PERFORMANCE DEMONSTRATION STATEMENT
YSI 9600 Nitrate Monitor

TECHNOLOGY TYPE:	Field portable nutrient analyzer
APPLICATION:	In situ estimates of dissolved nitrate + nitrite for moored deployments
GOALS:	Demonstrate the capabilities and potential of this instrument
TYPE OF EVALUATION:	Field Performance Demonstration at three ACT Partner sites
DATE OF EVALUATION:	Testing conducted from May through October 2007
EVALUATION PERSONNEL:	M. Carroll, D. Chigounis, J. Cook, S. Gilbert, T. Johengen, T. Koles, T. McKissack, D. Wells, M. McIntyre, A. Pinchuk H. Purcell, C. Robertson, D. Schar, G.J. Smith, M. Tamburri

NOTICE:

The goals of this ACT Performance Demonstration were to highlight the potential capabilities of in situ nutrient analyzers, to promote awareness of this emerging technology, and to provide field tests to aid in further instrument refinement. Unlike ACT Performance Verifications, the intent was NOT to verify manufacturer specifications for the technology ACT Demonstrations are based on an evaluation of technology performance under specific, agreed-upon protocols, criteria, and quality assurance procedures. ACT and its Partner Institutions do not certify that a technology will always operate as demonstrated and make no expressed or implied guarantee as to the performance of the technology or that a technology will always, or under circumstances other than those used in testing, operate at the levels demonstrated. ACT does not seek to determine regulatory compliance; does not rank technologies nor compare their performance; does not label or list technologies as acceptable or unacceptable; and does not seek to determine "best available technology" in any form. The end user is solely responsible for complying with any and all applicable federal, state, and local requirements, as well as manufacturer operational protocols.

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:

A key to the successful adoption, and transition to operational use, of new technologies is broad community awareness and confidence. The Alliance for Coastal Technologies (ACT) has therefore completed a Performance Demonstration of in situ nutrient analyzers/sensors with the goal of aiding in technology refinement and building user acceptance of these novel instruments. The fundamental objectives of this Performance Demonstration were to: (1) highlight the potential capabilities of in situ nutrient analyzers by demonstrating their utility in a broad range of coastal environments with varying nutrient concentrations, (2) promote the awareness of this emerging technology to the scientific and management community responsible for monitoring coastal environments, and (3) work with manufacturers that are presently developing new or improved sensor systems by providing a forum for rigorously evaluating their products using an objective, third-party, nationally distributed testing program.

We wish to highlight several fundamental differences in the protocols between an ACT Performance Demonstration and a Performance Verification. First, participating manufacturers were asked to perform all of the required set-up and calibration procedures prior to deployment and to extract the data from the test and submit it in a final concentration specific format. In addition, manufacturers facilitated the testing of laboratory reference standards (made in deionized water with certified SPEX nutrient standards) at the beginning and end of the test. Secondly, there was no laboratory component for directly testing the stated instrument performance capabilities under controlled conditions. Thirdly, field tests were conducted at a subset of four of the eight partner test sites. Lastly, we provided manufacturers with results of initial and final laboratory reference standards, on-board instrument standards and field reference samples to facilitate post-test correction of the in situ determined nutrient concentrations. This procedure is highly recommended for any application of these technologies and provides a better measure of the potential for in situ analyzers to capture accurate time series once appropriate calibrations and controls are applied.

In this Demonstration Statement, we present the performance results of the YSI 9600 Nutrient Monitor under diverse environmental conditions in moored deployment tests. A total of three different field sites were used for testing, including: estuary, coastal ocean, and riverine environments. Complete time series data were successfully retrieved in two or the three tests. A software/communication problem resulted in an unsuccessful test at Resurrection Bay, AK and no data will be presented for that test. At the estuarine site in Chesapeake Bay, the YSI 9600 reported 100% of expected data and generally tracked observed variations in nitrate concentrations that ranged between 0.03 – 0.19 mgN/L. Regressed instrument versus reference sample data produced an R^2 of 0.93, however, there was a significant calibration offset with YSI 9600 predicted concentrations equal to ca. 60% of lab determined concentrations, on average. A slight decay in the accuracy was observed over the course of the 4 week deployment during the test. At the riverine test site in Michigan, the YSI 9600 also reported 100% of expected data and generally tracked observed variations in nitrate concentrations that ranged between 0.5 – 2.7 mgN/L. Regressed instrument versus reference sample data produced an R^2 of 0.89, and the observed calibration offset was much less, with YSI 9600 predicted concentrations equal to ca. 83% of lab determined concentrations, on average.

We encourage readers to review the entire document for a comprehensive understanding of instrument performance and to discuss results with the instrument manufacturer. In general, however, it appears that the fundamental technology has the capability to successfully measure in situ nitrate concentrations under a variety of field conditions.

BACKGROUND:

There are a number of challenges in assessing nutrient concentrations in aquatic systems that point to the value of sustained in situ observations. High spatial horizontal variability is typical of many coastal, estuarine and fresh water systems, as are strong depth gradients. High temporal variability in natural background concentrations are typical of many locations, often in response to short-term forcing (e.g., vertical mixing) or input events (e.g., runoff, river discharge). Furthermore, in many aquatic ecosystems, assessing responses to nutrient inputs from various sources requires monitoring of multiple nutrient species. In situ nutrient analyzers can play an important role in addressing these challenges and offer promise for range of applications including: regulatory, applied, observing system and basic research. For any of these applications, users will be concerned about the traditional performance attributes including: accuracy, reliability, comparability, affordability, and ease of use.

A key to the successful adoption and transition to operational use of new technologies is broad community awareness and confidence. 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 (visit www.act-us.info).

This document summarizes the procedures used and results of an ACT Demonstration to examine the performance of the YSI 9600 nitrate analyzer. Detailed protocols, including QA/QC methods, are described in the ACT *Protocols for Demonstrating the Performance of In Situ Nutrient Analyzers* (ACT PD07-01), which can be downloaded from the ACT website (www.act-us.info/evaluation_reports.php). Appendix 1 is an interpretation of the Performance Demonstration results from the manufacturer's point of view.

TECHNOLOGY TYPE:

The YSI 9600 Nitrate Monitor allows continuous recording of nitrate levels at a variable sample interval and thus provides a much greater capability to detect nutrient events than the spot sampling activities that have historically been carried out. To measure nitrate, the 9600 uses flow injection technology combined with a chemical reaction sequence, which is defined in literature from both the USEPA and *Standard Methods for the Examination of Water and Wastewater*. The intensity of the color produced by the chemical reaction sequence is determined by standard absorbance spectroscopy and compared to that of a standard of known concentration in order to quantify the amount of nitrate in an environmental sample. The use of flow injection technology minimizes the amount of reagents required and the amount of waste generated.

The 9600 can be custom configured with regard to detector cell to provide maximum accuracy for the type of water that you normally monitor. For applications where typical nitrate levels are over 2 mgN/L (range 0 - 10 mgN/L), the 9600 should be configured with a 2 mm cell (designated 9600-02) and delivers a stated detection limit of 0.025 mgN/L. The stated accuracy of the 9600-02 is ± 5% of reading or 0.2 mgN/L, whichever is greater, within an operating the range of 0 - 6 mgN/L and ± 10% of reading within the operating range of 6 - 10 mgN/L. For applications where typical nitrate levels are less than 2.0 mgN/L, the monitor should be equipped with a 10 mm detector cell (designated 9600-10) and delivers a stated detection limit of 0.005 mgN/L. The stated accuracy of the 9600-10 is ± 5% of reading or 0.02 mgN/L, whichever is greater, within an operating the range of 0 - 2 mgN/L. The 9600-02 and 9600-10 are designed primarily for freshwater and estuarine/near coastal applications, respectively, but there may be some cases where the greater range of the 9600-02 will be required for brackish water studies and some cases where freshwater nitrate levels are consistently low enough to require the greater accuracy of the 9600-10. The 9600 system can be used at depths up to 200 feet with a temperature operating range of 1 - 45 ^{o}C.

The 9600 uses an individual pump based system with unidirectional flow injection which helps to eliminate volume and contamination errors from syringe type injections and offers automatic calibration at user-selected interval. Calibration points do not affect data continuity for sample intervals of 30 minutes and greater. The 9600 comes standard with its own waste collection system, which significantly reduces the risk of cadmium discharge into the environment. Reagent life is estimated at > 30 days at a 1 hour sampling interval.

The 9600 comes standard with the easy-to-use NUview™ PC software for simple set-up and deployment and the proven EcoWatch® for Windows® software for data analysis and custom presentations. It is equipped with SDI-12 and RS-232 as interface standards for use with computers and data collection platforms. The 9600 exhibits good battery life (approx. 70 days at a 1-hour sample interval at 20º C) with internal D-cell battery pack, and also can be powered externally by 12 V lead acid battery, AC adapter, or data collection platform for longer deployments. The 9600 is manufactured and distributed by YSI Inc. of Yellow Springs, OH, USA.

OBJECTIVES OF THE NUTRIENT ANALYZER PERFORMANCE DEMONSTRATION:

The fundamental objectives of this Performance Demonstration were to: (1) highlight the potential capabilities of in situ nutrient analyzers by demonstrating their utility in a broad range of coastal environments, (2) promote the awareness of this emerging technology to the scientific and management community responsible for monitoring coastal environments, and (3) work with manufacturers that are presently developing new or improved analyzer systems by providing a forum for rigorously testing their products using an objective, third-party, nationally distributed testing program.

ACT conducted two customer needs and use assessments and held two workshops on the topic of in situ nutrient analyzers to evaluate current patterns of use, perceived limitations and what criteria are most used when selecting a nutrient analyzer system. The results of these assessments were used to identify the main applications and key parameters to be considered in this Technology Demonstration. The majority of respondents use (or plan to use) in situ nutrient analyzers to measure time-series nitrate and phosphate concentrations from remote moored platforms in nearshore environments. There was also interest in underway surface mapping and vertical profiling applications. The performance characteristics that ranked highest included reliability, accuracy and precision. This ACT Performance Demonstration focused on these applications and criteria utilizing a series of field tests at three of the ACT Partner Institution sites, representing marine, estuarine and freshwater environments. Protocols were developed with the aid of manufacturers and the Technical Advisory Committee (listed at www.act-us.info/tech_evaluations.php) to evaluate these specific areas. Complete needs and use assessment and workshop reports can be found at www.act-us.info/customer_needs.php.

PARAMETERS INVESTIGATED:

Field tests focused on reliability/stability and the ability of the instrument to track natural changes in nutrient concentrations. The following definitions were agreed upon with the manufacturers as part of the demonstration protocols.

- **Accuracy** – a measure of the closeness of an estimated value to the true value (see below). For this demonstration, the accuracy of the test instruments was determined in field tests by comparing the difference between the in situ instrument's determined nutrient concentrations and laboratory measured concentrations of collected reference water samples using approved analytical methods. Laboratory analyses followed approved standard operating procedures and were checked against external certified reference standards to ensure they represented the best

possible measure of the nutrient concentration. All laboratory analyses were run in triplicate to assess the precision of these reference measurements.

- **Reliability** – the ability to maintain integrity or stability of the instrument and data collections over time. Reliability of instruments was determined in two ways. In field tests, comparisons were made of the percent of data recovered versus percent of data expected. In addition, instrument stability was determined by pre and post measurement of blanks and reference standards 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.

SUMMARY OF DEMONSTRATION PROTOCOLS:

The testing protocols were based on an amalgamation of standard procedures for calibrating and testing nutrient analyzers provided by the participating manufacturers, and protocols recommended by ACT personnel and an external Technical Advisory Committee. A consensus was reached that the testing protocols would: (A) utilize standard, approved laboratory analytical methods at a single certified laboratory to provide the best measure of 'true' nutrient concentration for field and laboratory reference samples, (B) include month-long moored deployments in a wide range of coastal environments and (C) employ a wide geographic distribution of test sites with varying nutrient concentrations and water quality characteristics. As defined by the protocols, manufacturer representatives directly assisted in the initial set-up and calibration of the instruments, instrument retrieval, and data management.

Laboratory Based Nutrient Analysis

All nutrient concentrations for laboratory reference standards and field reference samples were determined by the Nutrient Analytical Services Laboratory (NASL) at the Chesapeake Biological Laboratory following their Standard Operating Procedures Manual (CEES, UMD, Publication Series No. SS-80-04-CBL). The nitrate method employed is based on U.S. EPA Method 353.2, *in* Methods for chemical analysis of water and wastes. (United States Environmental Protection Agency, Office of Research and Development. Cincinnati, Ohio. Report No. EPA-600-4-79-020 March 1979), but modified to use an enzymatic reduction of nitrate instead of the traditional cadmium reduction method (Campbell, W.H. E.R. Campbell, and L. Egan 2006. Green Chemistry Nitrate Determination: An Alternative Nitrate Analysis Method. American Laboratory, February 2006). In brief, nitrate in the sample is reduced enzymatically to nitrite in a buffered solution. The nitrite is then determined by diazotizing with sulfanilamide and coupling with N-1-naphthylethylenediamine dihydrochloride to form a color azo dye. The absorbance measured at 540 nm is linearly proportional to the concentration of nitrate + nitrite in the sample. Nitrate concentrations are obtained by subtracting nitrite values, which have been separately determined without the enzyme reduction procedure. A comparative study on the methods has been performed and documentation is available from NASL or ACT. Results of direct comparisons were highly linear ($r2 = 0.97$ or higher) for a range of concentrations spanning 0.05 to 15 mgN/L.

All laboratory nutrient analyses were conducted on an Aquakem 250. A statistically determined detection limit for this method has been established at 0.0007 mgN/L and 0.0006 mgN/L for nitrate and nitrite respectively, by prior laboratory studies for a wide range of salinities. The typical working concentration range for the nitrate method and SOP is between 0.0049 – 5.6 mgN /L. The typical working concentration range for the nitrite method and SOP is between 0.0042 – 0.28 mgN /L. A sample reagent blank was analyzed in conjunction with every sample and all internal standards were verified and calibrated using certified external nutrient standards. Additional internal QAQC samples including laboratory duplicates and nutrient recovery spikes were analyzed with each analytical batch.

Field Deployment

Field demonstration tests of instrument performance in a moored application were conducted at three ACT Partner Institution sites including Chesapeake Bay, Solomons, MD; Resurrection Bay, Seward, AK; and Clinton River, Mt. Clemens, MI. The same model instrument was tested at all three sites. At each test site the instrument was deployed at a fixed depth of 1 m over four weeks. Prior to deployment, the instrument was set up and calibrated as required at the field sites by a manufacturer representative. The manufacturer was allowed to select a sampling interval of up to a maximum of 2 hours based on instrument settings needed to allow continued operation over a 30 day deployment.

The YSI 9600 was programmed to record in situ concentrations at 1 h intervals over the entire deployment. The test instrument was delivered a zero (deionized, Type 1 laboratory water (DIW)) and mid-range (ca. 0.3 mgN/L) nitrate and nitrite reference standard (made from certified SPEX nutrient solutions) both before and after deployment as an estimate performance drift over time. A photograph of the instrument and its sample inlet was taken just prior to deployment and just after recovery to provide a qualitative estimate of the extent of biofouling accumulating on the instrument during the field tests. Finally, a sub-sample of the on-board standard solution was collected both immediately before and after the deployment period for independent analysis by CBL-NASL to help account for any possible accuracy offset and degradation of the standard over time.

A standard 2-L Van Dorn water sampler was used at each test site to collect field reference samples for laboratory nutrient analysis. Reference samples were used to examine instrument performance and stability over time. The sampling frequency was structured to examine daily to weekly variations in nutrient concentrations at the test site. Specifically once each week an intensive sampling event was conducted consisting of 4 consecutive samples spaced at two-hour intervals. For the remaining 4 days of the week water was sampled only once per day. Reference sample collections were planned to occur during sample uptake of the test instrument.

Ancillary Environmental Data

A series of ancillary data were collected during field deployments to help characterize the variation in water quality conditions during testing. At each of the mooring test sites a calibrated CTD, in situ fluorometer and transmissometer were attached to the test rack and positioned at the same depth as the deployed test instrument to provide a time series of conductivity, temperature, fluorescence and transmissivity measured at 15-minute intervals. Optical instruments were cleaned daily during the work week to remove bio-fouling. After cleaning, an in-air value was recorded to assure that the instruments were performing consistently throughout the test period.

Personnel at each test site either established a meteorological station, or identified one in the vicinity, that continuously recorded air temperature, humidity, directional wind speed and precipitation. In addition field observations of natural or anthropogenic disturbances, tidal state, water clarity, water depth and any obvious problems or failures with instruments were noted during each sampling event. Observations were recorded on sampling log sheets along with the exact date and time of reference sample collection. Ancillary data are provided to help understand the history of changes in ambient water quality conditions. These data were not used for any direct calibration, correction, or statistical comparison to the nutrient concentration test data.

Quality Assurance / Quality Control

The ACT Nutrient Demonstration was implemented according to the test protocols and technical documents (e.g. Standard Operating Procedures) prepared during the planning stages of the test. Prescribed procedures and a sequence for the work were defined and all work performed during the

Demonstration followed those procedures and sequence. All implementation activities were documented and are traceable to the test/QA plan, SOPs and to test personnel.

Four levels of QA/QC were applied to the sampling and analytical procedures for each field test. First, ACT provided the companies with a laboratory blank (DIW) and laboratory reference standard both before and after the field test deployment. All concentrations were confirmed by analysis at NASL. Secondly, ACT sub-sampled an aliquot of the on board standard that was present in the nutrient analyzer at the beginning and end of the test to verify that it matched with its stated value and to assess whether there was any degradation during the deployment. Thirdly, field trip blanks were collected once a week during mooring tests to test for any measurable contamination resulting from sampling and analytical protocols. Field trip blanks consisted of carrying DIW through all of the collection, processing, storage and analysis steps. Lastly nutrient spikes of field reference samples were performed once a week during mooring tests. Spikes were created by adding a known amount of certified standard to a known volume of filtrate of an existing field reference sample.

DEMONSTRATION RESULTS:

The YSI 9600 Nitrate Monitor was successfully tested under a fixed, surface (1m) mooring deployment of 4 week duration at two field sites that included Chesapeake Bay, MD and the Clinton River, Mt. Clemens, MI (Table 1). A software/communication problem between the instrument and its external power supply compromised a third attempted mooring test in Resurrection Bay, AK. No data will be presented from that test site within this report.

Table 1. The ACT Partners sites, dates, and basic physical/chemical conditions observed during the moored deployment field tests for the YSI 9600 Nitrate Monitor. Temperature and Salinity (or Conductivity for the MI test site) were determined by a CTD, relative fluorescence was measured with a fluorometer and transmissivity was measured with a 25cm path length transmissometer.

SITES		Temp. (ºC)	Salinity/ Conductivity (μS/cm)	Fluorescence (mV)	% Transmission
Chesapeake Bay, MD (5/16/07 - 6/12/07)	Min	17.0	9.8	31	6.2
	Max	25.8	11.9	2549	52.1
	Mean	21.3	10.9	713	27.5
Clinton River, MI (10/01/07 - 10/26/07)	Min	11.5	259	No data	No data
	Max	24.7	895	No data	No data
	Mean	17.2	496	No data	No data

Results are reported out by test site and summarize the time series of nutrient concentrations predicted by the YSI 9600 during the deployment compared against concentrations of reference sample determined by NASL. A comparison of results for external blanks and standards performed immediately before and after the deployment, plus initial and final on-board standards are also presented to help resolve potential calibration offsets in instrument predicted concentrations.

Moored Deployment Results in Chesapeake Bay, MD

The moored deployment test in Chesapeake Bay took place at the end of a fixed pier located at the mouth of the Patuxent River (Fig.1) on the campus on the Chesapeake Biological Laboratory. The average water depth of the test site was 2.2 m. The site was brackish with salinity ranging from 9.8 - 11.9, and water temperature ranged from 17.7 - 25.8. (Table 1). Distinct diurnal cycles were apparent in temperature and to a lesser extent salinity, but there were no sharp variations indicative of large meteorological events (Fig. 2). The site water was quite turbid (mean % beam transmission = 27) with significant algal concentration (mean fluorescence = 713 mV) (Table 1 and Fig. 2).

Figure 1. Coastal environment and field test site for the Chesapeake Biological Lab. The test instrument was deployed on a mooring frame constructed within an Easy Dock platform. Collection of reference samples occurred within the middle opening of the platform less than 1m apart from the instruments sample intake.

Nitrate + nitrite concentrations generally declined over the course of the test, ranging from a maximum of 0.19 mgN/L to a minimum of 0.03 mgN/L based on field reference sample measurements. Nitrite accounted for between 2 - 7 percent (mean = 4%) of the two species and nitrite results are not presented individually. Field trip blanks (N=4) averaged 0.0062 (± 0.0005) mgN/L, accounting for less than 6% on average of the field reference sample concentrations.

The YSI 9600 determined in situ concentrations closely followed the overall pattern of field reference sample concentrations throughout the deployment (Fig.3). A linear regression of directly compared instrument versus field reference sample concentrations shows a very strong correlation (R^2 = 0.93) (Fig. 4a). However, a comparison of the ratio of corresponding instrument versus laboratory measurements indicates that there was a consistent calibration offset, with YSI 9600 concentrations averaging around 60% of the field reference sample measured concentrations (Fig 4b). A time series plot of this ratio indicates that the accuracy of in situ predictions decreased slightly during the deployment. In contrast, there was no change in the relative accuracy of the predictions for the pre- and post- exposure to certified standards (see Table 2). It is possible that some of decrease in performance during time may have been due to the presence of intensive bio-fouling near the sample intake. However, there was a slight trend for the amount of this offset to increase over the deployment.

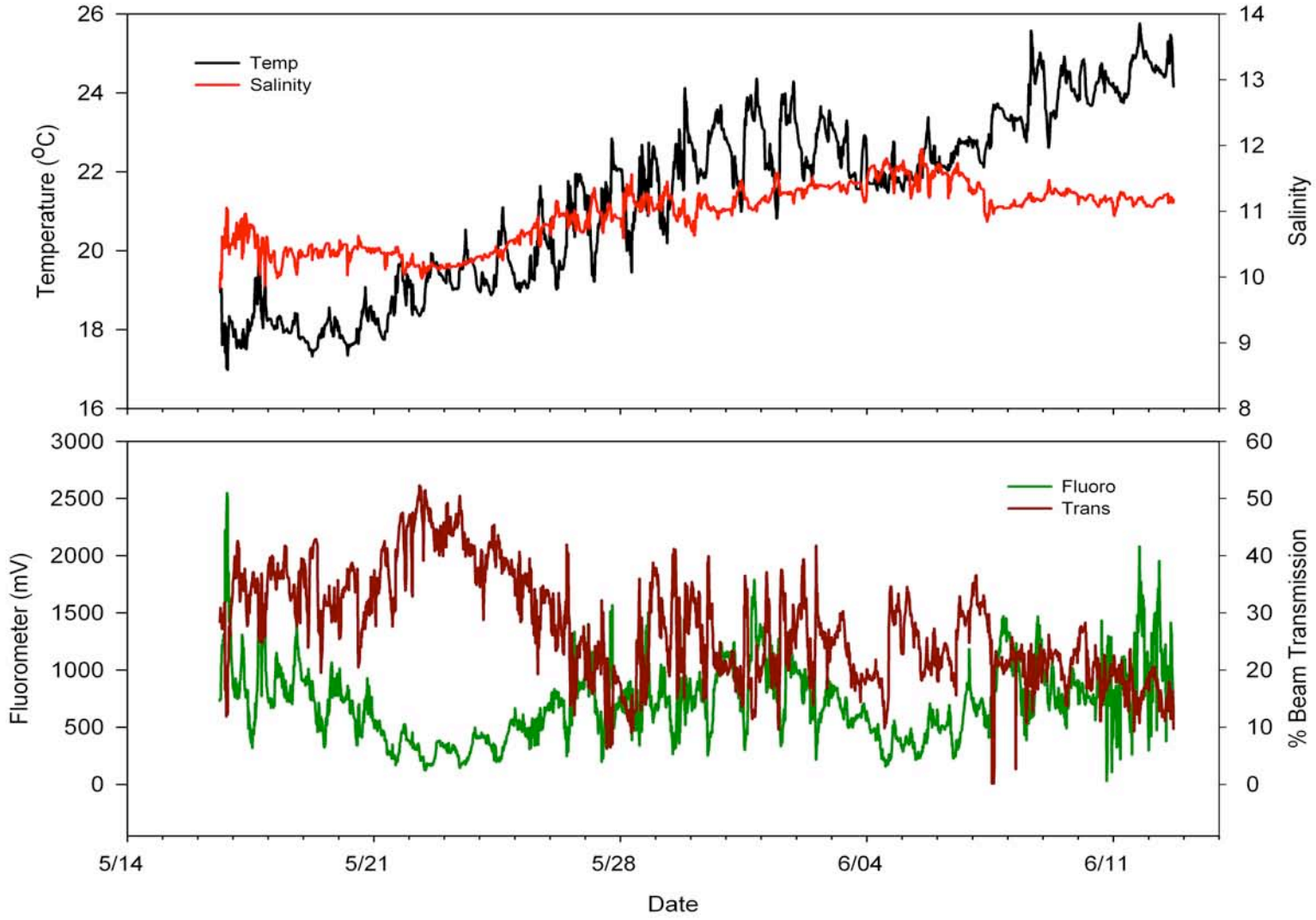


Figure 2. Ancillary data collected at the Chesapeake Bay, MD field test site describing conditions of temperature, salinity, algal fluorescence and water transparency.

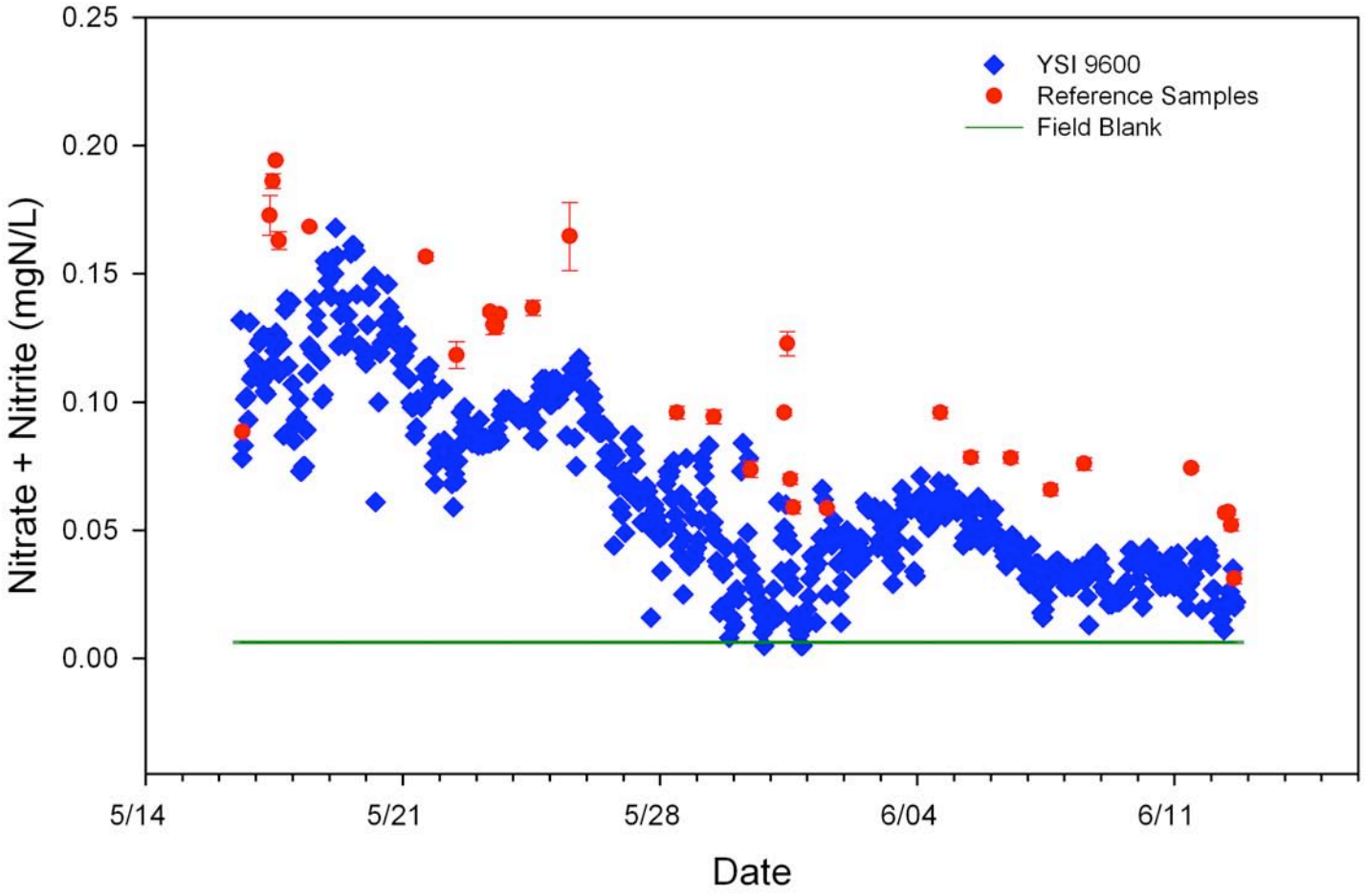


Figure 3. Time series comparison of YSI 9600 predicted nitrate+nitrite concentrations against laboratory measured reference samples and field trip blanks for the Chesapeake Bay moored deployment test. (Data from laboratory measurements represent mean ± standard deviation, n=3).

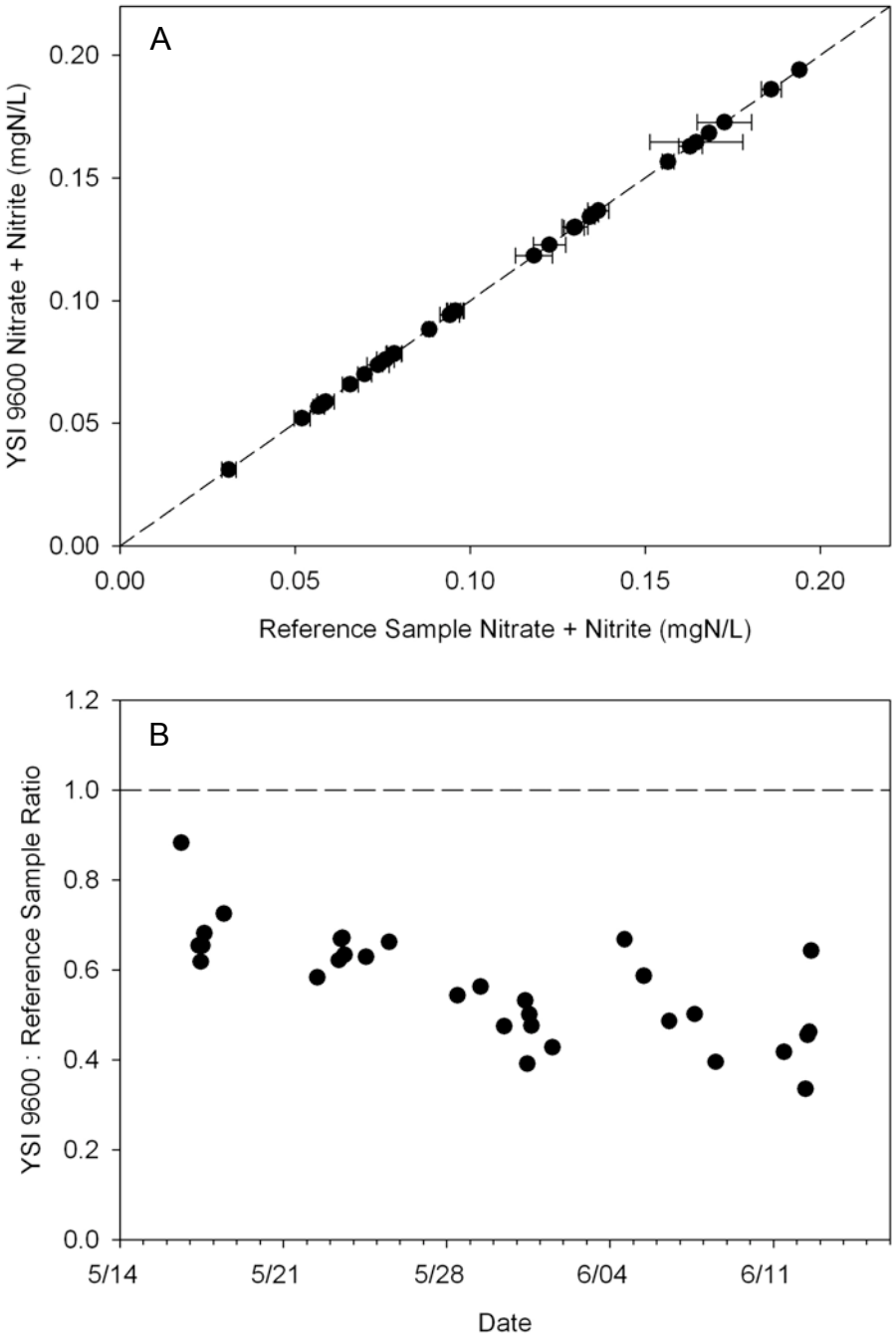


Figure 4. Analysis of test results from the Chesapeake Bay, MD test site. (A) A regression ($R^2 = 0.93$) of YSI 9600 predicted versus laboratory measured nitrate+nitrite concentrations for matching field reference samples. (B) Time series of the ratio of YSI 9600 predicted versus laboratory measured nitrate+nitrite concentrations for matching field reference samples.

Moored Deployment Results in Chesapeake Bay, MD (cont.)

A set of pre- and post-deployment exposures to blanks (DIW) and laboratory reference standards where completed back in the laboratory as part of each field test (Table 2). The YSI 9600 reported un-measureable nitrate levels on the blanks (slightly negative) for both the pre- and post-test exposures. The YSI measured concentration for a pure nitrate standard where 70% and 89% of the laboratory based measurement for the pre- and post-test exposures respectively (Table 2). This pattern is opposite the trend observed during the actual deployment where there was a decline in the ratio of instrument to reference sample concentration observed during the deployment itself. The YSI 9600 measured concentrations for a pure nitrite standard was 118% of the laboratory measured concentration. We were not able to report a post-deployment comparison for the nitrite standard due to a sample handling error. The difference in predicted concentration of the nitrate standard between the beginning and end of the deployment could suggest a change in the response or calibration factor of the YSI 9600 over time.

Table 2. Comparison of predicted blank, nitrate and nitrite values for pre-test and post-test exposure standards (mean, standard deviation for n=3 replicates for lab results; instruments reported between 1 - 3 results).

	Lab Result (mgN/L)	YSI 9600 Result (mgN/L)
Initial Lab Blank	0.0009 (0.0002)	- 0.003
Initial Nitrate Standard	0.2517 (0.0053)	0.177 (0.004)
Initial Nitrite Standard	0.2172 (.0078)	0.258 (0.002)
Final Lab Blank	0.0007 (0.0001)	- 0.002
Final Nitrate Standard	0.2371 (0.0056)	0.211 (.007)
Final Nitrite Standard	No data	No data

ACT also collected a sub-sample of the nutrient standard that was used within the instrument before and after deployment. The sample was handled and analyzed identically to all of the reference samples, with the exception that no filtration step was required. This value was compared against the reported concentration that was entered into the program of the YSI 9600 and used to predict in situ concentrations. The laboratory determined concentration of the on-board standard was 1.097 ± 0.018 mgN/L at the beginning of the test and 1.083 ± 0.010 mgN/L at the end of test indicating no significant change during the deployment (Table 3). The stated value of the standard used by the YSI 9600 to compute in situ nitrate concentrations was 1.0 mgN/L, which would be ca. 91-92% of the laboratory determined value.

Table 3. Comparison of onboard nitrate standard at beginning and end of field test as measured in the laboratory (mean, standard deviation for n=3 replicates) versus the stated 1.0 mgN/L concentration used by the YSI 9600 to predict in situ concentrations.

	Pre-test Lab Result (mgN/L)	Post-test Lab Result (mgN/L)
On-Board Standard	1.097 (0.018)	1.083 (0.010)

Lastly, nutrient spikes of field reference samples were performed once per week to examine the consistency of the NASL nutrient determinations (Table 4). The percent recovery for nitrate spikes ranged from 101 - 109 percent with a mean of 103.8 ± 3.7. The percent recovery for nitrite spikes ranged from 96 - 103 percent with a mean of 99.4 ± 2.7.

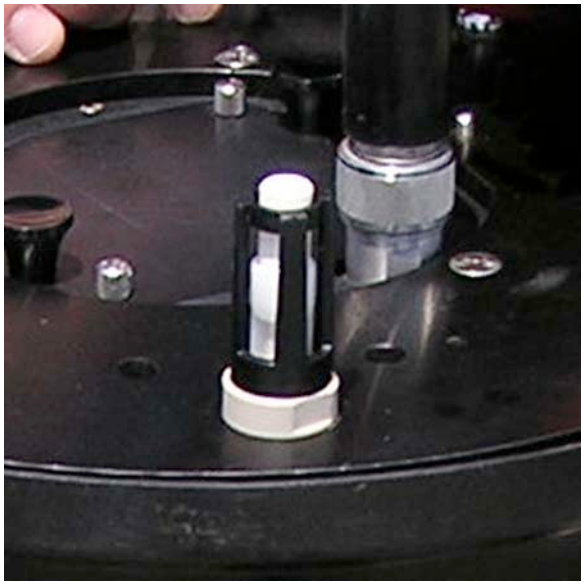
Table 4. Percent recovery of nitrate and nitrite added to field reference samples. Spikes were performed once each week at each of the test sites. All concentrations were determined on triplicates.

Week	Nitrate (% Recovery)	Nitrite (% Recovery)
1	102.5	96.3
2	109.3	98.7
3	101.4	99.9
4	101.9	102.8
Mean (std dev)	103.8 (3.7)	99.4 (2.7)

Instrument Photographs

Before and after photos were taken of the instrument package and its sample intake to examine the extent and possible impacts of bio-fouling (Fig. 5). A significant amount of 'hard' bio-fouling occurred at this test site, including on the sample intake but there was no indication of sample blockage during the test.

YSI 9600 canister prior to deployment



YSI 9600 sample inlet prior to deployment

YSI 9600 canister after the deployment

YSI 9600 sample inlet after the deployment

Figure 5. YSI 9600 Instrument Photos from Chesapeake Bay, MD before and after deployment

Moored Deployment Results in Clinton River, MI

The mooring test in Michigan took place at the end of a fixed pier located at the mouth of the Clinton River which drains into Lake St. Clair (Fig. 6). The water depth of the test site was 2.2 m. The site exhibited a fairly large fluctuation in conductivity, ranging from 300 - 900 uS/cm as shifting winds produce a varying mixture of river water and lake water and water temperature ranged from 11.5 - 24.7. (Table 1, Fig.7).

Figure 6. The Michigan field test site was located at the mouth of the Clinton River in Mt. Clemens, MI. The test instrument was deployed on a mooring frame attached to the end of a fixed pier. Collection of reference samples occurred at the same depth and less than 1m apart from the sample inlet. Samples were immediately filtered and frozen in the adjacent laboratory.

Nitrate + nitrite concentrations measured on field reference samples ranged from 0.51 - 2.7 mgN/L during the deployment. Nitrite accounted for only between 1 - 2 percent of the two species and is not presented individually. Field trips blanks averaged 0.009 (± 0.0009) mgN/L accounting for less than 1% on average of the field reference samples. The YSI 9600 in situ concentration measurements followed the overall pattern of concentration changes during events, as well as, changes over the entire course of the deployment (Fig.8). A regression of instrument versus field reference samples concentrations shows a very strong correlation ($R^2 = 0.89$)(Fig. 9a). There was a slight calibration offset, with predicted concentrations averaging around 83% of the reported lab measured concentrations (Fig 9b). The offset was consistent throughout the deployment and no decrease in performance was noted throughout the 26 day deployment. However on two occasions the YSI 9600 predictions dropped sharply and produced three values that deviated significantly from measured field reference samples (Fig 8, Fig. 9b).

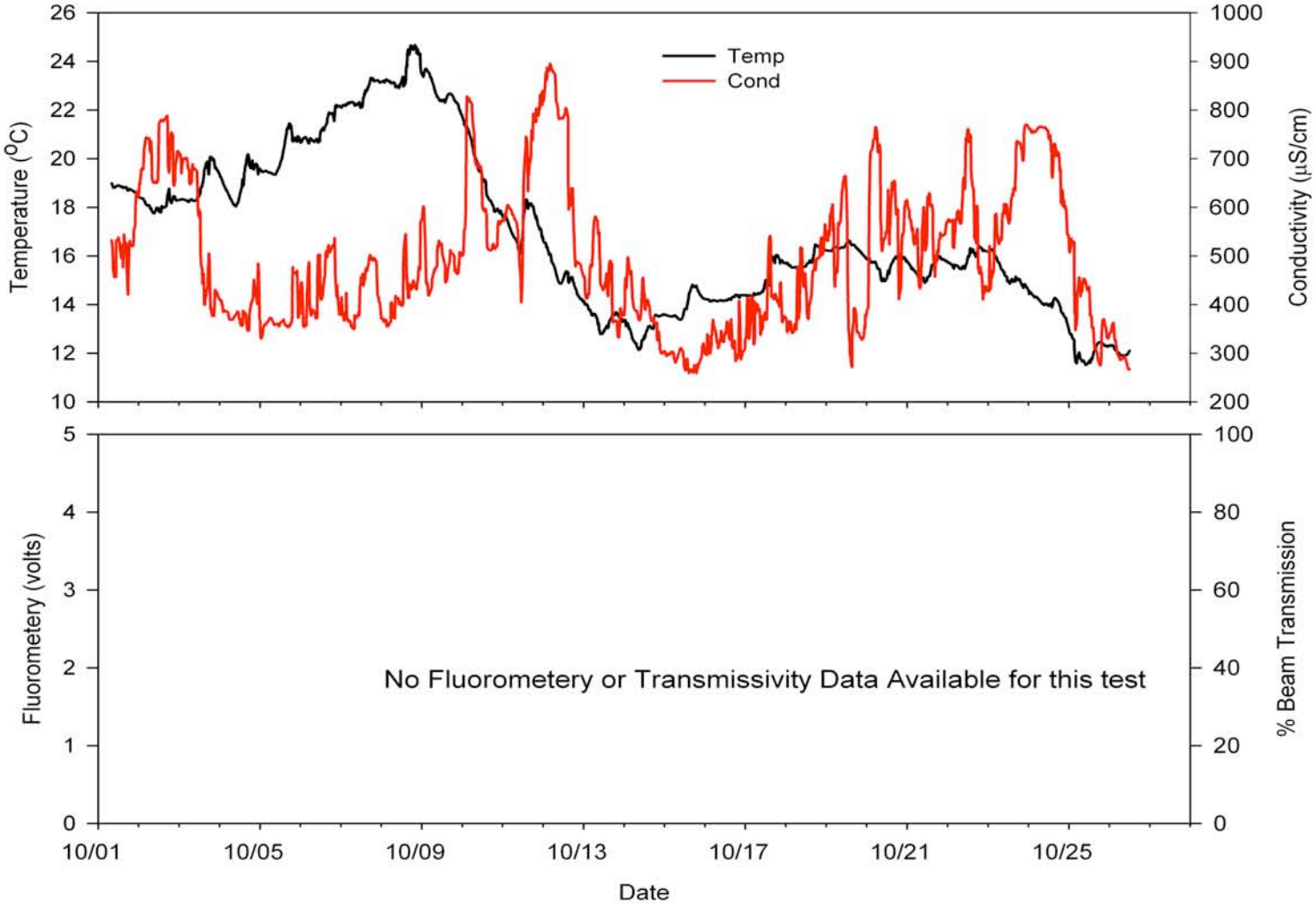


Figure 7. Ancillary data collected at the Clinton River, Michigan field test site describing conditions of temperature and conductivity. A data logging error resulted in the loss of the algal fluorescence and water transparency data.

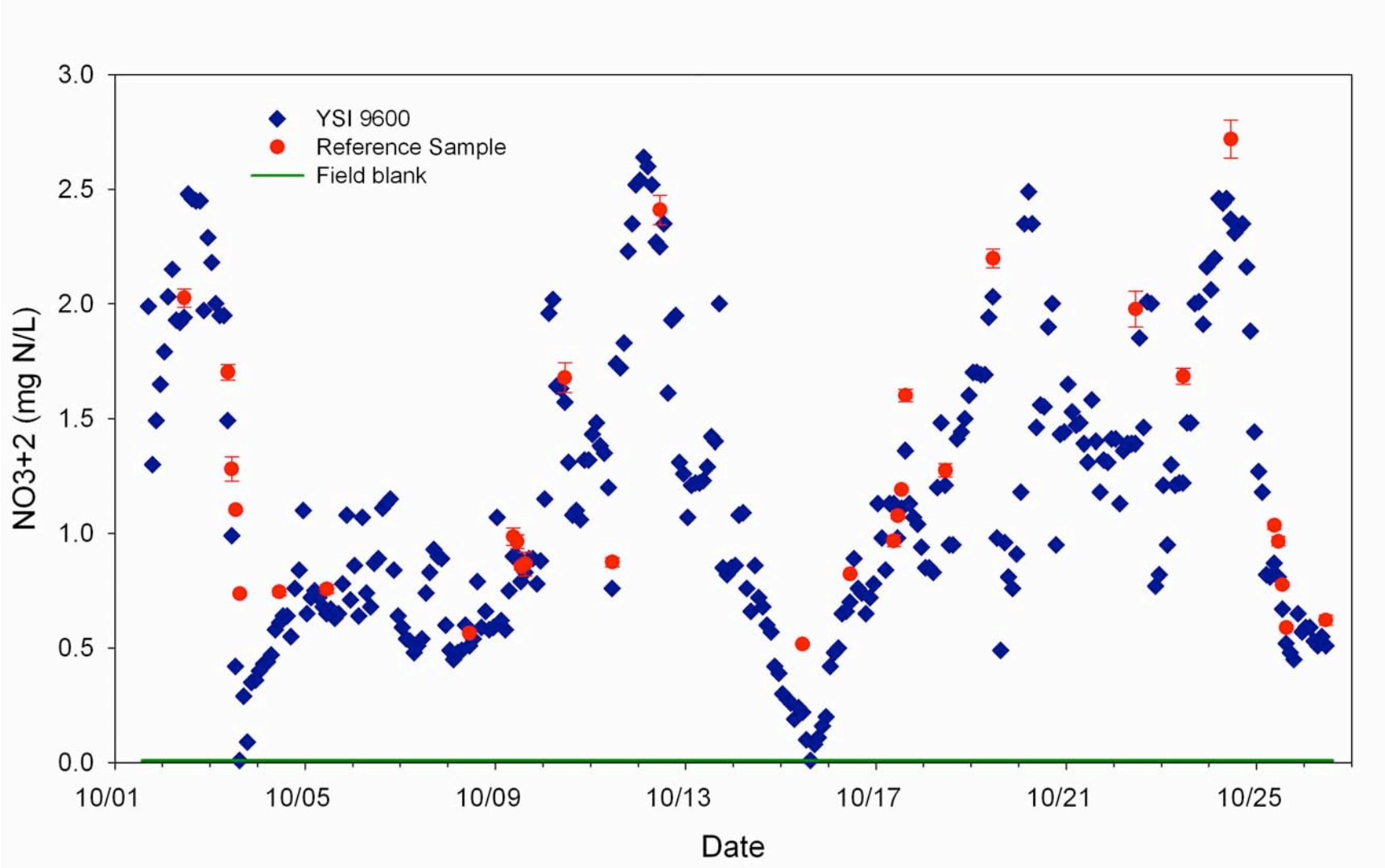


Figure 8. Time series comparison of YSI 9600 predicted nitrate+nitrite concentrations against laboratory measured field reference samples and field trip blanks for the Clinton River, MI moored deployment test. (Data from laboratory measurements represent mean ± standard deviation, n=3).

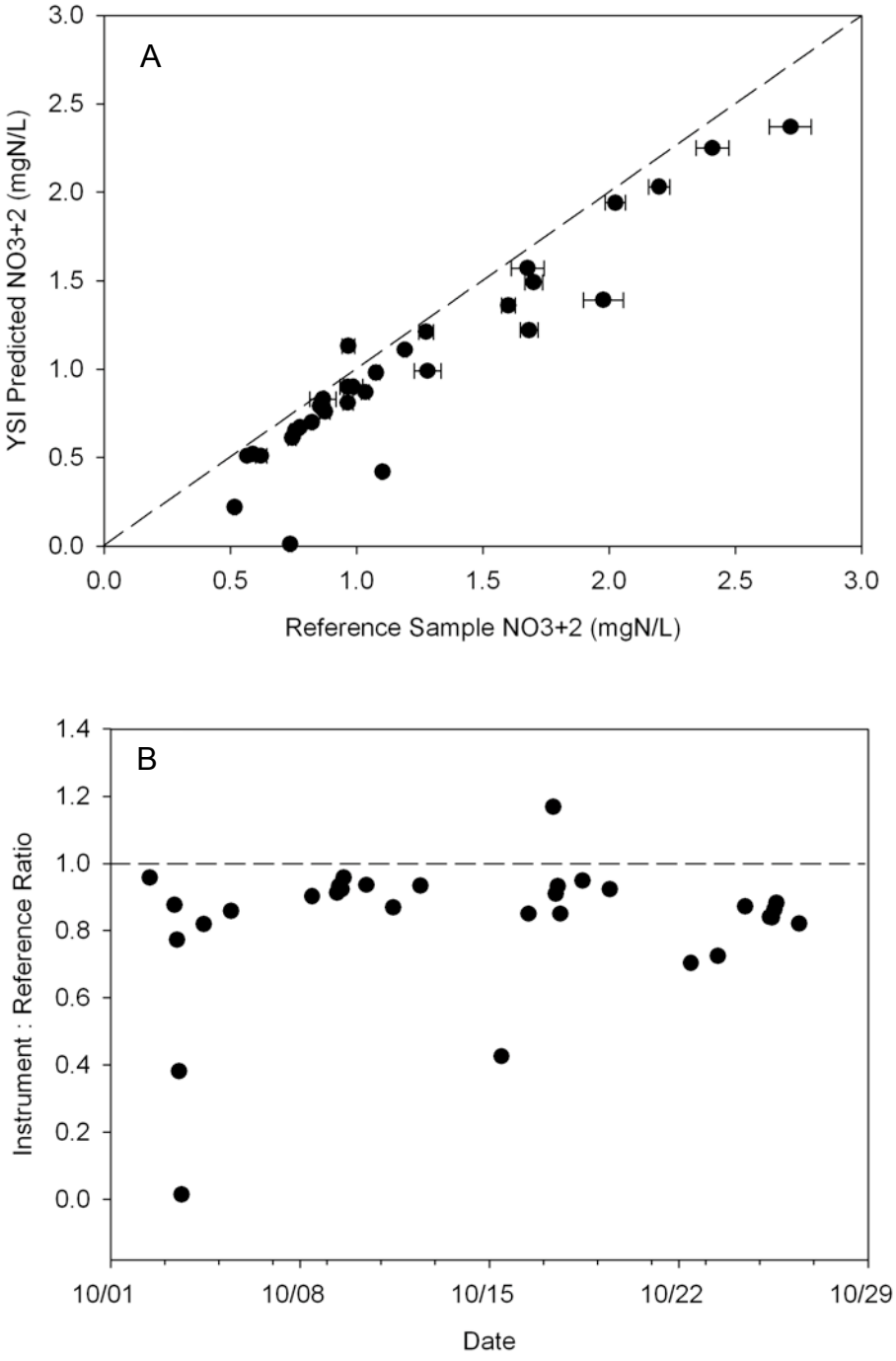


Figure 9. Analysis of test results from the Clinton River, MI test site. (A) A regression of YSI 9600 predicted versus laboratory measured nitrate+nitrite concentrations ($R^2 = 0.89$) for matching field reference samples. (B) Time series of the ratio of YSI 9600 predicted versus laboratory measured nitrate+nitrite concentrations for matching field reference samples.

Moored Deployment Results in Clinton River, MI (cont.)

A set of pre- and post- exposures to blanks (DIW) and laboratory reference standards where completed back in the laboratory as part of each deployment test (Table 5). An operating error with YSI 9600 resulted in no measurements of the blank and standards at the end of the deployment, even though the instrument was working at the time of retrieval. For the pre-deployment exposures, the YSI 9600 reported a slightly high value for the blank compared to the lab result. The predicted nitrate standard was 97 percent of the lab result and the predicted nitrite value was 146 percent of the measured lab value.

Table 5. Comparison of predicted nitrate and nitrite values for pre-test exposure standards (mean, standard deviation for n=3 replicates for the lab results; instrument reported only a single result). No results are available for the post-exposure test due to an operating error during the procedure.

	Lab Result (mgN/L)	YSI 9600 Result (mgN/L)
Initial Lab Blank	0.0007 (0.0000)	0.018
Initial Nitrate Standard	0.2411 (0.0128)	0.203
Initial Nitrite Standard	0.0970 (0.0002)	0.142

The stated concentration of the onboard standard used to predict in situ nitrate concentrations by the YSI 9600 was 2.0 mgN/L. The lab results for a pre- and post-test sample of this standard solution were 2.51 and 2.15 mgN/L, respectively (Table 6).

Table 6. Comparison of onboard nitrate standard at beginning and end of field test as measured in the laboratory (mean, standard deviation for n=3 replicates) versus stated value from manufacturer.

	Pre-test Lab Result (mgN/L)	Post-test Lab Result (mgN/L)
On-board Standard	2.5103 (0.0492)	2.1547 (0.0056)

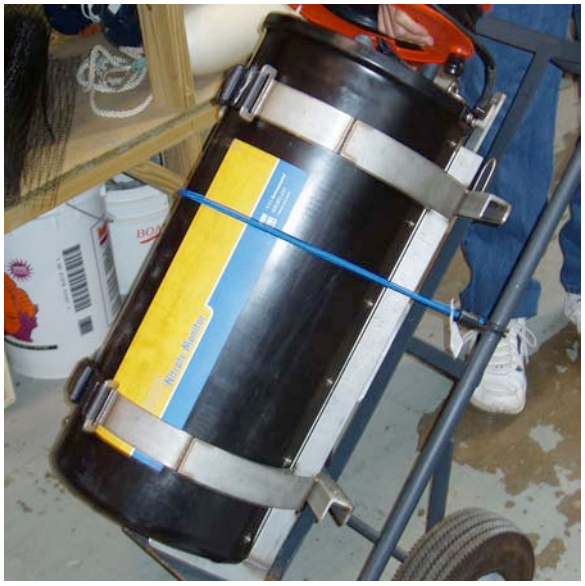
Lastly, nutrient spikes of field reference samples were performed once per week to examine the consistency of the NASL nutrient determinations (Table 7). The percent recovery for nitrate spikes during the MI test ranged from 95 - 103 percent with a mean of 97.9 ± 3.7. The percent recovery for nitrite spikes ranged from 95 - 101 percent with a mean of 99.0 ± 2.9.

Table 7. Percent recovery of nitrate and nitrite added to field reference samples. Spikes were performed once each week at each of the test sites. All concentrations were determined on triplicates.

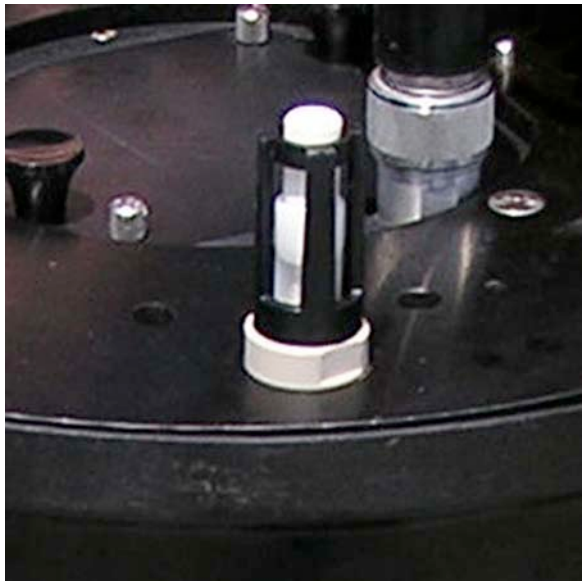
Week	Nitrate (% Recovery)	Nitrite (% Recovery)
1	95.5	100.7
2	94.5	100.9
3	102.7	99.6
4	98.8	94.8
Mean (std dev)	97.9 (3.7)	99.0 (2.9)

Instrument Photographs

Before and after photos were taken of the instrument package and its intake to examine the extent and possible impacts of bio-fouling (Fig. 10). No hard fouling occurred at this test site but evidence of high turbidity is seen on the filter of the intake after retrieval.



YSI 9600 prior to deployment



YSI 9600 intake prior to deployment

YSI 9600 after deployment

YSI 9600 intake after deployment

Figure 10. YSI 9600 Instrument Photos from Clinton River, MI before and after deployment

QUALITY ASSURANCE / QUALITY CONTROL:

Technical System Audits

Technical systems audits of the field work were conducted at the moored deployment test sites of Chesapeake Bay, MD (Chesapeake Biological Laboratory) on May 17, 2007 and at Resurrection Bay, AK (University of Alaska-Seward) on August 6, 2007, approximately 6 days after deployment. All steps of field work were observed, including water sample collection, ancillary environmental data, field log documentation, filtrations, handling and storage, blanks, sample preparation for transfer to NASL, and transmissometer and fluorometer cleaning. There were no significant negative findings at either site. One deviation was made at the Chesapeake Bay site. The protocols were revised with respect to the number of reference, field spike and blank reference samples collected – two additional vials were filled at each collection and held in reserve in a freezer in the laboratory for analysis if necessary. This revision was adopted for all subsequent field tests. In Alaska, meteorological data were not being collected at the site at the time of the audit due to malfunction of the meteorological sensor system, and data from the closest available site in Seward were recorded.

NASL nutrient analysis

With every analytical batch of field samples, NASL conducted internal laboratory checks on their accuracy and precision. QA performance checks included duplicate analysis of field samples, analytical spikes of field samples, internal lab standards, and external certified standards. A summary of the laboratory QA results for each of the test sites used in the Demonstration are presented in Table 8.

Table 8. Summary of NASL laboratory QA results during the analysis of nitrate+nitrite concentrations on reference samples from each of the ACT test sites. Data represent the mean and standard deviation of reported values for the number of observations given. Note: Test results for the YSI 9600 are only reported for the CBL and MI test sites.

Test Site	#	Lab Duplicates (% Diff)	#	Lab Spikes (% Rec)	#	Lab Stds (% Diff)	#	External Stds (% Diff)
MI	26	2.86 (2.38)	13	103.57 (3.84)	6	3.05 (1.37)	2	8.34 (1.94)
AK	24	4.64 (4.41)	17	104.24 (3.52)	10	2.83 (1.61)	2	7.95 (1.39)
MD	27	2.99 (3.84)	15	98.51 (3.67)	16	3.63 (3.14)	5	6.83 (5.66)

Reference Sample Analysis

The initial results of all reference samples nutrient concentration reported by NASL were evaluated statistically. Whenever the results of the triplicate measurements of reference samples produced a coefficient of variation greater than 15%, the test site sent the reserved 2 samples for re-analysis. The new values were added to the database and the three values from the five which gave the minimum standard deviation were selected to calculate the reference sample mean.

RELIABILITY:

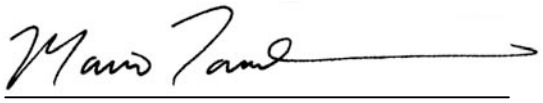
The YSI 9600 Nutrient Monitor was tested in a fixed mooring application at three different field sites including, estuary, coastal ocean, and riverine environments. Complete time series data were successfully retrieved for the Chesapeake Bay, MD and the Clinton River, MI tests. However, an operating error did occur at the MI test site when we the manufacturer attempted to run the post deployment laboratory reference standards. The error was apparently due to a software communication problem as the instrument was still functioning and the manufacturer was able to run standards successfully a day later on the same instrument back at their company. Lastly, a software/communication problem resulted in an unsuccessful test at Resurrection Bay, AK and no data was collected for that field test. In general, however, it appears that the fundamental technology has the capability to successfully measure in situ nitrate concentrations under a variety of field conditions.

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. Newton, C. D'Elia, L. Codispoti, P. Anderson, 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.

March 30, 2007

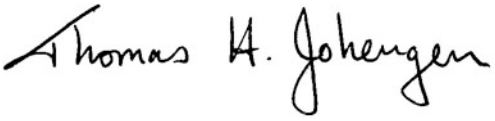
Date



Approved By: Dr. Mario Tamburri
ACT Executive Director

March 30, 2007

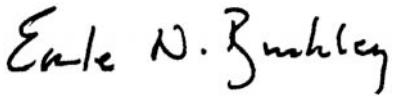
Date



Approved By: Dr. Tom Johengen
ACT Chief Scientist

March 30, 2007

Date



Approved By: Dr. Earle Buckley
Quality Assurance Supervisor