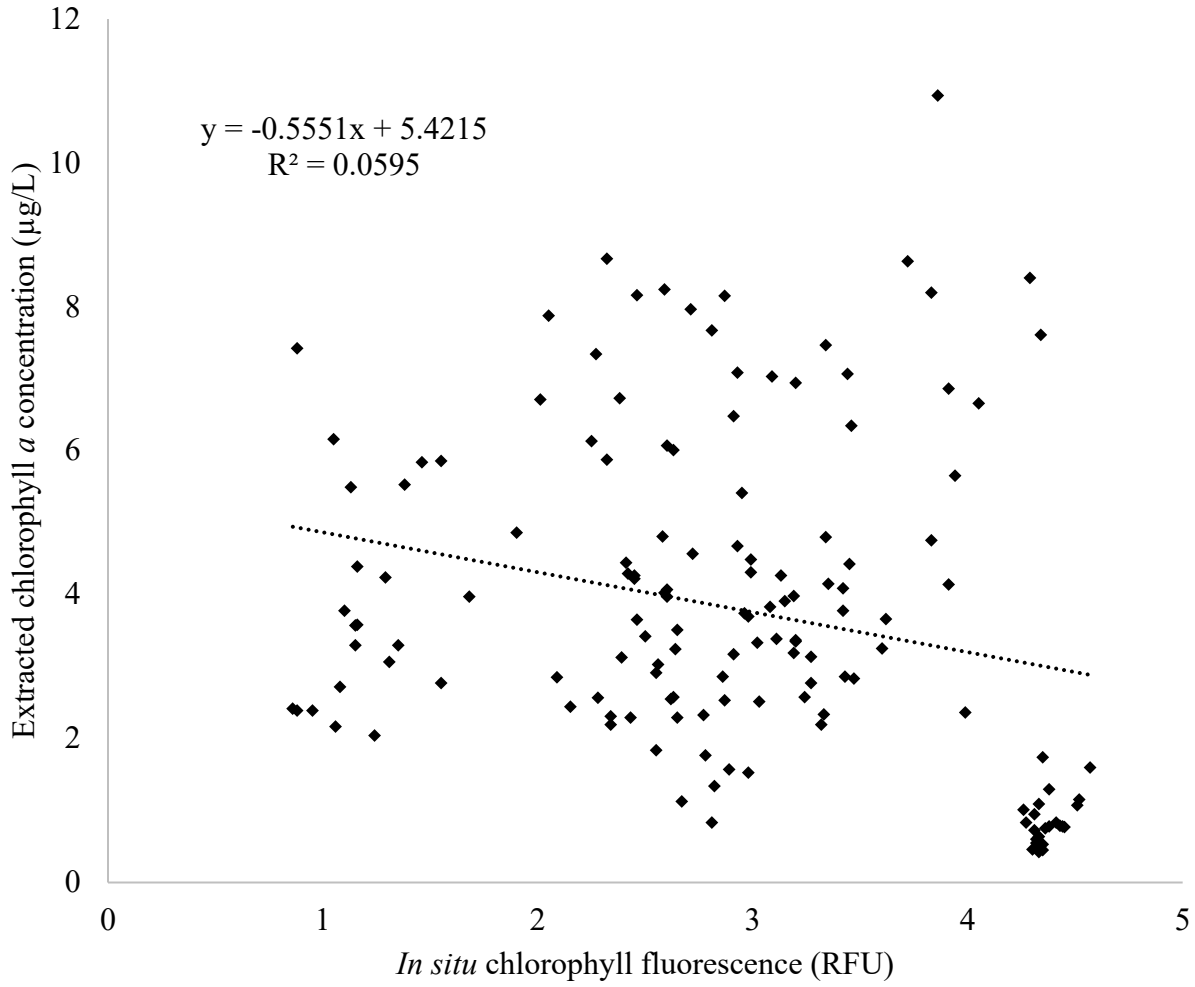

Figure 4 Linear regression of extracted chlorophyll concentrations on *in situ* chlorophyll fluorescence. Data collected using field-based (diamonds, n = 144, $R^2 = 0.0595$) method.

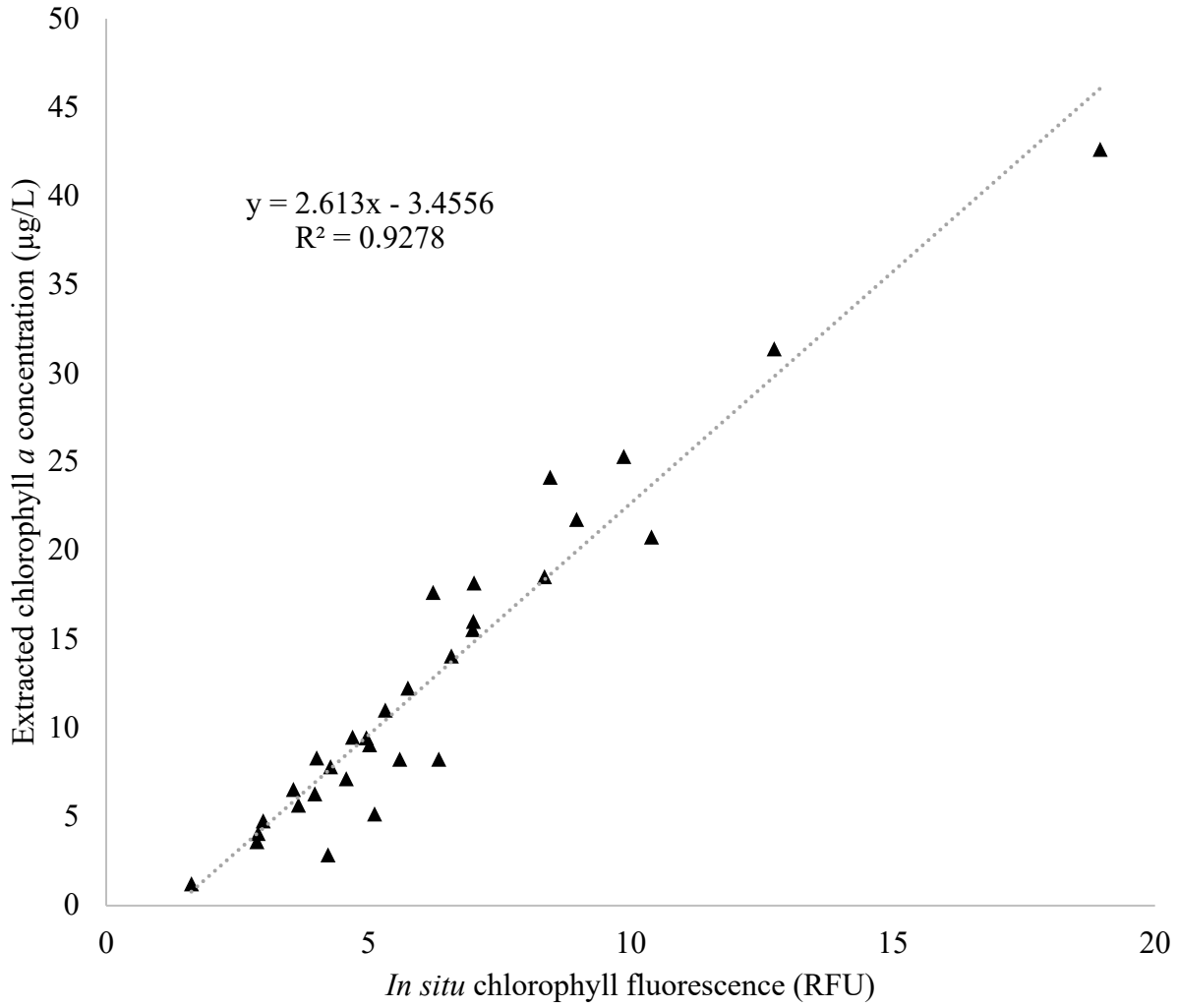

Figure 5 Linear regression of extracted chlorophyll concentrations on *in situ* chlorophyll fluorescence. Data collected using lab-based (triangles, n = 31, $R^2 = 0.9278$) method.

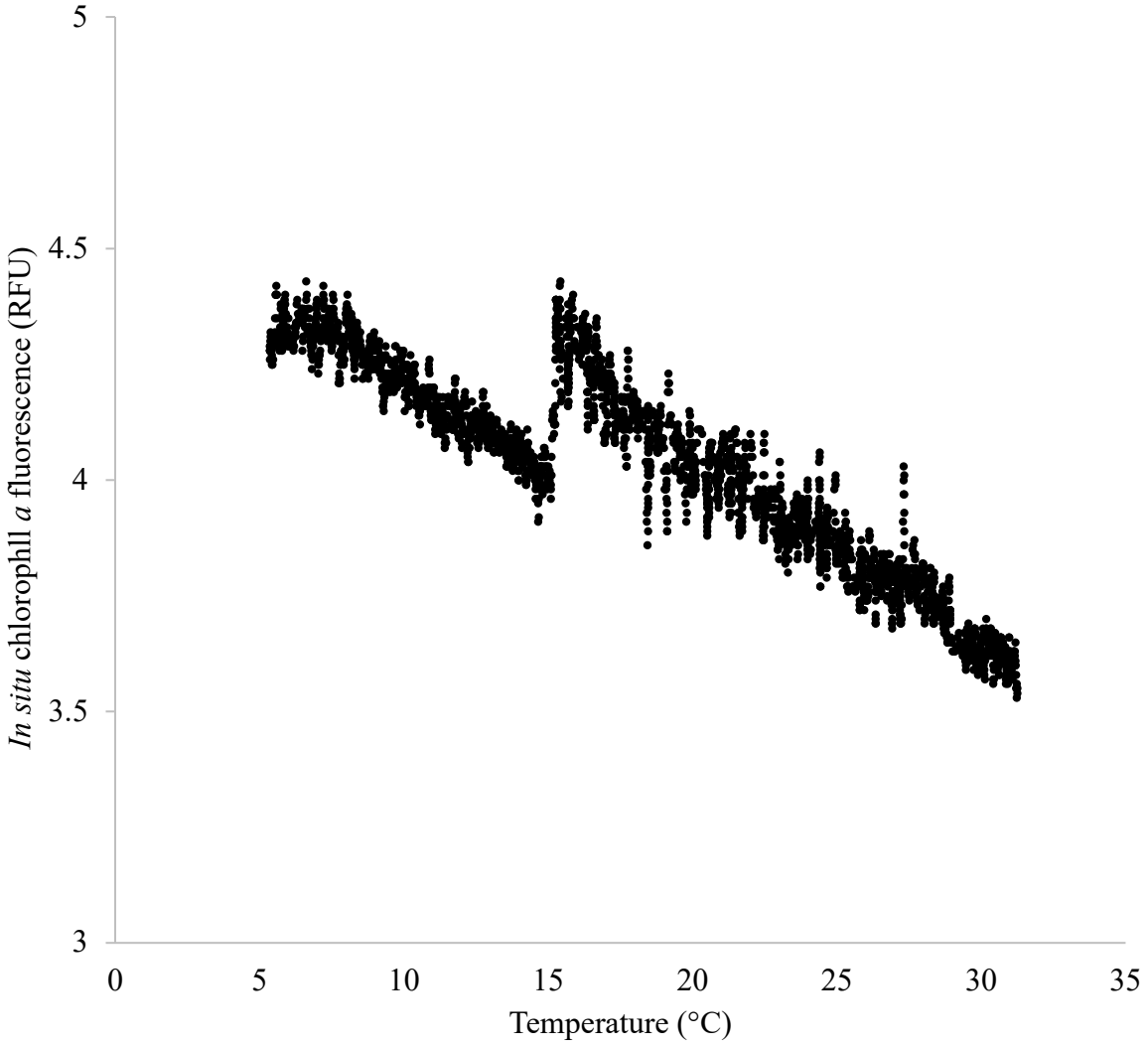

Figure 6 Attenuation of YSI EXO TAL sensor fluorescence as a function of temperature in ambient estuarine water from Pellicer Creek. Temperature was serially increased using heated stir plate.

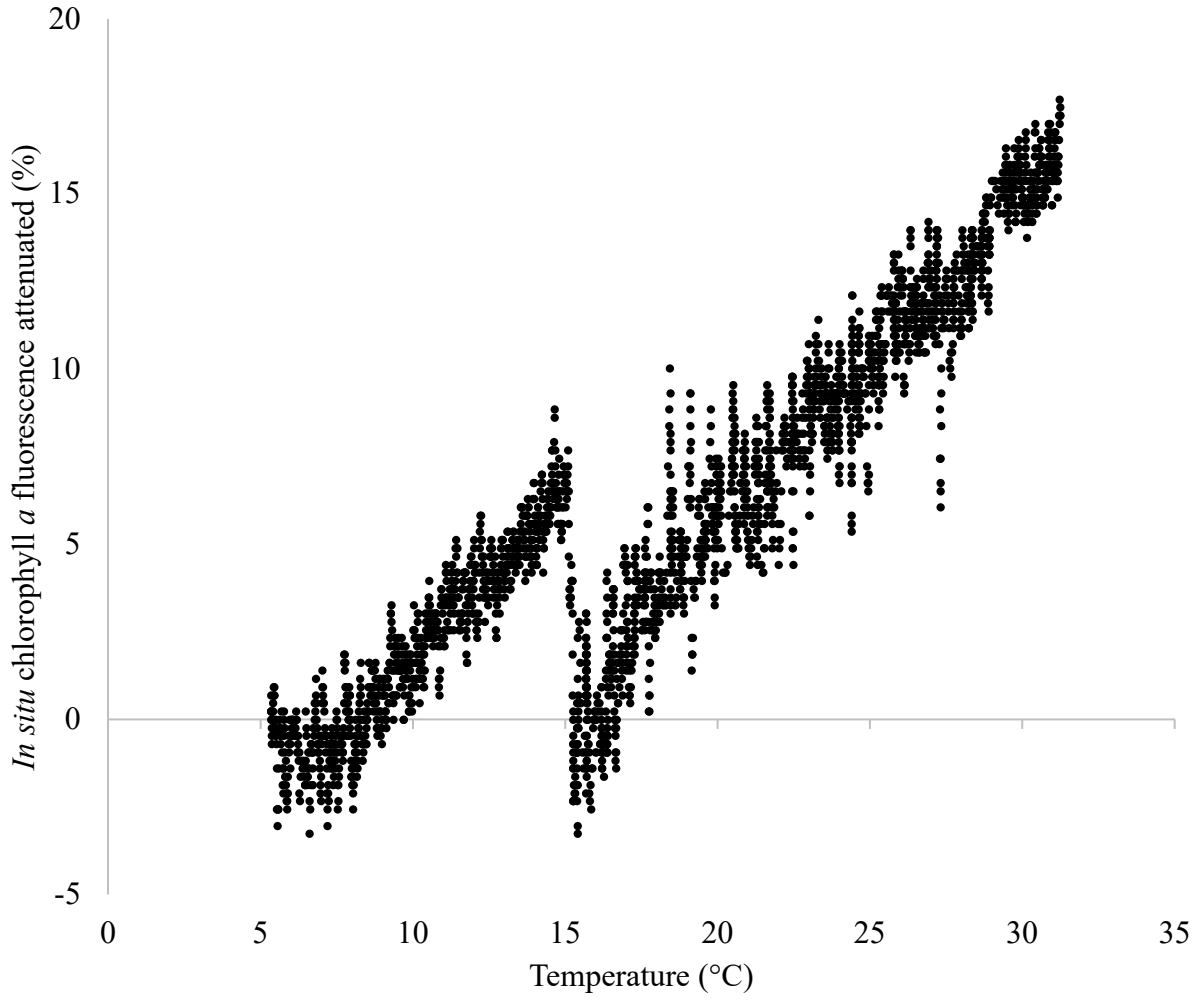

Figure 7 Percentage of attenuation of YSI EXO TAL sensor fluorescence as a function of temperature in ambient estuarine water from Pellicer Creek.

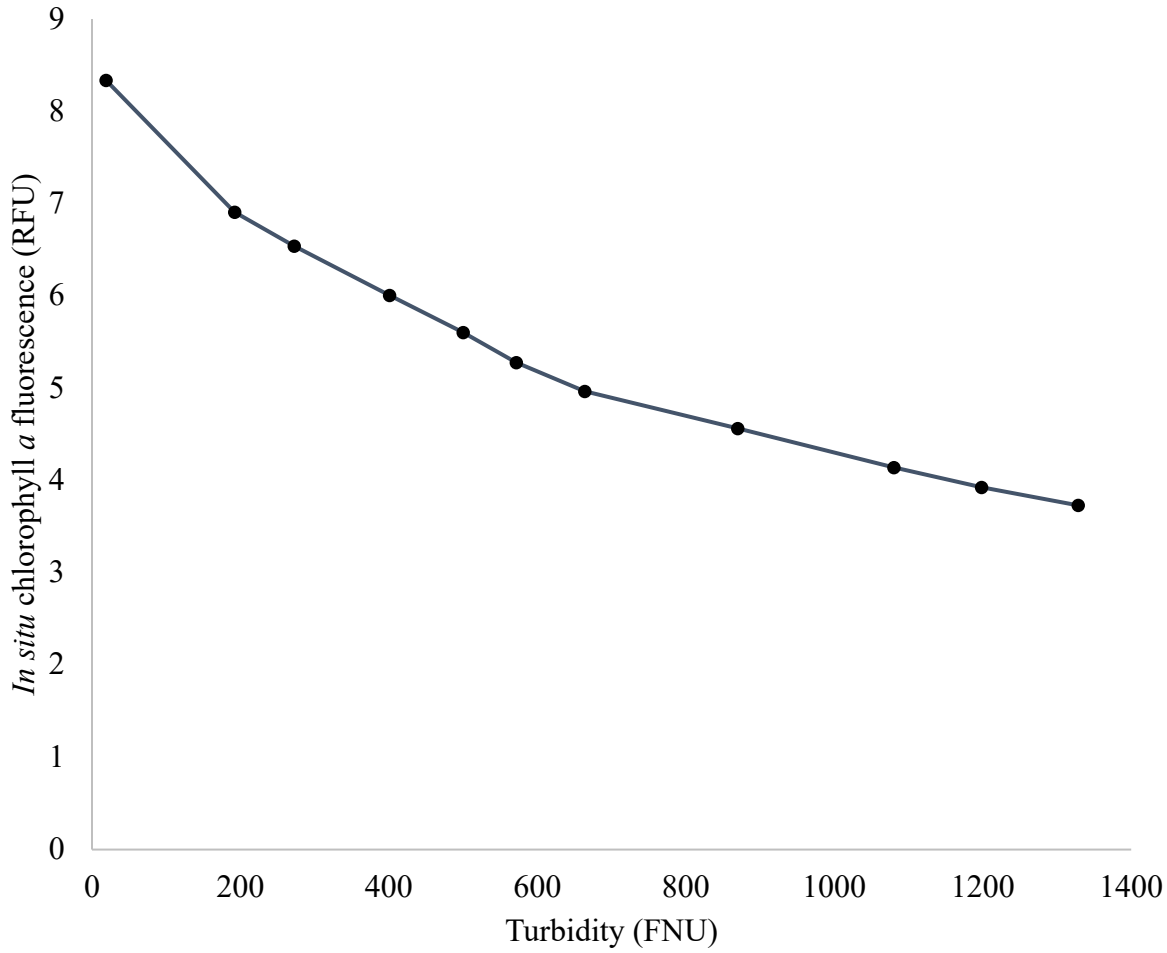

Figure 8 Attenuation of YSI EXO TAL sensor fluorescence as a function of turbidity in ambient estuarine water from Pellicer Creek. Turbidity was serially increased using combusted marsh sediment from North Inlet-Winyah Bay NERR. High frequency data (2-sec intervals) were averaged for 5-min following addition of suspended sediment surrogate (1 tsp).

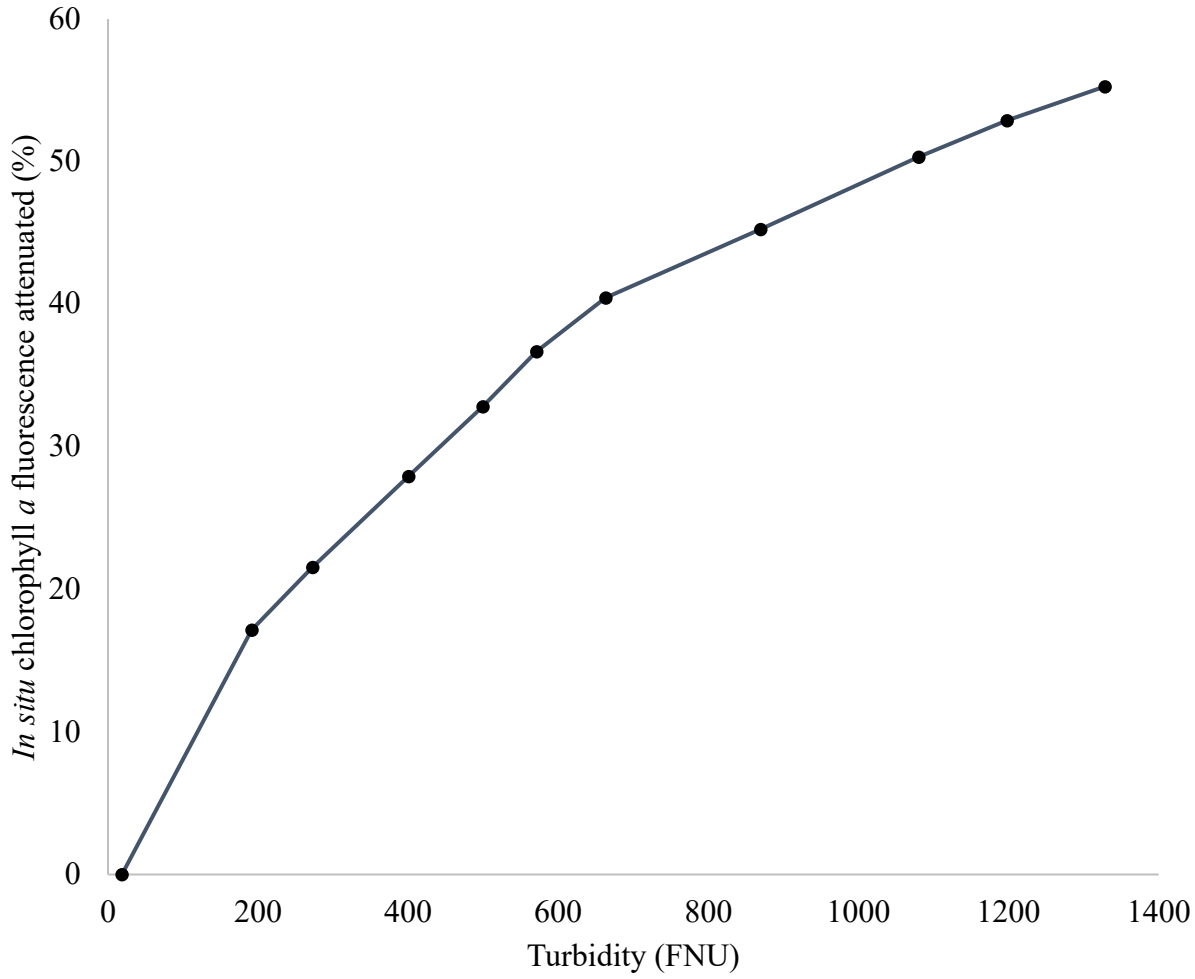

Figure 9 Percentage of attenuation of YSI EXO TAL sensor fluorescence as a function of turbidity in ambient estuarine water from Pellicer Creek.

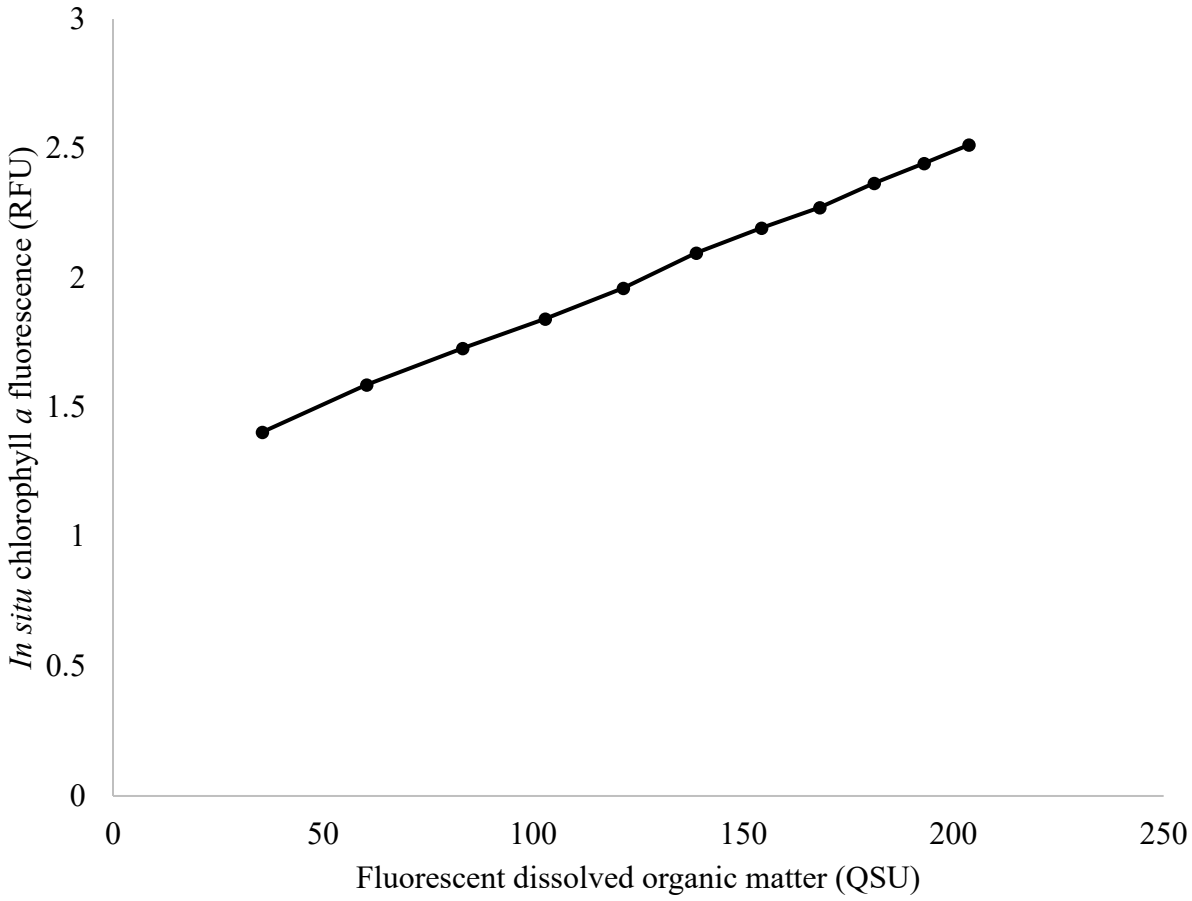

Figure 10 Amplification of YSI EXO TAL sensor fluorescence as a function of fluorescent dissolved organic matter (fDOM) in ambient water from the Tolomato River approximately 3 miles north of the Saint Augustine inlet. fDOM was serially increased using a surrogate created by filtering ambient estuarine water (0.2 µm) from Pellicer Creek and concentrating the filtrate on a heated stir plate (80 ºC) to increase the concentration of fDOM within the surrogate. High frequency data (2-sec intervals) was averaged for five minutes following addition of each surrogate (10 mL).

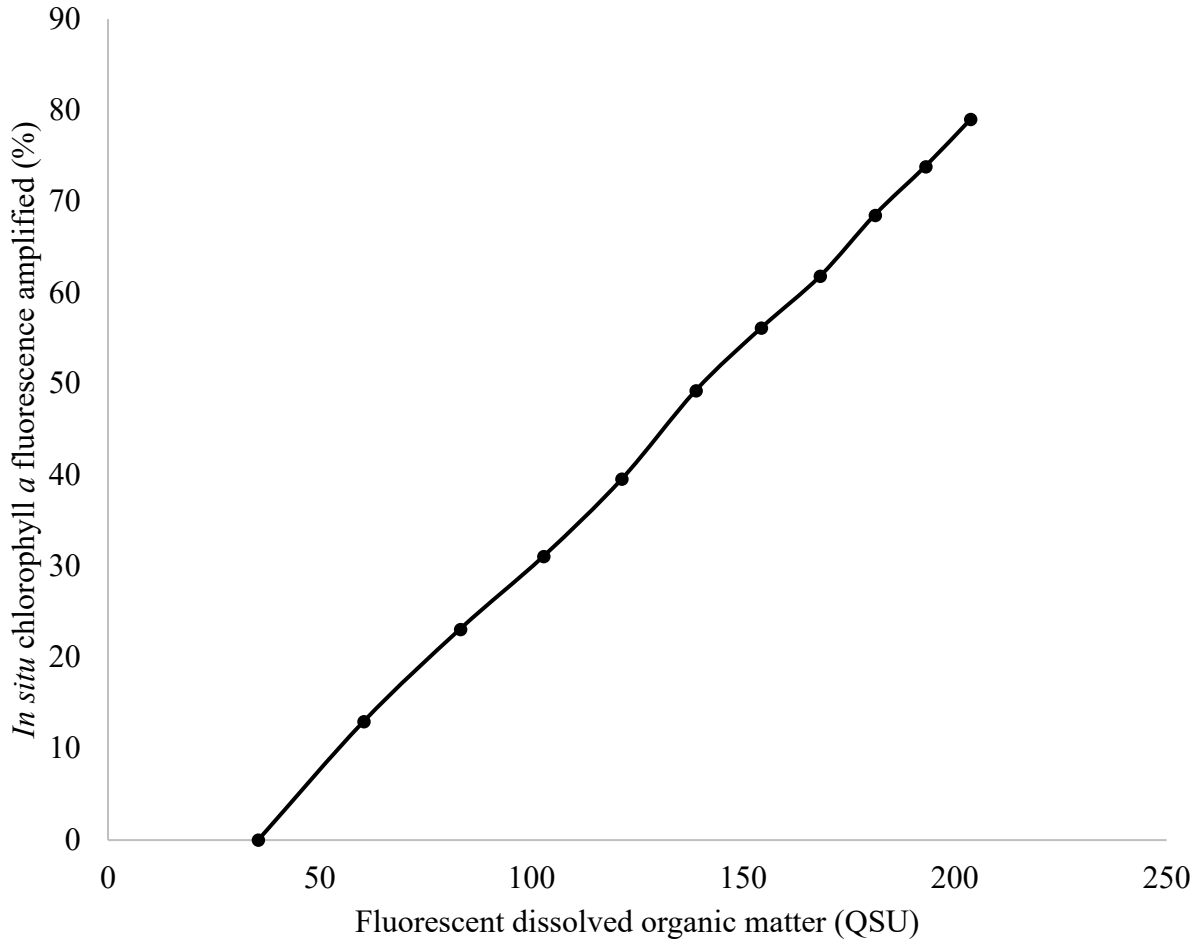

Figure 11 Percentage of amplification of YSI EXO TAL sensor fluorescence as a function of fDOM (Quinine sulfate units – QSU) using natural surrogate from Pellicer Creek in ambient estuarine water from the Tolomato River approximately 3 miles north of the Saint Augustine inlet.

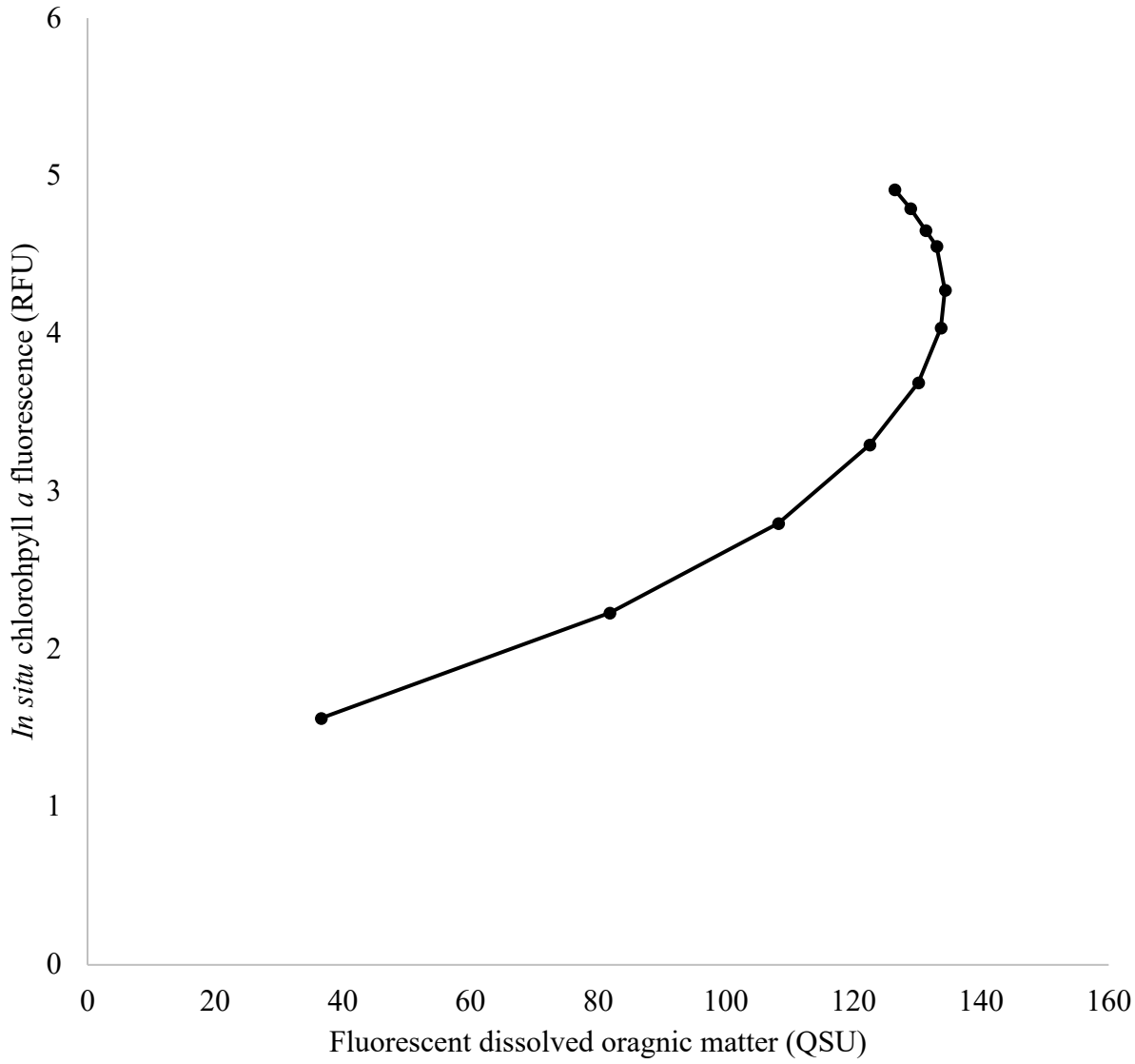

Figure 12 Amplification of YSI EXO TAL sensor fluorescence as a function of fluorescent dissolved organic matter (fDOM) in ambient water from the Tolomato River approximately 3 miles north of the Saint Augustine inlet. fDOM was serially increased using a surrogate provided by North Inlet-Winyah Bay NERR. High frequency data (2-sec intervals) were averaged for 5 min following addition of each surrogate (10 mL).

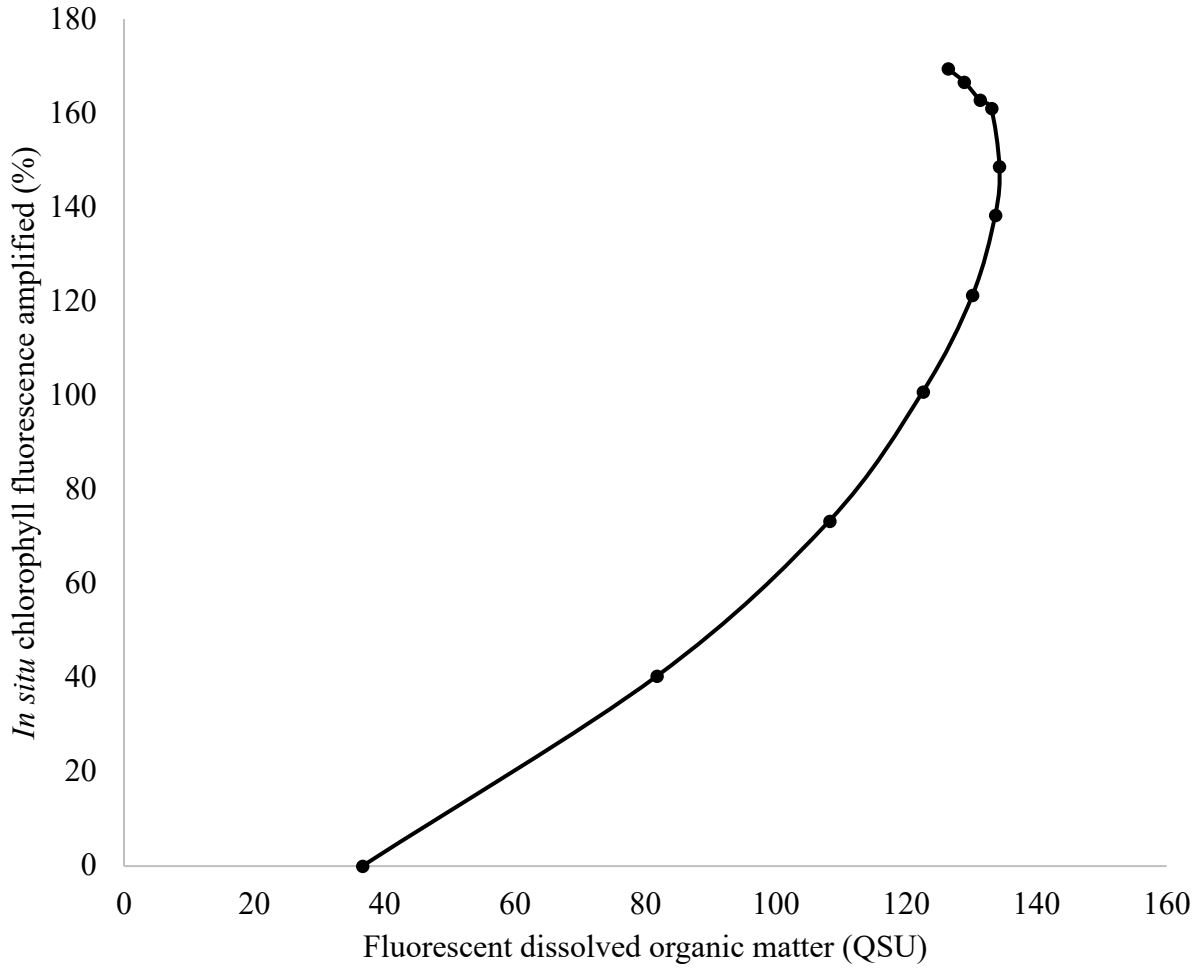

Figure 13 Percentage of amplification of YSI EXO TAL sensor fluorescence as a function of fDOM provided by North Inlet-Winyah Bay NERR in ambient estuarine water from the Tolomato River approximately 3 miles north of the Saint Augustine inlet.

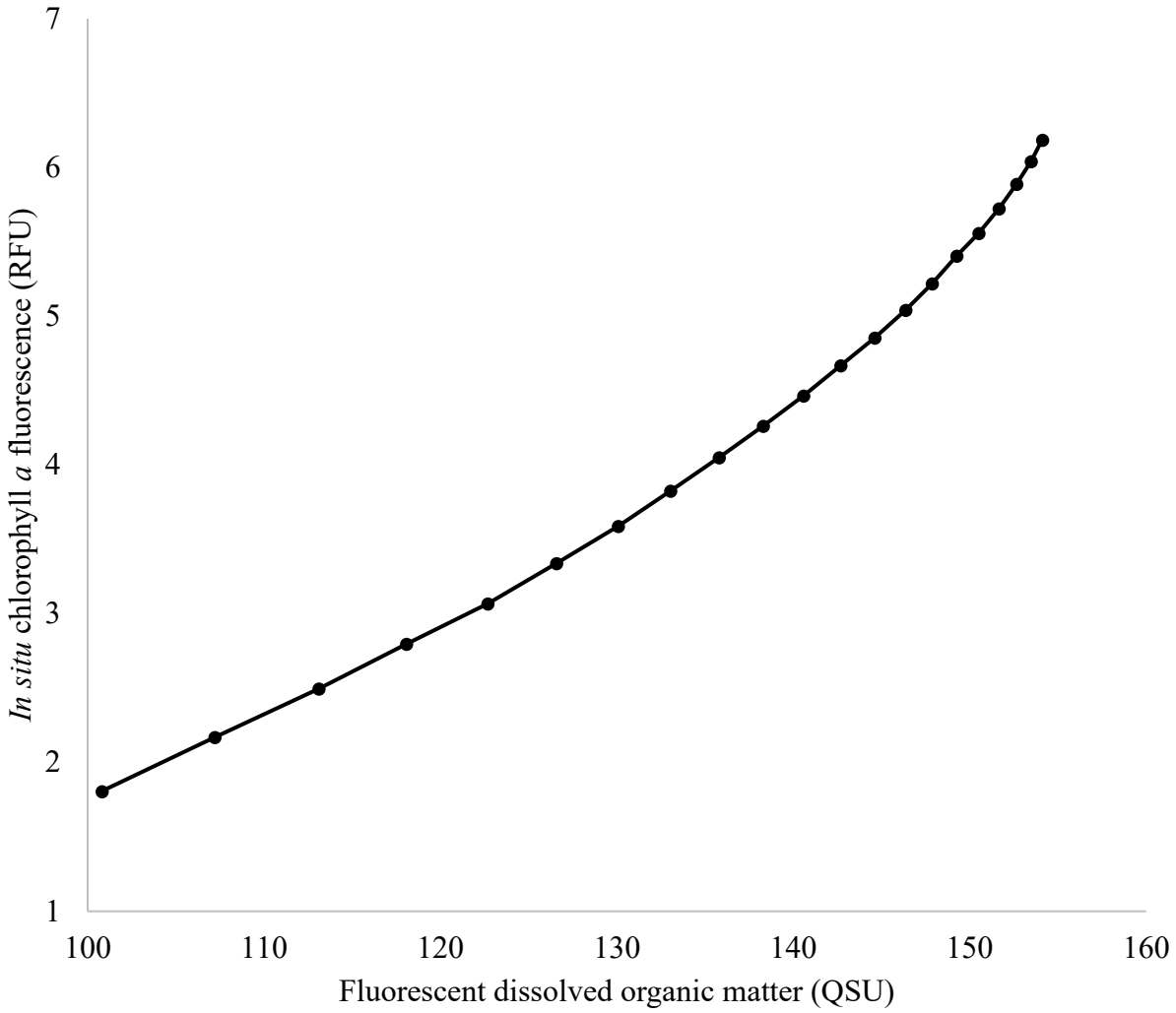

Figure 14 Amplification of YSI EXO TAL sensor fluorescence as a function of fluorescent dissolved organic matter (fDOM) in ambient water from the Pellicer Creek SWMP station. fDOM was serially increased using a surrogate created using commercially available powdered humic acid brought into solution. High frequency data (2-sec intervals) were averaged for 5 min following addition of each surrogate (5 mL).

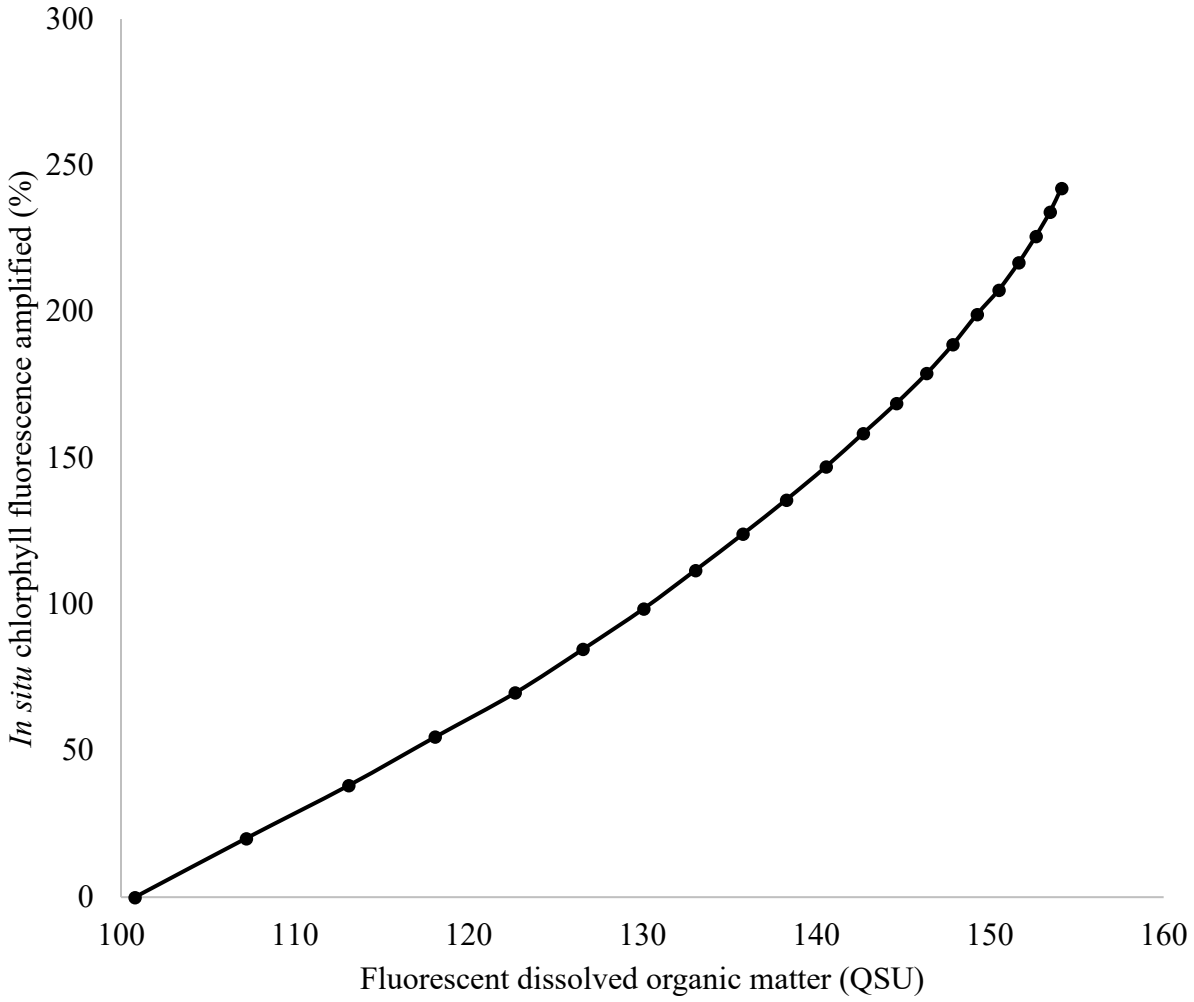

Figure 15 Percentage of amplification of YSI EXO TAL sensor fluorescence as a function of fDOM from humic acid (Quinine sulfate units – QSU) in ambient water matrix from the Pellicer Creek SWMP station.

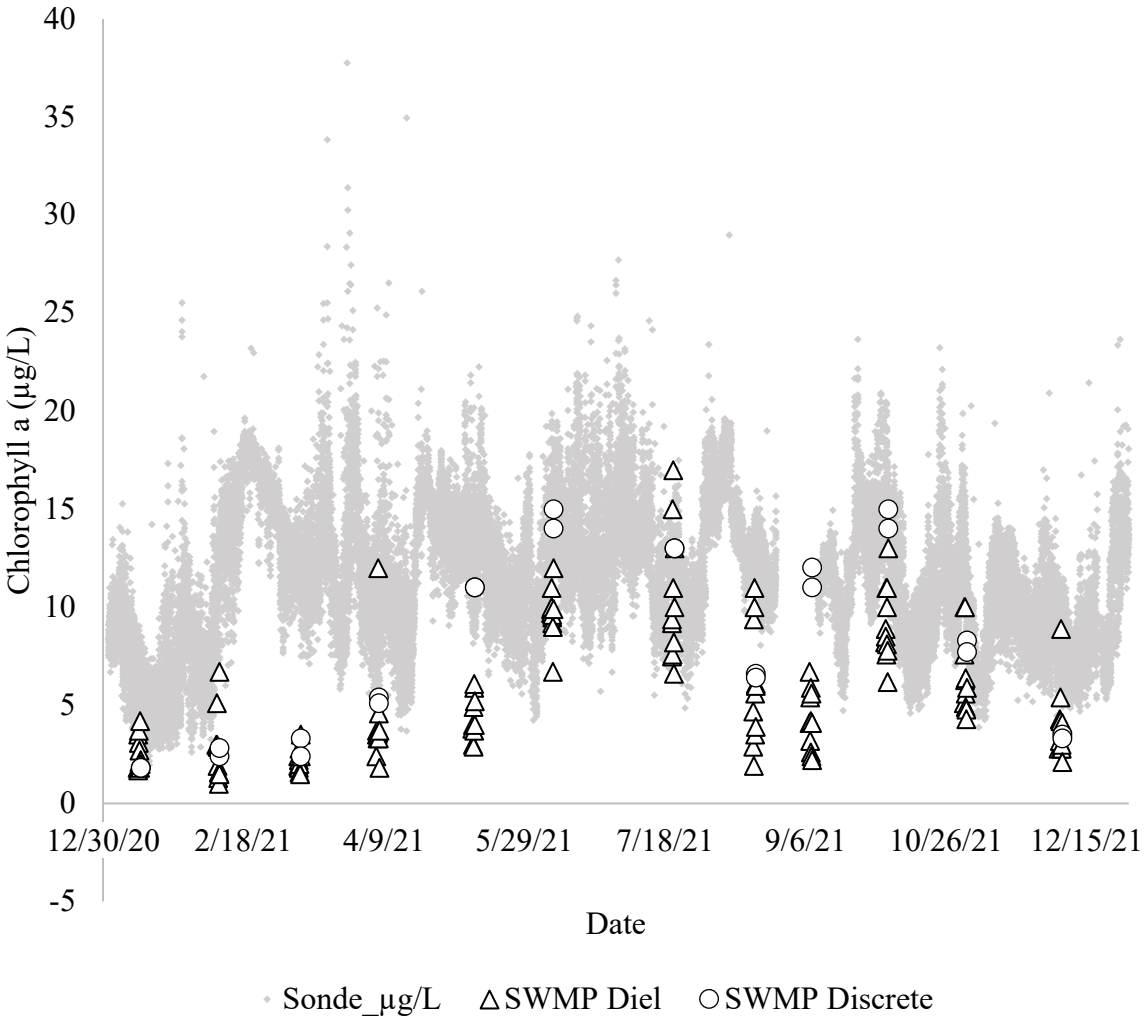

Figure 16 Time series of data collected at the Guana Tolomato Matanzas National Estuarine Research Reserve System-Wide Monitoring Program (SWMP) Pellicer Creek long-term monitoring site during 2021. Light grey diamonds represent stock *in situ* YSI EXO chlorophyll fluorescence data, white triangles represent extracted chlorophyll concentrations from samples taken by an automated diel water sampler, and white circles represent extracted chlorophyll concentrations from extractions of discrete grab samples.

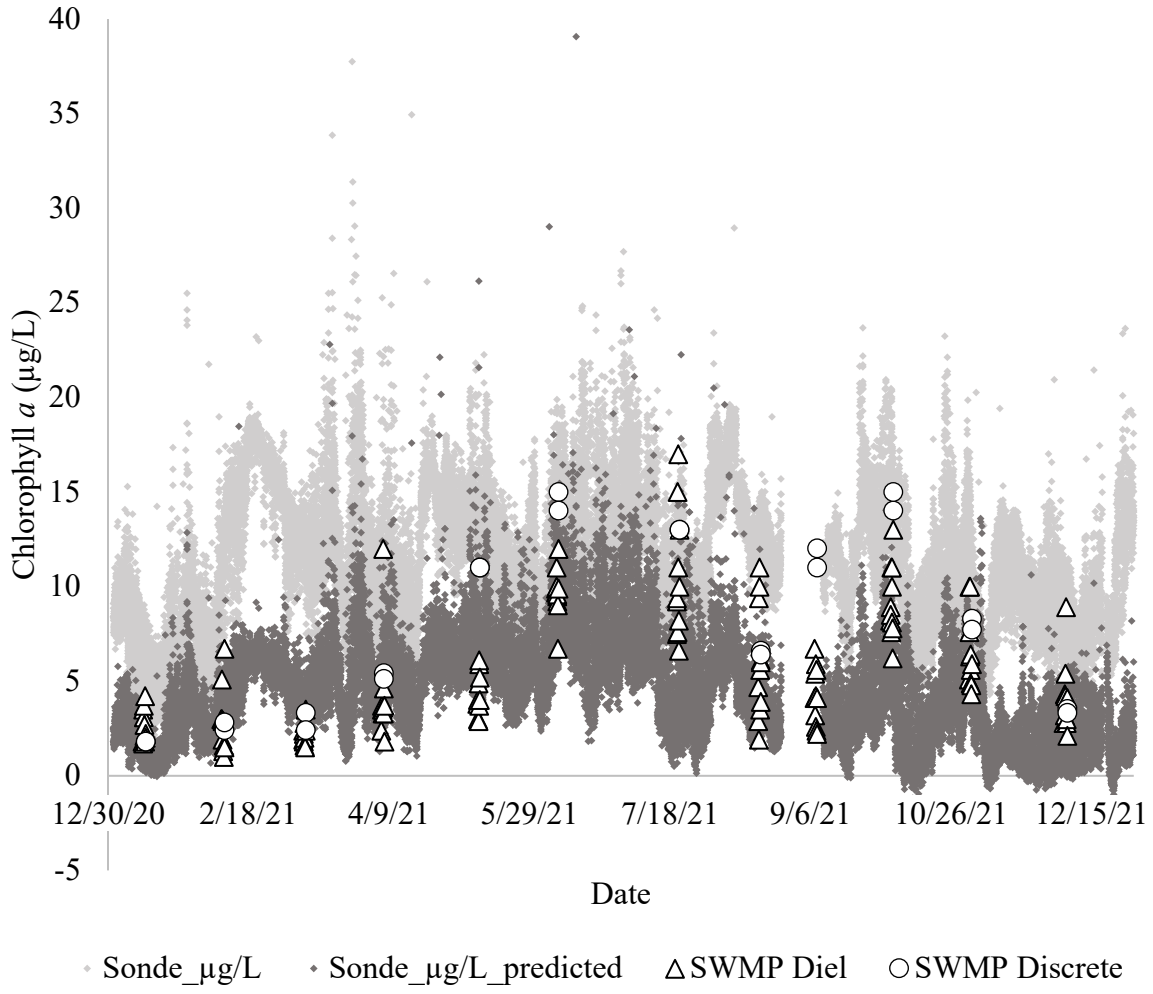

Figure 17 Time series of data collected at the Guana Tolomato Matanzas National Estuarine Research Reserve System-Wide Monitoring Program (SWMP) Pellicer Creek long-term monitoring site during 2021. Light grey diamonds represent *in situ* YSI EXO chlorophyll fluorescence data (µg/L), darker grey diamonds represent *in situ* YSI EXO chlorophyll fluorescence data (RFU) estimated using the hierarchical regression model, white triangles represent extracted chlorophyll concentrations from samples taken by an automated diel water sampler, and white circles represent extracted chlorophyll concentrations from extractions of discrete grab samples.

Tables

Table 1 Laboratory-based sample collection dates and times for data collected for comparisons between fCHLa and extracted chlorophyll from discrete water samples.

Site	Date Time Collected
Pellicer Creek	1/11/2021 9:45
Pellicer Creek	2/2/2021 8:15
Pellicer Creek	2/5/2021 9:00
Pellicer Creek	2/8/2021 9:45
Pellicer Creek	3/25/2021 11:09
Pellicer Creek	4/6/2021 8:51
Pellicer Creek	5/3/2021 12:03
Pellicer Creek	5/26/2021 11:32
Pellicer Creek	6/7/2021 8:11
Pellicer Creek	6/9/2021 12:30
Pellicer Creek	6/10/2021 13:48
Pellicer Creek	6/17/2021 12:30
Pellicer Creek	6/22/2021 9:25
Pellicer Creek	6/22/2021 12:55
Pellicer Creek	6/23/2021 12:20
Pellicer Creek	6/24/2021 10:20
Pellicer Creek	6/29/2021 12:10
Pellicer Creek	7/6/2021 12:00
Pellicer Creek	7/7/2021 8:30
Pellicer Creek	7/13/2021 12:10
Pellicer Creek	7/16/2021 8:15
Pellicer Creek	7/19/2021 9:05
Pellicer Creek	7/20/2021 8:30
Pellicer Creek	7/21/2021 10:00
Pellicer Creek	7/22/2021 7:30
Pellicer Creek	8/31/2021 9:57
Pellicer Creek	9/2/2021 10:31
Pellicer Creek	9/13/2021 9:03
Pellicer Creek	9/17/2021 10:38
Pellicer Creek	9/27/2021 6:52
Pellicer Creek	9/28/2021 8:59
Total (n)	31

Table 2 Field-based sample collection dates and times for data collected for comparisons between fCHLa and extracted chlorophyll from discrete water samples.

Site	Start Date Time	n
Pellicer Creek	12/7/2020 11:30	24
Pellicer Creek	1/19/2021 9:00	24
Pellicer Creek	2/22/2021 8:30	24
Pellicer Creek	5/3/2021 11:00	24
Pellicer Creek	7/22/2021 7:30	24
Pellicer Creek	9/28/2021 9:00	24
Total		144

Appendix

Supplemental Information:

Datasonde information for instruments used for data collection.

Method	Sonde Nickname	Calibration Date	Deployment Timeframe	Sample Collection
Field	Marlin	11/30/2020	12/1/2020 12:45 - 12/15/2020 11:45	12/7/2021 11:30 - 12/8/2021 10:30
Field	Marlin	1/5/2021	1/5/2021 13:30 - 1/27/2021 09:15	1/19/2021 09:00 - 1/20/2021 08:00
Field	Marlin	2/16/2021	2/17/2021 13:45 - 03/02/2021 13:15	2/22/2021 08:30 - 02/23/2021 07:30
Field	Nautilus	4/27/2021	4/28/2021 13:15 - 5/20/2021 10:00	5/3/2021 11:00 - 5/4/2021 10:00
Field	Osprey	7/13/2021	7/13/2021 12:15 - 7/27/2021 12:45	7/22/2021 07:30 - 07/23/2021 06:30
Field	Nautilus	9/20/2021	9/20/2021 10:00 - 10/05/2021 12:30	09/28/2021 09:00 - 09/29/2021 08:00
Lab	Egret	1/5/2021		1/11/2021 9:45
Lab	Egret	1/5/2021		2/2/2021 8:15
Lab	Egret	1/5/2021		2/5/2021 9:00
Lab	Egret	1/5/2021		2/8/2021 9:45
Lab	Egret	3/16/2021		3/25/2021 11:09
Lab	Egret	3/16/2021		4/6/2021 8:51
Lab	Egret	3/16/2021		5/3/2021 12:03
Lab	Egret	5/18/2021		5/26/2021 11:32
Lab	Egret	5/18/2021		6/7/2021 8:11
Lab	Egret	5/18/2021		6/9/2021 12:30
Lab	Egret	5/18/2021		6/10/2021 13:48
Lab	Egret	5/18/2021		6/17/2021 12:30
Lab	Egret	5/18/2021		6/22/2021 9:25
Lab	Egret	5/18/2021		6/22/2021 12:55
Lab	Egret	5/18/2021		6/23/2021 12:20
Lab	Egret	5/18/2021		6/24/2021 10:20
Lab	Egret	6/29/2021		6/29/2021 12:10
Lab	Egret	6/29/2021		7/6/2021 12:00
Lab	Egret	6/29/2021		7/7/2021 8:30
Lab	Egret	6/29/2021		7/13/2021 12:10

Lab	Egret	6/29/2021		7/16/2021 8:15
Lab	Egret	6/29/2021		7/19/2021 9:05
Lab	Egret	6/29/2021		7/20/2021 8:30
Lab	Egret	6/29/2021		7/21/2021 10:00
Lab	Egret	6/29/2021		7/22/2021 7:30
Lab	Egret	8/24/2021		8/31/2021 9:57
Lab	Egret	8/24/2021		9/2/2021 10:31
Lab	Egret	8/24/2021		9/13/2021 9:03
Lab	Egret	8/24/2021		9/17/2021 10:38
Lab	Egret	8/24/2021		9/27/2021 6:52
Lab	Egret	8/24/2021		9/28/2021 8:59

Limits of detection for chlorophyll extractions

Method Detection Limits (MDL), the lowest concentration of a parameter that an analytical procedure can reliably detect, were determined as 3 times the standard deviation of a minimum of 7 replicates of a single low concentration sample. Analysis of seven sample replicates of low chlorophyll concentration resulted in a calculated MDL_s of 0.26 µg/L. Analysis of seven blanks (DI water) resulted in a calculated MDL_b of 0.20 RFU. These values were reviewed and revised 01/11/2021.

Sensor specifications

YSI EXO Sonde:
All sondes used for this project were of the same model and same sensor configuration.

Parameter: Temperature
Units: Celsius (C)
Sensor Type: Wiped probe; Thermistor
Model#: 599827
Range: -5 to 50 C
Accuracy: ±0.2 C
Resolution: 0.001 C

Parameter: Conductivity
Units: milli-Siemens per cm (mS/cm)
Sensor Type: Wiped probe; 4-electrode cell with autoranging
Model#: 599827
Range: 0 to 100 mS/cm
Accuracy: ±1% of the reading or 0.002 mS/cm, whichever is greater
Resolution: 0.0001 to 0.01 mS/cm (range dependent)

Parameter: Salinity
Units: practical salinity units (psu)/parts per thousand (ppt)
Model#: 599827
Sensor Type: Wiped probe; Calculated from conductivity and temperature
Range: 0 to 70 ppt
Accuracy: ±2% of the reading or 0.2 ppt, whichever is greater
Resolution: 0.01 psu

Parameter: Dissolved Oxygen (% saturation)
Sensor Type: Optical probe w/ mechanical cleaning
Model#: 599100-01
Range: 0 to 500% air saturation
Accuracy: 0-200% air saturation: +/- 1% of the reading or 1% air saturation, whichever is greater 200-500% air saturation: +/- 5% or reading
Resolution: 0.1% air saturation

Parameter: Dissolved Oxygen (mg/L - Calculated from % air saturation, temperature, and salinity)
Units: milligrams/Liter (mg/L)
Sensor Type: Optical probe w/ mechanical cleaning
Model#: 599100-01
Range: 0 to 50 mg/L
Accuracy: 0-20 mg/L: +/-0.1 mg/l or 1% of the reading, whichever is greater
20 to 50 mg/L: +/- 5% of the reading
Resolution: 0.01 mg/L

Parameter: Non-vented Level - Shallow (Depth)
Units: feet or meters (ft or m)
Sensor Type: Stainless steel strain gauge
Range: 0 to 33 ft (10 m)
Accuracy: +/- 0.013 ft (0.004 m)
Resolution: 0.001 ft (0.001 m)

Parameter: pH
Units: pH units
Sensor Type: Glass combination electrode
Model#: 599702(wiped)
Range: 0 to 14 units
Accuracy: +/- 0.1 units within +/- 10° of calibration temperature, +/- 0.2 units for entire temperature range
Resolution: 0.01 units

Parameter: Turbidity
Units: formazin nephelometric units (FNU)
Sensor Type: Optical, 90 degree scatter

Model#: 599101-01
Range: 0 to 4000 FNU
Accuracy: 0 to 999 FNU: 0.3 FNU or +/-2% of reading (whichever is greater); 1000 to 4000 FNU +/-5% of reading
Resolution: 0 to 999 FNU: 0.01 FNU, 1000 to 4000 FNU: 0.1 FNU

Parameter: Chlorophyll
Units: micrograms/Liter
Sensor Type: Optical probe
Model#: 599102-01
Range: 0 to 400 ug/Liter
Accuracy: Dependent on methodology
Resolution: 0.1 ug/L CHL a, 0.1% FS

Parameter: Fluorescent Dissolved Organic Matter (fDOM)
Units: Quinine Sulfate Units (QSU), ppb
Sensor Type: Optical probe
Model#: 599104-01
Range: 0 to 300 ppb QSU
Accuracy: Dependent on methodology
Resolution: 0.01 ppb QSU