Recommendations for the National Estuarine Research Reserve System-Wide Monitoring Program Regarding the YSI EXO Total Algae Sensor

April 2022

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Funded by NERRS Science Collaborative Catalyst Grant #NA19NOS4190058
Refining techniques for high-frequency monitoring of chlorophyll a *in the NERRS*

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Suggested Citation Format
Dix, N., H. Ramage, J. Cianci-Gaskill, S. Dunnigan, J.S. Tanner, K. Cressman, S. McMurray, Y. Rii, R. Guy, R. Jeppesen, E. Smith. 2022. Recommendations for the National Estuarine Research Reserve System-Wide Monitoring Program regarding the YSI EXO Total Algae sensor. Technical Report: 35 pp.

Project Resources
https://nerrssciencecollaborative.org/project/Dix20

Executive Summary

Concentrations of the photosynthetic pigment chlorophyll *a* are used as a proxy for phytoplankton biomass by estuarine scientists and managers in order to study eutrophication, food web dynamics, and harmful algal blooms. Traditionally, chlorophyll has been measured by filtering a water sample and then extracting pigments from the filter in a laboratory, and this practice is still employed by the NERRS in monthly grab samples. However, these monthly measurements are not sufficient for tracking plankton dynamics, which fluctuate hourly. Recent sensor technology development allows high-frequency, *in situ* measurement of chlorophyll on the same YSI EXO sondes used in the NERRS System-Wide Monitoring Program (SWMP). While *in situ* measurements are related to extracted measurements, environmental variations create inconsistencies between the two. Before this project, the exact nature of these inconsistencies had not been tested for the EXO sensors, and SWMP practitioners were requesting this information so they could respond to local and national needs for algal bloom research.

Twelve biogeochemically diverse reserves participated in a one-year study funded by the NERRS Science Collaborative. Protocols for calibration and data management were standardized and both chlorophyll methods (extractions and *in situ* sensor readings) were conducted at various frequencies for approximately one year. Keeping technician experiences with preliminary sensor deployments and results from previous studies in mind, one objective of this project was to identify possible sensor interferences and develop standardized empirical correction procedures. A second objective was to test how accurately extracted CHL-A (µg/L) could be predicted from the suite of YSI EXO sensors. While there is value in the YSI EXO Total Algae (TAL) sensor's relative fluorescence units (RFU) for short-term or single-site chlorophyll measurements, reliable predictions of extracted CHL-A (µg/L) would provide a standard measure for comparison between sites and for comparison with historic data.

The effect of temperature on *in situ* fluorescence, as well as the potential for turbidity and FDOM to interfere with fluorescence, was investigated at select reserves. An EXO2 was submerged in a tank of estuarine water and set to record fluorescence while temperature, turbidity, or FDOM was manipulated. At the beginning and end of each interference test, an aliquot of water was withdrawn from the tank for extracted CHL-A determination. Participating reserves then used field- and lab-based approaches to compare this extracted CHL-A measurement to *in situ* CHL-A fluorescence in natural water samples. Both approaches ensured that the same water mass was sampled in each method. Although the sampling frequency and the number of samples collected for both approaches were not standardized across reserves, each reserve captured as much environmental variability as possible, for a total of 1255 comparison samples. The ability to predict extracted CHL-A (µg/L) from *in situ* sensor data was explored using linear regression models of comparison data at two spatial scales, national and site-specific.

Overall, sensor fluorescence was related to extracted chlorophyll measurements, but the strength and drivers of the relationship varied by site. Temperature, turbidity, and FDOM all influenced sensor readings independent of phytoplankton biomass. Hence, considerations for *in situ* sensor implementation depend on the chlorophyll monitoring goals of individual stations.

Based on our findings, **we recommend the following for the NERR SWMP**:

- Implement high-frequency, *in situ* chlorophyll monitoring where appropriate and where capacity exists.
- Acknowledge the *in situ* sensor is not a direct substitute for extractive chlorophyll analysis.
- To save time and provide the most accurate reflection of what the sensor detects, calibrate sensors to relative fluorescence units (RFU) only; integrate RFU into the SWMP manual; and, update CDMO submission requirements accordingly. This is consistent with previous study recommendations and the YSI EXO TAL manual.
- Calibrate the YSI EXO TAL sensor using a revised SOP (see draft). We have also drafted a SOP for calibrating the EXO FDOM sensor according to the YSI manual.
- The SWMP Data Management Committee develops standard metadata language and guidance on QAQC documentation for chlorophyll sensor deployment. Technician training could be improved with a "tips and tricks" document, supply list, and instructional videos.

With a proper understanding of their respective strengths and limitations, both *in situ* and extracted metrics can complement the NERRS mission. Extracted measurements contribute to our understanding of long-term change because they can be compared with historical data. High-frequency, *in situ* measurements are useful for a short-term variability, allowing us to examine, for example, how chlorophyll changes with tides, from day to night, seasonally, and after storms. The simultaneous use of both approaches adds value to Reserves as reference sites and as living laboratories for research on topics ranging from harmful algal blooms to ecosystem metabolism. High frequency, *in situ* chlorophyll data can also support management, education, and aquaculture, particularly when the monitoring stations are telemetered. Near real-time chlorophyll data can be used to detect algal blooms, assess food availability for aquaculturists' filter feeder crops, and educate students on data literacy, primary production, and food web dynamics.

Introduction

The National Estuarine Research Reserve System (NERRS) is an integrated network of estuaries representing a wide variety of biogeographic and environmental conditions. The NERRS has a long history of using standard monitoring methods to promote national syntheses and advance science-based coastal management. The hallmark System-Wide Monitoring Program (SWMP) was established in 1994 to track short-term variability and long-term change in all of the estuarine reserves. In 2002, chlorophyll *a* (CHL-A, a photosynthetic pigment used as an indicator of phytoplankton biomass) was added as a required parameter for monitoring due to increasing concerns about eutrophication (Kennish 2004). Since then, long-term CHL-A datasets produced by the SWMP have proven valuable for investigating biogeochemical patterns (Buzzelli et al. 2004), autotrophic and heterotrophic planktonic processes (Apple et al. 2008), mechanisms regulating phytoplankton biomass (Dix et al. 2013), effects of eutrophication (Jeppesen et al. 2018), and ocean acidification (Baumann and Smith 2018). CHL-A is also a crucial parameter for resource managers and regulatory agencies when defining numeric water quality standards and assessing impairments based on the Clean Water Act.

When CHL-A measurements were introduced into the SWMP, it was determined that measurements would be conducted with *in vitro* methods. Measuring chlorophyll 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 the water sample (Arar and Collins 1997). Since *in vitro* measurements are the traditional, widely accepted methods for estimating phytoplankton biomass in water, monthly *in vitro* measurements are the current practice in the NERRS SWMP. However, monthly measurements are not sufficient for tracking plankton dynamics, which can fluctuate hourly (Agawin et al. 2000). Incorporating *in situ* sensors capable of monitoring chlorophyll would increase the frequency of chlorophyll data collection from once a month to every 15 minutes, greatly enhancing the value of SWMPs,

Field-deployable chlorophyll sensors have been available for decades. They measure chlorophyll by emitting light, exciting the chlorophyll molecules inside cells (*in vivo*), and measuring the resulting fluorescence. Chlorophyll concentrations are measured by these sensors as relative fluorescence units (RFU) but can also be reported in the more traditional metric of µg/L by using an internal YSI proprietary conversion. To date, these sensors have not been incorporated into the SWMP, as past studies showed sensor results to be inconsistent across reserves and thus not reliable as a quantitative measure of chlorophyll (Lohrer 2000). With the transition to YSI's EXO datasonde in the SWMP and improvements in chlorophyll sensor detection limits and wavelength resolution, SWMP staff around the country began purchasing and deploying YSI EXO Total Algae (TAL) sensors.

Of the 55 attendees at the 2021 NERRS Technician Training Workshop, 63% had experience using these TAL sensors. According to attendees, the biggest potential impediments to deploying TAL sensors were cost, unknown sensor stability/reliability, result interpretation needs, and time commitment. Additionally, some reserve technicians had previously observed improbably high values of sensor fluorescence and expressed concerns about potential interferences. These potential interferences, caused by concentrations of light-absorbing or light-scattering material present during field measurements, are well documented among optical sensors, including chlorophyll fluorescence sensors like the EXO TAL (Cremella et al. 2018; Downing et al. 2012). The optical detection of fluorescent

material is further complicated by the fact that fluorescence intensity is temperature sensitive (Watras et al. 2017).

The ability of an early YSI chlorophyll fluorescence sensor (part of the 6-series line of YSI data sondes) to provide standardized measures of CHL-A across the range of water types represented within SWMP was the subject of a NERRS cross-system assessment in the late 1990s (Lohrer 2000). At the time, difficulties arose with standardizing for potential interfering substances and variable relationships between measured *in situ* fluorescence and extracted CHL-A concentrations across participating reserves. As a result, the decision was made to not recommend the inclusion of the YSI 6025 chlorophyll probe as a core parameter of SWMP water quality monitoring. However, given recent advances in optical sensor technology and guidance on assessing and potentially correcting for sensor interferences, as discussed above, a reassessment of the use of field-deployable chlorophyll sensors is clearly warranted.

The NERR system enables sensor performance to be evaluated in a novel way. Many current studies on *in situ* sensor performance are conducted at only a handful of sites (Kuha et al. 2020), over the course of only one season (Proctor and Roesler 2010), or in only a few habitat types (Xing et al. 2017). The NERR system is uniquely positioned to expand the conditions under which these *in situ* sensors can be assessed due to both the large geographic area covered by the reserves and the standardized protocols of the SWMP. SWMP data are publicly available in near-real time and are therefore well situated to be used as early detection of environmental stressors. High-frequency, *in situ* CHL-A measured as part of SWMP could leverage existing SWMP infrastructure and processes and increase the utility of SWMP data for stakeholders and partners.

The purpose of this project was to assess the YSI EXO TAL sensor performance and make recommendations for the NERRS regarding inclusion of high-frequency, *in situ* CHL-A measurements in the SWMP. Twelve biogeochemically diverse reserves (Fig. 1) participated in a one-year study funded by the NERRS Science Collaborative. Protocols for calibration and data management were standardized, and both chlorophyll methods (*in vitro* extractions and *in vivo* sensor readings) were conducted at various frequencies for approximately one year. Given technicians' past experiences with preliminary sensor deployments and the results of previous studies, the first objective of this project was to identify how temperature, turbidity, and FDOM interfere affect CHL-A fluorescence measurements and develop standardized empirical correction procedures. The second objective was to determine how to best predict extracted CHL-A (µg/L) using YSI EXO sensors. While there is value in the TAL sensor's relative fluorescence units (RFU) for short-term or single-site chlorophyll measurements, reliable predictions of CHL-A (µg/L) would provide a standard measure to compare among sites and with historic data produced by *in vitro* techniques. Specific research questions were as follows:

1) How do temperature, turbidity, and FDOM interfere with CHL-A fluorescence (RFU) measurements taken by the YSI EXO TAL sensor?
2) How can we best predict extracted CHL-A (µg/L) using the suite of YSI EXO sensors?

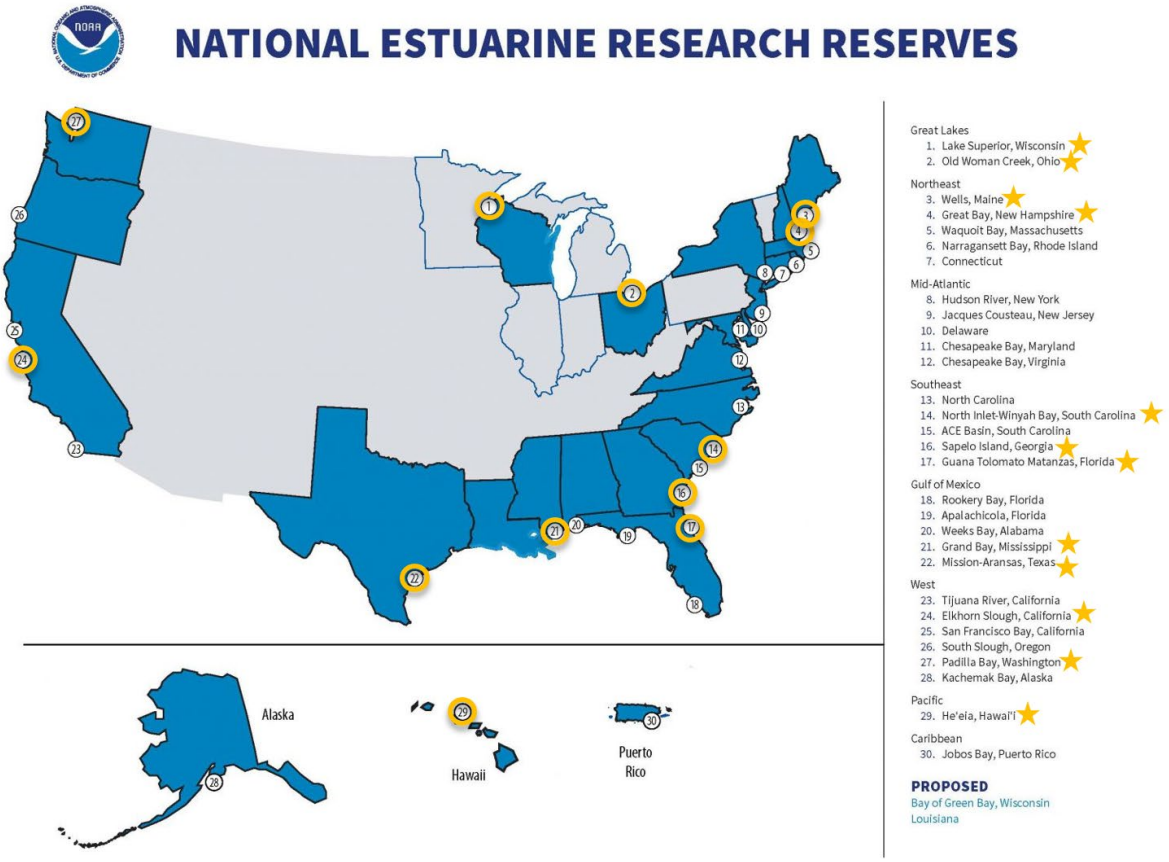

Figure 1. Research reserves that participated in the study: Lake Superior (LKS), Old Woman Creek (OWC), Wells (WEL), Great Bay (GRB), North Inlet-Winyah Bay (NIW), Sapelo Island (SAP), Guana Tolomato Matanzas (GTM), Grand Bay (GND), Mission-Aransas (MAR), Elkhorn Slough (ELK), Padilla Bay (PDB), and He'eia (HEE).

Methods

Sensor Calibration

YSI EXO Total Algae Sensors were calibrated in units of µg/L using a two-point calibration with DI water and a rhodamine WT dye standard, as described by current NERR SWMP SOPs. Sensors were similarly calibrated in RFU, as recommended in the latest version of the EXO manual. For each calibration, a separate 625 µg/L rhodamine dye standard was prepared by diluting a concentrated (125 mg/L) stock solution with deionized water. These standards were used for each calibration within 24 hours.

CHL-A Extraction

To achieve cell disruption and shortened extraction time, extracted CHL-A (µg/L) was quantified in a darkened laboratory following EPA method 445.0 minus the use of grinding. Samples were briefly filtered using a GF/F filter under low (< 6 Hg) pressure, during which the volume of each sample filtered was noted until color appeared on the filter. Filters were then folded, transferred to a scintillation vial or

test tube with a screw top, and frozen at -20°C for 24 hours in order to fracture cells. A known volume of 90% aqueous reagent-grade acetone was subsequently added to each vial, which were then gently agitated and placed in the freezer at -20°C for a 24- to 48-hour steeping period. Samples were gently agitated for a second time during this steeping period, after which they were inverted to resuspend any chlorophyll that may have settled. Once the samples had returned to ambient temperature, *In vitro* fluorescence was quantified on a fluorometer equipped with narrow band-pass filters using the non-acidification technique (Welschmeyer 1994).

Case Studies

Approximately one year of *in situ* CHL-A (µg/L) data measured in fifteen-minute increments and extracted CHL-A (µg/L) data measured montly were plotted for three reserve sites in order to illustrate the data characteristics produced by each method. Basic summary statistics were calculated (i.e., quantity, means, minima, maxima, and variation) for comparison.

Laboratory Assessments of Sensor Interferences and Corrections

Interference experiments

The effect of temperature on *in situ* fluorescence, as well as the potential for turbidity and FDOM to interfere with fluorescence, was investigated at select reserves (Table 1). For all experiments, a large volume (10 - 20 L) of natural water was sampled from a reserve SWMP site, returned to the lab, and transferred to a darkened vessel sitting atop a magnetic stirring hot plate in order to homogenize the sample volume. An EXO2 calibrated according to NERRS protocols (except for having a central wiper) was then submerged in the tank, with the rotation of the stir bar adjusted to avoid a vortex. The EXO2 was set to record fluorescence at the highest possible sampling frequency (1-2 sec) while temperature, turbidity, or FDOM was manipulated (Table 1). At the beginning and end of each interference test, an aliquot of water was withdrawn from the tank for extracted CHL-A determination, as previously described.

Table 1. Reserves that conducted experiments investigating potential effects of temperature, turbidity, and FDOM on *in situ* fluorescence measured by the EXO TAL sensor: Guana Tolomato Matanzas (GTM), He'eia (HEE), Lake Superior (LKS), North Inlet-Winyah Bay (NIW), Old Woman Creek (OWC), and Padilla Bay (PDB).

Reserve	Temperature	Turbidity	FDOM
GTM	X	X	X
HEE	X	X	
LKS			X
NIW	X	X	X
OWC			X
PDB	X		

Temperature

Potential temperature quenching was investigated following the protocols of Watras et al. (2017). Natural water (10-20 L) sampled from a reserve SWMP site was diluted with filtered (0.2 µm) sample water to four different concentrations of CHL-A (100, 50, 25, and 0% of ambient) in a darkened laboratory. Samples were collected at times and locations with high anticipated CHL-A concentrations in order to enable examination of potential quenching over a maximized range of CHL-A concentrations. After allowing water samples to acclimate to ~ 4 °C in an ice bath, each dilution was transferred to a darkened vessel atop a hot plate. Temperature and CHL-A fluorescence were then continuously measured with a submerged EXO2 sonde while the samples gradually warmed to approximately 30 °C over the course of roughly one hour.

Reserve-specific temperature corrections were derived for each of the four participating Reserves using the equations developed by Watras et al. (2017) to standardize fluorescence intensity to a reference temperature of 20 °C. Each Reserve's temperature correction was then evaluated by applying it to lab-based comparison data (methods below) and assessing whether it improved the modeled relationship between extracted CHL-A (µg/L) and *in situ* CHL-A (RFU). To assess the feasibility of a future system-wide temperature correction, a universal temperature correction based on the average of all trials across four Reserve's was applied to all lab-based comparison data.

Turbidity

The effect of turbidity on *in situ* CHL-A fluorescence was investigated following the general approaches used by Downing et al. (2012) for FDOM sensor assessment. Natural water sampled from a reserve site with naturally low turbidity was transferred to a darkened vessel in the lab and stirred at low speed. Aliquots of a turbidity standard, derived by combusting (450 °C for four hours) and homogenizing marsh mud collected in bulk from the North Inlet estuary, were then serially added to the tank every five minutes until turbidity reached approximately 1000 FNU. During this addition, a calibrated EXO2 sonde continuously measured turbidity and CHL-A fluorescence. The turbidity standard had a grain size distribution of 15.6 % clay, 42.7 % silt, and 41.7 % sand (as determined on a Beckman Coulter particle size analyzer). The percentage of the TAL sensor's RFU signal that was attenuated by turbidity was calculated following the example of Downing et al. (2012) to assess attenuation differences among sites and trials.

FDOM

The effect of FDOM on *in situ* fluorescence was investigated in a similar manner as Downing et al. (2012). Natural water from a reserve site that contained a measured amount of CHL-A was transferred to a darkened tank in the lab and stirred at low speed. Aliquots of a concentrated FDOM surrogate were then serially added to the tank every five minutes until FDOM reached 120-200 qsu, during which a calibrated EXO2 sonde continuously measured FDOM, turbidity, and CHL-A fluorescence. Reserves also performed trials in a DI water matrix. Reserves uniquely created their FDOM surrogate standards by collecting local ambient water naturally high in FDOM, filtering it through a 0.2-µm filter to remove chlorophyll-containing cells, and concentrating it by a factor of five to ten using a heated stir plate (80 °C).

OWC conducted six trials using an FDOM surrogate standard derived from Old Woman Creek.

Three trials were conducted in a deionized (DI) water matrix, and three in a Lake Erie ambient water matrix. NIW conducted one trial in a DI water matrix, two in a Crab Haul Creek ambient water matrix, and three using a FDOM surrogate standard derived from a forested wetland that drains into Crab Haul Creek in North Inlet. LKS conducted one trial in a DI matrix, another in a St. Louis River estuary matrix, and two trials with a FDOM surrogate standard derived from the St. Louis River Headwaters. GTM conducted two trials, one with a Pellicer Creek derived FDOM surrogate standard and one with a humic acid standard (Sigma Aldridge), both added to a Tolomato River ambient water matrix. Dissolved organic matter concentrations and light absorption of FDOM surrogate standards from GTM (Pellicer Creek), NIW (Crab Haul Creek headwater wetland), LKS (St. Louis River Headwaters), and OWC (Old Woman Creek) were assessed (Table 2) to provide context for results.

Table 2. Final concentration and light absorption of FDOM surrogates used in interference experiments. North Inlet-Winyah Bay (NIW), Lake Superior (LKS), Guana Tolomato Matanzas (GTM), and Old Woman Creek (OWC).

Reserve	DOC (mg L^{-1})	Abs @ 355 nm (m^{-1})	Abs/DOC
NIW	345.1	1485.9	4.3
LKS	216.4	378.9	1.8
GTM	79.1	177.0	2.2
OWC1	75.4	75.4	1.0
OWC2	95.8	144.7	1.5

Predicting Extracted CHL-A (µg/L) from *In Situ* CHL-A (RFU)

Sampling design

Two approaches were used by reserves to compare *in situ* CHL-A fluorescence to extracted CHL-A fluorescence for natural water samples. Both approaches were designed to ensure that their methods sampled the same water mass. First, simultaneous paired sampling was conducted by collecting discrete samples immediately adjacent to EXO2 sondes deployed in the field (field-based samples). For the second approach, a large volume (10-20 L) of natural water was sampled from a reserve SWMP site, returned to the lab, and transferred to a darkened mixing tank sitting atop a magnetic stir plate to homogenize the sample volume. An EXO2 was then submerged in the tank, and the rotation speed of the stir bar was set to avoid a vortex. The EXO2 was set to record CHL-A fluorescence at the highest possible sampling frequency (one to two seconds), and, after approximately five minutes of acclimation, an aliquot was withdrawn from the tank for extracted CHL-A determination.

For both field- and laboratory-based sampling, sondes were equipped with the full suite of required SWMP sensors, plus FDOM, to provide ancillary data for cross-system analysis, as well as data potentially necessary for CHL-A fluorescence *a posteriori* corrections. Water samples for extracted CHL-A determination were stored at 4°C in the dark and processed within twelve hours of the collection as described above. For every batch analysis or every 24 samples, a deionized water sample was included as a lab blank to identify potential contamination for both field- and lab-based samples.

The sampling frequency and the number of samples collected for both approaches were not standardized across reserves (Table 3), but each reserve attempted to capture as much environmental variability as possible.

Table 3. Site characteristics, the number of field and laboratory samples from each site, and project totals. Elkhorn Slough (ELK), Grand Bay (GND), Great Bay (GRB), Guana Tolomato Matanzas (GTM), He'eia (HEE), Lake Superior (LKS), Mission-Aransas (MAR), North Inlet-Winyah Bay (NIW), Old Woman Creek (OWC), Padilla Bay (PDB), Sapelo Island (SAP), and Wells (WEL).

Reserve	Site Name	Location	Salinity Range	Average Depth (m)	Tidal Range (m)	# Field-Based Samples	# Lab-Based Samples
ELK	South Marsh	36.81806, -121.73940	28-36	0.3	3.4	119	0
GND	Bangs Lake	30.35712, -88.46299	4-32	0.9	0.5	11	10
GRB	Adams Point	43.09208, -70.86428	10-32	2.5	3.0	34	9
GRB	Oyster River	43.13389, -70.91111	0-33	1.7	3.0	0	2
GRB	Squamscott River	43.04167, -70.92222	0-32	1.9	3.0	24	5
GRB	Lamprey River	43.08000, -70.93444	0-31	1.8	3.0	65	0
GRB	Great Bay East	43.06179, -70.85376	0-32	1.6	2.9	0	2
GRB	Adams Point Marsh	43.09208, -70.86428	Un-known	0.3	0.5	0	1
GRB	Upper Little Bay	43.10738, -70.86337	7-32	3.9	3.0	0	1
GTM	Pellicer Creek	29.66694, -81.2575	0-34	1.3	0.5	144	31

Table 3 (continued).

Reserve	Site Name	Location	Salinity Range	Average Depth (m)	Tidal Range (m)	# Field-Based Samples	# Lab-Based Samples
HEE	Kaho'okele	21.43582, -157.80524	27-35	1.1	0.7	84	28
HEE	Wai 2	21.43731, -157.81093	0-34	0.4	0.7	0	4
LKS	Barker's Island	46.721772, -92.06352	0.1-0.2	3.0	NA	58	77
MAR	Ship Channel	27.83818, -97.05252	18-37	6.1	1.0	0	18
NIW	Oyster Landing (Crab Haul Creek)	33.34944, -79.18889	0-37	2.0	1.4	0	65
NIW	Winyah Bay Surface	33.30944, -79.28861	0-25	5.0	1.1	72	0
OWC	Wetland Mouth	41.22570, -82.30530	0.1-0.9	0.5	0.04	130	0
PDB	Gong	48.5575, -122.5725	23-32	18.0	2.4	0	13
PDB	Bayview	48.49614, -122.50211	23-32	1.5	2.4	96	10
SAP	Lighthouse Creek	31.39728, -81.28156	18-29	Un-known	2.1	0	46
WEL	Inlet	43.32025, -70.56347	16-33	3.5	4.7	96	0
Total						*933*	*322*

Model development and selection

The ability to predict extracted CHL-A (µg/L) from *in situ* sensor data was explored using linear regression models. Models were created to describe relationships between

1) extracted CHL-A (µg/L) and *in situ* CHL-A (RFU) only;
2) extracted CHL-A (µg/L) and *in situ* CHL-A (RFU) plus FDOM, temperature, and turbidity (including all interactions); and,
3) extracted CHL-A (µg/L) and *in situ* CHL-A (RFU) plus temperature and turbidity (including all interactions).

The second and third set of models included "reserve" and "season" as fixed effects. The third set of models excluded FDOM to assess its value in predicting extracted CHL-A (µg/L). FDOM is currently not required in the SWMP, as purchasing FDOM sensors is a significant expense.

These three relationships were explored at two spatial scales, national and site-specific. National models were created using data from

1) all comparison tests (field- and lab-based) at all sites (n = 1076) and
2) only lab-based tests at all sites (n = 311).

Site-specific models were created with data from

1) all comparison tests (field- and lab-based) at that site and
2) only lab-based tests at that site.

Results specific to lab-based comparisons were of interest because the conditions were more controlled than in field-based comparisons.

Prior to analysis, observations missing any of the model input variables were removed and a correlation matrix was examined. No strong correlations among predictor variables were observed. Residuals were funnel shaped (more variable at higher chlorophyll concentrations), so the response variable (extracted CHL-A) was square-root transformed.

Model selection was performed on the full model with chlorophyll, temperature, turbidity, FDOM, and their interactions. The 'best' model from that subset was determined by lowest corrected Akaike Information Criterion (AICc). There may have been multiple models within two AICc of the top model (Burnham and Anderson 2002), but those were disregarded. If at least two seasons were present, the season was included in the model; otherwise, it was not.

The model with the lowest AICc was compared to (1) models with *in situ* CHL-A only and (2) models without FDOM by comparing model fit (R^2) and prediction errors based on either 10-fold cross-validation (national models) or leave-one-out cross-validation (site-specific models). Prediction error, after predictions were back-transformed to original units, was calculated as symmetric Median Absolute Percentage Error (Hyndman and Koehler 2006).

Results and Discussion

Case Study Comparisons of Data Characteristics

Three time series of fifteen-minute *in situ* CHL-A (µg/L) data and monthly extracted CHL-A (µg/L) data collected for the SWMP illustrate the data characteristics produced by each method (Fig. 2). A fifteen-minute data collection frequency provides approximately 3,000 data points per month and captures more variability than monthly discrete sample collection (Table 4).

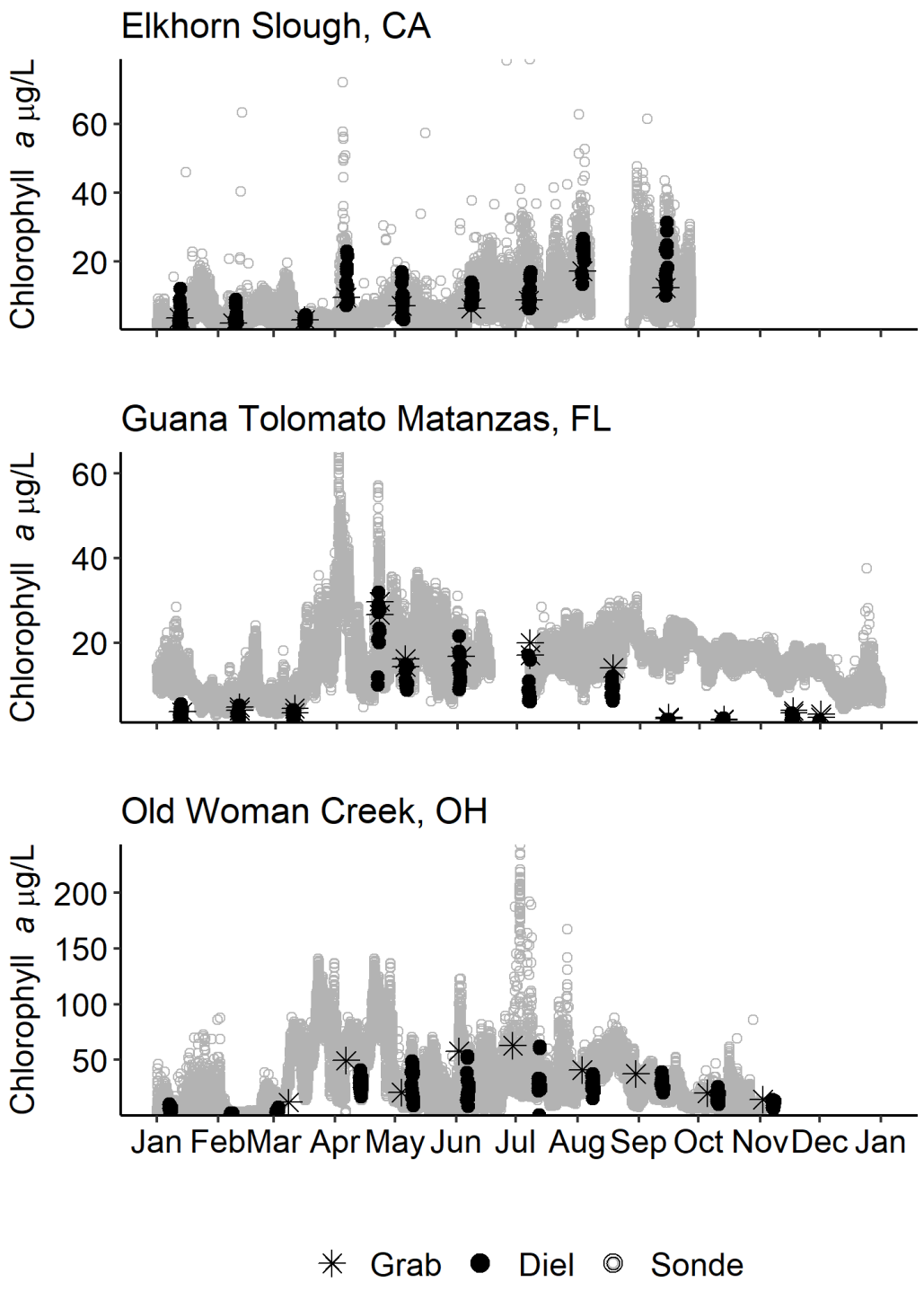

Figure 2. *In situ* chlorophyll *a* (µg/L) from the EXO2 sondes (gray open circles) and chlorophyll *a* (µg/L) from extracted discrete samples (black asterisks and closed circles).

Table 4. Data characteristics from Elkhorn Slough (ELK), Guana Tolomato Matanzas (GTM), and Old Woman Creek (OWC) reserve case studies.

Reserve (Site)	Date Range	15-min CHL-A	diel CHL-A	monthly CHL-A
ELK (South Marsh)	01/12/2021 - 09/15/2021	Mean = 7.5 µg/L N = 23726 SD = 6.2 µg/L	Mean = 10.9 µg/L N =119 SD = 7.3 µg/L	Mean = 7.9 µg/L N = 9 SD =4.8 µg/L
GTM (Pellicer Creek)	01/01/2020 - 12/31/2020	Mean = 15.2 µg/L N = 32660 SD = 6.6 µg/L	Mean = 8.2 µg/L N = 106 SD = 7.1 µg/L	Mean = 9.8 µg/L N = 12 SD = 8.7 µg/L
OWC (Wetland Mouth)	01/01/2021 - 11/02/2021	Mean = 29.2 µg/L N = 29265 SD = 26.3 µg/L	Mean = 18.6 µg/L N = 164 SD = 13.5 µg/L	Mean = 34.9 µg/L N = 9 SD = 18.1 µg/L

Laboratory Assessments of Interferences and Corrections

Temperature

Even though YSI has a temperature correction on the TAL sensor, we observed temperature quenching in experimental trials (Fig. 3). The effect of temperature quenching was somewhat less when CHL-A was low (Table 5).

Using an average slope from all sites to correct the total CHL-A (RFU) across all Reserves did not improve model fit or prediction (results not shown). More trials would be necessary to get a more accurate average, but regardless, the rho values were remarkably similar across sites (Table 5) and were notably close to the rho values derived from other sensors (Watras et al. 2007). Even though model fit and prediction error did not improve much, the corrections applied did adjust CHL-A (RFU) values significantly when there was a large temperature range.

In particular, temperature correction noticeably modified the slope between CHL-A (RFU) and extracted CHL-A (µg/L), especially for sites that experienced high variability in temperature (e.g., NIW, typical annual range = 8 - 34 °C; Fig. 4). Therefore, temperature corrections could improve the accuracy of the sensor's CHL-A (RFU) output, even if the corrections do not improve the amount of variation described by the predictive model.

Because all SWMP stations collect temperature data, it is feasible for any Reserve to make a site-specific temperature correction by replicating the methods employed here. This correction would be most needed at SWMP stations that experience high temperature variability and high chlorophyll concentrations. However, this dataset does not allow us to create a "NERRS-wide" temperature correction because the short project period did not allow sufficient time for replication. These trials were conducted with ambient water from different SWMP sites, so other sources of interference are possible. For example, studies have shown that different species of algae/bacteria have varying temperature-quenching effects (Watras et al. 2017). However, unlike CHL-A (RFU) responses to FDOM and turbidity (discussed below), responses to temperature were fairly consistent across different sites, raising the possibility of a universal correction factor. Hence, we recommend more trials be conducted

across the system throughout the year to obtain multiple species compositions and to establish a system-wide temperature ρ. We expect the standard deviation to improve with more replication.

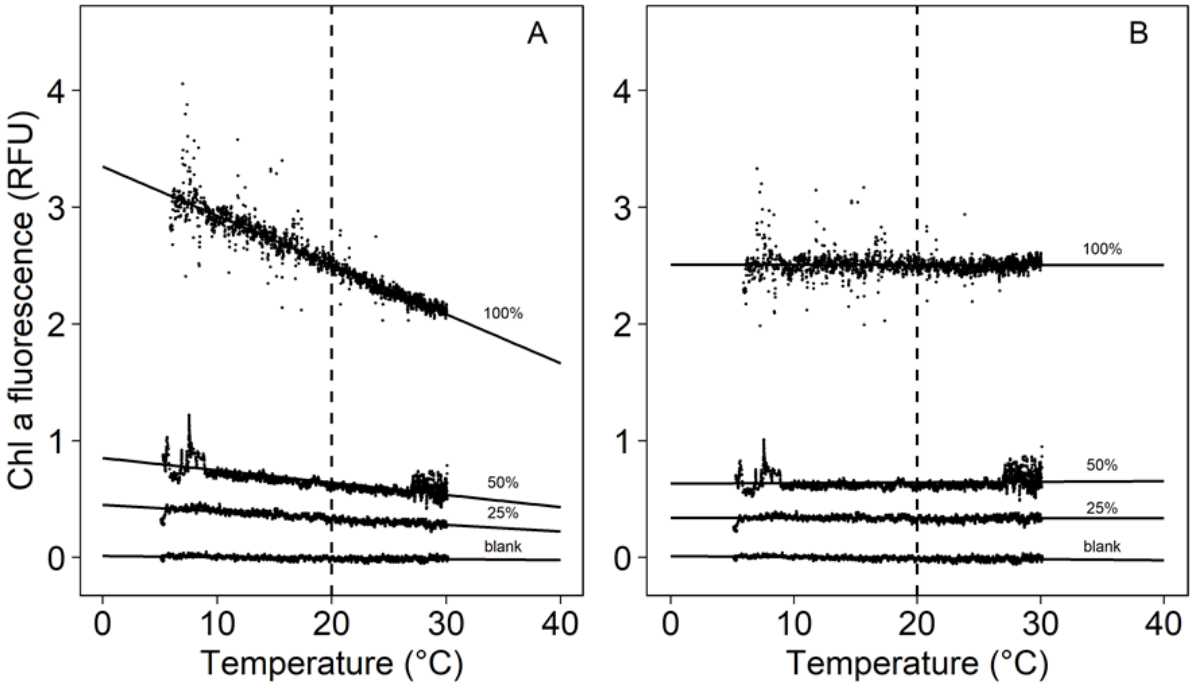

Figure 3. Example from Winyah Bay (North Inlet-Winyah Bay NERR) of the temperature-quenching effect on chlorophyll fluorescence as measured by the TAL sensor (A) and how it can be corrected (B) using Eq. 1 from Watras et al. (2017) with a standard reference temperature of 20°C.

Table 5. Temperature trial results at Guana Tolomato Matanzas (GTM), North Inlet-Winyah Bay (NIW), and He'eia (HEE) reserves; the waterbody name where the sample was collected (Matrix); the extracted CHL-A concentration in the matrix water at the beginning of the experiment (Matrix CHL-A); and the number of trials (n).

Reserve	Matrix	Date	Matrix CHL-A (µg/L)	n	Mean (± SD) Temperature Coefficient (ρ) ($°C^{-1}$)
GTM	Pellicer Creek	07/08/2021	5.48	3	-0.0142 (± 0.0064)
NIW	Crab Haul Creek	08/04/2021	20.4	3	-0.0167 (± 0.0003)
HEE	Kaho'okele	08/30-08/31/2021	1.06	3	-0.0098 (± 0.0025)

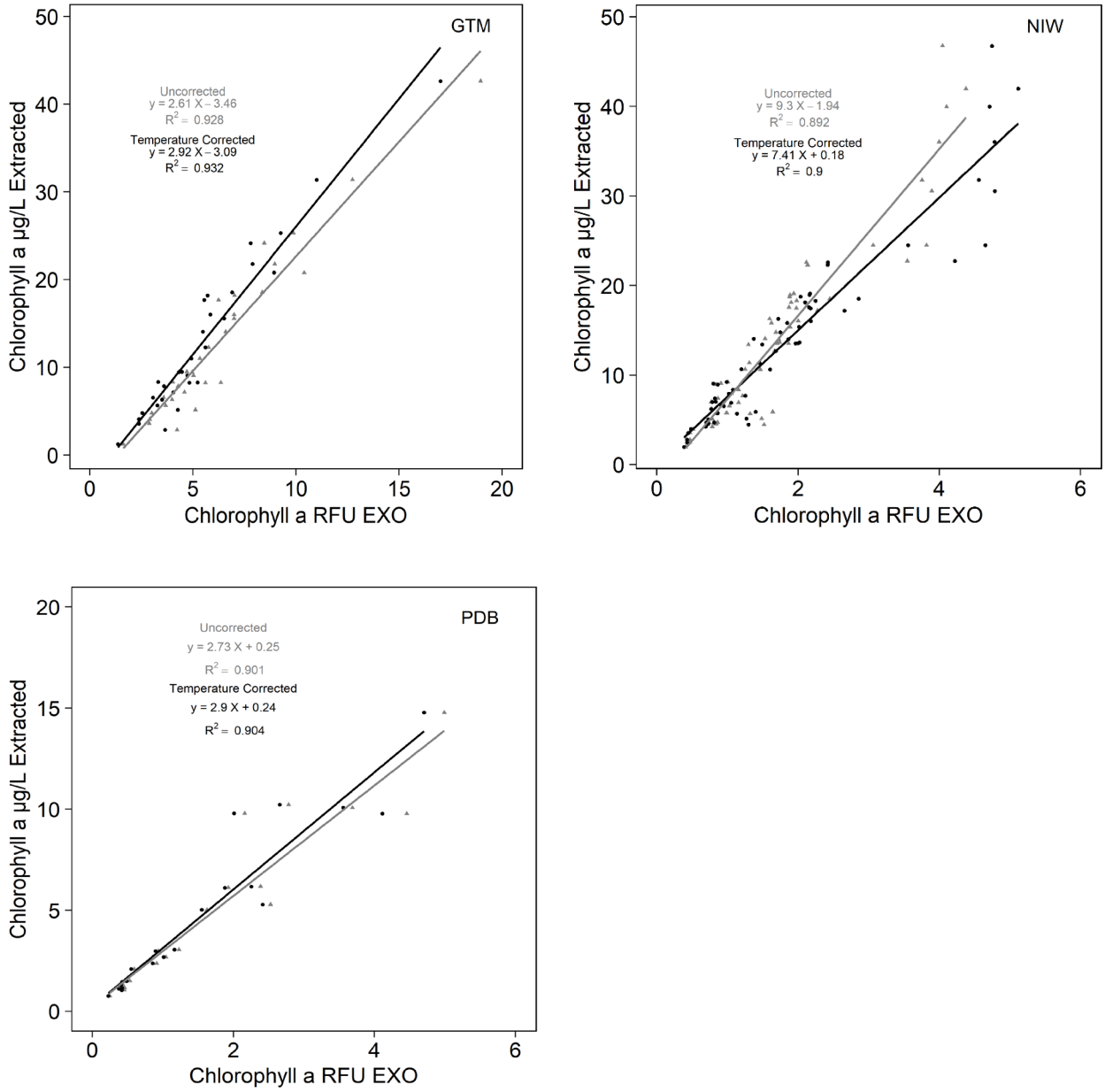

Figure 4. Temperature corrections derived from individual Reserve's interference trials were applied to tank data sets from three Reserves to assess the effect of the temperature correction on the model strength (R^2) and model slope (GTM = Guana Tolomato Matanzas, NIW = North Inlet-Winyah Bay, and PDB = Padilla Bay).

Turbidity

Manipulating the turbidity in water samples from 0 - 1500 FNU with chlorophyll-free, inorganic standard, caused a 0.3 - 2-fold decrease in CHL-A RFU (Fig. 5) at GTM and NIW. At those sites, turbidity caused 20-55% attenuation of CHL-A fluorescence (Fig. 6). At HEE, where CHL-A values were low and unrealistically high turbidity levels (for that site) were tested, CHL-A (RFU) increased with increasing

turbidity in a non-linear fashion (Fig. 5).

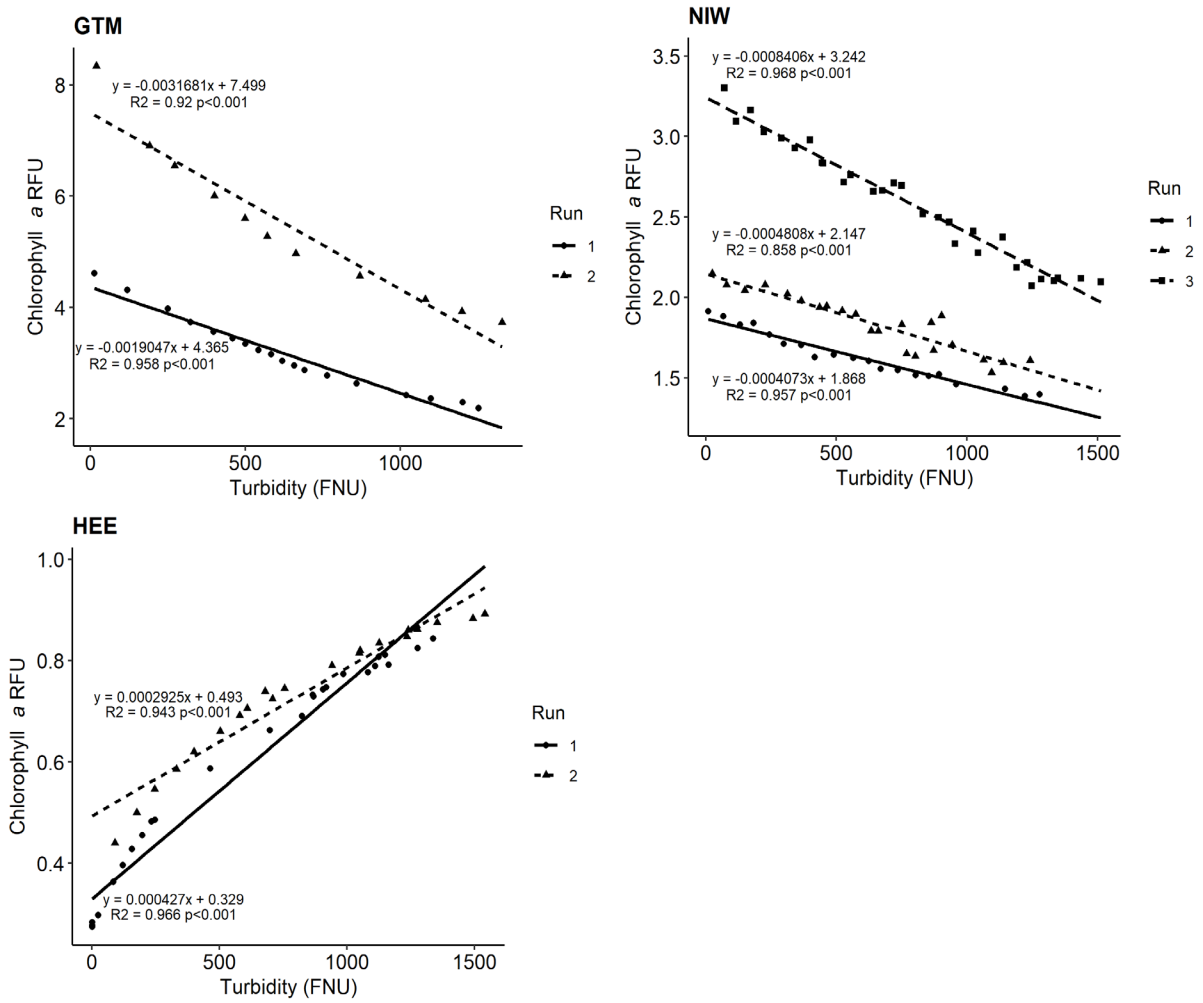

Figure 5. Turbidity trial results at Guana Tolomato Matanzas (GTM), North Inlet-Winyah Bay (NIW), and He'eia (HEE) reserves.

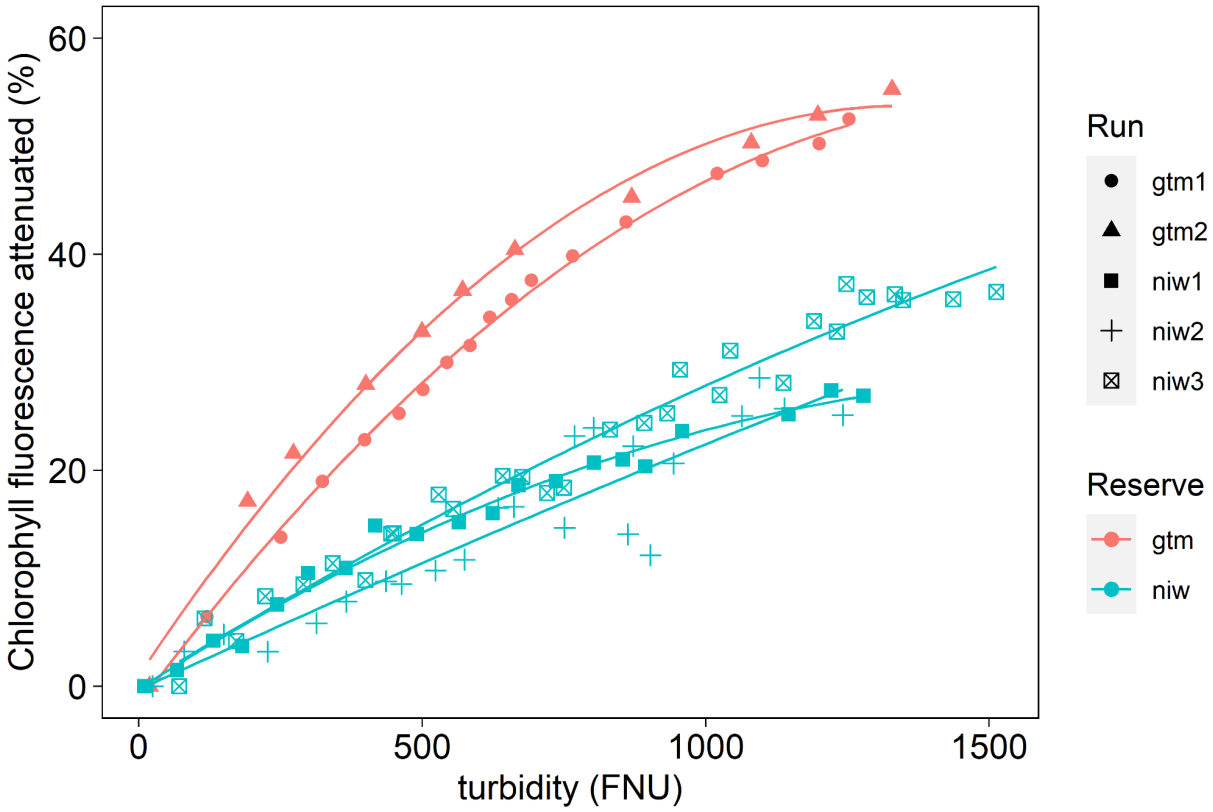

Figure 6. Attenuation of the CHL-A (RFU) signal as a function of turbidity. Data derived from interference trials performed at Guana Tolomato Matanzas (GTM) and North Inlet-Winyah Bay (NIW). Note that He'eia's turbidity trial data is not visualized due to an inadequate amount of CHL-A in their trials.

Table 6. Turbidity trial results from Guana Tolomato Matanzas (GTM), North Inlet-Winyah Bay (NIW), and He'eia (HEE) reserves.

Reserve	Matrix	Trial #	Date	CHL-A (µg/L)	Slope
GTM	Pellicer Creek	1	07/07/2021	10.7	-0.0019
GTM	Pellicer Creek	2	07/20/2021	14.1	-0.0032
NIW	Oyster Landing	1	07/01/2021	17.4	-0.0004
NIW	Oyster Landing	2	07/07/2021	19.2	-0.0005
NIW	Oyster Landing	3	07/08/2021	29.9	-0.0008
HEE	Stream Mouth	1	08/16/2021	0.88	0.0004
HEE	Wai 2	2	08/16/2021	1.42	0.0003

FDOM

Experimentally increasing FDOM in natural water samples and deionized water caused varying increases of *in situ* CHL-A (Fig. 7), the opposite direction of the effect of increasing turbidity and temperature. Relationships between *in situ* CHL-A and FDOM were not uniform across sites (Table 7), likely due to differences in the dissolved organic matter constituents used in the surrogate standard (Table 2).

FDOM varies widely across Reserves in both concentration and optical properties, and many Reserves do not measure it consistently. Therefore, using a standard FDOM correction across Reserves is not practical.

Table 7. FDOM experimental trial results conducted at Old Woman Creek (OWC), North Inlet-Winyah Bay (NIW), Lake Superior (LKS), and Guana Tolomato Matanzas (GTM) reserves.

Reserve	Matrix	n	FDOM surrogate source	Mean Slope +/- SD
OWC	Deionized water	1	Old Woman Creek	0.0039
OWC	Deionized water	2	Old Woman Creek	0.0063 +/- 0.0012
NIW	Deionized water	1	Crab Haul Creek headwater wetland	0.0172
LKS	Deionized water	1	St. Louis River Headwaters	0.0054
GTM	Tolomato River	1	St. Louis River Headwaters	0.0054
GTM	Tolomato River	1	Crab Haul Creek headwater wetland	0.0172
GTM	Tolomato River	1	Pellicer Creek	0.0066
GTM	Pellicer Creek	1	Humic acid standard	0.0816
OWC	Lake Erie	1	Old Woman Creek	0.0016
OWC	Lake Erie	2	Old Woman Creek	0.0027 +/- 0.0017
NIW	Crab Haul Creek	1	Crab Haul Creek headwater wetland	0.0188
LKS	St. Louis River Estuary	1	St. Louis River Headwaters	0.0059

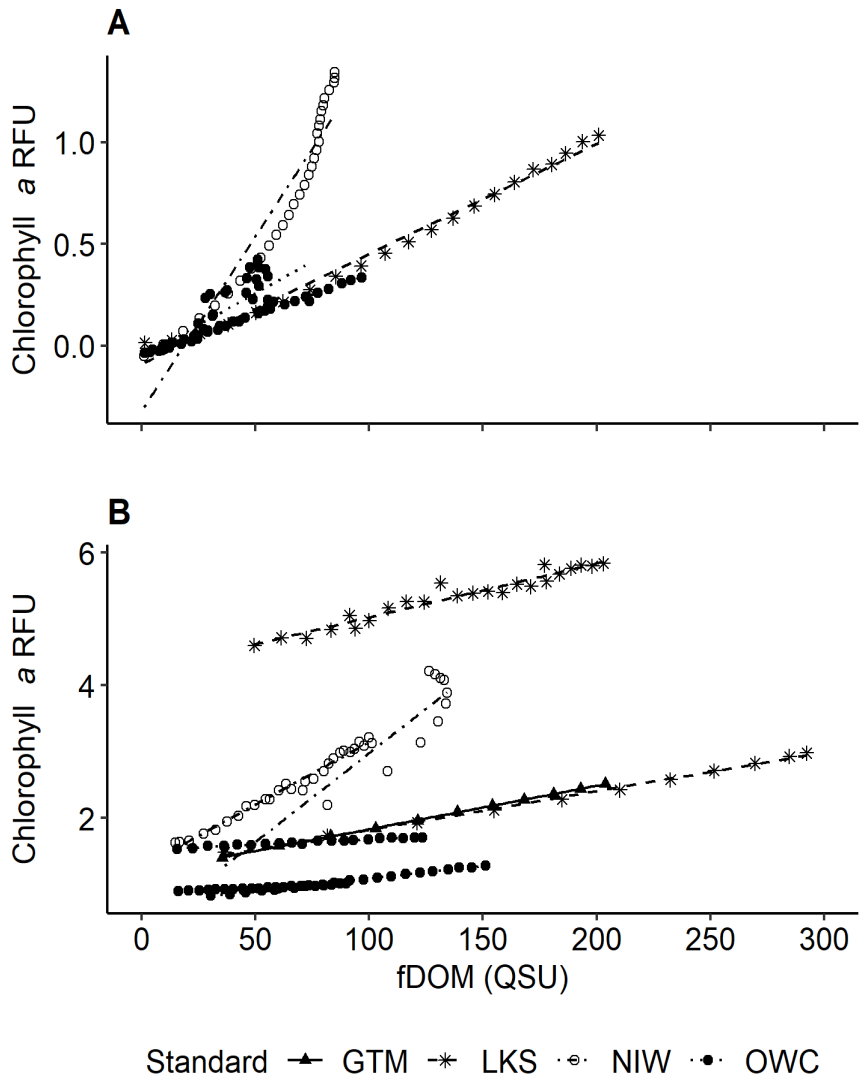

Figure 7. Fluorescent dissolved organic carbon (FDOM) effect on CHL-A (RFU) measured by the EXO Total Algae sensor. Experiments were conducted by Guana Tolomato Matanzas (GTM), Lake Superior (LKS), North Inlet-Winyah Bay (NIW), and Old Woman Creek (OWC) NERRs. Standards made using ambient water near each NERR site were added to deionized water (A) and local ambient water (B).

Predicting Extracted CHL-A from *In Situ* CHL-A

Comparisons between extracted and in situ *CHL-A*

When *in situ* CHL-A and extracted CHL-A were measured simultaneously in both field- and lab-based comparisons, they were significantly correlated, but *in situ* CHL-A only explained 35% of the variance in extracted CHL-A (Fig. 8). At individual sites, the relationship was tight for some (Fig. 9) and scattered for others (Fig. 10).

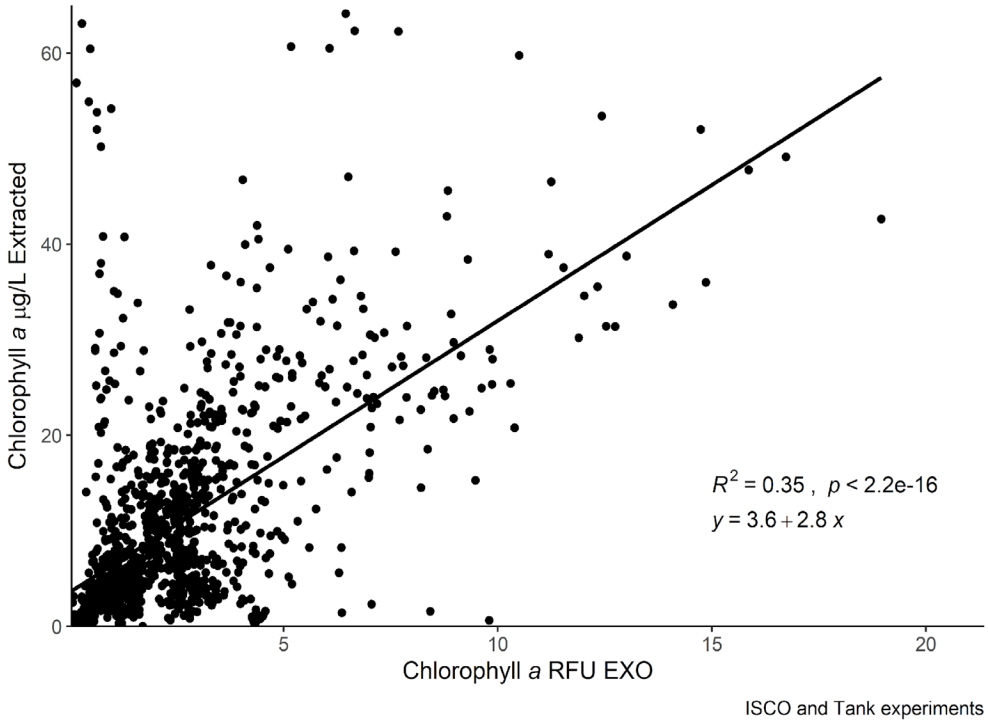

Figure 8. Relationship between *in situ* CHL-A (RFU) and extracted CHL-A (µg/L) using data from all comparisons (n = 1255).

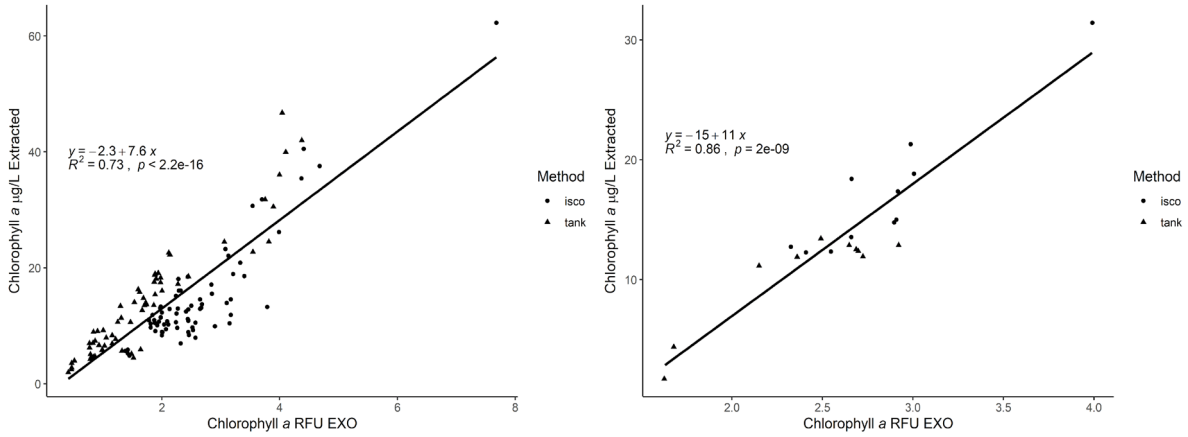

Figure 9. Examples of tight relationships between *in situ* CHL-A and extracted CHL-A at North Inlet-Winyah Bay (left; R^2 = 0.73) and Grand Bay (right; R^2 = 0.86) reserves.

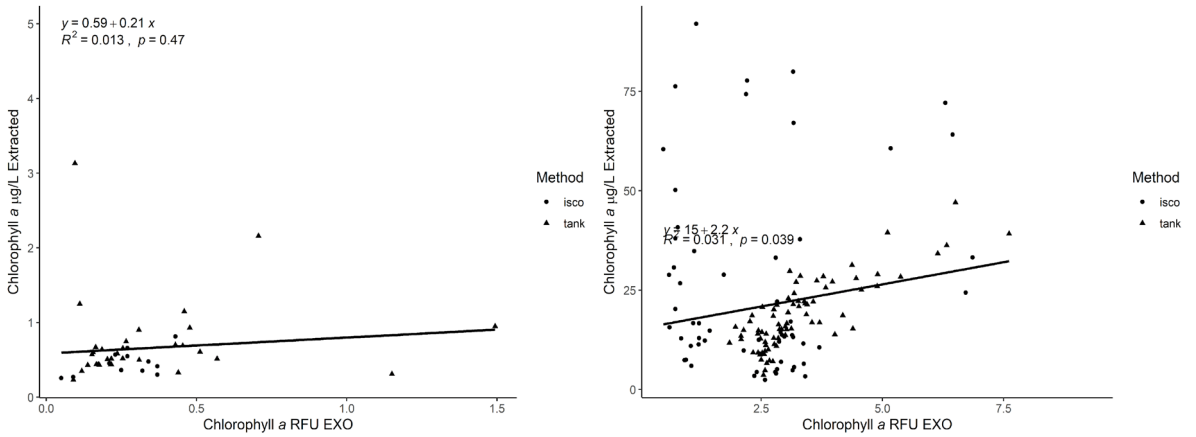

Figure 10. Examples of scattered relationships between *in situ* CHL-A and extracted CHL-A at Heʻeia (left; R^2 = 0.0013) and Lake Superior (right; R^2 = 0.031) reserves.

National models

Using data from lab-based comparisons only, phytoplankton biomass as estimated by extracted chlorophyll CHL-A was linearly related to *in situ* CHL-A, reserve, FDOM, temperature, and turbidity data with an R^2 = 0.786 and a median prediction error of 26% (Fig. 11 and Table 8). The model without FDOM explained only slightly less variance in the response (R^2 = 0.774) and had slightly higher prediction error (27%). The model with only *in situ* CHL-A performed worse (R^2 = 0.414, median prediction error = 50%).

When data from lab- and field-based comparisons were combined, extracted CHL-A was linearly related to *in situ* CHL-A, reserve, season, FDOM, temperature, and turbidity data from both field- and lab-based comparisons with an R^2 of 0.657 and a median prediction error of 36% (Fig. 11 and Table 9). The model without FDOM explained slightly less variance in the response (R^2 = 0.632) and had slightly higher prediction error (38%). The model with *in situ* CHL-A performed poorest (R^2 = 0.299, median prediction error = 56%).

Site-specific models

For most sites, site-specific models explained more variance and were more than or equally reliable as the national lab-based model (Fig. 11, Table 10, Table 11). In general, the models with *in situ* CHL-A, FDOM, temperature, and turbidity data performed better than (1) models without FDOM and (2) models with *in situ* CHL-A only. However, the models without FDOM had similar fits and prediction ability to the models with FDOM. At some sites, models with only *in situ* CHL-A explained less than 5-15% of the variance in the data. Some model overfitting was observed (R^2 = 1), indicating both a need for more data points at those sites and a need for caution using those models to predict extracted CHL-A.

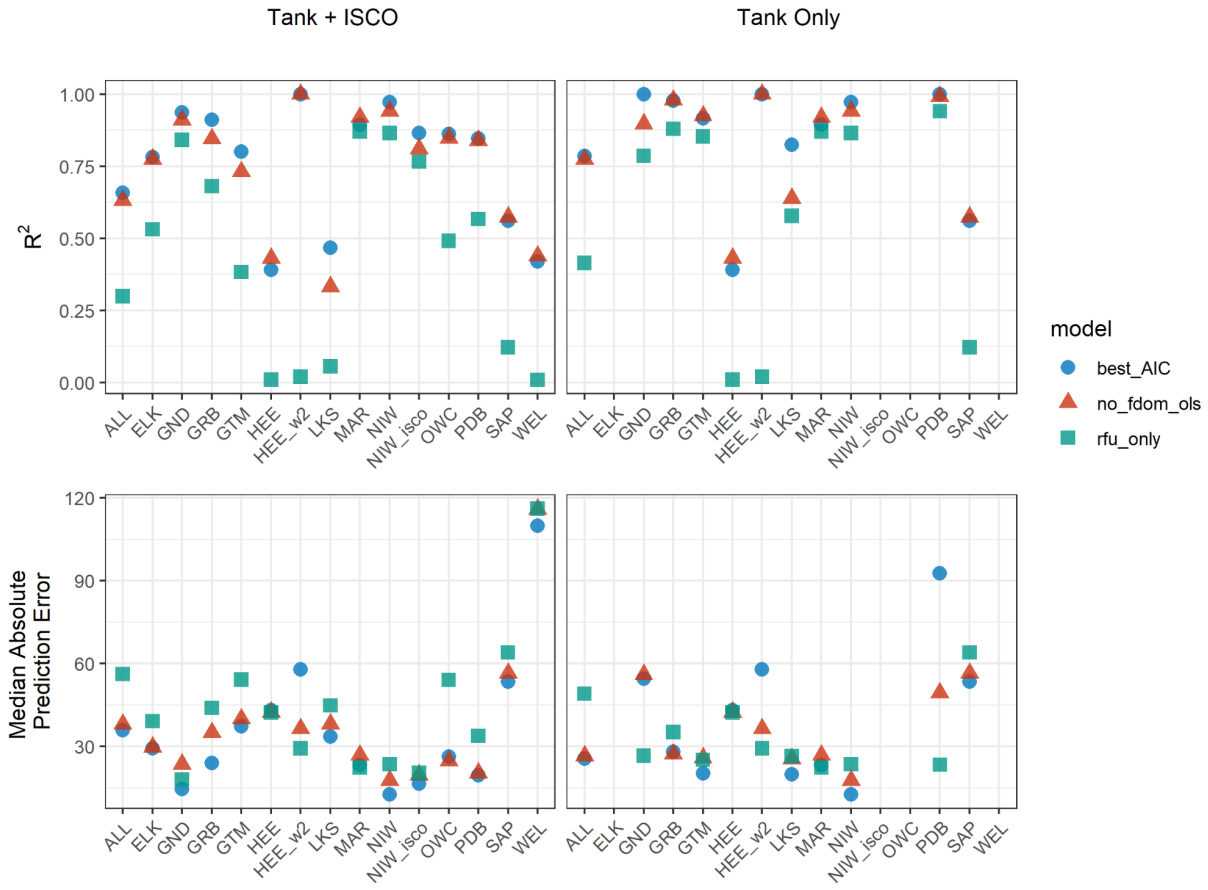

Figure 11. Model performance comparison.

Table 8. National model results using lab- and field-based comparisons. “best_AIC” models represent the model with the best AIC out of the selection process with the following sensors and interactions included: chlorophyll, FDOM, temperature, and turbidity. “no_fdom” models represent the output including data from temperature, chlorophyll, and turbidity sensors, plus interactions. If at least two seasons were present in “best_AIC” and “no_fdom” models, season was included in the model as well; otherwise, it was not. “rfu_only” models only included data from the chlorophyll sensor. No model selection was performed on “no_fdom” and “rfu_only” models. In the 'season' column, a plus sign (+) is present if season was significant in the model. All other potential coefficients are continuous variables (and thus have a slope coefficient) or are excluded if they were not significant or intentionally not included in the model. Interactions are represented by a period between variable names.

model	AICc	R^2	prediction_error	chl_rfu	fdom	turb	temp	reserve	season	X Intercept	fdom.chl_rfu	fdom.temp	fdom.turb	chl_rfu.temp	chl_rfu.turb	fdom.chl_rfu.turb
best_AIC	3047.55	0.657	35.96	0.557	7e-04	0.025	0.126	+	+	0.162	NA	-4e-04	NA	-0.012	-0.004	NA
no_fdom	3116.05	0.632	38.10	0.609	NA	0.025	0.092	+	+	0.565	NA	NA	NA	-0.014	-0.004	NA
rfu_only	3769.49	0.299	56.21	0.375	NA	NA	NA	NA	NA	2.013	NA	NA	NA	NA	NA	NA

Table 9. National model results using lab-based comparisons. “best_AIC” models represent the model with the best AIC out of the selection process with the following sensors and interactions included: chlorophyll, FDOM, temperature, and turbidity. “no_fdom” models represent the output including data from temperature, chlorophyll, and turbidity sensors, plus interactions. If at least two seasons were present in “best_AIC” and “no_fdom” models, season was included in the model as well; otherwise, it was not. “rfu_only” models only included data from the chlorophyll sensor. No model selection was performed on “no_fdom” and “rfu_only” models. In the 'season' column, a + is present if season was significant in the model. All other potential coefficients are continuous variables (and thus have a slope coefficient) or are excluded if they were not significant or intentionally not included in the model. Interactions are represented by a period between variable names.

model	AICc	R^2	prediction_error	chl_rfu	fdom	turb	temp	reserve	season	X Intercept	fdom.chl_rfu	fdom.temp	fdom.turb	chl_rfu.temp	chl_rfu.turb	fdom.chl_rfu.turb
best_AIC	704.48	0.786	25.65	0.231	0.003	0.125	0.027	+	NA	1.149	0.001	-6e-04	NA	0.017	-0.027	NA
no_fdom	721.17	0.774	26.66	0.414	NA	0.109	-0.021	+	+	1.334	NA	NA	NA	0.010	-0.024	NA
rfu_only	983.06	0.414	49.14	0.388	NA	NA	NA	NA	NA	2.178	NA	NA	NA	NA	NA	NA

Table 10. Site-specific model results using lab- and field-based comparisons. “best_AIC” models represent the model with the best AIC out of the selection process with the following sensors and interactions included: chlorophyll, FDOM, temperature, and turbidity. “no_fdom” models represent the output including data from temperature, chlorophyll, and turbidity sensors, plus interactions. If at least two seasons were present in “best_AIC” and “no_fdom” models, season was included in the model as well; otherwise, it was not. “rfu_only” models only included data from the chlorophyll sensor. No model selection was performed on “no_fdom” and “rfu_only” models. In the 'season' column, a + is present if season was significant in the model. All other potential coefficients are continuous variables (and thus have a slope coefficient) or are excluded if they were not significant or intentionally not included in the model. Interactions are represented by a period between variable names.

Reserve	model	AICc	R^2	prediction_error	chl_rfu	fdom	turb	temp	season	X Intercept	fdom.chl_rfu	fdom.temp	fdom.turb	chl_rfu.temp	chl_rfu.turb	fdom.chl_rfu.turb
ELK	best_AIC	164	0.782	29.31	0.365	0.024	-0.020	0.232	+	-1.216	-0.012	NA	NA	NA	NA	NA
ELK	no_fdom	167.15	0.775	29.86	0.497	NA	0.025	0.261	+	-1.448	NA	NA	NA	-0.011	-0.0166	NA
ELK	rfu_only	219.1	0.531	39.18	0.410	NA	NA	NA	NA	2.161	NA	NA	NA	NA	NA	NA
GND	best_AIC	16.05	0.936	14.57	4.200	0.102	0.043	NA	+	-6.832	-0.0431	NA	NA	NA	NA	NA
GND	no_fdom	28.46	0.911	23.63	-4.397	NA	0.206	-0.649	+	15.283	NA	NA	NA	0.244	-0.0709	NA
GND	rfu_only	19.89	0.842	18.12	1.569	NA	NA	NA	NA	-0.488	NA	NA	NA	NA	NA	NA
GTM	best_AIC	218.52	0.801	37.36	0.270	-0.010	-0.033	0.034	+	1.999	NA	NA	NA	-0.015	0.0118	NA
GTM	no_fdom	268.4	0.731	40.07	0.143	NA	-0.067	0.026	+	1.34	NA	NA	NA	-0.014	0.0235	NA
GTM	rfu_only	398.79	0.383	54.22	0.278	NA	NA	NA	NA	1.151	NA	NA	NA	NA	NA	NA
LKS	best_AIC	400.05	0.467	33.69	-0.438	0.004	NA	0.345	NA	1.776	0.0104	-0.0034	NA	NA	NA	NA
LKS	no_fdom	432.81	0.332	38.19	-0.158	NA	-0.523	0.048	+	5.139	NA	NA	NA	-0.015	0.1323	NA
LKS	rfu_only	462.74	0.056	44.9	0.254	NA	NA	NA	NA	3.821	NA	NA	NA	NA	NA	NA
NIW_isco	best_AIC	71.22	0.866	16.52	4.453	0.024	0.014	0.125	NA	-4.938	NA	NA	NA	-0.117	NA	NA
NIW_isco	no_fdom	96.52	0.81	19.76	3.762	NA	0.011	0.098	NA	-1.924	NA	NA	NA	-0.098	0.0034	NA
NIW_isco	rfu_only	102.31	0.766	20.54	0.922	NA	NA	NA	NA	1.313	NA	NA	NA	NA	NA	NA
OWC	best_AIC	287.83	0.862	26.37	0.265	0.006	-0.008	0.162	+	1.433	-0.005	0.0014	NA	NA	0.0047	NA
OWC	no_fdom	295.83	0.848	24.83	0.088	NA	-0.005	0.245	+	1.556	NA	NA	NA	-0.004	0.0038	NA
OWC	rfu_only	437.02	0.491	54.08	0.296	NA	NA	NA	NA	2.731	NA	NA	NA	NA	NA	NA
PDB	best_AIC	1.33	0.846	19.65	1.100	0.022	0.123	0.063	+	-0.026	NA	NA	NA	-0.036	-0.0855	NA
PDB	no_fdom	2.08	0.839	20.41	1.096	NA	0.128	0.067	+	0.159	NA	NA	NA	-0.035	-0.0834	NA
PDB	rfu_only	66.63	0.567	33.88	0.567	NA	NA	NA	NA	1.26	NA	NA	NA	NA	NA	NA
WEL	best_AIC	307.19	0.42	109.97	0.223	-0.067	NA	0.141	NA	0.198	NA	0.0051	NA	NA	NA	NA
WEL	no_fdom	312.19	0.439	115.92	3.126	NA	0.006	0.46	+	-2.051	NA	NA	NA	-0.203	-0.0797	NA
WEL	rfu_only	340.46	0.009	116.06	0.472	NA	NA	NA	NA	1.889	NA	NA	NA	NA	NA	NA
GRB	best_AIC	109.4	0.912	24.09	-0.943	-0.015	0.014	-0.038	+	2.703	0.0039	NA	NA	0.060	NA	NA
GRB	no_fdom	188.79	0.845	35.05	-1.700	NA	0.057	-0.211	+	4.942	NA	NA	NA	0.112	-0.0117	NA
GRB	rfu_only	278.53	0.681	44.01	0.471	NA	NA	NA	NA	1.301	NA	NA	NA	NA	NA	NA

Table 10 (continued).

Reserve	model	AICc	R^2	prediction_error	chl_rfu	fdom	turb	temp	season	X Intercept	fdom.chl_rfu	fdom.temp	fdom.turb	chl_rfu.temp	chl_rfu.turb	fdom.chl_rfu.turb
HEE_w2	best_AIC	-286.34	1	57.91	-8.174	0.029	-0.464	NA	NA	7.383	NA	NA	NA	NA	NA	NA
HEE_w2	no_fdom	-277.99	1	36.5	-7.889	NA	-0.468	-0.092	NA	11.55	NA	NA	NA	NA	NA	NA
HEE_w2	rfu_only	Inf	0.02	29.38	-0.320	NA	NA	NA	NA	1.003	NA	NA	NA	NA	NA	NA
HEE	best_AIC	11.76	0.39	43.13	0.079	-0.020	NA	NA	+	0.642	NA	NA	NA	NA	NA	NA
HEE	no_fdom	23.13	0.431	42.48	12.403	NA	0.411	0.06	+	-1.389	NA	NA	NA	-0.375	-2.5329	NA
HEE	rfu_only	12.72	0.01	42.28	0.084	NA	NA	NA	NA	0.783	NA	NA	NA	NA	NA	NA
MAR	best_AIC	26.65	0.895	23.35	1.046	NA	NA	0.052	NA	0.63	NA	NA	NA	NA	NA	NA
MAR	no_fdom	54.27	0.92	26.94	0.301	NA	-0.010	0.047	+	0.757	NA	NA	NA	0.020	0.0146	NA
MAR	rfu_only	26.81	0.87	22.38	1.079	NA	NA	NA	NA	1.959	NA	NA	NA	NA	NA	NA
NIW	best_AIC	10.66	0.972	12.74	2.316	-0.011	0.032	0.006	+	0.524	-0.0072	0.0015	-5.00E-04	-0.039	NA	NA
NIW	no_fdom	51.41	0.941	17.79	1.697	NA	0.019	0.1	+	-0.325	NA	NA	NA	-0.039	0.001	NA
NIW	rfu_only	89.74	0.865	23.58	1.158	NA	NA	NA	NA	1.535	NA	NA	NA	NA	NA	NA
SAP	best_AIC	141.16	0.561	53.51	1.146	NA	0.130	NA	+	0.195	NA	NA	NA	NA	-0.0346	NA
SAP	no_fdom	145.89	0.574	56.58	1.058	NA	0.148	0.097	+	-1.384	NA	NA	NA	0.007	-0.039	NA
SAP	rfu_only	162.66	0.122	64.05	0.248	NA	NA	NA	NA	2.49	NA	NA	NA	NA	NA	NA

Table 11. Model results using lab-based comparisons. “best_AIC” models represent the model with the best AIC out of the selection process with the following sensors and interactions included: chlorophyll, FDOM, temperature, and turbidity. “no_fdom” models represent the output including data from temperature, chlorophyll, and turbidity sensors, plus interactions. If at least two seasons were present in “best_AIC” and “no_fdom” models, season was included in the model as well; otherwise, it was not. “rfu_only” models only included data from the chlorophyll sensor. No model selection was performed on “no_fdom” and “rfu_only” models. In the 'season' column, a + is present if season was significant in the model. All other potential coefficients are continuous variables (and thus have a slope coefficient) or are excluded if they were not significant or intentionally not included in the model. Interactions are represented by a period between variable names.

Reserve	model	AICc	R^2	prediction_error	chl_rfu	fdom	turb	temp	season	X Intercept	fdom.chl_rfu	fdom.temp	fdom.turb	chl_rfu.temp	chl_rfu.turb	fdom.chl_rfu.turb
GND	best_AIC	-750.39	1	54.52	3.88	0.115	-0.889	-0.291	+	1.287	-0.088	0.000	0.013	0.102	NA	NA
GND	no_fdom	159.92	0.897	56.14	-6.19	NA	0.164	-0.808	+	20.103	NA	NA	NA	0.300	-0.050	NA
GND	rfu_only	17.23	0.786	26.65	1.57	NA	NA	NA	NA	-0.612	NA	NA	NA	NA	NA	NA
GRB	best_AIC	27.53	0.977	28.15	-0.961	0.012	NA	-0.064	NA	2.668	NA	NA	NA	0.069	NA	NA
GRB	no_fdom	35.46	0.98	27.26	-1.526	NA	0.089	-0.154	+	4.224	NA	NA	NA	0.116	-0.063	NA
GRB	rfu_only	49.24	0.88	35.24	0.397	NA	NA	NA	NA	1.981	NA	NA	NA	NA	NA	NA
GTM	best_AIC	32.21	0.917	20.32	0.292	NA	0.081	NA	NA	0.88	NA	NA	NA	NA	NA	NA
GTM	no_fdom	50.5	0.925	25.93	0.243	NA	0.006	0.002	+	1.157	NA	NA	NA	-0.001	0.006	NA
GTM	rfu_only	47.16	0.853	25.19	0.332	NA	NA	NA	NA	1.303	NA	NA	NA	NA	NA	NA

Table 11 (continued).

Reserve	model	AICc	R^2	prediction_error	chl_rfu	fdom	turb	temp	season	X Intercept	fdom.chl_rfu	fdom.temp	fdom.turb	chl_rfu.temp	chl_rfu.turb	fdom.chl_rfu.turb
HEE_w2	best_AIC	-286.34	1	57.91	-8.174	0.029	-0.464	NA	NA	7.383	NA	NA	NA	NA	NA	NA
HEE_w2	no_fdom	-277.99	1	36.5	-7.889	NA	-0.468	-0.092	NA	11.55	NA	NA	NA	NA	NA	NA
HEE_w2	rfu_only	Inf	0.02	29.38	-0.32	NA	NA	NA	NA	1.003	NA	NA	NA	NA	NA	NA
HEE	best_AIC	11.76	0.39	43.13	0.079	-0.020	NA	NA	+	0.642	NA	NA	NA	NA	NA	NA
HEE	no_fdom	23.13	0.431	42.48	12.403	NA	0.411	0.060	+	-1.389	NA	NA	NA	-0.375	-2.533	NA
HEE	rfu_only	12.72	0.01	42.28	0.084	NA	NA	NA	NA	0.783	NA	NA	NA	NA	NA	NA
LKS	best_AIC	108.77	0.824	19.93	-0.892	0.025	0.111	0.294	+	0.672	0.007	-0.002	-0.002	NA	0.086	NA
LKS	no_fdom	156.21	0.639	25.61	0.186	NA	-0.403	0.059	+	3.697	NA	NA	NA	-0.024	0.120	NA
LKS	rfu_only	156.35	0.578	26.57	0.686	NA	NA	NA	NA	1.942	NA	NA	NA	NA	NA	NA
MAR	best_AIC	26.65	0.895	23.35	1.046	NA	NA	0.052	NA	0.63	NA	NA	NA	NA	NA	NA
MAR	no_fdom	54.27	0.92	26.94	0.301	NA	-0.010	0.047	+	0.757	NA	NA	NA	0.020	0.015	NA
MAR	rfu_only	26.81	0.87	22.38	1.079	NA	NA	NA	NA	1.959	NA	NA	NA	NA	NA	NA
NIW	best_AIC	10.66	0.972	12.74	2.316	-0.011	0.032	0.006	+	0.524	-0.007	0.002	-0.001	-0.039	NA	NA
NIW	no_fdom	51.41	0.941	17.79	1.697	NA	0.019	0.100	+	-0.325	NA	NA	NA	-0.039	0.001	NA
NIW	rfu_only	89.74	0.865	23.58	1.158	NA	NA	NA	NA	1.535	NA	NA	NA	NA	NA	NA
PDB	best_AIC	-1021.23	1	92.6	0.904	-0.215	-2.820	-0.153	+	3.545	-0.131	0.027	0.133	NA	-0.252	0.169
PDB	no_fdom	102.7	0.992	49.48	0.361	NA	-0.039	0.029	+	0.554	NA	NA	NA	0.012	0.122	NA
PDB	rfu_only	6.44	0.941	23.42	0.643	NA	NA	NA	NA	0.951	NA	NA	NA	NA	NA	NA
SAP	best_AIC	141.16	0.561	53.51	1.146	NA	0.130	NA	+	0.195	NA	NA	NA	NA	-0.035	NA
SAP	no_fdom	145.89	0.574	56.58	1.058	NA	0.148	0.097	+	-1.384	NA	NA	NA	0.007	-0.039	NA
SAP	rfu_only	162.66	0.122	64.05	0.248	NA	NA	NA	NA	2.49	NA	NA	NA	NA	NA	NA

Conclusions

Based on the evidence from these experiments and models, varying levels of temperature, turbidity, and FDOM all influence *in situ* CHL-A readings from the YSI EXO TAL sensor. In manipulation experiments, *in situ* CHL-A fluorescence generally decreased when temperature was elevated, decreased when turbidity was elevated, and increased when FDOM was elevated. Therefore, while the raw sensor data provides much higher temporal resolution and captures variability missed by monthly discrete sampling, there is potential for erroneous, misleading sensor readings if they are not adjusted for the effects of temperature, turbidity, and FDOM. However, correcting *in situ* CHL-A using data from the accessory sensors is not a straightforward process. For example, fluorescence readings from the FDOM optical sensor are themselves influenced by temperature and turbidity (Downing et al. 2012). Even when the same standard was used for turbidity and FDOM treatment additions, *in situ* CHL-A responses were site-specific.

Temperature, turbidity, and FDOM corrections may be useful for some stations. The preliminary experiments in this project were helpful in highlighting potential errors in sensor readings due to light scattering and absorbance by those parameters, but more replication is needed to develop reliable corrections for those parameters. Based on the experimental trials from HEE, there may be a minimum ambient CHL-A concentration required for reliable results, and it may be important to keep manipulations to realistic levels.

The national linear model of *in situ* CHL-A explained 35% of the variance in extracted CHL-A. Predictive capability increased when both (1) other sensor data and (2) only lab-based comparison data were included (R^2 = 0.786 and prediction error = 26%). Given the results from our interference experiments, it is not surprising that temperature, turbidity, and FDOM were significant factors in the national models. The significance of the "reserve" parameter in these models suggests that site-specific factors beyond temperature, turbidity, and FDOM are also important and that it is not appropriate to use the national model to predict extracted CHL-A from *in situ* CHL-A measured at sites not included in this study. The amount of variance not explained by the model is likely a combination of species composition, chlorophyll degradation, light history, and interferences.

Site-specific models varied with respect to model performance and significant explanatory variables, which indicates that site-specific factors are important in determining the strength and the drivers of the relationship between *in situ* CHL-A and extracted CHL-A. A few site-specific models poorly predicted extracted CHL-A even when temperature, turbidity, and FDOM were considered. Among these models were He'eia and Wells sites, which experienced extremely low CHL-A levels, likely due to high noise-to-signal ratios. Additionally, the Lake Superior site experienced high temperature variability. In fact, *in situ* CHL-A was not a significant factor in this model, as extracted CHL-A was explained better by temperature.

Recommendations

The TAL sensor is clearly valuable, and we recommend NERRS begin implementing high-frequency chlorophyll monitoring system-wide. However, we also support the conclusions of manufacturers and previous studies (Lohrer 2000) that this sensor is not a direct substitute for

extractive CHL-A analysis. Due to the significant costs and time investment required to implement high-frequency chlorophyll monitoring, we do not recommend that reserves be required to do so unless staff and supply funding is dedicated.

Recommendations for whether and how reserves choose to implement the EXO TAL sensor depend on the chlorophyll monitoring goals for each individual station and the resources available (see considerations section below). If assessing short-term variability in algal abundance between monthly SWMP grab sampling events is desirable, then simply deploying a TAL sensor and recording chlorophyll fluorescence in RFU is useful and feasible. If early warning, rapid response to algal blooms is a goal (see considerations below), then the station needs to be telemetered.

If relating RFU to µg/L with better accuracy is a goal, then we recommend the following steps (Fig. 12):

- Step 1: Assess historical levels of extracted CHL-A.
    - If historic extracted CHL-A is always less than 2 µg/L, relationships with extracted CHL-A may not be feasible because of high noise to signal ratio.
        - This 2 µg/L benchmark was chosen based on HEE results.
        - In case of HEE, they are still interested in CHL-A peaks relating to rainfall and seasonal change, so they are still using TAL sensors with the caveat that they don't directly correlate with extracted CHL-A. Their sensor also requires extra maintenance due to siltation on the sensor face.
    - If historic extracted CHL-A ranges are more than 2 µg/L, move to Steps 2 and 3
- Step 2 (optional): Conduct a year of SWMP discrete and continuous sampling.
    - Develop a regression model.
    - If the relationship is poor, then move to Step 3.
- Step 3: Conduct site-specific comparison trials.
    - Conduct comparisons over multiple seasons and try to capture as much environmental variability as possible.
    - See lab-based comparison protocols.
    - Develop a regression model.
    - If the relationship is poor, then run trials based on your site characteristics (Step 4).
- Step 4: Conduct site-specific manipulation experiments and make any necessary corrections
    - Note: for maximum efficiency with supplies and effort, we recommend that this step be run at the same time as Step 3, but do acknowledge the extra level of effort required).
    - Assess historical levels of temperature, turbidity, and FDOM.
    - If the site has high and variable temp, run temperature experiments.
    - If the site has high and variable turbidity, run turbidity experiments.
    - If the site has high organic matter, run FDOM experiments.
    - See recommended protocols for details, but add more replication.
    - In both ambient water and experimental manipulations, sample/manipulate over the range of expected values specific to the site.
    - Develop correction equations.
        - Note: we also recommend the NERRS create a coordinated protocol for improved FDOM correction development. Our trials show that several more experiments that vary in matrices and carbon source standards are required to develop a robust correction method.

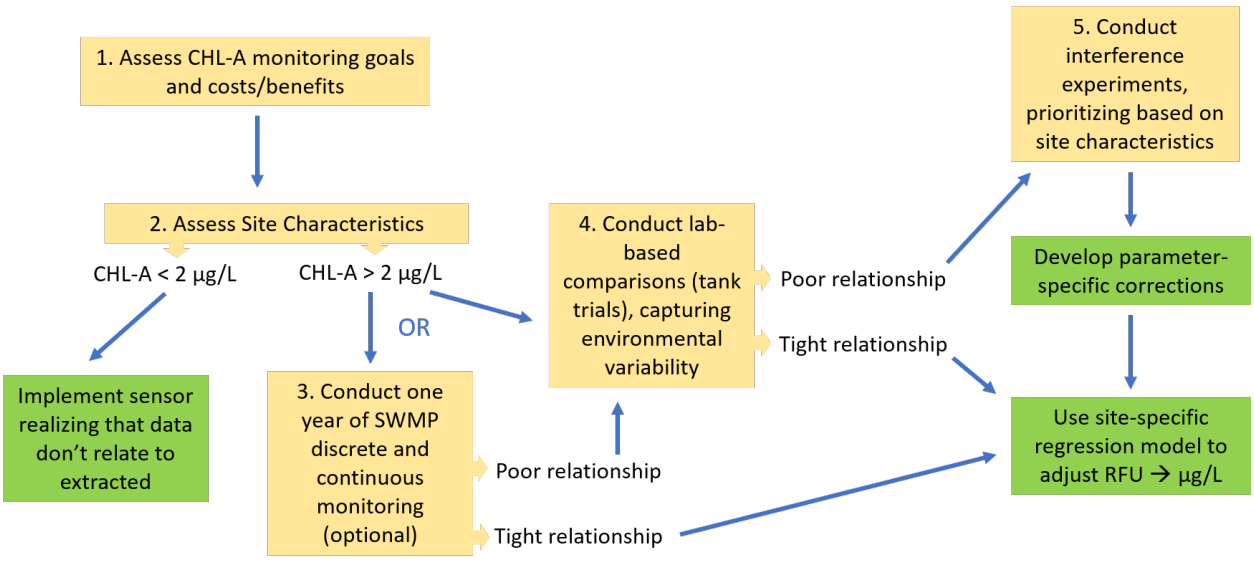

Figure 12. Decision tree for reserves considering chlorophyll sensor implementation.

Regardless of individual reserve monitoring goals and decisions about deploying the EXO TAL sensor, we recommend that the Centralized Data Management Office (CDMO) require data from the TAL sensor be submitted in RFU. Currently, CDMO requires data in µg/L. This recommendation supports previous recommendations by Lohrer (2000) that the data be reported and interpreted as fluorescence units (with no conversion to µg L^{-1}) and used as relative (not absolute) values. In addition, the YSI EXO manual itself does not recommend using µg/L:

> "The TAL sensors generate data in RFU or µg/L of pigment (chl, PC or PE) units, with RFU as the default … However, users are advised to use default RFU, which stands for Relative Fluorescence Units … RFU calibration allows for the best comparisons of data from sensor to sensor, and also enables users to monitor for sensor drift and edaphic factors such as biofouling or declining sensor optical performance over time as the LEDs age … The µg/L output generates an estimate of pigment concentration that is based upon correlations we built between sensor outputs and extracted pigments from laboratory-grown blue-green algae … Further, since algal populations can regulate their intracellular pigment concentrations, the µg/L of pigment per cell changes with season, time of day, and population dynamics. Thus the challenge with the µg/L unit is user expectations: it should not be expected that µg/L will necessarily correlate well with pigment extractions that customers perform themselves." (page 134; EXO User Manual Rev. K)

We recommend that TAL sensors be calibrated to and reported as RFU, rather than µg/L, because we believe RFU acknowledges the inherent uncertainty of the measurement, whereas the µg/L output of the sensor could be misleading. This nuance could be lost on end users who are interested in reserve chlorophyll datasets, and *in situ* CHL-A data can be potentially mischaracterized if they are reported in µg/L, as currently recommended by SWMP protocols. Therefore, given that the TAL requires separate calibrations for RFU and ug/L, we recommend that the SWMP SOP be revised to recommend calibrating to RFU only (see draft) in order to save time and provide the most objective reflection of

what the sensor is measuring. We have also drafted a SOP for calibrating the EXO FDOM sensor according to the YSI manual (see draft).

Based on feedback from the CDMO, we recommend the NERRS Data Management Committee develop standard metadata language and guidance on QAQC documentation for TAL sensor deployment. Technician training could also be improved by creating a "tips and tricks" document, a supply list, and instructional videos.

Considerations for Implementing High-Frequency *In Situ* CHL-A Monitoring

Using CHL-A as an indicator of phytoplankton biomass has several limitations to consider. Phytoplankton can adjust the amount of CHL-A in their chloroplasts based on light availability, a phenomenon termed photoacclimation (Falkowski and LaRoche 1991). Furthermore, they can emit heat instead of fluorescence to protect themselves from high light intensities by using a mechanism called non-photochemical quenching (Müller et al. 2001). These processes do not affect cell biomass, meaning that CHL-A measurements may over or underestimate actual phytoplankton biomass based on environmental conditions and cell physiology. These considerations are applicable to all methods that use CHL-A as a proxy for phytoplankton biomass, including *in situ* CHL-A sensors, but additional caveats related to traditional CHL-A extractions also exist. While extracted CHL-A measurements are made more quickly than quantifying phytoplankton biomass using microscopy, they still require water samples to be collected, transported, and analyzed in a laboratory by a technician. Since phytoplankton turnover times can be less than a day (Agawin et al. 2000), rapidly occurring phenomena such as weather events (Klug et al. 2012) can potentially create changes in phytoplankton that would be missed by infrequent water sample collections.

Another important consideration is that *in situ* CHL-A methods measure the instantaneous fluorescence of the "packet" of water immediately in front of the sensor, which can change by the second. Therefore, *in situ* CHL-A data will vary based on the time period over which the fluorescence is averaged (Chaffin et al. 2018), although this is not a consideration with extracted CHL-A measured from a grab sample. Furthermore, and as previously discussed, other components in water can fluoresce at a similar wavelength as CHL-A. This creates the potential for error *in situ*, but not with extracted CHL-A, where solvents bind specifically to CHL-A molecules, enabling fluorescence measurements to be specific to CHL-A. Another reason that both metrics differ is that *in situ* sensors measure fluorescence in RFUs, whose conversion to a concentration is not always accurate. However, extracted CHL-A does accurately report concentrations because of a known volume of water and a lack of potential interference. While there are instances when the relationship between *in situ* and extracted CHL-A is strong, the relationship between these metrics often changes over time, making it difficult to rely exclusively on one metric over the other, so care should be taken when making direct comparisons between the two. Both metrics are informative as long as proper consideration is given to what each is measuring.

Taken together, and with a proper understanding of the strengths and limitations to each metric, both extracted and *in situ* CHL-A can be informative for the SWMP. Extracted CHL-A data from discrete samples contribute to our understanding of long-term changes because they can be compared with historical measurements, while high-frequency, *in situ* CHL-A data are especially useful in assessing short-term variability (see Case Studies, Fig. 2 and Table 4). The most immediate benefit of high-

frequency chlorophyll data is that reserve staff and local stakeholders gain a better understanding of the natural dynamics in their system. With high-frequency data, researchers can study how chlorophyll changes with tides, from day to night, from season to season, and after events like hurricanes and nor'easters. They can also explore how patterns in phytoplankton biomass relate to other environmental variables like salinity, temperature, oxygen, nutrients, precipitation, and light. Even further, this foundational information on natural chlorophyll dynamics is essential for detecting change induced by humans. Reserves can then serve as reference sites for more impacted estuaries, sites within reserves with varying degrees of human impact can be compared, and long-term trends at each site can be more quickly detected. High-frequency chlorophyll data will encourage more research by the broader scientific community on the detailed mechanisms that drive blooms and general plankton dynamics, which will help prevent and manage harmful blooms in the future. Other related research topics include investigations of carbonate chemistry/coastal acidification, ecosystem metabolism, herbivorous fish diet, resource habitat niche partitioning, and effects of altered hydrology and upwelling on primary production. It is worth noting that during the COVID pandemic and subsequent lapse in fieldwork (due to restrictions preventing water sampling for extracted chlorophyll), continuous monitoring of *in situ* chlorophyll at the Padilla Bay NERR was the only way staff could confirm patterns in spring bloom dynamics and compare them to previous years.

With some effort, other management, education, and aquaculture applications for high-frequency chlorophyll data have great potential. For instance, coastal managers need more effective ways to track algal blooms over space and time. This could be done by verifying and contextualizing remote sensing products using chlorophyll sensor data from fixed, continuous stations. Where monitoring stations are telemetered, near-real-time chlorophyll data could be used for early detection of and rapid response to algal blooms, as monitoring stations can alert technicians when chlorophyll values reach a pre-defined threshold. These alerts could mobilize field crews to respond quickly, collect plankton and other environmental samples, and alert appropriate agencies and the public. In fact, this very need has recently been defined by Florida's Coastal Management Program Director and Chief Science Officer (personal communication). Aquaculture professionals could also use these data to assess food availability for the filter feeders they grow in estuaries, and teachers could use these data to enhance education efforts on data literacy, primary production, and food web dynamics.

Monetary cost is a primary factor each that reserve should consider when deciding whether to include TAL sensors in their monitoring program. Potential costs include the cost of purchasing the TAL and FDOM sensors, purchasing associated standards, and proper waste disposal of those standards. At the time of writing, the TAL from YSI costs $3,150, and the FDOM sensor costs $2,394. Per YSI, the minimum lifespan of the TAL and FDOM sensors is five years, though both are under warranty for two years. Both the TAL and FDOM sensors are optical, which means that consumable costs should be minimal over their lifespan. The standard for calibrating the TAL sensor requires rhodamine dye, while quinine sulfate dihydrate and 0.1N sulfuric acid are used to calibrate the FDOM sensor. Rhodamine dye is a small cost because the small quantity required to make the stock (and the one-year stability of that stock)means that it can be purchased infrequently. For the FDOM sensor, 0.1N sulfuric acid costs approximately $120 per year, while quinine sulfate dihydrate costs less than $100 per year. After sonde post deployment, calibration standards must be properly disposed of. Rhodamine dye disposal varies, with some entities requiring external disposal while others allow drain disposal. Quinine sulfate, the

standard for calibrating the FDOM sensor, should not be disposed via drain and requires proper disposal. Costs incurred from waste disposal will vary from reserve to reserve.

Time investment is the second major consideration for reserves considering including TAL sensors in their monitoring. We estimate that performing the two-point calibration required for both the TAL and FDOM sensors will take around thirty minutes per sonde for each pre-calibration. This estimate does not include the roughly thirty minutes it takes to prepare the rhodamine dye and quinine sulfate standards, which would only have to be performed once for all sondes with TAL and FDOM sensors. We estimate that post-calibration would take less than thirty minutes per sonde, which, if performed within the 24 hour lifespan of the rhodamine calibration standard, could use the standard prepared previously for pre-calibration. Quinine sulfate will need to be prepared separately for both pre- and post-deployment.

The third and final consideration for incorporating *in situ* chlorophyll monitoring is the lost opportunity of including an alternative sensor on a sonde. Reserves will need an EXO2 sonde (rather than an EXO1 or EXO3 sonde) because the EXO2 has enough sensor ports to accommodate the TAL and FDOM sensors in addition to all the sensors required for SWMP. The TAL and FDOM sensors would fill all the EXO2's ports, thereby excluding other non-required SWMP sensors (e.g., YSI's NITRALed sensor).

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