PERFORMANCE VERIFICATION STATEMENT for the TURNER Designs CYCLOPS-7 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.

Questions and comments should be directed to: Dr. Mario Tamburri
Alliance for Coastal Technologies
Chesapeake Biological Laboratory
PO Box 38 / One Williams Street
Solomons, Maryland 20688, USA
Email: tamburri@cbl.umces.edu

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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 Turner Designs CYCLOPS-7 fluorometer evaluated in the laboratory and under diverse field conditions 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.turnerdesigns.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 Turner Designs CYCLOPS-7 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 Turner Designs CYCLOPS-7 submersible fluorometer is a single channel, mini-fluorometer designed for monitoring fluorophores in water. It is intended to be integrated into a multi-parameter system to obtain its power, and to deliver an output voltage proportional to fluorescence to the system data logger. The excitation wavelength is 460 nm and the emission wavelength is 620 - 715 nm. Three user settable gain ranges provide a wide measurement dynamic range of 0.03 to 500 $\mu g\ L^{-1}$ for Chlorophyll *a*. The manufacturer's published performance specifications for the CYCLOPS-7 fluorometer include: Dynamic Range is dependent on gain setting (X1: 0 - 500 $\mu g\ L^{-1}$, X10: 0 - 50 $\mu g\ L^{-1}$, or X100: 0 - 5 $\mu g\ L^{-1}$), Minimum Detection Limit 0.03 $\mu g\ L^{-1}$, Linearity (in lab environment over dynamic range) 99% R^2, and Operating Depth of 0 to 600 meters. More information can be found at www.turnerdesigns.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.

The Turner Designs CYCLOPS-7 fluorometers tested, both in the laboratory and in the field, did not include a biofouling prevention system and were plugged into a Campbell datalogger for data recording and power. 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 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.

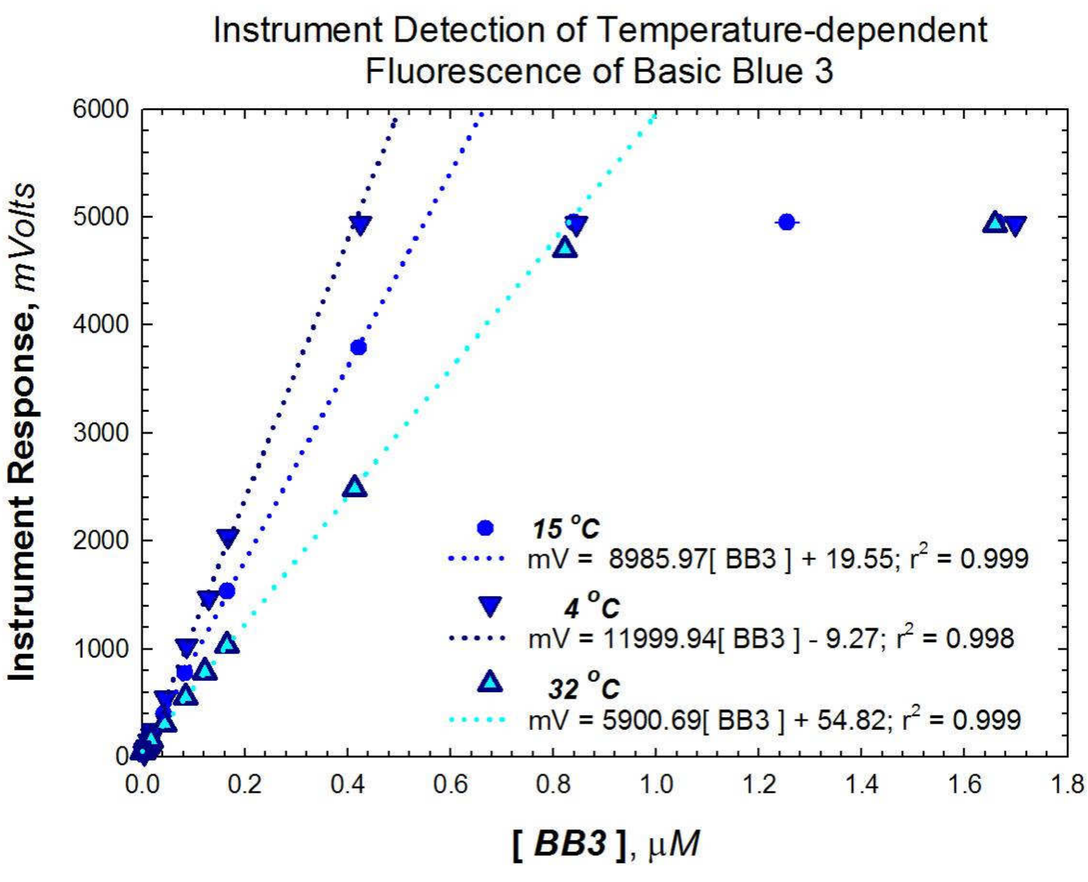
*** Data is presented as relative fluorescence units (RFU) as reported by the instrument. For additional corrections, interpretation and analysis of results, please visit www.turnerdesigns.com.**

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. Laboratory tests were conducted with the fluorometers set at a fixed gain (10X) that corresponded to chlorophyll values commonly found in coastal waters.

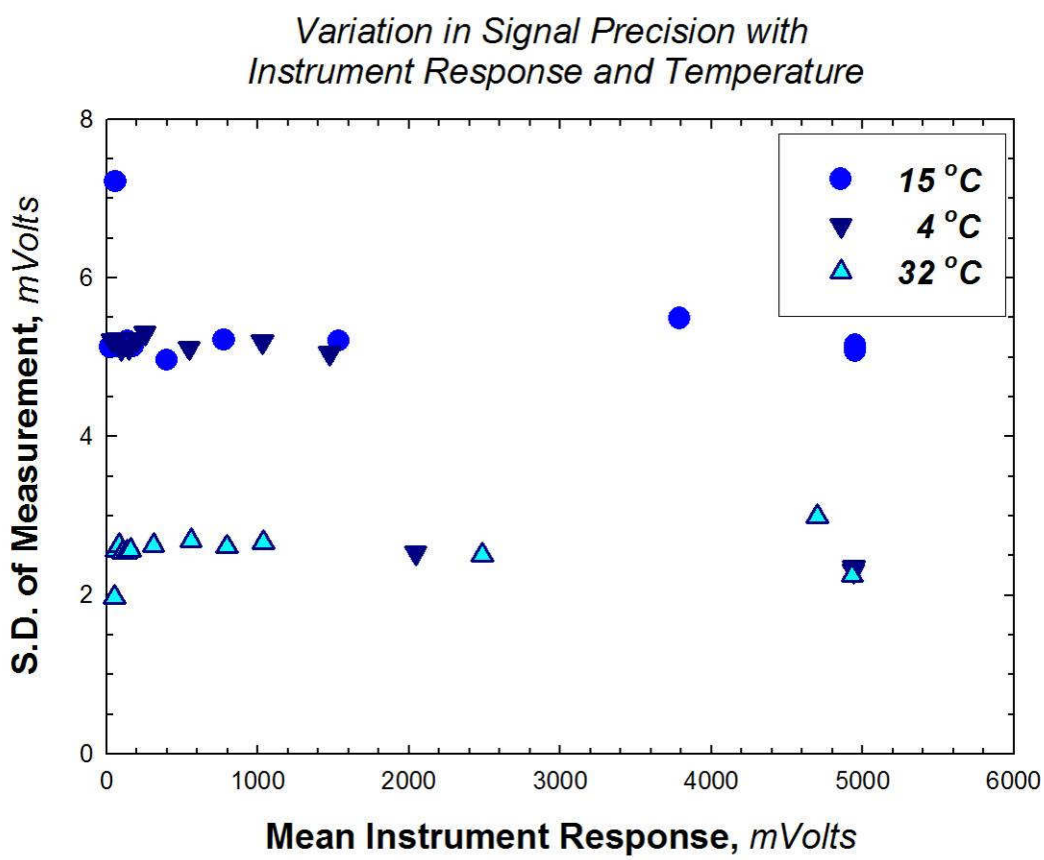
Response Linearity and Detection Range

Figure 1: Instruments were equilibrated in temperature regulated water baths and programmed to sample at 1 minute intervals while being exposed to sequential increases in BB3 concentrations. The Cyclops fluorometer output was highly linear through a BB3 concentration at least 0.5 µM and detector response saturated at higher concentrations with a maximum signal of ca. 4940 mV. The average instrument response in dye-free water was 37.37 ± 4.11 mV, indicating a limit of detection at 3 s.d. of 12.32 mV 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 Cyclops fluorometer sensor response did exhibit a slight temperature hysteresis, yielding a BB3 temperature-dependence of -1.80% ± 0.21% 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.
Note: Instrument gain (10X)



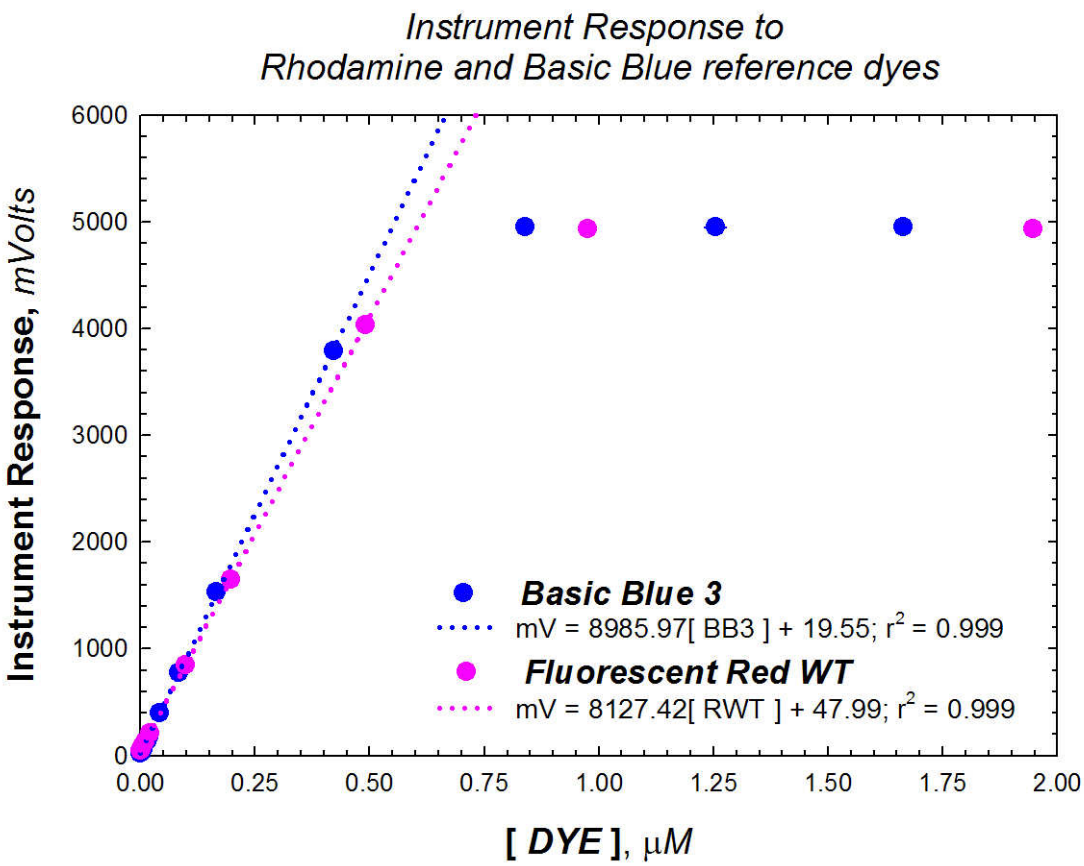
Response Precision

Figure 2: Detector noise, here expressed as the mean standard deviation of 10 sequential 1 minute averages of 1 sec samples at fixed temperature and BB3 dye concentrations, exhibited no significant trends with mean detector response below saturation. Over the instrument detection range of 0-0.5 μM BB3, the absolute signal noise was ± 4.22 (1.98 – 7.21) mV. Detector noise was about 50% lower at 32 ^{o}C. All data plotted as mean and standard deviation of both detector response and analyte concentration.
Note: Instrument gain (10X)



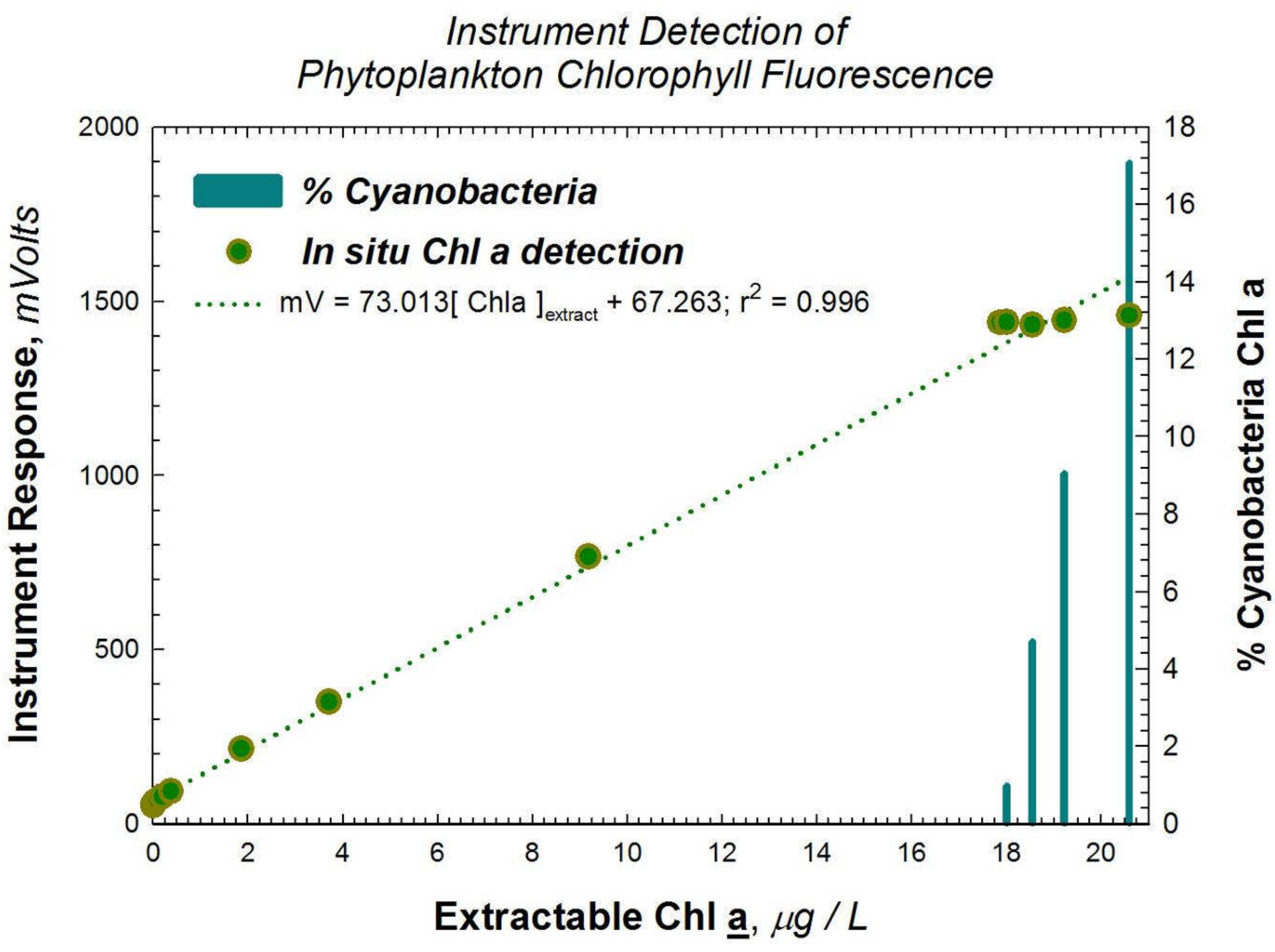
Response Linearity and Fluorochrome Response

Figure 3: The Cyclops 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). BB3 was detected with approximately 10% higher molar efficiency than RWT. All data plotted as mean and standard deviation of both detector response and analyte concentration.
Note: Instrument gain (10X)



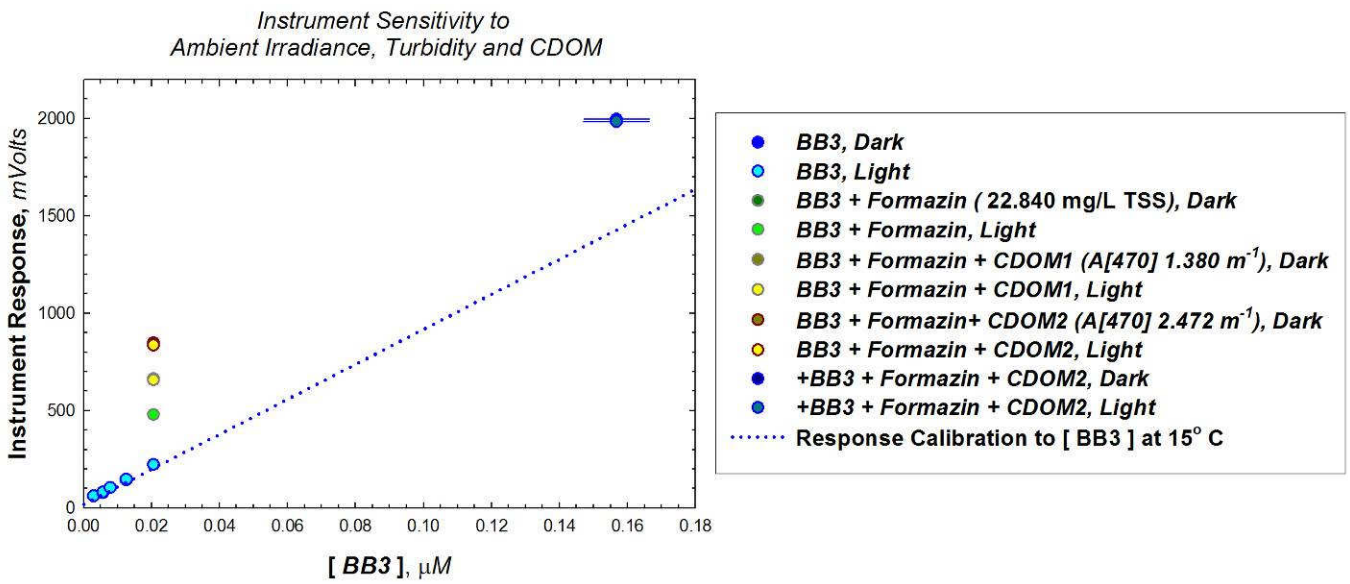
Response Linearity and Phytoplankton Chlorophyll Fluorescence

Figure 4: *Detection of Phytoplankton Chlorophyll Fluorescence.* Instruments were equilibrated f/2-enriched seawater in a temperature controlled tank at 15 °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 °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: RFU=77.14[Chl *a*]+ 60.86, r^2=0.999, p<0.001 whereas the response to subsequent cyanobacterial additions was ca. 70% lower: RFU=23.08[Chl *a*]-3.61, r^2=0.975, p<0.001. Instrument noise in the background seawater media was ± 1.83 mV. Significant instrument response was observed at an added dose of 0.018 µg L^{-1} of Chl *a*, better performance than the predicted limit of detection of 0.059 µg L^{-1} of Chl *a*.
Note: Instrument gain (10X)



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 ^oC. Instrument detection of added BB3 was in good agreement (+13%) with the prior, independent calibration to BB3 concentration (see Fig. 1). At low BB3 concentrations the Cyclops sensor appears to be sensitive to formazin, added as a proxy for turbidity, inducing a doubling (ca. 256 mV offset) of detector response. Coffee extract, used as a proxy for CDOM, induced a similar signal enhancement (ca 184 mV) 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 6% lower than the BB3 calibration response (8441.0 mV/ µM BB3 vs 8986.0 mV/ 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.
Note: Instrument gain (10X)



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 continuously at 1 sec intervals.

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 in vivo chlorophyll *a* fluorescence 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 µg 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.
The mooring tests were conducted with the fluorometers set at a fixed gain (10X), except for the Hawaii partner site (100X).

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 fluoromHeter 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)
Note: Instrument gain (10X)

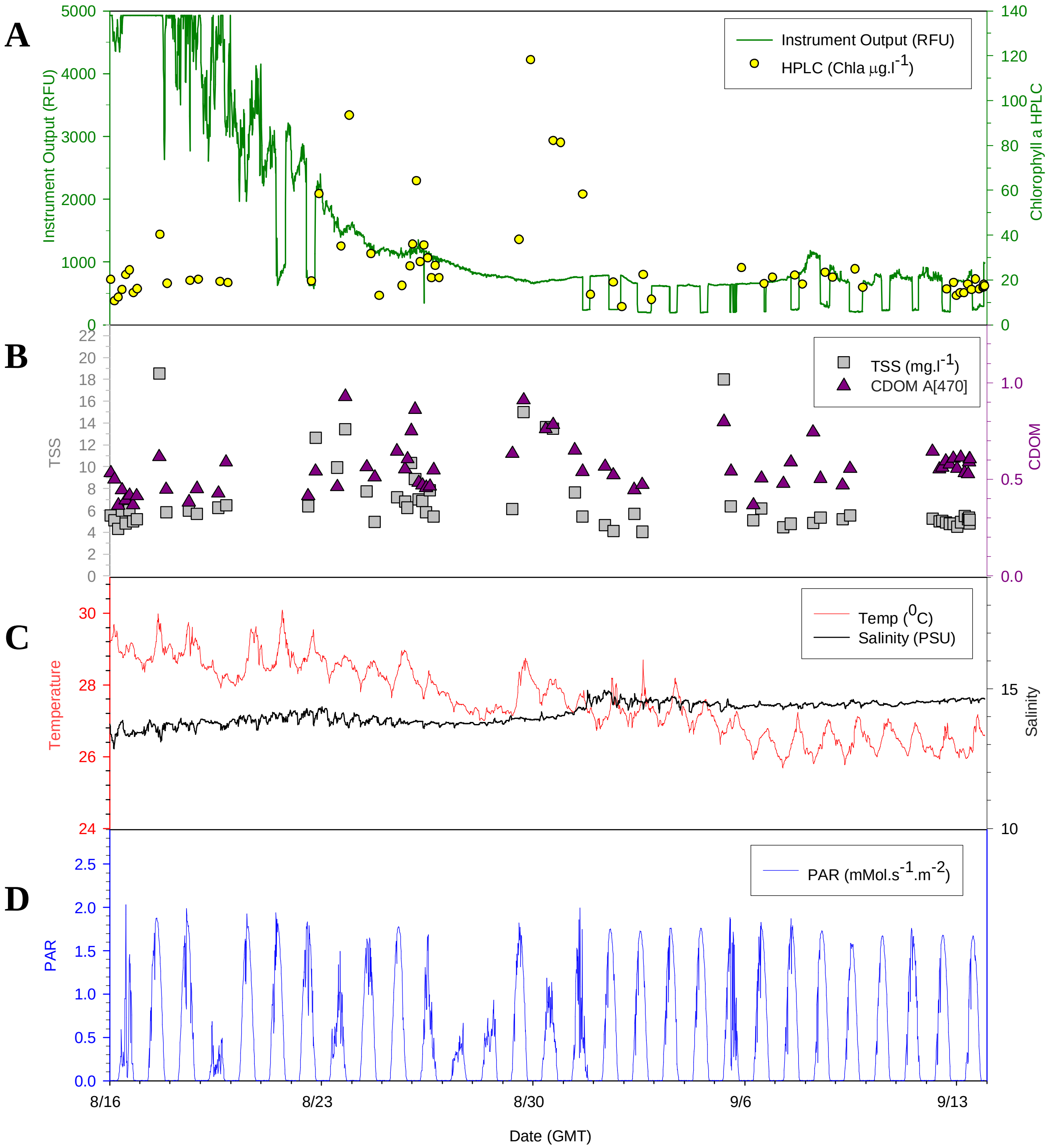
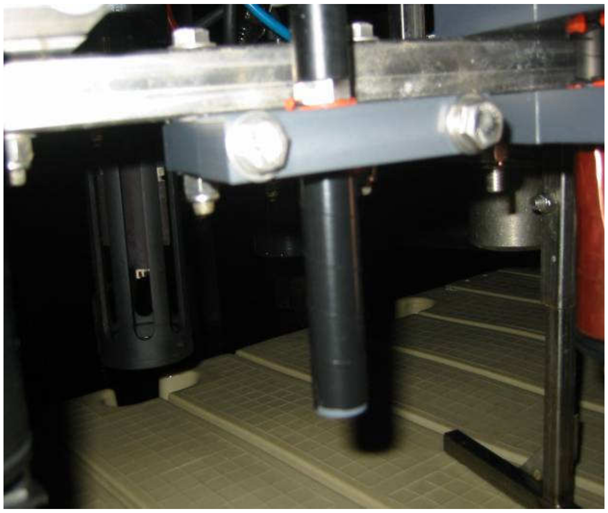


TABLE 2

	PRE		POST pre-cleaning		POST post-cleaning	
	Mean (RFU)	**STD ±**	**Mean (RFU)**	**STD ±**	**Mean (RFU)**	**STD ±**
Blk/DI	56.00	26.84	1009.77	33.06	112.71	10.85
BB3	3555.20	1189.22	1042.37	12.63	4927.27	0.12
Rhod	4926.93	0.35	1088.93	21.55	4927.17	0.06



Sensor before the four weeks deployment.

Sensor after the four weeks deployment.

Figure 7: Field Performance – Muskegon, Lake Michigan (freshwater)
Note: Instrument gain (10X)

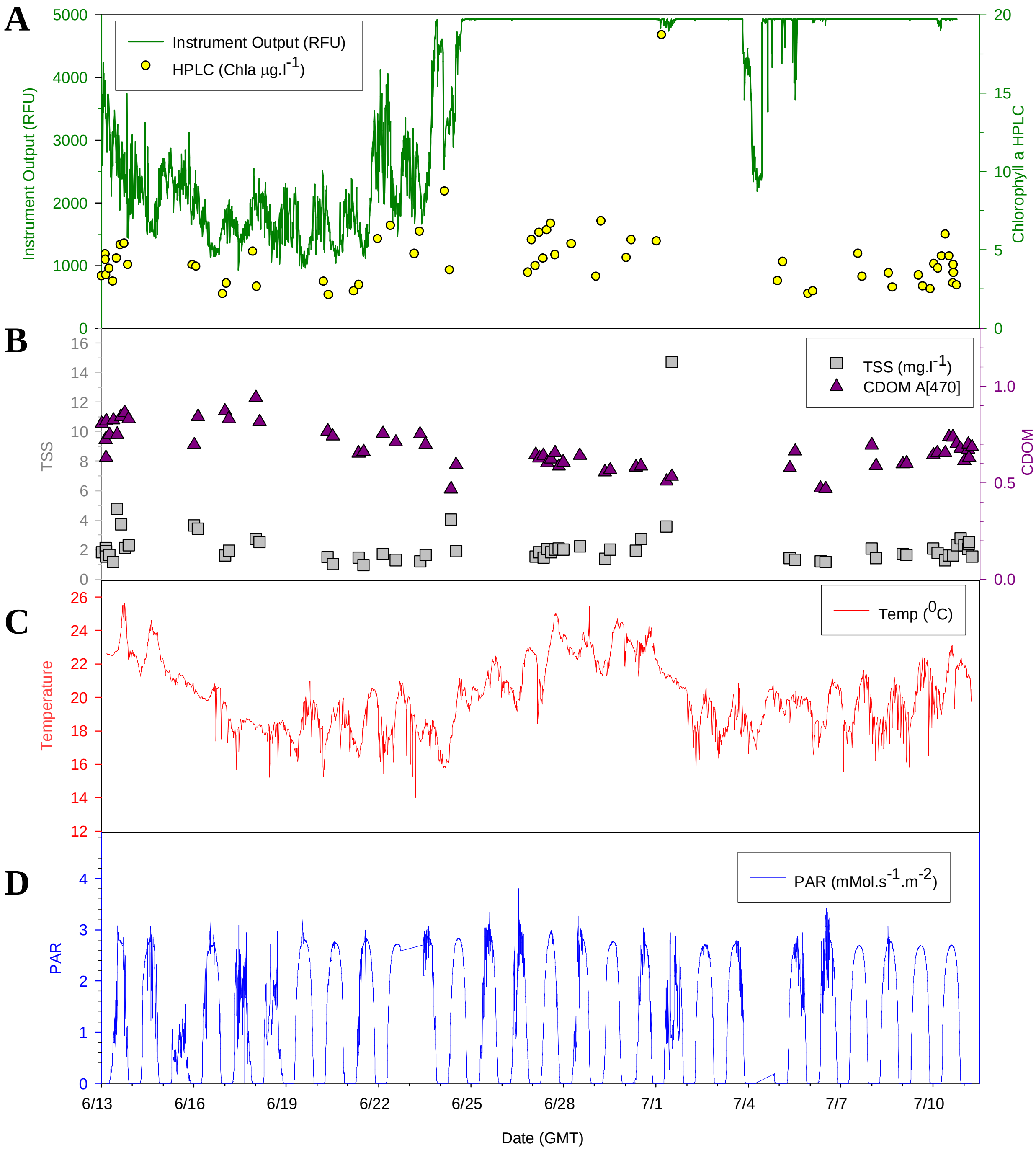


TABLE 3

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			4927.90	n/a	1346.00	n/a
BB3			4927.82	n/a	3176.08	n/a
Rhod			4916.87	n/a	3434.91	n/a

Sensor before the four weeks deployment.

Sensor after the four weeks deployment.

Figure 8: Field Performance – Coconut Island, Hawaii (coral reef)
Note a: The missing PAR and instrument data were due to a malfunction of the ACT datalogger.
Note b: The gain was set up at 100X for this location.

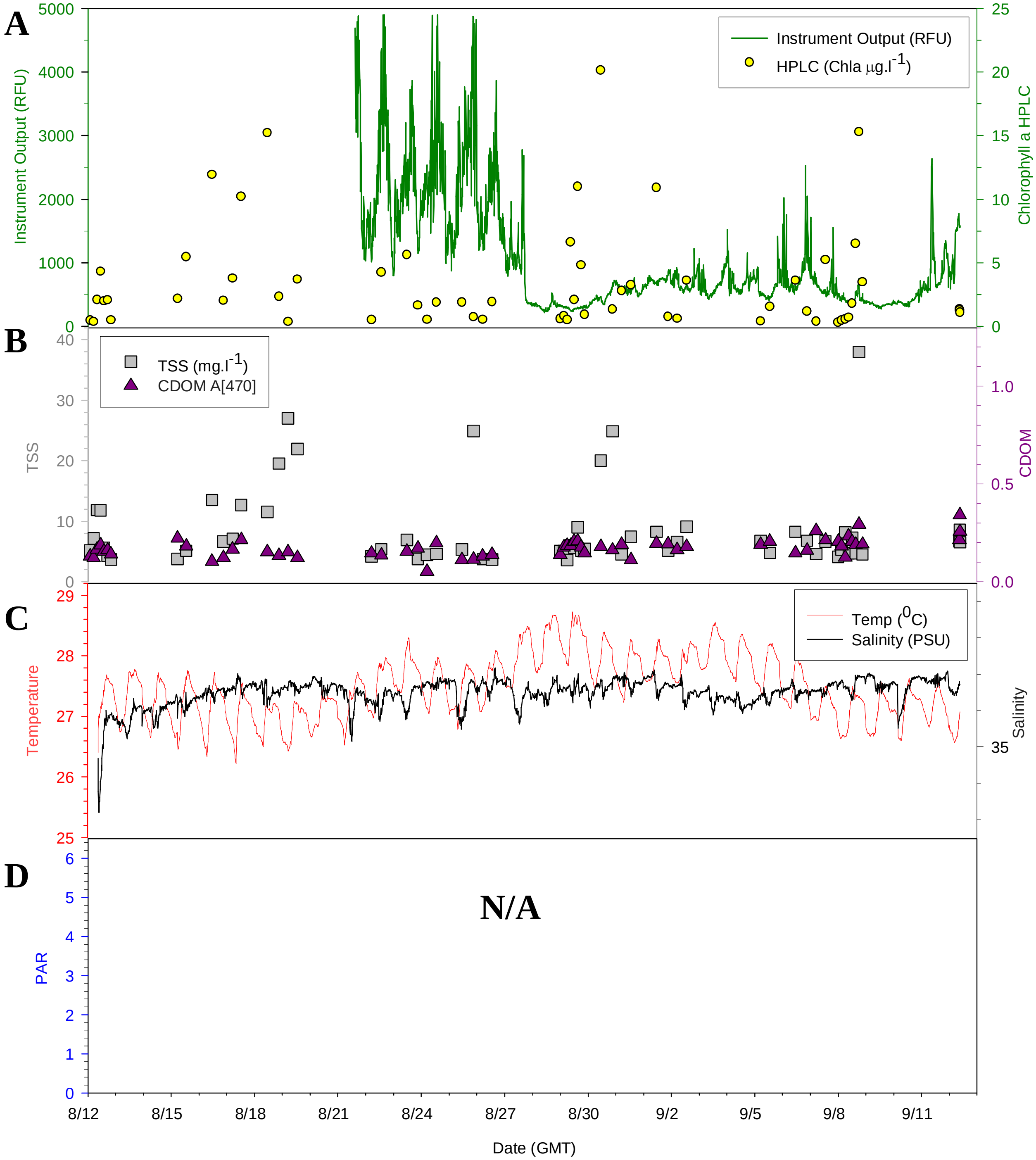


TABLE 4

Note: The missing data were due to data loss following a malfunction of the ACT datalogger.
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			2104.24	783.04	37.68	1.89
BB3			n/a	n/a	2982.95	97.99
Rhod			n/a	n/a	2752.77	68.93

Sensor before the four weeks deployment.

Sensor after the four weeks deployment.

Figure 9: Field Performance – Damariscotta River Estuary, Gulf of Maine (tidal embayment)
Note: Instrument gain (10X)

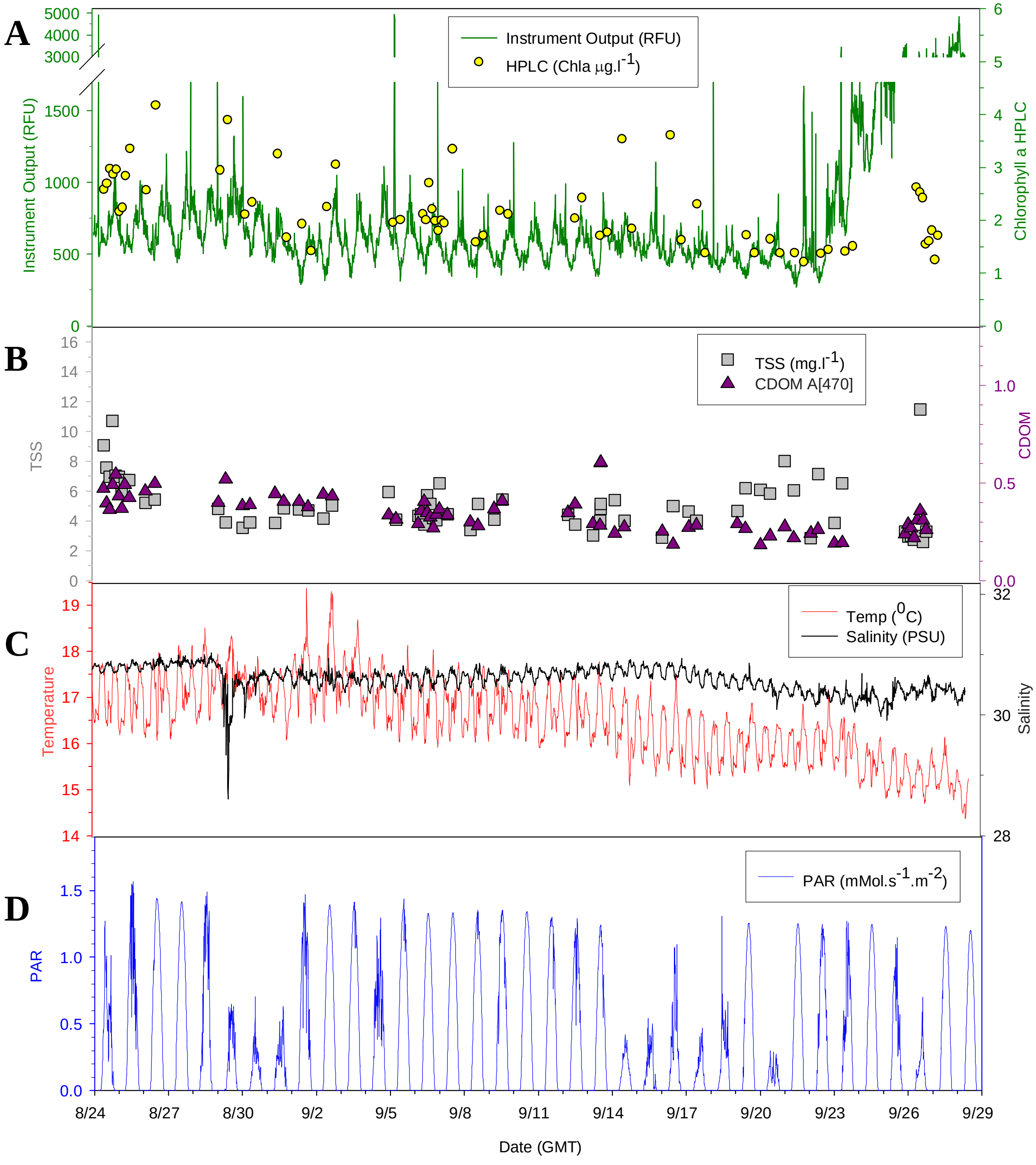
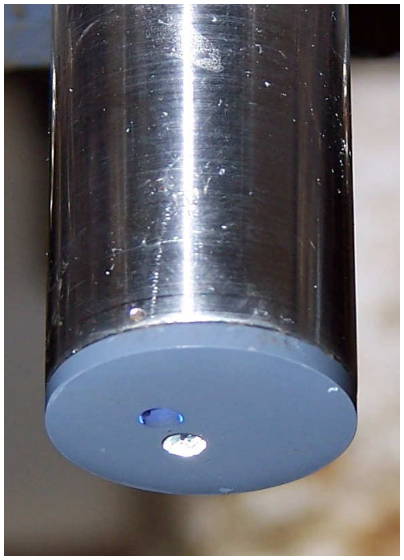


TABLE 5

	PRE		POST pre-cleaning		POST post-cleaning	
	Mean (RFU)	**STD ±**	**Mean (RFU)**	**STD ±**	**Mean (RFU)**	**STD ±**
Blk/DI	43.97	1.88	4375.57	504.26	41.93	2.22
BB3	3745.43	120.93	4161.89	499.56	3850.61	20.71
Rhod	3584.02	4.76	3381.39	53.74	3579.75	11.53



Sensor before the four weeks deployment.

Sensor after the four weeks deployment.

Figure 10: Field Performance – Moss Landing, California (estuary)
Note: Instrument gain (10X)

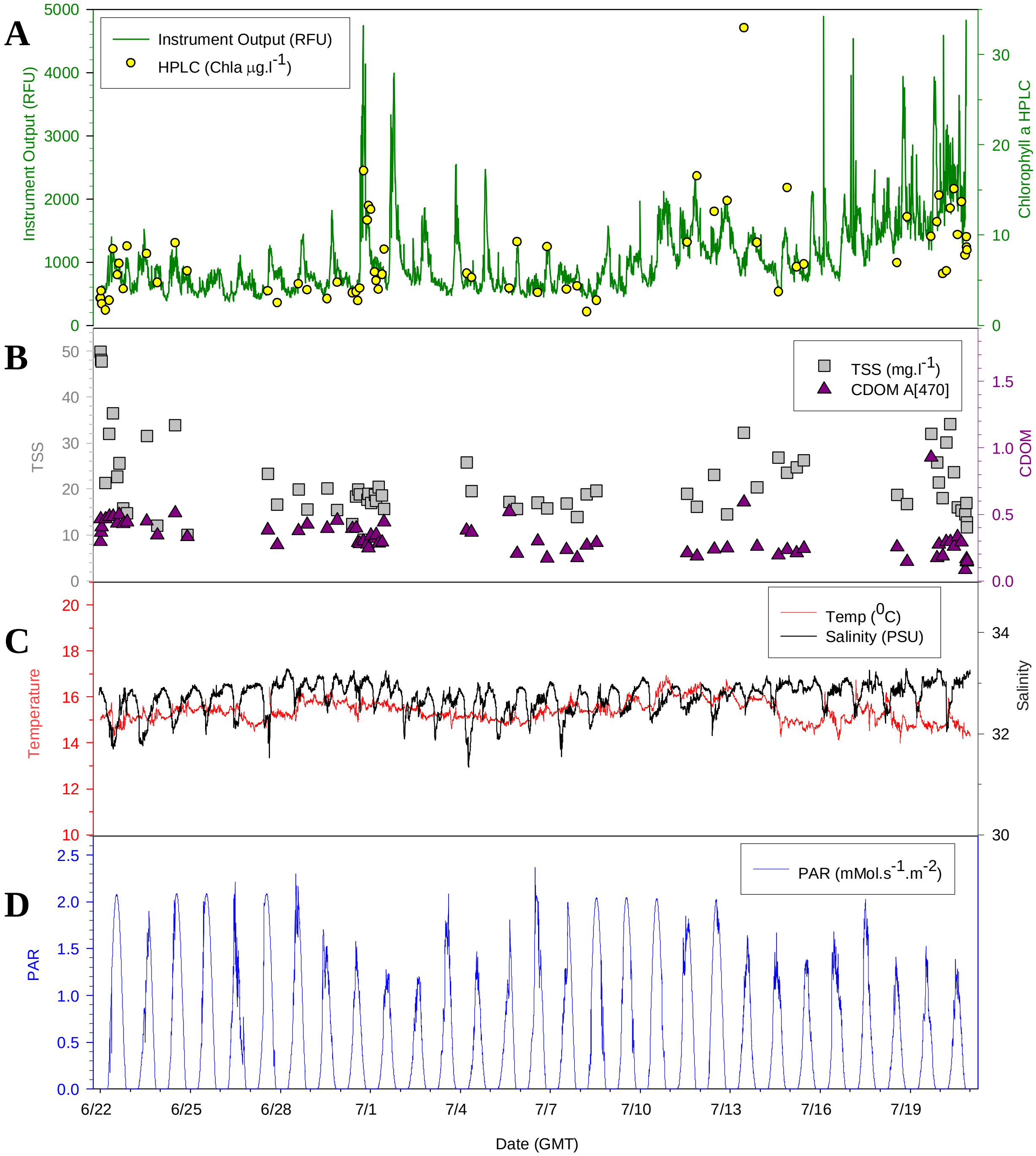
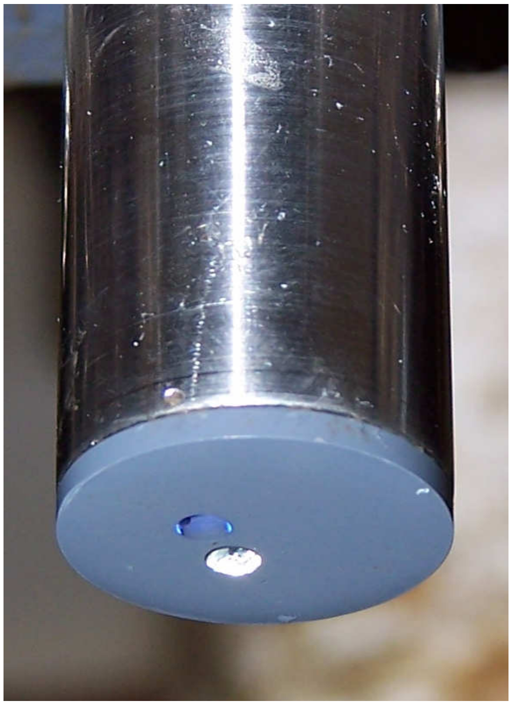


TABLE 6
n/a= non available due to biofouling, not an instrument malfunction.

	PRE		POST pre-cleaning		POST post-cleaning	
	Mean (RFU)	**STD ±**	**Mean (RFU)**	**STD ±**	**Mean (RFU)**	**STD ±**
Blk/DI	47.08	0.17	1057.79	106.40	41.49	0.11
BB3	3000.77	28.33	n/a	n/a	3597.03	3.2
Rhod	3568.23	1.14	n/a	n/a	1536.00	1.78



Sensor before the four weeks deployment.

Sensor after the four weeks deployment.

Figure 11: Field Performance – Skidaway Island, Georgia (estuary)
Note: Instrument gain (10X)

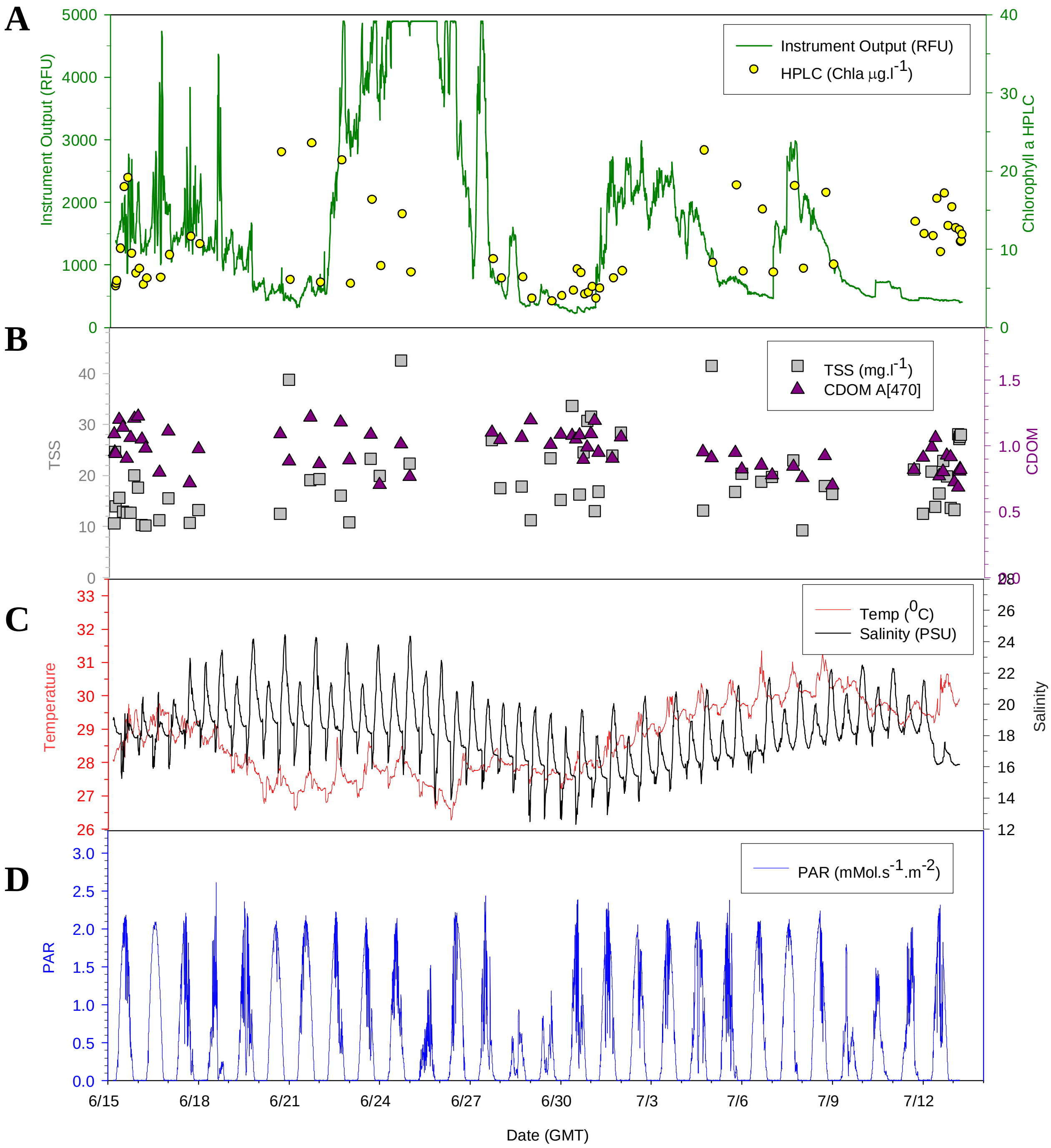


TABLE 7

	PRE		POST pre-cleaning		POST post-cleaning	
	Mean (RFU)	**STD ±**	**Mean (RFU)**	**STD ±**	**Mean (RFU)**	**STD ±**
Blk/DI	46.39	0.24	450.9	23.50	60.2	2.26
BB3	3021.01	80.48	411.8	4.25	3017.91	47.67
Rhod	3377.24	99.11	399.1	3.69	2553.96	8.57

Sensor before the four weeks deployment.

Sensor after the four weeks deployment.

Figure 12: Field Performance – Bayboro Harbor, Tampa Bay, Florida (estuary)
Note: Instrument gain (10X)

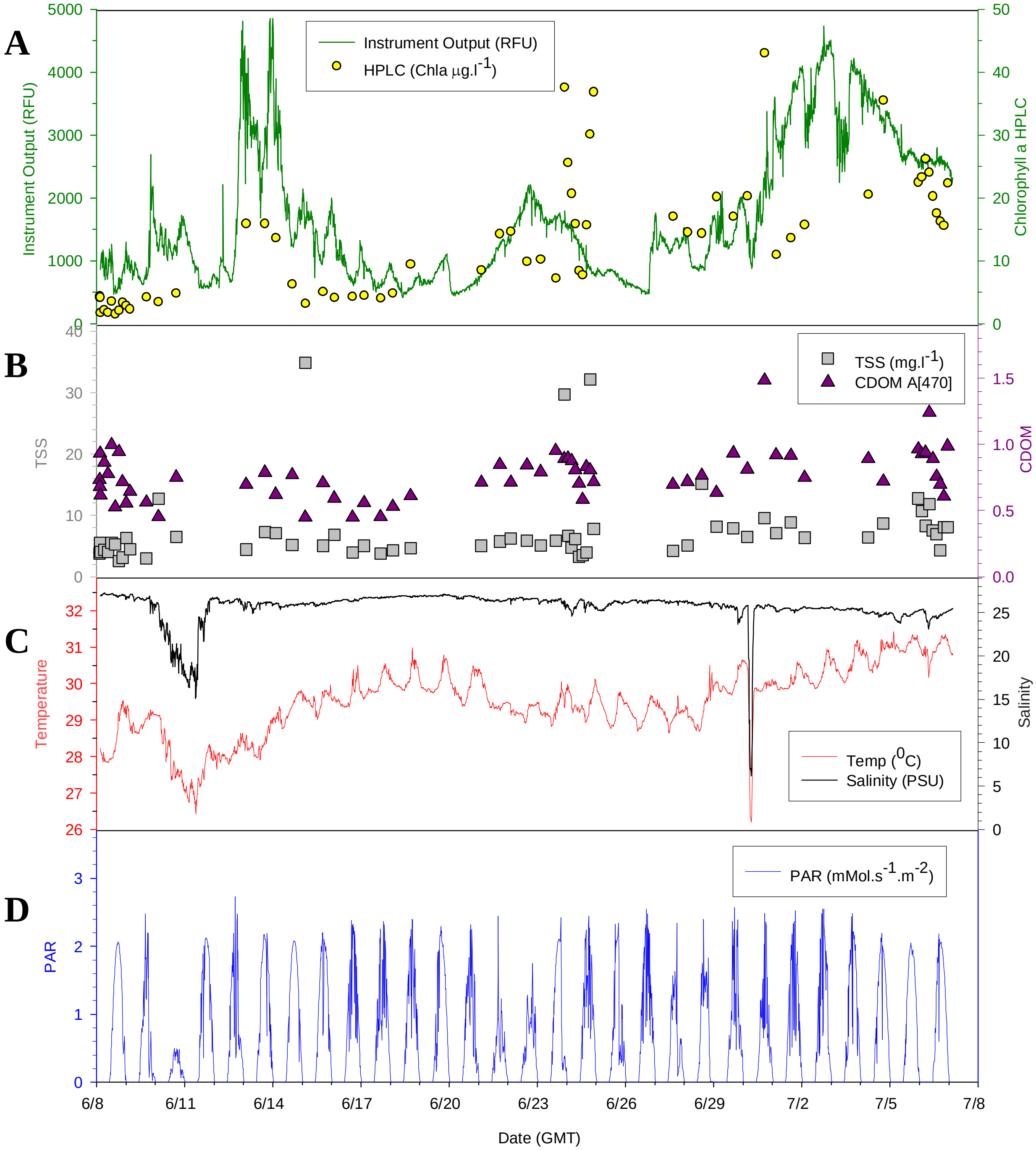


TABLE 8

	PRE		POST pre-cleaning		POST post-cleaning	
	Mean (RFU)	**STD ±**	**Mean (RFU)**	**STD ±**	**Mean (RFU)**	**STD ±**
Blk/DI	38.77	0.43	1403.17	31.05	233.18	6.96
BB3	3751.44	81.20	1465.66	15.35	1175.79	47.59
Rhod	4923.32	0.15	1532.32	20.32	2089.69	26.26

Sensor before the four weeks deployment.

Sensor after the four weeks deployment.

Moored Reliability

There were no major issues with this instrument and, for most of the site, 100% of the data was recovered from the field deployment. At the Hawaii site, the missing data for the first 10 days were due to an external malfunction of one of the ACT datalogger and not any instrument malfunction.

SUMMARY OF VERIFICATION RESULTS, FIELD PROFILING TESTS:

Figures 13A, 14A and 15A, display depth profiles of in vivo chlorophyll *a* fluorescence 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.
The profiling tests were conducted with the fluorometers set at a fixed gain (10X).

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 vivo chlorophyll *a* fluorescence 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.
The profiling tests were conducted with the fluorometers set at a fixed gain (10X).

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.
Note: Instrument gain (10X)

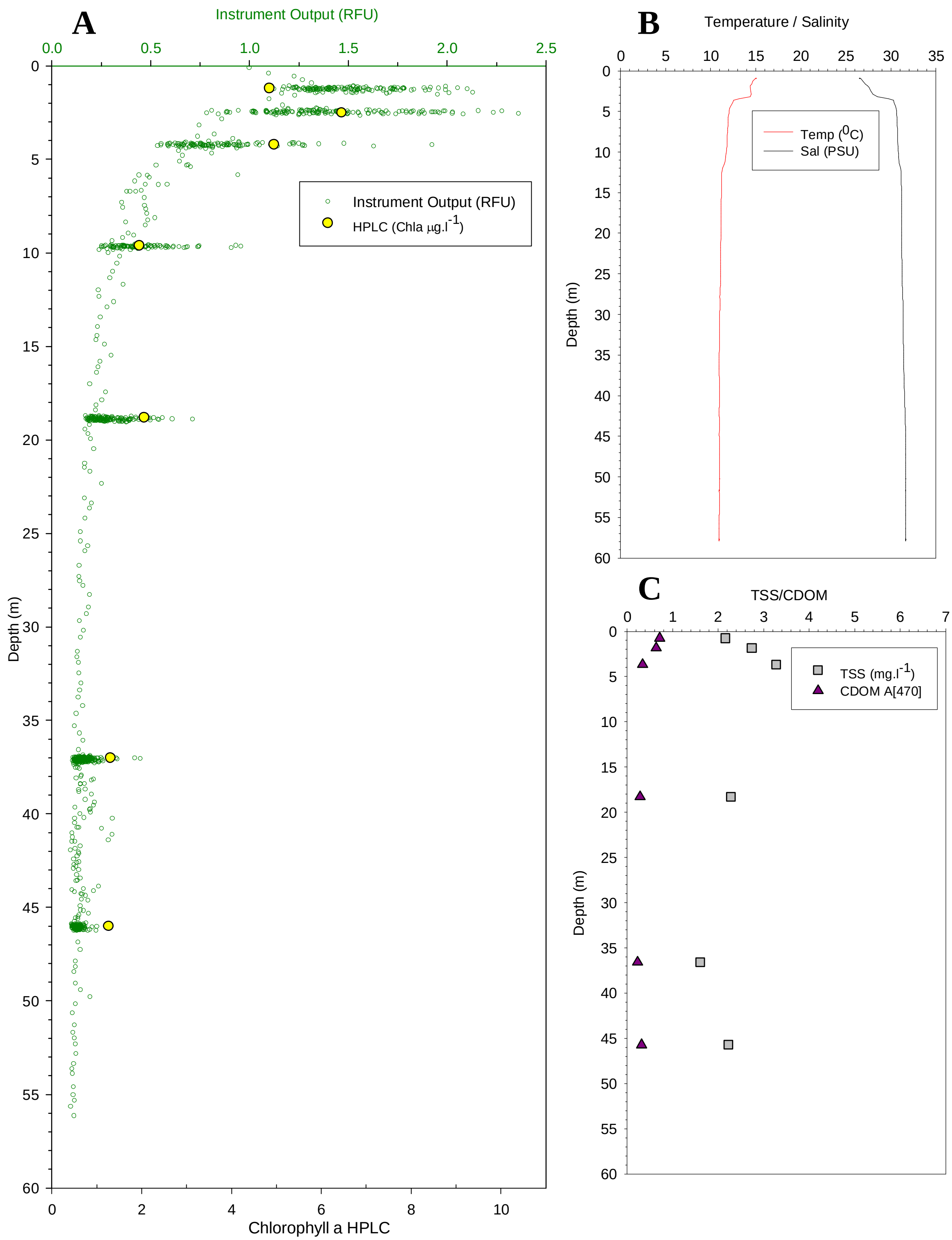


Figure 14: MAINE Profile 2 - Penobscot Bay, Bay Mouth Channel, Lat: 44 06.395, Lon: 68 59.447
Start. Down ~ 21:15:49 EST
Note: Instrument gain (10X)

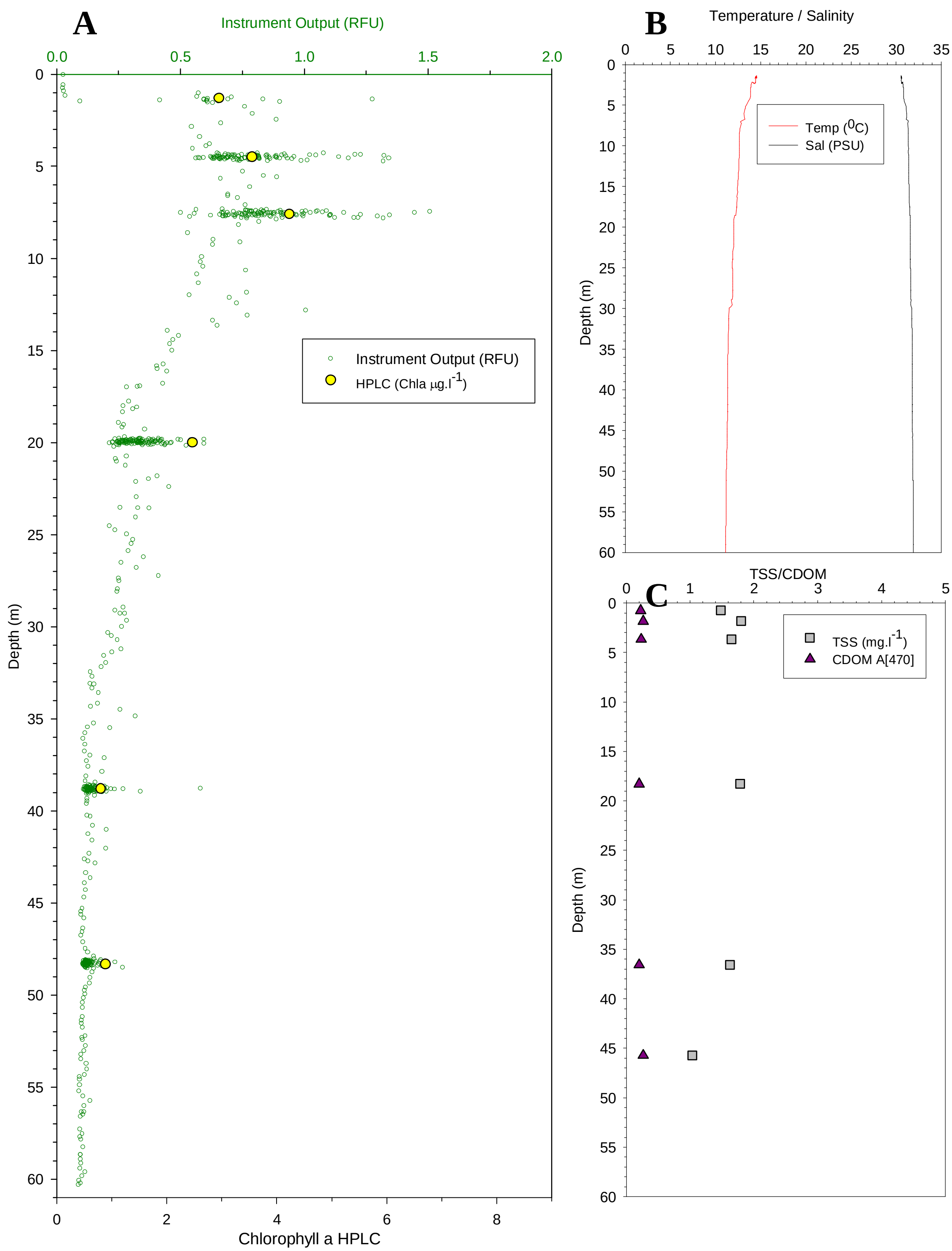


Figure 15: MAINE Profile 3 - Position: Penobscot Bay, Southern Passage, Lat: 44 19.850, Lon: 68 56.322. Start Down ~ 00:47:15 EST.
Note: Instrument gain (10X)

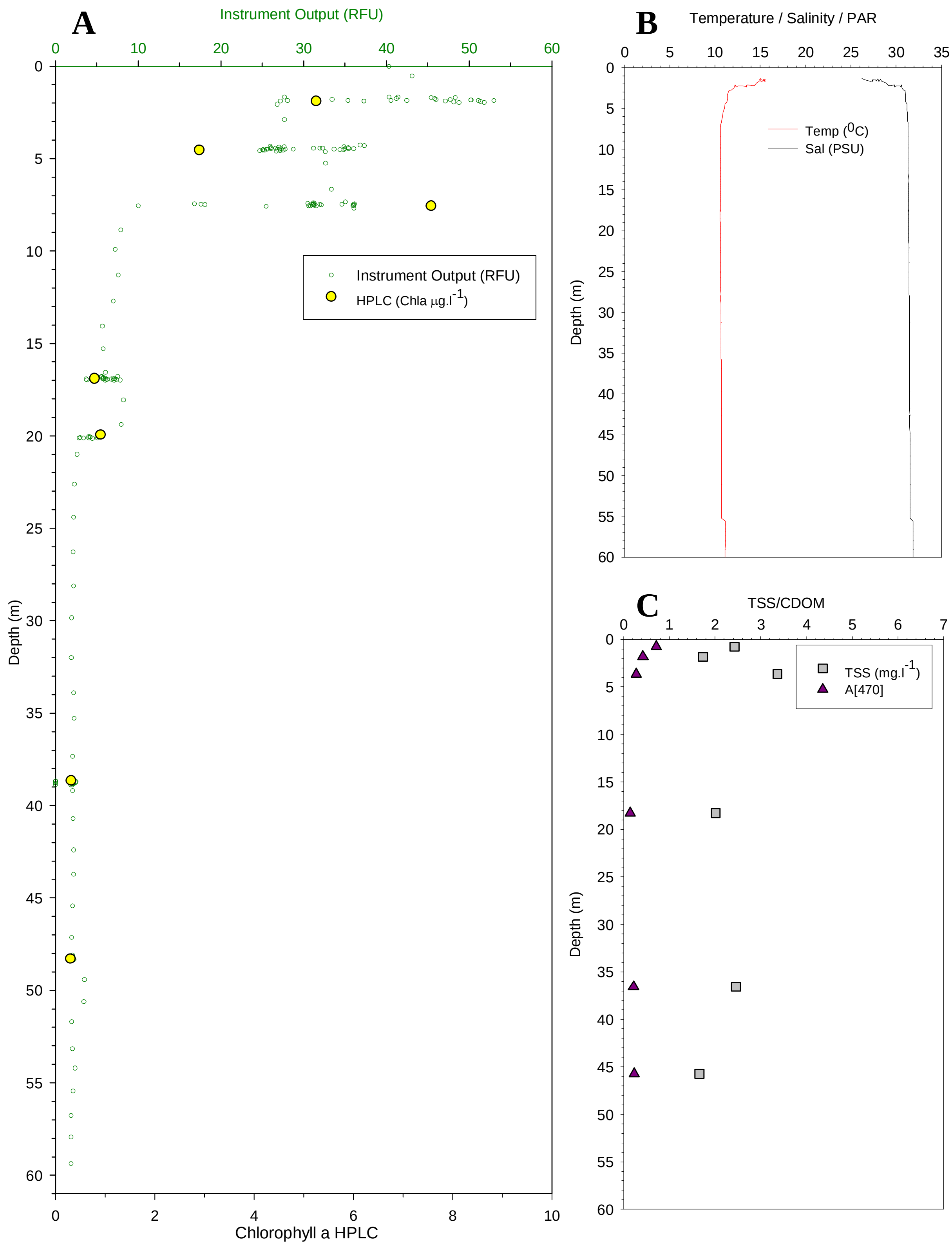


Figure 16: Michigan Profile 1 – Lake Michigan
Start Down ~ 7:00:00 EST
Note: Instrument gain (10X), missing instrument data due to the ACT datalogger malfunction.

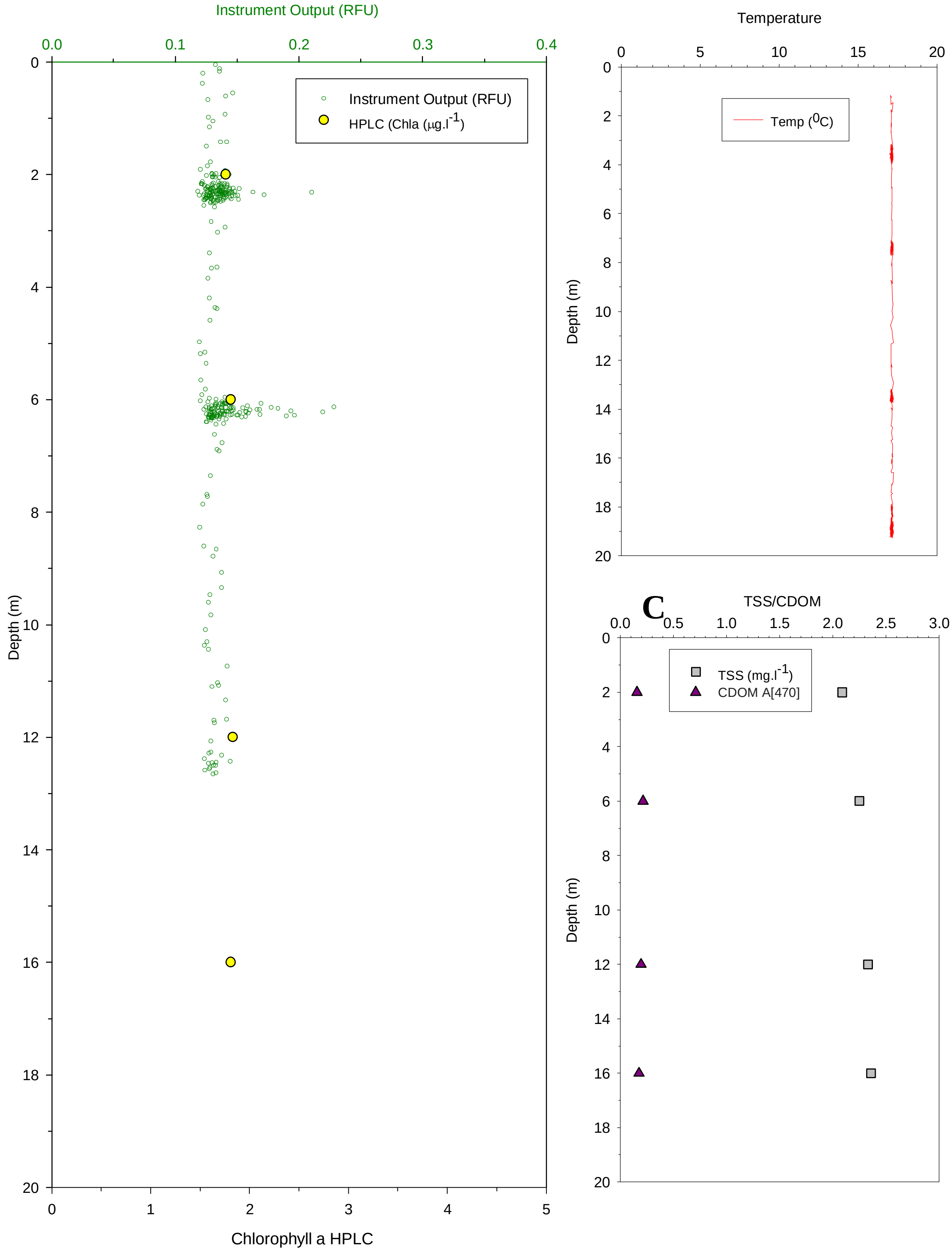


Figure 17: Michigan Profile 2 - Lake Michigan
Start Down ~ 9:10:04 EST
Note: Instrument gain (10X)

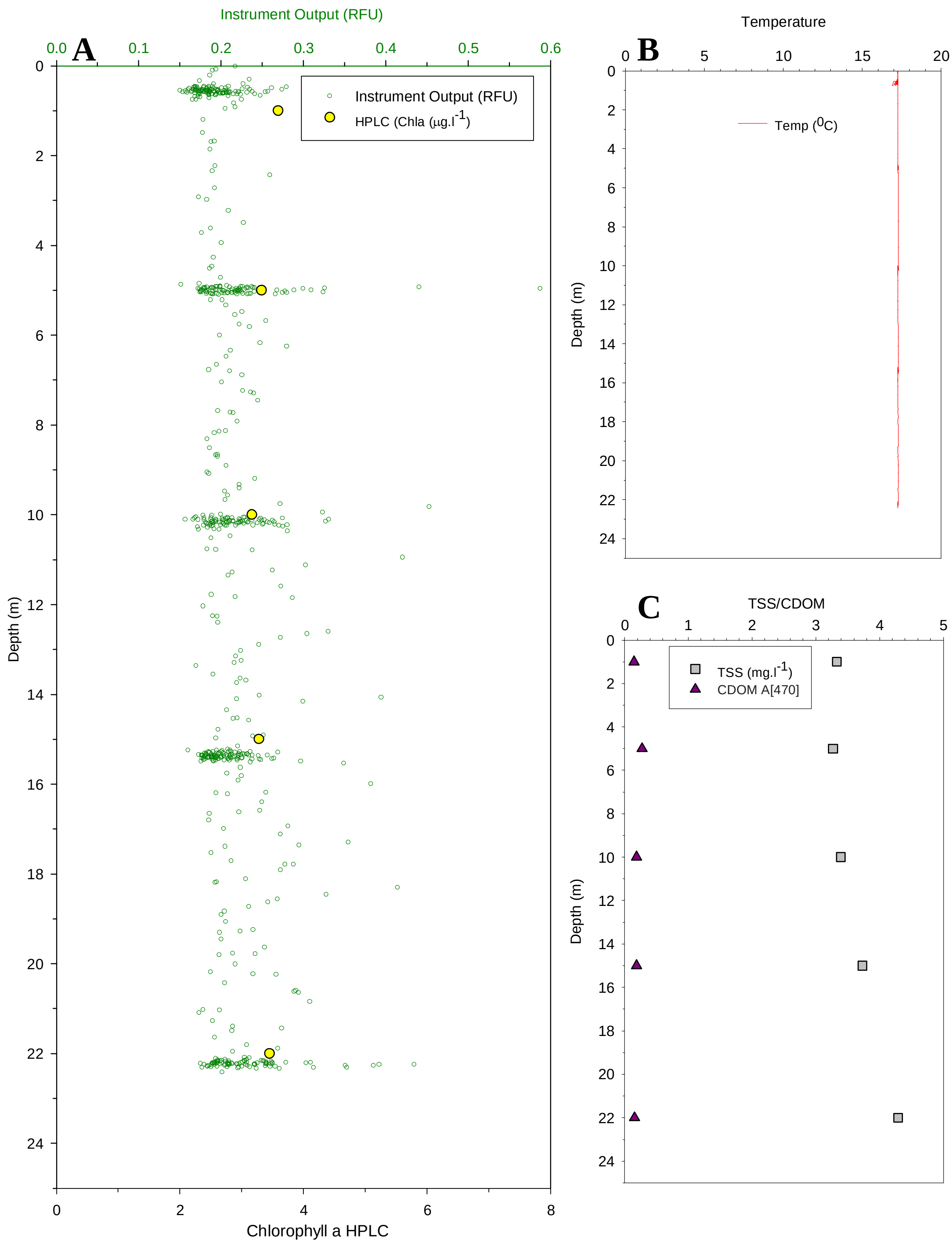
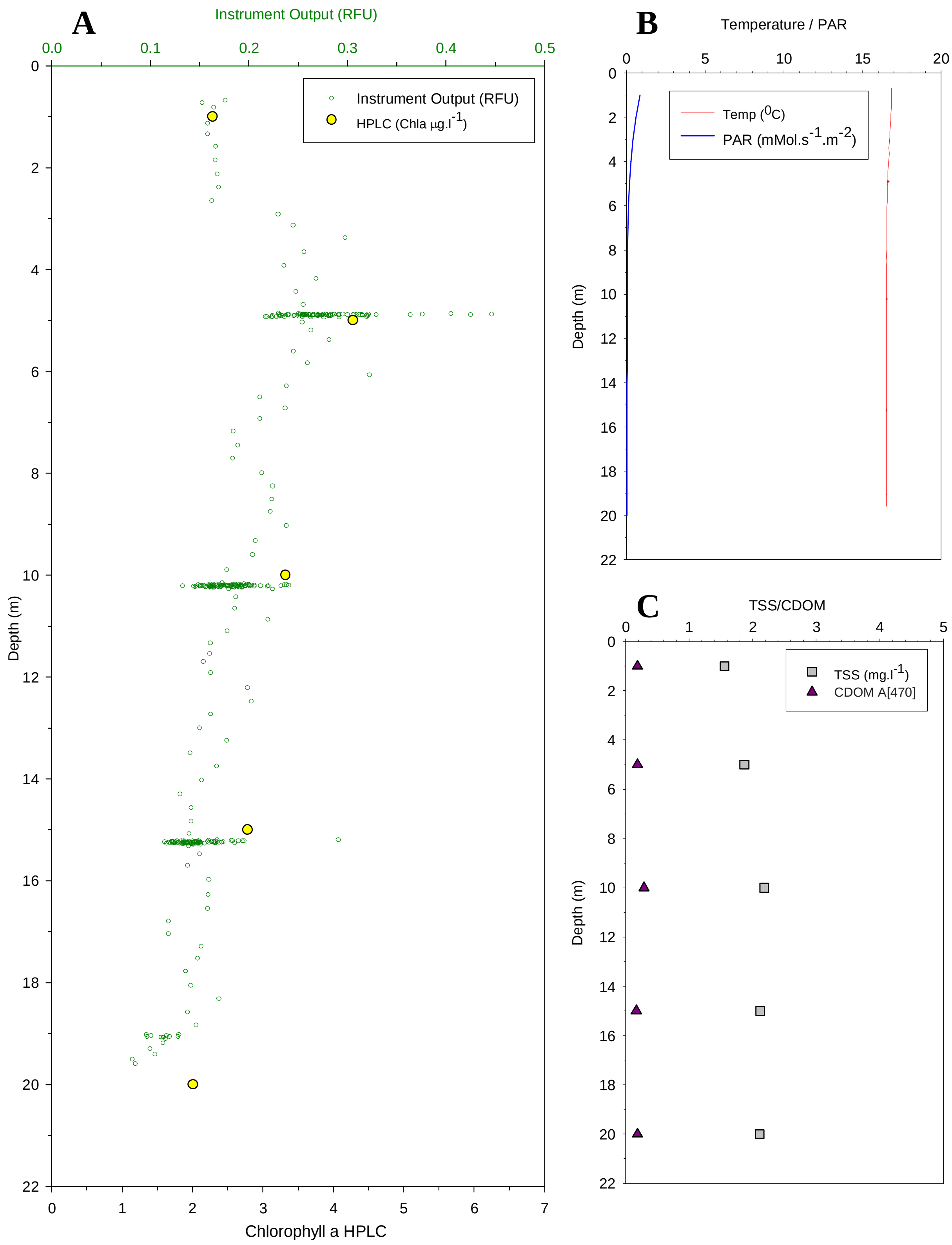


Figure 18: Michigan Profile 3 - Lake Michigan
Start Down ~ 17:27:49 EST
Note: Instrument gain (10X)



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April 24, 2006

Date

Approved By: Dr. Kenneth Tenore
ACT Director

April 24, 2006

Date

Approved By: Dr. Mario Tamburri
ACT Chief Scientist

April 24, 2006

Date

Approved By: Dr. Earle Buckley
Quality Assurance Supervisor

The ACT evaluation was a comprehensive suite of verification tests performed in laboratory, long-term field mooring, and profiling conditions. The design guidelines of all sensors used in this study need to be considered when evaluating results. The “stand-alone” Cyclops is intended to be integrated into a multi-parameter system that provides power, control gain switching, self-cleaning mechanisms, as well as convert analog into digital data.

Laboratory Tests:

Response Linearity and Detection Range: All results showed excellent linearity and accuracy reflecting the performance that we expect from the “stand-alone” *in vivo* chlorophyll *a* Cyclops during the temperature-dependent fluorescence of the dye tests. As expected fluorescence will decrease as temperature increases. A predetermined temperature coefficient correction factor can be applied to response values during post-calculations. It is important to note the “stand-alone” Cyclops was integrated into a Campbell datalogger at a fixed 10X gain setting (stated in the ACT Evaluation). Values above 5000 mVolts (Figure 1 and 3) can be determined when the gain is set at a 1X gain.

Response Precision: The Cyclops showed repeatable and precise measurements during precision tests. The noise variance was insignificant at varying dye concentrations and temperatures. The majority of samples (n=30) had standard deviations below 5 mVolts.

Response Linearity and Phytoplankton Chlorophyll a Fluorescence: The Cyclops displayed a strong linear correlation to extracted chlorophyll *a*. The _in vivo chlorophyll a Cyclops_ did not fully detect cyanobacteria at the same efficiency because the filters are configured for chlorophyll *a* specific wavelengths. Turner Designs has designed a phycocyanin and phycoerythrin Cyclops model specific to cyanobacteria accessory pigments. These models should be used as a more accurate estimate of cyanobacteria accessory pigment fluorescence.

Response Linearity and Sensitivity to Ambient Turbidity, CDOM and Irradiance: The Cyclops was able to detect BB3 dye at low to high range without significant interference from high concentrations of formazin (turbidity proxy, 22.8 mg/l) and coffee extract (CDOM proxy, 2.472 m^{-1}). It is important to note that formazin and coffee extracts are proxies, not absolute substitutes, used for natural turbidity and CDOM substances. Turbidity and CDOM proxies used in the laboratory experiments were well above typical averages found at the seven ACT sites (pg. 12). At very low BB3 levels the Cyclops did detect turbidity and CDOM proxies however it was minimal compared to the sensor’s range.

Field Tests:

Moored:

Data from all sites had a 100% recovery rate. The ACT performance evaluation was designed to represent a wide range of aquatic applications, and therefore, some instruments were deployed in environments subject to severe fouling. Our users have long recognized that some environments will require more frequent maintenance than the ACT test protocols allowed. When operated as intended users will obtain accurate and precise data. Complete pre and post data was not always available for all sites, due to the ACT datalogger malfunction, so it is difficult to accurately assess instrument drift. Data for the Maine (Table 5) and Moss Landing (Table 6) sites showed an instrument drift of 5-12% over a four week deployment.

Profiling: The profiling results showed excellent agreement between Cyclops *in vivo* chlorophyll *a* fluorescence and HPLC chlorophyll *a* processed samples.

A complete interpretation of the ACT Evaluation data can be found on the Turner Designs’ website www.turnerdesigns.com. Please do not hesitate to contact Turner Designs with any additional questions.

Chelsea Donovan
Environmental Marketing Manager

Turner Designs Inc.
845 W.Maude Ave Sunnyvale, CA 94085
toll free (877)316-8049 / (408)749-0994 / fax (408)749-0998
www.turnerdesigns.com