PERFORMANCE VERIFICATION STATEMENT for the WET Labs *ECO* FLNTUSB fluorometer

TECHNOLOGY TYPE:	Fluorometer
APPLICATION:	In situ estimates of chlorophyll concentrations
PARAMETERS EVALUATED:	Response linearity, precision, range, and reliability
TYPE OF EVALUATION:	Laboratory and Field Performance Verification at seven ACT Partner sites
DATE OF EVALUATION:	Testing conducted from May through September 2005
EVALUATION PERSONNEL:	M. Carroll, D. Chigounis, S. Gilbert, K. Gundersen, K. Hayashi, C. Janzen, T. Johengen, T. Koles, F. Laurier, T. McKissack, L. Meadows, C. Metcalfe, C. Robertson, D. Schar, J. Seiter, G.J. Smith, M. Tamburri and D. Wells.

NOTICE:

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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.

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EXECUTIVE SUMMARY:

Instrument performance verification is necessary so that effective existing technologies can be recognized and so that promising new technologies can become available to support coastal science, resource management, and ocean observing systems. The Alliance for Coastal Technologies (ACT) has therefore completed an evaluation of in situ fluorometers designed for measuring chlorophyll. Chlorophyll measurements are widely used by resource managers and researchers to estimate phytoplankton abundance and distribution. Chlorophyll is also the most important light-capturing molecule for photosynthesis and is an important variable in models of primary production. While there are various techniques available for chlorophyll determinations, in situ fluorescence is widely accepted for its simplicity, sensitivity, versatility, and economical advantages.

As described below in more detail, field tests that compare manufacturer's chlorophyll values to those determined by extractive HPLC analysis were designed only to examine an instrument's ability to track changes in chlorophyll concentrations through time or depth and NOT to determine how well the instrument's values matched those from extractive analysis. The use of fluorometers to determine chlorophyll levels in nature requires local calibration to take into account species composition, physiology and the effect of ambient irradiance, particularly photoquenching.

In this Verification Statement, we present the performance results of the WET Labs *ECO* FLNTUSB fluorometer evaluated in the laboratory and under diverse field conditions to in both moored and profiling tests. A total of nine different field sites or conditions were used for testing, including tropical coral reef, high turbidity estuary, open-ocean, and freshwater lake environments. Because of the complexity of the tests conducted and the number of variables examined, a concise summary is not possible. We encourage readers to review the entire document (and supporting material found at www. wetlabs.com) for a comprehensive understanding of instrument performance. However, specific subsection of parameters tested for and environments tested in can be more quickly identified using the Table of Contents below.

TABLE OF CONTENTS:

BACKGROUND:

Instrument performance verification is necessary so that effective existing technologies can be recognized and so that promising new technologies can become available to support coastal science, resource management, and ocean observing systems. To this end, the NOAA-funded Alliance for Coastal Technologies (ACT) serves as an unbiased, third party testbed for evaluating sensors and sensor platforms for use in coastal environments. ACT also serves as a comprehensive data and information clearinghouse on coastal technologies and a forum for capacity building through workshops on specific technology topics (for more information visit www.act-us.info).

This document summarizes the procedures used and results of an ACT Evaluation to verify manufacturer claims regarding the performance of the WET Labs *ECO* FLNTUSB fluorometer. Detailed protocols, including QA/QC methods, are described in the *Protocols for the ACT Verification of In Situ Fluorometers* (ACT TV05-01), which can be downloaded from the ACT website (www.act-us.info/evaluation_reports.php). Appendix 1 is an interpretation of the Performance Verification results from the manufacturer's point of view.

TECHNOLOGY TYPE:

Chlorophyll measurements are widely used by resource managers and researchers to estimate phytoplankton abundance and distribution and can be used as a tool in assessing eutrophication status. Chlorophyll is also the most important light-capturing molecule for photosynthesis and is an important variable in models of primary production. These data are used for numerous industrial applications as well, including water quality management, water treatment, ecosystem health studies, and aquaculture. There are various techniques available for chlorophyll determinations, including spectrophotometry, bench-top fluorometry and high performance liquid chromatography (HPLC) using samples collected on filters and extracted in solvent. However, chlorophyll measurement by in situ fluorescence is widely accepted for its simplicity, sensitivity, versatility, and economical advantages.

In situ fluorometers are designed to detect chlorophyll *a* in living algal and cyanobacterial cells in aquatic environments. The excitation light from the fluorometer passes through the water and excites photosynthetic pigments, including chlorophyll within the living cells of the algae present. A small fraction of this absorbed light is re-emitted by chlorophyll *a* as red fluorescence. As light absorption by chlorophyll and its accessory pigments and the fate of absorbed photons are biophysical events driving photosynthesis that are under physiological control, several factors make in situ fluorescence monitoring of chlorophyll, a semi-quantitative measure at best. Environmental conditions, phytoplankton community composition, physiological status, cell morphology and irradiance history all play a role in altering the relationship between fluorescence and the concentrations of chlorophyll *a*. Also interfering materials such as other plant pigments, degradation products and dissolved organic matter, can compete with light absorption or change the optical path of fluoresced light. Even with these diverse natural constraints, in situ fluorescence in a variety of deployment modes does supply valuable information on the relative temporal and/or spatial distribution of chlorophyll concentrations in the water column and under similar conditions correlates well with extracted chlorophyll *a* samples.

The *ECO* allows the user to measure chlorophyll fluorescence at 470 nm and to monitor chlorophyll concentration by directly by measuring the amount of chlorophyll-*a* fluorescence emission from a given sample volume of water. Two bright blue LEDs (centered at 455 nm and modulated at 1 kHz) provide the excitation source. A blue interference filter is used to reject the small amount of red light emitted by the LEDs. The blue light from the sources enters the water volume at an angle of approximately 55–60 degrees with respect to the end face of the unit. Fluoresced light is received by a detector positioned where the acceptance angle forms a 140-degree intersection with the source beam. A red interference filter is used to discriminate against the scattered blue excitation light. The red fluorescence emitted is synchronously detected by a silicon photodiode.The manufacturer's published performance specifications for the *ECO* fluorometer include: Sensitivity (per count) +/- 0.02 µg L^{-1}, Range 0.02 to 60 µg L^{-1} (0 to 100 % full scale), Linearity (both signals) 99 % R^2, and Operating Depth of 0 to 600 meters. More information can be found at www.wetlabs.com.

APPLICATION - OBJECTIVES AND FOCUS OF PERFORMANCE VERIFICATION:

The basic application and parameters evaluated were determined by surveying users of in situ fluorometers. Almost equal numbers of respondents to our needs and use assessment indicated in situ fluorometers were commonly deployed on remote platforms in estuarine and near shore environments and used in profiling applications, typically down to at least 100 meters depth. Therefore, this performance verification focused on these two applications. It was also clear from the user survey that accuracy, precision, range (i.e., detection limits), and reliability are the most important parameters guiding instrument selection decisions. Given that in vivo or in situ fluorometry is a relative measurement with no absolute "true value" reference (see discussion above), accuracy in the measurement of chlorophyll in vivo cannot be determined directly. Much of the variation in fluorescence as a measure of chlorophyll is due to physiological and taxonomic factors that have nothing to do with any particular instrument. Therefore, a surrogate for accuracy was used in this Performance Verification; response linearity or stability of the response/calibration factor to a defined reference (see below). Protocols were developed with the aid of manufacturers and Technical Advisory Committee to evaluate these specific areas.

PARAMETERS EVALUATED:

Definitions below were agreed upon with the manufacturer as part of the verification protocols.

Response Linearity – Stability of a predetermined response or calibration factor, computed as: (fluorometer measurement in sample solution – fluorometer measurement in blank solution) / [reference standard] over a range of reference standard concentrations. As relative fluorescence is temperature dependent, response factors were quantified in the laboratory for each test temperature and the influence of reference dye and algal concentrations, varying standard turbidity concentrations, and light conditions were assessed.

Precision – Precision is a measure of the repeatability of a measurement. Instrument precision was determined by calculating the coefficient of variation (STD/Mean x 100) of replicate fluorometer measurements at 3 different reference dye concentrations and a fixed temperature in the laboratory.

Range – Range or detection limit is a measure of the minimum and maximum concentration of specific reference dyes and in vivo chlorophyll *a* the instrument can accurately (see definition above) measure. Range and linearity were determined on a dilution series of dye and algal concentrations in water under total darkness.

Reliability – Reliability is the ability to maintain integrity or stability of the instrument and data collections over time. Reliability of instruments was determined in two ways. In both laboratory and field tests, comparisons were be made of the percent of data recovered versus percent of data expected. In field tests, instrument stability was determined by pre- and post-measures of blanks and reference dyes to quantify drift during deployment periods. Comments on the physical condition of the instruments (e.g., physical damage, flooding, corrosion, battery failure, etc.) were also recorded.

TYPE OF EVALUATIONS - SUMMARY OF VERIFICATION PROTOCOLS:

In conference with the participating instrument manufacturers and the Technical Advisory Committee, it was determined that the verification protocols would: (A) employ reference dyes and extractive chlorophyll a analysis through HPLC as the standards of reference for determining instrument performance characteristics; (B) include controlled laboratory tests; and (C) include field tests to evaluate performance under a variety of environmental conditions.

The HPLC method used for chlorophyll analysis follows that of Zapata et al. (2000, MEPS 195:29-45). Analyses were conducted by the laboratory of Dr. Nick Welschmeyer at Moss Landing Marine Laboratories (MLML, the West Coast ACT Partner Institution). All samples from Partner sites were frozen in liquid N_2 and shipped by overnight courier in liquid N_2 dry shippers to MLML. Frozen samples were logged in by ACT staff upon receipt and stored in liquid N_2 dewars along with the MLML samples. Samples were then extracted by physical grinding and in N_2-purged 90% acetone overnight, followed by autosampler HPLC processing commencing the following day. Extracts were simultaneously

analyzed by a standard fluorometric technique (Welschmeyer 1994, L&O 39: 1985-1992) to complement HPLC assays described above.

All laboratory tests of response linearity, precision, range, and reliability were also conducted at MLML in well-mixed (submersible circulating pumps), temperature controlled water baths. As the goal of the laboratory tests was to assess performance of the fluorescence detection systems rather than biologically based variation in chlorophyll fluorescence, an inert fluorochrome was employed as the reference standard. Basic Blue 3 (BB3, C.I. 51004, CAS 33203-82-6, M.W. 359.9) was selected as the primary fluorometric reference standard (Kopf and Heinze 1984 *Anal. Chem.* 56, 1931-1935). BB3 is readily soluble in both deionized and sea-water (>>1 $mg.mL^{-1}$ or > 2.8 mM) without substantial shifts in absorbance properties (λ_{max} = 654, $\varepsilon_{M,654}$ = 88954, λ_{em} = 661 nm). At the request of the participating manufactures and on recommendation of the scientific advisory panel, the dye Rhodamine WT (RWT, λ_{max} = 497, λ_{em} = 523 nm) was also used in a limited number of independent test conditions to permit cross calibration of BB3 and RWT fluorescence signals. Instrument output was first "calibrated" to BB3 and/or RWT concentration under standard reference conditions by immersion in one or two-point standardization solutions as suggested by each manufacturer.

Moored field tests were conducted by seven ACT Partner Institutes at a fixed depth of 1 m from secure deployment sites representing a range of environmental conditions, representative of the range of coastal environments in North America. Field sites included the Chesapeake Biological Laboratory (Solomons, University of Maryland), NOAA/GLERL Lake Michigan Field Station (Muskegon, Michigan, CILER/University of Michigan), Darling Marine Center (Walpole, Maine, GoMOOS/University of Maine), Moss Landing Harbor (Moss Landing, California, MLML), western shore of Skidaway Island (Skidaway, Georgia, SkIO), Kaneohe Bay Barrier Reef (Kaneohe Bay, Hawaii, University of Hawaii), and Bayboro Harbor (Tampa Bay, Florida, University of South Florida). Similar profiling tests were conducted at two sites, CILER/University of Michigan and GoMOOS/University of Maine.

Instruments tested, both in the laboratory and in the field, were incorporated in the WET Labs *ECO* FLNTUSB fluorometer, a stand-alone package, which included cooper tape and *Bio-wiper*™, a mechanical wiping system (biofouling prevention), data logging, and independent power, provided by the manufacturer. A total of four fluorometers were evaluated and all instruments were reconditioned by the manufacturer prior to the second set of deployments at the remaining ACT Partner test sites.

For moored tests, instruments were programmed to record data every 15 minutes and both prior to and after deployment, a series of blanks (DI water) and dyes (BB3 and RWT) were presented to the instruments at the field sites as baseline references. Water samples for HPLC chlorophyll analysis were collected (at the same depth and as close as possible to the sensor heads) at least twice a day, Mondays through Fridays during the four-week field test at the time instruments were programmed to sample. In conjunction with each water sample collection, site-specific conditions were also noted (e.g., date, time, weather conditions, natural or anthropogenic disturbances, and tidal state). Identical methods were used for profiling test with the instrument programmed to record at one second intervals and water sample collected at varying depths.

*** Detailed fluorometer performance verification protocols can be downloaded at: www.act-us.info/evaluation_reports.php**

Quality Assurance/Quality Control – This performance verification was implemented according to the test/QA plans and technical documents prepared during planning of the verification test. Prescribed procedures and a sequence for the work were defined during the planning stages, and work performed followed those procedures and sequence. Technical procedures included methods to assure proper handling and care of test instruments, samples, and data. Performance evaluation, technical system, and data quality audits were performed by QA personnel independent of direct responsibility for the verification test. All implementation activities were documented and are traceable to the test/QA plan and to test personnel.

The main component to the QA plan included technical systems audits (TSA), conducted by ACT Quality Assurance Specialists at four of the ACT Partner test sites selected at random (MLML; CILER/University of Michigan, SkIO, and University of Hawaii). These audits were designed to ensure that the verification test was performed in accordance with the test protocols and the ACT *Quality Assurance Guidelines*. (e.g., reviews of sample collection, analysis and other test procedures to those specified in the test protocols, and data acquisition and handling). During the verification tests, no deviations from the test protocols were necessary. The following QA/QC for the extractive HPLC analysis was performed at Moss Landing Marine Laboratory.

The environmental samples used for determination of total chlorophyll *a* content by HPLC analysis were subject to several levels of quality assurance control. First, addition of the internal standard (trans-beta-8-carotenal; Fluka) to the 90% acetone extracts was used to control for variation is injection volume and potential sample dilution/evaporation during tissue-grinding extraction. Second, HPLC chromatograms were visually inspected to ensure accuracy of peak and baseline calls and corrected as needed. Third, as an independent check on the accuracy of the HPLC chlorophyll *a* estimates, roughly two-thirds of the samples were selected from each field site and the extracts assayed on calibrated on lab-bench fluorometers using standard protocols (single-step fluorometry: Welschmeyer, 1994 and acidification fluorometry: Yentsch et al. 1965).

Sample discrepancies (*>50% difference in estimate*) identified by direct comparison of chlorophyll *a* estimates obtained by these independent methods were re-evaluated for accuracy by checks of the original chromatogram calls, spreadsheet entries and if necessary re-injection of the sample under consideration. When standardized against pure chlorophyll *a* in 90% acetone, the simple fluorometric assays inherently overestimate chlorophyll *a* in natural samples because of additional fluorescent compounds contained in the natural pigment matrix; this overestimate is typically ca. 10%, but can be greater when large portions of chl b, chl c1, chl c2, chl3 and pheopigments are present in natural samples.

HOW TO INTERPRET THE RESULTS:

As described above, fluorometers are sensors designed to detect the fluorescent energy emitted by certain molecules of interest, such as chlorophyll. When working with pure analyte solutions, the fluorescence value measured by an in situ fluorometer is typically proportional to the concentration of the molecules present. The laboratory tests therefore focused on instrument parameters such as response linearity to dye solutions under varying concentrations and conditions. However, the relationship between fluorescence and the concentration of chlorophyll *a* in living cells is strongly influenced by many biophysical and physiological factors. For example, chlorophyll fluorescence in vivo is a function of light absorbed by all photosynthetic pigments in the targeted sample, whereas in an extract, it is only the light absorbed by chlorophyll molecules. This makes fluorescence of chlorophyll in an extract a poor proxy of chlorophyll fluorescence in vivo. Field tests, which compare fluorometer values to those determined by extractive HPLC analysis, were therefore designed only to examine the instrument's ability to reliably track changes in chlorophyll concentrations through time or depth and NOT to determine how well the instrument's values match those form extractive analysis. Ancillary water quality measures taken during the field trials (CDOM and TSS) might be used to help assess the underlying cause (optical path interference versus instrument electronic noise or phytoplankton, physiology) of any deviations between measured fluorescence and extracted chlorophyll.

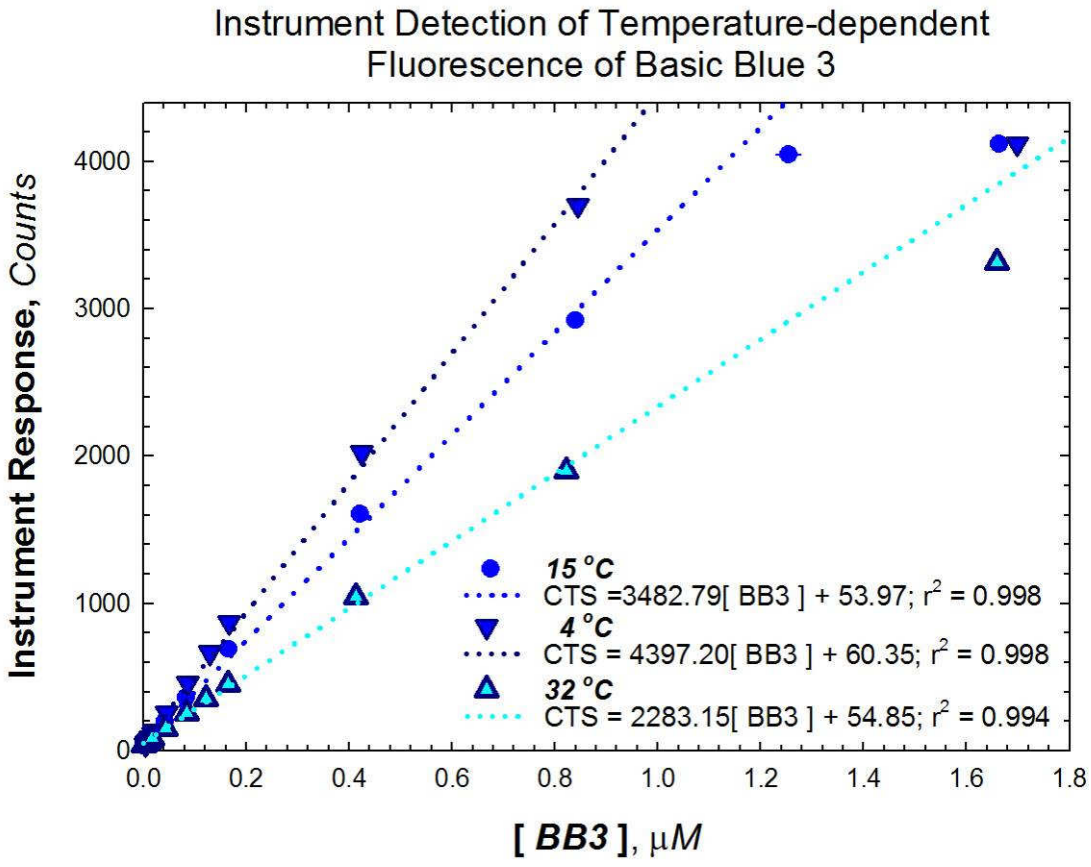
*** Data is presented as relative fluorescence units (RFU) as reported by the instrument. For additional corrections, interpretation and analysis of results, please visit www.wetlabs.com.**

SUMMARY OF VERIFICATION RESULTS, LABORATORY TESTS:

Because of the inherent limitations of in situ fluorometry and the inability to control various factors that can impact the data during field tests; response linearity, precision and range were determined in the laboratory only.

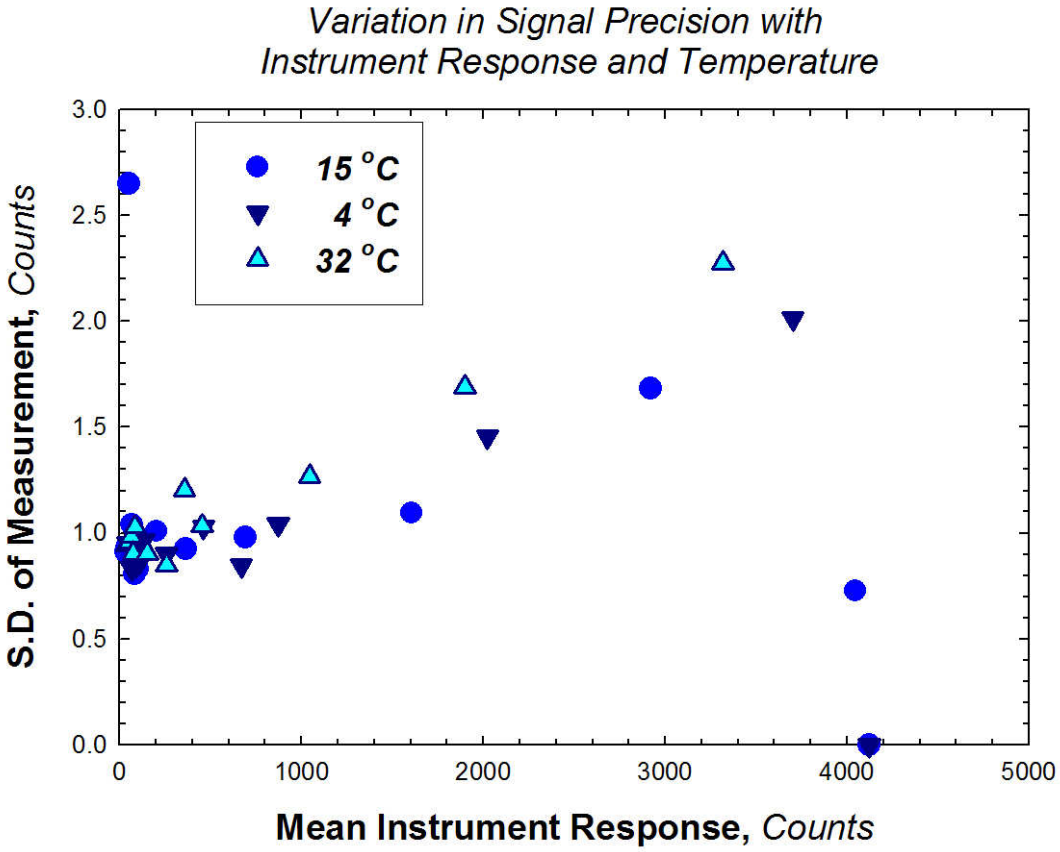
Response Linearity and Detection Range

Figure 1: Instruments were equilibrated in temperature regulated water baths and programmed to sample at 1 minute intervals, consisting of 6 consecutive 1 second sampling bursts, and exposed to sequential increases in BB3 concentrations. The WET Labs *ECO* fluorometer exhibited a linear response to BB3 concentrations through at least 1.0 µM, with detector response saturation occurring at the highest test concentrations with a maximum detector signal 4120 counts. The average instrument response in dye-free water was 40.43 ± 0.93 counts, indicating a limit of detection at 3 s.d. of 2.80 counts above the baseline reading. The fluorescence yield of BB3 is temperature-dependent (-1.56% ± 0.06% per ^{o}C, G. J. Smith, pers. Obs; Kopf and Heinz 1984). As deployed, the *ECO* fluorometer sensor response exhibited a slight temperature hysteresis, yielding a BB3 temperature-dependence of -1.84% ± 0.26% per ^{o}C. All data plotted as mean and standard deviation of both detector response and analyte concentration. Linear regression analysis was restricted to test dye concentrations less than 0.8 µM for all experiments reported. All data plotted as mean and standard deviation of both detector response and analyte concentration.



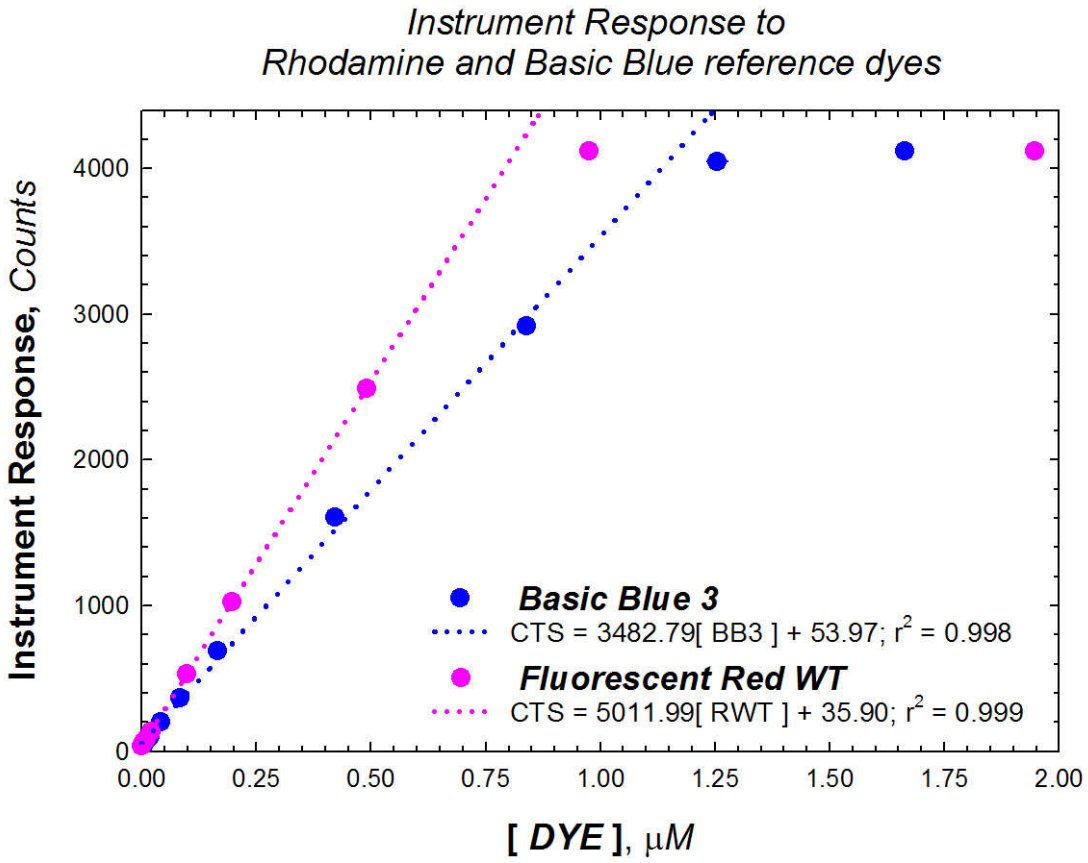
Response Precision

Figure 2: Detector noise, here expressed as the mean standard deviation of 10 sequential sets of 6x1 second sampling bursts space at ca. 1 minute intervals while held at fixed temperature and BB3 dye concentrations, increased by ca. 1 count across the mean detector response range below saturation. Over the instrument detection range of 0-1.0 μM BB3, the absolute signal noise was ± 1.13 (0.81 – 2.65) counts. Detector noise was slightly elevated at the highest test temperature. All data plotted as mean and standard deviation of both detector response and analyte concentration.



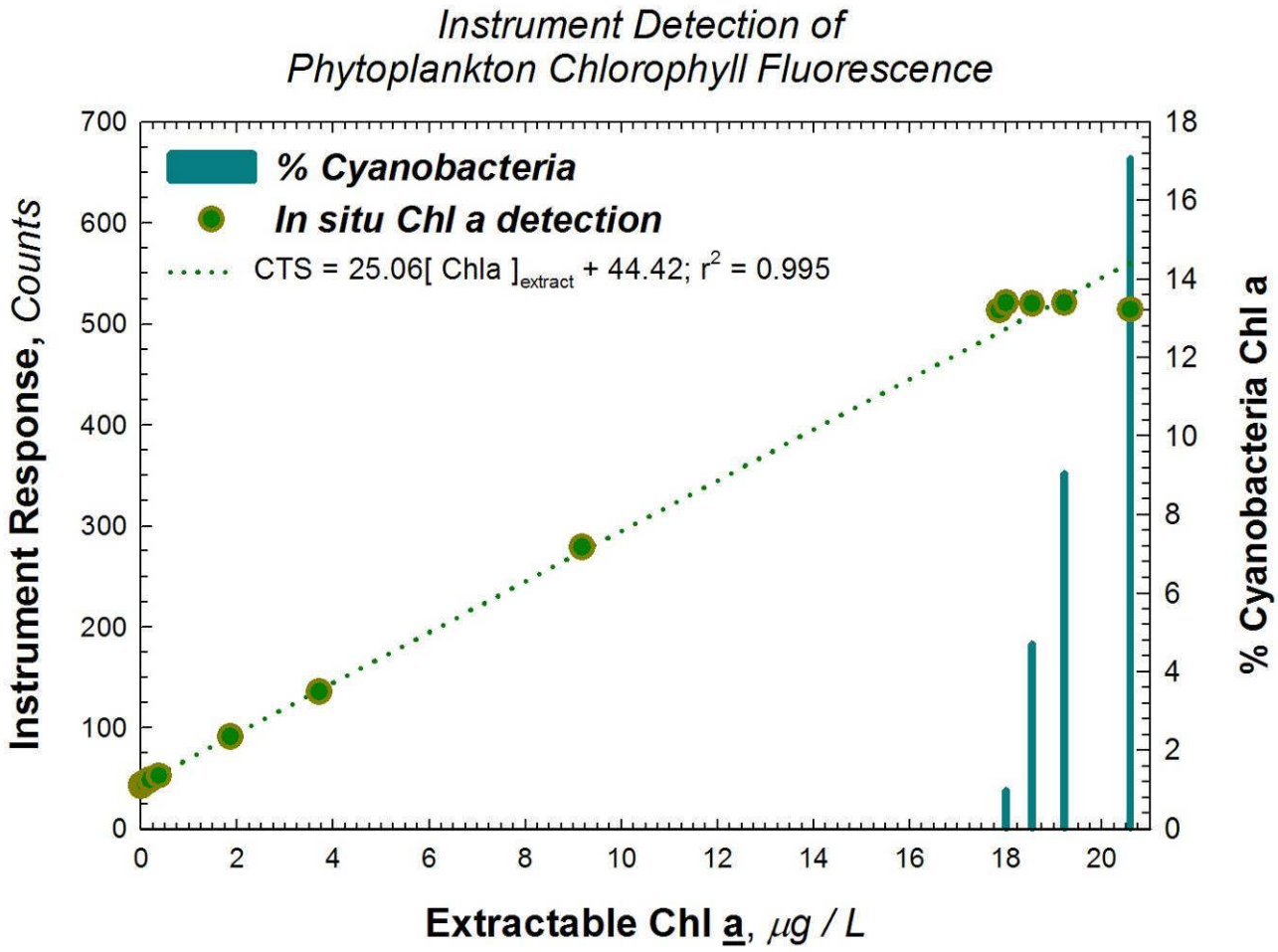
Response Linearity and Fluorochrome Response

Figure 3: The *ECO* fluorometer detector response was linear over comparable concentration ranges of two distinct test fluorochromes BB3 (λ_{max} 654 nm) and Fluorescent Red (Rhodamine) WT (λ_{max} 555 nm). RWT was detected with approximately 30% higher molar efficiency than BB3. All data plotted as mean and standard deviation of both detector response and analyte concentration.



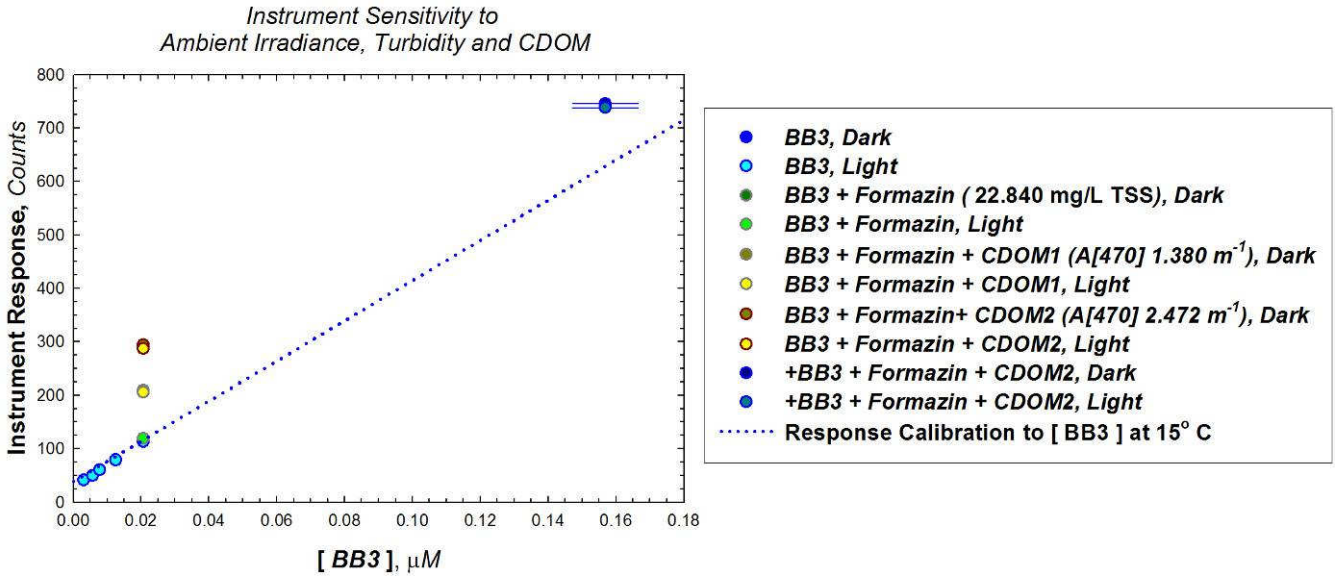
Response Linearity and Phytoplankton Chlorophyll Fluorescence

Figure 4: *Detection of Phytoplankton Chlorophyll Fluorescence.* Instruments were equilibrated f/2-enriched seawater in a temperature controlled tank at 15 ^{o}C in darkened conditions. Total chlorophyll *a* concentration in the media was manipulated by adding aliquots of late log-phase cultures (276.85. ± 19.88 µg L^{-1} of Chl *a*) of the diatom *Thalassiosira pseudonana* Clone 3H (CCMP 1335) which had been grown in f/2 enriched seawater under constant illumination at 15 ^{o}C. Instrument response was linear with total extractable diatom chlorophyll *a* concentrations through 18 µg L^{-1} of Chl *a*. Subsequently, media Chl *a* concentrations were amended by addition of log-phase cultures (80.94 ± 3.79 µg L^{-1} of Chl *a*) of the cyanobacterial strain *Synechococcus* sp. CCMP 1282 grown in parallel with the diatom cultures. The instrument did not detect the cyanobacterial packaged chlorophyll *a* with the same efficiency observed for the diatom packaged chlorophyll. Response regressions for diatom additions was: Counts=26.18[Chl *a*]+ 42.47, r^2=0.999, p<0.001 whereas the response to subsequent cyanobacterial additions was ca. 80% lower: Counts=5.07[Chl *a*]+8.34, r^2=0.787, p=0.045. Instrument noise in the background seawater media was ± 1.01 count. Significant instrument response was observed at an added dose of 0.076 µg L^{-1} of Chl *a*, performance consistent with the predicted limit of detection of 0.095 µg L^{-1} of Chl *a*.



Response Linearity and Sensitivity to ambient turbidity, CDOM and irradiance

Figure 5: Instrument response to the test fluorochrome BB3 was assessed in a temperature regulated bath at 15 °C. Instrument detection of added BB3 was in good agreement (-15%) with the prior, independent calibration to BB3 concentration (see Fig. 1). The *ECO* sensor was relative insensitive to formazin, added as a proxy for turbidity, which induced only a minor increase (ca. 5 counts offset) in detector response. Coffee extract, used as a proxy for CDOM, did induce a larger signal enhancement (ca 87 counts) likely due to organic fluorochromes in this extract. While both proxies of water quality components induced an offset in detector response, this represents a simple shift in instrument baseline that in subsequent additions of the test fluorochrome BB3 produced an incremental detector response only 2% higher than the BB3 calibration response (3311 counts / µM BB3 vs 3271 counts/ µM BB3). Exposure of the tanks to a downwelling surface irradiance of ca. 500 µmol quanta m^{-2} s^{-1} PAR (artificial light) induced no significant or consistent change in detector response under the above treatment conditions. All data plotted as mean and standard deviation of both detector response and analyte concentration.



Laboratory Reliability

There were no issues with this instrument and 100% of the data was recovered from all laboratory experiments. The instrument was set to sample at 6x1sec at 1minute intervals. Sample time drifted forward by at least 1 second every minute and jumped by several seconds if the last sample period was less than 10 seconds before the next clock minute.

SUMMARY OF VERIFICATION RESULTS, FIELD MOORED TESTS:

Field Conditions

TABLE 1. Lists the field conditions during the mooring testing (fw = freshwater).

SITES		**Temperature** ^{0}C	**Salinity** PSU	**TSS** mg.l^{-1}	**CDOM** A [470 nm], m^{-1}
Chesapeake Bay	Minimum	25.68	12.86	0.88	0.37
	Maximum	30.08	14.94	18.53	0.93
	Average	27.59	14.13	6.74	0.56
	STDev	1.00	0.38	3.32	0.13
Lake Michigan	Minimum	14.02	fw	0.94	0.47
	Maximum	26.56	fw	14.71	0.94
	Average	20.17	fw	2.21	0.68
	STDev	2.08	fw	1.79	0.11
Hawaii	Minimum	26.22	34.64	3.60	0.05
	Maximum	28.72	35.43	38.00	0.34
	Average	27.49	35.29	8.50	0.18
	STDev	0.51	0.08	6.60	0.05
Gulf of Maine	Minimum	14.37	28.61	2.58	0.18
	Maximum	22.78	31.02	11.48	0.54
	Average	16.61	30.59	5.03	0.34
	STDev	0.95	0.21	1.80	0.09
Moss Landing	Minimum	10.6	31.34	8.98	0.08
	Maximum	19.42	33.29	34.08	0.93
	Average	14.67	32.73	19.41	0.33
	STDev	1.59	0.29	5.22	0.12
Skidaway Island	Minimum	26.28	12.31	9.30	0.69
	Maximum	31.35	24.43	54.86	1.22
	Average	28.68	18.28	20.07	0.96
	STDev	1.09	2.03	8.79	0.15
Tampa Bay	Minimum	26.21	6.15	0.16	0.45
	Maximum	31.42	27.25	34.85	1.48
	Average	29.51	25.64	7.23	0.76
	STDev	0.93	1.90	6.12	0.18

Field Moored Tests

Field Performance:
Figures, 6A, 7A, 8A, 9A, 10A, 11A and 12A on the following pages display chlorophyll *a* concentrations in RFU (green line) measured by the instrument through time (month/day on x axis) with the corresponding mean chlorophyll *a* concentrations from extractive HPLC analysis (yellow dots in $\mu g\ L^{-1}$, n = 3, standard deviation is plotted although values are smaller than symbols used in graphs) taken periodically during the four-week field deployments.

Field Ancillary Data:
Figure, 6B, 7B, 8B, 9B, 10B, 11B and 12B display the total suspended solid (grey squares, TSS in mg L^{-1}) measured by weight and the colored dissolved organic matter (CDOM) estimated by spectrophotometric analysis (purple triangles, absorption coefficient at 470 nm) both derived from samples taken periodically during the four-week field deployments.

Field Ancillary Data:
Figure 6C, 7C, 8C, 9C, 10C, 11C and 12C shows the corresponding temperature (degree Celsius) and salinity (PSU) at field site during deployments.
Figure 6D, 7D, 8D, 9D, 10D, 11D and 12D features the Photosynthetically Active Radiation (PAR in mMol s^{-1} m^{-2}) at field site during deployments.

Pre and Post-deployment tests:
Table 2, 3, 4, 5, 6, 7 and 8. Instrument responses to blank (DI water) and dyes (BB3, RHOD) before deployment (PRE) and after deployment (POST). The instrument response to blank and dyes after the deployment was tested in two stages, pre-cleaning with the biofouling remaining on the instrument and post-cleaning with the biofouling removed. Please use caution when interpreting these results. While each test site attempted to remove all material that may influence fluorometer performance for the post-cleaning blank and dye readings, we can not guarantee that the instruments were restored completely to the pre-deployment state.

Figure 6: Field Performance – Patuxent River, Chesapeake Bay, Maryland (estuary)

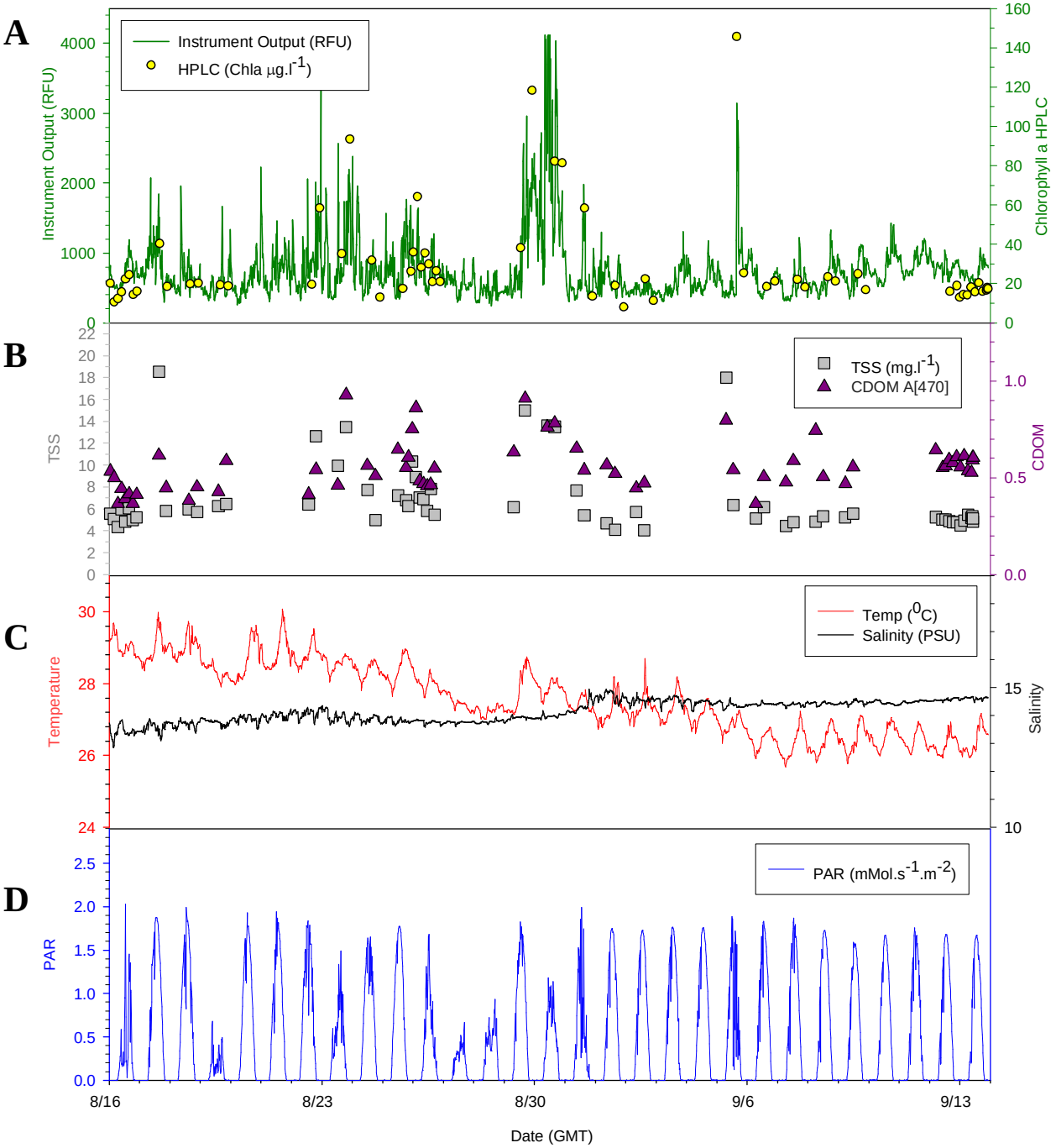


TABLE 2

	PRE		POST pre-cleaning		POST post-cleaning	
	Mean (RFU)	**STD ±**	**Mean (RFU)**	**STD ±**	**Mean (RFU)**	**STD ±**
Blk/DI	57.83	00.87	88.30	6.85	101.07	2.58
BB3	1299.73	25.06	1222.27	19.23	350.73	15.09
Rhod	3894.70	32.13	1870.23	48.78	516.77	12.55

Sensor before the four weeks deployment.

Sensor after the four weeks deployment.

Figure 7: Field Performance – Muskegon, Lake Michigan (freshwater)

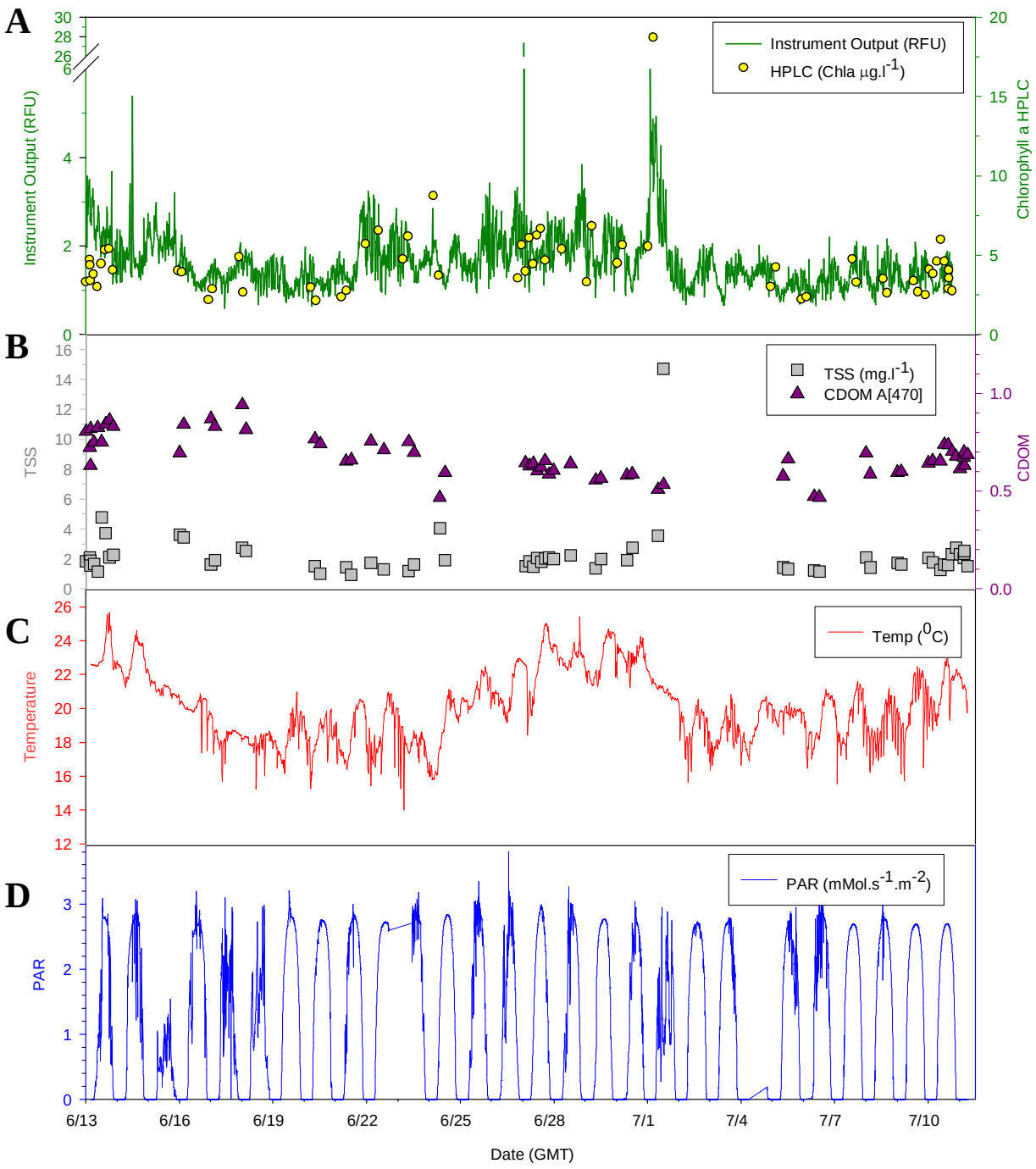
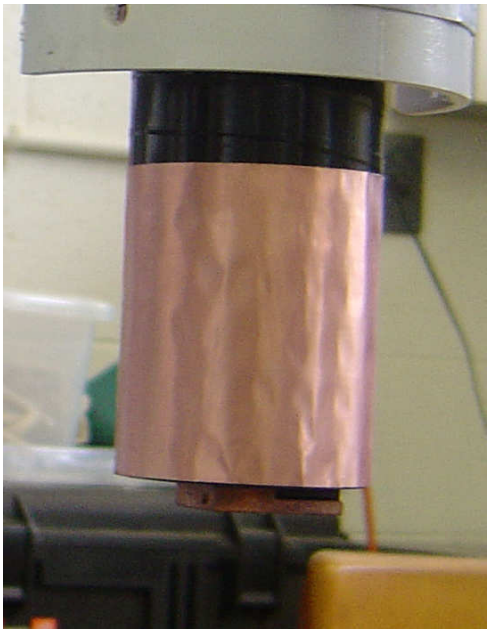


TABLE 3
Note: Missing values due to a problem with pre-deployment standard solutions, not an instrument malfunction.
n/a= non available since it was possible to take only one sample for the PRE and POST dye tests, not an instrument malfunction.

	PRE		POST pre-cleaning		POST post-cleaning	
	Mean (RFU)	**STD ±**	**Mean (RFU)**	**STD ±**	**Mean (RFU)**	**STD ±**
Blk/DI			-0.29	n/a	0.91	n/a
BB3			5.70	n/a	15.89	n/a
Rhod			24.87	n/a	24.77	n/a



Sensor before the four weeks deployment.

Sensor after the four weeks deployment.

Figure 8: Field Performance – Coconut Island, Hawaii (coral reef)
Note: The missing PAR data were due to data loss following a malfunction of the ACT datalogger.

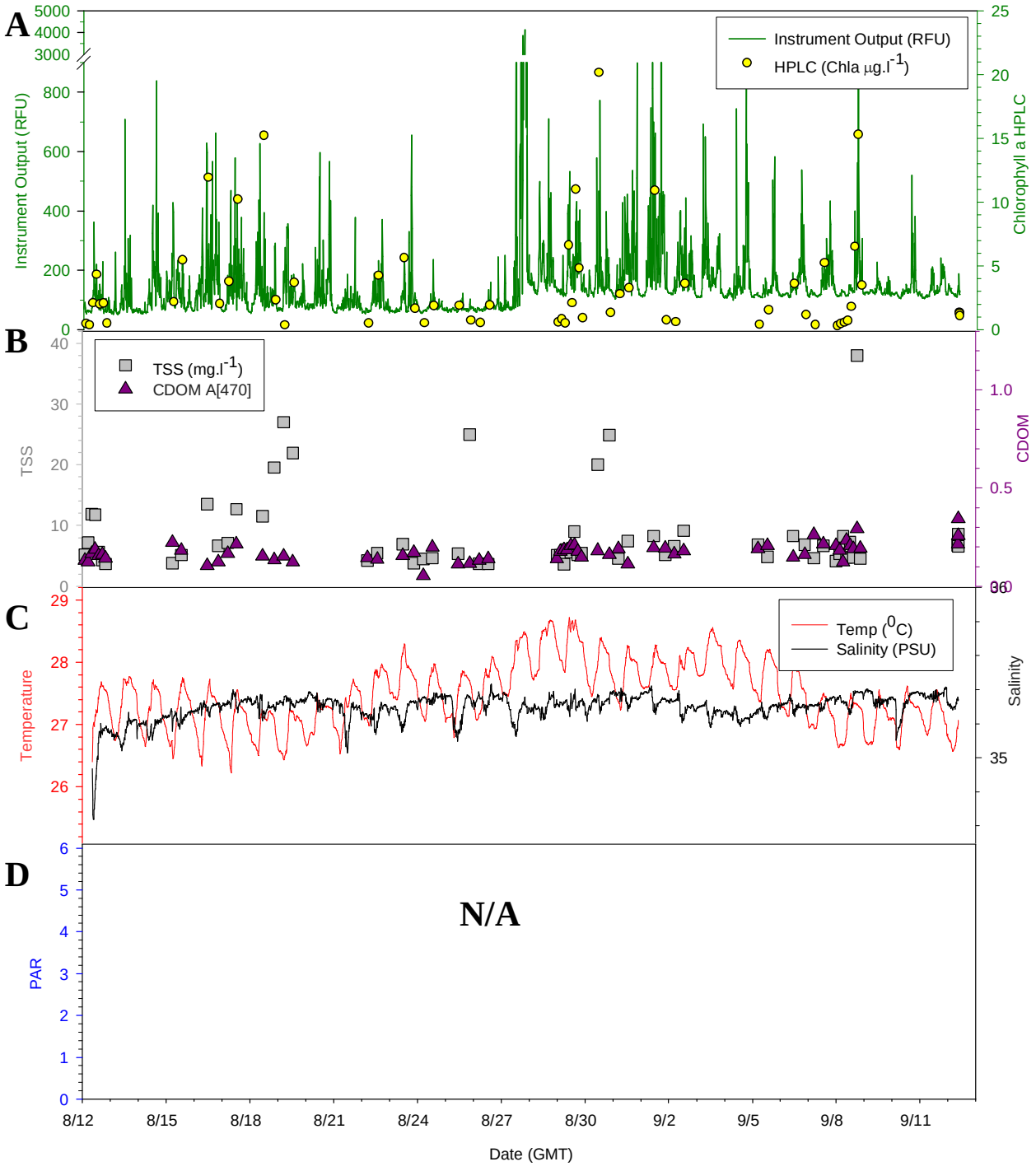
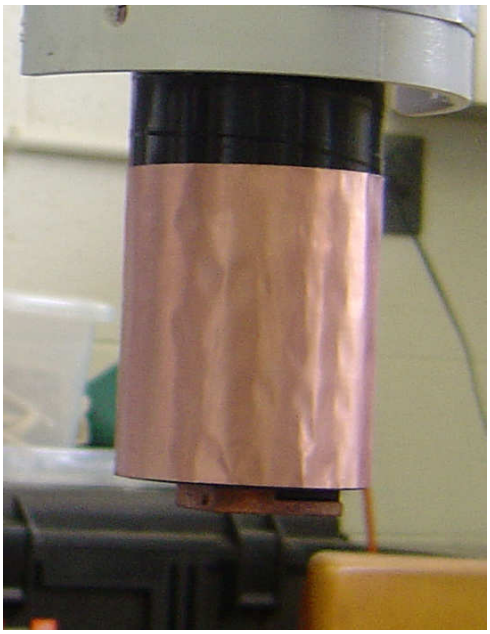


TABLE 4
n/a= non available due to biofouling, not an instrument malfunction.

	PRE		POST pre-cleaning		POST post-cleaning	
	Mean (RFU)	**STD ±**	**Mean (RFU)**	**STD ±**	**Mean (RFU)**	**STD ±**
Blk/DI	57.57	0.25	166.10	96.34	76.70	3.50
BB3	1831.97	56.28	n/a	n/a	1746.13	22.66
Rhod	2410.20	28.20	n/a	n/a	1062.53	11.25



Sensor before the four weeks deployment.

Sensor after the four weeks deployment.

Figure 9: Field Performance – Damariscotta River Estuary, Gulf of Maine (tidal embayment)

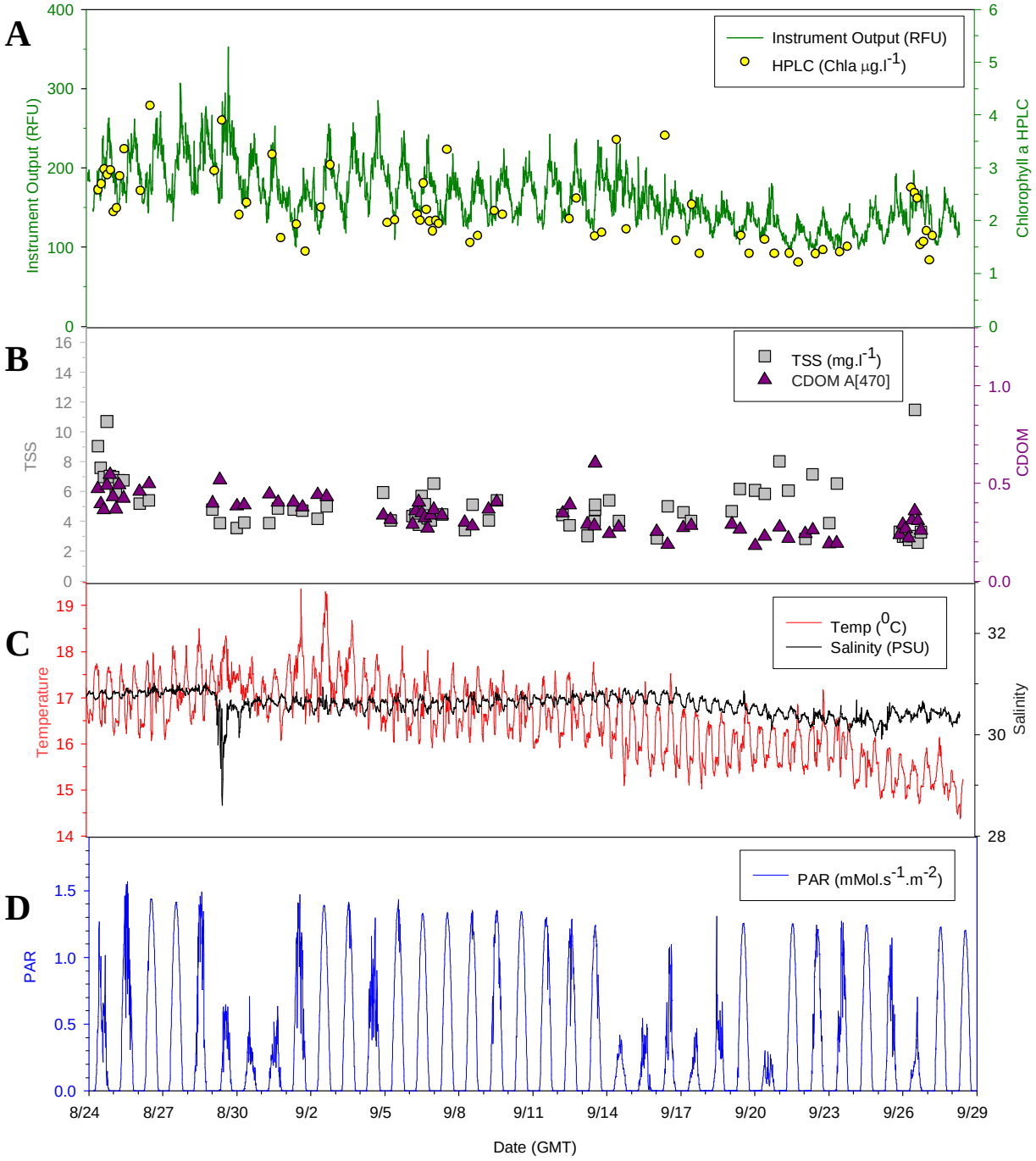


TABLE 5

	PRE		POST pre-cleaning		POST post-cleaning	
	Mean (RFU)	**STD ±**	**Mean (RFU)**	**STD ±**	**Mean (RFU)**	**STD ±**
Blk/DI	66.87	0.06	154.30	5.27	38.23	1.45
BB3	1459.97	72.09	1489.87	17.72	1527.03	2.11
Rhod	2338.57	16.80	2406.87	13.23	2304.53	10.83

Sensor before the four weeks deployment.

Sensor after the four weeks deployment.

Figure 10: Field Performance – Moss Landing, California (estuary)

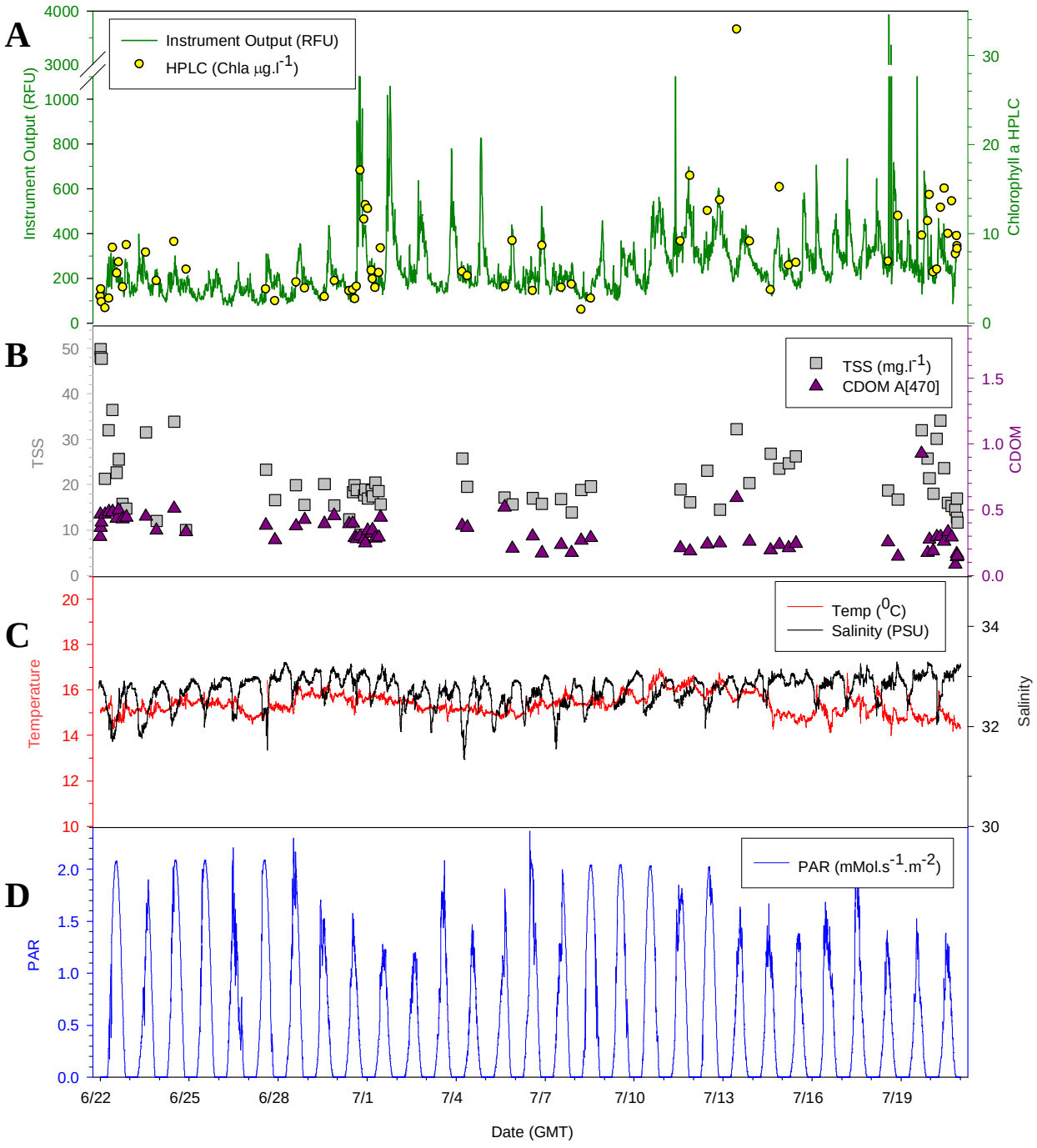


TABLE 6
n/a= non available due to biofouling, not an instrument malfunction.

	PRE		POST pre-cleaning		POST post-cleaning	
	Mean (RFU)	**STD ±**	**Mean (RFU)**	**STD ±**	**Mean (RFU)**	**STD ±**
Blk/DI	48.63	1.25	26.63	2.35	-13.17	0.64
BB3	1306.93	3.68	n/a	n/a	1968.10	20.23
Rhod	2296.43	27.65	n/a	n/a	771.93	2.5

Sensor before the four weeks deployment.

Sensor after the four weeks deployment.

Figure 11: Field Performance – Skidaway Island, Georgia (estuary)

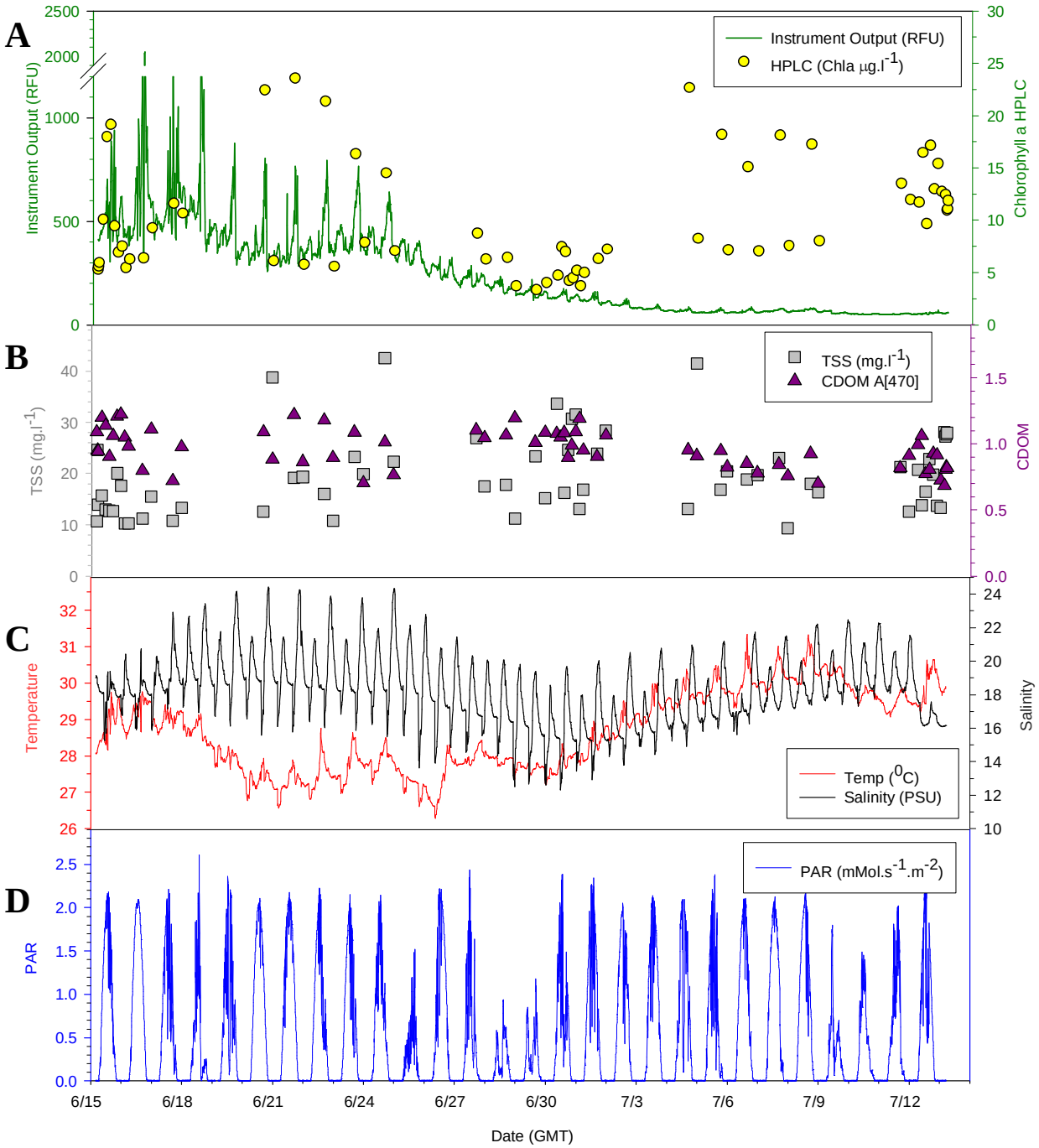


TABLE 7

	PRE		POST pre-cleaning		POST post-cleaning	
	Mean (RFU)	**STD ±**	**Mean (RFU)**	**STD ±**	**Mean (RFU)**	**STD ±**
Blk/DI	56.31	0.17	46.8	0.70	44.7	0.33
BB3	1112.77	33.05	70.5	1.72	115.40	3.54
Rhod	1758.30	28.43	95.0	0.72	159.90	4.35

Sensor before the four weeks deployment.

Sensor after the four weeks deployment.

Figure 12: Field Performance – Bayboro Harbor, Tampa Bay, Florida (estuary)

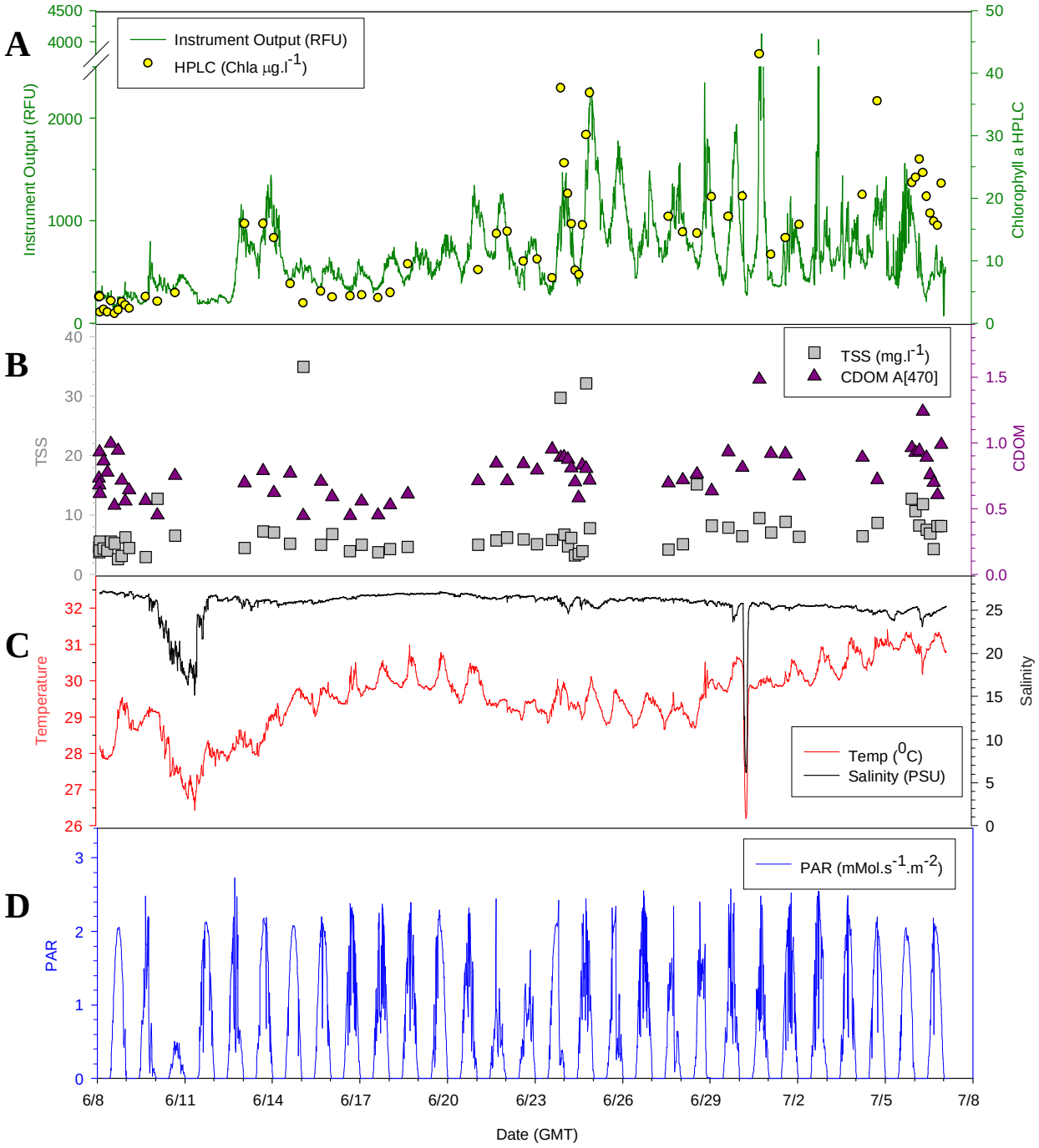


TABLE 8

	PRE		POST pre-cleaning		POST post-cleaning	
	Mean (RFU)	**STD ±**	**Mean (RFU)**	**STD ±**	**Mean (RFU)**	**STD ±**
Blk/DI	69.67	0.15	61.80	13.08	67.57	4.96
BB3	1302.50	6.68	81.30	29.30	152.03	70.36
Rhod	3906.73	55.78	66.97	1.00	500.25	15.34

Sensor before the four weeks deployment.

Sensor after the four weeks deployment.

Moored Reliability

The instrument performed as expected with 100% data recovery. One unit sent to SkIO required a battery replacement prior to the deployment. The instrument sample rate generated a slight drift in the time stamp.

SUMMARY OF VERIFICATION RESULTS, FIELD PROFILING TESTS:

Figures 13A, 14A and 15A, display depth profiles of chlorophyll *a* concentrations in RFU (green line) measured during the up-cast by the instrument with the corresponding chlorophyll *a* concentrations from extractive HPLC analysis (yellow dots in µg L^{-1}, n = 3, standard deviation is plotted although values are smaller than symbols used in graphs) taken at 6 discrete depth throughout the water column during the up-cast.

Figures 13C, 14C and 15C display the total suspended solid (grey squares, TSS in mg L^{-1}) measured by weight and the colored dissolved organic matter (CDOM) estimated by spectrophotometric analysis (purple triangles, absorption coefficient at 470 nm) both derived from samples taken at 6 discrete depth throughout the water column during the up-cast.

Figures 16A, 17A and 18A, display depth profiles of chlorophyll *a* concentrations in RFU (green line) measured during the down-cast by the instrument with the corresponding chlorophyll *a* concentrations from extractive HPLC analysis (yellow dots in µg L^{-1}, n = 3, standard deviation is plotted although values are smaller than symbols used in graphs) taken at 6 discrete depth throughout the water column during the down-cast.

Figures 16C, 17C and 18C display the total suspended solid (grey squares, TSS in mg L^{-1}) measured by weight and the colored dissolved organic matter (CDOM) estimated by spectrophotometric analysis (purple triangles absorption coefficient at 470 nm) both derived from samples taken at 6 discrete depth throughout the water column during the down-cast.

Figures 13B, 14B, 15B 16B, 17B, 18B display shows the corresponding temperature (degree Celsius) salinity (PSU when available) the Photosynthetically Active Radiation (PAR in mMol s^{-1} m^{-2} when available) throughout the water column during the down-cast.

Figure 13: MAINE Profile 1 - Position: Penobscot Bay, Upper Bay near Castine, 44 21.258, Lon: 68 50.062. Start Down ~ 17:58:00 EST.

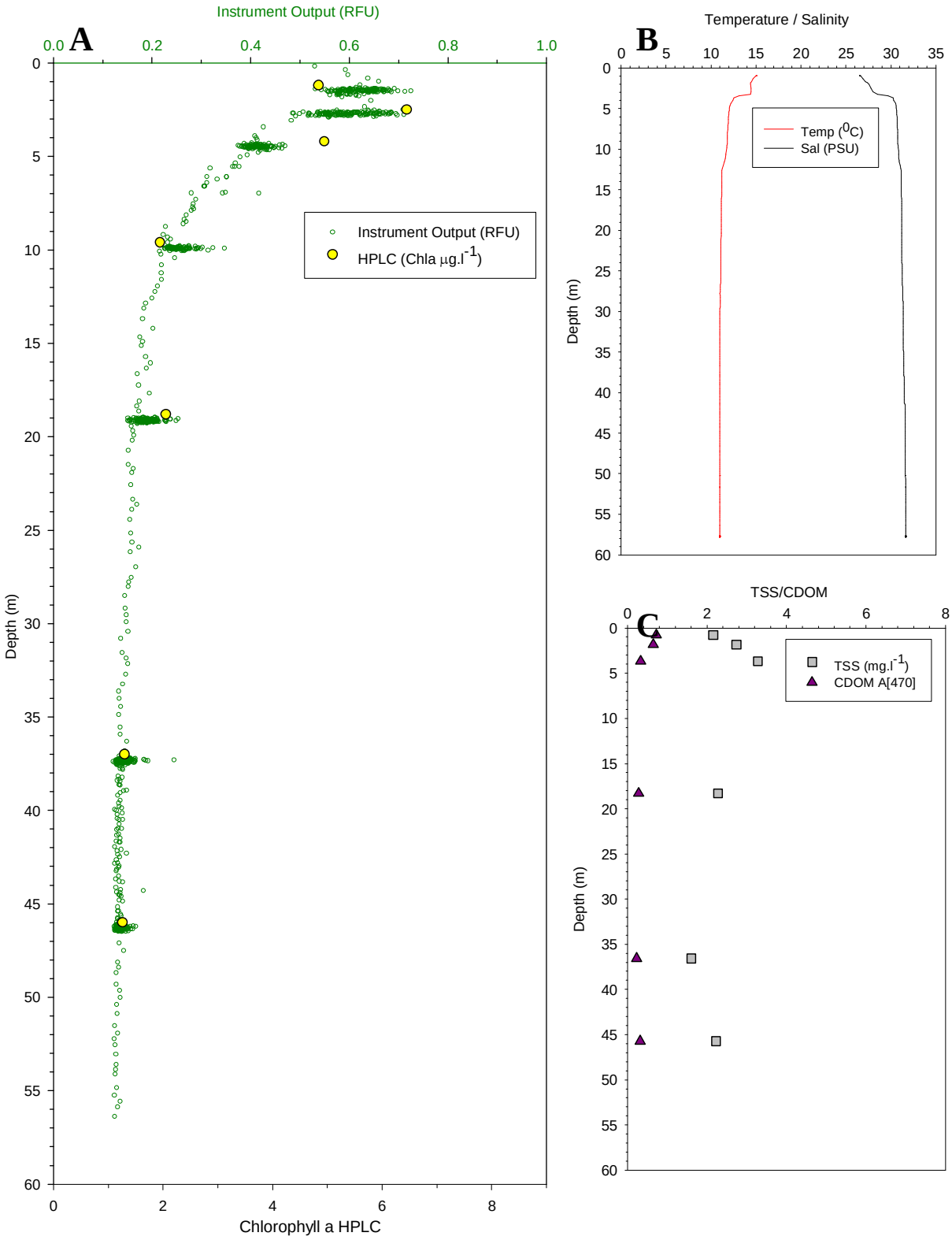


Figure 14: MAINE Profile 2 - Penobscot Bay, Bay Mouth Channel, Lat: 44 06.395, Lon: 68 59.447
Start Down ~ 21:15:49 EST

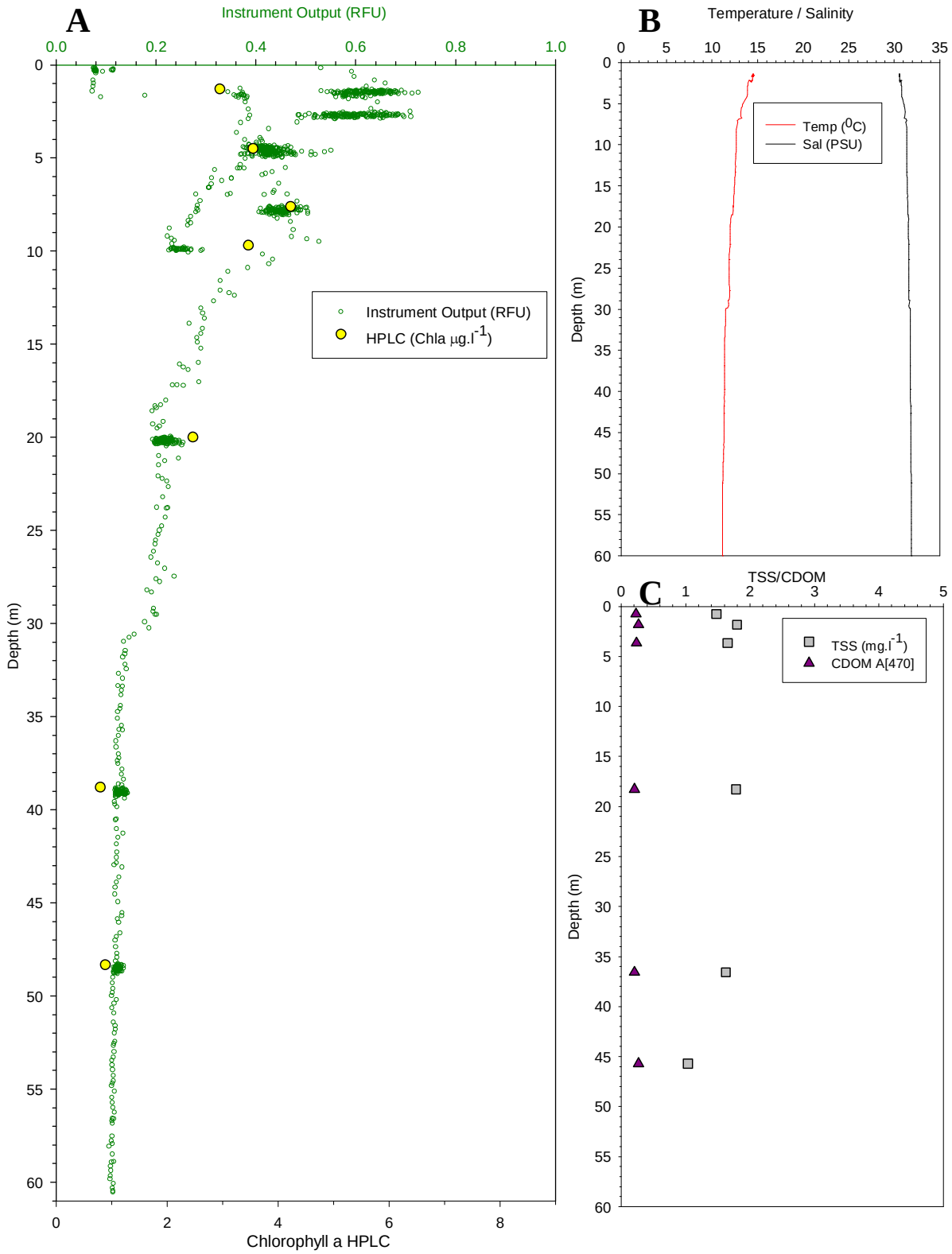


Figure 15: MAINE Profile 3 - Position: Penobscot Bay, Southern Passage, Lat: 44 19.850, Lon: 68 56.322. Start Down ~ 00:47:15 EST.

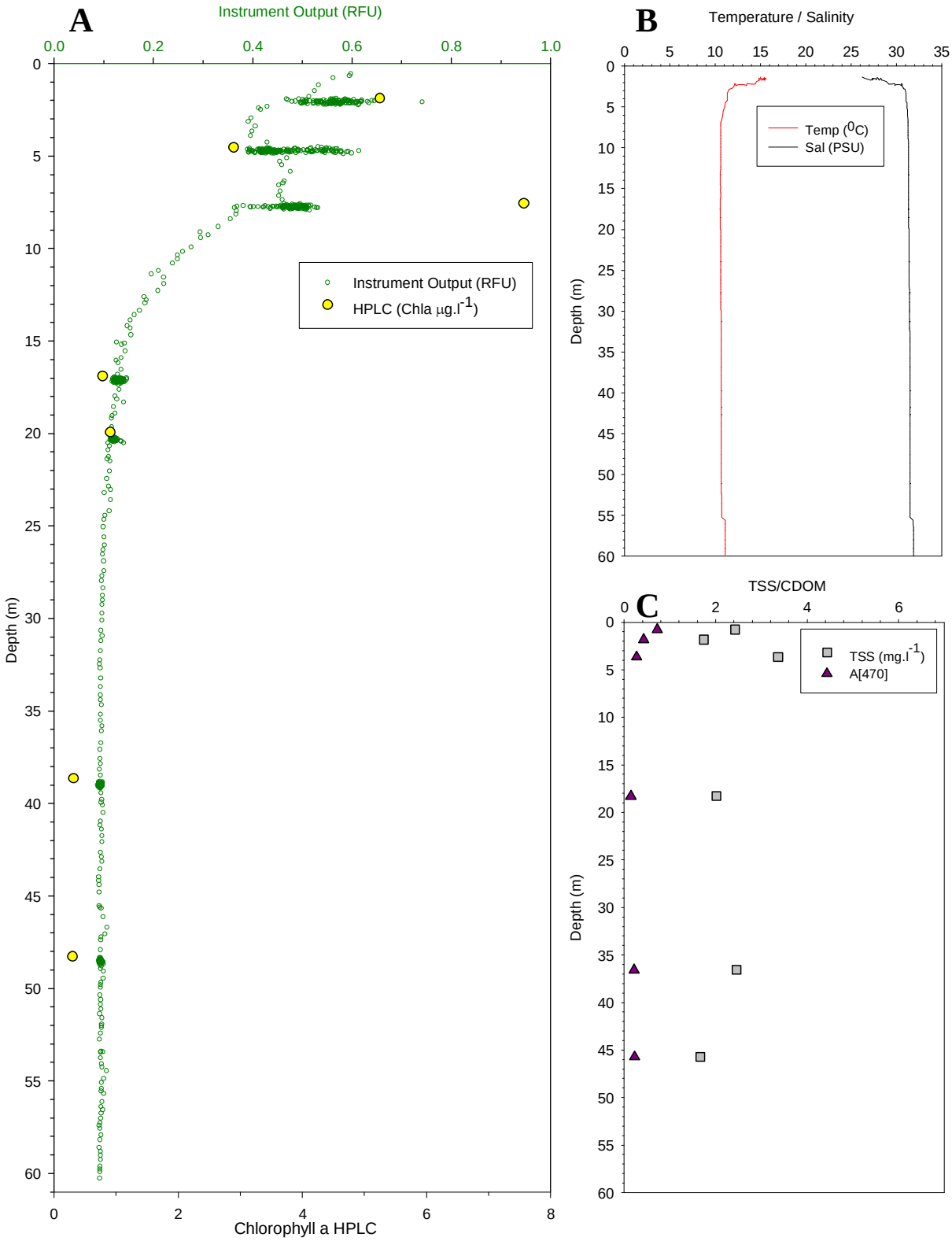


Figure 16: Michigan Profile 1 – Lake Michigan
Start Down ~ 7:00:00 EST

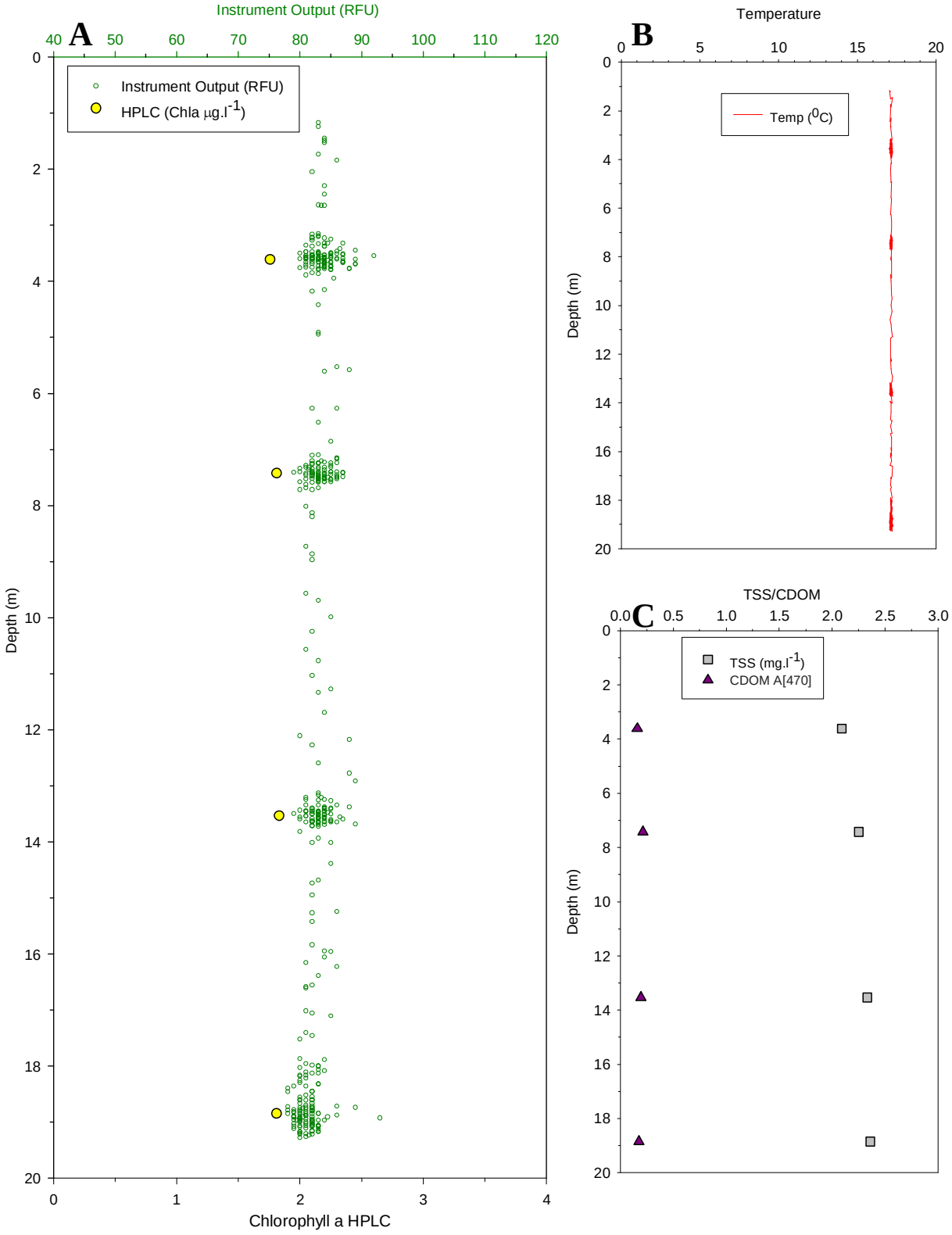


Figure 17: Michigan Profile 2 - Lake Michigan
Start Down ~ 9:10:04 EST

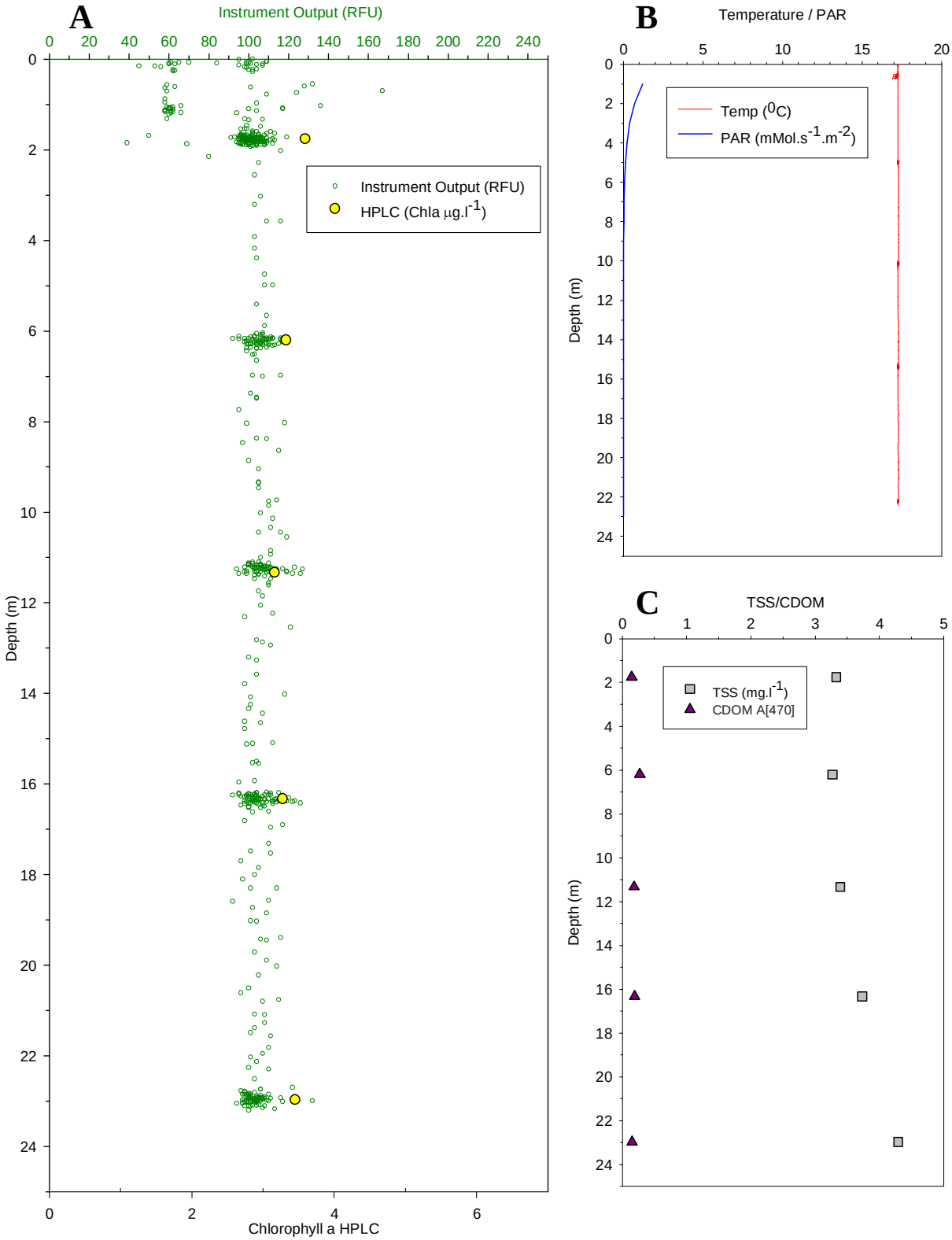
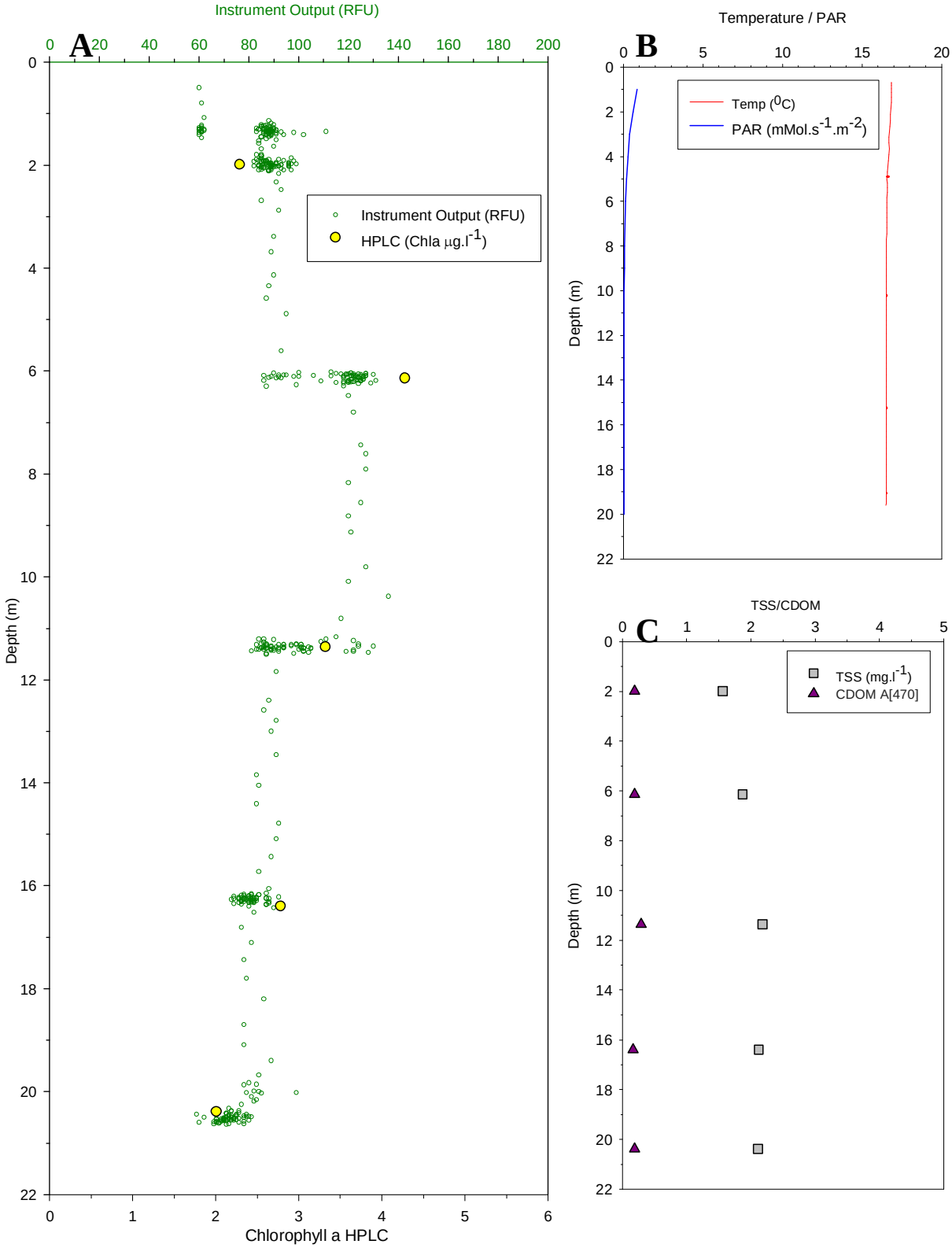


Figure 18: Michigan Profile 3 - Lake Michigan
Start Down ~ 17:27:49 EST



ACKNOWLEDGMENTS:

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, J. Cullen, Z. Kolber, S. McLean, H. MacIntyre, J. Newton, P. Pennington, M.J. Perry, C. Roesler, and N. Welschmeyer for their advice and direct participation in various aspects of this evaluation. E. 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, Coastal Services Center, Charleston, SC.

April 24, 2006

Date

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ACT Director

April 24, 2006

Date

Approved By: Dr. Mario Tamburri
ACT Chief Scientist

April 24, 2006

Date

Approved By: Dr. Earle Buckley
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WET Labs comments on ACT Fluorometer Verification Statement

WET Labs wholeheartedly endorses the ACT goals and processes and participated in the fluorometer verification with pleasure. The ACT protocols test instruments with respect to basic operation and more importantly how they work in the field. For long term monitoring programs the initial cost of an instrument is quickly dwarfed by the operational and maintenance costs, particularly in remote environments. It is paramount to be able to report good data for as long a period as possible. In the coastal environment where biofouling is extreme the cost of retrieving, refurbishing and replacing instruments is only exceeded by the cost of unreportable data due to poor data quality. In that respect, the ACT fluorometer verification results are similar to the results our users are finding around the world: the ECO sensors are stable and correlatable with natural variability. Since the ACT testing reported here we have improved the ECO line with copper face plates which further reduce fouling on the instrument and reduce fouling related wear and tear on the bio-wiper. Please see our website (www.wetlabs.com) for current information.

Field Results: The instruments tracked short and long term variability at all of the sites. Only at Skidaway did bio-fouling interfere with the data series. Our website has a detailed look at the results from each of the sites. This includes a discussion on calibration, data processing and the physical forcing functions that impact chlorophyll fluorescence monitoring.

Laboratory results of the ACT testing are in keeping with our specifications for the instrument: the instrument's response is linear with respect to chlorophyll (or the dye proxy).

- Temperature. We concur that the response linearity of the instrument is not a function of temperature apart from the change in fluorescence efficiency of the dye with respect to temperature.
- Turbidity. Our testing and field data from users agree with the ACT testing that the ECO fluorescence response is not a function of turbidity (non-fluorescent particle concentration).
- Ambient Light. The insensitivity of the ECO line to ambient light in the ACT testing is in keeping with our specifications.
- CDOM. The step-wise response of the ECO to the CDOM enrichment using coffee extract is in keeping with our testing. Coffee extract, while a useful and simply obtained proxy for CDOM does exhibit a stronger fluorescent response at 470 nm than most natural CDOM, and hence in the field there is little need for a CDOM correction. Note that in the field results CDOM variability was negligible relative to observed variability in both the chlorophyll fluorescence and extracted chlorophyll.

Timing issues: The timing issues noted in the report were discovered during the ACT testing and are alleviated by adjusting the data reporting rate to slightly higher than 1 Hz (typically 1.08 Hz).

Pre and post deployment tests: We concur with the report's statements that caution should be used in interpreting these results. Variability between the pre and post data is probably mostly a function of cleaning and particles introduced into the test fluid from the instrument. For stability over the test period it is more useful to look at the baseline data in-situ over the test period as well as covariance between the chlorophyll fluorescence and extracted data over time. On that basis the ECO's did not exhibit instrument drift at any site, in keeping with our long term experience of the stability of the instrument over far longer time periods of use.

Ian Walsh, V.P. Operations. 5/5/06