PERFORMANCE VERIFICATION STATEMENT for the BBE Moldaenke FluoroProbe 2

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.

At the manufactures request, and consistent with instrument design intent, the performance of the BBE Moldaenke Fluoroprobe 2 was assessed only in the laboratory and profiling tests. Two different field sites or conditions were used for testing including, an 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.bbe-moldaenke.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.

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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 BBE Moldaenke FluoroProbe 2. 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 BBE Moldaenke FluoroProbe 2 is a submersible profiling fluorometer which measures the emission intensity for excitation in five characteristic wavelength ranges employing pulsed light-emitting diodes. The LED's emit light at the following wavelengths: 450 nm, 525 nm, 570 nm, 590 nm and 610 nm. Fluorometric emission is measured at 680 nm by photomultiplier at an angle of 90 degrees to the exciting light source. The five-point excitation spectra (5 wavelength ranges) are deconvoluted on the basis of norm spectra, which have been obtained by analysis of several species of each spectral group. This enables an estimation of the mean deviation of the norm spectra. By means of the deconvolution approach, for each spectral algal group an estimate of the corresponding Chlorophyll a concentration is obtained (Chl *a* $\mu g\ L^{-1}$ per spectral algal group in the measuring volume at the given depth). The manufacturer's published performance specifications for the FluoroProbe 2 include: Range 0 -200 $\mu g\ L^{-1}$, Extended resolution 0.05 $\mu g\ L^{-1}$, and Operating Depth of 0 to 100 meters. More information can be found

at www.bbe-moldaenke.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. 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 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.

The profiling tests were conducted at two partner sites, CILER/University of Michigan and GoMOOS/University of Maine. Instruments tested, both in the laboratory and in the field, were incorporated in the The BBE Moldaenke FluoroProbe package, which included data logging, data transformation/conversion equations, and independent power, provided by the manufacturer. A total of two fluorometers were evaluated.

Instruments were programmed to record data every seconds 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 as close as possible to the sensor heads at varying depths throughout the water column.

***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-evaluted 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.

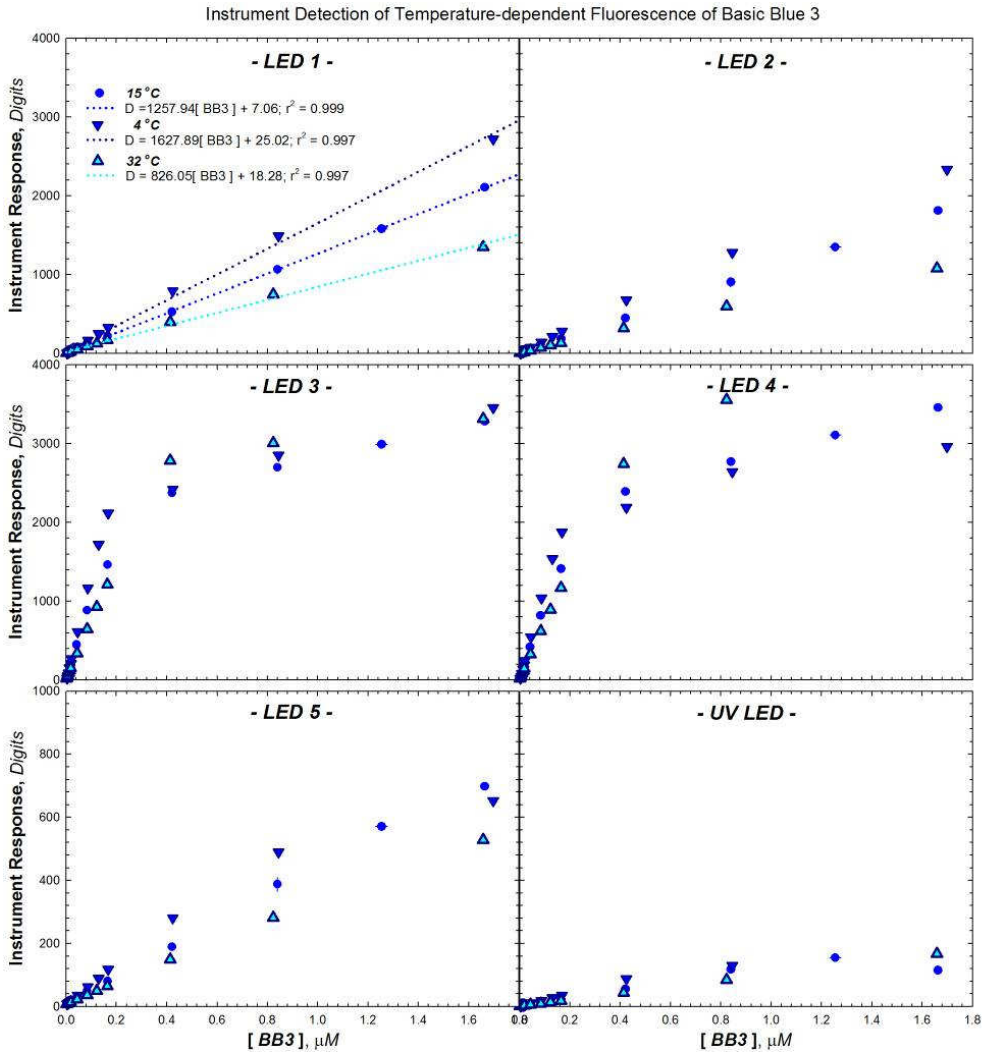
*** Data is presented as µg L^{-1} of Chl *a* as calculated and reported by the instrument. For additional corrections, interpretation and analysis of results, please visit www.bbe-moldaenke.com.**

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.

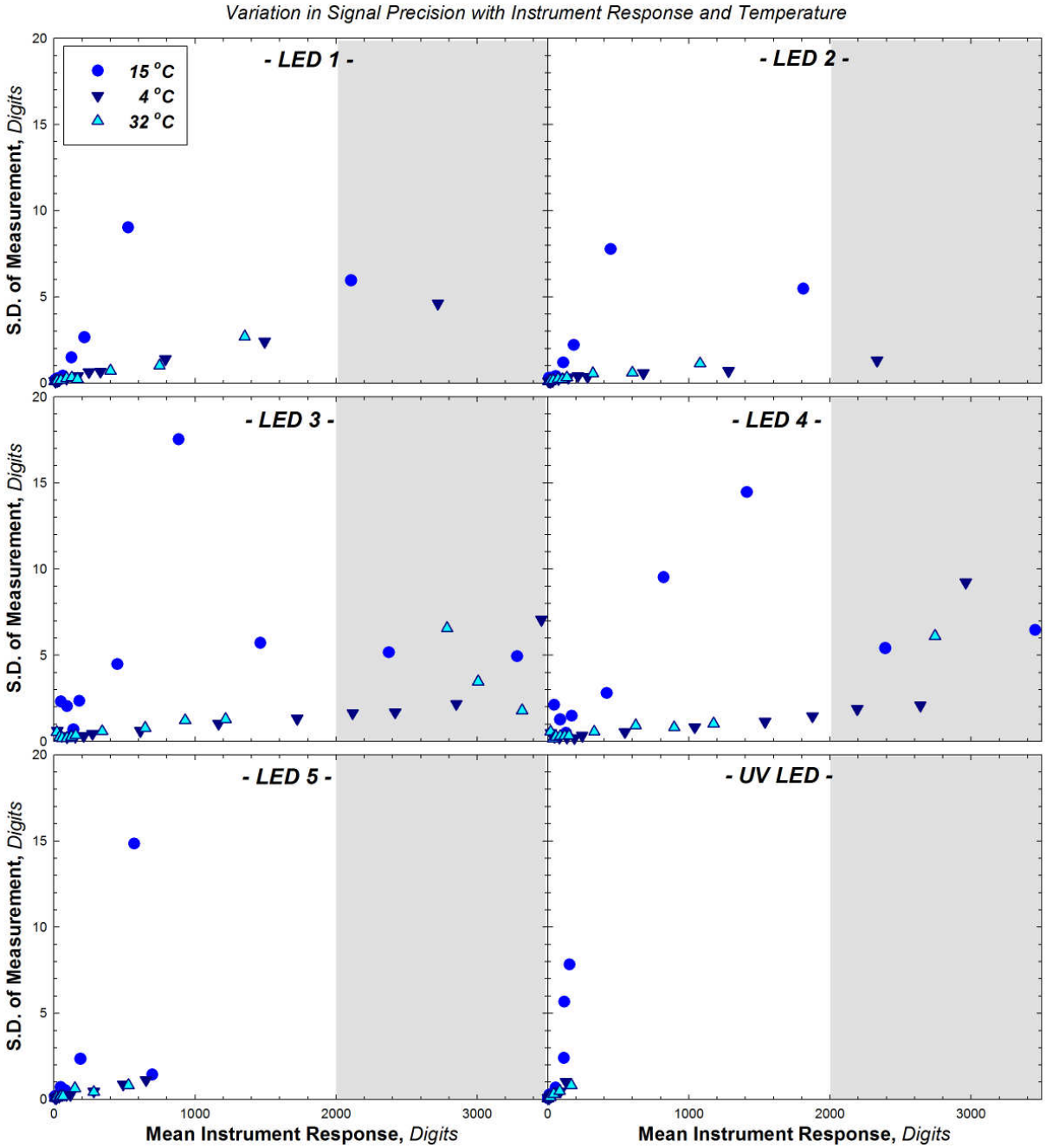
Response Linearity and Detection Range

Figure 1: Instruments were equilibrated in temperature regulated water baths and programmed to sample at 30 second intervals while being exposed to sequential increases in BB3 concentrations. The BBE Fluoroprobe uses 6 distinct LEDs for determining the spectral excitation characteristics of the target water mass; here individual LED excitation responses are plotted to visualize their performance in the presence of different fluorochrome concentrations. LED1 induced response will be used as the reference for comparison. The LED1 induced fluorescence exhibited a linear response to BB3 concentrations through at least 1.8 µM, detector saturation was only indicated with excitation by LEDs 3 & 4 for this dye. The average instrument response to LED1 in dye-free water was 6.07 ± 0.12 digits, indicating a limit of detection at 3 s.d. of 0.36 digits above the baseline reading. The fluorescence yield of BB3 is temperature-dependent (-1.56% ± 0.06% per ^{o}C). As deployed, the Fluoroprobe LED1 excitation response exhibited a slight temperature hysteresis, yielding a BB3 temperature-dependence of -1.76% ± 0.15% per ^{o}C. All data plotted as mean and standard deviation of both detector response and analyte concentration. Note the change in scale for LED5 and UVLED dependent responses. Regression analysis encompassed the entire test dye concentration range tested for all experiments reported. All data plotted as mean and standard deviation of both detector response and analyte concentration.
Note: Values exceeding 2000 digits are in excess of the tested instrument's normal operation range (200µg/L).



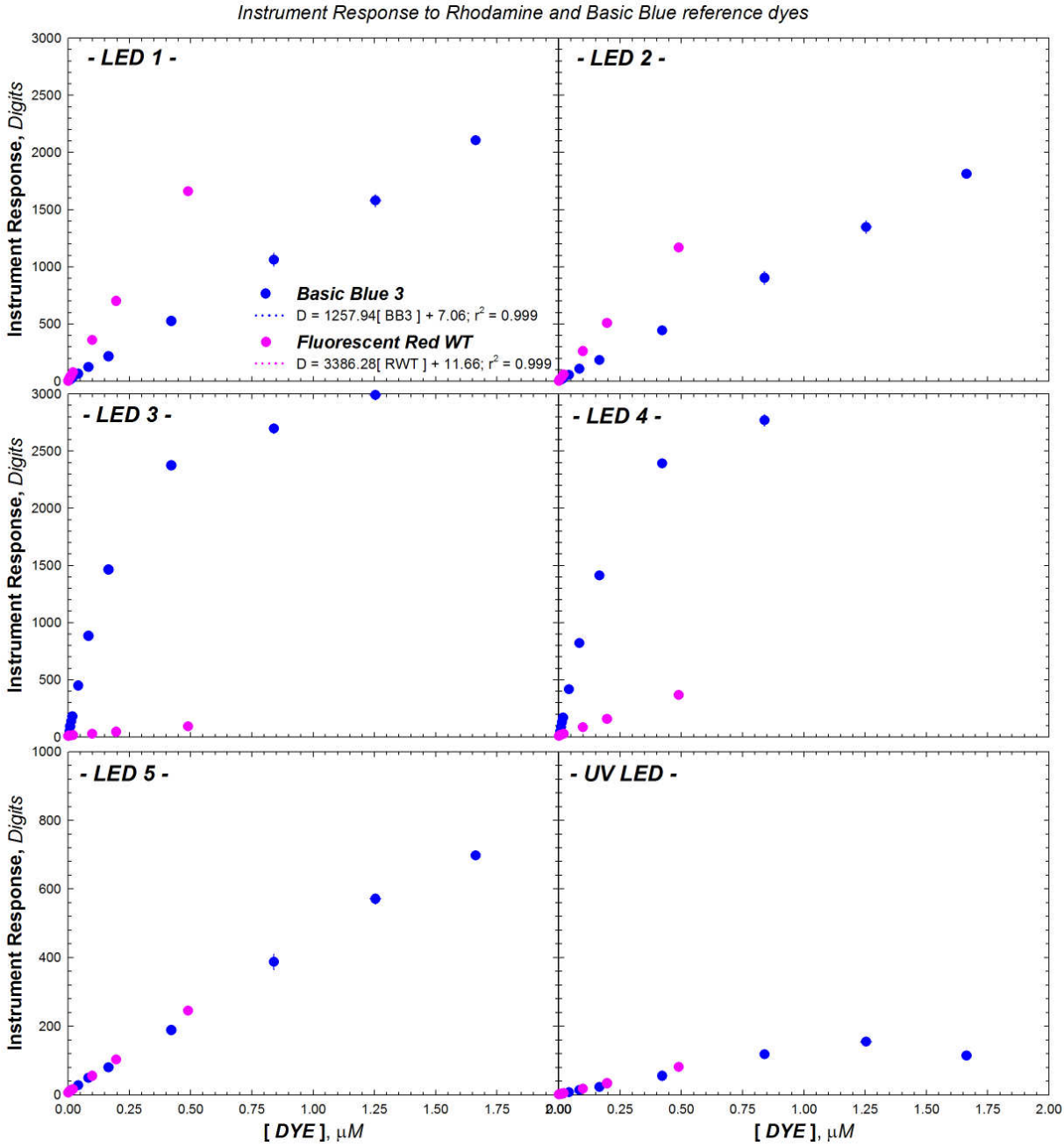
Response Precision

Figure 2: Detector noise, here expressed as the mean standard deviation of 10 sequential 30 second samples while held at fixed temperature and BB3 dye concentrations, generally increased with mean detector response, and was general less than 1% of maximum detector signal. LED1-dependent detector noise over the instrument detection range of 0-1.8 µM BB3, exhibited an absolute signal noise was ± 0.93 (0.07 – 6.60) digits. No consistent temperature-dependence in detector noise was evident. All data plotted as mean and standard deviation of both detector response and analyte concentration.
Note: Values exceeding 2000 digits (grey shaded areas) are in excess of the tested instrument's normal operation range (200µg/L).



Response Linearity and Fluorochrome Response

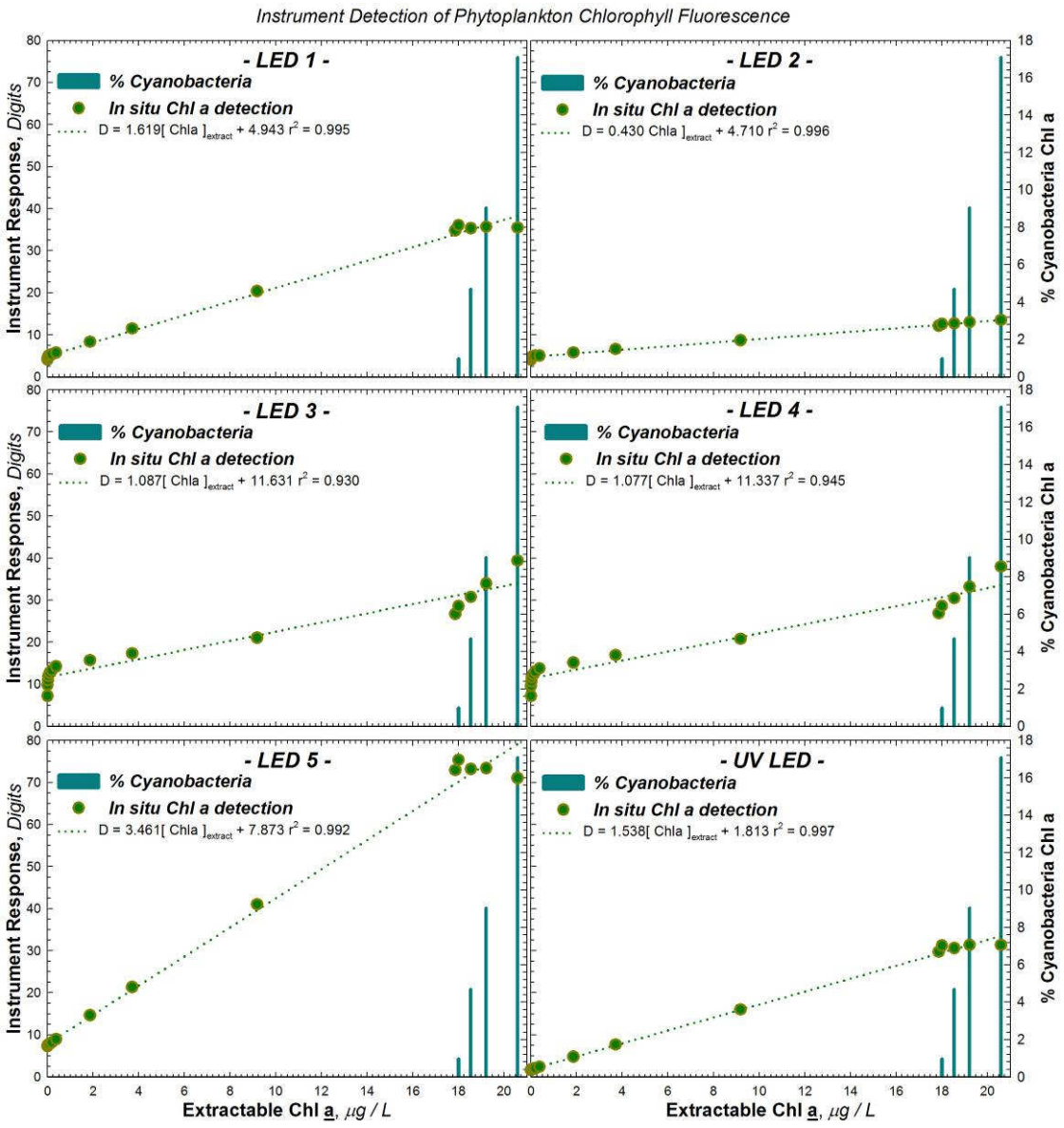
Figure 3: The Fluoroprobe detector response clearly resolves different fluorochromes through the combination of different excitation LEDs. For LED1 the fluorescence response was linear over comparable concentration ranges of two distinct test fluorochromes BB3 (λ_{max} 654 nm) and Fluorescent Red (Rhodamine) WT (λ_{max} 555 nm); however RWT exhibited a 2-times higher molar response than BB3. LEDs 3 &4 were more efficient at detecting the red-shifted absorbance of BB3 although response saturation was apparent above 1.0 µM BB3. LED1 induced response curves are provided as reference for all other excitation channels. All data plotted as mean and standard deviation of both detector response and analyte concentration.



Response Linearity and Phytoplankton Chlorophyll Fluorescence

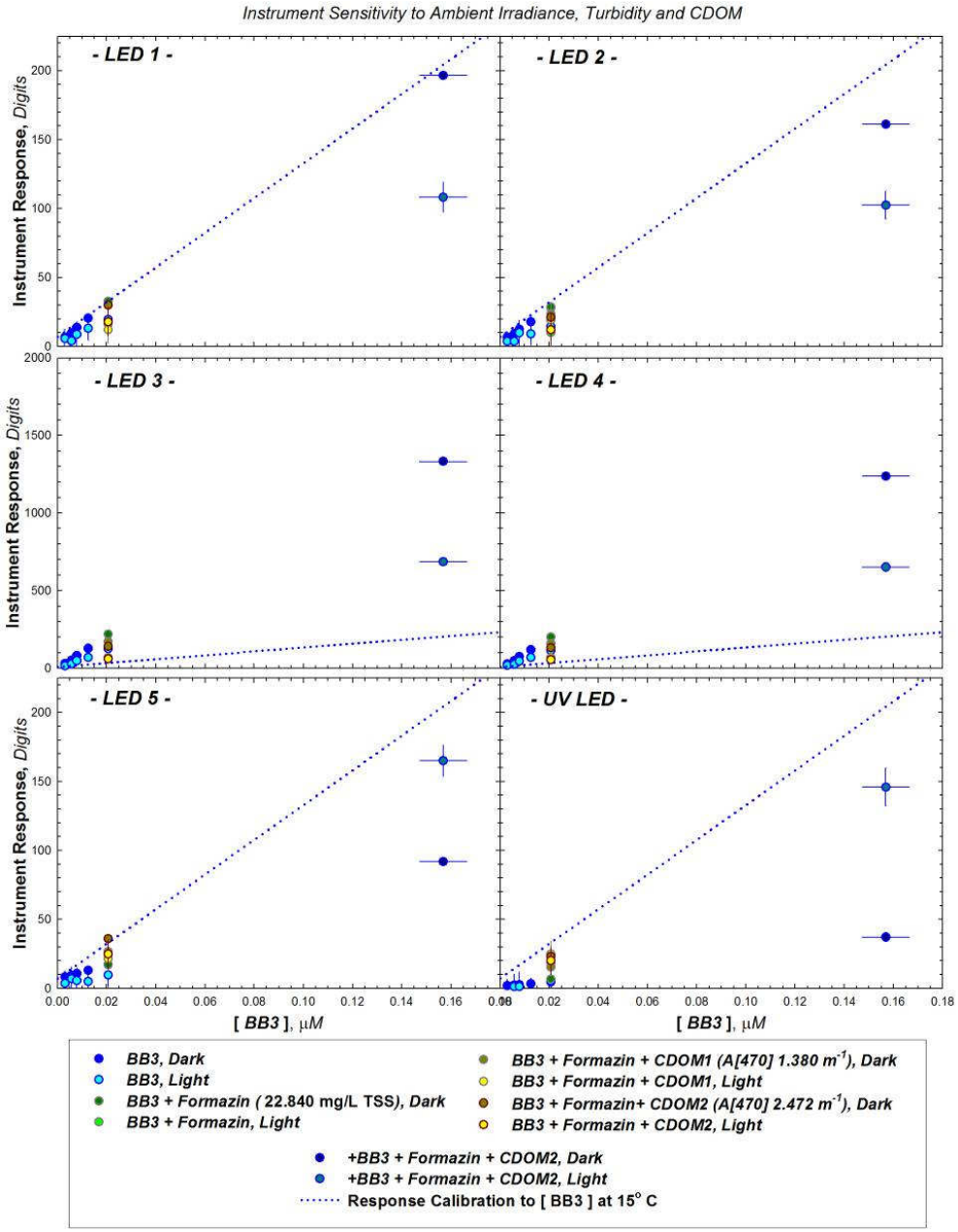
Figure 4: *Detection of Phytoplankton Chlorophyll Fluorescence.* Instruments were equilibrated f/2-enriched seawater in a temperature controlled tank at 15 ^{o}C in darkened conditions. Total chlorophyll *a* concentration in the media was manipulated by adding aliquots of late log-phase cultures (276.85 ± 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 different excitation LEDs in the Fluoroprobe provided discrimination between diatom and cyanobacterially derived chlorophyll fluorescence, with LEDs 3 & 4 inducing enhanced cyanobacterial fluorescence. Discrimination between diatom and cyanobacteria associated chlorophyll *a* based different excitation wavelengths is summarized in the following table of linear regression response coefficients for each LED channel. Instrument noise in the background seawater media based on LED1 excitation was ± 0.09 digits. Significant instrument response was observed at an added dose of 0.018 μg L^{-1} of Chl *a*.

Excitation LED	***Wave-length (nm)***	***Response to Diatom Chl a***			***Response to Cyanobacteria Chl a***		
		Slope	*Intercept*	R^2	*Slope*	*Intercept*	R^2
LED1	525 (blue-) green	1.69	4.82	0.998	0.41	0.42	0.640
LED2	570 (green)	0.42	4.72	0.988	0.44	0.07	0.952
LED3	610 (orange)	0.89	11.93	0.852	3.66	-0.38	0.999
LED4	590 (yellow)	0.92	11.59	0.875	3.23	-0.33	0.989
LED5	470 (blue)	3.66	7.53	0.999	-0.01	1.28	0.000
UVLED	370 (almost invisible)	1.57	1.75	0.999	0.66	0.72	0.752


Instrument Detection of Phytoplankton Chlorophyll Fluorescence
- LED 1 -
% Cyanobacteria
In situ Chl a detection
D = 1.619[Chla]extract + 4.943 r2 = 0.995
- LED 2 -
% Cyanobacteria
In situ Chl a detection
D = 0.430 Chla]extract + 4.710 r2 = 0.996
- LED 3 -
% Cyanobacteria
In situ Chl a detection
D = 1.087[Chla]extract + 11.631 r2 = 0.930
- LED 4 -
% Cyanobacteria
In situ Chl a detection
D = 1.077[Chla]extract + 11.337 r2 = 0.945
- LED 5 -
% Cyanobacteria
In situ Chl a detection
D = 3.461[Chla]extract + 7.873 r2 = 0.992
- UV LED -
% Cyanobacteria
In situ Chl a detection
D = 1.538[Chla]extract + 1.813 r2 = 0.997
Instrument Response, Digits
% Cyanobacteria Chl a
Extractable Chl a, µg / L

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 ^{o}C. Instrument detection of added BB3 was in good agreement (+12%) with the prior, independent calibration to BB3 concentration (see Fig. 1). The BBE Fluoroprobe sensor was highly sensitive to formazin, added as a proxy for turbidity, inducing a doubling (ca. 0.0136 V offset) of detector response. Coffee extract, used as a proxy for CDOM, induced a similar signal enhancement (ca 0.0125 V) 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 7% lower than the BB3 calibration response (0.5890 V/ µM BB3 vs 0.6324 V/ 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

The instrument stopped sampling (no flashes detected) logging about 4 hours into the RWT test series at 15 ^{o}C. Data logged up to that point was recovered but required external power. No other issues other than memory rollover, due to operator error were observed and all recorded data was recovered.

SUMMARY OF VERIFICATION RESULTS, FIELD PROFILING TESTS:

Figures 6A, 7A and 8A, display depth profiles of chlorophyll a concentrations in μg L^{-1} (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 6C, 7C and 8C 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 9A, 10A and 11A, display depth profiles of chlorophyll a concentrations in μg L^{-1} (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 9C, 10C, 11C 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 6B, 7B, 8B, 9B, 10B and 11B 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.

Note: The calculations of the total chlorophyll content by the test instrument in field profiling tests were made by the BBE FluoroProbe 2 for each of the detectable algae classes. The test instrument provides the capability to correlate its concentration values for each algae class to a specific external method but that capability was not tested. Therefore, the values plotted are based on the default correlation stored in the instrument. Further interpretation and corrections can be found at www.bbe-moldaenke.com.

Figure 6: MAINE Profile 1 - Position: Penobscot Bay, Upper Bay near Castine, 44 21.258, Lon: 68 50.062. Start Down ~ 17:58:00 EST.

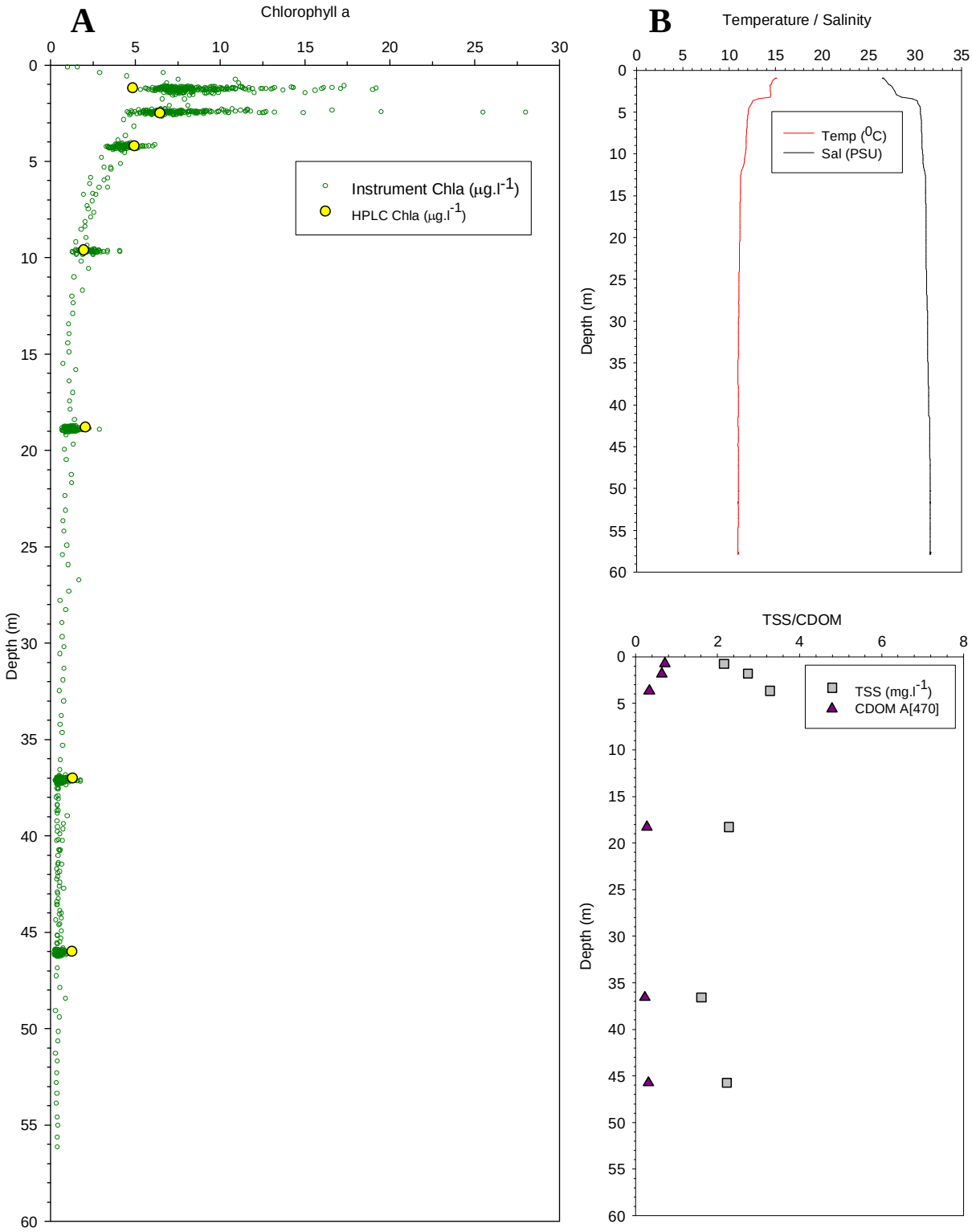


Figure 7: MAINE Profile 2 - Penobscot Bay, Bay Mouth Channel, Lat: 44 06.395, Lon: 68 59.447 Start. Down ~ 21:15:49 EST

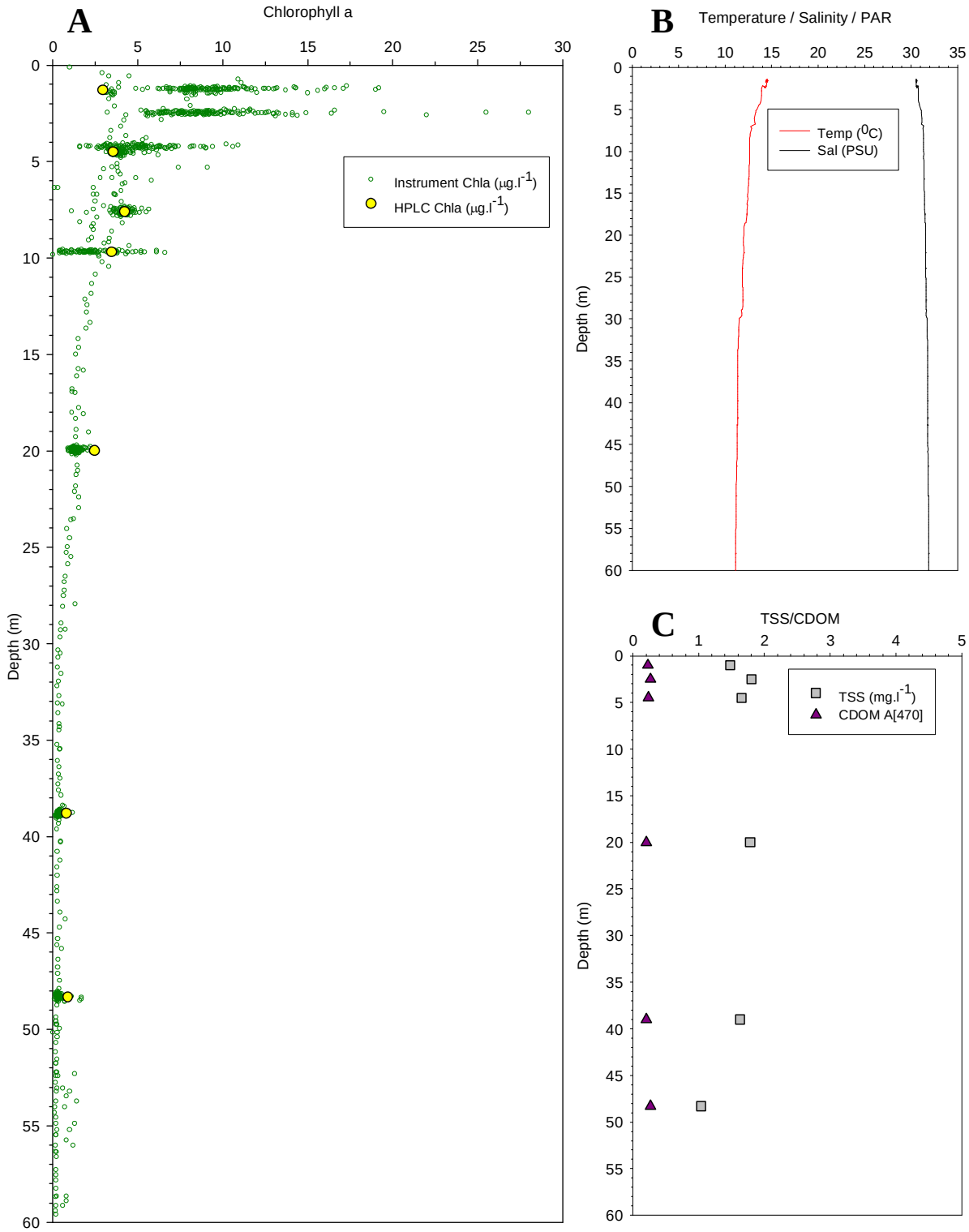


Figure 8: MAINE Profile 3 - Position: Penobscot Bay, Southern Passage, Lat: 44 19.850, Lon: 68 56.322. Start Down ~ 00:47:15 EST.

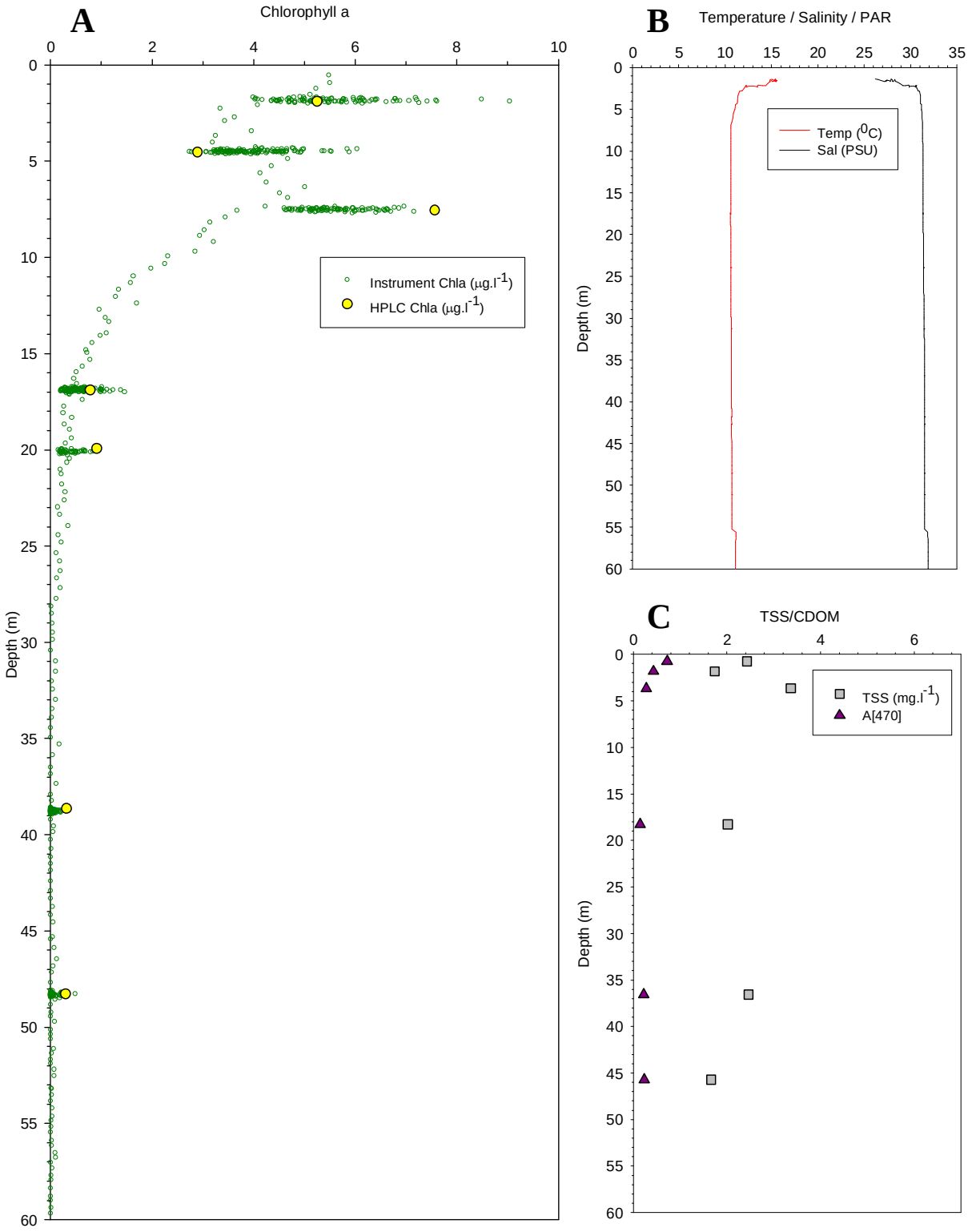


Figure 9: Michigan Profile 1 – Lake Michigan
Start Down ~ 7:00:00 EST

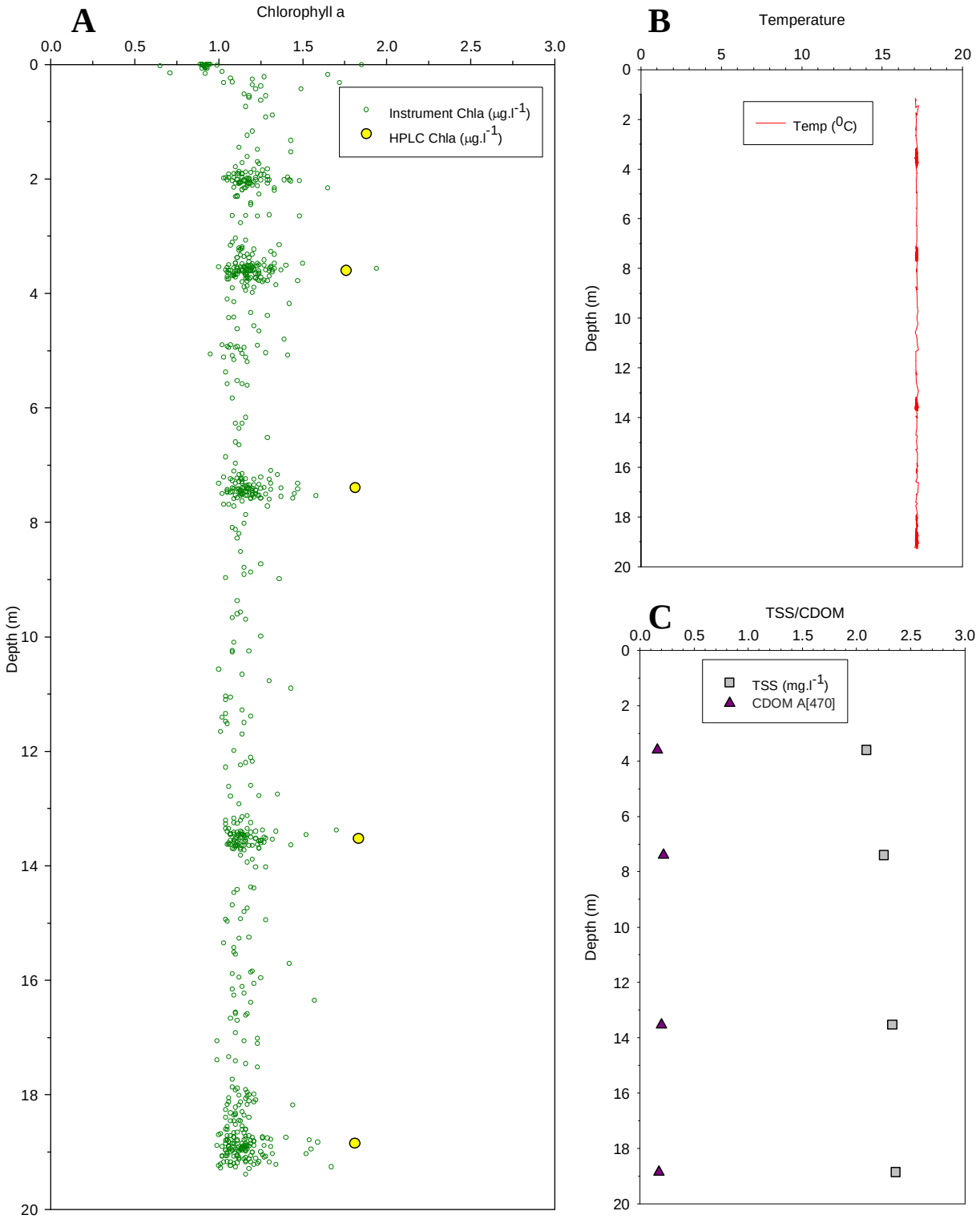


Figure 10: Michigan Profile 2 - Lake Michigan
Start Down ~ 9:10:04 EST

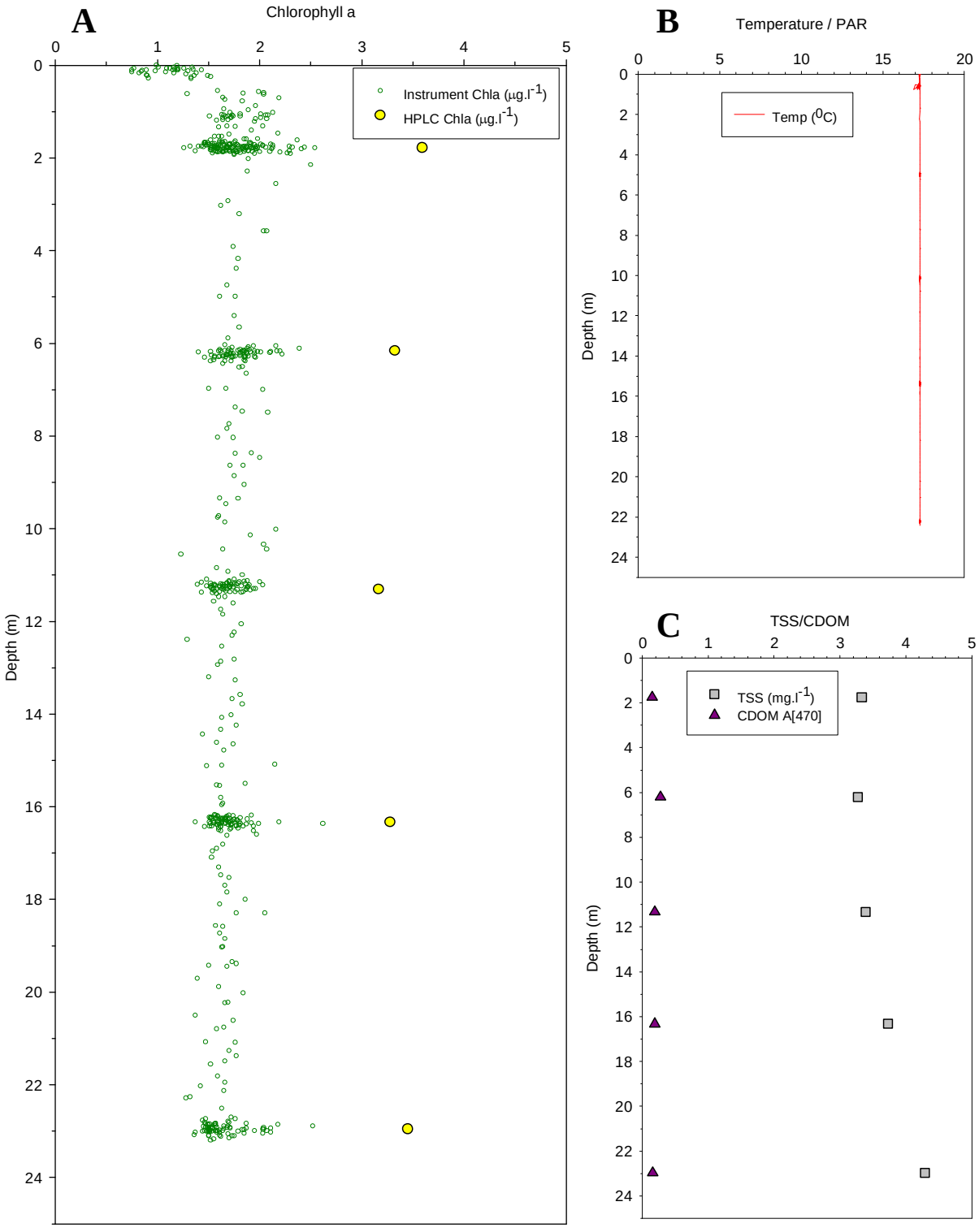
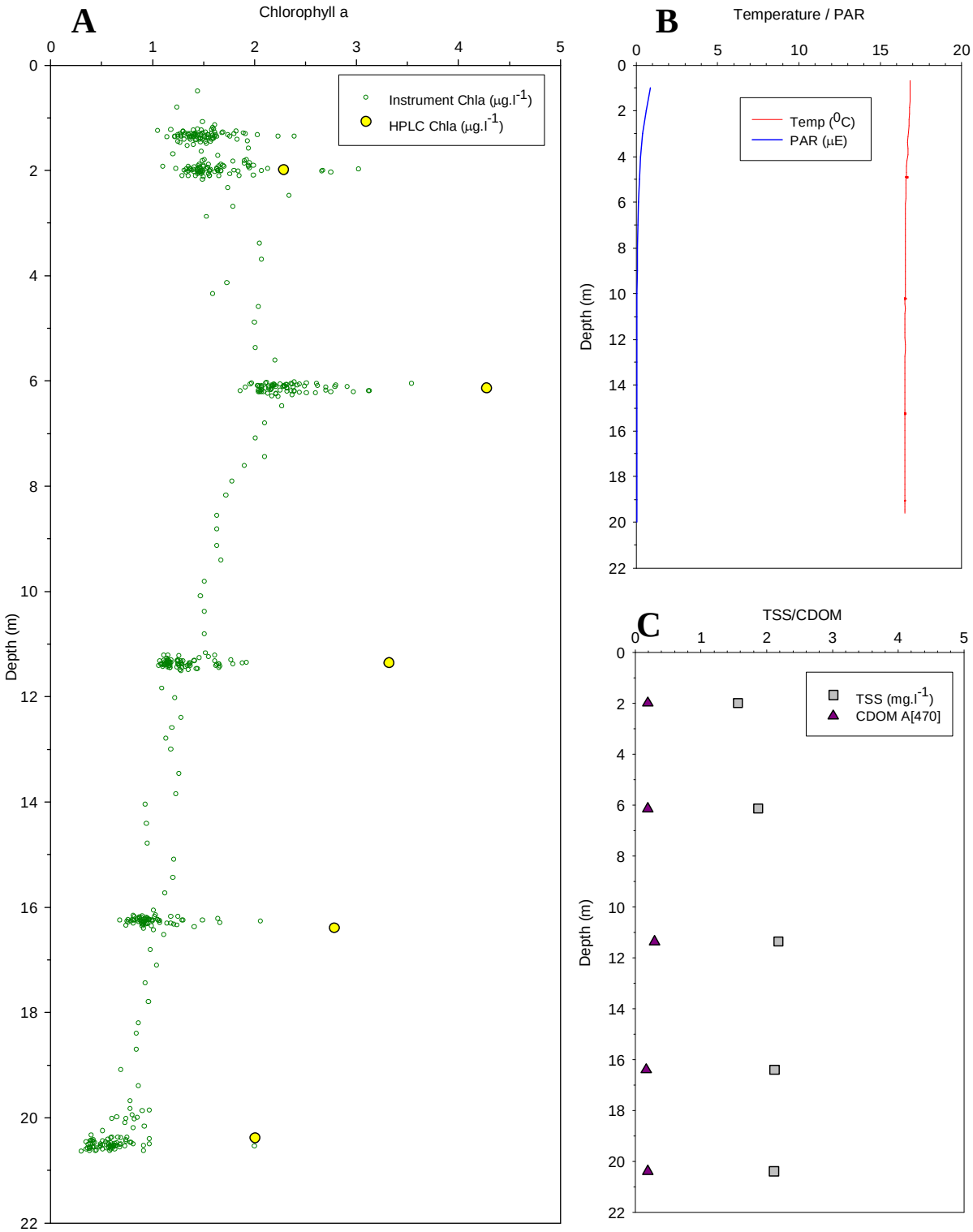


Figure 11: Michigan Profile 3 - Lake Michigan
Start Down ~ 17:27:49 EST



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

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Firstly, bbe Moldaenke would like to say thank you to the all staff involved in organizing and conducting the ACT fluorometer tests. Clearly, both the operational effort and data processing effort required to carry out the tests represented a significant undertaking and we congratulate ACT on completion of the project.

We believe that the test result clearly demonstrated advantages of the bbe FluoroProbe:

- Automatic discrimination of algae classes, shown here using diatoms and cyanobacteria
- Automatic detection of yellow substances and automatic offset determination
- Low disturbance by particles
- High instrument stability
- Direct reading of Chlorophyll concentrations

bbe Moldaenke entered the FluoroProbe 2 product in the lab tests and in the profiling tests. We decided not to participate in the mooring test because at the shallow depths proposed for the mooring tests we would normally recommend that customers use our related Algae Online Analyser rather than the FluoroProbe 2, thus avoiding a number of potential problems including bio-fouling and photo saturation of cells. Unfortunately, ACT was unable to test the Algae Online Analyser in addition to the FluoroProbe 2 during this test series due to logistical constraints. We hope to include the Algae Online Analyzer in a future ACT test.

Figure 1: The bbe FluoroProbe measures the chlorophyll fluorescence with 6 independent excitation LEDs at 6 different wavelengths. The fluorescence readings are limited to 2000 digits for each excitation LED, corresponding to an upper measuring limit of 200µg/L of Chlorophyll (other measuring limits are available to special order). If any excitation LED produces a fluorescence reading higher than 2000 the FluoroProbe 2 software clearly indicates that the derived Chlorophyll concentration values are invalid. The reader should therefore be aware that all values in excess of 2000 on the vertical axis are in excess of the tested instrument's normal operation range. Only values below 2000 are used by the instrument and only values below 2000 contribute to the instrument's linearity in its stated operational range.

Figure 2: Full examination of the data for the 15°C test clearly indicates that the somewhat higher standard deviation comes from unknown environmental conditions on site. Air bubbles are a possible suspect but in the absence of a camera we cannot be certain what occurred in the measurement aperture during this test. However, the tested FluoroProbe 2 was equipped with a transmission sensor and the corresponding transmission readings (recorded in the full instrument dataset) are clearly disturbed, so we can state with confidence that during the 15°C test the conditions in the test aperture were not as was intended by the test protocol. Furthermore, neither the tests at other temperatures nor the test with RodaminWT at 15° shows a similar effect adding further evidence that the 15°C test data must be considered unsafe due to some unknown interference introduced externally to the instrument. We conclude therefore that the higher deviation is not caused by temperature as inferred from the graph.
As in the earlier figure, data is plotted for fluorescence values greater than 2000 and such values are outside the 0 - 200µg/L of Chlorophyll operating range for the tested instrument. The new figure 2 (available at www.bbe-moldaenke.com) shows the same graph without the undefined values.
When calculating the theoretical noise limits we believe that only 1 excitation LED has been considered whereas the instrument actually uses 6 LEDs rendering the calculation incorrect. As noted subsequently in the report, the use of 6 excitation LEDs makes the instrument significantly more sensitive than the erroneous theoretical calculation would suggest. The total noise reduction would have been a factor

SQRT(6) = 2.45! In fact, during the standard deviation test, the instrument was set to make 1 measurement of 1s every 30s. As a result, the FluoroProbe 2 remained in standby mode most of the time. If the instrument had been set to make 1 measurement of 30s every 30s, the theoretical noise reduction would have been a factor SQRT(30) = 5.47. This should be noted when comparing the FluoroProbe 2 data with instruments which may have averaged values over such a period.
It can therefore be seen that it is actually quite possible to obtain higher sensitivity than that obtained in report. There is in fact evidence that the detector noise is no bigger than 2 digits. Taking the number of LEDs and the unintentional reduction of the averaging time into account the maximum noise level is smaller than 0.2 digits of 2000 digits full scale (~200µg/L) which is very roughly equivalently to 0.02 µg/L. This is valid for a 200µg/L Chlorophyll concentration equivalent. At 2 µg/l equivalent even lower values can be obtained.

Figure 3: As already stated for figure 1: the fluorescence values higher than 2000 are considered invalid by the instrument software and no Chlorophyll concentrations would be derived from such readings. The fluorescence measurements for the 6 excitation LEDs are independent measurements. The responses are fully expected to vary from one excitation LED to the other according the response of the algae spectral group or dye to the excitation.

Figure 4: The principle of the FluoroProbe 2 is to discriminate between algae spectral groups based on the different fluorescence yield obtained by excitation at 6 different LED wavelengths. When reading Figure 4 the reader should be aware that lower R^2 values do not indicate anything wrong with the instrument! The low R^2 values simply reflect the fact that different spectral groups respond differently to different excitation wavelengths. For example, it can be seen that cyanobacteria mainly react to LED 3 and 4. Cyanobacteria are not very sensitive to excitation at the other LED's wavelengths thus the corresponding excitation LED's low R^2 values are based on this fact and not on any negative property of the instrument. If one considers the 0.09 digits noise for LED 1 one can very roughly assume that 0.09 digit noise correspond to about 0.009 µg/L. As mentioned in fig 2, quality is improved by sqrt(30) by taking the averaging time into account. The FluoroProbe 2 also has 6 LED's which can theoretically improve the signal/noise ratio by sqrt(6). So the predicted limit is 'roughly' 0.009µg/L / 5.47 / 2,45 = 0.00067 µg/L. Nevertheless, for algae solutions the experimental value of 0.0018 µg/L corresponds to our experience.
For better understanding, we redrew the graph (available at www.bbe-moldaenke.com) but this time we implemented a second regression line, which emphasizes the effect of pigment properties on the fluorescence response of each LED.

Figure 5: The small depression of the signal in presence of irradiance is caused by an AC driven light source. The FluoroProbe 2 is indeed sensitive to this sort of light - but such light is never present in nature. DC drive light would reduce the effect on more than a factor of 10. We stated this fact before the beginning of the test and we had understood that the test would be carried out using DC drive light. The effects of the AC light would also have been far smaller if the light protection device had not been removed during the test!

Figure 6-11.: The correlation with the HPLC (ACT) measurement in the Maine profiles is very good. In the Maine profiles the chlorophyll content is dominated by diatoms or dinoflagellates. Obviously our calibration of diatoms/dinoflagellates fit to the ACT-HPLC. This is slightly different with the cryptomonades which are found in the Lake Michigan. Adjustment of the fingerprint with the HPLC (ACT) for cryptomonades would lead immediately to a very good correlation for the Lake Michigan profiles as well.

Please do not hesitate to contact bbe.

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