Ref. No. [UMCES] CBL 2019-006
ACT VS19-01

PERFORMANCE VERIFICATION STATEMENT
For BBE Moldaenke's PhycoProbe

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.

.

NOTICE:

ACT verifications are based on an evaluation of technology performance under specific, agreed-upon protocols, criteria, and quality assurance procedures. ACT and its Partner Institutions do not certify that a technology will always operate as verified and make no expressed or implied guarantee as to the performance of the technology or 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 nor 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. The end user is solely responsible for complying with any and all applicable federal, state, and local requirements.

This document has been peer reviewed by ACT Partner Institutions and a technology-specific advisory committee and was recommended for public release. Mention of trade names or commercial products does not constitute endorsement or recommendation by ACT for use.

Questions and comments should be directed to: Dr. Tom Johengen
ACT Chief Scientist
CIGLR - University of Michigan
4840 S. State Street
Ann Arbor, MI 48108 USA
Email: Johengen@umich.edu

TABLE OF CONTENTS

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 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 testing application as a measure of Quality Assurance.

This document presents the results of the Bbe PhycoProbe which measures a fluorescence excitation spectrum using seven LED excitation wavelengths of 370nm, 430nm, 470nm, 525nm, 570nm, 590nm and 610nm. The excitation wavelengths are adapted to the absorption wavelengths of the light-harvesting pigments of different algal classes: phycocyanin, phycoerythrin, fucoxanthin, peridinin and chlorophyll-a. A mean excitation spectrum per chlorophyll-a content (fingerprint) of an algal class is determined. Using these “fingerprints” and a mathematical operation (best-fit procedure) enables the instrument to calculate the chlorophyll-a concentration from a complex mixture and estimate the distribution of five different algal classes including cyanobacteria, chlorophytes, the brown group (diatoms, dinoflagellates, etc.), cryptophytes, and planktothrix. A single instrument was provided for the entire round of lab and field testing and all tests were conducted under the same calibration 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 PhycoProbe 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. Low response slopes occurred 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). The lowest 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 PhycoProbe total chlorophyll fluorometric response compared as regressed against extracted chlorophyll for each of the laboratory and field tests completed during the ACT technology evaluation along with a summary of potential reference comparisons and actual data returns.

Test	Response Slope	Regression R-squared	# Ref Samples	Instrument OBS	Data Return %	Distance or Duration
LAB Tests						
ML Day1	1.1	99	8	8	100	1 d
ML Day 2	1.1	98	10	10	100	1 d
ML Day 3	0.46	87	31	31	100	1 d
ML Day 4	1.24	96	19	19	100	1 d
ML Day 5	0.46	92	21	21	100	1 d
GLERL	0.61	78	10	10	100	1 d
Field Test Underway						
SF Bay	0.24	89	16	445	100	150 km
Monterey Bay	0.26	47	14	498	100	75 km
WLE	0.13	75	14	1971	100	75 km
Field Test Moored						
Maumee R	0.71	86	31	621	100	13 d
Chesapeake Bay	1.54	53	60	641	99	28 d

The accuracy of algal group classification by the PhycoProbe was compared graphically against algal group biovolume proportion estimates derived from microscopic counts and established shape formulas. Overall, the PhycoProbe responded to the presence of phycobilin pigments associated with Cyanobacteria, Cryptophytes and Planktothrix but the proportion of the total chlorophyll assigned to those groups did not always agree with distributions assigned by biovolume estimates. The addition of CDOM as a matrix caused a shift in classification of the algae present. In one lab test CDOM presented as Planktothrix and in a second caused a shift from reporting as Green algae to Brown algae. In the field tests, the PhycoProbe tended to over-assign the proportion of green algae and under-predict cyanobacteria and the brown group.

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

Bbe fluorometers are not only capable of measuring the chlorophyll-a content of phytoplankton in water samples, they can also distinguish between different microalgae classes as well as determine the photosynthetic activity. The instruments also measure turbidity and yellow substances to correct the chlorophyll-a content.

The measuring principle of the bbe fluorometers works as follows: Microalgae of a taxonomic class possess a similar composition of photosynthetic pigments and thus have a typical *in vivo* fluorescence-excitation spectrum, whereby the emission wavelengths of the measured fluorescent light are between 680 and 700 nm. It is thus possible to allocate an algal species to a spectral algal class due to its fluorescence spectrum. Additional information is provided by the measurement of the phycocyanin fluorescence emission in the range 640 – 660 nm. In order to obtain a meaningful fluorescence excitation spectrum, seven LEDs provide excitation wavelengths of 370nm, 430nm, 470nm, 525nm, 570nm, 590nm and 610nm, respectively. The excitation wavelengths of the LEDs are adapted to the absorption wavelengths of the light-harvesting pigments of

different algal classes: phycocyanin, phycoerythrin, fucoxanthin, peridinin and chlorophyll a, etc. The excitation of the algal pigments is performed after a dark adaptation by switching on the LEDs one after the other at high frequency. In the phases in between these pulses, the fluorescence emission of the chlorophyll is measured as an answer to the excitation. Spectra of different algal classes within a sample consisting of cyanobacteria, chlorophytes, the brown group, diatoms, dinoflagellates, etc. and cryptophytes are recorded. A mean excitation spectrum per chlorophyll-a content (fingerprint) of an algal class is determined. Using these "fingerprints" and a mathematical operation (best-fit procedure) enables the instrument to calculate the chlorophyll-a concentration from a complex mixture and the distribution of up to 4 different algal classes in a water sample. Additional algal classes can be added. The chlorophyll determination is quantitatively based on an established HPLC separation method of algal pigments. The integrated detection and correction of yellow substances eliminates the disturbing influence of fluorescent humic substances on the chlorophyll measurement and improves the quality of the measurement. Additional information on turbid matter is provided by the transmission measurement, which is also used for the compensation of turbidity. Bbe multispectral fluorometers are available in a variety of formats including the submersible PhycoProbe, the on-line continuous measuring AlgaeOnlineAnalyser and the cuvette based AlgaeLabAnalyser.

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 bbe Moldaenke GmbH and used in all subsequent testing without any further calibration or servicing by the company. 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 to the evaluation with instrument specific details for the PhycoProbe 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. For these small batch tests the PhycoProbe was inserted into a narrow, opaque PVC cylinder with the test solution completely covering the sensor window.

Field Testing

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, cyano*phytes, 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 ^{o}C.

A total of six replicates was filtered for each reference sample and stored at -80 ^{o}C 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 ^{o}C 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 OF 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). There was a small positive CHL fluorescence response to both DI and the media. The absolute difference between instrument estimation and reference measurement increased at the higher concentration level of both species. There was no apparent difference in accuracy across the two species, although this could not be tested statistically given the experimental design.

A cross plot of PhycoProbe 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 0.99 and a slope of 1.13.

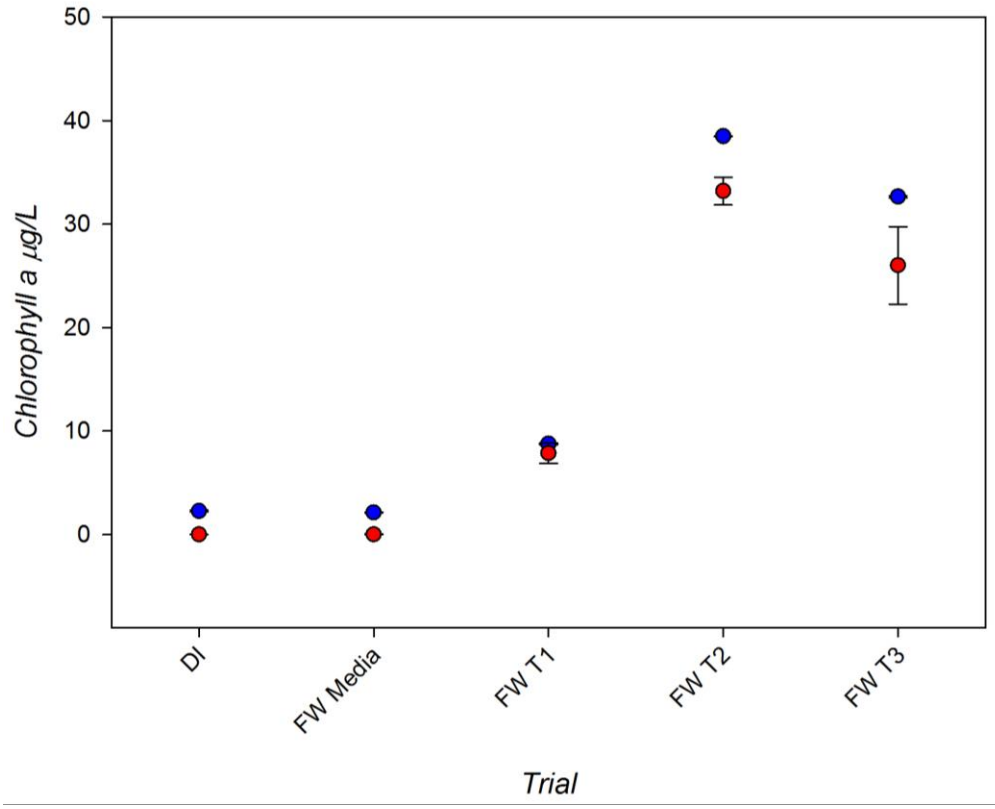


Figure 1. Plot of instrument (blue) and reference (red) measurements of chlorophyll a in the freshwater individual algae tests, including background readings for DI and the freshwater culture media. The plotted reference values represent the average and standard deviation of the two reference measurements 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.

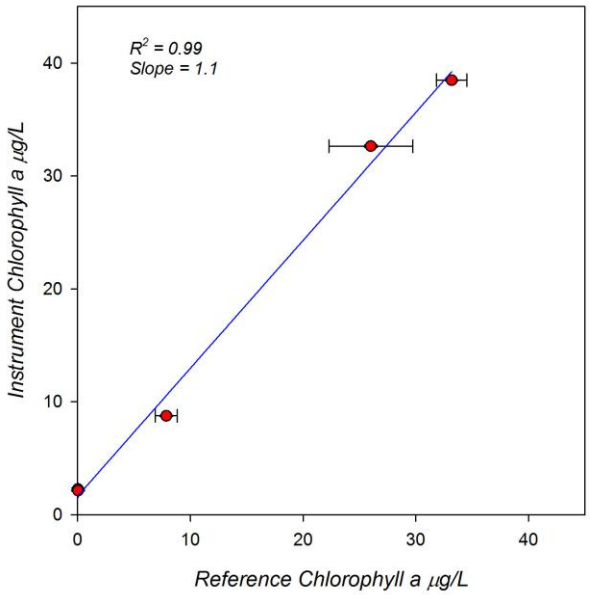


Figure 2. Cross plot of instrument readings versus extracted reference sample pigments for chlorophyll a during the freshwater individual algae lab trials. The blue line represents the linear regression of the data.

A comparison of algal classification from microscope counts on preserved reference samples against the PhycoProbe classifications is shown in figure 3. The PhycoProbe accurately assigned the fluorescence singal of *Coelastrum* to Green Algae. However, we did note some apparent contamination of *Microcystis* in the *Coelastrum* culture at the C2 concentration based on detectable phycocyanin measurements, but it was not picked up by either the PhycoProbe or in the microscopy analysis of the reference sub-samples. The PhycoProbe did not accurately classify the cyanobacterial culture during the *Microcystis* exposure in trial 3, but the reference sample extracted PC analysis was approximately 1 µg/L, therefore the exposure culture did not contain much signal.

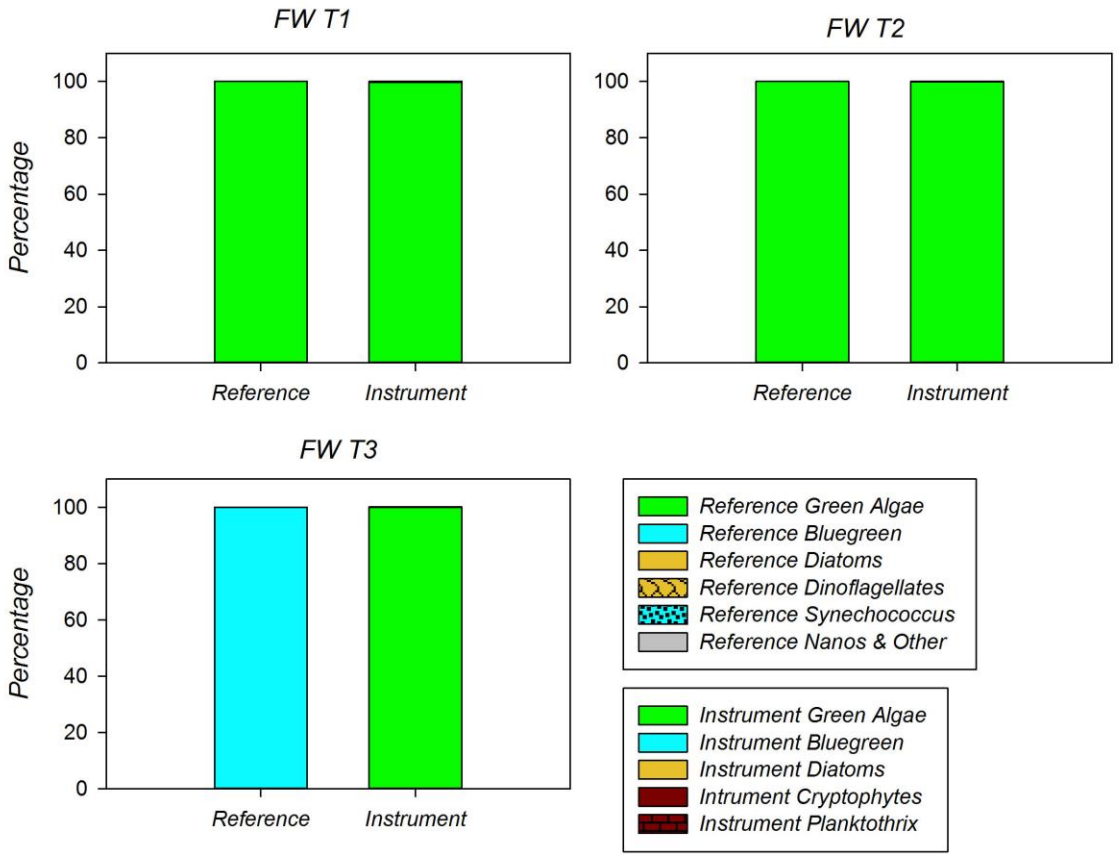


Figure 3. Algal classification from microscope counts on preserved reference samples against the PhycoProbe classifications as a percentage of biomass, with instrument algae categories in percentage of total chlorophyll a. Algal counts were grouped at the functional class level.

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 PhycoProbe showed similar, small positive CHL fluorescence responses to both DI and the saltwater media. The CHL fluorescence response tracked reference chorophyll levels over the range tested but the magnitude of difference increased at higher concentrations and in the present calibration was always higher than extracted CHL measurements. There was no obvious difference in fluorescence response across the two algal species tested, but this was not examined statistically given the experimental design.

A cross plot of the PhycoProbe chlorophyll a fluorescence estimation compared to reference sample extracted chlorophyll a measurements for the saltwater trials is shown in figure 5. The regression was highly significant ($p<0.001$) with an R^2 of 0.98 and a slope 1.1.

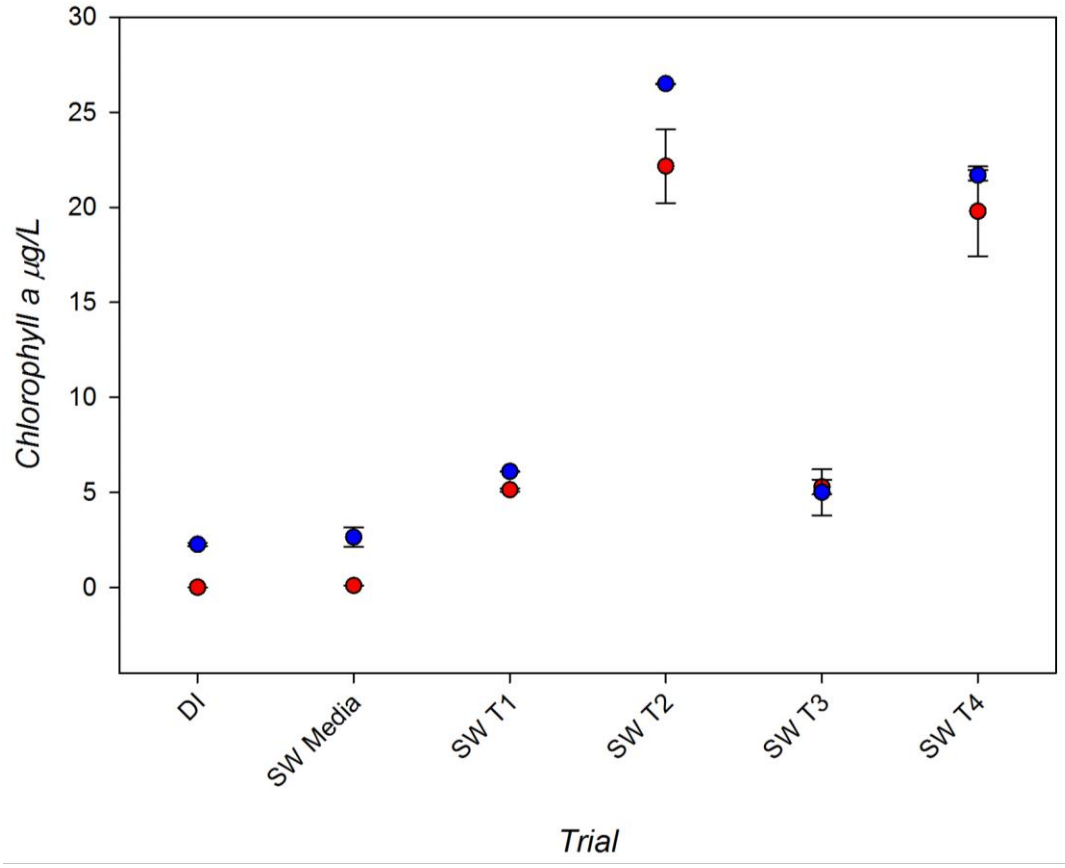


Figure 4. Plot of instrument (blue) and reference (red) CHL measurements in the saltwater individual algae tests including readings for DI and saltwater media. Plotted reference values represent the average and standard deviation of the two reference measurements taken at the beginning and end of the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

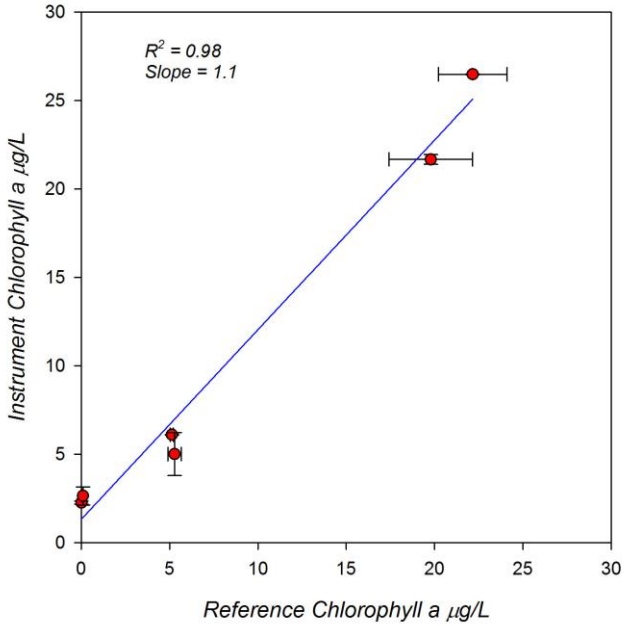


Figure 5. Cross plot of instrument readings versus extracted reference sample pigments for chlorophyll-a during the saltwater individual algae lab trials. The blue line represents the linear regression of the data.

A comparison of algal classification from microscope counts on preserved reference samples against the PhycoProbe classifications is shown in figure 6. The PhycoProbe largely classified the cultured sample in their brown category as expected, but also called out small contributions from cyanobacteria. We do recognize that some contamination may have been present that was not picked up in our microscopic analysis, however, no measureable phycobilins were detected in any of the reference samples for these trials.

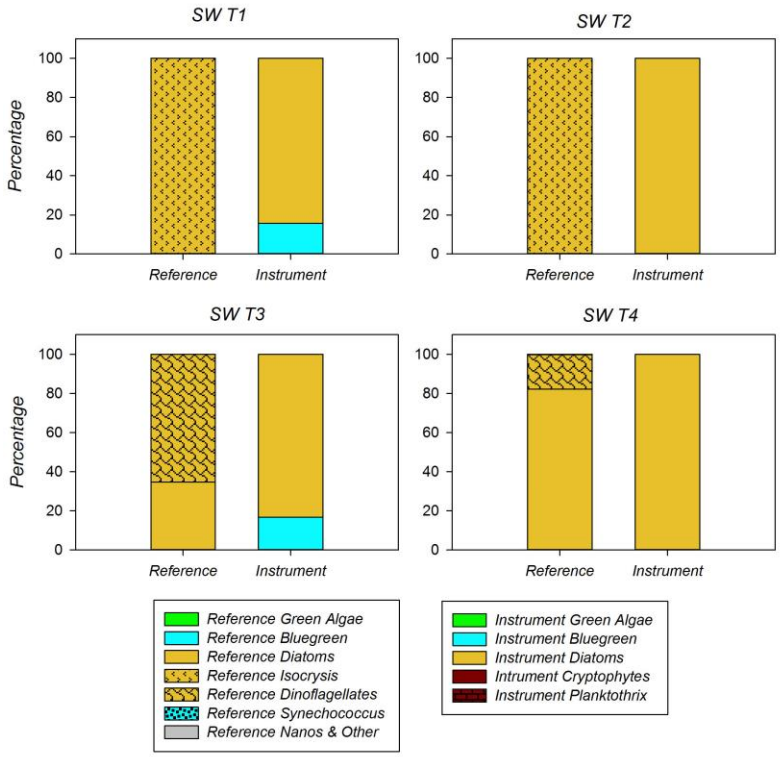


Figure 6. Algal classification from microscope counts on preserved reference samples against the PhycoProbe classifications as a percentage of biomass with instrument algae categories in percentage of total chlorophyll a. Algal counts were grouped at the functional class level. Estimations of Isocrysis 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 PhycoProbe 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, CHL levels ranged from 3.2 to 40 µg/L and PC levels ranged from 0 to 1.5 µg/L for the reference samples. The pigment ratios further confirm what we observed in our cells counts in terms of the level of contamination by diatoms. CDOM additions increased from background levels of approximately 0.25 up to 3.0, and turbidity additions increased levels from a background 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 PhycoProbe exhibited a high positive response for the seawater culture media, reading approximately 10 µg/L. This background reading seemed fairly consistent through the first five trials with CHL concentrations up to approximately 6 µg/L. When CHL was increased up to 40 µg/L in subsequent trials, the PhycoProbe began to under-predict concentrations. The under-prediction was exacerbated at the two highest turbidity levels of 23 and 50 NTU. There was no obvious impact of the CDOM additions looking across results between trials T3 - T5.

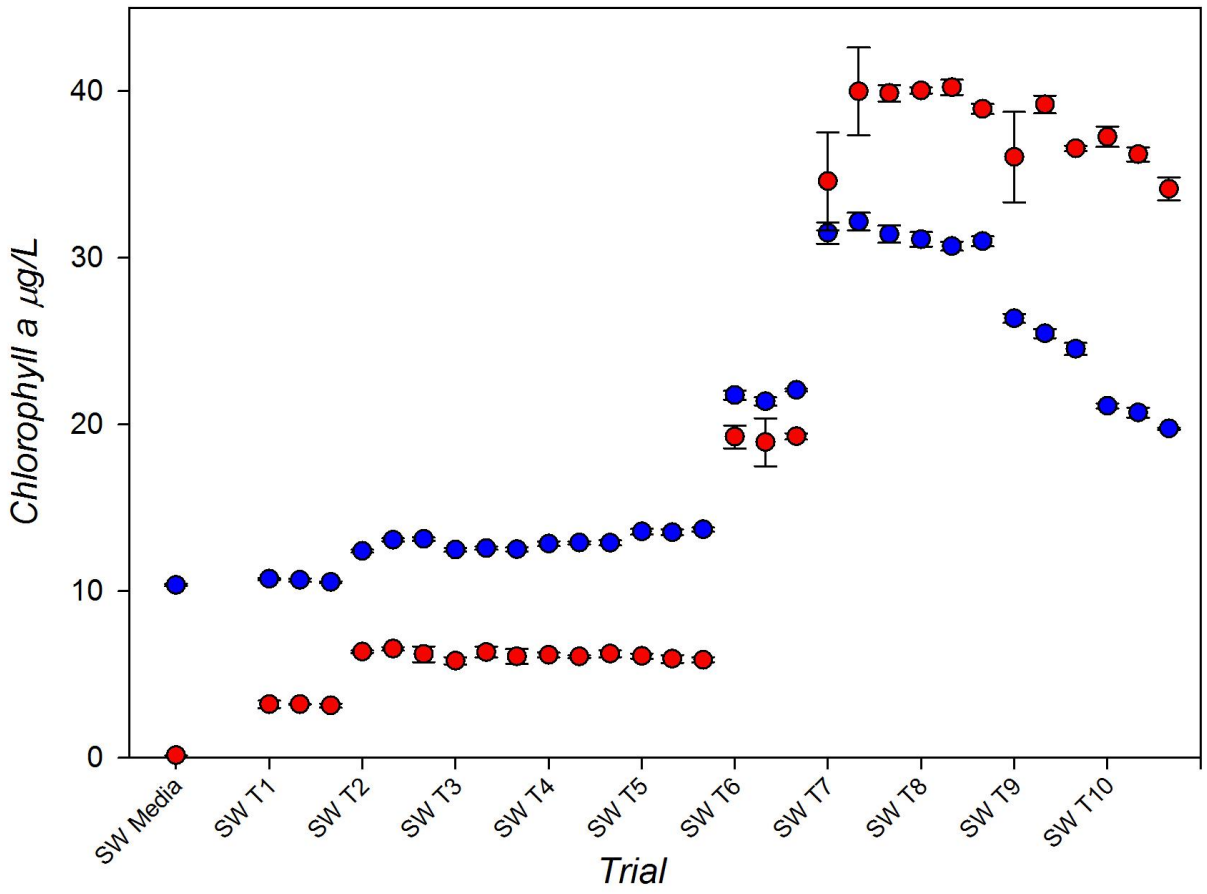


Figure 7. Plot of instrument (blue) and reference (red) measurements of chlorophyll a in the saltwater CDOM and turbidity addition trials covering 4 algae, 3 CDOM and 3 turbidity concentration levels. Three replicate reference measurements were made at each level with only one read in blank media. PhycoProbe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

A one-to-one cross plot of the PhycoProbe readings compared to reference sample measurements for CHL during the saltwater trials is shown in figure 8. The regression for the CHL response was highly significant ($p<0.001$) with an R^2 of 0.87 and a slope of 0.46. This response slope is noticeably lower than for the previous two lab tests and demonstrates a higher level of variation owing to the matrix challenge of very inorganic turbidity conditions.

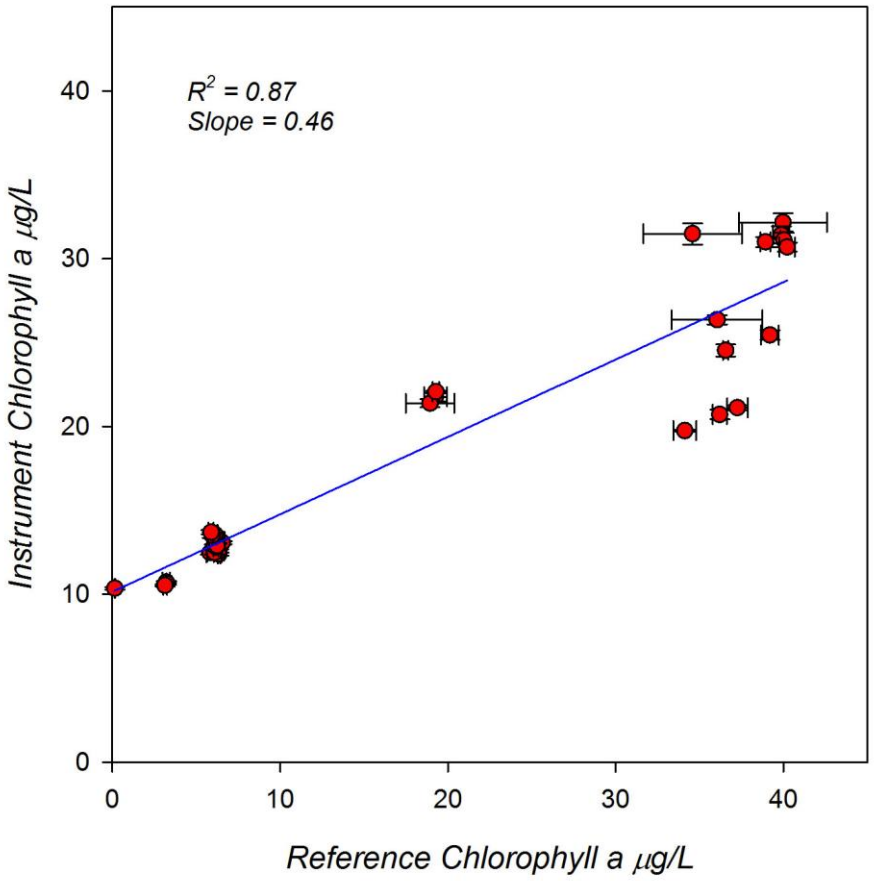


Figure 8. Cross plot of instrument readings versus extracted reference sample pigments for chlorophyll during the saltwater *Synechococcus* algae exposure with add in matrix challenges for CDOM and turbidity. The blue line represents the linear regression of the data.

A comparison of algal classification from microscope counts on preserved reference samples against the PhycoProbe classifications for the Lab test with *Synechococcus* with CDOM and turbidity additions is shown in figure 9. Instrument phytoplankton classification was consistent across all three timepoints (a,b,c) within each trial. There was, however, inconsistent classification of the bluegreen category as the algal concentration and matrix challenges were increased across trials. It is unclear why the PhycoProbe classification strongly favored cyanobacteria in trial 1. With each culture addition, more of the fluorescence was assigned to the Diatom category, which more accurately matched the cell counts. Some of the change in PhycoProbe classifications may have been due to the addition of CDOM, but the effect was inconsistent. The addition of CDOM in trial 3 resulted in a classification shift from cyanobacteria to diatoms for the same algal concentration, whereas the effect was not seen for a further CDOM addition at trial 5. There was no apparent effect on instrument classification in response to the turbidity additions across trials 8-10.

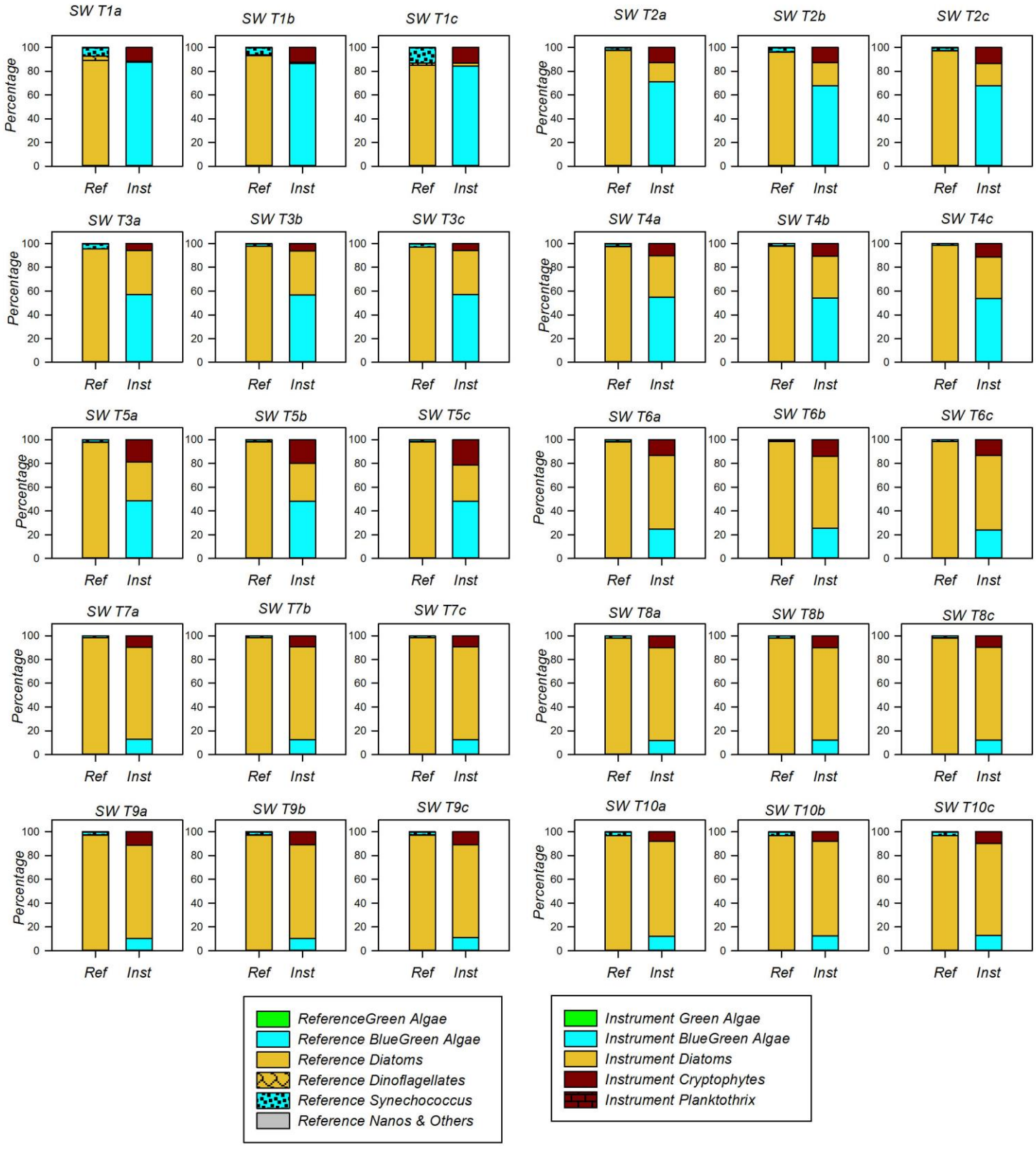


Figure 9. Reference cell counts as a percentage of biomass compared to instrument algae categories as a percentage of total chlorophyll a. Each row represents three timepoint replicates (a, b, c) of the same batch of algae and matrix conditions.

On June 28th, 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 PhycoProbe response to the freshwater media blank was near zero and generally tracked chorophyll levels over the test range from 4 to 25 µg/L across all mixtures of the three algal groups. However, at its tested calibration settings, the PhycoProbe over-predicted CHL concentration at levels above 20 µg/L.

A one-to-one cross plot of the PhycoProbe readings compared to reference sample measurements for CHL during the freshwater algal mixture trials are shown in figure 11. The regression line was highly significant ($p<0.001$) with an R^2 of 0.96 and a slope of 1.24.

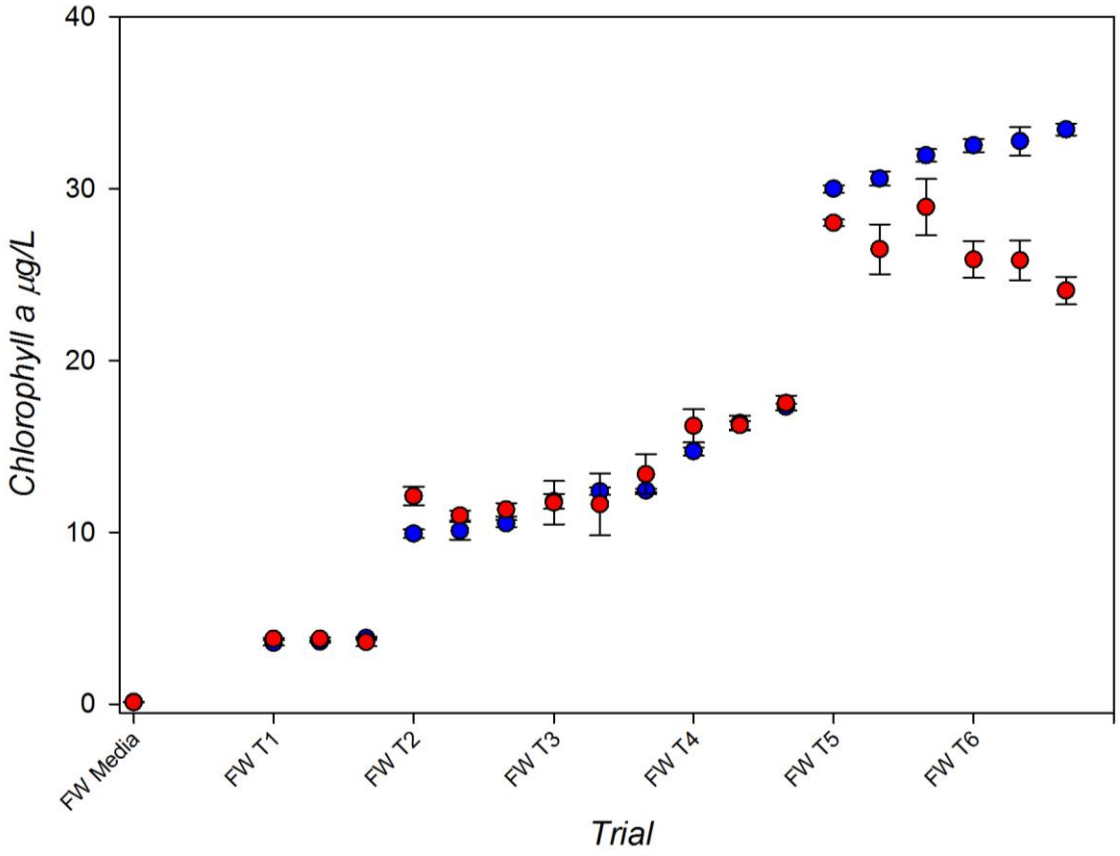


Figure 10. Plot of instrument (blue) and reference (red) measurements of chlorophyll a in the freshwater algal mixture trials covering 6 concentration ranges and mixtures of 3 different algae. Three replicate reference measurements were made at each level, and the PhycoProbe data were averaged over the 2 minutes bracketing each reference point.

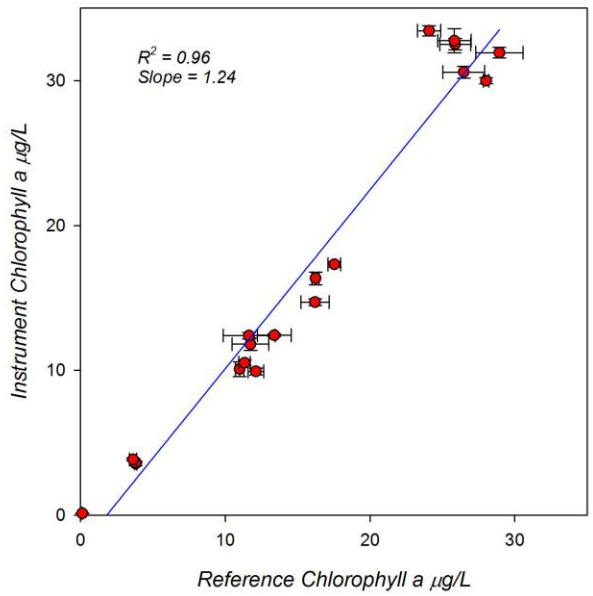


Figure 11. Cross plot of instrument readings versus extracted reference sample pigments for chlorophyll a during the June 28 freshwater algal mixtures lab trials. The blue line represents the linear regression of the data.

A comparison of algal classification from microscope counts on preserved reference samples against the PhycoProbe classifications for the freshwater algal mixtures is shown in figure 12. Instrument phytoplankton classification was again very consistent across all three timepoints (replicates a,b,c) within each trial. There was a small percentage (5-10%) of the fluorescent signature attributed to Cryptophytes during each one of trials, although no PE-containing species were used in the test. The PhycoProbe did not report any Bluegreen signal during trials 3 – 6, despite measurable PC levels of between 1.3 and 6.7 µg/L in the comparative reference samples. The addition of *Peridinium* at nearly 35% of the biovolume also did not produce a signal in the fluorescent characterization by the PhycoProbe. For this particular calibration set-up, the PhycoProbe seemed to inaccurately assign fluorescence contributions from Bluegreen algae into the Green algae category. Bluegreen cyanobacterial estimates did not correspond (in fact were negatively correlated) to culture additions nor microscopic counts for this set of trials.

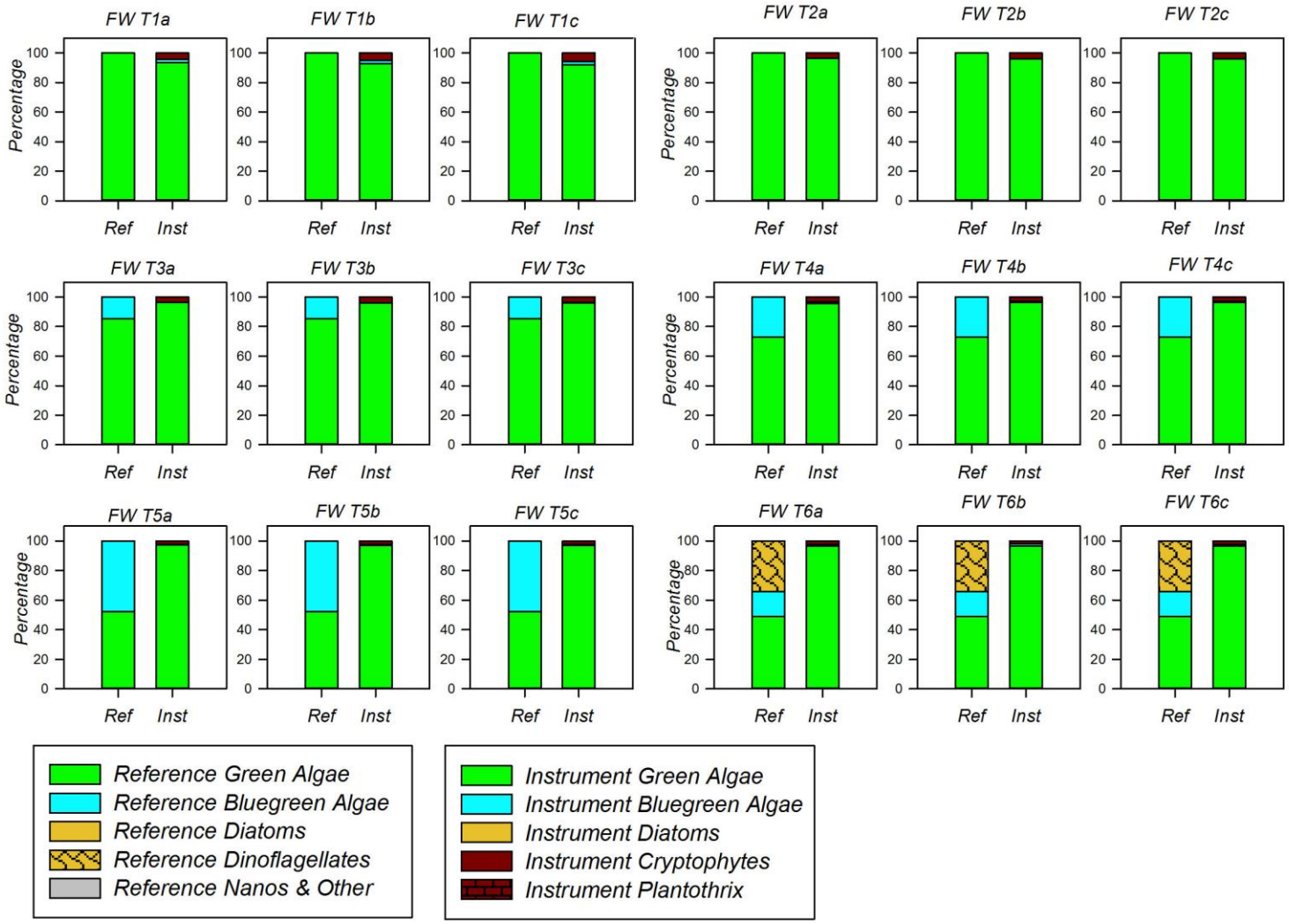


Figure 12. Reference cell counts as a percentage of biovolume compared to PhycoProbe partitioning of algal taxa as a percentage of total chlorophyll a.

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 (A_{400})	*Coelastrum*	Turbidity (NTU)
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). The PhycoProbe response to the freshwater media was near zero and the instrument accurately predicted overall CHL levels for the first five trials. Instrument measured CHL was underpredicted by approximately 40% for the highest added level of *Microcystis* in trial 6. The offset was in part related to the CDOM addition based on the effect seen in trial 5, but that could not explain the majority of the disagreement. The underprediction became much greater for trials 7 and 8 when additional algae were added in the form of *Coelastrum*. Similar offsets between trials 7 and 8 suggest a minimum effect from the large addition of inorganic turbidity. In general, the results from this lab test were quite different than previous tests since the PhycoProbe under-predicted concentrations at higher exposure levels. We do note there was significant variation in the two reference sample CHL estimates for trial 8 (Figure 13, grey symbol), but not for the PhycoProbe. Based on known additions of the cultures the CHL concentrations should have been very similar between trials 8 and 9 since only turbidity was added during trial 9. However, cell counts in the replicate timepoint for trial 8 were also 25% lower and suggest some patchiness in the tank during the sub-sampling.

A one-to-one cross plot of the PhycoProbe 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 0.92 and a slope of 0.46.

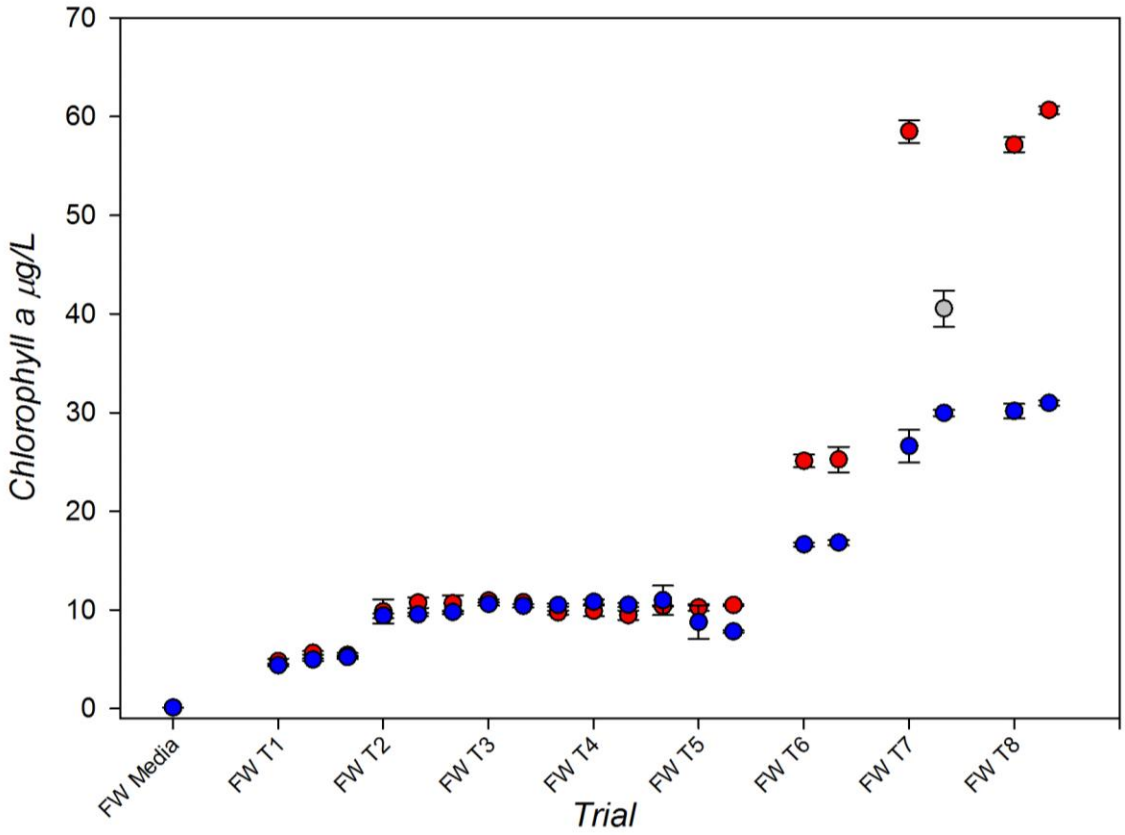


Figure 13. Plot of instrument (blue) and reference (red) measurements of chlorophyll a during the June 29th laboratory test with the freshwater algae *Microcystis* and *Coelastrum*, and challenge additions of CDOM and turbidity. Three replicate reference measurements were made for trials 1-5, two replicates were made for trials 6-8, and one replicate for the blank media. PhycoProbe data were averaged over the 3 minutes bracketing each reference sample.

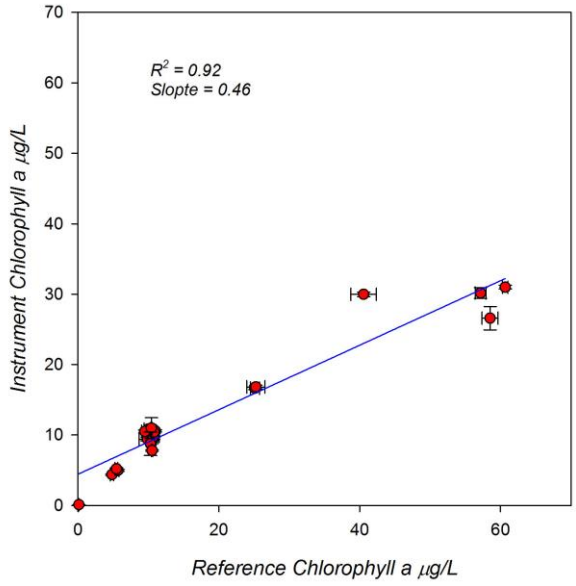


Figure 14. Response plot for PhycoProbe chlorophyll a compared to reference samples for the freshwater algae addition lab trials. The blue line represents the linear regression of the data.

A comparison of algal classification from microscope counts on preserved reference samples against the PhycoProbe classifications for the freshwater algal mixtures is shown in figure 15. While the PhycoProbe's chlorophyll-a estimates were in close agreement with the reference measures across the first two *Microcystis* exposure levels, the instrument consistently misclassified the algal composition. Microscopic counts confirmed cyanobacterial presence in the sample, but the PhycoProbe classified these exposures entirely as green algae. Instrument classification was sensitive to CDOM load, exhibited by the change in classification at the highest CDOM loading (T5) where diatoms are classified along with a minor cyanobacterial percentage. Instrument estimates of cyanobacterial relative abundance did increase when a higher challenge concentration was used (T6), but subsequent additions of the green algal species *Coelastrum spp.* (observable microscopically) were not reported by the PhycoProbe. The diatom classification by the PhycoProbe is attributable to the CDOM challenge level.

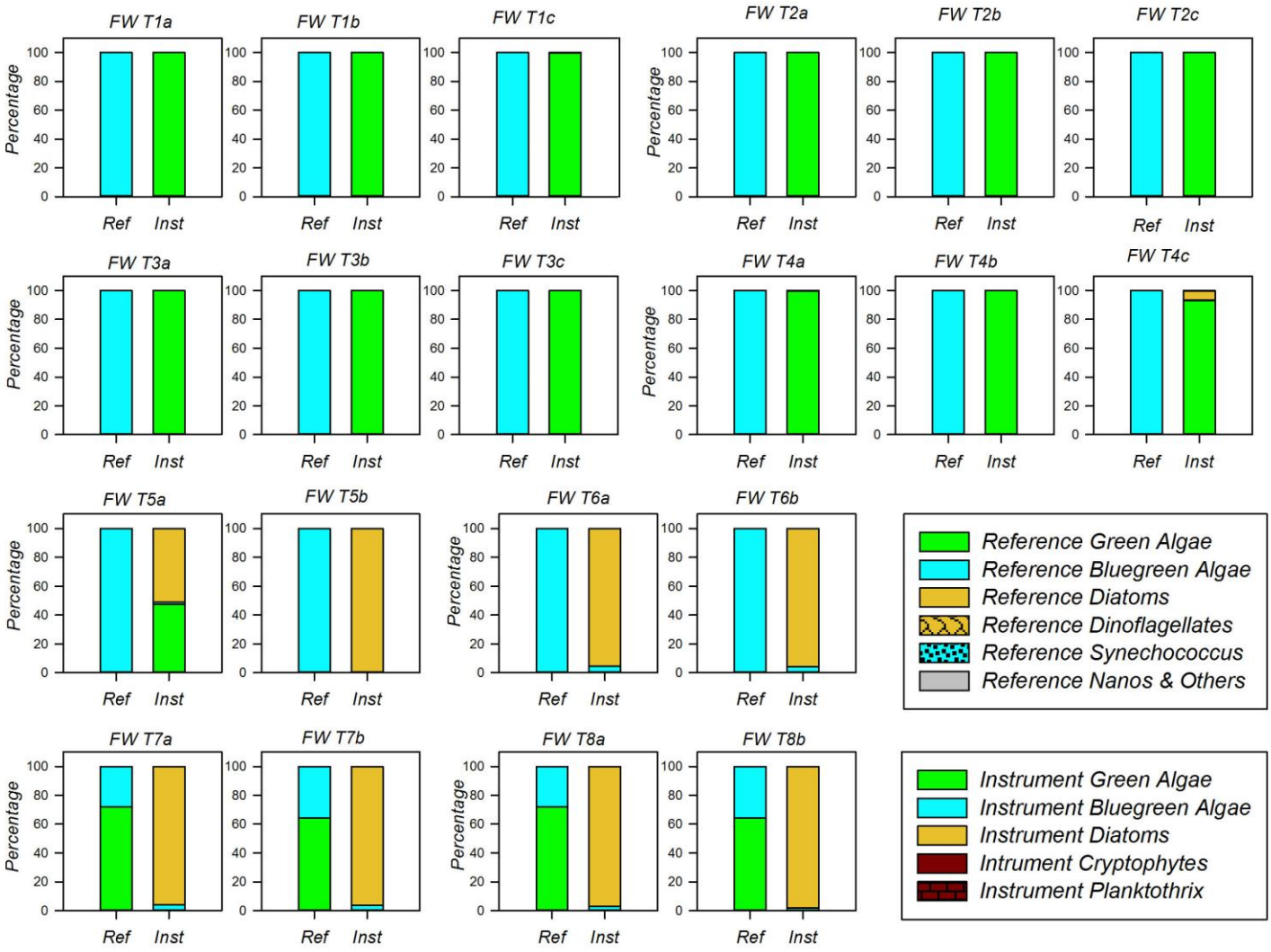


Figure 15. Reference cell counts as a percentage of biovolume compared to PhycoProbe taxonomic partitioning as a percentage of total chlorophyll-a.

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 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 involved 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 preceding 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. PhycoProbe results are taken as an average of 10 second readings around the 5th through 7th minutes of exposure.

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 denotes abundance at the higher C2 level and a lower case letter denotes the C1 abundance level which was 20% of C2. Reference sample CHL concentrations in µg/L for each culture addition (measured for monocultures and based on volumetric addition for mixtures) are provided in parenthesis.

Trial	*Chlorella* (ug/L)	*Cryptomonas* (ug/L)	*Aphanizomenon* (ug/L)	*Peridinium* (ug/L)	CDOM (A_{400})	Turbidity (NTU)
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 (Figure 16) as a time series of instrument measurements compared to extracted chlorophyll a concentrations in µg/L determined in the reference samples. The accuracy of the PhycoProbe estimations varied across the four different mono-cultures and hence the accuracy was affected by the proportion of the monoculture within the mixtures. In addition, the PhycoProbe tended to under-estimate CHL concentrations at the higher concentrations (approx. 60 µg/L) compared to the lower concentrations between 12-30 µg/L. Based on the monoculture exposures at a single concentration, the PhycoProbe most accurately estimated concentrations for *Cryptomonas* and *Peridinium*, over-estimated concentration for *Chlorella*, and under-estimated concentrations for *Aphanizomenon*. Individual response curves to the monocultures were not established to accurately quantify these biases.

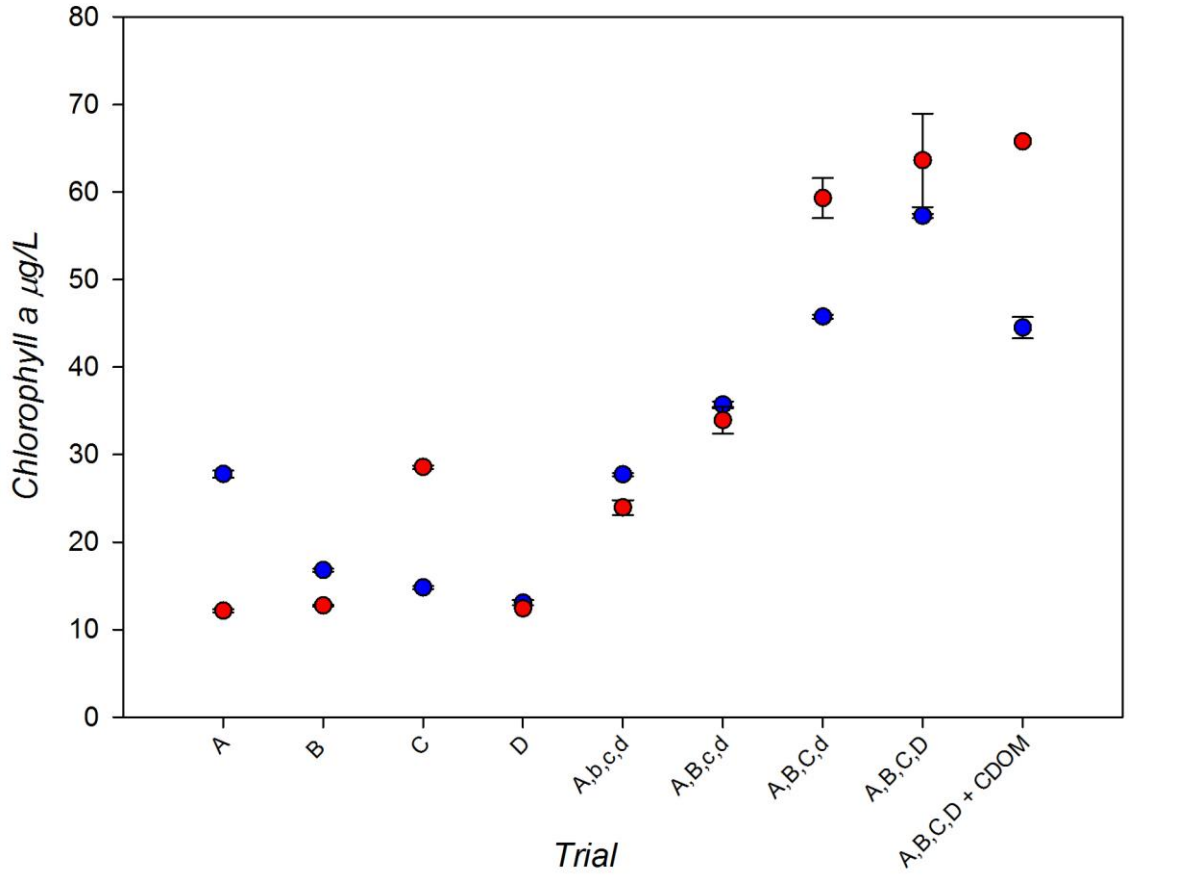


Figure 16. Plot of instrument (blue) and reference (red) measurements of chlorophyll a in the Great Lakes laboratory trial over 4 individual algae cultures, 4 mixtures and a CDOM and turbidity addition. One reference measurement was made at each level and instrument measurements were averaged over 5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

A one-to-one cross plot of the PhycoProbe readings compared to reference sample measurements during the freshwater algal mixture trials is shown in figure 17. Due to the species-specific response, the overall variation was greater, however, the regression line was highly significant ($p=0.001$) with an R^2 of 0.78 and a slope of 0.61. Also, this particular test instrument may not have been well suited to accurately measure these higher concentration levels.

A comparison of algal classification from microscope counts on preserved reference samples against the PhycoProbe classifications for the freshwater algal mixtures is shown in figure 18. The PhycoProbe misassigned the *Chlorella* culture as 65% Bluegreen and 35% Diatoms. The *Cryptomonas* culture was assigned as 60% Bluegreen and 30% Cryptophytes. The *Aphanizomenon* culture was correctly assigned in the Bluegreen category. The *Peridinium* was largely assigned to the correct golden-brown family but with about 10 % attributed to both Bluegreen and Cryptophytes. The algal mixtures were largely over-assigned to Green algae but the PhycoProbe did accurately assign the proportion of Bluegreens present. The addition of CDOM during the last trial caused Green algae to be reclassified in the golden-brown Diatom category.

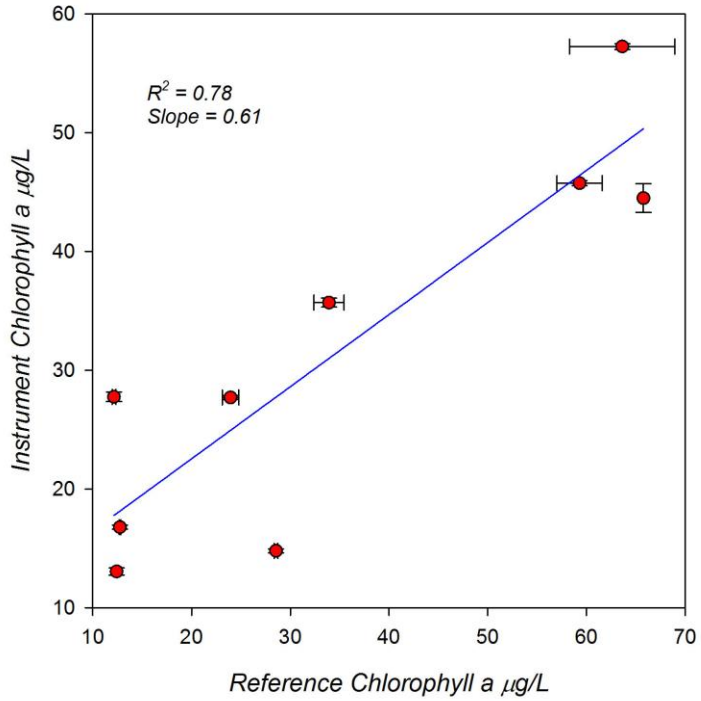


Figure 17. Response plot for the GL lab trial of the PhycoProbe compared to reference samples. The instrument values were obtained by averaging over 5 minutes. The blue line represents the linear regression of the data.

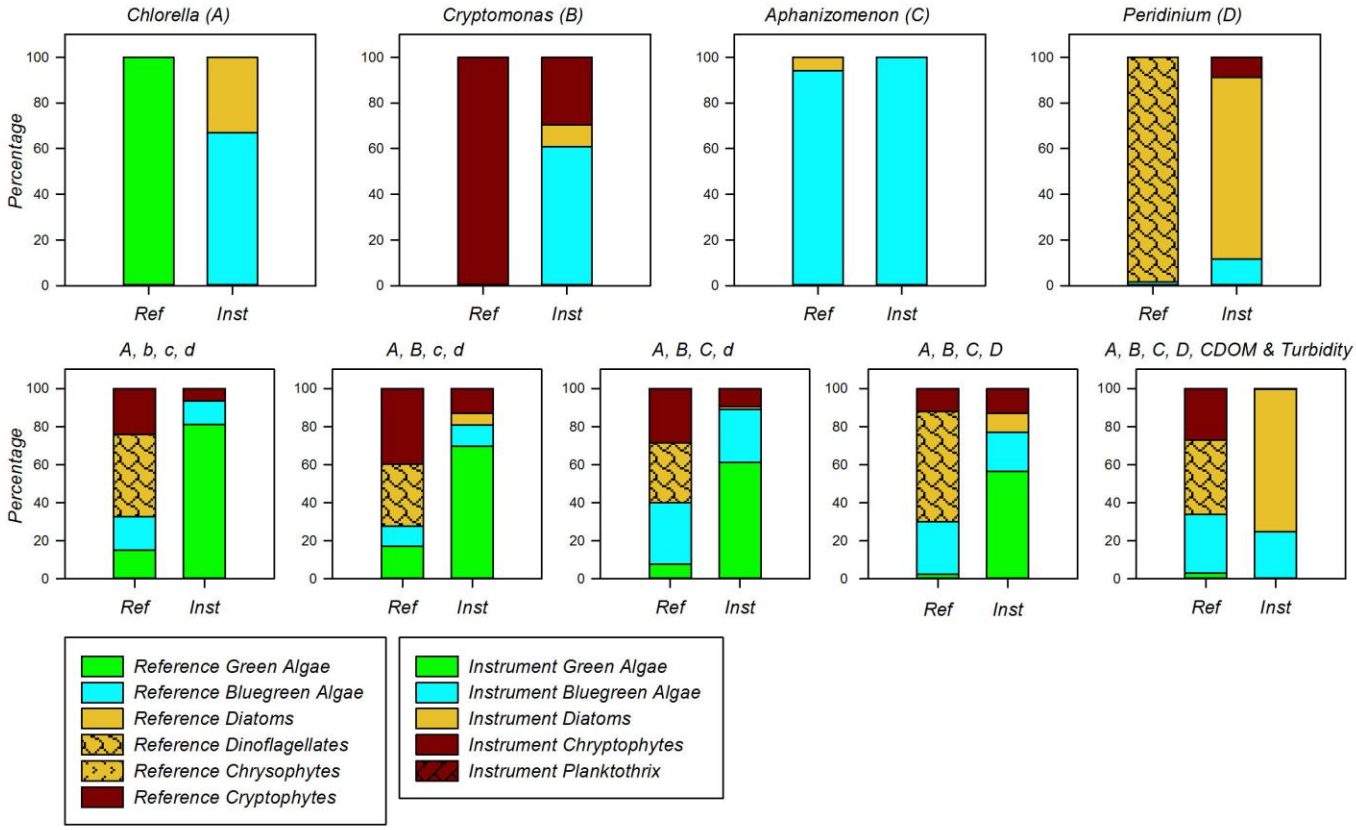


Figure 18. Reference cell counts as a percentage of biomass compared to instrument algae categories as a percentage of total chlorophyll a.

FIELD TESTS

Five field tests were conducted as part of the performance evaluation of the PhycoProbe 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 PhycoProbe and the consistency of its response over the time course of the evaluation.

Table 8. Results of the pre-deployment BB3 dye check for the PhycoProbe for each deployment 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**	0.0 ± 0.0	42.0 ± 0.28	351.2 ± 0.71
8/10/17	**UM**	0.01 ± 0.01	38.1 ± 0.16	322.3 ± 0.51
9/05/17	**CBL**	n.d.	38.3 ± 0.31	323.3 ± 0.31

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 plankton 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* (*photo at right*). 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 (Figure 19). During the 150 km underway mapping cruise eight stations were selected to make comparative reference sample measurements.

R/V David H. Peterson

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 with sensors oriented toward the bottom of the tank. Coordinated rotation of the rack and instruments was used to clear accumulated bubbles and debris. Port valves were open during between station transits 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.

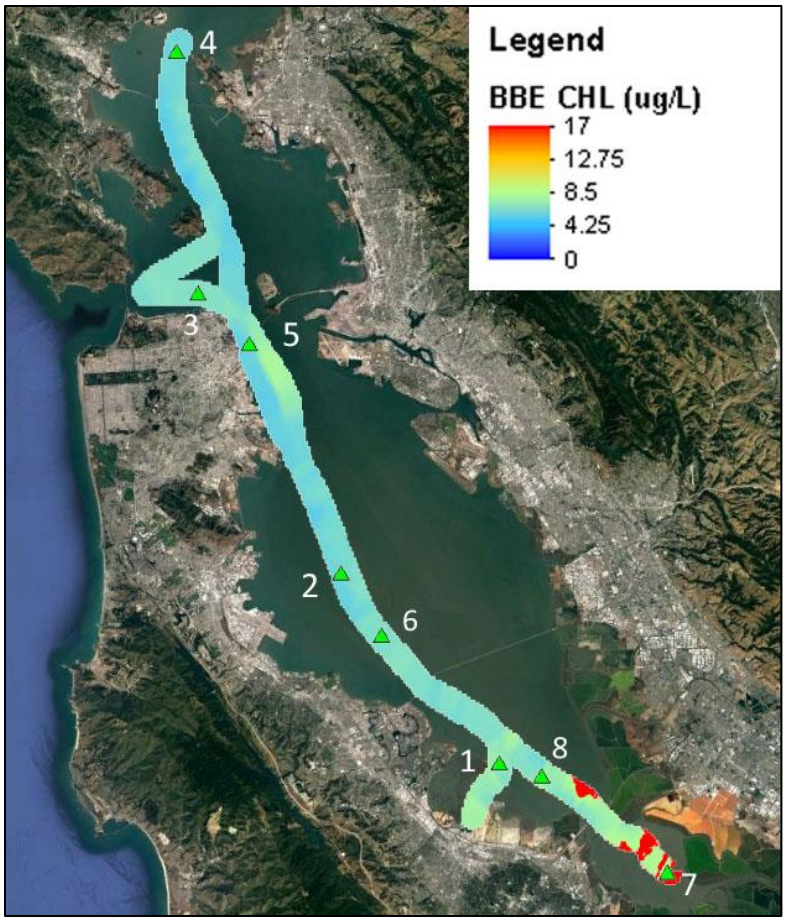


Figure 19. Chlorophyll data contours from the Phycoprobe during the underway surface mapping survey in San Francisco on the *USGS R/V Peterson*. Green triangles denote isolated, compartive sampling stations.

During the survey the PhycoProbe produced 445 readings, all of which were considered acceptable for a successful data return of 100% (Fig. 19). CHL estimations from the PhycoProbe ranged from 4.0 to 11.3 µg/L over the entire survey. Algal classifications during the survey 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 sample 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.

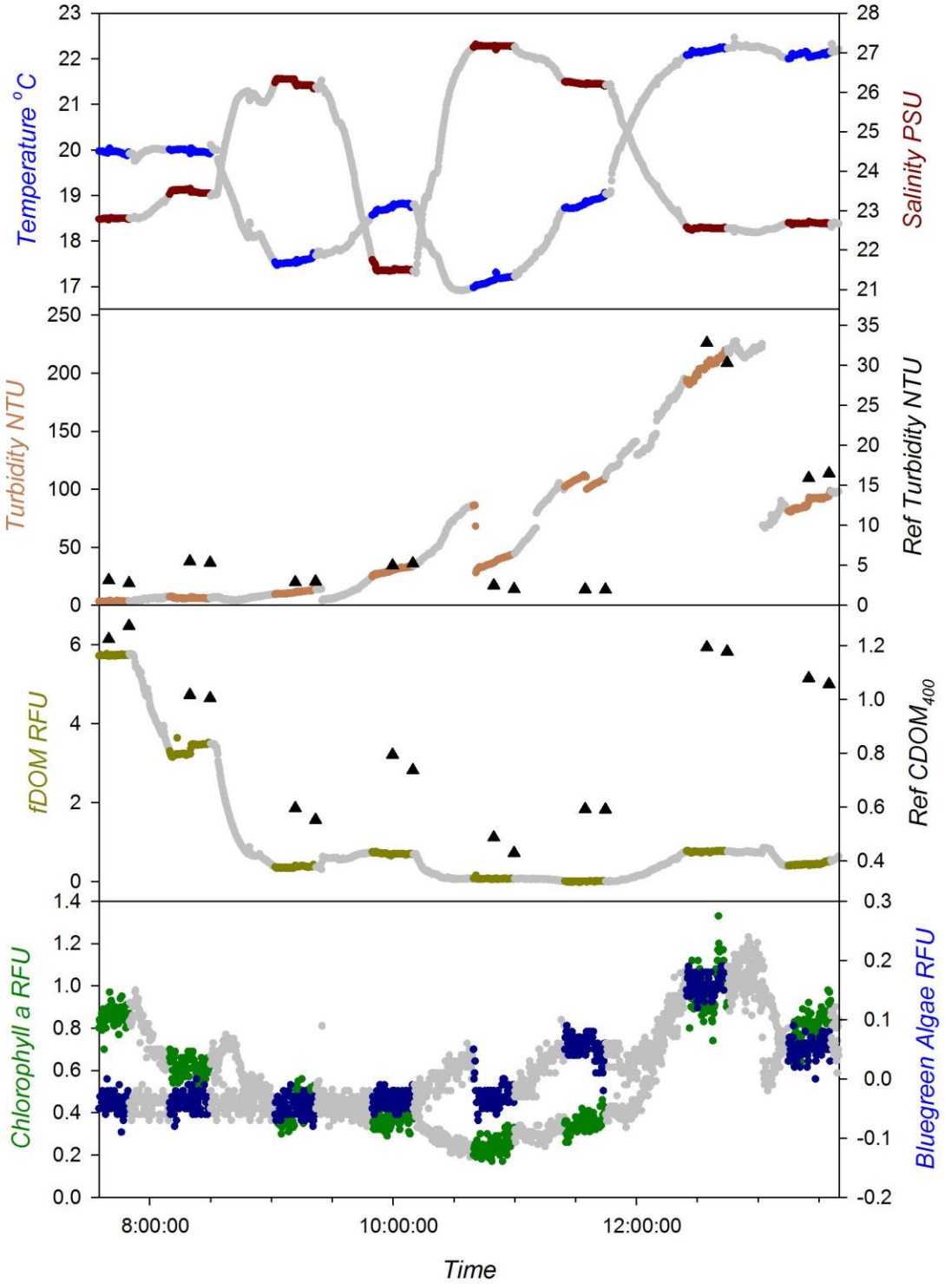


Figure 20. Time series of water conditions encountered during the surface mapping cruise in San Francisco Bay. *Top Panel:* Variation in temperature (blue) and salinity (red) at the depth of the sensors in the flow through tank, measured by an EXO 2 Sonde. *Second Panel:* Turbidity (brown) as measured 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 $CDOM_{400}$ absorbance (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 PhycoProbe 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 PhycoProbe CHL measurements ranged from 3.96 - 11.3 µg/L and were congruent with their corresponding reference samples. In contrast extractable phycocyanin, a proxy for cyanobacterial biomass, was low throughout the survey, ranging from 0 to 0.12 µg/L. The PhycoProbe estimation of cyanobacteria chlorophyll (% cyano X total CHL) was below 0.5 µg/L for stations 1 – 6, about 3 µg/L for station 7 and 1 µg/L for station 8 (data not shown).

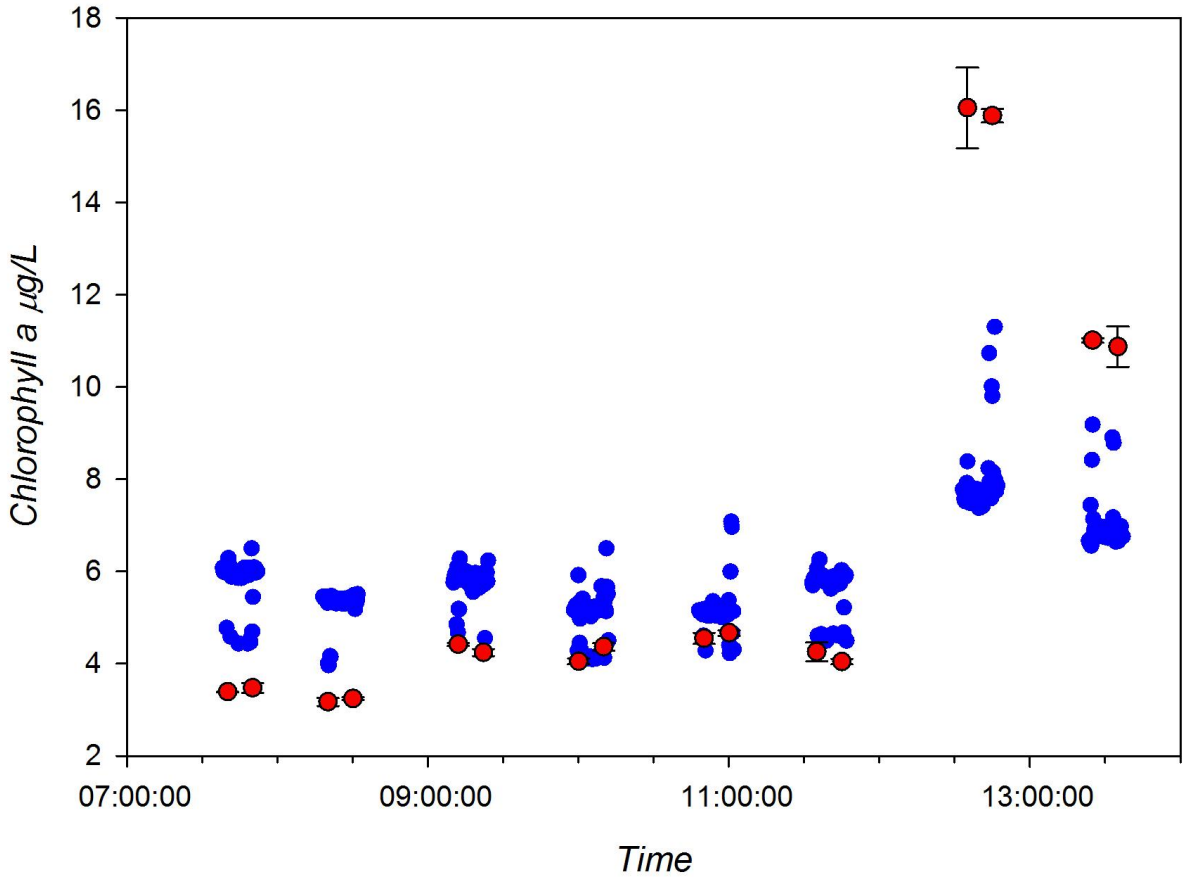


Figure 21. Plot of instrument (blue) and reference (red) measurements of chlorophyll a during San Francisco Bay surface mapping. Two reference measurements were made at each station, and the instrument data was averaged over two minutes bracketing the reference samples.

A linear regression of the PhycoProbe chlorophyll measurements against the extracted chlorophyll (Figure 22) was highly significant ($p<0.001$) with an $R^2=0.89$ and a slope of 0.24.

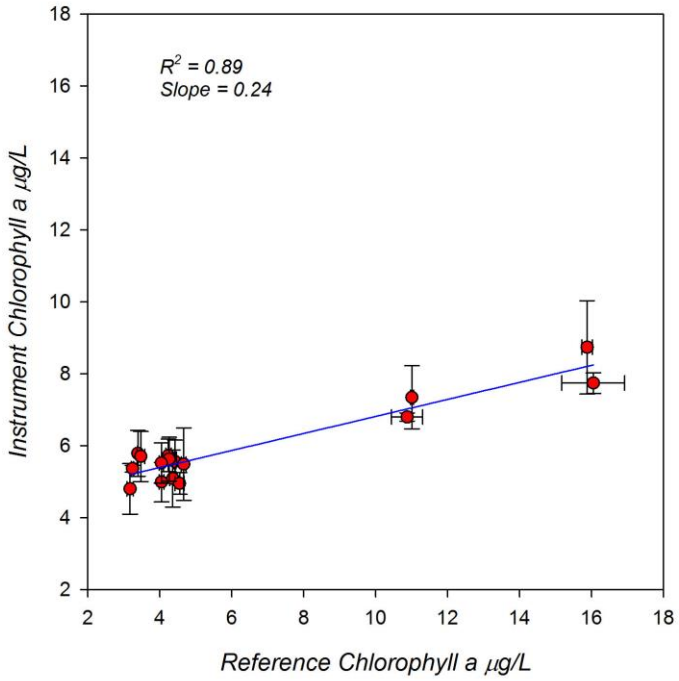


Figure 22. San Francisco Bay surface mapping response plot of the PhycoProbe chlorophyll measurements compared to reference chlorophyll measured. The blue line represents the linear regression of the data.

A comparison of algal classification from microscope counts on preserved reference samples from the survey transects relative to the PhycoProbe classifications 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. The PhycoProbe broadly captured the photosynthetic phytoplankton classifications although the relative biomass partition diverged relative to microscopic counts. It was not possible to distinguish whether ciliates were non-fluorescent or fluorescent due to recent consumption of phytoplankton prey. In general, the PhycoProbe overestimated the relative abundance of chlorophyte algae along the transect and underestimated relative diatom (=diatom+dinoflagellate in PhycoProbe). Other classifications provided by the PhycoProbe were consistent with the microscope observations.

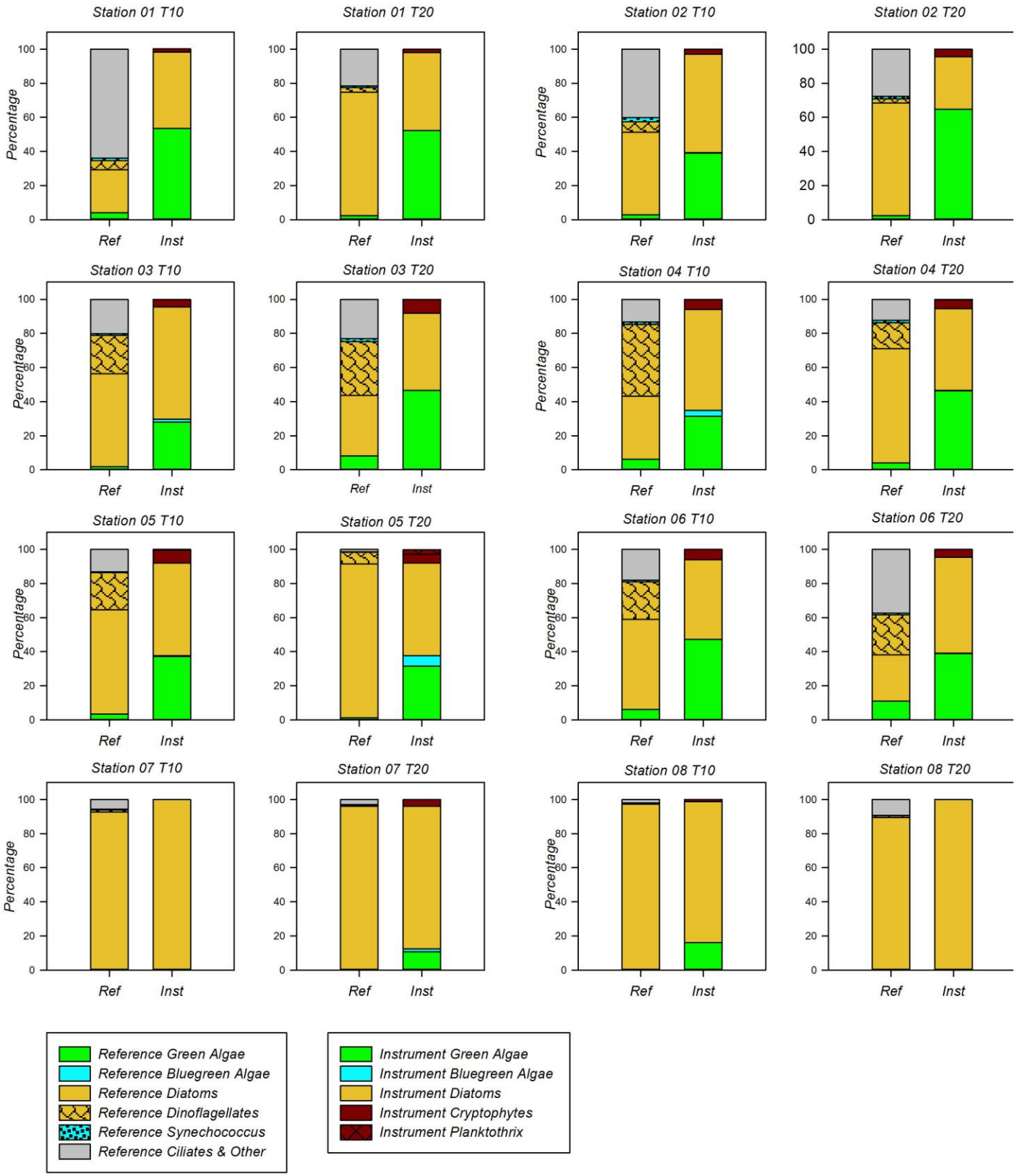


Figure 23. San Franscisco Bay surface mapping reference cell counts as a percentage of biovolume compared to PhycoProbe algal taxonomic categories as a percentage of total chlorophyll a.

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 (*photo below*). The *R/V JH Martin* was 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 was mixed manually during the sampling process. Comparative reference samples were 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, then along the 30 m isobath to assess near shore communities impacted by the combination of urban and agricultural watersheds feeding the coastal waters from Santa Cruz to Moss Landing Harbor.

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 a little 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 PSU) and lower temperature waters (14 – 16.5 °C). Reference sample analyses for CDOM (0.03 to 0.16 A_{400}) and turbidity (0.5 to 1.3 NTU) were also lower and covered a narrower range. Concentrations of extracted chlorophyll were similar in range (ca 5 – 15 µg/L) to the SF Bay observations 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.

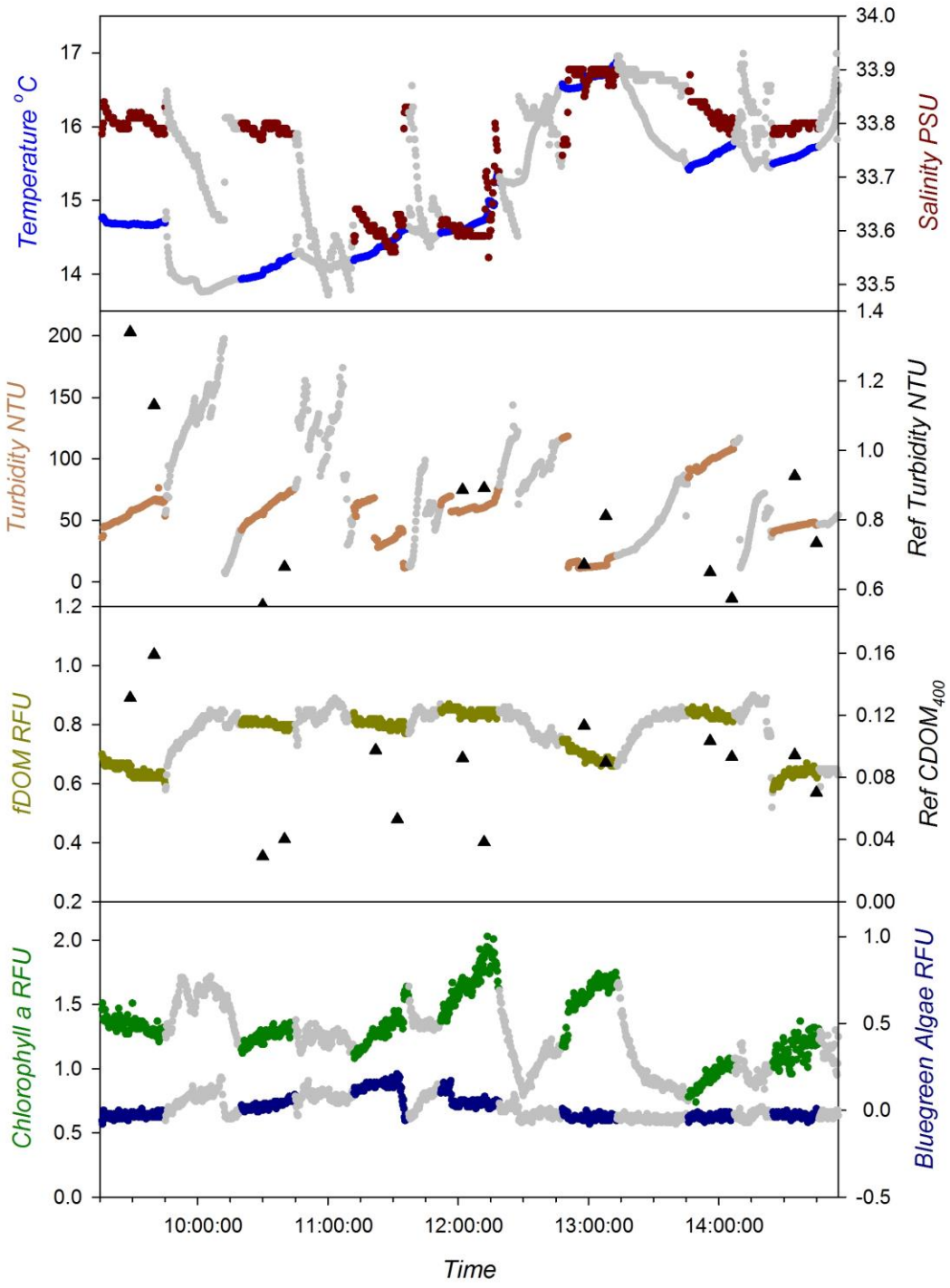


Figure 24. Water conditions encountered during the surface mapping in Monterey Bay. *Top Panel:* Variation in temperature (blue) and conductivity (red) at the depth of the sensors in the flow through tank, measured by an EXO 2 Sonde. *Second Panel:* Variation of turbidity (brown) as measured by the EXO 2 and discrete samples (black triangles) taken from the tank during reference sampling and measured on a HACH 2100AN Turbidimeter. Transients observed between reference sample periods in the EXO 2 time series for T, S and fDOM, reflect periods of partial draining of the exposure tank to promote enhanced water exchange for the next reference sample. *Third Panel:* fDOM (olive) as measured by the EXO 2, and $CDOM_{400}$ measured in discrete samples on an Agilent 8453 spectrometer (black triangles). *Bottom Panel:* Time series of dissolved chlorophyll a (green) and bluegreen algae (blue) as measured by the EXO 2 Sonde.

During the survey, the PhycoProbe produced 498 readings all of which were considered acceptable for a successful data return of 100%. CHL estimations by the PhycoProbe ranged from 7.4 to 15.2 µg/L over the entire survey (Figure 25). The range in extracted phycocyanin from reference samples was only 0.1 to 0.3 µg/L and extracted PE was undetectable. The PhycoProbe identified slightly higher contributions with a maximum of 0.5 µg/L as Cyanobacteria and 0.9 µg/L as Cryptophytes. Phytoplankton community compositions encountered along the survey transect are described below.

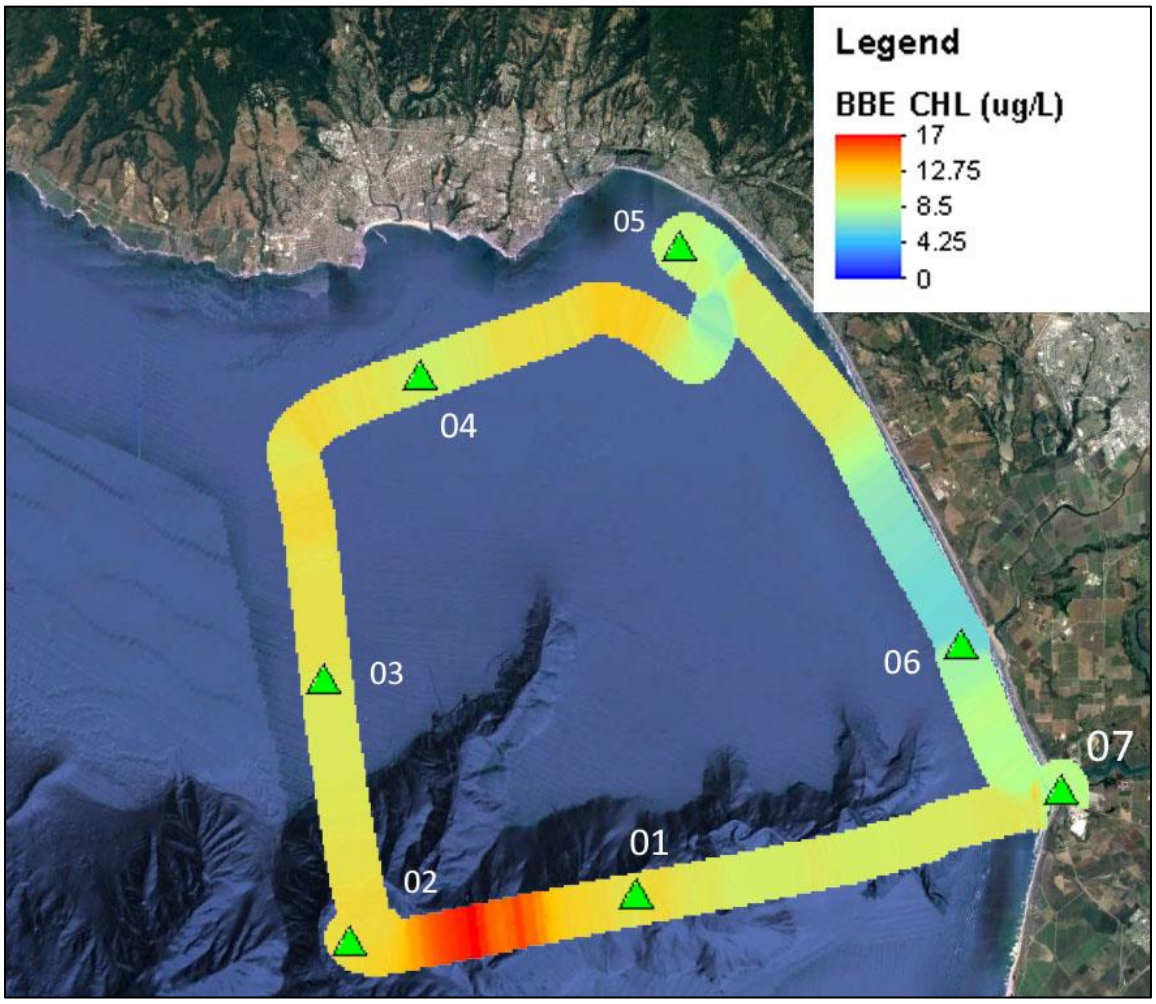


Figure 25. PhycoProbe chlorophyll data contours during the continuous underway surface mapping cruise in Monterey Bay onboard the *R/V JH Martin*. Triangles denote stations where the flow-through tank was isolated and comparative reference samples analyzed.

A time series of the PhycoProbe CHL measurements are plotted against the corresponding reference measurements during the seven isolation periods (Figure 26). During the isolated exposures CHL measurements for the PhycoProbe ranged from 7.4 to 15.2 µg/L compared to a range of 4.8 to 14.7 µg/L for the reference data. However, there was significant variability in both the PhycoProbe and reference sample grabs within the tank during the isolation period which limits the ability to accurately assess the response. Despite that variability, the linear regression for instrument versus reference CHL estimation was still significant ($p=0.007$) but with a lower $R^2=0.47$ and a slope of 0.26 (Fig. 27).

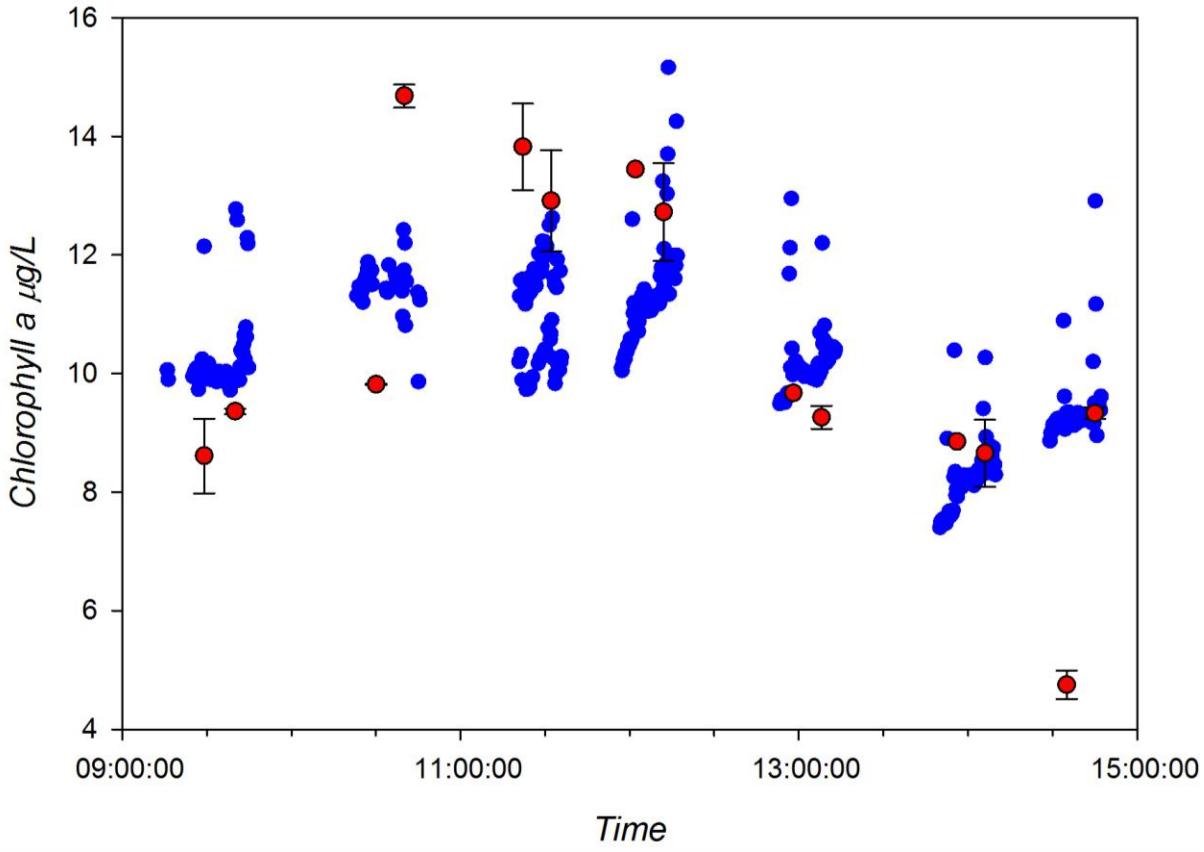


Figure 26. Plot of instrument (blue) and reference (red) measurements of chlorophyll a during Monterey Bay surface mapping. Two reference measurements were made at each station level while instrument measurements were averaged over 2 minutes bracketing the reference sample.

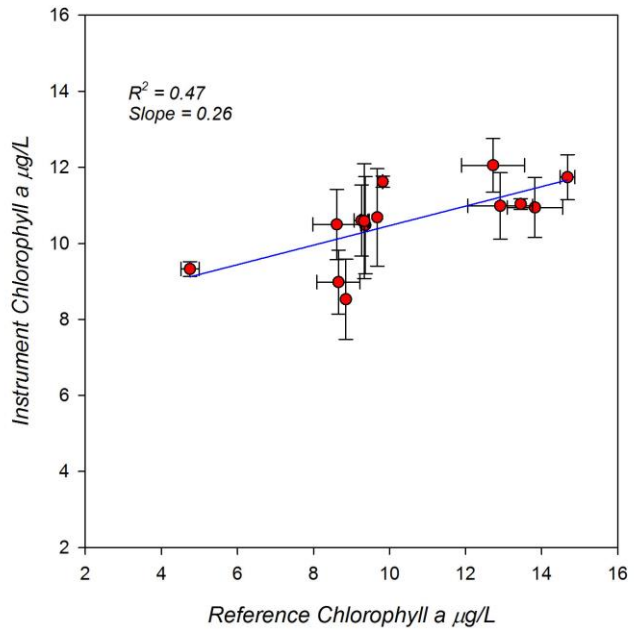


Figure 27. Monterey Bay surface mapping response plot of the PhycoProbe chlorophyll a measurements compared to reference chlorophyll a measured in µg/L. The blue line represents the linear regression of the data.

A comparison of algal classification from microscope counts on preserved reference samples from the survey transects relative to the PhycoProbe classifications 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 were generally dominated by diatoms and dinoflagellates. Present throughout the survey at lower abundance were ciliate grazers and nanoflagellates of unknown pigment composition along with euglenoid cell types. The PhycoProbe broadly captured the photosynthetic phytoplankton classifications although relative biomass partition diverged relative to microscopic counts. It was not possible to distinguish whether ciliates were non-fluorescent or fluorescent due to recent consumption of phytoplankton prey. In general, the PhycoProbe overestimated the relative abundance of chlorophyte algae along the transect (7/14 comparisons) and underestimated relative diatom abundance (=diatom+dinoflagellate in PhycoProbe). While marine cyanobacteria were observed at low abundance (<5% biovolume) in the majority of reference stations, this contribution was not detected by the PhycoProbe.

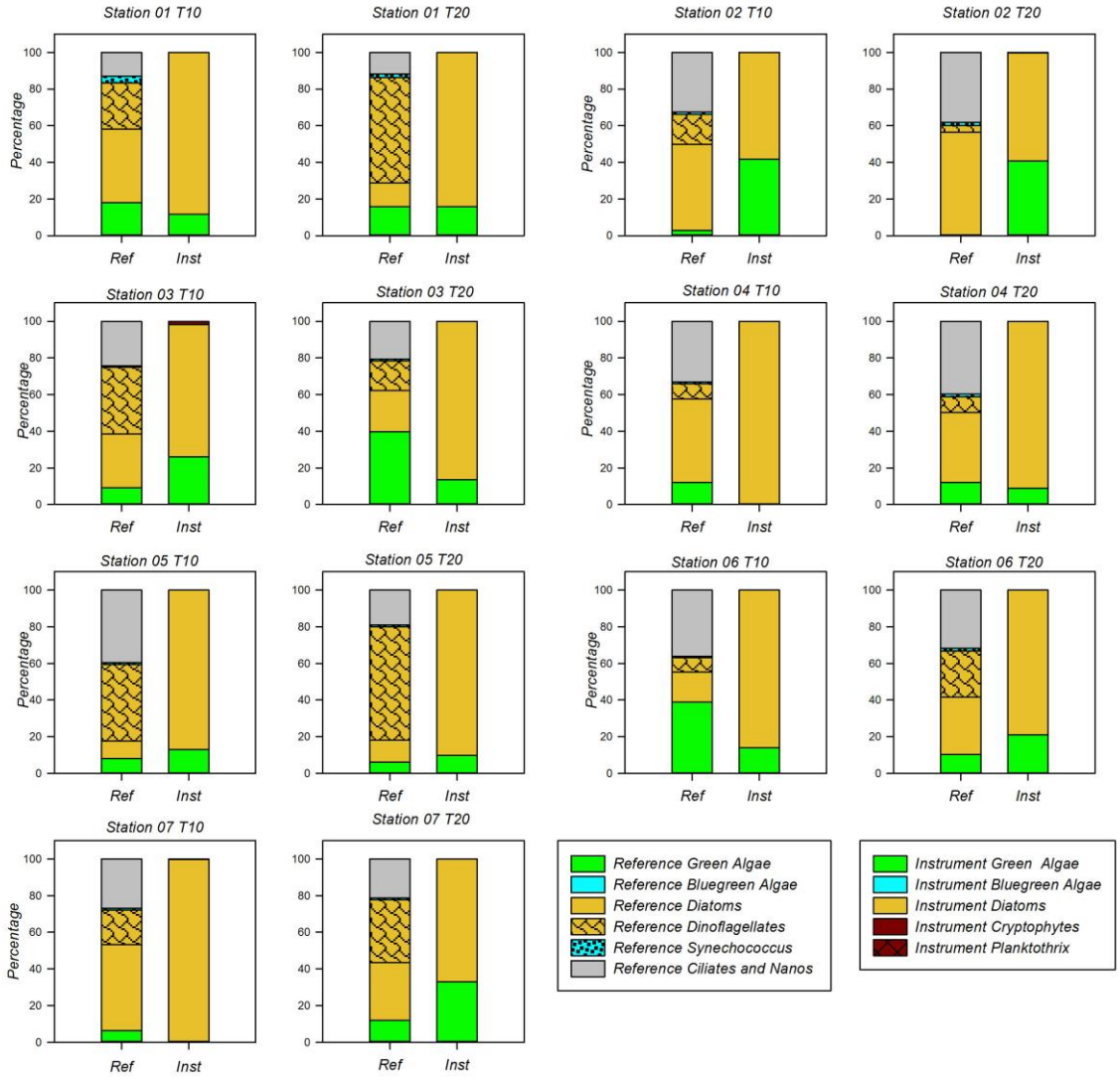
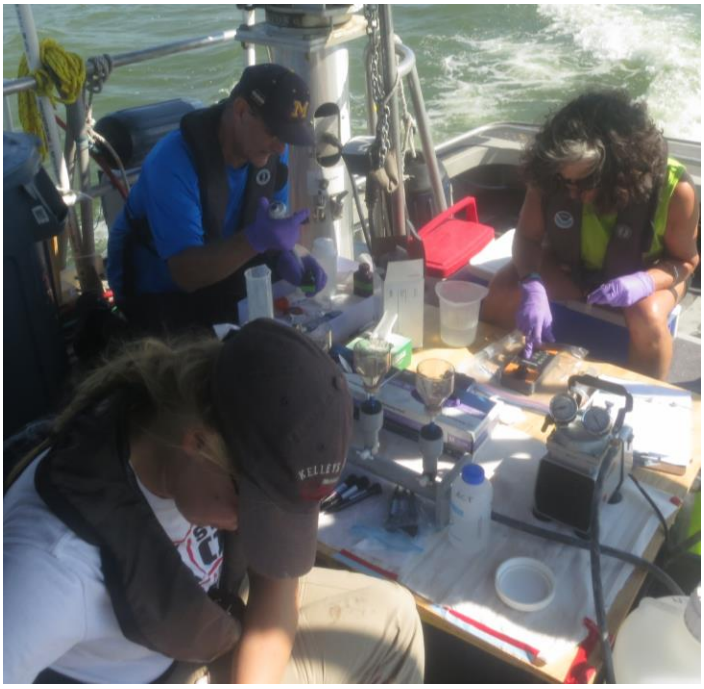


Figure 28. Monterey Bay surface mapping reference cell counts as a percentage of biovolume compared to PhycoProbe algal taxonomic categories as a percentage of total chlorophyll a.

Lake Erie Surface Mapping

A surface mapping cruise was conducted in the western basin of Lake Erie on August 16th onboard the NOAA GLERL *R/V4108* (*photo at right*). The survey covered a 75 km range, including sites from the mouth of the Maumee River out to open waters 20 km offshore. The survey occurred during an intense cyanobacterial bloom dominated by *Microcystis*. During the underway mapping cruise, 7 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, keeping it well mixed with manual stirring. After an initial equilibration period of 5 minutes, reference samples were taken at timepoints of 5 and 20 minutes from the time of isolation.



A YSI EXO2 sonde in the tank provided continuous monitoring results during the underway and isolated time periods with measurements taken every 15 seconds (Figure 29). Continuous measurements indicated that conditions in the tank during isolation were more variable at high bloom stations, likely reflecting the colonial nature of *Microcystis* and its high buoyancy when isolated. Reference sample analyses for CDOM and turbidity are plotted over the sonde data for comparison with CDOM absorbance ranging from 0.5 to 1.7 and turbidity ranging from 2.4 to 141 NTU. During the survey, temperature ranged from 24 to 25.2 ºC and specific conductivity ranged from 260 to 370 µS/cm, reflecting a gradient in nearshore to open lake conditions. The continuos CHL and BGA readings from the sonde showed significant spikes in cyanobacterial abudance as the survey transited nearshore, especially outside of Maumee Bay.

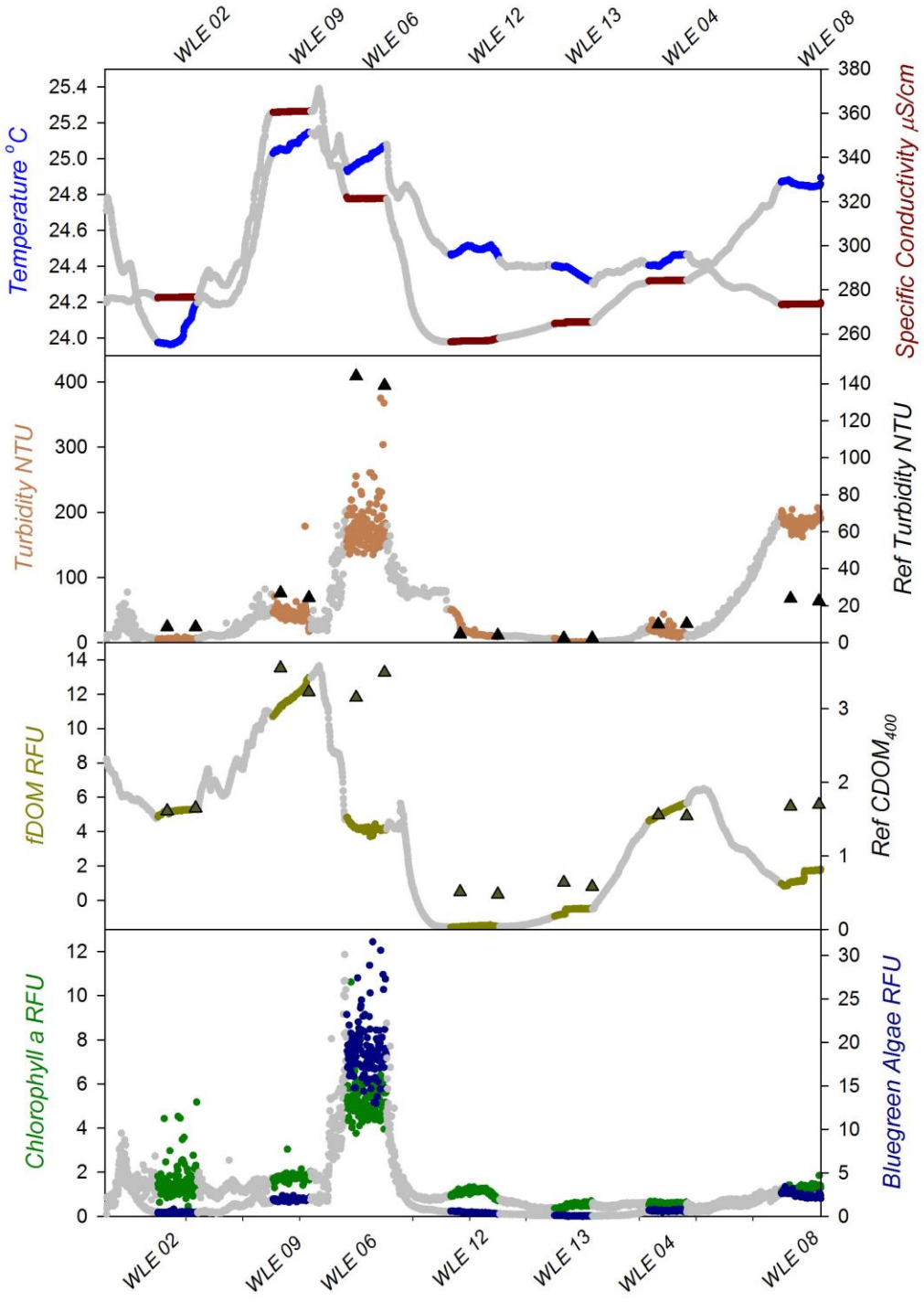


Figure 29. Water conditions encountered during the surface mapping in Western Lake Erie. *Top Panel:* Variation in temperature (blue) and conductivity (red) at the depth of the sensors in the flow through tank, measured by an EXO 2 Sonde. *Second Panel:* Variation of turbidity (brown) as measured by 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 $CDOM_{400}$ measured in discrete samples on an Agilent 8453 spectrometer (black triangles). *Bottom Panel:* Time series of dissolved chlorophyll a (green) and bluegreen algae (blue) as measured by the EXO 2 Sonde.

During the western Lake Erie survey the PhycoProbe produced 1971 readings all of which were considered acceptable values for a successful data return of 100% (Figure 30). CHL estimations by the PhycoProbe ranged from 4.5 to 103 µg/L over the entire survey compared to a range of 11 to 833 µg/L for extracted chlorophyll samples. The range in extracted phycocyanin from reference samples was 0.9 to 705 µg/L (data not shown) compared to the fluorescence contribution attributed to Cyanobacteria by the PhycoProbe of 0 to 12 µg/L. Algal classifications during the survey are described below.

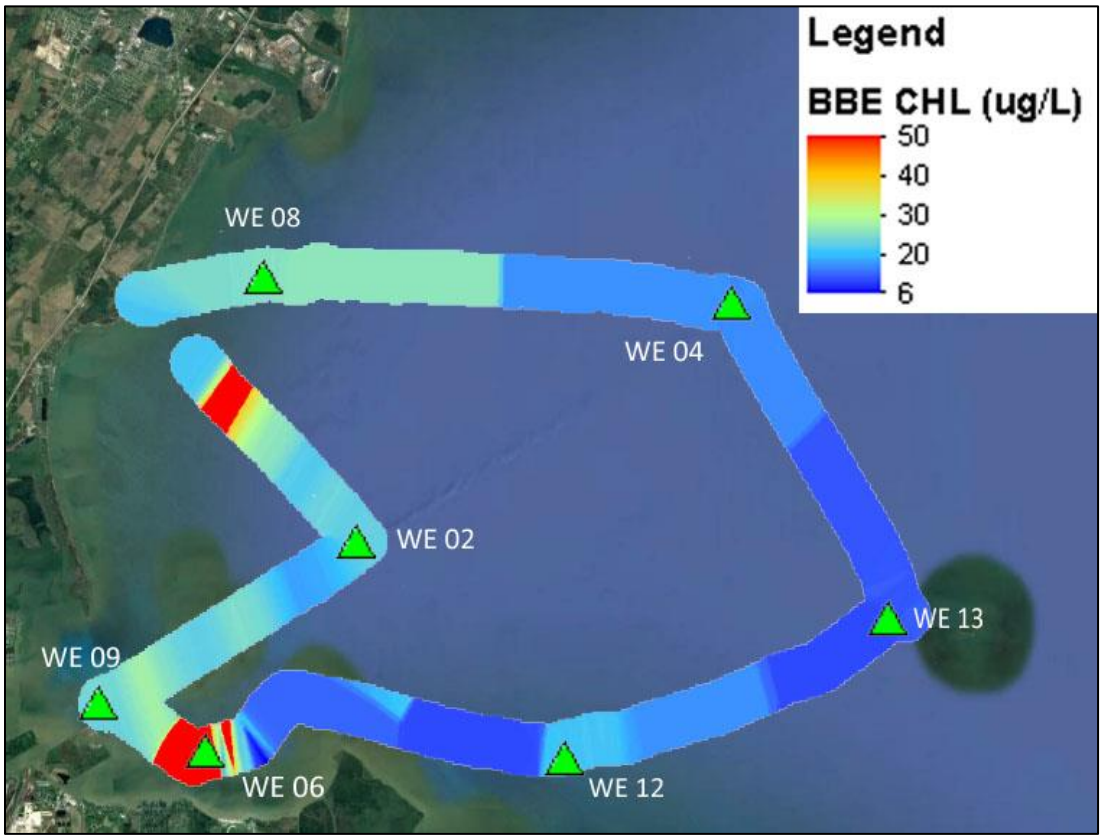


Figure 30. PhycoProbe continuous underway chlorophyll data during the surface mapping cruise in Western Lake Erie. Triangles denote stations where the flow-through tank was isolated and comparative reference samples analyzed.

Comparative chlorophyll estimations between the PhycoProbe and reference samples during the isolated sampling timepoints are plotted in figure 31. Chlorophyll measurements for the PhycoProbe during the isolation periods ranged from 4.5 to 72 µg/L and tracked the overall pattern, albiet at reduced concentrations relative to extracted reference sample concentrations which ranged from 11 to 833 µg/L.

A one-to-one cross plot of the PhycoProbe versus reference sample chlorophyll measurements, with data from WLE 06 omitted due to its extreme value, is shown in figure 32. The linear regression was significant ($p<0.001$) with an $R^2=0.75$, but with a slope of only 0.13. The much lower instrument response per unit of extracted chlorophyll was not unexpected based on the previous testing and calibration settings of the instrument as tested. Furthermore *Microcystis* colonies like those present during this bloom have very low fluorescent responses relative to their pigment content.

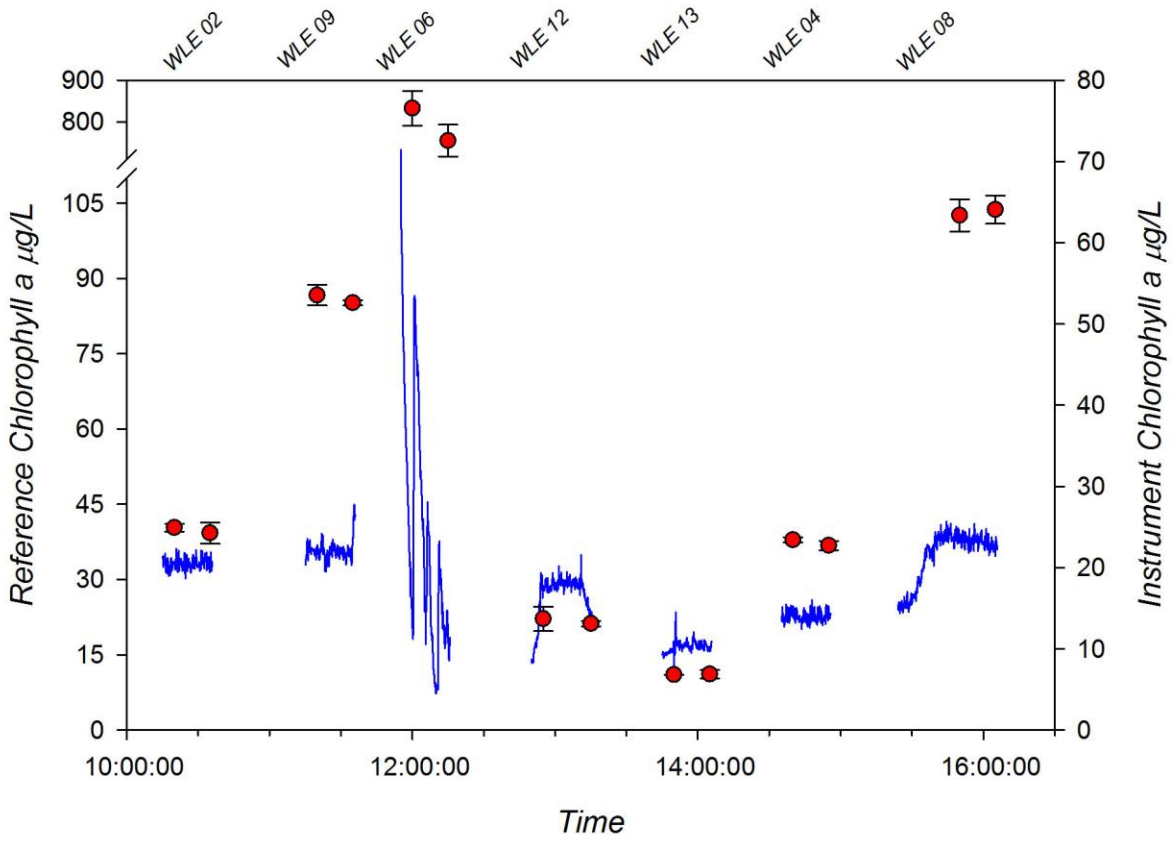


Figure 31. Time series plot of the PhycoProbe chlorophyll (blue) and reference (red) during surface mapping deployment on Lake Erie. Two reference measurements were made at each station, and instrument data were averaged over 2 minutes bracketing the reference sample time.

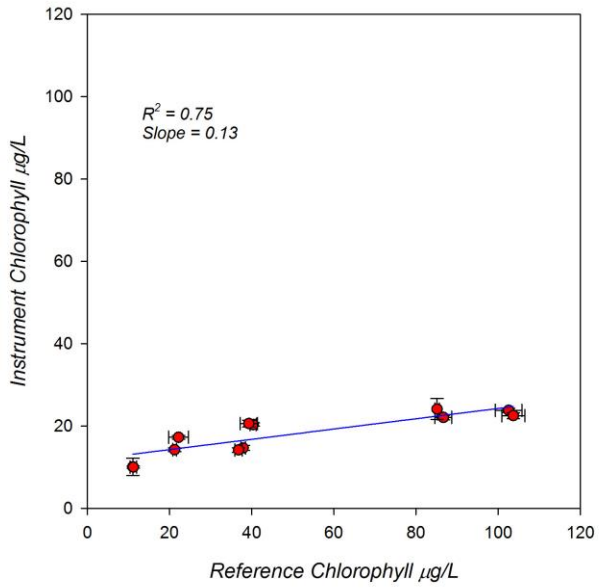


Figure 32**.** Lake Erie surface mapping response plot for the PhycoProbe chlorophyll measurements compared to reference chlorophyll a. The blue line represents the linear regression of the data.

A comparison of algal classification from microscope counts on preserved reference samples against the PhycoProbe classifications for the Lake Erie surface mapping samples is shown in figure 33. The PhycoProbe classifications for the two timepoints within each isolated station were quite consistent with a similar amount of variance noted in the reference sample microscope counts. In general, the PhycoProbe over-classified Green algae relative to microscopy counts which identified a much greater proportion of Diatoms. The relative abundance of Bluegreen algae was quite accurately predicted across the range of samples, with the one exception of WLE 06 where the biomass was extremely high. At this site, the PhycoProbe characterized the community composition as between 30 to 70% Cryptophythes/Planktothrix whereas microscopy identified 85 to 95% Cyanobacteria.

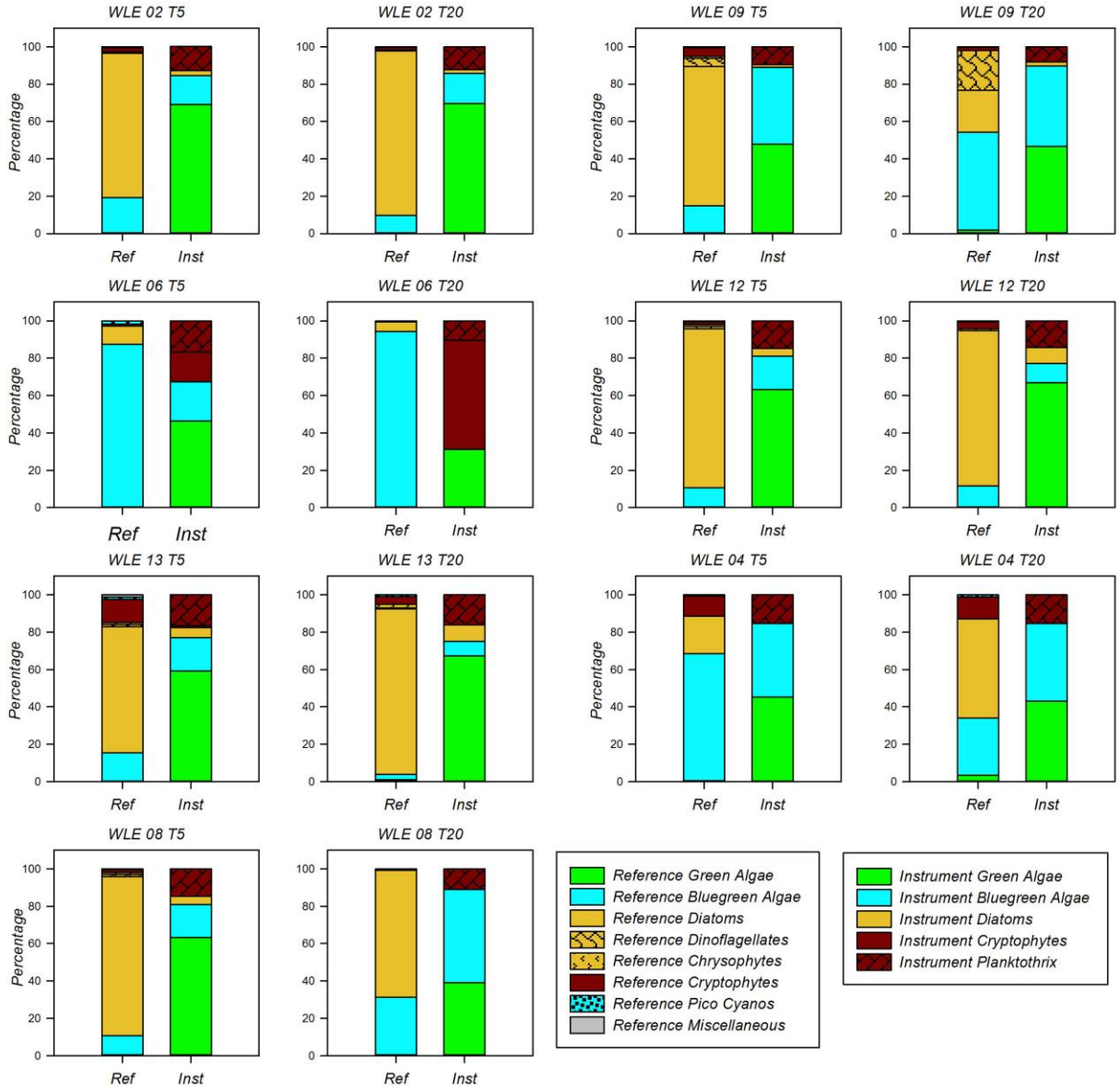


Figure 33. Reference cell counts as a percentage of biomass compared to instrument algae categories as a percentage of total chlorophyll a.

Field Deployment at Maumee River, Waterville, Ohio

A 13-day field deployment occurred from July 25 through August 7 in the Maumee River 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 upriver 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 moored deployment, the PhycoProbe collected 621 observations with all data accepted for a data return rate of 100%. The range of CHL measured by the PhycoProbe during the entire deployment was 4.5 to 131 µg/L and the fluorescence contribution attributed to Cyanobacteria ranged from 0 to 10.2 µg/L.

Time series results of ambient conditions for temperature, specific conductivity, turbidity, fDOM, chlorophyll and bluegreen algae measured in the flow-through tank by an EXO2 sonde are given in figure 35. Reference sample turbidity and $CDOM_{400}$ are overlaid for comparison with turbidity ranging from 21.6 to 78.3 NTU and CDOM absorbance ranging from 4.5 to 5.6. During the deployment, temperature ranged from 23.1 – 29.4ºC and discharge varied by a factor of 5x from 2000 to 10,000 cfs. The continuous sonde data indicated a 10-fold range in chlorophyll and phycocyanin RFU over the deployment, with noticeable patterns across diurnal cycles and river discharge cycles.

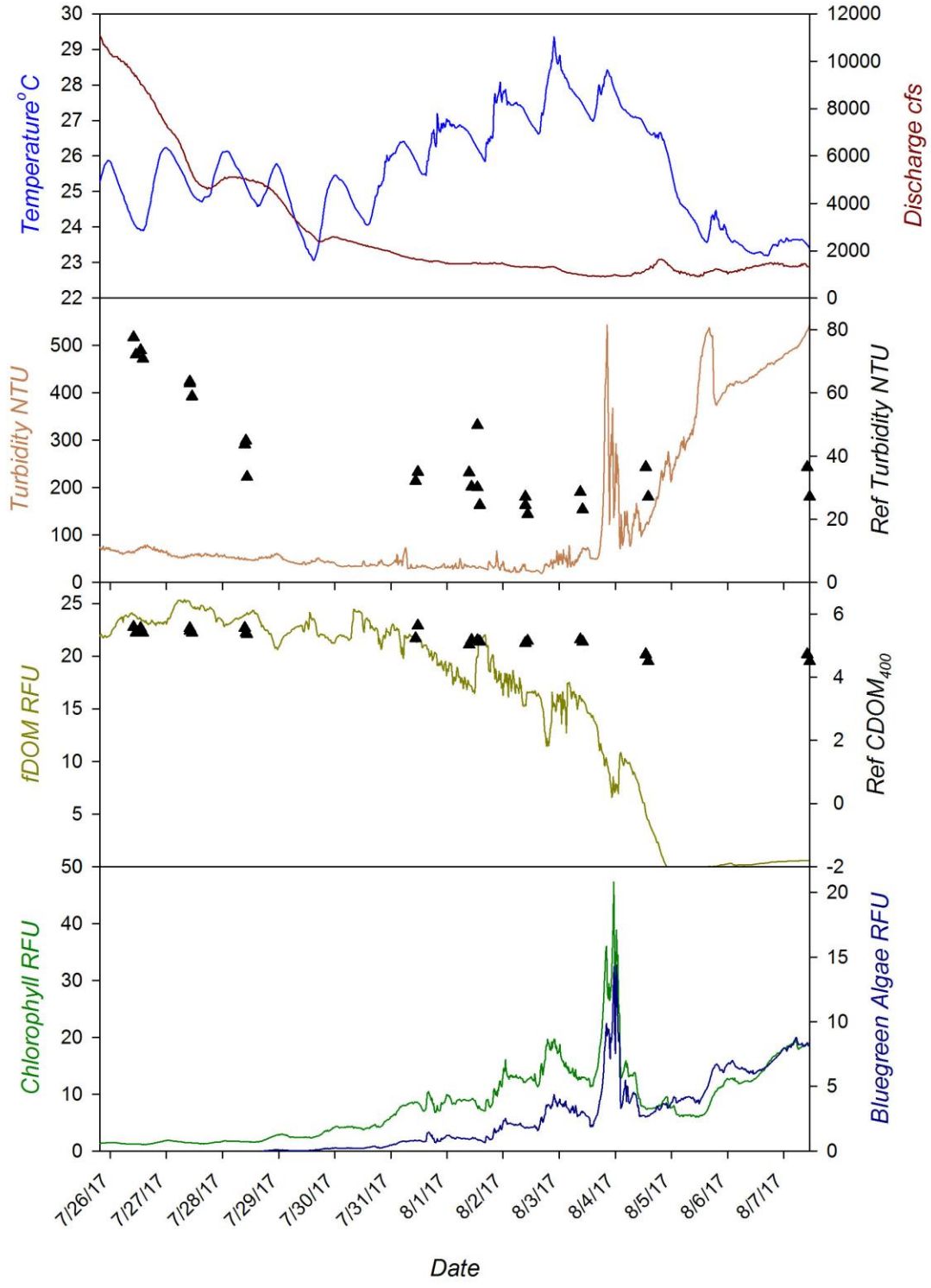


Figure 35. Environmental conditions encountered during the 13 day freshwater deployment in the Maumee River at Waterville, OH. *Top Panel:* Variation in temperature (blue) and Conductivity (red) at the depth of the sensors, measured by an EXO 2 Sonde. *Second and Third Panels:* Variation of turbidity (brown) and fDOM (olive) at the depth of the sensors, measured by an EXO2 Sonde and $CDOM_{400}$ measured in discrete samples on an Agilent 8453 spectrometer (black triangles). *Bottom Panel:* Time series of chlorophyll (green) and bluegreen algae (blue) as measured by the EXO 2 Sonde.

A time series of the PhycoProbe CHL measurements is plotted against the corresponding reference measurements for the Maumee River deployment in figure 36. Chlorophyll measurements by the PhycoProbe ranged from 4.5 to 131 µg/L during the entire deployment and from 4.5 to 107 µg/L during the isolated sampling periods. CHL concentrations from corresponding reference samples over that same period ranged from 9.5 to 119 µg/L, which was a significantly better agreement than seen for Lake Erie surface mapping.

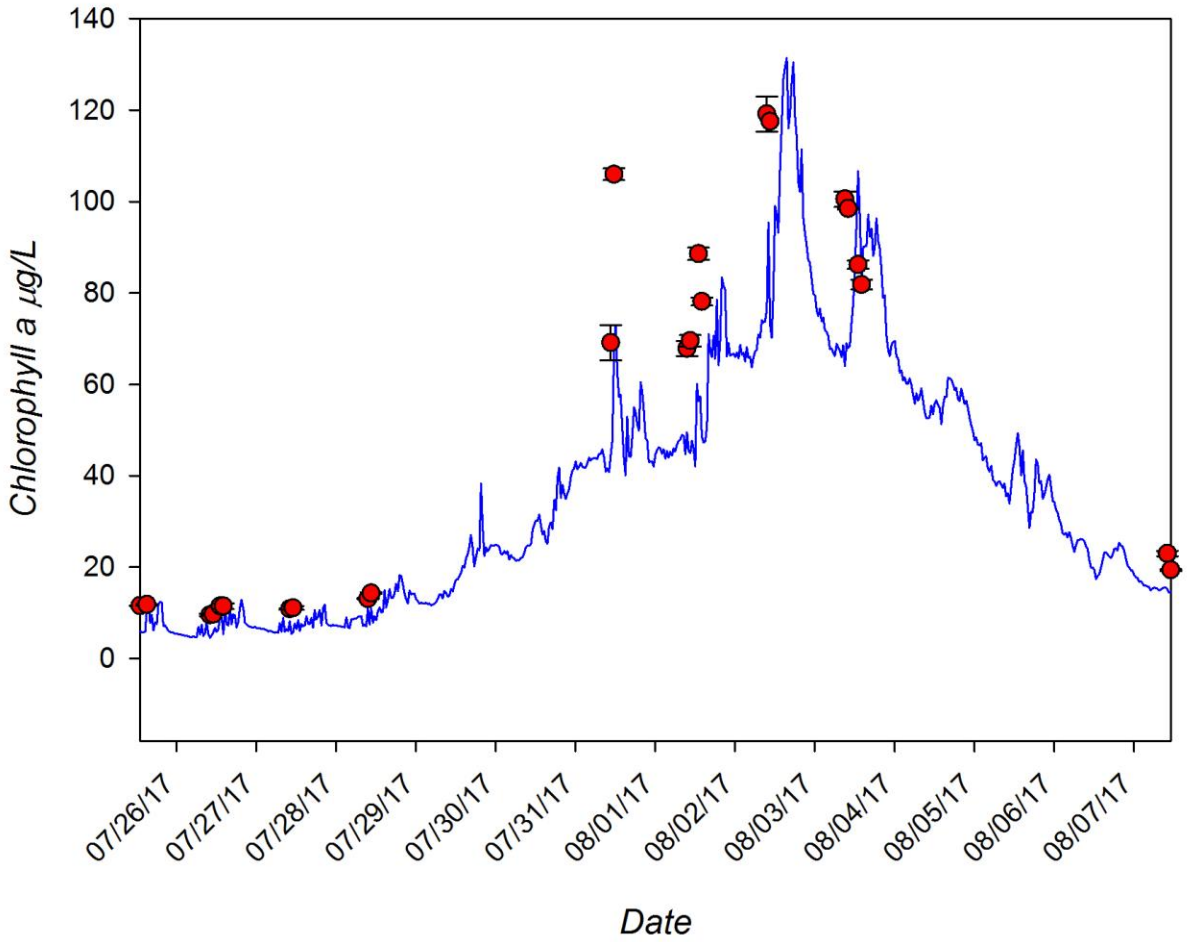


Figure 36. Time series plot of the PhycoProbe measurements (blue) and reference measurements (red) of chlorophyll a during the freshwater deployment in the Maumee River at Waterville, OH. Water samples were typically collected 1 hour apart, with either two or four samples on a given day.

A one-to-one cross plot of the PhycoProbe versus reference sample CHL measurements is shown in figure 37. The linear regression of the data was highly significant ($p<0.001$) with an $R^2=0.86$ and a slope of 0.71.

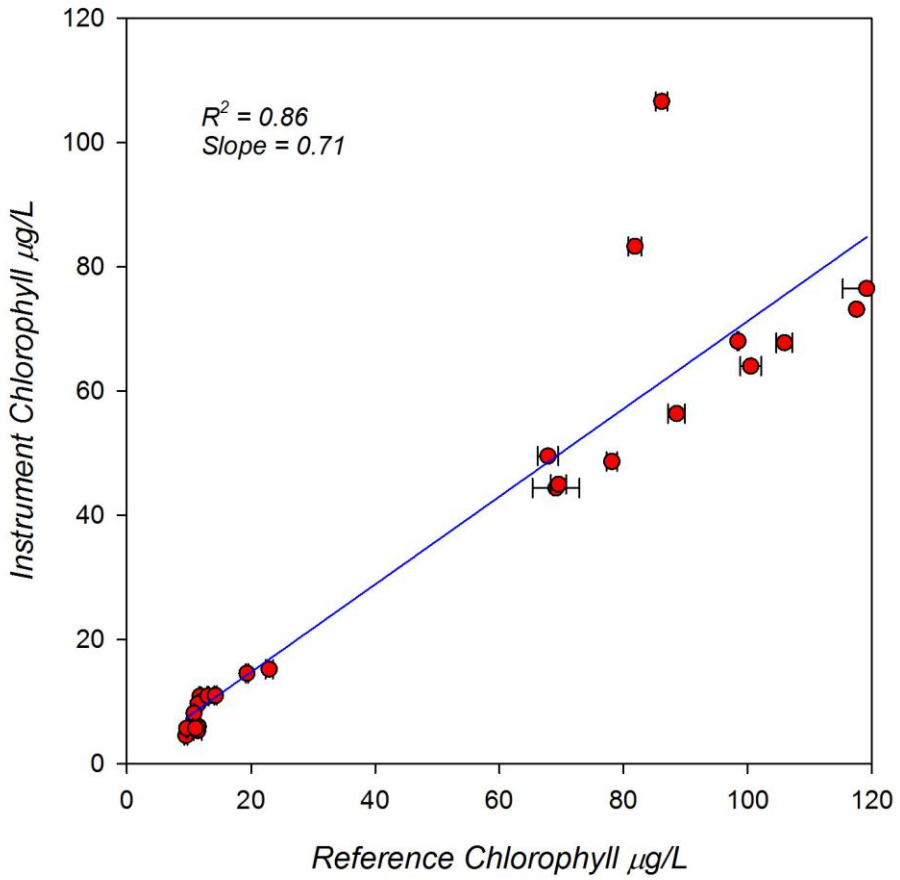


Figure 37. Cross plot of PhycoProbe and reference sample measurements during the Maumee River field deployment. The data represent observations from 6 days of a 13-day deployment. The blue line represents the linear regression of the data.

A comparison of algal classification from microscope counts on preserved reference samples against the PhycoProbe classifications for the Maumee River deployment is shown in figure 38. This test was one of the few times we saw a measurable contribution from Cryptophytes in our freshwater applications. This contribution was confirmed by low but measureable levels of phycoerythrin in the reference samples with concentrations ranging from 0 to 0.61 µg/L (not plotted). In general the PhycoProbe over-assigned the contribution of Green Algae relative to the golden-brown algal class as determined from the comparative cell counts. In addition, the PhycoProbe appeared to partition some of the measured Cryptophytes to Cyanobacteria.

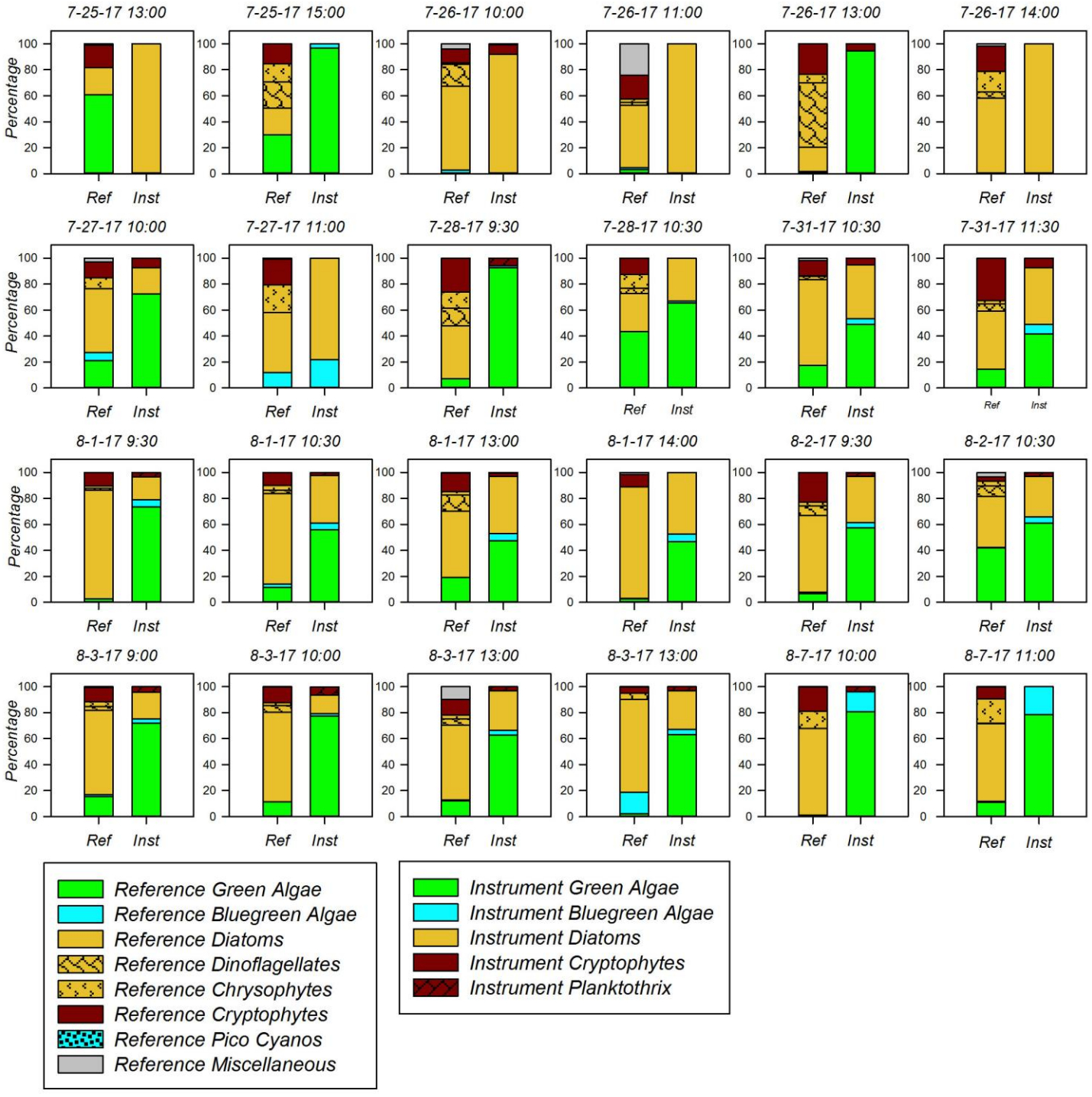


Figure 38. Reference cell counts as a percentage of biomass compared to instrument algae categories as a percentage of total chlorophyll-a estimated by the PhycoProbe for the Maumee River field deployment test.

Figure 39. Instrument photographs prior to deployment (*top*) and post deployment (*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 39). 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).

During the 28 day CBL deployment, the PhycoProbe collected 647 observations with 6 results coming back as all zeros for an accepted data return of over 99%. Over the entire deployment, the CHL measurements from the PhycoProbe ranged from 0.03 to 37.3 µg/L. This environment showed the greatest phytoplankton diversity with measured extracted phycocyanin concentration from 0.6 to 5.5 µg/L and extracted phycoerythrin concentrations from 0 to 3.4 µg/L. Indicative of this community diversity the maximum amount of fluorescence attributed to Cyanobacteria, Cryptophyta, and Planktothrix by the PhycoProbe was 7.9, 11.7, and 6.4 µg/L, respectively.

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. Reference sample turbidity and $CDOM_{400}$ are overlaid for comparison with turbidity ranging from 0.7 to 2.1 NTU and CDOM absorbance ranging from 0.9 to 1.7, indicating a much less optically challenging environment than the previous river deployment. During the deployment, temperature ranged from 22.4 to 26.1°C and salinity from 8.1 to 13.2 PSU. The continuous sonde data indicated a roughly 5-fold range in chlorophyll and phycocyanin over the deployment with very strong diurnal cycles from tidal flows and a small overall decline in phycocyanin as salinity decreased.

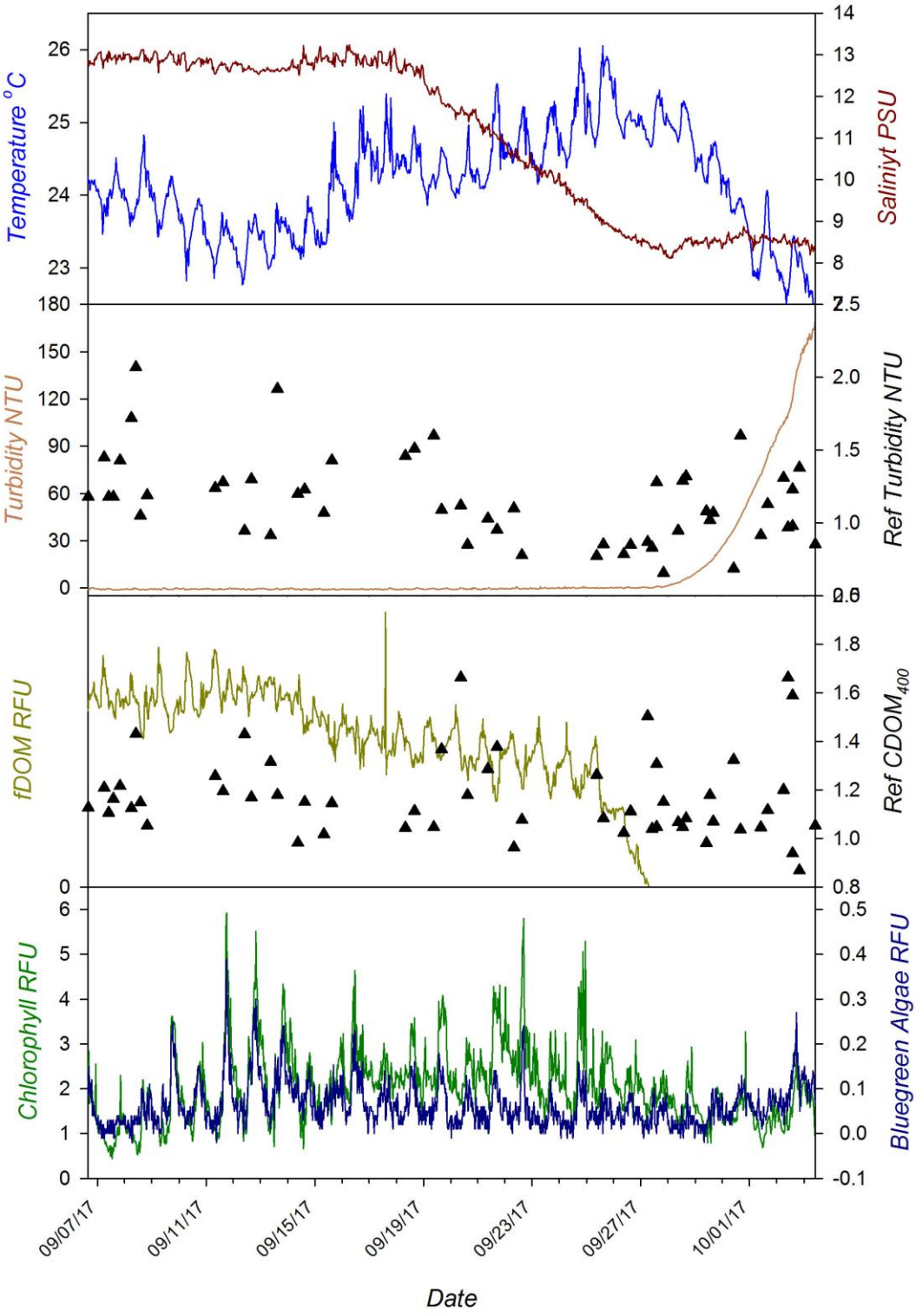


Figure 41. Environmental conditions encountered during the 28 day CBL moored deployment. *Top Panel:* Variation in temperature (green) and salinity (red) at depth of instrument sensor detected by an EXO2 sonde. *Second Panel*: Variation in turbidity (brown) as measured by the EXO 2 sonde and discrete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

A time series of the PhycoProbe CHL measurements are plotted against the corresponding reference sample CHL measurements in figure 42. Chlorophyll measurements for the PhycoProbe during comparative sampling periods ranged from 0.00 to 33 µg/L compared to the range in reference samples of 7.4 to 21.7 µg/L.

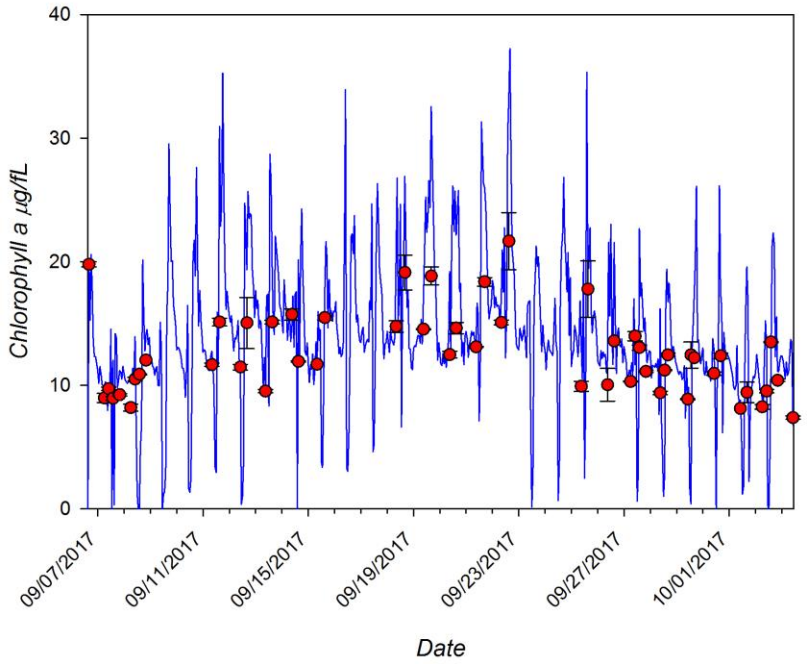


Figure 42. Time series plot of the PhycoProbe (blue) and reference measurements (red) of chlorophyll a during the CBL moored deployment in Solomons, MD.

A one-to-one cross plot of the PhycoProbe versus reference sample CHL measurements is shown in figure 43. The linear regression of the data was highly significant ($p<0.001$) with an $R^2=0.53$ and a slope of 1.54. The intercept of the regression (-4.0) was not significant..

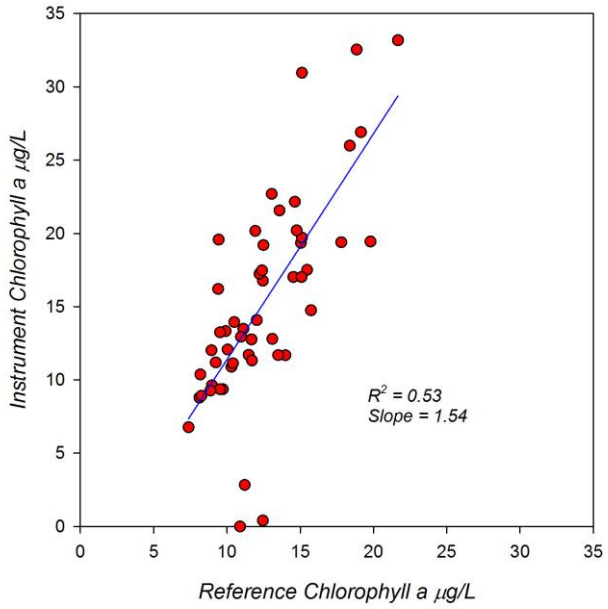
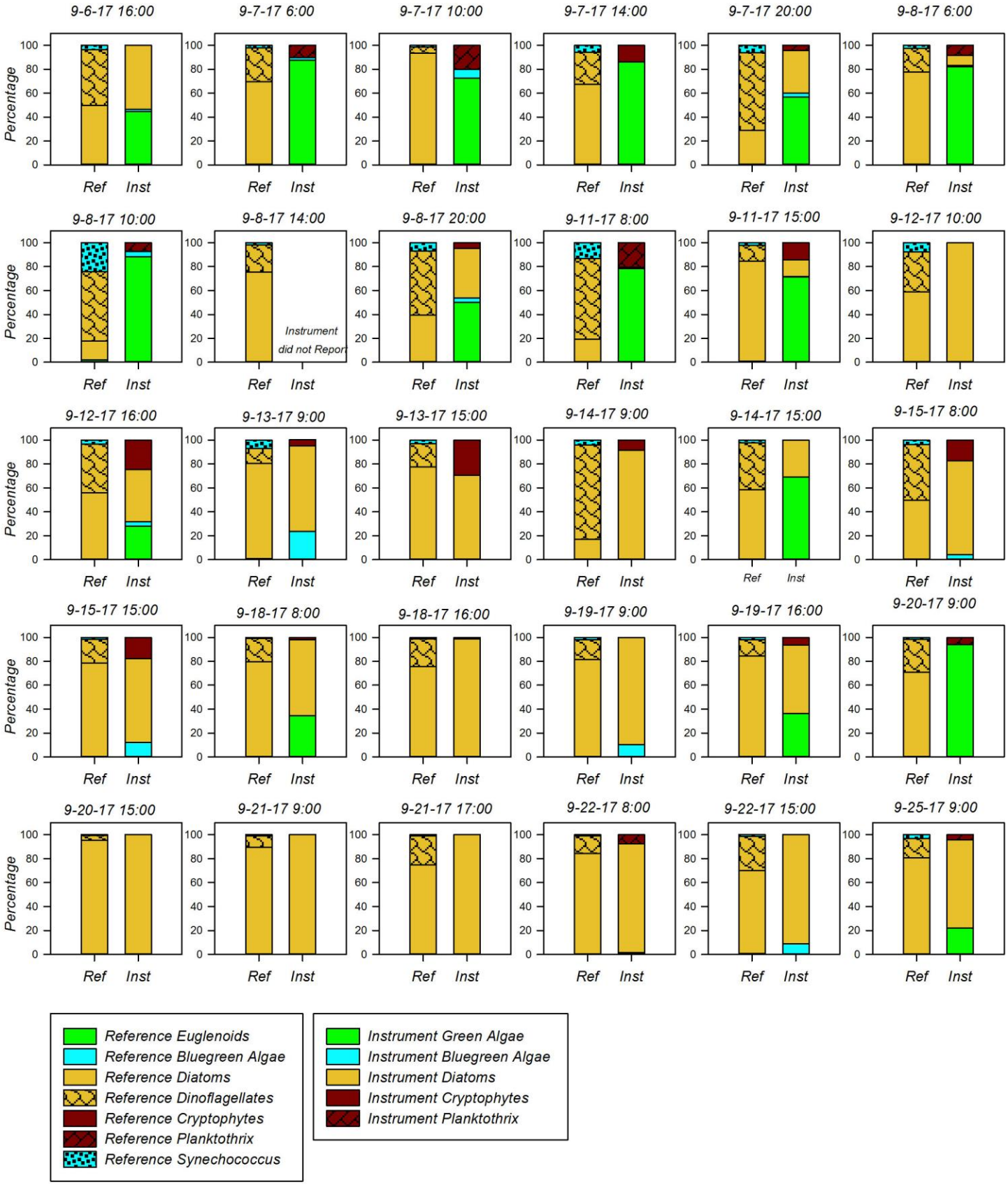


Figure 43. Cross plot of PhycoProbe and reference sample measurements during the CBL field deployment. The blue line represents the linear regression of the data.

A comparison of algal classification from microscope counts on preserved reference samples against the PhycoProbe classifications for the CBL deployment is shown in figure 44. As configured for this evaluation, the PhycoProbe over-assigned the contribution of Green Algae and the over-contribution usually came from the golden-brown algal classes as determined from cell counts. In addition, the PhycoProbe designated a much larger contribution of Cryptophytes and/or Planktothrix than was seen by our counts. Although we do recognize that extracted phycobilins were measureable there was not a clear quantitive relationship extracted PC or PE concentrations and the PhycoProbe classification of these classes.


9-6-17 16:00
9-7-17 6:00
9-7-17 10:00
9-7-17 14:00
9-7-17 20:00
9-8-17 6:00
9-8-17 10:00
9-8-17 14:00
Instrument
did not Report
9-8-17 20:00
9-11-17 8:00
9-11-17 15:00
9-12-17 10:00
9-12-17 16:00
9-13-17 9:00
9-13-17 15:00
9-14-17 9:00
9-14-17 15:00
9-15-17 8:00
9-15-17 15:00
9-18-17 8:00
9-18-17 16:00
9-19-17 9:00
9-19-17 16:00
9-20-17 9:00
9-20-17 15:00
9-21-17 9:00
9-21-17 17:00
9-22-17 8:00
9-22-17 15:00
9-25-17 9:00
Percentage
Ref
Inst
Reference Euglenoids
Reference Bluegreen Algae
Reference Diatoms
Reference Dinoflagellates
Reference Cryptophytes
Reference Planktothrix
Reference Synechococcus
Instrument Green Algae
Instrument Bluegreen Algae
Instrument Diatoms
Instrument Cryptophytes
Instrument Planktothrix

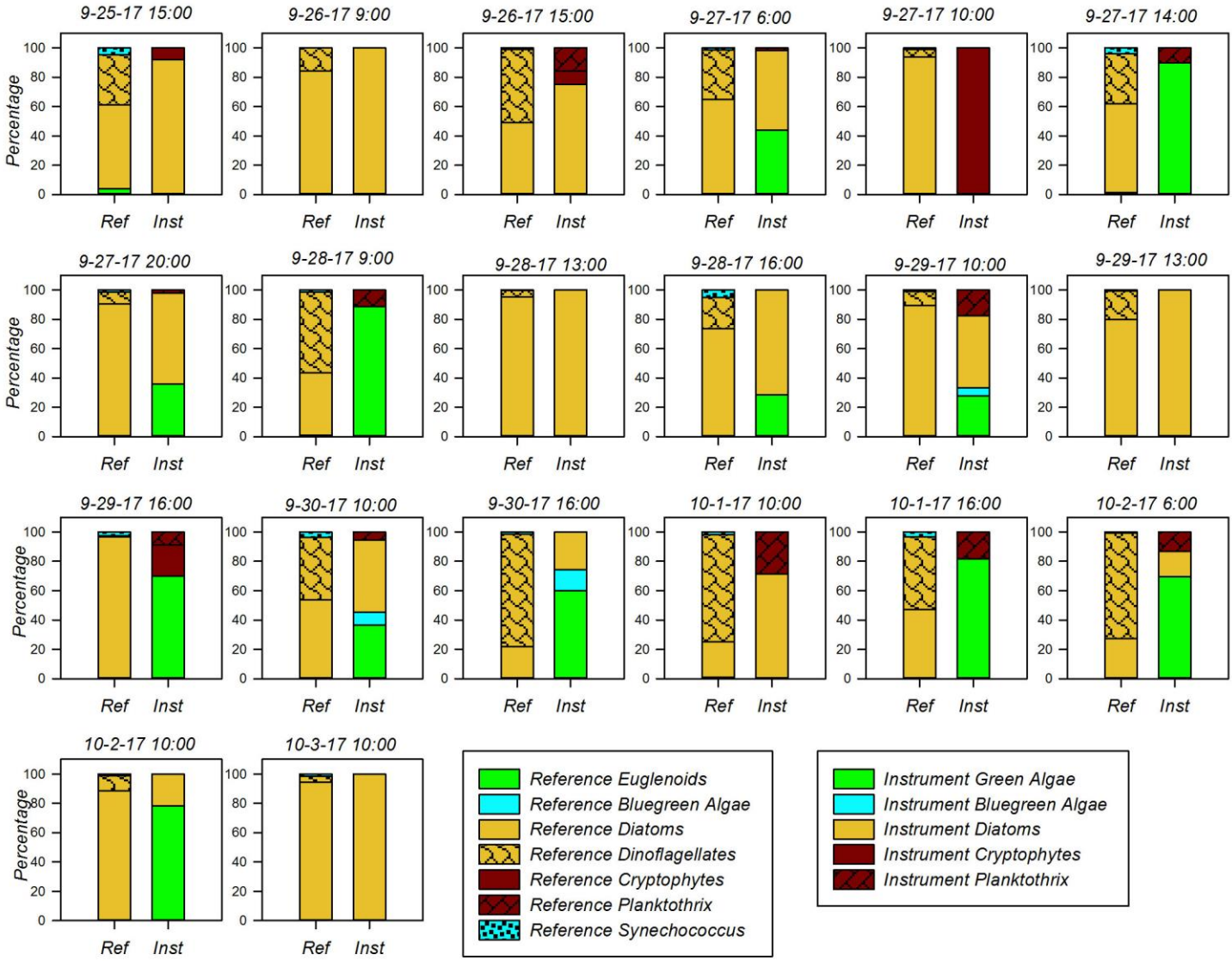


Figure 44. Reference cell counts as A percentage of biomass compared to instrument algae categories as A percentage of total chlorophyll estimated by the PhycoProbe for the Chesapeake Bay field deployment test.

Figure 45. Photographs prior to deployment (*top*) and upon retrieval (*bottom*) from beside the CBL pier.

GLOBAL RESPONSE

A cross plot of PhycoProbe versus reference chlorophyll for all field tests were combined into a single plot to examine how response linearity varied across the test environments (Figure 46). Data from each field test are color coded for each of the different environments. The PhycoProbe showed relatively good agreement across test sites with the exception of the Lake Erie surface mapping during an intense Cyanobacterial bloom. A single regression fit through all the data (excluding Lake Erie) resulted in a linear regression ($p < 0.001$) with a slope of 0.68 and $R^2 = 0.88$.

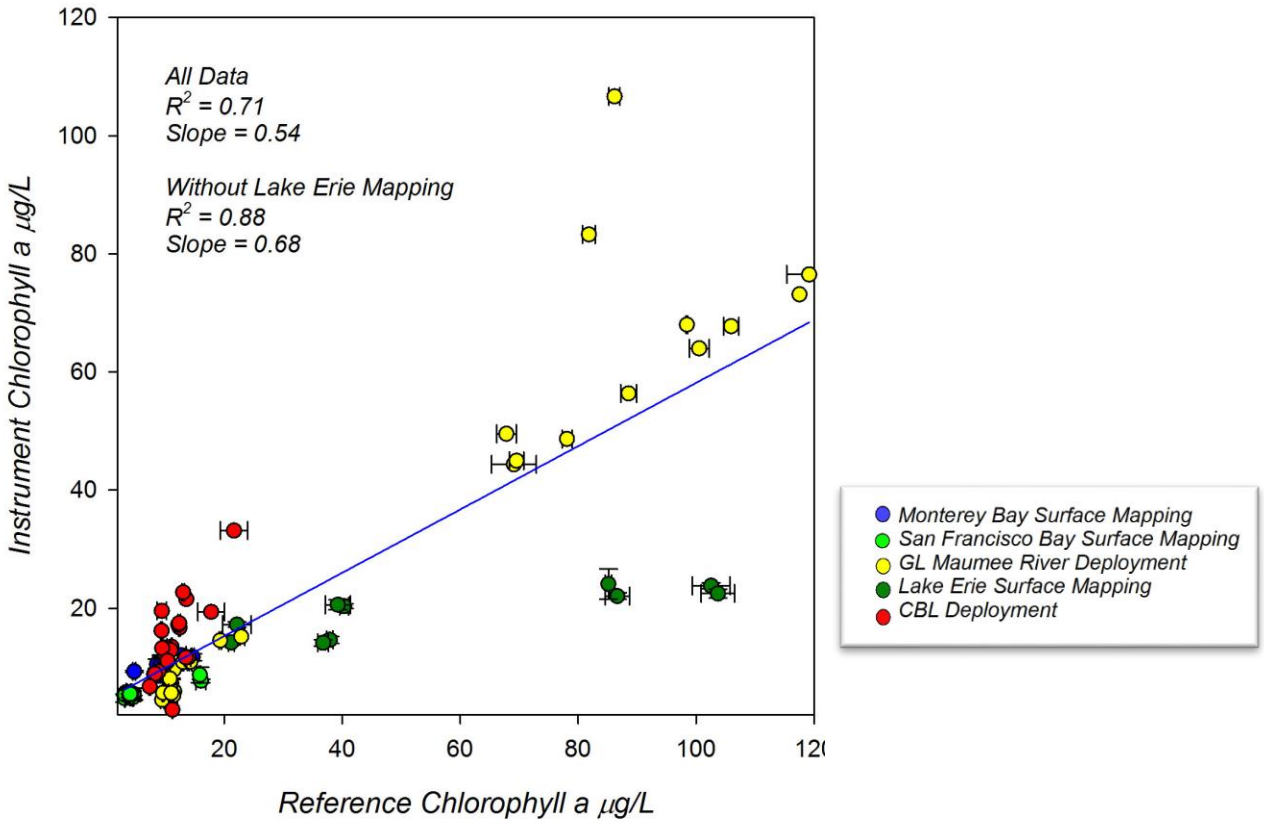


Figure 46. Global response plot for the PhycoProbe CHL estimation compared to extracted chlorophyll for all five ACT field trials. The blue line represents the linear regressions.

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 9-11) 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 8) 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 µm^3/mL)	St Dev		Biovolume (10^3 µm^3/mL)	Biovolume (10^3 µm^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

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's were conducted in accordance with the procedures described in the 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 following procedures. 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 TSA's 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 cultures. 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 tests 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, indicating 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 were 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.

REFERENCES

Speziale, B.J., Schreiner, S.P., Giammatteo, P.A., and Schindler, J.E. 1984. Comparison of *N,N*-Dimethylformamide, Dimethl Sulfoxide, and Acetone for Extraction of Phytoplankton Chlorophyll. Canadian Journal of Fisheries and Aquatic Sciences, 41:1519-1522.

Lawrenz, E., Fedewa, E.J. and Richardson, T.L. 2011. Extraction protocols for the quantification of phycobilins in aqueous phytoplankton extracts. Journal of Applied Phycology, 23:865.

ACKNOWLEDGEMENTS:

We wish to acknowledge the support of all those who helped plan and conduct the verification test, analyze the data, and prepare this report. In particular we would like to thank our Technical Advisory Committee, Dr. Brian Bergamaschi, U.S. Geological Survey, Dr. Thomas Bridgeman, University of Toledo, Dr. Christopher Gobler, Stony Brook University, Dr. Mary Jane Perry, University of Maine, Dr. Alan Wilson, Auburn University for their advice and direct participation in various aspects of this evaluation. Earle Buckley also provided critical input on all aspects of this work and served as the independent Quality Assurance Manager. This work has been coordinated with, and funded by, the National Oceanic and Atmospheric Administration, Integrated Ocean Observing System program.

January 2019

Date

Approved By: Dr. Mario Tamburri
ACT Executive Director

January 2019

Date

Approved By: Dr. Tom Johengen
ACT Chief Scientist

January 2019

Date

Approved By: Dr. Earle Buckley
Quality Assurance Supervisor

Thanks to all the organizers and participants for this extensive test of the submersible PhycoProbe. The PhycoProbe is a new product and was given to ACT for an evaluation test just after its introduction into the market. Intensive tests were performed under various conditions in the laboratory as well as in the field. For the lab tests, various cultured microalgae and cyanobacteria were used.

The selection of the algal cultures was suitable in regard of the performance evaluation test plan although some of the cultures were contaminated. The cyanobacteria *Microcystis* and *Synechococcus* are well known species in the bbe laboratory and are in use for calibration procedures of all bbe fluorometers with the extended algae differentiation.

For good performance, fluorometric analysis requires an offset or blank to obtain accurate results. A low offset is important for instrument sensitivity at low algal concentrations. In the laboratory one would expect the very best conditions to test the performance of the PhycoProbe in comparison to the reference. Figure 1 reflects the offset with deionized water and the FW media. The offset was clearly higher than the reference, although the correlation was high (R^2=0,99). The elevated offsets were carried forward to the other fresh and sea water tests (Figure 4 & Figure 5). At this point the skilled experimenter should consider why the values are elevated and take action: either perform an offset calibration or contact the manufacturer to diagnose the problem.
Although the PhycoProbe, like its precursor the PhycoProbe, is designed for the identification and classification of different types of algae, the comparison of biomass and chlorophyll a percentages gave unexpected results. The elevated offsets of the PhycoProbe were identified as cyanobacteria – a result which is typical when bubbles are in front of the fluorescence sensor. The presence of the cyanobacteria *Microcystis* was not shown by the PhycoProbe (Figure 3). This is unusual because the PhycoProbe not only analyzes the chlorophyll a content of cyanobacteria but also determines the phycocyanin content, which is an essential part of the light-harvesting pigments of the cyanobacteria.

The algal classification of brown algae Isochrysis and dinoflagellates is fairly good for the reference and the PhycoProbe (Figure 6). However, the lower concentration of microalgae results in the classification of 10% of biomass as cyanobacteria, which might also be caused by bubbles. At higher concentrations there is good agreement between the reference and PhycoProbe.
The experimental design with the marine *Synechococcus* test series is undefined (Table 4 and Figures 7-9). The culture was contaminated with diatoms to an unknown extent. The reference measurement recognized chlorophyll a, but this chlorophyll a was mostly due to diatoms. The PhycoProbe also indicated the presence of diatoms, but also measured higher percentages of cyanobacterial chlorophyll. A comparison between chlorophyll a and biovolume distribution is improper under these conditions. Although the PhycoProbe classification of cryptophytes and Planktothrix is unreliable, the chlorophyll content of the higher amount of diatoms might be reasonable. The effect of CDOM and/or turbidity remains unclear as there were no defined conditions for this test. In this case, bbe strongly recommends contacting the manufacturer and submitting the data for detailed analysis. A test with pure *Synechococcus* in the bbe lab measured Synechococcus at almost 100% without any cryptophytes or diatoms. The reported under-prediction at higher concentrations could not be confirmed. The test result is available from bbe on request.

Another test was performed with mixtures of chlorophytes, cyanobacteria and one addition of dinoflagellates. Although the chlorophyll contents were comparable between the reference and PhycoProbe, up to 25 µg/L chlorophyll a, the addition of the dinoflagellates to the mixture

decreased the determined reference value by 10%, whereas the PhycoProbe showed a slight increase. In contrast to the previous tests, the FW media resulted in zero chlorophyll for both the PhycoProbe and the reference. There must be a reason for the difference between this test and the previous tests. The allocation of chlorophyll a to the different algal classes is implausible. Although the reference recognized increasing amounts of cyanobacteria, the PhycoProbe determined almost all chlorophyll a as originating from the chlorophyte with a minor contribution from cryptophytes. This raises the question of which algal classes were activated and which were switched off. This result was completely unexpected and contradicts all prior experience bbe has with the PhycoProbe. A malfunction is also possible, as the next test with cyanobacteria and chlorophytes (Table 6, Figure 13, 14, 15) in the presence of low amounts of turbid particles revealed a fault in detection by the PhycoProbe. An additional indication of a possible malfunction is the change in the determined algal classes from green algae to diatoms when the amount of cyanobacteria is increased (T4 to T5). Moreover, in the same mixture (T5) the PhycoProbe reported a change from 50% chlorophytes to 100% diatoms within 10 minutes. This result is displayed in real-time so that the unexpected shift is obvious. At this point the experimenter is advised to contact the manufacturer to clarify the discrepancies of the obtained result. Subsequent analyses of results from lab or field test are of little significance especially with respect to the algal class determination. Even though the global response resulted in a “relatively good agreement across the test sites” with one exception, the capabilities of the PhycoProbe could not be shown. The manufacturer therefore recommends performing further test series with the assistance of bbe experts. Bbe is confident that the PhycoProbe with extended features and an additional detector will provide more accurate chlorophyll determinations with respect of cyanobacteria and an improved classification of different algae types.