Ref. No. [UMCES] CBL 2019-009
ACT VS19-04

PERFORMANCE VERIFICATION STATEMENT
For JFE Advantech's Multi-Excitation Chlorophyll Fluorometer

TECHNOLOGY TYPE: Multispectral Fluorometers

APPLICATION: In situ estimates of algae for coastal moored deployments

PARAMETERS EVALUATED: Accuracy, precision, range response and reliability

TYPE OF EVALUATION: Laboratory and Field Performance Verification

DATE OF EVALUATION: Testing conducted from June 2017 to November 2017

EVALUATION PERSONNEL: T.H. Johengen, H. Purcell, G.J. Smith, D. Schar, H. Bowers, M. Tamburri, D. Fyffe and G.W. Jeter.

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EXECUTIVE SUMMARY

The Alliance for Coastal Technology (ACT) conducted a sensor verification study of in situ multispectral fluorometers during 2017-2018 to characterize performance measures of accuracy and reliability in a series of controlled laboratory studies and field tests in diverse coastal environments. Laboratory tests using known algal cultures both individually and in various combinations along with add-in matrix challenges for turbidity and CDOM were conducted at Moss Landing Marine Laboratory (MLML) and NOAA Great Lakes Environmental Research Laboratory (GLERL). In total, 40 different exposure trials were conducted within these Lab tests. Five different field testing applications were conducted including three continuous underway surface mapping cruises and two moored deployments. Underway mapping cruises were conducted in San Francisco Bay, in Monterey Bay, and in western Lake Erie. Underway cruises covered between 75 – 150 km and each included seven isolated tank-exposure comparisons comprising two timepoints over 30 minutes. The first moored field test was conducted over 13 days in a flow-through tank using Maumee River source water at the Bowling Green Municipal Water Treatment Plant. The second mooring test was conducted for 28 days from a submerged rack deployed off the research pier of the Chesapeake Biological Research Lab in Solomons, MD. Instrument performance was evaluated against reference samples collected and analyzed by ACT staff or through sub-contracts at certified Phytoplankton counting laboratories at the University of Minnesota Duluth's Natural Resources Research Institute and the Smithsonian Environmental Research Center. Instrument performance was evaluated against extracted chlorophyll, extracted phycocyanin, and algal species classification at the functional group level on the basis of estimated biovolume contribution within each sample. A total of 243 reference samples were collected for direct instrument comparisons. For each reference sample six replicates were filtered for pigment analysis with two replicates analyzed for chlorophyll and three replicates analyzed for phycobilins. One filter was reserved in storage and used when the variance in analytical replicates exceeded a 10 percent threshold. Field duplicates and field trip blanks were collected during each test application as a measure of Quality Assurance.

This document presents the results of the JFE Multi-Exciter which makes use of nine wavelength excitation spectra to quantify the total phytoplankton biomass (chlorophyll-a) and estimate phytoplankton group composition. The Light Emitting Diodes (LED) hit the water with their peaks centered at 375, 400, 420, 435, 470, 505, 525, 570, and 590 nm. These excitation wavelengths were chosen considering the maximum absorption of photosynthetic pigments at wavelengths shorter than 600 nm. The Multi-Exciter detects fluorescence emitted from 630 nm to approximately 1000 nm, where phytoplankton commonly emits a distinguishable red fluorescence near 680 nm. This fluorometer was developed to have high sensitivity to chlorophyll-a, allowing for detection at concentrations of 0.1 µg/L and have low sensitivity to turbidity with a reduced noise-effect from reflectance of suspended particles A single instrument was provided for the entire round of lab and field testing and all tests were conducted under the same configuration, with no attempt to optimize response within a given environment or community composition.

Instrument performance across all lab and field tests based on linear regression of the JFE Multi-Exciter total chlorophyll estimation against extracted chlorophyll is given below in Table 1 along with a summary of successful data returns for each of the tests completed. Overall the Multi-Exciter under-estimated total chlorophyll and the response slopes decreased in Lab tests when CDOM additions were added as a matrix challenge (ML Day 3 and 5) or when cyanobacteria contributed a higher proportion of the biomass (GLERL). It is unclear why the chlorophyll estimation were so out of range for the underway mapping in San Francisco Bay and Monterey

Bay since the emission spectra appeared to respond to changes in species composition. The low response slope for the western Lake Erie underway test occurred in the presence of a significant colonial *Microcystis* bloom which is known to exhibit a low in situ fluorescence response.

Table 1. Summary of the JFE Multi-Exciter total chlorophyll fluorometric response compared as regressed against extracted chlorophyll measurements and data returns across all of the laboratory and field tests completed during the ACT technology evaluation.

Test	Response Slope	Regression R-squared	# Ref Samples	Instrument OBS	Data Return %	Distance or Duration
LAB Tests						
ML Test 1	0.66	98	8	8	100	1 d
ML Test 2	0.80	99	10	10	100	1 d
ML Test 3	0.33	85	31	31	100	1 d
ML Test 4	0.63	97	19	19	100	1 d
ML Test 5	0.39	96	21	21	100	1 d
GLERL	0.32	80	10	10	100	1 d
Field Test Underway						
SF Bay	-0.01	0.1	16	1604	100	150 km
Monterey Bay	-0.05	6.4	14	1418	100	75 km
WLE	0.05	52	14	2076	100	75 km
Field Test Moored						
Maumee River	0.32	84	31	623	100	13 d
Chesapeake Bay	0.25	15	60	1293	100	28 d

The Multi-Exciter as tested was not configured to predict specific algal group so emission spectra were simply compared graphically against algal group biovolume proportion estimates derived from microscopic counts and established shape formulas. We recognize that biovolume is not a direct equivalent for fluorescence contribution but provided the best proxy of community composition. The Multi-Exciter strongly responded to the presence of phycobilin pigments associated with Cyanobacteria and Cryptophytes with enhanced fluorescence emission at 570 nm. The instrument also strongly responded to the presence of CDOM with elevated emissions at 370 and 400 nm during both Laboratory add-in experiments and in natural waters during field testing. Phytoplankton in the green and brown algal groups showed typical emission maxima at the 420 and 435 nm wavelengths. Emission strength was well correlated with extracted CHL during all Lab tests but only for two of the five field tests. It is likely the flow-through tank design for underway field surveys strongly influenced instrument response.

The manufacturer was given the opportunity to respond to the findings and presentation of this evaluation and their response is provided at the end of the report.

BACKGROUND AND OBJECTIVES

ACT was established on the premise that instrument validation of existing and emerging technologies is essential to support both coastal science and resource management. The overall goal of ACT's verification program is to provide industry with an opportunity to have a third-party test their instruments in both controlled laboratory settings and in diverse field applications across a range of coastal environments in order to provide users of this technology with an independent and credible assessment of instrument performance. To this end, the data and information on performance characteristics were focused on the types of information users most need. It is important to note that ACT does not certify technologies or guarantee that a technology will always, or under circumstances other than those used in testing, operate at the levels verified. ACT does not seek to determine regulatory compliance; does not rank technologies or compare their performance; does not label or list technologies as acceptable or unacceptable; and does not seek to determine "best available technology" in any form.

As part of our service to the coastal community, ACT conducted a performance verification of commercially available, in situ multi-excitation fluorometers that are designed to discriminate among classes of phytoplankton and may be used to enhance the detection of harmful algae and cyanobacteria. The fundamental objectives of this Performance Verification were to: (1) highlight the potential capabilities of particular in situ fluorometers for monitoring harmful algal blooms; (2) verify the claims of manufacturers on the performance characteristics of these instruments when tested in a controlled laboratory setting, and (3) verify performance characteristics of these instruments when applied in real world applications in a diverse range of coastal environments.

INSTRUMENT TECHNOLOGY TESTED

The Multi-Exciter makes use of nine wavelength excitation spectra to quantify the total phytoplankton biomass (chlorophyll-a) and estimate phytoplankton group composition. This fluorometer was developed to have high sensitivity to chlorophyll-a, allowing for detection at concentrations of 0.1 µg/L or less. Also, the instrument has a low sensitivity to turbidity with a reduced noise-effect from reflectance of suspended particles in water (detection error of 0.6% FS or less when turbidity is at 109 FTU). Therefore, this instrument can be deployed in the open ocean (low phytoplankton concentration), as well as, in coastal waters, lakes and marshes (relatively high turbidity).

The Multi-Exciter has a depth rating of 500 m and it is available in two versions, the internal memory version that allows long-term autonomous observations and the wired version, which is easily integrated on real-time monitoring platforms. The Light Emitting Diodes (LED) hit the water with their peaks centered at 375, 400, 420, 435, 470, 505, 525, 570, and 590 nm. These excitation wavelengths were chosen considering the maximum absorption of photosynthetic pigments at wavelengths shorter than 600 nm. The Multi-Exciter detects fluorescence emitted from 630 nm to approximately 1000 nm,

where phytoplankton commonly emits a distinguishable red fluorescence near 680 nm. The detector of the instrument is a Si (silicon) photodiode and there are optical filters to intercept stray light generated through light scattering in the excitation LEDs as well as in the Si-photodiode detector. The Multi-Exciter has additional temperature, depth, and turbidity sensors and it is provided with a mechanical wiper to prevent fouling and/or bio-fouling on its optical window, which allows for stable and accurate optical data during long-term deployments.

The measured fluorescence excitation spectra can be applied to estimate phytoplankton group composition by multiple regression analysis. Each phytoplankton group has a distinctive spectral signature and by using those different signatures, it is possible to estimate phytoplankton composition by using a multiple regression algorithm.

PERFORMANCE EVALUATION TEST PLAN

Phytoplankton and cyanobacteria offer a range of inherent characteristics that enable their discrimination and classification. Their morphological and cell surface diversity enables broad discrimination through microscopic examination and light scattering properties. Photosynthetic pigment composition is also taxon specific and their inherent absorption and fluorescence properties provide an additional, sensitive target for *in situ* detection and discrimination. This verification study evaluated the field and laboratory performance of instruments leveraging the capacity for fluorescence-based parsing of phytoplankton community composition. Evaluations focused on the ability of these technologies to determine presence and abundance of cyanobacteria and potentially harmful eukaryotic phytoplankton (diatoms, dinoflagellates, prymnesiophytes) within mixed natural communities.

A single instrument was provided to ACT by JFE and used in all subsequent testing without any further calibration or servicing by the company or by ACT personnel. Prior to testing, all ACT personnel participated in a full day training session from the manufacturer in set-up and operations. Since testing was performed in many different environments and algal communities, no effort was made to optimize performance or calibration for any given test. At the start of testing at each of the three ACT facilities, instrument output was referenced to defined Basic Blue 3 (BB3) solutions at concentrations levels of 0.05 and 0.5 uM under standard conditions to ensure good working order and consistent operational response (see Table 8). The following text summarizes the test protocols used by ACT for all of the instruments submitted to the evaluation with instrument specific details for the JFE Multi-Exciter defined as appropriate.

Laboratory Tests

Laboratory tests of response linearity, precision, range, and reliability were conducted at Moss Landing Marine Laboratories (MLML). Instrument response to several individual freshwater and marine cultures was quantified at various concentration levels. Instruments were exposed to mixtures of different phytoplankton assemblages within freshwater or marine media. Lastly, matrix effects of turbidity and dissolved organic carbon were assessed through addition of specified concentrations to mixed algal assemblages. *It should be noted that many of the lab cultures were contaminated and we were not able to quantify specific response functions to individual algal taxon.*

The various test conditions were produced in mechanically mixed, temperature controlled water baths where instruments were submerged for testing. Test tanks were equipped with a multi-parameter YSI EXO2 sonde to continuously monitor temperature, salinity, turbidity, fDOM, pH, DO, CHL, and BGA during all laboratory testing. All laboratory tests were conducted at a fixed temperature and salinity level near the closest optimal growth temperature for all phytoplankton taxa utilized. Fluorometric response and discrimination were tested on both freshwater and marine algal species utilizing known mixtures and concentrations of live cultures added into a background matrix of filtered deionized water or seawater, supplemented with appropriate salt and nutrient additives (BG11+Si and L1 respectively). Freshwater and seawater were obtained from the MLML aquaculture facility.

Phytoplankton Taxa – Algal cultures came from a variety of sources including the traceable commercial entities UTEX and NCMA, however, when those stocks did not propagate well in large batch cultures, additional cultures from personal collections at the NOAA Great Lakes Environmental Research Lab were included. Freshwater taxa from NOAA collections included a cyanobacterium (*Microcystis* spp.), a chlorophyte (*Coelastrum*), and a dinoflagellate (*Peridinium*). Marine taxa generated from the NCMA collection included a diatom (*Thalassiosira* spp.), a dinoflagellate (*Amphidinium carterae*), and a cyanobacterium (*Synechococcus* spp.). Cultures were grown in large 20L batch cultures under cool LED light (ca 75 µmol quanta m^{-2} s^{-1}; 6-8 light dark cycles at 20^{o}C) using appropriate growth media as indicated above to mid-log phase (determined by cell counts).

Response Linearity and Range – For linearity or range tests, comparative measurements of instrument and reference samples were generated from instrument readings at 10-second intervals, after the instruments were allowed at least 15 minutes to equilibrate to each new test condition change. The instrument mean and standard deviation (SD) were computed from readings averaged around a one-minute interval for each reference sample timepoint. For each test condition two or three reference samples were taken at roughly five-minute intervals following the equilibration period. Each reference sample was analyzed for CHL, PC, PE, and algal biovolume as described below. Mixtures of phytoplankton taxa were titrated based on stock cultures' volumetric chlorophyll concentration. Given that taxa vary according to their pigment quotas, it is recognized that actual cell densities will not be present in the ratios defined, given that the ratios are based on pigment content. All additions and test conditions were maintained at low ambient light (< 75 umol photon m^{-2} s^{-1}). Individual algal species were added sequentially to produce different ratios and concentrations. The exact CHL concentrations tested varied depending on culture yields, however 'real-world' ranges were targeted. A regression of instrument fluorescence versus total CHL was examined to estimate the potential linear environmental detection range.

CDOM and Turbidity Challenges – Sensitivity to water clarity and natural fluorescence was assessed by exposing the test instruments to sequential additions of background CDOM (Pahokee Peat leachate reference material) and turbidity (Elliot Silt Loam reference material). Instruments were initially placed in a test bath at 20 ^{o}C and fluorescence response measured at three algal concentrations over 15minute exposures, after which, they were challenged with the CDOM and/or turbidity additions. For some tests, following the CDOM and turbidity additions, additional algal culture was added to examine instrument linearity under the matrix challenge conditions. For each challenge condition, the tank was equilibrated for 15 minutes to ensure uniform mixing (T0), followed by 15 minutes of instrument measurements (T15) for analysis against reference samples. Continuous monitoring of CDOM and turbidity within the test tank was conducted at one-minute intervals with the EXO sonde to verify the stability of the test conditions. Challenge CDOM

concentrations were increased from background to levels ranging from 2 - 20 mg/L (as DOC) and turbidity increased to levels between 10 - 100 NTU. Turbidity concentrations of the discrete reference samples were measured using a Hach 2100 benchtop turbidity sensor calibrated in NTU. CDOM concentrations on the discrete reference samples were measured on filtered reference samples analyzed by absorbance spectroscopy (see below).

Reliability – Instrument reliability during the laboratory test was determined by comparing percent of data recovered versus percent of data expected. Comments on problems or instrument failures were noted.

Due to contamination of several of the freshwater and marine cultures we were not able to conduct the intended single species responses and not all additions followed the exact described method due to time and handling constraints. For clarity, the actual conditions of each trial within a daily lab test are presented at the beginning of the results for each lab test. In addition, a second lab test was established at the NOAA Great Lakes Environmental Research Lab using clean, mono-culture freshwater algal cultures. For this test instruments were exposed to four individual species in small 2L batches, followed by mixtures of all species together at four different composition ratios, and finally a repeat of the last mixture with CDOM and turbidity enhancements at similar levels to the previous lab test.

Field Tests

A rigorous field testing component was conducted to provide a variety of algal composition and densities within various ecosystems including riverine, lake, estuarine, and marine. Exact environmental conditions were constrained by the available testing windows available at each site, but the schedule was designed to maximize the potential of including exposure to known harmful algal bloom communities within each field deployment. Instrument performance and reliability were determined in both moored and surface mapping applications. Instrument reliability for each of the field tests was assessed by comparing percent of data recovered versus percent of data expected. Comments on problems or instrument failures were recorded.

Moored Deployment

In situ evaluations of instrument performance in a moored application were conducted at two ACT Partner Institution sites. The first moored deployment was conducted in a flow-through tank sampling water from the Maumee River at a location adjacent to the City of Bowling Green, OH, public water utility. The deployment occurred over 13 consecutive days and provided a wide range of chlorophyll concentrations (10 to 120 µg/L), high turbidity (up to 100 NTU), and variable concentrations of cyanobacteria. A second moored application was conducted at the Chesapeake Biological Laboratory in Solomons, MD. Instruments were deployed on a dock-side mooring in Chesapeake Bay for 28 continuous days. Test conditions provided a range of salinity and temperature conditions and variable algal composition and abundance as a function of tidal cycle and variable riverine input. This environment is also known for high rates of both soft and hard biofouling, and an additional objective of this test application was to evaluate the ability of the *in situ* instruments to maintain performance levels under high biofouling.

Instrument Setup - Prior to deployment, all instruments were setup according to the recommendations and training by the manufacturer. The instruments were tested as supplied and no calibration procedures were applied by ACT staff. Fluorometers were programmed to record data at a minimum frequency of every 15 minutes during the entire field deployment. All internal

clocks were set to local time using www.time.gov as the time standard. Before deployment, all instruments were exposed to a DI blank and two concentrations of BB3 (0.05 and 0.5 µg/mL) dye produced from a common stock prepared and distributed by MLML. Responses to the dye exposure were used to ensure good working order and establish any calibration offset across different test applications. Photographs of instruments were taken just prior to deployment and just after recovery to provide a qualitative estimate of biofouling during the field tests.

Deployment Rack - All test instrument packages were deployed side-by-side on a common mooring rack such that all sensor measurement windows were at the same depth. Instrument sensor heads were deployed with a separation distance of at least one instrument-diameter to minimize the potential for cross interference. For the Maumee River test, instruments were deployed in a 500 L, 1 m deep flow-through tank with sensor heads at approximately 20cm off the bottom. For the CBL moored deployment, the rack was deployed so that all of the fluorometers remained a minimum of 1 m below the water surface, accounting for variance due to tidal state or river stage. For each deployment a calibrated CTD and/or a multi-parameter EXO2 sonde was attached to the mooring and programmed to provide an independent record of temperature, conductivity, CDOM, turbidity, CHL, and PC at the same depth and the same 15-minute intervals as the test instruments. For the CBL deployment, light intensity was also measured continuously with a LI-COR LI-193 underwater spherical PAR sensor mounted on a Seabird SBE911 CTD at the same depth as the sensors.

Sampling Schedule – For the Maumee River deployment we collected two references samples per day approximately one hour apart during the work week, however, once each week we sampled four times within a day to capture a larger daily range. When possible we varied the sampling timepoints between morning and afternoon on different days to capture some variation in light history. For the CBL deployment, we evaluated diurnal responses across the day-night spectrum on three occasions including day 2, day 3, and day 27 of the deployment. On those days we collected four reference samples throughout the day at instrument sampling timepoints: 06:00, 10:00, 15:00, and 20:00. During all other sampling events, reference samples were collected twice a day with one in the morning and one in the afternoon.

Water Samples – At the Maumee River test site reference samples were collected by dipping two 1 L polypropylene bottles directly into the tank. Bottles were rinsed a minimum of three times before final collection. At CBL reference samples were collected with a standard 4 liter Van Dorn bottle. The sampling bottle was lowered into the center of the sensor rack at the same depth and as close as safely possible to the fluorometers and allowed to incubate for one minute prior to sample collection. The bottle was triggered to close at the same time as instrument sampling to ensure that the same water mass was being evaluated. For each reference sample, six replicates (two for CHL, three for PC/PE, one reserve) were filtered under low light and low vacuum conditions, and stored in a -80 ^{o}C freezer until analysis (methods described below). Cell abundances of coarse taxonomic groupings (e.g. diatoms, dinoflagellates, chlorophytes, cyanophytes, others) and biovolumes were determined on Lugol's fixed sample aliquots. A whole water subsample was collected to measure turbidity using a Hach model2100AN Turbidometer. Lastly, filtrate was collected using acid-cleaned filters and shipped to MLML for CDOM analysis. Field duplicates were collected during one sampling event per week at each test site. Duplicates were collected by deploying two Van Dorn bottles (or two dipped 1 L bottles) side-by-side, and were processed in identical fashion.

Surface Mapping Deployment

In situ evaluations of instrument performance in surface mapping applications were conducted at three locations including freshwater, estuarine, and marine environments. On July 6th a surface mapping cruise was conducted in San Francisco Bay in collaboration with Dr. Raphe Kudela of UC Santa Cruz and Dr. Jim Cloern of USGS following their existing HAB survey program sampling over a 150km transect ranging from Palo Alto in the south to the Richmond bridge in the north. On July 13th a second surface mapping cruise was conducted in Monterey Bay over a transit distance of 75km covering a range from outside the harbor to open ocean environments. On August 13th the third surface mapping cruise was conducted in the western basin of Lake Erie during a known period of *Microcystis* blooms. The survey covered approximately 75 km of transit and included regions dominated by cyanobacteria near the mouth of the Maumee River to regions further offshore to the north and east with lower abundance and a more diverse composition.

Instrument Setup - For the underway surface mapping test instruments were programmed to record data at one second intervals. Submersible instruments were deployed in a flow-through tank with a known exchange rate (nominally 10-15 min). The tank was kept shaded under cover. A calibrated multi-parameter sonde was positioned within the tank to provide an independent record of temperature, conductivity, CDOM, turbidity, CHL, and PC continuously at 1 minute measurement intervals.

Water Samples – Seven or eight stations were selected during each surface mapping survey to make comparative reference sample measurements. Stations were selected to cover a diversity of phytoplankton abundance and composition. At each selected station, water in the flow-through tank was isolated for a period of 30 minutes, keeping it well mixed with manual stirring. After an initial equilibration period of 15 minutes, reference samples were taken at timepoints of 20 and 30 minutes from the point of isolation. Sub-samples of the composited sample draw were used to expose the one bench-top test instrument. Samples were collected under shade to minimize light exposure and immediately taken into a shipboard laboratory (or a shaded deck space for Lake Erie) and processed using the same protocols as defined for the field mooring deployments. Reference samples were analyzed for extractive chlorophyll *a* and phycobilins, fixed cell counts, CDOM, and turbidity as described below.

Reference Sample Analysis

Pigment Quantification

Water samples were collected onto 25 mm Whatman GF/F filters under low vacuum filtration (<5 in Hg). Filtered volumes (sufficient to discern coloration of filters) varied by sampling location (≥ 100 mL). Chlorophyll-a (CHL) content of the filtered material was determined by fluorescence analysis of dimethylformamide (DMF) extracts using the non-acidification method (Speziale et. al. 1984) on a Turner Designs 10 AU fluorometer calibrated against certified chlorophyll a standard (Turner Designs). Phycobilin (phycocyanin, PC; phycoerythrin, PE) content of filtered water samples was determined by fluorescence analysis of phosphate buffer (50 mM, pH 6.8) extracts following 3 freeze-thaw cycles and sonication to maximize pigment extraction (Lawrenz et al. 2011) on a Turner Aquaflor fluorometer calibrated with certified PC and PE standards (Prozyme Inc.). All sample handling for pigment extraction was

conducted under low light to minimize sample degradation. All fluorometer calibrations and extract readings were done at room temperature, typically controlled at 20 ± 1.0 ºC.

A total of six replicates was filtered for each reference sample and stored at -80 ºC immediately after processing. Filters for chlorophyll were stored and extracted in amber glass vials. Filters for PC/PE were stored and analyzed in 15 ml opaque, poly-carbonate centrifuge tubes. Pigment analysis was conducted on two replicates for chlorophyll and three replicates for phycobilins. One filter was reserved in storage at -80 ºC and subsequently analyzed when the variability in the initial results were above a threshold of 20% in coefficient of variation. All reference sample pigment analyses were performed by the same trained ACT personnel using the same instrumentation and procedures.

Species Identification, Abundance and Biovolume

Whole water samples (500 mL) were fixed with acidified Lugol's (1% final concentration, v/v) and concentrated as necessary by settling or gentle centrifugation (3000 rpm, 10 min). Total cell abundance was enumerated microscopically and assigned to coarse taxonomic groups (i.e. diatoms, dinoflagellates, chlorophytes, prymnesiophytes, and cyanobacteria), or to the lowest taxonomic category needed to assign appropriate biovolume conversions. Cell abundance was converted to biovolumes using site-specific dimensional relationships based on equivalent spherical diameter. Data are reported as total phytoplankton abundance and biovolume of each group after adjustment for volume dilutions.

For the surface mapping survey in San Francisco Bay, phytoplankton abundance was determined from image libraries generated with an Imaging FlowCytobot (IFCB) operated by UC Santa Cruz personnel. For field sampling in Monterey Bay and San Francisco Bay, additional sub-samples were preserved with paraformaldehyde at a final concentration of 0.24% and evaluated using flow cytometry. For these test sites all phytoplankton analysis and cytometric quantification was performed by ACT staff at MLML based on local knowledge and experience in these analyses.

For the Great Lakes tests, phytoplankton counting was conducted under a contract to Dr. Euan Reavie of the National Resources Research Institute in Duluth, MN. The SOPs for counting Great Lakes samples follow protocols of the USEPA Great Lakes National Program Office (GLNPO) Biological Surveillance Program which has been in place for over thirty years. Details of the SOPs may be found at: http://www3.epa.gov/greatlakes/monitoring/sop/chapter4/lg401.pdf. For the Chesapeake Bay tests, phytoplankton counting was conducted under a contract to Tim Mullady of the Smithsonian Environmental Research Center in Edgewater, MD. Phytoplankton analyses were conducted using an Utermohl settling chamber and inverted phase/fluorescent microscope following the Maritime Environmental Resource Center SOP entitled, Live Organisms ≥ 10 to < 50 um Standard Operating Procedures, Rev No. 4.0, Feb 02, 2017. Both contract Labs have performed microscopy services as part of previous ACT/Naval Research Lab fluorometer testing under a ballast water compliance monitoring study, and have undergone previous Technical Audits by ACT's Quality Assurance Manager and both maintain rigorous protocols and certifications.

Colored Dissolved Organic Matter (CDOM)

Approximately 40 ml of sample filtrate was used to rinse the collection flask and the 50 ml BD Falcon centrifuge tubes, and then discarded. Following the rinse, an additional 45 mls of the

CDOM designated sample was filtered using 47 mm GF/F filters (0.7 µm pore size) with low vacuum pressure (<5 in Hg). The filtrate was captured in the centrifuge tube, capped, wrapped with Parafilm, labeled, and stored in a refrigerator (4° C) until analysis. All samples were shipped to MLML on dry ice for analysis using a calibrated laboratory-grade spectrophotometer. The sample and MilliQ blank were equilibrated to room temperature and spectrophotometric scans were run between 250 and 800 nm at 1 nm intervals, with a 4-5 nm slit width. Absorption from optical density was calculated by subtracting the optical density at 750 nm to correct for residual scattering and reported as the absorption at wavelength 400.

Turbidity

Turbidity was measured on gently mixed raw water samples using a Hach 2100AN Turbidimeter, calibrated with certified turbimetric standards (Hach). In addition, continuous *in situ* turbidity measurements were generated during all testing with a calibrated EXO2 sonde.

Ancillary Data

In conjunction with each water sample collection, ACT personnel recorded site-specific conditions from nearby river and tide gauges, meteorological stations, and visual observations of the water. Sampling information was logged on standardized datasheets and transmitted weekly to the ACT Chief Scientist for data archiving and QA/QC performance checks.

Quality Management

All technical activities conducted by ACT comply with ACT's Quality Management System (QMS), which includes the policies, objectives, procedures, authority, and accountability needed to ensure quality in ACT's work processes, products, and services. The QMS provides the framework for quality assurance (QA) functions, which cover planning, implementation, and review of data collection activities and the use of data in decision-making, and quality control. The QMS also ensures that all ACT data collection and processing activities are carried out in a consistent manner, to produce data of known and documented quality that can be used with a high degree of certainty by the intended user to support specific decisions or actions regarding technology performance. ACT's QMS meets the requirements of ISO/IEC 17025:2005(E), *General requirements for the competence of testing and calibration laboratories*; the American National Standards Institute (ANSI)/American Society for Quality (ASQ) E4-2004 *Quality Systems for Environmental Data and Technology Programs*; and U.S. Environmental Protection Agency, quality standards for environmental data collection, production, and use.

RESULTS

All instrument test results are presented in units of µg/L (or ppb) but we emphasize that the Multi-Exciter was not calibrated for each test condition or environment, and acknowledge that for the most accurate quantification direct empirical calibration under the specific application would be required.

LABORATORY TEST

Instrument accuracy of chlorophyll and phycocyanin determinations, and their resulting ratios, was evaluated in two separate laboratory tests which took place at Moss Landing Marine Laboratory (MLML) and the NOAA Great Lakes Environmental Research Laboratory (GLERL). In both cases the tests involved a series of short-term exposures to various cultured phytoplankton species along with add-in matrix challenges for turbidity and CDOM.

Moss Landing Marine Lab

Four lab tests with cultured algae were conducted from June 26 – June 29, 2017. Each test was conducted over the course of one day and involved a series of individual 50 – 60 minute trials. The test conditions for each individual trial are defined in tables 2 – 6, immediately preceding the presentation of results for that day. For the June 26 lab test, trials were conducted on individual freshwater and saltwater algal species made up in discrete 3 L batches. Prior to the algal exposures, background readings were taken on DI and the freshwater or saltwater culture media. Three freshwater algal culture trials were conducted using two different levels of *Coelastrum* additions (ca. 10 and 20 µg/L CHL) and one level of *Microcystis* (ca. 25 µg/L CHL and 1 µg/L PC) (Table 2).

Table 2. Test conditions for each trial of the June 26 MLML laboratory tests. This lab test focused on instrument response to two different freshwater algal species, *Coelastrum* and *Microcystis*, as well as, responses to DI and freshwater culture media. (n = number of reference samples taken during the exposure; C1 and C2 refer to concentration level from additional culture addition).

Trial	*Coelastrum*	*Microcystis*
DI (n = 1)	-	-
FW Media (n = 2)	-	-
FW T1 (n = 2)	C1	-
FW T2 (n = 2)	C2	-
FW T3 (n = 2)	-	C1

Two reference samples were collected from each test batch including one immediately after sample preparation and the second at the end of all instrument exposures (approximately 45 minutes later). Results are plotted as a time series of instrument readings compared to extracted pigment concentrations in µg/L determined on the reference samples (Figure 1). The JFE showed no response in terms of estimated CHL fluorescence to background DI or the freshwater media. The absolute difference between instrument estimation and reference measurement increased at the higher concentration levels. The CHL estimation for the *Microcystis* culture addition was slightly more under-predicted than for the *Coelastrum* culture addition (Fig. 1 and 2), although this could not be tested statistically given the experimental design.

A one-to-one cross plot of JFE readings compared to reference sample measurements for

CHL is shown in figure 2. The regression for the CHL response over the tested range of 0 to 32 µg/L was statistically significant ($p<0.001$) with an R^2 of 0.98 and a slope of 0.66.

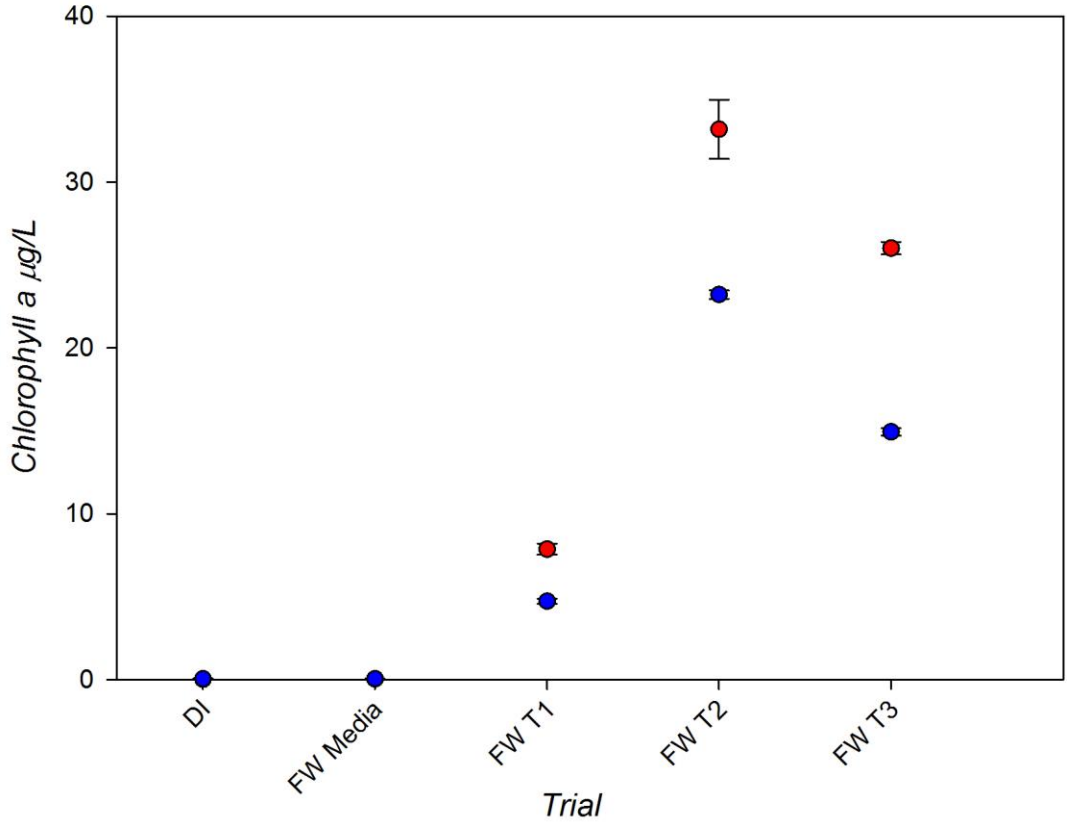


Figure 1. Plot of instrument (blue) and reference (red) measurements of chlorophyll a in the freshwater individual algae tests, including background readings for DI and the freshwater culture media. The plotted reference values represent the average and standard deviation of the two reference measurements taken at the beginning and end of the exposure period. Instrument estimations were generated from the 3 second readings averaged over 1 minute, following an equilibration time of 3 minutes.

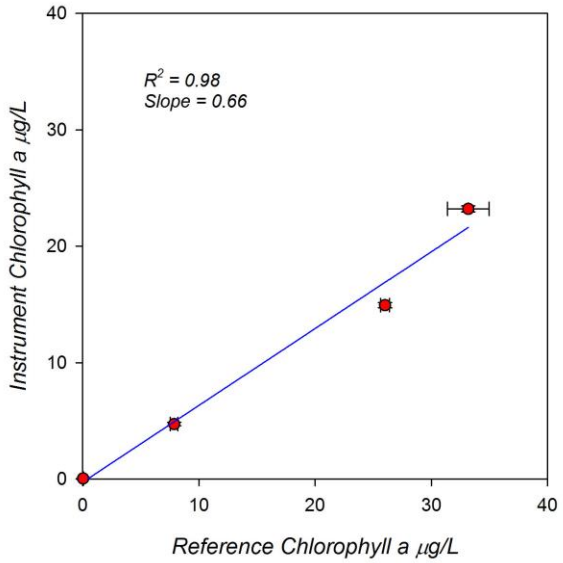


Figure 2. Cross plot of instrument readings versus extracted reference sample pigments for chlorophyll a during the freshwater individual algae lab trials.

A comparison of algal classification from microscope counts on preserved reference samples against the JFE emission measurements at each excitation wavelength are shown in figure 3. Emmision strength clearly matched algal concentration, and there was a noticeable decrease in the 470 nm response for the Cyanobacterial culture versus the Green algae culture. The wavelength emmision response at 570 nm was slightly less for this exposure than for a subsequent lab test exposure on June 29 based on comparing the proportion of the emission to either the peak emission at 435 of the total CHL estimate (see Fig. 15). We do note that some contamination by *Microcystis* in the *Coelastrum* culture was discovered in subsequent trials (and was seen from phycocyanin measurements) but was not picked up in the microscopy analysis of the reference sub-samples counted. So the emission response in trials 1 and 2 may not reflect a response to a pure chlorophyte culture.

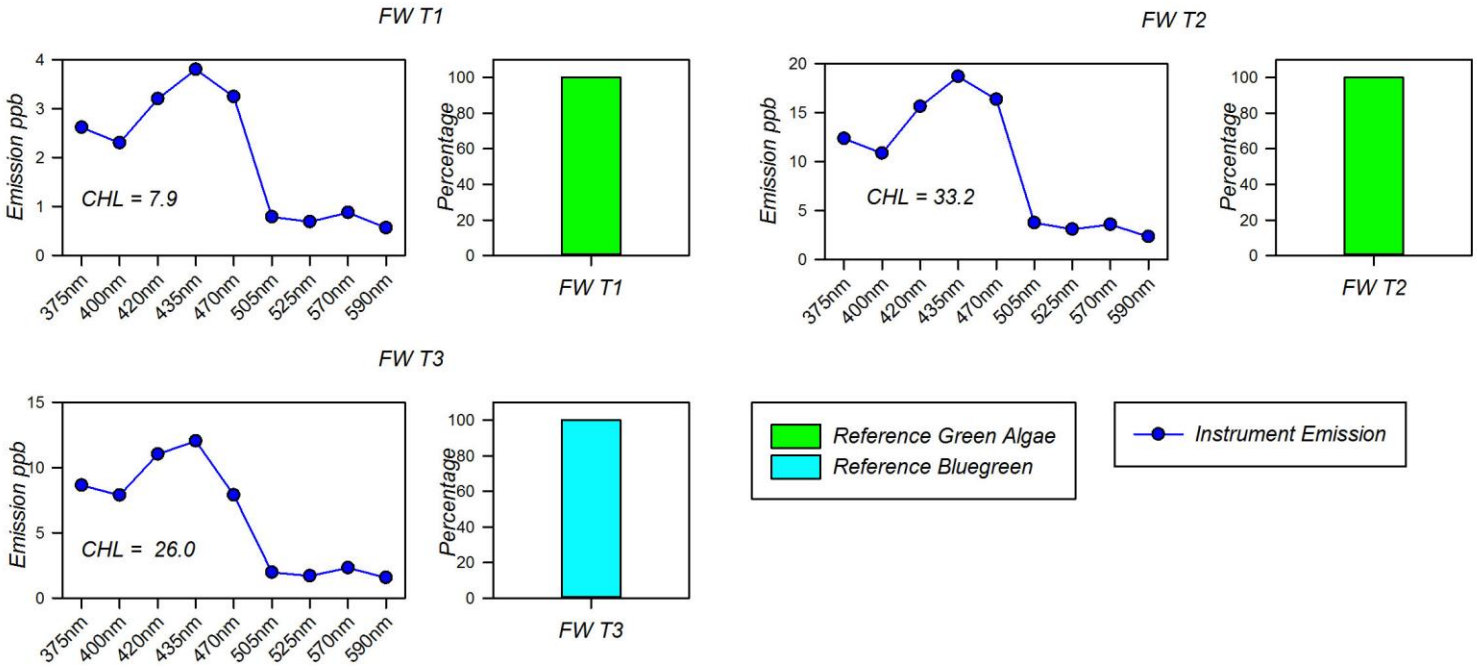


Figure 3. Algal classification from microscope counts on preserved reference samples as a percentage of biomass grouped at the functional class level, compared to the JFE emission measurements at each excitation wavelength. Algal counts were grouped at the functional class level.

Four individual trials were conducted using saltwater algal cultures along with the DI and saltwater media blanks (Table 3). Trials 1 and 2 used two different levels of the golden-brown haptophyte *Isochrysis* at approximately 5 and 22 µg/L CHL, respectively. Trials 3 and 4 used two different levels of the dinoflagellate *Amphidinium* added at concentrations of approximately 5 and 20 µg/L CHL, respectively. This culture is denoted as 'AC Mix' because microscopic examination indicated it was contaminated with other golden-brown diatoms that likely broke-through the seawater filtration system.

Table 3. Test conditions for the individual saltwater algal culture exposures for the June 26 MLML laboratory tests. The test examined instrument response to two different saltwater algal species, *Isochrysis* and *Amphidinium* (denoted as 'AC Mix' because it was not a pure culture) along with background readings of DI and the saltwater culture media. (n = number of reference samples collected during the trial; C1 and C2 refer to increasing concentrations from additional culture addition).

Trial	*Isochrysis*	AC Mix
DI (n = 1)	-	-
SW Media (n = 2)	-	-
SW T1 (n = 2)	C1	-
SW T2 (n = 2)	C2	-
SW T3 (n = 2)	-	C1
SW T4 (n = 2)	-	C2

Results of instrument readings compared to extracted pigment concentrations determined on the reference samples are plotted in figure 4. The JFE showed a small but positive response to the background seawater media response and tracked chorophyll levels more closely across the higher concentration range for these marine species than the previous freshwater species. Seawater media was made from DI and added salts so should not have contributed any unexpected cells to the culture additions. There was no apparent difference in response across the two marine algal species tested but this was not examined statistically given the experimental design.

A one-to-one cross plot of the JFE CHL estimations compared to reference sample measurements during the saltwater trials is shown in figure 5. The regression for the CHL response was highly significant ($p<0.001$) with an R^2 of 0.99 and a slope 0.80.

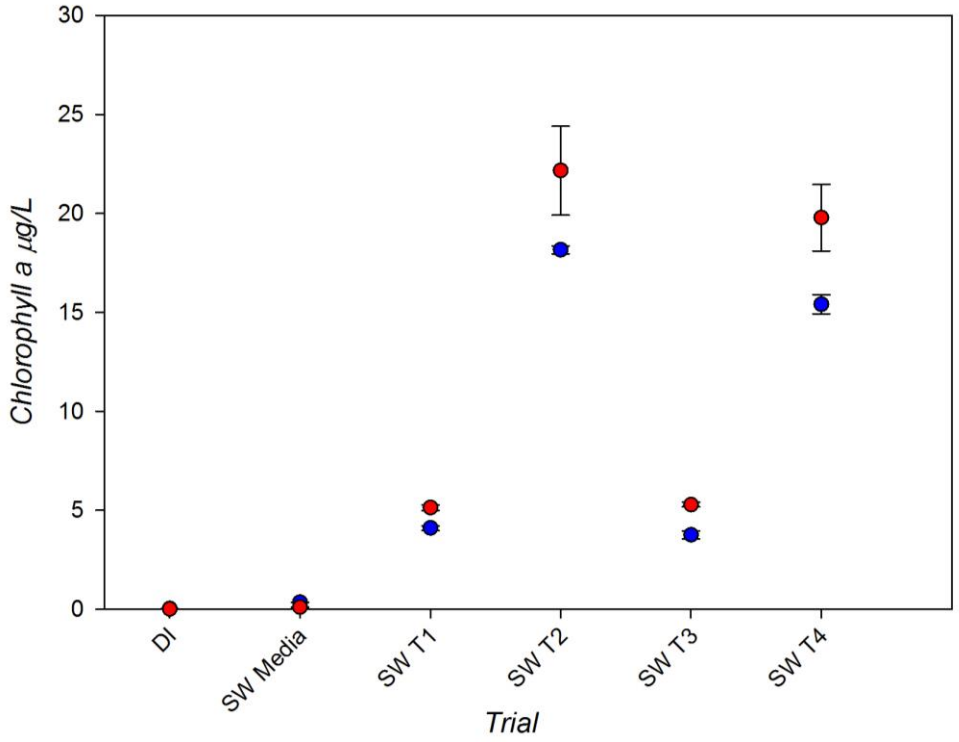


Figure 4. Plot of instrument (blue) and reference (red) measurements of chlorophyll-a in the saltwater individual algae tests including background readings for DI and saltwater media. The plotted reference values represent the average and standard deviation of the two reference measurements taken at the beginning and end of the exposure period. Instrument estimations were generated from the 1 second readings averaged over 1 minute, following an equilibration time of 3 minutes.

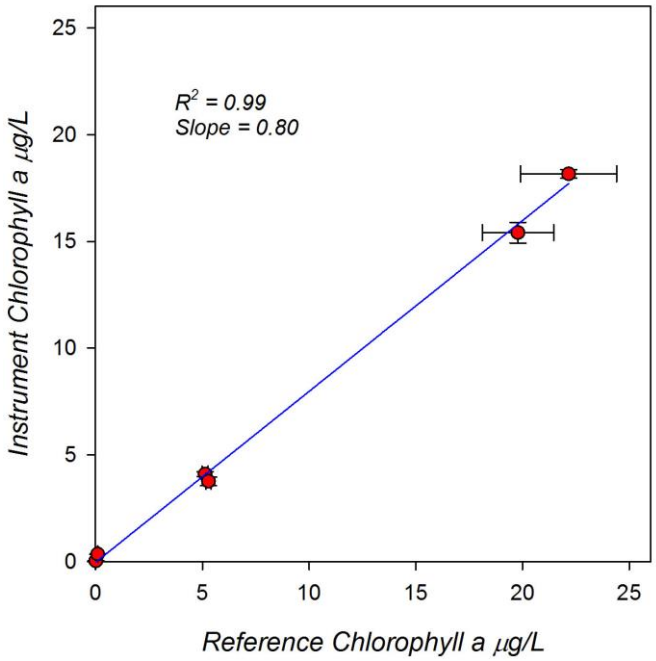


Figure 5. Cross plot of instrument readings versus extracted reference sample pigments for chlorophyll-a during the saltwater individual algae lab trials.

A comparison of algal classification from microscope counts on preserved reference samples against the JFE emission measurements at each excitation wavelength is shown in figure 6. We recognize that contamination was present in the AC mix and microscopic analysis indicate a significant presecence of diatoms in the culture. However, no phycobilins were detected in reference sample pigment extracts from these trials. Emission strength was well matched to algal abundance. The emission curves were quite similar across the two cultures, with a slight increasead response at 525 nm for the AC mixture relative to the Isochrysis culture. Emission spectra appeared quite consistent across the two concentrations of the same culture addition.

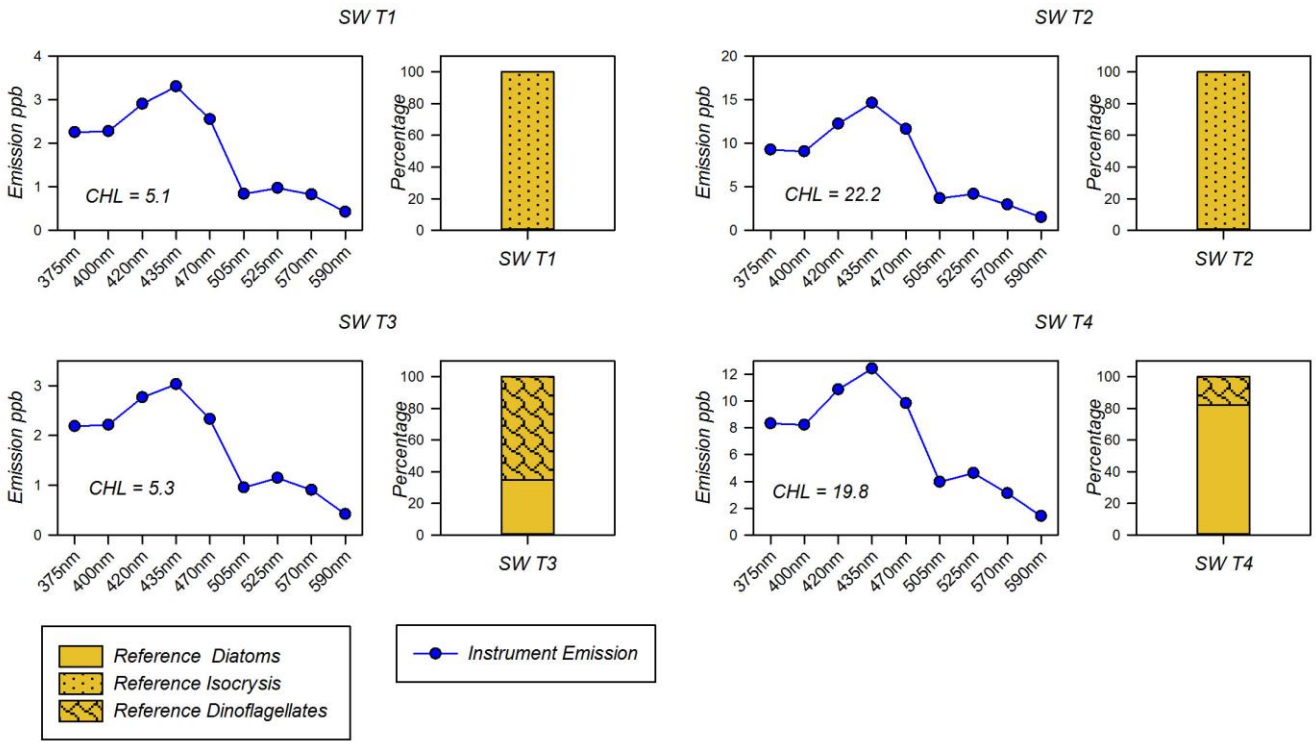


Figure 6. Algal classification from microscope counts on preserved reference samples compared to emission spectra of the JFE. Algal counts were grouped at the functional class level and represented as a percentage of biovolume. Estimations of Isocrysis in trials 1 and 2 were based on examination of the culture and known culture addition volumes and not directly confirmed with microscopic counts on the reference sample aliquots.

On June 27th, 11 different trials were conducted using four levels of *Synechococcus* with add-in matrix challenges of three CDOM levels and two turbidity levels, plus background culture media (Table 4). Each test condition was made up in a 40 L container and reference samples were withdrawn at three timepoints over the course of a 30 minute exposure period. Comparative JFE Multi-Exciter results were generated from 3 minute averages bracketing each reference point. It must be noted that the *Synechococcus* culture became contaminated with large marine Diatoms which ended up dominating the community in terms of biovolume (98%) even though the numerical abundance of the small *Synechococcus* cells was greater.

Table 4. June 27 test conditions with exposures to combinations of saltwater algal cultures at various concentrations with add-in challenges of turbidity and CDOM. (n = number of reference samples collected during the exposure and the values in parenthesis show averaged concentrations determined on the reference samples).

Trial	*Synechococcus*	CDOM (A_{400})	Turbidity (NTU)
SW Media (n = 1)		(0.25)	(0.69)
SW T1 (n = 3)	C1 (3.2)	(0.25)	(0.55)
SW T2 (n = 3)	C2 (6.4)	(0.24)	(0.54)
SW T3 (n = 3)	C2 (6.1)	C1 (0.83)	(0.34)
SW T4 (n = 3)	C2 (6.2)	C2 (1.76)	(0.48)
SW T5 (n = 3)	C2 (6.0)	C3 (3.25)	(0.44)
SW T6 (n = 3)	C3 (19)	C3 (3.05)	(0.65)
SW T7 (n = 3)	C4 (38)	C3 (2.86)	(1.0)
SW T8 (n = 3)	C4 (40)	C3 (2.90)	C1 (3.3)
SW T9 (n = 3)	C4 (37)	C3 (2.90)	C2 (23)
SW T10 (n = 3)	C4 (36)	C3 (2.97)	C3 (50)

Over the ten algal trials, reference sample CHL levels ranged from 3.2 to 40 µg/L and PC levels ranged from 0 to 1.5 µg/L. Across the trials, CDOM concentrations were increased from a background level of approximately 0.25 up to 3.0, and turbidity was increased from a background level of 0.5 up to 50 NTU.

Comparative results of instrument readings versus reference sample CHL concentrations in µg/L are plotted in figure 7. The JFE expressed a small positive response to background seawater media and closely estimated concentrations during the first five trials when CHL levels were less than 10 µg/L. There was a slight positive bias (higher prediction) in the JFE CHL estimates across the three levels of CDOM addition (SW trials T3 - T5). Based on its' set-up as tested, the JFE significantly under predicted CHL at the higher concentration and a new calibration would be required to appropriately measure these higher concentrations. The addition of turbidity at levels of 23 and 50 NTU (T9-10 versus T7-8) further decreased the JFE CHL estimation relative to reference CHL.

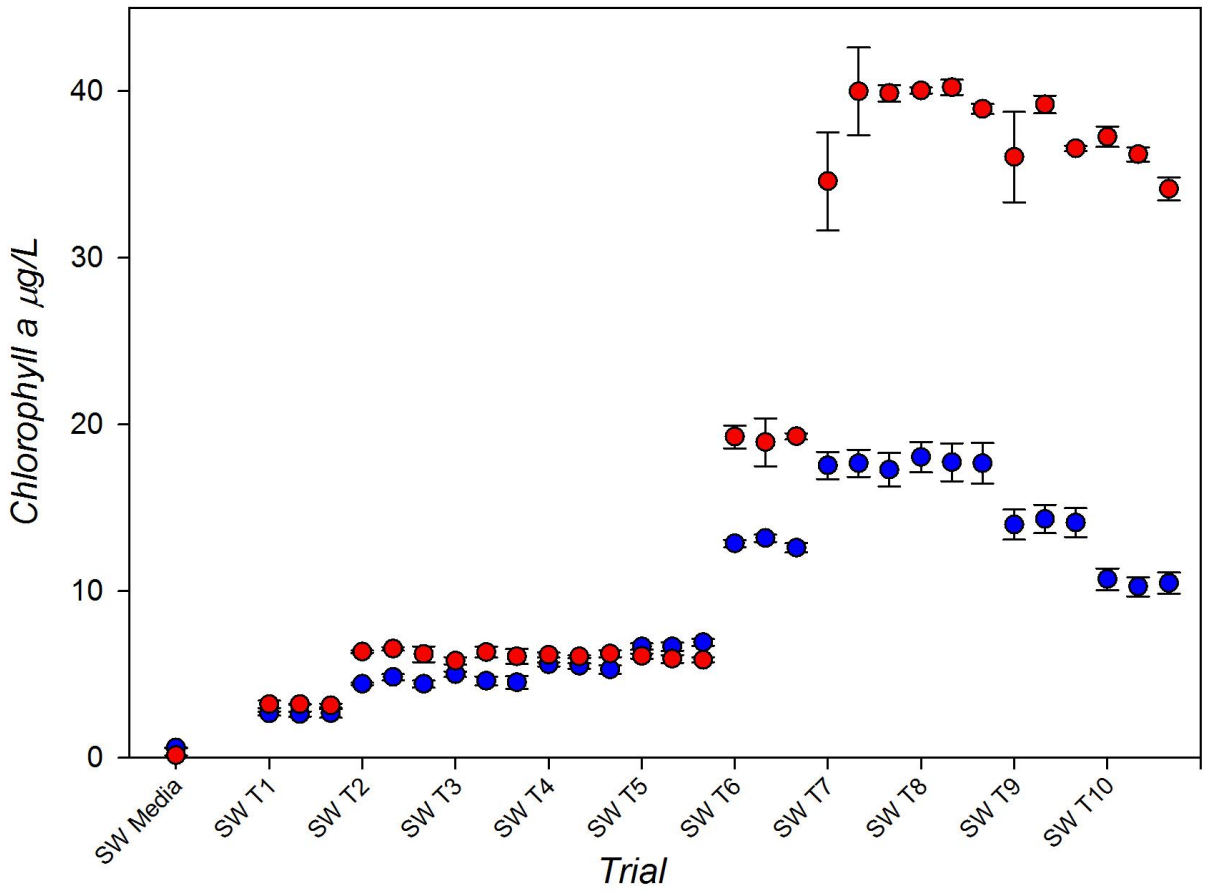


Figure 7. Plot of instrument (blue) and reference (red) measurements of chlorophyll a in the saltwater CDOM and turbidity addition trials covering 4 algae, 3 CDOM and 3 turbidity concentration levels. Three replicate reference measurements were made at each level with only one read in blank media, the JFE data was averaged over 1 minute, bracketing each reference point.

A one-to-one cross plot of the JFE CHL estimations compared to reference sample measurements during the saltwater matrix challenge test is shown in figure 8. Overall, the regression for the CHL response was highly significant ($p<0.001$) with an R^2 of 0.85 but with a slope of only 0.33. The overall response slope is noticeably lower than for the previous saltwater culture addition Lab test. The variability in instrument response at similar CHL levels for trials T7 – T10 (Fig. 8) indicate that the JFE CHL estimation decreased at the highest turbidity additions of 23 and 50 NTU (larger proportion of decline than for the reference CHL) but there was no noticeable impact in response to the turbidity increase between 1 and 3.3 NTU across trials 7 and 8.

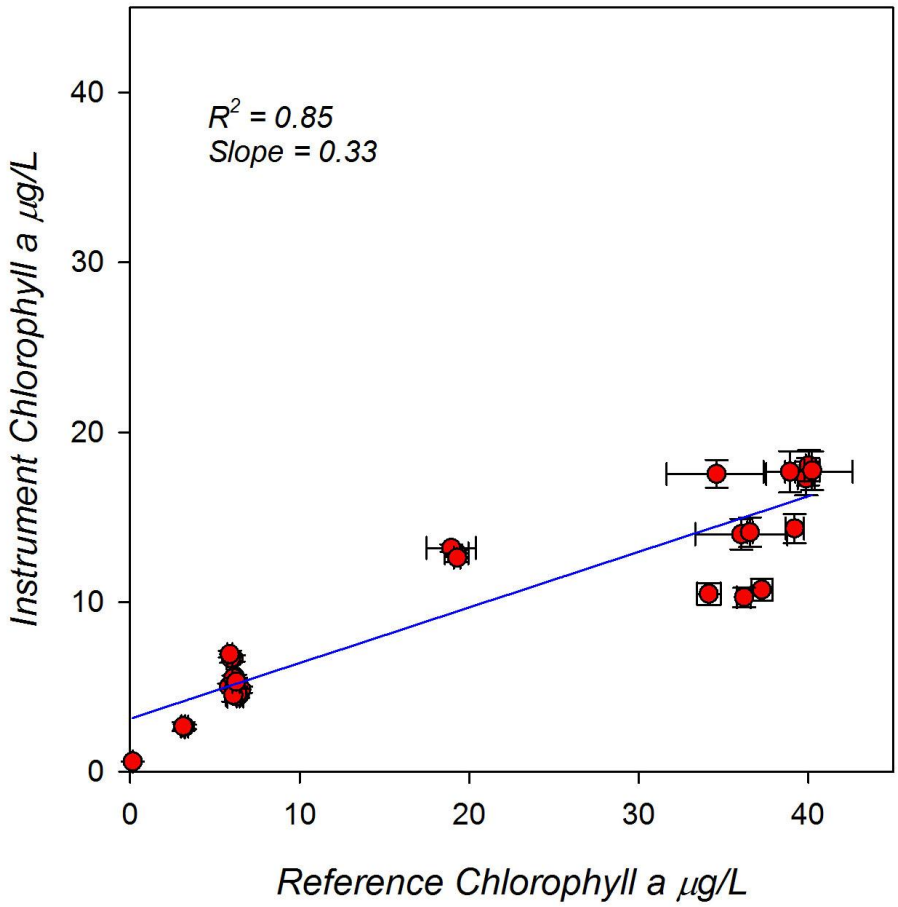


Figure 8. Cross plot of instrument readings versus extracted reference sample pigments for chlorophyll during the saltwater *Synechococcus* algae exposure with add in matrix challenges for CDOM and Turbdity. The blue line represents the linear regression of the data.

A comparison of algal classification from microscope counts on preserved reference samples against the JFE emission measurements at each excitation wavelength is shown in figure 9. Prior to the experimental we discovered that the *Synechococcus* culture was heavily contaminated with marine diatom species which dominated the biovolume proportion and cyanobacterial biovolume only represent less than two percent. For each of the ten trials the JFE emission spectra were very consistent across the three replicate measurements made over the 20 minute incubation test. The JFE emission curves were responsive to the increased CDOM concentration across trials 3 – 5, with an increased emission contribution at the lower wavelengths. As CHL concentration was subsequently increased against the CDOM level at trial 5, the emission peak at 435 became more prevelant. The cyanobacterial responsive emission at 570 nm remained similar throughout all trials with most of the response differences occuring at wavelengths less than 505 nm. The addition of turbidity to similar phytoplankton concentrations in trials 8-10 indicate that turbidity reduced the overall emission strength but produced very similar spectral curves.

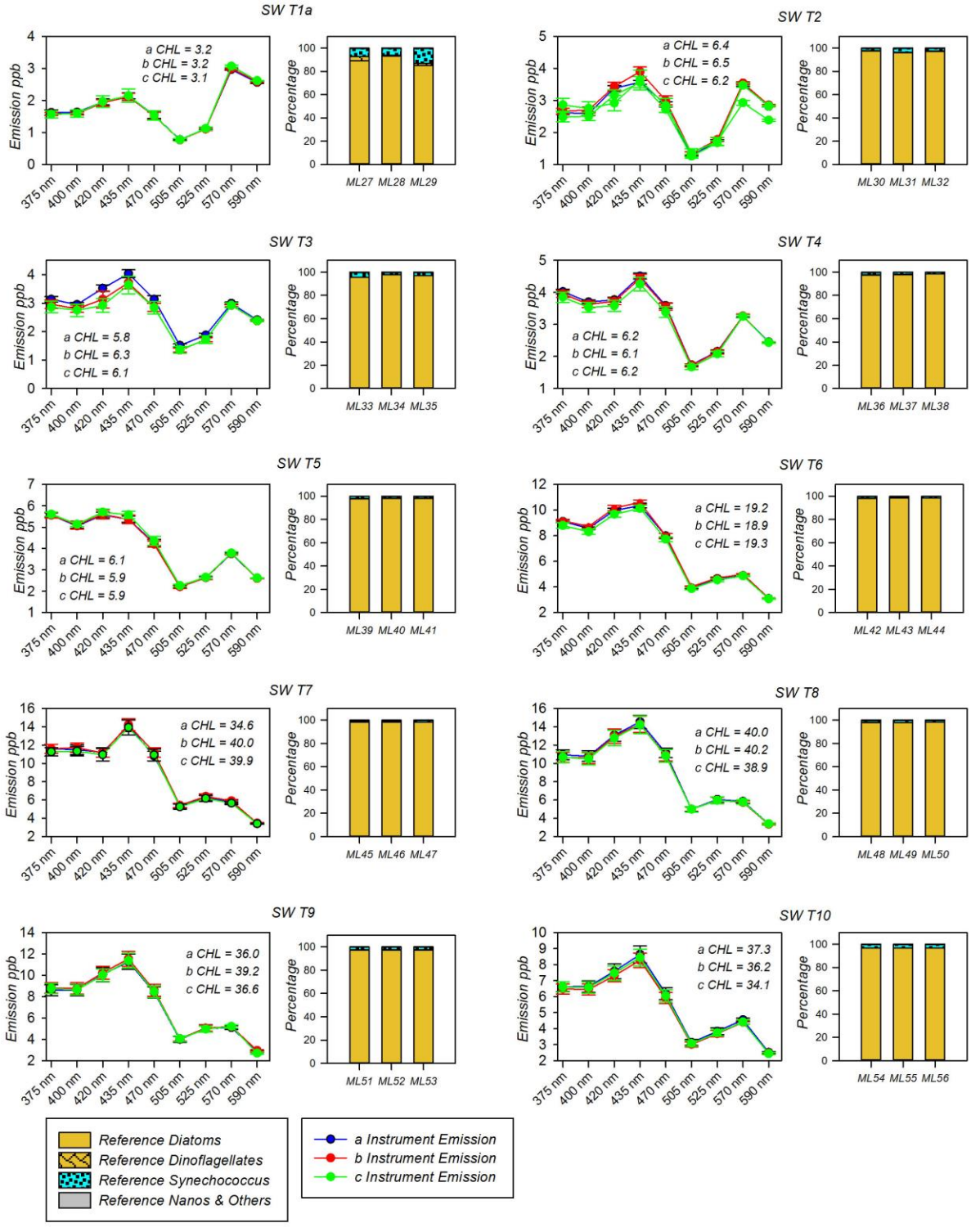


Figure 9. Algal classification from microscope counts on preserved reference samples compared to emission spectra of the JFE. Algal counts were grouped at the functional class level and represented as a percentage of biovolume. Each row represents three timepoint replicates of the same batch of algae and matrix conditions.

For the June 28 lab test, 6 different trials were conducted using various mixtures of *Coelastrum*, *Microcystis* and *Peridinium* at varying concentrations, along with a blank for the freshwater media (Table 5). Each test condition was made up in a 40 L container and reference samples were withdrawn at three timepoints over the course of a 30 minute exposure period. Over the six algal trials, CHL levels ranged from 3.8 to 25 µg/L and PC levels ranged from 1.3 to 6.7 µg/L.

Table 5. June 28 test conditions with exposures to combinations of freshwater algal cultures at various concentrations. (n = number of reference samples taken during the exposure; C1 and C2 represent concentrations levels from culture additions).

Trial	*Coelastrum*	*Microcystis*	*Peridinium*
FW Media (n = 1)	-	-	-
FW T1 (n = 3)	C1	-	-
FW T2 (n = 3)	C2	-	-
FW T3 (n = 3)	C2	C1	-
FW T4 (n = 3)	C2	C2	-
FW T5 (n = 3)	C2	C3	-
FW T6 (n = 3)	C2	C3	C1

Comparative results of instrument readings versus reference sample CHL concentrations in µg/L for the June 28 lab test are plotted in figure 10. The JFE showed no background response to the freshwater media and the agreement to reference CHL measurements was again proportional to the concentration with a very strong linear response across the range tested. There were no obvious differences in response across the three species tested.

A one-to-one cross plot of the JFE readings versus reference sample CHL measurements for the June 28 freshwater algal mixture test is shown in figure 11. The regression of the JFE CHL response was highly significant ($p<0.001$) with an R^2 of 0.97 and a slope of 0.63.

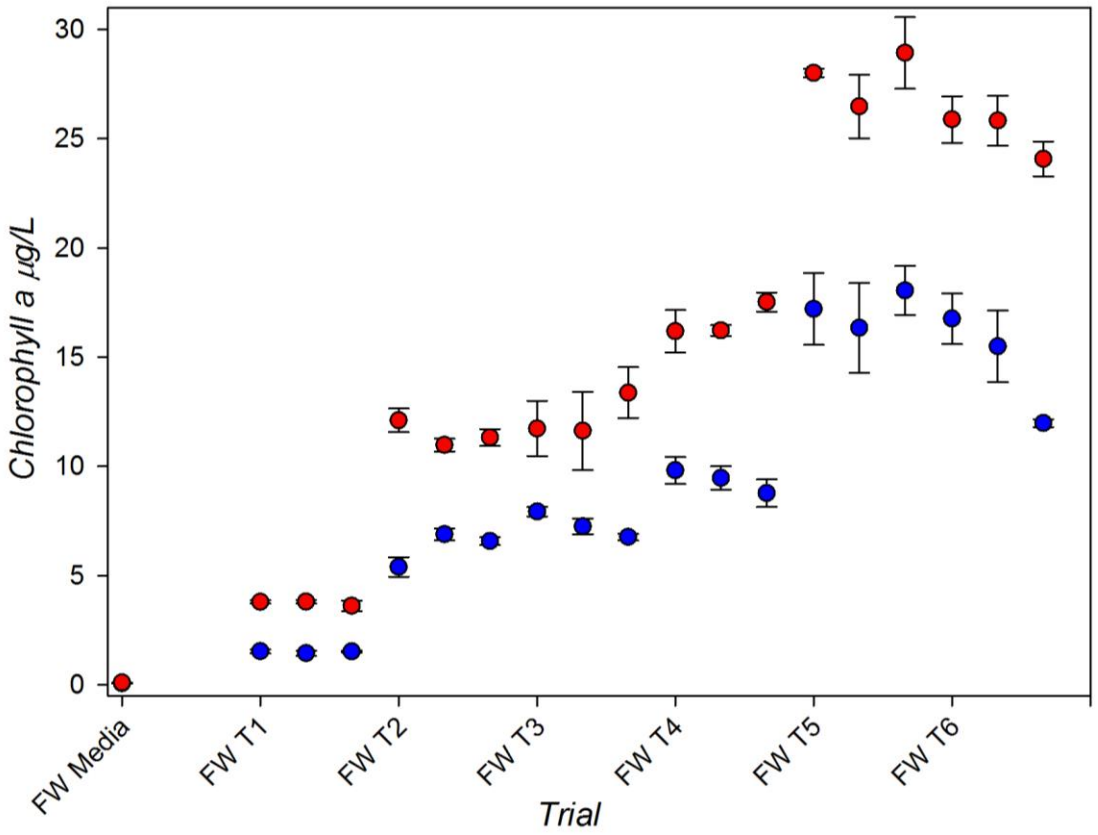


Figure 10. Plot of instrument (blue) and reference (red) measurements of chlorophyll a in the freshwater algal mixture trials covering 6 concentration ranges and mixtures of 3 different algae. Three replicate reference measurements were made at each level, the JFE data was averaged over 1 minutes, bracketing each reference point.

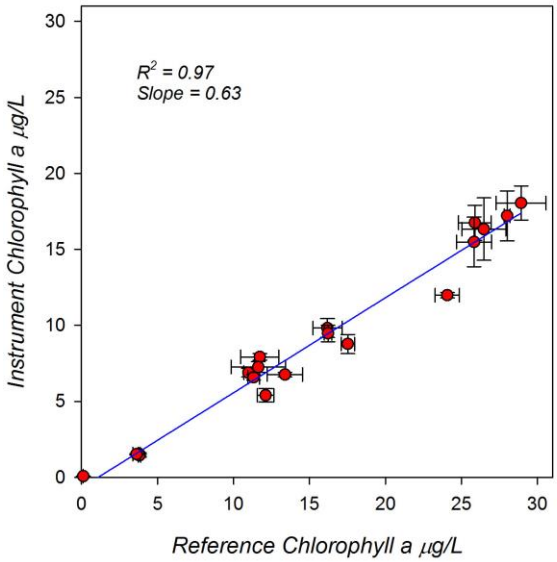


Figure 11. Cross plot of instrument readings versus extracted reference sample pigments for chlorophyll a during the June 28 freshwater algal mixtures lab trials. The blue lines represent the linear regression of the data.

A comparison of algal classification from microscope counts on preserved reference samples against the JFE emission measurements at each excitation wavelength is shown in figure 12. There was more variance in the replicate readings across the 30 minute exposure which may reflect some heterogeneity in algae distributions within the tank. Emission strength at the 435 nm peak was well matched to overall abundance as estimated by CHL extracts. The JFE exhibited an elevated response at 570 nm in trial 1 for the *Coelastrum* only addition. We recognize that the *Coelastrum* culture was not pure even though we did not pick it up in our counts. Although we found no measurable PC in trial 1, we did find about 1 µg/L PC in trial 2 when higher levels of *Coelastrum* were added. There was not a strong response to the *Microcystis* additions in the subsequent trials 3-6 when added in combination with *Coelastrum* even though reference sample PC levels were up to 6.7 µg/L. The most notable pattern was for increase in the emission at 420 nm and a decline in the emission at 470 nm, especially with the addition of the dinoflagellate *Peridinium*.

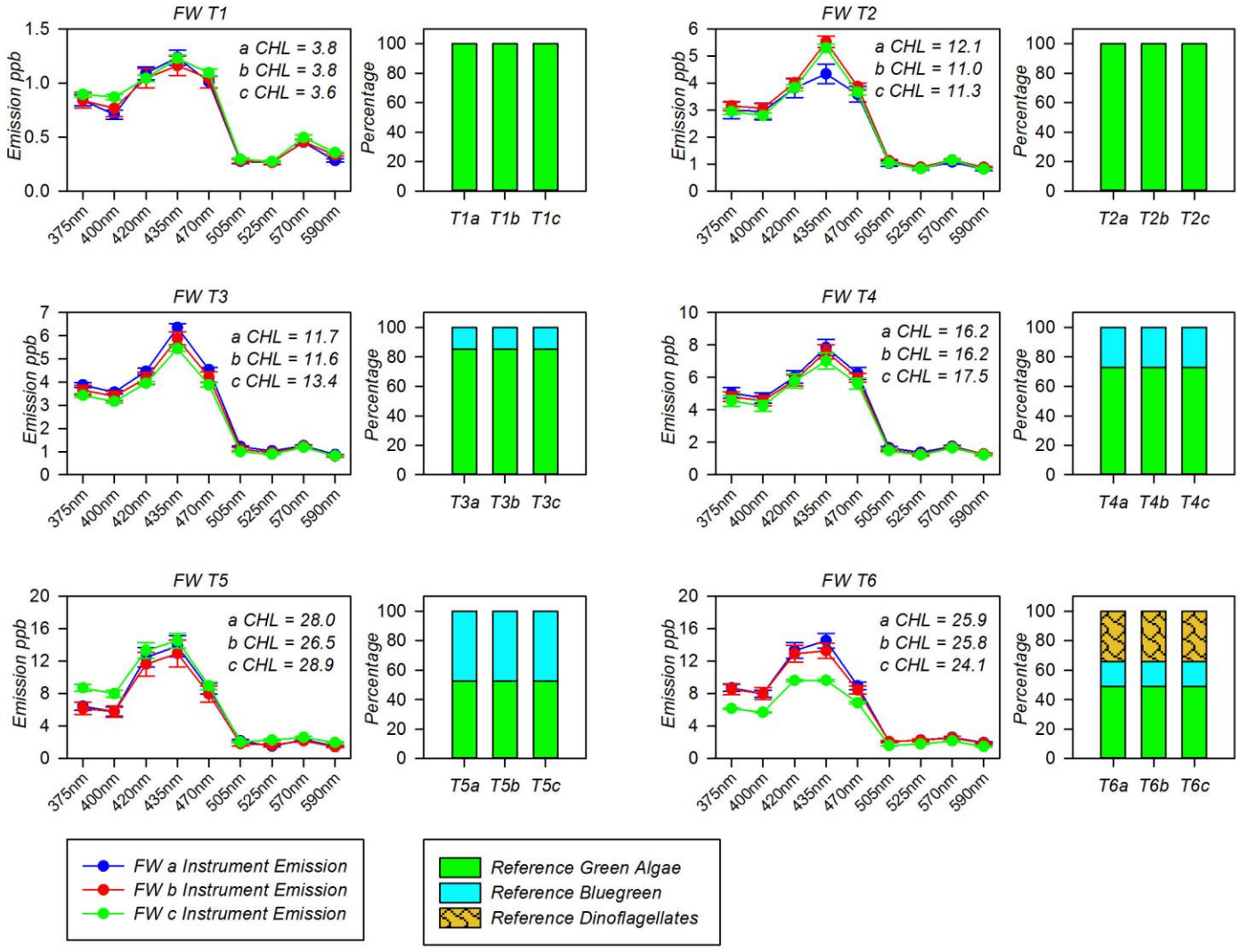


Figure 12. Algal classification from microscope counts on preserved reference samples compared to emission spectra of the JFE. Algal counts were grouped at the functional class level and represented as a percentage of biovolume. Each row represents three timepoint replicates of the same batch of algae and matrix conditions.

For the June 29 lab test, eight different trials (plus a media blank) were conducted using three levels of *Microcystis* with add-in matrix challenges of three CDOM levels, an addition of *Coelastrum,* and lastly an addition of turbidity (Table 6). Each test condition was made up in a 40 L container and reference samples were withdrawn at two or three timepoints over the course of a 30 minute exposure period. Over the eight algae trials, CHL levels ranged from 5.3 to 59 µg/L and PC levels ranged from 0.2 to 9.8 µg/L. CDOM additions increased concentrations from a background level of 0.43 up to 4.7, and the turbidity additions increased concentrations from a background of 1 NTU up to 25 NTU.

Table 6. June 29 test conditions with exposures to combinations of freshwater algal cultures at various concentrations with add-in challenges of turbidity and CDOM. Designations with C# indicate additions, or concentration levels of the specific parameter. Measured concentrations of CDOM and turbidity for reference samples are provided in parenthesis.

Trial	*Microcystis*	CDOM	*Coelastrum*	Turbidity
FW Media (n = 1)	-	-	-	-
FW T1 (n = 3)	C1	(0.43)	-	(0.25)
FW T2 (n = 3)	C2	(0.43)	-	(0.39)
FW T3 (n = 3)	C2	C1 (1.5)	-	(0.38)
FW T4 (n = 3)	C2	C2 (3.3)	-	(0.45)
FW T5 (n = 2)	C2	C3 (6.4)	-	(0.56)
FW T6 (n = 2)	C3	C3 (4.6)	-	(0.86)
FW T7 (n = 2)	C3	C3 (4.6)	C1	(1.1)
FW T8 (n = 2)	C3	C3 (4.7)	C1	C1 (25)

Results for the June 29 lab test with freshwater algal mixtures and CDOM and turbidity additions are plotted as a time series of instrument readings compared to reference sample CHL in µg/L (Figure 13). As was seen during the June 27 saltwater species lab test, the addition of CDOM produced a small positive bias in the JFE estimation of CHL (see comparison across trials 2-5). JFE estimations of CHL were again more under-predicted for the higher CHL concentrations near 25 and 60 µg/l resulting from the particular set-up that came with the instrument and re-calibration would be required to quantify these higher concentrations. In this Lab test there did not appear to be any large effect from the turbidity addition at a concentration of 25 NTU, i.e there was not a big change across in response or accuracy across trial 7 and 8. We note there was significant variation in the two reference sample CHL estimates for trial 7 (grey symbol), but not for the JFE. Cell counts in two replicates for trial 7 were also 25% lower so suggest some patchiness in the tank during the sub-sampling, but we consider the second reference sample replicate to be un-representative of the trial conditions based on known additions and should not be interpreted as a missed response by the JFE.

A one-to-one cross plot of the JFE readings compared to reference sample measurements for CHL during the freshwater algal mixture trials are shown in figure 14, with the suspect reference measurement noted above omitted. The regression line was highly significant ($p<0.001$) with an R^2 of 0.96 and a slope of 0.39.

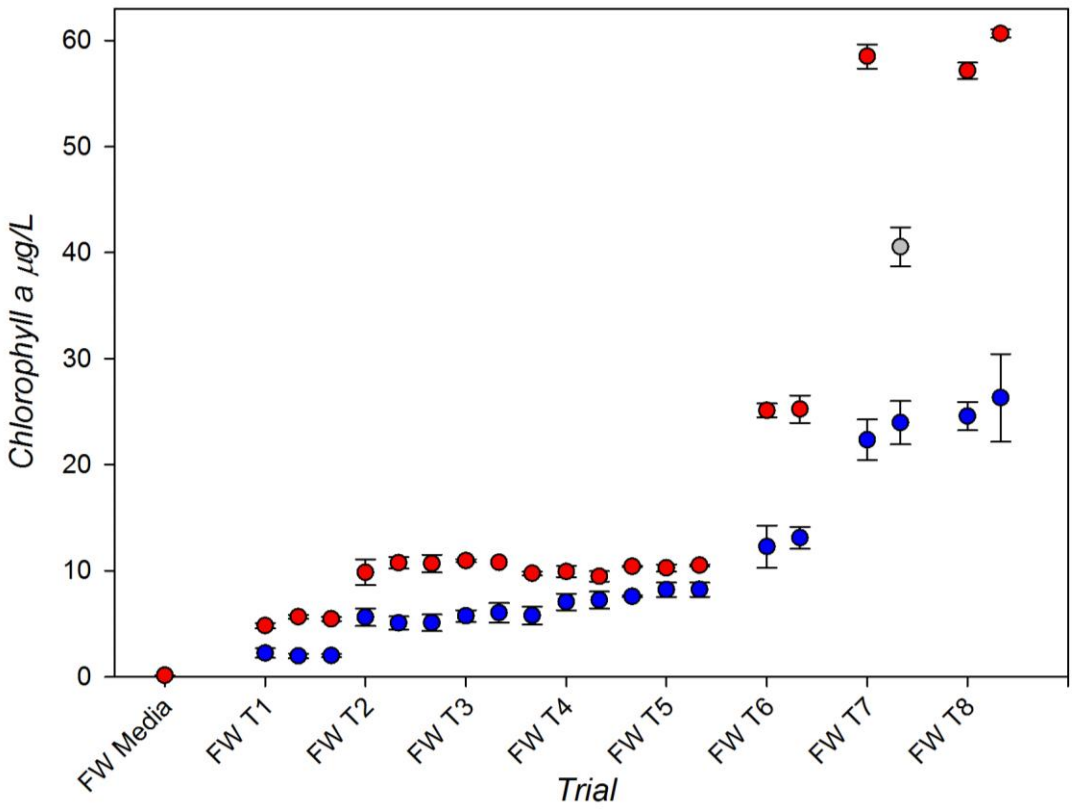


Figure 13. Plot of instrument (blue) and reference (red) measurements of chlorophyll a during the June 29th laboratory test with freshwater algae *Microcystis* and *Coelastrum*, and challenge additions of CDOM and turbidity. Three replicate reference measurements were made for trials 1-5, two replicates were made for trials 6-8, and one replicate for the blank media. JFE data was averaged over 1 minute bracketing each reference sample.

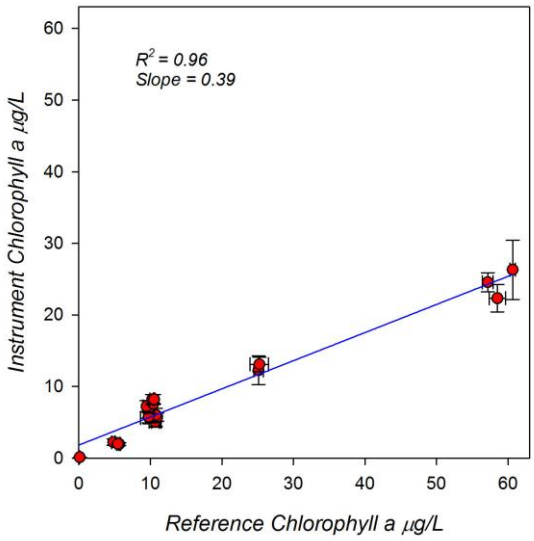


Figure 14. Response plot for the freshwater algae addtion lab trial of the JFE chlorophyll a compared to reference samples. The blue lines represent the linear regression of the data.

A comparison of algal classification from microscope counts on preserved reference samples against the JFE emission measurements at each excitation wavelength is shown in figure 15. For trial 1 and 2 with only *Microcystis* present there was a recognizable increased response at 570 nm. As CDOM was added to the *Microcystis* in increasing amounts during trials 3-6, there was a notable increase in the emission strength at 375 and 400 nm. The addition of more *Microcystis* during trials 6-8, brought back the signal of an elevated 570 nm emission and dampened the proportional strength of the lower wavelengths due to the increased CHL. The addition of *Coelastrum* during trials 7 and 8 produced the most noticeable change in the emission at 470 nm. The further addition of turbidity in trial 8 did not produce any obvious shifts or decreases in emission strength across the the wavelength spectra, but conditions in the tank seemed more variable and made it slightly harder to interpret the response patterns.

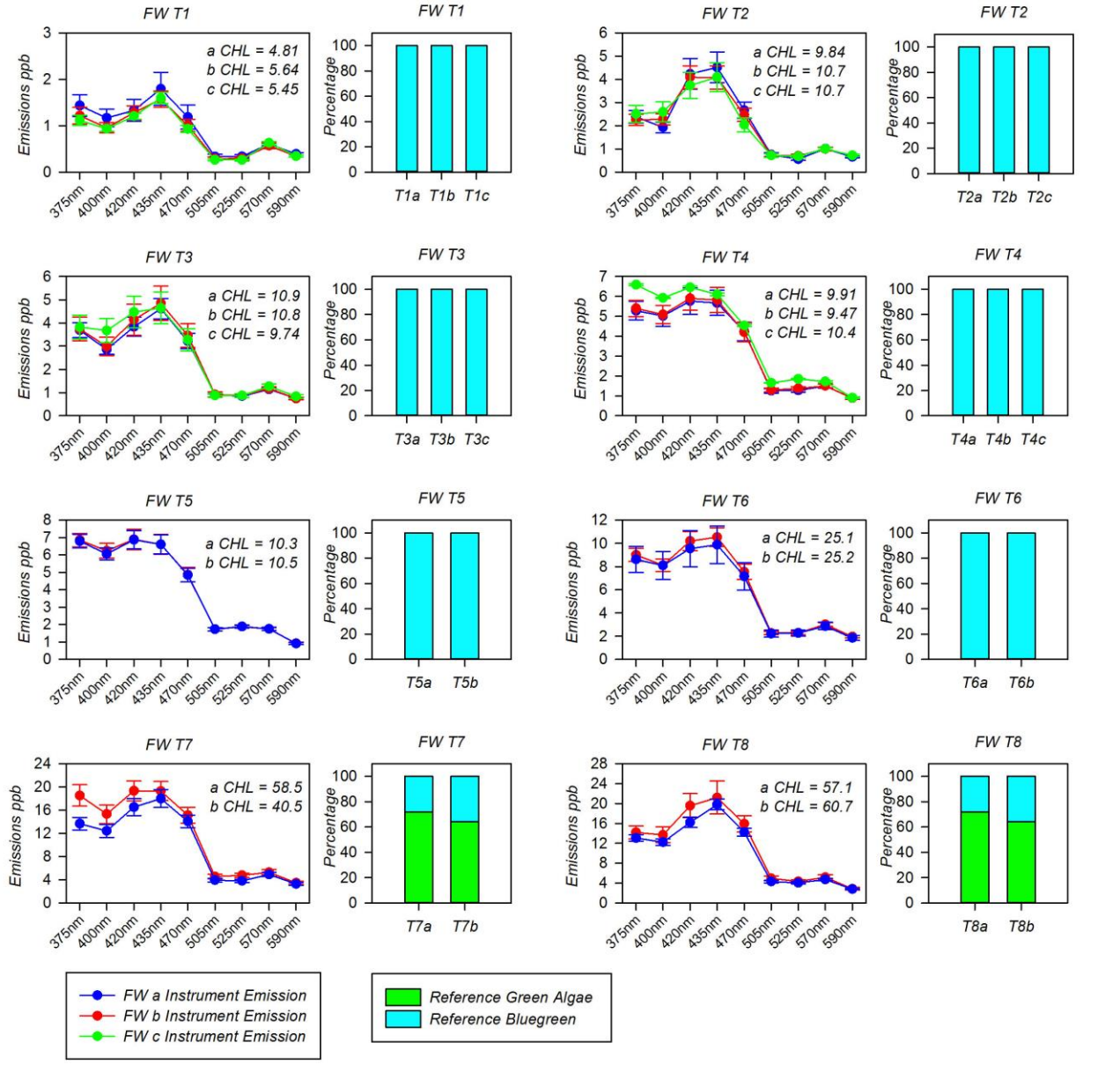


Figure 15. Algal classification from microscope counts on preserved reference samples compared to emission spectra of the JFE. Algal counts were grouped at the functional class level and represented as a percentage of biovolume. Each row represents two timepoint replicates of the same batch of algae and matrix conditions.

Great Lakes Freshwater Lab Test

A second laboratory test with freshwater algal cultures was conducted at the NOAA Great Lakes Environmental Research Lab (GLERL) on August 10, 2017. This test was added after the development of the original test protocols to help address the contamination that occurred in the MLML lab cultures and to test at higher levels of cyanobacteria and PC. The GLERL freshwater lab test was conducted over the course of one day and involved a series of nine individual trials (Table 7). The first four trials were exposures to single algae monocultures of *Chlorella, Cryptomonas, Aphanizomenon*, and *Peridinium* at concentrations levels defined in table 7. The next four trials used a mixture of all four algal species to access the ability to discriminate among the distinct algal taxa when present in different ratios. The last trial incorporated challenge additions of both CDOM and turbidity to the same composition as the proceeding trial. Each test solution was made up in discrete 2 L batches by combining known quantities of the cultures into a fixed volume of freshwater media.

Table 7. Great Lakes lab test conditions conducted on August 10th with exposures to four individual freshwater algal cultures followed by four mixtures of all 4 algal species at various ratios, followed by an add-in challenge of turbidity and CDOM. For the mixtures, a capital letter in the trial ID denotes abundance at the higher C2 level which was about five times higher than the C1 level. Reference sample CHL concentrations in µg/L for each culture addition (directly measured for monocultures and based on volumetric addition for mixtures) are provided in parenthesis.

Trial	*Chlorella*	*Cryptomonas*	*Aphanizomenon*	*Peridinium*	CDOM	Turbidity
A	C2 (12.1)	-	-	-	-	-
B	-	C2 (12.7)	-	-	-	-
C	-	-	C2 (28.6)	-	-	-
D	-	-	-	C2 (12.4)	-	-
Abcd	C2 (12.1)	C1 (2.5)	C1 (5.7)	C1 (2.5)	-	-
ABcd	C2 (12.1)	C2 (12.7)	C1 (5.7)	C1 (2.5)	-	-
ABCd	C2 (12.1)	C2 (12.7)	C2 (28.6)	C1 (2.5)	-	-
ABCD	C2 (12.1)	C2 (12.7)	C2 (28.6)	C2 (12.4)	-	-
ABCD +Turb+CDOM	C2 (12.1)	C2 (12.7)	C2 (28.6)	C2 (12.4)	C1 (6.0)	C1 (33)

Results for this Lab test are plotted as a time series of instrument measurements compared to extracted chlorophyll a concentrations in µg/L determined on the reference samples in figure 16. As with previous test the JFE under-predicted total CHL at higher concentrations due to the response slope within the unit as tested and re-calibration would be required to better quantify the test exposures. The relative estimations by the JFE were consistent for individual species test with *Chlorella*, *Cryptomonas*, and *Peridinium*; however, the amount of under-estimation was nearly three times greater with *Aphanizomenon.* Subsequently, the accuracy of the total CHL prediction in the following five algal mixtures trials was dependent on the relative contribution of *Aphanizomenon* within the mixture. The addition of CDOM and turbidity in the last trial did not produce any noticeable effect on the JFE total CHL prediction.

A cross plot of the JFE readings compared to reference sample measurements during the freshwater algal mixture trials is shown in figure 17. The regression line was highly significant ($p=0.001$) with an R^2 of 0.80 and a slope of 0.32. The greater variation in instrument response again reflected differences in the proportions of specific algal species.

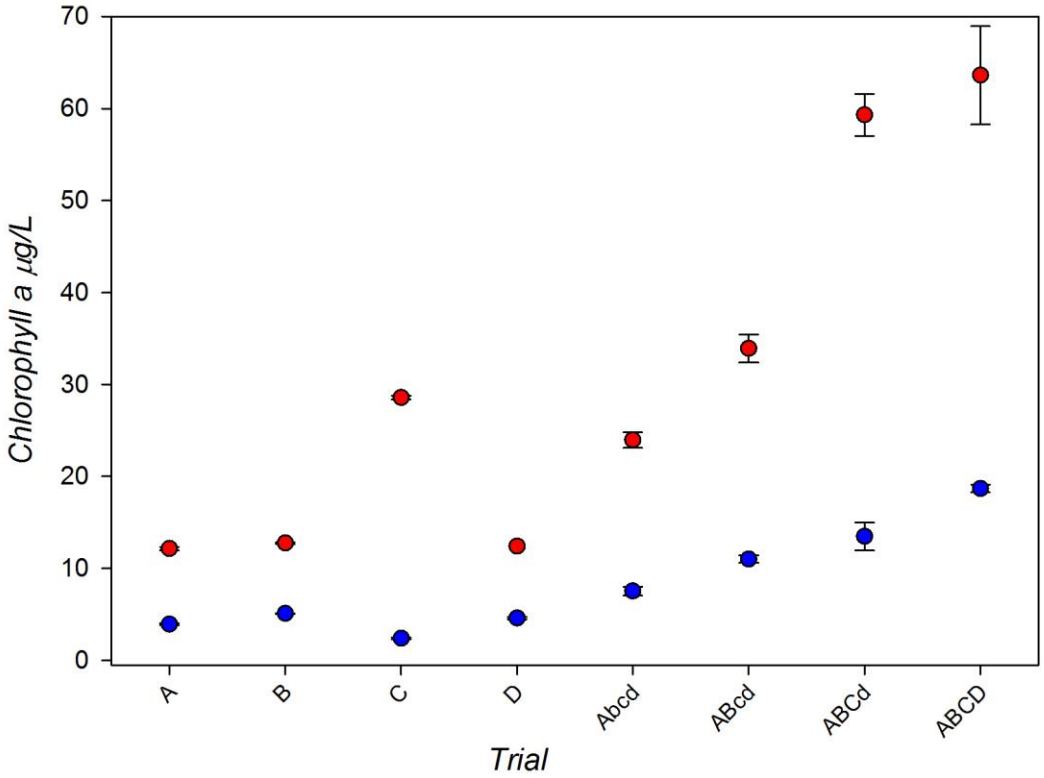


Figure 16. Plot of instrument (blue) and reference (red) measurements of chlorophyll-a in the Great Lakes laboratory trial over 4 individual algae cultures, 4 mixtures and a CDOM and turbidity addition. One reference measurement was made at each level and instrument was averaged over 5 minute periods.

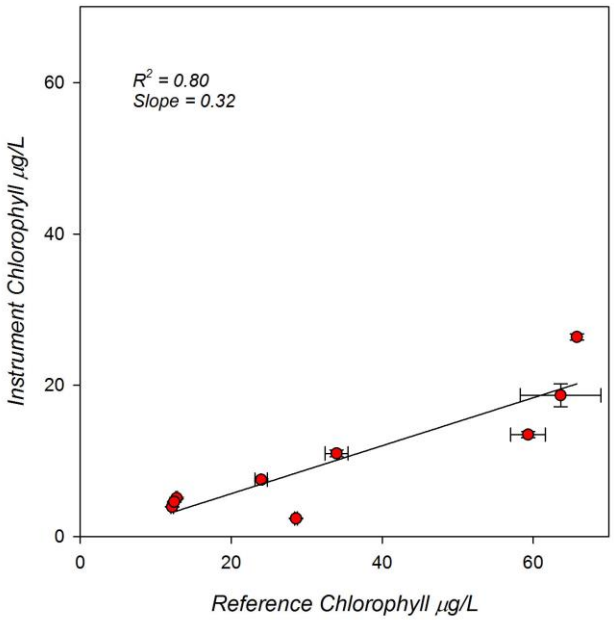


Figure 17. Response plot for the Great Lakes lab test of the JF compared to reference samples. The instrument values were obtained by averaging over 2 minutes. The blue lines represent the linear regression of the data.

A comparison of algal classification from microscope counts on preserved reference samples against the JFE emission spectra for this Lab test is shown in figure 18. There was a strong emission response at 570 nm for both the *Cryptomonas* and *Aphanizomenon* species. The relatively low response in the CHL emission wavelengths for *Aphanizomenon* was distinguished by 570:435 Chl excitation ratios >>1, whereas for *Crytomonas* the ration was closer to one. In contrast green and brown algae exhibited 570:435 ratios < 1. Dual peaks at 435 and 570 nm were observed for all mixed algal exposures with their relative strengths proportional to the percent of cyanobacteria and cryptophytes present. For all tests, emission strength was proportional to overall abundance as estimated by total CHL.

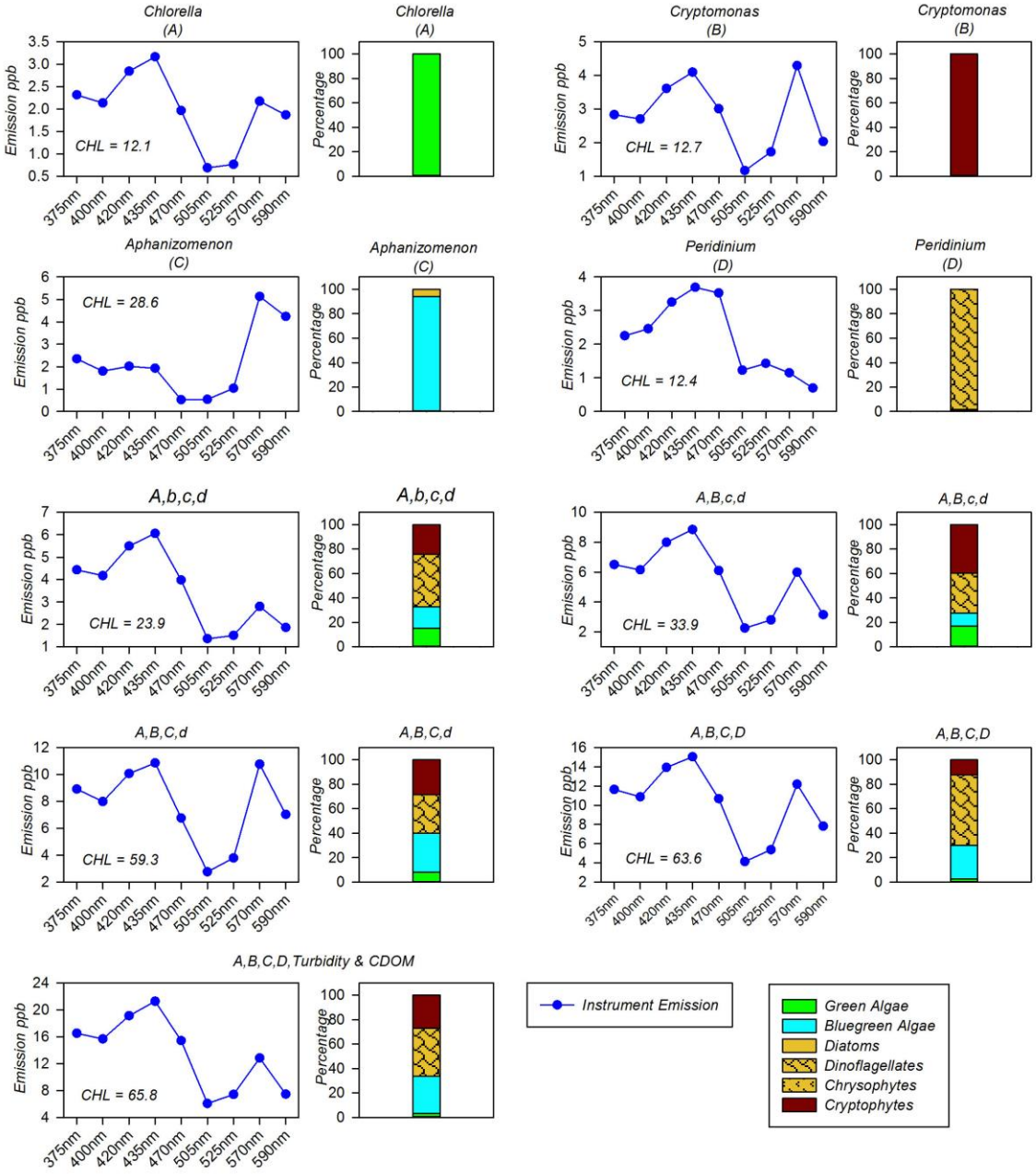


Figure 18. Algal classification from microscope counts on preserved reference samples compared to emission spectra of the JFE. Algal counts were grouped at the functional class level and represented as a percentage of biovolume.

FIELD TESTS

Five field tests were conducted as part of the performance evaluation of the JFE including three underway surface mapping applications and two mooring applications. The three surface mapping applications were conducted in San Francisco Bay, Monterey Bay, and western Lake Erie. The two moored deployment tests were conducted in the Maumee River, Waterville, OH and in Chesapeake Bay, Solomons Island, MD. Further descriptions of each test are provided below. Before the beginning of field testing at each site, the local ACT Partner performed a reference dye test using two concentrations of a commonly prepared BB3 dye from MLML, and a DI reading. The dye readings were done to check the working order of the JFE and the consistency of its response over the duration of the evaluation.

Table 8. Results of the pre-deployment DI and BB3 dye check for the JFE for each testing site. (n.d. denotes no data for that observation.)

Date	Deployment Site	DI	BB3 0.05 µM	BB3 0.50 µM
6/25/17	**MLML**	0.03 ± 0.01	1.30 ± 0.01	12.3 ± 0.01
8/10/17	**UM**	0.04 ± 0.01	1.52 ± 0.01	14.0 ± 0.01
9/05/17	**CBL**	n.d.	n.d.	n.d.

Surface Mapping Applications

San Francisco Bay, CA

USGS Menlo Park has conducted monthly water quality surveys along the axis of South San Francisco Bay, through the central bay, San Pablo and Susuin Bay and into the Sacramento delta since 1968 (https://sfbay.wr.usgs.gov/access/wqdata/index.html). This historical and ongoing set of observations has revealed tremendous plankon diversity along the transect ranging from protistan grazer dominated communities in the shallow warmer South Bay, to oceanic influenced communities in the Central Bay through the Golden Gate, and freshwater influenced communities eastward through the northern bays and Sacramento River. ACT's ongoing collaboration with USGS enabled us to leverage their transect design and research platform for a dedicated surface mapping cruise on 6 July 2017 onboard the *R/V David H. Peterson.* The cruise departed from the berth at the Redwood City Yacht Club on Redwood Creek, north along the axis of the South Bay, transited north to the Golden Gate Bridge in the west Central Bay, north into San Pablo Bay and returned southward below Redwood Creek to sample the shallow, warm and lower salinity waters of the southern reach of the South Bay before returning to dock (Fig. 16). During the 150 km underway mapping cruise, eight stations were selected to make comparative reference sample measurements.

The *R/V Peterson* is equipped with a flow-through seawater system powered by a Headhunter Stingray continuous flow pump (20GPM) drawing from a through hull port at approximately 1 m depth near the bow. A 40 gal black polyethylene trash can was plumbed with one-inch PVC inflow ports 1 inch from the bottom and 4 inches below the top and these were attached to valves which allowed us to control flow rates into the tank. Flow rate was sufficient to fill the exposure tank to the overflow port within 2 min. Instruments were hung from a PVC frame within the tank with sensors oriented toward the bottom. Coordinated rotation of the rack and intruments was used to clear instruments of accumulated bubbles and debris. Port valves were open between station transit to permit continuous turnover of the contained water. The tank lid was kept closed except when sampling and to mix exposure water. Once on station, the inflow port valve was closed after 2 min and instruments were allowed to equilibrate for 10 min, then two reference samples were withdrawn at 10 and 20 minutes after isolation. Sampling was below the water surface near the sensor depth. After the 20 min sampling period, the tank was reopened to flow through while transiting to the next station.

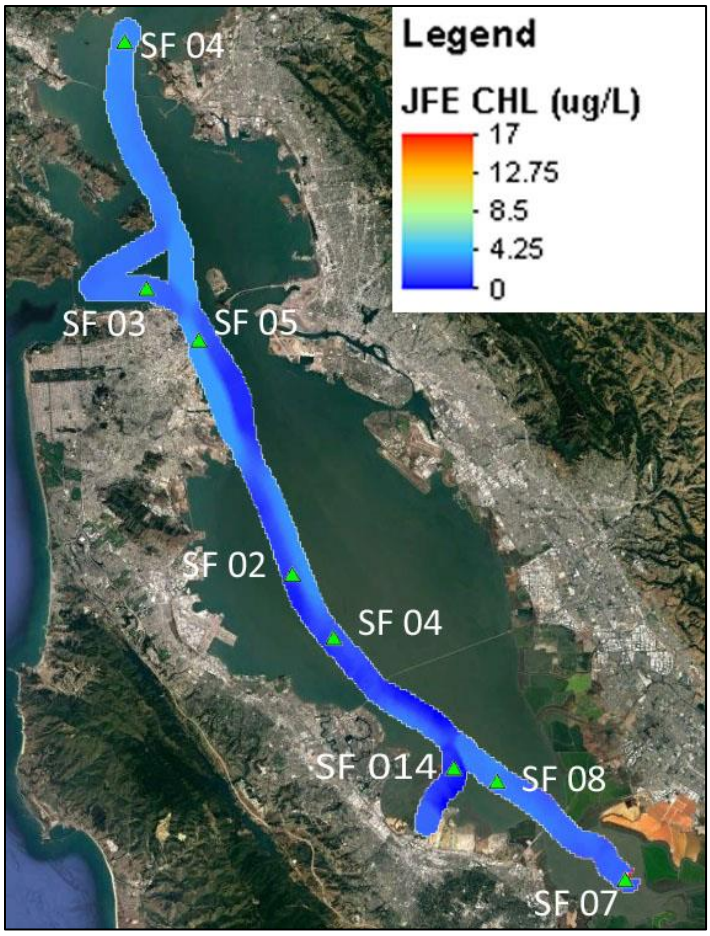


Figure 19. Chlorophyll data contours from the JFE during the underway surface mapping survey in San Francisco on the USGS *R/V Peterson*. Green triangles denote isolated, comparative sampling stations.

During the San Francisco Bay survey the JFE produced 1604 measurements all of which were consider acceptable values for a successful data return of 100% (Figure 19). CHL concentrations determined from reference samples ranged from 4.4 to 20.8 µg/L over the entire survey. Other water quality conditions and descriptions of algal classifications are described below.

A YSI EXO2 sonde in the tank provided continuous monitoring results during both underway and isolated time periods with measurements taken every 15 seconds (Figure 20). Continuous measurements indicated that conditions in the tank during isolation periods were relatively stable. Reference samples analyses for CDOM and turbidity are plotted over the sonde data for consistency with other tests. During the San Francisco Bay cruise temperature ranged from 17 to 22 ^{o}C and salinity ranged from 21.5 to 27 PSU.

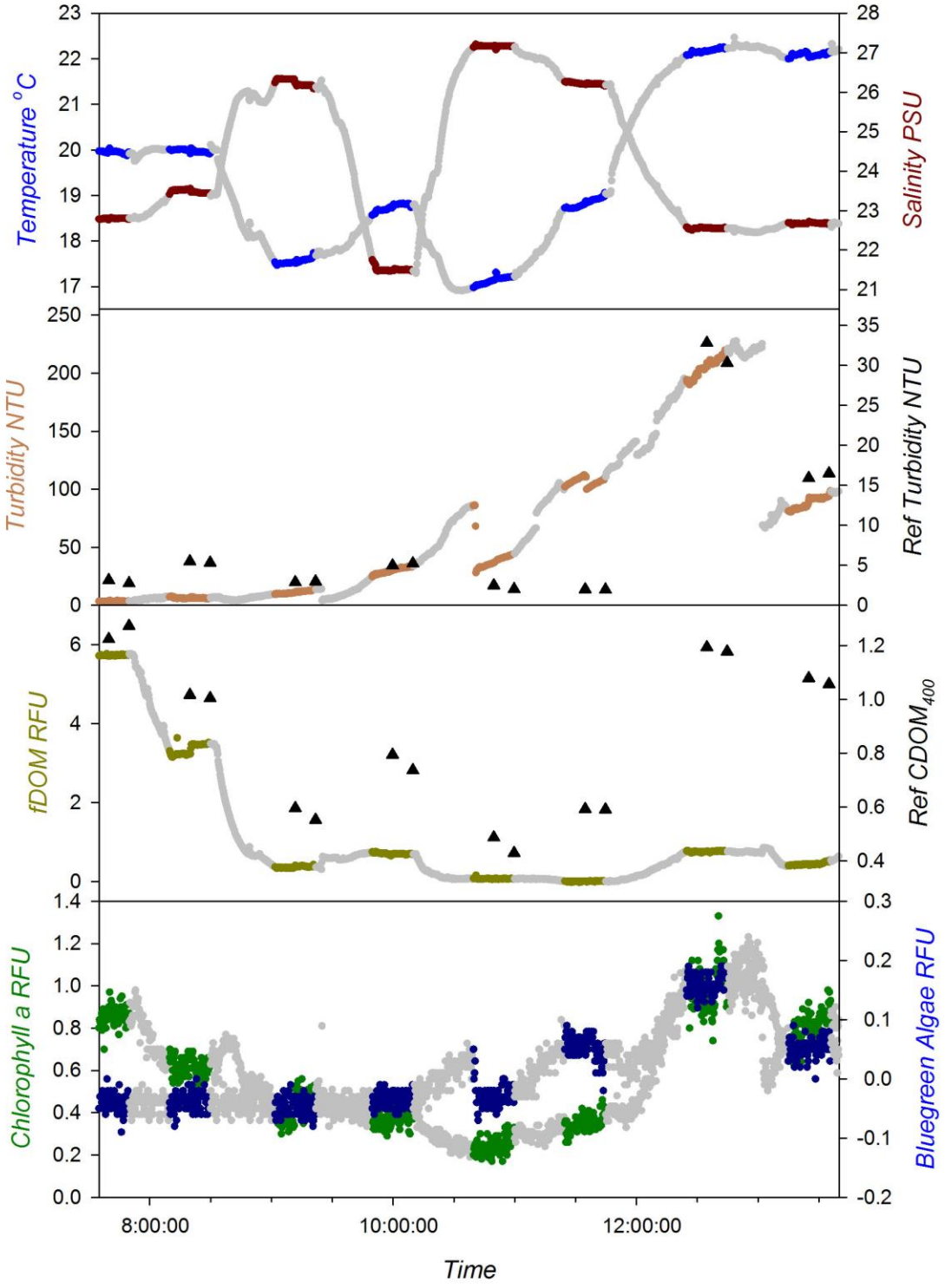


Figure 20. Time series of water conditions encountered during the surface mapping cruise in San Francisco Bay *Top Panel:* Variation in temperature (blue) and salinity (red) at the depth of the sensors in the flow through tank, measured by an EXO 2 Sonde. *Second Panel:* Turbidity (brown) as measured by the EXO 2 and HACH 2100AN Turbidimeter analysis of reference grab samples (black triangles) taken from the exposure tank. *Third Panel:* Continuous fluorescent DOM (fDOM, olive) measured by the EXO 2,and CDOM absorptance (black triangles) measured on reference samples. *Bottom Panel:* Time series of dissolved chlorophyll-a (green) and cyanobacterial (blue) fluorescence measured by the EXO 2 Sonde.

A time series of the CHL measurements from the JFE and reference samples during the isolated exposures is shown figure 21. Extractable chlorophyll, a proxy for total phytoplankton biomass, ranged from *ca*. 3 to 16 µg/L along the sampling transect with highest concentrations encountered in the southern end of South Bay (station 7). The JFE CHL measurements ranged from 1 to 4.4 µg/L and overall underestimated the corresponding reference samples at higher concentrations. Extractable phycocyanin, a proxy for cyanobacterial biomass, was also low throughout the survey, ranging only from 0 to 0.12 µg/L.

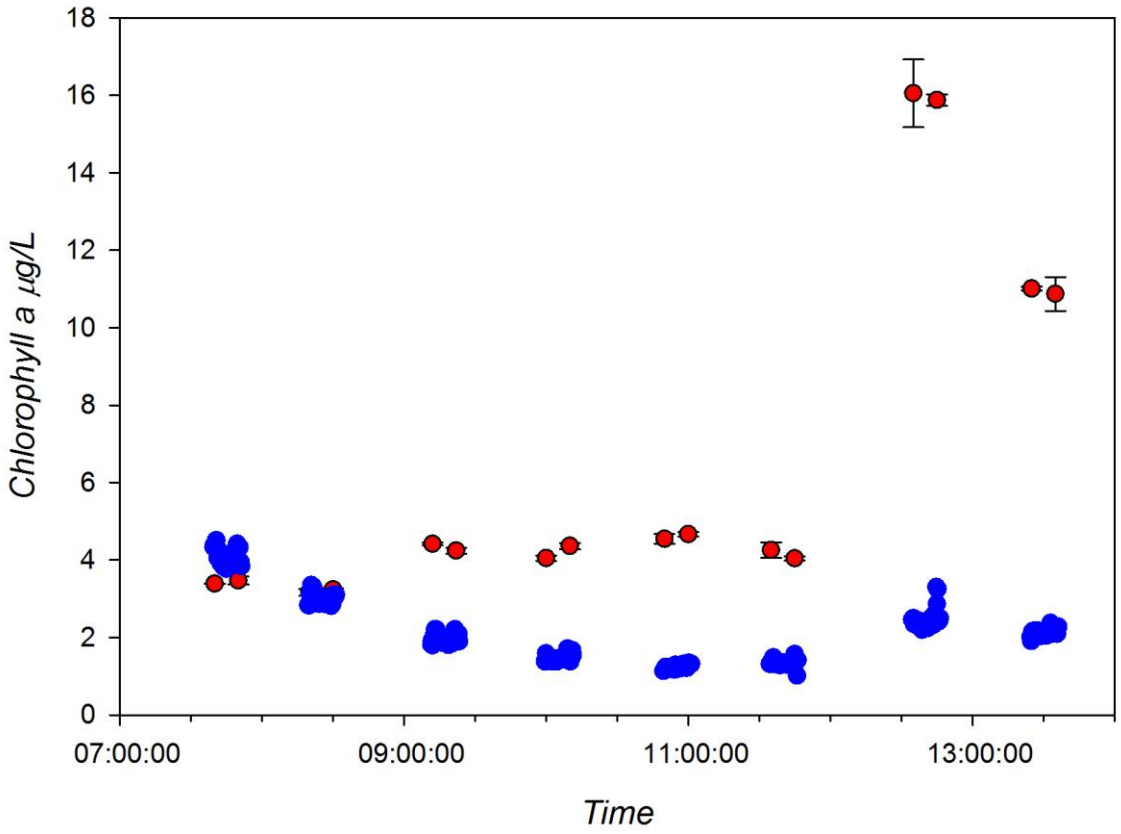


Figure 21. Plot of instrument (blue) and reference (red) measurements of chlorophyll a during San Francisco Bay surface mapping. Two reference measurements were made at each station, the instrument data was averaged over 2 minutes bracketing the reference samples.

A linear regression of the JFE chlorophyll measurements against the extracted chlorophyll (Fig. 22) was not significant (p=0.91) with an R^2=0.001 and a slope of -0.01.

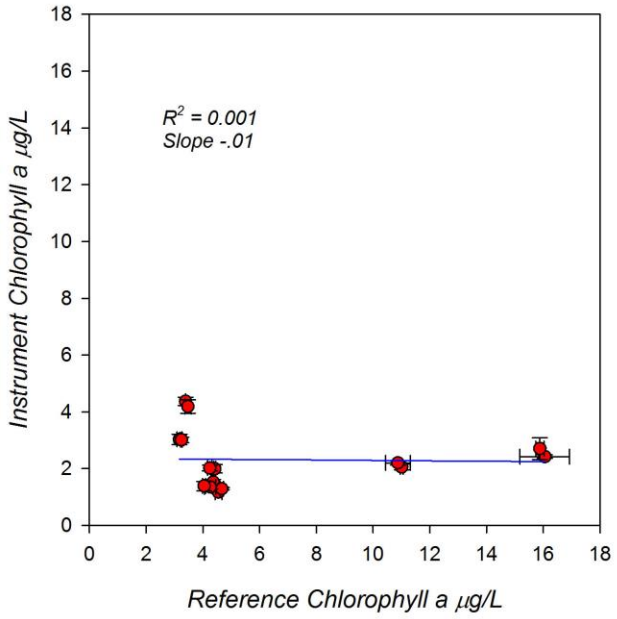


Figure 22. San Francisco Bay surface mapping response plot of the JFE chlorophyll measurements compared to reference chlorophyll measured.

A comparison of algal classification from microscope counts on preserved reference samples from the survey transects relative to the JFE Multi-Exciter chlorophyll excitation spectra for the phytoplankton communities encountered along the San Fransico Bay survey track are shown in figure 23. Microscopic analysis of preserved reference samples indicate a dominance of ciliates in South Bay with diatoms and dinoflagellates dominating in central San Fransico Bay (Stations 1,2,6). The JFE spectral shapes were consistent with a dominance of Chlorophyll c containing algae in these communities (see figure 9 for reference spectra). In general there was a relative increases in the 570 nm excitation contribution in stations were chlorophyte algae were observed microscopically. The high 570:435 signal observed at station 7, in the southern most reaches of the bay are attributable to higher CDOM loads and low chlorophyll biomass in the water. It was not possible to distinguish whether ciliates were non fluorescent or fluorescent due to recent consumption of phytoplankton prey.

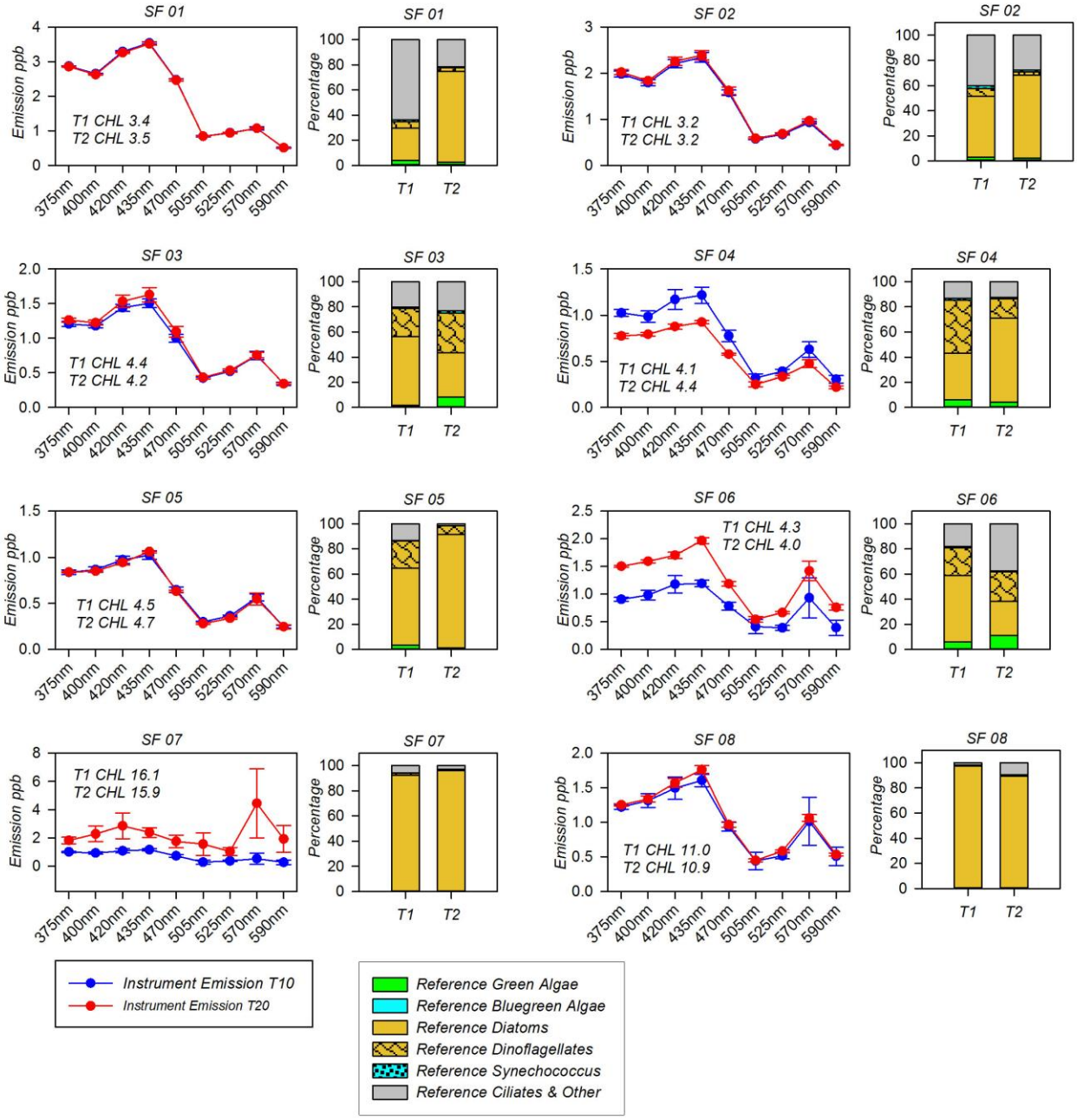


Figure 23. Algal classification from microscope counts on preserved reference samples compared to emission spectra of the JFE during the San Francisco Bay underway mapping survey. Algal counts were grouped at the functional class level and represented as a percentage of biovolume. Each row represents two timepoint replicates of the same batch of algae and matrix conditions.

Monterey Bay, CA

A 75 km surface mapping cruise was undertaken in Monterey Bay, CA on 13 July 2017 using MLML's coastal research vessel the *R/V JH Martin*, to assess instrument performance in near-shore to oceanic water conditions. The *R/V JH Martin* was also equipped with a Headhunter – StingRay continuous flow pump which drew water via a through-hull port near the bow and supplied the vessel's underway data acquisition system and was plumbed into the same exposure tank setup as described above. Flow to the tank was stopped during reference sampling and tank water mixed by the sampling process with samples being taken 10 and 20 min after isolation. The cruise headed out of Moss Landing Harbor, with intial samples taken near the entry to the Elkhorn Slough estuary, continuing WSW along the Monterey Bay Canyon axis to the western, oceanic edge of the bay, then NNW, back onto the shelf toward Santa Cruz, to assess potential impacts from urban and agricultural runoff into the coastal waters (Fig. 23).

A YSI EXO2 sonde in the tank provided continuous monitoring results during both underway and isolated time periods with measurements taken every 15 seconds (Figure 24). Continuous measurements indicated that conditions in the tank during isolation periods were more variable than for the previous survey. Water quality conditions along this sampling transect were in sharp contrast to the SF Bay observations and encompassed higher salinities over a narrow range (33.6 – 33.9 S) and lower temperature waters (14 – 16.5 °C). Reference samples analyses for CDOM (0.03 to 0.11) and turbidity (0.5 to 1.3 NTU) were also lower and less variable. Concentrations of extracted chlorophyll were similar in range (ca 5 – 15 μg/L) to SF Bay and phycocyanin was detected at low but measurable levels (0.05 – 0.3 μg/L) at all stations indicating the presence of small marine cyanobacterial populations.

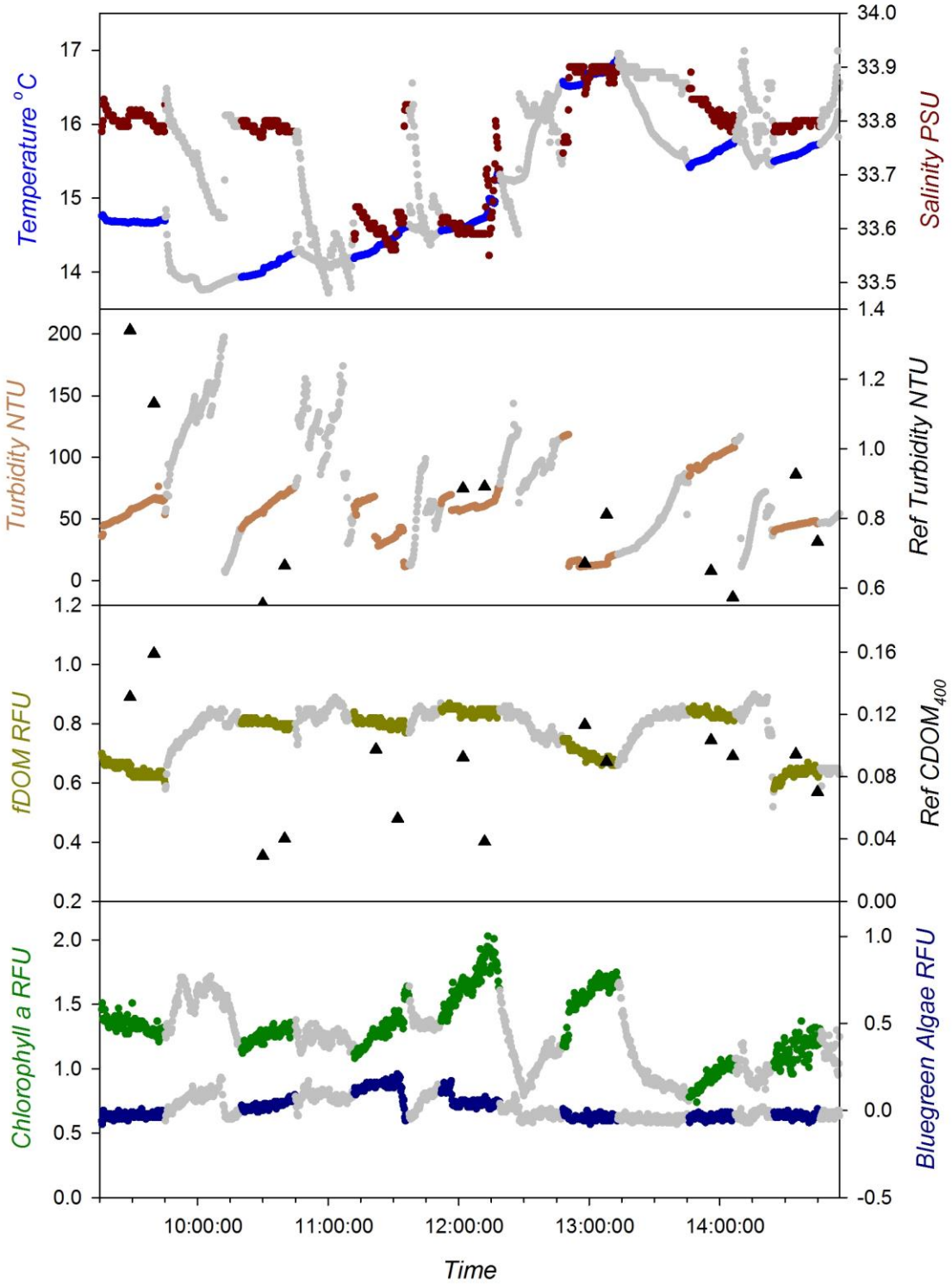


Figure 24. Water conditions encountered during the surface mapping in Monterey Bay *Top Panel:* Variation in temperature (blue) and Conductivity (red) at the depth of the sensors in the flow through tank, measured by an EXO 2 Sonde. *Second Panel:* Variation of turbidity (brown) as measured by the EXO 2 and discrete samples (black triangles) taken from the tank during reference sampling and measured on a HACH 2100AN Turbidimeter. *Third Panel:* fDOM (olive) as measured by the EXO 2,and CDOM measured in discrete samples on an Agilent 8453 spectrometer *Bottom Panel:* Time series of dissolved chlorophyll a (green) and bluegreen algae (blue) as measured by the EXO 2 Sonde.

During the Monterey Bay survey the JFE produced 1418 measurements all of which were considered acceptable values for a successful data return of 100% (Fig. 25). CHL concentrations determined from reference samples ranged from 4.8 to 14.7 µg/L over the entire survey. The range in extracted phycocyanin from reference samples was only 0.1 to 0.3 µg/L and did not represent a good environment to evaluate fluorescence response to Cyanobacteria. Other water quality conditions and descriptions of algal classifications are described below.

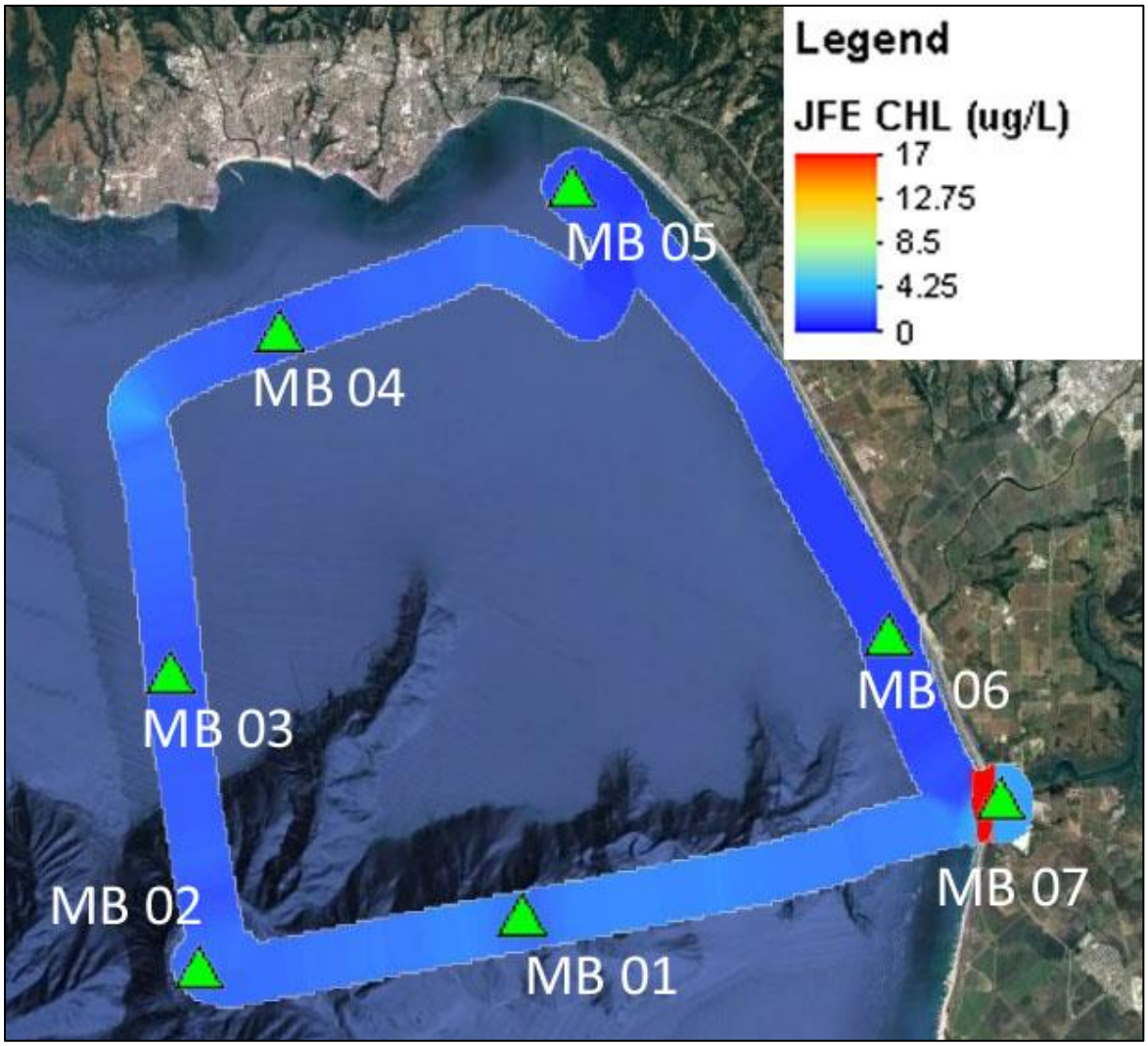


Figure 25. JFE continuous underway chlorophyll data during the surface mapping cruise in Monterey Bay. Triangles denote stations where the flow-through tank was isolated and comparative reference samples analyzed.

A time series of the JFE CHL measurements are plotted against the corresponding reference measurements for the isolated exposure stations (Figure 26). For the isolated exposures, JFE CHL measurements ranged from ca. 2 to 4 µg/L compared to a range of 4.7 to 14.7 µg/L for the reference data. It was unclear why there was high variability in reference sample CHL at station 2 and 7 which does affect the ability to accurately assess the response. The low overall response by the JFE and variable reference values resulting in a non-significant linear regression for instrument versus reference CHL estimation ($p=0.38$) with an $R^2=0.06$ and a slope of -0.05 (Figure 27).

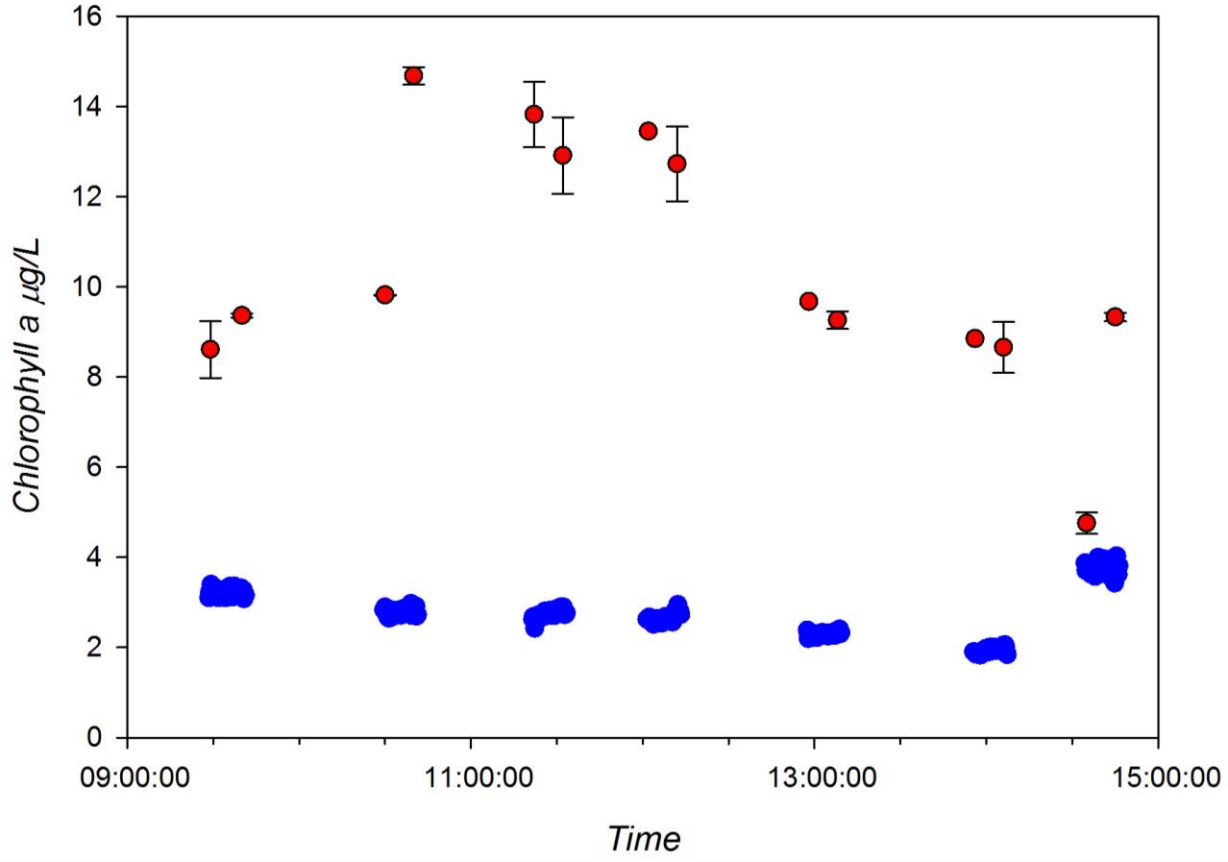


Figure 26. Plot of instrument (blue) and reference (red) measurements of chlorophyll a during Monterey Bay surface mapping. Two reference measurements were made at each station level while instrument measurements were averaged over 2 minutes bracketing the reference sample.

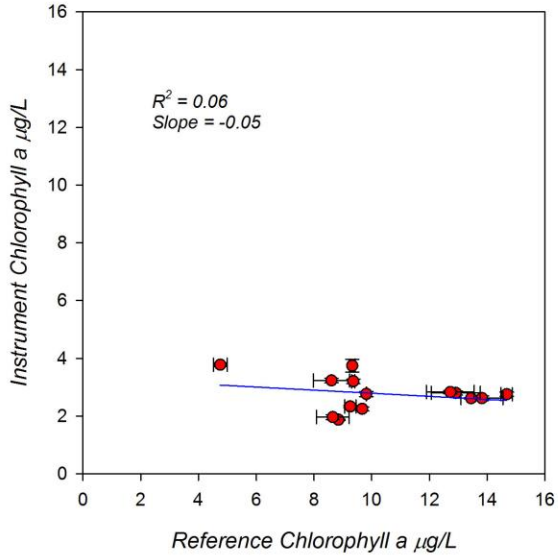


Figure 27. Monterey Bay surface mapping response plot of the JFE chlorophyll a measurements compared to reference chlorophyll a measured in µg/L.

A comparison of algal classification from microscope counts on preserved reference samples from the survey transects relative to the JFE Multi-Exciter spectra for the phytoplankton communities encountered along the Monterey Bay survey track are shown in figure 28. Microscopic analysis of preserved reference samples indicate that surface waters in this region where generally dominated by chlorophyll c containing diatoms and dinoflagellates. Present throughout the survey at lower abundance were ciliate grazers and nannoflagellates of unknown pigment composition along with chlorophyll b containing euglenoid cell types. The JFE chlorophyll excitation spectra broadly captured the photosynthetic phytoplankton classifications being dominated by a diatom/dinoflagellate signature with a declining linear shoulder being prevalent in dinoflagellate dominated waters. The 570nm excitation again was responsive to presence of chlorophytes above 10% biovolume. It was not possible to distinguish whether ciliates were non fluorescent or fluorescent due to recent consumption of phytoplankton prey.

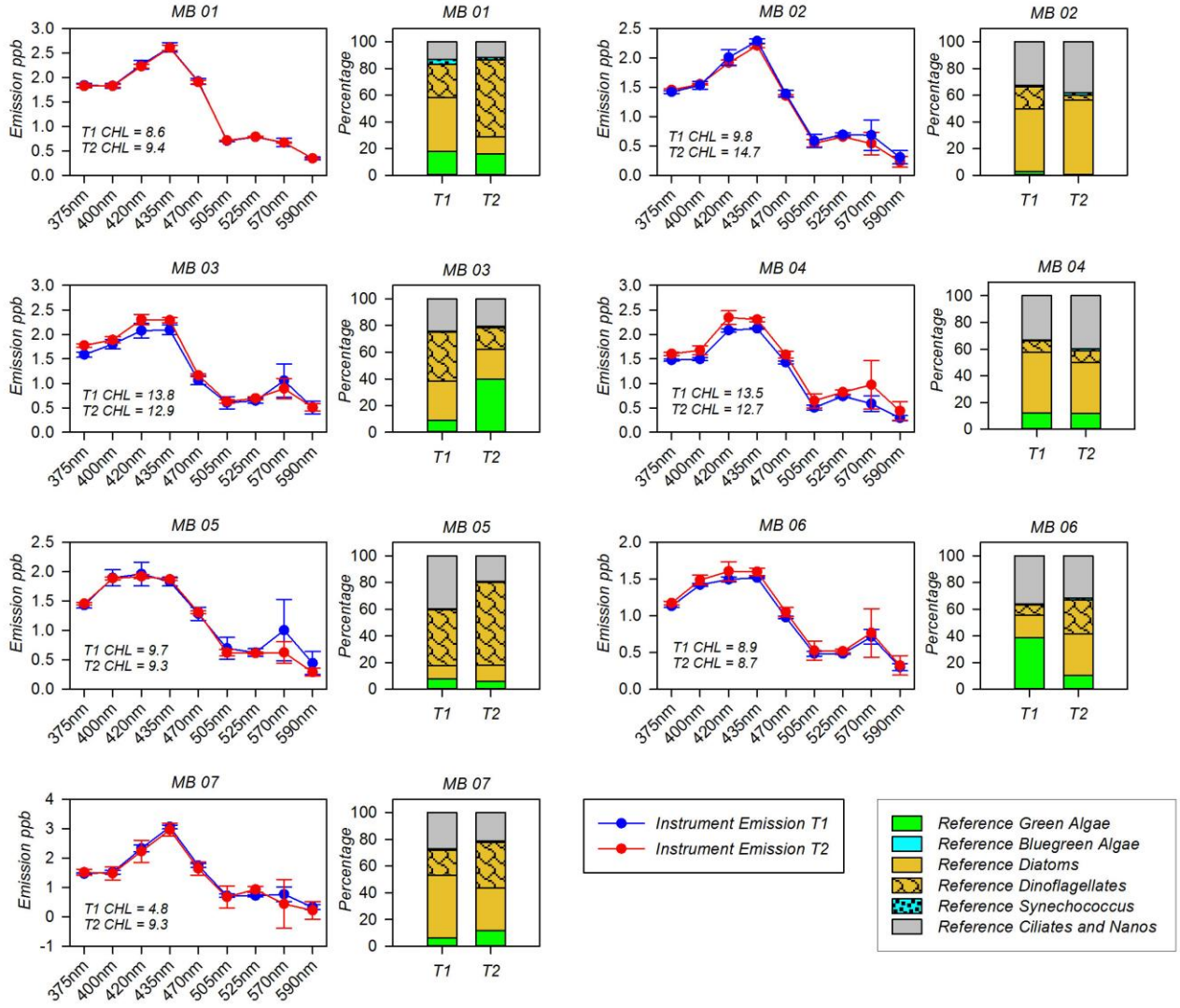
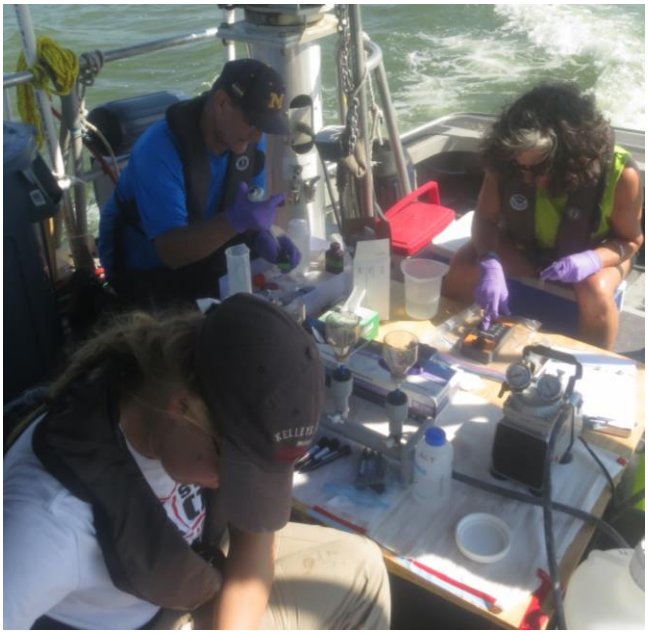


Figure 28. Algal classification from microscope counts on preserved reference samples compared to emission spectra of the JFE during the Monterey Bay underway mapping survey. Algal counts were grouped at the functional class level and represented as a percentage of biovolume. Each row represents two timepoint replicates of the same batch of algae and matrix conditions.

Lake Erie Surface Mapping

The western Lake Erie surface mapping cruise was conducted on August 16th on board the NOAA GLERL *R/V4108* (*photo below*) and covered a 75 km range including sites near the mouth of the Maumee River out to open waters 20 km offshore. The survey occurred during an intense *Microcystis* cyanobacterial bloom. During the underway mapping survey, seven stations were selected to make comparative reference sample measurements. At each selected station, water in the tank was isolated for a period of 25 minutes, and kept well mixed with manually stirring. After an initial equilibration, reference samples were taken at timepoints of 5 and 20 minutes from the point of isolation.



A YSI EXO2 sonde in the tank provided continuous monitoring results during both underway and isolated time periods with measurements taken every 15 seconds (Figure 29). Continuous measurements indicated that conditions in the tank during isolation periods were more variable for stations with high amounts of cyanobacteria. This result likely reflects their highly buoyant nature and clumpiness of large colonies. Reference samples analyses for CDOM and turbidity are plotted over the sonde data for consistency with other tests. During the western Lake Erie cruise temperature ranged from 24 to 25.2 ºC and specific conductivity ranged from 260 to 370 µS/cm. Reference sample analysis showed that CHL ranged from 11 to 833 µg/L, PC ranged from 0.9 to 705 µg/L, turbidity ranged from 2.4 to 141 NTU, and CDOM ranged from 0.5 to 1.7.

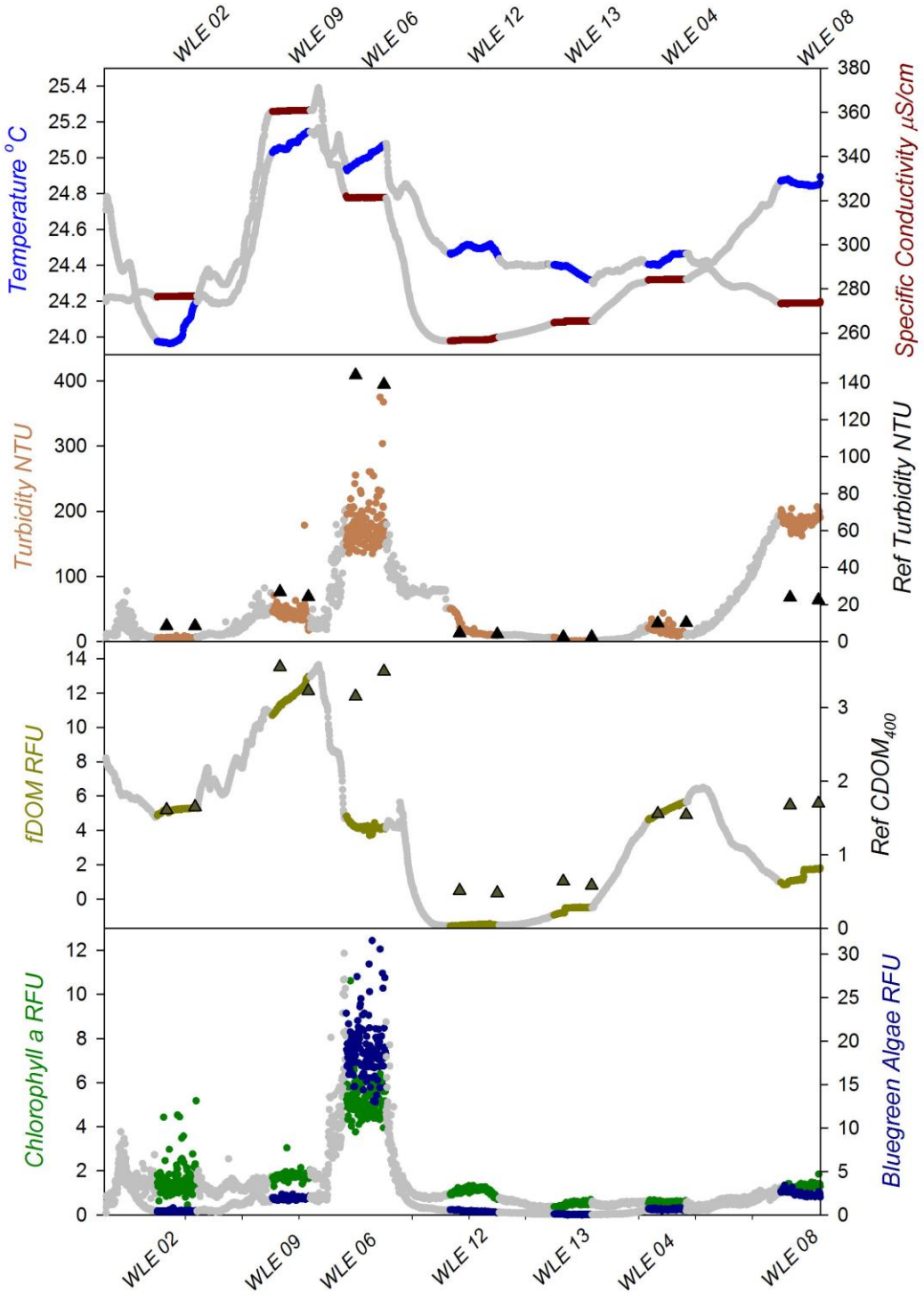


Figure 29. Water conditions encountered during the surface mapping in western Lake Erie. *Top Panel:* Variation in temperature (blue) and conductivity (red) at the depth of the sensors in the flow through tank, measured by an EXO 2 Sonde. *Second Panel:* Variation of turbidity (brown) as measured by the EXO 2 and discrete samples (black triangles) taken from the tank during reference sampling and measured on a HACH 2100AN Turbidimeter. *Third Panel:* fDOM (olive) as measured by the EXO 2,and CDOM measured in discrete samples on an Agilent 8453 spectrometer. *Bottom Panel:* Time series of dissolved chlorophyll a (green) and bluegreen algae (blue) as measured by the EXO 2 Sonde.

During the survey the JFE produced 2076 readings all of which were considered acceptable values for a successful data return of 100% (Fig. 30). CHL estimations from the JFE ranged from 1.5 to 24 µg/L over the entire survey compared to a range of 11 to 833 µg/L for the extracted chlorophyll reference samples. The range in extracted phycocyanin from reference samples was 0.8 to 705 µg/L. Algal classifications during the survey are described below.

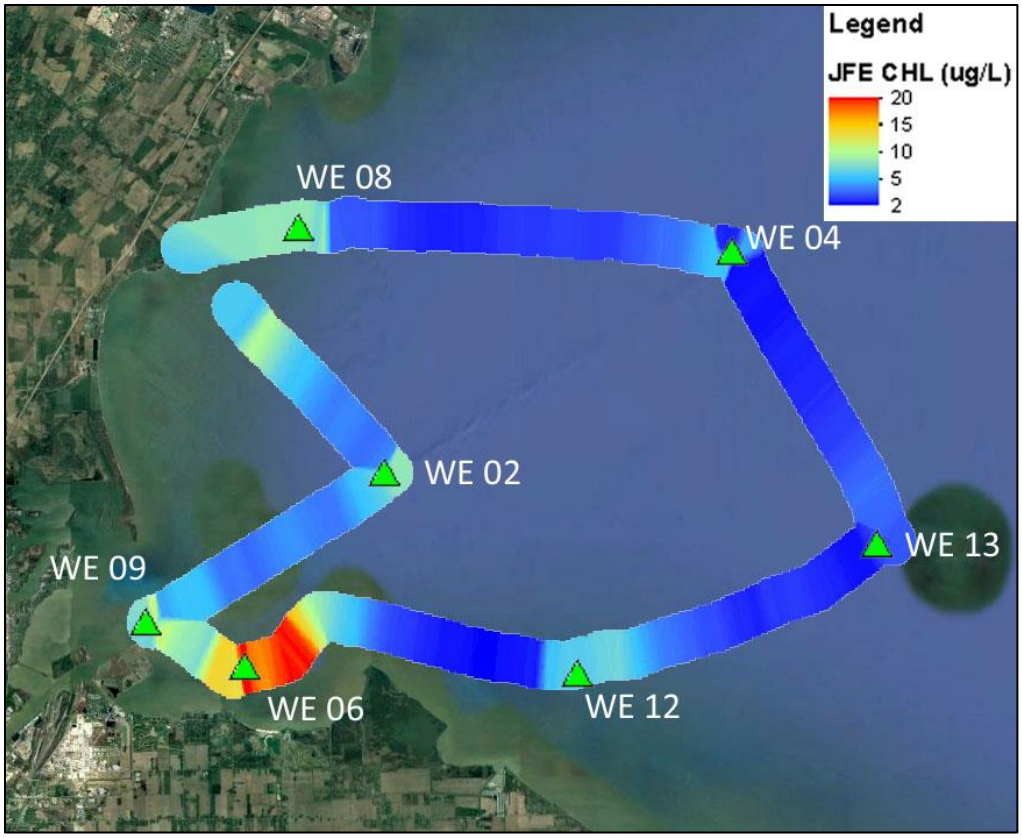


Figure 30. JFE continuous underway chlorophyll data during the surface mapping cruise in western Lake Erie. Triangles denote stations where the flow-through tank was isolated and comparative reference samples analyzed.

A time series of the JFE and corresponding reference sample chlorophyll measurements for the isolated exposures is plotted in figure 31. CHL measurements for the JFE during the isolated sampling periods ranged from 2.5 to 18.7 µg/L and were significantly lower than the observed reference sample concentrations which averaged from 11 to 793 µg/L over the isolation timepoints. Pigment concentrations at station WLE06 were clearly out of range for any meaningful in situ fluorescence measurement.

A one-to-one cross plot of the JFE versus reference sample measurements, with data from WLE06 ommitted, is shown in figure 32. The linear regression was significant ($p=0.008$) with an $R^2=0.52$, but with a slope of only 0.050. The much lower instrument response per unit of extracted chlorophyll was not unexpected based on the calibration set-up and further impacted by large *Microcystis* colonies that are known to produce very low fluorescent responses.

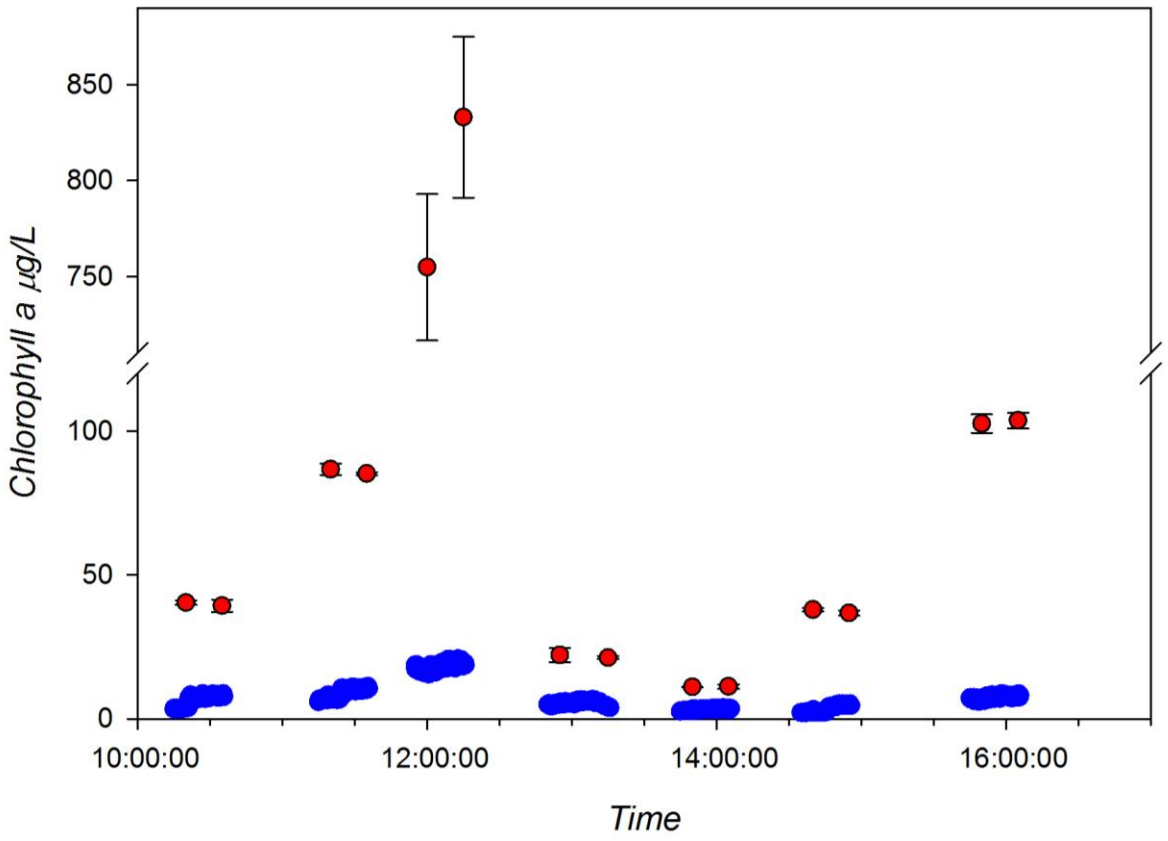


Figure 31. Time series plot of the JFE chlorophyll (blue) and reference (red) during surface mapping deployment on Lake Erie. Two reference measurements were made at each station, instrument data was averaged over 2 minutes bracketing the reference sample time.

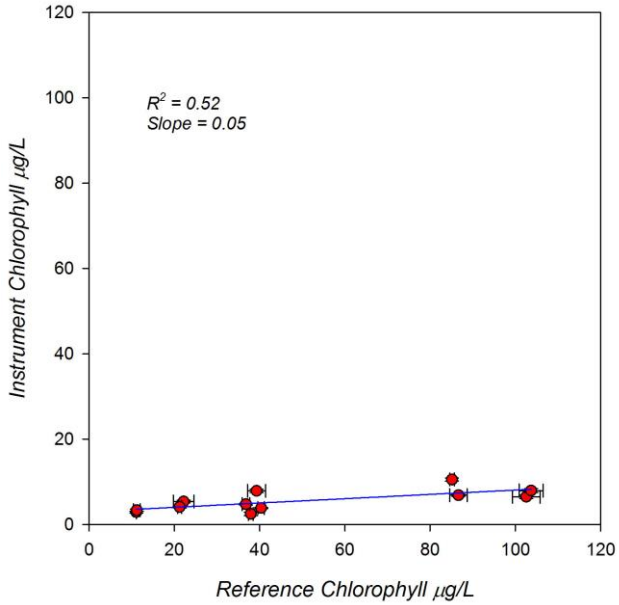


Figure 32. Lake Erie surface mapping response plot for the JFE chlorophyll measurements compared to reference chlorophyll a.

A comparison of algal classification from microscope counts on preserved reference samples against the JFE emission spectra for the Lake Erie underway surface mapping is shown in figure 33. There was substantial variance among the timepoint replicates at four of the seven stations despite the constant manual mixing. We note that *Microcystis* colonies were extremely buoyant and difficult to keep evenly distributed. Emission spectra clearly responded to variations in the amount of cyanobacteria present as noted by the relative strength of the 570 nm emission compared to emissions between 420 and 470 nm. At stations where diatoms dominated the biovolume (WE2, WE12, WE13) peak emissions occurred at 420 and 435, but the presence of cyanobacteria was still clearly distinguishable at the higher wavelengths.

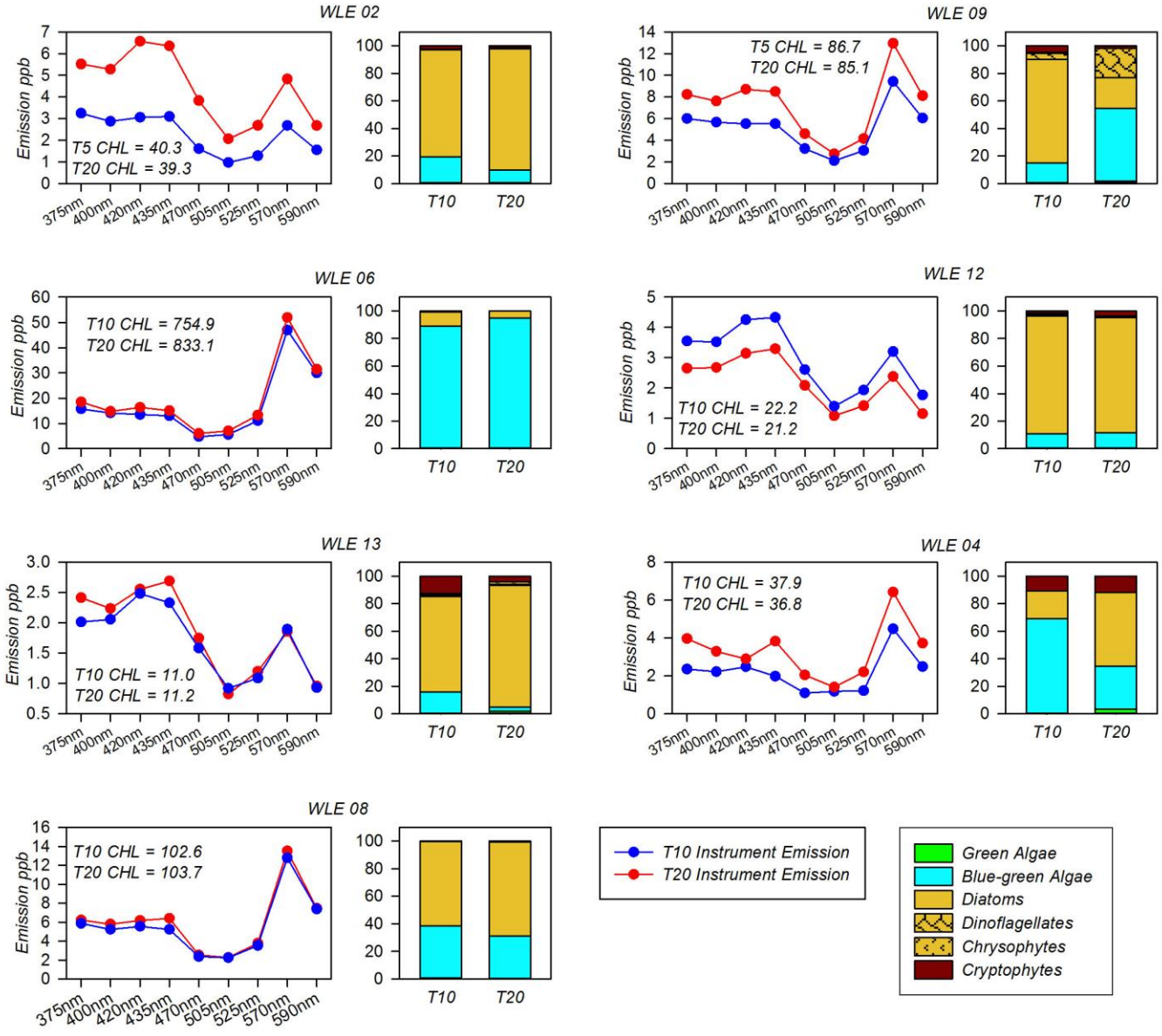


Figure 33. Reference cell counts as percentage of biomass compared to instrument algae categories as percentage of total chlorophyll a.

Field Deployment at Maumee River, Waterville, Ohio

A 13 day field deployment in the Maumee River occurred from July 25 through August 7, at the facilities of the Bowling Green, Ohio Water Treatment Plant (Figure 34). The deployment site was located at 41.48° N, 83.74° W, in a flow-through tank located in the water treatment plant pump house. The pump house is located above the Maumee, approximately 200 m up river from the water treatment intake and approximately 35 km from the Maumee outflow into Lake Erie. River water was continuously pumped into a 180 gallon test tank where it was mixed using a shaft propeller. The residence time in the tank was approximately 10 minutes. For comparative reference samples the flow was isolated and mixed for 5 minutes prior to an instrument measurement and reference grab sample.

Figure 34. Aerial view of the Maumee River and Bowling Green Water Treatment plant (left) and the flow-through deployment tank servicing the supply of river water to the test instruments (right).

During the Maumee River moored deployment test the JFE collected 623 observations all of which were accepted values for a data return of 100% of the planned test. Time series results of ambient conditions for temperature, specific conductivity, turbidity, fDOM, chlorophyll and bluegreen algae measured by the YSI EXO2 are given in figure 35. During the deployment, temperature ranged from 23.1 – 29.4ºC and discharge covered a 5-fold range from 2,000 to 10,000 cfs. Reference sample turbidity and $CDOM_{400}$ are overlaid for better comparison across tests, with turbidity ranging from 21.6 to 78.3 NTU and CDOM absorbance ranging from 4.5 to 5.6. The continuous sonde data indicated a 10-fold range in chlorophyll and phycocyanin fluorescence over the deployment with noticeable patterns across diurnal cycles and river discharge cycles.

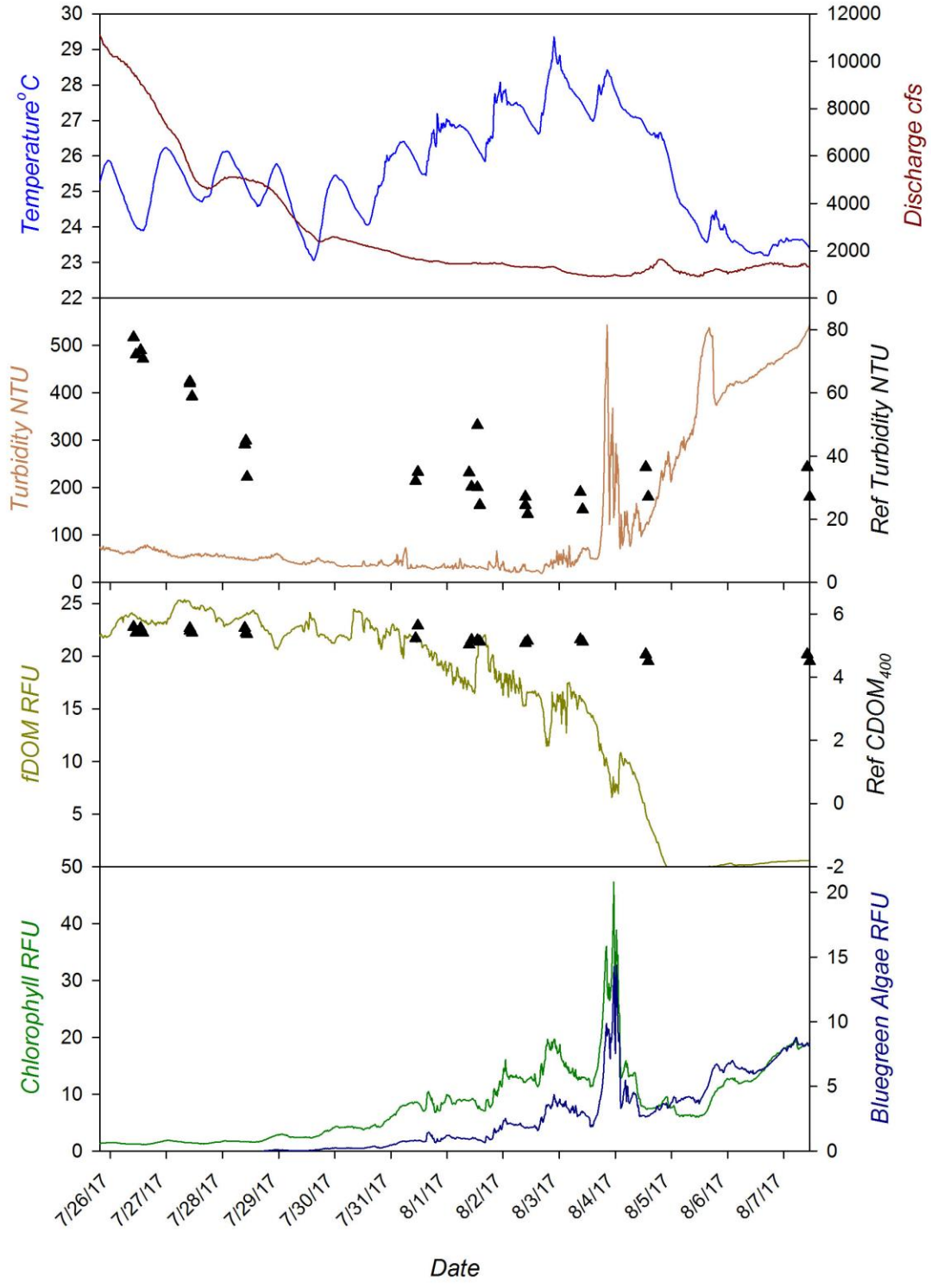


Figure 35. Environmental conditions encountered during the 12 day freshwater deployment in the Maumee River at Waterville, OH. *Top Panel:* Variation in temperature (blue) and conductivity (red) at the depth of the sensors, measured by an EXO 2 Sonde. *Second and Third Panels:* Variation of turbidity (brown) and fDOM (olive) at the depth of the sensors, measured by an EXO2 Sonde and CDOM measured in discrete samples on an Agilent 8453 spectrometer. *Bottom Panel:* Time series of dissolved oxygen (blue) and chlorophyll (green) as measured by the EXO 2 Sonde.

A time series of the JFE CHL measurements is plotted against the corresponding reference measurements for the Maumee River deployment in figure 36. Chlorophyll measurements for the JFE ranged from 4.0 to 62 µg/L during the deployment, while CHL from corresponding reference samples ranged from 9.5 to 119 µg/L.

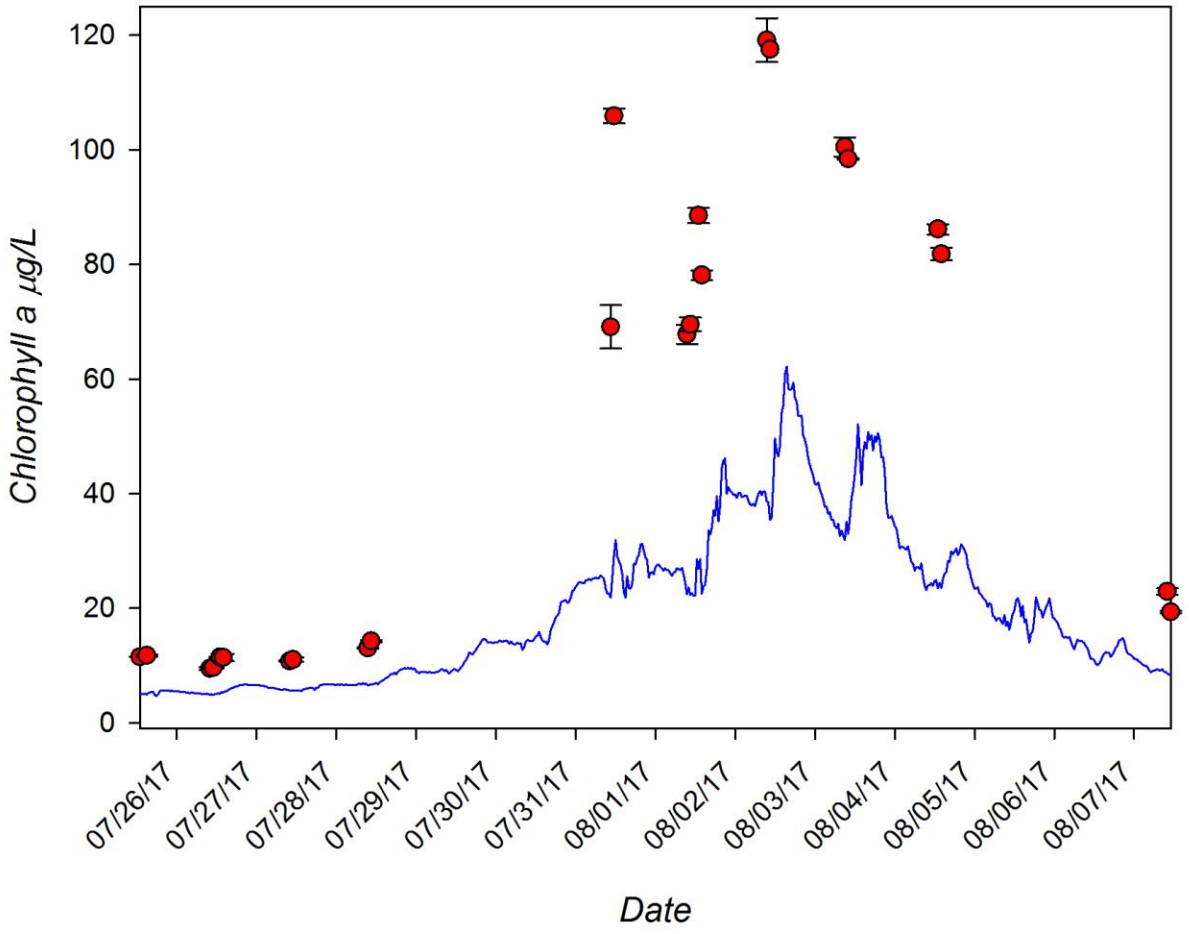


Figure 36. Time series plot of the JFE measurements (blue) and reference measurements (red) of chlorophyll a during the freshwater deployment in the Maumee River at Waterville, OH. Water samples were typically collected 1 hour apart, with either two or four samples on a given day.

A one-to-one cross plot of the JFE measurements versus reference sample measurements is shown in figure 37. The linear regression of the paired data was highly significant ($p<0.001$) with an $R^2=0.84$ and a slope of 0.32.

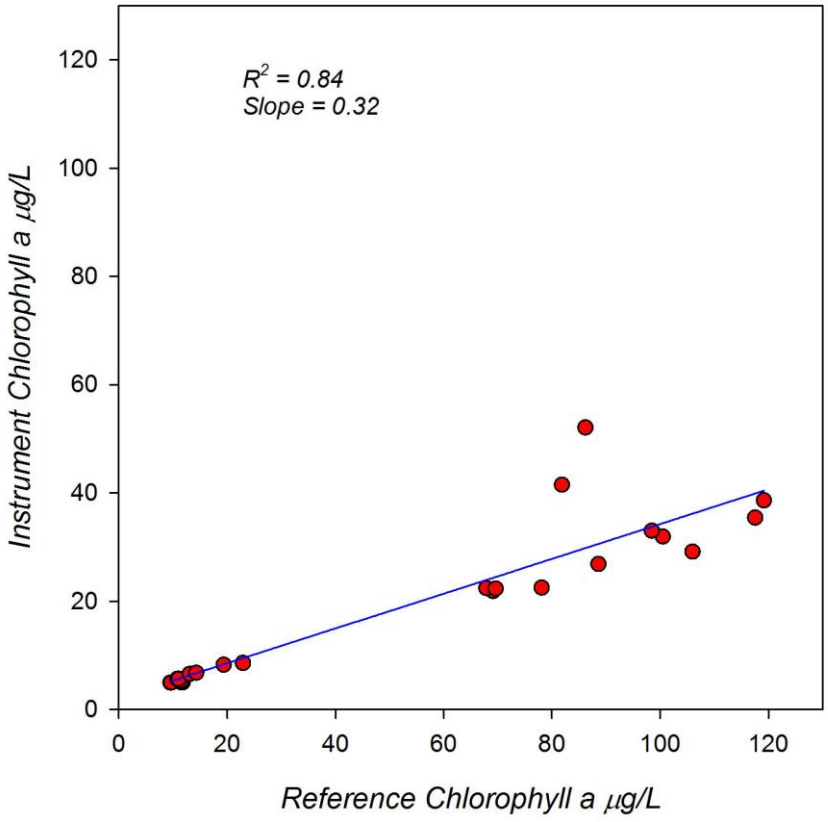


Figure 37. Cross plot of JFE and reference sample chlorophyll measurements during the Maumee River field deployment and resulting linear regression.

A comparison of algal classification from microscope counts on preserved reference samples against the JFE emission spectra for the Maumee River field test is shown in figure 38. As was seen in individual species Lab testing, the prescence of either Cyanobacteria or Cryptophytes produced measureable increases in the 570 nm emission. Similarly the prescence of Chlorophytes produced measureable increases in the 420 and 435 nm emission. It was not possible to compute specific proportions of algal classifications from the emission spectra for this test, but patterns and signal strength clearly reflected differences in abundance and composition.

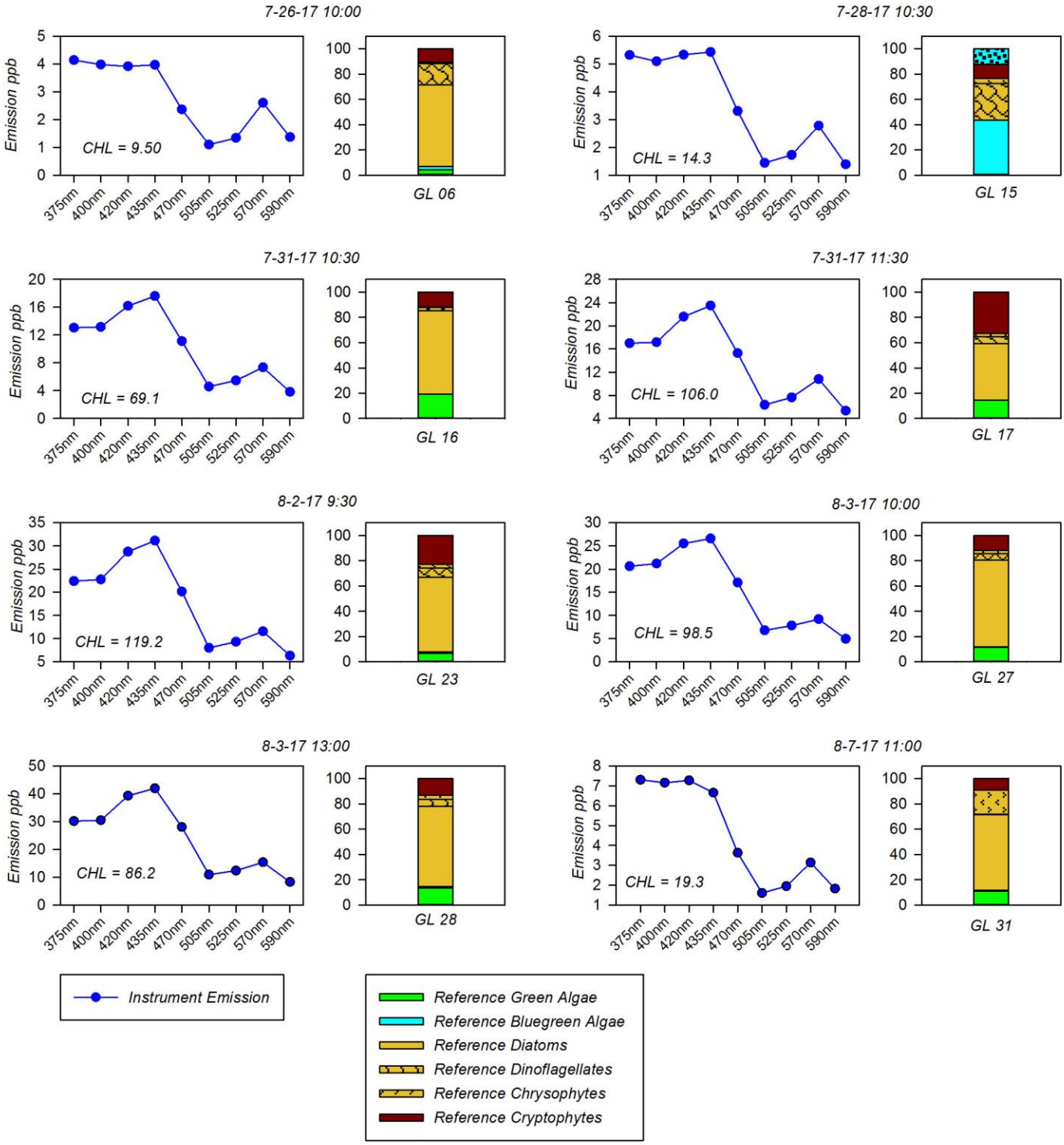


Figure 38. Reference cell counts as percentage of biomass compared to instrument algae categories as percentage of total CHL estimated by the JFE for the Maumee River field deployment test.

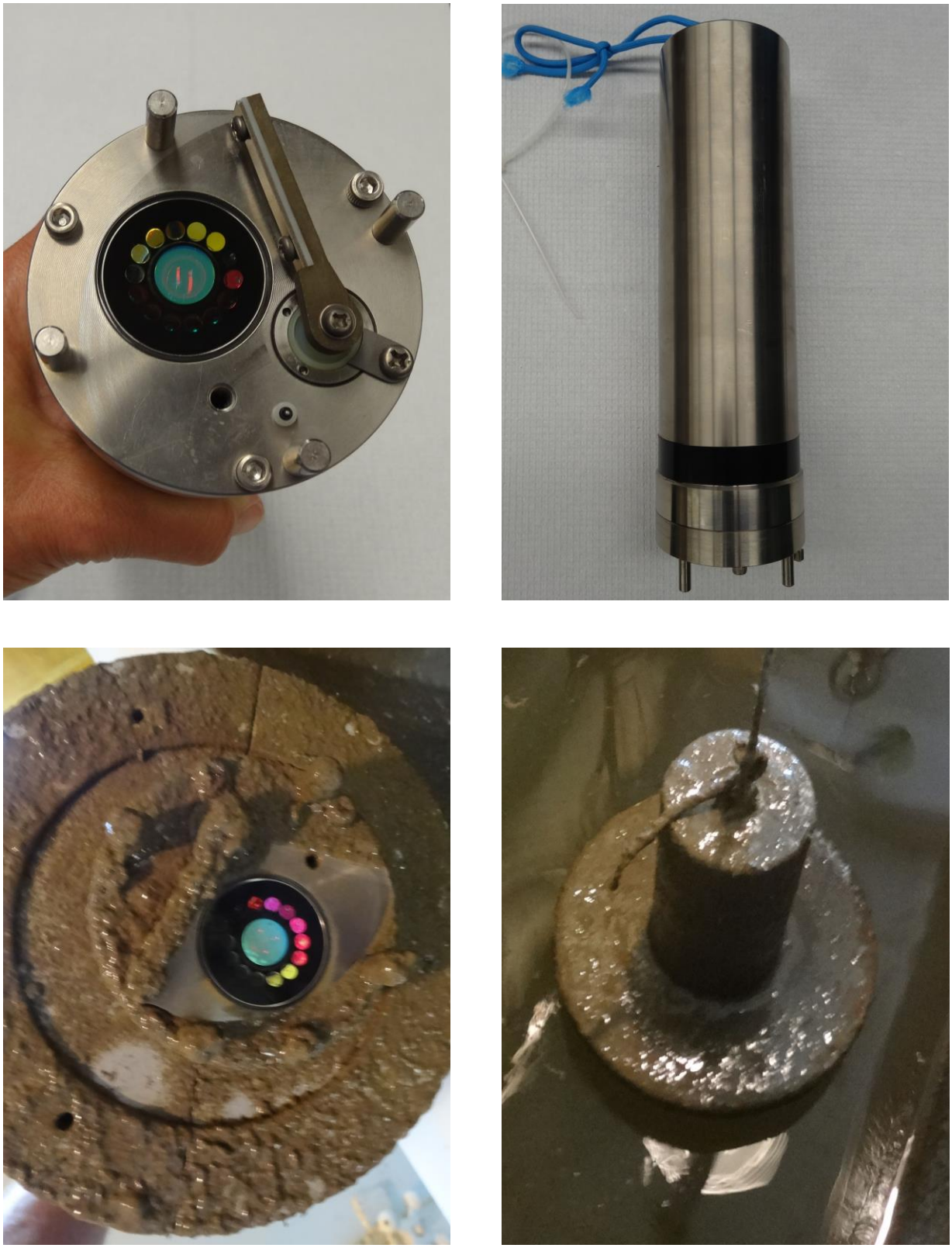


Figure 39. Instrument photographs prior to deployment (top) and upon retrieval (bottom).

Field Deployment at Chesapeake Biological Laboratory (CBL)

A 28 day moored field test was conducted in Chesapeake Bay from September 6 to October 3, 2017. The deployment was located at 38.32°N, 76.45°W attached to the side of a floating pier at the mouth of the Patuxent River within Chesapeake Bay (Figure 40) The site was brackish with an average water depth of 2.2 m at the test site.

Figure 40. Aerial view of CBL deployment site (*left*) and instrument deployment rack located next to CBL dock (*right*).

The JFE operated for the entire deployment, collecting 1293 accepted observations for a data return of 100% of the planned test. Continuous monitoring of ambient conditions for temperature, salinity, turbidity, fDOM, chlorophyll and bluegreen algae measured by an EXO 2 sonde at 15 minute intervals are given in figure 41. During the deployment, temperature ranged from 22.4 to 26.1°C and salinity from 8.1 to 13.2 PSU. Reference sample turbidity and $CDOM_{400}$ are overlaid for better comparison across tests, with turbidity ranging narrowly from 0.6 to 1.5 NTU and CDOM absorbance ranging from 0.9 to 1.5. The continuous sonde data indicated a roughly 5-fold range in chlorophyll and phycocyanin over the deployment with very strong diurnal cycles and a small overall decline in phycocyanin as salinity decreased.

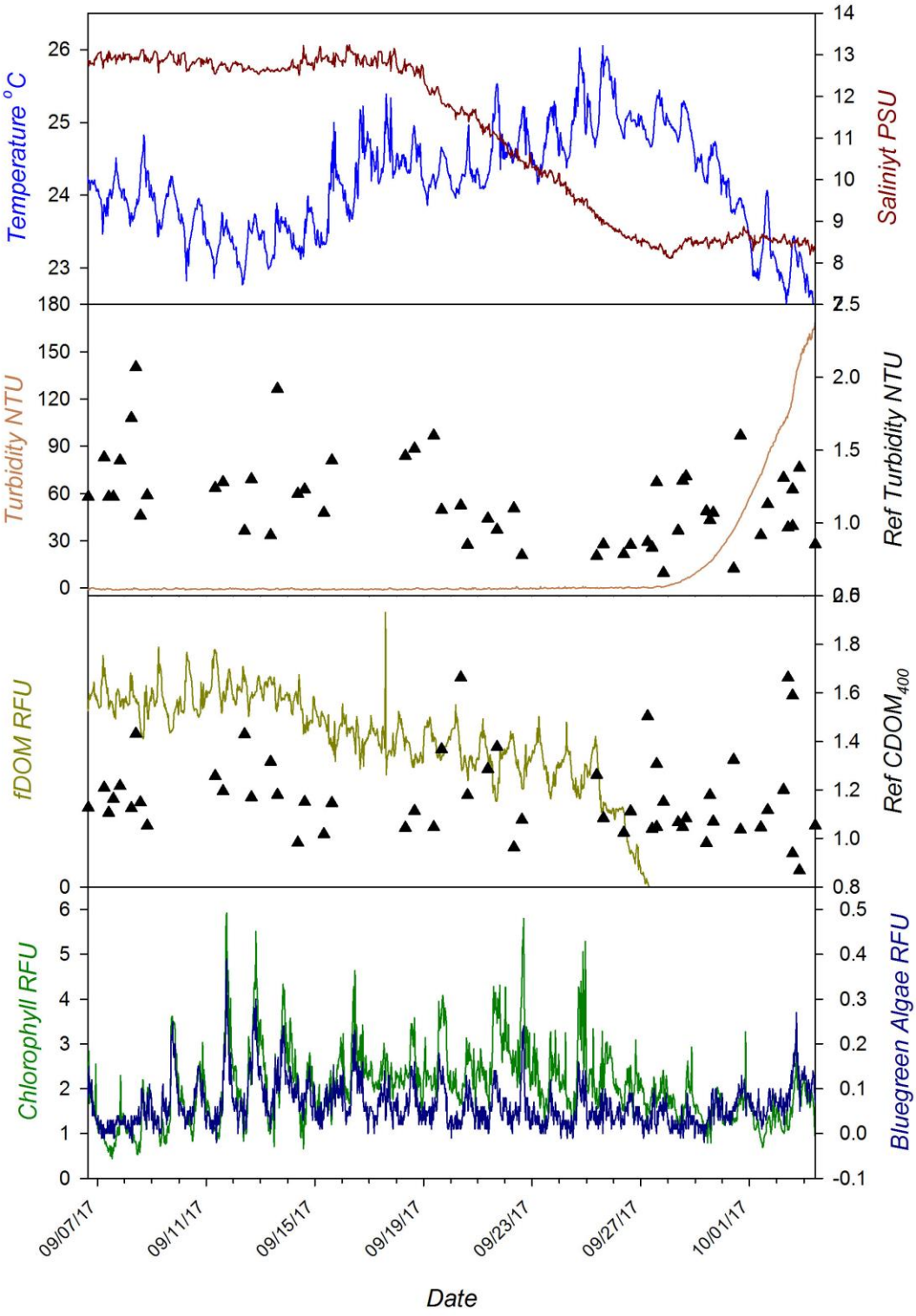


Figure 41. Environmental conditions encountered during the 28 day CBL moored deployment. *Top Panel:* Variation in temperature (green) and salinity (red) at depth of instrument sensor detected by an EXO2 sonde. *Second Panel*: Variation in turbidity (brown) as measured by the EXO 2 sonde and discrete samples measured on a HACH 2100AN (black.) *Third Panel:* fDOM (dark yellow) as measured by the EXO 2 and $CDOM_{400}$ measured on an Agilent 8453 spectrometer. *Bottom Panel:* Chlorophyll (green) and bluegreen algae (blue).

A time series of the JFE measurements of chlorophyll are plotted against the corresponding reference measurements in figure 42. Chlorophyll measurements for the JFE ranged from 0.13 to 12.7 µg/L compared to the range in reference samples of 7.4 to 21.7 µg/L for the entire test period. Phycocyanin concentrations from reference samples ranges from 0.6 to 5.5 µg/L and it should be noted that this was the only site where we also saw measureable levels of phycoerithythrin in the reference samples which concentrations ranging from 0 to 3.4 µg/L (data not plotted). The JFE showed a diminished diurnal range and diminished magnitude of response after the first week and it is likely that the wiping system did not completely prevent the impact of biofouling (see instrument photos, Fig. 45,). The ratio of instrument CHL to extracted CHL declined from nearly 70% at the beginning of the deployment to less than 15% at the final week.

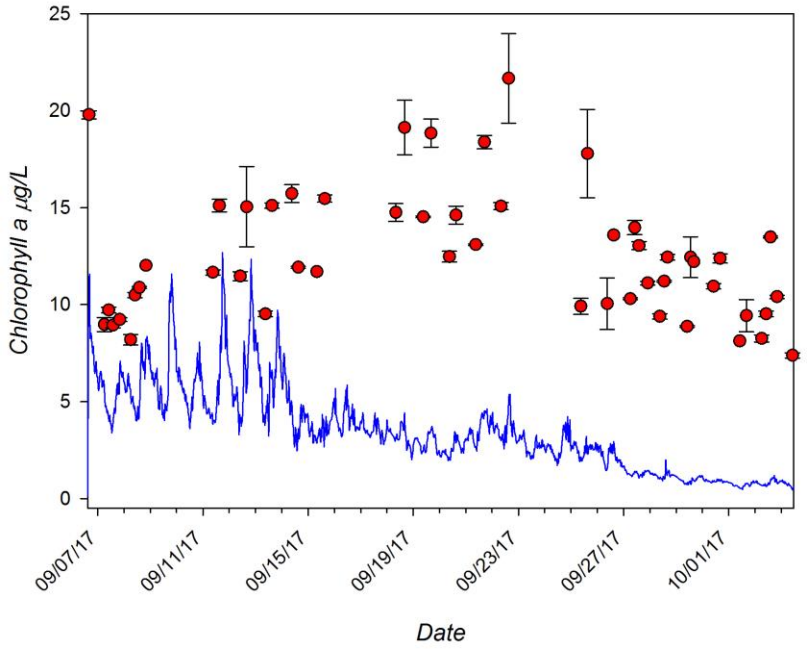


Figure 42. Time series plot of the JFE (blue) and reference measurements (red) of chlorophyll a during the CBL moored deployment in Solomons, MD.

A one-to-one cross plot of the JFE versus reference sample measurements of chlorophyll for the Chesapeake Bay field test is shown in figure 43. Despite rather high variability, a linear regression of the data was significant ($p=0.0045$) but with an $R^2=0.15$ and a slope of 0.25. Again biofouling appears to have impacted the response over time and this regression does not represent a full characterization of the potential response in this environment. For example, the regression over the first 8 days of the deployment show an improved relationship with an $R^2=0.42$ and a slope of 0.34.

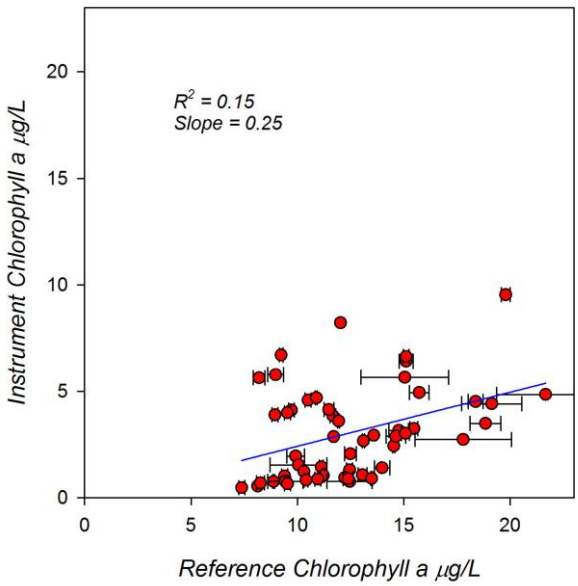


Figure 43. Cross plot of JFE and reference sample measurements during the CBL field deployment.

A comparison of algal classification from microscope counts on preserved reference samples against the JFE emission spectra for the Chesapeake Bay field test is shown in figure 44. The prescence of the cyanobacterial species, *Synechococcus* produced measureable increases in the 570 nm emission. It was more difficult to discern consistent differences in the pattern of the emission spectra based on the relative proportion of Diatoms versus Dinoflagellates. Both groups caused measureable increases in emission at lower wavelengths, most notably at 375 nm. Under the current set-up of the instrument, it is impossible to compute specific proportions of algal classifications from the emission spectra, but patterns and signal strength are reflecting differences in abundance and composition.

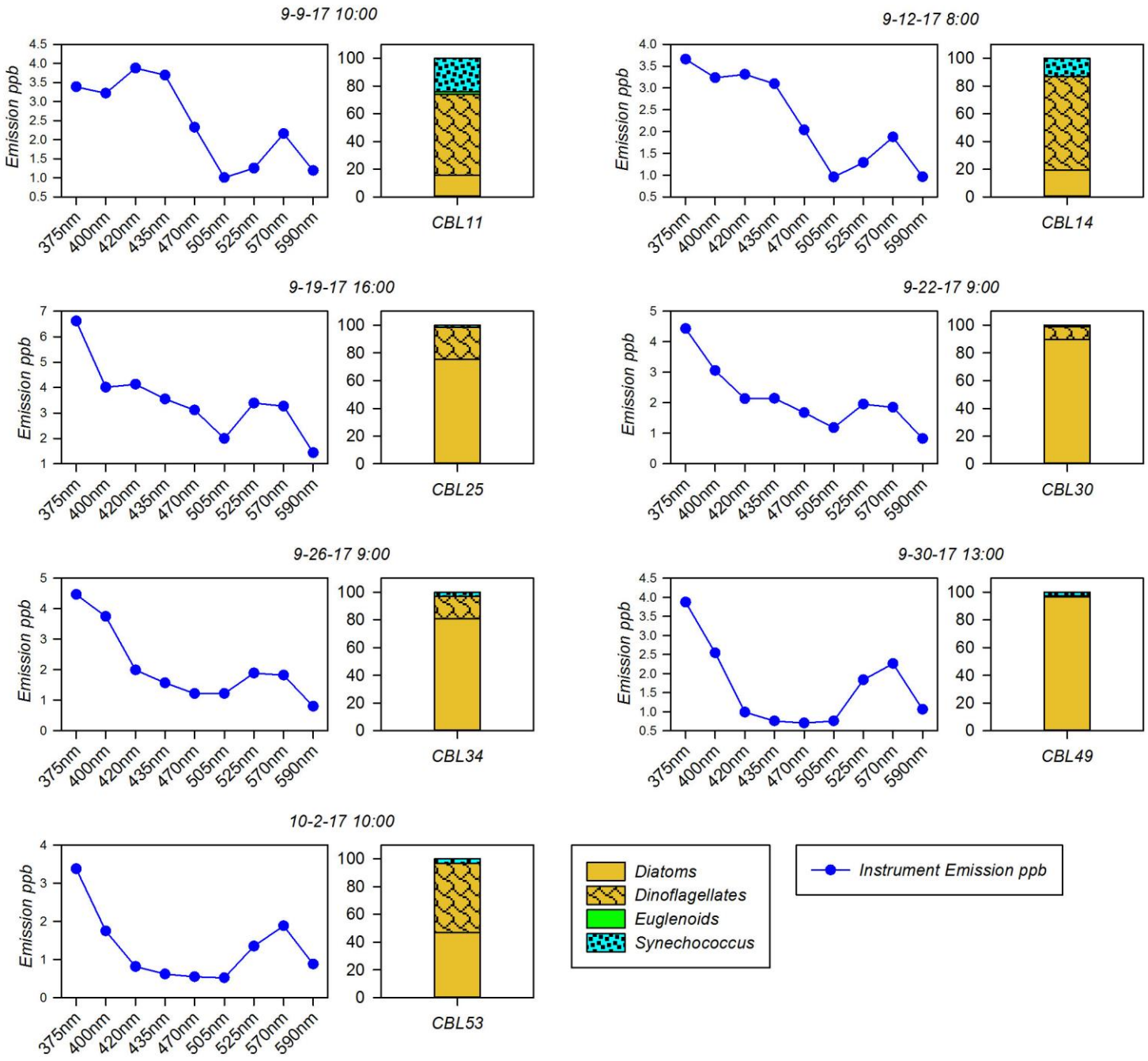


Figure 44. Reference cell counts as percentage of biomass compared to emission measured by the JFE for the Chesapeake Bay field deployment test.

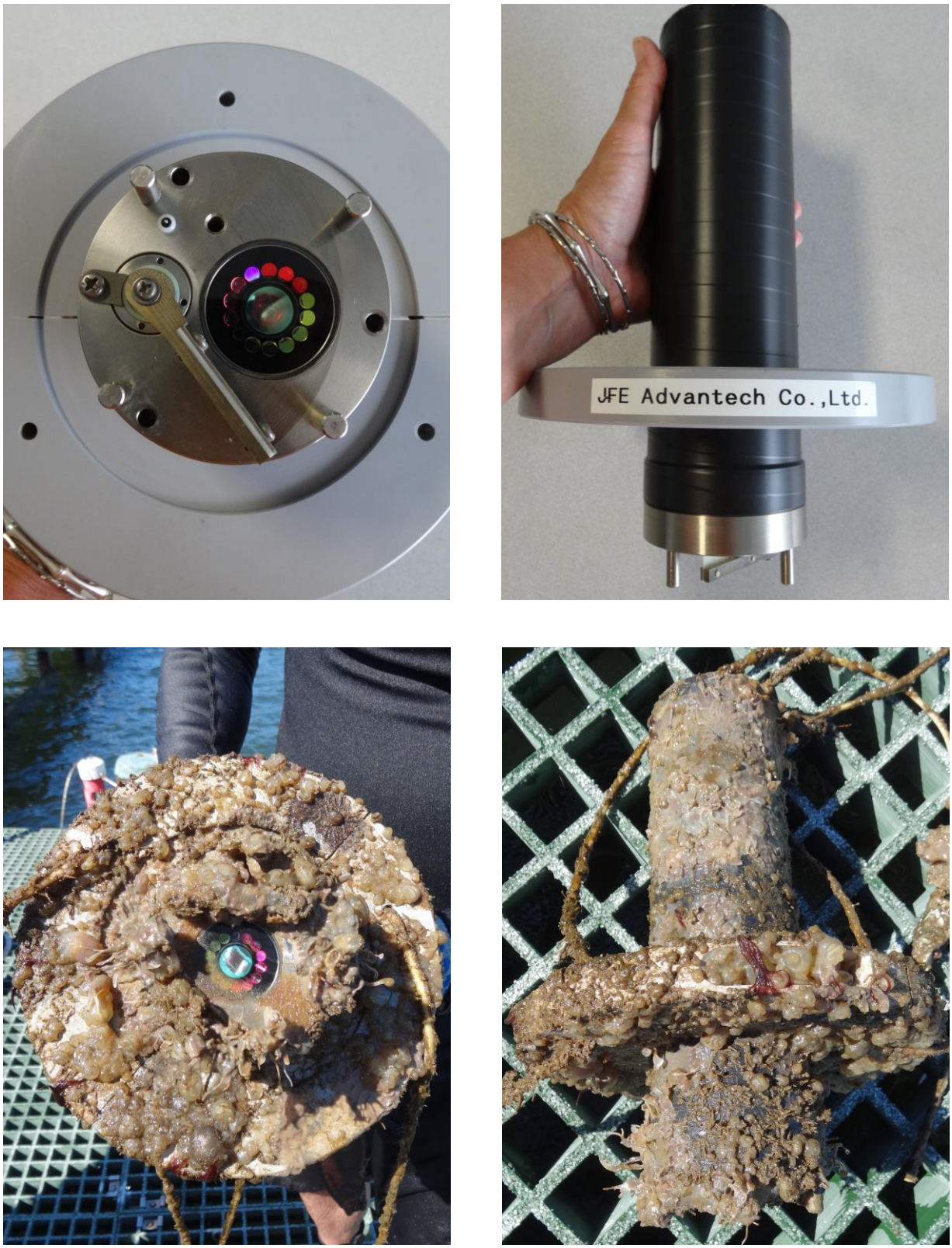


Figure 45. Instrument photographs prior to deployment (*top*) and upon retrieval (*bottom*).

GLOBAL RESPONSE

A one-to-one cross plot of JFE versus reference chlorophyll measurements for all field tests were combined into a single plot (Figure 46). Data from each field test are color coded so that the variance in fluorescence response across different environments and phytoplankton communites can be observed. With the exception of the Lake Erie surface mapping test, the JFE response for CHL estimation was quite consistent across the diverse range of environments and concentrations. A linear regression against extracted CHL with the Lake Erie data excluded revealed a significant relationship ($p<0.001$ and $R^2=0.88$) with a slope of 0.35 under the specific configuration provided with the instrument. A more appropriate calibration matched to the environmental test conditions would be required to provide better quantitative measurements. The JFE total CHL estimations remained linear across a wide concentration range spanning from 4 to over 120 µg/l showing a good dynamic range and broad applicability.

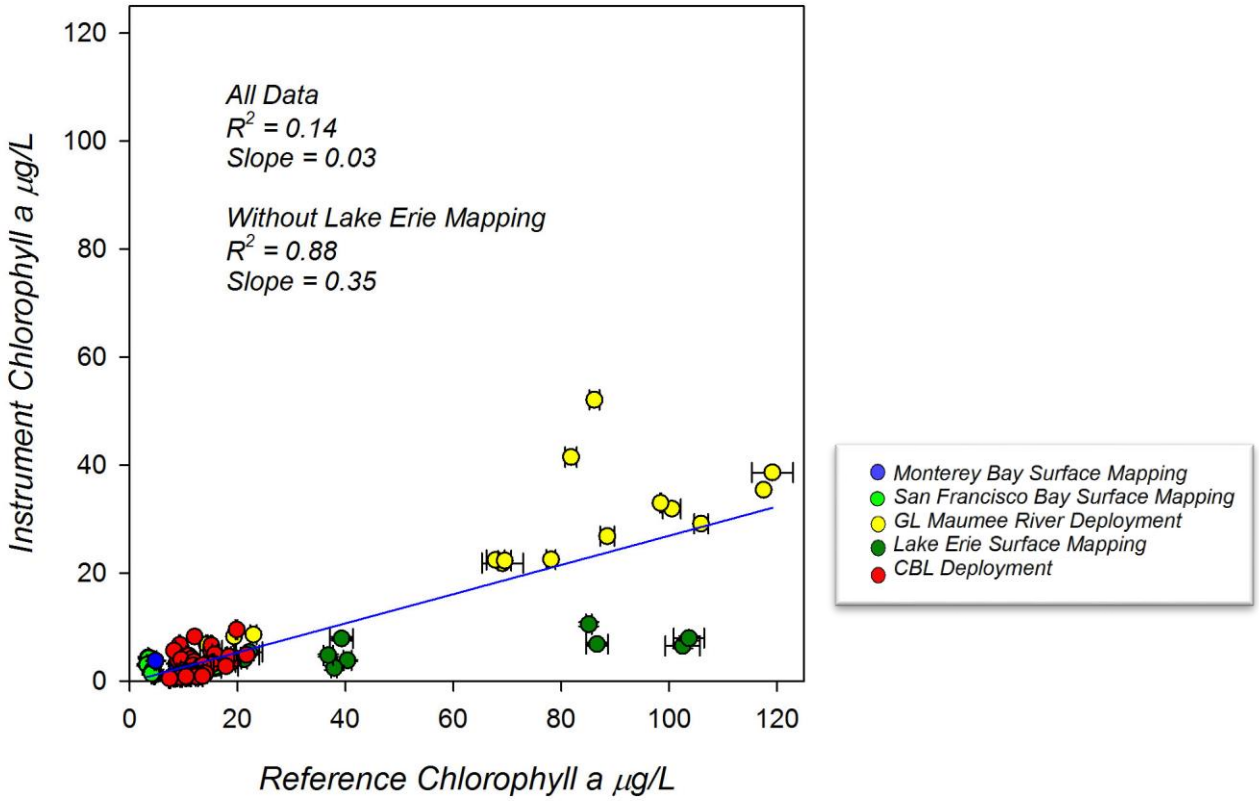


Figure 46. Global response plot for the JFE CHL estimation compared to extracted chlorophyll for all five ACT field trials. The blue lines represent the linear regression excluding the data from the Lake Erie Surface Mapping (GL SM, denoted with dark green circles).

Quality Assurance and Quality Control

All technology evaluations conducted by ACT comply with its Quality Management System (QMS), which includes the policies, objectives, procedures, authority, and accountability needed to ensure quality in work processes, products, and services. A QMS provides the framework for quality assurance (QA) functions, which cover planning, implementation, and review of data collection activities and the use of data in decision making, and quality control. The QMS also ensures that all data collection and processing activities are carried out in a consistent manner, to produce data of known and documented quality that can be used with a high degree of certainty by the intended user to support specific decisions or actions regarding technology performance. ACT's QMS meets U.S. Environmental Protection Agency quality standards for environmental data collection, production, and use, and the requirements of ISO/IEC 17025:2005(E), *General Requirements for the Competence of Testing and Calibration Laboratories*.

Quality Control Sample Analysis

As part of the sample analysis quality control evaluation two field blank samples (Table 9) and two field duplicate samples (Tables 10-12) were collected during each of the moored field testing applications in the Maumee River and in Chesapeake Bay. Results of the reference sample field blanks (Table 9) were quite consistent across all samples at both sites and did not indicate the presence of any contamination or bias associated with sample processing or analysis.

Table 9. Results of reference sample Field Blank analysis.

Sample ID	Collection	Turbidity (NTU)	CDOM (A_{400})	CHLa (µg/L) (stdev)	PC (µg/L) (stdev)	PE (µg/L) (stdev)
GL14	7/28/17 10:00	0.097	0.02	0.02 (0.02)	0.20 (0.22)	-0.23 (.002)
GL24	8/2/17 09:30	0.08	0.08	0.07 (0.03)	-0.06 (0.08)	-0.28 (.002)
CBL39	9/27/17 10:00	0.23	0.07	0.04 (0.01)	-0.17 (0.15)	-0.14 (0.002)
CBL55	10/2/17 10:00	0.18	0.06	0.03 (0.01)	-0.17 (0.15)	-0.06 ((0.08)

Results of the laboratory analysis for reference sample field duplicates (Table 10) were quite consistent across all samples at both sites and did not indicate the presence of any contamination or bias associated with sample processing or analysis. Coefficients of variance were elevated when concentrations were low or near detection limits.

Table 10. Comparison of reference sample analysis for Field Duplicates from the Maumee River, OH and Chesapeake Biological Laboratory, MD mooring tests.

Sample ID	Sample Type	Turbidity (NTU)	CDOM (A_{400})	CHLa (µg/L)	PC (µg/L)	PE (µg/L)
GL10	Ref	63.6	5.46	10.9	0.23	0.036
GL11	Field Dup	63.0	5.54	10.7	0.42	0.046
	Mean (stdev)	**63.3 (0.42)**	**5.51 (0.06)**	**10.8 (0.10)**	**0.32 (0.13)**	**0.04 (0.01)**
	Coeff Var	**0.67**	**1.08**	**0.94**	**41.9**	**16.9**
GL20	Ref	30.3	5.17	87.6	10.1	0.14
GL21	Field Dup	30.1	5.15	89.5	8.7	0.11
	Mean (stdev)	**30.2 (0.14)**	**5.16 (0.01)**	**88.6 (1.3)**	**9.4 (1.0)**	**0.12 (0.03)**
	Coeff Var	**0.47**	**0.20**	**1.5**	**10.3**	**20.7**
CBL41	Ref	1.28	1.31	12.7	1.62	1.39
CBL42	Field Dup	1.28	1.05	13.4	1.18	0.96
	Mean (stdev)	**1.28 (0.00)**	**1.18 (0.18)**	**13.1 (0.44)**	**1.40 (0.31)**	**1.18 (0.3)**
	Coeff Var	**0.00**	**15.6**	**3.4**	**22.4**	**25.5**
CBL57	Ref	0.98	1.59	13.4	2.01	3.25
CBL58	Field Dup	1.23	0.94	13.6	2.33	2.75
	Mean (stdev)	**1.10 (0.18)**	**1.26 (0.46)**	**13.5 (0.18)**	**2.17 (0.23)**	**3.00 (0.35)**
	Coeff Var	**16.1**	**36.4**	**1.4**	**10.6**	**11.8**

A comparison of microscopy results for field duplicate reference samples collected during the Maumee moored deployment test is shown in Table 11. Total biovolume differed by a factor of 2 at low cell abundance (GL10 and GL11), but with similar ratios of composition across species. At higher abundance (GL20 and GL21) total abundance agreed to within approximately 20% and relative species composition was consistent between the two replicates.

Table 11. Comparison of reference sample analysis for Field Duplicates from the Maumee River, OH mooring test.

Sample ID	GL10	GL11			GL20	GL21	
Sample Type	Ref	Field Dup			Ref	Field Dup	
	Biovolume (10^3 µm^3/mL)	Biovolume (10^3 µm^3/mL)	St Dev		Biovolume (10^3 µm^3/mL)	Biovolume (10^3 µm^3/mL)	St Dev
Greens	141	298	111		2081	1081	707
Diatoms	332	696	257		3794	5187	985
Bluegreens	1	181	127		0	5	na
Chrysophytes	1	229	161		150	343	137
Cryptophytes	165	0	na		731	1891	820
Dinoflagellates	0	0	na		875	1258	270
Miscellaneous	40	2	27		0	68	na
Syn/Picos	0.32	0.24	0.06		0.27	0.37	0.07
Total	**680**	**1406**	**513**		**7631**	**9833**	**1557**

A comparison of microscopy results for field duplicate reference samples collected during the Chesapeake Bay moored deployment test is shown in Table 12. For the first set of field replicates (CBL 41 and 42) total biovolume differed by a factor of 3 but the species composition ratios were fairly consistent. For the second set of field replicates (CBL 57 and 58) total biovolume differed by only 15% and the species composition ratios were very consistent.

Table 12. Comparison of reference sample analysis for Field Duplicates from the Chesapeake Bay, MD mooring test.

Sample ID	CBL41	CBL42			CBL57	CBL58	
Sample Type	Ref	Field Dup			Ref	Field Dup	
	Biovolume (10^3 µm^3/mL)	Biovolume (10^3 m^3/mL)	St Dev		Biovolume (10^3 m^3/mL)	Biovolume (10^3 m^3/mL)	St Dev
Diatoms	656	1596	665		1713	1676	26.2
Dinoflagellates	232	1323	772		122	475	249
Euglenoids	21.4	21.4	0		0	0	0
Syn/Picos	54.0	79.8	18.3		57.8	54.3	2.5
Total	**964**	**3021**	**1455**		**1894**	**2206**	**220**

Technical System Audits

An effective assessment program is an integral part of a quality system. The ACT Quality Assurance (QA) Manager independently conducted Technical Systems Audits (TSA) of the laboratory test at Moss Landing Marine Laboratories on June 25-28, 2017, and two field tests, at the Monterey Bay surface mapping test during July 11-12, 2017 and at the Chesapeake Biological Laboratory during September 5-7, 2017; and data quality reviews of the reference data sets from all tests.

A TSA is a thorough, systematic, on-site qualitative audit of sampling and measurement processes and procedures associated with a specific technology evaluation. The objectives of the TSAs conducted during this evaluation were to assess and document the conformance of on-site testing procedures with the requirements of the Test Protocols, the ACT Quality Assurance Project Plan (QAPP), and associated Standard Operating Procedures (SOPs).

The TSA were conducted in accordance with the procedures described in n EPA's *Guidance on Technical Audits and Related Assessments for Environmental Data Operations (EPA QA/G-7)* and ISO 19011, *Guidelines for Quality and/or Environmental Management Systems Auditing*. A TSA checklist based on the Test Protocols was prepared prior to the audits and reviewed by the ACT Director and Senior Scientist. The TSA assessed ACT personnel, the test and analytical facilities, equipment maintenance and calibration procedures, sample collection, analytical activities, record keeping, and QC procedures. Reference sample handling and chain-of-custody were observed during each audit.

During the audits, the QA Manager met with ACT technical staff involved in the evaluation and asked them to describe the procedures followed. All procedures were observed; and logbooks, data forms, and other records were reviewed.

Key components of the audit included:

- Assessment of Quality Assurance/Quality Control:
  - Adequacy of procedures, and
  - Adherence to procedures.
- Assessment of Sample System:
  - Sample collection,
  - Analytical procedures, and
  - Documentation.
- Assessment of Data and Document Control:
  - Chain of custody, and
  - Documentation.

The TSAs' findings were positive for the two field tests, which were implemented consistent with the Test Protocols, QAPP, and SOPs. Minor deviations were documented in laboratory records. There were no deviations which may have had an effect on data quality for these tests.

Prior to the start of the laboratory test, the stock cultures of individual species of marine algae were contaminated. The algal cultures were grown in a semi-continuous culture system. The culture vessels were filled with filtered seawater pumped in through the MLML seawater system. The pore size of the filters allowed other species of algae to enter the culture. Corrective action, replacing the existing filters with filters with a smaller pore size, was taken immediately and effectively resolved the problem. This resulted in a number of deviations in the Test Protocols. The deviations and corrective action altered the type of data results but did not have an effect on data quality.

For all tests, the implementation of the audited tests was performed in a manner consistent with ACT data quality goals. All samples were collected as described in the Test Protocols and SOPs. Examination of maintenance and calibration logs provided evidence of recent and suitable calibration of sampling and analytical equipment. The overall quality assurance objectives of the test were met.

ACT personnel are well-qualified to implement the evaluation and demonstrated expertise in pertinent procedures. Communication and coordination among all personnel was frequent and effective. Internal record keeping and document control was well organized. The ACT staff understands the need for QC, as shown in the conscientious development and implementation of a variety of QC procedures.

Data Quality Review

Quality Control. Quality control samples collected included periodic duplicate field samples and field blanks to determine the adequacy and control of field collection and processing procedures of analytical laboratory processing and analysis procedures. QC samples were treated identically to routine samples in terms of sample identification, custody, request for analytical services, and data processing.

Results from field blanks showed no contamination indicate that field procedures were adequate for accomplishing data quality objectives. If the concentration observed in a replicate did not meet the criteria for precision and accuracy, the value was rejected and a back-up filter was processed and analyzed.

Calibration data was reviewed at a cursory level and was determined to be acceptable. No data qualification was required based on the calibration review.

Custody for all reference samples, was adequately maintained throughout the collection, processing, and delivery of samples to the analytical laboratories. Chain-of-custody documentation was complete. All analysis holding times were met as described in SOPs for the method or the Test Protocols.

Overall, data quality for the reference water samples was acceptable.

Data Verification, Validation, and Quality Assessment. Data review is conducted to ensure that only sound data that are of known and documented quality and meet technology evaluation quality objectives are used in making decisions about technology performance. Data review processes are

based in part on two EPA guidance documents: *Guidance on Environmental Data Verification and Data Validation* (QA/G-8) [EPA, 2002] and *Guidance on Technical Audits and Related Assessments for Environmental Data Operations* (QA/G-7) [EPA, 2000].

The data were verified and validated to evaluate whether the data have been generated according to the Test Protocols and satisfied acceptance criteria. Data verification evaluates the completeness, correctness, and consistency of the data sets against the requirements specified in the Test Protocols, measurement quality objectives (MQOs), and any other analytical process requirements contained in SOPs. Data validation assesses and documents compliance with methods and procedures and determines the quality of the data based on the quality objectives defined in the Test Protocols and QAPP.

The ACT QA Manager reviewed the reference data sets from all field and laboratory tests. The number of reference samples collected at each site and the laboratory tests are in Table 13. A total of 243 reference samples were collected for the field and laboratory tests. Each reference sample was split into replicates for pigment analysis. Distinct grab samples were taken for phytoplankton cell counts, CDOM, and turbidity.

Table 13. Summary of samples replicates and number of analyses for each lab and field site.

Site	No. of Samples[1/]	No. of Replicates per Sample[2/]	No. of Measurements (Pigments)[3/]
MLML - Lab	98	5	490
SF Bay - Surface	16	5	80
Monterey Bay - Surface	14	5	70
Maumee River	31	5	155
Lake Erie - Surface	14	5	70
UM - Lab	10	5	50
CBL – Field	60	5	300
Total	243		1,215

1/ Includes replicate samples
2/ A total of six replicates were filtered for each reference sample. Pigment analysis was conducted on two replicates chlorophyll and three replicates for phycobilins. One filter was reserved in storage.
3/ Does not include phytoplankton cell counts and biovolume, CDOM, and turbidity, which also were verified and validated.

The data verification determined that the sampling and analysis protocols specified in the Test Protocols were followed, and that the ACT measurement and analytical systems performed in accordance with approved methods, based on:

- The raw data records were complete, understandable, well-labeled, and traceable;
- All data identified in the Test Protocols were collected;
- QC criteria were achieved; and
- Data calculations were accurate.

Data validation uses the outputs from data verification and included inspection of the verified field and laboratory data to determine the analytical quality of the data set. Validation of the data sets established:

- Required sampling methods were used;
- Sampling procedures and field measurements met performance criteria; and
- Required analytical methods were used.

The data validation also confirmed that the data were accumulated, transferred, summarized, and reported correctly. There is sufficient documentation of all procedures used in the data collection and analysis to validate that the data were collected in accordance with the evaluation's quality objectives.

Data Quality Assessment, sometimes referred to as a Data Usability Assessment is a scientific and statistical evaluation of validated data to determine if the data are of the right type, quality, and quantity to support conclusions on the performance of the technologies. The DQA determined that the test's data quality objectives, described in Section 7.2 of the Test Protocols and Section 3.4 and Appendix B of the ACT QAPP (ACT, 2016), were achieved. This evidence supports conclusions that:

- The sampling design performed very well and is very robust with respect to changing conditions.
- Sufficient samples were taken to enable the reviewer to see an effect if it were present.
- The complete data set was fit for its intended use for determining the performance of the test instruments.

Audit of Data Quality. The ACT QA Manager also conducted an Audit of Data Quality (ADQ) on verified data to document the capability of ACT's data management system to collect, analyze, interpret, and report data as specified in the Test Protocols, QAPP, and SOPs. An ADQ involves tracing data through their processing steps and duplicating intermediate calculations. A representative set of approximately 10% of the data was traced in detail from 1) raw data from field and laboratory logs, 2) data transcription, 3) data reduction and calculations, to 4) final reported data.

The ADQ determined that the data were accumulated, transferred, reduced, calculated, summarized, and reported correctly. There is sufficient documentation of all procedures used in the data collection and analysis to verify that the data have been collected in accordance with ACT quality objectives defined in the ACT QMS.

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March 21, 2019

Date

Approved By: Dr. Mario Tamburri
ACT Executive Director

March 21, 2019

Date

Approved By: Dr. Tom Johengen
ACT Chief Scientist

March 21, 2019

Date

Approved By: Dr. Earle Buckley
Quality Assurance Supervisor

JFE Advantech Co., Ltd.

We would like to thank ACT's scientists and staff for their efforts in making this important evaluation available. In general, we consider the performance of the Multi-Exciter presented in this report as satisfactory. The instrument showed consistent results and behaved as expected (considering the current calibration settings, with which it was deployed) in different environments. However, in our opinion, there are some points regarding the data collected by the instrument that deserve to be explained further. We are addressing these issues in the effort to elucidate the performance of our instrument.

As pointed out in the report, the Multi-Exciter was not calibrated for each one of the tests, and we should highlight that due to the lack of proper calibration, none of the tests performed by ACT showed the true capabilities of the instrument in identifying phytoplankton classes and their respective concentrations. Thus, it is expected that chlorophyll concentration estimated by the instrument will differ from the reference. However, one may ask why such difference increases at high chlorophyll concentrations as shown in this report. It is widely known that chlorophyll and other fluorescent pigments lose linearity at high concentrations. Consequently, chlorophyll estimations following the calibration coefficients written in the instrument will not be adequate. This can be properly addressed using polynomial calibration methods at JFE Advantech Co., Ltd. With the current standard calibration settings, we believe that the performance would be better in conditions where phytoplankton concentration is relatively low. The instrument is capable of detecting very low pigment concentrations.

Still, taking into account what was discussed above, we were expecting a better relationship between the instrument and the reference during the surface mapping test on San Francisco Bay, since diatoms were predominant and chlorophyll concentrations were relatively low. However, we observed that chlorophyll estimated by Multi-Exciter decreased as the noon approached; while the reference estimated relatively constant chlorophyll concentration during the same period (stations SF01, SF02, SF03, SF04, SF05 and SF06). We are wondering if this decay in fluorescence emission would be linked to non-photochemical quenching (NPQ) occurring in the first meters of the water column, where solar radiation is more intense, particularly in the hottest hours of the day during summer, the period in which the experiment was carried out. We acknowledge that there was an acclimation period of 10 minutes, but is not clear if that was enough time to allow relaxation of NPQ components related to photosynthesis inhibition.

Another issue that we would like to discuss further is the supposed turbidity effect pointed out during June 27th 2017 experiment at Moss Landing Marine Lab. The report argues that turbidity additions of 23 and 50 NTU caused a reduction of chlorophyll estimations larger decrease than observed in the reference. However, we consider that direct comparisons between the Multi-Exciter and the reference are compromised, particularly at high concentrations, where the current calibration settings are not ideal – as discussed in the previous paragraph. We believe that turbidity did not play any role here and the instrument is reflecting the exact same thing that was happening in the reference samples, which is a decrease in chlorophyll concentration as shown in Figure 7. An important fact that corroborates with us is that 2 experiments conducted by ACT, where turbidity

increases from 1.1 to 25 NTU (June 29th 2017 experiment at Moss Landing Marine Lab) and from 0 to 33 NTU (August 10th 2017 experiment at the Great Lakes lab) did not show any effect of turbidity. Note that those two experiments had larger additions of turbidity than that from the SW – T9 trial in the June 27th experiment, where turbidity was considered responsible for decreasing chlorophyll estimations from the Multi-Exciter.